Aus der Klinik für Frauenheilkunde und Geburtshilfe
der Heinrich-Heine-Universitat Düsseldorf
Direktorin: Professor Dr. med. Tanja Fehm

Impact of Progesterone Receptor Membrane Component 1 on Lipid Metabolism and Oncogenic Signaling in Breast Cancer

Dissertation

zur Erlangung des Grades eines Doktors der Medizin
der Medizinischen Fakultät der Heinrich-Heine-Universität Düsseldorf

vorgelegt von
Hannah Asperger

2023

Als Inauguraldissertation gedruckt mit Genehmigung der
Medizinischen Fakultät der Heinrich-Heine-Universität Düsseldorf

gez.:

Dekan:	Prof. Dr. med. Nikolaj Klöcker
Erstgutachter:	Prof. Dr. rer. nat. Hans Neubauer
Zweitgutachter:	Prof. Dr. rer. nat. Björn Stork

Für meine Eltern

Parts of this work have been published:

H Asperger, N Stamm, B Gierke, M Pawlak, U Hofmann, U Zanger, A Marton, R Katona, A Buhala, C Vizler, J-P Cieslik, Z Kovacevic, D Richardson, E Ruckhäberle, D Niederacher, T Fehm, H Neubauer☯, M Ludescher☯
"Progesterone receptor membrane component 1 regulates lipid homeostasis and drives oncogenic signaling resulting in breast cancer progression." **Breast Cancer Research** 13.7 (2020): s13058-020-01312-8.

☯These authors contributed equally

I Summary

English

PGRMC1, a highly conserved heme binding protein, is comprehensively involved in cell signaling and metabolism and often upregulated in cancer. In breast cancer, PGRMC1 is associated with enhanced tumor growth, increased metastasis, therapy resistance and poor prognosis, which indicates the protein's role in carcinogenesis. Overexpression of PGRMC1 promotes proliferation in luminal A breast cancer cells, indicating that the mode of action of PGRMC1 depends on the ERα status of these cells. However, the mechanisms by which PGRMC1 drives tumor progression are not well understood. Previous research showed that PGRMC1 is involved in processes related to cholesterol and fatty acid synthesis, which are often disrupted during cancer progression. The aim of this study was to investigate the involvement of PGRMC1 in lipid homeostasis to detect new mechanisms by which PGRMC1 alters cancer metabolism and signaling. In an extension of our previous results on the interplay of PGRMC1 with SCD1 and FDFT1, we examined the interaction of PGRMC1 with CYP51A1 in different breast cell lines. In addition, we determined the mRNA and protein expression of essential genes involved in lipid metabolism by real-time RT-PCR and western blot analysis. To explore the functional impact of PGRMC1 overexpression on lipid homeostasis, IF-staining for lipid droplets followed by flow cytometry was used to assess cellular lipid content. Additionally, mass spectrometry was used to quantify levels of cholesterol and lathosterol. With regard to the role of cholesterol as a precursor for steroid hormones, we studied the effect of PGRMC1 on E2 levels and ERα downstream signaling among others using ELISA analysis, IF-staining and real-time RT-PCR. Supplementary, a possible dependence of cholesterol and E2 content on PGRMC1 phosphorylation-status was investigated. Moreover, the influence of PGRMC1 on lipid raft formation was analyzed via flow cytometry. Since many studies show that growth hormone receptors are enriched in lipid rafts, IF-staining was used to determine co-localization of HER2 and lipid rafts. Furthermore, alteration of EGFR/HER2 signaling through PGRMC1 overexpression was explored via western blot. Finally, the impact of PGRMC1 on cell viability upon depletion of cholesterol and fatty acids induced by simvastatin was investigated. This work demonstrated that PGRMC1 interacts with enzymes of the mevalonate pathway and alters the expression of pivotal proteins involved in lipid homeostasis, lipid uptake and lipid synthesis, possibly leading to higher levels of neutral lipids, cholesterol, steroid hormones and lipid rafts in HR+ breast cancer. Furthermore, PGRMC1 modifies ERα and EGFR signaling. Overexpression of PGRMC1 results in a greater sensitivity to a treatment with simvastatin, suggesting PGRMC1 as a target for lipid lowering therapeutic approaches. In summary, PGRMC1 may play an important role in the proliferation and progression of cancer by altering lipid metabolism and activating key oncogenic signaling pathways, such as ERα and EGFR signaling. This work underlines the potential of PGRMC1 as a target for anti-cancer therapy.

German

PGRMC1 ist ein hoch konserviertes, Häm bindendes Protein, das in unterschiedliche Signalwege und metabolische Vorgänge involviert und in Krebszellen hochreguliert ist. Im Mammakarzinom ist PGRMC1 unter anderem mit erhöhtem Tumorwachstum, verstärkter Metastasierung, Therapieresistenz und einer schlechten Prognose assoziiert. Überexpression von PGRMC1 steigert die Zellproliferation im Mammakarzinom des Luminal A Subtyps, was darauf hinweist, dass die Wirkungsweise von PGRMC1 zumindest teilweise mit dem Hormonrezeptorstatus der Zelle zusammenhängt. Die Mechanismen, durch die PGRMC1 die Tumorprogression vorantreibt, sind jedoch nach wie vor unklar. Ziel dieser Arbeit ist es, Funktionen von PGRMC1 im Bereich der Lipidhomöostase zu identifizieren und neue Prozesse zu erschließen, mittels derer PGRMC1 den Krebsstoffwechsel und die Signalübertragung verändert. In Fortsetzung unserer früheren Arbeiten zur Rolle von PGRMC1 im Mevalonatweg wurde die Interaktion von PGRMC1 mit CYP51A1 in verschiedenen Zelllinien untersucht. Außerdem wurde die mRNA- und Protein-Expression von wichtigen Genen des Lipidstoffwechsels mittels real-time RT-PCR und Western Blot in Brustkrebszellen bestimmt. Um funktionelle Auswirkungen der PGRMC1-Überexpression auf die Lipid-Homöostase zu ermitteln, wurde der zelluläre Lipidgehalt mittels Durchflusszytometrie analysiert und der Cholesterin- und Lathosterolgehalt mittels Massenspektrometrie quantifiziert. Im Hinblick auf die Rolle von Cholesterin als Vorläufer für Steroidhormone wurde die Wirkung von PGRMC1 auf den E2-Spiegel und die ERα-Signalgebung unter anderem durch ELISA-Analysen, IF-Färbung und real-time RT-PCR untersucht. Verschiedene Studien konnten die regulierende Wirkung unterschiedlicher Phosphorylierungen von PGRMC1 auf dessen Funktionsweise zeigen, sodass im Weiteren untersucht wurde, ob der zelluläre Cholesterin- und E2-Gehalt mit dem Phosphorylierungsstatus von PGRMC1 zusammenhängt. Der Einfluss von PGRMC1 auf die Bildung von Lipid Rafts wurde anschließend mittels Durchflusszytometrie beurteilt. Da Wachstumshormonrezeptoren häufig in Lipid Rafts angereichert sind, wurde die Co-Lokalisation von HER2 und Lipid Rafts mit Hilfe von IF-Färbungen ermittelt. Außerdem konnte gezeigt werden, dass PGRMC1 die EGFR/HER2-Signalübertragung im Mammakarzinom verändert. Anknüpfend wurde die Auswirkung von PGRMC1 auf die Viabilität von Brustkrebszellen im Rahmen einer Simvastatin-Behandlung analysiert. Zusammenfassend konnte im Rahmen dieser Arbeit gezeigt werden, dass PGRMC1 mit Enzymen des Mevalonatstoffwechsels interagiert und die Expression von Schlüsselproteinen der Lipidhomöostase, Lipidaufnahme und Lipidsynthese verändert, was verbunden ist mit höheren zellulären Spiegeln von neutralen Lipiden, Cholesterin, Steroidhormonen und Lipid Rafts im ER+ Mammakarzinom. Darüber hinaus verändert PGRMC1 die Signalübertragung von ERα und EGFR. PGRMC1-Überexpression erhöht die Empfindlichkeit gegenüber der Behandlung mit Simvastatin, sodass PGRMC1 als Target für lipidsenkende Therapieansätze im Rahmen multimodaler Behandlungsregime in den Fokus rückt.

II List of abbreviations

AC	adenylate cyclase
ACC	acetyl-CoA carboxylase
ACLY	ATP Citrat Lyase
ACSL	acyl-CoA synthetase long chain family
AKT	protein kinase B
APOE	apolipoprotein E
ATF	activating transcription factor
ATM	ataxia telangiectasia mutated
ATP	adenosine triphosphate
BDNF	brain derived neurotrophic factor
BMI	body mass index
BRCA	breast cancer gene
CaMK	calcium/calmodulin-dependent protein kinase
CCND	cyclin D
CD	cluster of differentiation
CE	cholesteryl ester
CI	confidence interval
CoA	coenzyme A
CRE	cAMP response element
CREB	cAMP response element-binding protein
CYP	cytochromes P450 superfamily of enzymes
CYP51A1	lanosterol 14 alpha demethylase
cytb5	cytochrome b5
DAG	diacylglycerol
DAP	DAmage resistance protein
DFS	disease free survival
DNA	deoxyribonucleic acid
E2	17β-estradiol
EGF	epidermal growth factor
EGFR	epidermal growth factor receptor
ELISA	enzyme linked immunosorbent assay
EMT	epithelial mesenchymal transition
ErbB	protein receptor family

ERE	estrogen response element
ERK	extracellular signal regulated kinase
ERα	estrogen receptor alpha
ESR	estrogen receptor
EVC	protein mutated in chondroectodermal dysplasia Ellis-van Creveld syndrome
FA	fatty acid
FABP	fatty acid binding protein
FAD	flavin adenine dinucleotide
FADH	flavin adenine dinucleotide
FAK	focal adhesion kinase
FASN	fatty acid synthase
FAT/CD36	fatty acid translocase
FATP	fatty acid transport protein
FDFT1	farnesyl diphosphate farnesyltransferase 1
FFA	free fatty acid
FPP	farnesyl pyrophosphate
GGPP	geranylgeranyl pyrophosphate
GLUT	glucose transporter 1/4
GM1	monosialotetra-hexosylganglioside
GPCR	G protein-coupled receptor
GTP	nucleotide guanosine triphosphate
HCT	hydroxycinnamoyl-transferase
HEPG2	human liver cancer cell line
HER	human epidermal growth factor receptor
HK	hexokinase 1/2
HMG	3-hydroxy-3-methylglutaryl
HMGCR	3-hydroxy-3-methylglutaryl-CoA reductase
HMGCS	hydroxymethylglutaryl-CoA synthase
HR+	hormon receptor positive
HR-	hormon receptor negative
IL	interleukin
iNOS	cytokine inducible nitric oxide synthase
INSIG	insulin induced gene

IP3	inositol-1,4,5-trisphosphate
IR	insulin receptor
JAK	Janus kinase
kDa	kilodalton
Ki-67	marker of proliferation
LCFA	long chain fatty acids
LDL	low density lipoprotein
LDLR	low density lipoprotein receptor
LR	lipid raft
LTED	long term estrogen deprivation
MAPR	membrane associated progesterone recept
MCF7	epithelial cells isolated from metastatic adenocarcinoma breast tissue
MDA	epithelial, human breast cancer cell line
MEK	mitogen activated protein kinase kinase 1/2
mRNA	messenger RNA
mTOR	mammalian target of rapamycin
MTT	thiazolyl blue tetrazolium bromide
NAD	nicotinamide adenine dinucleotide
NADH	nicotinamidadenin-dinukleotid dehydrogenase
NF-kB	nuclear factor kappa-light-chain-enhancer of activated B cells
nm	nanometre
nM	nanomolar
NSCLC	non small cell lung carcinoma
OS	overall survival
p	p-value/ probability value
P4	progesterone
P450	cytochrome P450 enzyme superfamily
P53	cellular tumor antigen p53
PARP	poly ADP ribose polymerase
PC	phosphatidylcholin
PCR	polymerase chain reaction
PD-1	programmed cell death protein 1

PDK1	phosphoinositide dependent kinase 1
PGR	progesterone receptor
PGRMC1	progesterone receptor membrane component 1
PI3K	phosphoinositide-3-kinase
PIP2	phosphatidylinositol-4,5-bisphosphate
PIP3	phosphatidylinositol-3,4,5-trisphosphat
PKA	protein kinase A
PKB	protein kinase B
PKC	protein kinase C
PLA	proximity ligation assay
PLCγ	phospholipase C
PPAR	peroxisome proliferator activated receptor
PPRE	peroxisome proliferator responsive element
PR	progesterone receptor
PSD	phophosite database
PTEN	phosphatase and tensin homolog
PTM	post translational modification
RAF	proto-oncogene serine/threonine-protein kinase
RAS	GTPase protein family related to rat sarcoma virus
ROS	reactive oxygen species
RR	relative risk
RTK	receptor tyrosine kinase
S, G1, G2, M	phases of the cell cycle Synthesis, Gap1, Gap2, Mitosis
S6	S6 ribosomal protein
SCAP	sterol regulatory element binding protein cleavage activating protein
SCD	stearoyl-CoA desaturase
SERD	selective estrogen receptor degrader
SERM	selective estrogen receptor modulators
SFA	saturated fatty acids
SH	src homology 2/3

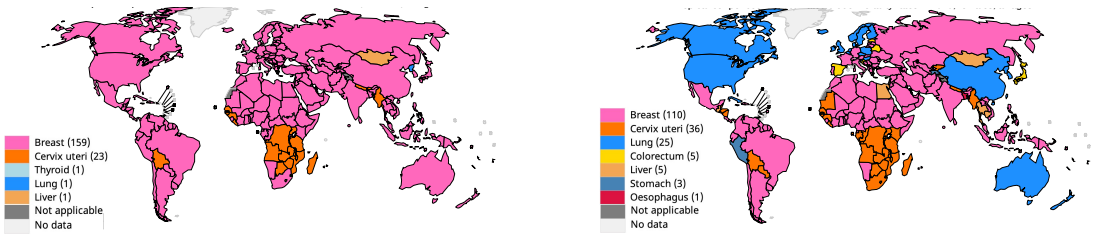
siRNA	small interfering RNA
SRC	proto-oncogene tyrosine protein kinase
SREBF	sterol regulatory element binding transcription factor
SREBP	sterol regulatory element binding protein
STAT	signal transducer and activator of transcription 1/3
T47D	epithelial cells isolated from a pleural effusion in infiltrating ductal carcinoma of the breast
TAG	triacylglycerol
TCA	tricarboxylic acid cycle
TCF/LEF	T cell factor/lymphoid enhancer factor family
TF	transcription factor
TFRE	transcription factor regulatory element
TGF-β	transforming growth factor beta
TMEM	transmembrane protein family
TNBC	triple negative breast cancer
TNF	tumor necrosis factor
TNM	classification system of malignant tumors
UFA	unsaturated fatty acids
VEM-1	mammalian ventral midline antigen
VLDL	very low density lipoprotein
VLDLR	very low density lipoprotein receptor
WHO	World Health Organization
WNT/β-catenin	group of signal transduction pathways
xCT	cystine/glutamate transporter
µM	micromolar

III Index

1 Introduction

1.1 Breast Cancer Epidemiology

Despite the inevitable progress of scientific research, cancer is still one of the most dreaded diseases and its diagnosis and treatment pose major challenges for many physicians and scientists. The WHO estimates that approximately 10 million deaths in 2020 were due to malignancies, making cancer a leading cause of death worldwide [1,2]. While in countries with poor medical care and nutrition (mainly due to infectious diseases spread by deficient sanitation and drinking water) and a lack of preventative health services like vaccines, people are at greater risk of developing certain types of cancer like malignant tumors of the liver, stomach and cervix, higher-income countries tend to have higher rates of prostate, breast and colorectal cancer [1]. With about 2.3 million cases and 685,000 deaths in 2020, breast cancer has surpassed lung cancer to become the most common form of cancer and also the leading cause of death from cancer globally in women (Figure 1) and it is even estimated that case numbers will reach 4.4 million in 2070 [1,3]. The annual increase of breast cancer incidence by about 0.5%, among other things, is due to the growing proportion of older people in first world countries, the ongoing decline in fertility rates and the increase in obesity in affluent societies [4].



(a) (b)

Figure 1: **Top cancer per country in females**
Estimated crude rates of **(a)** incidence and **(b)** mortality, worldwide in 2020.
Data source: GLOBOCAN 2020, Graph production: Cancer Today © International Agency for Research on Cancer 2021

While breast cancer is the most common malignant tumor in women, it is also one of the tumor types for which the best screening and treatment options exist. The 5 year relative survival rate in breast cancer is about 90% in high-income countries (67% for all cancer types); survival is only higher for prostate cancer (98%) and melanoma of the skin (93%) [1]. Since there are more lost disability-adjusted life years (DALYs) by women to breast cancer than any other cancer type,

and women in all countries and at all ages can be affected, many researchers explore cancer development, e.g., by focusing on molecular, clinical, or social epidemiological processes in an effort to improve diagnostics and treatment.

1.2 Breast Cancer Risk Factors

Breast cancer is influenced by numerous factors including demography, heredity, lifestyle, cancer subtype, reproductive and hormonal history, pre-existing diseases, breast histology, and environment. Although especially external factors account for the increase in breast cancer incidence, the risk factors with the largest impact on breast cancer are gender and age. Thus, breast cancer primarily affects menopausal women; even so, younger women tend to have larger and more aggressive tumors. Approximately 1% of all cases occur in men. Hormonal imbalance, exposure to radiation, family history of breast cancer, and mutation of the BRCA2 gene are some of the major risk factors associated with male breast cancer [5]. Furthermore, hormonal regulation plays a significant part in cancer development. Accordingly, younger age during menarche, age of menopause over 50 years, nulliparity or first childbirth in older age, and different pregnancy characteristics (e.g., first pregnancy placental abruption or preterm delivery) increase the risk of developing breast cancer [6,7,8,9]. The importance of hormonal regulation in carcinogenesis is also evidenced by many studies working out the link between breast cancer risk and hormonal therapy, e.g., in the form of contraceptive pills [10,11], ovulation-stimulating drugs [12], or postmenopausal hormone replacement therapy [13]. Another detrimental factor regarding breast cancer development and prognosis is a history of radiation or chemotherapy exposure [14,15]. While the majority of breast cancer cases are due to acquired somatic genetic and epigenetic alterations, about 10% of all breast cancers have a hereditary condition, caused by inherited germline mutations in high-penetrance, moderate-penetrance, and low-penetrance breast cancer susceptibility genes [16]. Family anamnesis plays a decisive role in tumor development and progression [17]. While several genetic alterations result in an increase in breast cancer incidence and mortality, about 40% of hereditary breast cancer cases are related to mutations in the tumor suppressor genes BRCA1 and BRCA2, and by the age of 80 the cumulative risk amounts to 72% for BRCA1 and 69% for BRCA2 carriers [18,19]. Furthermore, many studies have addressed how lifestyle factors like alcohol consumption, smoking, diet and nutrition, physical activity, and Vitamin D intake affect breast cancer risk [20,21,22,23,24].

1.3 Obesity and Breast Cancer

Obesity has been recognized as a major issue for breast cancer development, outcome and management. The WHO has defined that obesity as measured by the individually calculated BMI is an excess accumulation of adipose tissue and divided in class I-III with a BMI of 30 or more at baseline [25]. With 600 million obese adults worldwide, the health implications of obesity are becoming a major concern in both developed and developing countries [26]. Obesity is the most important modifiable risk factor especially for HR+ breast cancer in post-menopausal women [27]. In contrast, some studies and meta-analyses indicate no change or even a decrease in breast cancer risk for overweight or obese pre-menopausal women [27,28,29,30]. Nevertheless, different studies show that high weight during adulthood increases post-menopausal breast cancer risk and obesity at diagnosis of early pathogenesis correlates with reduced breast cancer survival also in pre-menopausal women [31,-32,33]. In comparison to women with normal weight, relative risk for breast cancer is increased by 12% in overweight women (BMI 25–29.9kg/m^2) and even by 16% in obese women (BMI $\geq$ 30kg/m^2) [29]. Interestingly, post-menopausal hormone replacement therapy attenuates the relationship between obesity and breast cancer development, possibly by reducing adipose tissue estrogen production [34]. With reference to hormone replacement, the Women's Health Initiative demonstrated that estrogen–progestogen preparations increase the risk of post-menopausal breast cancer compared with estrogen alone [35,36].

1.4 Breast Cancer Classification and Therapy

Breast cancer is extremely heterogeneous and can be classified in many ways, e.g., based on clinical behavior, histology, or biological features. Since recent therapeutic approaches build on molecular biological tumor characteristics, molecular stratification, e.g., regarding genomic drivers, is crucial for effective clinical management. One commonly used classification relies upon molecular biological categorization based on gene expression profiles (Figure 2). This system classifies breast cancers into four subtypes, mostly based on HR and HER2 status [37,38]. By using the Ki-67 labeling index assessed by immunohistochemical assays, breast cancer subtypes can be further graduated. Ki-67 was identified in the early 1980s as a nuclear antigen associated with cell proliferation. Except from G0 phase, it is expressed in certain phases of the cell cycle namely S, G1, G2, and M phases [39,40].

Subtype	ER	PR	HER2	Ki-67
Luminal A	+	+/-	-	<14%
Luminal B	+	+/-	+/-	$\geq$ 14%
Non luminal, HER2+	-	-	+	$\geq$ 14%
Triple negativ	-	-	-	$\geq$ 14%

Figure 2: **Molecular breast cancer subtypes**
Breast cancer can be subtyped into four distinct subtypes: luminal A, luminal B, non luminal (HER2+) and triple negative breast cancer. This is based on (IHC-)expression levels of Ki-67 and ER, PR, and HER2 status [37,41].

The above classification addresses molecular heterogeneity of breast tumors in accordance with their clinical and pathological characteristics and provides prognostic and predictive information for specific therapies. While the more differentiated luminal A tumors are often associated with lobular histology, luminal B tumors mostly present as invasive ductal carcinoma. Tumors with high expression of HER2 comparatively exhibit larger tumor size and higher rates of nodal metastasis. Compared to other subtypes of breast cancer, the most undifferentiated triple negative breast cancers (TNBC) tend to occur at a younger age and, in Black or Hispanic women, present as medullary or metaplastic carcinoma, are larger in size (but with less nodal metastasis), and show a high proliferative index [42,43]. In a descending order from above classification, molecular subtypes also have a different locoregional and systemic recurrence pattern. Luminal A, the subtype with the lowest rate of relapse, often recurs in the bones or soft tissues. In contrast the non-luminal types frequently metastasize to visceral organs. There is also a high proportion of bone metastasis in luminal A and HER2-enriched tumors. While TNBC often present with lung metastasis, they rarely metastasize to bones. The central nervous system is a preferred site of recurrence for HER2-enriched tumors. Luminal A subtypes are associated with longer DFS and OS than the more proliferative and less differentiated other subtypes [42,43].

Treatment options are determined by different factors including the tumor specific molecular signature, TNM stage, and grading. Treatment for nonmetastatic cancer consists of local therapy in form of surgical resection, removal of axillary lymph nodes, and, when required, radiation and systemic therapy, which can be neoadjuvant, adjuvant, or both. Based on the molecular subtype of tumors, standard therapy comprises endocrine treatment (e.g., aromatase inhibitors for women after menopause, SERMS such as tamoxifen, or SERDs such as fulvestrant), HER2-directed antibody therapy (e.g., trastuzumab) and conventional chemotherapy (e.g.,

combinations like cyclophosphamide, doxorubicin and, where appropriate, docetaxel or cyclophosphamide, methotrexate, and fluorouracil). While HR+ breast tumors undergo endocrine treatment, the only effective treatment for TNBC is chemotherapy [44]. Presently, metastatic breast cancer has usually no curative treatment options, so that achieving symptom palliation and prolonging life are primary treatment goals. Systemic therapy of metastatic breast cancer typically follows the same principles as non-metastatic breast cancer. Surgery and radiation only see use for palliation. In addition to conventional therapies, newer types of treatment are targeted toward a tumor's unique genetic code. Examples of such therapies include inhibition of PARP enzyme, e.g., by olaparib specifically in BRCA-deficient cells used for treatment of refractory metastatic breast cancer [45] and checkpoint inhibitor immunotherapy, e.g., the PD-1 antibody pembrolizumab, for tumors with mismatch repair deficiency or high microsatellite instability [46].

1.5 Breast Cancer Signaling

Deregulation of growth and survival signaling are key factors in breast cancer development, progression, and metastasis. Many different signaling pathways contribute to the heterogeneous pathogenesis of breast tumors including ATM, ERK, P53, PI3K/AKT, PPAR, PTEN, hedgehog and Wnt/β-catenin. Furthermore, different cytokines and inflammatory pathways are modified in cancer cells, e.g., NF-kB, TGF-β, TNF, iNOS, IL6, IL9 and IL15 [47,48,49,50,51,52,53]. The ERα along with the receptor tyrosine kinase signaling are two signaling cascades frequently altered in breast cancer (Figure 3).

1.5.1 ERα Signaling

The highly conserved nuclear HR is involved in a variety of functions in different organs, e.g., as estrogen dependent inhibition of apoptosis and necrosis in cardiac and endothelial cells or modification of glucose and lipid metabolism in hepatocytes [54,55]. ERα activation occurs primarily when the main estrogen hormone – 17β-estradiol (E2) – binds to the globular ligand binding domain of the ERα containing a hormone binding site, dimerization interface and section for coactivation or repression, leading to a translocation of both to the nucleus. The E2-ERα complex, on the one hand, can further bind to estrogen response elements (EREs) in the DNA, leading to the transcription of specific genes or, on the other hand, can recruit coactivator complexes, resulting in chromatin remodeling via acetylation of histones [56]. Since the ligand binding domain exhibits a wider ligand cavity, not only can E2 activate the receptor but also, e.g., other hormones, metabolic molecules, and synthetic structures [57,58]. There are distinct mechanisms for ERα activation even in

the absence of E2 [58,59]. For example, cross coupling of cellular kinases and ERα signaling results in phosphorylation of the ER on serine or/and tyrosine residues [60,61,62].

1.5.2 ErbB Signaling

The ErbB family of receptor tyrosine kinases includes a total of four members: ErbB1/EGFR, ErbB2/HER2, ErbB3, and ErbB4 [63]. These members form homo- and heterodimeric cell-surface receptors with special extracellular domains that confer individual ligand-binding specificity, leading to downstream signaling via tyrosine phosphorylation [64]. Various polypeptide hormones containing a 6-kDa domain which is homologous to EGF are able to modulate ErbB activation and signaling, including amphiregulin, betacellulin, EGF, epiregulin, neuregulin (neuregulin-1, -2, -3, and -4), and TGF-α [65]. Unlike other ErbB receptors, HER2 does not have known ligands and is activated either by heterodimerization with ligand bound EGFR, ErbB3, or ErbB4 family receptors or via ligand independent homodimerization. Several pathways are activated downstream of ErbB receptor activation leading to cell proliferation, growth, and survival, as well as invasion and angiogenesis [66]. Among the most important signaling cascades are the PI3K/AKT (PKB), RAS/RAF/MEK/ERK1/2 and the phospholipase C (PLCγ) pathways [67]. EGFR and HER2 are particularly involved in cell transformation, and mutations, amplifications and overexpression occur in several types of carcinoma, including breast cancer.

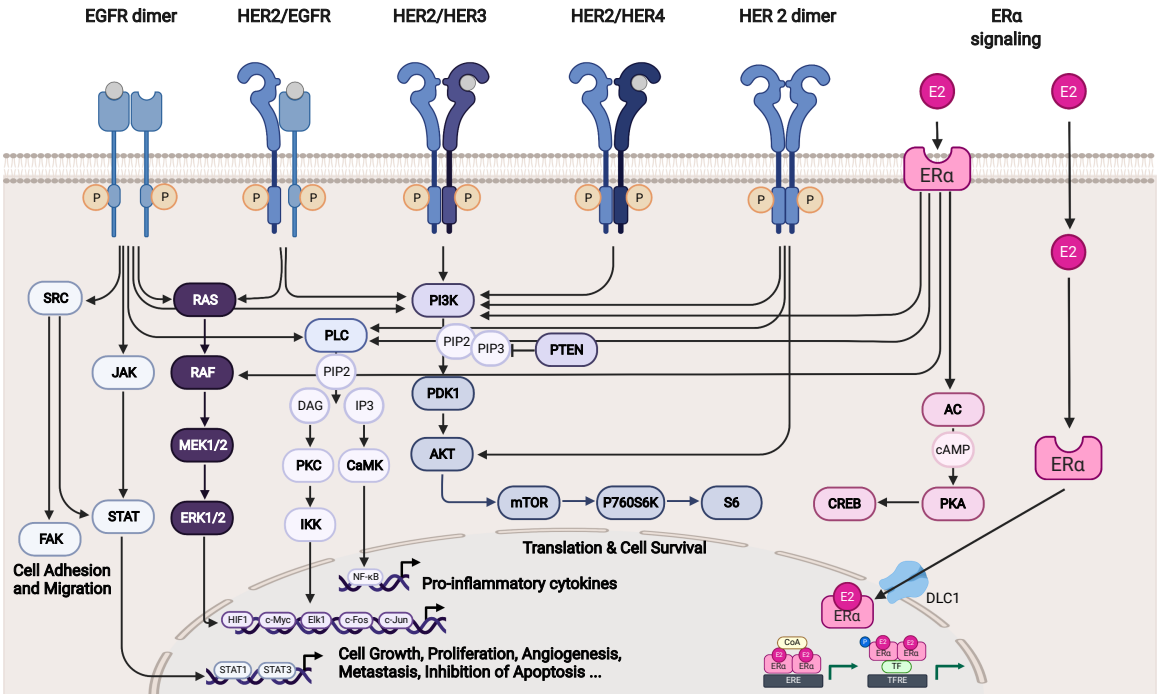


Figure 3: **: Overview of HER and ERα signaling**

The figure illustrates the downstream signaling of the human epidermal growth factor receptor (HER) family and ERα, including MAPK and PI3K/Akt pathways.

Abbreviation: AC: adenylate cyclase; AKT: protein kinase B; CaMK: Ca2+/calmodulin-dependent protein kinase; CREB: cAMP response element-binding protein; DAG: diacylglycerol; DLC1: deleted in liver cancer 1; EGFR: epidermal growth factor receptor; Elk1: ETS domain-containing protein; ERα: estrogen receptor α; ERE: estrogen-response element; ERK: extracellular signal-regulated kinase; E2: estradiol; FAK: focal adhesion kinase; HER: human epidermal growth factor receptor; HIF1: hypoxia-inducible factor 1; IKK: IκB kinase; IP3: Inositol-1,4,5-trisphosphat; JAK: Janus kinase; MAPK: mitogen-activated protein kinase; MEK: mitogen-activated protein kinase kinase; mTOR: mammalian target of rapamycin; NF-κB: nuclear factor NF-κ-B; P: indicates phosphorylation; PDK1: 3-phosphoinositide-dependent protein kinase-1; PIP2: phosphatidylinositol-4,5-bisphosphate; PIP3: phosphatidylinositol-3,4,5-trisphosphate; PI3K: phosphoinositide 3-kinase; PKA: protein kinase A; PKC: protein kinase C; PTEN: phosphatidylinositol-3,4,5-trisphosphate 3-phosphatase; RTK: receptor tyrosine kinase; STAT: signal transducer and activator of transcription, TF: transcription factor; TFRE: transcription factor regulatory element

1.6 Lipids

Energy production within cancer cells differs from that of non-malignant cells. Depending on tumor type and differentiation, malignant cells use numerous mechanisms for energy production. In addition to the Warburg effect, which outlines the metabolic change from oxidative phosphorylation to aerobic glycolysis, cancer cells can take advantage of alterations in lipid metabolism for energy demand, e.g., involving changes in lipid-synthesis/-catabolism, and fatty acid oxidation [68,69].

1.6.1 Lipid Classification

While most mammalian cells meet their lipid requirements through the uptake of free fatty acids (FFAs) and lipoproteins, such as low-density lipoproteins (LDLs), the de novo biosynthesis of fatty acids and cholesterol is restricted to certain cells, including cells from the liver, adipose tissue, and lactating breast tissue, as well as cancer cells. Lipids are classified into seven categories (Figure 4): fatty acids, triglycerides, waxes, phospholipids, sphingolipids, lipopolysaccharides, and isoprenoids (e.g., steroids, carotenoids) [70].

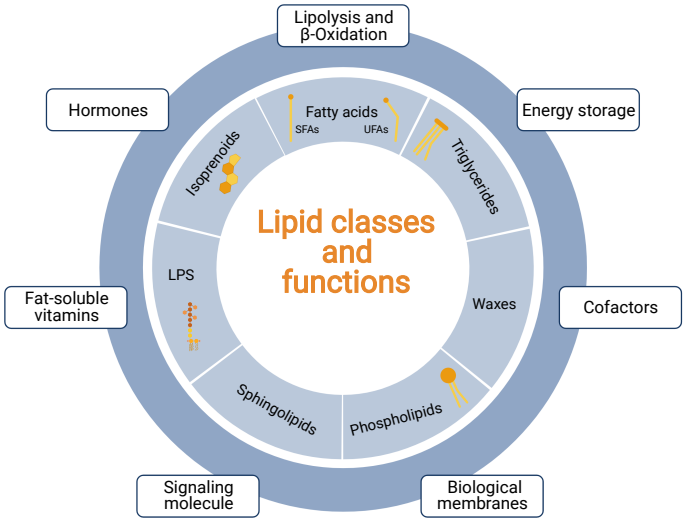


Figure 4: **: Lipid classes overview**
Lipids can be categorized into seven groups: fatty acids, triglycerides, waxes, phospholipids, sphingolipids, lipopolysaccharides and isoprenoids. Lipids have a variety of functions, including energy production or storage, and are components of vitamins, hormones, cofactors, signaling molecules and membranes.

Fatty acids are usually unbranched monocarboxylic hydrocarbons with a carboxylic acid at the end of a hydrocarbon chain. Saturated fatty acids (SFAs) contain no double bonds, and their carbon chain is completely filled with hydrogen atoms. Unsaturated fatty acids (UFAs) have one or more double bonds, which cause the carbon chain to bend [70]. Triacylglycerols are composed of glycerol and three fatty acids esterified with glycerol. When they are liquid at room temperature, they are called oils; when they are solid, they are called fats. Membrane forming lipids, including phospholipids, sphingolipids, and glycolipids, consist of a hydrophilic and a hydrophobic part [70]. The amphiphilic property of these lipids enables them to form into micelles or bilayers in polar solvents such as water. Isoprenoids, e.g., steroids, terpenes, and terpenoids, are compounds made up of isoprene units [70]. The basic structure of all steroids is a system of four, usually trans-connected carbon rings, three hexagonal and one pentagonal. Cholesterol, which is used to create

steroid hormones in the body, is the most well known example. Cholesterol is an important component in all human cell membranes with the exception of the inner membrane of mitochondria. It is usually present in esterified form as the cholesterol ester of fatty acids [70].

1.6.2 Lipid Metabolism

Lipid metabolism is a dynamic balance between the synthesis and degradation of lipids in cells, including the breakdown and storage of fats for energy and the synthesis of structural and functional lipids. Figure 5 provides a schematic illustration of the basic components in the lipid metabolism of eukaryotic cells. Because of their hydrophobic nature triglycerides and cholesterol must be transported through the blood using amphipathic lipoproteins, e.g., chylomicrons, LDLs or VLDLs. LDL receptors (LDLRs) bind to LDLs, which deliver cholesterol to most peripheral tissues. LDLRs are located on the plasma membrane of most cells, capture LDLs, and rapidly degrade them in the lysosome [71]. Lipoprotein lipase breaks down chylomicron particles to release triglycerides, which are broken down into fatty acids and glycerol by enzymes before entering cells. Three groups of proteins facilitate LCFA transport: fatty-acid translocase (FAT/CD36), plasma membrane-associated fatty-acid binding protein (FABP), and fatty-acid transport proteins (FATP) [72]. Fatty acid catabolism takes place in different cell compartments. After ATP-dependent reaction of the fatty acid and a coenzyme A, acyl-CoA can traverse the mitochondrial membrane and can be used for β-oxidation, mainly generating acetyl-CoA, NADH, and FADH, which in turn contribute in the citric acid cycle to produce energy [73]. Besides lipid catabolism, triacylglycerols, membrane lipids, and cholesterol are synthesized through a variety of pathways. Acetyl-CoA is the main precursor for both fatty acids and cholesterol. Acetyl-CoA can emerge via intramitochondrial metabolism (β-oxidation or glycolysis) or different extramitochondrial pathways, e.g., via the ATP citrate lyase (ACLY), which converts citrate derived from the TCA cycle to acetyl-CoA. In the synthesis of fatty acids, acetyl-CoA carboxylase (ACC) converts acetyl-CoA to malonyl-CoA. Fatty acid synthase (FASN) catalyzes the repeated condensation of acetyl-CoA and malonyl-CoA to form a 16-carbon fatty acid chain. The 16-carbon fatty acid chain is then cleaved to generate long-chain fatty acids, such as palmitic acid. Adding a double bond by stearoyl-CoA desaturase (SCD) produces monounsaturated fatty acids [74]. After elongation and desaturation of the initial fatty acids, there is a pool of fatty acids with varying degrees of saturation. These essential fatty acids can be acquired through dietary means as well. Further modifications result in the formation of different lipid classes, for example sphingolipids, eicosanoids, or phospholipids [70]. Choles-

terol biosynthesis is governed by the series of addition and conversion processes catalyzed by 3-hydroxy-3-methylglutarate-CoA synthase (HMGCS) and 3-hydroxy-3-methylglutaryl-CoA reductase (HMGCR). The conversion of HMG-CoA to mevalonic acid and its subsequent addition of acyl groups creates farnesyl-pyrophosphate, an important intermediate for protein prenylation [75].

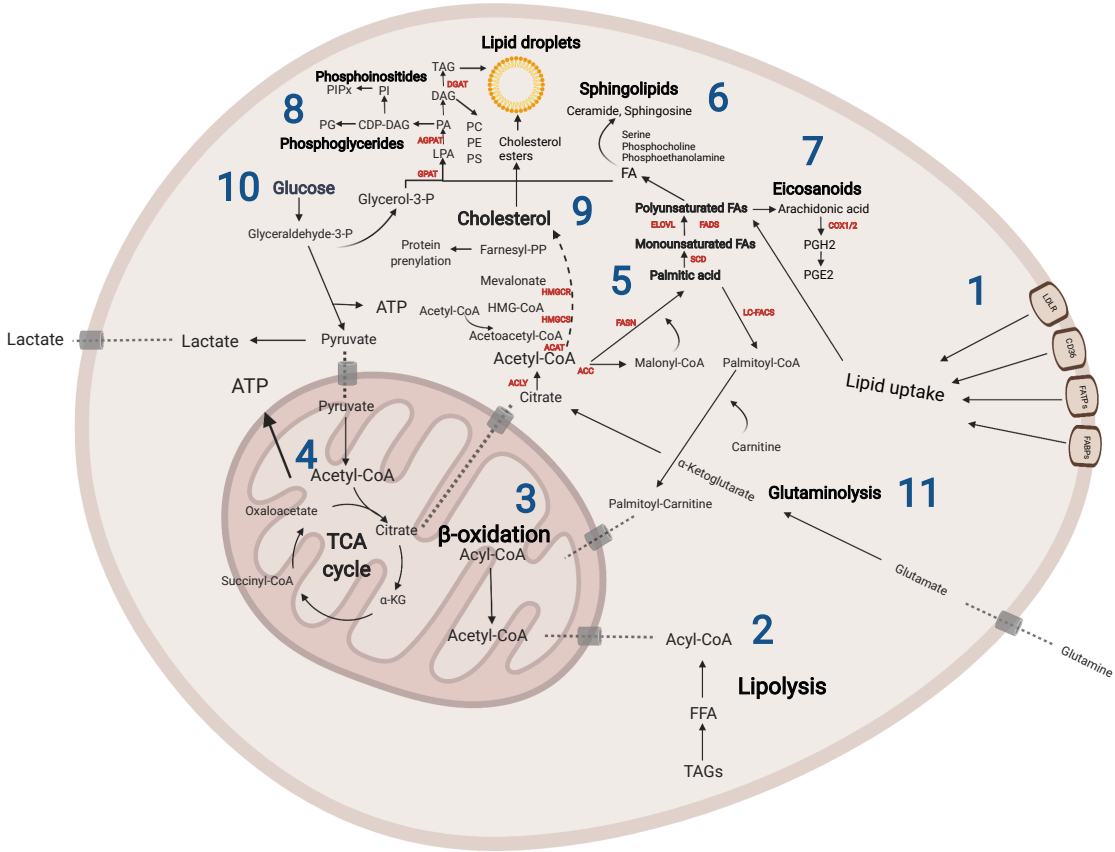


Figure 5: **: Energy metabolism overview**

Rough overview of energy metabolism in human cells. **1** lipid uptake **2** lipolysis **3** β-oxidation **4** TCA cycle **5** fatty acid synthesis **6** sphingolipid synthesis **7** eicosanoid synthesis **8** phospholipid synthesis **9** mevalonate pathway and cholesterol de novo synthesis **10** glycolysis **11** glutaminolysis Abbreviation: ACAT: acetyl-CoA acetyltransferase; ACC: acetyl-CoA carboxylase; ACLY: ATP-citrate synthase; AGPAT: 1-acylglycerophosphate acyltransferase; ATP: adenosine triphosphate; CDP-DAG: cytidine diphosphate diacylglycerol; CD36: cluster of differentiation 36; COX: cytochrome c oxidase; DAG: diacylglycerol; DGAT: diglyceride acyltransferase; ELOVL: fatty acid elongase; FA: fatty acid; FABPs: fatty acid binding proteins; FASN: fatty acid synthase; FATPs: fatty acid transport proteins; FFA: free fatty acids; GPAT: glycerol-3-phosphate O-acyltransferase; HMGCR: 3-hydroxy-3-methylglutaryl-coenzyme A reductase; HMGCS: hydroxymethylglutaryl-CoA synthase; LC-FACS: long chain fatty acyl-CoA synthetase; LDLR: low density lipoprotein receptor; LPA: lysophosphatidic acid; PA: phosphatidic acid; PC: phosphatidylcholine; PE: phosphatidylethanolamine; PG: phosphoglycerat; PGE2: prostaglandin E2; PGH2: prostaglandin H2; PI: phosphatidylinositol; PS: phosphatidylserine; SCD: stearoyl-CoA desaturase; TAG: triacylglycerol; α-KG: α-ketoglutarate

While levels of cholesterol are normal, a protein complex consisting of INSIG1/-SREBP1A/SCAP1 is bound to the endoplasmic reticulum in an inactive form. In situations of low intracellular sterol, decreased cholesterol level in endoplasmic reticulum membranes, and phosphatidylcholine level in golgi membranes activate SREBPs, which bind to the promoters of the genes implicated in de novo fatty acid synthesis and cholesterol biosynthesis [76].

