Einfluss des Arylhydrocarbon Rezeptors auf Plattenepithelkarzinome

Inaugural-Dissertation

Zur Erlangung des Doktorgrades
der Mathematisch-Naturwissenschaftlichen Fakultät
der Heinrich-Heine-Universität Düsseldorf

vorgelegt von

Marius Pollet

aus Langenfeld (Rhld.)

Düsseldorf, Mai 2019

aus dem Leibniz-Institut für Umweltmedizinische Forschung (IUF)

an der Heinrich-Heine-Universität Düsseldorf gGmbH

Gedruckt mit der Genehmigung der
Mathematisch-Naturwissenschaftlichen Fakultät der

Heinrich-Heine-Universität Düsseldorf

Referent: Prof. Dr. Dr. h.c. Peter Proksch

Korreferent: Prof. Dr. med. Jean Krutmann

Tag der mündlichen Prüfung: 17.09.2019

I. Zusammenfassung

Ultraviolette Strahlung kann zur Induktion maligner Hauterkrankungen führen und stellt, aufgrund ansteigender Fallzahlen, sowohl medizinisch als auch ökonomisch ein wachsendes Problem für das Gesundheitssystem dar. Trifft ultraviolette B (UVB) Strahlung auf die Haut, wird diese zu einem Großteil von der DNA epidermaler Keratinozyten absorbiert. Die daraus resultierenden Schäden im Erbgut werden durch Initiation spezialisierter Signalkaskaden erkannt. Der Körper versucht diese Schäden durch eigene Reparaturmechanismen zu beheben und somit die Manifestierung der Schäden in Form von Mutationen zu vermeiden. Verläuft dieser Reparaturprozess nicht oder fehlerhaft ab, wird der programmierte Zelltod eingeleitet. Durch Mutationen in Protoonkogenen und Tumorsuppressorgenen, sowie der Akkumulation weiterer DNA-Schäden werden besagte Schutzmechanismen inaktiviert und der Prozess der Hautkarzinogenese vorangetrieben.

Im Rahmen dieser Dissertation soll der Einfluss des Arylhydrocarbon Rezeptors (AHR) auf die Entstehung von kutanen Plattenepithelkarzinomen (SCC) untersucht werden. Die in dieser kumulativen Dissertation aufgeführten Veröffentlichungen konnten unter anderem zeigen, dass eine erhöhte Aktivität des AHR einen negativen Einfluss auf das Tumorsuppressorprotein $p27^{KIP1}$ (im folgenden p27) hat. Neben bereits beschriebenen zellzyklusregulierenden Eigenschaften konnten wir zeigen, dass p27 einen positiven Effekt auf die Nukleotidexzisionsreparatur (NER) in humanen Keratinozyten ausübt. Die *in vivo*-Relevanz konnten wir in SKH1-Mäusen mit ausgeschaltetem AHR Gen dokumentieren. Diese Tiere weisen 48 Stunden nach UVB-Bestrahlung 50% weniger CPDs auf als ihre Wildtyp-Wurfgeschwister.

Auch in der Therapie von Hautkrebs ist der AHR bedeutsam. Ein Beispiel ist der Proteinkinase-Inhibitor (PKI) Vemurafenib der zur Therapie des malignen Melanoms eingesetzt wird. Nebenwirkungen der Therapie sind Hautausschläge und die verstärkte Bildung von SCCs bei behandelten Patienten. Wir konnten zeigen, dass Vemurafenib zu einer Inhibierung des AHR und zur gesteigerten Expression von pro-inflammatorischen Cytokinen sowie Chemokinen führt, wodurch die Ausbildung der Hautauschläge begünstigt zu werden scheint.

Neben vemurafenib werden weitere Proteinkinase-Inhibitoren in der Krebstherapie wie auch in der Forschung eingesetzt. Mittels Literaturrecherche konnten wir ein breites Spektrum an PKI erfassen und deren zellspezifischen Einfluss auf den AHR dokumentieren. Je nach chemischer Struktur können sie den AHR aktivieren oder inhibieren. Veränderungen im Enzymhaushalt und der Metabolisierung von Medikamenten sind die Folge, was einen Einfluss auf den therapeutischen Erfolg haben kann. Eine veränderte Aktivität des AHR resultiert zudem in unterschiedlichen biologischen Endpunkten wie der Apoptose- oder Proliferationsrate von Zellen oder auch der Immunantwort. Diese wissenschaftlichen Daten sind von höchster Relevanz in der Grundlagenforschung.
Zusammenfassend liefert diese Arbeit Grundgedanken für die Prävention von solarem Hautkrebs und kann dazu beitragen Nebenwirkungen bei der Therapie des malignen Melanoms zu minimieren.

II. Summary

Exposure to ultraviolet radiation is the major risk factor for the development of various types of skin cancers, whose incidence is rapidly increasing. Therefore, these skin malignancies are a growing medical and economic burden for health care systems. When ultraviolet B (UVB) radiation penetrates the skin, most of it is absorbed by the DNA of epidermal keratinocytes. The resulting DNA damage is recognized and fixed by specialized DNA repair systems to avoid its mitotic manifestation. If case DNA repair fails, programmed cell death is initiated to maintain the genomic integrity within the tissue. Mutations in proto-oncogenes and tumor suppressor genes, as well as the accumulation of further DNA damage, may contribute to the inactivation of these protective mechanisms, thereby fostering the process of skin carcinogenesis.

In this dissertation, the functional importance of the aryl hydrocarbon receptor (AHR) for the development of cutaneous squamous cell carcinoma (SCC) was investigated. The results from the publications presented in this cumulative dissertation show, among other things, that increased activity of the AHR has a negative influence on the tumor suppressor protein p27. This was associated with a repression of nucleotide excision repair and apoptosis in UVB-exposed keratinocytes. Accordingly, AHR inhibition stabilized the p27 protein level and accelerated CPD repair at early time-points after UVB irradiation, whereas at later time-points, keratinocyte apoptosis was elevated. The in vivo relevance of these findings was confirmed in a chronic UVB irradiation study on SKH1 hairless mice, showing that AHR-deficient animals developed 50% less cutaneous SCCs than their wild type littermates. These findings underscore the potential of AHR as a suitable target for the chemoprevention of skin cancer.

Besides skin cancer prevention, AHR may also play a crucial role in skin cancer treatment. One example is the protein kinase inhibitor (PKI) vemurafenib, which is used for the treatment of malignant melanoma. Side effects of the therapy among others are skin rashes and the increased formation of SCCs in treated patients. We could show that vemurafenib inhibits cutaneous AHR signaling and thereby increases the expression of pro-inflammatory cytokines and chemokines, which seem to favor the formation of UV-unrelated skin rashes.

Besides vemurafenib, there are several other protein kinase inhibitors that are used in both cancer therapy and basic research. By means of literature studies, we were able to identify a broad spectrum of PKI and document their cell-specific influence on the AHR. Depending on their chemical structure, they can activate or inhibit the AHR. Changes in the enzyme balance and the metabolism of drugs are the results which can have an influence on therapeutic success. A change in the activity of the AHR also results in different biological endpoints such as the apoptosis or proliferation rate of cells or the immune response. Such scientific data are of the utmost relevance in basic research.

In conclusion, this thesis provides further concepts for the prevention of solar induced skin cancer und might help minimizing side effects regarding the therapy of malignant melanoma.

III. Abkürzungsverzeichnis

AD	atopische Dermatitis
AHR	Arylhydrocarbon Rezeptor
AKR	Aldo-Keto-Reduktasen
Apaf-1	Apoptose-auslösender Faktor-1
AREG	Amphiregulin
ARNT	AHR nuclear translocator
BAD	Bcl-2-Antagonist for Cell Death
BaP	Benzo(a)pyren
BAX	Bcl-2-assoziiertes X Protein
CDK2	cyclin dependent kinase 2
CHK1	Checkpoint Kinase-1
COX-2	Cyclooxygenase 2
CPD	Cyclobutanpyrimidin Dimere
CS	Cockayne Syndrom
CYP1A1	Cytochrom P450 1A1
DISC	Death-inducing signaling complex
EGFR	Epidermaler Wachstumsfaktor Rezeptor
EREG	Epiregulin
ERK	Extracellular signal-regulated kinase
FADD	Fas-associated death domain
FICZ	6-Formylindol[3,2b]carbazol
GGR	Globale Genome Reparatur
IARC	International Agency for Research on Cancer
KC	Keratinozyten
MAPK	Mitogen aktivierte Proteinkinase
NER	Nucleotidexzisionsreparatur
NF-kB	Nuclear factor kappa B
PGD2	Prostaglandin D_2
PKI	Proteinkinase Inhibitor
PPARγ	peroxisome proliferator-activated receptor gamma
RAF	Rapidly accelerated fibrosarcoma
RAS	Rat sarcoma
ROS	Reaktive Sauerstoffspezies
SCC	Plattenepithelkarzinom
SCF2	Skp1/cullin/F-box protein
TCDD	2,3,7,8-Tetrachlorodibenzo-p-dioxin
TCR	Transkriptionsgekoppelte Reparatur
TNF	Tumor-Nekrosefaktor Rezeptor
UVB	Ultraviolette B Strahlung
VIR	Vemurafenib vermittelte Hautausschläge
XP	Xeroderma Pigmentosum
XRE	Xenobiotika-responsive Elemente

Inhaltsverzeichnis

1 Einleitung

1.1 Die menschliche Haut

Die Haut stellt mit ihren knapp 2 m² Fläche eines der größten Organe des menschlichen Körpers dar. Wie in Abbildung 1 dargestellt wird sie in Epidermis (Oberhaut), Dermis (Lederhaut) und Subkutis (Unterhaut) unterteilt (Fritsch 1998). Die Haut bildet eine mechanische Barriere vor chemischen, biologischen und physikalischen Umwelteinflüssen. Des Weiteren unterstützt sie bei der Thermoregulation und verhindert das Austrocknen des Organismus. Freie Nervenendigungen und Tastkörperchen befähigen die Haut zur Aufnahme von Berührungsreizen und Vermittlung von Schmerzempfinden. Zudem verfügt sie über Kälte- und Wärmerezeptoren zur Aufnahme von Temperaturreizen.

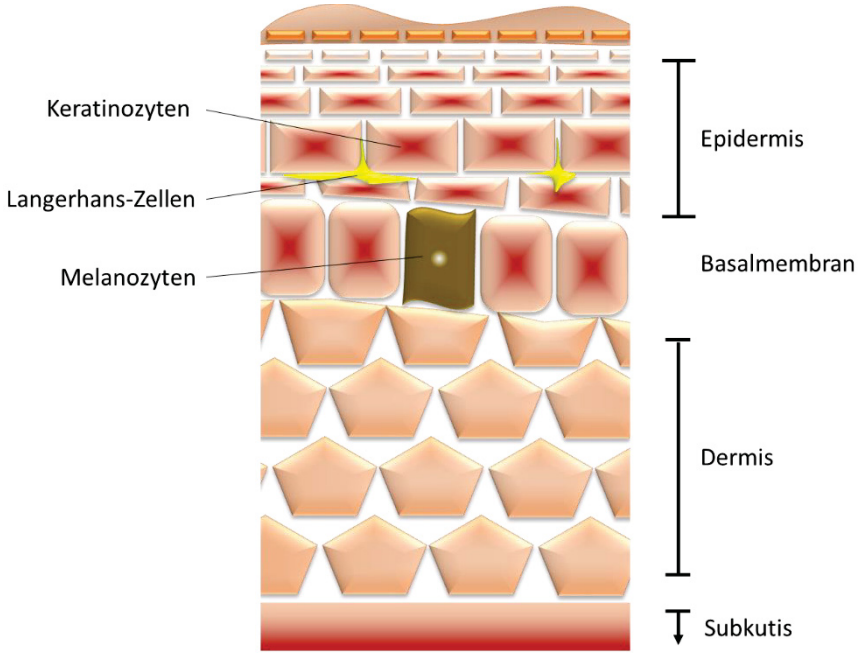

Abbildung 1 Schematischer Aufbau der Haut mit den wichtigsten Vertretern der Epidermis

Die oberste Schicht der Haut, die Epidermis, ist ein gefäßfreies verhornendes Plattenepithel, dessen Versorgung mit Nährstoffen und Sauerstoff über Diffusion erfolgt. Die größte Zellpopulation der Epidermis bilden die Keratinozyten mit ca. 90%. Sie werden im Stratum basale gebildet, differenzieren auf dem Weg zur Hautoberfläche zu Korneozyten aus und bilden vermehrt Keratin. Im Stratum Basale finden sich zudem

pigmentbildende Melanozyten (Rassner 2013). Im darüber liegenden Stratum Spinosum befinden sich die Langerhans-Zellen. Sie machen ca. 3 – 6% der Epidermis aus. Bei Kontakt mit Viren oder Bakterien, aber auch chemischen Agentien oder physikalischer Strahlung initiieren sie eine entsprechende Immunreaktion (Chomiczewska et al. 2009). Die Dermis liegt unterhalb der Epidermis und wird in Stratum papillare und Stratum reticulare unterteilt. Das Stratum papillare bildet zapfenförmige Zellstrukturen aus welche die Dermis mit der Epidermis verbinden. Das Stratum reticulare besteht aus Haarfollikeln, Blutgefäßen, Nervenendigungen und Lymphgefäßen. Zelluläre Vertreter sind Fibroblasten, Mastzellen und Makrophagen. Mastzellen speichern eine Vielzahl von Botenstopfen z.B. Histamin und Heparin. Sie spielen eine wichtige Rolle bei der Abwehr von Bakterien und Parasiten. Zudem vermitteln sie die rasch eintretende Immunantwort beim Kontakt mit Allergenen. Makrophagen sind Phagozyten und mit Aufnahme und Abbau von exogenen Organismen sowie körpereigenen Proteinen betraut. Fibroblasten produzieren Kollagenfasern und Proteoglykane für eine erhöhte Festigkeit der extrazellulären Matrix (Rassner 2013).

1.2 Wirkung ultravioletter Strahlung auf den Menschen

Das von der Sonne emittierte Licht kann in verschiedene Spektren unterschiedlicher Wellenlänge unterteilt werden. Die Infrarotstrahlung bildet den Bereich mit der höchsten Wellenlänge ab (1000 – 780 nm), gefolgt von sichtbarem Licht mit einer Wellenlänge zwischen 780 nm – 380 nm. Das ultraviolette Licht lässt sich noch einmal in die Bereiche UV-A, UV-B und UV-C unterteilen. Die UV-C Strahlung wird von der Stratosphäre vollständig absorbiert, daher kommen Strahlen mit einer Wellenlänge von 280 – 200 nm nicht auf der Erdoberfläche an. UV-A Strahlen (380 – 320 nm) interagieren mit verschiedenen Molekülen in der Dermis und führen zur Bildung von radikalen Verbindungen (Moseley 1988). Diese reaktiven Sauerstoffspezies (ROS) schädigen Membranen, Proteine aber auch die DNA (Cadet und Douki 2011). UV-B Strahlen (320 – 280 nm) werden in der Epidermis von Keratinozyten absorbiert. Das stärkste Chromophor in den Keratinozyten ist die DNA. Die absorbierte Energie regt die doppelgebundenen Kohlenstoffatome der Pyrimidinbasen an. Liegt eine Pyrimidinbase in unmittelbarer Nachbarschaft, kommt es zur Ausbildung zweier kovalenter Bindungen zwischen den Basen. Es bildet sich ein Cyclobutan-Pyrimidin-Dimer (CPD). Formiert sich lediglich zwischen dem 6. Kohlenstoffatom und dem 4. Kohlenstoffatom zweier Pyrimidinbasen eine kovalenten Bindung, spricht man von einem 6-4-Photoprodukt (Goodsell 2001). Der Vorgang ist schematisch in Abbildung 2 dargestellt.

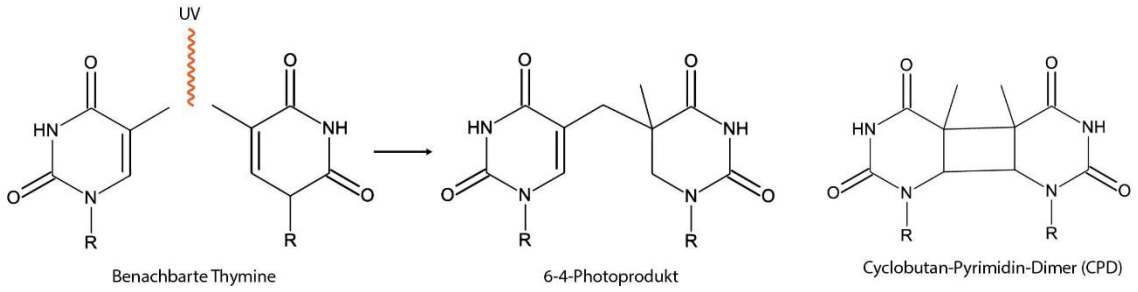

Abbildung 2 Bildung von CPDs und 6-4-Photoprodukten zweier benachbarter Thyminbasen (Zur Verfügung gestellt von Georg Wodarz)

CPDs und 6-4-Photoprodukte können zu einem fehlerhaften ablesen des Erbguts führen. Der Schaden muss schnellstmöglich repariert werden um eine Manifestierung der Veränderung nach der Zellteilung zu vermeiden. Werden diese Schäden von der Zelle erkannt, hält sie den Zellzyklus an (Kim et al. 2002). Proteine der Nukleotid Exzisionsreparatur (NER) werden rekrutiert und der Schaden behoben. Sind die

hervorgerufenen DNA-Schäden zu stark, leitet die Zelle den programmierten Zelltod ein (Kulms et al. 1999; Assefa et al. 2005).

