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The envelope protein of the human endogenous retrovirus type W (HERV-W ENV) differentially modulates the glioblastoma microenvironment

Dissertation

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

vorgelegt von
Benedikt Oliver Plaack
2025

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

gez.:
Dekan: Univ.- Prof. Dr. med. Nikolaj Klöcker
Erstgutachter: PD Dr. med. David Kremer
Zweitgutachter: Univ.- Prof. Dr. med. Ulrich Germing

Parts of this work have been published:

L. Reiche, B. Plaack, M. Lehmkuhl, V. Weyers, J. Gruchot, D. Picard, H. Perron, M. Remke, C. Knobbe-Thomsen, G. Reifenberger, P. Küry and D. Kremer (2024). "HERV-W envelope protein is present in microglial cells of the human glioma tumor microenvironment and differentially modulates neoplastic cell behavior." Microbes and Infection: 105460.

Zusammenfassung

Das Glioblastom (GBM) ist der aggressivste primäre Hirntumor mit einer medianen Überlebenszeit von 14-17 Monaten (Reifenberger et al. 2017). Die schlechte Prognose, verbunden mit der raschen Verschlechterung neurologischer Funktionen und der Lebensqualität, hat zu enormen Forschungsanstrengungen geführt, um die dem GBM zugrunde liegenden Mechanismen zu erforschen und neue therapeutische Strategien zu entwickeln. Ein neuer Forschungsbereich in diesem Gebiet betrifft die humanen endogenen Retroviren (HERVs), eine Klasse pathogener Elemente, die bis zu 8% des menschlichen Genoms ausmachen. Diese Retroviren gingen ursprünglich vor Millionen von Jahren aus Keimbahninfektionen von Säugetieren hervor und sind in der Regel epigenetisch inaktiviert. Umweltfaktoren wie Virusinfektionen können HERVs jedoch reaktivieren, was zur Produktion von viralen Proteinen, insbesondere der HERV-W- und HERV-K-Familien, führt. Das Hüllprotein des humanen endogenen Retrovirus vom Typ-W (HERV-W ENV) beeinträchtigt bei der Multiplen Sklerose (MS) die Reparatur von Läsionen, indem es Mikroglia (MG), Zellen des angeborenen Immunsystems im ZNS, aktiviert. Nun gelang es jedoch unserer Arbeitsgruppe in ihren Vorarbeiten HERV-W ENV auch in malignem menschlichen Gliomgewebe nachzuweisen (Reiche et al. 2024). Hierbei ist die Präsenz von HERV-W ENV jedoch nicht nur auf Gliomzellen beschränkt, sondern findet sich auch in den Zellen der sogenannten Tumor-Mikroumgebung (*tumor microenvironment*; TME), wie beispielsweise den vorgenannten Mikrogliazellen. Diese Dissertation untersucht die genauen Auswirkungen von HERV-W ENV auf das Verhalten von Glioblastom- und Mikrogliazellen. Die Ergebnisse deuten darauf hin, dass HERV-W ENV eine proinflammatorische Genexpression in Tumor- und Mikrogliazellen induziert, was einen aggressiveren Phänotyp fördert. Die daraus resultierende Modulation funktioneller Parameter neoplastischer Zellen wie Proliferation, Migration und Invasion hängt dabei maßgeblich von der Interaktion mit Mikroglia ab. Zudem könnte HERV-W ENV die Sensitivität gegenüber Temozolomid, dem in der klinischen Therapie standardmäßig eingesetzten Chemotherapeutikum, erhöhen. Zusammenfassend untermauert diese Dissertation, dass HERV-W ENV ein vielversprechendes Ziel für künftige therapeutische Ansätze in der Glioblastomtherapie sein könnte.

Abstract

Glioblastoma (GBM) is the most aggressive primary brain tumor with a median survival time of 14-17 months (Reifenberger et al. 2017). This dismal prognosis and the rapid decline in neurological function and quality of life have driven extensive research efforts to clarify the molecular mechanisms underlying GBM pathology and to identify new therapeutic strategies. Among emerging areas of interest are human endogenous retroviruses (HERVs), a class of pathogenic elements that account for up to 8% of the human genome. These retroviruses originated from mammalian germline infections millions of years ago and are typically epigenetically silenced. However, environmental factors such as viral infections can reactivate HERVs, leading to the production of viral proteins, particularly of the HERV-W and HERV-K families. In multiple sclerosis (MS), the most prevalent chronic inflammatory and demyelinating autoimmune disease of the human central nervous system (CNS), the human endogenous retrovirus type-W envelope protein (HERV-W ENV) interferes with lesion repair by activating microglia (MG), the innate immune cells of the CNS. Recently, clinical trials investigated the use of temelimab, a monoclonal antibody neutralizing HERV-W ENV, supporting the potential use of this agent for patients with progressive MS by reducing neurodegenerative effects. The previous studies of our research group found HERV-W ENV both in tumor cells and infiltrating microglia of human glioma tissue (Reiche et al. 2024). However, HERV-W ENV expression is not limited to glioblastoma but is scattered throughout gliomas of all WHO grades. The experiments presented in this dissertation investigated the effects of HERV-W ENV on neoplastic glioma and microglial cell behavior. The results suggest that HERV-W ENV stimulation induces a proinflammatory gene expression shift in both tumor and microglial cells, promoting a more aggressive cell phenotype. In this context, the crucial finding of this study is that HERV-W ENV-mediated modulation of functional neoplastic cell parameters, such as proliferation, migration, and invasion, depends on the presence of microglia. In addition, it was found that HERV-W ENV stimulation may increase the sensitivity of neoplastic cells to temozolomide, the standard chemotherapeutic agent used in clinical glioblastoma therapy. In summary, this dissertation suggests that HERV-W ENV could be a promising target for future therapeutic approaches in glioblastoma therapy.

Abbreviations

°C Degrees celsius
5-ALA 5-aminolevulinic acid
ANG Angiopoetin
ATCC American Type Culture Collection
ATRX Alpha thalassemia/mental retardation syndrome X-linked
BAX Bcl-2-associated X protein
BMDM Bone marrow-derived macrophage
BSA Bovine serum albumin
CC3 Caspase-3
CD Cluster of differentiation
CDH1 Cadherin-1
CDKN1A Cyclin dependent kinase inhibitor 1A
CDKN2A/B Cyclin-dependent kinase inhibitor 2A/B
cDNA Complementary deoxyribonucleic acid
CNS Central nervous system
COVID-19 Coronavirus disease 2019
CSF Cerebrospinal fluid
CSF-1 Colony-stimulating factor 1
CTC Circulating tumor cell
CX3CL C-X3-C motif chemokine ligand
DMEM Dulbecco's Modified Eagle's Medium
DMF Dimethylformamide
DNA Deoxyribonucleic acid
EBV Epstein-Barr-Virus
ECM Extracellular matrix
EGFR Epidermal growth factor receptor
EMT Epithelial-to-mesenchymal transition
ENA Epithelial-derived neutrophil-activating protein
FBS Fetal bovine serum
FET O-(2-[18F] fluoroethyl)-L-tyrosine
FGF Fibroblast Growth Factor
gag Group-specific antigen gene
GBM Glioblastoma
GSC Glioblastoma stem cell
GTR Gross total resection
HERV Human endogenous retrovirus
HERV-W ENV Human endogenous retrovirus type-W envelope protein
HIV-1 Human immunodeficiency virus 1
HTLV-1 Human t-cell lymphotropic virus type 1
I-309 Inflammatory Cytokine I-309
IDH Isocitrate dehydrogenase
IL Interleukin
iNOS Inducible nitric oxide synthase
ITGA Integrin alpha
ITGB Integrin beta
KPS Karnofsky performance score
LAL Limulus amebocyte lysate
Lcn-2 Lipocalin-2
LGG Low grade gliomas
LPS Lipopolysaccharide
LTR Long terminal repeat
MACS Magnetic activated cell sorting

MDC Macrophage-derived chemokine

merTK MER Proto-Oncogene, Tyrosine Kinase

MG Microglia

MGMT O6-methylguanine-DNA methyltransferase

MHC Major histocompatibility complex class

MIG Monokine induced by Gamma-Interferon

MIP Macrophage Inflammatory Protein

MMP Matrix metalloproteinase

MMR Mismatch repair

MRI Magnetic resonance imaging

MSRV Multiple sclerosis-associated retrovirus

MTT 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide

ncRNA Noncoding ribonucleic acids

NDS Normal donkey serum

NF-kB Nuclear factor kappa light chain enhancer of activated B-cells

NGS Normal goat serum

NO Nitric oxide

NSC Neural stem cell

OPC Oligodendrocyte precursor cell

ORF Open reading frame

PAMP Pathogen-associated molecular pattern

PBMC Peripheral blood mononuclear cell

PBS Phosphate-buffered saline

PDGF Platelet-derived growth factor

PET Positron emission tomography

pol Polymerase gene

POSTN Periostin

pro Protease gene

PRR Pattern-recognition receptor

PsP Pseudoprogression

PWI Perfusion-weighted imaging

qRT-PCR Quantitative reverse transcription-polymerase chain reaction

RANTES Regulated on activation, normal T-cell expressed and secreted

RIPK Receptor-interacting serine/threonine-protein kinase

RNA Ribonucleic acid

RTK Receptor tyrosine kinase

S100a10 S100 calcium-binding protein A10

S1PR Sphingosine-1-phosphate receptor, Sphingosine-1-phosphate receptor

SARS-CoV-2 Severe acute respiratory syndrome coronavirus 2

SCF Stem cell factor

SDF-1 Stromal cell-derived factor 1

SDS Sodium dodecyl sulfate

SU Subunit

T1D Type-1 diabetes

TAM Tumor-associated macrophage

TARC Thymus- and activation-regulated chemokine

TE Transposable elements

TGF Transforming Growth Factor

Th1 T helper cell type 1

TLR4 Toll-like receptor 4

TME Tumor microenvironment

TMZ Temozolomide

TNFa Tumor necrosis factor alpha

TREM2 Triggering receptor expressed on myeloid cells 2

tRNA Transfer ribonucleic acid

TTFields Tumor therapy fields

VEGF Vascular Endothelial Growth Factor

WHO World Health Organization

ΔΔCt Comparative cycle threshold

Table of Content

1. Introduction

1.1. Tumors of the central nervous system

In general, tumors can be divided into two categories: benign and malignant. Benign tumors are defined as a noncancerous abnormal growth of cells that grow slowly and are less likely to recur after treatment (Fritz 2000). In contrast, malignant tumors have the ability to invade tissues locally and spread to different parts of the body (Fritz 2000). In the central nervous system (CNS), most tumors are metastases from other malignant tumors such as lung cancer, breast cancer, and melanoma (Barnholtz-Sloan et al. 2004; Berghoff et al. 2016; Nayak et al. 2012). Primary brain tumors, on the other hand, arise from the brain's own or adjacent tissue. In adults, primary brain tumors account for 2% of all cancers, of which the most common benign CNS tumor is meningioma, and the most common malignant tumor is glioma (Ostrom et al. 2017). This results in an overall incidence of primary brain tumors of 22 per 100.000 (Lapointe et al. 2018).

As primary brain tumors encompass a heterogeneous group of tumors of different cellular origins, all brain and spinal cord tumors are systemically classified by the World Health Organization (WHO) Classification of Tumors of the Central Nervous System 2021 (Louis et al. 2021). Recently, as molecular and genetic tumor characteristics gained increasing importance, they replaced the previously used histological criteria in the WHO Classification of 2021. The resulting revised terminology and grading led to new tumor types and subtypes. In this context, novel diagnostic technologies such as DNA methylome profiling facilitate a precise description of each tumor tissue in addition to the histological and immunohistochemical classification based on features like mitotic rate, the presence of tumor necrosis, and pathological vascular proliferation. This enables a more objective classification since the histological classification of CNS tumors suffered from considerable inter-observer variation (Capper et al. 2018; Louis 2012). Of note, due to the above-mentioned changes in the WHO Classification of 2021, the epidemiological data presented in this thesis refer to the previous classifications, as more recent, reliable data have not yet been published. The most recent WHO Classification stratifies CNS tumors from grades 1 to 4, with WHO Grade 1 indicating the least malignant type (Louis

et al. 2021). Grade 1 tumors are benign, mostly curable, and commonly found in children, whereas grade 2 tumors are still slow-growing but may show invasive growth. Grade 1 and 2 gliomas are considered low-grade gliomas (LGG) that eventually progress to high-grade tumors. On the other hand, high-grade tumors of grade 3 are usually malignant, more aggressive, and infiltrating, while grade 4 tumors represent the most aggressive and malignant tumors (Chatterjee et al. 2022). The group of gliomas, glioneuronal and neuronal tumors is further subdivided into six families: adult-type diffuse gliomas, pediatric-type diffuse low-grade gliomas, pediatric-type diffuse high-grade gliomas, circumscribed astrocytic gliomas, glioneuronal and neuronal tumors, and ependymomas (Louis et al. 2021; Park et al. 2023). Therefore, the term glioma does not imply the cellular origin of the respective tumor but rather refers to a cytological similarity of the tumor cells compared to different glial cell types (Zong et al. 2012).

1.2. Glioblastoma

1.2.1. Epidemiology and etiology

Gliomas are the most common malign primary CNS tumors, accounting for 75% of malignant brain tumors (Ostrom et al. 2017). In total, glioblastoma (GBM), the most aggressive and lethal type of gliomas, accounts for 17.9% of all primary brain tumors in recent years and occurs more frequently in males than in females with a median age of 64 at diagnosis (Price et al. 2024). Most GBM are localized in the brain's frontal lobe, whereas the brain stem and cerebellum are the rarest locations (Ghosh et al. 2017). Initially, GBM was described to originate from astrocytic glial cells (Perry and Wesseling 2016), but recent research points to different cellular origins that affect tumor behavior (Yao et al. 2018). Gene profiling analysis revealed that GBM is derived from neural stem cells (NSCs), NSC-derived astrocytes, and oligodendrocyte precursor cells (OPCs), which carry tumor-initiating genetic alterations (Yao et al. 2018). GBM is one of the deadliest tumors in humans. Only about 5% of patients survive five years after the initial diagnosis (Jaoude et al. 2019). This poor survival is a result of the highly invasive behavior of tumor cells and their resistance to therapy (Seker-Polat et al. 2022). Compared to other malignancies, peripheral metastases of GBM are very rare (Armstrong et al. 2011), even though recent studies detected circulating tumor cells (CTCs) in the blood of glioma

patients (Zhang et al. 2021). Regarding the development of GBM, the exact underlying mechanisms are not yet well understood. Various studies have investigated different risk factors, but no identifiable risk factors have been found for the majority of patients (Lapointe et al. 2018; Yoshikawa et al. 2023). Of note, studies did not find any relation between smoking as a carcinogen and glioma risk. On the other hand, a history of allergies and atopic diseases was identified as a protective characteristic regarding the risk of developing glioma (Lachance et al. 2011). In addition, obesity at the age of 18 increases the risk of glioma nearly fourfold compared to normal weight (Moore et al. 2009). Additionally, in recent years, studies have also focused on ionizing radiation and the long-term usage of mobile phones as carcinogenic factors, showing an increased risk of developing malignant brain gliomas (Braganza et al. 2012; Yang et al. 2017). Additionally, in 5% of all cases, gliomas are associated with hereditary syndromes such as Neurofibromatosis type 1 and 2, Li-Fraumeni, or Von Hippel-Lindau syndrome (Blumenthal and Cannon-Albright 2008). In summary, most GBM risk factors remain unclear, and many associations are controversial (Grochans et al. 2022; Lapointe et al. 2018; Wrensch et al. 2002). Currently, the only definitive risk factor is advanced age, although the underlying biology of this phenomenon has not yet been clarified (Ostrom et al. 2021).

1.2.2. Clinical course and treatment

The urgent need for new treatment options for GBM is underlined by a median patient survival of only 12 months in population-based studies (Reifenberger et al. 2017). This low median survival results from its fast, progressive, and destructive growth. Initially, as the tumor tissue shows rapid, destructive growth, symptoms can range from headaches, limb paresis, and nausea to epileptic seizures (Wang et al. 2023a). In this regard, the clinical presentation of patients depends on the specific tumor location (Urbanska et al. 2014). In magnetic resonance imaging (MRI), most GBM present with a characteristic single ring-enhancing lesion with central necrosis and peritumoral edema (Smirniotopoulos et al. 2007). This lesion is hypointense on T1 weighted precontrast imaging and hyperintense on T2/FLAIR imaging sequences (Lundy et al. 2020). Due to neoangiogenesis and blood-brain-barrier (BBB) disruption, perfusion-weighted imaging (PWI) shows increased cerebral blood flow (Gilard et al. 2021). Additional imaging using

positron emission tomography (PET) in combination with amino acid tracers such as O-(2-[^{18}F] fluoroethyl)-L-tyrosine (FET) can even better distinguish between neoplastic and non-neoplastic lesions, with tracer uptake increasing with the degree of malignancy (Galldiks et al. 2022). Importantly, other differential diagnoses such as abscess, lymphoma, or tumor metastases feature similar MRI characteristics, ultimately often resulting in the need for histopathological confirmation to diagnose GBM. The ensuing analysis of formalin-fixed and paraffin-embedded tissue typically shows a diffuse astrocytic glioma with a high mitosis rate, pleomorphism, microvascular proliferation, and necrosis (Lan et al. 2024). Historically, since the last century, the diagnosis and classification of primary brain tumors have been primarily based on histopathology, which classifies tumors according to their microscopic abnormalities and their cells of origin. However, in 2016, the WHO updated its brain tumor classification by incorporating molecular features such as the IDH mutation status (Louis et al. 2016). The new WHO Classification of 2021 further emphasizes the molecular classification of brain tumors by incorporating even more molecular markers to enhance the objectivity of the diagnostic process (Louis et al. 2021). This precise classification of molecular features in GBM enables an accurate characterization of each patient's tissue, leading to more personalized treatment approaches (Tan et al. 2020; Verdugo et al. 2022). Generally, the current therapy paradigm for glioblastoma involves surgical tumor resection as a first step, which is then combined with an accurate histological examination for diagnosis. If possible, gross total resection (GTR) should be pursued as the extent of tumor resection has been positively correlated with survival time (Oszvald et al. 2012). Survival time after GTR is increased regardless of the molar characteristics of the tumor (Molinaro et al. 2020). However, as a general approach, preventing new permanent neurological deficits is prioritized over achieving GTR (Weller et al. 2021). Of note, surgical intervention faces many challenging problems, such as the microscopic infiltration of surrounding brain tissue in tumor margins, which complicates tumor resection (Tripathi et al. 2022). To reduce complications and further enhance patient survival, surgical tumor resection is supported by the application of 5-aminolevulinic acid (5-ALA), which accumulates in the tumor cells, causing a red fluorescence signal when exposed to blue light (Stummer et al. 2000; Stummer et al. 2006). Current clinical practice after surgical resection includes a postoperative MRI to verify the surgical outcome and to create a baseline for monitoring and detecting progression (Weller et al. 2021). After histological confirmation and precise

classification of the resected tissue, the standard treatment regimen includes pharmacological and radiation therapy. The most commonly used drug in glioma treatment is the alkylating agent temozolomide (TMZ). Although TMZ was first introduced for the treatment of melanoma, it is now mainly used in glioma therapy due to its oral bioavailability and ability to cross the BBB (Newlands et al. 1997; Thomas et al. 2013). Since the introduction of TMZ for glioma treatment in 2005, no other chemotherapeutic agent has been more effective in glioma therapy (Oraiopoulou et al. 2024). Its cytotoxic effect is based on the methylation of purine in the DNA (mainly O6-guanine). This toxic modification then leads to the activation of DNA mismatch repair (MMR) enzymes and, ultimately, apoptosis (Zhang et al. 2012). In this context, the O6-methylguanine-DNA methyltransferase (MGMT) can repair this DNA modification by transferring the O6-methyl group to its cysteine, which leads to the inactivation of this enzyme (Pegg et al. 1995). Therefore, the response to TMZ therapy is based on functional MMR enzymes and low levels of MGMT. Patients usually receive 75 mg/m^2 body surface of TMZ daily throughout radiotherapy for six weeks and six cycles of maintenance TMZ for five days every 28 days in a higher concentration (150–200 mg/m^2; (Stupp et al. 2005)). Regarding side effects, TMZ is considered to have a generally favorable safety profile even though it can interfere with hematopoiesis, leading to myelosuppression or thrombocytopenia (Gerber et al. 2007). Other side effects are less common and include non-hematologic toxicities such as nausea, anorexia, fatigue, and hepatotoxicity (Dixit et al. 2012). Radiation therapy, on the other hand, is usually conducted within 3-5 weeks of surgery and is commonly administered at 50-60 Gray in 1,8-2 Gray daily fractions (Cabrera et al. 2016; Press et al. 2020; Weller et al. 2021). In this context, further classification of the tumor and prognostic values influence the exact dosing and timing of the radiation and chemotherapeutical treatment (Aiyappa-Maudsley et al. 2022). In general, aggressive antitumor therapy and its side effects must be balanced with optimizing quality of life.

Regarding its disease course, glioblastoma recurrences are observed in almost all cases. Accordingly, current guidelines recommend close monitoring of patients with follow-up examinations every 2-3 months, including neurological examination and imaging (Wen et al. 2010). The treatment of recurrences remains an area of ongoing research, largely due to the lack of standards caused by, *inter alia*, pseudoprogression (PsP). PsP refers to new or progressive contrast-enhanced MRI signals, that are typically caused by treatment-induced inflammation, edema, or necrosis rather than true tumor growth (Young et al.

2023). However, even advanced imaging techniques have difficulty distinguishing between actual cancer progression and PsP (Radbruch et al. 2015; Wang et al. 2023b). Another critical factor is primary or secondary (i.e. acquired) tumor cell resistance to TMZ (Teraiya et al. 2023). In such cases, further treatment options are selected by an interdisciplinary tumor board according to the Karnofsky performance score (KPS), neurological function, and previous therapy (Weller et al. 2021). Since recently, GBM therapy also includes the use of tumor therapy fields (TTFields), leading to prolonged progression-free and overall survival (Guo et al. 2022). TTFields use weak electromagnetic alternating fields in the long-wave range directed at the resected tumor area, disrupting the mitosis of residual tumor cells, which induces cancer cell autophagy (Glas et al. 2022; Taphoorn et al. 2018). Several ongoing clinical trials further are currently evaluating the safety and efficiency of TTFields in GBM and the combination with other therapy options to improve patient survival. In summary, glioblastoma is a difficult-to-treat tumor with a comparatively short survival time. As a result, there is a high demand for new therapeutic strategies aiming, for instance, at molecular tumor biology and the interaction between neoplastic cells and the immune system.

1.2.3. Molecular Pathology

As mentioned above, the characterization of brain tumors primarily involves histological tissue evaluation, which is the basis for further classification and grading. Diffuse gliomas are usually described by molecular markers such as IDH1, H3, and ATRX, whose expression is investigated via immunohistochemical staining followed by additional molecular analyses of the tumor tissue (van der Meulen et al. 2022). As molecular markers have become well-established in the diagnostic process in recent years, the following section provides an overview of the most important markers. As mentioned above, a detailed molecular description and distinction of glioblastoma was first included in the WHO classification of CNS tumors in 2016, classifying glioblastoma by their isocitrate dehydrogenase 1/2 (IDH) mutation status (Louis et al. 2016). In the past, IDH mutations were associated with better disease outcomes and prolonged survival in GBM with 15 months in the IDH-wild type and 31 months in the IDH-mutant type, respectively (Yan et al. 2009). IDH-wild type GBM was clinically defined as primary GBM appearing *de novo* while IDH-mutant GBM was termed secondary GBM, resulting from progression from

lower-grade gliomas (Lan et al. 2024). However, since the above-mentioned changes in the latest WHO classification of CNS tumors, only adult-type diffuse gliomas with an IDH-wild type status are referred to as glioblastoma. In contrast, IDH-mutant astrocytic gliomas are now referred to as astrocytoma, IDH-mutant, and can be further subclassified into different CNS-WHO grades depending on histological features (Louis et al. 2021). Additionally, due to its prognostic role, a recent phase 3 study investigated using vorasidenib, an oral dual inhibitor of the mutant IDH1 and IDH2 enzymes, in patients with grade 2 IDH-mutant glioma. Results were promising and showed that vorasidenib significantly improved progression-free survival with only a low rate of serious adverse events (Mellinghoff et al. 2023). Other important glioma markers include alpha thalassemia/mental retardation syndrome X-linked (ATRX) gene (Suzuki et al. 2015) , the tumor suppressor gene TP53 (Noor et al. 2021), the cyclin-dependent kinase inhibitor 2A/B (CDKN2A/B; (Huang 2022)), the growth factor receptor (EGFR; (Huang et al. 2009; Rodriguez et al. 2023)) and the methylation status of the DNA repair enzyme O^6-methylguanine-DNA-methyltransferase (MGMT; (Reifenberger et al. 2017; Weller et al. 2010)) which are crucial factors for prognosis and therapy response.

1.3. The tumor microenvironment of glioblastoma

The tumor microenvironment (TME) of glioblastoma is an essential component of the tumor tissue, which consists of both cancerous and non-cancerous cells (Quail and Joyce 2013). As a result, the past years have seen an increasing focus on molecular targets in the TME, which is a highly immuno-suppressive environment facilitating and promoting cancer progression (Pombo Antunes et al. 2020). The primary cell population in the TME are tumor-associated macrophages (TAMs) that account for 30-50% of cells (Graeber et al. 2002; Hambardzumyan et al. 2016). TAMs can originate from the yolk sac as brain-resident microglia or from the bone marrow as monocytes that, conditioned by proinflammatory cytokines or growth factors, traffic into tissue during inflammation (Bowman et al. 2016; Chen and Hambardzumyan 2018; Shi and Pamer 2011). Several cell surface markers can distinguish between brain macrophage populations, such as TMEM119 for microglia or ITGA4 for bone marrow-derived macrophages (BMDMs), respectively (Bowman et al. 2016). Since BDMDs do not occur in the healthy brain and the two cell types differ not only in their surface markers but also in their properties and

behavior, it can be assumed that they contribute differentially to the progression of tumors (Hambardzumyan et al. 2016). In low-grade glioma, the number of TAMs correlates positively with tumor malignancy (Komohara et al. 2008) and negatively with the recurrence-free survival of patients with pilocytic astrocytoma (Dorward et al. 2010). In general, in high-grade glioma, immune cells are characterized by an immense diversity, but TAMs are generally associated with tumor aggressiveness and shorter survival (Sørensen et al. 2018). Additionally, TAMs play an important role in glioblastoma invasiveness (Darmanis et al. 2017) and therapy resistance (Osuka and Van Meir 2017). On the cellular level, the still widely used dichotomic classification of microglia into M1 tumor-suppressive ("pro-inflammatory") and M2 tumor-supportive ("anti-inflammatory") microglia is today considered to be somewhat oversimplified (Paolicelli et al. 2022). In this regard, recent studies using single-cell RNA sequencing suggested various TAM subpopulations (Hambardzumyan et al. 2016; Khan et al. 2023) characterized by different transcriptomes depending on tumor type and IDH mutation status, highlighting the complexity of the microenvironmental landscape (Klemm et al. 2020). In this context, the crosstalk between neoplastic tumor cells and TAMs induces TAM recruitment and polarization toward a tumor-supportive phenotype, facilitating malignant progression (Andersen et al. 2021). To this end, glioblastoma cells can secrete various cytokines, including chemo-attractants (CX3CL1, CCL2, MIC-1, and CSF-1) and interleukins (IL-6, IL-33; (Andersen et al. 2021)). The polarization of TAMs towards a tumor-supportive (M2) phenotype mainly leads to the secretion of cytokines and growth factors, which promote critical biological functions in tumor progression (Hambardzumyan et al. 2016). In addition, via the secretion of TGF-ß, TAMs induce MMP-9 expression of glioblastoma stem cells (GSCs), leading to GSC invasiveness (Ye et al. 2012). GSCs are considered a subpopulation of neoplastic cells in GBM that support tumor development, recurrence, and therapeutic resistance (Gimple et al. 2019; Prager et al. 2020). Because of their ability to drive tumor growth and treatment resistance, they are a significant focus of cancer research (Chu et al. 2024). Another critical characteristic of GBM tissue is extensive hypoxia, which is based on its rapid growth and progression (Monteiro et al. 2017). Overall, hypoxia is consistently associated with poor prognosis in multiple cancer types (Jing et al. 2019; Walsh et al. 2014) by facilitating an immunosuppressive microenvironment (Lin et al. 2020). This immunosuppressive environment is characterized by the presence of numerous cell types, such as regulatory T cells, as well

as high levels of immunosuppressive cytokines (TGF-ß, IL-10, VEGFa, and ARG1) that hinder the activity of other immune effector cells (Terry et al. 2017). Another relevant cell population of the TME are lymphoid cells that mainly consist of $CD4^+$ T helper (Th), $CD8^+$ T cytotoxic (Tc), and regulatory T cells (Treg), accounting for ca. 2% of the tumor (Martinez-Lage et al. 2019). Glioblastoma cells are known to secrete several cytokines, such as TGF-ß and IL-10, which suppress the T-cell response (Gieryng et al. 2017). In this context, glioblastomas are considered to be immunologically “cold”, which refers to a restricted T-cell response, the lack of antigens, and defects in antigen presentation. These factors may explain why immunotherapeutic therapies do not lead to a significant survival benefit (Bonaventura et al. 2019; Frederico et al. 2021). Turning “cold tumors” into “hot” ones is considered a promising approach in modern therapy strategies and may, in the future, be achieved by vaccination, oncolytic viruses, or the combination of different immune-checkpoint inhibitors (Lim et al. 2018). Finally, non-immune TME cells like astrocytes, neurons, and endothelial cells produce proteins and biomolecules such as hormones or nitric oxide (NO*)* supporting tumor growth (Schiffer et al. 2019). In this context, astrocytes buffer the metabolic environment (Matyash and Kettenmann 2010) and thereby support the immunosuppressive environment in gliomas (Henrik Heiland et al. 2019).

1.4. The human endogenous retrovirus family

1.4.1. HERVs and diseases

HERVs are ancient retroviral elements accounting for up to 8% of the human genome (Lander et al. 2001). They originated from mammalian germline retroviral infections millions of years ago (Dolei 2006) but are usually epigenetically silenced by DNA methylation and histone modifications (Groh and Schotta 2017; Lavie et al. 2005). Overall, HERVs are class 1 transposable elements (TEs; (Rebollo et al. 2012)) that structurally resemble their endogenous retroviral origin, containing open reading frames (ORFs) for the viral genes gag (group-specific antigen gene), pro (protease gene), pol (polymerase gene), and env (envelope gene; (Griffiths 2001)). Long terminal repeats (LTRs) flanking the viral genome portions consist of silencing and enhancing sequences, enabling the modulation of retroviral gene expression via the interaction with transcription factors

(Durnaoglu et al. 2021; Villesen et al. 2004). In total, the human genome consists of more than 40 different HERV families that are classified by their specific type of transfer ribonucleic acid (tRNA), which is used for initiating reverse transcription (Nelson et al. 2003). In this context, elements of the HERV-K family are the most intact and biologically active HERVs (Marchi et al. 2014). Infections with viruses like the Epstein-Barr-Virus (EBV; (Perez-Perez et al. 2022)), herpesviruses (Perron et al. 1993), or other exogenous retroviruses like the human immunodeficiency virus 1 (HIV-1) and the human t-cell lymphotropic virus type 1 (HTLV-1) can transactivate HERVs (Kury et al. 2018). These pathogenic HERVs may then contribute to the pathogenesis of different diseases as they are expressed in tissue and blood, producing viral particles and proteins (Feschotte and Gilbert 2012). Notably, in recent years, HERVs have been found to be involved in complex and multifactorial diseases whose pathogeneses and etiologies are driven by many variables (Charvet et al. 2021). In general, HERVs are supposed to be mainly associated with autoimmune diseases by modulating the human immune response (Posso-Osorio et al. 2021). However, the precise mechanisms and extent of their impact on the course of these diseases remain an intensely debated issue, and it is still unclear whether they initiate disease onset, trigger disease progression, or potentially even both.

1.4.2. The human endogenous retrovirus type W

HERVs were first reported to be associated with multiple sclerosis (MS), the most common chronic inflammatory autoimmune disease of the central nervous system (CNS), in 1997 (Perron et al. 1997). Perron et al. identified retroviral particles in leptomeningeal cell cultures from MS patients, which they eventually named multiple sclerosis-associated retrovirus (MSRV). This retrovirus was later renamed HERV-W due to its tryptophan (W) tRNA primer for reverse transcription (Blond et al. 1999). Further studies then demonstrated that the pathogenic envelope protein of HERV-W (pHERV-W ENV) could be detected in the serum, peripheral blood mononuclear cells (PBMCs), and cerebrospinal fluid (CSF) of MS patients (Perron et al. 1997; Perron et al. 2012). In fact, the protein expression of HERV-W ENV correlates with the activity and the progression of this disease (Sotgiu et al. 2010). Moreover, HERV-W ENV levels were found to correlate with inflammation and demyelination and were detected in considerable amounts in active MS lesions (Mameli et al. 2007). Interestingly, regarding the still unclear pathogenesis and

etiology of MS, researchers were also able to correlate the reactivation of retroviral elements in MS with EBV infection (see above chapter 1.4.1.; (Latifi et al. 2022; Mameli et al. 2012)). In addition, other exogenous factors such as influenza A or herpesvirus were shown to activate HERV-W ENV protein expression, underlining the importance of viral infections for HERV-W ENV expression (Charvet et al. 2018; Li et al. 2014). Further research focused on the various cellular effects and altered cellular interactions caused by the HERV-W ENV protein. Regarding cellular surface interaction, HERV-W ENV protein binds to toll-like receptor 4 (TLR4) and its co-receptor CD14, which leads to the activation of the nuclear factor kappa light chain enhancer of activated B-cells (NF-kB) signaling pathway, eventually resulting in the secretion of proinflammatory molecules (Rolland et al. 2006). In addition, HERV-W ENV protein-based activation of TLR4 leads to the production of various molecules associated with nitrosative stress, which inhibits the differentiation and proliferation of oligodendroglial precursor cells (OPCs; (Kremer et al. 2013)). In microglia, HERV-W ENV induces a proinflammatory phenotype, promotes cell proliferation, and induces ameboid microglial morphologies, thus creating a neurodegenerative environment near axons in MS tissue (Kremer et al. 2019). In addition, HERV-W ENV alters the behavior of dendritic cells by promoting the differentiation of T helper cell type 1 (Th1), which is involved in the anti-tumor immune response (Shang et al. 2024). More recently, several studies have found that HERV-W ENV is also associated with the onset and progression of other immunological and neurological diseases such as type-1 diabetes (T1D), chronic inflammatory demyelinating polyneuropathy (CIDP), schizophrenia, amyotrophic lateral sclerosis (ALS), or coronavirus disease 2019 (COVID-19; (Balestrieri et al. 2021; Kury et al. 2018)). Studies in T1D indicated that HERV-W ENV contributes to the pathogenesis of this complex autoimmune disease, as it is found in the blood and pancreas of patients (Levet et al. 2017). However, in the pancreas, HERV-W ENV protein is expressed in acinar cells near the islets of Langerhans rather than in immune cells. Additionally, HERV-W ENV expression was found in lymphocytes of patients with severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection (Balestrieri et al. 2021). In this study, Balestrieri et al. described a potential contribution of HERV-W ENV to the pathogenic features of SARS-CoV-2 as HERV-W ENV expression was found to correlate with inflammatory markers and pneumonia severity. In this context, HERV-W ENV was proposed as a biomarker for COVID-19 predicting respiratory outcome and clinical progression (Garcia-Montojo and Nath 2021). Corroborating this link, further

studies then found HERV-W ENV to be expressed in various postmortem tissues of COVID-19 patients (Charvet et al. 2023). In summary, HERV-W ENV is associated with various neurological and immunological diseases where it contributes to progression by increasing inflammatory stress.