1.6.3 Deregulation of Lipid Metabolism in Cancer

Following on the observation that neoplastic tissues showed aberrant activation of de novo lipogenesis, numerous studies have confirmed that inhibition of different enzymes within the fatty acid biosynthesis pathway can block cancer cell growth. However, there are numerous individual differences in the regulation of lipid metabolism. Using gene expression data from breast cancer samples and a genome-scale human metabolic model, Jerby et al. [77] found that fatty acid biosynthesis is a feature of early stages of cancer development. In contrast, later stage tumors show reduced proliferation as well as activated antioxidant pathways. Lipid droplets are subcellular organelles composed of a monolayer of phospholipid that covers a hydrophobic core. They contain neutral lipids, mainly triacylglycerol (TAG) and cholesteryl esters (CEs), as well as proteins, which vary depending on the cell type and external stimuli [78,79]. Lipid droplets, formerly believed to serve only as a storage system for energy, have been found to have diverse roles within cells, including ER stress, ROS detoxification, and protein dynamics. Furthermore, expression of lipid droplets are correlated to stemness features in breast cancer cell lines [80].
Lipid rafts are low density, detergent resistant sphingolipid- and cholesterol-rich microdomains, in which a variety of membrane proteins, e.g., caveolins and flotillins are enriched and compartmentalized. Because of their small size (100-200 nm), lipid rafts present a challenge to visual observation. However, for example, fluorescence microscopy provides an opportunity to locate lipid rafts by using dyes like Laurdan or Filipin, which intercalate between domains or head-labeled dyes, such as Texas Red [81,82]. Furthermore, lipid rafts can be visualized via the B subunit of the cholera toxin, which binds to GM1, a ganglioside which is highly enriched in lipid rafts [83]. Proteins often found in lipid rafts include proteins with a hydrophobic membrane-spanning sequence or transmembrane domain, glycosylphosphatidylinositol-anchored proteins, doubly acylated proteins (e.g., SRC family kinases), palmitoylated type-I transmembrane proteins (e.g., CD44), and receptor tyrosine kinases with two transmembrane subunits (e.g., insulin receptor or EGFR) [84,85,86,87,88,89]. Several growth factor receptors, T-cell receptors, and the TNF receptor superfamily have been shown to interact with lipid rafts. Consequently, some signaling

molecules are redistributed after the activation of those receptors. Therefore, these microdomains act as platforms for cellular signaling and mediate transport of raft-associated molecules via internalization and cell trafficking. Further, lipid rafts influence various cellular functions, among others the regulation of apoptosis and cell proliferation [90,91,92,93]. Different research groups found elevated levels of lipid rafts in cancer cells. Li et al. [94] showed that different prostate and breast cancer cell lines stain much more intensively for cholesterol and GM1 than their non-cancerous counterpieces. In addition, higher numbers of lipid rafts have been found in malignant melanoma and renal carcinoma cells [95,96]. Moreover, lipid rafts regulate metastasis mechanisms, such as cell adhesion, migration and EMT, in a complex manner. For example, two research groups showed that disruption of lipid rafts leads to reversion of TGF-β1 induced EMT in breast cancer and gastric cancer [97,98]. Another group found an attenuation of EMT and EMT-associated paclitaxel resistance in NSCLC via simvastatin induced inhibition of integrin-β3/FAK signaling [99]. Furthermore, Tisza et al. [100] showed that in breast cancer lipid raft destabilization enhances stem cell properties and EMT-induced remodeling.

1.7 Progesterone Receptor Membrane Component 1

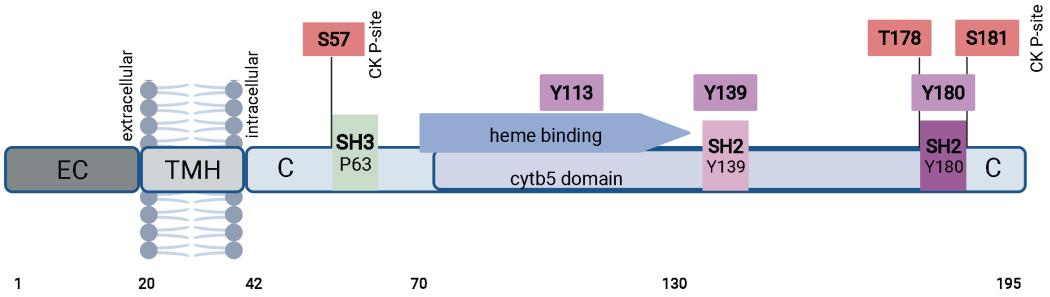
Progesterone Receptor Membrane Component 1 (PGRMC1) belongs to the membrane-associated progesterone receptor (MAPR) family (including the homologous proteins PGRMC1, PGRMC2, Neudesin and Neuferricin), which share a cytochrome b5 (cytb5) related heme-binding domain. Dependent on factors like dimerization/multimerization or posttranslational modifications, PGRMC1 plays a role in various cellular mechanisms and pathways and is expressed not only in different tissues and cells but also in diverse compartments, such as the cytoplasm, the plasma membrane, the inner acrosomal membrane, the nucleus/nucleolus, and the mitochondria [101,102,103,104,105,106,107,108]. In high concentrations PGRMC1 has been found in the liver and kidneys, as well as the brain, breast, heart, lung, pancreas and reproductive tissues [109,110,111]. Studies done in 1996 by Meyer et al. [112] and Selmin et al. [113], respectively, discovered PGRMC1 independently of each other. While Meyer et al. [112] isolated the protein as high affinity progesterone binding site from porcine liver membranes, the group of Selmin [113] purified the upregulated PGRMC1 from the livers of rats treated with dioxin and termed it 25-Dx. PGRMC1 has a highly conserved structure. Homologous proteins have also been discovered in other eukaryotes, among others the yeast homolog DAP1 in S. cerevisiae, the nematode homolog VEM-1 in C. elegans, and the plant homolog AT2G24940 in A. thaliana [114,115,116,117].

1.7.1 Structure and Modifications

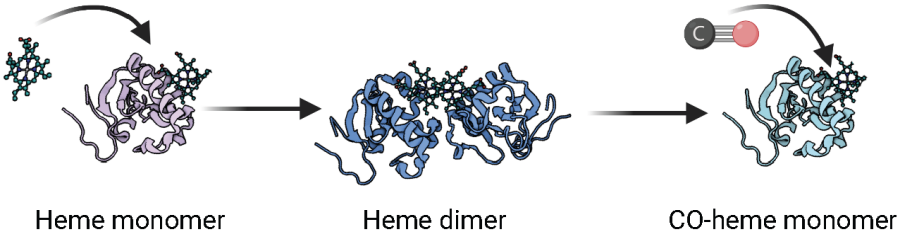
The main splice variant (195 amino acids) of PGRMC1 has a calculated mass of 21.7 kDa and an experimental mass of 25 kDa, which accounts to different post-translational modifications (Figure 6a). PGRMC1 comprises a short N-terminal luminal or extracellular domain (residues 1–20), a single membrane spanning domain (residues 21–42), and a long cytoplasmic domain (residues 43–195) [101,102,-105,118] (Figure 6b). The C-terminal region contains a cytochrome b5-like motif heme-binding domain (amino acids 70–130). While in most cytochrome b5 proteins the heme iron molecule is bound via histidine residues in a hexacoordinate fashion, in PGRMC1 heme is coordinated in a pentacoordinate fashion with the hydroxyl group of a tyrosine residue [110,119,120,121,122,123]. Via heme-heme stacking PGRMC1 is able to form homodimers, which leads to interaction with other proteins, e.g., receptor proteins or cytochrome P450 enzymes. Carbon monoxide (CO) binds to the sixth coordination site of the heme in PGRMC1 and thus interferes with dimerization (Figure 6c). Phosphorylation of Y113 may facilitate membrane trafficking functions of PGRMC1, but concurrently impedes heme binding and homodimerization due to steric interference, indicating a reciprocal regulation [123,124]. The numerous functions of PGRMC1 seem to be regulated by a variety of PTM, including phosphorylation, ubiquitination, acetylation, and SUMOylation [107,124,125]. The phosphosite database (PSD) indicates that S57, Y113, Y180 and S181 are the most commonly detected phosphorylation sites in PGRMC1 [126]. Differential phosphorylation of PGRMC1 in HR+ and HR- breast cancer indicates that not only the expression level but also PTM of PGRMC1 may play a role in (breast cancer) cell homeostasis [127]. PGRMC1 includes predicted binding site motifs for SH2 and SH3 domain containing proteins, with several other phosphorylation sites, e.g., at S57, T178, and S181 thought to regulate these sites. PGRMC1 contains two target sequences (Y139, Y180) that require tyrosine phosphorylation for SH2 domain containing proteins to bind, thereby inducing conformational changes in the receptor and subsequent facilitating downstream signaling [120,124]. Phosphorylation of S181 and T178, by contrast, may sterically inhibit phosphorylation of Y180 and attenuate protein interaction. Furthermore, PGRMC1 phosphorylation of S57 might impair binding of SH3 domain containing proteins to the PGRMC1 SH3 target sequence P63 [124]. Potential proximity stimulated tripartite signaling platforms are formed by juxtaposition of the SH3 target motif adjacent to S57 and the SH2 target motifs containing Y139 and T178/Y180/S181 on the folded protein surface [120,-125].

```
         10         20         30         40         50
MAAEDVVATG ADPSDLESGG LLHEIFTSPL NLLLLGLCIF LLYKIVRGDQ
         60         70         80         90        100
PAASGDSDDD EPPPLPRLKR RDFTPAELRR FDGVQDPRIL MAINGKVFDV
        110        120        130        140        150
TKGRKFYGPE GPYGVFAGRD ASRGLATFCL DKEALKDEYD DLSDLTAAQQ
        160        170        180        190
ETLSDWESQF TFKYHHVGKL LKEGEEPTVY SDEEEPKDES ARKND
```

(a)



(b)



(c)

Figure 6: **PGRMC1 structure**
(a) Primary structure of PGRMC1. The leading splice variant of PGRMC1 contains 195 amino acids, each letter represents one amino acid. A 1-letter code is used to denote each amino acid [128,129]. **(b)** Secondary structure of PGRMC1. The protein consists of a N-terminal extracellular domain (EC), an helical transmembrane domain (TMH) and a cytoplasmic domain (C) with a cytochrome b5-like domain (cytb5 domain) which incorporates a heme binding element (heme binding). Following peptide motifs are depicted: Src homology 3 (SH3) target sequence at P63, Src homology 2 (SH2) target sequences at Y139 and Y180 [120,124]. The most commonly detected phosphorylation sites S57, Y113, Y139, T178, Y180 and S181 according to the phosphosite database are also shown [130]. **(c)** Crystallographic structure of the cytosolic domain of PGRMC1 [123]. The dimerization of PGRMC1 is mediated by stacking of two heme domains. The open surface of the heme, whose iron is five-coordinated by Y113, mediates dimerization. Heme-dependent dimerization of PGRMC1 is disrupted by CO binding to the sixth coordination site of the heme.

1.7.2 General Functions

PGRMC1 has a plethora of functions, including heme and progesterone binding, regulation of cytochrome P450 enzymes, cell proliferation and migration, involvement in apoptosis and cell cycle control, cholesterol/steroid synthesis, angiogenesis, hypoxic biology, autophagy promotion, and vesicle trafficking. PGRMC1 has diverse

effects based on cell type, cellular location of the protein and post-translational modifications. As previously described, one of the better-understood functions of PGRMC1 is binding of heme and the interaction with cytochrome P450 enzymes (CYPs) [111,131,132,133,134]. Crystallographic analyses of Kabe et al. [123] showed that dimerization of PGRMC1 via heme-heme stacking is responsible for interaction with different cytochrome P450 enzymes and activation of the EGFR. They further showed, that by facilitating its degradation, PGRMC1 leads to resistance to the chemotherapeutic agent doxorubicin [123]. Via altering enzymatic activity of CYPs, PGRMC1 might also influence susceptibility of cancer cells to cytostatic agents such as paclitaxel and cisplatin [132,135,136,137]. In addition, PGRMC1 regulates cholesterol and steroid synthesis by interacting with CYPs [110,111,138,-139]. Furthermore, a recent study revealed that PGRMC1 binds and stabilizes a broad range of cytochromes P450 in a heme independent manner [140]. PGRMC1 has been implicated in the regulation of intracellular protein translocation among other things via its YXXψ motifs, which are associated with vesicle transport and endocytosis [115,141]. Although the name of PGRMC1 would suggest that it is primarily involved in progesterone metabolism, the overall role of this molecule in progesterone signaling is comparatively small. Progesterone (P4), a steroid primarily synthesized from cholesterol by steroidogenic tissues in the gonads, placenta, adrenal cortex, and brain, regulates carbohydrate, lipid, and protein metabolism [142,143]. There is some debate over whether P4 is the natural ligand for PGRMC1 [120,144]. However, spectroscopic and mutagenesis studies showed that progesterone binds to PGRMC1 in both the ferric and deoxyferrous states, preferably at a site located within a segment composed of a part of the transmembrane domain and the initial segment of the C-terminal domain. Thereby, P4 binding introduces changes in the heme, possibly due to its colocalization with the heme in the putative heme-/ligand-binding cleft [112,118,122,145]. PGRMC1 has been linked to P4-dependent activities in many cell systems. For instance, PGRMC1 has been shown to induce P4-dependent anti-apoptotic action in different tissue types, e.g., via interaction with serpin 1 mRNA binding protein 1 (SERBP1) in granulosa cells [135,146,147,-148]. In addition, PGRMC1 interacts with the extracellular signal-regulated kinase 5 (ERK5) to regulate P4-induced secretion of brain-derived neurotrophic factor (BDNF) in glial cells [149]. Furthermore, SUMOylated PGRMC1 has been shown to inhibit T cell factor/lymphoid enhancer factor TCF/LEF-mediated transcriptional activity by P4 [150]. PGRMC1 directly interacts with tubulin and stabilizes microtubules. Via its effect on microtubule dynamics PGRMC1 influences mitosis and cell motility [151]. In human pluripotent stem cells, PGRMC1 inhibits the p53 and Wnt/β-catenin pathways to enhance self-renewal and suppress early differentiation [152]. In addition, PGRMC1 provoked autophagy, inter alia, via interaction with

microtubule-associated proteins 1 light chain 3 (LC3), which is a crucial component of the degradative activity of autophagy [153].

1.7.3 Involvement in Cell Metabolism

Metabolic functions of PGRMC1 in lipid homeostasis and glycolysis have been shown to be important by previous studies. Mass spectrometric analysis identified hexokinases, which are the first rate-limiting enzymes of glycolysis and the pentose phosphate pathway, as potential PGRMC1 interacting proteins [154]. The different isoenzymes of hexokinase (HK1-4) differ in substrate specificity, tissue-specific expression, and intracellular localization [155,156]. Sabbir et al. [157] noted that P4-PGRMC1 signaling causes rapid induction of aerobic glycolysis in line with the Warburg effect in human embryonic kidney-derived cells (HEK293), which was associated with the post-translational modification of a 70 kDa fraction of PGRMC1 protein predominantly located in the ER and mitochondria. In contrast, P4 treatment in HEPG2 cells decreased glycolysis and PGRMC1 was not degraded. Interaction of PGRMC1 with hexokinases and translocation of HK1/2 to the endoplasmic reticulum, mitochondria, and nuclear compartments following P4 treatment are cell type specific. Dependent on cell type, there is also a difference in PGRMC1 half-life and PTMs under basal conditions and after P4 treatment. Moreover, PGRMC1 has been shown to mediate the placental P4-dependent shift from aerobic towards anaerobic glucose metabolism in gestational diabetes [158]. Further, PGRMC1 has been reported to maintain plasma membrane pools of the insulin receptor (IR) and to modulate IR signaling and function. PGRMC1 interacts with the IR and decreases insulin binding at the cell surface in cancer cells. PGRMC1 also elevates the plasma membrane levels of GLUT4 and GLUT1, which are two principle glucose transporters [159]. Similar results were obtained in a study of PGRMC1 in adipose tissue, which showed that the heme-dimerized PGRMC1 interacts with low-density lipoprotein receptors (VLDLR and LDLR) or GLUT4 and regulate their translocation to the plasma membrane, facilitating lipid uptake and accumulation, as well as de-novo fatty acid synthesis. In addition, in insulin induced adipogenesis PGRMC1 gene expression is transactivated by transcription factors such as ATF/CREB and PPARγ [160]. Contrary to these findings, other groups reported that in hepatic cells PGRMC1 inhibits de novo lipogenesis, suppresses fatty liver development, and promotes pancreatic insulin secretion [161,162]. By forming a complex with the Sigma-2 Receptor/Transmembrane Protein 97 (TMEM97) and LDLR, PGRMC1 leads to efficient uptake of lipoproteins such as LDL and apolipoprotein E (apoE) [163,164]. The TMEM97, PGRMC1, and LDLR complex mediates cellular uptake of Aβ42 via apoE dependent and independent mechanisms and seems to have impli-

cations in neurodegenerative diseases such as Alzheimer's disease [163,164,165,166]. Studies by Thejer et al. [167,168] revealed that phosphorylation of PGRMC1 causes pleiotropic plasticity-related changes in mitochondrial form and function, as well as in PI3K/AKT activity, migration, and glucose consumption. PGRMC1 also has functions related to the synthesis of sex hormones and the metabolism of cholesterol and drugs [141]. Suchanek et al. [169] found that PGRMC1 directly interacts with INSIG and SCAP, thereby contributing to cholesterol homeostasis. In addition, PGRMC1/INSIG2 signaling exerts regulatory effects in atypical antipsychotics-induced lipid disturbances in the liver [170]. Furthermore, PGRMC1 interacts with and activates the CYP-monooxygenase lanosterol demethylase (CYP51A1), which is induced by SREBP2 as part of the cholesterol synthesis mechanism [171]. PGRMC1 also influences the ATP-independent incorporation of cholesterol into the membrane and its stepwise conversion into estrogens by aromatase [139].

1.7.4 Role in Breast Cancer

PGRMC1 mRNA and protein is overexpressed in many solid cancer types, including colon, pancreas, lung, ovary, cervix and breast cancer, and it contributes to tumor progression through induction of cancer cell proliferation, metastasis, and chemoresistance [123,131,135,147,172,173,174,175,176,177]. Different studies showed the considerable role of PGRMC1 in breast cancer. Indeed, mechanisms behind its regulatory function are complex and affected by multiple factors. Interestingly, PGRMC1 expression and phosphorylation differ between HR+ and HR- breast cancer. While PGRMC1 expression is elevated in HR- tumors, phosphorylation of PGRMC1 occurs predominantly in HR+ tumors [127]. In line with this, PGRMC1 expression correlates with lymph node metastasis, larger tumor size and poorer overall and tumor-free survival [178,179,180]. In vitro and in vivo analysis showed that higher expression of PGRMC1 fuels cancer cell proliferation and metastasis [146]. In addition, PGRMC1 is linked to resistance to chemotherapeutics like doxorubicin, cisplatin, and paclitaxel, for instance via interaction with EGFR and CYP enzymes, leading to degradation of the drugs [123,135,136,147,181,182]. Furthermore, PGRMC1 induction is linked to hypoxia and PGRMC1 is overexpressed in hypoxic areas surrounding necrotic tumor tissue. Corresponding cells also abundantly express GLUT1, leading to enhanced rates of anaerobic metabolism [127,171,183]. Another mechanism by which PGRMC1 impacts carcinogenesis is its activation of intracellular signaling pathways, e.g., via the AKT kinase [184].

1.8 Aims of Thesis

The objective of this work is to get a deeper insight into the mechanisms by which PGRMC1 regulates lipid homeostasis and oncogenic signaling in breast cancer. PGRMC1 has been implicated in signaling pathways for proliferation, differentiation, invasion, migration, and cell survival in prior studies. Nevertheless, the role of PGRMC1 in lipid metabolism and oncogenic signaling, particularly in breast cancer, remains unclear. Previous studies from our laboratory have focused on the interaction between PGRMC1 with proteins from the mevalonate pathway. In this study, we specifically focused on how PGRMC1 affects lipid synthesis, uptake and regulation. We performed the experiments in two HR+ breast cancer cell lines (MCF7 and T47D) and in one triple negative breast cancer cell line (MDA-MB-231). In addition to the interaction of PGRMC1 with proteins involved in lipid metabolism, we sought to investigate the influence of PGRMC1 on downstream signaling in the context of altered lipid metabolism. We further examined whether the transcriptomic and proteomic alterations induced by PGRMC1 were accompanied by changes in metabolite levels. Therefore, levels of cholesterol and E2 were measured in PGRMC1 overexpressing and control cells also under consideration of post-translational modifications. A special focus was placed on PGRMC1-dependent expression of lipid droplets and lipid rafts. In light of this, we assessed the alteration of levels and localization of cell growth associated receptors such as HER2 and ERα in PGRMC1 expressing cells. Furthermore, effects of PGRMC1 expression on EGFR signaling were examined. In order to investigate the effect of lipid depletion on breast cancer cells and to confirm our hypothesis, we treated the cell lines with simvastatin and measured treatment response by quantification of viability. Understanding the way PGRMC1 interferes with key metabolic regulatory mechanisms could provide valuable information on how we can better diagnose, classify, and then treat diseases such as breast cancer in the future.

2 Publication

Progesterone receptor membrane component 1 regulates lipid homeostasis and drives oncogenic signaling resulting in breast cancer progression

Journal: Breast Cancer Research
Year: 2020

Relevant publication:
Asperger, H., Stamm, N., Gierke, B. et al. Progesterone receptor membrane component 1 regulates lipid homeostasis and drives oncogenic signaling resulting in breast cancer progression. Breast Cancer Res 22, 75 (2020).

Link to publication: https://doi.org/10.1186/s13058-020-01312-8

Asperger et al. Breast Cancer Research (2020) 22:75
https://doi.org/10.1186/s13058-020-01312-8

Breast Cancer Research

RESEARCH ARTICLE Open Access

Progesterone receptor membrane component 1 regulates lipid homeostasis and drives oncogenic signaling resulting in breast cancer progression

Hannah Asperger[1], Nadia Stamm[1], Berthold Gierke[2], Michael Pawlak[2], Ute Hofmann[3], Ulrich M. Zanger[3], Annamaria Marton[4], Robert L. Katona[5], Andrea Buhala[4], Csaba Vizler[4], Jan-Philipp Cieslik[1], Eugen Ruckhäberle[1], Dieter Niederacher[1], Tanja Fehm[1], Hans Neubauer[1*†] and Marina Ludescher[1†]

Abstract

Background: PGRMC1 (progesterone receptor membrane component 1) is a highly conserved heme binding protein, which is overexpressed especially in hormone receptor-positive breast cancer and plays an important role in breast carcinogenesis. Nevertheless, little is known about the mechanisms by which PGRMC1 drives tumor progression. The aim of our study was to investigate the involvement of PGRMC1 in cholesterol metabolism to detect new mechanisms by which PGRMC1 can increase lipid metabolism and alter cancer-related signaling pathways leading to breast cancer progression.

Methods: The effect of PGRMC1 overexpression and silencing on cellular proliferation was examined in vitro and in a xenograft mouse model.
Next, we investigated the interaction of PGRMC1 with enzymes involved in the cholesterol synthesis pathway such as CYP51, FDFT1, and SCD1. Further, the impact of PGRMC1 expression on lipid levels and expression of enzymes involved in lipid homeostasis was examined. Additionally, we assessed the role of PGRMC1 in key cancer-related signaling pathways including EGFR/HER2 and ERα signaling.

Results: Overexpression of PGRMC1 resulted in significantly enhanced proliferation. PGRMC1 interacted with key enzymes of the cholesterol synthesis pathway, alters the expression of proteins, and results in increased lipid levels. PGRMC1 also influenced lipid raft formation leading to altered expression of growth receptors in membranes of breast cancer cells. Analysis of activation of proteins revealed facilitated ERα and EGFR activation and downstream signaling dependent on PGRMC1 overexpression in hormone receptor-positive breast cancer cells. Depletion of cholesterol and fatty acids induced by statins reversed this growth benefit.

(Continued on next page)

* Correspondence: Hans.Neubauer@med.uni-duesseldorf.de
†Hans Neubauer and Marina Ludescher contributed equally to this work.
[1]Department of Obstetrics and Gynecology, University Hospital and Medical Faculty of the Heinrich-Heine University Duesseldorf, Life Science Center, Merowingerplatz 1A, 40225 Düsseldorf, Germany
Full list of author information is available at the end of the article

(Continued from previous page)
Conclusion: PGRMC1 may mediate proliferation and progression of breast cancer cells potentially by altering lipid metabolism and by activating key oncogenic signaling pathways, such as ERα expression and activation, as well as EGFR signaling. Our present study underlines the potential of PGRMC1 as a target for anti-cancer therapy.

Keywords: PGRMC1, Breast cancer, Tumor progression, Cholesterol, Lipids, Estrogen receptor α, HER2, EGFR, Breast cancer signaling pathway

Background

With approximately 25% of all new cancer cases, breast cancer is the most common cancer in women [1] and responsible for the highest fraction of cancer death [2]. Therefore, the investigation of underlying mechanisms on molecular levels and the discovery of new therapy approaches are research goals of utmost significance.

Progesterone receptor membrane component 1 (PGRMC1) is a highly conserved protein, which is primarily found in the liver and kidney but also expressed in various tissues such as brain, breast, lung, pancreas, and reproductive tissues [3–5].

PGRMC1 has been confirmed to play a role in carcinogenesis especially in breast cancer and may therefore represent a target for cancer therapy [6]. In many studies, upregulation of PGRMC1 protein and mRNA was detected in malignancies including colon, lung, ovary, cervix, and breast [7–11]. Besides, PGRMC1 expression correlates with metastasis to lymph nodes, larger tumor size, and poorer overall- and tumor-free survival [9, 12]. Further, interactions of PGRMC1 or its homologous proteins with cytochrome P450 enzymes (CYPs) have been reported, for example by stably binding heme in its cytb5 related domain [3, 5, 13–15]. PGRMC1 leads to resistance against chemotherapeutic agents like doxorubicin, cisplatin, and paclitaxel [13, 16, 17]. Moreover, different authors discuss an involvement of PGRMC1 in cholesterol synthesis via interaction with CYPs [3, 5, 18]. The role of cholesterol in cancer is still not fully evaluated. Many studies describe an association of high plasma and endogenous cholesterol levels with (breast) cancer development and progression [19–21], pointing towards a major role in cancer. Elevated cholesterol and steroid levels may affect carcinogenesis in different ways, e.g., in saturating the increased requirement for membrane components due to abundant cell growth [22]. Furthermore, high cholesterol levels result in an increase in the size and number of lipid rafts. Since lipid rafts contain several signaling molecules, differences in lipid rafts are modulating signaling cascades [23, 24], such as EGFR and HER2 signaling and expression [25]. In addition, cholesterol is the precursor of steroid hormones like estradiol (E2), the important growth factor for hormone receptor-positive breast cancer [26].

The aim of the present study was to investigate the impact of PGRMC1 on lipid metabolism, lipid raft formation, and its contribution to breast cancer progression and cancer-associated signaling pathways in hormone receptor-positive (MCF7) and hormone receptor-negative (MDA-MB-231) cells. For this purpose, interaction of PGRMC1 with enzymes of the mevalonate pathway was evaluated. Subsequently, effects of PGRMC1 expression on cholesterol and lipid levels were investigated. A special focus was placed on PGRMC1-dependent expression and signaling of ERα and EGFR/HER2. To explore the impact of modified lipid and steroid metabolism (due to PGRMC1 expression), breast cancer cell growth was further explored by PGRMC1 overexpression and -silencing.

Methods

Cells and cell culture

MCF7, T47D, and MDA-MB-231 cells were purchased from the ATCC (Manassas, Virginia). Cells were maintained in RPMI 1640 medium (Thermo Fisher Scientific, Waltham, Massachusetts), supplemented with 10% (v/v) fetal bovine serum (Thermo Fisher Scientific, Waltham, Massachusetts), 100 units/mL penicillin/streptomycin (Thermo Fisher Scientific, Waltham, Massachusetts), and 0.025 mol/L HEPES (Thermo Fisher Scientific, Waltham, Massachusetts) in a humidified incubator at 37 °C with 5% CO_2. Cells (passage number ≤ 25) were authenticated regularly by Microsynth AG (Balgach, Switzerland) using STRS analysis. The last authentication was performed on May 22, 2018.

Transfection of cell lines

Cells were transfected with the expression vector pcDNA3.1/Hygro(+) (Thermo Fisher Scientific, Waltham, Massachusetts), containing 3x HA-tagged (3x human influenza hemagglutinin-tagged) PGRMC1, using Lipofectamine™ 2000 transfection reagent (Thermo Fisher Scientific, Waltham, Massachusetts) (MCF7/PGRMC1, T47D/PGRMC1 and MDA-MB-231/PGRMC1). As a control, we used cells transfected with the "empty" vector (MCF7/EVC, T47D/EVC, and MDA-MB-231/EVC). Stable transfection was verified by PCR, western blot, and immunofluorescence staining, to isolate PGRMC1-over-expressing clones.

siRNA silencing of endogenous PGRMC1

For silencing of endogenous PGRMC1 in MCF7 cells, FlexiTube GeneSolution for PGRMC1 (Qiagen, Hilden, Germany) was used, containing four siRNAs that specifically target human PGRMC1 mRNA. Cells were harvested after cultivation for 24 h, 48 h, and 72 h at 37 °C to verify silencing by western blot analysis.

For MTT assays, cells were pre-incubated with siRNA against PGRMC1 for 24 h at 37 °C in cell culture flasks to silence the endogenous protein. Subsequently, the cells were seeded in 96-well plates and again treated with siRNA. Cell viability was measured after 24 h, 48 h, and 72 h at 37 °C of incubation.

MTT assay

Cells (5×10^4 cells per well) were seeded in triplicates in 96-well plates in complete medium. Cells were either grown (for different timespans) in full medium without or with treatment. Afterwards cells were incubated with 0.25 mg/ml MTT solution for 3 h. After 1 h of incubation with DMSO, absorption at 540 nm was determined with TECAN Spark®.

Quantification of lathosterol and cholesterol

Cholesterol and lathosterol were quantified by gas chromatography-mass spectrometry analysis as described previously (Maier et al., 2009), with minor modifications.

Western blot analysis

Samples for western blot analysis and the respective molecular weight marker were loaded onto Mini-PROTEAN® Precast Gel and separated via SDS-Page at 150 V. We activated the PVDF membrane with methanol. Transmission of proteins was performed for 16 h at 4 °C and 10 mA in blotting buffer. Afterwards, unspecific binding was blocked by incubation of the PVDF membrane with the transferred proteins with blocking solution for 1 h at room temperature. Primary antibody in respective concentration was added in blocking solution and incubated for 16 h at 4 °C. Subsequently, a secondary antibody was applied in 20% blocking solution at room temperature. Proteins were detected using Amersham™ ECL™ Western Blotting Detection Reagent.

Co-immunoprecipitation

Immunoprecipitation of HA-tagged PGRMC1 and HA-tagged PGRMC1-variants was performed using the Pierce™ HA-Tag IP/Co-IP Kit according to the manufacturer's instructions. Cells overexpressing GFP-tagged PGRMC1 were used as a negative control. Cell pellets were resuspended in Co-IP lysis buffer. An amount of 500-μg protein was incubated with anti-HA agarose slurry at 4 °C overnight. For elution, proteins were denatured in sample buffer at 95 °C for 5 min and the eluent was supplemented with 1 M DTT. The elution of PGRMC1 and mutual interaction partners was analyzed directly via mass spectrometry (explained in the supplements), SDS-PAGE, and western blot.

Proximity ligation assay (PLA)

The PLA procedure was performed using the Duolink® PLA Kit. Cells were grown in chamber slides. Incubation with the primary antibody cocktail containing anti-PGRMC1 antibody and antibody against one of the possible interaction partners (or rabbit isotype IgG as negative control) was performed overnight at 4 °C.

Additionally, staining with anti-cytokeratin antibody for 1 h was performed after amplification. Afterwards, cells were stained with DAPI for 10 min and analyzed by fluorescence microscopy within 1 week.

Reverse phase protein array (RPPA)

RPPA using Zeptosens technology was used for analysis of signaling protein expression and activity profiling.

RPPA assay images were analyzed using ZeptoVIEW Pro 3.1 array analysis software. Sample signals were quantified as protein-normalized, blank-corrected mean fluorescence intensities (NFI) of the single spots applying linear fits and interpolation to the mean of the four printed sample dilutions (eight spots per sample).

qRT-PCR

RNA was isolated from a cell pellet of 0.5×10^6 cells using the RNeasy Mini Kit (Qiagen, Hilden, Germany) according to the manufacturer's specifications.

Reverse transcription of RNA into cDNA was performed with the Omniscript RT kit (Qiagen, Hilden, Germany) according to the manufacturer's instructions. For qPCR, QuantiFast SYBR Green PCR kit (Qiagen, Hilden, Germany) and RT [2] qPCR Primer assays for ESR1, HER2, TFF1, Myc, CCND1, PGR, SCD, FASN, HMGS1, SREBF1, SREBF2, LDLR, ACAT1, and PDH (Qiagen, Hilden, Germany) were used according to the manufacturer's specifications. qPCR was performed using the LightCycler® 480 System (Roche, Penzberg, Germany).

Estradiol ELISA

Supernatants of MCF7/EVC and MCF7/PGRMC1 cells were analyzed for 17β-Estradiol (E2) concentrations using a commercially available kit (ab108667, Abcam plc, Cambridge, UK) according to the manufacturer's specifications.

Staining for lipid rafts and HER2

Co-staining of HER2 with lipid rafts was performed in PGRMC1 overexpressing MCF7 and MDA-MB-231 cells

and their respective empty vector controls. Cells were seeded in a chamber slide for 24 h. Afterwards, the medium was removed, and the cells were incubated for another 24 h with medium containing stripped FCS and were then incubated for 24 h with medium containing normal FCS. Staining of lipid rafts was performed using Vybrant™ Alexa Fluor™ 488 Lipid Raft Labeling Kit. Afterwards, cells were fixed with 4% formaldehyde for 10 min. DAKO® protein block was used to block unspecific binding sites for 1 h. Following this, cells were stained with antibodies specific for HER2 (ab16901) over night at 4 °C followed by an anti-mouse secondary-antibody (Alexa Fluor 549 labeled) for 1 h. As negative control mouse isotype IgG was used. After this, staining with DAPI was performed. Subsequently cells were examined by fluorescence microscopy using Axioplan 2 Imaging (Carl Zeiss Microscopy GmbH, Jena, Germany). For analyzing the amounts of lipid rafts and HER2 via flow cytometry, cells were seeded in culture flasks and synchronized as described above. Staining and fixation was performed as described above. The emission (488 nm wavelength) was detected via high throughput flow cytometry (CyAn, Beckman Coulter, Brea, USA).

Staining of lipid droplets

For visualizing of lipid droplets in PGRMC1 overexpressing MCF7, T47D, and MDA-MB-231 cells and their respective empty vector controls via fluorescence microscopy, the cells were grown in chamber slides for 24 h. Afterwards cells were stained with BODIPY™ 493/503 (Sigma-Aldrich, St. Louis, Missouri) solubilized in FCS-free medium and 2% BSA for 30 min. Cells were fixed with 4% formaldehyde for 10 min, stained with DAPI, and examined by fluorescence microscopy. For analyzing amounts of lipid droplets via flow cytometry, cells were grown for 24 h and harvested with trypsin. Staining was performed as described above. The emission (488-nm wavelength) was detected via high throughput flow cytometry (CyAn, Beckman Coulter, Brea, USA).

Scatter plots of breast cancer microarray data

We obtained normalized microarray data (Affymetrix Human Genome U133A Array) from the Gene Expression Omnibus (GEO, NCBI) [27]. The samples were normalized using global scaling by the data set authors. We confirmed the value distribution using mean values and boxplots. Technical replicates were averaged. The values of a selected panel of reporters were correlated against a PGRMC1 reporter utilizing Spearman's correlation.

Xenograft models

NOD.CB17-Prkdc^{scid} (SCID) mice (female, 6-weeks old) were obtained from the Jackson Laboratory (Bar Harbor, Maine) and were bred in the SPF animal facility of the Institute of Genetics at the Biological Research Centre, Szeged, Hungary. Young adult SCID female mice were transplanted subcutaneously in the flank with 17β-estradiol pellet (containing biodegradable carrier-binder, 1.7 mg/pellet, 60-day release; SE-121, Innovative Research of America, Sarasota, Florida) under pentobarbital anesthesia. The next day, the mice were injected subcutaneously with 3×10^6 tumor cells in the opposite flank. The mice were checked daily, and the tumor size was measured twice weekly. At the end of the experiment, the animals were euthanized, by pentobarbital overdose, and the tumors dissected.

Treatment with simvastatin

For treatment with simvastatin, cells (10^5 cells per well) were seeded in 96-well plates in complete medium for 24 h/37 °C. Afterwards, the medium was removed and the cells were incubated with 100, 50, 25, 12.5, 6.25, and 3.125 μg/mL simvastatin for MCF7 cells and 20, 10, 5, 2.5, 1.25, and 0.625 μg/mL simvastatin for MDA-MB-231. MTT assays were performed after 24 h, 48 h, and 72 h.

Statistical analysis

All experiments were performed with several independent biological replicates and repeated a minimum of three iterations. Results are reported as means with standard deviation. The data were tested for normal distribution using Shapiro-Wilk and Kolmogorov-Smirnov test. Differences between groups were determined by unpaired Student's t test. Statistical analysis was performed using R (RStudio) and IBM SPSS. Spearman's ρ was calculated in R using normalized microarray data and was plotted as a scatterplot using the ggpubr R library. $p < 0.05$ was considered as statistically significant.