Bei einer zu intensiven oder chronischen Bestrahlung können diese Schutzmechanismen versagen und der Zellzyklus läuft vor der Schadensbeseitigung weiter. Die Zelle teilt sich und gibt die Erbgutveränderung an die Tochterzellen weiter (D'Orazio et al. 2013). Treten diese Veränderungen im kodierenden Bereich von Onkogenen, Reparaturgenen oder Tumorsuppressorgene auf, kann dies zum Funktionsverlust oder zur Funktionsveränderung der daraus synthetisierten Proteine führen. Die Bildung von DNA-Photoprodukten ist damit das initiale Ereignis für die Entwicklung von Hauttumoren (Brash 1988).

Neben der direkten Interaktion mit der DNA führt UV-B Strahlung auch zur Aktivierung verschiedener Signalkaskaden. Zwei dieser Signalwege laufen über die *mitogen-aktivierten Proteinkinasen* (MAPK) und den *Nuclear factor-kappa B* (NF-kB) (Herrlich et al. 2008; Krutmann et al. 2012). Beide wirken anti-apoptotisch und fördern tumorpromovierende Eigenschaften in den bestrahlten Zellen.

Damit vermag UV Strahlung nicht nur das Erbgut zu schädigen, es fördert auch die Tumorpromotion (Bode und Dong 2003; Liu et al. 2013). Aufgrund dieser Eigenschaften wird die nicht-ionisierende Strahlung der Sonne von der *International Agency for Research on Cancer* (IARC) als vollständiges Humankarzinogen der Gruppe 1 eingestuft (IARC 2012).

1.3 Nukleotidexzisionsreparatur

Der menschliche Körper ist Zeit seines Lebens DNA-schädigenden Einflüssen ausgesetzt. Neben physikalischer Strahlung können chemische Substanzen, virale Infektionen, aber auch die zelleigene Replikation zur Schädigung des Erbguts führen (Friedberg et al. 2004). Um diese Schäden zu beheben, verfügt die Zelle über verschiedene Reparatursysteme. Die durch UV-Strahlung entstehenden Läsionen werden mittels Nukleotidexzisionsreparatur (NER) entfernt (Abbildung 3). Diese Reparaturmethode lässt sich in die globale Genomreparatur (GGR) und die Transkriptionsgekoppelte Reparatur (TCR) unterteilen (Schärer 2013).

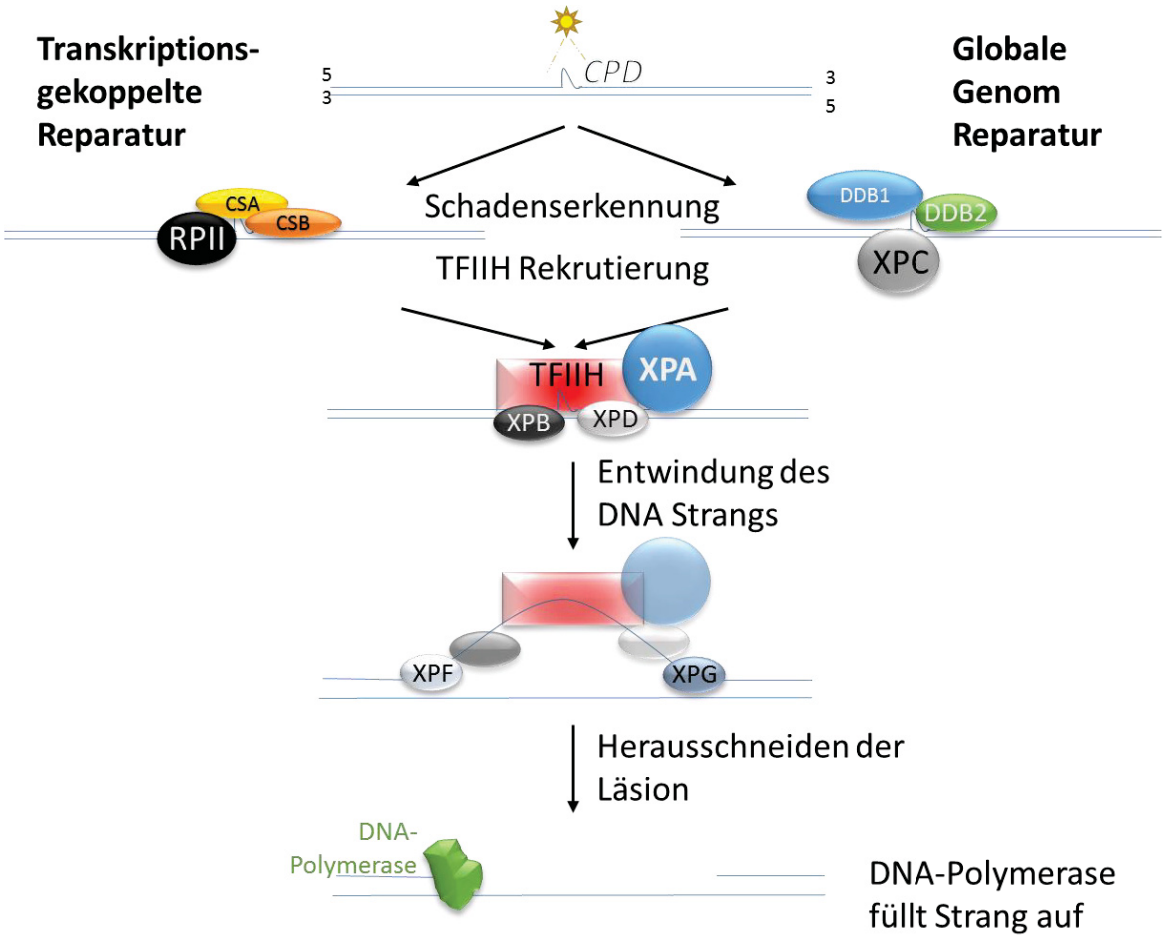

Abbildung 3 Schematische Darstellung der Nukleotidexzisionsreparatur

Sie unterscheiden sich in der Schadenserkennung. Kommt es beim Ablesen der DNA durch die RNA Polymerase II aufgrund eines geschädigten Bereichs zum Stillstand, wird diese durch die TCR Proteine CSA und CSB (Cockayne Syndrom Group) erkannt und entfernt. Bei der GGR erkennt ein Proteinkomplex aus DDB1 und DDB2 (DNA-damage-binding Protein) mit dem Xeroderma Pigmentosum Protein C (XPC) den DNA-Schaden. Ab diesem Punkt laufen beide Reparaturmechanismen identisch ab (Schärer 2013). Nach Bindung von XPA und RPA zur weiteren Schadenserkennung, werden die Helikasen XPB und XPD rekrutiert. Diese sind Teil des TFIIH-Komplexes und entwinden den DNA Strang unter Verbrauch von ATP. Anschließend schneiden die Endonukleasen XPG und XPF ein ca. 30 Basen umfassendes Oligonukleotid, das den DNA Schaden aufweist, heraus. Der geschädigte Strang wird anhand des komplementären Stranges durch die DNA Polymerasen δ und ε aufgefüllt. Die Ligation des synthetisierten Abschnitts wird durch die DNA-Ligase komplementiert.

1.4 Apoptose

Apoptose ist eine hoch konservierte und stark regulierte Form des Zelltods und wichtig für das Ausschalten von infizierten, beschädigten oder transformierten Zellen. Die Apoptose spielt zudem in vielen natürlichen Prozessen des Körpers wie der Embryogenese, Metamorphose oder der Regeneration von Geweben eine Rolle. Die meisten apoptose-induzierenden Signale beruhen auf der Aktivierung der Caspase (cystein-aspartic-acid-proteasen)-Signalkaskade welche zu einer verstärkten Spaltung zelleigener Proteine führt. Dabei wird zwischen intrinsischer und extrinsischer Apoptose unterschieden. UVB-induzierte Schäden der DNA rekrutieren das Tumorsuppressorprotein p53, das je nach Stärke des Schadens Zellzyklusarrest oder, durch gesteigerte Expression der Proteine BAX (Bcl-2-assoziiertes X Protein) und BAD (Bcl-2-Antagonist of Cell Death), Apoptose initiiert. Reaktive Sauerstoffspezies (ROS) und DNA Schäden triggern die Translokation der Proteine an die mitochondriale Membran wo sie „apoptotische Poren“ induzieren, die zum Austritt von Cytochrom C führen. Apaf-1 (*Apoptose-auslösender Faktor-1*) und Cytochrom C bilden einen Heterodimer, das Apoptosom und rekrutieren die Proform der Inititatorcaspase-9 (siehe Abbildung 4). Die Bindung geht mit einer Konformationsänderung einher und befähigt die Procaspase-9 zur Autokatalyse. Die aktive Form der Caspase-9 spaltet die Procaspase-3 in ihre aktive Form.

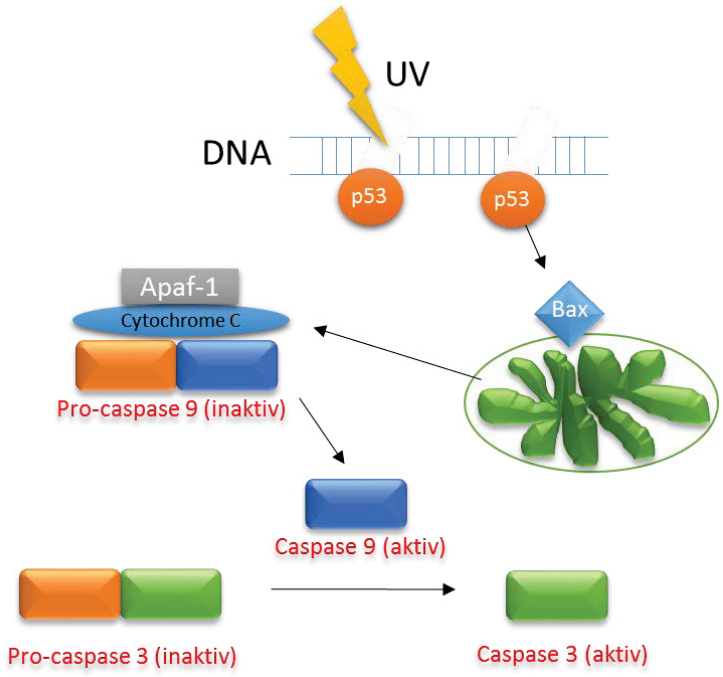

Abbildung 4 Intrinsischer Weg der Caspase-3 Aktivierung

Bei der extrinsischen Aktivierung binden Liganden an die Todesrezeptoren in der Zellmembran. Die wichtigsten Vertreter dieser Todesrezeptoren sind der Fas-Rezeptor und der Tumor-Nekrosefaktor (TNF) Rezeptor. Nach der Trimerisierung der Rezeptoren lagert sich das FADD (Fas-associated death domain) Protein an die zytosolische Seite der Rezeptoren an und bildet zusammen mit der Procaspase-8 den *Death-inducing signaling complex* (DISC). Die Procaspase-8 wird in ihre aktive Form geschnitten und ist damit in der Lage die Caspase-3 zu aktivieren (siehe Abbildung 5). Aktive Caspase-3 schneidet eine Vielzahl von Zielproteinen und erzwingt den Zelltod.

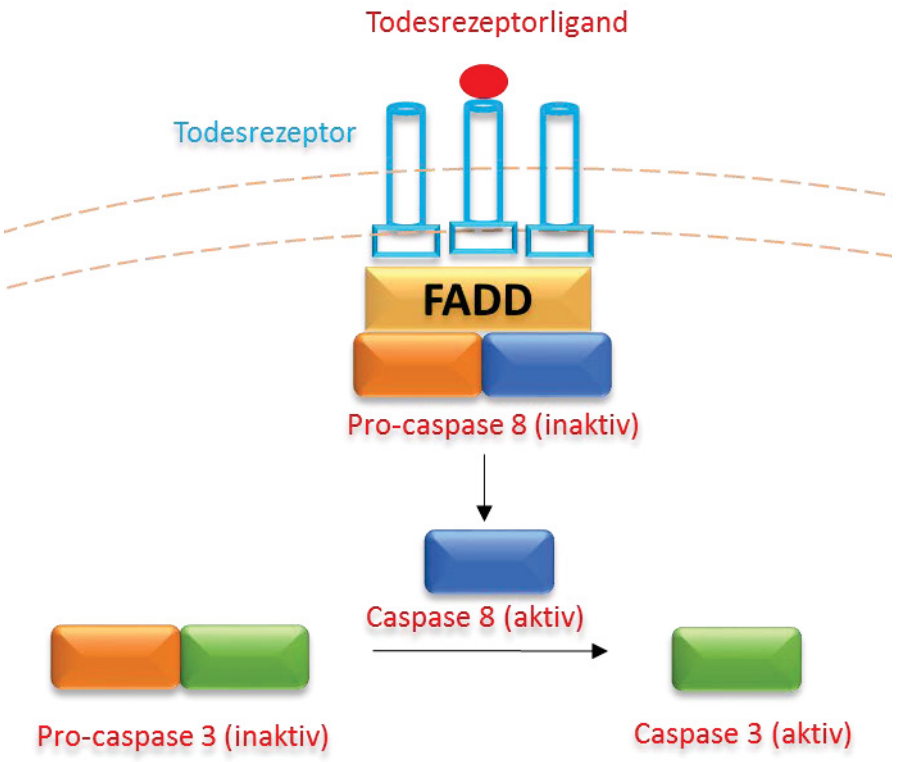

Abbildung 5 Extrinsischer Weg der Caspase-3 Aktivierung

1.5 Der Arylhydrocarbon Rezeptor

Der AHR ist ein liganden-aktivierter Transkriptionsfaktor, der die toxischen Effekte von Umweltgiften, wie 2,3,7,8-Tetrachlordibenzo-*p*-dioxin (TCDD) und Benzo(*a*)pyren (BaP), vermittelt (Abel und Haarmann-Stemmann 2010). Wie in Abbildung 6 dargestellt, liegt der AHR im inaktiven Zustand in Form eines Multiproteinkomplexes, bestehend aus Hitzeschockprotein 90, p23, XAP2 und der Tyrosinkinase c-Src, im Zytoplasma der Zelle vor. Bindet ein Ligand, zerfällt dieser Komplex. Der AHR dringt in den Zellkern ein und dimerisiert dort mit seinem Partnermolekül ARNT (*AHR nuclear translocator*). Das resultierende Heterodimer erkennt und bindet an spezifische DNA-Motive, sogenannte xenobiotisch-responsive Elemente (XRE), in der Promotorsequenz von Zielgenen und verstärkt deren Expression (Abel und Haarmann-Stemmann 2010).

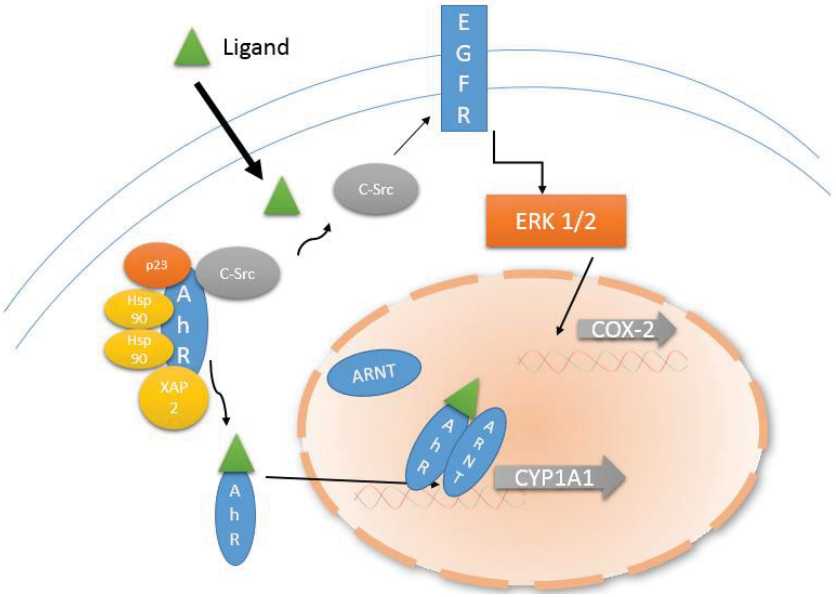

Abbildung 6 Schematische Darstellung des AHR Signalwegs

Die am besten untersuchten Zielgene dieses AHR/ARNT-Transkriptionsfaktors sind das fremdstoffmetabolisierende Enzym Cytochrom P450 1A1 (CYP1A1) und die für den AhR-Repressor kodierende Sequenz. Der AHRR konkurriert im Zellkern um den Bindungspartner ARNT und führt damit zu einer reduzierten AHR/ARNT Aktivität (Mimura et al. 1999; Evans et al. 2008). Neben der AHR Aktivität im Zellkern resultiert der Zerfall des Multiproteinkomplexes in einer Freisetzung der Tyrosinkinase c-Src, was

eine Aktivierung des EGFR und nachgeschalteter MAPK-Signalwege zur Folge hat (Xie et al. 2012). Eine transkriptionelle Induktion XRE-unabhängiger Gene, wie beispielsweise der Cyclooxygenase-2 (COX-2), ist die Folge (Köhle et al. 1999; Fritsche et al. 2007). In epidermalen Keratinozyten kann der AHR auch durch UVB-Exposition aktiviert werden. Verantwortlich hierfür ist die intrazelluläre Bildung des Photoprodukts 6-Formylindol[3,2-*b*]carbazol (FICZ) aus der Aminosäure Tryptophan (Fritsche et al. 2007). Dieses Photoprodukt kann mit hoher Affinität an den AHR binden und die oben beschriebenen Signalwege aktivieren (Fritsche et al. 2007; Wincent et al. 2012). Durch diese Arbeiten konnte erstmals die UVB-induzierte und DNA-schadensunabhängige Aktivierung des EGFR und nachgeschalteter MAPK mechanistisch erklärt werden (Devary et al. 1992).