As HERV-W ENV seems to play a significant role in various diseases, it is considered as a suitable therapeutic target. For this purpose, the neutralizing humanized IgG4 monoclonal antibody temelimab (GNbAC1) was developed, which specifically targets the subunit (SU) of HERV-W ENV, as well as the full-length HERV-W ENV-T protein (Curtin et al. 2015). Preclinical studies demonstrated its efficacy in a mouse model of MS, with no adverse events at therapeutic doses (Curtin et al. 2015). Clinical trials in MS patients then revealed that temelimab was well tolerated, reduced HERV-W ENV transcripts, and showed potential neuroprotective effects, including fewer T1-hypointense lesions (Curtin et al. 2012; Derfuss et al. 2015; Hartung et al. 2022). Recently, the ProTect-MS trial (ClinicalTrials.gov: NCT04480307) evaluated higher doses of temelimab in combination with rituximab, an anti-CD20 antibody commonly used off-label in MS treatment (Granqvist et al. 2018). The combination of both agents was well tolerated, with brain MRI scans demonstrating a reduction in neurodegenerative features, suggesting that it could be a promising strategy for treating progressive MS in the future (Piehl 2022). Additionally, temelimab was also evaluated in post-COVID-19 neuropsychiatric syndromes but did not significantly improve fatigue, despite being well tolerated (GeNeuro 2024).

1.4.3. The oncogenic potential of human endogenous retroviruses

Cancer cells are distinguished by a set of characteristics called the hallmarks of cancer, first described by Douglas Hanahan and Robert Weinberg in 2000 (Hanahan and Weinberg 2000). Providing a framework for understanding the complexities of cancer biology, the authors identified six main hallmarks: self-sufficiency in growth signals, insensitivity to anti-growth signals, tissue invasion and metastasis, limitless replicative potential, sustained angiogenesis, and evasion of apoptosis. In 2011, the authors then added two more hallmarks: deregulated metabolism and evasion of the immune system

(Hanahan and Weinberg 2011). Furthermore, they described two enabling characteristics, genome instability and mutation, and tumor-promoting inflammation, that lead to the acquisition of the hallmarks. These hallmarks can also be used as a basis for the investigation of the oncogenic potential of HERVs in human malignancies. While HERVs are associated with various cancers, it is not known how HERVs specifically interfere with the development and progression of these malignancies (Kitsou et al. 2023). More precisely, aberrant HERV expression was found in malignancies such as breast cancer (Tavakolian et al. 2019), prostate cancer (Ibba et al. 2018), lung cancer (Zare et al. 2018), leukemia (Bergallo et al. 2017), lymphoma (Barth et al. 2019), and Kaposi's sarcoma (Dai et al. 2018). In these cancers, HERV expression is regulated by exogenous virus infection, chemical or physical damage, or epigenetic regulation (Zhang et al. 2019). Especially in glioma, epigenetic modifications contribute to the complex heterogeneity of neoplastic cells and allow cancer stem cells to gain self-renewal and drug-resistance properties (Toh et al. 2017; Wainwright and Scaffidi 2017; Wu et al. 2021). In this context, HERV-K expression was found to correlate with glioma stem-cell markers and to be modulated through treatment with reverse transcriptase inhibitors (Argaw-Denboba et al. 2017). However, the functional role of HERVs in carcinogenesis remains complex and still largely elusive. HERV transcripts directly interact with cellular signaling pathways and alter tumor cell behavior (Bannert et al. 2018). In addition, researchers found an association between HERVs and noncoding ribonucleic acids (ncRNA), which were dysregulated in several tumors and were shown to regulate various biological processes (Kelley and Rinn 2012; Pan et al. 2016). In summary, HERVs may alter both oncogenic processes and the antitumor response, making their impact on tumor progression a double-edged sword (Bannert et al. 2018; Zhang et al. 2019).

1.5. Aim of this thesis

In the context of our previous investigations regarding HERV-W and glioblastoma, our research group was the first to detect HERV-W ENV in malignant tissue isolated from glioma patients in both tumor cells and infiltrating macrophages (Reiche et al. 2024). As previously described, HERV-W ENV is known to affect the behavior of various cell entities such as microglia, OPCs, and astrocytes but has not yet been studied in the context of glioblastoma. Therefore, this study aimed to investigate the potential impact of HERV-W

ENV on neoplastic cells, with a particular focus on the tumor microenvironment (TME). Since the TME is crucial for glioblastoma survival and growth, this study investigated the effects of HERV-W ENV protein on both glioblastoma and TME cells, concentrating on molecular and cellular parameters such as proliferation, migration, and invasiveness. In doing so, it sought to clarify whether in the future HERV-W ENV might be a therapeutic target in the treatment of glioma potentially using temelimab.

2. Materials and Methods

2.1. Materials

2.1.1. Organisms

Species	**Line**	**Supplier**
Rattus norvegicus	Wistar	Janvier Labs, Saint-Berthevin, France

2.1.2. Human glioblastoma cell lines

Cell line	**Sex**	**Age (y)**	**Supplier**
A172	Male	53	American Type Culture Collection, Manassas, Virginia, United States
LN229	Female	60	American Type Culture Collection, Manassas, Virginia, United States
T98	Male	61	American Type Culture Collection, Manassas, Virginia, United States
U87	Male	Unknown	American Type Culture Collection, Manassas, Virginia, United States

2.1.3. Reagents, enzymes and media

Substance	**Supplier**
2-propanol	Merck, Darmstadt, Germany
4',6-Diamidino-2-phenylindole	Roche Diagnostic GmbH, Mannheim, Germany
Accutase	PAA Laboratories, Pasching, Austria
Albumin BSA-V	ThermoFisher Scientific, Life Technologies, Darmstadt, Germany

ß-Mercaptoethanol	Sigma-Aldrich Chemie GmbH, München, Germany
Bovine serum albumin (BSA) Fraktion V 7,5%	Lab intern production
Rat-CD11b magnetic microbeads	Miltenyi Biotec, Bergisch Gladbach, Germany
Citifluor mounting medium	Citifluor, London, UK
Dulbecco's modified eagle medium (DMEM); low glucose	ThermoFisher Scientific, Life Technologies, Darmstadt, Germany
Dulbecco's modified eagle medium (DMEM); high glucose	ThermoFisher Scientific, Life Technologies, Darmstadt, Germany
DNase I	Cell Systems, Lakewood, USA
Dulbecco´s phosphate buffered saline (PBS)	Sigma-Aldrich, St. Louis, USA
Ethanol ≥ 96%	Merck, Darmstadt, Germany
Fetal calf serum (FCS)	Lonza, Basel, Switzerland
Isoflurane	Piramal-Healthcare, Mumbai, India
L-cysteine	Sigma-Aldrich, St. Louis, USA
LiChrosolv® water	Merck, Darmstadt, Germany
L-glutamine	ThermoFisher Scientific, Life Technologies, Darmstadt, Germany
Normal goat serum	Sigma-Aldrich, St. Louis, USA
Normal donkey serum	Sigma-Aldrich, St. Louis, USA
Paraformaldehyde	Merck, Darmstadt, Germany
Penicillin / Streptomycin	ThermoFisher Scientific, Life Technologies, Darmstadt, Germany
RNase-free water	Qiagen, Hilden, Germany
Triton X-100	Sigma-Aldrich, St. Louis, USA
Trypan blue 0,4%	Sigma-Aldrich, St. Louis, USA
Trypsin inhibitor	Sigma-Aldrich, St. Louis, USA

2.1.4. Laboratory equipment and software

Equipment	**Supplier**
7900HT Fast Real-Time PCR System	ThermoFisher Scientific, Life Technologies, Darmstadt, Germany
Autoclave GLA30	Fritz Gössner GmbH, Hamburg, Germany
Axioplan 2 Fluorescence microscope	Carl Zeiss, Oberkochen, Germany
Axiovision 4.2 software	Carl Zeiss, Oberkochen, Germany
BBD 6220 CO_2 incubator	ThermoFisher Scientific, Life Technologies, Darmstadt, Germany
Centrifuge	Heraeus Holding GmbH, Hanau, Germany
Endnote X21.3.0	Clarivate Analytics, Philadelphia, USA
Eppendorf centrifuge 5804	Eppendorf, Wesseling-Berzdorf, Germany
Excella E24 incubator shaker	New Brunswick Scientific, Nürtingen, Germany
GraphPad PRISM software 10.1.1	GraphPad Prism, San Diego, USA
Herasafe HSP 12 sterile bench	Heraeus Holding GmbH, Hanau, Germany
ImageJ software	National Institute of Health, Rockville, USA
LSM 510 Confocal laser scanning microscope (CLSM)	Carl Zeiss, Oberkochen, Germany
MACS MultiStand	Miltenyi Biotec, Bergisch Gladbach, Germany
MiniMACS™ Separator	Miltenyi Biotec, Bergisch Gladbach, Germany
Minishaker MS2; vortexer	IKAR Works, Inc. Wilmington, USA
MS Office 365	Microsoft Corporation, Redmond, USA
Nanodrop ND 1000	PeqLab, Erlangen, Germany
Primer Express 3.0.1	ThermoFisher Scientific, Life Technologies, Darmstadt, Germany

Sequence Detection System (Version 2.3)	ThermoFisher Scientific, Life Technologies, Darmstadt, Germany
Tecan i-control (Version 1.7.1.12)	Tecan, Männedorf, Switzerland
Tecan Microplate reader Infinite 200pro	Tecan, Männedorf, Switzerland
Thoma counting chamber (Depth: 0.100 mm; Area 0.0025 mm^2)	Optik Labor, Görlitz, Germany
Veriti thermocycler	ThermoFisher Scientific, Life Technologies, Darmstadt, Germany
Water bath	GFL, Burgwedel, Germany

2.1.5. RNA preparation, cDNA synthesis and PCR reagents

Reagent	**Supplier**
ß-Mercaptoethanol	Sigma-Aldrich Chemie, Taufkirchen, Germany
RNeasy Mini Kit RNeasy Mini Spin Columns Collection Tubes (1.5 ml) Collection Tubes (2 ml) Buffer RLT Buffer RW1 Buffer RPE (concentrate) RNase-free water	Qiagen, Hilden, Germany

High-Capacity cDNA Reverse Transcription Kit 10x RT Buffer 10x RT Random Primers 25x dNTP Mix (100mM) MultiScribe Reverse Transcriptase 1 tube (1.0ml) 2 tubes (0.1ml) RNase Inhibitor	ThermoFisher Scientific, Life Technologies, Darmstadt, Germany
Power SYBR Green PCR Master Mix SYBR® Green I dye AmpliTaq Gold® DNA Polymerase dNTPs Passive reference dye Optimized buffer components	ThermoFisher Scientific, Life Technologies, Darmstadt, Germany

2.1.6. Antibodies

Reagent	**Dilution**	**Supplier**
Anti-Ki-67 monoclonal rabbit antibody (ab16667)	1:250	Abcam, Cambridge, UK
Anti-Ki67 monoclonal rat antibody (cat#14-5698-82)	1:250	ThermoFisher Scientific, Life Technologies, Darmstadt, Germany
Anti-Iba1 polyclonal goat antibody (ab5076)	1:500	Abcam, Cambridge, UK
Anti-TLR4 polyclonal rabbit antibody (ab13556)	1:200	Abcam, Cambridge, UK
Anti-CC3 polyclonal rabbit antibody (#9661)	1:500	Cell Signaling Technology, Cambridge, UK

Anti-beta3-tubulin chicken antibody (AB_2313564)	1:500	Aves Labs, Davis, USA
Goat anti rabbit Alexa Fluor 488	1:500	ThermoFisher Scientific, Life Technologies, Darmstadt, Germany
Goat anti rabbit Alexa Fluor 594	1:500	ThermoFisher Scientific, Life Technologies, Darmstadt, Germany
Donkey anti rabbit Alexa Fluor 488	1:500	ThermoFisher Scientific, Life Technologies, Darmstadt, Germany
Donkey anti goat Alexa Fluor 594	1:500	ThermoFisher Scientific, Life Technologies, Darmstadt, Germany
CruzFluorTM 594 Conjugate	1:1000	Santa Cruz Biotechnology, Dallas, USA

2.1.7. Primer sequences for human qRT-PCR analysis

Name	**Sequence forward**	**Sequence reverse**
ARF1	GAC CAG ATC CTC TAC AAG GC	TCC CAC ACA GTG AAG CTG ATG
BAX	ATG GAC GGG TCC GGG G	CGA TCC TGG ATG AAA CCC TGA A
CSF-1	TTC AGC AAG AAC TGC AAC AAC A	TCA GGC TTG GTC ACC ACA TC
CX3CL1	CGG CAA ACG CGC AAT C	CGG GTC GGC ACA GAA CAG
HERV-W ENV	TTT ACT CCT CTT TGG ACC CT	ATC TGG GGT TCC ATT TGA AG
IL-1β	ACG ATG CAC CTG TAC GAT CAC T	CAC CAA GCT TTT TTG CTG TGA GT
IL-6	GCT GCA GGC ACA GAA CCA	GCT GCG CAG AAT GAG ATG AG
INOS	TGG ATG CAA CCC CAT TGT C	CGC TGC CCC AGT TTT TGA T

MCP-1	CAA GCA GAA TG GGT TCA GGA T	TCT TCG GAG TTT GGG TTT GC
MCP-3	TGT GCT GAC CCC ACA CAG A	TTT GGA GTT TGG GTT TTC TTG TC
MLKL	CCT GGG CAC AGG AAG ATC AG	AGG ACG ATT CCA AAG ACT GCC
MMP9	CGC CAG TCC ACC CTT GTG	CAG CTG CCT GTC GGT GAG A
RIPK3	TGC GTC AAG TTA TGG CCC AG	AGC CCC ACT TCC TAT GTT GC
TNFα	TGC TCC TCA CCC ACA CCA T	GGA GGT TGA CCT TGG TCT GGT A
Trail	CAC AGT GTC TGC TGG GAC C	AGG AGT CAA AGG GCA CGA TG
VEGF	CGA GGG CCT GGA GTG TGT	CGC ATA ATC TGC ATG GTG ATG

2.1.8. Primer sequences for rat qRT-PCR analysis

Name	**Sequence forward**	**Sequence reverse**
ANG2	GAT GCC AGA TAC TGC GAA AGC	CTC TTT GCA GGG CGA GGT T
CD274	CAG CTA TGG TGG AGC GGA CTA	TGC GGT ATG GAG CGT TGA
EGF	TCC CGT TGA AGA CGA TCA AAA	AAC GTC TTG ACT TCG GTT TCT GA
TGFb	AAA CGG AAG CGC ATC GAA	TGG CGA GCC TTA GTT TGG A
TNFa	AGC CCT GGT ATG AGC CCA TGT A	CCG GAC TCC GTG ATG TCT AAG T
CSF-1	CGA GGT GTC GGA GCA CTG TA	TCA ACT GCT GCA AAA TCT GTA GGT

IL6	GTT GTG CAA TGG CAA TTC TGA	TCT GAC AGT GCA TCA TCG CTG
Arg1	GGT GAC CCC CTG CAT ATC TG	TCT CGC AAG CCG ATG TAC AC
IL10	CCC AGA AAT CAA GGA GCA TTT G	CAG CTG TAT CCA GAG GGT CTT CA
S1PR	AAA TGC CCC AAC GGA GAC T	TCC ATG CCC GGG ATG AT
VEGFa	GCA CTG GAC CCT GGC TTT ACT	TGC AGC CTG GGA CCA CTT
FGF-2	TGG TAT GTG GCA CTG AAA CGA	CCA GGC CCC GTT TTG G
IGF1	AGA CGG GCA TTG TGG ATG A	ACA TCT CCA GCC TCC TCA GAT C
Serping1	GAC AGC CTG CCC TCT GAC A	GCA CTC AAG TAG ACG GCA TTG A
MerTK	TCT GAC AGA GAC CGC AGT CTT C	TGG ACA CCG TCA GTC CTT TG
S100a10	GCC ATC CCA AAT GGA GCA T	CCC CTG CAA ACC TGT GAA AT
TREM2	CCA AGG AGC CAA TCA GGA AA	GGC CAG GAG GAG AAG AAT GG
C3	GGT CTG CGG AAG TGT TGT GA	GGC GCT GGC AGC TGT ACT
Lcn2	GGG CAG GTG GTT CGT TGT C	AGC GGC TTT GTC TTT CTT TCT G
ODC	GGT TCC AGA GGC CAA ACA TC	GTT GCC ACA TTG ACC GTG AC

2.1.9. Immunocytochemical staining

Reagent	**Contents and concentration**
Paraformaldehyde	• Dulbecco´s phosphate buffered saline (PBS) • PFA (4%)

Blocking solution I	• Dulbecco´s phosphate buffered saline (PBS) • Normal goat serum (NGS, 10%) • Triton X-100 (0.1%)
Blocking solution II	• Dulbecco´s phosphate buffered saline (PBS) • Normal donkey serum (NDS, 10%) • Triton X-100 (0.1%)
Antibody solution I	• Dulbecco´s phosphate buffered saline (PBS) • Normal goat serum (NGS, 10%) • Triton X-100 (0.03%)
Antibody solution II	• Dulbecco´s phosphate buffered saline (PBS) • Normal donkey serum (NDS, 10%) • Triton X-100 (0.03%)

2.1.10. Reagents for cell culture stimulation

Reagent	**Supplier**
Recombinant HERV-W ENV-T protein	GeNeuro, Plan-les-Ouates, Switzerland
HERV-W ENV-T buffer	GeNeuro, Plan-les-Ouates, Switzerland
Lipopolysaccharide (LPS)	Sigma-Aldrich, St. Louis, USA
MACS buffer	• Dulbecco´s phosphate buffered saline (PBS) • Bovine serum albumin (BSA) Fraktion V (0.5%)

2.1.11. Reagents for MTT assay

Reagent	**Supplier**
Methylthiazolyldiphenyl-tetrazoliumbromide	Sigma-Aldrich, St. Louis, USA
Dimethylformamide (DMF)	Sigma-Aldrich, St. Louis, USA
Glacial acetic acid	Sigma-Aldrich, St. Louis, USA
Sodium dodecyl sulfate (SDS)	Sigma-Aldrich, St. Louis, USA
Sodium hydroxide	Sigma-Aldrich, St. Louis, USA

2.2. Cell culture methods

2.2.1. Human glioblastoma cell line cultivation

The human glioblastoma (GBM) cell lines A172, LN229, U87MG, and T98G were obtained from the American Type Culture Collection (ATCC). All cell lines were cultured according to ATCC'S guidelines in T-75 flasks with GBM culture medium consisting of Dulbecco's Modified Eagle's Medium (DMEM) supplemented with 10% fetal bovine serum (FBS) and 50 U/ml of penicillin/streptomycin maintained in a cell incubator with an atmosphere of 5% CO_2 at 37°C. The culture medium was exchanged once the cells were grown confluently. The cell culture was passaged and seeded to prepare experimental procedures. To this end, the cells were detached from the flask's surface with trypsin-EDTA for 3 minutes, and the reaction was stopped with GBM culture medium. After spinning down for 5 min at 300 x g and 4°C, the cells were either plated directly into 24-well plates at a density of 10^4 cells per well for quantitative reverse transcription-polymerase chain reaction (qRT-PCR) or plated onto 13 mm glass coverslips placed within 24-well plates at a density of 5 x 10^3 cells per well for immunocytochemical staining. Regardless of the further experimental procedure, the glioblastoma cells were cultured with glioblastoma medium for 24 hours in a cell incubator. After 24 hours, depending on the experimental procedure, the cells were either cocultured with microglia to establish cocultures of glioblastoma and microglia cells, or they were stimulated with 1000 ng/ml HERV-W ENV protein, the appropriate buffer solution, or lipopolysaccharide (LPS). Regarding the high proliferation rate of glioblastoma cells, the cell cultures were restimulated every 48 hours for longer experimental procedures.

2.2.2. Preparation of primary rat microglial cell culture

Primary microglial cells were generated and purified according to the McCarthy and de Vellis protocol (McCarthy and de Vellis 1980). All animal procedures were performed in compliance with the experimental guidelines approved by the regional authorities (State Agency for Nature, Environment and Consumer Protection of North Rhine Westphalia) and ZETT (Zentrale Einrichtung für Tierforschung und wissenschaftliche Tierschutz-aufgaben) with the licenses O69/11 and O82/12. Briefly, postnatal (P0 and P1) Wister rats

were anesthetized using isoflurane and decapitated. Then, the cortices were released from the meninges and vessels. After cutting the cortices into small pieces, they were collected in MEM-Hepes medium and centrifuged for 30 seconds at 1200 rpm. After discarding the medium, fresh MEM-Hepes medium containing 30 U/ml papain, 0.24 mg/ml L-cysteine, and 40 µg/ml DNAse I type IV was added. Following an incubation period (45 min at 37°C and 5% CO_2), 1 ml trypsin inhibitor solution (1 mg/ml ovomucoid trypsin inhibitor, 50 mg/ml BSA V, and 40 µg/ml DNAse I type IV in 1 ml L-15 medium) was added at room temperature for 5 min to stop digestion. After that, the supernatant was discarded, and another 1 ml trypsin inhibitor solution was added and homogenized through trituration with a glass pipette. After adding 10 ml of DMEM medium containing 10% FCS and centrifuging the solution for 10 min at 1500 rpm, the supernatant was discarded, and the cell pellet was resuspended in 20 ml of DMEM medium containing 10% FCS and 1% Penicillin-Streptomycin. The cell suspension was added to 75 cm^2 tissue-culture-treated (TC-treated) T-75 cell culture flasks and incubated for 10 days at 37°C, 98% humidity, and 5% CO_2. During this incubation period, the culture medium was changed every 2 days. After 10 days, flasks were shaken at 180 rpm for 2 hours. Microglia-containing supernatants were transferred to bacterial dishes and kept in the incubator, allowing cell attachment to the surface. Culture flasks were again loaded with fresh DMEM and shaken for another 24 hours to increase the final cell yield. Afterward, supernatants were again transferred to bacterial dishes to allow cell attachment.

2.2.3. Isolation of microglia cells via Magnetic Activated Cell Sorting (MACS)

Further isolation of the primary microglia cells from the cell suspension was performed using Magnetic Activated Cell Sorting (MACS) with CD11 b/c microbeads according to the manufacturer's instructions (Miltenyi Biotec). First, the viability of the microglia-containing dishes from the first and the second shaking steps was verified via bright-field microscopy. The medium was discarded, and cells were rinsed with PBS. Cells were incubated for 5 min with accutase to detach the cells from the dish. The reaction was stopped by adding FCS-containing DMEM. After centrifuging the cell suspension for 5 min at 1200 rpm and 4°C, the supernatant was aspired, and the cell pellet was resuspended in 80 µl of MACS

buffer containing 0,5% bovine serum albumin (BSA) in PBS. Then, 20 µl of CD11b/c Microbeads were added. Following an incubation period of 15 minutes at 2-8°C, 2 ml MACS buffer was added, and cells were centrifuged under the same conditions as above. The supernatant was discarded, and cells were resuspended in 500 µl MACS buffer. A column was placed in the magnetic field of the MACS Separator, and 500 µl MACS buffer was added. Cell suspension was then applied onto the column. The column was washed 3 times with each 500 µl MACS buffer. After that, the column was removed from the separator and placed on a collection tube. 1 ml MACS buffer was added to the column, and a plunger was pushed into the column immediately to flush out the magnetically labeled microglia. Cells were centrifuged again, the supernatant was discarded, and the pellet was resuspended in 1 ml medium. Trypan blue staining was performed to quantify the cells in a Thoma chamber. Additionally, the purity of the microglial cell culture was verified regularly by Iba1/GFAP staining, which showed a 99% purity rate for microglia. To establish cocultures of human GBM cells with primary rat microglia (GBM/MG cocultures), microglia (10^5 cells/well) were either directly seeded with the GBM cells (+MG) or placed in a hanging Millicell® cell culture insert (*MG; pore size 0.3 µM; Merck, PCSP24H48) preventing direct cell-cell interaction of GBM and MG. After 24 hours, GBM/MG cocultures were treated with 1000 ng/ml HERV-W ENV protein or the appropriate buffer solution as described above.

2.2.4. HERV-W ENV protein stimulation

The recombinant full-length HERV-W ENV protein was produced by Protein'eXpert (Grenoble, France) according to the quality control specifications of GeNeuro (Geneva, Switzerland). Using the limulus amebocyte lysate (LAL) test, endotoxin levels were quantified and found to be below the detection threshold (<5EU/ml). Human and rat primary cells were stimulated by adding the recombinant HERV-W ENV protein to the culture medium at a concentration of 1000 ng/ml. All control stimulation experiments were conducted using a recombinant ENV buffer solution (20 mM Tris-HCl, pH 7.5, 150 mM NaCl, 1.5% SDS, 10 mM DTT) with an equal dilution.

2.2.5. Immunocytochemistry

The immunocytochemical staining of the paraformaldehyde-fixed cell culture was performed as described below. Cultured cells were rinsed thrice with PBS at room temperature and then fixed with 4% paraformaldehyde (PFA) for 10 min. After rinsing again three times with PBS, cells were incubated for 45 min with 10% normal goat serum (NGS) or normal donkey serum (NDS) and 0.1% Triton X-100 to block non-specific antibody binding. Cells were then incubated with the primary antibody solution containing 10% NGS or NDS in PBS with 0.03% Triton X-100 overnight at 4°C. The dilution of primary antibodies is illustrated in Chapter 2.1.6. After 24 hours, cells were rinsed three times with PBS and then incubated with secondary fluorescent antibodies labeled with Alexa Fluor488 or Alexa Fluor594 for 2 hours at room temperature. Nuclei were stained with DAPI. For visualization of GBM cell line clustering and morphologies in GBM/+MG cocultures, F-actin was stained by adding Phalloidin CruzFluorTM 594 conjugate to the secondary antibody solution. Following three more rinsing steps with PBS, the cell culture immunostainings were mounted in Citifluor and analyzed with an Axioplan 2 fluorescence microscope. The expression of Ki67 was analyzed using ImageJ BioVoxxel software (Schindelin et al. 2012). Nine images per coverslip (with two coverslips per condition) were captured at 20x magnification, and the exposure times across all experiments were maintained consistently. The ratio of marker-positive cells to the total number of DAPI-stained nuclear cells was calculated for quantification.

2.2.6. Wound healing assay

To assess cell migration of the glioblastoma cells, predefined gaps with a width of 500 µm were created in the 24 well plates using culture inserts (ibidi culture-insert 2-well, ibidi GmbH, Martinsried, Germany). After seeding $5*10^4$ glioblastoma cells in 35 µl medium on each side of the insert and adding $5*10^4$ microglia per side 24 hours later, depending on the experimental setup, cells were cultured overnight in a cell incubator, allowing cell attachment. The culture inserts were then removed carefully using sterile tweezers, and the well was rinsed with PBS to discard any non-adherent cells. Then, the medium was replaced with HERV-W ENV protein or buffer-supplemented GBM medium, respectively. Three different but defined positions per well were captured using a Zeiss CLSM 510

microscope system in 10x magnification via live cell imaging to monitor the progression of gap closure. Images of each position reflecting gap closure were taken every 4 hours. Each gap was identified and marked manually using ImageJ BioVoxxel software (Schindelin et al. 2012). The analysis of gap closure was then performed using a specific ImageJ plugin (Suarez-Arnedo et al. 2020). To calculate gap closure, the initial wound area was compared to the remaining wound area after each time point, with results expressed as the percentage of the initial gap closed.

2.2.7. Matrigel Invasion assay

To further measure invasion and migration capability, a Matrigel invasion assay was performed. 10^5 microglia per well were seeded in a 24-well plate for this experimental procedure. 24 hours later, Millicell® cell culture inserts with a pore size of 0.8 µM were coated with 166µg/ml Matrigel according to the manufacturer's protocol, imitating the extracellular matrix. After that, $5*10^4$ glioblastoma cells in 100 µl glioblastoma medium were added to the upper chamber. The next day, the medium in both chambers was replaced with either HERV-W ENV protein- or buffer-supplemented medium. Again, 24 hours later, the lower chamber was rinsed thrice with PBS and then fixed with 4% PFA for 10 minutes. After that, the lower chamber was rinsed again three times with PBS. The upper chamber was rinsed once with PBS, fixed with 4% PFA, and then rinsed twice with PBS. The invasion of glioblastoma cells was quantified using the Crystal Violet Cell Cytotoxicity Assay Kit (BioVision). Inserts were removed from the 24-well plate and scraped off carefully from the inside with a cotton swab to remove non-migrating glioblastoma cells. After that, the insert membrane was cut out using a scalpel, carefully divided into two halves, and put in a 96-well plate. Next, the membrane was washed with 200 µl 1X Washing solution. Then, 50 µl of the Crystal Violet Staining Solution was added to each well, and the staining procedure was performed for 20 min at room temperature. After this incubation period, the staining solution was removed. Following a last washing step with each 200µl 1X Washing solution four times, the membranes were airdried for 3-4 min. To solubilize the migrated glioblastoma cells, 100 µl of the Solubilization Solution was added to each well, and the plate was put on a shaker for 20 min at room temperature. The optical density was measured at 9 different positions and in duplets at 570 nm in an Infinite M200 Pro using the Tecan i-control software.

2.2.8. Quantitative analysis of cytokine secretion dynamics

For measuring cytokine secretion dynamics, GBM/+MG cell culture medium was collected after 3 days of stimulation with either buffer or HERV-W ENV protein to assess cytokine secretion dynamics. As the cell medium was renewed every 48 hours, the results of this experiment particularly reflect the cytokine dynamics between day 2 and day 3 after the first stimulation with HERV-W ENV. For further analysis, three technical replicates were pooled, resulting in a total of 1.5 ml of collected supernatant. This supernatant was centrifuged at 300 x g for 5 minutes at 4°C to eliminate cell debris or floating cells. Following centrifuging, pure supernatants were immediately frozen on dry ice and preserved at -80°C until further analysis. To assess cytokine levels, supernatants were thawed gently on ice and analyzed using the Human Cytokine Antibody Assay (ab133997, Abcam) following the manufacturer's instructions. First, array membranes were blocked for 30 minutes at room temperature. Subsequently, they were incubated overnight at 4°C with 1 ml of supernatant from each condition and GBM cell line, along with fresh, uncultured GBM medium (used as a "blank" for baseline comparison with cultured supernatant samples) on a rocking platform shaker. Following a 30-minute wash with buffer W1 and subsequent 5-minute washes with W1 and W2 (each repeated three times), the membranes were incubated overnight at 4°C on a rocking platform shaker with biotin-conjugated anti-cytokines. After three required washing steps with W1 and W2, the array membranes were incubated overnight at 4°C on a rocking platform shaker with HRP-conjugated streptavidin. Following the last set of washing steps, chemiluminescence detection reagents were applied for 2 minutes at room temperature without agitation. Images were captured using a Fusion FX imaging system (Vilber Lourmat), and densitometric quantification of cytokine levels was conducted using ImageJ BioVoxxel software (Schindelin et al. 2012). For further data processing, the background was subtracted, and the mean pixel densities of the 42 cytokines were standardized against the mean pixel densities of the positive controls (totaling 6) on a buffer-treated array membrane for each specific GBM cell line group (e.g. A172/+MG, buffer vs. ENV). In this analysis, Pixel densities averaging below 1 after background subtraction were deemed insignificant and excluded from the study. The data are expressed as means ± SEM from two independent experiments.

2.2.9. Temozolomide treatment and cell viability assay

To assess the efficacy of chemotherapeutic treatment on glioblastoma cells, cells were treated with temozolomide (TMZ) at different concentrations, and a 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT)-assay was performed. The results of this assay, which measures the metabolic activity of cells, correlate strongly with cell viability. This experimental procedure was performed with glioblastoma cells in monoculture and compared glioblastoma cells treated with microglia-conditioned, HERV-W ENV stimulated medium (**MG). To this end, primary rat microglia were isolated as described above. Subsequently, microglia were seeded in a 6-well format in 3 ml medium with a concentration of $4*10^5$ cells per well. 24 hours later, glioblastoma cells were passaged as described above, and cells were seeded in a 96-well plate with $5*10^3$ cells per well in 100 µl medium. Due to their high proliferative activity and to make results viable, LN229 cells were seeded in a concentration of $2,5*10^3$ cells per well. Additionally, negative controls consisted of medium alone, with no cells seeded into these wells. On the same day, as usual, the microglia cells were stimulated with either 1000 ng/ml of recombinant HERV-W ENV or buffer. For the remainder of this experiment, microglia were restimulated every 48 hours with fresh medium containing HERV-W ENV protein or buffer solution. Again, 24 hours later, the glioblastoma cells were treated with GBM medium containing TMZ in different concentrations (1 µM, 10 µM, 100 µM, 200 µM, 400 µM, 600 µM, 700 µM, 800 µM, and 1000 µM). The HERV-W ENV or buffer-stimulated microglia medium was also aspirated and filtered with a 0.2 µm filter to discard cells or cell debris. Then, the same temozolomide concentrations were created by diluting the microglial medium in a 4:1 ratio. In both conditions, the medium was discarded, and 100 µl of fresh, temozolomide-containing medium was added to the glioblastoma cells. The procedure to supplement the HERV-W ENV stimulated, and microglia-conditioned medium with TMZ was repeated every 48 hours, and the medium of the glioblastoma cells was thus replaced.

After glioblastoma cells were treated with TMZ for 3 or 5 cumulative days, respectively, an MTT assay was performed. To this end, MTT solution was generated by dissolving MTT in PBS to a concentration of 5 mg/ml. This solution was then filter-sterilized through a 0.2 µM filter into a sterile, light-protected container. The MTT solution was stored at 4°C for frequent use or at -20°C for long-term storage. To create the solubilization solution, a 40%

solution of dimethylformamide (DMF) was dissolved in 2% glacial acetic acid. Then, 16% of sodium dodecyl sulfate (SDS) was added and again dissolved. Using sodium hydroxide, the pH value was adjusted to 4.7. The solubilization solution was stored at room temperature to avoid SDS precipitation. MTT assays were performed by adding 10 µl of MTT solution per well. After an incubation period of 2 hours, 100 µl of the solubilization solution was added to dissolve formazan crystals. The medium solution was then incubated overnight at room temperature to ensure complete solubilization. Then, optical density was measured at 9 different positions and in triplets at 570 nm and at 650 nm in an Infinite M200 Pro using the Tecan i-control software. For further analysis of cell viability, absorbance values at 650 nm were subtracted from the 570 nm value. Then, the average of the negative controls was subtracted from all wells, and the relative absorption was calculated for each well, using the buffer (-MG) condition as a control.

2.3. Molecular biological methods

2.3.1. Ribonucleic acid isolation

The ribonucleic acid (RNA) isolation from cell culture was performed using the Biogen RNEasy Mini Kit according to the manufacturer's protocol. Cells were lysed in 350 µl lysis Buffer containing ß-Mercaptoethanol and RDD Buffer in the ratio of 1:100 and were frozen on dry ice immediately. Lysates were transferred into the QIAshredder spin columns and centrifuged for 1 min at 14000 rpm at 4°C. 350 µl of 70% ethanol was added to the flow through, and the spin column was discarded. After homogenizing the liquid, it was transferred into the RNeasy spin column and centrifuged for 1 min at 10000 rpm. The flow-through was discarded, and 350 µl RW1 Buffer was added and centrifuged for 1 min. The DNase and RDD Buffer solution containing 10 µl DNase and 70 µl RDD Buffer was added directly to the filter and then incubated for 15 min at room temperature. After discarding the flow-through, two further centrifugation steps followed using 350 µl RW1 Buffer and then 500 µl RPE Buffer, each of them performed for 1 min at 10000 rpm. The last centrifugation step followed using 500 µl RPE Buffer for 2 min at 10000 rpm. Finally, the spin columns were dry centrifuged for 1 min at 14000 rpm, the collection tubes were discarded, and filters were put on fresh collection tubes. 21 µl RNase-free water was added directly to the filter, incubated for 3 minutes at room temperature, and then

centrifuged for 1 min at 10000 rpm. RNA quality and its concentration were assessed using a Nanodrop 1000 spectrophotometer with samples stored at -80°C until further analysis.

2.3.2. Reverse transcription of ribonucleic acids

Isolated RNA was reverse transcribed using the High-Capacity cDNA Reverse Transcription Kit (Thermo Fisher) following the manufacturer's protocol. The isolated RNA was diluted with RNAse-free water to reach 250 ng of cDNA within 20 µl of volume. Depending on the number of samples, a master mix was prepared, whereof 10 µl were added to each sample, resulting in a total volume of 30 µl. The exact details of the master mix are provided in Table 1, along with the volume of each reagent. The samples were then reverse transcribed in a thermocycler: First step for 10 min at 25°C, second step for 120 min at 37°C, third step for 5 seconds at 85°C, and lastly at 4°C until process termination. The samples were stored at -20°C until further analysis.