Results

PGRMC1 promotes viability of breast cancer cells and growth of xenograft tumors while PGRMC1 inhibition and downregulation reduce viability of breast cancer cells

As already shown in previous studies by us and others, PGRMC1 overexpression results in increased proliferation of tumor cells [28–30]. In accordance with these results, in our study, MCF7/PGRMC1 and T47D/PGRMC1 cells also profit from a significantly higher viability compared to the respective empty vector control cells (Fig. 1b, supplemental Figure 1A). For MDA-MB-231 cells overexpressing PGRMC1, no such effects can be observed (Fig. 1b). To further strengthen our theory, we examined the impact of PGRMC1 silencing on tumor proliferation by knocking down endogenous PGRMC1 expression. As hypothesized, the knockdown of PGRMC1 led to significantly decreased viability of MCF7 and T47D

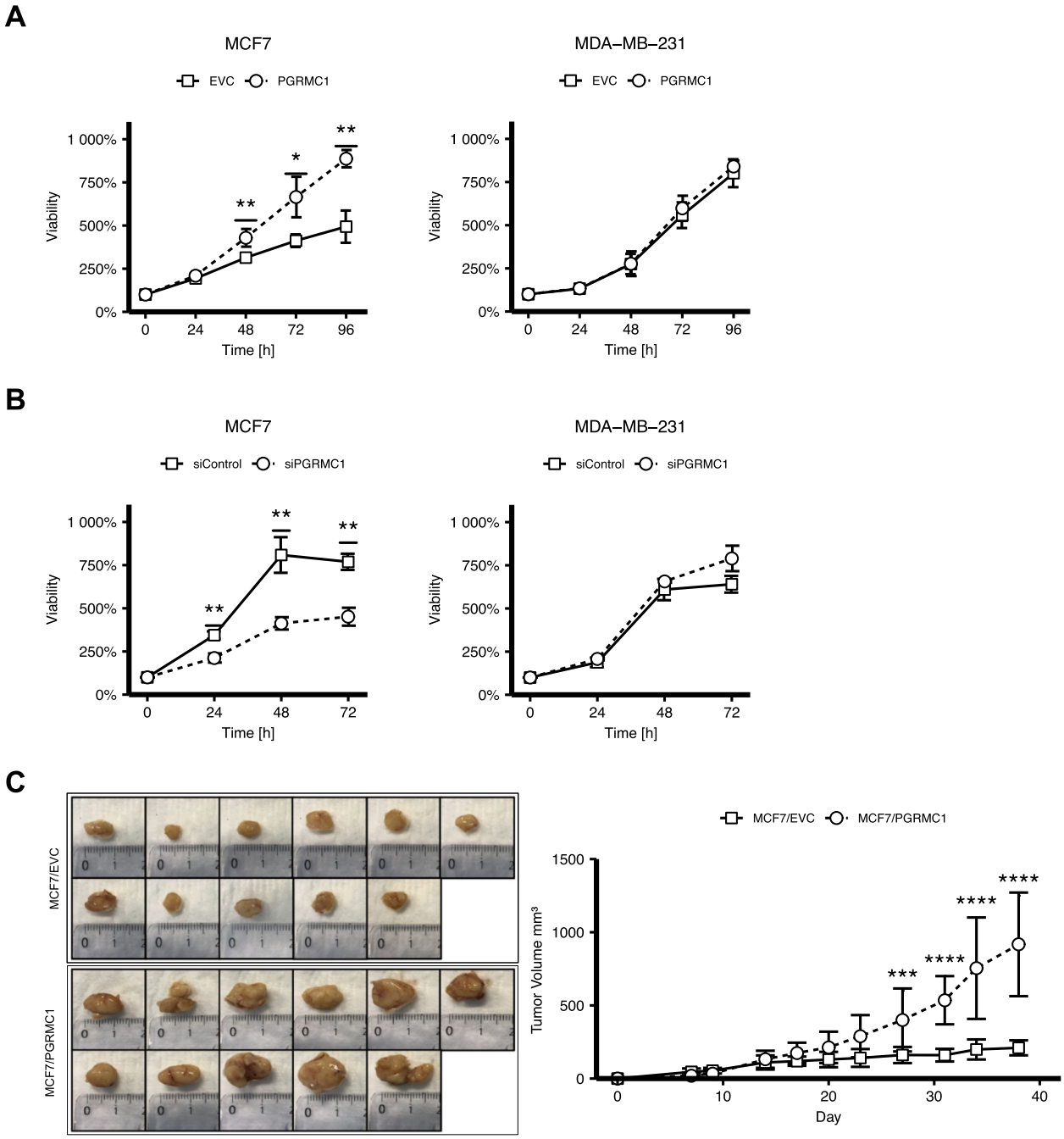


Fig. 1 **a** Cell viability of MCF7/EVC and MCF7/PGRMC1 cells as well as MDA-MB-231/EVC and MDA-MB-231/PGRMC1 cells ($n = 3$). Viability was analyzed by MTT assay at $t = 0$ h, 24 h, 48 h, 72 h, and 96 h/37 °C. Values were normalized to $t = 0$ (100%). *$p \leq 0.05$, **$p \leq 0.01$ (Student's t test, $n = 3$). **b** Cell viability of MCF7 and MDA-MB-231 cells, treated with siRNA against *PGRMC1* (siPGRMC1) and scrambled siRNA (siControl) (Student's t test, $n = 3$). Viability was analyzed at $t = 0$ h, 24 h, 48 h, and 72 h/37 °C. Values were normalized to $t = 0$ (100%). *$p \leq 0.05$, **$p \leq 0.01$ (Student's t test, $n = 3$). **c** Tumor volumes of immunodeficient mice bearing human breast cancer MCF7/EVC and MCF7/PGRMC1 xenografts. ***$p \leq 0.001$, ****$p \leq 0.0001$ (Student's t test, $n = 11$ mice each group). Images of tumor tissue dissected from each mouse

cells but not of MDA-MB-231 cells (Fig. 1a, supplemental Figure 1B).

To validate and strengthen the in vivo findings of Ruan et al. [30], to verify "our" cell models but also to extend the data to other ER-positive BC cells, we investigated effects of PGRMC1 overexpression on MCF7 and T47D breast cancer cell growth in a xenograft model. On that account, MCF7/PGRMC1 and T47D/PGRMC1 cells were injected into the flanks of immunodeficient mice. As control, we used EVC cells.

Subsequently, the size of the developed tumor mass was measured. As assumed, mice injected with PGRMC1 overexpressing breast cancer cells matured significantly larger tumor masses, than mice injected with the respective EVC cells (Fig. 1c, supplemental Figure 1C).

PGRMC1 interacts with proteins of the mevalonate pathway

As already shown in previous studies from different research groups, PGRMC1 might regulate cholesterol synthesis in different ways, e.g., by activating enzymes of the mevalonate pathway like CYP51/lanosterol demethylase or by binding to the proteins Insig and Scap, which span the endoplasmic reticulum and sense cholesterol levels [31, 32]. In our present study, we focused on this regulating influence and its possible involvement in PGRMC1-induced breast cancer promotion.

In order to get a broader view about the role of PGRMC1 in this context, we screened for potential PGRMC1 interaction partners by mass spectrometry analysis of proteins co-immunoprecipitated from whole cell lysates of MCF7 cells that had been transfected with PGRMC1-HA, utilizing an antibody directed against the HA-tag (Fig. 2a). Among proteins with higher significance, we found various potential interaction partners involved in the mevalonate pathway (e.g., SCD1, FDFT1, and CYP51A1) and cellular transport processes such as vesicle trafficking (e.g., Coatomer subunit beta and Coatomer subunit gamma-1) and nuclear export or import (e.g., Exportin-1, Exportin-2, Exportin-5, Exportin-7 or Importin-4 and Importin-5) processes. Since SCD1, FDFT1, and CYP51A1 indicate a high evidence for protein interaction with PGRMC1 and since they play an important role in cholesterol metabolism, we scrutinized these interactions. Interaction of PGRMC1 with SCD1, FDFT1, and CYP51A1 was confirmed by immunoprecipitating PGRMC1-HA in MCF7/PGRMC1 cells and by subsequently visualizing the respective interaction partners via western blot (Fig. 2b). To verify the observed interactions in different cell lines independently of PGRMC1 overexpression and immunoprecipitation, we performed proximity ligation assay of candidate proteins with endogenous PGRMC1 in MCF7 (Fig. 2c) and MDA-MB-231 cells (supplemental Figure 1B). Interactions between PGRMC1 and the respective enzymes are represented by single spots in fluorescence microscopy. While in MCF7 cells, a high number of spots per cell were visible for the interaction with CYP51, FDFT1, and SCD1, the low number of spots in MDA-MB-231 cells indicated no or little interaction (Fig. 2d). Interactions of PGRMC1 with FDFT1 and SCD1 were also observed in T47D cells (supplemental Figure 2B,C). Western blot analysis of protein expression of SCD1, FDFT1, and CYP51 revealed higher CYP51 and SCD1 protein levels in MCF7/PGRMC1 cells compared to MCF7/EVC, while no difference in MDA-MB-231/PGRMC1 cells could be observed compared to MDA-MB-231/EVC cells (Fig. 2e). These results implicate not only a direct interaction of PGRMC1 with SCD1, FDFT1, and CYP51, but also an increased PGRMC1-driven upregulation of these enzymes in estrogen receptor-positive cells, that appeared absent in hormone receptor-negative cells.

Overexpression of PGRMC1 leads to higher levels of cholesterol in hormone receptor-positive breast cancer cells

We hypothesized that the interaction of PGRMC1 with enzymes of the mevalonate pathway might alter their function and thus affects cholesterol synthesis, resulting in elevated cholesterol levels, which may provide energy and components supporting cancer metabolism. Therefore, we measured intracellular cholesterol levels in synchronized PGRMC1 overexpressing and empty vector control MCF7 and MDA-MB-231 cells via mass spectrometry (Fig. 2f). Overexpression of PGRMC1 in MCF7 cells caused a significant increase ($p < 0.05$) of intracellular cholesterol levels compared to the empty vector control, while no difference in MDA-MB-231/PGRMC1 cells was observed (Fig. 2f). Additionally, levels of lathosterol, a precursor of cholesterol, were measured (Fig. 2f). For MCF7/PGRMC1 cells, we detected a significantly decreased ratio compared to MCF7/EVC cells. Interestingly, a significantly decreased ratio of lathosterol/cholesterol in MDA-MB-231/PGRMC1 cells was observed compared to MDA-MB-231/EVC cells, pointing towards a small influence of PGRMC1 on cholesterol de novo synthesis in these cells. The data reveal an impact of PGRMC1 on de novo synthesis of cholesterol regarding cholesterol levels and enzymatic turnover.

Upregulation of ERα, ERα downstream targets, and E2 levels mediated by PGRMC1

Since cholesterol is the precursor for steroid hormones, we assumed that enhanced cholesterol synthesis may affect E2 levels. E2 plays an essential role in hormone receptor-positive breast cancer, e.g., by activating ERα which is leading to tumor proliferation. E2 levels were determined in the supernatant of MCF7/PGRMC1 cells by ELISA (Fig. 3a). Consistent with the higher amounts of cholesterol in MCF7/PGRMC1 cells, we found significantly increased levels of E2 in the supernatant of MCF7/PGRMC1 cells in comparison to MCF7/EVC cells. To analyze the effect of higher E2 levels in MCF7/PGRMC1 cells on breast cancer signaling, we determined the expression of different proteins known to play a role in key signaling cascades in breast cancer via reverse phase protein array technology (RPPA) (Fig. 3b). RPPA analysis revealed significantly ($p < 0.05$) elevated

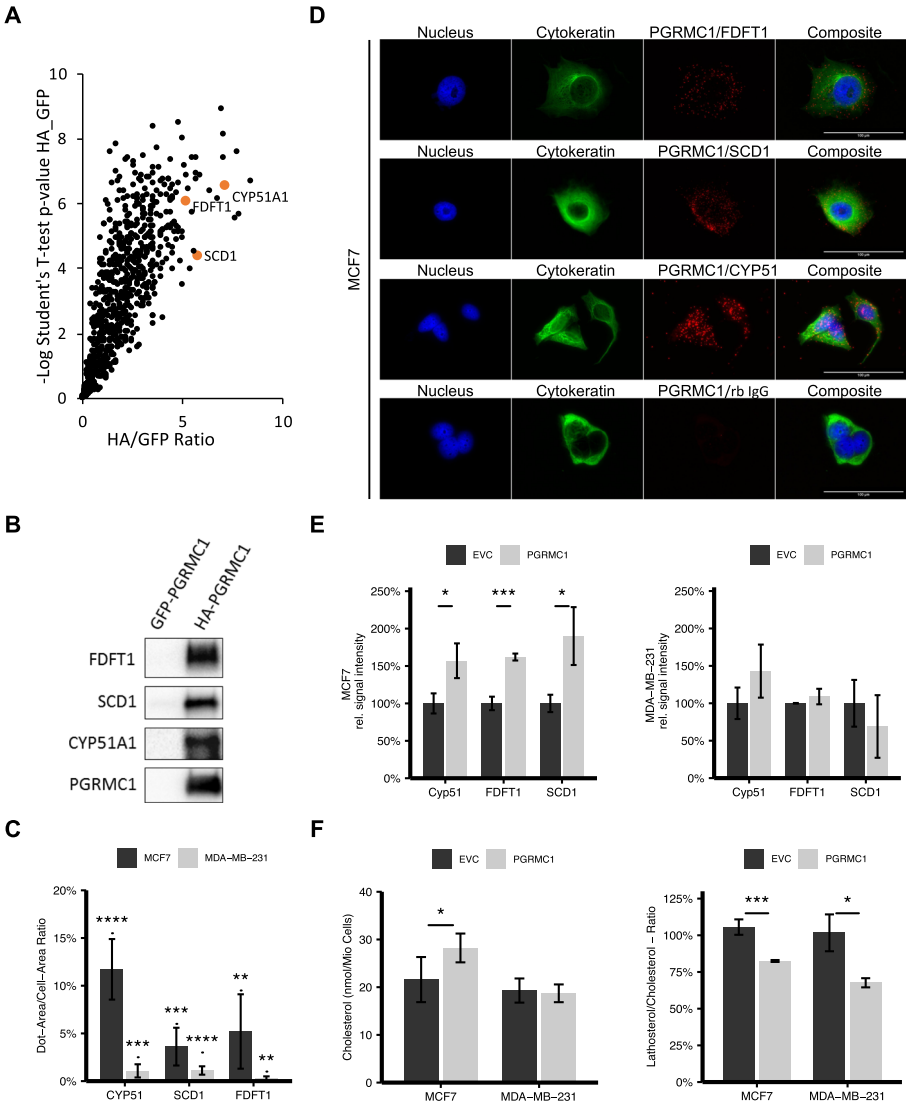


Fig. 2 **a** Scatter plot of proteins with significantly higher intensities in PGRMC1-HA samples compared to PGRMC1-GFP samples identified by mass spectrometry. The most significant proteins exhibit very high value for Student's *t* test difference HA_GFP and –log Student's *t* test *p* value HA_GFP and are found in the upper right corner. Highlighted are proteins with important functions in steroid synthesis. **b** Detection of co-immunoprecipitated proteins CYP51A1, Stearoyl-CoA desaturase (SCD1), and FDFT1 by western blot. **c** Verification of the interactions via proximity ligation assay. Quantification of dots per cell. **d** Visualization via immunofluorescence microscopy. **e** Quantification of protein expression of CYP51, SCD1, and FDFT1 in MCF7/PGRMC1 cells and MDA-MB-231/PGRMC1 cells compared to their respective empty vector control by western blot. $^{*}p \leq 0.05$, $^{***}p \leq 0.001$. **f** Detection of cholesterol and its precursor lathosterol in PGRMC1 overexpressing cells compared to the empty vector control cells with mass spectrometry $^{*}p \leq 0.05$, $^{***}p \leq 0.001$ (Student's *t* test, $n = 3$)

expression of ERα in MCF7/PGRMC1 cells compared to MCF7/EVC cells (Fig. 3b). Subsequently higher levels of HER2 and c-Myc proteins, whose expression depend on the transcriptional activity of ERα, were observed while c-Fos and PR levels were not altered (Fig. 3b). To verify the results from RPPA, western blots were performed to detect protein expression of ERα, HER2, and c-Myc (Fig. 3c). In MCF7/PGRMC1 cells, expression of ERα, HER2, and c-Myc is increased. Because E2 activates ERα and our previous studies have demonstrated higher E2 levels in MCF7/PGRMC1 cells compared to MCF7/EVC (Fig. 3a), we analyzed ERα phosphorylation at S118 (ERα-P-S118), which was also significantly increased ($p < 0.01$) in MCF7/PGRMC1 cells compared to MCF7/EVC (Fig. 3c). Additionally, we performed qPCR analysis of mRNA expression for ESR1, Tff1, HER2, CCND1, Myc, and PGR in the PGRMC1 overexpressing cell lines in comparison to the empty vector control (Fig. 3d, supplemental Figure 3B). In MCF7/PGRMC1 and T47D/PGRMC1 we detected higher mRNA levels for ESR1 and

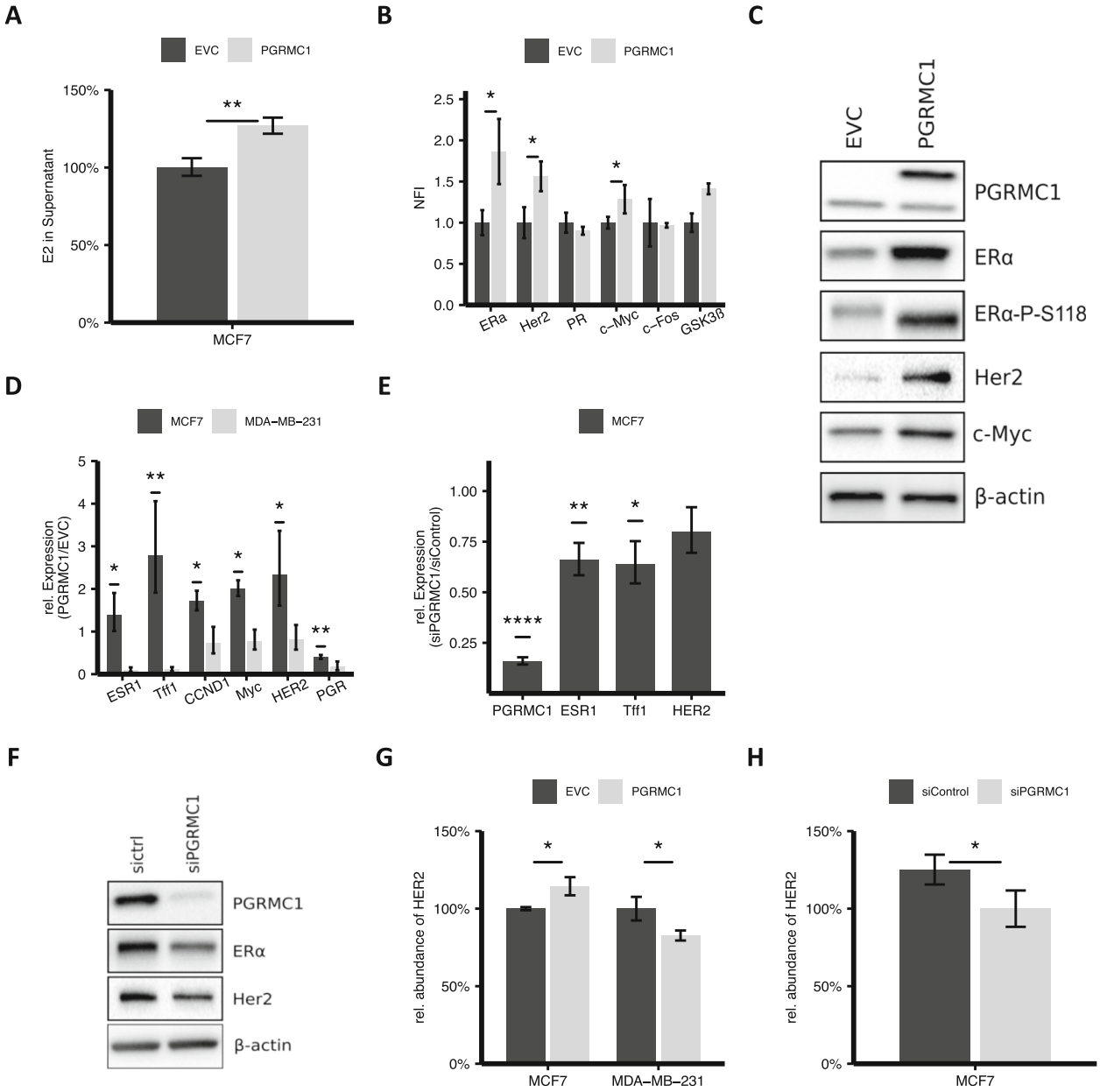


Fig. 3 **a** Amount of E2 in the supernatant of MCF7/PGRMC1 cells compared to the empty vector control after 48 h, detected with ELISA. $^{**}p \leq 0.01$. **b** NFI (blank-corrected mean fluorescence intensity) ratio of protein expression of ERα, Her2, PR, c-Myc, and c-Fos analyzed by RPPA. Protein expression was normalized to MCF7/EVC and protein expression measured in MCF7/EVC cells was set to 1. Up-/downregulation of protein expression in MCF7/PGRMC1 cells were calculated. $^{*}p \leq 0.05$ (Student's *t* test, $n = 3$). **c** Western blot analysis of ERα, Her2, and c-Myc protein levels in MCF7/EVC and MCF7/PGRMC1 cells. Representative picture of 3 independent analyses. **d** qRT-PCR analysis of *ESR1*, *TFF1*, *HER2*, *CCND1*, *Myc*, and *PGR* mRNA expression in MCF7/EVC and MCF7/PGRMC1 cells, MDA-MB-231/EVC and MDA-MB-231/PGRMC1 cells. $^{*}p \leq 0.05$, $^{***}p \leq 0.001$ (Student's *t* test, $n = 3$). **e** qRT-PCR analysis of *PGRMC1*, *ESR1*, *HER2*, and *TFF1* mRNA expression in MCF7 siCtrl and MCF7 siPGRMC1 cells. $^{*}p \leq 0.05$, $^{**}p < 0.01$, $^{****}p \leq 0.0001$ (Student's *t* test, $n = 3$). **f** Western blot analysis of ERα and Her2 protein levels in MCF7 siCtrl and MCF7 siPGRMC1 cells. Representative blot from 3 independent analyses. **g** Quantification of HER2 protein in membranes of unpermeabilized MCF7/EVC and MCF7/PGRMC1 cells, MDA-MB-231/EVC and MDA-MB-231/PGRMC1 cells, and MCF7 siCtrl and MCF7 siPGRMC1 cells **(h)** via flow cytometry. $^{*}p \leq 0.05$ (Student's *t* test, $n = 3$)

the ERα-dependent gene trefoil factor 1 (Tff1), CCND1 and Myc as reporter genes for ERα activation compared to MCF7/EVC and T47D/EVC. Interestingly, mRNA levels of PGR were significantly lower in the PGRMC1 overexpressing cells compared to their empty vector control. To further consolidate our hypothesis, we significantly silenced ($p < 0.01$) PGRMC1 expression by siPGRMC1 (Fig. 3e). As expected, the expression of ERα,

ESR1, and Tff1 were significantly downregulated (Fig. 3e), albeit no significant upregulation was detected for mRNA levels of HER2 pointing towards a post-transcriptional regulation of HER2 levels by PGRMC1 (Fig. 3e). In accordance, western blot analysis revealed decreased expression of ERα and HER2 in MCF7/siPGRMC1 (Fig. 3f). Previous studies revealed that HER2 overexpression causes deformation of the cell membrane and a subsequent disruption of epithelial features independent of receptor signaling [25, 33]. We demonstrated higher HER2 expression on the surface of non-permeabilized MCF7/PGRMC1 cells compared to MCF7/EVC cells using flow cytometry (Fig. 3g). Similarly, HER2 levels were reduced on the surface of MCF7/siPGRMC1 cells (Fig. 3h). MDA-MB-231/PGRMC1 cells even showed lower expression of HER2 compared to MDA-MB-231/EVC cells (Fig. 3g).

PGRMC1 overexpressing breast cancer cells show higher amounts of neutral lipids and lipid droplets

Lipid droplets recently emerged as new organelles not only due to their role in energy storage, but also as modulators of cell signaling and lipid homeostasis in several diseases including breast cancer [34–36].

By altering cholesterol levels in breast cancer cells, PGRMC1 could have a major influence on tumor growth via an enhanced lipid droplet formation in hormone receptor-positive breast cancer. To quantify the amount of neutral lipids, PGRMC1 overexpressing cell lines and their respective empty vector control were examined by BODIPY® staining of neutral lipids respectively lipid droplets. Subsequent flow cytometry analysis showed that PGRMC1 overexpressing hormone receptor-positive cells have a significantly higher amount of neutral lipids in comparison to the empty vector control (Fig. 4a, supplemental Figure 4A). Interestingly, we found significantly lower levels of lipids in MDA-MB-231/PGRMC1 cells compared to MDA-MB-231/EVC (Fig. 4a). Our results point towards an upregulation of lipid synthesis due to PGRMC1 overexpression in hormone receptor-positive breast cancer, which might lead to enhanced tumor growth.

PGRMC1 fuels endogenous lipid synthesis and lipid uptake and upregulates enzymes of the cholesterol metabolism

Besides the direct interaction of PGRMC1 with enzymes of the mevalonate pathway, the influence of PGRMC1 on lipid metabolism might be explained by increased mRNA expression of enzymes involved in endogenous and exogenous lipid metabolism.

Quantitative PCR analysis revealed increased levels of mRNA for SREBF1, SREBF2, LDLR, HMGS1, SCD, FASN, and ACAT1 in MCF7/PGRMC1 cells compared to MCF7/EVC cells (Fig. 4b, supplemental Figure 4B). These enzymes are not only key players in cholesterol and fatty acid synthesis, but also upregulated in breast cancer and they are associated with a worse outcome. In MDA-MB-231 cells, PGRMC1 overexpression did not result in higher expression of the abovementioned proteins (Fig. 4b). To show the increasing effect of PGRMC1 on expression of enzymes of the lipid metabolism, we obtained normalized microarray data of 63 hormone receptor-positive breast cancers tissue samples [37]. Spearman's correlation between the PGMRC1 expression level and various expression levels of proteins (FASN, FDFT1, HMGCS1, HMGCR, LDLR, SCD) indicated positive correlations between PGRMC1 and the respective enzymes in luminal A breast cancer tissue samples (Fig. 4c). Our findings advert to a complex and diverse impact of PGRMC1 on lipid homeostasis in breast cancer.

PGRMC1 enhances expression of lipid rafts in cell membranes of breast cancer cells

Lipid rafts are cholesterol-rich microdomains in cell membranes, which have functions in cell proliferation and growth, membrane trafficking, metastasis, and apoptosis [23, 24, 38]. Furthermore, lipid raft formation in cell membranes is influenced by FDFT1 activity [39]. Since lipid rafts play a role in breast cancer progression and due to the fact that (a) PGRMC1 overexpressing hormone receptor-positive breast cancer cells have higher amounts of cholesterol and that (b) PGRMC1 interacts with FDFT1, we determined the abundance of lipid rafts in MCF7 and MDA-MB-231 cells with PGRMC1 overexpression and respective empty vector control as well as in MCF7 cells treated with siRNAs directed against PGRMC1, to knockdown PGRMC1 (Fig. 4d). Cells were stained with Vybrant™ Alexa Fluor™ 488 Lipid Raft Labeling Kit and detected by flow cytometry. MCF7/PGRMC1 cells showed significantly higher levels of lipid rafts compared to the respective empty vector control (Fig. 4d, upper). In addition, we found significantly lower expression of lipid rafts when endogenous PGRMC1 was knocked down in MCF7 cells (Fig. 4d, lower). Interestingly, lipid rafts were decreased in PGRMC1 overexpressing MDA-MB-231 cells (Fig. 4d).

Elevated proliferation mediated by lipid rafts is, among others, attributed to modulation of signaling functions of growth factor receptors like the ErbB (HER) receptor family.

Since we found higher expression of HER2 in the membrane of PGRMC1 overexpressing MCF7 cells (Fig. 3g), we analyzed the HER2 expression in lipid rafts in more detail.

PGRMC1 overexpressing MCF7 and MDA-MB-231 cells and respective empty vector control cells were co-

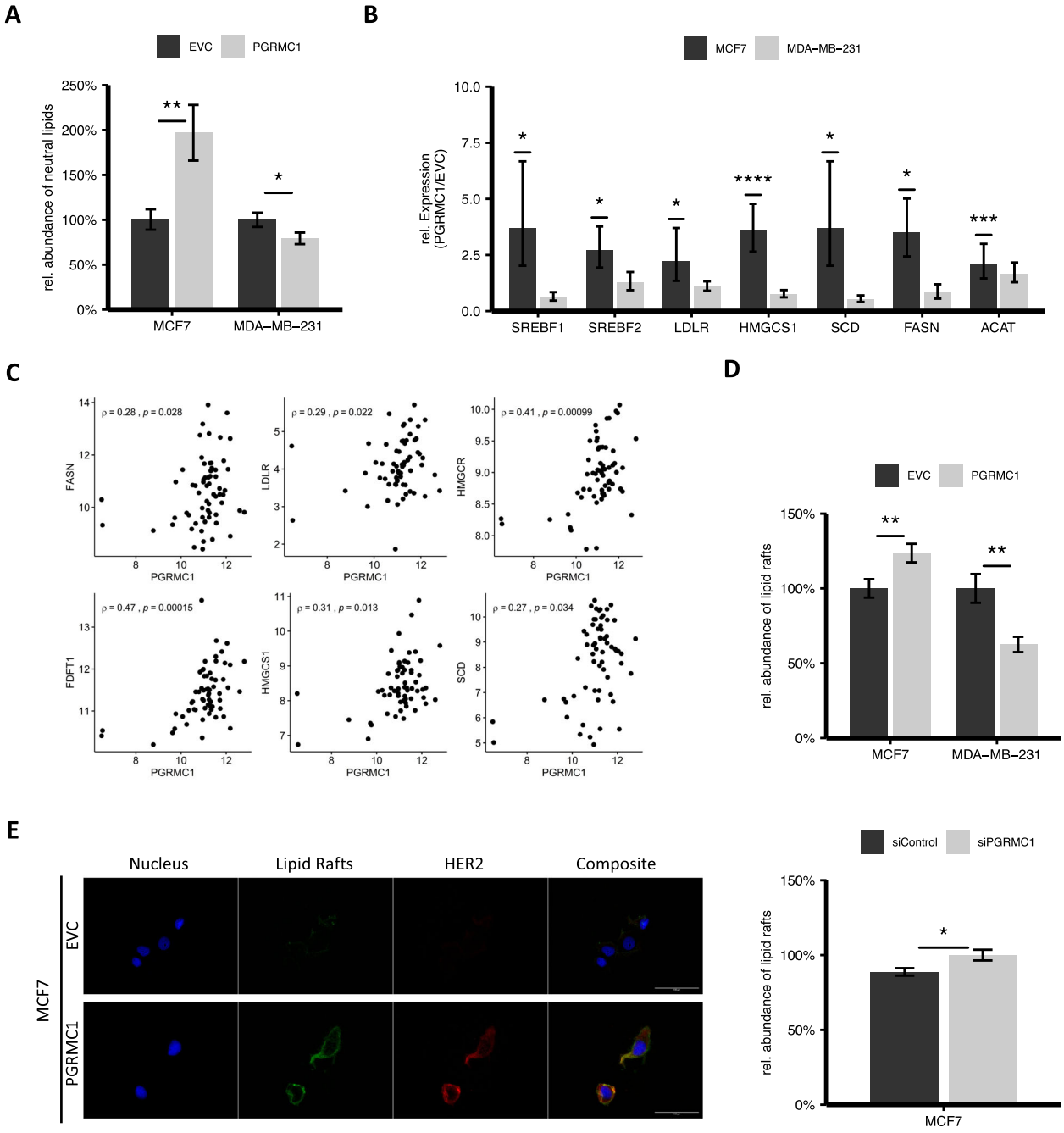


Fig. 4 a Detection of neutral lipids and lipid droplets in MCF7/EVC and MCF7/PGRMC1, MDA-MB-231/EVC and MDA-MB-231/PGRMC1 cells by BODIPY® staining and quantification via flow cytometry. *$p \leq 0.05$, **$p \leq 0.01$. (Student's *t* test, $n = 3$). **b** qRT-PCR analysis of SREBF1, SREBF2, LDLR, HMGS1, SCD, FASN, ACAT mRNA expression in MCF7/PGRMC1 and MDA-MB-231/PGRMC1 cells compared to the respective EVC cells. *$p \leq 0.05$, **$p \leq 0.01$, ***$p \leq 0.001$ (Student's *t* test, $n = 3$). **c** Spearman's correlation between the PGMRC1 expression level and various expression levels of proteins (FASN, FDFT1, HMGCS1, HMGCR, LDLR, SCD) involved in lipid metabolism. Data obtained from normalized microarray data (Affymetrix Human Genome U133A Array) of 63 hormone receptor-positive breast cancer tissue samples. **d** Detection of lipid rafts in cell membranes of MCF7/EVC and MCF7/PGRMC1 cells, MDA-MB-231/EVC and MDA-MB-231/PGRMC1 cells, and MCF7 siCtrl and MCF7 siPGRMC1 cells by Vybrant™ Alexa Fluor™ 488 and subsequent quantification via flow cytometry. *$p \leq 0.05$, **$p \leq 0.01$ (Student's *t* test, $n = 3$). **e** Immunofluorescence staining with Vybrant™ Alexa Fluor™ 488, fluorescence immunocytochemistry for HER2, and nuclear staining with DAPI. 63-fold magnification. Cells were grown on chamber slides for 24 h

stained for HER2 and lipid rafts (Fig. 4e, supplemental Figure 3C). Especially in MCF7/PGRMC1 cells, we found a strong co-localization of HER2 in lipid rafts (Fig. 4e).

PGRMC1 influences activation of EGFR signaling

Another important member of the ErbB receptor family, which plays a major role in breast cancer signaling, is the EGFR. Several studies suggest that PGRMC1 may promote EGFR phosphorylation and activation [8, 9, 13, 40]. The hypothesis of PGRMC1 enhancing EGFR signaling was investigated by reverse phase protein array (RPPA) with a focus on phosphorylation of EGFR and its downstream targets in MCF7/PGRMC1 and MCF7/EVC cells (Fig. 5a). Our results point towards an increased phosphorylation of EGFR (p-Tyr1068), Akt (p-Ser473 and p-Thr308), MEK1/2 (p-Ser217/Ser221), ERK1/2 (p-Thr202/Tyr204), and S6 (p-Ser240/Ser244) in PGRMC1/MCF7 cells compared to EVC cells (Fig. 5a). In combination with our results from immunofluorescence staining, this suggests that there might exist a powerful link between PGRMC1 expression and activation of oncogenic signaling pathways in MCF7 cells (Fig. 5c).

To verify the RPPA results, we performed western blot analysis of EGFR signaling induced with EGF (Fig. 5b). Phosphorylation of EGFR, Akt, MEK1/2, and ERK1/2 was observed (Fig. 5b). Compatible, significantly elevated levels of EGFR (p-Tyr1068), Akt (p-Ser473), MEK1/2 (p-Ser217/Ser221), and ERK1/2 (p-Thr202/Tyr204) were monitored in MCF7/PGRMC1 cells. In contrast, expression levels of total protein did not vary significantly (Fig. 5c). MDA-MB-231 showed no difference in expression levels of EGFR (p-Tyr1068), Akt (p-Ser473), MEK1/2 (p-Ser217/Ser221), and ERK1/2 (p-Thr202/Tyr204), suggesting a subordinated role of PGRMC1 in EGFR signaling in triple-negative breast cancer (supplemental Figure 4A, 4B).

Cholesterol and fatty acid depletion induced by statins reverses the growth benefit interceded by PGRMC1

Our findings suggest a complex and broad role of PGRMC1 in cholesterol and lipid metabolism (Fig. 5d). Based on our research concerning the influence of PGRMC1 on lipid homeostasis and increased viability of PGRMC1 overexpressing cells, we hypothesized that a higher lipid synthesis might lead to a survival benefit of PGRMC1 overexpressing cells.

To verify this hypothesis, we treated PGRMC1 overexpressing MCF7 and MDA-MB-231 cells and the respective controls with different concentrations of simvastatin, a competitive inhibitor of HMG-CoA reductase, and performed subsequent viability assays (Fig. 5d).

Interestingly, contrary to expectations, inhibition of HMG-CoA reductase and following depletion of cholesterol not only assimilated viability in MCF7/PGRMC1 cells compared to MCF7/EVC cells, but even led to inferior viability. This suggests a higher dependence of PGRMC1 overexpressing cells on cholesterol. Intriguingly, MDA-MB-231 cells with PGRMC1 overexpression reacted similar to MCF7 cells (Fig. 5d).

Discussion

Although previous studies report on the proliferative effect of PGRMC1 in breast cancer, little is known about the mechanisms by which PGRMC1 effects carcinogenesis. Therefore, our present study focuses on the modifying function of PGRMC1 on lipid metabolism and oncogenic signaling. Evidence is pointing towards a meaningful impact of modified lipid metabolism in breast cancer progression and metastasis [41–44]. Although one of the most relevant mechanisms of energy usage of cancer cells is their increase in glucose uptake and their use of non-oxidative glycolysis, also known as Warburg effect, breast cancer cells upregulate lipid de novo synthesis and the uptake of free fatty acids and low-density lipoproteins [44, 45]. Our findings suggest the function of PGRMC1 as an important enhancer especially of lipid synthesis resulting in oncogenic signaling and tumor progression. For the first time, we detected enhanced mRNA expression of proteins regulating lipid synthesis and uptake in PGRMC1 overexpressing hormone receptor-positive MCF7 and T47D cells resulting in significantly higher lipid levels in MCF7/PGRMC1 and T47D/PGRMC1 cells compared to the empty vector control cells. Further, we could demonstrate that PGRMC1 interacts with CYP51, FDFT1, and SCD1, which are major players in lipogenesis. Interestingly, these interactions are less pronounced in MDA-MB-231 cells. An explanation for the lower interaction might be that triple-negative breast cancer cells have been reported to cover their needs for lipids via the uptake of exogenous fatty acids in contrast to performing lipid de novo synthesis [44, 46].

A possible result of the detected interactions between PGRMC1 and CYP51, FDFT1, and SCD1 could be the increase of cholesterol and neutral lipid levels in MCF7/PGRMC1 and T47D/PGRMC1 cells. Since cholesterol is the precursor of steroid hormones like estradiol, elevated levels of cholesterol may subsequently lead to higher levels of estradiol as indicated by our measurements in the supernatant of MCF7/PGRMC1 cells of this scenario. One consequence could be that PGRMC1 promotes tumor progression by upregulation of ERα protein and ESR1 mRNA directly via a transcriptional mechanism or indirectly via elevated steroid synthesis. Since various studies showed an upregulation of steady-

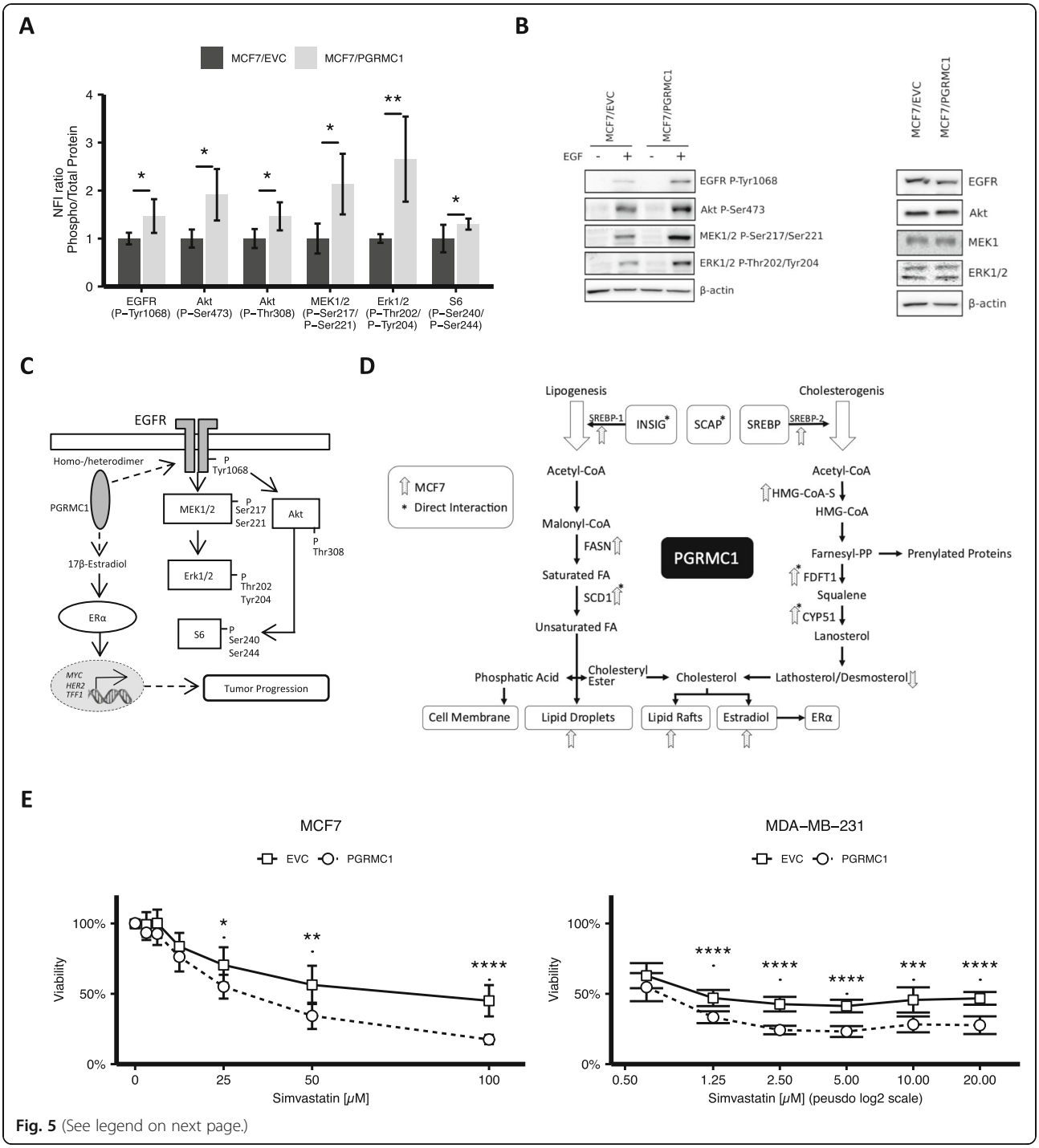


Fig. 5 (See legend on next page.)