Eine Vielzahl von Untersuchungen insbesondere an Tumorzelllinien haben gezeigt, dass der AHR neben Enzymen des Fremdstoffwechsels auch die Genexpression zahlreicher Proteine reguliert, die zelluläre Prozesse wie Proliferation, Migration und Apoptose steuern (Abel und Haarmann-Stemmann 2010; Frauenstein et al. 2013; Pollet et al. 2018).

Diverse Studien an Mausmodellen demonstrierten darüber hinaus, dass der AHR für Prozesse der Tumorigenese relevant ist. So sind AHR-defiziente Tiere gegen die Hautkarzinogenität von BaP und verwandten polyzyklischen aromatischen Kohlenwasserstoffen (PAK) geschützt (Shimizu et al. 2000), während transgene Mäuse, die einen konstitutiv-aktiven AHR exprimieren, eine verstärkte Tendenz zur Entstehung von hepatozellulärem Karzinom aufweisen (Moennikes et al. 2004).

Die Überexpression eines konstitutiv aktiven AHR in der Haut führt hingegen zur Ausbildung entzündlicher Hautläsionen (Tauchi et al. 2005). Der AHR übt in UVB-exponierten Keratinozyten eine anti-apoptotische Funktion aus. Sowohl in vitro als auch in vivo führte eine Hemmung des AHR zu einem signifikant erhöhten Proteinlevel des Tumorsuppressorproteins p27 und einer erhöhten Apoptoserate in bestrahlten Keratinozyten (Frauenstein et al. 2013; Pollet et al. 2018). Da dieser Zelltyp in der Epidermis kontinuierlich neu entsteht, wird eine Eliminierung UV-geschädigter Keratinozyten, durch eine erhöhte Apoptoserate, als Chemoprävention gegen die Entstehung von umweltinduziertem Hautkrebs diskutiert (Conney et al. 2013).

1.6 Tumorsuppressorprotein p27

Das Protein p27 hat eine entscheidende Rolle im Zellzyklus und steht im Fokus der Tumorforschung. Mäuse mit p27 *knockout* weisen eine Hyperplasie der Organe, gesteigerte Körpergröße und eine erhöhte Anfälligkeit für die Bildung von Tumoren auf. Dies lässt darauf schließen, dass p27 als Tumorsuppressor fungiert und einen Einfluss auf die Proliferation und Größe von Geweben hat (Kiyokawa et al. 1996).
Ein geringes Level des Proteins in Tumorgewebe geht mit einer schlechten Überlebensprognose des Patienten einher (Lloyd et al. 1999). Die Überexpression von p27 in Krebszellen führt zu einer gesteigerten Apoptoserate (Wang et al. 1997). Der Tumorsuppressor bindet die ATP-Bindestelle von *cyclin dependent kinase* 2 (CDK2), verhindert dessen Aktivierung und hält die Zelle in der G1 Phase des Zellzyklus. Das p27-Level wird nur bedingt transkriptionell reguliert. Einen weitaus größeren Einfluss hat die post-translationale Modifikation. Zwischen G1 und S-Phase wird p27 am Serin 10 phosphoryliert, was zum Export aus dem Nukleus führt (Boehm et al. 2002). Die Bindung in der ATP-Bindestelle von CDK2 wird aufgehoben, CDK2 kann aktiviert werden und phosphoryliert p27 am Tyrosin 88. Diese Stelle kann auch durch Src-Kinasen phosphoryliert werden (Hara et al. 2001; Chu et al. 2008; Chen et al. 2009). Proteinkinase-B/AKT initiiert die Akkumulation im Zytoplasma und die anschließende Degradierung von p27 über die Phosphorylierung am Threonin 157 (Viglietto et al. 2002). Eine weitere Phosphorylierung am Threonin 187 führt zu einem SCF2 (*Skp1/cullin/F-box protein*) vermittelten Abbau des Tumorsuppressorproteins und einem Übergang in die S-Phase des Zellzyklus (Grimmler et al. 2007). Daten aus unserem Institut konnten bereits aufzeigen, dass eine Herabregulation des AHR in Keratinozyten zu einem gesteigerten p27 Proteinlevel führt (Frauenstein et al. 2013).Dies gilt auch für Medulloblastom-Zellen (Dever und Opanashuk 2012). Diese Effekte scheinen zellspezifisch zu sein, da die Aktivierung des AHR in Leberzellen der Ratte zu einer gesteigerten Transkription des p27-Gens führte (Kolluri et al. 1999). Ein Ausschalten des AHR in HepG2 Zellen zeigte keinerlei Einfluss auf das Proteinlevel von p27 (Abdelrahim et al. 2003). Aufgrund der beschriebenen Eigenschaft ist eine Stabilisierung des Tumorsuppressorproteins von großem Interesse für die Medizin.

1.7 Aufbau und Inhibition der MAPK Signalkaskade

Der MAPK Signalweg, auch als RAS-RAF-MEK-ERK Kaskade beschrieben, beeinflusst zellbiologische Endpunkte wie Proliferation, Differenzierung sowie Inhibition und Initiation von Apoptose. Über die Ligandenbindung an Rezeptor-Tyrosinkinasen wird der Signalweg aktiviert. Es folgt die Bindung von GTP an das RAS (*rat sarcoma*) Protein. RAS phosphoryliert RAF (*rapidly accelerated fibrosarcoma*), welches in der Signalkaskade das Protein MEK1/2 aktiviert. MEK phosphoryliert ERK (*Extracellular signal-regulated kinase*), das eine Vielzahl von Transkriptionsfaktoren aktivieren kann, die für die Regulation von Proliferation und das Überleben der Zelle verantwortlich sind. Die Signalkaskade ist schematisch in Abbildung 7 dargestellt.

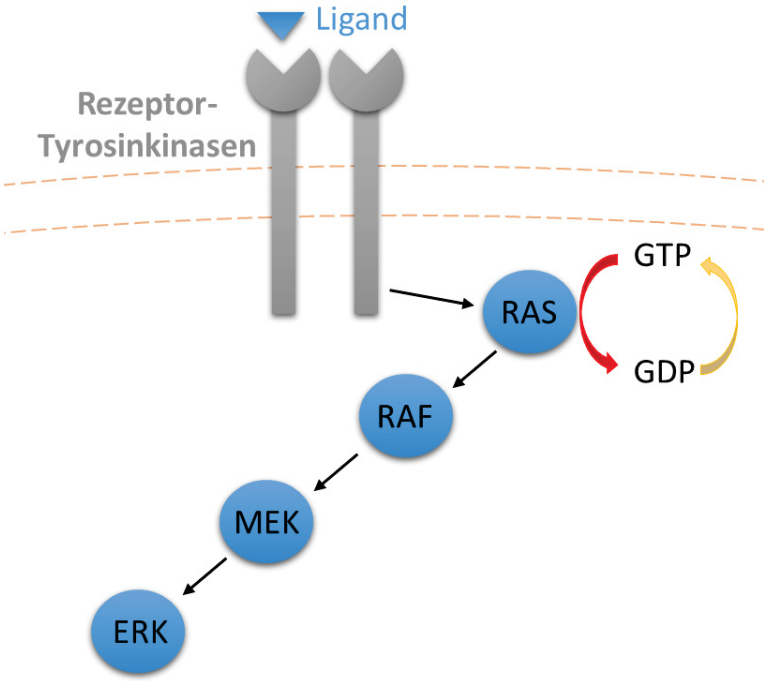

Abbildung 7 Vereinfachte Darstellung des MAP-Kinase Signalwegs

Diese Signalkaskade ist in Karzinomzellen der am häufigsten von Mutationen betroffene Signalweg. Alleine RAS ist in 15% aller menschlichen Tumorerkrankungen verändert (Davies et al. 2002). Eine Inhibierung innerhalb der Kaskade ist ein erfolgsversprechendes Ziel in der Therapie dieser Erkrankungen. BRAF und MEK Inhibitoren werden sehr erfolgreich im klinischen Umfeld eingesetzt, jedoch reduziert die

hohe Mutationsfrequenz in vielen Tumoren die Wirkweise der Substanzen erheblich (Liu et al. 2018). Einige dieser spezifischen Inhibitoren vermitteln die Aktivierung anderer Proteinkinasen der MAPK-Signalkaskade. Beispielsweise führt die Blockade des p38-Proteins mittels SB203580 zur gesteigerten Phosphorylierung von ERK1/2 (Meurer und Weiskirchen 2018). Interessanterweise binden einige dieser Proteinkinase-Inhibitoren den AHR (Korashy et al. 2011) und beeinflussen die Aktivität des Rezeptors.

1.8 BRAF-Inhibitor Vemurafenib

Patienten mit malignem Melanom haben in über 60% aller Fälle eine Mutation im B-RAF Gen. Es kodiert an der Aminosäureposition 600 für Glutaminsäure anstelle von Valin (V600E). Die Substitution führt zu einer erhöhten Kinase-Aktivität, die autark von vorgeschalteten Stimuli erfolgt (siehe Abbildung 7). BRAF Mutationen werden in allen Stadien des malignen Melanoms gefunden, was darauf schließen lässt, dass sie sowohl auf die Tumorprogression als auch die Metastasierung einen Einfluss haben (Davies et al. 2002).
Das Arzneimittel Vemurafenib bindet und inhibiert das mutierte BRAF, allerdings nicht dessen Wildtyp-Variante. Es wird bei Patienten eingesetzt, die inoperabel am malignen Melanom erkrankt sind. Die Progression des Tumors kann im Vergleich zu herkömmlichen Behandlungsmethoden um ca. 74% verringert werden. Unerwünschte Nebenwirkungen bei der Therapie mit Vemurafenib sind unter anderem Hautausschlag, Photosensibilität, Übelkeit, Durchfall und eine rasante Entwicklung von Plattenepithelkarzinomen (Chapman et al. 2011). Letzteres wird mit einer gesteigerten Aktivität von RAS in Hautproben der Patienten in Verbindung gebracht (Su et al. 2012), die über eine Aktivierung des AHR vermittelt werden könnte (Corre et al. 2018).

Interessanterweise steigert sich die CYP1A1 Expression bei einer Behandlung mit Vemurafenib nicht, was gegen eine klassische Aktivierung des AHR spricht (Kaya et al. 2018). Beide Studien zeigen deutlich, wie wichtig weitere Anstrengungen sind, Vemurafenib vermittelten molekularen Mechanismen zu entschlüsseln und eine nebenwirkungsärmere Therapie von Patienten mit malignem Melanom zu gewährleisten.

1.9 Ziel der Thesis

Das Ziel dieser Dissertation ist es einen Beitrag zum besseren Verständnis über den Einfluss des AHR auf die Entstehung und Progression des kutanen Plattenepithelkarzinoms zu leisten. Ein wichtiger Faktor ist hierbei die Abhängigkeit des Proteinlevels des Tumorsuppressorproteins p27 von der Aktivität des AHR und dessen Einfluss auf die NER nach UVB-Bestrahlung von Keratinozyten. Die in Vorbereitung dieser Arbeit erhobenen Daten deuten auf eine Regulation von p27 über den MAP-Kinase Signalweg hin. Eine Vielzahl von Inhibitoren insbesondere in der Therapie von Hautkarzinomen zielt auf diese Signalkaskade ab.

Die Interaktion zwischen den Inhibitoren und dem AHR ist ein weiterer Bestandteil der vorliegenden Arbeit. Ein starker Fokus wird dabei speziell auf den BRAF Inhibitor Vemurafenib gelegt, der in der Therapie des malignen Melanoms eingesetzt wird. Bei der Behandlung treten hautassoziierte Nebenwirkungen wie Ausschläge und eine rasche Entwicklung von kutanen Plattenepithelkarzinomen auf. Untersuchungen sollen zeigen, ob Vemurafenib mit dem AHR interagiert, sodass auf Basis dessen die Therapie verbessert und Nebenwirkungen reduziert werden können.

2 Publikationen

Die vorgelegte Arbeit besteht aus drei Manuskripten.

Manuskript 2.1 *”The AHR represses nucleotide excision repair and apoptosis and contributes to UV induced skin carcinogenesis”*

Manuskript 2.2 *“Commentary: Usage of Mitogen-Activated Protein Kinase Small Molecule Inhibitors: More Than Just Inhibition!”*

Manuskript 2.3 *“Vemurafenib acts as an aryl hydrocarbon receptor antagonist: Implications for inflammatory cutaneous adverse events”*

2.1 The AHR represses nucleotide excision repair and apoptosis and contributes to UV induced skin carcinogenesis

Journal:	Cell Death and Differentiation
Impact factor:	8,0
Beitrag zur Publikation:	Allgemeine Zellkultur, FACS Analysen, WesternBlot, siRNA-Experimente, SouthwesternBlot COMET-Assay, Klonierung, Überexpressionsstudien, Caspase3-Assays, Statistische Auswertung
Status der Publikation:	Veröffentlicht: 16. Juli 2018

Marius Pollet, Siraz Shaik, Melina Mescher, Katrin Frauenstein, Julia Tigges, Stephan A. Braun, Kevin Sondenheimer, Mana Kaveh, Anika Bruhs, Stephan Meller, Bernhard Homey, Agatha Schwarz, Charlotte Esser, Thierry Douki, Christoph F.A. Vogel, Jean Krutmann, Thomas Haarmann-Stemmann

Abstract

Ultraviolet B (UVB) radiation induces mutagenic DNA photoproducts, in particular cyclobutane pyrimidine dimers (CPDs), in epidermal keratinocytes (KC). To prevent skin carcinogenesis, these DNA photoproducts must be removed by nucleotide excision repair (NER) or apoptosis. Here we report that the UVB-sensitive transcription factor aryl hydrocarbon receptor (AHR) attenuates the clearance of UVB-induced CPDs in human HaCaT KC and skin from SKH-1 hairless mice. Subsequent RNA interference and inhibitor studies in KC revealed that AHR specifically suppresses global genome but not transcription-coupled NER. In futher experiments we found that the accelerated repair of CPDs in AHR-compromised KC depended on a modulation of the p27 tumor suppressor protein. Accordingly, p27 protein levels were increased in AHR-silenced KC and skin biopsies from $AHR^{-/-}$ mice, and functionally critical for the beneficial effect on NER. Besides increasing NER activity, AHR inhibition was accompanied by an enhanced occurrence of DNA double-strand breaks triggering KC apoptosis at later time points after irradiation. The UVB-activated AHR thus acts as a negative regulator of both early defence systems against carcinogenesis, NER and apoptosis, implying that it exhibits tumorigenic functions in UVB-exposed skin. In fact, $AHR^{-/-}$ mice developed 50% less UVB-induced cutaneous squamous cell carcinomas in a chronic photocarcinogenesis study than their $AHR^{+/+}$ littermates. Taken together, our data reveal that AHR influences DNA damage-dependent responses in UVB-irradiated KC and critically contributes to skin photocarcinogenesis in mice.

Introduction

Exposure to ultraviolet B (UVB) radiation is the major risk factor for cutaneous squamous cell carcinoma (SCC), one of the most frequent malignancies in humans [1, 2]. When skin is exposed to solar radiation, high-energy UVB rays penetrate into the epidermis where they are mainly absorbed by the DNA of keratinocytes (KC). The resulting DNA photoproducts, in particular cyclobutane pyrimidine dimers (CPDs), are highly mutagenic and critically involved in the development of SCC [1, 2]. Depending on the extent of DNA damage, KC either initiate nucleotide excision repair (NER) or apoptosis to preserve genomic integrity [3, 4]. Failure of these early defense mechanisms may give rise to initiated cells, whose spread is further promoted by UVB-activated signaling pathways facilitating proliferation, apoptosis resistance and impairment of host immune responses [1, 2].

NER is divided into two distinct sub-pathways: Transcription-coupled repair (TCR), which quickly removes DNA adducts in actively transcribed genes, and global genome repair (GGR), which removes DNA lesions throughout the entire genome [3]. Both subpathways only differ in their way of DNA damage recognition. In case of TCR, Cockayne Syndrome (CS)-A and CSB proteins recognize the stalled RNA polymerase II and serve as damage sensors. In GGR, a complex consisting of XPC, RAD23B and centrin-2 is responsible for DNA damage recognition. Upon damage verification by XPA, DNA is unwinded by the helicases XPB and XPD, two components of the general transcription factor TFIIH. Subsequently, the damage-containing DNA fragment is excised by the endonucleases XPF-ERCC1 and XPG and the remaining gap is filled by DNA polymerases. The pivotal role of NER in skin photocarcinogenesis is illustrated by the autosomal recessive NER disorder *Xeroderma Pigmentosum* (XP). XP patients with a deficiency in GGR have a dramatically increased risk of developing SCC and other skin cancers [5]. In contrast to the repair process, the precise regulation of NER in response to UVB radiation is still not completely understood.