Reagent	**Volume in µl**
10x RT Buffer	3
10x RT Random Primers	3
25x dNTP Mix (100 mM)	1,2
Reverse Transcriptase	1,5
RNase Inhibitor	1,5

Table 1. cDNA synthesis master mix. Components of the reverse transcription master mix and their volumes.

2.3.3. Quantitative reverse transcription polymerase chain reaction (qRT-PCR)

The amplification of targeted DNA molecules was measured using a 7900HT Sequence Detection System in combination with SYBRGreen Universal Master Mix (Thermo Fisher Scientific). All primers were designed using the PrimerExpress 2.0 software and were

used at a final concentration of 0.30 pmol. ARF1 was identified as the most accurate and stable normalization gene compared to ODC, 18s, or GAPDH for glioblastoma cells. For rat microglial gene measurements, the normalization gene ODC was used. Each sample was measured in duplicates, and the relative gene expression levels were determined according to the comparative cycle threshold (ΔΔCt) method.

2.4. Statistics

All data are expressed as mean values ± standard error of the mean (SEM) or as Z-scores. Graphs and statistical analyses were conducted using Microsoft Excel and GraphPad Prism software (version 8.0.2; GraphPad Prism, San Diego, CA, RRID: SCR_002798). The normality of the datasets was confirmed using the Shapiro-Wilk test, indicating a Gaussian distribution. A two-tailed Student's *t*-test was used for comparisons between two groups, while one-way or two-way ANOVA followed by Tukey's post-test was used for multiple group comparisons involving three or more groups. Statistical significance was determined as follows: *$p \leq 0.05$, ** $p \leq 0.01$, *** $p \leq 0.001$, and *ns* for not significant. The term "n" refers to the number of independent experiments.

3. Results

3.1. Human glioblastoma cell lines express toll-like receptor 4

Previous studies showed that HERV-W ENV interacts with dendritic cells, microglia, and oligodendroglial precursor cells (OPCs) via toll-like receptor 4 (TLR4; (Kremer et al. 2019; Kremer et al. 2013; Rolland et al. 2006)). The activation of this receptor leads, *inter alia*, to the induction of proinflammatory cytokines in microglia and increases their proliferation rate (Kremer et al. 2019). In general, toll-like receptors are part of the group of pattern-recognition receptors (PRRs) and play an essential role in the innate immune response against microorganisms by recognizing pathogen-associated molecular patterns (PAMPs (Trinchieri and Sher 2007)). However, in glioblastoma and the surrounding tumor microenvironment (TME), TLR4 receptors are downregulated and contribute to the immune escape of these tumors (da Cruz et al. 2021). To validate the experimental setup described in this thesis, it was essential to confirm that all investigated human glioblastoma cell lines expressed TLR4 (Fig. 1). To this end, immunocytochemical staining was performed which demonstrated that the cell lines LN229 (Fig. 1A), T98 (Fig. 1B), and U87 (Fig. 1C) expressed TLR4. Additionally, compared to the expression of the microtubule element beta3-tubulin (ß3-tubulin), TLR4 was found to be evenly expressed on the entire cell surface.

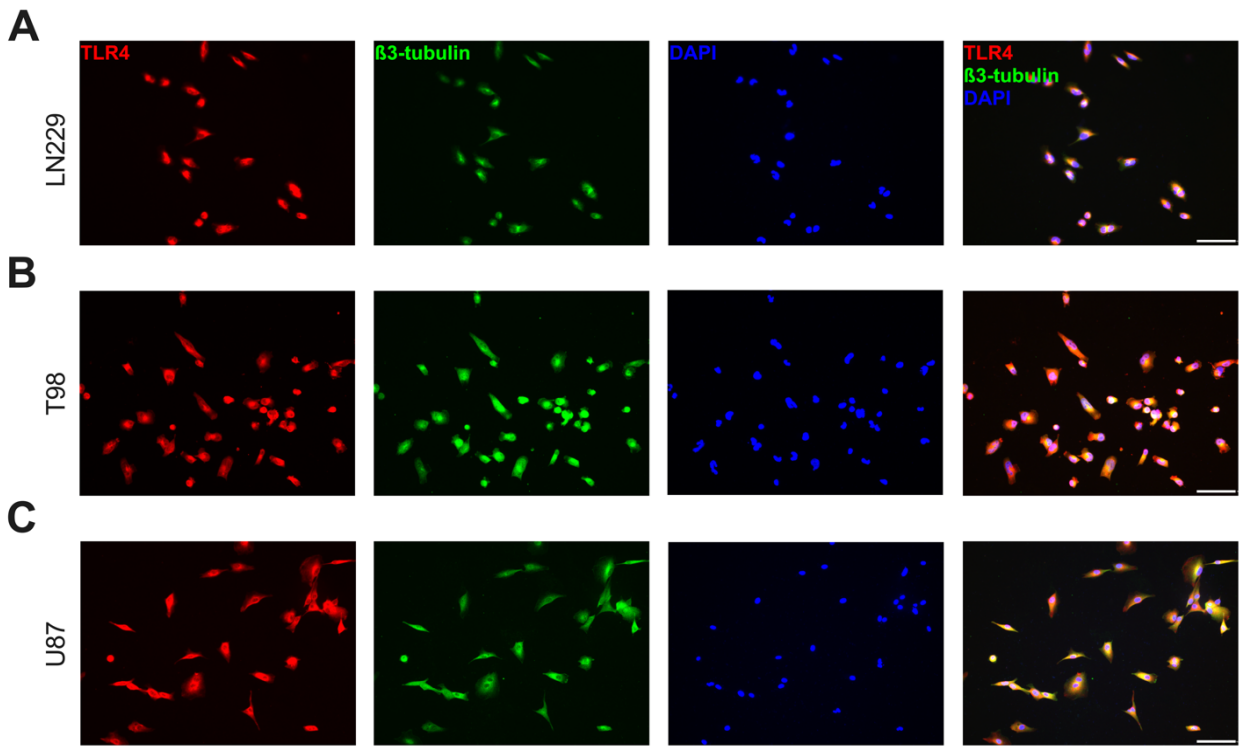


Figure 1. Toll-like receptor 4 (TLR4) expression by the human glioblastoma cell lines LN229, T98, and U87. (A-C) Immunocytochemical staining of human glioblastoma cell lines LN229 (A), T98 (B), and U87 (C) showed the expression of the toll-like receptor 4 (TLR4) on the glioblastoma cell surface. (TLR4: red; beta3-tubulin: green; DAPI: blue) Scale bars: 100 µm.

3.2. Stimulation with HERV-W ENV protein alone does not alter glioblastoma gene expression profile

In order to assess the potential impact of HERV-W ENV protein on tumor cell behavior, we first investigated its effect on the gene expression profile of human glioblastoma cells (Fig. 2, 3, 4, 5). To this end, human glioblastoma cell lines were stimulated with either 1000 ng/ml of recombinant HERV-W ENV or buffer. As a positive control, in a third experimental condition, glioblastoma cells were stimulated with 1000 ng/ml of the TLR4 agonist lipopolysaccharide (LPS), which is an essential component of the outer membrane of gram-negative bacteria (Poltorak et al. 1998). In doing so, we found significant LPS-mediated gene expression changes in A172 cells, while stimulation with HERV-W ENV did not modify the gene expression in this cell line. More precisely, A172 showed an

upregulation of MCP1 expression after 6 hours upon LPS stimulation, which substantially weakened after 12 hours (Fig. 2E and 2E'). In contrast, the upregulation of CSF-1 and TNFa was more substantial after 6 hours than after 12 hours but not statistically significant (Fig. 2A, 2A' and 2D, 2D'). On the other hand, IL-6 and TLR4 expression were neither regulated upon LPS nor HERV-W ENV stimulation in this cell line (Fig. 2B and 2B').

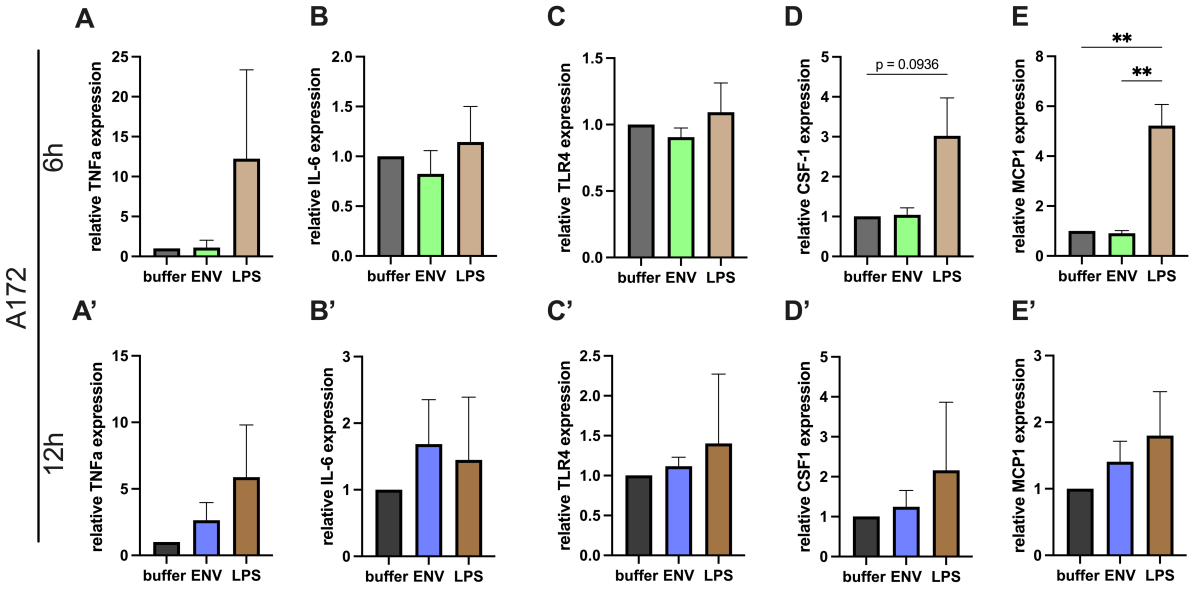


Figure 2. Modulation of gene expression in the human glioblastoma cell line A172 upon LPS stimulation but not in response to stimulation with HERV-W ENV. (A-E') The human glioblastoma cell line A172 was stimulated with either HERV-W ENV, LPS, or buffer for 6 or 12 hours, respectively. Results showed significant gene expression modulation by LPS after 6 hours, while stimulation with HERV-W ENV protein did not modify the gene expression of A172 glioblastoma cells. Data are mean values ± SEM and were considered significantly different at *$p \leq 0.05$, and ** $p \leq 0.01$ assessed with ANOVA. N=3.

In LN229 cells, gene expression was modulated most significantly by LPS after 6 hours (Fig. 3A-3B') with an induction of TNFa and IL-6 expression, which then weakened after 12 hours. In addition, the gene expression of MCP1 was upregulated upon LPS stimulation after 6 and 12 hours but was also significantly induced by HERV-W ENV stimulation after 12 hours (Fig. 3E and 3E'). None of the other genes showed expression changes upon HERV-W ENV stimulation. Additionally, CSF-1 and TLR4 expression

showed no modulation upon LPS or HERV-W ENV stimulation (Fig. 3C-3D'). Of note, the expression of TLR4 in this cell line was investigated in only one independent experiment.

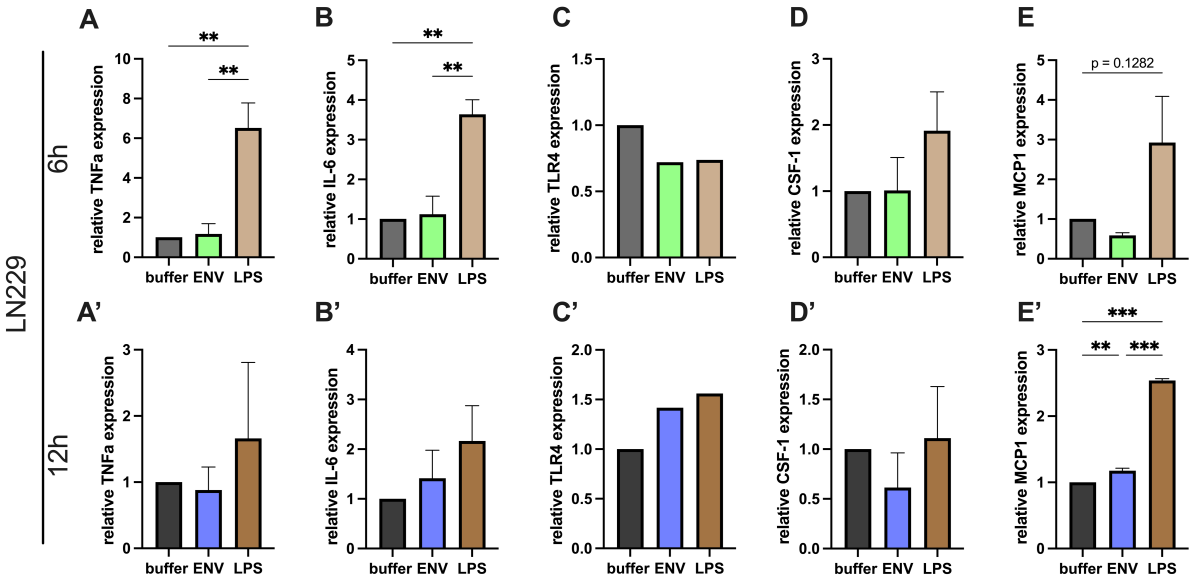


Figure 3. Gene expression changes in the human glioblastoma cell line LN229 upon HERV-W ENV and LPS stimulation. (A-E') The human glioblastoma cell line LN229 was stimulated with either HERV-W ENV, LPS, or buffer for 6 hours or 12 hours, respectively. Results showed gene expression modulation by LPS most significantly after 6 hours. Additionally, stimulation with HERV-W ENV protein significantly increased the expression of MCP1 after 12 hours compared to buffer control. Data are mean values ± SEM and were considered significantly different at *$p \leq 0.05$, ** $p \leq 0.01$, and *** $p \leq 0.001$ assessed with ANOVA. N=3 (except TLR4: N=1).

In T98 cells, LPS led to a similar gene expression modulation as in A172 cells, i.e., an upregulation of TNFa, IL-6, CSF-1, and MCP-1, which again weakened after 12 hours (Fig. 4A-4E'). However, stimulation with HERV-W ENV protein did not significantly modulate the gene expression of T98 glioblastoma cells.

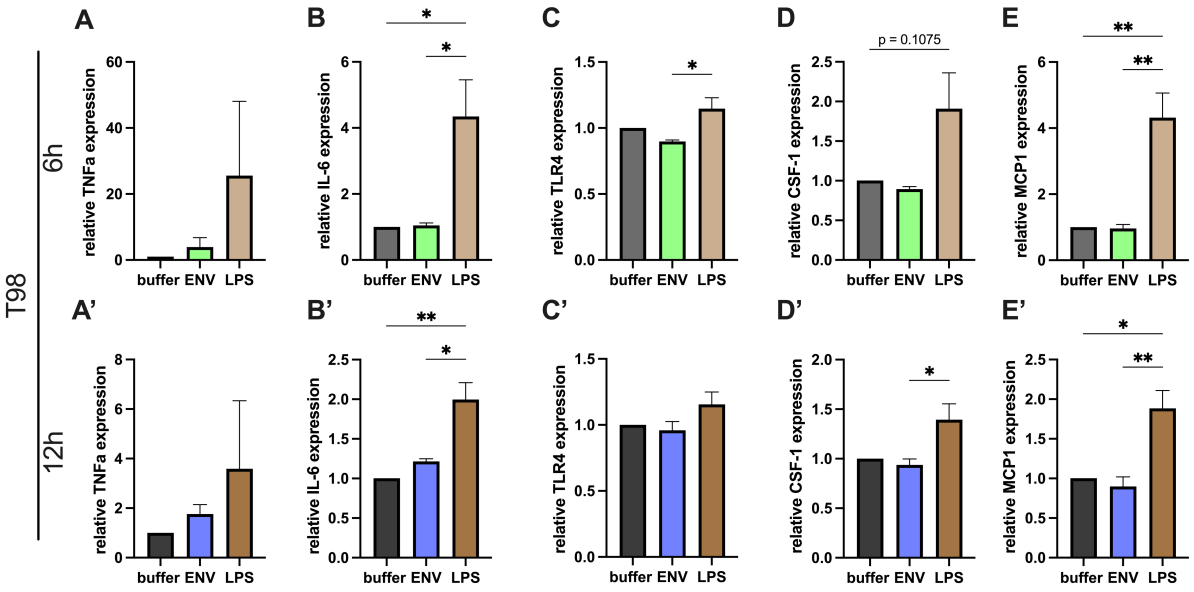


Figure 4. Gene expression modulation in the human glioblastoma cell line T98 upon LPS stimulation but not in response to HERV-W ENV stimulation. (A-E') The human glioblastoma cell line T98 was stimulated with either HERV-W ENV, LPS, or buffer for 6 hours or 12 hours, respectively. Results showed gene expression changes by LPS both after 6 and 12 hours. However, stimulation with HERV-W ENV protein did not modulate the gene expression of T98 glioblastoma cells. Data are mean values ± SEM and were considered significantly different at *$p \leq 0.05$, and ** $p \leq 0.01$, and assessed with ANOVA. N=3.

In contrast to the other cell lines, the U87 glioblastoma cell line did not show any significant changes in its gene expression profile, neither upon HERV-W ENV nor LPS stimulation except for a marginal upregulation of MCP-1 after 6 hours (Fig 5E). Of note, the gene expression in this cell line was investigated in only two independent experiments, thus, no appropriate statistical analysis could be conducted.

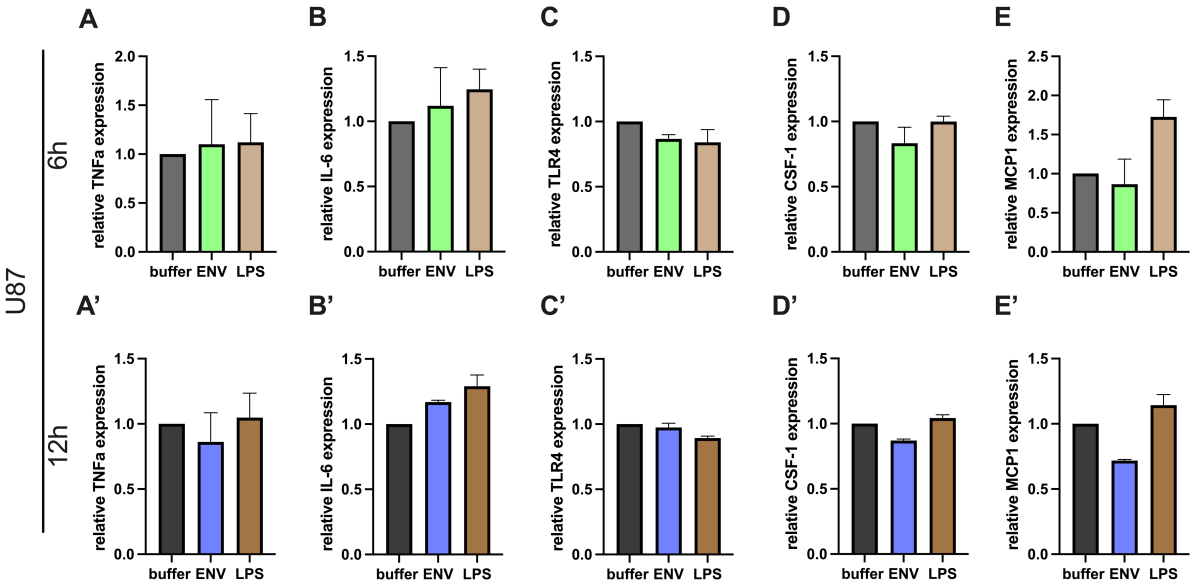


Figure 5. No change in gene expression in the human glioblastoma cell line U87 upon LPS and HERV-W ENV stimulation. (A-E') The human glioblastoma cell line U87 was stimulated with either HERV-W ENV, LPS, or buffer for 6 hours or 12 hours, respectively. Results showed that HERV-W ENV or LPS stimulation induced no gene expression changes after 6 or 12 hours. Data are mean values ± SEM. N=2.

In summary, HERV-W ENV stimulation was found to significantly induce MCP1 expression after 12 hours in LN229 cells (Fig. 3E'). Other measured genes such as TNFa, IL-6, TLR4, and CSF-1 underwent no modulation in any glioblastoma cell line upon HERV-W ENV stimulation. Interestingly, glioblastoma cell lines featured different temporal patterns of gene expression regulation in response to LPS stimulation, exhibiting a more significant modulation after 6 hours. In this regard, upregulation of MCP-1 was the strongest in A172, LN229, and T98 cells followed by IL-6. Notably, U87 glioblastoma cells showed no changes in gene expression upon LPS nor HERV-W ENV stimulation.

3.3. HERV-W ENV protein modulates glioblastoma gene expression profile via microglia

As it is known that HERV-W ENV exerts considerable effects on myeloid cells (Kremer et al. 2019) and since HERV-W ENV had failed to alter glioblastoma gene expression in monoculture (see 3.2) a coculture system was implemented to investigate whether HERV-W ENV would be able to indirectly modulate the gene expression of glioblastoma cells via microglia (Fig. 6). Since neoplastic cells and cells of the TME can communicate paracrinnaly and/or via direct cell contact, primary rat microglia were either directly cocultured on glioblastoma cells (+MG) or indirectly cultivated in cell culture inserts (*MG), preventing direct cell-cell interaction between microglia and tumor cells. In a first step, in order to investigate the gene expression changes of human glioblastoma cells following HERV-W ENV stimulation, the cells were either stimulated with 1000 ng/ml of recombinant HERV-W ENV or buffer in the absence (-MG) or presence (+MG and *MG) of rat microglia for 1d, 3d or 5d, respectively. Gene expression was then measured by qRT-PCR. As microglial viability in the *MG condition was found to be decreased after day one, the obtained results were not considered accurate and, therefore, not included. In general, HERV-W ENV stimulation of monocultured glioblastoma cells led to no significant gene regulation compared to controls, confirming the results of the previous chapter. However, gene expression profiles of tumor cells stimulated with HERV-W ENV were altered when they were in direct or indirect contact with microglia, leading to increased expression of genes linked to tumor progression, aggressiveness, survival (TNFα, IL-6, IL-1β, iNOS), tumor invasiveness and tumor microenvironment modulation (MCP-1, MCP-3, MMP9), and tumor growth (CSF-1). In this context, glioblastoma cell lines demonstrated a varying spatio-kinetic responsiveness to HERV-W ENV stimulation. In A172 cells, there was a significant regulation of genes on days 1 and 3 under the +MG condition, which notably diminished by day 5 (Fig. 6A). In contrast, LN229 cells exhibited a more scattered gene regulation, reaching significance for several genes on day 1 under both the +MG and *MG conditions (Fig. 6B). T98 cells displayed an even more scattered yet still significant gene induction on day 1 under the +MG condition, with a decrease in regulation by day 3 and further weakening by day 5 (Fig. 6C). Notably, in the *MG condition on day 1, A172 cells showed the overall most pronounced gene regulation, followed by LN229 and T98 cells. MCP1 consistently displayed the strongest upregulation across all cell lines and time

points, followed by IL-6, CSF-1, MMP9, and IL-1β, with MCP-3, TNFα, iNOS, and VEGF. Interestingly and in contrast, CX3CL1 tended to be downregulated upon stimulation with HERV-W ENV.

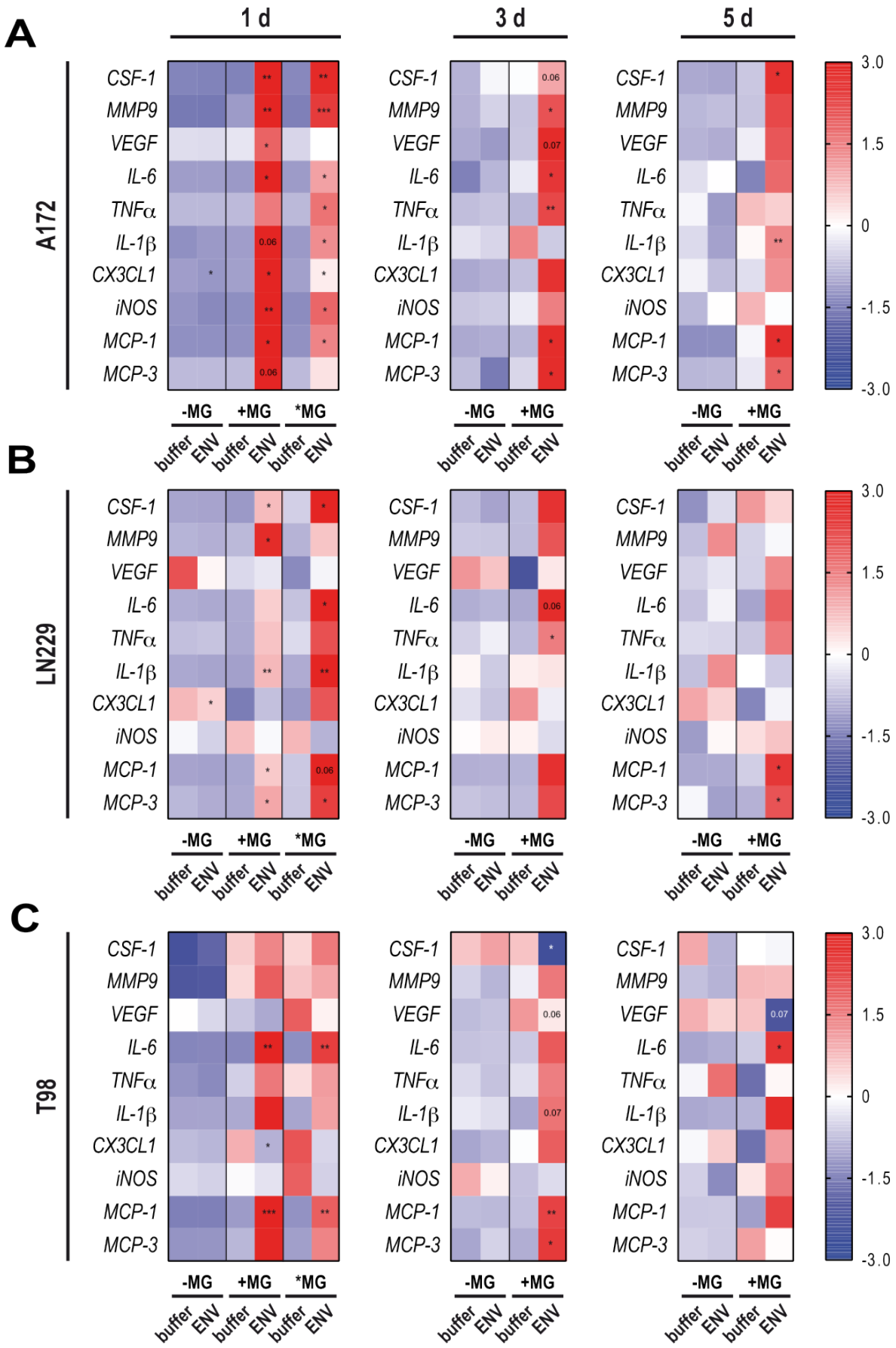

A
1 d
3 d
5 d
A172
CSF-1
MMP9
VEGF
IL-6
TNFα
IL-1β
CX3CL1
iNOS
MCP-1
MCP-3
-MG
+MG
*MG
buffer
ENV
3.0
1.5
0
-1.5
-3.0
B
LN229
C
T98

Figure 6. HERV-W ENV protein induces a microglia-dependent modulation of glioblastoma gene expression. (A-C) Human glioblastoma cell lines A172 (A), LN229 (B), and T98 (C) were stimulated with either HERV-W ENV protein or buffer in the absence (-MG) or presence (+MG and *MG) of rat microglia for 1, 3, or 5 days, respectively. This resulted in an increased expression of genes associated with tumor progression, aggressiveness, and survival (TNFα, IL-6, IL-1β, iNOS), genes associated with tumor invasiveness and TME modulation (MCP-1, MCP-3, MMP9), as well as genes associated with tumor growth (CSF-1). Data are presented as z-scores and were considered significantly different at *$p \leq 0.05$, ** $p \leq 0.01$, and *** $p \leq 0.001$ assessed with the one-sample t-test. The figure was modified according to Reiche et al. 2024.

3.4. HERV-W ENV protein modulates glioblastoma cytokine secretion via microglia

Further investigation of the impact of HERV-W ENV protein on human glioblastoma cells aimed at clarifying whether corresponding protein levels mirrored the previously found and above-described gene expression changes. To this end, the human glioblastoma cell lines A172, LN229, and T98 were stimulated with either 1000 ng/ml of recombinant HERV-W ENV protein or buffer in the presence (+MG) of rat microglia (Fig. 7). Of note, this experiment was not performed in glioblastoma monoculture due to the above-described lack of changes in gene expression in this setup. After 3 days, the cell culture medium was collected, and cytokine secretion was measured using array membranes detecting 42 distinct human cytokines, which enabled a comprehensive evaluation of cytokine secretion dynamics in response to HERV-W ENV stimulation. The results demonstrated that, in general, cytokine secretion changes mirrored the previously observed gene expression changes, although the respective dynamics differed significantly depending on the cell line.

In A172 cells (Fig. 7A), we found an induction of MCP-1 secretion and, even though generally modest, of MCP-3 and MCP-2, it increased consistently in response to HERV-W ENV, aligning with our gene regulation data. Interestingly, HERV-W ENV-stimulated cells featured an increase of IL-8 secretion, while IL-6 secretion decreased compared to controls. Other interleukins displayed only modest secretion levels, with IL-13 even being completely absent. The anti-inflammatory cytokine IL-10 was secreted at low levels,

though it tended to increase slightly upon HERV-W ENV stimulation. In contrast, TNFα levels tended to decrease compared to controls. The secretion of growth factors varied, with epidermal growth factor (EGF) secretion slightly decreasing and insulin-like growth factor 1 (IGF-1) remaining stable. Among factors associated with neoangiogenesis, angiogenin secretion showed a slight tendency to decrease, while VEGF and other angiogenic markers remained unaffected. Additionally, GM-CSF (CSF-2) secretion was undetectable.

LN229 cells displayed similar changes upon HERV-W ENV stimulation (Fig. 7B) with a slightly decreased secretion of MCP-1 but increased MCP-3 secretion, which was in line with gene regulation data. Furthermore, HERV-W ENV stimulation increased the secretion of proinflammatory cytokines such as IL-8 and IL-6 mirroring gene expression changes. On the other hand, the secretion of other interleukins remained modest, while there was no secretion of IL-13 at all, and IL-10 secretion increased compared to controls. Chemokines, including GRO α and the GRO mix (CXCL1-3), as well as growth factors such as epidermal growth factor (EGF) showed a notable secretion increase with the exception of insulin-like growth factor 1 (IGF-1), whose secretion remained unchanged. On the other hand, TNFα secretion was decreased. Regarding neoangiogenesis, angiogenin and VEGF levels showed a slight decrease upon HERV-W ENV stimulation. The secretion of GM-CSF (CSF-2) was increased, but no differential regulation of other angiogenesis-related proteins such as ENA-78, SDF-1, Leptin, and others was found.

In parallel to the other cell lines described above in T98 cells, HERV-W ENV stimulation resulted in an increased secretion of MCP-1 and MCP-3 (Fig. 7C). The secretion of the proinflammatory cytokine IL-8 was increased, while the secretion of IL-6 remained unchanged upon HERV-W ENV stimulation. The secretion of other interleukins was modest, with no IL-13 detected at all. Chemokines like GRO α and the GRO mix (CXCL1-3) showed increased secretion, while TNFα levels increased. The level of the secretion of growth factors such as epidermal growth factor (EGF) were elevated, but insulin-like growth factor 1 (IGF-1) levels remained unchanged compared to controls. Regarding neoangiogenesis, angiogenin secretion tended to decrease while VEGF levels showed no significant changes upon HERV-W ENV stimulation. Additionally, GM-CSF (CSF-2) secretion was increased, and G-CSF secretion was abundant in this cell line.

In summary, upon HERV-W ENV stimulation, A172 cells showed strong increases of MCP-1 and MCP-3 secretion, while T98 cells demonstrated only slight increases, and LN229 displayed a slightly decreased MCP-1 secretion but higher MCP-3 secretion. The secretion of the proinflammatory cytokines IL-8 increased across all three cell lines, reflecting gene expression changes. On the other hand, IL-6 secretion strongly decreased in A172, strongly increased in LN229, and remained unchanged in T98 cells. In contrast, TNFα levels decreased in A172 and LN229 cells and increased in T98 cells stimulated with HERV-W ENV. IL-10 secretion was low in A172 cells but tended to increase, while the high base levels of IL-10 secretion in LN229 cells further increased, and T98 cells remained unchanged. The secretion of growth factors showed varying responses: EGF increased in LN229 and T98 cells but slightly decreased in A172 cells, while IGF-1 remained unchanged across all three cell lines. Angiogenin levels decreased in all cell lines, and VEGF levels remained unchanged in A172 and T98 cells but decreased slightly in LN229 cells. GM-CSF secretion increased in LN229 and T98 cells but was absent in A172 cells. Other protein levels of ENA-78, I-309, SDF-1, Leptin, MDC, MIG, MIP-1, Oncostatin, PDGF, RANTES, SCF, and TARC were not altered in any of the investigated cell lines upon HERV-W ENV stimulation or showed inconclusive fluctuating secretion levels across different cell lines.

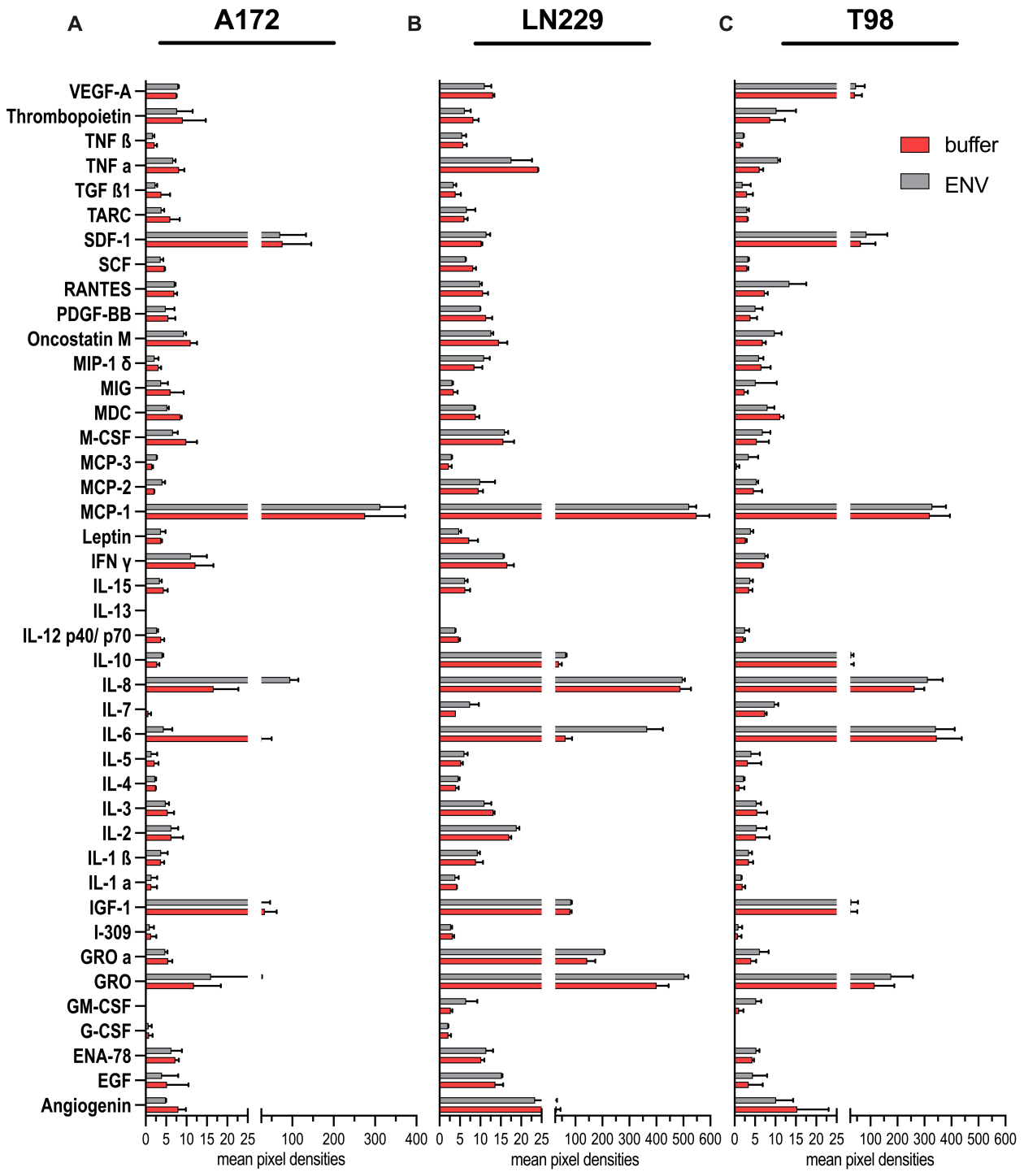


Figure 7. HERV-W ENV stimulation modulates the protein secretion of human glioblastoma cell lines in the presence of microglia. (A-C) Human glioblastoma cell lines (A) A172, (B) LN229, and (C) T98 were stimulated with either HERV-W ENV or buffer in the presence (+MG) of rat microglia for 3 days. Results showed a microglia-mediated modulation of glioblastoma cytokine secretion after HERV-W ENV protein stimulation, mirroring the previous gene expression results.