(See figure on previous page.)
Fig. 5 a Protein phosphorylation of EGFR P-Tyr1068, Akt P-Ser473, Akt P-Thr308, MEK1/2 P-Ser217/Ser221, Erk1/2 P-Thr202/Tyr204, and S6 P-Ser240/Ser244 analyzed by RPPA. NFI (blank-corrected mean fluorescence intensity) ratio of phospho-protein/total protein was calculated, normalized to MCF7/EVC, and ratio in MCF7/EVC cells was set to 1. Up-/downregulation of protein phosphorylation in MCF7/PGRMC1 cells was calculated. *$p \leq 0.05$, **$p \leq 0.01$ (Student's t test, $n = 3$). **b** Protein phosphorylation of EGFR P-Tyr1068, Akt P-Ser473, MEK1/2 P-Ser217/Ser221, and Erk1/2 P-Thr202/Tyr204 verified by western blot analysis. Cells were treated with EGF (10 ng/mL) for 10 min/37 °C. Representative blot of 3 independent analyses**.** Total protein expression of EGFR, Akt, MEK1/2, and Erk1/2 verified by western blot analysis. Representative blot of 3 independent analyses shown. **c** PGRMC1 mediates phosphorylation of EGFR and its downstream targets and upregulates E2 levels, ERα expression, and ERα-target genes. EGFR phosphorylation activates the MAPK signaling cascade (including MEK1/2-, ERK1/2-, and S6-phosphorylation) and PI3K signaling cascade (including Akt- and S6-phosphorylation). Phosphorylation of S6 induces transcription of genes, involved in the regulation of cell cycle progression, cell proliferation, and glucose homeostasis. ERα translocates into the nucleus upon ligand-dependent or ligand-independent activation and acts as a transcription factor to transcribe genes involved in tumor progression. **d** Overview of the influence of PGRMC1 in cholesterol and lipid metabolism. **e** MCF7/EVC and MCF7/PGRMC1 cells were treated with 100 μM, 50 μM, 25 μM, 12.5 μM, 6.25 μM, and 3.175 μM simvastatin and respective DMSO control. MDA-MB-231/EVC and MDA-MB-231/PGRMC1 cells were treated with 20 μM, 10 μM, 5 μM, 2.5 μM, 1.25 μm, and 0.625 μM simvastatin and respective DMSO control. Viability was analyzed by MTT assay at $t = 24$ h, $t = 48$ h, $t = 72$ h and 37 °C. Depicted are results after 48 h of treatment. Viability is normalized on the DMSO control. p values were adjusted using the Bonferroni correction ($n_{doses} = 6$; $n_{replicates} = 9$)

state ERα levels by long-term exposure to E2 [47], increased levels of ERα plus a simultaneous autocrine/paracrine activation by E2 may trigger a proliferative cycle support in tumor growth. For the first time, we also observed that PGRMC1 impacts on lipid rafts, another regulator of cancer progression. Lipid rafts are important, e.g., in modulation of membrane geometry, lateral movement of molecules, and signal transduction [23, 48]. We observed increased lipid raft formation in PGRMC1 overexpressing hormone receptor-positive breast cancer cells. The co-localization of HER2 in lipid rafts, also reported by other research groups [49, 50], may influence EGFR signaling. Zhuang et al. reported an EGF-induced and constitutive signaling via the Akt serine-threonine kinase and subsequent survival in cancer cells [51]. Furthermore, EGFR and HER2 localization in lipid rafts is discussed to play a role in cancer cell drug resistance, e.g., regarding treatment with trastuzumab or tyrosine kinase inhibitors [49, 50]. On the other hand, Orr et al. showed that altered cholesterol levels modify the mobility of EGFR in the cell membrane leading to its decreased activation due to reduced dimerization of EGFR monomeres [25]. The relevant role of PGRMC1 in promoting phosphorylation and activation of receptors for example by heme-dependent PGRMC1 dimerization has already been reported [8, 9, 13, 40]. Here, elevated phosphorylation levels of EGFR and its downstream targets in MCF7/PGRMC1 cells were discovered. The crosstalk between EGFR/Her2 and ERα signaling cascades has often been reported, whereby ERα can induce the E2-dependent activation of the EGFR signaling pathway by promoting phosphorylation of Akt (P-Ser473) via the non-genomic pathway. Alternatively, ERα activation can be accomplished independently of estrogens by EGFR-activated MAPK-signaling or PI3K pathway [52, 53]. In the current study, we demonstrate that both MAPK and PI3K pathway components (i.e., MEK1/2, ERK1/2, and AKT) are activated in PGRMC1 overexpressing MCF7 cells. This may lead to increased ERα activation and finally to increased cancer proliferation. Additionally, ERα and HER2 correlate positively in HER2 non-overexpressing breast cancer [54, 55]. Hence, higher levels of ER in MCF7/PGRMC1 cells could lead to higher expression of HER2. However, the influence of PGRMC1 on EGFR/HER2 signaling in lipid rafts and its impact on tumor progression requires further studies.

Due to the role of the mevalonate pathway and its dual role in cholesterol synthesis and prenylation of signaling proteins, statins have been tested as anti-cancer drugs. Statins block the HMG-CoA reductase, the gatekeeper of the mevalonate pathway. We speculated due to increased activation of the mevalonate pathway and due to higher cholesterol and neutral lipids production that PGRMC1 overexpressing cells may be more dependent on the mevalonate pathway. Hence, they might be more susceptible to statin treatment [56–60]. For the first time, we detected that MCF7/PGRMC1 and MDA-MB-231/PGRMC1 cells are more sensitive to treatment with simvastatin compared to the respective controls. We assume that PGRMC1 overexpression leads to higher dependence on cholesterol and fatty acids of cancer cells due to an alteration of fatty acid metabolism, by enhanced driving of the mevalonate pathway and related synthesis of the isoprenoids geranylgeranyl pyrophosphate (GGPP) and farnesyl pyrophosphate (FPP) [61, 62], e.g., leading to inhibition of small Rho GTPase prenylation [63].

Indeed, PGRMC1 might also reduce viability of breast cancer cells under treatment with statins, because PGRMC1 is known to interact with CYP enzymes [3, 5, 13–15]. Specifically, inhibition of cytochromes P450 could increase the concentration of simvastatin, since statins are metabolized by CYP3A4.

Hence, PGRMC1 overexpressing tumors may be an interesting target for additional cholesterol lowering therapy.

Conclusion

We demonstrate that PGRMC1 mediates progression of breast cancer cells potentially by altering cholesterol and lipid metabolism and activating key drivers of tumor progression in breast cancer, namely ERα expression and activation, as well as EGFR signaling. Our data underline the contribution of PGRMC1 to especially hormone receptor-positive breast cancer pathogenesis in vitro and in vivo and suggest its potential as a target for anti-cancer therapy.

Supplementary information

Supplementary information accompanies this paper at https://doi.org/10.1186/s13058-020-01312-8.

Additional file 1.

Abbreviations

ACAT1: Acetyl-Coenzyme A acetyltransferase 1; Akt: Protein kinase B; BODIPY: Difluoro {2-[1-(3,5-dimethyl-2H-pyrrol-2-ylidene-N)ethyl]-3,5-dimethyl-1H-pyrrolatoN} boron; BSA: Bovine serum albumin; CK-2: Casein kinase-2; CYP: Cytochromes P450; DAPI: 4,6-Diamidin-2-phenylindol; E2: 17β-Estradiol; EGF: Epidermal growth factor; EGFR: Epidermal growth factor receptor; ELISA: Enzyme-linked immunosorbent assay; Erk1/2 (MAPK p44/42): extracellular signal-regulated kinases; ERα: Estrogen receptor α; FASN: Fatty acid synthase; FCS: Fetal calf serum; GFP: Green fluorescent protein; HA: Hemagglutinin; Her2: Human epidermal growth factor receptor-2; HMGS: 3-Hydroxy-3-methylglutaryl coenzyme A synthase; INSIG: Insulin-induced gene protein; LDLR: Low-density lipoprotein receptor; MEK1 (MAP2K1): Mitogen-activated protein kinase kinase 1; MEK2 (MAP2K2): Mitogen-activated protein kinase kinase 2; NFI: Blank-corrected mean fluorescence intensity; PGRMC1: Progesterone receptor membrane component-1; PR: Progesterone receptor; RPPA: Reverse phase protein array; SCAP: SREBP cleavage-activating protein; SCD1: Stearoyl-CoA desaturase1; FDFT1: Farnesyl-diphosphate farnesyltransferase 1 (Squalene Synthase); SREBP1/2: Sterol regulatory element-1/2; TFF1: Trefoil factor 1

Acknowledgements

This study contains results of the dissertation "Role of PGRMC1 in breast cancer progression and increased breast cancer risk upon progestin-based hormone therapy" submitted to the Faculty of Mathematics and Natural Sciences at the Heinrich-Heine-University Duesseldorf on 30 September 2017 by Marina Ludescher (née Willibald).

Authors' contributions

HA, NS, ML, and HN were responsible for conceptual design of the study. HA, NS, ML, MP, and CV designed the experiments. HA, ML, NS, BG, AM, AB, and UH performed the experiments. HA, ML, MP, CV, JC, and UH analyzed the data. HA, NS, ML, HN, MP, and CV interpreted the findings. HA wrote the original draft. HA, ML, MP, CV, RLK, UH, UMZ, and HN critically revised the manuscript. RLK, ER, DN, TF, UZ, and HN contributed to the project administration. All authors read and approved the final manuscript.

Funding

This work was supported by the German Research Foundation (Deutsche Forschungsgemeinschaft, DFG, NE 805/6-1), by the German Academic Exchange Service (ID-57215625), and by GINOP grants (GINOP-2.3.2-15-2016-00001 and GINOP-2.3.2-15-2016-00039). Parts of this project were also supported by the Wilhelm Sander Foundation (2013.126.1), by the Robert Bosch Foundation (Stuttgart, Germany), and by the Brigitte and Dr. Konstanze Wegener Foundation (Duesseldorf, Germany).

Availability of data and materials

The microarray data analyzed during the current study are available in the Gene Expression Omnibus under the ID GSE129560. [https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE129560]. The remaining datasets used and analyzed during the current study are available from the corresponding author on reasonable request.

Ethics approval and consent to participate

All animal experiments were performed in accordance with animal experimentation and ethics guidelines of the EU (2010/63/EU). Experimental protocols were approved by the Review Committee of Biological Research Centre of the Hungarian Academy of Sciences and the responsible governmental agency (clearance number: CSI/01/1489-5/2014).

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

Author details

[1]Department of Obstetrics and Gynecology, University Hospital and Medical Faculty of the Heinrich-Heine University Duesseldorf, Life Science Center, Merowingerplatz 1A, 40225 Düsseldorf, Germany. [2]NMI TT Pharmaservices, Protein Profiling, Reutlingen, Germany. [3]Dr. Margarete Fischer-Bosch Institute of Clinical Pharmacology and University of Tübingen, Stuttgart, Germany. [4]Institute of Biochemistry, Biological Research Centre, Szeged, Hungary. [5]Institute of Genetics, Biological Research Centre, Szeged, Hungary.

Received: 11 March 2020 Accepted: 30 June 2020
Published online: 13 July 2020

References

1. Bray F, Ferlay J, Soerjomataram I, Siegel RL, Torre LA, Jemal A. Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA Cancer J Clin. 2018;68(6):394–424. https://doi.org/10.3322/caac.21492.
2. Ferlay J, Soerjomataram I, Dikshit R, et al. Cancer incidence and mortality worldwide: sources, methods and major patterns in GLOBOCAN 2012. Int J Cancer. 2015;136(5):E359–86. https://doi.org/10.1002/ijc.29210.
3. Kimura I, Nakayama Y, Konishi M, et al. Functions of MAPR (membrane-associated progesterone receptor) family members as heme/steroid-binding proteins. Curr Protein Pept Sci. 2012;13(7):687–96. https://doi.org/10.2174/138920312804142110.
4. Gerdes D, Wehling M, Leube B, Falkenstein E. Cloning and tissue expression of two putative steroid membrane receptors. Vol 379; 1998.
5. Ryu CS, Klein K, Zanger UM. Membrane associated progesterone receptors: promiscuous proteins with pleiotropic functions - focus on interactions with cytochromes P450. Front Pharmacol. 2017;8. https://doi.org/10.3389/fphar.2017.00159.
6. Cahill MA, Jazayeri JA, Catalano SM, Toyokuni S, Kovacevic Z, Richardson DR. The emerging role of progesterone receptor membrane component 1 (PGRMC1) in cancer biology. Biochim Biophys Acta - Rev Cancer. 2016;1866(2):339–49. https://doi.org/10.1016/j.bbcan.2016.07.004.
7. Zhang Y, Ruan X, Willibald M, et al. May progesterone receptor membrane component 1 (PGRMC1) predict the risk of breast cancer? Gynecol Endocrinol. 2016;32(1):58–60. https://doi.org/10.3109/09513590.2015.1078303.
8. Ahmed IS, Rohe HJ, Twist KE, Craven RJ. Pgrmc1 (progesterone receptor membrane component 1) associates with epidermal growth factor receptor and regulates erlotinib sensitivity. J Biol Chem. 2010;285(32):24775–82. https://doi.org/10.1074/jbc.M110.134585.
9. Hampton KK, Stewart R, Napier D, Claudio PP, Craven RJ. PGRMC1 elevation in multiple cancers and essential role in stem cell survival. Adv Lung Cancer. 2015;04(03):37–51. https://doi.org/10.4236/alc.2015.43006.
10. Mir SUR, Ahmed ISA, Arnold S, Craven RJ. Elevated progesterone receptor membrane component 1/sigma-2 receptor levels in lung tumors and plasma from lung cancer patients. Int J Cancer. 2012;131(2). https://doi.org/10.1002/ijc.26432.
11. Willibald M, Wurster I, Meisner C, et al. High level of progesteron receptor membrane component 1 (PGRMC 1) in tissue of breast cancer patients is associated with worse response to anthracycline-based neoadjuvant therapy. Horm Metab Res. 2017;49(8):595–603. https://doi.org/10.1055/s-0043-113635.

12. Ruan X, Zhang Y, Mueck AO, et al. Increased expression of progesterone receptor membrane component 1 is associated with aggressive phenotype and poor prognosis in ER-positive and negative breast cancer. Menopause. 2017;24(2):203–9. https://doi.org/10.1097/GME.0000000000000739.
13. Kabe Y, Nakane T, Koike I, et al. ARTICLE Haem-dependent dimerization of PGRMC1/Sigma-2 receptor facilitates cancer proliferation and chemoresistance. Nat Commun. 2016;6. https://doi.org/10.1038/ncomms11030.
14. Ahmed IS, Rohe HJ, Twist KE, Mattingly MN, Craven RJ. Progesterone receptor membrane component 1 (Pgrmc1): a heme-1 domain protein that promotes tumorigenesis and is inhibited by a small molecule. J Pharmacol Exp Ther. 2010;333(2):564–73. https://doi.org/10.1124/jpet.109.164210.
15. Mansouri MR, Schuster J, Badhai J, et al. Alterations in the expression, structure and function of progesterone receptor membrane component-1 (PGRMC1) in premature ovarian failure. Hum Mol Genet. 2008;17(23):3776–83. https://doi.org/10.1093/hmg/ddn274.
16. Lin ST, May EWS, Chang JF, Hu RY, Wang LHC, Chan HL. PGRMC1 contributes to doxorubicin-induced chemoresistance in MES-SA uterine sarcoma. Cell Mol Life Sci. 2015;72(12):2395–409. https://doi.org/10.1007/s00018-014-1831-9.
17. Friel AM, Zhang L, Pru CA, et al. Progesterone receptor membrane component 1 deficiency attenuates growth while promoting chemosensitivity of human endometrial xenograft tumors. Cancer Lett. 2015;356(2):434–42. https://doi.org/10.1016/j.canlet.2014.09.036.
18. Cahill MA, Medlock AE. Thoughts on interactions between PGRMC1 and diverse attested and potential hydrophobic ligands. J Steroid Biochem Mol Biol. 2017;171:11–33. https://doi.org/10.1016/j.jsbmb.2016.12.020.
19. Antalis CJ, Uchida A, Buhman KK, Siddiqui RA. Migration of MDA-MB-231 breast cancer cells depends on the availability of exogenous lipids and cholesterol esterification. Clin Exp Metastasis. 2011;28(8):733–41. https://doi.org/10.1007/s10585-011-9405-9.
20. Marwarha G, Raza S, Hammer K, Ghribi O. 27-hydroxycholesterol: a novel player in molecular carcinogenesis of breast and prostate cancer. Chem Phys Lipids. 2017;207:108–26. https://doi.org/10.1016/j.chemphyslip.2017.05.012.
21. Menendez JA, Lupu R. Fatty acid synthase and the lipogenic phenotype in cancer pathogenesis. Nat Rev Cancer. 2007;7(10):763–77. https://doi.org/10.1038/nrc2222.
22. Nelson ER, Chang C Yi, McDonnell DP. Cholesterol and breast cancer pathophysiology. Trends Endocrinol Metab 2014;25(12):649–655. doi:https://doi.org/10.1016/j.tem.2014.10.001.
23. Mollinedo F, Gajate C. Lipid rafts as major platforms for signaling regulation in cancer. Adv Biol Regul. 2015;57:130–46. https://doi.org/10.1016/j.jbior.2014.10.003.
24. Li YC, Park MJ, Ye SK, Kim CW, Kim YN. Elevated levels of cholesterol-rich lipid rafts in cancer cells are correlated with apoptosis sensitivity induced by cholesterol-depleting agents. Am J Pathol. 2006;168(4):1107–18. https://doi.org/10.2353/ajpath.2006.050959.
25. Orr G, Hu D, Özçelik S, Opresko LK, Wiley HS, Colson SD. Cholesterol dictates the freedom of EGF receptors and HER2 in the plane of the membrane. Biophys J. 2005;89(2):1362–73. https://doi.org/10.1529/biophysj.104.056192.
26. Fox EM, Andrade J, Shupnik MA. Novel actions of estrogen to promote proliferation: integration of cytoplasmic and nuclear pathways. Steroids. 2009;74(7):622–7. https://doi.org/10.1016/j.steroids.2008.10.014.
27. Barrett T, Wilhite SE, Ledoux P, et al. NCBI GEO: Archive for functional genomics data sets - Update. Nucleic Acids Res. 2013;41(D1). doi:https://doi.org/10.1093/nar/gks1193.
28. Zhao Y, Ruan X, Wang H, et al. The presence of a membrane-bound progesterone receptor induces growth of breast cancer with norethisterone but not with progesterone: a xenograft model. Maturitas. 2017;102:26–33. https://doi.org/10.1016/j.maturitas.2017.05.007.
29. Schneck H, Ruan X, Seeger H, et al. Membrane-receptor initiated proliferative effects of dienogest in human breast cancer cells. Gynecol Endocrinol. 2013;29(2):160–3. https://doi.org/10.3109/09513590.2012.730572.
30. Ruan X, Gu M, Cai G, et al. Progestogens and PGRMC1-dependent breast cancer tumor growth: an in-vitro and xenograft study. Maturitas. 2019;123:1–8. https://doi.org/10.1016/j.maturitas.2019.01.015.
31. Rohe HJ, Ahmed IS, Twist KE, Craven RJ. PGRMC1 (progesterone receptor membrane component 1): a targetable protein with multiple functions in steroid signaling, P450 activation and drug binding. Pharmacol Ther. 2009;121(1):14–9. https://doi.org/10.1016/j.pharmthera.2008.09.006.
32. Xu J, Zeng C, Chu W, et al. Identification of the PGRMC1 protein complex as the putative sigma-2 receptor binding site. Nat Commun. 2011;2(1). https://doi.org/10.1038/ncomms1386.
33. Chung I, Reichelt M, Shao L, et al. High cell-surface density of HER2 deforms cell membranes. Nat Commun. 2016;7. https://doi.org/10.1038/ncomms12742.
34. Koizume S, Miyagi Y. Lipid droplets: a key cellular organelle associated with cancer cell survival under normoxia and hypoxia. Int J Mol Sci. 2016;17(9):1–23. https://doi.org/10.3390/ijms17091430.
35. Baenke F, Peck B, Miess H, Schulze A. Hooked on fat: the role of lipid synthesis in cancer metabolism and tumour development. Dis Model Mech. 2013;6(6):1353–63. https://doi.org/10.1242/dmm.011338.
36. Blücher C, Zilberfain C, Venus T, et al. Single cell study of adipose tissue mediated lipid droplet formation and biochemical alterations in breast cancer cells. Analyst. 2019;144(18):5558–70. https://doi.org/10.1039/c9an00816k.
37. Sinn BV, Fu C, Lau R, et al. SET ER/PR: a robust 18-gene predictor for sensitivity to endocrine therapy for metastatic breast cancer. npj Breast Cancer. 2019;5(1). https://doi.org/10.1038/s41523-019-0111-0.
38. de Laurentiis A, Donovan L, Arcaro A. Lipid rafts and caveolae in signaling by growth factor receptors. Open Biochem J. 2007;1:12–32. https://doi.org/10.2174/1874091X00701010012.
39. Brusselmans K, Timmermans L, Van De Sande T, et al. Squalene synthase, a determinant of raft-associated cholesterol and modulator of cancer cell proliferation. J Biol Chem. 2007;282(26):18777–85. https://doi.org/10.1074/jbc.M611763200.
40. Aizen J, Thomas P. Role of Pgrmc1 in estrogen maintenance of meiotic arrest in zebrafish oocytes through Gper/Egfr. J Endocrinol. 2015;225(1). https://doi.org/10.1530/JOE-14-0576.
41. OF K, Noory MA, Robertson GP. The role of cholesterol in cancer. Cancer Res. 2016;76(8):2063–70. https://doi.org/10.1158/0008-5472.CAN-15-2613.
42. Gorin A, Gabitova L, Astsaturov I. Regulation of cholesterol biosynthesis and cancer signaling. Vol 12. Elsevier Ltd; 2012:710–716. doi:https://doi.org/10.1016/j.coph.2012.06.011.
43. McDonnell DP, Park S, Goulet MT, et al. Obesity, cholesterol metabolism, and breast cancer pathogenesis. Cancer Res. 2014;74(18):4976–82. https://doi.org/10.1158/0008-5472.CAN-14-1756.
44. Monaco ME. Fatty acid metabolism in breast cancer subtypes. Oncotarget. 2017;8(17). https://doi.org/10.18632/oncotarget.15494.
45. Medes G, Thomas A, Weinhouse S. Metabolism of neoplastic tissue. IV. A study of lipid synthesis in neoplastic tissue slices in vitro. Cancer Res. 1953;13(1):27–9.
46. Antalis CJ, Arnold T, Rasool T, Lee B, Buhman KK, Siddiqui RA. High ACAT1 expression in estrogen receptor negative basal-like breast cancer cells is associated with LDL-induced proliferation. Breast Cancer Res Treat. 2010;122(3):661–70. https://doi.org/10.1007/s10549-009-0594-8.
47. Pinzone JJ, Stevenson H, Strobl JS, Berg PE. Molecular and cellular determinants of estrogen receptor alpha expression. Mol Cell Biol. 2004;24(11):4605–12. https://doi.org/10.1128/MCB.24.11.4605-4612.2004.
48. Lingwood D, Simons K. Lipid rafts as a membrane-organizing principle. Science. 2010;327(5961):46–50. https://doi.org/10.1126/science.1174621.
49. Menendez JA, Vellon L, Lupu R. Targeting fatty acid synthase-driven lipid rafts: a novel strategy to overcome trastuzumab resistance in breast cancer cells. Med Hypotheses. 2005;64(5):997–1001. https://doi.org/10.1016/J.MEHY.2004.09.027.
50. Irwin ME, Mueller KL, Bohin N, Ge Y, Boerner JL. Lipid raft localization of EGFR alters the response of cancer cells to the EGFR tyrosine kinase inhibitor gefitinib. J Cell Physiol. 2011;226(9):2316–28. https://doi.org/10.1002/jcp.22570.
51. Zhuang L, Lin J, Lu ML, Solomon KR, Freeman MR. Cholesterol-rich lipid rafts mediate Akt-regulated survival in prostate cancer cells. Cancer Res. 2002;62(8):2227–31.
52. Egloff AM, Rothstein ME, Seethala R, Siegfried JM, Grandis JR, Stabile LP. Cross-talk between estrogen receptor and epidermal growth factor receptor in head and neck squamous cell carcinoma. Clin Cancer Res. 2009;15(21):6529–40. https://doi.org/10.1158/1078-0432.CCR-09-0862.
53. Stoica GE, Franke TF, Moroni M, et al. Effect of estradiol on estrogen receptor-α gene expression and activity can be modulated by the ErbB2/PI 3-K/Akt pathway. Oncogene. 2003;22(39):7998–8011. https://doi.org/10.1038/sj.onc.1206769.
54. Alqaisi A, Chen L, Romond E, et al. Impact of estrogen receptor (ER) and human epidermal growth factor receptor-2 (HER2) co-expression on breast

cancer disease characteristics: implications for tumor biology and research. Breast Cancer Res Treat. 2014;148(2):437–44. https://doi.org/10.1007/s10549-014-3145-x.
55. Pinhel I, Hills M, Drury S, et al. ER and HER2 expression are positively correlated in HER2 non-overexpressing breast cancer. Breast Cancer Res. 2012;14(2). https://doi.org/10.1186/bcr3145.
56. Swanson K, Hohl R. Anti-cancer therapy: targeting the mevalonate pathway. Curr Cancer Drug Targets. 2006;6(1):15–37. https://doi.org/10.2174/156800906775471743.
57. Ahern TP, Pedersen L, Tarp M, et al. Statin prescriptions and breast cancer recurrence risk: a Danish nationwide prospective cohort study. J Natl Cancer Inst. 2011;103(19):1461–8. https://doi.org/10.1093/jnci/djr291.
58. Murtola TJ, Visvanathan K, Artama M, Vainio H, Pukkala E. Statin use and breast cancer survival: a nationwide cohort study from Finland. PLoS One. 2014;9(10). https://doi.org/10.1371/journal.pone.0110231.
59. Borgquist S, Bjarnadottir O, Kimbung S, Ahern TP. Statins: a role in breast cancer therapy? J Intern Med. 2018;284(4):346–57. https://doi.org/10.1111/joim.12806.
60. Beckwitt CH, Brufsky A, Oltvai ZN, Wells A. Statin drugs to reduce breast cancer recurrence and mortality. Breast Cancer Res. 2018;20(1):144. https://doi.org/10.1186/s13058-018-1066-z.
61. Liao JK. Isoprenoids as mediators of the biological effects of statins. J Clin Invest. 2002;110(3):285–8. https://doi.org/10.1172/jci200216421.
62. Ahern TP, Lash TL, Damkier P, Christiansen PM, Cronin-Fenton DP. Statins and breast cancer prognosis: Evidence and opportunities. Lancet Oncol. 2014;15(10). https://doi.org/10.1016/S1470-2045(14)70119-6.
63. Alizadeh J, Zeki AA, Mirzaei N, et al. Mevalonatecascade inhibition by simvastatin induces the intrinsic apoptosis pathway via depletion of isoprenoids in tumor cells. Sci Rep. 2017;7. https://doi.org/10.1038/srep44841.

Publisher's Note

Supplements

Methods

Quantification of lathosterol and cholesterol

Cholesterol and lathosterol were quantified by GC-MS analysis as described previously (Maier et al., 2009), with minor modifications.

Briefly, the cell pellet was extracted with hexane:2-propanol 3:2 v/v, spiked with internal standards [$^2H_5$]cholesterol and [$^2H_7$]lathosterol and evaporated to dryness under nitrogen. The residue was saponified with 1 M NaOH in 90% ethanol for 1 h at 70 °C and subsequently extracted with cyclohexane. The organic phase was evaporated and the *tert*-butyldimethylsilyl derivatives prepared by addition of 20 µl of N-*tert*-butyldimethylsilyl-N-methyltrifluoroacetamide (MBDSTFA) and 20 µl of DMF. Cholesterol and lathosterol were measured by GC-MS on a 5975C inert XL MSD, coupled to a 7890A GC (Agilent) in selected ion monitoring (SIM) mode at m/z 443.4, [$^2H_5$]cholesterol at m/z 448.4, and [$^2H_7$]lathosterol at m/z 450.4. Calibration samples prepared directly from the working solutions, were worked up as described above, and analyzed together with the unknown samples. Calibration curves based on internal standard calibration were obtained by weighted (1/x) linear regression for the peak-area ratio of the analyte to the respective internal standard against the amount of the analyte. The concentration of the analytes in unknown samples was obtained from the regression line.

PGRMC1 was immunoprecipitated from four individual replicates of MCF7/PGRMC1-HA cells. As a negative control, GFP-labelled MCF7/PGRMC1 cells (MCF7/PGRMC1-GFP) were used. Resulting protein preparations were shortly separated in a 4-12% polyacrylamide gel (about 4 mm running distance), silver stained and processed as

Mass spectrometry of PGRMC1 interaction partners

PGRMC1 was immunoprecipitated from four individual replicates of MCF7/PGRMC1-HA cells. As a negative control, GFP-labelled MCF7/PGRMC1 cells (MCF7/PGRMC1-GFP) were used. Resulting protein preparations were shortly separated in a 4-12% polyacrylamide gel (about 4 mm running distance), silver stained and processed as
previously described. Briefly, samples were destained, reduced with dithiothreitol, alkylated with iodoacetamide, digested with trypsin peptides extracted from the gel and finally resuspended in 0.1 % trifluoroacetic acid. Subsequently, the samples were analyzed on a liquid chromatography coupled electrospray ionization Orbitrap mass spectrometer. An Ultimate 3000 Rapid Separation Liquid Chromatography System was used for peptide separation: peptides were initially pre-concentrated on a trap column (Acclaim PepMap100, 3 µm C18 particle size, 100 Å pore size, 75 µm inner diameter, 2 cm length) at a flow rate of 6 µl/min for ten minutes using 0.1 % TFA as mobile phase and thereafter separated on an analytical column (Acclaim PepMapRSLC, 2 µm C18 particle size, 100 Å pore size, 75 µm inner diameter, 25 cm length) at a flow rate of 300 nl/min at 60°C using a 2 h gradient from 4 to 40 % solvent B (0.1 % (v/v) formic acid, 84 % (v/v) acetonitrile in water) in solvent A (0.1 % (v/v) formic acid in water). The liquid chromatography system was online coupled to an Orbitrap Elite mass spectrometer via a nano electrospray ionization source and peptides injected by distal coated Silica Tip emitters using a spray voltage of 1.45 kV. The mass spectrometer was operated in positive, data-dependent mode with capillary temperature set to 225 °C. First, full scans (350-1700 m/z, resolution 60,000) were recorded in the

Orbitrap analyzer of the instrument with a maximal ion time of 200 ms and the target value for automatic gain control set to 1,000,000. In the linear ion trap part of the instrument subsequently up to twenty double- and triple-charged precursors with a minimal signal of 500 were isolated (isolation window 2 m/z), fragmented by collision induced dissociation (CID) and analyzed with a maximal ion time of 50 ms and the target value for automatic gain control set to 3000 (available mass range 50-2000 m/z, resolution 5400). Already analyzed precursors were excluded from further isolation and fragmentation for 45 sec.
For data analysis, the MaxQuant environment (version 1.5.3.8, was used with standard parameters if not otherwise stated. Spectra were searched against 20187 Swiss-Prot entries from the Homo sapiens proteome (UP000005640, downloaded on 18 th November 2015 from UniProt KB). Label-free quantification was enabled as well as the match between runs option. Tryptic cleavage specificity was chosen, as well as carbamidomethyl at cysteines as fixed and methionine oxidation, phosphorylation (threonine, serine and tyrosine), acetylation at protein n-termini and ubiquitination at lysine (GlyGly, +114.0429) as variable modifications. Mass tolerances were 20 ppm (first search) and 4.5 ppm (second search after recalibration) for precursor masses and 0.5 Da for fragment masses. Phosphorylation sites were reported showing the highest probability calculated form an MS/MS spectrum peak matches. Peptides and proteins were accepted at a false discovery rate of 1 %. For relative quantification of phosphorylated peptides, peptide intensities were normalized to progesterone receptor amounts by dividing them by the total progesterone receptor intensity.

Reverse Phase Protein Assay (RPPA)
The following primary antibodies were used (provider and product number): Akt (CST 4685), Akt-P-Ser473 (CST 4060), Akt-P-Thr308 (CST 9275), c-Fos (CST 4384), c-myc (CST 9402), c-myc-P-Thr58/Ser62 (Abcam ab32029), EGFR (ErB-1, HER1) (CST 4405), EGFR (ErB-1, HER1)-P-Tyr1068 (CST 2234), ER (estrogen receptor) (Thermo RM-9101-S), Erk1/2 (MAPK p44/42) (CST 4695), Erk1/2 (MAPK p44/42)-P-Thr202/Tyr204 (CST 4370), Her2 (Dako A0485), Her2-P-Tyr1221/Tyr1222 (CST 2243), MEK1 (CST 9124), MEK1/2-P-Ser217/Ser221 (CST 9154), MEK2 (CST 9125), Rb-P-Ser807/Ser811 (CST 8516), S6 ribosomal protein (CST 2217), S6 ribosomal protein-P-Ser235/Ser236 (CST 2211), S6 ribosomal protein-P-Ser240/Ser244 (CST 2215), PR (progesterone receptor) (sc-810).
The antibodies were purchased from CST (Cell Signaling Technology, Danvers, Massachusetts), Abcam (Abcam plc, Cambridge, UK), Thermo (Thermo Fisher Scientific, Waltham, Massachusetts), Dako (Dako Products, Hamburg, Germany), Santa Cruz (Santa Cruz Biotechnology, Dallas, USA).

Western Blot analysis and Immunofluorescence
The following primary antibodies were used (provider and product number): Akt (CST 4685), Akt-P-Ser473 (CST 4060), β-Actin (sc-2004), c-Fos (CST 4384), c-myc (CST 9402), CYP51A1 (ab210792), EGFR (ErB-1, HER1) (CST 4405), EGFR (ErB-1, HER1)-P-Tyr1068 (CST 2234), ERα (CST 8644), ERα (p-Ser118) (CST 2511), Erk1/2 (MAPK p44/42) (CST 4695), Erk1/2 (MAPK p44/42)-P-Thr202/Tyr204 (CST 4370), HER2 (CST 2165), Her2-P-Tyr1221/Tyr1222 (CST 2243), MEK1 (CST 9124), MEK1/2-P-Ser217/Ser221 (CST 9154), PGRMC1 (ab48012), PGRMC1 (13856), PR (progesterone receptor) (sc-810).
The antibodies were purchased from CST (Cell Signaling Technology, Danvers, Massachusetts), Abcam (Abcam plc, Cambridge, UK), Thermo (Thermo Fisher Scientific, Waltham, Massachusetts), and Santa Cruz (Santa Cruz Biotechnology, Dallas, USA).

qRT-PCR

We evaluated the qPCR results as follows: At first, we calculated the mean of all matching technical replicates [C_T]. Then we calculated ΔC_T with $\Delta C_T = C_{T\ Target\ Gene} - C_{T\ Reference\ Gene}$. Our reference was the expression of PDH, we subtracted its value from the other expression levels. Afterwards we determined $\Delta\Delta C_T$ with $\Delta\Delta C_T = \Delta C_{T\ Sample} - \Delta C_{T\ Reference\ Sample}$.

The standard deviation of ΔC_T is defined as $\sigma = \sqrt{\sigma^2_{Target\ Gene} + \sigma^2_{Reference\ Gene}}$ [with σ as the standard deviation]. At last the fold-difference, which is defined as the range: $[2^{-\Delta\Delta C_T+\sigma}; 2^{-\Delta\Delta C_T-\sigma}]$, was calculated.

Scatter Plots of breast cancer microarray data

The normalized data was obtained from the Gene Expression Omnibus (GEO, NCBI) and analysed via a R script. At first, we utilized GeoQuery for the GSE download. Afterwards we plotted the per sample expression values to ensure proper normalization. As the data contained technical replicates, we generated the mean value per sample. Finally, we calculated Spearman's correlation and plotted a scatterplot with a regression line in R.