The aryl hydrocarbon receptor (AHR) is a ligand-activated transcription factor that mediates the toxic effects of dioxins, polycyclic aromatic hydrocarbons and related environmental chemicals [6, 7]. In its inactive state, AHR rests in a cytosolic multiprotein complex. Upon ligand-binding, AHR shuttles in the nucleus, dimerizes with AHR nuclear translocator and binds to xenobiotic-responsive elements in the enhancer of target genes to initiate their transcription. AHR target genes encode drug-metabolizing enzymes, such as cytochrome P450 1A1, as well as proteins controlling cell division, differentiation and apoptosis [6, 7]. Another target is the AHR repressor (AHRR), a negative feedback regulator, which, depending on cell-type and tissue, may compete with AHR for both AHR nuclear translocator- and xenobiotic-responsive element-binding [8]. Beside the canonical AHR pathway, AHR activation often affects other signal transduction pathways, including NF-κB and EGFR signaling [6, 7]. AHR is expressed in all cutaneous cell-types and contributes to physiological as well as pathophysiological processes [9]. In epidermal KC, AHR activation results from the absorbance of UVB rays by tryptophan and the subsequent generation of 6-formylindolo[3,2-*b*]carbazole [10]. This tryptophan photoproduct is a high-affinity ligand for AHR [11] and, among others, induces the expression of cytochrome P450 1A1 and cyclooxygenase-2 [9, 10]. As these enzymes play critical roles in tumor initiation and promotion, it was proposed that the UVB-activated AHR contributes to photocarcinogenesis [12]. This idea is supported by our recent observations that AHR triggers immunosuppression [13] and anti-apoptosis in response to UVB exposure [14]. Interestingly, independent reports revealed that AHR-antagonizing polyphenols, such as epigallocatechin-3-gallate [15], stimulate the repair of UVB-induced CPDs [16], suggesting that AHR signaling may influence NER. In the present study, we directly tested this hypothesis by assessing the impact of AHR on the removal of UVB-induced CPDs in human HaCaT KC and SKH-1 hairless mice. We found that AHR inhibition enhances the removal of CPDs *in vitro* and *in vivo*. Mechanistic studies revealed that AHR specifically dampens GGR activity by decreasing the protein level of

the tumor suppressor $p27^{KIP1}$ (p27), which probably affects DNA repair independently from its main function, i.e. induction of cell-cycle arrest. In addition to its positive effect on NER, AHR inhibition enhances the apoptotic clearance of remaining CPD-positive KC at later time points and thus enforces both early defense mechanisms against photocarcinogenesis. Accordingly, chronic UVB irradiation experiments conducted in $AHR^{+/+}$ and $AHR^{-/-}$ SKH-1 mice revealed a dramatically reduced SCC formation in the $AHR^{-/-}$ animals.

Results

AHR inhibition accelerates the removal of UVB-induced CPDs in human KC and murine skin

To assess if AHR affects the removal of CPDs, we irradiated human HaCaT KC with 200 J/m^2 UVB and monitored CPD clearance by southwestern slot blot (SWB) analysis over time. CPD content stayed elevated in irradiated cells for the first 4 h after irradiation and then gradually declined to only 10% after 24 h (Fig. 1A). As expected (and true for all of the following CPD SWB analyses), we did not detect a CPD signal in sham-exposed KC. Next, we exposed HaCaT KC to 50 J/m^2 and 200 J/m^2 UVB (approximately equivalent to 0.25 and 1 minimal erythema dose for a fair-skinned individual [17]) and subsequently treated the cells with the AHR antagonist 3'-methoxy-4'-nitroflavone (MNF, 20 μM) to exclude putative UV-filtering effects. Four hours after irradiation, the CPD content in MNF-treated cells was 37% and 33% lower than in solvent controls (Figure 1B). Similar results were obtained when primary human epidermal KC were treated with MNF (data not shown). In stable AHR-knockdown cells (HaCaT-shAHR), the amount of UVB-induced CPDs was reduced by 37% 4 h after irradiation, as compared to irradiated empty vector control cells (HaCaT-EV) (Figure 1C), thus excluding putative off-target effects of MNF. In addition, ectopic overexpression of AHR's feedback inhibitor AHRR accelerated the removal of UVB-induced CPDs (Figure 1D). To confirm an influence of AHR on CPD removal *in vivo*, AHR$^{+/+}$ and AHR$^{-/-}$ SKH-1 hairless mice were irradiated once with 185 mJ/cm^2 UVB and 0.5 h and 48 h thereafter skin biopsies were taken. CPD content was quantified in complete biopsies by HPLC-MS/MS. In the skin of both genotypes, the distribution between TT, TC and CT CPDs was approximately 55:35:10 (Supplementary Figure S1), which is in accordance with previously published results [18]. Immediately after UVB exposure, CPD content did not significantly differ between AHR genotypes. However, after 48 h approximately 50% less CPDs were present in the skin of AHR$^{-/-}$

mice as compared to AHR$^{+/+}$ mice (Figure 1E), thus demonstrating that the negative effect of AHR on CPD clearance is also present *in vivo*.

AHR regulates NER

In all of the *in vitro* experiments, CPD content was determined in adherent KC. Also, co-exposure of MNF-treated HaCaT KC to Ac-DEVD-CHO (20 μM), an inhibitor of effector caspases, did not affect the MNF-mediated acceleration of CPD removal (Figure 1F). These results indicate that the reduced CPD levels observed in AHR-compromised UVB-irradiated human KC were not due to an early apoptotic demise of damaged cells, but likely resulted from altered NER. To assess whether acceleration of CPD removal induced by AHR inhibition was NER-dependent, we silenced the expression of XPA. In contrast to control cells, MNF exposure of UVB-exposed XPA-silenced KC failed to affect CPD removal (Figure 1G), strongly indicating that AHR modulates NER activity. We next investigated whether AHR antagonism affects GGR and/or TCR. Therefore, we transiently transfected HaCaT KC with siRNAs targeted against XPC and CSB. After 24 h, transfected cells were irradiated with 200 J/m^2 UVB and treated with 20 μM MNF or solvent. We found that MNF significantly increased CPD clearance in CSB-knockdown KC (Figure 1H), but not in XPC-silenced KC (Figure 1I). These results provide evidence that AHR inhibition accelerates CPD removal by specifically modulating GGR.

p27 is causally involved in the AHR-dependent inhibition of NER

We have previously reported that AHR-compromised KC contain an increased level of the CDK inhibitor p27 [14]. Accordingly, the p27 protein level was strongly elevated in HaCaT-shAHR KC as compared to HaCaT-EV KC, and associated with a reduced phosphorylation of CDK2 (T-160) (Figure 2A). As enhanced p27 levels were reported to correlate with DNA repair capacity in human peripheral blood lymphocytes [19], we assessed whether elevated p27 levels may be causative for the acceleration of NER.

We silenced p27 expression using transient RNAi and investigated if MNF exposure still accelerates NER. We found that in contrast to respective control cells, knockdown of p27 abolished the MNF-mediated increase in CPD removal (Figure 2B). In addition, transient overexpression of p27 in HaCaT KC resulted in an enhanced clearance of UVB-induced CPDs (Figure 2C), demonstrating that p27 can *per se* stimulate NER activity.

p27 enhances GGR activity independently from cell-cycle arrest

Interestingly, FACS analyses of UVB-exposed HaCaT KC treated with DMSO or MNF did not show any significant differences in the cell-cycle profiles, at least within the first 4 h after irradiation (supplementary figure S2), suggesting that p27 affects GGR independently from CDK2/CDK4 inhibition. To elucidate if p27 alters GGR activity in a CDK-dependent manner, we tested the potential of various chemical CDK inhibitors on their ability to alter CPD removal in HaCaT KC. In fact, treatment of UVB-exposed KC with roscovitine, an inhibitor of CDK1, CDK2, CDK5 and CDK7, resulted in an enhanced removal of CPDs 4 h after UVB irradiation (Figure 2D). In contrast, exposure to SU9516, an inhibitor of CDK1, CDK2 and CDK4, had no effect on the cellular amount of UVB-induced CPDs (Figure 2D), again supporting our notion that p27 alters GGR activity independently from CDK2 and CDK4. Interestingly, treatment of UVB-irradiated cells with BS-181, a specific inhibitor of CDK7, mimicked the positive effect of p27 overexpression on CPD repair (Figure 2D). Although CDK7 is known to be involved in the regulation of NER [20], subsequent co-immunoprecipitation analyses in sham- and UVB-exposed HaCaT KC did not reveal a direct protein-protein interaction of p27 and CDK7 (data not shown). Thus, p27 may either affect GGR independently from CDK7 or through an indirect modulation of CDK7 function.

AHR regulates the proteasomal degradation of p27

To assess the *in vivo* relevance of the observed AHR-dependent effects on p27 level, we determined the p27 protein content in the skin of two strains of $AHR^{+/+}$ and $AHR^{-/-}$ mice. As expected, protein levels of p27 were higher in the skin of $AHR^{-/-}$ SKH-1 mice (Figure 2E) and $AHR^{-/-}$ B6 mice (Figure 2F) as compared to littermate controls. Also, overexpression of AHRR increased p27 protein content in the skin of transgenic B6 (AHRR Tg) mice [21] (Figure 2F) as well as in transiently transfected HaCaT KC (Figure 2G). We next tested whether transcriptional or post-transcriptional events were responsible for these effects. As expected, immunofluorescence (IF) staining of HaCaT KC revealed a nuclear accumulation of AHR in response to UVB irradiation, which was attenuated by MNF treatment (Supplementary Figure S3A). Exposure of HaCaT KC to MNF alone resulted in an increase of nuclear p27 (Figure 3A). In comparison to irradiated HaCaT KC, an increased amount of nuclear p27 was also present in MNF-treated HaCaT KC 2 h and 3 h after exposure to 200 J/m^2 UVB (Figure 3A). The increased levels of p27 were accompanied by a reduced phosphorylation of CDK2 (Supplementary Figure S3B). The differences in nuclear p27 were reflected by IF stainings using a phospho-p27 (T-187) antibody (Figure 3B). The amino acid T-187 serves as substrate for CDK2 and targets p27 to proteolysis [22]. Further mechanistic studies in HaCaT cells using chemical AHR ligands confirmed that AHR activity affected p27 on the protein level. Treatment of HaCaT KC with the AHR agonist benzo[*a*]pyrene (BaP) reduced the p27 protein level, whereas exposure to MNF increased it (Figure 3C). These changes were not retrievable on the mRNA level (Figure 3D). Accordingly, the BaP-induced reduction of p27 protein was abrogated by co-treatment with the proteasome inhibitor MG-132 (Figure 3E). Interestingly, exposure of HaCaT-shAHR KC to EGF attenuated the elevated p27 protein level (Figure 3F), whereas treatment of HaCaT-EV KC with the EGFR inhibitor PD153035 was sufficient to increase it (Figure 3G). Importantly, treatment of HaCaT KC with inhibitors for EGFR (PD153035) and its effector pathways PI3K/AKT (Wortmannin)

and MEK/ERK (PD98059) accelerated the CPD removal 4 h after UVB irradiation (Figure 3H). These results are consistent with the assumption that AHR attenuates GGR in UVB-irradiated KC by promoting proteolysis of p27, most likely involving EGFR and downstream signal transduction.

AHR inhibition increases UVB-induced apoptosis in KC by enhancing DNA double strand break formation

An increase in NER should partially reduce UVB-induced KC apoptosis [23]. This is in contrast to our previous report that AHR inhibition enhances UVB-induced KC apoptosis [14]. Accordingly, FACS-based analyses of Annexin V/PI-stained cells revealed a stronger apoptotic response in HaCaT-shAHR KC exposed to 200 J/m^2 UVB as compared to HaCaT-EV cells (Figure 4A, Supplementary Figure S4A). The AHR-dependent difference in UVB-induced apoptosis was confirmed on the level of caspase-3 activity (Supplementary Figure S4B). Also, UVB irradiation of AHRR-overexpressing HaCaT KC resulted in a more pronounced apoptotic response, as compared to control cells (Figure 4B, Supplementary Figure S4C). In our previous study we showed that the anti-apoptotic action of AHR is due to the loss of checkpoint kinase-1 (CHK1) expression [14]. CHK1 is a stress kinase that is directly activated in response to DNA damage to prevent cell-cycle progression [24]. In fact, exposure of HaCaT KC to 20 µM MNF resulted in a reduced expression of CHK1 protein, both in sham and UVB-irradiated cells (Figure 4C). As CHK1 is also required for homologous recombination repair (HRR) [25], we next asked if DNA double-strand breaks (DSBs) may be responsible for the enhancement of apoptosis observed in AHR-compromised KC. Accordingly, phosphorylation of histone H2AX (γH2AX), an established marker for DSBs, was significantly stronger in UVB-exposed MNF-treated HaCaT as compared to irradiated control KC (Figure 4D). Moreover, neutral comet assay analyses demonstrated an enhanced occurrence of DSBs in MNF-treated HaCaT KC 18 h after UVB irradiation (Figure 4E). The DSBs still occurred when the cells

were co-exposed to a caspase inhibitor, indicating that they were not produced by apoptosis-related DNases (Figure 4E). These results indicate that the enhanced UVB-induced apoptosis in AHR-compromised cells is driven by an increased formation of DSBs and that it occurs independently of NER, thus reconciling our seemingly contradictory observations on NER and apoptosis in AHR-deficient KC.

Reduced photocarcinogenesis in AHR$^{-/-}$ mice

Our results demonstrate that the UVB-activated AHR is a negative regulator of GGR and apoptosis. We therefore speculated that AHR-deficiency would protect mice against the UVB-induced development of SCC. To test this hypothesis, we conducted a chronic photocarcinogenesis study in AHR$^{+/+}$ and AHR$^{-/-}$ SKH-1 mice. As shown in figure 5A, AHR$^{-/-}$ mice developed approximately 50% less skin tumors than their AHR$^{+/+}$ littermates. The animals started to develop skin tumors after 15 to 17 weeks of UVB exposure (Figure 5B). There were no genotype-dependent differences in tumor histology (Figure 5C). As previously reported for hairless mice [26] all UVB-induced skin tumors were SCC ranging from well to poorly differentiated *in situ* carcinomas to deeply infiltrating tumors. This was confirmed by immunohistochemical analyses revealing an accumulation of mutant p53 protein, a hallmark of UVB-induced SCCs [27, 28], in lesional but not adjacent non-lesional skin from mice of both AHR genotypes (Figure 5D). In addition, immunoblot analyses exhibited an elevated activation of STAT3 (phosphorylation at Y-705), an established key driver of UVB-induced SCC development [29], in tumor samples from both, AHR$^{+/+}$ and AHR$^{-/-}$ mice, as compared to irradiated non-lesional skin (Figure 5E). Notably, sham-exposed control animals of both genotypes did not develop any skin tumors (data not shown). Taken together, these data reaveal a crucial role of AHR in UVB-induced skin carcinogenesis.

Discussion

The major findings of the present study are that AHR attenuates the clearance of UVB-induced CPDs by specifically repressing GGR in a p27-dependent manner, and that AHR-deficiency largely protects mice against UVB-induced skin carcinogenesis.

CPDs are primarily responsible for the onset of UVB-induced skin carcinogenesis [28] and their forced repair has been demonstrated to efficiently reduce the incidence of skin cancer in mice [30] and humans [31]. We therefore believe that the reduced SCC development in $AHR^{-/-}$ mice is, at least to a major extent, the consequence of elevated NER activity. This assumption is further supported by the fact that AHR inhibition specifically increased GGR, which is the pivotal DNA repair system restraining photocarcinogenesis. In fact, XP patients suffering from GGR-deficiency have a greatly increased risk of developing skin cancer [5]. In contrast, TCR-deficiency (CS) is not associated with an increased incidence of skin cancer, which is probably due to an enhanced cytotoxicity (but not mutagenicity) in response to UV exposure [32]. Since CPDs are the major trigger for UVB-induced immunosuppression [33], their accelerated repair in $AHR^{-/-}$ mice may have also amplified antitumor immune responses. In addition to the beneficial effect on GGR, the observed increase in UVB-induced apoptosis and the associated clearance of damaged cells may have also contributed to the reduced SCC development in $AHR^{-/-}$ mice. Indeed, an enhancement of epidermal apoptosis, for instance by topical application of caffeine or resveratrol, has been shown to reduce UVB-induced skin carcinogenesis in mice [34, 35]. Thus, an enhanced stimulation of both defense mechanisms, GGR and apoptosis, is most probably responsible for the reduced SCC occurrence in $AHR^{-/-}$ mice.

Our results are consistent with the view that an upregulation of the cutaneous p27 protein is causative for the increased GGR in AHR-compromised KC (Figure 6). The underlying molecular mechanism is quite enigmatic and seems to be independent from p27's capability to inhibit CDK2/CDK4 and induce cell-cycle arrest. Interestingly, an inhibition

of CDK7 seemed to mimic the positive effect of p27 overexpression on NER. CDK7 is the active subunit of the CDK-activating complex, which is part of the multifaceted transcription factor TFIIH. It has been shown that upon UV exposure the CDK-activating complex dissociates from the TFIIH core complex [36], which then switches its function from transcription factor to NER factor [20, 37]. In fact, chemical inhibition of CDK7 has been shown to specifically increase GGR activity [20], thus making it a likely candidate being involved in the stimulation of this repair pathway in AHR-compromised KC. However, in accordance with a previous report [38], we were not able to show a direct protein-protein interaction between CDK7 and p27 in our cell system, indicating that both proteins either interact indirectly *via* an additional yet to be identified factor or affect GGR through independent mechanisms.

The p27 gene (CDKN1B) is haplo-insufficient for tumor suppression [39, 40], indicating that low p27 levels correlate with cancer proneness. Hence, downregulation of p27 by oncogenic signal transduction occurs frequently in various solid cancers [41], including SCC [42]. Although an effect on p27 transcription was observed in extracutaneous tissues and cells [43], we propose that AHR affects p27 post-translationally, i.e. by activating signal transduction pathways that phosphorylate p27 and target it to the proteasome. The precise underlying mechanism has not yet been identified, however our data clearly point to an involvement of EGFR and downstream PI3K/AKT and MEK/ERK signal transduction. Importantly, AKT [44, 45] and ERK [46, 47] are known to phosphorylate p27 and thereby affect its sub-cellular localization and associated proteasomal degradation. As indicated by our IF stainings, the latter process may involve a CDK2-mediated phosphorylation of p27 at T-187 [22]. Our hypothesis is further supported by previous reports from others and us, showing that the UVB-activated AHR stimulates EGFR and downstream MEK/ERK and PI3K/AKT signaling [10, 48]. A role of EGFR in p27 regulation is also underscored by clinical studies reporting elevated cutaneous p27 protein levels in cancer patients under systemic EGFR inhibitor therapy [49].