Data are shown as mean (±SEM) pixel densities of two biological replicates. The figure was modified according to Reiche et al. 2024.

3.5. HERV-W ENV protein leads to a microglia-dependent increase of glioblastoma migration velocity

The migration of tumor cells is a key factor in the formation and development of gliomas, leading to an infiltrative growth of neoplastic cells into the healthy brain (Demuth and Berens 2004). To assess the migration behavior of glioblastoma cells upon stimulation with HERV-W ENV in monoculture and coculture with microglia, human glioblastoma cell lines were either stimulated with 1000 ng/ml of recombinant HERV-W ENV or buffer in the absence (-MG) or presence (+MG) of rat microglia, respectively (Fig. 8). Of note, the U87 cells not only migrated within the cell insert, but also moved upwards along the cell insert, leading to the detachment of the cells from the well surface when the insert was eventually removed for analysis. As a result, no reliable results could be generated using this cell line. Beyond that, results showed that the A172 and LN229 cells did not change their migration velocity upon HERV-W ENV stimulation at any measured time regardless of the presence of microglia (Fig. 8A-B’’’). In T98, HERV-W ENV stimulation without microglia (-MG) did not significantly change migration velocity (Fig. 8C and 8C’). However, when microglia were present and cells were stimulated with HERV-W ENV, migration velocity was significantly increased (+MG), leading to a gap closure of ca. 80% after 16 hours compared to buffer controls (Fig. 8C’’). These changes could already be observed in the first analysis after 4 hours. Interestingly, in all cell lines, it was found that the mere presence of microglia (+MG) without any additional stimulation decreased wound closure and migration velocity. In contrast to T98 cells, in A172 and LN229 cells, stimulation with HERV-W ENV even led to a marginal, non-significant decrease in wound closure (Fig. 8A’’ and 8B’’).

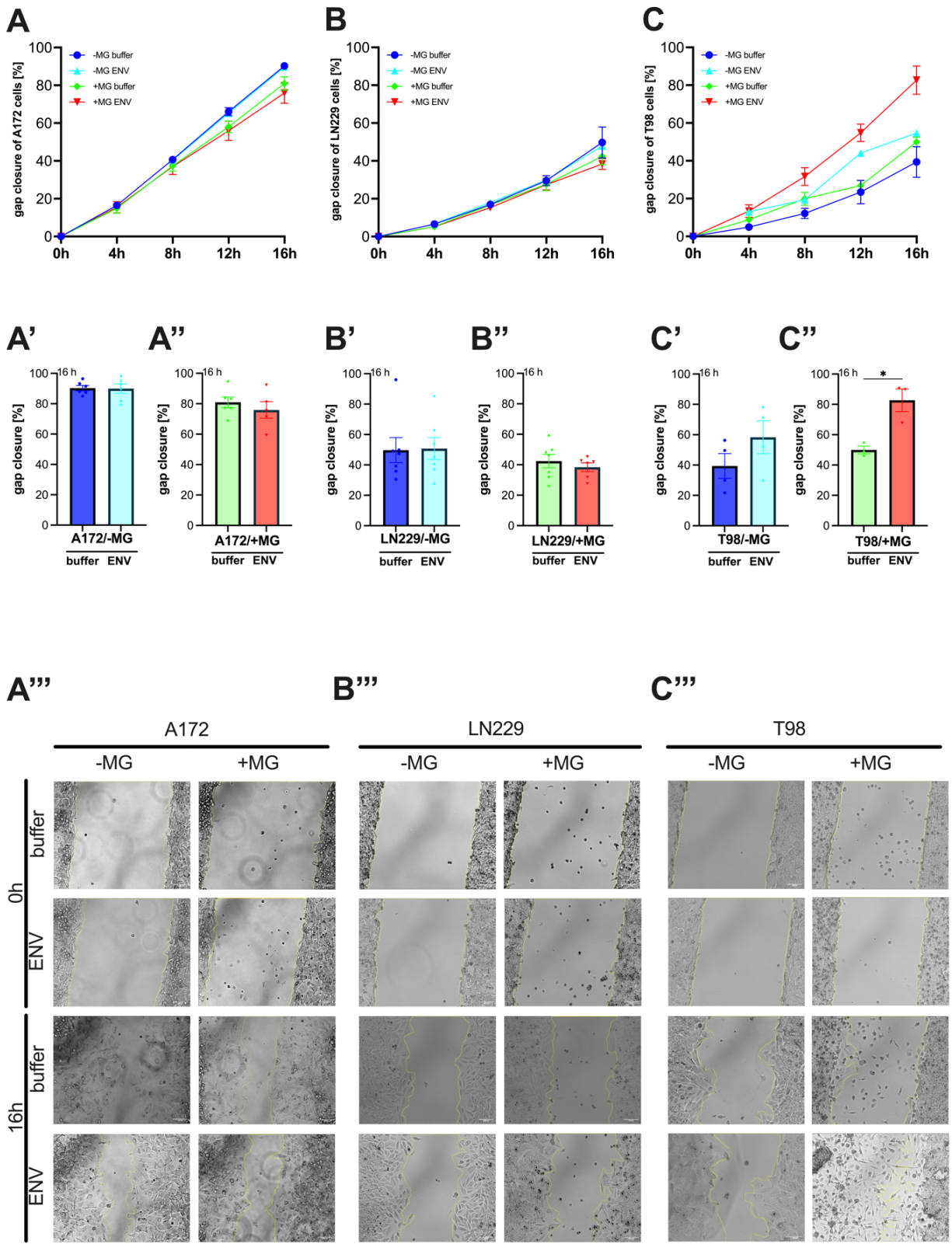


Figure 8. HERV-W ENV stimulation leads to a microglia-dependent increase in migration velocity of human glioblastoma cells. (A-C''') Human glioblastoma cell lines A172 (A-A'''),

LN229 (B-B’’’), and T98 (C-C’’’) were stimulated with either 1000 ng/ml of recombinant HERV-W ENV or buffer in the absence (-MG) or presence (+MG) of rat microglia, respectively. Culture inserts were used to create a predefined gap of 500 µm, and gap closure was measured after 4, 8, 12, and 16h. The gap closure rate over all time points is shown in (A, B, C). In contrast, the final gap closure after 16 hours is shown in (A’, A’’, B’, B’’, C’, C’’). Representative pictures of the gap closure after 16 hours are shown in (A’’’, B’’’, C’’’). Results demonstrated that HERV-W ENV stimulation led to a slight increase of migration velocity of T98 glioblastoma cells in monoculture (-MG). In contact with microglia (+MG), migration velocity of T98 cells increased significantly (C-C’’’). In contrast, the A172 and LN229 cells did not change their migratory behavior after HERV-W ENV (A-B’’’) stimulation. Data are displayed in percentage of the initial gap closed and shown as means (±SEM). Statistical significance was calculated using the Student’s two-sided, unpaired t-test: *p ≤ 0.05. N≥3. Scale bars: 100 µm. The figure was modified according to Reiche et al. 2024.

3.6. HERV-W ENV protein does not significantly modulate glioblastoma invasiveness

The invasion of glioma cells into the surrounding healthy brain tissue is another key characteristic of human glioma, which complicates surgical tumor resection, leading to less effective treatment (So et al. 2021). To investigate the impact of HERV-W ENV on the invasion behavior of glioma, human glioblastoma cell lines and rat microglia were stimulated with either 1000 ng/ml of recombinant HERV-W ENV or buffer for 24 hours, and transwell migration assays were performed. Results showed that neither the LN229 nor the T98 and U87 cell lines altered their invasion behavior in response to HERV-W ENV stimulation (Fig. 9A, B, C). Of note, the U87 cells showed a 20% decrease in their invasion rate upon stimulation with HERV-W ENV compared to controls (Fig. 9C) narrowly missing significance (p-value: 0.07). In summary, HERV-W ENV stimulation led to no significant modulation of the invasion behavior of glioblastoma cells.

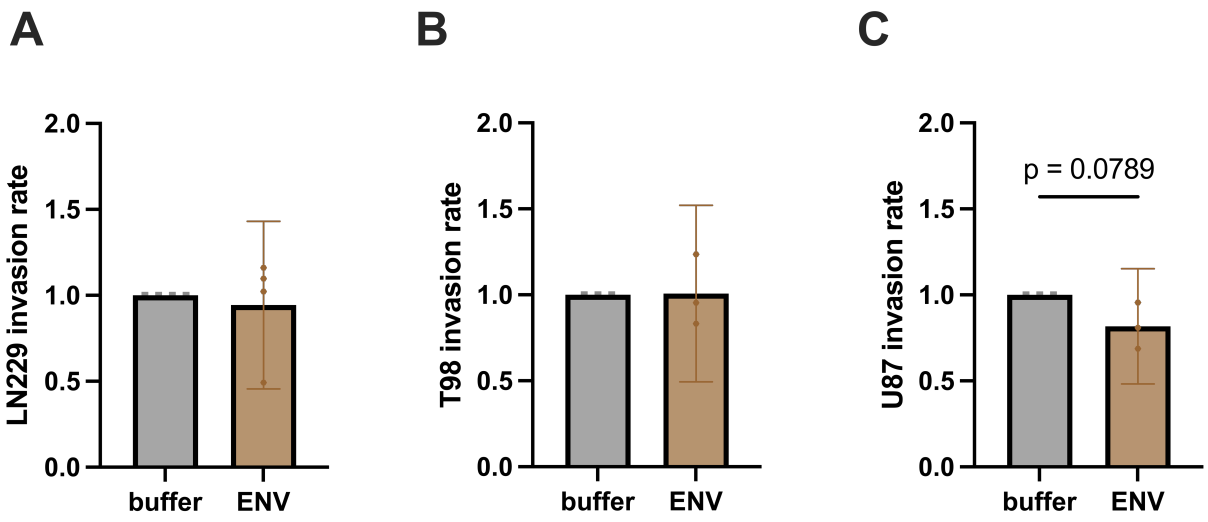


Figure 9. HERV-W ENV stimulation does not significantly modulate the invasion rate of human glioblastoma cells. (A-C) Human glioblastoma cell lines LN229 (A), T98 (B), and U87 (C) in the upper chamber and rat primary microglia in the lower chamber of a cell culture insert coated with Matrigel were stimulated with either HERV-W ENV or buffer for 24 hours. Results showed that HERV-W ENV stimulation exerted no significant effect on the invasiveness of LN229 and T98 cells (A, B) even though a decrease in the invasion rate of U87 glioblastoma cells was found (C). Data are shown as mean ± SEM relative to the buffer solution. Statistical significance was calculated using the Mann-Whitney U test. N=3.

3.7. HERV-W ENV protein promotes glioblastoma cell proliferation

Routine pathological analysis of glioma specimens includes the quantification of cell proliferation by labeling of the nuclear protein Ki-67 (Li et al. 2015). While not expressed by a resting cell in phase G0 (Gerdes et al. 1984), Ki-67 is expressed during all active phases of the cell cycle (Menon et al. 2019). Although the Ki-67 status *per se* is not a predictor of survival in glioblastoma patients (Alkhaibary et al. 2019), it has been a widely used proliferation marker for decades. To investigate how HERV-W ENV may change proliferation, human glioblastoma cell lines were either stimulated with 1000 ng/ml of recombinant HERV-W ENV or buffer in the absence (-MG) or presence (+MG and *MG) of rat microglia for 24 hours, respectively. Then, the proliferation rate of glioblastoma cells was assessed by immunocytochemical double-staining of Ki-67 and beta3-tubulin, while staining of beta3-tubulin was performed to investigate potential morphological changes of

glioblastoma cells upon HERV-W ENV stimulation. Our results demonstrated that in monoculture experiments, HERV-W ENV stimulation of A172 cells led to a significant decrease in Ki-67 expression (Fig. 10A). In contrast, stimulation with HERV-W ENV did not change the proliferation rate when A172 were in direct contact with microglia (+MG, Fig. 10A'). Finally, proliferation of A172 was nearly doubled in response to HERV-W ENV stimulation when tumor cells were in indirect contact with microglia (*MG; Fig. 10A''). In contrast, HERV-W ENV protein stimulation of the cell lines LN229 and T98 did not affect proliferation behavior either in the absence (-MG) or presence of microglia (+MG and *MG; Fig. 10B-C''). Of note, beta3-tubulin staining did not demonstrate morphological changes in any investigated cell line.

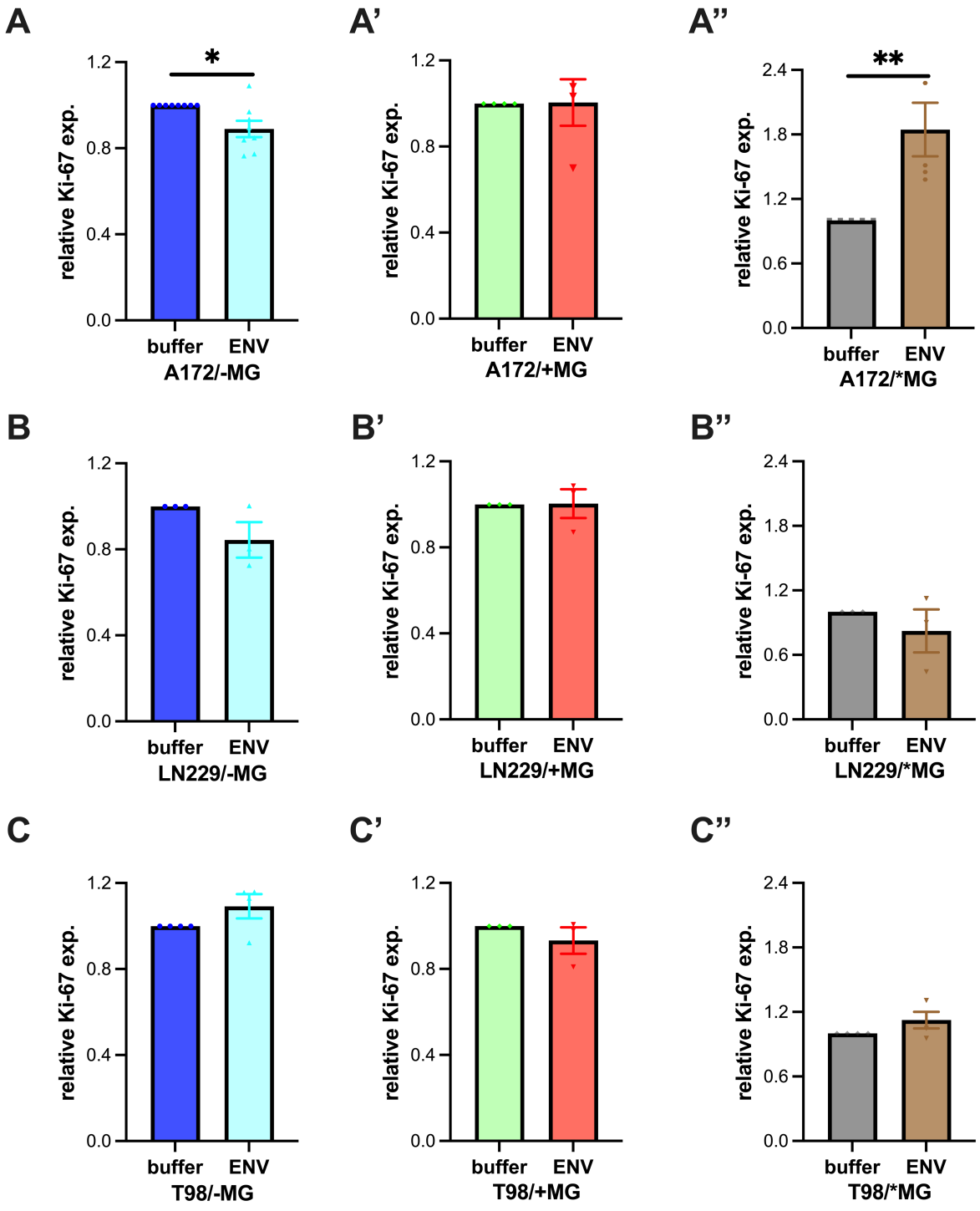
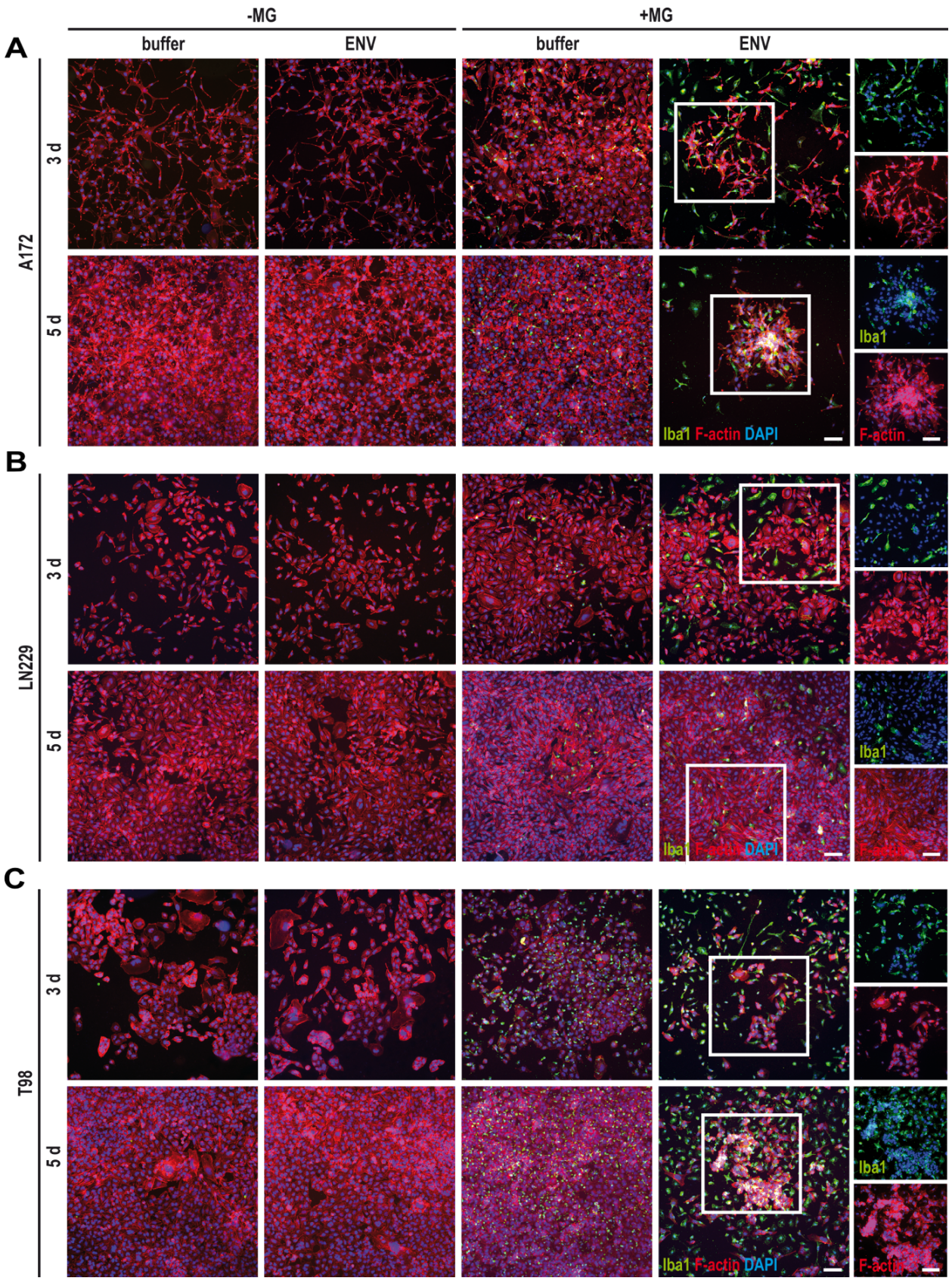


Figure 10. HERV-W ENV stimulation modulates the proliferation rate of human glioblastoma cells. (A-C'') Human glioblastoma cell lines A172 (A-A''), LN229 (B-B''), and T98 (C-C'') were stimulated with either 1000 ng/ml of recombinant HERV-W ENV or buffer for 24 hours in the absence (-MG) or presence (+MG and *MG) of rat microglia, respectively. The proliferation rate of glioblastoma cells was quantified by immunocytochemical staining of the nuclear marker Ki-67,

and the percentage of Ki-67-positive cells was calculated in relation to the nuclear marker 4′,6-Diamidin-2-phenylindol (DAPI). Results demonstrated that in monoculture experiments, HERV-W ENV stimulation of A172 cells led to a significant decrease in Ki-67 expression. In contrast, the proliferation rate of A172 cells almost doubled when interacting indirectly with microglia (*MG) upon stimulation with HERV-W ENV. In contrast, HERV-W ENV protein did not modify cell proliferation of LN229 and T98 cell lines. Data are shown as mean ± SEM relative to the buffer solution. Statistical significance was calculated using the Mann-Whitney U test: *$p \leq 0.05$, and ** $p \leq 0.01$. N≥3.

3.8. HERV-W ENV protein induces microglia-dependent clustering of glioblastoma cells

Since previous studies had shown that HERV-W ENV induces an amoeboid phenotype in microglia (Kremer et al. 2019), it was of interest to investigate whether such or comparable changes would also occur in glioblastoma cells. To this end, tumor cells were either stimulated with 1000 ng/ml of recombinant HERV-W ENV or buffer in the absence (-MG) or presence (+MG) of rat microglia for 3d or 5d, respectively. Immunocytochemical staining of the cytoskeletal marker F-actin and the microglial marker Iba1 was performed to analyze cell morphology and to differentiate between neoplastic and microglial cells based on cytoskeletal structure. In doing so, it was found that HERV-W ENV or buffer stimulation of T98 and A172 cells in the absence of microglia (-MG) did not change the spatial distribution or the morphology of tumor cells after 3 or 5 days (Fig. 11A and C). In contrast, direct microglial contact (+MG) combined with HERV-W ENV stimulation resulted in a progressive clustering of T98 and A172 tumor cells after 3 and 5 days, respectively, compared to buffer controls. Interestingly, LN229 cells did not show this clustering (Fig. 11B). On close inspection, it was then found that cell density after 3 and 5 days of HERV-W ENV stimulation in the presence of microglia was noticeably lower compared to controls. As elucidated above, stimulation with HERV-W ENV did not change the proliferation rate when tumor cells were in direct contact with microglia. Accordingly, in the next step, we wanted to investigate whether the effect on cell density could be explained by an upregulation of apoptosis or a downregulation of proliferation after three or five days of HERV-W ENV stimulation, respectively.


-MG
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B
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C
T98
3 d
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Iba1 F-actin DAPI
F-actin

Figure 11. HERV-W ENV stimulation leads to microglia-dependent clustering of human glioblastoma cell lines. (A-C) Human glioblastoma cell lines A172 (A), LN229 (B), and T98 (C) were stimulated with either HERV-W ENV or buffer in the absence (-MG) or presence (+MG) of rat microglia for 3 or 5 days, respectively. Results showed that HERV-W ENV stimulation of A172 and T98 cells (A, C) in contact with microglia results in progressive clustering of tumor cells after 3 and 5 days, respectively. In contrast, this effect could not be observed in the absence of microglia. Additionally, LN229 glioblastoma cells did not show any tendency of clustering (B). Regarding cell density, it was observed that in A172 and T98 cells, the number of tumor cells was reduced under the same experimental conditions. (F-actin: red; Iba1: green; DAPI: blue) All scale bars: 100 µm. The figure was modified according to Reiche et al. 2024.

3.9. HERV-W ENV protein does not affect glioblastoma cell apoptosis

In order to assess the potential role of apoptosis or proliferation in the above-described clustering of tumor cells, human glioblastoma cell lines A172, LN229, and T98 were either stimulated with 1000 ng/ml of recombinant HERV-W ENV or buffer in the presence (+MG) of rat microglia. After 5 days of stimulation, immunocytochemical staining of the proliferation marker Ki-67 and the apoptosis marker CC3 were performed to further study the cell clusters described in Fig. 11. Results showed that all cell lines expressed the nuclear proliferation marker Ki67, verifying the absence of a proliferation stop after 5 days of HERV-W ENV stimulation. However, the quantification of Ki-67 expression was technically unfeasible due to the above-described clustering of A172 and T98 cells. On the other hand, CC3-staining was neither detected in glioblastoma cells nor microglia (Fig. 12A-C).

To further clarify these observations, qRT-PCR was performed to study the gene expression of different markers associated with apoptosis, necroptosis, and glioma stem cells (GSCs). In doing so, it was found that in A172 cells (+MG), the apoptosis marker BAX was significantly upregulated after 5 days of stimulation with HERV-W ENV (Fig. 12A') but was downregulated under the same conditions in LN229 cells (Fig. 12B'). A similar result, but without statistical significance, was obtained for the CDKN1A gene, which was upregulated in A172 cells after 3 and 5 days but showed an upregulation after

3 days and a downregulation after 5 days in T98 and LN229 cells. Additionally, the necroptosis marker RIPK3 expression was upregulated in contact with microglia (+MG) in A172 and T98 cells after 5 days but downregulated in LN229 cells under the same conditions. However, results also showed that the expression of PTGS2, also known as Cyclooxygenase-2, was upregulated upon HERV-W ENV stimulation in all investigated cell lines in the presence of microglia. Again, there was evidence for an inflammatory phenotype shift, as the expression of CXCL8 (Interleukin 8) was found to be stimulated by HERV-W ENV in the presence of microglia. Regarding the observed clustering phenomenon, it was also of relevance to study the gene expression levels of integrins. However, the expression of the integrins ITGA5, ITGB3, and the cadherin CDH1 underwent only a slight, non-significant modulation following HERV-W ENV protein stimulation. In contrast, periostin (POSTN), which functions as a ligand for various integrins to support the adhesion and migration of epithelial cells, was downregulated in all investigated cell lines but only showed statistical significance in A172 cells stimulated with HERV-W ENV for 5 days. Lastly, there was no evidence of increased expression of the tumor stem cell marker CD133 and the chemokine CXCL12.

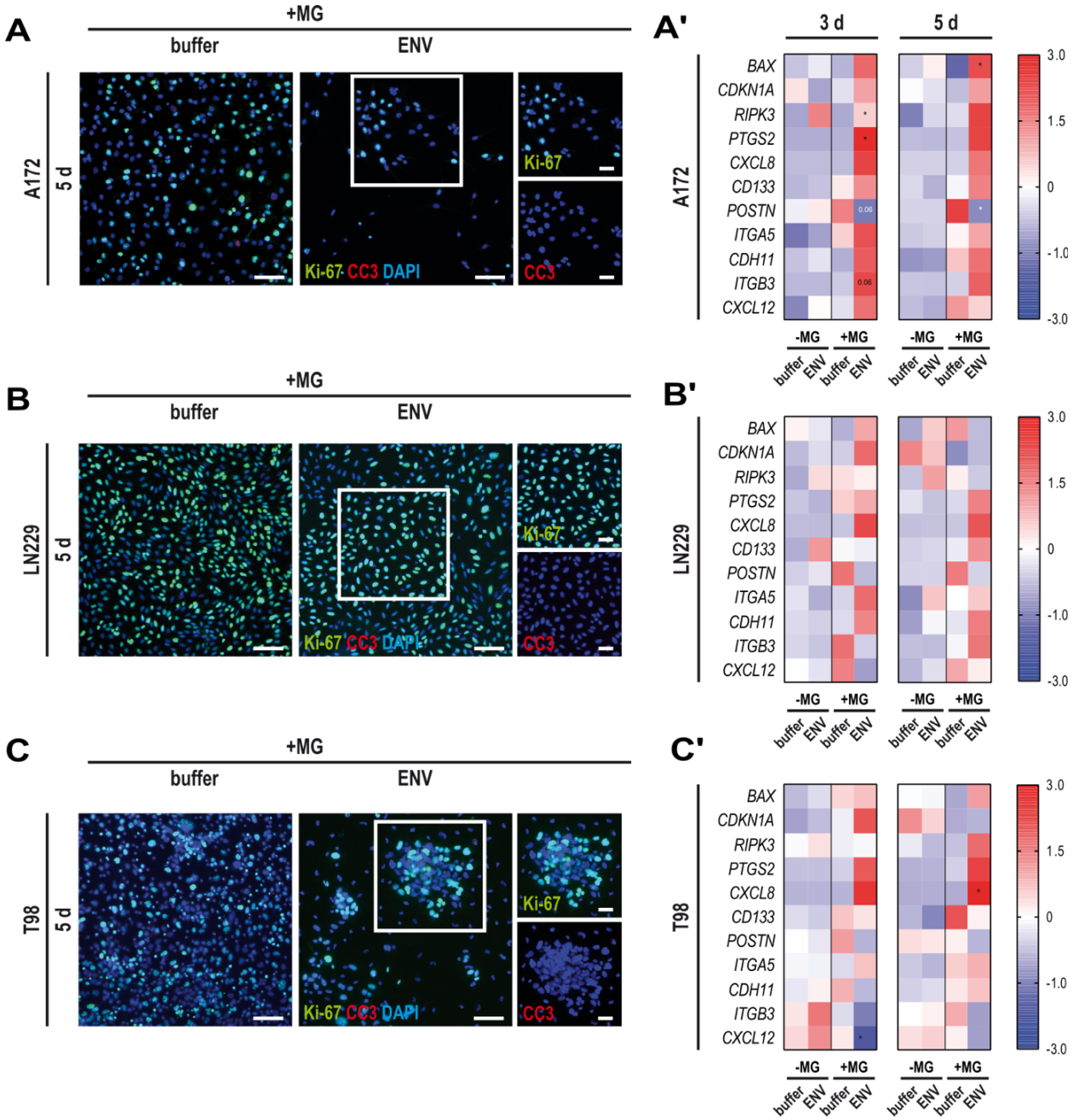

Figure 12. HERV-W ENV stimulation does not induce apoptosis of glioblastoma cells but inconsistently modulates apoptosis and necroptosis gene marker expression. (A-C') Human glioblastoma cell lines A172 (A, A'), LN229 (B, B'), and T98 (C, C') were stimulated with either HERV-W ENV or buffer in the absence (-MG) or presence (+MG) of rat microglia for 3 or 5 days, respectively. Results showed that glioblastoma cells still expressed Ki-67 after 5 days of stimulation with HERV-W ENV, but CC3-Staining was neither detected in glioblastoma cells nor microglia (A, B, C). Additionally, qRT-PCR of glioblastoma cells (+MG) showed that HERV-W ENV stimulation led to an inconsistent modulation of the expression of genes associated with apoptosis

and necroptosis (A', B', C'). Statistical significance was calculated using the one-sample t-test: *p ≤ 0.05. N=3. Scale bars: 100 µm and 50 µm for insets. (Ki67: green; CC3: red)

3.10. HERV-W ENV protein modulates glioblastoma cell survival upon temozolomide treatment

Temozolomide (TMZ) is the first-choice chemotherapeutic agent for glioblastoma and part of the standard therapy (Weller et al. 2021) where it induces DNA damage and, subsequently, the death of tumor cells (Tolcher et al. 2003). However, the treatment of glioblastomas is limited by TMZ resistance which has resulted in intensive research into the respective underlying molecular mechanisms (Singh et al. 2021). To investigate if the response of tumor cells to TMZ is modulated by HERV-W ENV, glioblastoma cells were first treated in monoculture with increasing concentrations of this agent (1 µM, 10 µM, 100 µM, 200 µM, 400 µM, 600 µM, 800 µM, 1000 µM). Three- and five-day analysis time points were chosen as the typical clinical treatment cycle is 5 consecutive days of therapy. Since microglia expectedly showed reduced viability under the influence of even low concentrations of TMZ, we could not follow the same co-culture approaches as used in the other above-described experiments. Accordingly, in order to mimic the previously observed paracrine effects of the microglial secretome (i.e. under the *MG condition), microglia were now stimulated separately in cell culture plates with HERV-W ENV or buffer without TMZ. After 24 hours, the microglia-conditioned cell culture medium was removed and filtered. TMZ was added to the medium, which was then used to treat glioblastoma cells (**MG). In general, and as expected, the obtained results demonstrated a dose-dependent effect of TMZ on glioblastoma cell viability, showing that cell survival was lower at higher concentrations of TMZ (Fig. 13A-D, 13A''-D''). In a next step, cells were treated with 1000 µM TMZ and simultaneously stimulated with 1000 ng/ml of recombinant HERV-W ENV or buffer, respectively.

Following this approach, in A172 cells, HERV-W ENV stimulation further reduced cell viability in both monoculture and in contact with microglia-conditioned medium upon TMZ treatment for 3 days. In more detail, combining microglia-conditioned medium (**MG) with TMZ treatment led to a 50% decrease of cell viability (Fig. 13A'). However, after 5 days

of treatment, cell viability remained unchanged regarding both stimulation with HERV-W ENV stimulation and/or microglia-conditioned medium compared to controls (Fig. 13A”’).

In LN229 cells, HERV-W ENV stimulation also led to a decrease of cell viability in monoculture under TMZ treatment similar to A172 cells. Adding microglia-conditioned medium then even further decreased cell viability by 20%, with a further slight decrease upon HERV-W ENV stimulation (Fig. 13B’). After 5 days, results demonstrated a slight decrease of cell viability in monoculture in response to HERV-W ENV stimulation. When adding microglia-conditioned medium, cell survival remained unchanged but was again decreased slightly upon HERV-W ENV stimulation (Fig. 13B”’).

In T98 cells, viability remained unchanged upon HERV-W ENV stimulation and TMZ treatment in monoculture but decreased modestly when cells were exposed to microglia-conditioned medium (**MG) after 3 days. Similar to A172 cells, combining microglia-conditioned medium and TMZ treatment, regardless of HERV-W ENV stimulation, resulted in a 50% reduction of cell viability. However, after 5 days of treatment, the results showed more variability. Here, HERV-W ENV stimulation of the monoculture was found to increase cell survival under TMZ treatment, with no significant changes in cell viability observed for either HERV-W ENV stimulation, **MG exposure, or their combination compared to controls.

Finally, in U87 cells, HERV-W ENV stimulation showed no effect on cell viability under TMZ treatment in monoculture. Adding microglia-conditioned medium reduced cell viability by 50% compared to controls, with an additional slight decrease following HERV-W ENV stimulation (Fig. 13D’). HERV-W ENV stimulation of U87 glioblastoma cells treated with TMZ for 5 days showed a slight increase of cell viability. When microglia-conditioned medium was added, cell survival was further increased compared to controls but decreased slightly again upon HERV-W ENV stimulation (Fig. 13D”’).

In summary, A172, T98, and U87 cells showed a similar behavior with a 50% cell viability reduction under **MG exposure after 3 days of TMZ treatment, which was further decreased by HERV-W ENV stimulation. LN229 cells showed a more modest 20% reduction upon **MG exposure, which was again slightly decreased following HERV-W

ENV stimulation. After 5 days of TMZ treatment, results were more scattered across all cell lines. Unlike the other cell lines, U87 cells demonstrated an increase of cell viability after 5 days of TMZ treatment with HERV-W ENV stimulation and **MG exposure. However, all experiments missed statistical significance.