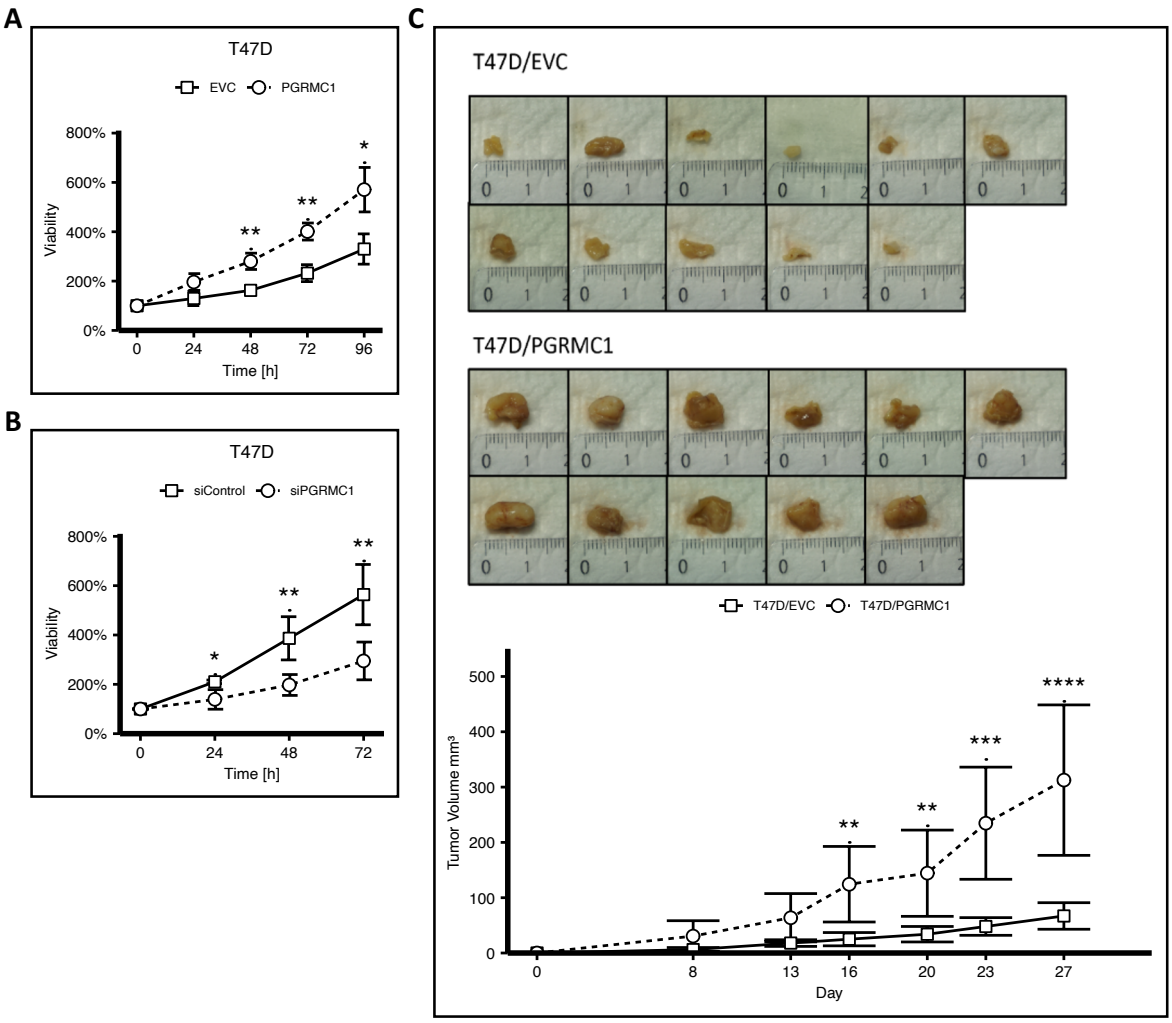
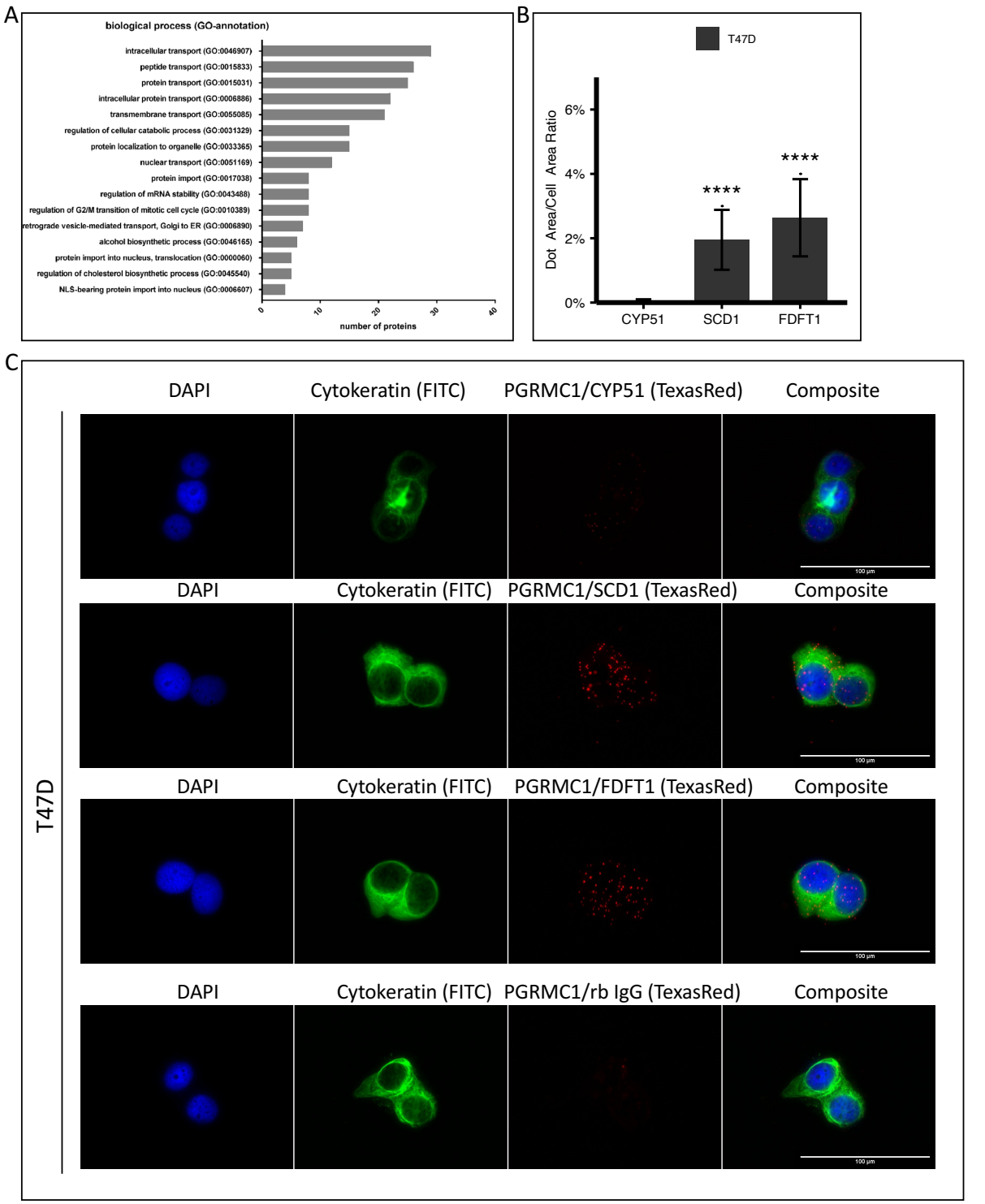


*Figure 1: (A) Cell viability of T47D/EVC and T47D/PGRMC1 cells (n = 3). Viability was analyzed by MTT assay at t = 0 h, 24h, 48h, 72h and 96h/37°C. Values were normalized to t = 0 (100%). *: p ≤ 0.05, **: p ≤ 0.01. (Student's t-test, n = 3). (B) Cell viability of T47D cells, treated with siRNA against PGRMC1 (siPGRMC1) and scrambled siRNA (siControl). (Student's t-test, n = 3). Viability was analyzed at t = 0h, 24h, 48h and 72h/37°C. Values were normalized to t = 0 (100%). *: p ≤ 0.05, **: p ≤ 0.01. (Student's t-test, n = 3). (C) Tumor volumes of immunodeficient mice bearing human breast cancer T47D/EVC and T47D/PGRMC1 xenografts. ***: p ≤ 0.001, ****: p ≤ 0.0001. (Student's t-test, n = 11 mice each group). Images of tumor tissue dissected from each mouse.*


A
biological process (GO-annotation)
intracellular transport (GO:0046907)
peptide transport (GO:0015833)
protein transport (GO:0015031)
intracellular protein transport (GO:0006886)
transmembrane transport (GO:0055085)
regulation of cellular catabolic process (GO:0031329)
protein localization to organelle (GO:0033365)
nuclear transport (GO:0051169)
protein import (GO:0017038)
regulation of mRNA stability (GO:0043488)
regulation of G2/M transition of mitotic cell cycle (GO:0010389)
retrograde vesicle-mediated transport, Golgi to ER (GO:0006890)
alcohol biosynthetic process (GO:0046165)
protein import into nucleus, translocation (GO:0000060)
regulation of cholesterol biosynthetic process (GO:0045540)
NLS-bearing protein import into nucleus (GO:0006607)
0
10
20
30
40
number of proteins
B
T47D
Dot Area/Cell Area Ratio
6%
4%
2%
0%
****
****
CYP51
SCD1
FDFT1
C
DAPI
Cytokeratin (FITC)
PGRMC1/CYP51 (TexasRed)
Composite
T47D
DAPI
Cytokeratin (FITC)
PGRMC1/SCD1 (TexasRed)
Composite
DAPI
Cytokeratin (FITC)
PGRMC1/FDFT1 (TexasRed)
Composite
DAPI
Cytokeratin (FITC)
PGRMC1/rb IgG (TexasRed)
Composite
100 µm

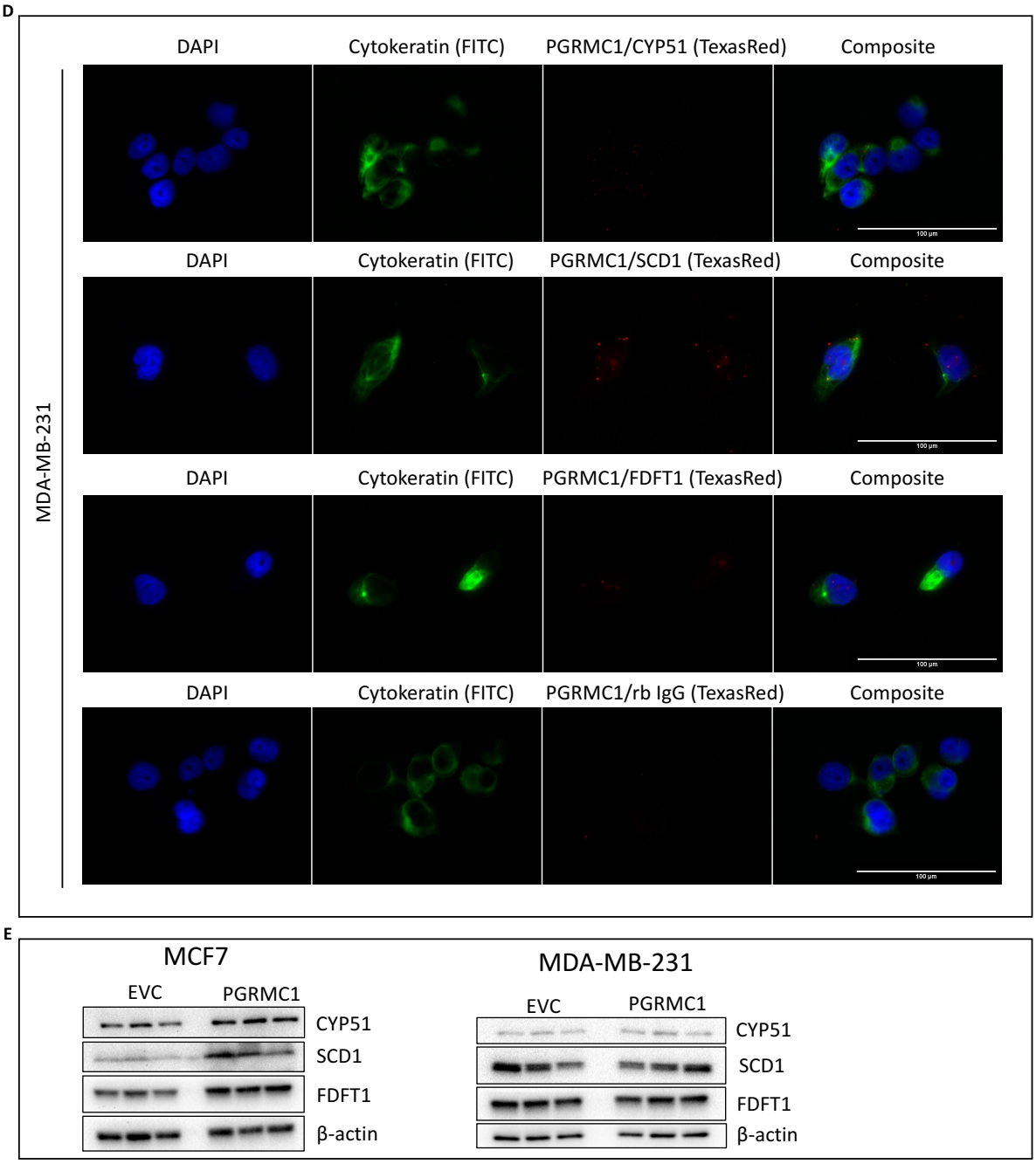


Figure 2: (A) PGRMC1 interaction partners associated with GO-annotations for biological functions. Number of proteins assigned to the respective biological function. The dataset was analyzed using Gene Ontology (geneontology.org). Total number of proteins in the dataset: 100. (B) PLA of PGRMC1 and Cyp51, FDFT1, SCD1 SCD1 and negative control rabbit isotype IgG (TexasRed) in T47D. Subsequent staining of Cytokeratin (FITC) and DAPI. Quantification of dots per cell and (C) Visualization via immunofluorescence microscopy. Magnification: 63 x. (D) PLA for protein interactions between PGRMC1 and CYP51, FDFT1, SCD1 and negative control rabbit isotype IgG (TexasRed) in MDA-MB-231 cells. Subsequent staining of Cytokeratin (FITC) and DAPI. Magnification: 63 x. (E) Western Blot analysis of CYP51, SCD1 and FDFT1 in MCF7/PGRMC1 cells and MDA-MB-231/PGRMC1 cells compared to their respective empty vector control.

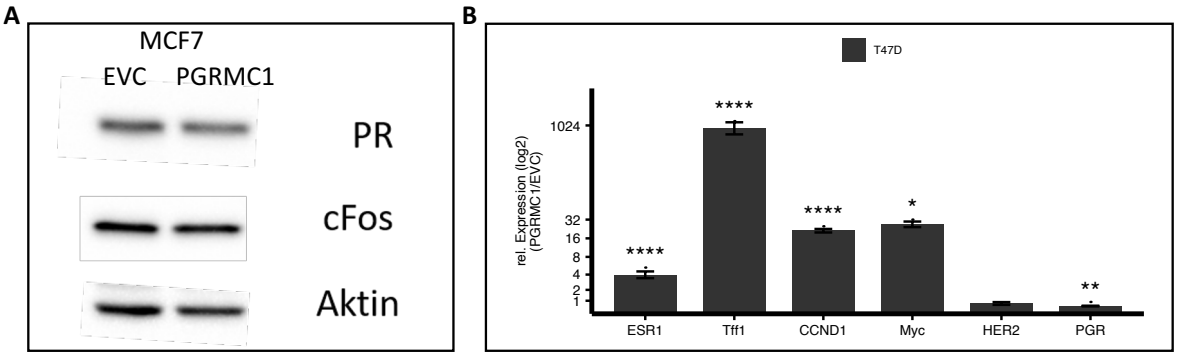
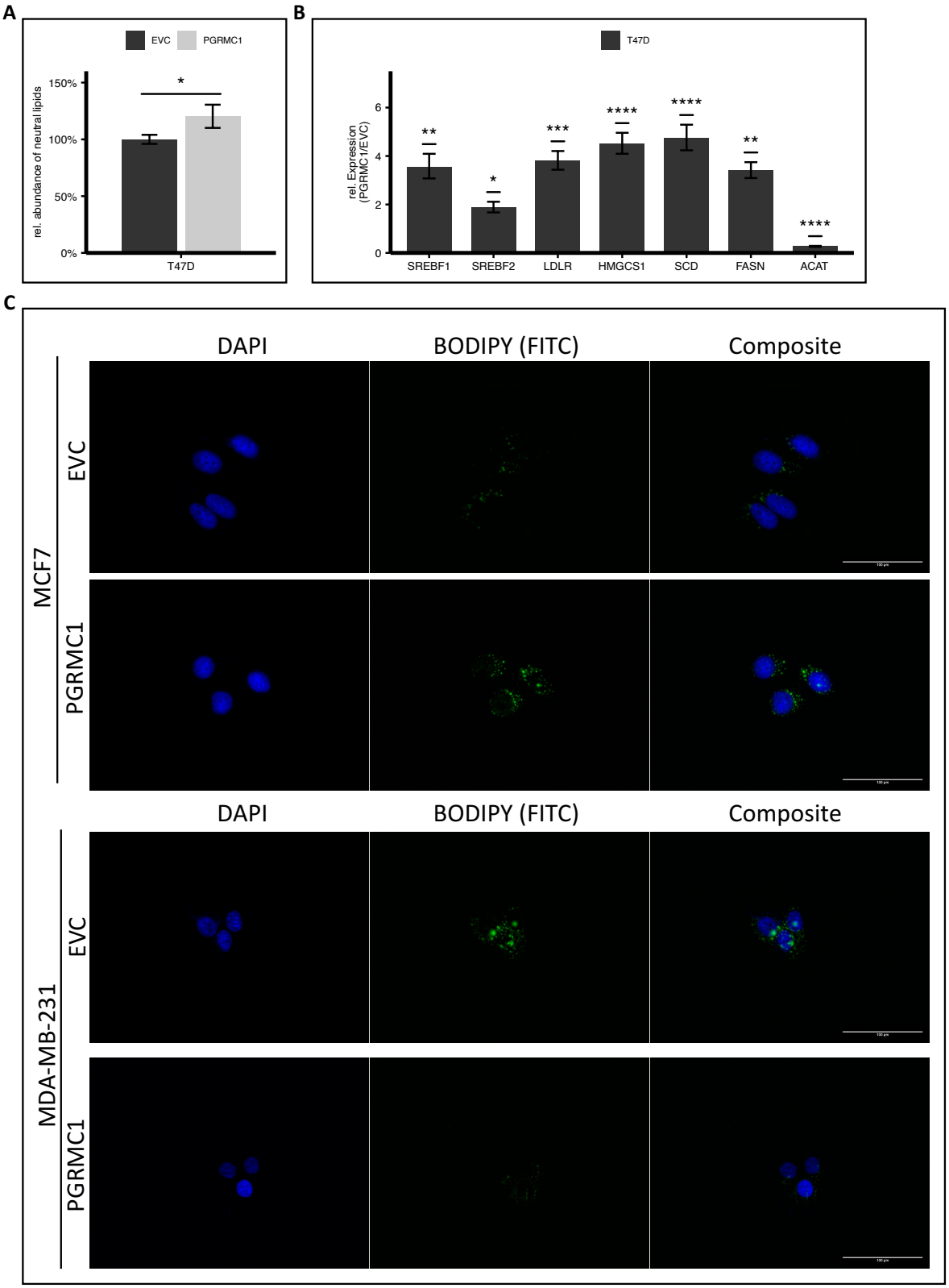


*Figure 3: (A) Western blot analysis of PR and cfos protein levels in MCF7/EVC and MCF7/PGRMC1 cells. (B) qRT-PCR analysis of ESR1, TFF1, HER2, CCND1, Myc and PR mRNA expression in T47D/EVC and T47D/PGRMC1 cells *: $p \leq 0.05$, ***: $p \leq 0.001$. (Student's t-test, n = 3)*


A
EVC
PGRMC1
rel. abundance of neutral lipids
150%
100%
50%
0%
*
T47D
B
T47D
rel. Expression (PGRMC1/EVC)
6
4
2
0
**
*
***
****
****
**
****
SREBF1
SREBF2
LDLR
HMGCS1
SCD
FASN
ACAT
C
DAPI
BODIPY (FITC)
Composite
MCF7
EVC
PGRMC1
DAPI
BODIPY (FITC)
Composite
MDA-MB-231
EVC
PGRMC1

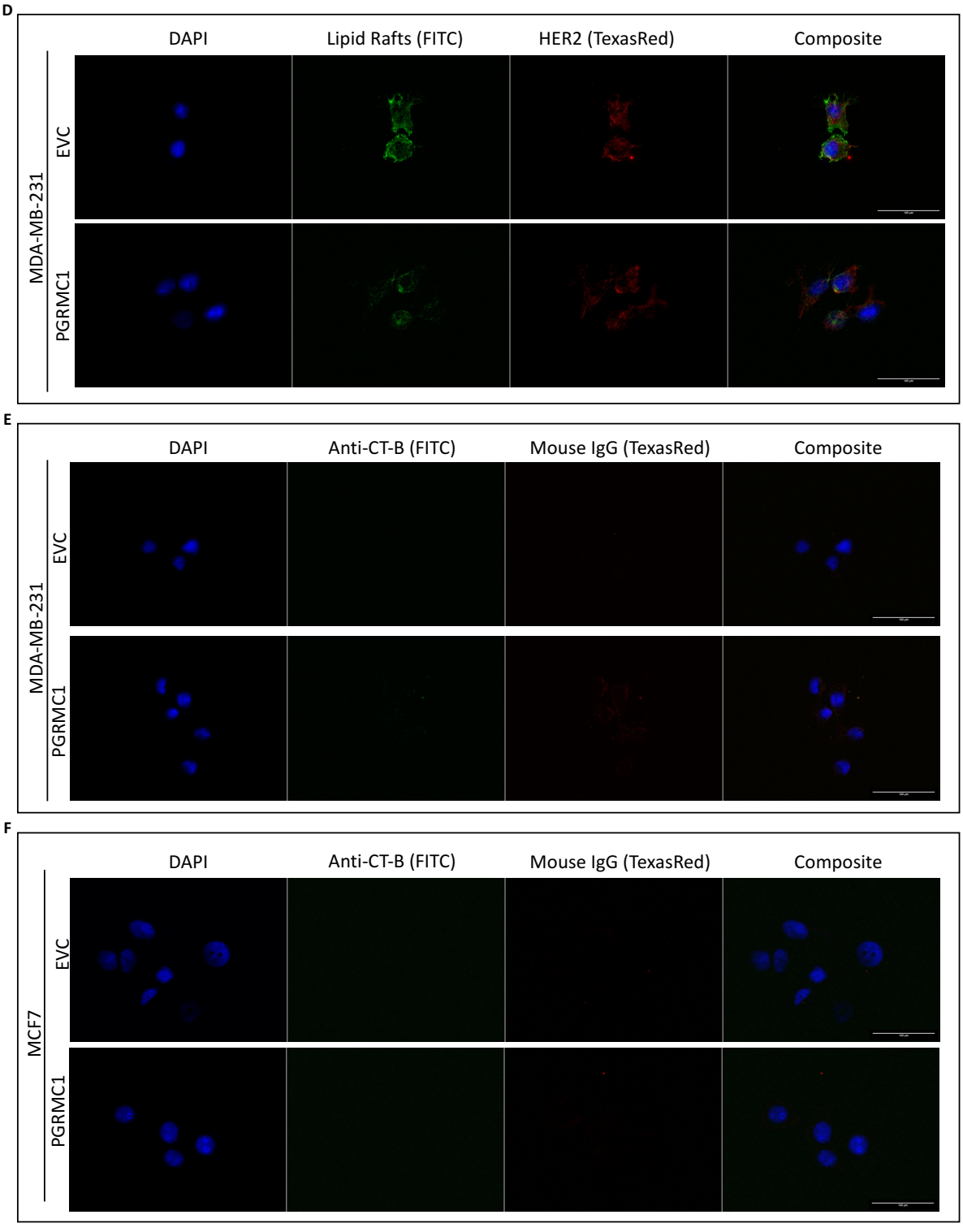


*Figure 4: (A) Detection of neutral lipids and lipid droplets in T47D/EVC and T47D/PGRMC1 cells by BODIPY® staining and quantification via flow cytometry. *: p ≤ 0.05, **: p ≤ 0.01. (Student's t-test, n = 3) (B) qRT-PCR analysis of SREPF1, SREBF2, LDLR, HMGS1, SCD, FASN, ACAT mRNA expression in T47D/PGRMC1 cells compared to the respective EVC cells. *: p ≤ 0.05, **: p ≤ 0.01, ***: p ≤ 0.001. (Student's t-test, n = 3). (C) Staining of lipid droplets in MCF7/EVC and MCF7/PGRMC1, MDA-MB-231/EVC and MDA-MB-231/PGRMC1 with BODIPY™ 493/503. Nuclear stain with DAPI. Magnification: 63 x. (D) Immunofluorescence staining in MDA-MB-231/EVC and MDA-MB-231/PGRMC1 with Vybrant™ Alexa Fluor™ 488, fluorescence immunocytochemistry for HER2 and nuclear staining with DAPI. Magnification: 63 x. Negative control staining with rabbit isotype IgG (TexasRed) and Anti-CT-B only (FITC) in (E) MDA-MB-231/EVC and MDA-MB-231/PGRMC1 and (F) MCF7/EVC and MCF7/PGRMC1.*

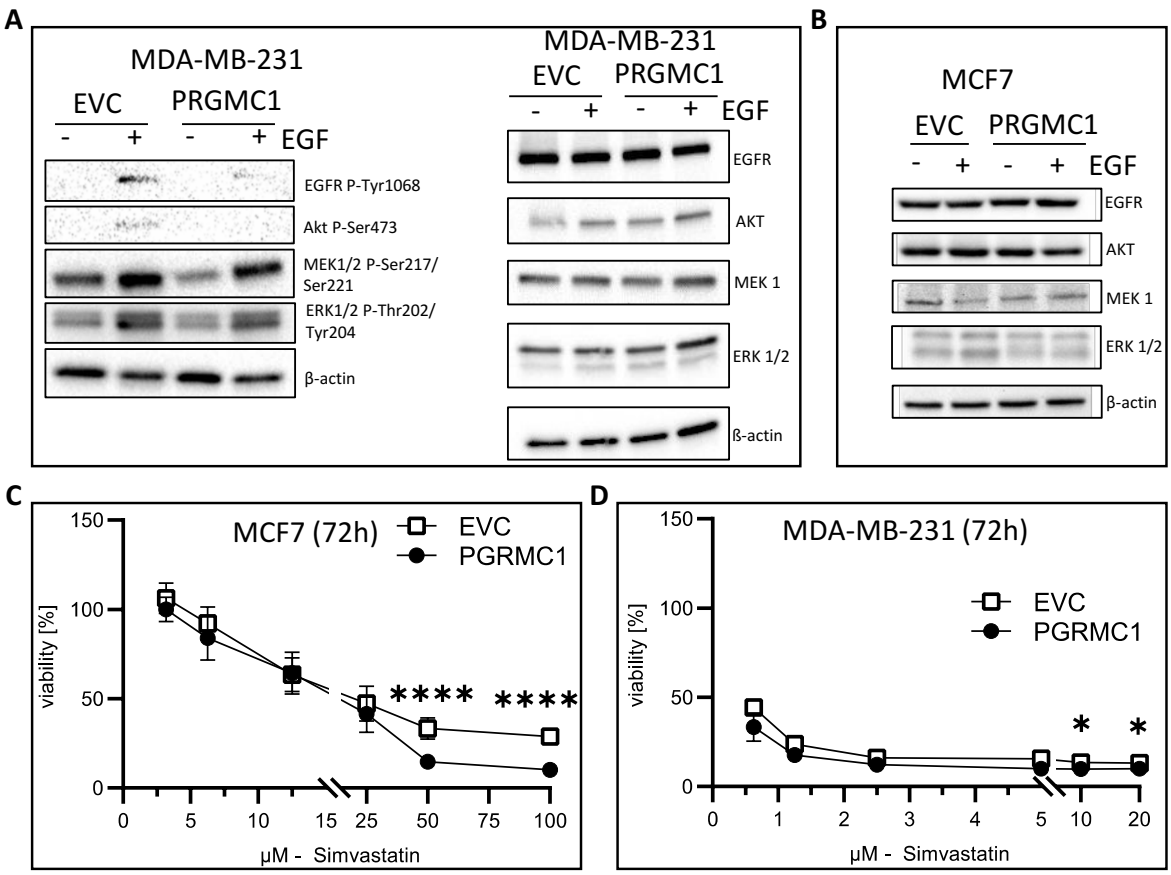
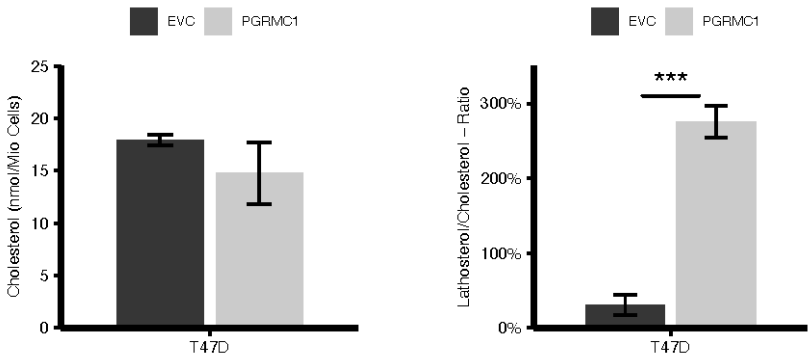


Figure 5 (A) Protein phosphorylation of EGFR P-Tyr1068, Akt P-Ser473, MEK1/2 P-Ser217/Ser221 and Erk1/2 P-Thr202/Tyr204) and total protein expression of EGFR, Akt, MEK1/2 and Erk1/2 in MDA-MB-231/EVC and MDA-MB-231/PGRMC1 analyzed by Western blot analysis. Cells were treated with EGF (10 ng/mL) for 10 min/37°C. (B) Total protein expression of EGFR, Akt, MEK1/2 and Erk1/2 in MCF7/EVC and MCF7/PGRMC1 analyzed by western blot analysis. MCF7/EVC and MCF7/PGRMC1 cells were treated with 100μM, 50μM, 25μM, 12.5μM, 6.25 μM and 3.175 μM simvastatin and respective DMSO control. MDA-MB-231/EVC and MDA-MB-231/PGRMC1 cells were treated with 20μM, 10μM, 5μM, 2.5μM, 1.25μm and 0.625μM simvastatin and respective DMSO control. Viability was analyzed by MTT assay at t=24h, t=48h, t=72h and 37°C. Depicted are results after 72 h (C, D) of treatment. Viability is normalized on the DMSO control. P-Values were adjusted using Bonferroni correction ($n_{doses} = 6$; $n_{replicates} = 9$)

3 Additional Research

3.1 Complementary Studies in T47D Cells

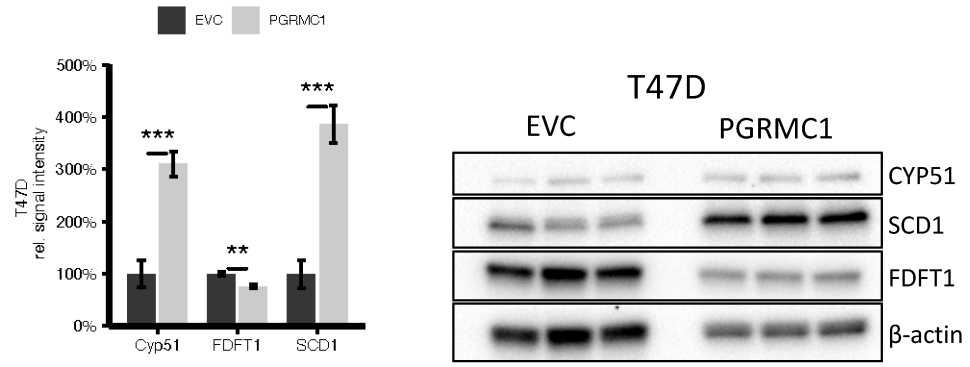
Additional experiments were performed in T47D cell lines that were not reported in the paper.
As in the published results from the GC-MS analysis in MCF7 and MDA-MB-231 cells, intracellular contents of cholesterol and its intermediate lathosterol were investigated in synchronized T47D/PGRMC1 and T47D/EVC. While no significant difference in the total amount of cholesterol per cell was observed between T47D/PGRMC1 and T47D/EVC (Figure 7a), overexpression of PGRMC1 in T47D cells resulted in a substantial shift toward a positive lathosterol/cholesterol proportion (Figure 7b), suggesting that PGRMC1 might boost especially the initial steps in the mevalonat pathway in this cell line.



(a) (b)

Figure 7: **Intracellular content of cholesterol and its precursor lathosterol** Quantification of intracellular cholesterol and its precursor lathosterol via mass spectrometry in T47D/EVC and T47D/PGRMC1. Absolute levels of intracellular cholesterol and significant decrease of lathosterol to cholesterol ratio. *: $p \leq 0.05$, ***: $p \leq 0.001$

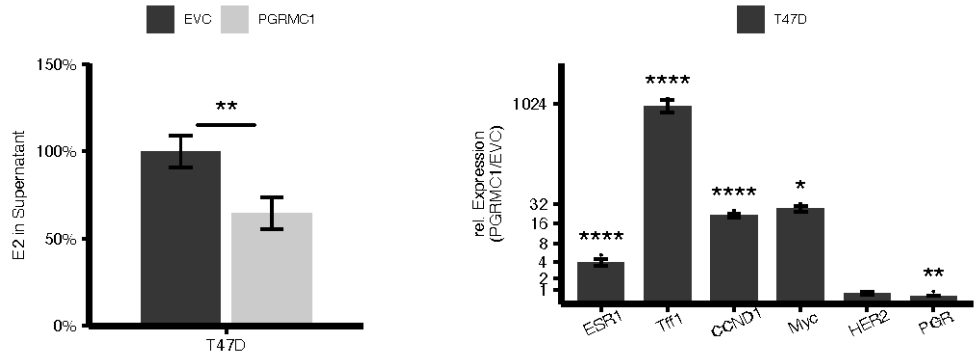
Similar to our results from western blot analysis in MCF7 cells, we found that PGRMC1 overexpression in T47D cells leads to higher protein expression of CYP51 and SCD1 compared to the EVC, whereas the protein expression of FDFT1 was slightly lower upon PGRMC1 overexpression (Figure 8).



(a) (b)

Figure 8: **Protein expression of CYP51, SCD1, and FDFT1**
Quantification of protein expression of CYP51, SCD1, and FDFT1 in T47D/PGRMC1 cells compared to their respective empty vector control by western blot. **: p≤0.005, ***: p≤0.001

We hypothesized that cholesterol synthesis is related to the level of E2, which plays a key role in HR+ breast cancer, e.g., due to its effects on ERα. In contrast to our studies in the modified MCF7 cells, we found a significantly lower E2 content in the supernatant of T47D/PGRMC1 cells than in that of the EVC (Figure 9a). Nonetheless, PGRMC1 overexpression in T47D cells also resulted in upregulation of the ERα downstream targets ESR1, TFF1, CCND1, and MYC, which were analyzed via real-time RT-PCR analysis. However, no significant upregulation was detected for mRNA levels of HER2, and PGR levels were even lower in T47D/PGRMC1 in relation to T47D/EVC (Figure 9b).

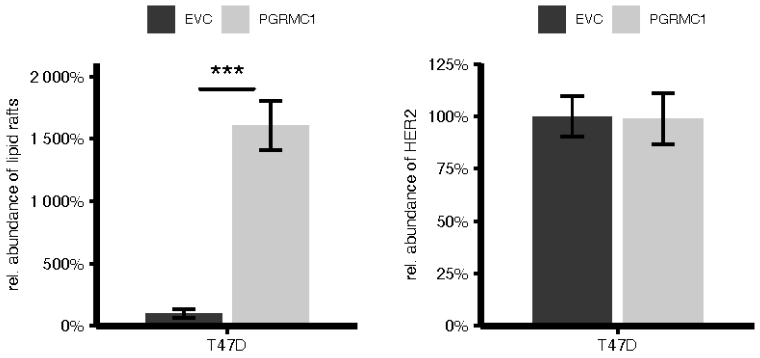


(a) (b)

Figure 9: **ERα signaling in T47D/EVC and T47D/PGRMC1**
(a) ELISA analysis of E2 in the supernatant of PGRMC1 overexpressing T47D cells compared to the EVC after 48h normalized to cell count. **(b)** qRT-PCR analysis of ESR1, TFF1, HER2, CCND1, MYC and PR mRNA expression *: p ≤ 0.05, **: p≤0.01, ***: p ≤ 0.001 (Student's t test, n=3)

We further studied formation of lipid rafts in the modified T47D cells. Therefore, not permeabilized T47D/PGRMC1 and T47D/EVC were stained for lipid rafts and

analyzed by flow cytometry. We observed that PGRMC1 overexpression resulted in a significant increase in lipid raft expression (Figure 10a). We accessorily investigated whether PGRMC1 overexpression affects HER2 expression in the cell membrane, which could result in a change of the membrane conformation. As with the results for mRNA expression, there was no significant difference in the outer membrane HER2 level in T47D/PGRMC1 cells and the respective EVC (Figure 10b).



(a) (b)

Figure 10: **Lipid raft and HER2 expression in T47D/EVC and T47D/PGRMC1**

(a) Detection of lipid rafts in cell membranes by Vybrant™ Alexa Fluor™ 488 and subsequent quantification via flow cytometry. **(b)** Quantification of HER2 protein in unpermeabilized T47D/EVC and T47D/PGRMC1 via flow cytometry. ***: $p \leq 0.001$ (Student's t test, n=3)

The PGRMC1 overexpressing T47D cells in particular showed co-localization of HER2 with lipid rafts, as visualized via fluorescence microscopy (Figure 11).

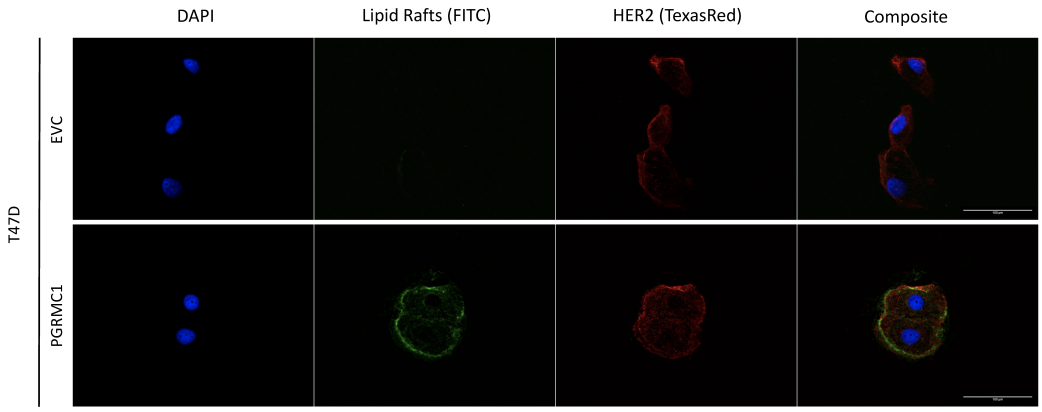


Figure 11: **Co-staining of lipid rafts and HER2 in T47D/EVC and T47D/PGRMC1**

IF staining with Vybrant™ Alexa Fluor™ 488, fluorescence IC for HER2 and nuclear staining with DAPI. Magnification: 63 x

Based on our research concerning the influence of PGRMC1 on lipid homeostasis

in breast cancer cells, we suggested that vulnerability of cell proliferation to inhibition of cholesterol synthesis might be related to PGRMC1 expression. Accordingly, PGRMC1 overexpressing cells and the respective controls were treated with different concentrations of simvastatin, a competitive inhibitor of HMG-CoA reductase, and subsequent viability assays were performed at different time points. In contrast to MCF7 and MDA-MB-231 cells, in T47D cells, we found no significant difference in viability between PGRMC1 overexpressing cells and their EVC, pointing towards a different adjustment mechanism under inhibition of de novo cholesterol synthesis (Figure 12).

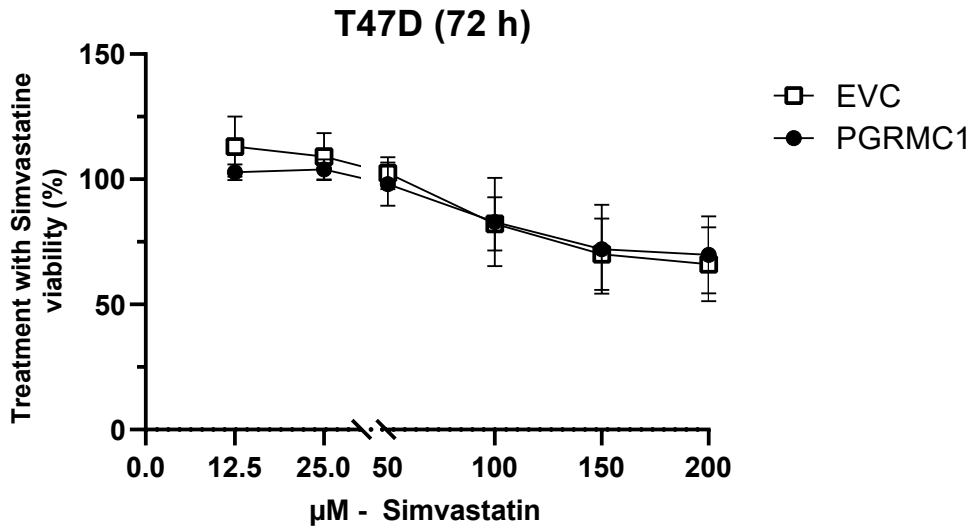


Figure 12: **Simvastatin treatment of T47D/EVC and T47D/PGRMC1**
T47D/EVC and T47D/PGRMC1 were treated with 100 µM, 50 µM, 25 µM, 12.5 µM, 6.25 µM and 3.175 µM simvastatin and respective DMSO control. Viability was analyzed by MTT assay at t=24 h, t=48 h, and t=72 h and 37 °C. Depicted are results after 72 h of treatment. Viability was normalized on the DMSO control. P-Values were adjusted using Bonferroni correction (ndoses = 6; nreplicates = 9)

PGRMC1 mediated activation and enhancement of EGFR signaling has already been shown in previous studies. Nevertheless, contrary to MCF7 cells, PGRMC1 overexpression in T47D cells did not eventuate in enhanced phosphorylation of EGFR, AKT, MEK1/2 and ERK1/2. EGFR activation by EGF treatment entailed elevated levels of EGFR (p-Y1068 p-Y1068), AKT (p-Ser473), MEK1/2 (p-S217/S221) and ERK1/2 (p-T202/Y204) mainly in T47D/EVC, whereas no or only scarce phosphorylation effect was observed in T47D/PGRMC1 (Figure 13a). In addition, the PGRMC1 overexpressing T47D cells showed significantly lower protein expression of total EGFR, while protein expression of total AKT protein was higher than in the EVC (Figure 13a).

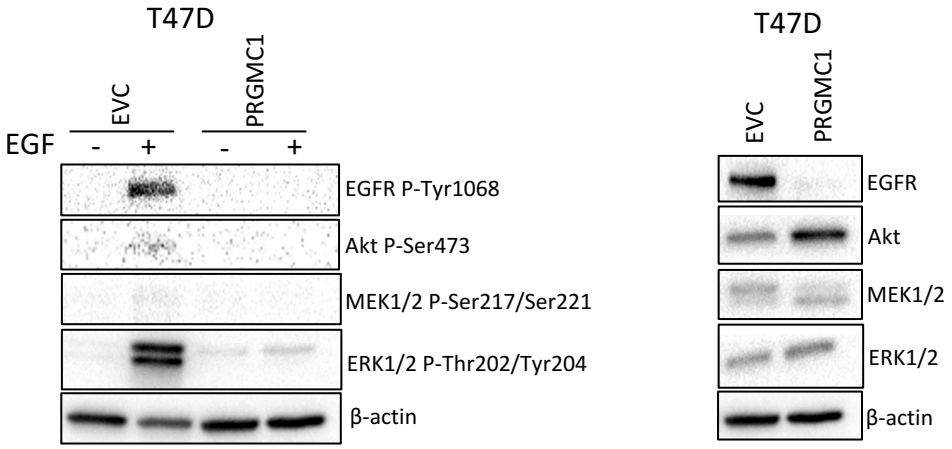


Figure 13: **Influence of PGRMC1 overexpression on EGFR signaling in T47D cells**

(a) Protein phosphorylation of EGFR p-Y1068, Akt p-S473, MEK1/2 p-S217/S221 and ERK1/2 p-T202/Y204 verified by western blot analysis. Cells were treated with EGF (10 ng/mL) for 10 min/37°C. Representative blot of 3 independent analysis. **(b)** Total protein expression of EGFR, AKT, MEK1/2 and ERK1/2 in T47D/EVC and T47D/PGRMC1 analyzed by Western blot analysis. Representative blot of 3 independent analysis.