As reported earlier [14], AHR inhibition did not reduce but rather enhance UVB-induced apoptosis in HaCaT KC (Figure 6). The increase of p27 protein upon AHR antagonism resulted in a decreased expression of CHK1, which is required for the initiation of DNA damage responses, i.e. cell-cycle arrest [24] and HRR [25]. Inhibition of CHK1 was shown to enhance UVB-induced KC apoptosis and to prevent photocarcinogenesis [34]. In fact, we have previously observed reduced CHK1 protein levels not only in AHR-compromised human KC, but also in the skin of $AHR^{-/-}$ mice [14]. When UVB-irradiated KC enter mitosis, remaining CPDs may cause a collapse of the replication fork leading to the formation of DSBs [50, 51]. It is therefore highly likely that AHR-compromised KC, due to reduced CHK1 levels, do not properly repair these potent apoptosis-inducing DNA lesions [4], resulting in elevated cell death at later time points (Figure 6). Accordingly, exposure of CHO cells to dioxin has been shown to accelerate HRR in an AHR-dependent manner [52]. In addition, we have previously reported that AHR is required for proper repair of DSBs induced by ionizing radiation [53] and that reconstitution of CHK1 expression neutralizes the enhanced susceptibility of AHR-silenced KC towards UVB-induced apoptosis [14]. Accordingly, the increased DSB formation observed in AHR-deficient KC is most probably due to an attenuated HRR. Thus, AHR inhibition prevents photocarcinogenesis by accelerating GGR as well as by promoting lethal DSBs in remaining CPD-positive KC (Figure 6).

Overexpression of AHRR increased p27 protein level, CPD removal and apoptosis, implying that AHRR not only attenuates AHR/xenobiotic-responsive element-dependent responses but also AHR-triggered protein kinase activities. In fact, we have recently reported that the dioxin-induced nuclear accumulation and DNA-binding of C/EBPβ and NF-κB, which is mediated via non-canonical AHR signaling, is reduced in tissues of AHRR Tg mice [21]. However, as AHRR abrogates the growth and malignancy of various human cancers [8], a further elucidation of the link between AHRR and p27 may help to better understand AHRR's tumor suppressive properties.

In summary, we provide evidence that AHR represses GGR and apoptosis in UVB-exposed KC and critically contributes to skin photocarcinogenesis. The translational relevance of these findings is highlighted by a recent two-stage genome-wide association study identifying AHR as a novel susceptibility locus for SCC in humans [54]. As we have previously shown that AHR antagonism in human skin *in vivo* is feasible [55], AHR may be a suitable target for topical chemoprevention of UVB-induced skin malignancies.

Material and Methods

Cell culture, UVB irradiation and treatment

HaCaT KC were provided by P. Boukamp (DKFZ/IUF) and authenticated by the German Collection of Microorganisms and Cell Cultures (Braunschweig, Germany). The cultivation of HaCaT KC and the generation and cultivation of HaCaT-EV and HaCaT-shAHR KC has been previously described [10]. The source for UVB irradiation was a TL20W/12RS lamp (Philips, Eindhoven, The Netherlands), which emits most of its energy in the UVB range (290–320 nm) with an emission peak at 310 nm. For both UVB and sham exposure, culture medium was replaced by PBS. For cell treatment, Wortmannin, PD153035, PD98059, MG-132, BaP (all from Sigma-Aldrich, Munich, Germany), roscovitine (Enzo Life Sciences, Loerrach, Germany), BS-181 (Selleckchem, Houston, TX, USA), SU9516 (Tocris Bioscience, Bristol, UK) and MNF (provided by I. Meyer, Symrise AG, Holzminden, Germany) were dissolved in DMSO. EGF (Sigma-Aldrich) and Ac-DEVD-CHO (Enzo Life Sciences) were dissolved in water.

Southwestern slot-blot analyses

Equal amounts of isolated DNA were diluted in $TE_{10/1}$-buffer (pH 8), incubated for 5 min in boiling water and cooled down for 2 min on ice. Samples were spotted on positively charged nitrocellulose membrane (GE Healthcare, Little Chalfont, UK) using a slot-blot

chamber coupled to a vacuum manifold. The membrane-bound DNA was denatured for 45 min on Whatmann paper soaked with 0.4 N NaOH. Membranes were blocked overnight in 5% skim milk in TBS-Tween-20 (0.5%; TBS-T) at 4°C. Membranes were incubated for 2 h at 4°C with a HRP-conjugated thymine dimer antibody (Kamiya Biomedical Company, Tukwila, WA, USA) in 5% skim milk/TBS-T. Membranes were washed and signals were detected using the WesternBright ECL substrate (Advansta, Menlo Park, CA, USA). The signal intensity of the irradiated control sample was defined as 100%. A serial dilution of this sample was spotted and stained for CPDs to ensure linearity of the signal intensity (example shown in Supplementary Figure S5). The SWB-based CPD detection in sham-exposed KC did not produce any detectable signals and therefore is not shown.

SDS-PAGE and western blot analyses

Protein isolation, SDS-PAGE and western blot analyses were carried out as described previously [14]. Primary antibodies used in this study were: p27, CDK2, γH2AX, β-actin, GAPDH, STAT3, pSTAT3 Y-705 (all from Cell Signaling Technology, Dancers, MA, USA), XPA (Sigma-Aldrich), α-tubulin (ExBio, Vestec, Czech Republic), AHRR (Novoprotein Scientific, Summit, NJ, USA), AHR, pp27 T-187, pCDK2 T-160, XPC and CSB (all from Santa Cruz Biotechnology).

Immunohistochemistry

Hematoxylin and eosin (H&E) staining and immunohistochemistry of skin tumors were performed on paraformaldehyde-fixed 7-µm paraffin sections. H&E stainings were mounted in Vectashield medium (Biozol, Eching, Germany). For immunohistochemical staining of tissues, paraffin sections were deparaffinized. Antigens were retrieved by boiling in pH 6 citrate buffer for 12 min. After washing, endogenous mouse Ig´s were blocked using M.O.M Blocking Reagent (Biozol) according to the manufacturer´s protocol. Primary antibody (mutant p53, clone PAb

240, Thermo Scientific, Dreieich, Germany) was applied overnight at 4°C in a humidified chamber. Next day, staining was visualized using a peroxidase–based detection kit (Vector AEC Substrate Kit, Biozol) before mounting in gelatine.

Immunofluorescence staining of cells

KC were grown on collagen I-coated coverslips until subconfluency and fixed with either ice-cold MeOH or 70% EtOH for 10 min at -20°C. EtOH-fixed cells were permeabilized with 0.1% Triton/PBS for 10 min at RT. Cells were blocked with 5% BSA for 1 h at RT and subsequently incubated with primary antibodies (AHR, p27, pp27 T-187, pCDK2 T-160) diluted in blocking solution. Next day, cells were incubated with AlexaFluor 488- and 568-conjugated secondary antibodies and DAPI for 1 h at RT. Antibodies were diluted in blocking solution and immunostained cells were mounted in Mowiol (Sigma-Aldrich).

Quantiative real-time PCR

RNA isolation, cDNA synthesis, quantitative real-time PCR and primer sequences were described previously [14].

Transient RNA interference

Transient transfection of HaCaT KC with XPA, XPC, CSB, p27 and non-silencing siRNA (all from Santa Cruz Biotechnology) was done using INTERFERin reagent (Polyplus Transfection, Illkirch, France).

Overexpression experiments

Transient transfection of HaCaT KC with pCMV5p27 [provided by J. Massagué, Addgene plasmid #14049, [56]], pcDNA5-rAHRR [provided by Y. Inouye, [57]] and respective empty

vectors was done using JetPEI reagent (Polyplus Transfection). Efficiency of rat AHRR overexpression was assessed by semi-quantitative PCR.

Apoptosis assay

Apoptosis was determined by using the Annexin V-FITC Apoptosis kit (BioVision, Mountain View, CA, USA) and a FACSCalibur II device (BD Biosciences, San Jose, CA, USA). In addition, caspase-3 activity was measured by using the Caspase-3 Fluorometric Assay Kit (PromoCell, Heidelberg, Germany) according to the manufacturer's instructions.

Neutral comet assay

HaCaT KC were detached with trypsin/EDTA and 20 µl of the cell suspension (approx. 1,000 cells) plus 120 µl of 0.5% low melting agarose solution were pipetted on pre-coated superfrost slides, sealed with a cover slip and incubated for 5 min on ice. Slides were transferred to vertical staining jars containing lysis buffer (pH 9.5; 2.5 M NaCl, 100 mM EDTA, 10 mM Tris, 10% DMSO, 1% Triton X-100). After 1 h, slides were washed 3-times with dH_2O. Next, slides were placed in electrophoresis buffer (pH 8.5; 300 mM NaOH, 1 mM, EDTA) for 20 min followed by 10 min electrophoresis. Slides were washed 3-times with ice-cold neutralization buffer (pH 7.5; 0.4 M Tris), kept in absolute ethanol for 5 min and then air-dried. Slides were stained with ethidium bromide and sealed with cover slips. Fifty cells per treatment were analyzed using a camera-connected microscope (Olympus BX60). Tail moment was used as parameter to characterize the extent of DNA damage.

Animals and chronic UVB irradiation

Generation, breeding and genotyping of $AHR^{-/-}$ and $AHR^{+/+}$ SKH-1 hairless mice was described previously [14]. The generation, breeding and characterization of transgenic

AHRR B6 (AHRR Tg) mice is described in [21]. All animals were housed in our specific pathogen-free animal facility. For the photocarcinogenesis study, 8 $AHR^{-/-}$ SKH-1 mice and 10 $AHR^{+/+}$ littermates were irradiated over a period of 24 weeks. Sham-exposed mice of each genotype were used as control groups: In week 1 the animals were irradiated 3-times with 90 mJ/cm^2 UVB, followed by one irradiation-free week. From week 3 on, the mice were irradiated 3-times / week with an initial dose of 60 mJ/cm^2 UVB, followed by a weekly increase of 10 mJ/cm^2 until a maximum dose of 150 mJ/cm^2 UVB was reached (week 12). Mice were further irradiated with this dose until the end of week 24. Two weeks later, animals were sacrificed and tumor numbers were assessed. Tumor samples were embedded in paraffin and the resulting slices were H&E stained and number-coded for blinded histopathological evaluation. Animal experiments were performed according to the national animal care guidelines.

Acute UVB exposure of mice

For CPD analyses, $AHR^{+/+}$ and $AHR^{-/-}$ SKH-1 mice (except ctrl. animals) were exposed to a single dose of 185 mJ/cm^2 UVB, and 30 min and 48 h later mice were sacrificed and the dorsal skin of each animal was prepared. Total DNA was isolated from 4 mm punch biopsies using a standard phenol/chloroform extraction protocol.

Quantification of CPD by HPLC/MS-MS

The HPLC-MS/MS-based detection of CPD in DNA isolated from UVB-irradiated murine skin was performed as described previously [18].

Statistical analyses

All data shown are mean (± standard deviation) from three or more independent experiments, if not indicated otherwise. In some cases, representative results are shown. Differences were considered significant at $p \leq 0.05$. A comparison of two groups was

made with an unpaired, two-tailed Student's T-test. A comparison of multiple groups was made with analysis of variance followed by a Sidak's multiple comparison test.

Acknowledgment

We thank Joan Massagué and Yoshio Inouye for providing critical plasmids, and Imke Meyer for donating MNF. We thank Petra Boukamp and Ellen Fritsche for providing cell-lines and Ragnhild Wirth, Julia Kolling, Jaqueline Hofmann and Kerstin Fischer for technical support. This work was supported by grants of the Wilhelm Sander Foundation (to T.H.-S.; 2014.073.1), the National Institute of Environmental Health Sciences (to C.V.; R01 ES019898), and the Deutsche Forschungsgemeinschaft (to T.H.-S.; HA 7346/2-1).

Conflict of interest

The authors declare no competing financial interests.

References

1. Elmets CA, Athar M. Milestones in photocarcinogenesis. J Invest Dermatol 2013, 133(E1): E13-E17.
2. Ratushny V, Gober MD, Hick R, Ridky TW, Seykora JT. From keratinocyte to cancer: the pathogenesis and modeling of cutaneous squamous cell carcinoma. J Clin Invest 2012, 122(2): 464-472.
3. Marteijn JA, Lans H, Vermeulen W, Hoeijmakers JH. Understanding nucleotide excision repair and its roles in cancer and ageing. Nat Rev Mol Cell Biol 2014, 15(7): 465-481.
4. Roos WP, Thomas AD, Kaina B. DNA damage and the balance between survival and death in cancer biology. Nat Rev Cancer 2016, 16(1): 20-33.
5. DiGiovanna JJ, Kraemer KH. Shining a light on xeroderma pigmentosum. J Invest Dermatol 2012, 132(3 Pt 2): 785-796.
6. Denison MS, Soshilov AA, He G, DeGroot DE, Zhao B. Exactly the same but different: promiscuity and diversity in the molecular mechanisms of action of the aryl hydrocarbon (dioxin) receptor. Toxicol Sci 2011, 124(1): 1-22.
7. Nebert DW. Aryl hydrocarbon receptor (AHR): "pioneer member" of the basic-helix/loop/helix per-Arnt-sim (bHLH/PAS) family of "sensors" of foreign and endogenous signals. Prog Lipid Res 2017, 67: 38-57.
8. Vogel CFA, Haarmann-Stemmann T. The aryl hydrocarbon receptor repressor – More than a simple feedback inhibitor of AhR signaling: Clues for its role in inflammation and cancer. Curr Opin Toxicol 2017, 2: 109-119.
9. Esser C, Bargen I, Weighardt H, Haarmann-Stemmann T, Krutmann J. Functions of the aryl hydrocarbon receptor in the skin. Semin Immunopathol 2013, 35(6): 677-691.
10. Fritsche E, Schafer C, Calles C, Bernsmann T, Bernshausen T, Wurm M, et al. Lightening up the UV response by identification of the arylhydrocarbon receptor as a

cytoplasmatic target for ultraviolet B radiation. Proc Natl Acad Sci USA 2007, 104(21): 8851-8856.

11. Rannug A, Rannug U, Rosenkranz HS, Winqvist L, Westerholm R, Agurell E, et al. Certain photooxidized derivatives of tryptophan bind with very high affinity to the Ah receptor and are likely to be endogenous signal substances. J Biol Chem 1987, 262(32): 15422-15427.
12. Agostinis P, Garmyn M, Van Laethem A. The Aryl hydrocarbon receptor: an illuminating effector of the UVB response. Sci STKE 2007, 2007(403): e49.
13. Navid F, Bruhs A, Schuller W, Fritsche E, Krutmann J, Schwarz T, et al. The Aryl hydrocarbon receptor is involved in UVR-induced immunosuppression. J Invest Dermatol 2013, 133(12): 2763-2770.
14. Frauenstein K, Sydlik U, Tigges J, Majora M, Wiek C, Hanenberg H, et al. Evidence for a novel anti-apoptotic pathway in human keratinocytes involving the aryl hydrocarbon receptor, E2F1, and checkpoint kinase 1. Cell Death Differ 2013, 20(10): 1425-1434.
15. Williams SN, Shih H, Guenette DK, Brackney W, Denison MS, Pickwell GV, et al. Comparative studies on the effects of green tea extracts and individual tea catechins on human CYP1A gene expression. Chem Biol Interact 2000, 128(3): 211-229.
16. Meeran SM, Mantena SK, Elmets CA, Katiyar SK. (-)-Epigallocatechin-3-gallate prevents photocarcinogenesis in mice through interleukin-12-dependent DNA repair. Cancer Res 2006, 66(10): 5512-5520.
17. Autier P, Dore JF, Reis AC, Grivegnee A, Ollivaud L, Truchetet F, et al. Sunscreen use and intentional exposure to ultraviolet A and B radiation: a double blind randomized trial using personal dosimeters. Br J Cancer 2000, 83(9): 1243-1248.
18. Douki T, Cadet J. Individual determination of the yield of the main UV-induced dimeric pyrimidine photoproducts in DNA suggests a high mutagenicity of CC photolesions. Biochemistry 2001, 40(8): 2495-2501.

19. Fan YH, Hu Z, Li C, Wang LE, Guo Z, Qiao Y, et al. In vitro expression levels of cell-cycle checkpoint proteins are associated with cellular DNA repair capacity in peripheral blood lymphocytes: a multivariate analysis. J Proteome Res 2007, 6(4): 1560-1567.
20. Coin F, Oksenych V, Mocquet V, Groh S, Blattner C, Egly JM. Nucleotide excision repair driven by the dissociation of CAK from TFIIH. Mol Cell 2008, 31(1): 9-20.
21. Vogel CF, Chang WL, Kado S, McCulloh K, Vogel H, Wu D, et al. Transgenic Overexpression of Aryl Hydrocarbon Receptor Repressor (AhRR) and AhR-Mediated Induction of CYP1A1, Cytokines, and Acute Toxicity. Environ Health Perspect 2016, 124(7): 1071-1083.
22. Sheaff RJ, Groudine M, Gordon M, Roberts JM, Clurman BE. Cyclin E-CDK2 is a regulator of p27Kip1. Genes Dev 1997, 11(11): 1464-1478.
23. Kulms D, Poppelmann B, Yarosh D, Luger TA, Krutmann J, Schwarz T. Nuclear and cell membrane effects contribute independently to the induction of apoptosis in human cells exposed to UVB radiation. Proc Natl Acad Sci USA 1999, 96(14): 7974-7979.
24. Sanchez Y, Wong C, Thoma RS, Richman R, Wu Z, Piwnica-Worms H, et al. Conservation of the Chk1 checkpoint pathway in mammals: linkage of DNA damage to Cdk regulation through Cdc25. Science 1997, 277(5331): 1497-1501.
25. Sorensen CS, Hansen LT, Dziegielewski J, Syljuasen RG, Lundin C, Bartek J, et al. The cell-cycle checkpoint kinase Chk1 is required for mammalian homologous recombination repair. Nat Cell Biol 2005, 7(2): 195-201.
26. Winkelmann R, Zollman P, Baldes E. Squamous cell carcinoma produced by ultraviolet light in hairless mice. J Invest Dermatol 1963, 40: 217-224.
27. Berg RJ, van Kranen HJ, Rebel HG, de Vries A, van Vloten WA, van Kreijl CF, et al. Early p53 alterations in mouse skin carcinogenesis by UVB radiation:

immunohistochemical detection of mutant p53 protein in clusters of preneoplastic epidermal cells. Proc Natl Acad Sci USA 1996, 93(1): 274-278.