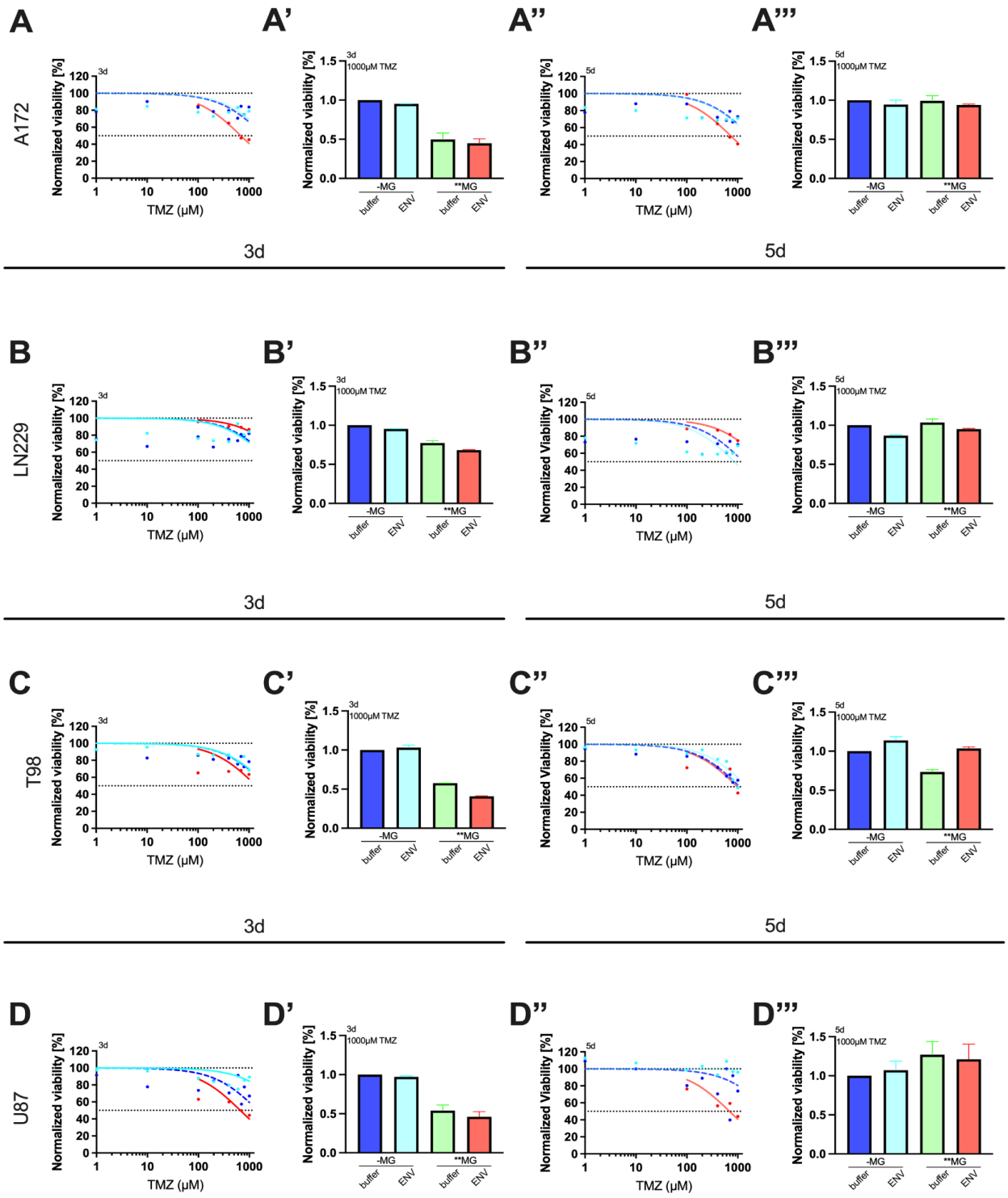

Figure 13. HERV-W ENV modulates the viability rate of glioblastoma cells upon temozolomide (TMZ) treatment. (A-D) Human glioblastoma cell lines A172 (A-A'''), LN229 (B-B'''), T98 (C-C'''), and U87 (D-D''') were treated with the chemotherapeutic agent temozolomide (TMZ) and stimulated with either HERV-W ENV or buffer in the absence of microglia (-MG) or with

microglia-conditioned medium (**MG) for 3 or 5 days, respectively. Viability was measured via MTT assay. Results showed an increased effectiveness of temozolomide treatment for 3 days in A172, T98, and U87 glioblastoma cells additionally treated with HERV-W ENV stimulated- and microglia-conditioned medium (Fig. 13A', C', D'). However, this effect was more scattered after 5 days of TMZ treatment. Statistical significance was calculated using the Student's two-sided, unpaired t-test. N=3.

3.11. HERV-W ENV protein modulates the gene expression profile of microglia in contact with glioblastoma cells

As already elucidated above, different cell types in the glioma TME play crucial roles in the survival, proliferation, migration, and invasion of neoplastic cancer cells and their response to therapy (Plaks et al. 2015). In this context, recent research not only focused on the behavior of tumor cells alone but also on the reciprocal effects between them and the TME (Sharma et al. 2023). Therefore, it was of great interest to investigate if HERV-W ENV is able to modulate the interplay between glioblastoma cells and the microglia of the TME and in how far microglial gene expression may change in this context. To this end, rat primary microglia were cocultured in direct contact (+MG) with the human glioblastoma cell lines A172, LN229, and T98 and stimulated with 1000 ng/ml of recombinant HERV-W ENV protein or buffer for 3 or 5 days, respectively. Then, qRT-PCR was used to investigate the gene expression changes of the rat microglia which revealed a strong modulation (Fig. 14). Notably, the interaction with different glioblastoma cell lines led to different microglial patterns of spatio-kinetic responsiveness. In response to contact with A172 cells and stimulation with HERV-W ENV for 3 days, microglia increased their expression of proinflammatory genes such as IL-6, TNFa, C3, and TGF-b with a more modest increase of gene regulation after 5 days. Other genes such as CD274 (PD-L1), FGF-2, MerTK, CSF-1, TREM2, and S100a10, which support a proinflammatory shift were also increased upon HERV-W ENV stimulation. On the other hand, HERV-W ENV was found to induce anti-inflammatory genes such as IL-10, Lipocalin-2 (Lcn-2), Arg1 and Serping-1 after 3 and 5 days. Regarding vascularization factors, HERV-W ENV increased the expression of ANG2 and VEGF both after 3 days, but ANG2 was downregulated after 5 days. Growth factors such as EGF and IGF-1 were induced after 5 days. However, the expression of the sphingosine-1-phosphate receptor (S1PR) was downregulated upon

HERV-W ENV stimulation. Similar to contact with A172 cells, microglia in contact with LN229 and stimulated with HERV-W ENV showed a pronounced upregulation of proinflammatory genes such as IL-6, TNFa, C3, and TGF-b with a more scattered regulation after 5 days. Additionally, genes supporting a proinflammatory profile, including CD274, FGF-2, and MerTK, were also upregulated after 3 days but again showed a more scattered regulation after 5 days. Conversely and also similar to contact with A172 cells, HERV-W ENV also induced anti-inflammatory genes like IL-10, Lcn-2, Arg1 and Serping-1. Regarding the vascularization factors, ANG2 was downregulated by day 3 and the expression of ANG2 and VEGF was increased after 5 days. Growth factors, including EGF and IGF-1, showed only modest regulation upon HERV-W ENV stimulation. In contrast, the expression of S1PR was downregulated following HERV-W ENV stimulation. Finally, microglia in contact with T98 cells and stimulated with HERV-W ENV also upregulated proinflammatory genes IL-6, TNFa, C3, and TGF-b after both 3 and 5 days. CD274, FgF-2, MerTK, TREM2, and S100a10, were also elevated following HERV-W ENV stimulation. At the same time and similar to the other cell lines, HERV-W ENV stimulation induced anti-inflammatory genes, such as IL-10, Lcn-2, Arg1 and Serping-1. ANG2 and VEGF were upregulated after both 3 and 5 days of HERV-W ENV stimulation and the growth factors EGF and IGF-1 were induced after 3 days. In contrast to the other investigated cell lines, the expression of S1PR was only downregulated following 3 days of HERV-W ENV stimulation and remained unchanged after 5 days.

Overall, HERV-W ENV stimulation was found to consistently lead to an increased microglial expression of proinflammatory genes (i.e. IL-6, TNFa, C3, TGF-ß) when cells were in direct contact with different glioblastoma cell lines. On the other hand, and at the same time, HERV-W ENV stimulation also induced anti-inflammatory genes (IL-10, Lcn-2, Arg 1 and Serping-1). Vascularization factors (ANG2, VEGF) were consistently upregulated in contact with T98 cells but showed scattered expression in contact with A172 and LN229 cells upon HERV-W ENV stimulation. Similarly, growth factors (EGF, IGF-1) were notably induced in contact with T98 cells while being regulated modestly in contact with A172 and LN229 cells. However, uniquely, the expression of S1PR was constantly downregulated in microglia.

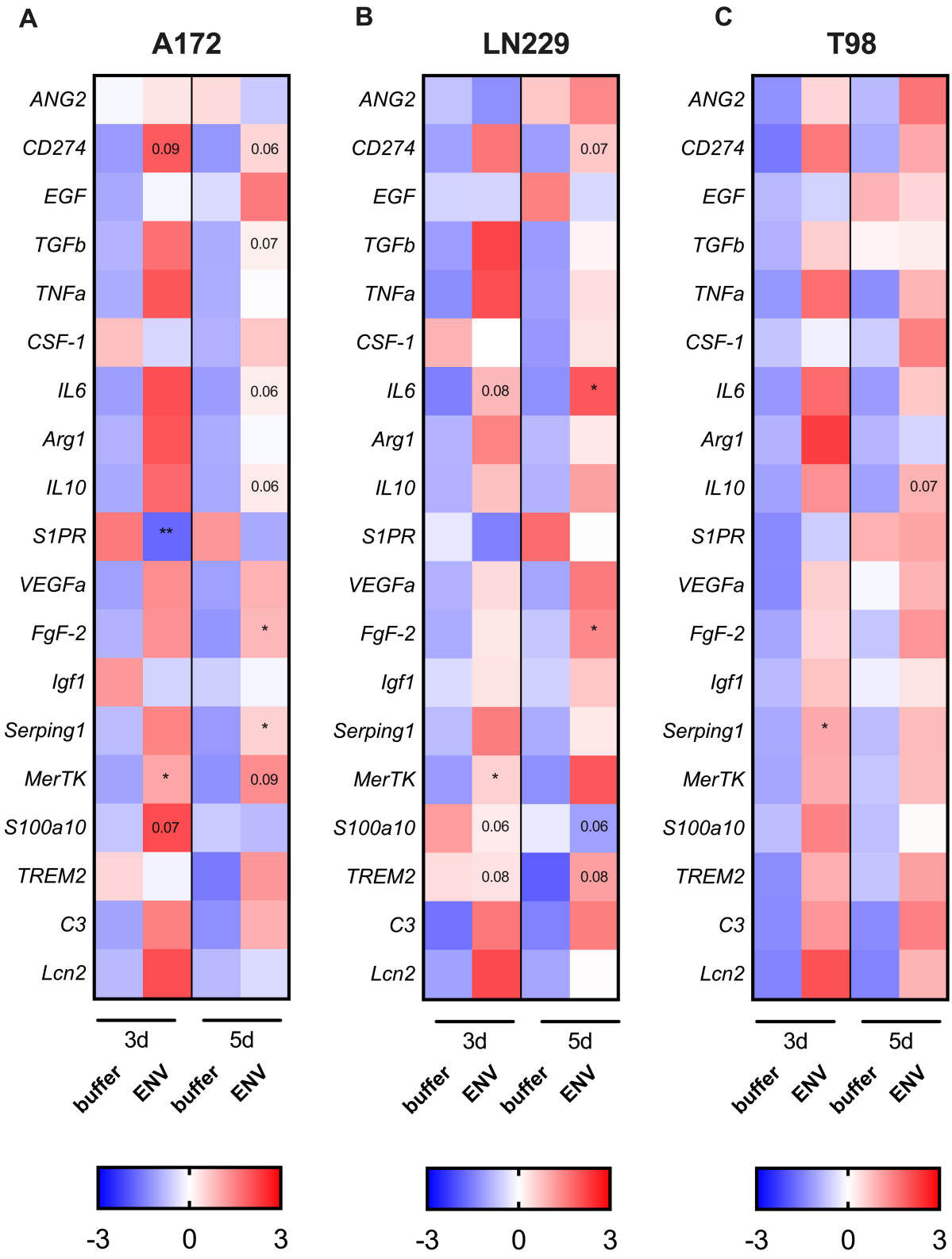

Figure 14. HERV-W ENV stimulation modulates gene expression of microglia cocultured with glioblastoma cells. (A-C) Primary rat microglia were cocultured in direct contact (+MG) with the human glioblastoma cell lines A172 (A), LN229 (B), and T98 (C) and stimulated with either

HERV-W ENV or buffer for 3 days or 5 days, respectively. Results showed an increased expression of genes associated with inflammation, growth factors, and vascularization factors. Data are presented as z-scores and were considered significantly different at *$p \leq 0.05$, and ** $p \leq 0.01$ assessed with the one-sample t-test. N=3.

4. Discussion

Glioblastomas, the most lethal human CNS tumors, are characterized by a highly infiltrative growth pattern of tumor cells and a dynamic interaction between the tumor and its TME. This study builds on previous results, which showed for the first time that the retroviral HERV-W ENV protein is expressed in glioma and, in particular, in the microglial cells of the TME (Reiche et al. 2024). HERV-W ENV was first described to be expressed in MS tissue, but over the course of the last decade, researchers identified other diseases in which it played a role in inflammation and disease progression. This is even the case for COVID-19, where researchers have demonstrated that HERV-W ENV expression correlates with disease outcomes. Interestingly, our research group found that HERV-W ENV expression in glioma is not associated with a higher WHO grade (Reiche et al. 2024), suggesting that the presence of HERV-W ENV does not result in a more malignant tumor phenotype. As a result, and in summary, the exact role of HERV-W ENV in gliomas still remains to be clarified.

4.1. HERV-W ENV modulates neoplastic cell behavior

In contrast to previously published studies that investigated the effects of HERV-W ENV stimulation on OPCs and MG (Förster 2020; Kremer et al. 2019; Kremer et al. 2013; Weyers 2024), this study showed that glioblastoma cells do not alter their gene expression upon HERV-W ENV protein stimulation in monoculture (Fig. 2-5), despite their expression of TLR4 (Fig.1). This somewhat unexpected finding may result from specific tumor cell characteristics. As glioblastoma cells show frequent genetic abnormalities due to chromosomal instability, various cellular functions as well as regulatory and oncological pathways are severely disrupted (Mazzoleni et al. 2024), which may result in less responsiveness to certain external stimuli. This argument is supported by the observation that even stimulation with the “classical” TLR4 agonist LPS resulted in only a modest gene regulation in glioblastoma cells compared to microglia (Fig. 2-5). Interestingly, the U87 cells show no changes in gene expression after stimulation with LPS at all, pointing to its degree of abnormality (Fig. 5). In this context, it should also be noted that this cell line has been strongly criticized in recent years as it was found not to be identical with its origin. Consequently, there is substantial doubt in the literature whether U87 cells are at all

suitable for modeling the *in vivo* behavior of glioblastomas (see 4.4.; (Allen et al. 2016)). Against this backdrop, this thesis focused primarily on the cell lines A172, LN229, and T98, although most experiments were also conducted with U87 cells. Another explanation for the above-mentioned lacking reaction of glioblastoma to HERV-W ENV protein stimulation in monoculture could involve a differential pathway activation in immune/neural precursor cells in comparison to neoplastic cells. This could be driven by the (relative) lack of additional or distinct functional receptors required for HERV-W ENV/TLR4 signaling such as, for instance, CD14, TLR4's co-receptor. Additionally, GBM cells are well-known to create an immunosuppressive microenvironment, characterized by a reduced response to proinflammatory signals. In this context, it is conceivable that autocrine signaling could inhibit the tumor cell response to HERV-W ENV (Lin et al. 2024).

With regard to the validity of the *in vitro* model chosen in this study, the glioblastoma TME is composed of a multitude of different cell types. These include, for instance, immune cells, microglia, astrocytes, and neurons, so that investigating glioblastoma monocultures would have constituted an oversimplification of the *in vivo* situation. Therefore, in order to at least partly address this problem, coculturing glioblastoma cells with primary neonatal rat microglia was performed. This approach was even more relevant as it had been shown previously that *in vivo* HERV-W ENV is mainly found in the microglia of the TME (Reiche et al. 2024). In this context, it was of particular interest to specifically investigate whether the effects of HERV-W ENV differ when microglia and glioblastoma cells are able to communicate directly through cell-cell interaction (+MG) versus via paracrine signaling (*MG). This difference is critical for a better understanding of the role of HERV-W ENV in the TME as paracrine signaling is based on soluble mediators such as cytokines or chemokines while direct signaling may also involve membrane-bound mediators such as integrins etc.. However, the data presented in this study demonstrate that these two modes of interaction did not result in substantially different transcription patterns (Fig. 6). The only difference found was a quantitative one, depending on the cell line: A172 and T98 cells showed a stronger reaction to direct cell-cell interaction (+MG) while LN229 cells reacted more vigorously to the indirect setting (*MG).
In general, the stimulation of GBM cells of all cell lines with HERV-W ENV in the direct or indirect presence of microglia (+MG and *MG, respectively), significantly increased their expression of the proinflammatory cytokines IL-1ß, IL-6, TNFa, and iNOS. As mentioned

above, the inflammatory environment in gliomas plays a crucial role in disabling anti-tumor responses and contributes to tumor invasion as well as progression (Alghamri et al. 2021; Yeo et al. 2021). In particular, the molecule Interleukin-6 produced and secreted by various cell types promotes glioblastoma growth, cell invasion, and angiogenesis (Goswami et al. 1998; Liu et al. 2010; Shan et al. 2015). Notably, elevated levels of IL-6 were found in the CSF, serum, and tissue of glioma patients, while higher IL-6 levels correlate with the WHO grade of gliomas and are therefore associated with poor survival (Cheng et al. 2016; Shan et al. 2015). As inflammation is a hallmark of cancer, mediators, such as reactive oxygen species (ROS), further contribute to the inflammatory milieu in glioblastoma tissue (Diakos et al. 2014; Grivennikov et al. 2010; Hanahan 2022). The overexpression of the inducible nitric oxide synthase (iNOS), which catalyzes the production of nitric oxide (NO) and therefore induces DNA damage (Aktan 2004; Khan et al. 2020), is associated with aggressiveness and chemoresistance of gliomas. Similarly, IL-1ß expression is associated with tumor progression, invasiveness, and reduced patient survival by activating the transcription factor NFκB (nuclear factor kappa-light-chain-enhancer of activated B-cells), which leads to the induction of even more proinflammatory genes (Griffin and Moynagh 2006; Tarassishin et al. 2014). In addition, interleukin-1ß triggers various tumorigenic processes by activating different cell types and inducing the upregulation of key molecules such as TGF-ß (Grochans et al. 2022; Tarassishin et al. 2014). In turn, TGF-ß is considered to play various roles in the TME, such as angiogenesis and immune evasion (Han et al. 2015), but also directly affects neoplastic cell behavior by inducing migration, extravasation, and an epithelial-to-mesenchymal transition (EMT) (Huber et al. 2005). Tumor necrosis factor alpha (TNFa) is yet another important cytokine in the glioma TME whose gene expression was upregulated after HERV-W ENV stimulation. This cytokine also stimulates tumor development and angiogenesis and modulates the immune response by increasing the expression of the major histocompatibility complex class (MHC-I) and by transcriptional activation (Șovrea et al. 2022; Yoshida et al. 1997). Additionally, TNFa is known to increase the activity of the epidermal growth factor receptor (EGFR) (Kore and Abraham 2014), whose abnormal signaling is widespread in GBM, and its inhibition is considered a promising therapeutic strategy (Ezzati et al. 2024).

The proinflammatory cytokines upregulated by HERV-W ENV protein stimulation also increase the expression of matrix metalloproteases (MMPs) such as MMP-9 that represent the majority of the extracellular matrix (ECM) components (Xue et al. 2017) and play a pivotal role in the degradation of the ECM (Ferrer et al. 2018; Mondal et al. 2020). The upregulation of these molecules leads to a higher invasion of neoplastic cells and correlates positively with tumor grade and negatively with patient survival (Zhou et al. 2019). In this context, it is important to underline the strong interdependence between gene expression and protein secretion of the proinflammatory cytokines, which are regulated in a mutually dependent fashion. In glioblastoma cells, this cycle is primarily sustained through autocrine secretion but can also be influenced by HERV-W ENV stimulation. Notably, HERV-W ENV can also regulate the expression of specific genes independently of this cycle. This is particularly evident in the expression of MMP9, which is strongly upregulated in the presence of microglia following HERV-W ENV stimulation and shows stronger expression in LN229 cells compared to the aforementioned cytokines. In addition, HERV-W ENV stimulation was found to increase the expression and secretion of Monocyte Chemotactic Protein 1 (MCP-1) and MCP-3. These cytokines/chemokines are associated with tumor development, invasion, metastasis, and angiogenesis (Vakilian et al. 2017). Notably, they are also involved in immune cell chemotaxis, which includes the recruitment of microglial cells into the TME (Friedmann-Morvinski and Hambardzumyan 2023; Maas et al. 2020; Matias et al. 2018).

Lastly, VEGF was found to be modulated upon HERV-W ENV stimulation in glioblastoma cell lines. This signal protein is the primary stimulator of angiogenesis, which is crucial for meeting the high metabolic demands of GBM (Șovrea et al. 2022). Through the interaction with its receptors, VEGFR1 and VEGFR2, VEGF plays a pivotal role in tumor survival, invasiveness, and progression (Melincovici et al. 2018; Șovrea et al. 2022).

Another aspect investigated in the context of HERV-W ENV stimulation was how it may alter neoplastic cell behavior by affecting functional parameters, such as migration, invasion, and proliferation. Glioblastoma cell migration, a key mechanism for tumor progression and invasion (Rosén et al. 2023), is mediated by cytoskeletal activity and integrin gene expression (Anderson et al. 2024), which showed only a slight modulation upon HERV-W ENV stimulation (Fig. 12A’, 12B’, 12C’). Results showed that HERV-W

ENV stimulation increased migration velocity in T98 glioblastoma cells, especially in the presence of microglia. However, no HERV-W ENV-mediated migration rate changes were observed in A172 or LN229 cells (Fig. 8). Of note, other studies found that the presence of microglia leads to a general increase of the migration velocity of glioma cells (Bettinger et al. 2002), which in this study could only be confirmed for T98 cells once more emphasizing cell line heterogeneity. Furthermore, tissue invasion represents one of the hallmarks of cancer (Hanahan and Weinberg 2000). The invasiveness of GBM cells not only leads to local destruction of healthy tissue and, therefore, complicates surgical treatment, but is also the main source of recurrence and considered a main factor for the bad prognosis (Demuth and Berens 2004). However, there was no significant modulation of the invasion rate of GBM cell lines upon HERV-W ENV stimulation, with only U87 cells showing a slight yet unsignificant decrease in their invasiveness (Fig. 9). In this context, these results do not only reflect tumor cell invasion behavior but also measure the chemoattracting ability of HERV-W ENV-stimulated microglia seeded in the lower chamber. In this regard, these experiments only mimic the invasion of neoplastic cells through the ECM towards HERV-W ENV-positive microglia as they were not carried out with direct contact between tumor cells and microglia. However, it is very conceivable that the ability of tumor cells to invade and degrade the ECM would be modulated differently by HERV-W ENV through direct cell-to-cell contact with microglia. Since this experimental setup was technically unfeasible in this study, future research could address this aspect by fluorescently labelling glioblastoma cells (e.g., with GFP) and quantifying only the labeled cells that invaded the Matrigel-coated cell culture insert. Additionally, this study also did not investigate the effect of HERV-W ENV on the invasion behavior of microglia, which are themselves known to promote ECM degradation and migration of glioblastoma cells (Andersen et al. 2021). Furthermore, it should also be noted that the transwell migration assays used here are a rather artificial simulation of the *in vivo* setting. In this context, three-dimensional models may be more suitable and could, for instance, also better investigate perivascular invasion of glioblastoma cells (Vollmann-Zwerenz et al. 2020).

Regarding proliferation behavior, the mitotic activity of neoplastic cells is considered a key factor contributing to the progression of gliomas (Xie et al. 2014). To this end, the overexpression of the nuclear protein Ki-67 can predict the overall survival of glioma

patients (Chen et al. 2015). This study showed that HERV-W ENV increased Ki-67 expression in A172 cells but only when they were in indirect contact with microglia (*MG). This once again showcases the microglial-mediated effect of the HERV-W ENV protein on glioblastoma cells but also demonstrates the heterogeneity of glioblastoma cell lines. On the other hand, prolonged HERV-W ENV stimulation decreased cell density without consistently and significantly triggering apoptosis or necroptosis, suggesting alternative mechanisms such as, for instance, cell fusion, a known effect of the HERV-W ENV protein associated with tumor development and progression (Grandi and Tramontano 2018). In more detail, the observed tumor cell clusters may result from a modulation of integrin gene expression and other proteins associated with tumor cell morphology. However, whether tumor cell fusion may mediate reduced therapy response and support tumor progression is still speculative. Further studies are therefore required to clarify the biology of tumor cell clusters - all the more so as similar structures were found circulating in the blood (circulating tumor cells; CTCs; (Krol et al. 2018)).

In conclusion, HERV-W ENV stimulation of GBM cells in the presence of microglia leads to an increased expression/secretion of several genes/proteins associated with tumor development, tumor aggressiveness, tumor progression, and immune evasion, which have been linked to worse overall patient survival. On a functional level, these changes result in an increase of migration velocity and proliferation, while invasion and apoptosis seem to remain unaffected. In this context, the results presented here prove that microglia are key for the effects of HERV-W ENV on glioblastoma cells. Moreover, they demonstrate that these effects do not require direct cell-to-cell interaction between microglia and tumor cells but can also be mediated by paracrine signaling. As the naturally occurring complex cellular interactions in the TME had to be significantly simplified by focusing only on microglia-glioblastoma interactions, further experiments analyzing the impact of HERV-W ENV on the TME in greater detail will need to also include other cell types, such as, for instance, astrocytes and T-cells.

4.2. HERV-W ENV induces a proinflammatory phenotype in microglia

Tumor-associated macrophages (TAMs) represent the biggest immune cell population in the TME of human glioma (Hambardzumyan et al. 2016). As evidenced in multiple sclerosis (MS) research, microglia undergo several changes upon HERV-W ENV stimulation, such as increased proliferation and the induction of a proinflammatory phenotype contributing to axonal damage (Kremer et al. 2019). Against this backdrop, it was critical to investigate potential HERV-W ENV-induced microglial phenotype changes in the human glioma TME in order to clarify its impact on gliomagenesis. Traditionally, microglia were categorized into an M1 and an M2 phenotype, with M2 microglia being associated with poor survival and aggressive glioblastoma (Sørensen et al. 2018). This rather artificial classification of microglia has recently attracted more and more criticism and is now considered insufficient to represent the great diversity of microglial phenotypes (Cassetta and Pollard 2018; Paolicelli et al. 2022). In this context, this study found that HERV-W ENV induces a proinflammatory gene expression profile in microglia/ glioblastoma coculture experiments (Fig. 14) as evidenced by the induction of the proinflammatory genes IL-6, TNFa, C3, and TGF-ß. TGF-ß, in turn, induces glioblastoma cell mesenchymal transition by activating the TNFa/NFκB signaling pathway, promoting glioma progression (Chao et al. 2021; Yan et al. 2022). In addition, and as described above, TNFa supports the creation of a tumor-supportive environment and induces angiogenesis (Șovrea et al. 2022). Regarding the latter aspect, the vascularization of glioblastoma is crucial for tumor progression and is further promoted by the expression of VEGF and ANG2 (Ahir et al. 2020), both of which were found to be significantly upregulated in microglia stimulated with HERV-W ENV. Moreover, HERV-W ENV stimulation also resulted in an upregulation of the expression of other growth factors, such as epidermal growth factor (EGF) and insulin-like growth factor 1 (IGF-1). These growth factors are considered to affect the cellular metabolism of neoplastic cells and promote malignancy (Liu et al. 2024; Tsuchihashi et al. 2016). Furthermore, HERV-W ENV stimulation induced the microglial expression of C3, which is a key component of the complement system and, therefore, a relevant factor for the effectiveness of the innate immune system (Ricklin et al. 2016). It is associated with more aggressive tumor behavior in general but has not yet been investigated systematically regarding glioma (Zhu et al.

2021). The expression of IL-10, which is, *per se,* an anti-inflammatory protein, was also found to be upregulated upon HERV-W ENV stimulation. However, in the context of glioma, TAMs secrete Il-10 to promote glioma cell proliferation (Qi et al. 2016). Notably, anti-inflammatory proteins, in general, may suppress the patient's immune response and can, therefore, contribute to glioma progression (Huettner et al. 1995). Additionally, Serping-1, a serine protease inhibitor gene that physiologically inhibits the complex system cascade (Gorelik et al. 2017), was upregulated upon HERV-W ENV stimulation, but its expression in glioblastoma is still elusive. Additionally, Lipocalin-2 (LCN2) is also a key marker for neuroinflammation and leads to the activation of neurotoxic microglia (Kim et al. 2023). However, regarding glioma, the overexpression of this gene in neoplastic cells is associated with reduced migration, invasion, and proliferation (Hsieh et al. 2021).

Another gene whose expression tended to be upregulated upon HERV-W ENV stimulation was triggering receptor expressed on myeloid cell 2 gene (TREM2). TREM2 is a myeloid cell-specific signaling molecule mostly associated with neurogenerative disorders such as Alzheimer's disease, which physiologically controls essential functions of microglia such as phagocytosis (Ulland et al. 2017). In glioma, the expression of TREM2 is associated with poor prognosis and promotes tumor angiogenesis (Chen et al. 2023; Yu et al. 2022). Upon HERV-W ENV stimulation, microglia also tend to downregulate their sphingosine-1-phosphate receptor (S1PR) gene expression. This might contribute to a more favorable patient outcome (Arseni et al. 2023) as pathways activated by S1PR are considered to play an oncogenic role in various human malignancies (Mahajan-Thakur et al. 2017). The cellular processes modulated by the activation of this receptor lead to more aggressive tumor behavior and represent a therapeutic target (Mahajan-Thakur et al. 2017; Wang et al. 2019). In addition, HERV-W ENV led to an increase of the expression of fibroblast growth factor 2 (FGF-2), which plays a crucial role in the self-renewal of various stem cell types by regulating signaling pathways maintaining tissue homeostasis (Jimenez-Pascual et al. 2020). Consequently, FGF-2 upregulation is linked to increased proliferation of astrocytes and glioma cells (Joy et al. 1997), and targeting this factor could enhance glioblastoma therapy (Jimenez-Pascual et al. 2020). The same applies to MerTK, a type I Receptor Tyrosine Kinase (RTK), whose inhibition has shown therapeutic benefits in preclinical mouse models (Wu et al. 2018). MerTK, crucial for signal transduction, functions homeostatically in normal cells and pathophysiologically in both tumor-

associated macrophages and malignant cells (Lahey et al. 2022). Its overexpression promotes tissue invasion (Wang et al. 2013) and is associated with tumor recurrence (Wu et al. 2018). Lastly, even though S100A10 showed a scattered gene expression modulation upon HERV-W ENV stimulation, its expression is *per se* linked to malignant pathological subtypes and poorer prognosis, as well as to immunosuppressive immune cell infiltration. This makes S100A10 a promising biomarker and potential target for the diagnosis, treatment, and prognostic evaluation of gliomas (Ma et al. 2022).

In summary, the HERV-W ENV-mediated gene expression profile of microglia in coculture with glioblastoma cells appears to be similar to the one previously described in microglia monoculture. As already elucidated, in glioblastoma, the local resident immune cells are known to express genes associated with glioma progression and aggressiveness. In this context, both proinflammatory and anti-inflammatory genes are upregulated, which, at first glance, seems to be contradictory. However, in reality, these genes act synergistically as the pro-inflammatory cytokines lead to faster and more aggressive tumor progression while the anti-inflammatory cytokines lead to immunosuppression, which also supports tumor progression. In this study, it was found that HERV-W ENV leads to a modulation of the gene expression of both tumor cells and immune cells of the TME. However, since only the average expression values were calculated in this study, HERV-W ENV may very well exert differential effects on different microglial subpopulations *in vivo,* including the alteration of their overall ratio. Further experiments are therefore required to investigate the specific effects of HERV-W ENV on different microglial populations and to identify which populations in the TME express HERV-W ENV at all. To investigate this in more detail, microglia could first be polarized toward either an M1 or M2 phenotype before being separately stimulated with HERV-W ENV. Additionally, it remains unclear whether HERV-W ENV is actively expressed by cells within the TME or if its presence is merely coincidental, with other mechanisms or cells driving its reactivation.

4.3. Does HERV-W ENV modulate glioblastoma treatment?

Glioblastomas are usually treated by surgical removal of the tumor tissue followed by a combination of chemotherapy with temozolomide (TMZ) and radiotherapy (Weller et al. 2021). In this context, the results presented in this study suggest that HERV-W ENV may

affect the response of glioblastoma cells to TMZ therapy (Fig. 13). This is based on the observation that in most cell lines exposed to TMZ overall cellular viability was found to decrease further upon contact with HERV-W ENV – notably without reaching statistical significance. However, the interpretation of these results is hampered by the restrictions of the *in vitro* setting, which is difficult to translate to clinical reality. In this regard, due to the *in vitro* instability of microglia, only one 3/5-day treatment cycle with TMZ could be simulated while, clinically, TMZ is applied for 5 days over several cycles and combined with radiotherapy as pointed out above (Stupp et al. 2005). As a result, more experiments are required, as there is the possibility that direct microglial cell contact is decisive for HERV-W ENV-mediated effects. This aspect could be investigated in more detail by blocking cell-cell interaction with antibodies or small molecule inhibitors targeting integrins, cadherins, or other adhesion molecules to disrupt direct contact in the glioblastoma/microglia coculture. Therefore, further research is needed to investigate to what extent HERV-W ENV-stimulated glioblastoma cells may alter their gene expression following prolonged TMZ exposition. Furthermore, the probably greatest challenge in glioblastoma therapy consists in the treatment of TMZ-resistant and recurrent glioblastoma (Osuka and Van Meir 2017), which was not analyzed in this thesis. Another aspect future research should address is the effect of TMZ on HERV-W ENV-stimulated microglia as the microglial secretome was shown to modulate chemotherapy response. Whether HERV-W ENV-stimulated microglia may have a survival advantage over controls under TMZ exposition and whether this could lead to a lower response to therapy remains currently unknown.

With regard to emerging treatment options, immunotherapy of malignancies is increasingly considered a therapeutic strategy that may have a significant impact on patient survival (Pardoll 2012). However, in glioblastoma, recent studies show that tumors are immunologically “cold” and therefore rarely respond to immunotherapy. Accordingly, immune checkpoint inhibitors showed no improved survival (Lim et al. 2022), which is likely to result from the immunosuppressive properties of the TME (Jackson et al. 2019). In this regard, researchers have suggested overcoming the “cold” TME with immunocytokines (Look et al. 2023; Weiss et al. 2020) or combining multiple therapy strategies (Weller et al. 2024). It is therefore tempting to speculate that HERV-W ENV could modulate the response of glioblastoma to immunotherapy by increasing the

expression of proinflammatory genes in the tumor and microglial cells of the TME – a question that certainly merits future research.

In summary, HERV-W ENV may slightly increase temozolomide's effectiveness by modulating the TME. Further studies should, therefore, investigate the extent to which this may lead to a better response to the standard therapy regimen or modern immunotherapeutic agents.