3.2 PGRMC1 Overexpression in MCF7 Cells Leads to Redistribution of the ERα

Because our previous experiments showed that PGRMC1 overexpression is associated with elevated ERα expression at least in MCF7 cells, we investigated whether there were also differences in the localization of ERα in MCF7/PGRMC1 and MCF7/EVC. To explore a possible redistribution and co-localisation, a co-staining for ERα with PGRMC1 was performed. In the MCF7/EVC cells, ERα was predominantly displayed in the nucleus, whereas in PGRMC1 overexpressing MCF7 cells it was more likely to be distributed in the cell membrane region. In addition, a partly overlapping staining pattern of PGRMC1 and ERα was evident in the PGRMC1 overexpressing cells (Figure 14). Redistribution of ERα and co-localisation with PGRMC1 could have influence on cell signalling and behaviour.

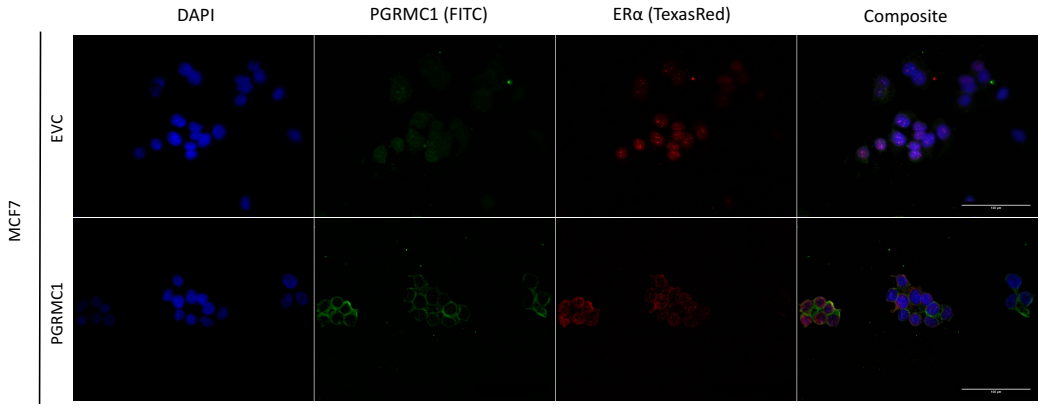
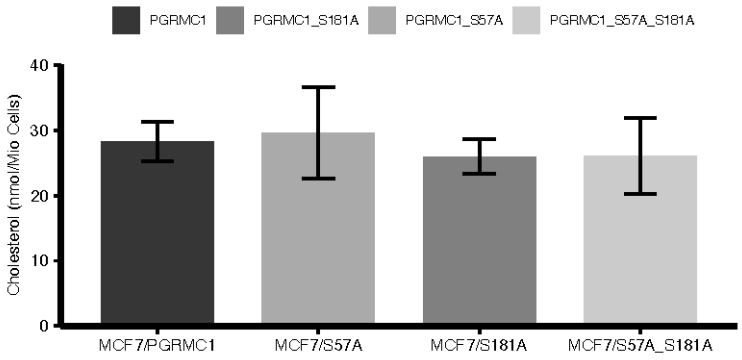


Figure 14: **Co-staining of PGRMC1 and ERα in MCF7/EVC and MCF7/PGRMC1**

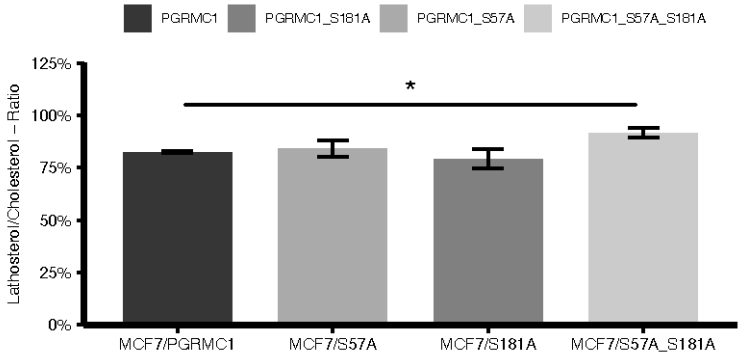
IF staining with Vybrant™ Alexa Fluor™ 488, fluorescence IC for HER2 and nuclear staining with DAPI. Magnification: 63 x

3.3 Effects of PGRMC1 Phosphorylation on Cholesterol and Steroid Hormone Synthesis

The diverse roles of PGRMC1 in cell metabolism and cell signaling have been linked to different PTM. Phosphorylation of PGRMC1 in particular might affect its properties and regulate its function, e.g., via altering the affinity of protein interactions and ligand binding. In previous work from our laboratory, several phosphorylation sites of PGRMC1 were recognized via mass spectrometry in MCF7/PGRMC1 cells. The phosphorylation sites S57 and S181 were anticipated to be phosphorylated by casein kinase 2 and were closely located to the SH3 and SH2 domains of PGRMC1 [185]. We explored whether S57 and S181 phosphorylation also functionally impacts cholesterol synthesis. Therefore in addition to the MCF7 cells overexpressing the HA-tagged PGRMC1 and the respective EVC, cholesterol and lathosterol were quantificated in the variants MCF7/PGRMC1_S57A, MCF7/PGRMC1_S181A and MCF7/PGRMC1_S57A_S181A. Concerning absolute levels of cholesterol no significant difference was observed between the groups (Figure 15a). Nevertheless, MCF7/PGRMC1_S57 and MCF7/PGRMC1_S181 exhibited a lathosterol/cholesterol ratio similar to MCF7/PGRMC1, while the ratio of MCF7/PGRMC1_S57A_S181A differed significantly from that of MCF7/PGRMC1 and MCF7/EVC (Figure 15b). These results suggest that the influence of PGRMC1 on cholesterol synthesis and turnover in MCF7 cells depend at least partly on phosphorylation of S57 and S181.



(a)



(b)

Figure 15: **Intracellular content of cholesterol and its precursor lathosterol in MCF7 cells dependent on the phosphorylation status of PGRMC1**
Mass spectrometry analysis indicated **(a)** no significant difference in absolute cholesterol levels but **(b)** a significant difference in lathosterol/cholesterol ratios related to the modification of PGRMC1 phosphorylation sites S57 and S181. *: $p \leq 0.05$

We also investigated levels of E2 in the supernatant of MCF7/PGRMC1 cells and the MCF7 cells overexpressing the HA-tagged PGRMC1 variants via ELISA. The detected concentration of E2 in the supernatant of MCF7/PGRMC1 was significantly higher than in the EVC. Notably, E2 levels were also significantly higher in the in MCF7/PGRMC1_S181 than in the EVC. There was no significant difference between MCF7/PGRMC1 and the different phosphorylation site mutated variants. These results suggest that phosphorylation at site S181 may inhibit E2 synthesis. In addition, our analysis indicated a trend towards phosphorylation site S57 as a negative regulator of E2 production.

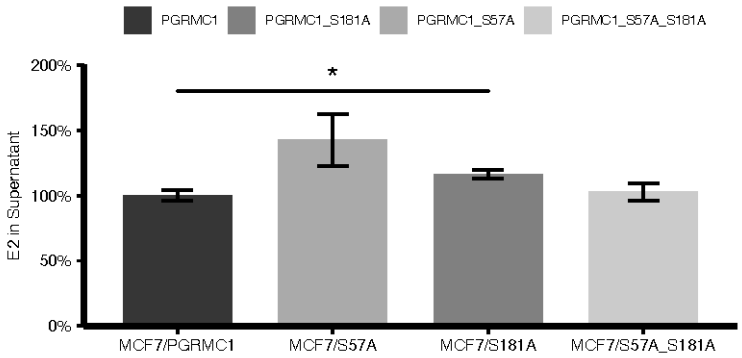


Figure 16: **E2 levels in the supernatant of MCF7 cells dependent on the phosphorylation status of PGRMC1**
ELISA analysis of E2 in the supernatant of MCF7/PGRMC1, MCF7/PGRMC1_S57A, MCF7/PGRMC1_S181A, and MCF7/PGRMC1_S57A_S181A (normalized to cell count) after 48 h showed a significant difference in E2 content related to the modification of PGRMC1 phosphorylation site S181. *: $p \leq 0.05$

3.4 Tamoxifen and Exemestane Treatment of PGRMC1 Overexpressing MCF7 Cells Moderately Reduces Viability Compared to the EVC

Our studies show an upregulation of ERα, ERα downstream targets, and E2 levels in MCF7/PGRMC1 cells compared to MCF7/EVC cells. Since tamoxifen, a SERM, and aromatase inhibitors are routinely used in the treatment of HR+ breast cancer, we investigated whether PGRMC1 expression might have a role in treatment response. We treated PGRMC1 overexpressing MCF7 cells and the respective control with different concentrations of tamoxifen and exemestane, a steroidal aromatase inhibitor. Viability assays were performed to assess the extent of cell growth inhibition. Although in the main our results did not indicate significant viability differences between MCF7/PGRMC1 and MCF7/EVC cells in our tamoxifen and exemestane experiments, we did note some exceptions. At some concentrations, PGRMC1 overexpression was associated with significantly lower viability compared to MCF7/EVC cells. This suggests that interference with estrogen metabolism and ERα signaling may have a greater impact on PGRMC1 overexpressing cells, at least related to MCF7 cells and in the short term.

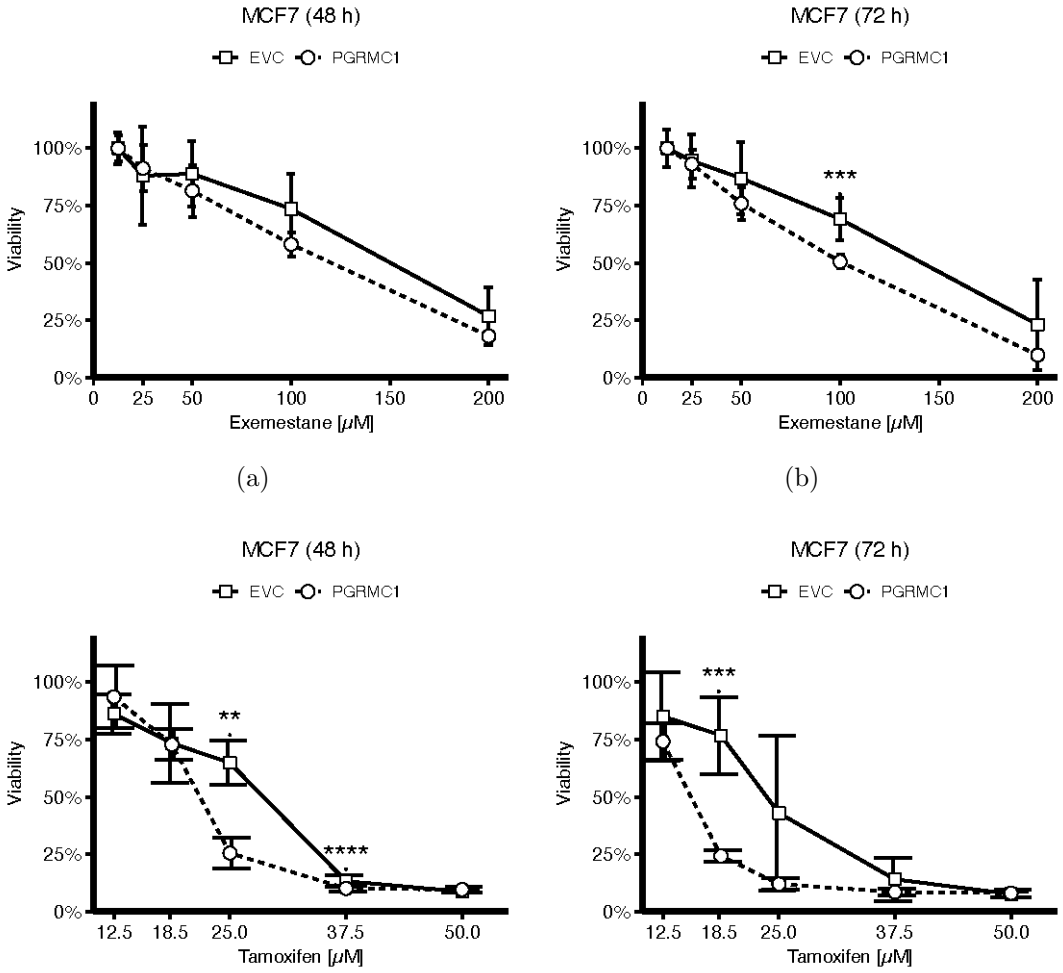


Figure 17: **Exemestane and Tamoxifen Treatment of MCF7/EVC and MCF7/PGRMC1**

MCF7/EVC and MCF7/PGRMC1 cells were treated with **(a)+(b)** 200 µM, 100 µM, 50 µM, 25 µM, 12.5 µM Exemestane and **(c)+(d)** 50 µM, 37.5 µM, 25 µM, 18.75 µM, 12.5 µM Tamoxifen. Viability was analyzed by MTT assay at t = 24 h, t = 48 h, and t = 72 h and 37°C. Viability values were normalized to the respective DMSO control. Depicted are results after 48 h and 72 h of treatment. P-Values were adjusted using Bonferroni correction (ndoses = 5; nreplicates = 9) *: $p \leq 0.05$, **: $p \leq 0.01$, ***: $p \leq 0.001$

4 Discussion

Although numerous studies show that PGRMC1 is involved in cancer development and progression, the mechanisms of how PGRMC1 influences signaling pathways are not fully understood. Reprogramming lipid metabolism allows cancer cells to evolve new mechanisms that satisfy their increased energy demands and drive the EMT, or even achieve therapy resistance. Although PGRMC1 has been identified as involved in various metabolic pathways, its role in the lipid metabolism of breast cancer is poorly studied. In the present study, we aimed to unveil mechanisms by which PGRMC1 changes lipid metabolism and oncogenic pathways in different breast cancer types.

4.1 PGRMC1 as Driver for Proliferation

In vitro experiments conducted in our laboratory demonstrated that PGRMC1 overexpressing breast cancer cell lines proliferate faster than their respective control cells and that knockdown of PGRMC1 with siRNA leads to the opposite effect. Interestingly, according to our research, only the HR+ cell lines (MCF7 and T47D) exhibit this behavior. Since we found no difference in proliferation upon PGRMC1 expression in the triple negative cell line (MDA-MB-231), only MCF7 and T47D cells were used to validate the impact of PGRMC1 overexpression in a xenograft mouse model. Most other in vitro and in vivo studies show that PGRMC1 expression has an enhancing effect on cancer cell proliferation [131,135,146,173,181,186,187,188,-189,190,191,192,193,194]. However, similar to our results on MDA-MB-231 cells, Kabe et al. [123] found no effect of PGRMC1 overexpression or silencing on proliferation of HCT116 cells.

4.2 Impact on Lipid Metabolism

The present study determined the effect of PGRMC1 on lipid metabolism in breast cancer cells. Our findings suggest that PGRMC1 is an important enhancer of lipid synthesis in HR+ breast cancer resulting in oncogenic signaling and tumor progression.

4.2.1 PGRMC1 Fuels Lipid Synthesis and Uptake in HR+ Breast Cancer

In this work, we reported enhanced mRNA expression of proteins involved in lipid regulation, synthesis, and uptake in PGRMC1 overexpressing MCF7 and T47D cells. Furthermore, we found a positive Spearman's correlation between the PGRMC1 ex-

pression level and various expression levels of proteins involved in cholesterol and fatty acid synthesis by analyzing microarray data from HR+ breast cancer tissue samples. Recent studies demonstrated that a high fat diet, insulin, or thiazolidine (a PPARγ agonist) increase PGRMC1 expression in adipocytes, e.g., via mechanisms involving transcription factors such as PPARγ and CREB/ATF via PPRE or CRE of the regulatory regions of the PGRMC1 gene. Additionally, PGRMC1 KO decreases PPARγ and FABP4 expression [160]. The transcription factor PPARγ transactivates fatty acid transport proteins, like FABP4 or CD36, which in turn enable insulin signaling and glucose uptake, e.g., via IRS2 and GLUT4 [195,196,-197]. Furthermore, PPARγ enhances expression of lipogenic proteins, such as FASN, SCD, ACSL1, SREBF1 and SREBF2 [198,199,200]. A similar cross-talk between PGRMC1 and PPARγ in breast cancer cells might contribute to the mRNA increase of lipogenic proteins in PGRMC1 overexpressing MCF7 and T47D cells. Interestingly, most studies found that PPARγ activation and accumulation inhibits cancer cell proliferation. However, in HER+ cancer cells PPARγ facilitates the formation of a lipogenesis/lipolysis joining point via conversion and storage of excess fatty acids to TAGs to prevent palmitate toxicity. Concurrently, palmitate can be incorporated into fat stores to avoid feedback inhibition on FASN [201]. In addition, interaction of heme-dimerized PGRMC1 with VLDLR, LDLR, or GLUT4 facilitates the translocation of these receptors to the plasma membrane, leading to de novo fatty acid synthesis and lipid uptake in adipocytes [160]. Other research groups also showed that PGRMC1 interacts with the IR and increases plasma membrane levels of IR, GLUT4 and GLUT1 in lung cancer cell lines [159]. Thereby PGRMC1 might elevate the intracellular glucose concentration, especially in terms of high fat diet and insulin resistance. Under conditions of high intracellular glucose concentration, stabilization of SCAP via N-glycosylation promotes its dissociation from INSIG1, leading to translocation of SCAP/SREBP1 to the golgi. Proteolytic activation of SREBP1 in turn leads to conversion of glucose into fatty acids [202,203].

4.2.2 PGRMC1 Influences Lipid Accumulation

The surplus lipids can be stored in the core of lipid droplet, which are a characteristic of hypoxic cancer cells, and released through a combination of lipolysis and lipophagy [204]. In line with this, we detected higher amounts of lipid droplets in MCF7 and T47D overexpressing cells compared to their EVC, while PGRMC1 overexpression in MDA-MB-231 cells decreased lipid droplet expression. Lipid droplet are utilized by cancer cells to adjust their metabolism, ensure energy production and redox balance, modulate autophagy, and control their membrane composition in response to nutrient and oxygen supply. Various studies showed, that lipid droplet

accumulation correlates with a more aggressive cancer phenotype, increased migration and stemness features [205,206,207,208,209,210,211,212]. Commensurately, the HR- and less differentiated MDA-MB-231/EVC exhibited higher lipid droplet formation than the HR+ MCF7/EVC and T47D/EVC. The heterogeneity of breast cancer subtypes is reflected in specific lipid profiles. In principle, HR- breast cancer subtypes concentrate on uptake and storage of exogeneous fatty acids and HR+ breast cancer subtypes enhance de novo fatty acid synthesis and oxidation [213,214,-215,216]. The capacity of MDA-MB-231 cells to accumulate further lipid droplets may be limited by the large number of lipid droplets already present in the basal state, so overexpression of PGRMC1 might have no or a negative impact on lipid accumulation. Furthermore, lipid overload tends to provoke the autophagy of cancer cells [217].

4.2.3 Cholesterol Metabolism is Altered upon PGRMC1 Overexpression

Prior research groups found that PGRMC1 interacts with both INSIG1 and SCAP. In prior work from our lab, we found a strong interaction of PGRMC1 with FDFT1 and SCD1 in MCF7 and T47D cells. Interestingly, interactions were barely detectable in MDA-MB-231 cells. Lower levels of FDFT1 and SCD1 were observed via immunofluorescence in these cells, suggesting that lower numbers of interactions might be influenced by decreased numbers of these proteins. However, the consequences of interactions between PGRMC1 and FDFT1 and SCD1 were not fully understood. Different authors investigated the role of PGRMC1 on CYP51, finding that downregulation of PGRMC1 leads to decreased sterol synthesis [171,-218,219]. We also demonstrated that PGRMC1 interacts with CYP51 in MCF7 cells. Surprisingly, in the T47D cells, levels of interaction were substantially lower. One explanation might be, that CYP51 and PGRMC1 expression are comparatively lower in T47D than in MCF7 cells. Different research groups showed that the dimerization of PGRMC1 via heme facilitates interaction with diverse subclasses of cytochrome P450 enzymes. CYP enzymes interacting with PGRMC1 via heme dependent dimerization are, among others, CYP1A2, CYP3A4, and CYP51 [123]. Alternatively, it is possible that the interaction of CYP51 with PGRMC1 is less common in T47D cells because PGRMC1 is more often present in its monomeric state in this cell line. More research in this field is necessary. By performing mass spectrometry analysis, we found higher amounts of cholesterol in contrast to its precursor lathosterol in MCF7/PGRMC1 compared to MCF7/EVC. The decreased lathosterol/cholesterol ratio in MCF7/PGRMC1 compared to MCF7/EVC points towards a higher turnover of lathosterol. Interestingly, overexpression of PGRMC1

in T47D cells had different effects on the terminal branch of the mevalonate pathway. Although the T47D/PGRMC1 and T47D/EVC cells exhibited no significant differences in their levels of absolute cholesterol, the PGRMC1 overexpressing cells manifested an increase in the level of lathosterol and, by inference, a corresponding rise in the ratio between cholesterol and lathosterol. Further research is needed to determine the impact of this marked increase in lathosterol in T47D/PGRMC1 cells. Our western blot results showed that FDFT1, SCD1, and CYP51 protein levels in PGRMC1 overexpressing MCF7 cells were significantly higher than in the MCF7 control group. In the triple negative MDA-MB-231 cells, overexpression of PGRMC1 did not significantly alter the levels of these proteins. Surprisingly, in T47D/PGRMC1 cells we found significantly lower expression of FDFT1 compared to the EVC. FDFT1 serves as a critical enzyme, directing the flow of the metabolite farnesyl pyrophosphate (FPP) to either sterol or non sterol biosynthetic branches [220]. A possible consequence of the lower expression of FDFT1 in PGRMC1 overexpressing cells could be that the metabolites are increasingly used for prenylation of proteins such as RAS, thus possibly contributing to the cellular transformation. Since cholesterol is the precursor of steroid hormones like estradiol, we determined levels of E2 in the supernatant of PGRMC1 overexpressing cancer cells and their respective EVC. Consistent with our previous findings, we found that E2 levels were significantly higher in the supernatant of MCF7/PGRMC1 than in the MCF7/EVC, whereas we determined no difference in the T47D cells.

4.2.4 PGRMC1 Phosphorylation Regulates Cholesterol and E2 Levels

Because the influence of PGRMC1 in cell signaling and metabolism depends not only on its protein expression but also on PTMs, especially phosphorylation, we investigated whether inhibiting CK2 phosphorylation sites S57 and/or S181 of PGRMC1 affects cholesterol and estradiol synthesis, respectively. No significant differences in total intracellular cholesterol levels were found between PGRMC1_S57A, PGRMC1_S181A, and PGRMC1_S57A_S181A cells compared to MCF7/PGRMC1 cells. Interestingly, for lathosterol/cholesterol ratio, which reflects the efficiency of the late stages of cholesterol biosynthesis, we found a significant disparity between, on the one hand, MCF7/PGRMC1, MCF7/PGRMC1_S57A and -/PGRMC1_S181A and, on the other hand, the MCF7/EVC and MCF7/PGRMC1_S57A_S181A, indicating that phosphorylation at S57 and S181 are at least partly involved in cholesterol de novo biosynthesis. These results are consistent with preliminary data from our laboratory, which demonstrated that phosphorylation of PGRMC1 at S57 and/or S181 are at least partly responsible for its interaction with CYP51, FDFT1 and SCD1. Interestingly, mutation at the S57 phosphorylation site and

also to a lesser degree mutation at the S181 phosphorylation site resulted in significantly higher E2 levels in the cell supernatant compared to MCF7/PGRMC1 and MCF7/PGRMC1_S57A_S181A.

4.3 PGRMC1 Influences EGFR and ERα Signaling

We suggested that PGRMC1 promotes tumor progression by upregulation of ERα and ESR1 mRNA via a transcriptional mechanism and via elevated steroid synthesis. Studies on ERα levels indicate that long term exposure of breast cancer cells to estrogen leads to an increase in ERα levels [221]. Together with the autocrine/paracrine activation by E2, increased levels of ERα may trigger a proliferative cycle support in tumor growth. Cross talk between ERα and EGFR signaling cascades has been described, whereby ERα stimulates E2 dependent activation of the EGFR pathway by promoting p-S473 phosphorylation of AKT through the non genomic pathway. Alternatively, ERα activation can occur independently of estrogens by EGFR activated MAPK signaling or PI3K pathway [222,223]. The involvement of PGRMC1 in promoting phosphorylation and activation of receptors, e.g., through heme-dependent PGRMC1 dimerization, has already been reported [123,131,179,224]. In this study, elevated phosphorylation levels of EGFR and its downstream targets in MCF7/PGRMC1 cells were discovered for the first time. Subsequent investigations from other laboratories also demonstrated that PGRMC1 alters PI3K/AKT/mTOR and EGFR signalling mechanisms in breast cancer cell lines [186,225]. We demonstrated enhanced activation in several components of the MAPK and PI3K pathways (i.e., MEK1/2, ERK1/2, and AKT) in MCF7/PGRMC1 cells. It is possible that these changes contribute to the increase in proliferation and ability of these cells to form tumors among others via interaction with ERα signaling. Growth factor signaling has been linked to transcriptional repression of ERα expression in breast cancer cells, resulting in endocrine resistance. A positive correlation between expression of ERα and HER2 has been demonstrated in HER2 non overexpressing HR+ breast cancer, taking into account that E2/ERα stimulation decreased the expression of HER2 [226,227]. Interestingly, ERα knockdown in MCF7 cells resulted in an aggressive phenotype with downregulation of HER2 and upregulation of EGFR followed by increased phosphorylation of ERK1/2 (predominantly ERK2), indicating a subsidiary role for EGFR/HER2 heterodimerization compared to EGFR homodimerization for cancer cell behavior [228,229]. Elevated ERα levels could at least partly explain the higher HER2 expression in the MCF7/PGRMC1 cells compared to their EVC despite increased E2 levels. Considering that in MCF7/PGRMC1 cells EGFR and HER2 signaling is also enhanced, it is possible that in MCF7 cells PGRMC1 simultaneously amplifies

ligand-dependent and -independent ERα as well as EGFR/HER2 signaling without a negative feedback. Astonishingly, the T47D cells displayed different behavior depending on PGRMC1 overexpression. On the one hand, EGF treatment only induced a distinct phosphorylation of EGFR and subsequent activation of AKT and ERK in T47D/EVC compared to T47D/PGRMC1. This could be due in part to the fact that T47D/EVC cells had significantly higher EGFR expression levels than PGRMC1 overexpressing cells. On the other hand, T47D/PGRMC1 cells do not show higher levels of HER2 protein despite higher mRNA expression of ESR1 and its target genes compared to their EVC. Since ERα silencing decreased expression and activation of the β5-subunit of the proteasome, which is involved in EGFR endocytosis, lysosomal trafficking and degradation [228,230,231], inhibition or degradation of ERα might increase EGFR protein levels via protein stabilization. Via exaggerated ERα signaling, PGRMC1 overexpression in T47D cells might fuel proteasomal degradation of EGFR leading to mitigated MAPK and PI3K downstream signaling. In addition, interaction of PGRMC1 with the ERα modulators prohibitin-1 (PHB1) and prohibitin-2 (PHB2) diminished binding of PHBs to ERα inhibiting subsequent ERα activation. Interaction of PHBs and PGRMC1, which is dependent on S181 phosphorylation of PGRMC1, could drive ERα signaling in T47D/PGRMC1 cells further, alleviating EGFR signaling [232]. Furthermore, interaction of PHB with RAS located to the cell membrane is inalienable for the EGF induced activation of the RAF/MEK/ERK pathway [233,234]. It is possible that PGRMC1 interferes with this signaling cascade via directly interacting with PHB. However, further research is needed to examine the role of PGRMC1 in ERα and ErbB signaling to understand the mechanisms behind the observed cell specific differences better.

4.4 PGRMC1 Impacts Lipid Raft Formation and Signaling

For the first time, we observed that PGRMC1 rises lipid raft formation in HR+ breast cancer cells. Interestingly, PGRMC1 overexpression caused multiple lipid rafts to form in T47D breast cancer cells even despite unchanged cholesterol content compared to the EVC. Several biochemical trials revealed that late precursors of cholesterol containing 7-8 double bonds such as lathosterol and 7-dehydrocholesterol stabilize lipid rafts to a greater degree than cholesterol [235,236,237,238]. The greater lipid raft formation in T47D/PGRMC1 cells might therefore be attributed to the also significantly elevated lathosterol levels found within these cells compared to the EVC. Since lipid rafts are important among others in modulation of membrane geometry, lateral movement of molecules, and signal transduction, they are suggested as major regulators in cancer progression. Although a lot of RTKs are localized to

lipid rafts, ligand-dependant and -independent effects are highly variable. To date, it is not possible to predict conclusively the function of HER signaling in lipid rafts and some systems seem to be more reliant on lipid rafts for suppressing than for supporting signaling. Upon activation by ligand, unlike other RTK, EGFR typically moves out of lipid rafts [239]. The effects of cholesterol depletion on receptor localization and downstream signaling are influenced by different aspects, determined by the specific RTK. Altered cholesterol levels modify the mobility of EGF receptors in the plane of membranes leading to a decrease of activation through less dimerization of EGFR monomers. Hence, depletion of cholesterol resulted in increased EGF binding, enhanced dimer formation and autophosphorylation with subsequent MAPK activation and decreased receptor internalization and down regulation [240,-241,242,243,244,245], whereas phosphatidylinositol turnover was inhibited [246,247]. Lower lipid raft formation in T47D/EVC cells than in PGRMC1 overexpressing cells might, at least in part, explain the greater expression of EGFR and enhanced activation after EGF binding observed in these cells. Notwithstanding, EGF receptors are also distributed in the plasma membrane and subcellular compartments outside of rafts and their activation and downstream signaling depends on further factors [248]. In addition, cholesterol depletion unequally affected EGFR autophosphorylation, so that phosphorylation of Y992 and Y1173 was enhanced while phosphorylation at Y1045 and Y1068 was unchanged [243]. Thus, our results on enhanced phosphorylation of EGFR at Y1068 after EGF treatment do not contradict the likewise higher lipid raft expression in MCF7/PGRMC1 compared with the EVC. In other RTKs, such as the IR, cholesterol depletion tends to inhibit downstream signaling and cell metabolism, e.g., leading to suppression of IRS1 and ACLY phosphorylation followed by decreased glucose uptake and oxidation as well as PKB/AKT activation [88,249,250,251]. Different studies, among others in breast cancer, showed the association of HER2, which is comparatively mobile in the plane of the membrane, with lipid rafts, where a low calcium and PIP2-enriched microenvironment facilitates HER2 membrane maintenance and downstream PI3K/AKT signaling [244,252,253]. HER2 colocalized with extranuclear ERα in membrane signaling domains, leading to proliferation of breast cancer cells [254]. Lipid rafts are necessary for the localization of the ERα in the plasma membrane and its membrane initiated effects [255,256]. A limitation of this study is that downstream signaling of RTKs was not examined in specific dependence on their localization in lipid rafts. One possibility is to isolate the raft and nonraft membranes and after stimulation with growth factors determinate in which compartment a specific signaling event has occurred. Furthermore, function of lipid rafts can be studied after cholesterol depletion, e.g., by treating cells with agents such as filipin and methyl-β-cyclodextrin that sequester or remove cholesterol or by treating cells with cholesterol lowering agents like statins.

In contrast to membrane fractionation, cholesterol depletion allows the analysis of the impact of lipid rafts in cell signaling in intact cells. Further research is needed to understand how PGRMC1 expression and modification affect the activation and downstream signaling of RTKs in lipid rafts.

4.5 PGRMC1 and Therapy Options

Researchers in the PGRMC1 field face the challenge of translating their findings from the lab to the clinic. In addition to supporting the use of PGRMC1 in diagnostics and prognostics, researchers are investigating how the protein could play a role in therapeutic approaches, such as the treatment of cancer, metabolic diseases, or neurodegenerative diseases [165,166,257,258]. Several studies have demonstrated the influence of PGRMC1 in pharmacological contexts. PGRMC1 has been shown to promote chemoresistance against classical chemotherapeutics, such as doxorubicin or paclitaxel, as well as newer agents such as EGFR tyrosine kinase inhibitors, e.g., erlotinibin in xenograft tumors and in vitro cancer cell line experiments [123,-135,181,259,260]. In previous studies among others conducted in our laboratory, PGRMC1 conferred resistance to doxorubicin and epirubicin in breast cancer cell lines when treated simultaneously with P4 [146,261]. Furthermore, patients who did not respond to anthracycline based therapy had significantly higher PGRMC1 expression than patients who achieved partial remission, implying that there is a correlation between the expression of PGRMC1 and response to cancer therapy. No differences were found in PGRMC1 expression of tumor cells between therapy responders and non-responders using aromatase inhibitors [16].

4.5.1 Endocrine Therapy

Recent clinical strategies for HR+ breast cancer treatment include the use of endocrine therapy to block ERα signaling. Aromatase inhibitors prevent the conversion of androgens to estradiol via inhibition of aromatase activity, whereas antiestrogen therapy with tamoxifen antagonizes the binding of estrogen to its receptor by competing with estrogen for binding sites on the receptor and by recruiting corepressors. Although the endocrine treatment regimen of tamoxifen and aromatase inhibitors like exemestane, either alone or in combination with tamoxifen, has been found to be an important option for (postmenopausal) women with HR+ breast cancer, patients often relapse with either de-novo or acquired resistance [262,263,264]. The mechanisms underlying endocrine resistance have been aided by the development of cellular models for resistant breast cancers and include cross-talk between ERα and HER signaling or upregulation of the cholesterol biosynthesis pathway [265,266,267,268]. We treated MCF7/PGRMC1 and MCF7/EVC cells with tamox-

ifen and exemestane to test whether PGRMC1 expression could play a role in treatment resistance. Only at a few concentrations did MCF7/PGRMC1 cells differ significantly from EVC cells; although interestingly, MCF7/PGRMC1 cells showed a tendency toward decreased viability in the context of endocrine therapy. An explanation for the decreased viability of MCF7/PGRMC1 cells in response to short term estrogen signaling suppression could be that upregulation of E2 levels, ERα protein expression, and ERα downstream targets in MCF7/PGRMC1 compared to the EVC simultaneously results in a temporarily increased dependency on estrogen signaling. One limitation of our experimental approach is that we suppressed estrogen synthesis and ERα signaling only for a short period of time. However, long term adaptation to estrogen deprivation occurs in many different ways, so that definitive conclusions about long term treatment resistance should not be drawn from these viability assays. Future studies should examine the effect of PGRMC1 on long term estrogen deprived cells and whether the protein influences specific LTED signaling types.

4.5.2 Statins

Statins are the first-line agents for the treatment of hypercholesterolemia or hyperlipidemia in a cardiovascular setting. However, recently they have also been used in anti-cancer research [269,270,271,272]. The antiproliferative effect of statins is attributed to many mechanisms [273,274,275,276]. Besides reducing cholesterol levels, blocking the mevalonate pathway leads to apoptosis in different ways, e.g., by decreasing the synthesis of the isoprenoids geranylgeranyl pyrophosphate (GGPP) and farnesyl pyrophosphate (FPP), resulting, e.g., in inhibition of small Rho GTPase prenylation [277,278,279]. We speculated that PGRMC1 overexpressing cells might be more susceptible to statin treatment because of their increased activity in the mevalonate pathway and lipid production. Therefore, we treated the PGRMC1 overexpressing cells with simvastatin to investigate the influence of PGRMC1 on tumor viability under cholesterol depletion. Surprisingly, we detected, for the first time, that MCF7/PGRMC1 and MDA-MB-231/PGRMC1 cells are more sensitive to treatment with simvastatin than their respective controls. Previous studies showed, that HR+ breast cancer cell lines are differentially affected by statins than HR- cell lines [280]. MCF7 cells are more resistant to a statin treatment than MDA-MB-231 cells, possibly because of their ability to induce HMGCR, their expression of estrogen receptor, and the lower levels of activated NF-kB [280,281]. Since statins act in a pleiotropic way, they may also affect HR+ and negative cells differently. Cancer cells have a higher demand on lipids, cholesterol, and its metabolites, which makes them much more sensitive to a cholesterol-depleting therapy. A similar mechanism

was supposed in paclitaxel-tolerant persister cancer cells which became vulnerable to ferroptosis by xCT inhibitors and had markedly elevated expression of PGRMC1, possibly contributing to the development of drug resistance [282]. In MCF7 cells, overexpression of PGRMC1 leads to higher levels of cholesterol, but also fuels activation of MAPK signaling. By blocking the mevalonate pathway, important proliferation advantages are inhibited. In MDA-MB-231 cells PGRMC1 overexpression does not change levels of free cholesterol but led to decreased lipid droplet levels. MDA-MB-231 cells cover their lipid requirement more by lipid uptake than de novo synthesis [283]. The significantly decreased lathosterol/cholesterol ratio might be because of increased de novo synthesis through PGRMC1 overexpression. Consequently, cholesterol uptake might decrease through feedback inhibition. By blocking the mevalonate pathway MDA-MB-231 cells might be more vulnerable, e.g., because of their smaller lipid storage. The sensitizing influence of PGRMC1 on a statin treatment in both cell lines might strengthen the hypothesis of a grand and complex involvement and cross link of the mevalonate pathway not only on cholesterol/lipid levels but also on oncogenic signaling. Simvastatin induces expression and activation of antioxidant enzymes like the heme oxygenase-1 (HO-1), an enzyme that degrades heme, producing CO, biliverdin, and ferrous iron (Fe2+) [284,285,286]. Kabe et al. [123] showed that heme dependent dimerization of PGRMC1 is essential for its interactions with EGFR and that CO binds to to the sixth coordination site of the heme, preventing dimerization of PGRMC1. Enhanced CO synthesis in the context of HO-1 induction by simvastatin treatment is consistent with the hypothesis that PGRMC1 dimerization and associated interactions with EGFR and CYP enzymes is inhibited. This effect, in turn, could particularly explain the decrease in viability of PGRMC1 overexpressing cells. This work did not investigate the effect of simvastatin treatment on cell signaling and metabolism. Accordingly, future studies should further investigate how PGRMC1 expression affects proteomic and metabolomic profiles under treatment conditions. In addition, future studies should investigate whether PGRMC1 dimerization affects potential treatment response. In breast cancer cell lines, EGFR localization to lipid rafts correlated with EGFR tyrosin kinase inhibitor resistance and depletion of cholesterol, e.g., via repression of cholesterol biosynthesis after treatment with lovastatin, sensitized resistant breast cancer cells to therapeutic tyrosin kinase inhibiton [287]. Therefore, a combination of chemotherapeutic agents and statins—especially when considering the PGRMC1 protein expression—may play an important role in future cancer treatment plans.

4.6 General Limitations of this Study

In this work, we investigate the influence of PGRMC1 on lipid metabolism and associated signaling pathways in a cell model. Most of the experiments in this work were performed in cells stably transfected with the expression vector pcDNA3.1/Hygro(+), containing 3x human influenza hemagglutinin-tagged PGRMC1. As a control, cells were transfected with an empty vector. One drawback of using this cell line as a model is that the cells also express endogenous PGRMC1, so they are still subject to influences of PGRMC1; another is that the function of the overexpressed PGRMC1 may be altered by the hemagglutinin tag. For this reason, we also conducted some of the experiments in MCF7 cells with knock-down of PGRMC1 by using siRNAs. To elucidate the role of PGRMC1 in breast cancer, we investigated its expression in different HR+ and negative breast cancer cell lines. Nevertheless, to account for the heterogeneity of breast cancer cells, additional cell lines, for example with HER2 overexpression, should be investigated. The experimental investigation in different breast cancer cell lines offers a good foundation for understanding the role of PGRMC1 in metabolism and signaling. However, the heterogeneity of tumor tissue and the fluidity of different metabolic conditions can only be inadequately represented. Furthermore, the media used in tissue culture differ significantly from in vivo environments. Additionally, we did not use dynamic measures of metabolic and proteomic flux. Thus, only steady-state levels of lipids and signaling proteins were examined. Moreover, in this study, only a few lipid metabolites were investigated. A more thorough understanding of PGRMC1 may be gained by examining the large scale changes in the context of its expression and post-translational modifications. Our viability analysis of breast cancer cell lines treated with simvastatin identified PGRMC1 as a potential biomarker for future therapeutic approaches in multimodal cancer therapy. However, this hypothesis is largely built on in vitro studies. Clinical investigations and trials are necessary to establish PGRMC1 as a breast cancer biomarker.