28. Brash DE, Rudolph JA, Simon JA, Lin A, McKenna GJ, Baden HP, et al. A role for sunlight in skin cancer: UV-induced p53 mutations in squamous cell carcinoma. Proc Natl Acad Sci USA 1991, 88(22): 10124-10128.
29. Kim DJ, Angel JM, Sano S, DiGiovanni J. Constitutive activation and targeted disruption of signal transducer and activator of transcription 3 (Stat3) in mouse epidermis reveal its critical role in UVB-induced skin carcinogenesis. Oncogene 2009, 28(7): 950-960.
30. Yarosh D, Alas LG, Yee V, Oberyszyn A, Kibitel JT, Mitchell D, et al. Pyrimidine Dimer Removal Enhanced by DNA Repair Liposomes Reduces the Incidence of UV Skin Cancer in Mice. Cancer Res 1992, 52(15): 4227-4231.
31. Yarosh D, Klein J, O'Connor A, Hawk J, Rafal E, Wolf P. Effect of topically applied T4 endonuclease V in liposomes on skin cancer in xeroderma pigmentosum: a randomised study. Xeroderma Pigmentosum Study Group. Lancet 2001, 357(9260): 926-929.
32. Reid-Bayliss KS, Arron ST, Loeb LA, Bezrookove V, Cleaver JE. Why Cockayne syndrome patients do not get cancer despite their DNA repair deficiency. Proc Natl Acad Sci USA 2016, 113: 10151-10156.
33. Applegate LA, Ley RD, Alcalay J, Kripke ML. Identification of the molecular target for the suppression of contact hypersensitivity by ultraviolet radiation. J Exp Med 1989, 170(4): 1117-1131.
34. Conney AH, Lu YP, Lou YR, Kawasumi M, Nghiem P. Mechanisms of Caffeine-Induced Inhibition of UVB Carcinogenesis. Front Oncol 2013, 3: 144.
35. Aziz MH, Reagan-Shaw S, Wu J, Longley BJ, Ahmad N. Chemoprevention of skin cancer by grape constituent resveratrol: relevance to human disease? FASEB J 2005, 19(9): 1193-1195.

36. Adamczewski JP, Rossignol M, Tassan JP, Nigg EA, Moncollin V, Egly JM. MAT1, cdk7 and cyclin H form a kinase complex which is UV light-sensitive upon association with TFIIH. EMBO J 1996, 15(8): 1877-1884.
37. Svejstrup JQ, Wang Z, Feaver WJ, Wu X, Bushnell DA, Donahue TF, et al. Different forms of TFIIH for transcription and DNA repair: holo-TFIIH and a nucleotide excision repairosome. Cell 1995, 80(1): 21-28.
38. Kato JY, Matsuoka M, Polyak K, Massague J, Sherr CJ. Cyclic AMP-induced G1 phase arrest mediated by an inhibitor (p27Kip1) of cyclin-dependent kinase 4 activation. Cell 1994, 79(3): 487-496.
39. Fero ML, Randel E, Gurley KE, Roberts JM, Kemp CJ. The murine gene p27Kip1 is haplo-insufficient for tumour suppression. Nature 1998, 396(6707): 177-180.
40. LeToriellec E, Despouy G, Pierron G, Gaye N, Joiner M, Bellanger D, et al. Haploinsufficiency of CDKN1B contributes to leukemogenesis in T-cell prolymphocytic leukemia. Blood 2008, 111(4): 2321-2328.
41. Chu IM, Hengst L, Slingerland JM. The Cdk inhibitor p27 in human cancer: prognostic potential and relevance to anticancer therapy. Nat Rev Cancer 2008, 8(4): 253-267.
42. Oh CW, Penneys N. P27 and mib1 expression in actinic keratosis, Bowen disease, and squamous cell carcinoma. Am J Dermatopathol 2004, 26(1): 22-26.
43. Kolluri SK, Weiss C, Koff A, Gottlicher M. p27(Kip1) induction and inhibition of proliferation by the intracellular Ah receptor in developing thymus and hepatoma cells. Genes Dev 1999, 13(13): 1742-1753.
44. Fujita N, Sato S, Katayama K, Tsuruo T. Akt-dependent phosphorylation of p27Kip1 promotes binding to 14-3-3 and cytoplasmic localization. J Biol Chem 2002, 277(32): 28706-28713.
45. Sun H, Lesche R, Li DM, Liliental J, Zhang H, Gao J, et al. PTEN modulates cell cycle progression and cell survival by regulating phosphatidylinositol 3,4,5,-trisphosphate

and Akt/protein kinase B signaling pathway. Proc Natl Acad Sci USA 1999, 96(11): 6199-6204.

46. Delmas C, Manenti S, Boudjelal A, Peyssonnaux C, Eychene A, Darbon JM. The p42/p44 mitogen-activated protein kinase activation triggers p27Kip1 degradation independently of CDK2/cyclin E in NIH 3T3 cells. J Biol Chem 2001, 276(37): 34958-34965.
47. Lenferink AE, Simpson JF, Shawver LK, Coffey RJ, Forbes JT, Arteaga CL. Blockade of the epidermal growth factor receptor tyrosine kinase suppresses tumorigenesis in MMTV/Neu + MMTV/TGF-alpha bigenic mice. Proc Natl Acad Sci USA 2000, 97(17): 9609-9614.
48. Wu R, Zhang L, Hoagland MS, Swanson HI. Lack of the aryl hydrocarbon receptor leads to impaired activation of AKT/protein kinase B and enhanced sensitivity to apoptosis induced via the intrinsic pathway. J Pharmacol Exp Ther 2007, 320(1): 448-457.
49. Lacouture ME. Mechanisms of cutaneous toxicities to EGFR inhibitors. Nat Rev Cancer 2006, 6(10): 803-812.
50. Dunkern TR, Kaina B. Cell proliferation and DNA breaks are involved in ultraviolet light-induced apoptosis in nucleotide excision repair-deficient Chinese hamster cells. Mol Biol Cell 2002, 13(1): 348-361.
51. Garinis GA, Mitchell JR, Moorhouse MJ, Hanada K, de WH, Vandeputte D, et al. Transcriptome analysis reveals cyclobutane pyrimidine dimers as a major source of UV-induced DNA breaks. EMBO J 2005, 24(22): 3952-3962.
52. Chan CY, Kim PM, Winn LM. TCDD affects DNA double strand-break repair. Toxicol Sci 2004, 81(1): 133-138.
53. Dittmann KH, Rothmund MC, Paasch A, Mayer C, Fehrenbacher B, Schaller M, et al. The nuclear aryl hydocarbon receptor is involved in regulation of DNA repair and cell

survival following treatment with ionizing radiation. Toxicol Lett 2016, 240(1): 122-129.

54. Chahal HS, Lin Y, Ransohoff KJ, Hinds DA, Wu W, Dai HJ, et al. Genome-wide association study identifies novel susceptibility loci for cutaneous squamous cell carcinoma. Nat Commun 2016, 7: 12048.
55. Tigges J, Haarmann-Stemmann T, Vogel CF, Grindel A, Hubenthal U, Brenden H, et al. The new aryl hydrocarbon receptor antagonist E/Z-2-benzylindene-5,6-dimethoxy-3,3-dimethylindan-1-one protects against UVB-induced signal transduction. J Invest Dermatol 2014, 134(2): 556-559.
56. Luo Y, Hurwitz J, Massague J. Cell-cycle inhibition by independent CDK and PCNA binding domains in p21Cip1. Nature 1995, 375(6527): 159-161.
57. Nishihashi H, Kanno Y, Tomuro K, Nakahama T, Inouye Y. Primary structure and organ-specific expression of the rat aryl hydrocarbon receptor repressor gene. Biol Pharm Bull 2006, 29(4): 640-647.

Figures

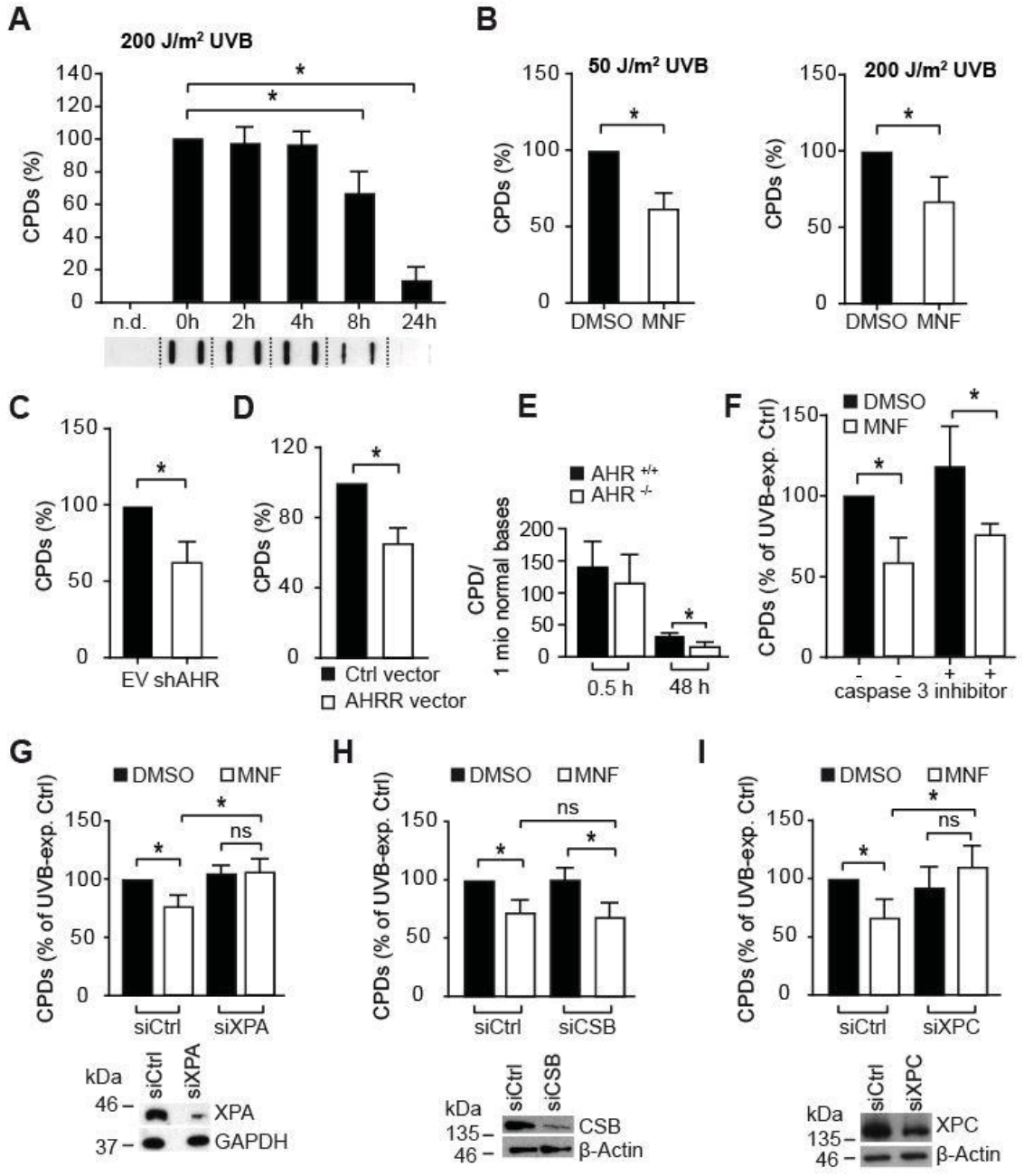

Figure 1. Chemical and genetic inhibition of AHR enhances the removal of UVB-induced CPDs by modulating GGR. **(A)** Time-dependent clearance of CPDs in HaCaT KC irradiated with 200 J/m^2 UVB. Cells were harvested at 0 (directly), 2, 4, 8 and 24 h after irradiation. Below the diagram, a representative SWB result is shown. **(B)** HaCaT KC were irradiated with 50 J/m^2 (left panel) and 200 J/m^2 (right panel) UVB and immediately

treated with 0.1% DMSO or 20 µM MNF. After 4 h CPD content was determined. **(C)** HaCaT-EV and HaCaT-shAHR KC were exposed to 200 J/m^2 UVB and 4 h later the CPD content was measured. **(D)** HaCaT KC were transfected with Ctrl. vector or an AHRR overexpression vector and 24 h later irradiated with 200 J/m^2 UVB. After 4 h the amount of CPDs was determined. **(E)** HPLC-MS/MS-based analysis of CPDs in the DNA of skin samples from AHR$^{+/+}$ and AHR$^{-/-}$ SKH-1 mice 0.5 h and 48 h after exposure to a single dose of 185 mJ/cm^2 UVB. **(F)** HaCaT KC were exposed to 200 J/m^2 and treated with MNF (20 µM) and caspase inhibitor Ac-DEVD-CHO (20 µM) alone or in combination. After 4 h, CPD content was measured. To assess if AHR affects TCR and/or GGR, we transiently silenced the expression of XPA **(G)**, CSB **(H)** and XPC **(I)** and 24 h later irradiated the cells with 200 J/m^2 UVB. Subsequently, the KC were treated for 4 h with MNF (20 µM) or 0.1% DMSO and CPD content was analyzed. Measurement of CPD content was carried out by SWB. For each experiment, the CPD content of the respective UVB-exposed controls was set to 100%. *p ≤ 0.05 and ns = not significant.

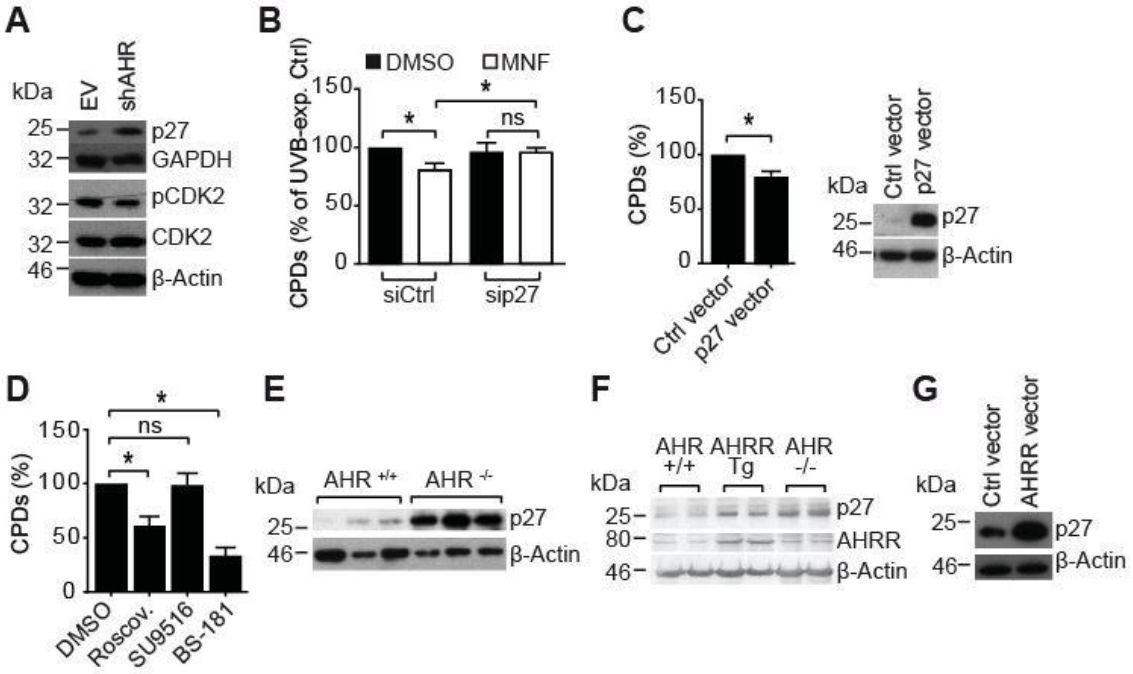

Figure 2. AHR inhibits GGR by modulating the protein level of the tumor suppressor p27. **(A)** Western blot analysis of p27, pCDK2 and CDK2 in untreated HaCaT-EV and HaCaT-

shAHR KC (representative blots). **(B)** HaCaT KC were transiently transfected with *p27*-targeted siRNA and Ctrl. siRNA. After 24 h, the cells were irradiated with 200 J/m^2 UVB and treated with 20 µM MNF or 0.1% DMSO. After 4 h, the CPD content was compared by SWB. **(C)** HaCaT KC were transiently transfected with Ctrl. vector or a p27 expression plasmid. After 24 h, the cells were exposed to 200 J/m^2 UVB and 4 h later the CPD content was determined. **(D)** HaCaT KC were irradiated with 200 J/m^2 UVB and subsequently treated with 1 µM roscovitine, 500 nM SU9516, 125 nM BS-181 or 0.1% DMSO. After 4 h, the CPD content was analyzed by SWB. **(E)** Protein lysates from skin samples of AHR$^{+/+}$ and AHR$^{-/-}$ SKH-1 mice were analyzed for p27 protein content by SDS-PAGE/western blot. **(F)** Protein lysates from skin samples of AHR$^{+/+}$, AHRR Tg and AHR$^{-/-}$ B6 mice were analyzed for p27 and AHRR protein content by SDS-PAGE/western blotting. **(G)** HaCaT KC were transiently transfected with an overexpression plasmid for rat *AHRR* or empty vector. After 24 h, the p27 protein level was compared by SDS-PAGE/western blot (representative blot). *p ≤ 0.05 and ns = not significant.