4.4. Limitations of glioblastoma cell line culture

Tumor cell lines are regularly used to model and investigate tumor cell behavior *in vitro*. To ensure validity, we selected a panel of four different cell lines that displayed differences in gene expression, protein secretion, and cell behavior upon HERV-W ENV stimulation. Notably, the selection of experimental glioblastoma cell lines, in general, constitutes a challenge in glioma/glioblastoma research. There is a wide range of commercially available glioma cell lines, including A172, T98, LN229, SNB19, U87, U251, TS543, SF188, SNB-19, U373, and U251. These cell lines consist of clonal cell populations, which do not represent the intratumoral heterogeneity observed in patient tumors. Additionally, patient-derived lines are commonly used to decrease the risk of genetically modified cells that may result from frequent and prolonged cultivation and propagation. In general, the choice of cell line should primarily be based on its biological relevance and similarity to the original tissue. However, the field is currently facing somewhat of a reproducibility crisis in this regard. For example, Allen et al. reported that the widely used U87 cell line no longer matches the cells from which it was originally derived nearly 50 years ago, having been established from a Swedish female but now exhibiting a Y chromosome (Allen et al. 2016). Similarly, the U-251 cell line has been identified to be identical with the U-373 cell line - probably due to contamination in the past, with multiple subclones exhibiting variations in both genotype and phenotype (Torsvik et al. 2014). This aspect is further complicated by the well-documented heterogeneity of GBM cell lines, which exhibit significant variations in their characteristics, as also reflected in this study. To date, no study has directly compared A172, LN229, T98, and U87 cells in terms of their omics profiles and functional behavior. Furthermore, the differences between glioblastoma cell lines raise the question of how translatable our findings are to human tumors, given the

diverse responses of the investigated cell lines to HERV-W ENV. The same concern applies to future studies exploring the effects of HERV-W ENV in mouse tumor models. This issue is highly significant in glioblastoma research, where many preclinical studies have shown promising results, yet the translation into clinical trials has frequently failed as early as Phase I.

4.5. Future perspectives

Glioblastomas feature a highly proliferative and dynamic network of different cell types in variable cellular states. In this study, the effect of HERV-W ENV on human glioma cells and rat microglia was investigated with a clear focus on the short-term effects of this protein. In contrast, its long-term impact could not be studied due to technical reasons associated with the fragile nature of microglial cells *in vitro*. However, this aspect is of relevance as this study, with its relatively short stimulation periods, already pointed to complex temporal reaction patterns. In addition, and even more importantly, the previous study by Reiche and colleagues found HERV-W ENV in glioblastoma tissue where it is highly likely to exert long-term effects on the surrounding cell populations. Notably, since Reiche and colleagues and this study are the first to investigate HERV-W ENV in glioblastoma, nothing is known about the temporospatial dynamics of this protein in the brain parenchyma – including its exact cellular origin. Future *ex vivo* studies could, therefore, be useful in investigating how and where exactly HERV-W ENV is expressed in/imported into the tumor and how long this expression is sustained. In this regard, future *in vitro* studies should also investigate the potential differences between an endogenous expression of HERV-W ENV in glioblastoma cell lines versus an exogenous stimulation as performed in this study. This could be achieved by either inducing an endogenous expression of the ENV protein in glioblastoma cells via transfection/transduction or by using a genetically modified animal model expressing the HERV-W ENV protein (Gruchot et al. 2023). In such an animal model, genetically modified glioblastoma cells could be injected into the brain, providing insights into how HERV-W ENV-negative tumor cells behave in a HERV-W ENV-positive TME. In addition, the above-mentioned long-term effects on all resident CNS cells could be easily investigated. Moreover, such data could then be compared to results from other research groups that investigated the expression of other HERVs in gliomas. In this context, full-length HERV-K expression was found in

both patient tissue samples and glioma cell lines, but the absence of detectable splice products initially suggested that HERV-K did not contribute to glioma malignancy (Kessler et al. 2014). However, recently, the subtype HML-2 of HERV-K was detected in patients with malignant gliomas in both cerebrospinal fluid and tumor tissue and was identified to be associated with a cancer stem cell phenotype (Shah et al. 2023). Epigenetic regulation seems to drive the differential expression of this subtype, with high expression leading to reduced overall patient survival (Shah et al. 2022). However, regarding HERV-W, experiments of the research group investigated the regulation of many other genes such as GSC markers like Nestin or SOX2, which showed no regulation (Reiche et al. 2024). In principle, as HERV-W and HERV-K both seem to modify glioblastoma tissue, this suggests that there is a high likelihood that several other HERVs are also expressed in gliomas. Consequently, it is also plausible that additional splice products of HERV-W could be detected in human gliomas. In addition, it would be interesting to investigate whether HERV-W ENV can be measured in the peripheral blood or cerebrospinal fluid (CSF) of glioblastoma patients and whether higher levels correlate with bigger tumor volumes. However, due to the fact that, as pointed out further above, HERV-W ENV is associated with many neurological diseases, the specificity of peripherally measured HERV-W ENV is probably rather low.

Notably, studying the co-prevalence of diseases associated with HERV-W ENV is of both epidemiological significance and scientific interest. In this regard, only a few studies have so far examined the clinical course of MS patients with glioma (Sahm et al. 2023). Overall, MS does not affect the risk of developing gliomas, but when present concurrently, it is linked to a poorer prognosis in IDH-mutant glioma patients (Sahm et al. 2023). In such patients, it would be interesting to study and compare the expression of HERV-W ENV in the tumor versus in MS lesions, blood, and CSF in order to gain a better insight into the distribution of HERV-W ENV expression.

Furthermore, the question arises how exactly HERV-W ENV correlates with the prognosis of brain tumors. As illustrated by the research group's results (Reiche et al. 2024), HERV-W ENV is expressed in gliomas regardless of their WHO grade, and so far, there is no data on different survival rates that should be investigated in future studies.

Finally, given the considerable changes in the classification of gliomas in recent years, this study is limited to investigating cell lines derived from patients in the last century. Accordingly, as mentioned above, future studies should further validate these findings using patient-derived tumor cells. Moreover, regarding cell origin, this study used neonatal primary rat microglia, which may differ in their properties and behavior from adult human microglia. However, in multiple sclerosis research, *in vitro* results using neonatal primary rat microglia have been shown to be transferable to adult human microglia (Weyers 2024).

5. Conclusion

The treatment of glioblastomas remains a significant challenge in today's medical care. Despite the discovery of numerous new mechanisms and treatment approaches in recent years, the standard treatment regimen has remained unchanged in the past two decades while prognosis is still abysmal. This highlights the urgent need for new therapeutic options. In this regard, the research group identified HERV-W ENV as a potential new target protein in glioblastoma (Reiche et al. 2024). This is of particular interest, as the neutralization of HERV-W ENV using the monoclonal antibody temelimab has already demonstrated safety in clinical trials for multiple sclerosis. In summary, this dissertation demonstrates that HERV-W ENV modulates molecular and cellular parameters in neoplastic glioblastoma cells and cells of the TME. While limitations persist, particularly regarding the timing, localization, duration, and mechanisms of HERV-W ENV expression in the tumor tissue, this study reveals that this retroviral protein induces a proinflammatory shift in gene and protein expression of glioblastoma cells. This, in turn, modulates key functional parameters such as proliferation, migration, and invasion, promoting an overall more aggressive neoplastic phenotype. Microglia function as mediators of these effects through both direct and indirect cell-cell interaction, although the precise mechanisms remain currently unclear. Furthermore, HERV-W ENV contributes to a proinflammatory TME by altering the gene expression of microglia, the predominant cell subtype within the glioblastoma TME. Moreover, this thesis suggests a potentially increased response to temozolomide treatment following HERV-W ENV stimulation, although - likely due to technical limitations - consistent effects were not observed. Future research will have to clarify whether HERV-W ENV neutralization with temelimab can be applied therapeutically in the treatment of GBM – which would be a first-in-class and entirely novel approach.

6. References

Ahir, B. K., Engelhard, H. H. and Lakka, S. S. (2020). **Tumor Development and Angiogenesis in Adult Brain Tumor: Glioblastoma**. Mol Neurobiol *57 (5)*, 2461-2478, doi: 10.1007/s12035-020-01892-8.

Aiyappa-Maudsley, R., Chalmers, A. J. and Parsons, J. L. (2022). **Factors affecting the radiation response in glioblastoma**. Neurooncol Adv *4 (1)*, vdac156, doi: 10.1093/noajnl/vdac156.

Aktan, F. (2004). **iNOS-mediated nitric oxide production and its regulation**. Life Sci *75 (6)*, 639-653, doi: 10.1016/j.lfs.2003.10.042.

Alghamri, M. S., McClellan, B. L., Hartlage, C. S., Haase, S., Faisal, S. M., Thalla, R., Dabaja, A., Banerjee, K., Carney, S. V., Mujeeb, A. A., Olin, M. R., Moon, J. J., Schwendeman, A., Lowenstein, P. R. and Castro, M. G. (2021). **Targeting Neuroinflammation in Brain Cancer: Uncovering Mechanisms, Pharmacological Targets, and Neuropharmaceutical Developments**. Front Pharmacol *12*, 680021, doi: 10.3389/fphar.2021.680021.

Alkhaibary, A., Alassiri, A. H., AlSufiani, F. and Alharbi, M. A. (2019). **Ki-67 labeling index in glioblastoma; does it really matter?** Hematology/Oncology and Stem Cell Therapy *12 (2)*, 82-88, doi: 10.1016/j.hemonc.2018.11.001.

Allen, M., Bjerke, M., Edlund, H., Nelander, S. and Westermark, B. (2016). **Origin of the U87MG glioma cell line: Good news and bad news**. Sci Transl Med *8 (354)*, 354re353, doi: 10.1126/scitranslmed.aaf6853.

Andersen, R. S., Anand, A., Harwood, D. S. L. and Kristensen, B. W. (2021). **Tumor-Associated Microglia and Macrophages in the Glioblastoma Microenvironment and Their Implications for Therapy**. Cancers (Basel) *13 (17)*, doi: 10.3390/cancers13174255.

Anderson, S. M., Kelly, M. and Odde, D. J. (2024). **Glioblastoma Cells Use an Integrin- and CD44-Mediated Motor-Clutch Mode of Migration in Brain Tissue**. Cell Mol Bioeng *17 (2)*, 121-135, doi: 10.1007/s12195-024-00799-x.

Argaw-Denboba, A., Balestrieri, E., Serafino, A., Cipriani, C., Bucci, I., Sorrentino, R., Sciamanna, I., Gambacurta, A., Sinibaldi-Vallebona, P. and Matteucci, C. (2017). **HERV-K activation is strictly required to sustain CD133+melanoma cells with stemness features**. Journal of Experimental & Clinical Cancer Research *36*, 1-17, doi: ARTN 20
10.1186/s13046-016-0485-x.

Armstrong, T. S., Prabhu, S., Aldape, K., Hossan, B., Kang, S., Childress, A., Tolentino, L. and Gilbert, M. R. (2011). **A case of soft tissue metastasis from glioblastoma and review of the literature**. J Neurooncol *103 (1)*, 167-172, doi: 10.1007/s11060-010-0370-y.

Arseni, L., Sharma, R., Mack, N., Nagalla, D., Ohl, S., Hielscher, T., Singhal, M., Pilz, R., Augustin, H., Sandhoff, R., Herold-Mende, C., Tews, B., Lichter, P. and Seiffert, M. (2023). **Sphingosine-1-Phosphate Recruits Macrophages and Microglia and Induces a Pro-Tumorigenic Phenotype That Favors Glioma Progression**. Cancers *15 (2)*, 479.

Balestrieri, E., Minutolo, A., Petrone, V., Fanelli, M., Iannetta, M., Malagnino, V., Zordan, M., Vitale, P., Charvet, B., Horvat, B., Bernardini, S., Garaci, E., di Francesco, P., Sinibaldi Vallebona, P., Sarmati, L., Grelli, S., Andreoni, M., Perron, H. and Matteucci, C. (2021). **Evidence of the pathogenic HERV-W envelope expression in T lymphocytes in association with the respiratory outcome of COVID-19 patients**. EBioMedicine *66*, 103341, doi: 10.1016/j.ebiom.2021.103341.

Bannert, N., Hofmann, H., Block, A. and Hohn, O. (2018). **HERVs New Role in Cancer: From Accused Perpetrators to Cheerful Protectors**. Front Microbiol *9*, 178, doi: 10.3389/fmicb.2018.00178.

Barnholtz-Sloan, J. S., Sloan, A. E., Davis, F. G., Vigneau, F. D., Lai, P. and Sawaya, R. E. (2004). **Incidence proportions of brain metastases in patients diagnosed (1973 to 2001) in the**

Metropolitan Detroit Cancer Surveillance System. J Clin Oncol *22 (14)*, 2865-2872, doi: 10.1200/JCO.2004.12.149.

Barth, M., Groger, V., Cynis, H. and Staege, M. S. (2019). **Identification of human endogenous retrovirus transcripts in Hodgkin Lymphoma cells**. Mol Biol Rep *46 (2)*, 1885-1893, doi: 10.1007/s11033-019-04640-x.

Bergallo, M., Montanari, P., Mareschi, K., Merlino, C., Berger, M., Bini, I., Daprà , V., Galliano, I. and Fagioli, F. (2017). **Expression of the gene of human endogenous retroviruses HERV-K and -W in leukemia patients**. Archives of Virology *162 (12)*, 3639-3644, doi: 10.1007/s00705-017-3526-7.

Berghoff, A. S., Schur, S., Fureder, L. M., Gatterbauer, B., Dieckmann, K., Widhalm, G., Hainfellner, J., Zielinski, C. C., Birner, P., Bartsch, R. and Preusser, M. (2016). **Descriptive statistical analysis of a real life cohort of 2419 patients with brain metastases of solid cancers**. ESMO Open *1 (2)*, e000024, doi: 10.1136/esmoopen-2015-000024.

Bettinger, I., Thanos, S. and Paulus, W. (2002). **Microglia promote glioma migration**. Acta Neuropathol *103 (4)*, 351-355, doi: 10.1007/s00401-001-0472-x.

Blond, J. L., Beseme, F., Duret, L., Bouton, O., Bedin, F., Perron, H., Mandrand, B. and Mallet, F. (1999). **Molecular characterization and placental expression of HERV-W, a new human endogenous retrovirus family**. J Virol *73 (2)*, 1175-1185, doi: 10.1128/JVI.73.2.1175-1185.1999.

Blumenthal, D. T. and Cannon-Albright, L. A. (2008). **Familiality in brain tumors**. Neurology *71 (13)*, 1015-1020, doi: 10.1212/01.wnl.0000326597.60605.27.

Bonaventura, P., Shekarian, T., Alcazer, V., Valladeau-Guilemond, J., Valsesia-Wittmann, S., Amigorena, S., Caux, C. and Depil, S. (2019). **Cold Tumors: A Therapeutic Challenge for Immunotherapy**. Front Immunol *10*, 168, doi: 10.3389/fimmu.2019.00168.

Bowman, R. L., Klemm, F., Akkari, L., Pyonteck, S. M., Sevenich, L., Quail, D. F., Dhara, S., Simpson, K., Gardner, E. E., Iacobuzio-Donahue, C. A., Brennan, C. W., Tabar, V., Gutin, P. H. and Joyce, J. A. (2016). **Macrophage Ontogeny Underlies Differences in Tumor-Specific Education in Brain Malignancies**. Cell Rep *17 (9)*, 2445-2459, doi: 10.1016/j.celrep.2016.10.052.

Braganza, M. Z., Kitahara, C. M., Berrington de Gonzalez, A., Inskip, P. D., Johnson, K. J. and Rajaraman, P. (2012). **Ionizing radiation and the risk of brain and central nervous system tumors: a systematic review**. Neuro Oncol *14 (11)*, 1316-1324, doi: 10.1093/neuonc/nos208.

Cabrera, A. R., Kirkpatrick, J. P., Fiveash, J. B., Shih, H. A., Koay, E. J., Lutz, S., Petit, J., Chao, S. T., Brown, P. D., Vogelbaum, M., Reardon, D. A., Chakravarti, A., Wen, P. Y. and Chang, E. (2016). **Radiation therapy for glioblastoma: Executive summary of an American Society for Radiation Oncology Evidence-Based Clinical Practice Guideline**. Pract Radiat Oncol *6 (4)*, 217-225, doi: 10.1016/j.prro.2016.03.007.

Capper, D., Jones, D. T. W., Sill, M., Hovestadt, V., Schrimpf, D., Sturm, D., Koelsche, C., Sahm, F., Chavez, L., Reuss, D. E., Kratz, A., Wefers, A. K., Huang, K., Pajtler, K. W., Schweizer, L., Stichel, D., Olar, A., Engel, N. W., Lindenberg, K., Harter, P. N., Braczynski, A. K., Plate, K. H., Dohmen, H., Garvalov, B. K., Coras, R., Holsken, A., Hewer, E., Bewerunge-Hudler, M., Schick, M., Fischer, R., Beschorner, R., Schittenhelm, J., Staszewski, O., Wani, K., Varlet, P., Pages, M., Temming, P., Lohmann, D., Selt, F., Witt, H., Milde, T., Witt, O., Aronica, E., Giangaspero, F., Rushing, E., Scheurlen, W., Geisenberger, C., Rodriguez, F. J., Becker, A., Preusser, M., Haberler, C., Bjerkvig, R., Cryan, J., Farrell, M., Deckert, M., Hench, J., Frank, S., Serrano, J., Kannan, K., Tsirigos, A., Bruck, W., Hofer, S., Brehmer, S., Seiz-Rosenhagen, M., Hanggi, D., Hans, V., Rozsnoki, S., Hansford, J. R., Kohlhof, P., Kristensen, B. W., Lechner, M., Lopes, B., Mawrin, C., Ketter, R., Kulozik, A., Khatib, Z., Heppner, F., Koch, A., Jouvet, A., Keohane, C., Muhleisen, H., Mueller, W., Pohl, U., Prinz, M., Benner, A., Zapatka, M., Gottardo, N. G., Driever, P. H., Kramm, C. M., Muller, H. L., Rutkowski, S., von Hoff, K., Fruhwald, M. C., Gnekow, A., Fleischhack, G., Tippelt, S., Calaminus, G., Monoranu, C. M., Perry, A., Jones, C., Jacques, T. S., Radlwimmer, B., Gessi, M., Pietsch, T., Schramm, J., Schackert, G., Westphal, M., Reifenberger, G.,

Wesseling, P., Weller, M., Collins, V. P., Blumcke, I., Bendszus, M., Debus, J., Huang, A., Jabado, N., Northcott, P. A., Paulus, W., Gajjar, A., Robinson, G. W., Taylor, M. D., Jaunmuktane, Z., Ryzhova, M., Platten, M., Unterberg, A., Wick, W., Karajannis, M. A., Mittelbronn, M., Acker, T., Hartmann, C., Aldape, K., Schuller, U., Buslei, R., Lichter, P., Kool, M., Herold-Mende, C., Ellison, D. W., Hasselblatt, M., Snuderl, M., Brandner, S., Korshunov, A., von Deimling, A. and Pfister, S. M. (2018). **DNA methylation-based classification of central nervous system tumours**. Nature *555 (7697)*, 469-474, doi: 10.1038/nature26000.

Cassetta, L. and Pollard, J. W. (2018). **Targeting macrophages: therapeutic approaches in cancer**. Nature reviews Drug discovery *17 (12)*, 887-904.

Chao, M., Liu, N., Sun, Z., Jiang, Y., Jiang, T., Xv, M., Jia, L., Tu, Y. and Wang, L. (2021). **TGF-β Signaling Promotes Glioma Progression Through Stabilizing Sox9**. Frontiers in Immunology *11*, doi: 10.3389/fimmu.2020.592080.

Charvet, B., Brunel, J., Pierquin, J., Iampietro, M., Decimo, D., Queruel, N., Lucas, A., Encabo-Berzosa, M. D. M., Arenaz, I., Marmolejo, T. P., Gonzalez, A. I., Maldonado, A. C., Mathieu, C., Kury, P., Flores-Rivera, J., Torres-Ruiz, F., Avila-Rios, S., Salgado Montes de Oca, G., Schoorlemmer, J., Perron, H. and Horvat, B. (2023). **SARS-CoV-2 awakens ancient retroviral genes and the expression of proinflammatory HERV-W envelope protein in COVID-19 patients**. iScience *26 (5)*, 106604, doi: 10.1016/j.isci.2023.106604.

Charvet, B., Pierquin, J., Brunel, J., Gorter, R., Quétard, C., Horvat, B., Amor, S., Portoukalian, J. and Perron, H. (2021). **Human Endogenous Retrovirus Type W Envelope from Multiple Sclerosis Demyelinating Lesions Shows Unique Solubility and Antigenic Characteristics**. Virologica Sinica *36 (5)*, 1006-1026, doi: 10.1007/s12250-021-00372-0.

Charvet, B., Reynaud, J. M., Gourru-Lesimple, G., Perron, H., Marche, P. N. and Horvat, B. (2018). **Induction of Proinflammatory Multiple Sclerosis-Associated Retrovirus Envelope Protein by Human Herpesvirus-6A and CD46 Receptor Engagement**. Front Immunol *9*, 2803, doi: 10.3389/fimmu.2018.02803.

Chatterjee, S., Nizamani, F. A., Nurnberger, A. and Speck, O. (2022). **Classification of brain tumours in MR images using deep spatiospatial models**. Sci Rep *12 (1)*, 1505, doi: 10.1038/s41598-022-05572-6.

Chen, W.-J., He, D.-S., Tang, R.-X., Ren, F.-H. and Chen, G. (2015). **Ki-67 is a valuable prognostic factor in gliomas: evidence from a systematic review and meta-analysis**. Asian Pacific Journal of Cancer Prevention *16 (2)*, 411-420.

Chen, X., Zhao, Y., Huang, Y., Zhu, K., Zeng, F., Zhao, J., Zhang, H., Zhu, X., Kettenmann, H. and Xiang, X. (2023). **TREM2 promotes glioma progression and angiogenesis mediated by microglia/brain macrophages**. Glia *71 (11)*, 2679-2695, doi: 10.1002/glia.24456.

Chen, Z. and Hambardzumyan, D. (2018). **Immune Microenvironment in Glioblastoma Subtypes**. Front Immunol *9*, 1004, doi: 10.3389/fimmu.2018.01004.

Cheng, W., Ren, X., Zhang, C., Cai, J., Liu, Y., Han, S. and Wu, A. (2016). **Bioinformatic profiling identifies an immune-related risk signature for glioblastoma**. Neurology *86 (24)*, 2226-2234, doi: 10.1212/WNL.0000000000002770.

Chu, X., Tian, W., Ning, J., Xiao, G., Zhou, Y., Wang, Z., Zhai, Z., Tanzhu, G., Yang, J. and Zhou, R. (2024). **Cancer stem cells: advances in knowledge and implications for cancer therapy**. Signal Transduction and Targeted Therapy *9 (1)*, 170, doi: 10.1038/s41392-024-01851-y.

Curtin, F., Lang, A. B., Perron, H., Laumonier, M., Vidal, V., Porchet, H. C. and Hartung, H.-P. (2012). **GNbAC1, a Humanized Monoclonal Antibody Against the Envelope Protein of Multiple Sclerosis—Associated Endogenous Retrovirus: A First-in-Humans Randomized Clinical Study**. Clinical Therapeutics *34 (12)*, 2268-2278, doi: https://doi.org/10.1016/j.clinthera.2012.11.006.

Curtin, F., Perron, H., Kromminga, A., Porchet, H. and Lang, A. B. (2015). **Preclinical and early clinical development of GNbAC1, a humanized IgG4 monoclonal antibody targeting**

endogenous retroviral MSRV-Env protein. MAbs *7 (1)*, 265-275, doi: 10.4161/19420862.2014.985021.

da Cruz, L. L. P., de Souza, P. O., Dal Prá, M., Falchetti, M., de Abreu, A. M., Azambuja, J. H., Bertoni, A. P. S., Paz, A. H. R., Araújo, A. B., Visioli, F., Fazolo, T., da Silva, G. G., Worm, P. V., Wink, M. R., Zanotto-Filho, A. and Braganhol, E. (2021). **TLR4 expression and functionality are downregulated in glioblastoma cells and in tumor-associated macrophages: A new mechanism of immune evasion?** Biochimica et Biophysica Acta (BBA) - Molecular Basis of Disease *1867 (8)*, 166155, doi: 10.1016/j.bbadis.2021.166155.

Dai, L., Del Valle, L., Miley, W., Whitby, D., Ochoa, A. C., Flemington, E. K. and Qin, Z. Q. (2018). **Transactivation of human endogenous retrovirus K (HERV-K) by KSHV promotes Kaposi's sarcoma development**. Oncogene *37 (33)*, 4534-4545, doi: 10.1038/s41388-018-0282-4.

Darmanis, S., Sloan, S. A., Croote, D., Mignardi, M., Chernikova, S., Samghababi, P., Zhang, Y., Neff, N., Kowarsky, M., Caneda, C., Li, G., Chang, S. D., Connolly, I. D., Li, Y. M., Barres, B. A., Gephart, M. H. and Quake, S. R. (2017). **Single-Cell RNA-Seq Analysis of Infiltrating Neoplastic Cells at the Migrating Front of Human Glioblastoma**. Cell Reports *21 (5)*, 1399-1410, doi: 10.1016/j.celrep.2017.10.030.

Demuth, T. and Berens, M. E. (2004). **Molecular mechanisms of glioma cell migration and invasion**. J Neurooncol *70 (2)*, 217-228, doi: 10.1007/s11060-004-2751-6.

Derfuss, T., Curtin, F., Guebelin, C., Bridel, C., Rasenack, M., Matthey, A., Du Pasquier, R., Schluep, M., Desmeules, J., Lang, A. B., Perron, H., Faucard, R., Porchet, H., Hartung, H. P., Kappos, L. and Lalive, P. H. (2015). **A phase IIa randomised clinical study of GNbAC1, a humanised monoclonal antibody against the envelope protein of multiple sclerosis-associated endogenous retrovirus in multiple sclerosis patients**. Mult Scler *21 (7)*, 885-893, doi: 10.1177/1352458514554052.

Diakos, C. I., Charles, K. A., McMillan, D. C. and Clarke, S. J. (2014). **Cancer-related inflammation and treatment effectiveness**. Lancet Oncol *15 (11)*, e493-503, doi: 10.1016/S1470-2045(14)70263-3.

Dixit, S., Baker, L., Walmsley, V. and Hingorani, M. (2012). **Temozolomide-related idiosyncratic and other uncommon toxicities: a systematic review**. Anticancer Drugs *23 (10)*, 1099-1106, doi: 10.1097/CAD.0b013e328356f5b0.

Dolei, A. (2006). **Endogenous retroviruses and human disease**. Expert Rev Clin Immunol *2 (1)*, 149-167, doi: 10.1586/1744666X.2.1.149.

Dorward, I. G., Luo, J., Perry, A., Gutmann, D. H., Mansur, D. B., Rubin, J. B. and Leonard, J. R. (2010). **Postoperative imaging surveillance in pediatric pilocytic astrocytomas**. J Neurosurg Pediatr *6 (4)*, 346-352, doi: 10.3171/2010.7.PEDS10129.

Durnaoglu, S., Lee, S. K. and Ahnn, J. (2021). **Human Endogenous Retroviruses as Gene Expression Regulators: Insights from Animal Models into Human Diseases**. Mol Cells *44 (12)*, 861-878, doi: 10.14348/molcells.2021.5016.

Ezzati, S., Salib, S., Balasubramaniam, M. and Aboud, O. (2024). **Epidermal Growth Factor Receptor Inhibitors in Glioblastoma: Current Status and Future Possibilities**. International Journal of Molecular Sciences *25 (4)*, 2316.

Ferrer, V. P., Moura Neto, V. and Mentlein, R. (2018). **Glioma infiltration and extracellular matrix: key players and modulators**. Glia *66 (8)*, 1542-1565, doi: 10.1002/glia.23309.

Feschotte, C. and Gilbert, C. (2012). **Endogenous viruses: insights into viral evolution and impact on host biology**. Nature Reviews Genetics *13 (4)*, 283-U288, doi: 10.1038/nrg3199.

Förster, M. (2020) **HERV-W ENV protein leads to a differentiation blockade in oligodendroglial precursor cells via nitrosative stress**.

Frederico, S. C., Hancock, J. C., Brettschneider, E. E. S., Ratnam, N. M., Gilbert, M. R. and Terabe, M. (2021). **Making a Cold Tumor Hot: The Role of Vaccines in the Treatment of Glioblastoma**. Front Oncol *11*, 672508, doi: 10.3389/fonc.2021.672508.

Friedmann-Morvinski, D. and Hambardzumyan, D. (2023). **Monocyte-neutrophil entanglement in glioblastoma**. J Clin Invest *133 (1)*, doi: 10.1172/jci163451.

Fritz, A. G. (2000). **International classification of diseases for oncology: ICD-O**, World Health Organization

Galldiks, N., Langen, K. J., Albert, N. L., Law, I., Kim, M. M., Villanueva-Meyer, J. E., Soffietti, R., Wen, P. Y., Weller, M. and Tonn, J. C. (2022). **Investigational PET tracers in neuro-oncology-What's on the horizon? A report of the PET/RANO group**. Neuro Oncol *24 (11)*, 1815-1826, doi: 10.1093/neuonc/noac131.

Garcia-Montojo, M. and Nath, A. (2021). **HERV-W envelope expression in blood leukocytes as a marker of disease severity of COVID-19 Comment**. Ebiomedicine *67*, 103363, doi: ARTN 103363
10.1016/j.ebiom.2021.103363.

GeNeuro (2024). **GeNeuro Announces Results of the GNC-501 study in post-Covid-19 syndrome**. URL: https://geneuro.ch/wp-content/uploads/GeNeuro_GNC501-Results_28062024_EN-3.pdf [as of20.01.2025].

Gerber, D. E., Grossman, S. A., Zeltzman, M., Parisi, M. A. and Kleinberg, L. (2007). **The impact of thrombocytopenia from temozolomide and radiation in newly diagnosed adults with high-grade gliomas**. Neuro Oncol *9 (1)*, 47-52, doi: 10.1215/15228517-2006-024.

Gerdes, J., Lemke, H., Baisch, H., Wacker, H. H., Schwab, U. and Stein, H. (1984). **Cell cycle analysis of a cell proliferation-associated human nuclear antigen defined by the monoclonal antibody Ki-67**. J Immunol *133 (4)*, 1710-1715.

Ghosh, M., Shubham, S., Mandal, K., Trivedi, V., Chauhan, R. and Naseera, S. (2017). **Survival and prognostic factors for glioblastoma multiforme: Retrospective single-institutional study**. Indian J Cancer *54 (1)*, 362-367, doi: 10.4103/ijc.IJC_157_17.

Gieryng, A., Pszczolkowska, D., Walentynowicz, K. A., Rajan, W. D. and Kaminska, B. (2017). **Immune microenvironment of gliomas**. Laboratory Investigation *97 (5)*, 498-518, doi: 10.1038/labinvest.2017.19.

Gilard, V., Tebani, A., Dabaj, I., Laquerriere, A., Fontanilles, M., Derrey, S., Marret, S. and Bekri, S. (2021). **Diagnosis and Management of Glioblastoma: A Comprehensive Perspective**. J Pers Med *11 (4)*, doi: 10.3390/jpm11040258.

Gimple, R. C., Bhargava, S., Dixit, D. and Rich, J. N. (2019). **Glioblastoma stem cells: lessons from the tumor hierarchy in a lethal cancer**. Genes Dev *33 (11-12)*, 591-609, doi: 10.1101/gad.324301.119.

Glas, M., Ballo, M. T., Bomzon, Z., Urman, N., Levi, S., Lavy-Shahaf, G., Jeyapalan, S., Sio, T. T., DeRose, P. M., Misch, M., Taillibert, S., Ram, Z., Hottinger, A. F., Easaw, J., Kim, C. Y., Mohan, S. and Stupp, R. (2022). **The Impact of Tumor Treating Fields on Glioblastoma Progression Patterns**. Int J Radiat Oncol Biol Phys *112 (5)*, 1269-1278, doi: 10.1016/j.ijrobp.2021.12.152.

Gorelik, A., Sapir, T., Woodruff, T. M. and Reiner, O. (2017). **Serping1/C1 Inhibitor Affects Cortical Development in a Cell Autonomous and Non-cell Autonomous Manner**. Front Cell Neurosci *11*, 169, doi: 10.3389/fncel.2017.00169.

Goswami, S., Gupta, A. and Sharma, S. K. (1998). **Interleukin-6-mediated autocrine growth promotion in human glioblastoma multiforme cell line U87MG**. J Neurochem *71 (5)*, 1837-1845, doi: 10.1046/j.1471-4159.1998.71051837.x.

Graeber, M. B., Scheithauer, B. W. and Kreutzberg, G. W. (2002). **Microglia in brain tumors**. Glia *40 (2)*, 252-259, doi: 10.1002/glia.10147.

Grandi, N. and Tramontano, E. (2018). **HERV Envelope Proteins: Physiological Role and Pathogenic Potential in Cancer and Autoimmunity**. Front Microbiol *9*, 462, doi: 10.3389/fmicb.2018.00462.

Granqvist, M., Boremalm, M., Poorghobad, A., Svenningsson, A., Salzer, J., Frisell, T. and Piehl, F. (2018). **Comparative effectiveness of rituximab and other initial treatment choices for multiple sclerosis**. JAMA neurology *75 (3)*, 320-327.

Griffin, B. D. and Moynagh, P. N. (2006). **Persistent interleukin-1β signaling causes long term activation of NFκB in a promoter-specific manner in human glial cells**. Journal of Biological Chemistry *281 (15)*, 10316-10326, doi: 10.1074/jbc.M509973200.

Griffiths, D. J. (2001). **Endogenous retroviruses in the human genome sequence**. Genome Biol *2 (6)*, REVIEWS1017, doi: 10.1186/gb-2001-2-6-reviews1017.

Grivennikov, S. I., Greten, F. R. and Karin, M. (2010). **Immunity, inflammation, and cancer**. Cell *140 (6)*, 883-899, doi: 10.1016/j.cell.2010.01.025.

Grochans, S., Cybulska, A. M., Siminska, D., Korbecki, J., Kojder, K., Chlubek, D. and Baranowska-Bosiacka, I. (2022). **Epidemiology of Glioblastoma Multiforme-Literature Review**. Cancers *14 (10)*, doi: ARTN 2412
10.3390/cancers14102412.

Groh, S. and Schotta, G. (2017). **Silencing of endogenous retroviruses by heterochromatin**. Cell Mol Life Sci *74 (11)*, 2055-2065, doi: 10.1007/s00018-017-2454-8.

Gruchot, J., Lewen, I., Dietrich, M., Reiche, L., Sindi, M., Hecker, C., Herrero, F., Charvet, B., Weber-Stadlbauer, U., Hartung, H.-P., Albrecht, P., Perron, H., Meyer, U. and Küry, P. (2023). **Transgenic expression of the HERV-W envelope protein leads to polarized glial cell populations and a neurodegenerative environment**. Proceedings of the National Academy of Sciences *120 (38)*, e2308187120, doi: doi:10.1073/pnas.2308187120.

Guo, X., Yang, X., Wu, J., Yang, H., Li, Y., Li, J., Liu, Q., Wu, C., Xing, H., Liu, P., Wang, Y., Hu, C. and Ma, W. (2022). **Tumor-Treating Fields in Glioblastomas: Past, Present, and Future**. Cancers (Basel) *14 (15)*, doi: 10.3390/cancers14153669.

Hambardzumyan, D., Gutmann, D. H. and Kettenmann, H. (2016). **The role of microglia and macrophages in glioma maintenance and progression**. Nat Neurosci *19 (1)*, 20-27, doi: 10.1038/nn.4185.

Han, J., Alvarez-Breckenridge, C. A., Wang, Q. E. and Yu, J. (2015). **TGF-β signaling and its targeting for glioma treatment**. Am J Cancer Res *5 (3)*, 945-955.

Hanahan, D. (2022). **Hallmarks of Cancer: New Dimensions**. Cancer Discov *12 (1)*, 31-46, doi: 10.1158/2159-8290.CD-21-1059.

Hanahan, D. and Weinberg, R. A. (2000). **The hallmarks of cancer**. Cell *100 (1)*, 57-70, doi: 10.1016/s0092-8674(00)81683-9.

Hanahan, D. and Weinberg, R. A. (2011). **Hallmarks of cancer: the next generation**. Cell *144 (5)*, 646-674, doi: 10.1016/j.cell.2011.02.013.