4.7 Conclusion and Outlook

In this study, we were able to achieve a better understanding of how PGRMC1 is involved in lipid metabolism and important breast cancer signaling pathways (Figure 18). PGRMC1 fuels cell proliferation and cancer progression, potentially via higher levels of neutral lipids, cholesterol and E2 in HR+ breast cancer. It can be hypothesized that several factors account for this including interaction of PGRMC1 with key enzymes of the mevalonat pathway and altered expression of proteins responsible for lipid homeostasis, lipid uptake, and lipid synthesis. Nevertheless, the

exact molecular mechanisms involved are not yet fully understood. Future research should address how PGRMC1 affects cell proliferation and signaling under different metabolic conditions. Moreover, the effect of degree of differentiation and hormone status on the mode of action of PGRMC1 in breast cancer should be examined. We found that PGRMC1 overexpressing MCF7 cells display higher amounts of E2. We demonstrated that phosphorylation of PGRMC1 affects cholesterol and E2 level. Further investigation is necessary to characterize the phosphorylation of PGRMC1 and its associated function. In addition, signaling pathways facilitating ERα expression and activation are enhanced in PGRMC1 overexpressing HR+ breast cancer cells. We also investigated the impact of PGRMC1 expression on breast cancer cells' response to endogenous hormone therapy. Our experiments suggest that interference with estrogen metabolism and ERα signaling may have a greater impact on PGRMC1 overexpressing MCF7 cells in the short term. In particular, future research should investigate the role of PGRMC1 in the development of resistance to endogenous therapy. One interesting avenue of research could be to explore the expression and mode of action of PGRMC1 in long term estrogen deprived cells. Furthermore, PGRMC1 altered EGFR signaling and HER2 expression. The present study showed that overexpression of PGRMC1 results in significantly higher lipid raft formation. However, the underlying mechanisms of how PGRMC1 influences lipid raft expression and signaling are not fully understood. We demonstrated that inhibition of HMG-CoA reductase and following depletion of cholesterol not only assimilated viability of PGRMC1 overexpressing breast cancer cells but even result in inferior viability. Further examination and analysis of these findings in vivo is needed. Concluding, PGRMC1 may promote proliferation and progression of breast cancer cells potentially via modification of lipid homeostasis and key oncogenic signaling pathways. This study emphasizes the potential of PGRMC1 as a target for multimodal anti-cancer therapy.

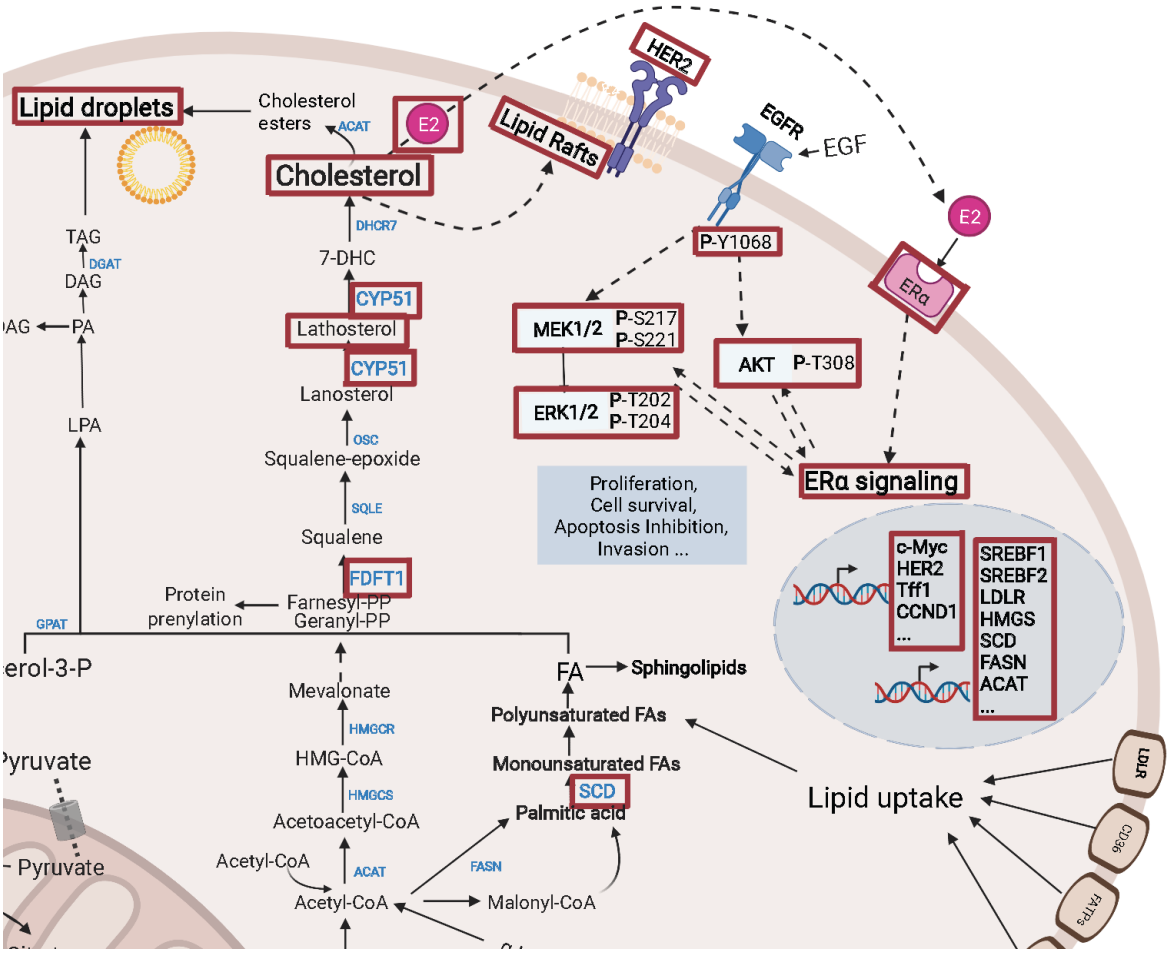


Figure 18: **Interaction of PGRMC1 in lipid metabolism and oncogenic signaling pathways**

Schematic model for the regulation of lipid metabolism and signaling pathways that contribute to proliferation, invasion, cell survival, and apoptosis inhibition by PGRMC1. Targets of PGRMC1 that are involved in lipid and signaling homeostasis are boxed in red.

Abbreviation: ACAT: acetyl-CoA acetyltransferase; AKT: protein kinase B; CCND1: cyclin D1; CD36: cluster of differentiation 36; CYP51: lanosterol 14 alpha-demethylase; DGAT: diglyceride acyltransferase; DHCR7: 7-dehydrocholesterol reductase; EGFR: epidermal growth factor receptor; ERK: extracellular signal-regulated kinase; ERα: estrogen receptor α; E2: estradiol; FA: fatty acid; FABPs: fatty acid binding proteins; FASN: fatty acid synthase; FDFT1: farnesyl-diphosphate farnesyltransferase 1; GPAT: glycerol-3-phosphate O-acyltransferase; HER: human epidermal growth factor receptor; HMGCR: 3-hydroxy-3-methylglutaryl-coenzyme A reductase; HMGCS: hydroxymethylglutaryl-CoA synthase; LDLR: low density lipoprotein receptor; LPA: lysophosphatidic acid; MEK: mitogen-activated protein kinase kinase; OSC: oxidosqualene cyclases; PA: phosphatidic acid; SCD: stearoyl-CoA desaturase; SQLE: squalene epoxidase; SREBF: sterol regulatory element binding transcription factor; TAG: triacylglycerol; TFF: trefoil factor

5 References

[1] Hyuna Sung et al. "Global cancer statistics 2020: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries." In: *CA: a cancer journal for clinicians* 71.3 (2021), pp. 209–249.

[2] J Ferlay et al. *Global cancer observatory: cancer today. Lyon: International Agency for Research on Cancer; 2018*. 2020.

[3] Isabelle Soerjomataram and Freddie Bray. "Planning for tomorrow: global cancer incidence and the role of prevention 2020–2070." In: *Nature Reviews Clinical Oncology* (2021), pp. 1–10.

[4] Ruth M Pfeiffer et al. "Proportion of US trends in breast cancer incidence attributable to long-term changes in risk factor distributions." In: *Cancer Epidemiology and Prevention Biomarkers* 27.10 (2018), pp. 1214–1222.

[5] Ali Jad Abdelwahab Yousef. "Male breast cancer: epidemiology and risk factors." In: *Seminars in oncology*. Vol. 44. 4. Elsevier. 2017, pp. 267–272.

[6] Françoise Clavel-Chapelon et al. "Reproductive factors and breast cancer risk: Effect of age at diagnosis." In: *Annals of epidemiology* 5.4 (1995), pp. 315–320.

[7] Huiyan Ma et al. "Reproductive factors and breast cancer risk according to joint estrogen and progesterone receptor status: a meta-analysis of epidemiological studies." In: *Breast Cancer Research* 8.4 (2006), pp. 1–11.

[8] Mads Melbye et al. "Preterm delivery and risk of breast cancer." In: *British Journal of Cancer* 80.3 (1999), pp. 609–613.

[9] Theodore M Brasky et al. "Pregnancy-related characteristics and breast cancer risk." In: *Cancer Causes & Control* 24.9 (2013), pp. 1675–1685.

[10] Polly A Marchbanks et al. "Oral contraceptives and the risk of breast cancer." In: *New England journal of medicine* 346.26 (2002), pp. 2025–2032.

[11] Elisabeth F Beaber et al. "Recent oral contraceptive use by formulation and breast cancer risk among women 20 to 49 years of age." In: *Cancer research* 74.15 (2014), pp. 4078–4089.

[12] Robabeh Taheripanah et al. "Breast cancer and ovulation induction treatments." In: *Clinical Breast Cancer* 18.5 (2018), pp. 395–399.

[13] V Beral et al. "Breast cancer and hormone replacement therapy: collaborative reanalysis of data from 51 epidemiological studies of 52,705 women with breast cancer and 108,411 women without breast cancer." In: *Lancet* 350.9084 (1997), pp. 1047–1059.

[14] Tara O Henderson et al. “Breast cancer risk in childhood cancer survivors without a history of chest radiotherapy: A report from the Childhood Cancer Survivor Study.” In: *Journal of Clinical Oncology* 34.9 (2016), p. 910.

[15] Chaya S Moskowitz et al. “Breast cancer after chest radiation therapy for childhood cancer.” In: *Journal of Clinical oncology* 32.21 (2014), p. 2217.

[16] Patrick J Willems. “Susceptibility genes in breast cancer: more is less?” In: *Clinical genetics* 72.6 (2007), pp. 493–496.

[17] KA Metcalfe et al. “Breast cancer risks in women with a family history of breast or ovarian cancer who have tested negative for a BRCA1 or BRCA2 mutation.” In: *British journal of cancer* 100.2 (2009), pp. 421–425.

[18] Erin F Cobain, Kara J Milliron, and Sofia D Merajver. “Updates on breast cancer genetics: Clinical implications of detecting syndromes of inherited increased susceptibility to breast cancer.” In: *Seminars in oncology*. Vol. 43. 5. Elsevier. 2016, pp. 528–535.

[19] Karoline B Kuchenbaecker et al. “Risks of breast, ovarian, and contralateral breast cancer for BRCA1 and BRCA2 mutation carriers.” In: *Jama* 317.23 (2017), pp. 2402–2416.

[20] Isabelle Romieu et al. “Alcohol intake and breast cancer in the E uropean prospective investigation into cancer and nutrition.” In: *International journal of cancer* 137.8 (2015), pp. 1921–1930.

[21] Juhua Luo et al. “Association of active and passive smoking with risk of breast cancer among postmenopausal women: a prospective cohort study.” In: *Bmj* 342 (2011).

[22] Sigrid Hatse et al. “Vitamin D status at breast cancer diagnosis: correlation with tumor characteristics, disease outcome, and genetic determinants of vitamin D insufficiency.” In: *Carcinogenesis* 33.7 (2012), pp. 1319–1326.

[23] Allison Soucise et al. “Sleep quality, duration, and breast cancer aggressiveness.” In: *Breast cancer research and treatment* 164.1 (2017), pp. 169–178.

[24] Sarah P Megdal et al. “Night work and breast cancer risk: a systematic review and meta-analysis.” In: *European journal of cancer* 41.13 (2005), pp. 2023–2032.

[25] Expert Panel on the Identification et al. *Clinical guidelines on the identification, evaluation, and treatment of overweight and obesity in adults: the evidence report*. 98. National Institutes of Health, National Heart, Lung, and Blood Institute, 1998.

[26] GBD 2015 Obesity Collaborators. “Health effects of overweight and obesity in 195 countries over 25 years.” In: *New England Journal of Medicine* 377.1 (2017), pp. 13–27.

[27] Steven K Clinton, Edward L Giovannucci, and Stephen D Hursting. “The World Cancer Research Fund/American Institute for Cancer Research third expert report on diet, nutrition, physical activity, and cancer: impact and future directions.” In: *The Journal of nutrition* 150.4 (2020), pp. 663–671.

[28] Zahra Cheraghi et al. “Effect of body mass index on breast cancer during premenopausal and postmenopausal periods: a meta-analysis.” In: *PloS one* 7.12 (2012), e51446.

[29] Andrew G Renehan et al. “Body-mass index and incidence of cancer: a systematic review and meta-analysis of prospective observational studies.” In: *The lancet* 371.9612 (2008), pp. 569–578.

[30] Yanzi Chen et al. “Body mass index had different effects on premenopausal and postmenopausal breast cancer risks: a dose-response meta-analysis with 3,318,796 subjects from 31 cohort studies.” In: *BMC Public Health* 17.1 (2017), pp. 1–11.

[31] NaNa Keum et al. “Adult weight gain and adiposity-related cancers: a dose-response meta-analysis of prospective observational studies.” In: *Journal of the National Cancer Institute* 107.2 (2015), djv088.

[32] World Cancer Research Fund and American Institute for Cancer Research. *Food, nutrition, physical activity, and the prevention of cancer: a global perspective*. Vol. 1. Amer Inst for Cancer Research, 2007.

[33] DSM Chan et al. “Body mass index and survival in women with breast cancer—systematic literature review and meta-analysis of 82 follow-up studies.” In: *Annals of Oncology* 25.10 (2014), pp. 1901–1914.

[34] Zsuzsanna Suba. “Circulatory estrogen level protects against breast cancer in obese women.” In: *Recent patents on anti-cancer drug discovery* 8.2 (2013), pp. 154–167.

[35] Zhiping Huang et al. “Dual effects of weight and weight gain on breast cancer risk.” In: *jama* 278.17 (1997), pp. 1407–1411.

[36] Libby M Morimoto et al. “Obesity, body size, and risk of postmenopausal breast cancer: the Women’s Health Initiative (United States).” In: *Cancer Causes & Control* 13.8 (2002), pp. 741–751.

[37] Charles M Perou et al. “Molecular portraits of human breast tumours.” In: *nature* 406.6797 (2000), pp. 747–752.

[38] Therese Sørlie et al. “Gene expression patterns of breast carcinomas distinguish tumor subclasses with clinical implications.” In: *Proceedings of the National Academy of Sciences* 98.19 (2001), pp. 10869–10874.

[39] Johannes Gerdes et al. “Cell cycle analysis of a cell proliferation-associated human nuclear antigen defined by the monoclonal antibody Ki-67.” In: *The journal of immunology* 133.4 (1984), pp. 1710–1715.

[40] Thomas Scholzen and Johannes Gerdes. “The Ki-67 protein: from the known and the unknown.” In: *Journal of cellular physiology* 182.3 (2000), pp. 311–322.

[41] Anna Britten et al. “Genomic classifications and radiotherapy for breast cancer.” In: *European journal of pharmacology* 717.1-3 (2013), pp. 67–70.

[42] Seho Park et al. “Characteristics and outcomes according to molecular subtypes of breast cancer as classified by a panel of four biomarkers using immunohistochemistry.” In: *The Breast* 21.1 (2012), pp. 50–57.

[43] William D Foulkes, Ian E Smith, and Jorge S Reis-Filho. “Triple-negative breast cancer.” In: *New England journal of medicine* 363.20 (2010), pp. 1938–1948.

[44] Rolf Kreienberg et al. “Interdisziplinäre S3-Leitlinie für die Diagnostik, Therapie und Nachsorge des Mammakarzinoms.” In: *Senologie-Zeitschrift für Mammadiagnostik und-therapie* 10.03 (2013), pp. 164–192.

[45] Mark Robson et al. “Olaparib for metastatic breast cancer in patients with a germline BRCA mutation.” In: *New England Journal of Medicine* 377.6 (2017), pp. 523–533.

[46] I Cortes-Ciriano et al. *A molecular portrait of microsatellite instability across multiple cancers. Nat. Commun. 8: 15180.* 2017.

[47] PDP Pharoah, NE Day, and C Caldas. “Somatic mutations in the p53 gene and prognosis in breast cancer: a meta-analysis.” In: *British journal of cancer* 80.12 (1999), pp. 1968–1973.

[48] Makoto Kubo et al. “Hedgehog signaling pathway is a new therapeutic target for patients with breast cancer.” In: *Cancer research* 64.17 (2004), pp. 6071–6074.

[49] Gunnar Schramm, Nandakumar Kannabiran, and Rainer König. “Regulation patterns in signaling networks of cancer.” In: *BMC systems biology* 4.1 (2010), pp. 1–12.

[50] David W Craig et al. “Genome and transcriptome sequencing in prospective metastatic triple-negative breast cancer uncovers therapeutic vulnerabilities.” In: *Molecular cancer therapeutics* 12.1 (2013), pp. 104–116.

[51] Musaffe Tuna, Arturo Chavez-Reyes, and Ana M Tari. “HER2/neu increases the expression of Wilms’ Tumor 1 (WT1) protein to stimulate S-phase proliferation and inhibit apoptosis in breast cancer cells.” In: *Oncogene* 24.9 (2005), pp. 1648–1652.

[52] Seoung Wan Chae et al. “Overexpressions of Cyclin B1, cdc2, p16 and p53 in human breast cancer: the clinicopathologic correlations and prognostic implications.” In: *Yonsei medical journal* 52.3 (2011), pp. 445–453.

[53] Evan C Merkhofer, Patricia Cogswell, and Albert S Baldwin. “Her2 activates NF-κB and induces invasion through the canonical pathway involving IKKα.” In: *Oncogene* 29.8 (2010), pp. 1238–1248.

[54] Anne A Knowlton and AR Lee. “Estrogen and the cardiovascular system.” In: *Pharmacology & therapeutics* 135.1 (2012), pp. 54–70.

[55] Minqian Shen and Haifei Shi. “Sex hormones and their receptors regulate liver energy homeostasis.” In: *International journal of endocrinology* 2015 (2015).

[56] Donald P McDonnell and John D Norris. “Connections and regulation of the human estrogen receptor.” In: *Science* 296.5573 (2002), pp. 1642–1644.

[57] Randall Bolger et al. “Rapid screening of environmental chemicals for estrogen receptor binding capacity.” In: *Environmental health perspectives* 106.9 (1998), pp. 551–557.

[58] Marc Ruff et al. “Estrogen receptor transcription and transactivation structure-function relationship in DNA-and ligand-binding domains of estrogen receptors.” In: *Breast Cancer Research* 2.5 (2000), pp. 1–7.

[59] Cristian G Bologa et al. “Virtual and biomolecular screening converge on a selective agonist for GPR30.” In: *Nature chemical biology* 2.4 (2006), pp. 207–212.

[60] Bruno Cenni and Didier Picard. “Ligand-independent activation of steroid receptors: new roles for old players.” In: *Trends in Endocrinology & Metabolism* 10.2 (1999), pp. 41–46.

[61] Simak Ali et al. “Modulation of transcriptional activation by ligand-dependent phosphorylation of the human oestrogen receptor A/B region.” In: *The EMBO journal* 12.3 (1993), pp. 1153–1160.

[62] Giuseppe Bunone et al. “Activation of the unliganded estrogen receptor by EGF involves the MAP kinase pathway and direct phosphorylation.” In: *The EMBO journal* 15.9 (1996), pp. 2174–2183.

[63] Stanley Cohen, Roy A Fava, and Stephen T Sawyer. “Purification and characterization of epidermal growth factor receptor/protein kinase from normal mouse liver.” In: *Proceedings of the National Academy of Sciences* 79.20 (1982), pp. 6237–6241.

[64] Yosef Yarden and Mark X Sliwkowski. “Untangling the ErbB signalling network.” In: *Nature reviews Molecular cell biology* 2.2 (2001), pp. 127–137.

[65] David J Riese and David F Stern. “Specificity within the EGF family/ErbB receptor family signaling network.” In: *Bioessays* 20.1 (1998), pp. 41–48.

[66] A Dittrich et al. “The HER2 signaling network in breast cancer—like a spider in its web.” In: *Journal of mammary gland biology and neoplasia* 19.3 (2014), pp. 253–270.

[67] Yosef Yarden and Gur Pines. “The ERBB network: at last, cancer therapy meets systems biology.” In: *Nature Reviews Cancer* 12.8 (2012), pp. 553–563.

[68] Otto Warburg. “On the origin of cancer cells.” In: *Science* 123.3191 (1956), pp. 309–314.

[69] Rafael Moreno-Sánchez et al. “Energy metabolism in tumor cells.” In: *The FEBS journal* 274.6 (2007), pp. 1393–1418.

[70] Eoin Fahy et al. “A comprehensive classification system for lipids1.” In: *Journal of lipid research* 46.5 (2005), pp. 839–861.

[71] Michael S Brown and Joseph L Goldstein. “A receptor-mediated pathway for cholesterol homeostasis.” In: *Science* 232.4746 (1986), pp. 34–47.

[72] Ira J Goldberg, Robert H Eckel, and Nada A Abumrad. “Regulation of fatty acid uptake into tissues: lipoprotein lipase-and CD36-mediated pathways.” In: *Journal of lipid research* 50 (2009), S86–S90.

[73] Bernadette S Jakobs and Ronald JA Wanders. “Fatty acid β-oxidation in peroxisomes and mitochondria: the first, unequivocal evidence for the involvement of carnitine in shuttling propionyl-CoA from peroxisomes to mitochondria.” In: *Biochemical and biophysical research communications* 213.3 (1995), pp. 1035–1041.

[74] Bernadette Sylvia Jakobs et al. “Function and Dysfunction of the Mitochondrial and Peroxisomal [beta]-oxidation Systems in Human Cells and Their Functional Interaction.” PhD thesis. Universiteit van Amsterdam [Host], 1999.

[75] Peter A Mayes and Kathleen M Botham. "Cholesterol synthesis, transport and excretion." In: *Harper's illustrated biochemistry* 26 (2003), pp. 219–227.

[76] Jay D Horton. "Sterol regulatory element-binding proteins: transcriptional activators of lipid synthesis." In: *Biochemical Society Transactions* 30.6 (2002), pp. 1091–1095.

[77] Livnat Jerby et al. "Metabolic associations of reduced proliferation and oxidative stress in advanced breast cancer." In: *Cancer research* 72.22 (2012), pp. 5712–5720.

[78] Kumi Tauchi-Sato et al. "The surface of lipid droplets is a phospholipid monolayer with a unique fatty acid composition." In: *Journal of Biological Chemistry* 277.46 (2002), pp. 44507–44512.

[79] Tobias C Walther and Robert V Farese Jr. "Lipid droplets and cellular lipid metabolism." In: *Annual review of biochemistry* 81 (2012), pp. 687–714.

[80] Benjamin J Hershey et al. "Lipid droplets define a sub-population of breast cancer stem cells." In: *Journal of clinical medicine* 9.1 (2020), p. 87.

[81] Luis A Bagatolli. "To see or not to see: lateral organization of biological membranes and fluorescence microscopy." In: *Biochimica et Biophysica Acta (BBA)-Biomembranes* 1758.10 (2006), pp. 1541–1556.

[82] Gerald Gimpl and Katja Gehrig-Burger. "Cholesterol reporter molecules." In: *Bioscience reports* 27.6 (2007), pp. 335–358.

[83] Palmer A Orlandi and Peter H Fishman. "Filipin-dependent inhibition of cholera toxin: evidence for toxin internalization and activation through caveolae-like domains." In: *The Journal of cell biology* 141.4 (1998), pp. 905–915.

[84] Kai Simons and Elina Ikonen. "Functional rafts in cell membranes." In: *nature* 387.6633 (1997), pp. 569–572.

[85] Michael F Hanzal-Bayer and John F Hancock. "Lipid rafts and membrane traffic." In: *FEBS letters* 581.11 (2007), pp. 2098–2104.

[86] Patrick Lajoie et al. "Lattices, rafts, and scaffolds: domain regulation of receptor signaling at the plasma membrane." In: *Journal of Cell Biology* 185.3 (2009), pp. 381–385.

[87] Sabiha N Zaman, Mary E Resek, and Stephen M Robbins. "Dual acylation and lipid raft association of Src-family protein tyrosine kinases are required for SDF-1/CXCL12-mediated chemotaxis in the Jurkat human T cell lymphoma cell line." In: *Journal of leukocyte biology* 84.4 (2008), pp. 1082–1091.

[88] Saara Vainio et al. "Dynamic association of human insulin receptor with lipid rafts in cells lacking caveolae." In: *EMBO reports* 3.1 (2002), pp. 95–100.

[89] Linda J Pike, Xianlin Han, and Richard W Gross. "Epidermal growth factor receptors are localized to lipid rafts that contain a balance of inner and outer leaflet lipids: a shotgun lipidomics study." In: *Journal of Biological Chemistry* 280.29 (2005), pp. 26796–26804.

[90] Ferruccio Galbiati, Babak Razani, and Michael P Lisanti. "Emerging themes in lipid rafts and caveolae." In: *Cell* 106.4 (2001), pp. 403–411.

[91] Robert Gniadecki. "Depletion of membrane cholesterol causes ligand-independent activation of Fas and apoptosis." In: *Biochemical and biophysical research communications* 320.1 (2004), pp. 165–169.

[92] Graham Carpenter. "The EGF receptor: a nexus for trafficking and signaling." In: *Bioessays* 22.8 (2000), pp. 697–707.

[93] Thomas Harder. "Lipid raft domains and protein networks in T-cell receptor signal transduction." In: *Current opinion in immunology* 16.3 (2004), pp. 353–359.

[94] Ying Chun Li et al. "Elevated levels of cholesterol-rich lipid rafts in cancer cells are correlated with apoptosis sensitivity induced by cholesterol-depleting agents." In: *The American journal of pathology* 168.4 (2006), pp. 1107–1118.

[95] Uliana Levin-Gromiko et al. "Amplified lipid rafts of malignant cells constitute a target for inhibition of aberrantly active NFAT and melanoma tumor growth by the aminobisphosphonate zoledronic acid." In: *Carcinogenesis* 35.11 (2014), pp. 2555–2566.

[96] Anup D Shah et al. "Integrative analysis of subcellular quantitative proteomics studies reveals functional cytoskeleton membrane–lipid raft interactions in cancer." In: *Journal of proteome research* 15.10 (2016), pp. 3451–3462.

[97] Yifan Wu et al. "Hydroxypropyl-β-cyclodextrin attenuates the epithelial-to-mesenchymal transition via endoplasmic reticulum stress in MDA-MB-231 breast cancer cells." In: *Molecular Medicine Reports* 21.1 (2020), pp. 249–257.

[98] Yu Cheng et al. "The chemokine receptor CXCR4 and c-MET cooperatively promote epithelial-mesenchymal transition in gastric cancer cells." In: *Translational oncology* 11.2 (2018), pp. 487–497.

[99] Hongyue Jin et al. "Targeting lipid metabolism to overcome EMT-associated drug resistance via integrin β3/FAK pathway and tumor-associated macrophage repolarization using legumain-activatable delivery." In: *Theranostics* 9.1 (2019), p. 265.

[100] Michael J Tisza et al. “Motility and stem cell properties induced by the epithelial-mesenchymal transition require destabilization of lipid rafts.” In: *Oncotarget* 7.32 (2016), p. 51553.

[101] E Falkenstein et al. “Localization of a putative progesterone membrane binding protein in porcine hepatocytes.” In: *Cellular and Molecular Biology (Noisy-le-Grand, France)* 44.4 (1998), pp. 571–578.

[102] Ralf Lösel et al. “Porcine spermatozoa contain more than one membrane progesterone receptor.” In: *The international journal of biochemistry & cell biology* 36.8 (2004), pp. 1532–1541.

[103] Christopher J Krebs et al. “A membrane-associated progesterone-binding protein, 25-Dx, is regulated by progesterone in brain regions involved in female reproductive behaviors.” In: *Proceedings of the National Academy of Sciences* 97.23 (2000), pp. 12816–12821.

[104] Zaklina Kovacevic et al. “PGRMC1 regulation by phosphorylation: Potential new insights in controlling biological activity!” In: (2016).

[105] Li Min et al. “Characterization of the adrenal-specific antigen IZA (inner zone antigen) and its role in the steroidogenesis.” In: *Molecular and cellular endocrinology* 215.1-2 (2004), pp. 143–148.

[106] Val Leel et al. “Identification of a truncated ratp28-related protein expressed in kidney.” In: *Biochemical and biophysical research communications* 316.3 (2004), pp. 872–877.

[107] Sean A Beausoleil et al. “Large-scale characterization of HeLa cell nuclear phosphoproteins.” In: *Proceedings of the National Academy of Sciences* 101.33 (2004), pp. 12130–12135.

[108] Robert B Piel III et al. “A novel role for progesterone receptor membrane component 1 (PGRMC1): a partner and regulator of ferrochelatase.” In: *Biochemistry* 55.37 (2016), pp. 5204–5217.

[109] Dirk Gerdes et al. “Cloning and tissue expression of two putative steroid membrane receptors.” In: *Biological chemistry* 379.7 (1998), pp. 907–912.

[110] Ikuo Kimura et al. “Functions of MAPR (membrane-associated progesterone receptor) family members as heme/steroid-binding proteins.” In: *Current Protein and Peptide Science* 13.7 (2012), pp. 687–696.

[111] Chang S Ryu, Kathrin Klein, and Ulrich M Zanger. “Membrane associated progesterone receptors: promiscuous proteins with pleiotropic functions–focus on interactions with cytochromes P450.” In: *Frontiers in pharmacology* 8 (2017), p. 159.

[112] Christiane Meyer et al. “Purification and partial sequencing of high-affinity progesterone-binding site (s) from porcine liver membranes.” In: *European Journal of Biochemistry* 239.3 (1996), pp. 726–731.

[113] Ornella Selmin et al. “Isolation and characterization of a novel gene induced by 2, 3, 7, 8-tetrachlorodibenzo-p-dioxin in rat liver.” In: *Carcinogenesis* 17.12 (1996), pp. 2609–2615.

[114] Erik Runko and Zaven Kaprielian. “Expression of Vema in the developing mouse spinal cord and optic chiasm.” In: *Journal of Comparative Neurology* 451.3 (2002), pp. 289–299.

[115] Erik Runko and Zaven Kaprielian. “Caenorhabditis elegans VEM-1, a novel membrane protein, regulates the guidance of ventral nerve cord-associated axons.” In: *Journal of Neuroscience* 24.41 (2004), pp. 9015–9026.

[116] Randal A Hand et al. “Saccharomyces cerevisiae Dap1p, a novel DNA damage response protein related to the mammalian membrane-associated progesterone receptor.” In: *Eukaryotic cell* 2.2 (2003), pp. 306–317.

[117] Naoei Yoshitani et al. “A structure-based strategy for discovery of small ligands binding to functionally unknown proteins: Combination of in silico screening and surface plasmon resonance measurements.” In: *Proteomics* 5.6 (2005), pp. 1472–1480.

[118] John J Peluso, Jonathan Romak, and Xiufang Liu. “Progesterone receptor membrane component-1 (PGRMC1) is the mediator of progesterone’s antiapoptotic action in spontaneously immortalized granulosa cells as revealed by PGRMC1 small interfering ribonucleic acid treatment and functional analysis of PGRMC1 mutations.” In: *Endocrinology* 149.2 (2008), pp. 534–543.

[119] Stephen G Sligar et al. “Alteration of heme axial ligands by site-directed mutagenesis: a cytochrome becomes a catalytic demethylase.” In: *Journal of the American Chemical Society* 109.25 (1987), pp. 7896–7897.

[120] Michael A Cahill. “Progesterone receptor membrane component 1: an integrative review.” In: *The Journal of steroid biochemistry and molecular biology* 105.1-5 (2007), pp. 16–36.

[121] Alisha M Thompson et al. “Measurement of the heme affinity for yeast dap1p, and its importance in cellular function.” In: *Biochemistry* 46.50 (2007), pp. 14629–14637.

[122] Daniel Kaluka et al. “Spectroscopic and mutagenesis studies of human PGRMC1.” In: *Biochemistry* 54.8 (2015), pp. 1638–1647.

[123] Yasuaki Kabe et al. “Haem-dependent dimerization of PGRMC1/Sigma-2 receptor facilitates cancer proliferation and chemoresistance.” In: *Nature communications* 7.1 (2016), pp. 1–13.

[124] Michael A Cahill et al. “PGRMC1 regulation by phosphorylation: potential new insights in controlling biological activity!” In: *Oncotarget* 7.32 (2016), p. 50822.

[125] Michael A Cahill et al. “The emerging role of progesterone receptor membrane component 1 (PGRMC1) in cancer biology.” In: *Biochimica et Biophysica Acta (BBA)-Reviews on Cancer* 1866.2 (2016), pp. 339–349.

[126] Peter V Hornbeck et al. “PhosphoSite: A bioinformatics resource dedicated to physiological protein phosphorylation.” In: *Proteomics* 4.6 (2004), pp. 1551–1561.

[127] Hans Neubauer et al. “Breast cancer proteomics reveals correlation between estrogen receptor status and differential phosphorylation of PGRMC1.” In: *Breast Cancer Research* 10.5 (2008), pp. 1–16.

[128] *PGRMC1 progesterone receptor membrane component 1 [Homo sapiens (human)] Gene - NCBI*. `https://www.ncbi.nlm.nih.gov/gene/10857`. Accessed: 2022-10-01.

[129] *PGRMC1 progesterone receptor membrane component 1 [Homo sapiens (human)] - UniProt*. `http://www.uniprot.org/uniprot/O00264`. Accessed: 2022-10-01.

[130] *PGRMC1 progesterone receptor membrane component 1 [Homo sapiens (human)] - PTM sites*. `https://www.phosphosite.org/proteinAction.action?id=5744`. Accessed: 2022-10-01.

[131] Ikhlas S Ahmed et al. “Progesterone receptor membrane component 1 (Pgrmc1): a heme-1 domain protein that promotes tumorigenesis and is inhibited by a small molecule.” In: *Journal of Pharmacology and Experimental Therapeutics* 333.2 (2010), pp. 564–573.

[132] Anthony Bruce and Adrian P Rybak. “CYB5D2 requires heme-binding to regulate HeLa cell growth and confer survival from chemotherapeutic agents.” In: *PLoS One* 9.1 (2014), e86435.

[133] Christian Albrecht et al. “In vitro inhibition of SKOV-3 cell migration as a distinctive feature of progesterone receptor membrane component type 2 versus type 1.” In: *Steroids* 77.14 (2012), pp. 1543–1550.

[134] Mahmoud Reza Mansouri et al. “Alterations in the expression, structure and function of progesterone receptor membrane component-1 (PGRMC1) in premature ovarian failure.” In: *Human molecular genetics* 17.23 (2008), pp. 3776–3783.

[135] Anne M Friel et al. “Progesterone receptor membrane component 1 deficiency attenuates growth while promoting chemosensitivity of human endometrial xenograft tumors.” In: *Cancer letters* 356.2 (2015), pp. 434–442.

[136] Xiaofei Zhu et al. “Progesterone protects ovarian cancer cells from cisplatin-induced inhibitory effects through progesterone receptor membrane component 1/2 as well as AKT signaling.” In: *Oncology reports* 30.5 (2013), pp. 2488–2494.

[137] Xiaofei Zhu et al. “PGRMC1-dependent autophagy by hyperoside induces apoptosis and sensitizes ovarian cancer cells to cisplatin treatment.” In: *International journal of oncology* 50.3 (2017), pp. 835–846.

[138] Michael A Cahill and Amy E Medlock. “Thoughts on interactions between PGRMC1 and diverse attested and potential hydrophobic ligands.” In: *The Journal of steroid biochemistry and molecular biology* 171 (2017), pp. 11–33.

[139] Ikhlas SA Ahmed, Cora Chamberlain, and Rolf J Craven. “S2rpgrmc1: the cytochrome-related sigma-2 receptor that regulates lipid and drug metabolism and hormone signaling.” In: *Expert opinion on drug metabolism & toxicology* 8.3 (2012), pp. 361–370.

[140] Meredith R McGuire et al. “Progesterone receptor membrane component 1 (PGRMC1) binds and stabilizes cytochromes P450 through a heme-independent mechanism.” In: *Journal of Biological Chemistry* 297.5 (2021).

[141] Hannah J Rohe et al. “PGRMC1 (progesterone receptor membrane component 1): a targetable protein with multiple functions in steroid signaling, P450 activation and drug binding.” In: *Pharmacology & therapeutics* 121.1 (2009), pp. 14–19.

[142] Ronald K Kalkhoff. “Metabolic effects of progesterone.” In: *American journal of obstetrics and gynecology* 142.6 (1982), pp. 735–738.

[143] Michael Schumacher et al. “Progesterone synthesis in the nervous system: implications for myelination and myelin repair.” In: *Frontiers in neuroscience* 6 (2012), p. 10.

[144] John J Peluso. “Progesterone receptor membrane component 1 and its role in ovarian follicle growth.” In: *Frontiers in neuroscience* 7 (2013), p. 99.

[145] Christiane Meyer et al. “Characterization of high affinity progesterone-binding membrane proteins by an anti-peptide antiserum.” In: *Steroids* 63.2 (1998), pp. 111–116.

[146] Nicole C Clark et al. “Progesterone receptor membrane component 1 promotes survival of human breast cancer cells and the growth of xenograft tumors.” In: *Cancer biology & therapy* 17.3 (2016), pp. 262–271.

[147] John J Peluso et al. “Progesterone receptor membrane component-1 regulates the development and Cisplatin sensitivity of human ovarian tumors in athymic nude mice.” In: *Endocrinology* 150.11 (2009), pp. 4846–4854.

[148] John J Peluso et al. “Plasminogen activator inhibitor 1 RNA-binding protein interacts with progesterone receptor membrane component 1 to regulate progesterone’s ability to maintain the viability of spontaneously immortalized granulosa cells and rat granulosa cells.” In: *Biology of reproduction* 88.1 (2013), pp. 20–1.

[149] Chang Su et al. “Progesterone increases the release of brain-derived neurotrophic factor from glia via progesterone receptor membrane component 1 (Pgrmc1)-dependent ERK5 signaling.” In: *Endocrinology* 153.9 (2012), pp. 4389–4400.

[150] John J Peluso, Valentina Lodde, and Xiufang Liu. “Progesterone regulation of progesterone receptor membrane component 1 (PGRMC1) sumoylation and transcriptional activity in spontaneously immortalized granulosa cells.” In: *Endocrinology* 153.8 (2012), pp. 3929–3939.

[151] Valentina Lodde and John J Peluso. “A novel role for progesterone and progesterone receptor membrane component 1 in regulating spindle microtubule stability during rat and human ovarian cell mitosis.” In: *Biology of reproduction* 84.4 (2011), pp. 715–722.

[152] Ji Yea Kim et al. “Progesterone Receptor Membrane Component 1 suppresses the p53 and Wnt/β-catenin pathways to promote human pluripotent stem cell self-renewal.” In: *Scientific reports* 8.1 (2018), pp. 1–16.

[153] Shakeel UR Mir et al. “Progesterone receptor membrane component 1/Sigma-2 receptor associates with MAP1LC3B and promotes autophagy.” In: *Autophagy* 9.10 (2013), pp. 1566–1578.

[154] Mohammad Golam Sabbir. “Progesterone induced Warburg effect in HEK293 cells is associated with post-translational modifications and proteasomal degradation of progesterone receptor membrane component 1.” In: *The Journal of steroid biochemistry and molecular biology* 191 (2019), p. 105376.

[155] Howard M Katzen and Robert T Schimke. "Multiple forms of hexokinase in the rat: tissue distribution, age dependency, and properties." In: *Proceedings of the National Academy of Sciences of the United States of America* 54.4 (1965), p. 1218.