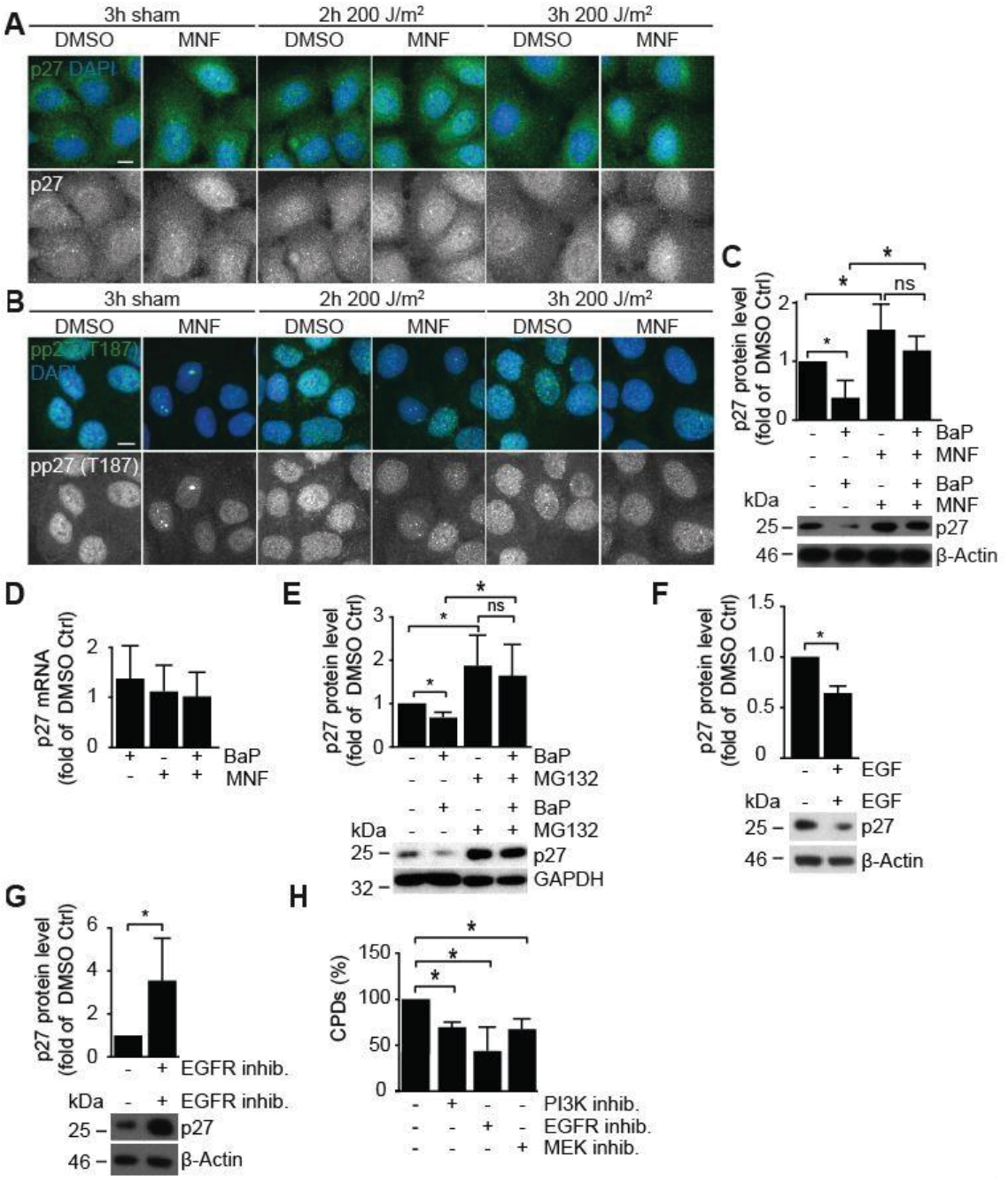

Figure 3. AHR-dependent modulation of the p27 protein level. HaCaT KC were irradiated with 0 and 200 J/m^2 UVB and treated with 20 µM MNF or 0.1% DMSO. After 2 h and 3 h, cells were fixed and antibody stained for **(A)** p27 and **(B)** pp27 T-187 (scale: 10 µm). **(C)** HaCaT KC were treated with 2.5 µM BaP and 20 µM MNF alone or in combination. Control cells were treated with 0.2% DMSO. Protein content of p27 was assessed by SDS-PAGE/western blot (right). **(D)** HaCaT cells were exposed to 2.5 µM BaP, 20 µM

MNF or both. After 24 h, copy numbers of *p27* were determined by qPCR. Data are shown as fold of DMSO ctrl. **(E)** HaCaT KC were treated with 2.5 µM BaP, 10 µM MG132 and 0.2% DMSO alone or in combination. Protein content of p27 was assessed by SDS-PAGE/western blot (top: quantification, bottom: representative WB). **(F)** HaCaT-shAHR KC were treated for 2 h with 50 ng/ml EGF or solvent. Subsequently, p27 protein level was determined by SDS-PAGE/western blot (top: quantification, bottom: representative WB). **(G)** HaCaT-EV KC were treated for 2 h with 10 µM of the EGFR inhibitor PD153035 or 0.1% DMSO. Subsequently, p27 protein level was determined by SDS-PAGE/western blot (top: quantification, bottom: representative WB). **(H)** HaCaT KC were irradiated with 200 J/m^2 UVB and immediately treated with 0.1% DMSO, 10 µM PD153035 (EGFR inhibitor), 1 µM Wortmannin (PI3K inhibitor) or 10 µM PD98059 (MEK inhibitor). After 4h CPD content was determind by SWB. *$p \leq 0.05$.

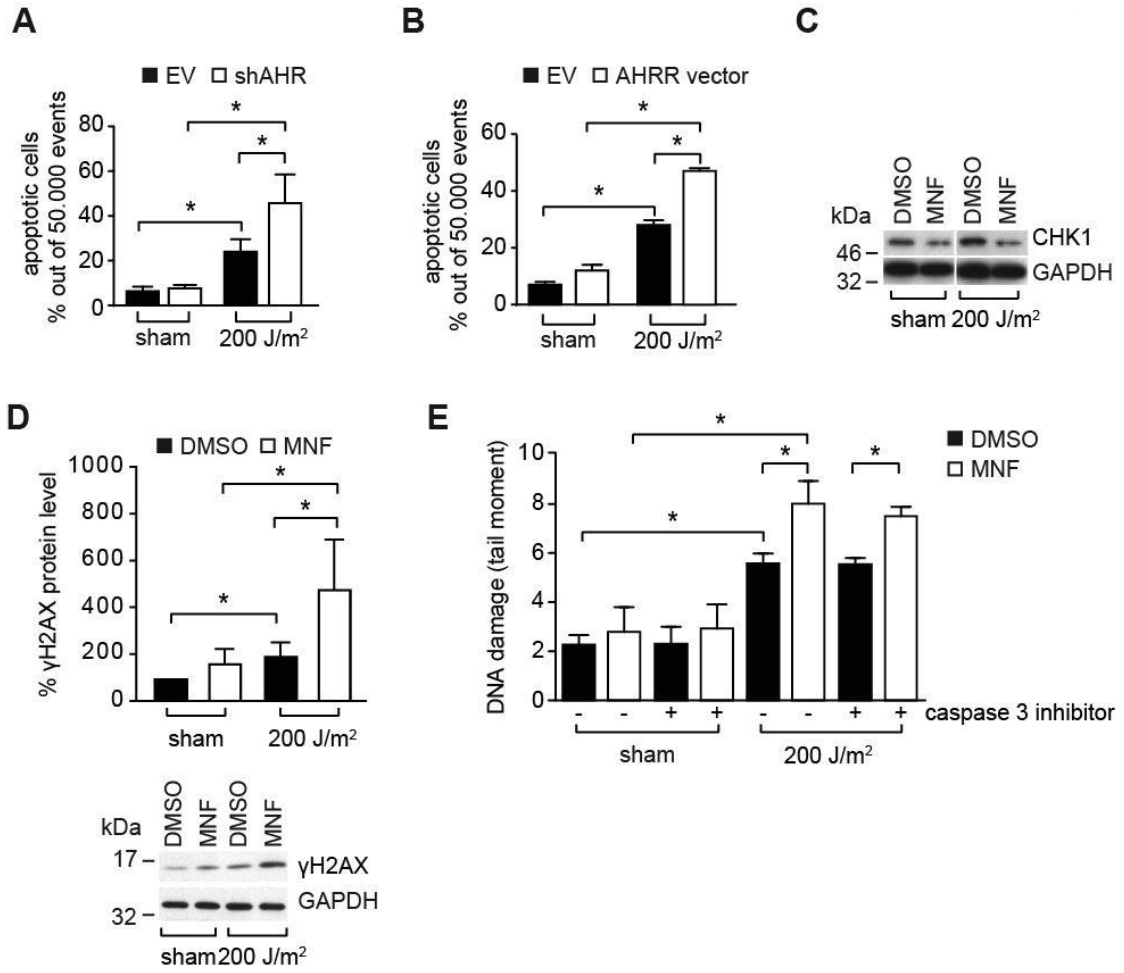

Figure 4. AHR inhibition increases UVB-induced apoptosis and is associated with DSB formation. **(A)** HaCaT-EV and HaCaT-shAHR KC were irradiated with 0 and 200 J/m^2 UVB. After 24 h the amount of dead cells was analyzed by Annexin V/PI staining. **(B)** HaCaT KC were transiently transfected with an expression vector for rat AHRR or empty vector. After 24 h, the KC were exposed to 200 J/m^2 UVB and another 24 h later, the amount of dead cells was determined by annexin V/PI staining. **(C)** Western blot analysis of CHK1 in HaCaT KC 24 h after irradiation with 0 and 200 J/m^2 UVB (representative blot). **(D)** HaCaT KC were irradiated with 0 and 200 J/m^2 UVB. After 18 h, γH2AX levels were assessed by SDS-PAGE/western blot. **(E)** HaCaT KC were irradiated with 0 and 200 J/m^2 UVB and immeadiately treated with MNF (20 µM) and DMSO alone or in combination with the caspase inhibitor Ac-DEVD-CHO (20 µM). After 18 h, DSBs were detected by neutral comet assay analyses. *p ≤ 0.05.

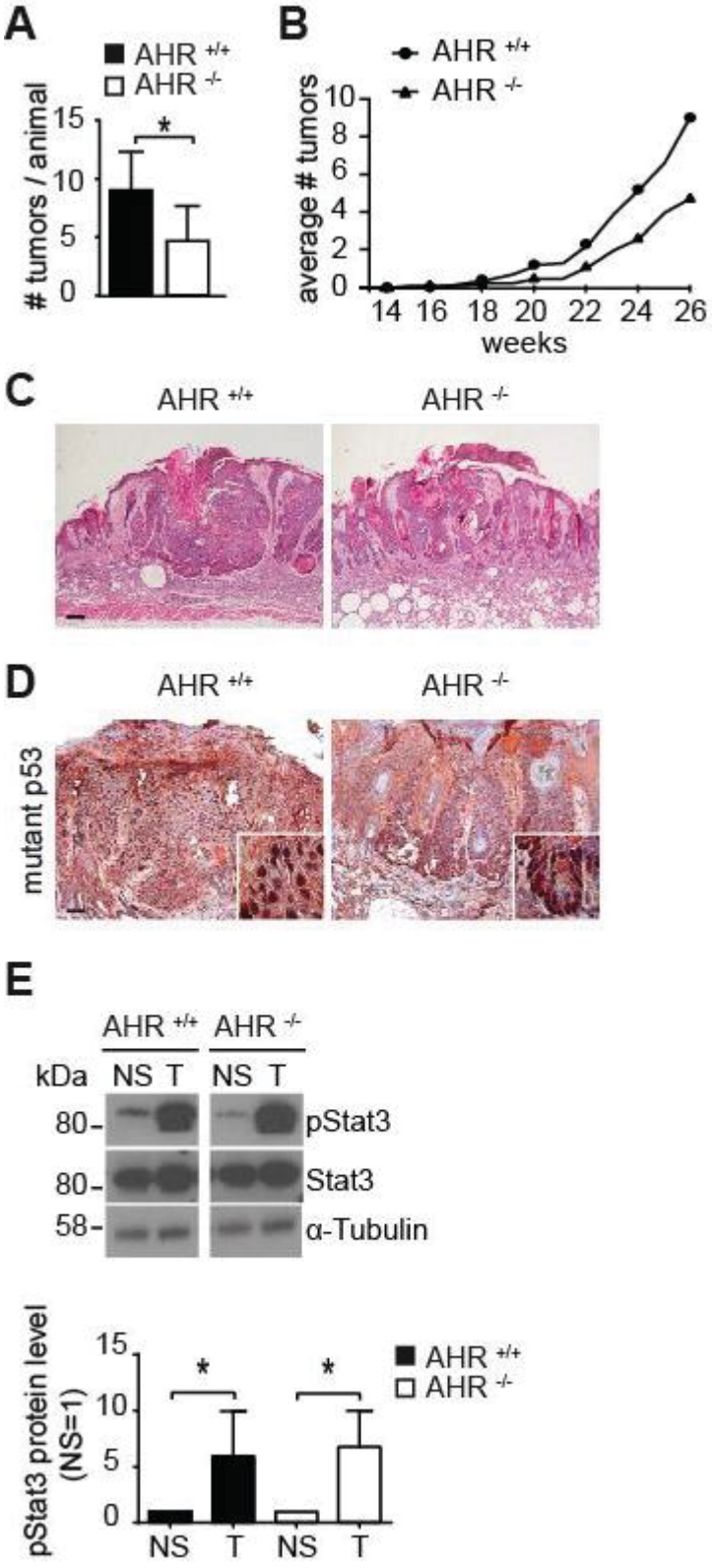

Figure 5. AHR contributes to UVB-induced skin carcinogenesis in SKH-1 hairless mice.

(A) Ten AHR$^{+/+}$ and eight AHR$^{-/-}$ SKH-1 mice were chronically exposed to UVB radiation

as described in *Material and Methods*. Two weeks after the last irradiation, total numbers of SCCs were compared. **(B)** Time-course of UVB-induced SCC development in the skin of AHR$^{+/+}$ and AHR$^{-/-}$ SKH-1 mice. **(C)** Representative histological pictures from primary SCCs from AHR$^{+/+}$ and AHR$^{-/-}$ mice (scale: 200 µm). *p ≤ 0.05. **(D)** Immunohistochemical staining of lesional and adjacent non-lesional skin of AHR$^{+/+}$ and AHR$^{-/-}$ SKH-1 mice with an antibody detecting mutant p53 protein (scale: 300 µm). **(E)** Protein lysates from lesional (T) and irradiated non-lesional (NS) skin of AHR$^{+/+}$ and AHR$^{-/-}$ SKH-1 mice were analyzed for phosphorylation (at Y-705) and expression of STAT3 by SDS-PAGE/western blotting. *p ≤ 0.05.

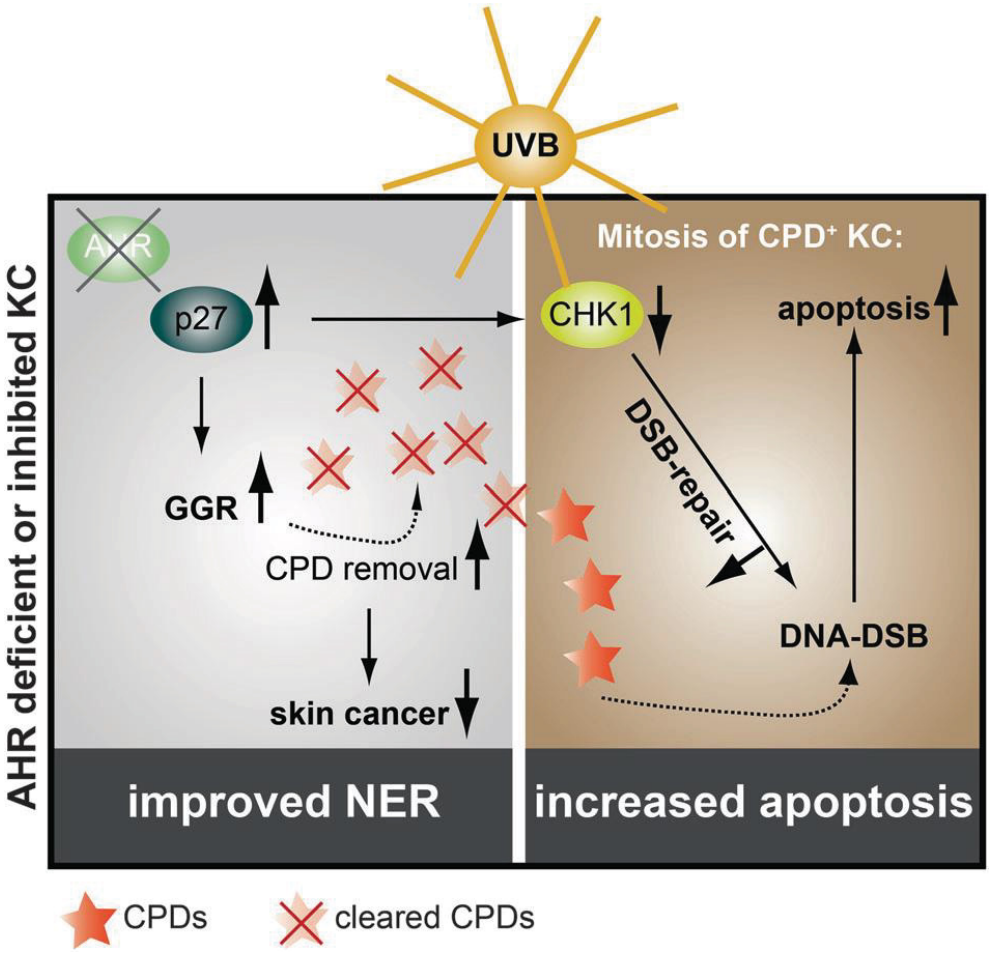

Figure 6. Hypothetical model: AHR inhibition increases p27 protein content resulting in an acceleration of GGR and reduction of mutagenic CPDs and associated SCC development. The increased p27 level results in a reduced CHK1 expression and an

attenuation of HRR. Accordingly, CPD-positive AHR-compromised KC are sensitive towards mitosis-related DSBs and subsequent apoptotic cell death.