Hartung, H.-P., Derfuss, T., Cree, B. A., Sormani, M. P., Selmaj, K., Stutters, J., Prados, F., MacManus, D., Schneble, H.-M., Lambert, E., Porchet, H., Glanzman, R., Warne, D., Curtin, F., Kornmann, G., Buffet, B., Kremer, D., Küry, P., Leppert, D., Rückle, T. and Barkhof, F. (2022). **Efficacy and safety of temelimab in multiple sclerosis: Results of a randomized phase 2b and extension study**. Multiple Sclerosis Journal *28 (3)*, 429-440, doi: 10.1177/13524585211024997.

Henrik Heiland, D., Ravi, V. M., Behringer, S. P., Frenking, J. H., Wurm, J., Joseph, K., Garrelfs, N. W. C., Strahle, J., Heynckes, S., Grauvogel, J., Franco, P., Mader, I., Schneider, M., Potthoff, A. L., Delev, D., Hofmann, U. G., Fung, C., Beck, J., Sankowski, R., Prinz, M. and Schnell, O. (2019). **Tumor-associated reactive astrocytes aid the evolution of immunosuppressive environment in glioblastoma**. Nat Commun *10 (1)*, 2541, doi: 10.1038/s41467-019-10493-6.

Hsieh, Y. H., Tsai, J. P., Yu, C. L., Lee, C. C., Hsu, J. C. and Chen, J. C. (2021). **Overexpression of Lipocalin-2 Inhibits Proliferation and Invasiveness of Human Glioblastoma Multiforme Cells by Activating ERK Targeting Cathepsin D Expression**. Biology (Basel) *10 (5)*, doi: 10.3390/biology10050390.

Huang, L. E. (2022). **Impact of CDKN2A/B Homozygous Deletion on the Prognosis and Biology of IDH-Mutant Glioma**. Biomedicines *10 (2)*, doi: 10.3390/biomedicines10020246.

Huang, P. H., Xu, A. M. and White, F. M. (2009). **Oncogenic EGFR signaling networks in glioma**. Science signaling *2 (87)*, re6-re6.

Huber, M. A., Kraut, N. and Beug, H. (2005). **Molecular requirements for epithelial-mesenchymal transition during tumor progression**. Curr Opin Cell Biol *17 (5)*, 548-558, doi: 10.1016/j.ceb.2005.08.001.

Huettner, C., Paulus, W. and Roggendorf, W. (1995). **Messenger RNA expression of the immunosuppressive cytokine IL-10 in human gliomas**. Am J Pathol *146 (2)*, 317-322.

Ibba, G., Piu, C., Uleri, E., Serra, C. and Dolei, A. (2018). **Disruption by SaCas9 endonuclease of HERV-K Env, a retroviral gene with oncogenic and neuropathogenic potential, inhibits molecules involved in cancer and amyotrophic lateral sclerosis**. Viruses *10 (8)*, 412.

Jackson, C. M., Choi, J. and Lim, M. (2019). **Mechanisms of immunotherapy resistance: lessons from glioblastoma**. Nat Immunol *20 (9)*, 1100-1109, doi: 10.1038/s41590-019-0433-y.

Jaoude, D. A., Moore, J. A., Moore, M. B., Twumasi-Ankrah, P., Ablah, E. and Moore, D. F., Jr. (2019). **Glioblastoma and Increased Survival with Longer Chemotherapy Duration**. Kans J Med *12 (3)*, 65-69.

Jimenez-Pascual, A., Mitchell, K., Siebzehnrubl, F. A. and Lathia, J. D. (2020). **FGF2: a novel druggable target for glioblastoma?** Expert Opin Ther Targets *24 (4)*, 311-318, doi: 10.1080/14728222.2020.1736558.

Jing, X., Yang, F., Shao, C., Wei, K., Xie, M., Shen, H. and Shu, Y. (2019). **Role of hypoxia in cancer therapy by regulating the tumor microenvironment**. Mol Cancer *18 (1)*, 157, doi: 10.1186/s12943-019-1089-9.

Joy, A., Moffet, J., Neary, K., Mordechai, E., Stachowiak, E. K., Coons, S., Rankin-Shapiro, J., Florkiewicz, R. Z. and Stachowiak, M. K. (1997). **Nuclear accumulation of FGF-2 is associated with proliferation of human astrocytes and glioma cells**. Oncogene *14 (2)*, 171-183, doi: 10.1038/sj.onc.1200823.

Kelley, D. and Rinn, J. (2012). **Transposable elements reveal a stem cell-specific class of long noncoding RNAs**. Genome Biol *13 (11)*, R107, doi: 10.1186/gb-2012-13-11-r107.

Kessler, A. F., Wiesner, M., Denner, J., Kämmerer, U., Vince, G. H., Linsenmann, T., Löhr, M., Ernestus, R.-I. and Hagemann, C. (2014). **Expression-analysis of the human endogenous retrovirus HERV-K in human astrocytic tumors**. BMC Research Notes *7 (1)*, 159, doi: 10.1186/1756-0500-7-159.

Khan, F., Pang, L., Dunterman, M., Lesniak, M. S., Heimberger, A. B. and Chen, P. (2023). **Macrophages and microglia in glioblastoma: heterogeneity, plasticity, and therapy**. J Clin Invest *133 (1)*, doi: 10.1172/JCI163446.

Khan, F. H., Dervan, E., Bhattacharyya, D. D., McAuliffe, J. D., Miranda, K. M. and Glynn, S. A. (2020). **The Role of Nitric Oxide in Cancer: Master Regulator or NOt?** Int J Mol Sci *21 (24)*, doi: 10.3390/ijms21249393.

Kim, J.-H., Kang, R. J., Hyeon, S. J., Ryu, H., Joo, H., Bu, Y., Kim, J.-H. and Suk, K. (2023). **Lipocalin-2 Is a Key Regulator of Neuroinflammation in Secondary Traumatic and Ischemic Brain Injury**. Neurotherapeutics *20 (3)*, 803-821, doi: 10.1007/s13311-022-01333-5.

Kitsou, K., Lagiou, P. and Magiorkinis, G. (2023). **Human endogenous retroviruses in cancer: Oncogenesis mechanisms and clinical implications**. J Med Virol *95 (1)*, e28350, doi: 10.1002/jmv.28350.

Klemm, F., Maas, R. R., Bowman, R. L., Kornete, M., Soukup, K., Nassiri, S., Brouland, J. P., Iacobuzio-Donahue, C. A., Brennan, C., Tabar, V., Gutin, P. H., Daniel, R. T., Hegi, M. E. and Joyce, J. A. (2020). **Interrogation of the Microenvironmental Landscape in Brain Tumors Reveals Disease-Specific Alterations of Immune Cells**. Cell *181 (7)*, 1643-1660 e1617, doi: 10.1016/j.cell.2020.05.007.

Komohara, Y., Ohnishi, K., Kuratsu, J. and Takeya, M. (2008). **Possible involvement of the M2 anti-inflammatory macrophage phenotype in growth of human gliomas**. J Pathol *216 (1)*, 15-24, doi: 10.1002/path.2370.

Kore, R. A. and Abraham, E. C. (2014). **Inflammatory cytokines, interleukin-1 beta and tumor necrosis factor-alpha, upregulated in glioblastoma multiforme, raise the levels of CRYAB in exosomes secreted by U373 glioma cells**. Biochem Biophys Res Commun *453 (3)*, 326-331, doi: 10.1016/j.bbrc.2014.09.068.

Kremer, D., Gruchot, J., Weyers, V., Oldemeier, L., Gottle, P., Healy, L., Ho Jang, J., Kang, T. X. Y., Volsko, C., Dutta, R., Trapp, B. D., Perron, H., Hartung, H. P. and Kury, P. (2019). **pHERV-W envelope protein fuels microglial cell-dependent damage of myelinated axons in multiple sclerosis**. Proc Natl Acad Sci U S A *116 (30)*, 15216-15225, doi: 10.1073/pnas.1901283116.

Kremer, D., Schichel, T., Forster, M., Tzekova, N., Bernard, C., van der Valk, P., van Horssen, J., Hartung, H. P., Perron, H. and Kury, P. (2013). **Human endogenous retrovirus type W envelope protein inhibits oligodendroglial precursor cell differentiation**. Ann Neurol *74 (5)*, 721-732, doi: 10.1002/ana.23970.

Krol, I., Castro-Giner, F., Maurer, M., Gkountela, S., Szczerba, B. M., Scherrer, R., Coleman, N., Carreira, S., Bachmann, F., Anderson, S., Engelhardt, M., Lane, H., Evans, T. R. J.,

Plummer, R., Kristeleit, R., Lopez, J. and Aceto, N. (2018). **Detection of circulating tumour cell clusters in human glioblastoma**. Br J Cancer *119 (4)*, 487-491, doi: 10.1038/s41416-018-0186-7.

Kury, P., Nath, A., Creange, A., Dolei, A., Marche, P., Gold, J., Giovannoni, G., Hartung, H. P. and Perron, H. (2018). **Human Endogenous Retroviruses in Neurological Diseases**. Trends Mol Med *24 (4)*, 379-394, doi: 10.1016/j.molmed.2018.02.007.

Lachance, D. H., Yang, P., Johnson, D. R., Decker, P. A., Kollmeyer, T. M., McCoy, L. S., Rice, T., Xiao, Y., Ali-Osman, F., Wang, F., Stoddard, S. M., Sprau, D. J., Kosel, M. L., Wiencke, J. K., Wiemels, J. L., Patoka, J. S., Davis, F., McCarthy, B., Rynearson, A. L., Worra, J. B., Fridley, B. L., O'Neill, B. P., Buckner, J. C., Il'yasova, D., Jenkins, R. B. and Wrensch, M. R. (2011). **Associations of high-grade glioma with glioma risk alleles and histories of allergy and smoking**. Am J Epidemiol *174 (5)*, 574-581, doi: 10.1093/aje/kwr124.

Lahey, K. C., Gadiyar, V., Hill, A., Desind, S., Wang, Z., Davra, V., Patel, R., Zaman, A., Calianese, D. and Birge, R. B. (2022). **Mertk: An emerging target in cancer biology and immuno-oncology**. Int Rev Cell Mol Biol *368*, 35-59, doi: 10.1016/bs.ircmb.2022.04.004.

Lan, Z., Li, X. and Zhang, X. (2024). **Glioblastoma: An Update in Pathology, Molecular Mechanisms and Biomarkers**. Int J Mol Sci *25 (5)*, 3040, doi: 10.3390/ijms25053040.

Lander, E. S., Linton, L. M., Birren, B., Nusbaum, C., Zody, M. C., Baldwin, J., Devon, K., Dewar, K., Doyle, M., FitzHugh, W., Funke, R., Gage, D., Harris, K., Heaford, A., Howland, J., Kann, L., Lehoczky, J., LeVine, R., McEwan, P., McKernan, K., Meldrim, J., Mesirov, J. P., Miranda, C., Morris, W., Naylor, J., Raymond, C., Rosetti, M., Santos, R., Sheridan, A., Sougnez, C., Stange-Thomann, Y., Stojanovic, N., Subramanian, A., Wyman, D., Rogers, J., Sulston, J., Ainscough, R., Beck, S., Bentley, D., Burton, J., Clee, C., Carter, N., Coulson, A., Deadman, R., Deloukas, P., Dunham, A., Dunham, I., Durbin, R., French, L., Grafham, D., Gregory, S., Hubbard, T., Humphray, S., Hunt, A., Jones, M., Lloyd, C., McMurray, A., Matthews, L., Mercer, S., Milne, S., Mullikin, J. C., Mungall, A., Plumb, R., Ross, M., Shownkeen, R., Sims, S., Waterston, R. H., Wilson, R. K., Hillier, L. W., McPherson, J. D., Marra, M. A., Mardis, E. R., Fulton, L. A., Chinwalla, A. T., Pepin, K. H., Gish, W. R.,

Chissoe, S. L., Wendl, M. C., Delehaunty, K. D., Miner, T. L., Delehaunty, A., Kramer, J. B., Cook, L. L., Fulton, R. S., Johnson, D. L., Minx, P. J., Clifton, S. W., Hawkins, T., Branscomb, E., Predki, P., Richardson, P., Wenning, S., Slezak, T., Doggett, N., Cheng, J. F., Olsen, A., Lucas, S., Elkin, C., Uberbacher, E., Frazier, M., Gibbs, R. A., Muzny, D. M., Scherer, S. E., Bouck, J. B., Sodergren, E. J., Worley, K. C., Rives, C. M., Gorrell, J. H., Metzker, M. L., Naylor, S. L., Kucherlapati, R. S., Nelson, D. L., Weinstock, G. M., Sakaki, Y., Fujiyama, A., Hattori, M., Yada, T., Toyoda, A., Itoh, T., Kawagoe, C., Watanabe, H., Totoki, Y., Taylor, T., Weissenbach, J., Heilig, R., Saurin, W., Artiguenave, F., Brottier, P., Bruls, T., Pelletier, E., Robert, C., Wincker, P., Smith, D. R., Doucette-Stamm, L., Rubenfield, M., Weinstock, K., Lee, H. M., Dubois, J., Rosenthal, A., Platzer, M., Nyakatura, G., Taudien, S., Rump, A., Yang, H., Yu, J., Wang, J., Huang, G., Gu, J., Hood, L., Rowen, L., Madan, A., Qin, S., Davis, R. W., Federspiel, N. A., Abola, A. P., Proctor, M. J., Myers, R. M., Schmutz, J., Dickson, M., Grimwood, J., Cox, D. R., Olson, M. V., Kaul, R., Raymond, C., Shimizu, N., Kawasaki, K., Minoshima, S., Evans, G. A., Athanasiou, M., Schultz, R., Roe, B. A., Chen, F., Pan, H., Ramser, J., Lehrach, H., Reinhardt, R., McCombie, W. R., de la Bastide, M., Dedhia, N., Blocker, H., Hornischer, K., Nordsiek, G., Agarwala, R., Aravind, L., Bailey, J. A., Bateman, A., Batzoglou, S., Birney, E., Bork, P., Brown, D. G., Burge, C. B., Cerutti, L., Chen, H. C., Church, D., Clamp, M., Copley, R. R., Doerks, T., Eddy, S. R., Eichler, E. E., Furey, T. S., Galagan, J., Gilbert, J. G., Harmon, C., Hayashizaki, Y., Haussler, D., Hermjakob, H., Hokamp, K., Jang, W., Johnson, L. S., Jones, T. A., Kasif, S., Kaspryzk, A., Kennedy, S., Kent, W. J., Kitts, P., Koonin, E. V., Korf, I., Kulp, D., Lancet, D., Lowe, T. M., McLysaght, A., Mikkelsen, T., Moran, J. V., Mulder, N., Pollara, V. J., Ponting, C. P., Schuler, G., Schultz, J., Slater, G., Smit, A. F., Stupka, E., Szustakowki, J., Thierry-Mieg, D., Thierry-Mieg, J., Wagner, L., Wallis, J., Wheeler, R., Williams, A., Wolf, Y. I., Wolfe, K. H., Yang, S. P., Yeh, R. F., Collins, F., Guyer, M. S., Peterson, J., Felsenfeld, A., Wetterstrand, K. A., Patrinos, A., Morgan, M. J., de Jong, P., Catanese, J. J., Osoegawa, K., Shizuya, H., Choi, S., Chen, Y. J., Szustakowki, J. and International Human Genome Sequencing, C. (2001). **Initial sequencing and analysis of the human genome**. Nature *409 (6822)*, 860-921, doi: 10.1038/35057062.

Lapointe, S., Perry, A. and Butowski, N. A. (2018). **Primary brain tumours in adults**. Lancet *392 (10145)*, 432-446, doi: 10.1016/S0140-6736(18)30990-5.

Latifi, T., Zebardast, A. and Marashi, S. M. (2022). **The role of human endogenous retroviruses (HERVs) in Multiple Sclerosis and the plausible interplay between HERVs, Epstein-Barr virus infection, and vitamin D**. Mult Scler Relat Disord *57*, 103318, doi: 10.1016/j.msard.2021.103318.

Lavie, L., Kitova, M., Maldener, E., Meese, E. and Mayer, J. (2005). **CpG methylation directly regulates transcriptional activity of the human endogenous retrovirus family HERV-K(HML-2)**. J Virol *79 (2)*, 876-883, doi: 10.1128/JVI.79.2.876-883.2005.

Levet, S., Medina, J., Joanou, J., Demolder, A., Queruel, N., Reant, K., Normand, M., Seffals, M., Dimier, J., Germi, R., Piofczyk, T., Portoukalian, J., Touraine, J. L. and Perron, H. (2017). **An ancestral retroviral protein identified as a therapeutic target in type-1 diabetes**. JCI Insight *2 (17)*, doi: 10.1172/jci.insight.94387.

Li, F., Nellaker, C., Sabunciyan, S., Yolken, R. H., Jones-Brando, L., Johansson, A. S., Owe-Larsson, B. and Karlsson, H. (2014). **Transcriptional derepression of the ERVWE1 locus following influenza A virus infection**. J Virol *88 (8)*, 4328-4337, doi: 10.1128/JVI.03628-13.

Li, L. T., Jiang, G., Chen, Q. and Zheng, J. N. (2015). **Ki67 is a promising molecular target in the diagnosis of cancer (review)**. Mol Med Rep *11 (3)*, 1566-1572, doi: 10.3892/mmr.2014.2914.

Lim, M., Weller, M., Idbaih, A., Steinbach, J., Finocchiaro, G., Raval, R. R., Ansstas, G., Baehring, J., Taylor, J. W., Honnorat, J., Petrecca, K., De Vos, F., Wick, A., Sumrall, A., Sahebjam, S., Mellinghoff, I. K., Kinoshita, M., Roberts, M., Slepetis, R., Warad, D., Leung, D., Lee, M., Reardon, D. A. and Omuro, A. (2022). **Phase III trial of chemoradiotherapy with temozolomide plus nivolumab or placebo for newly diagnosed glioblastoma with methylated MGMT promoter**. Neuro Oncol *24 (11)*, 1935-1949, doi: 10.1093/neuonc/noac116.

Lim, M., Xia, Y., Bettegowda, C. and Weller, M. (2018). **Current state of immunotherapy for glioblastoma**. Nat Rev Clin Oncol *15 (7)*, 422-442, doi: 10.1038/s41571-018-0003-5.

Lin, H., Liu, C., Hu, A., Zhang, D., Yang, H. and Mao, Y. (2024). **Understanding the immunosuppressive microenvironment of glioma: mechanistic insights and clinical perspectives**. Journal of Hematology & Oncology *17 (1)*, 31, doi: 10.1186/s13045-024-01544-7.

Lin, W., Wu, S., Chen, X., Ye, Y., Weng, Y., Pan, Y., Chen, Z., Chen, L., Qiu, X. and Qiu, S. (2020). **Characterization of Hypoxia Signature to Evaluate the Tumor Immune Microenvironment and Predict Prognosis in Glioma Groups**. Front Oncol *10*, 796, doi: 10.3389/fonc.2020.00796.

Liu, Q., Li, G., Li, R., Shen, J., He, Q., Deng, L., Zhang, C. and Zhang, J. (2010). **IL-6 promotion of glioblastoma cell invasion and angiogenesis in U251 and T98G cell lines**. J Neurooncol *100 (2)*, 165-176, doi: 10.1007/s11060-010-0158-0.

Liu, Z., Yang, L., Wu, W., Chen, Z., Xie, Z., Shi, D., Cai, N. and Zhuo, S. (2024). **Prognosis and therapeutic significance of IGF-1R-related signaling pathway gene signature in glioma**. Frontiers in Cell and Developmental Biology *12*, doi: 10.3389/fcell.2024.1375030.

Look, T., Puca, E., Buhler, M., Kirschenbaum, D., De Luca, R., Stucchi, R., Ravazza, D., Di Nitto, C., Roth, P., Katzenelenbogen, Y., Weiner, A., Rindlisbacher, L., Becher, B., Amit, I., Weller, M., Neri, D., Hemmerle, T. and Weiss, T. (2023). **Targeted delivery of tumor necrosis factor in combination with CCNU induces a T cell-dependent regression of glioblastoma**. Sci Transl Med *15 (697)*, eadf2281, doi: 10.1126/scitranslmed.adf2281.

Louis, D. N. (2012). **The next step in brain tumor classification:"Let us now praise famous men"… or molecules?** Acta neuropathologica *124 (6)*, 761-762.

Louis, D. N., Perry, A., Reifenberger, G., von Deimling, A., Figarella-Branger, D., Cavenee, W. K., Ohgaki, H., Wiestler, O. D., Kleihues, P. and Ellison, D. W. (2016). **The 2016 World Health Organization Classification of Tumors of the Central Nervous System: a summary**. Acta Neuropathol *131 (6)*, 803-820, doi: 10.1007/s00401-016-1545-1.

Louis, D. N., Perry, A., Wesseling, P., Brat, D. J., Cree, I. A., Figarella-Branger, D., Hawkins, C., Ng, H. K., Pfister, S. M., Reifenberger, G., Soffietti, R., von Deimling, A. and Ellison, D. W. (2021). **The 2021 WHO Classification of Tumors of the Central Nervous System: a summary**. Neuro Oncol *23 (8)*, 1231-1251, doi: 10.1093/neuonc/noab106.

Lundy, P., Domino, J., Ryken, T., Fouke, S., McCracken, D. J., Ormond, D. R. and Olson, J. J. (2020). **The role of imaging for the management of newly diagnosed glioblastoma in adults: a systematic review and evidence-based clinical practice guideline update**. J Neurooncol *150 (2)*, 95-120, doi: 10.1007/s11060-020-03597-3.

Ma, K., Chen, S., Chen, X., Yang, C. and Yang, J. (2022). **S100A10 Is a New Prognostic Biomarker Related to the Malignant Molecular Features and Immunosuppression Process of Adult Gliomas**. World Neurosurg *165*, e650-e663, doi: 10.1016/j.wneu.2022.06.124.

Maas, S. L. N., Abels, E. R., Van De Haar, L. L., Zhang, X., Morsett, L., Sil, S., Guedes, J., Sen, P., Prabhakar, S., Hickman, S. E., Lai, C. P., Ting, D. T., Breakefield, X. O., Broekman, M. L. D. and El Khoury, J. (2020). **Glioblastoma hijacks microglial gene expression to support tumor growth**. J Neuroinflammation *17 (1)*, 120, doi: 10.1186/s12974-020-01797-2.

Mahajan-Thakur, S., Bien-Moller, S., Marx, S., Schroeder, H. and Rauch, B. H. (2017). **Sphingosine 1-phosphate (S1P) signaling in glioblastoma multiforme-A systematic review**. Int J Mol Sci *18 (11)*, doi: 10.3390/ijms18112448.

Mameli, G., Astone, V., Arru, G., Marconi, S., Lovato, L., Serra, C., Sotgiu, S., Bonetti, B. and Dolei, A. (2007). **Brains and peripheral blood mononuclear cells of multiple sclerosis (MS) patients hyperexpress MS-associated retrovirus/HERV-W endogenous retrovirus, but not Human herpesvirus 6**. J Gen Virol *88 (Pt 1)*, 264-274, doi: 10.1099/vir.0.81890-0.

Mameli, G., Poddighe, L., Mei, A., Uleri, E., Sotgiu, S., Serra, C., Manetti, R. and Dolei, A. (2012). **Expression and activation by Epstein Barr virus of human endogenous retroviruses-**

W in blood cells and astrocytes: inference for multiple sclerosis. PLoS One *7 (9)*, e44991, doi: 10.1371/journal.pone.0044991.

Marchi, E., Kanapin, A., Magiorkinis, G. and Belshaw, R. (2014). **Unfixed endogenous retroviral insertions in the human population**. J Virol *88 (17)*, 9529-9537, doi: 10.1128/JVI.00919-14.

Martinez-Lage, M., Lynch, T. M., Bi, Y., Cocito, C., Way, G. P., Pal, S., Haller, J., Yan, R. E., Ziober, A., Nguyen, A., Kandpal, M., O'Rourke, D. M., Greenfield, J. P., Greene, C. S., Davuluri, R. V. and Dahmane, N. (2019). **Immune landscapes associated with different glioblastoma molecular subtypes**. Acta Neuropathol Commun *7 (1)*, 203, doi: 10.1186/s40478-019-0803-6.

Matias, D., Balca-Silva, J., da Graca, G. C., Wanjiru, C. M., Macharia, L. W., Nascimento, C. P., Roque, N. R., Coelho-Aguiar, J. M., Pereira, C. M., Dos Santos, M. F., Pessoa, L. S., Lima, F. R. S., Schanaider, A., Ferrer, V. P., Tania Cristina Leite de Sampaio e, S. and Moura-Neto, V. (2018). **Microglia/Astrocytes-Glioblastoma Crosstalk: Crucial Molecular Mechanisms and Microenvironmental Factors**. Front Cell Neurosci *12*, 235, doi: 10.3389/fncel.2018.00235.

Matyash, V. and Kettenmann, H. (2010). **Heterogeneity in astrocyte morphology and physiology**. Brain Res Rev *63 (1-2)*, 2-10, doi: 10.1016/j.brainresrev.2009.12.001.

Mazzoleni, A., Awuah, W. A., Sanker, V., Bharadwaj, H. R., Aderinto, N., Tan, J. K., Huang, H. Y. R., Poornaselvan, J., Shah, M. H., Atallah, O., Tawfik, A., Elmanzalawi, M. E. A. E., Ghozlan, S. H., Abdul-Rahman, T., Moyondafoluwa, J. A., Alexiou, A. and Papadakis, M. (2024). **Chromosomal instability: a key driver in glioma pathogenesis and progression**. European Journal of Medical Research *29 (1)*, 451, doi: 10.1186/s40001-024-02043-8.

McCarthy, K. D. and de Vellis, J. (1980). **Preparation of separate astroglial and oligodendroglial cell cultures from rat cerebral tissue**. J Cell Biol *85 (3)*, 890-902, doi: 10.1083/jcb.85.3.890.

Melincovici, C. S., Boşca, A. B., Şuşman, S., Mărginean, M., Mihu, C., Istrate, M., Moldovan, I. M., Roman, A. L. and Mihu, C. M. (2018). **Vascular endothelial growth factor (VEGF) - key factor in normal and pathological angiogenesis**. Rom J Morphol Embryol *59 (2)*, 455-467.

Mellinghoff, I. K., Bent, M. J. v. d., Blumenthal, D. T., Touat, M., Peters, K. B., Clarke, J., Mendez, J., Yust-Katz, S., Welsh, L., Mason, W. P., Ducray, F., Umemura, Y., Nabors, B., Holdhoff, M., Hottinger, A. F., Arakawa, Y., Sepulveda, J. M., Wick, W., Soffietti, R., Perry, J. R., Giglio, P., Fuente, M. d. l., Maher, E. A., Schoenfeld, S., Zhao, D., Pandya, S. S., Steelman, L., Hassan, I., Wen, P. Y. and Cloughesy, T. F. (2023). **Vorasidenib in IDH1- or IDH2-Mutant Low-Grade Glioma**. New England Journal of Medicine *389 (7)*, 589-601, doi: doi:10.1056/NEJMoa2304194.

Menon, S. S., Guruvayoorappan, C., Sakthivel, K. M. and Rasmi, R. R. (2019). **Ki-67 protein as a tumour proliferation marker**. Clinica Chimica Acta *491*, 39-45, doi: 10.1016/j.cca.2019.01.011.

Molinaro, A. M., Hervey-Jumper, S., Morshed, R. A., Young, J., Han, S. J., Chunduru, P., Zhang, Y., Phillips, J. J., Shai, A. and Lafontaine, M. (2020). **Association of maximal extent of resection of contrast-enhanced and non–contrast-enhanced tumor with survival within molecular subgroups of patients with newly diagnosed glioblastoma**. JAMA oncology *6 (4)*, 495-503.

Mondal, S., Adhikari, N., Banerjee, S., Amin, S. A. and Jha, T. (2020). **Matrix metalloproteinase-9 (MMP-9) and its inhibitors in cancer: A minireview**. Eur J Med Chem *194*, 112260, doi: 10.1016/j.ejmech.2020.112260.

Monteiro, A. R., Hill, R., Pilkington, G. J. and Madureira, P. A. (2017). **The Role of Hypoxia in Glioblastoma Invasion**. Cells *6 (4)*, 45, doi: 10.3390/cells6040045.

Moore, S. C., Rajaraman, P., Dubrow, R., Darefsky, A. S., Koebnick, C., Hollenbeck, A., Schatzkin, A. and Leitzmann, M. F. (2009). **Height, body mass index, and physical activity in**

relation to glioma risk. Cancer Res *69 (21)*, 8349-8355, doi: 10.1158/0008-5472.CAN-09-1669.

Nayak, L., Lee, E. Q. and Wen, P. Y. (2012). **Epidemiology of brain metastases**. Curr Oncol Rep *14 (1)*, 48-54, doi: 10.1007/s11912-011-0203-y.

Nelson, P. N., Carnegie, P. R., Martin, J., Davari Ejtehadi, H., Hooley, P., Roden, D., Rowland-Jones, S., Warren, P., Astley, J. and Murray, P. G. (2003). **Demystified. Human endogenous retroviruses**. Mol Pathol *56 (1)*, 11-18, doi: 10.1136/mp.56.1.11.

Newlands, E. S., Stevens, M. F., Wedge, S. R., Wheelhouse, R. T. and Brock, C. (1997). **Temozolomide: a review of its discovery, chemical properties, pre-clinical development and clinical trials**. Cancer Treat Rev *23 (1)*, 35-61, doi: 10.1016/s0305-7372(97)90019-0.

Noor, H., Briggs, N. E., McDonald, K. L., Holst, J. and Vittorio, O. (2021). **TP53 Mutation Is a Prognostic Factor in Lower Grade Glioma and May Influence Chemotherapy Efficacy**. Cancers (Basel) *13 (21)*, doi: 10.3390/cancers13215362.

Oraiopoulou, M. E., Tzamali, E., Psycharakis, S. E., Tzedakis, G., Makatounakis, T., Manolitsi, K., Drakos, E., Vakis, A. F., Zacharakis, G., Papamatheakis, J. and Sakkalis, V. (2024). **The Temozolomide-Doxorubicin paradox in Glioblastoma in vitro-in silico preclinical drug-screening**. Scientific Reports *14 (1)*, 3759, doi: ARTN 3759
10.1038/s41598-024-53684-y.

Ostrom, Q. T., Cioffi, G., Waite, K., Kruchko, C. and Barnholtz-Sloan, J. S. (2021). **CBTRUS Statistical Report: Primary Brain and Other Central Nervous System Tumors Diagnosed in the United States in 2014-2018**. Neuro Oncol *23 (12 Suppl 2)*, iii1-iii105, doi: 10.1093/neuonc/noab200.

Ostrom, Q. T., Gittleman, H., Liao, P., Vecchione-Koval, T., Wolinsky, Y., Kruchko, C. and Barnholtz-Sloan, J. S. (2017). **CBTRUS Statistical Report: Primary brain and other central nervous system tumors diagnosed in the United States in 2010-2014**. Neuro-Oncology *19 (suppl_5)*, V1-V88, doi: 10.1093/neuonc/nox158.

Osuka, S. and Van Meir, E. G. (2017). **Overcoming therapeutic resistance in glioblastoma: the way forward**. J Clin Invest *127 (2)*, 415-426, doi: 10.1172/JCI89587.

Oszvald, A., Guresir, E., Setzer, M., Vatter, H., Senft, C., Seifert, V. and Franz, K. (2012). **Glioblastoma therapy in the elderly and the importance of the extent of resection regardless of age**. J Neurosurg *116 (2)*, 357-364, doi: 10.3171/2011.8.JNS102114.

Pan, Y., Li, C., Chen, J., Zhang, K., Chu, X., Wang, R. and Chen, L. (2016). **The Emerging Roles of Long Noncoding RNA ROR (lincRNA-ROR) and its Possible Mechanisms in Human Cancers**. Cell Physiol Biochem *40 (1-2)*, 219-229, doi: 10.1159/000452539.

Paolicelli, R. C., Sierra, A., Stevens, B., Tremblay, M. E., Aguzzi, A., Ajami, B., Amit, I., Audinat, E., Bechmann, I., Bennett, M., Bennett, F., Bessis, A., Biber, K., Bilbo, S., Blurton-Jones, M., Boddeke, E., Brites, D., Brone, B., Brown, G. C., Butovsky, O., Carson, M. J., Castellano, B., Colonna, M., Cowley, S. A., Cunningham, C., Davalos, D., De Jager, P. L., de Strooper, B., Denes, A., Eggen, B. J. L., Eyo, U., Galea, E., Garel, S., Ginhoux, F., Glass, C. K., Gokce, O., Gomez-Nicola, D., Gonzalez, B., Gordon, S., Graeber, M. B., Greenhalgh, A. D., Gressens, P., Greter, M., Gutmann, D. H., Haass, C., Heneka, M. T., Heppner, F. L., Hong, S., Hume, D. A., Jung, S., Kettenmann, H., Kipnis, J., Koyama, R., Lemke, G., Lynch, M., Majewska, A., Malcangio, M., Malm, T., Mancuso, R., Masuda, T., Matteoli, M., McColl, B. W., Miron, V. E., Molofsky, A. V., Monje, M., Mracsko, E., Nadjar, A., Neher, J. J., Neniskyte, U., Neumann, H., Noda, M., Peng, B., Peri, F., Perry, V. H., Popovich, P. G., Pridans, C., Priller, J., Prinz, M., Ragozzino, D., Ransohoff, R. M., Salter, M. W., Schaefer, A., Schafer, D. P., Schwartz, M., Simons, M., Smith, C. J., Streit, W. J., Tay, T. L., Tsai, L. H., Verkhratsky, A., von Bernhardi, R., Wake, H., Wittamer, V., Wolf, S. A., Wu, L. J. and Wyss-Coray, T. (2022). **Microglia states and nomenclature: A field at its crossroads**. Neuron *110 (21)*, 3458-3483, doi: 10.1016/j.neuron.2022.10.020.

Pardoll, D. M. (2012). **The blockade of immune checkpoints in cancer immunotherapy**. Nat Rev Cancer *12 (4)*, 252-264, doi: 10.1038/nrc3239.

Park, Y. W., Vollmuth, P., Foltyn-Dumitru, M., Sahm, F., Ahn, S. S., Chang, J. H. and Kim, S. H. (2023). **The 2021 WHO Classification for Gliomas and Implications on Imaging Diagnosis: Part 1-Key Points of the Fifth Edition and Summary of Imaging Findings on Adult-Type Diffuse Gliomas**. Journal of Magnetic Resonance Imaging *58 (3)*, 677-689, doi: 10.1002/jmri.28743.

Pegg, A. E., Dolan, M. E. and Moschel, R. C. (1995). **Structure, function, and inhibition of O6-alkylguanine-DNA alkyltransferase**. Prog Nucleic Acid Res Mol Biol *51*, 167-223, doi: 10.1016/s0079-6603(08)60879-x.

Perez-Perez, S., Dominguez-Mozo, M. I., Garcia-Martinez, M. A., Ballester-Gonzalez, R., Nieto-Ganan, I., Arroyo, R. and Alvarez-Lafuente, R. (2022). **Epstein-Barr Virus Load Correlates with Multiple Sclerosis-Associated Retrovirus Envelope Expression**. Biomedicines *10 (2)*, doi: 10.3390/biomedicines10020387.

Perron, H., Garson, J. A., Bedin, F., Beseme, F., Paranhos-Baccala, G., Komurian-Pradel, F., Mallet, F., Tuke, P. W., Voisset, C., Blond, J. L., Lalande, B., Seigneurin, J. M. and Mandrand, B. (1997). **Molecular identification of a novel retrovirus repeatedly isolated from patients with multiple sclerosis. The Collaborative Research Group on Multiple Sclerosis**. Proc Natl Acad Sci U S A *94 (14)*, 7583-7588, doi: 10.1073/pnas.94.14.7583.

Perron, H., Germi, R., Bernard, C., Garcia-Montojo, M., Deluen, C., Farinelli, L., Faucard, R., Veas, F., Stefas, I., Fabriek, B. O., Van-Horssen, J., Van-der-Valk, P., Gerdil, C., Mancuso, R., Saresella, M., Clerici, M., Marcel, S., Creange, A., Cavaretta, R., Caputo, D., Arru, G., Morand, P., Lang, A. B., Sotgiu, S., Ruprecht, K., Rieckmann, P., Villoslada, P., Chofflon, M., Boucraut, J., Pelletier, J. and Hartung, H. P. (2012). **Human endogenous retrovirus type W envelope expression in blood and brain cells provides new insights into multiple sclerosis disease**. Mult Scler *18 (12)*, 1721-1736, doi: 10.1177/1352458512441381.