[156] John E Wilson. "Isozymes of mammalian hexokinase: structure, subcellular localization and metabolic function." In: *Journal of Experimental Biology* 206.12 (2003), pp. 2049–2057.

[157] Mohammad Golam Sabbir, Carla G Taylor, and Peter Zahradka. "Progesterone-induced Warburg Effect is Regulated by Cell-type-specific Interaction of Progesterone Receptor Membrane Component 1 and Hexokinases." In: (2020).

[158] F Gras et al. "Progesterone impairs cell respiration and suppresses a compensatory increase in glucose transport in isolated rat skeletal muscle: a non-genomic mechanism contributing to metabolic adaptation to late pregnancy?" In: *Diabetologia* 50.12 (2007), pp. 2544–2552.

[159] Kaia K Hampton et al. "Insulin receptor plasma membrane levels increased by the progesterone receptor membrane component 1." In: *Molecular pharmacology* 94.1 (2018), pp. 665–673.

[160] Ryogo Furuhata et al. "Progesterone receptor membrane associated component 1 enhances obesity progression in mice by facilitating lipid accumulation in adipocytes." In: *Communications biology* 3.1 (2020), pp. 1–13.

[161] Sang R Lee et al. "Loss of progesterone receptor membrane component 1 promotes hepatic steatosis via the induced de novo lipogenesis." In: *Scientific reports* 8.1 (2018), pp. 1–11.

[162] Ming Zhang et al. "Progesterone receptor membrane component 1 is a functional part of the glucagon-like peptide-1 (GLP-1) receptor complex in pancreatic β cells." In: *Molecular & cellular proteomics* 13.11 (2014), pp. 3049–3062.

[163] Aladdin Riad et al. "Sigma-2 receptor/TMEM97 and PGRMC-1 increase the rate of internalization of LDL by LDL receptor through the formation of a ternary complex." In: *Scientific reports* 8.1 (2018), pp. 1–12.

[164] Aladdin Riad et al. "The sigma-2 receptor/TMEM97, PGRMC1, and LDL receptor complex are responsible for the cellular uptake of Aβ42 and its protein aggregates." In: *Molecular neurobiology* 57.9 (2020), pp. 3803–3813.

[165] Nicholas J Izzo et al. "Alzheimer's therapeutics targeting amyloid beta 1–42 oligomers I: Abeta 42 oligomer binding to specific neuronal receptors is displaced by drug candidates that improve cognitive deficits." In: *PloS one* 9.11 (2014), e111898.

[166] Nicholas J Izzo et al. "Alzheimer's therapeutics targeting amyloid beta 1–42 oligomers II: Sigma-2/PGRMC1 receptors mediate Abeta 42 oligomer binding and synaptotoxicity." In: *PloS one* 9.11 (2014), e111899.

[167] Bashar M Thejer et al. "PGRMC1 phosphorylation affects cell shape, motility, glycolysis, mitochondrial form and function, and tumor growth." In: *BMC molecular and cell biology* 21.1 (2020), pp. 1–24.

[168] Bashar M Thejer et al. "PGRMC1 effects on metabolism, genomic mutation and CpG methylation imply crucial roles in animal biology and disease." In: *BMC molecular and cell biology* 21.1 (2020), pp. 1–19.

[169] Monika Suchanek, Anna Radzikowska, and Christoph Thiele. "Photo-leucine and photo-methionine allow identification of protein-protein interactions in living cells." In: *Nature methods* 2.4 (2005), pp. 261–268.

[170] HL Cai et al. "A potential mechanism underlying atypical antipsychotics-induced lipid disturbances." In: *Translational psychiatry* 5.10 (2015), e661–e661.

[171] Adam L Hughes et al. "Dap1/PGRMC1 binds and regulates cytochrome P450 enzymes." In: *Cell metabolism* 5.2 (2007), pp. 143–149.

[172] Rolf J Craven. *PGRMC1: a new biomarker for the estrogen receptor in breast cancer*. 2008.

[173] Dan Zhang et al. "PGRMC1 is a novel potential tumor biomarker of human renal cell carcinoma based on quantitative proteomic and integrative biological assessments." In: *PloS one* 12.1 (2017), e0170453.

[174] Gerard Crudden, Ralf Loesel, and Rolf J Craven. "Overexpression of the cytochrome p450 activator hpr6 (heme-1 domain protein/human progesterone receptor) in tumors." In: *Tumor Biology* 26.3 (2005), pp. 142–146.

[175] John J Peluso et al. "Regulation of ovarian cancer cell viability and sensitivity to cisplatin by progesterone receptor membrane component-1." In: *The Journal of Clinical Endocrinology & Metabolism* 93.5 (2008), pp. 1592–1599.

[176] John R Hornick et al. "Therapeutic targeting of pancreatic cancer utilizing sigma-2 ligands." In: *Surgery* 152.3 (2012), S152–S156.

[177] Shakeel UR Mir et al. “Elevated progesterone receptor membrane component 1/sigma-2 receptor levels in lung tumors and plasma from lung cancer patients.” In: *International Journal of Cancer* 131.2 (2012), E1–E9.

[178] Xiangyan Ruan et al. “Increased expression of progesterone receptor membrane component 1 is associated with aggressive phenotype and poor prognosis in ER-positive and negative breast cancer.” In: *Menopause* 24.2 (2017), pp. 203–209.

[179] Kaia K Hampton et al. “PGRMC1 elevation in multiple cancers and essential role in stem cell survival.” In: *Advances in lung cancer (Irvine)* 4.3 (2015), p. 37.

[180] Shufeng Ji, Aiguo Wu, and Huafeng Yang. “Expression of progesterone receptor membrane component-1 is associated with the malignant phenotypes of breast cancer.” In: *Nan fang yi ke da xue xue bao= Journal of Southern Medical University* 32.5 (2012), pp. 635–638.

[181] Szu-Ting Lin et al. “PGRMC1 contributes to doxorubicin-induced chemoresistance in MES-SA uterine sarcoma.” In: *Cellular and molecular life sciences* 72.12 (2015), pp. 2395–2409.

[182] Gerard Crudden, Rachel E Chitti, and Rolf J Craven. “Hpr6 (heme-1 domain protein) regulates the susceptibility of cancer cells to chemotherapeutic drugs.” In: *Journal of Pharmacology and Experimental Therapeutics* 316.1 (2006), pp. 448–455.

[183] Holly K Dressman et al. “Gene expression profiles of multiple breast cancer phenotypes and response to neoadjuvant chemotherapy.” In: *Clinical Cancer Research* 12.3 (2006), pp. 819–826.

[184] Randal A Hand and Rolf J Craven. “Hpr6. 6 protein mediates cell death from oxidative damage in MCF-7 human breast cancer cells.” In: *Journal of cellular biochemistry* 90.3 (2003), pp. 534–547.

[185] Marina Willibald et al. “Progesterone receptor membrane component 1 is phosphorylated upon progestin treatment in breast cancer cells.” In: *Oncotarget* 8.42 (2017), p. 72480.

[186] L Zhang et al. “E2+ norethisterone promotes the PI3K–AKT pathway via PGRMC1 to induce breast cancer cell proliferation.” In: *Climacteric* (2022), pp. 1–9.

[187] Hans Neubauer et al. “Overexpression of progesterone receptor membrane component 1: possible mechanism for increased breast cancer risk with norethisterone in hormone therapy.” In: *Menopause* 20.5 (2013), pp. 504–510.

[188] Guiju Cai et al. “Association of circulating Progesterone Receptor Membrane Component-1 (PGRMC1) with PGRMC1 expression in breast tumour tissue and with clinical breast tumour characteristics.” In: *Maturitas* 140 (2020), pp. 64–71.

[189] Xiangyan Ruan et al. “Association of circulating Progesterone Receptor Membrane Component-1 (PGRMC1) with breast tumor characteristics and comparison with known tumor markers.” In: *Menopause* 27.2 (2020), pp. 183–193.

[190] Sang R Lee et al. “Absence of progesterone receptor membrane component 1 reduces migration and metastasis of breast cancer.” In: *Cell Communication and Signaling* 19.1 (2021), pp. 1–11.

[191] H Neubauer et al. “Possible role of PGRMC1 in breast cancer development.” In: *Climacteric* 16.5 (2013), pp. 509–513.

[192] X Li et al. “PGRMC1 can trigger estrogen-dependent proliferation of breast cancer cells: estradiol vs. equilin vs. ethinylestradiol.” In: *Climacteric* 22.5 (2019), pp. 483–488.

[193] H Neubauer et al. “Membrane-initiated effects of progesterone on proliferation and activation of VEGF in breast cancer cells.” In: *Climacteric* 12.3 (2009), pp. 230–239.

[194] Diego A Pedroza et al. “Crosstalk between progesterone receptor membrane component 1 and estrogen receptor α promotes breast cancer cell proliferation.” In: *Laboratory Investigation* 101.6 (2021), pp. 733–744.

[195] Yajun Duan et al. “Peroxisome proliferator-activated receptor γ activation by ligands and dephosphorylation induces proprotein convertase subtilisin kexin type 9 and low density lipoprotein receptor expression.” In: *Journal of Biological Chemistry* 287.28 (2012), pp. 23667–23677.

[196] Béatrice Desvergne and Walter Wahli. “Peroxisome proliferator-activated receptors: nuclear control of metabolism.” In: *Endocrine reviews* 20.5 (1999), pp. 649–688.

[197] Michal Armoni et al. “Peroxisome proliferator-activated receptor-γ represses GLUT4 promoter activity in primary adipocytes, and rosiglitazone alleviates this effect.” In: *Journal of Biological Chemistry* 278.33 (2003), pp. 30614–30623.

[198] AKG Kadegowda et al. "Peroxisome proliferator-activated receptor-γ activation and long-chain fatty acids alter lipogenic gene networks in bovine mammary epithelial cells to various extents." In: *Journal of Dairy Science* 92.9 (2009), pp. 4276–4289.

[199] HB Shi et al. "Peroxisome proliferator-activated receptor-γ stimulates the synthesis of monounsaturated fatty acids in dairy goat mammary epithelial cells via the control of stearoyl-coenzyme A desaturase." In: *Journal of Dairy Science* 96.12 (2013), pp. 7844–7853.

[200] Anastasia P Nesterova et al. *Disease pathways: an atlas of human disease signaling pathways*. Elsevier, 2019.

[201] Javier A Menendez. "Fine-tuning the lipogenic/lipolytic balance to optimize the metabolic requirements of cancer cell growth: molecular mechanisms and therapeutic perspectives." In: *Biochimica et Biophysica Acta (BBA)-Molecular and Cell Biology of Lipids* 1801.3 (2010), pp. 381–391.

[202] Chunming Cheng et al. "Glucose-mediated N-glycosylation of SCAP is essential for SREBP-1 activation and tumor growth." In: *Cancer cell* 28.5 (2015), pp. 569–581.

[203] Chunming Cheng et al. "Analysis of SCAP N-glycosylation and Trafficking in Human Cells." In: *JoVE (Journal of Visualized Experiments)* 117 (2016), e54709.

[204] Xiaodong Zhang et al. "Inhibition of intracellular lipolysis promotes human cancer cell adaptation to hypoxia." In: *Elife* 6 (2017), e31132.

[205] Cyril Corbet et al. "TGFβ2-induced formation of lipid droplets supports acidosis-driven EMT and the metastatic spreading of cancer cells." In: *Nature communications* 11.1 (2020), pp. 1–15.

[206] Benjamin J Hershey et al. "Lipid droplets define a sub-population of breast cancer stem cells." In: *Journal of clinical medicine* 9.1 (2019), p. 87.

[207] Kenta Kuramoto et al. "Inhibition of the Lipid Droplet–Peroxisome Proliferator-Activated Receptor α Axis Suppresses Cancer Stem Cell Properties." In: *Genes* 12.1 (2021), p. 99.

[208] Luca Tirinato et al. "Lipid droplets: a new player in colorectal cancer stem cells unveiled by spectroscopic imaging." In: *Stem cells* 33.1 (2015), pp. 35–44.

[209] O Darragh et al. "Mega-stokes pyrene ceramide conjugates for STED imaging of lipid droplets in live cells." In: *Analyst* 144.5 (2019), pp. 1608–1621.

[210] Sanjica Rak et al. “FTIR spectroscopy reveals lipid droplets in drug resistant laryngeal carcinoma cells through detection of increased ester vibrational bands intensity.” In: *Analyst* 139.13 (2014), pp. 3407–3415.

[211] Junjie Li et al. “Lipid desaturation is a metabolic marker and therapeutic target of ovarian cancer stem cells.” In: *Cell stem cell* 20.3 (2017), pp. 303–314.

[212] Jennifer Man, Marina Pajic, and Anthony M Joshua. “Fats and Mets, KRAS-driven lipid dysregulation affects metastatic potential in pancreatic cancer.” In: *Cancer Research* 80.22 (2020), pp. 4886–4887.

[213] Han Sung Kang et al. “Protein and lipid MALDI profiles classify breast cancers according to the intrinsic subtype.” In: *BMC cancer* 11.1 (2011), pp. 1–9.

[214] Vera Cappelletti et al. “Metabolic footprints and molecular subtypes in breast cancer.” In: *Disease markers* 2017 (2017).

[215] Marie E Monaco. “Fatty acid metabolism in breast cancer subtypes.” In: *Oncotarget* 8.17 (2017), p. 29487.

[216] Xiangjian Luo et al. “Emerging roles of lipid metabolism in cancer metastasis.” In: *Molecular cancer* 16.1 (2017), pp. 1–10.

[217] Marta Bosch, Robert G Parton, and Albert Pol. “Lipid droplets, bioenergetic fluxes, and metabolic flexibility.” In: *Seminars in Cell & Developmental Biology*. Vol. 108. Elsevier. 2020, pp. 33–46.

[218] Elzbieta Szczesna-Skorupa and Byron Kemper. “Progesterone receptor membrane component 1 inhibits the activity of drug-metabolizing cytochromes P450 and binds to cytochrome P450 reductase.” In: *Molecular pharmacology* 79.3 (2011), pp. 340–350.

[219] Rolf J Craven, Julia C Mallory, and Randal A Hand. “Regulation of iron homeostasis mediated by the heme-binding protein Dap1 (damage resistance protein 1) via the P450 protein Erg11/Cyp51.” In: *Journal of biological chemistry* 282.50 (2007), pp. 36543–36551.

[220] Jacob Grünler, Johan Ericsson, and Gustav Dallner. “Branch-point reactions in the biosynthesis of cholesterol, dolichol, ubiquinone and prenylated proteins.” In: *Biochimica et Biophysica Acta (BBA)-Lipids and Lipid Metabolism* 1212.3 (1994), pp. 259–277.

[221] Joseph J Pinzone et al. “Molecular and cellular determinants of estrogen receptor α expression.” In: *Molecular and cellular biology* 24.11 (2004), pp. 4605–4612.

[222] Gerald E Stoica et al. "Effect of estradiol on estrogen receptor-α gene expression and activity can be modulated by the ErbB2/PI 3-K/Akt pathway." In: *Oncogene* 22.39 (2003), pp. 7998–8011.

[223] Ann Marie Egloff et al. "Cross-talk between estrogen receptor and epidermal growth factor receptor in head and neck squamous cell carcinoma." In: *Clinical Cancer Research* 15.21 (2009), pp. 6529–6540.

[224] Joseph Aizen and Peter Thomas. "Role of Pgrmc1 in estrogen maintenance of meiotic arrest in zebrafish oocytes through Gper/Egfr." In: *J Endocrinol* 225.1 (2015), pp. 59–68.

[225] Diego A Pedroza et al. "Progesterone receptor membrane component 1 promotes the growth of breast cancers by altering the phosphoproteome and augmenting EGFR/PI3K/AKT signalling." In: *British journal of cancer* 123.8 (2020), pp. 1326–1335.

[226] Isabel Pinhel et al. "ER and HER2 expression are positively correlated in HER2 non-overexpressing breast cancer." In: *Breast Cancer Research* 14.2 (2012), pp. 1–12.

[227] Abeer Alqaisi et al. "Impact of estrogen receptor (ER) and human epidermal growth factor receptor-2 (HER2) co-expression on breast cancer disease characteristics: implications for tumor biology and research." In: *Breast cancer research and treatment* 148.2 (2014), pp. 437–444.

[228] Panagiotis Bouris et al. "Estrogen receptor alpha mediates epithelial to mesenchymal transition, expression of specific matrix effectors and functional properties of breast cancer cells." In: *Matrix Biology* 43 (2015), pp. 42–60.

[229] Lesley-Ann Martin et al. "Enhanced estrogen receptor (ER) α, ERBB2, and MAPK signal transduction pathways operate during the adaptation of MCF-7 cells to long term estrogen deprivation." In: *Journal of Biological Chemistry* 278.33 (2003), pp. 30458–30468.

[230] Karianne E Longva et al. "Ubiquitination and proteasomal activity is required for transport of the EGF receptor to inner membranes of multivesicular bodies." In: *The Journal of cell biology* 156.5 (2002), pp. 843–854.

[231] Maria S Melikova, Kirill A Kondratov, and Elena S Kornilova. "Two different stages of epidermal growth factor (EGF) receptor endocytosis are sensitive to free ubiquitin depletion produced by proteasome inhibitor MG132." In: *Cell biology international* 30.1 (2006), pp. 31–43.

[232] Yingxue Bai et al. “PGRMC1 Promotes Progestin-Dependent Proliferation of Breast Cancer Cells by Binding Prohibitins Resulting in Activation of ERα Signaling.” In: *Cancers* 13.22 (2021), p. 5635.

[233] Krishnaraj Rajalingam et al. “Prohibitin is required for Ras-induced Raf–MEK–ERK activation and epithelial cell migration.” In: *Nature cell biology* 7.8 (2005), pp. 837–843.

[234] Jie Yang, Bin Li, and Qing-Yu He. “Significance of prohibitin domain family in tumorigenesis and its implication in cancer diagnosis and treatment.” In: *Cell death & disease* 9.6 (2018), pp. 1–10.

[235] Thomas KM Nyholm, Matts Nylund, and J Peter Slotte. “A calorimetric study of binary mixtures of dihydrosphingomyelin and sterols, sphingomyelin, or phosphatidylcholine.” In: *Biophysical journal* 84.5 (2003), pp. 3138–3146.

[236] Sari Lusa, Sanna Heino, and Elina Ikonen. “Differential mobilization of newly synthesized cholesterol and biosynthetic sterol precursors from cells.” In: *Journal of Biological Chemistry* 278.22 (2003), pp. 19844–19851.

[237] Megha Jiwei Wang and Erwin London. “Relationship between sterol/steroid structure and participation in ordered lipid domains (lipid rafts): implications for lipid raft structure and function.” In: *Biochemistry* 43.4 (2004), pp. 1010–1018.

[238] Omar Bakht, Erwin London, et al. “Cholesterol precursors stabilize ordinary and ceramide-rich ordered lipid domains (lipid rafts) to different degrees: implications for the Bloch hypothesis and sterol biosynthesis disorders.” In: *Journal of Biological Chemistry* 281.31 (2006), pp. 21903–21913.

[239] Chieko Mineo, Gordon N Gill, and Richard GW Anderson. “Regulated migration of epidermal growth factor receptor from caveolae.” In: *Journal of Biological Chemistry* 274.43 (1999), pp. 30636–30643.

[240] Xu Chen and Marilyn D Resh. “Cholesterol depletion from the plasma membrane triggers ligand-independent activation of the epidermal growth factor receptor.” In: *Journal of Biological Chemistry* 277.51 (2002), pp. 49631–49637.

[241] Kirstine Roepstorff et al. “Sequestration of epidermal growth factor receptors in non-caveolar lipid rafts inhibits ligand binding.” In: *Journal of Biological Chemistry* 277.21 (2002), pp. 18954–18960.

[242] Linda J Pike and Laurieann Casey. “Cholesterol levels modulate EGF receptor-mediated signaling by altering receptor function and trafficking.” In: *Biochemistry* 41.32 (2002), pp. 10315–10322.

[243] Emily J Westover et al. “Cholesterol depletion results in site-specific increases in epidermal growth factor receptor phosphorylation due to membrane level effects: studies with cholesterol enantiomers.” In: *Journal of Biological Chemistry* 278.51 (2003), pp. 51125–51133.

[244] Galya Orr et al. “Cholesterol dictates the freedom of EGF receptors and HER2 in the plane of the membrane.” In: *Biophysical journal* 89.2 (2005), pp. 1362–1373.

[245] Takemitsu Furuchi and Richard GW Anderson. “Cholesterol depletion of caveolae causes hyperactivation of extracellular signal-related kinase (ERK).” In: *Journal of Biological Chemistry* 273.33 (1998), pp. 21099–21104.

[246] Linda J Pike and Joanne M Miller. “Cholesterol depletion delocalizes phosphatidylinositol bisphosphate and inhibits hormone-stimulated phosphatidylinositol turnover.” In: *Journal of Biological Chemistry* 273.35 (1998), pp. 22298–22304.

[247] TF McGuire, SJ Corey, and SM Sebti. “Lovastatin inhibits platelet-derived growth factor (PDGF) stimulation of phosphatidylinositol 3-kinase activity as well as association of p85 subunit to tyrosine-phosphorylated PDGF receptor.” In: *Journal of Biological Chemistry* 268.30 (1993), pp. 22227–22230.

[248] Tove Ringerike et al. “Cholesterol is important in control of EGF receptor kinase activity but EGF receptors are not concentrated in caveolae.” In: *Journal of cell science* 115.6 (2002), pp. 1331–1340.

[249] Linda J Pike. “Lipid rafts: bringing order to chaos.” In: *Journal of lipid research* 44.4 (2003), pp. 655–667.

[250] Soazig Le Lay et al. “Cholesterol, a Cell Size-dependent Signal That Regulates Glucose Metabolism and Gene Expression in Adipocytes* 210.” In: *Journal of Biological Chemistry* 276.20 (2001), pp. 16904–16910.

[251] Johanna Gustavsson et al. “Localization of the insulin receptor in caveolae of adipocyte plasma membrane.” In: *The FASEB Journal* 13.14 (1999), pp. 1961–1971.

[252] Peter Nagy et al. “Lipid rafts and the local density of ErbB proteins influence the biological role of homo-and heteroassociations of ErbB2.” In: *Journal of cell science* 115.22 (2002), pp. 4251–4262.

[253] Jaekwang Jeong et al. “MAL2 mediates the formation of stable HER2 signaling complexes within lipid raft-rich membrane protrusions in breast cancer cells.” In: *Cell reports* 37.13 (2021), p. 110160.

[254] Diana C Márquez et al. "Estrogen receptors in membrane lipid rafts and signal transduction in breast cancer." In: *Molecular and cellular endocrinology* 246.1-2 (2006), pp. 91–100.

[255] Raquel Marin et al. "Modulation of Aβ-induced neurotoxicity by estrogen receptor alpha and other associated proteins in lipid rafts." In: *steroids* 73.9-10 (2008), pp. 992–996.

[256] Filippo Acconcia and Maria Marino. "The effects of 17b-estradiol in cancer are mediated by estrogen receptor signaling at the plasma membrane." In: *Frontiers in PHYSIOLOGY* 2 (2011), p. 30.

[257] Yong Shi et al. "Simultaneous detection of CA15-3 and PGRMC1 on a microfluidic chip for early diagnosis of breast cancer." In: *Journal of Liquid Chromatography & Related Technologies* (2021), pp. 1–10.

[258] Ting Cao et al. "Clozapine Induced Disturbances in Hepatic Glucose Metabolism: The Potential Role of PGRMC1 Signaling." In: *Frontiers in endocrinology* 12 (2021).

[259] Ying Lin et al. "Progesterone receptor membrane component 1 leads to erlotinib resistance, initiating crosstalk of Wnt/β-catenin and NF-κB pathways, in lung adenocarcinoma cells." In: *Scientific reports* 10.1 (2020), pp. 1–10.

[260] Yasuaki Kabe et al. "Glycyrrhizin Derivatives Suppress Cancer Chemoresistance by Inhibiting Progesterone Receptor Membrane Component 1." In: *Cancers* 13.13 (2021), p. 3265.

[261] Marina Willibald. "Role of PGRMC1 in breast cancer progression and increased breast cancer risk upon progestin-based hormone therapy." In: (2017). URL: `https://docserv.uni-duesseldorf.de/servlets/DerivateServlet/Derivate-48668/Marina_Willibald.pdf`.

[262] Cornelis JH Van de Velde et al. "Adjuvant tamoxifen and exemestane in early breast cancer (TEAM): a randomised phase 3 trial." In: *The Lancet* 377.9762 (2011), pp. 321–331.

[263] Bernard Fisher et al. "Tamoxifen for prevention of breast cancer: report of the National Surgical Adjuvant Breast and Bowel Project P-1 Study." In: *JNCI: Journal of the National Cancer Institute* 90.18 (1998), pp. 1371–1388.

[264] JF Forbes et al. "Arimidex, Tamoxifen, Alone or in Combination (ATAC) Trialists' Group. Effect of anastrozole and tamoxifen as adjuvant treatment for early-stage breast cancer: 100-month analysis of the ATAC trial." In: *Lancet Oncol* 9.1 (2008), pp. 45–53.

[265] Grazia Arpino et al. “HER-2 amplification, HER-1 expression, and tamoxifen response in estrogen receptor-positive metastatic breast cancer: a southwest oncology group study.” In: *Clinical cancer research* 10.17 (2004), pp. 5670–5676.

[266] Lesley-Ann Martin et al. “Elevated ERK1/ERK2/estrogen receptor cross-talk enhances estrogen-mediated signaling during long-term estrogen deprivation.” In: *Endocrine Related Cancer* 12 (2005), S75.

[267] Michele De Laurentiis et al. “A meta-analysis on the interaction between HER-2 expression and response to endocrine treatment in advanced breast cancer.” In: *Clinical cancer research* 11.13 (2005), pp. 4741–4748.

[268] Nikiana Simigdala et al. “Cholesterol biosynthesis pathway as a novel mechanism of resistance to estrogen deprivation in estrogen receptor-positive breast cancer.” In: *Breast Cancer Research* 18.1 (2016), pp. 1–14.

[269] Kelly M Swanson and Raymond J Hohl. “Anti-cancer therapy: targeting the mevalonate pathway.” In: *Current cancer drug targets* 6.1 (2006), pp. 15–37.

[270] Thomas P Ahern et al. “Statin prescriptions and breast cancer recurrence risk: a Danish nationwide prospective cohort study.” In: *Journal of the National Cancer Institute* 103.19 (2011), pp. 1461–1468.

[271] Teemu J Murtola et al. “Statin use and breast cancer survival: a nationwide cohort study from Finland.” In: *PloS one* 9.10 (2014), e110231.

[272] Chris R Cardwell et al. “Statin use after colorectal cancer diagnosis and survival: a population-based cohort study.” In: *J Clin Oncol* 32.28 (2014), pp. 3177–3183.

[273] Christophe Denoyelle et al. “Cerivastatin, an inhibitor of HMG-CoA reductase, inhibits the signaling pathways involved in the invasiveness and metastatic properties of highly invasive breast cancer cell lines: an in vitro study.” In: *Carcinogenesis* 22.8 (2001), pp. 1139–1148.

[274] Srigiridhar Kotamraju, Carol L Willams, and Balaraman Kalyanaraman. “Statin-induced breast cancer cell death: role of inducible nitric oxide and arginase-dependent pathways.” In: *Cancer research* 67.15 (2007), pp. 7386–7394.

[275] Claudia A Sánchez et al. “Statin-induced inhibition of MCF-7 breast cancer cell proliferation is related to cell cycle arrest and apoptotic and necrotic cell death mediated by an enhanced oxidative stress.” In: *Cancer investigation* 26.7 (2008), pp. 698–707.

[276] Yuanyuan Shen et al. "Synergistic effects of combined treatment with simvastatin and exemestane on MCF-7 human breast cancer cells." In: *Molecular Medicine Reports* 12.1 (2015), pp. 456–462.

[277] James K Liao et al. "Isoprenoids as mediators of the biological effects of statins." In: *The Journal of clinical investigation* 110.3 (2002), pp. 285–288.

[278] Thomas P Ahern et al. "Statins and breast cancer prognosis: evidence and opportunities." In: *The lancet oncology* 15.10 (2014), e461–e468.

[279] Javad Alizadeh et al. "Mevalonate cascade inhibition by simvastatin induces the intrinsic apoptosis pathway via depletion of isoprenoids in tumor cells." In: *Scientific reports* 7.1 (2017), pp. 1–14.

[280] Andy Göbel et al. "Induction of 3-hydroxy-3-methylglutaryl-CoA reductase mediates statin resistance in breast cancer cells." In: *Cell death & disease* 10.2 (2019), pp. 1–12.

[281] Michael J Campbell et al. "Breast cancer growth prevention by statins." In: *Cancer research* 66.17 (2006), pp. 8707–8714.

[282] Ji Hyeon You, Jaewang Lee, and Jong-Lyel Roh. "PGRMC1-dependent lipophagy promotes ferroptosis in paclitaxel-tolerant persister cancer cells." In: *Journal of Experimental & Clinical Cancer Research* 40.1 (2021), pp. 1–18.

[283] Seher Balaban et al. "Heterogeneity of fatty acid metabolism in breast cancer cells underlies differential sensitivity to palmitate-induced apoptosis." In: *Molecular oncology* 12.9 (2018), pp. 1623–1638.

[284] Hyun Joo Jang et al. "Simvastatin induces heme oxygenase-1 via NF-E2-related factor 2 (Nrf2) activation through ERK and PI3K/Akt pathway in colon cancer." In: *Oncotarget* 7.29 (2016), p. 46219.

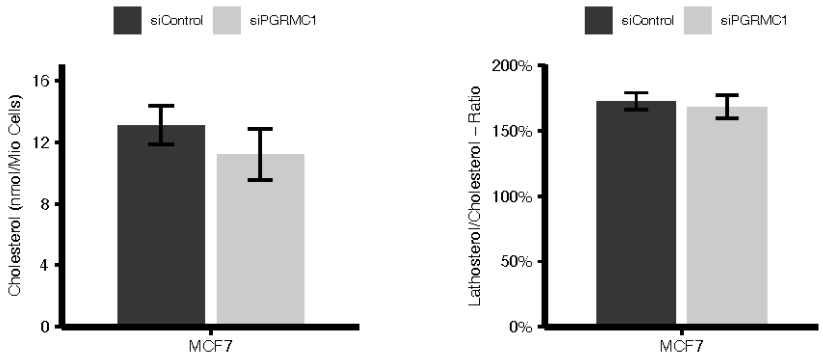
[285] Yu Zhang et al. "Simvastatin attenuates renal ischemia/reperfusion injury from oxidative stress via targeting Nrf2/HO-1 pathway." In: *Experimental and Therapeutic Medicine* 14.5 (2017), pp. 4460–4466.

[286] Aleksandra Piechota-Polanczyk et al. "Simvastatin treatment upregulates HO-1 in patients with abdominal aortic aneurysm but independently of Nrf2." In: *Oxidative Medicine and Cellular Longevity* 2018 (2018).

[287] Mary E Irwin et al. "Lipid raft localization of EGFR alters the response of cancer cells to the EGFR tyrosine kinase inhibitor gefitinib." In: *Journal of cellular physiology* 226.9 (2011), pp. 2316–2328.

6 Appendix

6.1 Supplements



(a) (b)

Figure 19: **Intracellular content of cholesterol and its precursor lathosterol** Quantification of intracellular cholesterol and its precursor lathosterol via mass spectrometry in MCF7/siControl and MCF7/siPGRMC1. Absolute levels of intracellular cholesterol and significant decrease of lathosterol to cholesterol ratio. *: $p \leq 0.05$, ***: $p \leq 0.001$

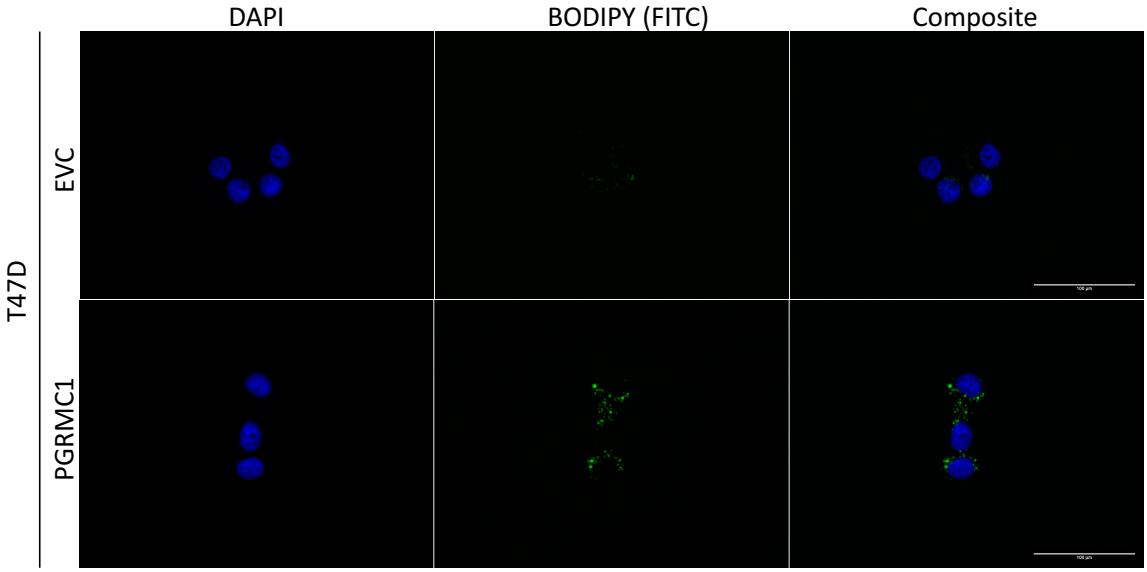


Figure 20: **IF staining of lipid droplets in T47D/EVC and T47D/PGRMC1** Staining of lipid droplets in T47D/EVC and T47D/PGRMC1 with BODIPY™ 493/503. Nuclear stain with DAPI. Magnification: 63 x.

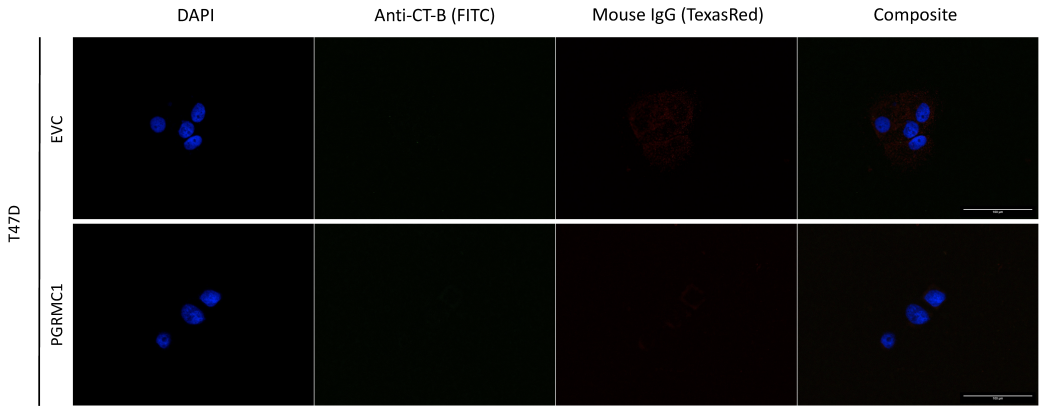
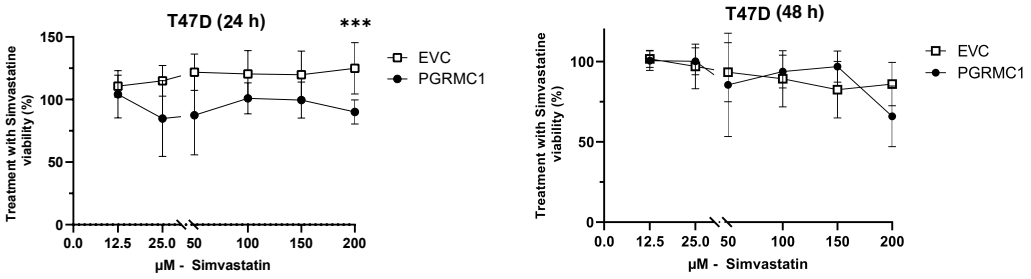


Figure 21: **Negative control for lipid raft and HER2 staining in T47D/EVC and T47D/PGRMC1 cells**

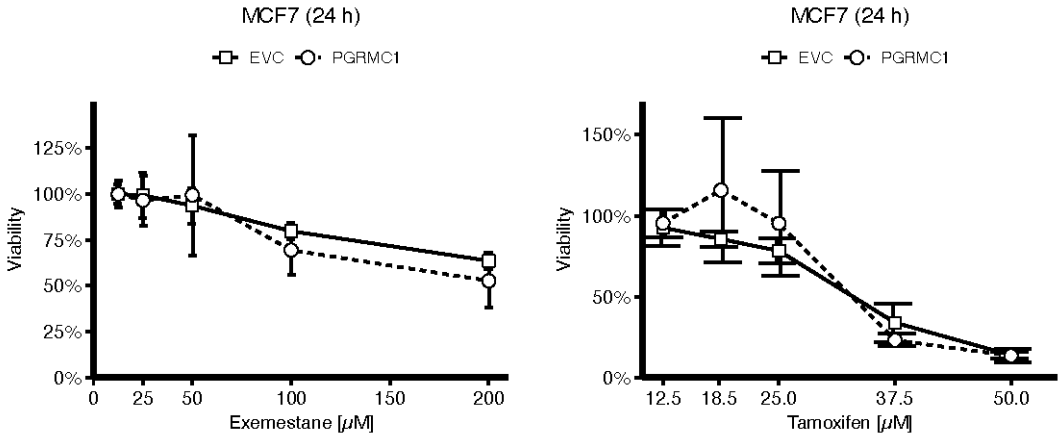
Negative control staining with rabbit isotype IgG (TexasRed) and Anti-CT-B only (FITC) in T47D/EVC and T47D/PGRMC1. Nuclear stain with DAPI. Magnification: 63 x.



(a) (b)

Figure 22: **Simvastatin treatment of T47D/EVC and T47D/PGRMC1**

T47D/EVC and T47D/PGRMC1 were treated with 100 µM, 50 µM, 25 µM, 12.5 µM, 6.25 µM and 3.175 µM simvastatin and respective DMSO control. Viability was analyzed by MTT assay at t = 24 h, t = 48 h, and t = 72 h and 37°C. Depicted are results after 24 h and 48 h of treatment. Viability was normalized on the DMSO control. P-Values were adjusted using Bonferroni correction (ndoses = 6; nreplicates = 9) ***: $p \leq 0.001$



(a) (b)

Figure 23: **Exemestane and Tamoxifen Treatment of MCF7/EVC and MCF7/PGRMC1**

MCF7/EVC and MCF7/PGRMC1 cells were treated with **(a)** 200 µM, 100 µM, 50 µM, 25 µM, 12.5 µM Exemestane and **(b)** 50 µM, 37.5 µM, 25 µM, 18.75 µM, 12.5 µM Tamoxifen. Viability was analyzed by MTT assay at t = 24 h, t = 48 h, and t = 72 h and 37°C. Viability values were normalized to the respective DMSO control. Depicted are results after 24 h of treatment. P-Values were adjusted using Bonferroni correction (ndoses = 5; nreplicates = 9) *: $p \leq 0.05$, **: $p \leq 0.01$, ***: $p \leq 0.001$

6.2 List of Figures

Danksagung

An dieser Stelle möchte ich allen beteiligten Personen danken, die mich bei der Durchführung und Erstellung meiner Dissertation unterstützt haben.

Zunächst möchte ich mich bei meinem Doktorvater Prof. Dr. Hans Neubauer für die Überlassung des Themas dieser Dissertation, die gute Betreuung dieser Arbeit, seinen fachlichen Rat und die persönliche Unterstützung bedanken. Insbesondere bedanke ich mich auch für das große mir entgegengebrachte Vertrauen und die Chancen, die mir im Labor ermöglicht wurden.

Des Weiteren gilt mein Dank Herrn Prof. Dr. Björn Stork für die freundliche Übernahme der Zweitkorrektur meiner Arbeit.

Bei Marina Ludescher möchte ich mich bedanken für die Einarbeitung und Unterstützung im Labor.

Dem gesamten Laborteam danke ich für die kleineren und größeren Hilfen im Laboralltag und für die angenehme Arbeitsatmosphäre.

Ein besonderer Dank geht an Jan Cieslik für seine ausdauernde Unterstützung und Gesellschaft im Labor.

Mein tiefer Dank gilt zudem Lukas Kowejsza, der mir bei allen Entscheidungen und Hürden zur Seite steht.

Zuletzt möchte ich mich bei meinen Eltern und Geschwistern für ihre Geduld, Ermutigungen und Zusprüche während dieser Doktorarbeit und ihre Unterstützung in allen Phasen meines Lebens bedanken.