Perron, H., Suh, M., Lalande, B., Gratacap, B., Laurent, A., Stoebner, P. and Seigneurin, J. M. (1993). **Herpes simplex virus ICP0 and ICP4 immediate early proteins strongly enhance expression of a retrovirus harboured by a leptomeningeal cell line from a**

patient with multiple sclerosis. J Gen Virol *74 (Pt 1)*, 65-72, doi: 10.1099/0022-1317-74-1-65.

Perry, A. and Wesseling, P. (2016). **Histologic classification of gliomas**. Handb Clin Neurol *134*, 71-95, doi: 10.1016/B978-0-12-802997-8.00005-0.

Piehl, F. (2022). **Primary analysis of ProTEct-MS, a randomised, placebo-controlled, phase 2 study of temelimab for the prevention of neurodegenerationin rituximab-treated patients with relapsing multiple sclerosis**. Multiple Sclerosis Journal *28 (3_suppl)*, 3-129, doi: 10.1177/13524585221123685.

Plaks, V., Kong, N. and Werb, Z. (2015). **The cancer stem cell niche: how essential is the niche in regulating stemness of tumor cells?** Cell stem cell *16 (3)*, 225-238.

Poltorak, A., He, X., Smirnova, I., Liu, M. Y., Van Huffel, C., Du, X., Birdwell, D., Alejos, E., Silva, M., Galanos, C., Freudenberg, M., Ricciardi-Castagnoli, P., Layton, B. and Beutler, B. (1998). **Defective LPS signaling in C3H/HeJ and C57BL/10ScCr mice: mutations in Tlr4 gene**. Science *282 (5396)*, 2085-2088, doi: 10.1126/science.282.5396.2085.

Pombo Antunes, A. R., Scheyltjens, I., Duerinck, J., Neyns, B., Movahedi, K. and Van Ginderachter, J. A. (2020). **Understanding the glioblastoma immune microenvironment as basis for the development of new immunotherapeutic strategies**. Elife *9*, doi: 10.7554/eLife.52176.

Posso-Osorio, I., Tobon, G. J. and Canas, C. A. (2021). **Human endogenous retroviruses (HERV) and non-HERV viruses incorporated into the human genome and their role in the development of autoimmune diseases**. J Transl Autoimmun *4*, 100137, doi: 10.1016/j.jtauto.2021.100137.

Prager, B. C., Bhargava, S., Mahadev, V., Hubert, C. G. and Rich, J. N. (2020). **Glioblastoma Stem cells: Driving Resilience through Chaos**. Trends in Cancer *6 (3)*, 223-235, doi: 10.1016/j.trecan.2020.01.009.

Press, R. H., Shafer, S. L., Jiang, R., Buchwald, Z. S., Abugideiri, M., Tian, S., Morgan, T. M., Behera, M., Sengupta, S., Voloschin, A. D., Olson, J. J., Hasan, S., Blumenthal, D. T., Curran, W. J., Eaton, B. R., Shu, H. G. and Zhong, J. (2020). **Optimal timing of chemoradiotherapy after surgical resection of glioblastoma: Stratification by validated prognostic classification**. Cancer *126 (14)*, 3255-3264, doi: 10.1002/cncr.32797.

Price, M., Neff, C., Nagarajan, N., Kruchko, C., Waite, K. A., Cioffi, G., Cordeiro, B. B., Willmarth, N., Penas-Prado, M., Gilbert, M. R., Armstrong, T. S., Barnholtz-Sloan, J. S. and Ostrom, Q. T. (2024). **CBTRUS Statistical Report: American Brain Tumor Association & NCI Neuro-Oncology Branch Adolescent and Young Adult Primary Brain and Other Central Nervous System Tumors Diagnosed in the United States in 2016-2020**. Neuro-Oncology *26 (Supplement_3)*, iii1-iii53, doi: 10.1093/neuonc/noae047.

Qi, L., Yu, H. Q., Zhang, Y., Zhao, D. H., Lv, P., Zhong, Y. and Xu, Y. (2016). **IL-10 secreted by M2 macrophage promoted tumorigenesis through interaction with JAK2 in glioma**. Oncotarget *7 (44)*, 71673-71685, doi: 10.18632/oncotarget.12317.

Quail, D. F. and Joyce, J. A. (2013). **Microenvironmental regulation of tumor progression and metastasis**. Nat Med *19 (11)*, 1423-1437, doi: 10.1038/nm.3394.

Radbruch, A., Fladt, J., Kickingereder, P., Wiestler, B., Nowosielski, M., Baumer, P., Schlemmer, H. P., Wick, A., Heiland, S., Wick, W. and Bendszus, M. (2015). **Pseudoprogression in patients with glioblastoma: clinical relevance despite low incidence**. Neuro Oncol *17 (1)*, 151-159, doi: 10.1093/neuonc/nou129.

Rebollo, R., Romanish, M. T. and Mager, D. L. (2012). **Transposable elements: an abundant and natural source of regulatory sequences for host genes**. Annu Rev Genet *46 (Volume 46, 2012)*, 21-42, doi: 10.1146/annurev-genet-110711-155621.

Reiche, L., Plaack, B., Lehmkuhl, M., Weyers, V., Gruchot, J., Picard, D., Perron, H., Remke, M., Knobbe-Thomsen, C., Reifenberger, G., Küry, P. and Kremer, D. (2024). **HERV-W envelope protein is present in microglial cells of the human glioma tumor**

microenvironment and differentially modulates neoplastic cell behavior. Microbes and Infection, 105460, doi: https://doi.org/10.1016/j.micinf.2024.105460.

Reifenberger, G., Wirsching, H. G., Knobbe-Thomsen, C. B. and Weller, M. (2017). **Advances in the molecular genetics of gliomas - implications for classification and therapy**. Nature Reviews Clinical Oncology *14 (7)*, 434-452, doi: 10.1038/nrclinonc.2016.204.

Ricklin, D., Reis, E. S., Mastellos, D. C., Gros, P. and Lambris, J. D. (2016). **Complement component C3 - The "Swiss Army Knife" of innate immunity and host defense**. Immunol Rev *274 (1)*, 33-58, doi: 10.1111/imr.12500.

Rodriguez, S. M. B., Kamel, A., Ciubotaru, G. V., Onose, G., Sevastre, A. S., Sfredel, V., Danoiu, S., Dricu, A. and Tataranu, L. G. (2023). **An Overview of EGFR Mechanisms and Their Implications in Targeted Therapies for Glioblastoma**. Int J Mol Sci *24 (13)*, doi: 10.3390/ijms241311110.

Rolland, A., Jouvin-Marche, E., Viret, C., Faure, M., Perron, H. and Marche, P. N. (2006). **The Envelope Protein of a Human Endogenous Retrovirus-W Family Activates Innate Immunity through CD14/TLR4 and Promotes Th1-Like Responses**. The Journal of Immunology *176 (12)*, 7636-7644, doi: 10.4049/jimmunol.176.12.7636.

Rosén, E., Mangukiya, H. B., Elfineh, L., Stockgard, R., Krona, C., Gerlee, P. and Nelander, S. (2023). **Inference of glioblastoma migration and proliferation rates using single time-point images**. Communications Biology *6 (1)*, 402, doi: 10.1038/s42003-023-04750-0.

Sahm, K., Kessler, T., Eisele, P., Ratliff, M., Sperk, E., König, L., Breckwoldt, M. O., Seliger, C., Mildenberger, I., Schrimpf, D., Herold-Mende, C., Zeiner, P. S., Tabatabai, G., Meuth, S. G., Capper, D., Bendszus, M., von Deimling, A., Wick, W., Sahm, F. and Platten, M. (2023). **Concurrent gliomas in patients with multiple sclerosis**. Communications Medicine *3 (1)*, 186, doi: 10.1038/s43856-023-00381-y.

Schiffer, D., Annovazzi, L., Casalone, C., Corona, C. and Mellai, M. (2019). **Glioblastoma: Microenvironment and Niche Concept**. Cancers *11 (1)*, 5, doi: ARTN 5

10.3390/cancers11010005.

Schindelin, J., Arganda-Carreras, I., Frise, E., Kaynig, V., Longair, M., Pietzsch, T., Preibisch, S., Rueden, C., Saalfeld, S., Schmid, B., Tinevez, J. Y., White, D. J., Hartenstein, V., Eliceiri, K., Tomancak, P. and Cardona, A. (2012). **Fiji: an open-source platform for biological-image analysis**. Nat Methods *9 (7)*, 676-682, doi: 10.1038/nmeth.2019.

Seker-Polat, F., Pinarbasi Degirmenci, N., Solaroglu, I. and Bagci-Onder, T. (2022). **Tumor Cell Infiltration into the Brain in Glioblastoma: From Mechanisms to Clinical Perspectives**. Cancers (Basel) *14 (2)*, doi: 10.3390/cancers14020443.

Shah, A. H., Govindarajan, V., Doucet-O'Hare, T. T., Rivas, S., Ampie, L., DeMarino, C., Banasavadi-Siddegowda, Y. K., Zhang, Y., Johnson, K. R., Almsned, F., Gilbert, M. R., Heiss, J. D. and Nath, A. (2022). **Differential expression of an endogenous retroviral element [HERV-K(HML-6)] is associated with reduced survival in glioblastoma patients**. Sci Rep *12 (1)*, 6902, doi: 10.1038/s41598-022-10914-5.

Shah, A. H., Rivas, S. R., Doucet-O'Hare, T. T., Govindarajan, V., DeMarino, C., Wang, T., Ampie, L., Zhang, Y., Banasavadi-Siddegowda, Y. K., Walbridge, S., Maric, D., Garcia-Montojo, M., Suter, R. K., Lee, M. H., Zaghloul, K. A., Steiner, J., Elkahloun, A. G., Chandar, J., Seetharam, D., Desgraves, J., Li, W., Johnson, K., Ivan, M. E., Komotar, R. J., Gilbert, M. R., Heiss, J. D. and Nath, A. (2023). **Human endogenous retrovirus K contributes to a stem cell niche in glioblastoma**. J Clin Invest *133 (13)*, doi: 10.1172/jci167929.

Shan, Y., He, X., Song, W., Han, D., Niu, J. and Wang, J. (2015). **Role of IL-6 in the invasiveness and prognosis of glioma**. Int J Clin Exp Med *8 (6)*, 9114-9120.

Shang, Q., Yu, X., Sun, Q., Li, H., Sun, C. and Liu, L. (2024). **Polysaccharides regulate Th1/Th2 balance: A new strategy for tumor immunotherapy**. Biomedicine & Pharmacotherapy *170*, 115976, doi: 10.1016/j.biopha.2023.115976.

Sharma, N., Mallela, A. N., Shi, D. D., Tang, L. W., Abou-Al-Shaar, H., Gersey, Z. C., Zhang, X., McBrayer, S. K. and Abdullah, K. G. (2023). **Isocitrate dehydrogenase mutations in**

gliomas: A review of current understanding and trials. Neurooncol Adv *5 (1)*, vdad053, doi: 10.1093/noajnl/vdad053.

Shi, C. and Pamer, E. G. (2011). **Monocyte recruitment during infection and inflammation**. Nat Rev Immunol *11 (11)*, 762-774, doi: 10.1038/nri3070.

Singh, N., Miner, A., Hennis, L. and Mittal, S. (2021). **Mechanisms of temozolomide resistance in glioblastoma - a comprehensive review**. Cancer Drug Resist *4 (1)*, 17-43, doi: 10.20517/cdr.2020.79.

Smirniotopoulos, J. G., Murphy, F. M., Rushing, E. J., Rees, J. H. and Schroeder, J. W. (2007). **Patterns of contrast enhancement in the brain and meninges**. Radiographics *27 (2)*, 525-551, doi: 10.1148/rg.272065155.

So, J. S., Kim, H. and Han, K. S. (2021). **Mechanisms of Invasion in Glioblastoma: Extracellular Matrix, Ca**

Signaling, and Glutamate. Frontiers in Cellular Neuroscience *15*, doi: ARTN 663092 10.3389/fncel.2021.663092.

Sørensen, M. D., Dahlrot, R. H., Boldt, H. B., Hansen, S. and Kristensen, B. W. (2018). **Tumour-associated microglia/macrophages predict poor prognosis in high-grade gliomas and correlate with an aggressive tumour subtype**. Neuropathology and applied neurobiology *44 (2)*, 185-206.

Sotgiu, S., Mameli, G., Serra, C., Zarbo, I. R., Arru, G. and Dolei, A. (2010). **Multiple sclerosis-associated retrovirus and progressive disability of multiple sclerosis**. Mult Scler *16 (10)*, 1248-1251, doi: 10.1177/1352458510376956.

Șovrea, A. S., Boșca, B., Melincovici, C. S., Constantin, A.-M., Crintea, A., Mărginean, M., Dronca, E., Jianu, M. E., Suflețel, R., Gonciar, D., Bungărdean, M. and Crivii, C.-B. (2022). **Multiple Faces of the Glioblastoma Microenvironment**. International Journal of Molecular Sciences *23 (2)*, 595.

Stummer, W., Novotny, A., Stepp, H., Goetz, C., Bise, K. and Reulen, H. J. (2000). **Fluorescence-guided resection of glioblastoma multiforme utilizing 5-ALA-induced porphyrins: a prospective study in 52 consecutive patients**. Journal of Neurosurgery *93 (6)*, 1003-1013, doi: 10.3171/jns.2000.93.6.1003.

Stummer, W., Pichlmeier, U., Meinel, T., Wiestler, O. D., Zanella, F., Reulen, H. J. and Group, A. L.-G. S. (2006). **Fluorescence-guided surgery with 5-aminolevulinic acid for resection of malignant glioma: a randomised controlled multicentre phase III trial**. Lancet Oncol *7 (5)*, 392-401, doi: 10.1016/S1470-2045(06)70665-9.

Stupp, R., Mason, W. P., van den Bent, M. J., Weller, M., Fisher, B., Taphoorn, M. J., Belanger, K., Brandes, A. A., Marosi, C., Bogdahn, U., Curschmann, J., Janzer, R. C., Ludwin, S. K., Gorlia, T., Allgeier, A., Lacombe, D., Cairncross, J. G., Eisenhauer, E., Mirimanoff, R. O., European Organisation for, R., Treatment of Cancer Brain, T., Radiotherapy, G. and National Cancer Institute of Canada Clinical Trials, G. (2005). **Radiotherapy plus concomitant and adjuvant temozolomide for glioblastoma**. N Engl J Med *352 (10)*, 987-996, doi: 10.1056/NEJMoa043330.

Suarez-Arnedo, A., Torres Figueroa, F., Clavijo, C., Arbelaez, P., Cruz, J. C. and Munoz-Camargo, C. (2020). **An image J plugin for the high throughput image analysis of in vitro scratch wound healing assays**. PLoS One *15 (7)*, e0232565, doi: 10.1371/journal.pone.0232565.

Suzuki, H., Aoki, K., Chiba, K., Sato, Y., Shiozawa, Y., Shiraishi, Y., Shimamura, T., Niida, A., Motomura, K. and Ohka, F. (2015). **Mutational landscape and clonal architecture in grade II and III gliomas**. Nature genetics *47 (5)*, 458-468.

Tan, A. C., Ashley, D. M., Lopez, G. Y., Malinzak, M., Friedman, H. S. and Khasraw, M. (2020). **Management of glioblastoma: State of the art and future directions**. CA Cancer J Clin *70 (4)*, 299-312, doi: 10.3322/caac.21613.

Taphoorn, M. J. B., Dirven, L., Kanner, A. A., Lavy-Shahaf, G., Weinberg, U., Taillibert, S., Toms, S. A., Honnorat, J., Chen, T. C., Sroubek, J., David, C., Idbaih, A., Easaw, J. C., Kim, C. Y., Bruna, J., Hottinger, A. F., Kew, Y., Roth, P., Desai, R., Villano, J. L., Kirson, E. D., Ram,

Z. and Stupp, R. (2018). **Influence of Treatment With Tumor-Treating Fields on Health-Related Quality of Life of Patients With Newly Diagnosed Glioblastoma: A Secondary Analysis of a Randomized Clinical Trial**. JAMA Oncol *4 (4)*, 495-504, doi: 10.1001/jamaoncol.2017.5082.

Tarassishin, L., Lim, J., Weatherly, D. B., Angeletti, R. H. and Lee, S. C. (2014). **Interleukin-1-induced changes in the glioblastoma secretome suggest its role in tumor progression**. J Proteomics *99*, 152-168, doi: 10.1016/j.jprot.2014.01.024.

Tavakolian, S., Goudarzi, H. and Faghihloo, E. (2019). **Evaluating the expression level of HERV-K env, np9, rec and gag in breast tissue**. Infect Agent Cancer *14*, 42, doi: 10.1186/s13027-019-0260-7.

Teraiya, M., Perreault, H. and Chen, V. C. (2023). **An overview of glioblastoma multiforme and temozolomide resistance: can LC-MS-based proteomics reveal the fundamental mechanism of temozolomide resistance?** Frontiers in Oncology *13*, doi: 10.3389/fonc.2023.1166207.

Terry, S., Buart, S. and Chouaib, S. (2017). **Hypoxic Stress-induced tumor and immune plasticity, suppression, and impact on tumor heterogeneity. Front Immunol 8: 1625** (

Thomas, R. P., Recht, L. and Nagpal, S. (2013). **Advances in the management of glioblastoma: the role of temozolomide and MGMT testing**. Clin Pharmacol *5*, 1-9, doi: 10.2147/CPAA.S26586.

Toh, T. B., Lim, J. J. and Chow, E. K. (2017). **Epigenetics in cancer stem cells**. Mol Cancer *16 (1)*, 29, doi: 10.1186/s12943-017-0596-9.

Tolcher, A., Gerson, S., Denis, L., Geyer, C., Hammond, L., Patnaik, A., Goetz, A., Schwartz, G., Edwards, T. and Reyderman, L. (2003). **Marked inactivation of O6-alkylguanine-DNA alkyltransferase activity with protracted temozolomide schedules**. British journal of cancer *88 (7)*, 1004-1011.

Torsvik, A., Stieber, D., Enger, P., Golebiewska, A., Molven, A., Svendsen, A., Westermark, B., Niclou, S. P., Olsen, T. K., Chekenya Enger, M. and Bjerkvig, R. (2014). **U-251 revisited: genetic drift and phenotypic consequences of long-term cultures of glioblastoma cells**. Cancer Med *3 (4)*, 812-824, doi: 10.1002/cam4.219.

Trinchieri, G. and Sher, A. (2007). **Cooperation of Toll-like receptor signals in innate immune defence**. Nat Rev Immunol *7 (3)*, 179-190, doi: 10.1038/nri2038.

Tripathi, S., Vivas-Buitrago, T., Domingo, R. A., De Biase, G., Brown, D., Akinduro, O. O., Ramos-Fresnedo, A., Sherman, W., Gupta, V., Middlebrooks, E. H., Sabsevitz, D. S., Porter, A. B., Uhm, J. H., Bendok, B. R., Parney, I., Meyer, F. B., Chaichana, K. L., Swanson, K. R. and Quiñones-Hinojosa, A. (2022). **IDH-wild-type glioblastoma cell density and infiltration distribution influence on supramarginal resection and its impact on overall survival: a mathematical model**. Journal of Neurosurgery *136 (6)*, 1567-1575, doi: 10.3171/2021.6.Jns21925.

Tsuchihashi, K., Okazaki, S., Ohmura, M., Ishikawa, M., Sampetrean, O., Onishi, N., Wakimoto, H., Yoshikawa, M., Seishima, R., Iwasaki, Y., Morikawa, T., Abe, S., Takao, A., Shimizu, M., Masuko, T., Nagane, M., Furnari, F. B., Akiyama, T., Suematsu, M., Baba, E., Akashi, K., Saya, H. and Nagano, O. (2016). **The EGF Receptor Promotes the Malignant Potential of Glioma by Regulating Amino Acid Transport System xc(—)**. Cancer Research *76 (10)*, 2954-2963, doi: 10.1158/0008-5472.Can-15-2121.

Ulland, T. K., Song, W. M., Huang, S. C. C., Ulrich, J. D., Sergushichev, A., Beatty, W. L., Loboda, A. A., Zhou, Y. Y., Caims, N. J., Kambal, A., Loginicheva, E., Gilfillan, S., Cella, M., Virgin, H. W., Unanue, E. R., Wang, Y. M., Artyomov, M. N., Holtzman, D. M. and Colonna, M. (2017). **TREM2 Maintains Microglial Metabolic Fitness in Alzheimer's Disease**. Cell *170 (4)*, 649-+, doi: 10.1016/j.cell.2017.07.023.

Urbanska, K., Sokolowska, J., Szmidt, M. and Sysa, P. (2014). **Glioblastoma multiforme - an overview**. Contemp Oncol (Pozn) *18 (5)*, 307-312, doi: 10.5114/wo.2014.40559.

Vakilian, A., Khorramdelazad, H., Heidari, P., Sheikh Rezaei, Z. and Hassanshahi, G. (2017). **CCL2/CCR2 signaling pathway in glioblastoma multiforme**. Neurochemistry International *103*, 1-7, doi: https://doi.org/10.1016/j.neuint.2016.12.013.

van der Meulen, M., Ramos, R. C., Mason, W. P., Von Deimling, A. and Maas, S. L. N. (2022). **Opinion & Special Article: Glioma Classification**. Neurology *99 (20)*, 903-908, doi: doi:10.1212/WNL.0000000000201262.

Verdugo, E., Puerto, I. and Medina, M. A. (2022). **An update on the molecular biology of glioblastoma, with clinical implications and progress in its treatment**. Cancer Commun (Lond) *42 (11)*, 1083-1111, doi: 10.1002/cac2.12361.

Villesen, P., Aagaard, L., Wiuf, C. and Pedersen, F. S. (2004). **Identification of endogenous retroviral reading frames in the human genome**. Retrovirology *1*, 32, doi: 10.1186/1742-4690-1-32.

Vollmann-Zwerenz, A., Leidgens, V., Feliciello, G., Klein, C. A. and Hau, P. (2020). **Tumor Cell Invasion in Glioblastoma**. Int J Mol Sci *21 (6)*, doi: 10.3390/ijms21061932.

Wainwright, E. N. and Scaffidi, P. (2017). **Epigenetics and Cancer Stem Cells: Unleashing, Hijacking, and Restricting Cellular Plasticity**. Trends Cancer *3 (5)*, 372-386, doi: 10.1016/j.trecan.2017.04.004.

Walsh, J. C., Lebedev, A., Aten, E., Madsen, K., Marciano, L. and Kolb, H. C. (2014). **The clinical importance of assessing tumor hypoxia: relationship of tumor hypoxia to prognosis and therapeutic opportunities**. Antioxid Redox Signal *21 (10)*, 1516-1554, doi: 10.1089/ars.2013.5378.

Wang, L. M., Englander, Z. K., Miller, M. L. and Bruce, J. N. (2023a). **Malignant Glioma**. In: Human Brain and Spinal Cord Tumors: From Bench to Bedside. Volume 2, eds. Rezaei, N. and Hanaei, S., Springer International Publishing, Cham, pp. 1-30.

Wang, P., Yuan, Y., Lin, W., Zhong, H., Xu, K. and Qi, X. (2019). **Roles of sphingosine-1-phosphate signaling in cancer**. Cancer Cell International *19 (1)*, 295, doi: 10.1186/s12935-019-1014-8.

Wang, W., Tugaoen, J. D., Fadda, P., Toland, A. E., Ma, Q., Elder, J. B., Giglio, P., James Cancer Center Integrated Neuro-Oncology, T. and Otero, J. J. (2023b). **Glioblastoma pseudoprogression and true progression reveal spatially variable transcriptional differences**. Acta Neuropathol Commun *11 (1)*, 192, doi: 10.1186/s40478-023-01587-w.

Wang, Y., Moncayo, G., Morin, P., Xue, G., Grzmil, M., Lino, M. M., Clément-Schatlo, V., Frank, S., Merlo, A. and Hemmings, B. A. (2013). **Mer receptor tyrosine kinase promotes invasion and survival in glioblastoma multiforme**. Oncogene *32 (7)*, 872-882, doi: 10.1038/onc.2012.104.

Weiss, T., Puca, E., Silginer, M., Hemmerle, T., Pazahr, S., Bink, A., Weller, M., Neri, D. and Roth, P. (2020). **Immunocytokines are a promising immunotherapeutic approach against glioblastoma**. Sci Transl Med *12 (564)*, doi: 10.1126/scitranslmed.abb2311.

Weller, M., Stupp, R., Reifenberger, G., Brandes, A. A., van den Bent, M. J., Wick, W. and Hegi, M. E. (2010). **MGMT promoter methylation in malignant gliomas: ready for personalized medicine?** Nat Rev Neurol *6 (1)*, 39-51, doi: 10.1038/nrneurol.2009.197.

Weller, M., van den Bent, M., Preusser, M., Le Rhun, E., Tonn, J. C., Minniti, G., Bendszus, M., Balana, C., Chinot, O., Dirven, L., French, P., Hegi, M. E., Jakola, A. S., Platten, M., Roth, P., Ruda, R., Short, S., Smits, M., Taphoorn, M. J. B., von Deimling, A., Westphal, M., Soffietti, R., Reifenberger, G. and Wick, W. (2021). **EANO guidelines on the diagnosis and treatment of diffuse gliomas of adulthood**. Nat Rev Clin Oncol *18 (3)*, 170-186, doi: 10.1038/s41571-020-00447-z.

Weller, M., Wen, P. Y., Chang, S. M., Dirven, L., Lim, M., Monje, M. and Reifenberger, G. (2024). **Glioma**. Nat Rev Dis Primers *10 (1)*, 33, doi: 10.1038/s41572-024-00516-y.

Wen, P. Y., Macdonald, D. R., Reardon, D. A., Cloughesy, T. F., Sorensen, A. G., Galanis, E., Degroot, J., Wick, W., Gilbert, M. R., Lassman, A. B., Tsien, C., Mikkelsen, T., Wong, E. T., Chamberlain, M. C., Stupp, R., Lamborn, K. R., Vogelbaum, M. A., van den Bent, M. J. and Chang, S. M. (2010). **Updated response assessment criteria for high-grade gliomas: response assessment in neuro-oncology working group**. J Clin Oncol *28 (11)*, 1963-1972, doi: 10.1200/JCO.2009.26.3541.

Weyers, V. (2024) **Proinflammatory effects of the pHERV-W envelope protein on primary neonatal microglia of the Wistar rat related to multiple sclerosis**.

Wrensch, M., Minn, Y., Chew, T., Bondy, M. and Berger, M. S. (2002). **Epidemiology of primary brain tumors: current concepts and review of the literature**. Neuro Oncol *4 (4)*, 278-299, doi: 10.1093/neuonc/4.4.278.

Wu, J., Frady, L. N., Bash, R. E., Cohen, S. M., Schorzman, A. N., Su, Y. T., Irvin, D. M., Zamboni, W. C., Wang, X., Frye, S. V., Ewend, M. G., Sulman, E. P., Gilbert, M. R., Earp, H. S. and Miller, C. R. (2018). **MerTK as a therapeutic target in glioblastoma**. Neuro Oncol *20 (1)*, 92-102, doi: 10.1093/neuonc/nox111.

Wu, W., Klockow, J. L., Zhang, M., Lafortune, F., Chang, E., Jin, L., Wu, Y. and Daldrup-Link, H. E. (2021). **Glioblastoma multiforme (GBM): An overview of current therapies and mechanisms of resistance**. Pharmacol Res *171*, 105780, doi: 10.1016/j.phrs.2021.105780.

Xie, Q., Mittal, S. and Berens, M. E. (2014). **Targeting adaptive glioblastoma: an overview of proliferation and invasion**. Neuro Oncol *16 (12)*, 1575-1584, doi: 10.1093/neuonc/nou147.

Xue, Q., Cao, L., Chen, X. Y., Zhao, J., Gao, L., Li, S. Z. and Fei, Z. (2017). **High expression of MMP9 in glioma affects cell proliferation and is associated with patient survival rates**. Oncol Lett *13 (3)*, 1325-1330, doi: 10.3892/ol.2017.5567.

Yan, H., Parsons, D. W., Jin, G., McLendon, R., Rasheed, B. A., Yuan, W., Kos, I., Batinic-Haberle, I., Jones, S., Riggins, G. J., Friedman, H., Friedman, A., Reardon, D., Herndon, J., Kinzler,

K. W., Velculescu, V. E., Vogelstein, B. and Bigner, D. D. (2009). **IDH1 and IDH2 mutations in gliomas**. N Engl J Med *360 (8)*, 765-773, doi: 10.1056/NEJMoa0808710.

Yan, T., Tan, Y., Deng, G., Sun, Z., Liu, B., Wang, Y., Yuan, F., Sun, Q., Hu, P., Gao, L., Tian, D. and Chen, Q. (2022). **TGF-β induces GBM mesenchymal transition through upregulation of CLDN4 and nuclear translocation to activate TNF-α/NF-κB signal pathway**. Cell Death & Disease *13 (4)*, 339, doi: 10.1038/s41419-022-04788-8.

Yang, M., Guo, W., Yang, C., Tang, J., Huang, Q., Feng, S., Jiang, A., Xu, X. and Jiang, G. (2017). **Mobile phone use and glioma risk: A systematic review and meta-analysis**. PLoS One *12 (5)*, e0175136, doi: 10.1371/journal.pone.0175136.

Yao, M., Li, S., Wu, X., Diao, S., Zhang, G., He, H., Bian, L. and Lu, Y. (2018). **Cellular origin of glioblastoma and its implication in precision therapy**. Cell Mol Immunol *15 (8)*, 737-739, doi: 10.1038/cmi.2017.159.

Ye, X. Z., Xu, S. L., Xin, Y. H., Yu, S. C., Ping, Y. F., Chen, L., Xiao, H. L., Wang, B., Yi, L., Wang, Q. L., Jiang, X. F., Yang, L., Zhang, P., Qian, C., Cui, Y. H., Zhang, X. and Bian, X. W. (2012). **Tumor-Associated Microglia/Macrophages Enhance the Invasion of Glioma Stem-like Cells via TGF-β1 Signaling Pathway**. Journal of Immunology *189 (1)*, 444-453, doi: 10.4049/jimmunol.1103248.

Yeo, E. C. F., Brown, M. P., Gargett, T. and Ebert, L. M. (2021). **The Role of Cytokines and Chemokines in Shaping the Immune Microenvironment of Glioblastoma: Implications for Immunotherapy**. Cells *10 (3)*, doi: 10.3390/cells10030607.

Yoshida, S., Ono, M., Shono, T., Izumi, H., Ishibashi, T., Suzuki, H. and Kuwano, M. (1997). **Involvement of interleukin-8, vascular endothelial growth factor, and basic fibroblast growth factor in tumor necrosis factor alpha-dependent angiogenesis**. Mol Cell Biol *17 (7)*, 4015-4023, doi: 10.1128/MCB.17.7.4015.

Yoshikawa, M. H., Rabelo, N. N., Telles, J. P. M. and Figueiredo, E. G. (2023). **Modifiable risk factors for glioblastoma: a systematic review and meta-analysis**. Neurosurg Rev *46 (1)*, 143, doi: 10.1007/s10143-023-02051-y.

Young, J. S., Al-Adli, N., Scotford, K., Cha, S. and Berger, M. S. (2023). **Pseudoprogression versus true progression in glioblastoma: what neurosurgeons need to know**. J Neurosurg *139 (3)*, 748-759, doi: 10.3171/2022.12.Jns222173.

Yu, M., Chang, Y., Zhai, Y., Pang, B., Wang, P., Li, G., Jiang, T. and Zeng, F. (2022). **TREM2 is associated with tumor immunity and implies poor prognosis in glioma**. Front Immunol *13*, 1089266, doi: 10.3389/fimmu.2022.1089266.

Zare, M., Mostafaei, S., Ahmadi, A., Azimzadeh Jamalkandi, S., Abedini, A., Esfahani-Monfared, Z., Dorostkar, R. and Saadati, M. (2018). **Human endogenous retrovirus env genes: Potential blood biomarkers in lung cancer**. Microb Pathog *115*, 189-193, doi: 10.1016/j.micpath.2017.12.040.

Zhang, H., Yuan, F., Qi, Y., Liu, B. and Chen, Q. (2021). **Circulating Tumor Cells for Glioma**. Front Oncol *11*, 607150, doi: 10.3389/fonc.2021.607150.

Zhang, J., Stevens, M. F. and Bradshaw, T. D. (2012). **Temozolomide: mechanisms of action, repair and resistance**. Curr Mol Pharmacol *5 (1)*, 102-114, doi: 10.2174/1874467211205010102.

Zhang, M., Liang, J. Q. and Zheng, S. (2019). **Expressional activation and functional roles of human endogenous retroviruses in cancers**. Rev Med Virol *29 (2)*, e2025, doi: 10.1002/rmv.2025.

Zhou, W., Yu, X., Sun, S., Zhang, X., Yang, W., Zhang, J., Zhang, X. and Jiang, Z. (2019). **Increased expression of MMP-2 and MMP-9 indicates poor prognosis in glioma recurrence**. Biomedicine & Pharmacotherapy *118*, 109369, doi: https://doi.org/10.1016/j.biopha.2019.109369.

Zhu, H., Yu, X., Zhang, S. and Shu, K. (2021). **Targeting the Complement Pathway in Malignant Glioma Microenvironments**. Front Cell Dev Biol *9*, 657472, doi: 10.3389/fcell.2021.657472.

Zong, H., Verhaak, R. G. and Canoll, P. (2012). **The cellular origin for malignant glioma and prospects for clinical advancements**. Expert Rev Mol Diagn *12 (4)*, 383-394, doi: 10.1586/erm.12.30.

Danksagung

An dieser Stelle möchte ich mich bei allen bedanken, die mich auf dem Weg zu dieser Dissertation begleitet und unterstützt haben.

Mein besonderer Dank gilt meinem Doktorvater PD Dr. med. David Kremer, der mich zu jeder Zeit mit wertvollen Anregungen, konstruktivem Feedback und fachlicher Expertise unterstützt und motiviert hat. Seine Geduld und Unterstützung haben wesentlich zum Gelingen dieser Arbeit beigetragen. Ich bin froh, dass ich mit diesem Projekt ein großes und interessantes Thema hoher medizinischer Relevanz erforschen konnte.

Ebenso möchte ich Univ.-Prof. Dr. phil. Patrick Küry danken, der mir die Möglichkeit gegeben hat, in der Arbeitsgruppe Neuroregeneration zu forschen. Seine Unterstützung und Begleitung während meiner Zeit im Labor waren von unschätzbarem Wert.

Ein besonderer Dank gilt Frau M.Sc. Laura Reiche. Liebe Laura, ohne deine großartige Betreuung wäre diese Arbeit nicht entstanden. Du hast mir nicht nur das wissenschaftliche Arbeiten und Denken beigebracht, sondern warst stets eine unverzichtbare Unterstützung mit einem offenen Ohr für alle Fragen und Herausforderungen. Deine Geduld, deine Hilfsbereitschaft und dein Engagement sind alles andere als selbstverständlich – dafür bin ich dir unendlich dankbar.

Auch dem gesamten wissenschaftlichen Team der AG Neuroregeneration möchte ich danken. Die angenehme Arbeitsatmosphäre und die großartige Zusammenarbeit haben meine Forschungszeit zu einer wertvollen Erfahrung gemacht. Besonders danke ich den technischen Assistentinnen Brigida Ziegler, Birgit Blomenkamp und Julia Jadasz, die mich mit ihrer Expertise und Hilfsbereitschaft jederzeit unterstützt haben.

Ein großes Dankeschön gilt auch meinen Freundinnen und Freunden, die mich in stressigen Zeiten ermutigt und für den nötigen Ausgleich gesorgt haben. Ohne Euch wären die letzten Jahre um einiges schwieriger gewesen.

Meinen tiefsten Dank widme ich meiner Familie, insbesondere meinen Eltern Regina und Oliver. Mit eurer Liebe, eurem Vertrauen und eurer bedingungslosen Unterstützung konnte ich mich immer auf euch verlassen. Ohne euch wäre ich nicht hier, und ohne eure Ermutigung und euren Rückhalt wäre diese Dissertation nicht möglich gewesen.

Abschließend möchte ich mich auch bei meiner Schwester Ann-Sophie bedanken, die mir in den letzten Jahren nicht nur eine liebevolle Schwester, sondern auch eine verlässliche Stütze war.