2.2 Commentary: Usage of Mitogen-Activated Protein Kinase Small Molecule Inhibitors: More Than Just Inhibition!

Marius Pollet, Jean Krutmann, Thomas Haarmann-Stemmann

Journal:	Frontiers in Pharmacology
Impact factor:	3,8
Beitrag zur Publikation:	Literaturrecherche, tabellarische Übersicht
Status der Publikation:	Veröffentlicht: 20. August 2018

A Commentary on
Usage of Mitogen-Activated Protein Kinase Small Molecule Inhibitors: More Than Just Inhibition!

By Meurer, S. K., and Weiskirchen, R. (2018). Front. Pharmacol. 9:98. doi: 10.3389/fphar.2018.00098

Steffen Meurer and Ralf Weiskirchen recently published an interesting and important study concerning the off-target effects of so-called “specific” protein kinase inhibitors (PKI) that are frequently applied in both, basic research and clinical applications (Meurer and Weiskirchen,2018). A PKI-dependent inhibition of non-targeted protein kinases may occur due to the usage of concentrations that exceed the respective IC50 value by multiple factors. Meurer and Weiskirchen instead describe a PKI-mediated activation of non-targeted protein kinases. Specifically, the authors observed that a treatment of hepatic stellate cells, hepatocytes and portal myofibroblasts with a chemical inhibitor for a certain mitogen-activated protein kinase (MAPK) led to an activation of other members of the MAPK network. The authors called this phenomenon “activation by inhibition” and “cross-activation” (Meurer and Weiskirchen, 2018), terms which well describe the experimental observations but not the underlying molecular mechanism. Regarding the latter one, we realized that four of the five MAPK inhibitors tested in the respective study are known to interfere with the activity of the aryl hydrocarbon receptor (AHR), a ligand-activated transcription factor and key regulator of xenobiotic metabolism (Murray et al., 2014). In its inactive form, the AHR is trapped in a cytosolic multiprotein complex.Upon binding of small

molecular weight compounds, this complex dissociates and the AHR shuttles in the nucleus, dimerizes with its partner molecule ARNT and induces gene expression (Murray et al., 2014). The probably best-examined AHR target genes encode for the xenobioticmetabolizing monooxygenases cytochrome P450 (CYP) 1A1, CYP1A2, and CYP1B1, which, in most cases, oxidize the invading chemicals to enhance their polarity and facilitate their excretion (Mescher and Haarmann-Stemmann, 2018). Importantly, the ligand-driven activation of AHR is frequently accompanied by a stimulation of other cellular signaling pathways, including NF-kB, epidermal growth factor receptor (EGFR) and MAPK signal transduction (Haarmann-Stemmann et al., 2009; Puga et al., 2009; Tian, 2009). The list of AHR ligands encompasses infamous environmental pollutants, such as 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) and benzo[a]pyrene, plant polyphenols, microbiota-derived indoles and phenazines as well as several pharmaceuticals (Murray et al., 2014). Interestingly, more than a dozen PKI, including the four MAPK inhibitors SB203580, U0126, PD98059, and SP600125 tested by Meurer and Weiskirchen, have been identified to date to modulate AHR activity and downstream gene expression (e.g., of CYP1A1) in either a positive or a negative manner (Table 1). Several PKI, such as the phosphoinositide 3-kinase inhibitor LY294002, bind to the AHR protein and antagonize its activation by the prototype ligand TCDD (Guo et al., 2000), whereas others, including the aforementioned U0126 and SB203580, were shown to interact with AHR and increase its transcriptional activity (Andrieux et al., 2004; Korashy et al., 2011). In this context, it is interesting to know that an activation of AHR by different ligands has been reported to stimulate the phosphorylation of ERK1/2, p38 MAPK, JNK, and upstream receptor tyrosine kinases in various human and

rodent cells (Haarmann-Stemmann et al., 2009; Puga et al., 2009). In fact, Fumio Matsumura and his team have been among the first reporting a direct impact of AHR activation on protein kinase activity. Specifically, they observed an increased activity of protein kinase C and EGFR in hepatic tissue of rodents treated with TCDD (Madhukar et al., 1984; Bombick et al., 1985). Further examples for an impact of AHR activation on the signal transduction network, are the TCDDinduced phosphorylation of p38 MAPK observed in hepatoma cells (Weiss et al., 2005) and macrophages (Park et al., 2005), as well as the activation of EGFR and downstreamERK1/2 signaling by the AHR agonists 6-formylindolo[3,2-b]carbazole and TCDD in keratinocytes and colon cancer cells, respectively (Fritsche et al., 2007; Xie et al., 2012). It is thus tempting to speculate that at least some of the PKI-induced off-target effects observed by Meurer and Weiskirchen, such as the phosphorylation of ERK1/2 and JNK by the p38 MAPK inhibitor SB203580 or the activation of JNK and p38 MAPK by the MEK1/2 blocker U0126, were due to a stimulation of AHR activity. One may describe this phenomenon as an effect of a certain PKI on a non-kinase target (Munoz, 2017) or simply as the recognition of a foreign compound by the cellular defense system against xenobiotics. This notion is supported by the fact that some PKI have been found to interact with other xenobiotic receptors as well. The MEK1/2 inhibitor U0126, for instance, was shown to induce the expression of CYP3A4 in human hepatoma cells by binding to the pregnane X receptor (PXR) (Smutny et al., 2014). In addition, five out of nine tested clinically relevant PKI (erlotinib, gefitinib, nilotinib, sorafenib, and vandetanib) induced the expression of the ATP-binding cassette transporter P-glycoprotein in a PXR-dependent manner in human colon cancer cells (Harmsen et al., 2013). The majority of PKI interact

with the ATP-binding cleft of the target enzyme (Bain et al., 2007; Wu et al., 2015). Given that the 3D structure of the ATP-binding cleft is highly conserved amongst eukaryotic protein kinases, these inhibitors are limited in both their size and structural diversity (Bain et al., 2007; Wu et al., 2015). As implied by the growing list of PKI identified to interact with AHR and PXR, the structural prerequisites to bind to the ATP-binding cleft of protein kinases seemto resemble those required to interact with the ligand-binding domain of the xenobiotic receptors. Indeed, AHR and PXR contain a relatively large ligand-binding domain with a cavity volume of _840 Å3 (Denison et al., 2002) and _1,150 Å3 (Watkins et al., 2001), respectively, and share an extreme structural diversity of ligands (Denison and Faber, 2017). Notably, the known IC50/EC50 values of some PKI to modulate AHR activity are indeed in the range of the IC50 for their target kinases (Table 1). However, apart from ligand-binding, certain PKI may indirectly interfere with AHR signaling, for instance by inhibiting protein kinases contributing to AHR's nuclear translocation (Haarmann-Stemmann et al., 2009). We agree with the authors that a detailed knowledge of the off-target effects induced by widely used PKI is urgently required, not only for the proper interpretation of experimental data, but in particular, to better forecast potential drug-drug interactions during therapy.When working with so-called "specific" PKI, one should be aware that these compounds will be at first recognized by the chemical defense system, i.e. by xenobiotic receptors, drugmetabolizing enzymes and drug transporters, of the exposed cells. The interaction with one or more xenobiotic receptor(s) is determined by the structural and physiochemical properties of a given PKI and may not only affect its own metabolism, but also the metabolism of eventually co-

administered drugs, and the activity of other signaling pathways that might be tightly interconnected with the addressed chemosensory receptor.

Inhibitor	Target kinase	IC_{50} target kinase [μM][a]	AHR modulation	Tested cells/cell-lines/tissue	IC_{50}/EC_{50} AHR-specific endpoint [μM]	References
AG-494	EGFR	1	Inhibitor[b]	Human Caco-2 colon cancer cells	Not available	Kasai and Kikuchi, 2010
Akti-1/2	AKT1 AKT2	0.05 0.21	Inhibitor[c]	Human MCF-7 breast cancer cells	IC_{50}: 5.86	Gilot et al., 2010
LY294002	PI3Kα PI3Kβ PI3Kδ	0.5 0.97 0.57	Antagonist	Human MCF-10A mammary epithelial cells	IC_{50}: 35	Guo et al., 2000
PD98059	MEK	2	Antagonist	MCF-10A cells	IC_{50}: 1–4	Reiners et al., 1998
PP2	SFK Fyn SFK Hck SFK Lck SFK Src	0.005 0.005 0.004 0.1	Agonist	Human HepG2 hepatoma cells, human NCTC 2544 keratinocytes	Not available	Frauenstein et al., 2015
SB203580	p38	0.0003–0.0005	Agonist	Murine Hepa1c1c7 hepatoma cells, HepG2	Not available	Korashy et al., 2011
SB216763	GSK3	0.034	Partial agonist	Hepa1c1c7 cells, murine PW531 hepatoma cells, murine primary hepatocytes	Not available	Braeuning and Buchmann, 2009
SP600125	JNK1/2 JNK3	0.04 0.09	Antagonist/partial agonist	Hepa1c1c7 cells, HepG2 cells, rat liver, human primary hepatocytes	IC_{50}: 1.5–7 EC_{50}: 0.005–1.89	Joiakim et al., 2003 Dvorak et al., 2008
STO-609	CaMKKα/β	0.027	Agonist	MCF-7 cells, human primary macrophages, human A549 lung cancer cells	EC_{50}: 0.043–3.4	Monteiro et al., 2008
SU11248	c-Kit CSF1R FGFR1 FLT3 PDGFRα PDGFRβ RET VEGFR1/2	0.001–0.01 0.05–0.1 0.88 0.25 0.069 0.039 0.05 0.004	Inducer[c]	MCF-7 cells	Not available	Maayah et al., 2013
TSU-16/SU5416	VEGFR2	1.2	Agonist	HepG2 cells, human primary hepatocytes, human 101L hepatoma cells, rat 5L hepatoma cells, murine primary splenocytes	EC_{50}: 0.007–9.8	Mezrich et al., 2012 Matsuoka-Kawano et al., 2010
TSU-68/SU6668	AURKB AURKC FGFR1 PDGFRβ VEGFR2	0.035 0.21 3 0.06 2.43	Inducer[b]	Human primary hepatocytes, rat liver	Not available	Kitamura et al., 2008a Kitamura et al., 2008b
U0126	MEK1 MEK2	0.07 0.06	Agonist	Human B16A2 hepatoma cells, rat primary hepatocytes	EC_{50}: 2.5	Andrieux et al., 2004

[a] *IC_{50} values for target kinase(s) and respective references are provided by Cayman Chemical (www.caymanchem.com) and Selleckchem (www.selleckchem.com), respectively. The IC_{50} values for SU11248 are from Heng and Kollmannsberger (2010).*

[b] *Mode of action not clear.*

[c] *Ligand-independent mode of action.*

AURKB, aurora kinase B; AURKC, aurora kinase C; AKT, protein kinase B; CaMKK, calcium/calmodulin-dependent protein kinase kinase; CSF1R, colony stimulating factor 1 receptor; c-kit, mast/stem cell growth factor receptor; EGFR, epidermal growth factor receptor; FGFR, fibroblast growth factor receptor; FLT3, Fms-like tyrosine kinase 3; GSK3, glycogen synthase kinase 3; JNK, c-Jun N-terminal kinase; MEK, mitogen-activated protein kinase kinase; PI3K, phosphoinositide-3-kinase; RET, rearranged during transfection; SFK, Src family kinases; p38 MAPK, p38 mitogen-activated protein kinase; PDGFR, platelet-derived growth factor receptor; VEGFR, vascular endothelial growth factor receptor.

AUTHOR CONTRIBUTIONS

MP performed literature research and created the table. JK revised the manuscript critically for important intellectual content. TH-S performed literature research and wrote the manuscript. All authors approved the final version of the manuscript.

REFERENCES

Andrieux, L., Langouët, S., Fautrel, A., Ezan, F., Krauser, J. A., Savouret, J. F., et al. (2004). Aryl hydrocarbon receptor activation and cytochrome P450 1A induction by the mitogen-activated protein kinase inhibitor U0126 in hepatocytes. Mol. Pharmacol. 65, 934–943. doi: 10.1124/mol.65.4.934

Bain, J., Plater, L., Elliott,M., Shpiro, N., Hastie, C. J.,Mclauchlan, H., et al. (2007). The selectivity of protein kinase inhibitors: a further update. Biochem. J. 408, 297–315. doi: 10.1042/BJ20070797

Bombick, D. W., Madhukar, B. V., Brewster, D. W., and Matsumura, F. (1985). TCDD (2,3,7,8-tetrachlorodibenzo-p-dioxin) causes increases in protein kinases particularly protein kinase C in the hepatic plasma membrane of the rat and the guinea pig. Biochem. Biophys. Res. Commun. 127, 296–302. doi: 10.1016/S0006-291X(85)80158-3

Braeuning, A., and Buchmann, A. (2009). The glycogen synthase kinase inhibitor 3-(2,4-dichlorophenyl)-4-(1-methyl-1H-indol-3-yl)-1H-pyrrole-2,5-dione (SB216763) is a partial agonist of the aryl hydrocarbon receptor. Drug Metab. Dispos. 37, 1576–1580. doi: 10.1124/dmd.109.027821

Denison, M. S., and Faber, S. C. (2017). And now for something completely different: diversity in ligand-dependent activation of Ah receptor responses. Curr Opin Toxicol 2, 124–131. doi: 10.1016/j.cotox.2017.01.006

Denison, M. S., Pandini, A., Nagy, S. R., Baldwin, E. P., and Bonati, L. (2002).

Ligand binding and activation of the Ah receptor. Chem. Biol. Interact. 141, 3–24. doi: 10.1016/S0009-2797(02)00063-7

Dvorak, Z., Vrzal, R., Henklova, P., Jancova, P., Anzenbacherova, E., Maurel, P., et al. (2008). JNK inhibitor SP600125 is a partial agonist of human aryl hydrocarbon receptor and induces CYP1A1 and CYP1A2 genes in primary human hepatocytes. Biochem. Pharmacol. 75, 580–588. doi: 10.1016/j.bcp.2007.09.013

Frauenstein, K., Tigges, J., Soshilov, A. A., Kado, S., Raab, N., Fritsche, E., et al. (2015). Activation of the aryl hydrocarbon receptor by the widely used Src family kinase inhibitor 4-amino-5-(4-chlorophenyl)-7-(dimethylethyl)pyrazolo[3,4-d]pyrimidine (PP2). Arch. Toxicol. 89, 1329–1336. doi: 10.1007/s00204-014-1321-8

Fritsche, E., Schäfer, C., Calles, C., Bernsmann, T., Bernshausen, T., Wurm, M., et al. (2007). Lightening up the UV response by identification of thearylhydrocarbon receptor as a cytoplasmatic target for ultraviolet B radiation. Proc. Natl. Acad. Sci. U.S.A. 104, 8851–8856. doi: 10.1073/pnas.0701764104

Gilot, D., Giudicelli, F., Lagadic-Gossmann, D., and Fardel, O. (2010). Akti-1/2, an allosteric inhibitor of Akt 1 and 2, efficiently inhibits CaMKIalpha activity and aryl hydrocarbon receptor pathway. Chem. Biol. Interact. 188, 546–552. doi: 10.1016/j.cbi.2010.08.011

Guo, M., Joiakim, A., and Reiners, J. J., Jr. (2000). Suppression of 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD)-mediated aryl hydrocarbon receptor transformation and CYP1A1 induction by the phosphatidylinositol 3-kinase inhibitor 2-(4-morpholinyl)-8-phenyl-4H-1- benzopyran-4-one (LY294002). Biochem. Pharmacol. 60, 635–642. doi: 10.1016/S0006-2952(00)00379-8

Haarmann-Stemmann, T., Bothe, H., and Abel, J. (2009). Growth factors, cytokines and their receptors as downstream targets of arylhydrocarbon

receptor (AhR) signaling pathways. Biochem. Pharmacol. 77, 508–520. doi: 10.1016/j.bcp.2008.09.013

Harmsen, S., Meijerman, I., Maas-Bakker, R. F., Beijnen, J. H., and Schellens, J. H. (2013). PXR-mediated P-glycoprotein induction by small molecule tyrosine kinase inhibitors. Eur. J. Pharm. Sci. 48, 644–649. doi: 10.1016/j.ejps.2012.12.019

Heng, D. Y. C., and Kollmannsberger, C. (2010). “Sunitinib” in Small Molecules in Oncology ed U. M. Martens (Berlin: Springer), 71–82.

Joiakim, A., Mathieu, P. A., Palermo, C., Gasiewicz, T. A., and Reiners, J. J. Jr. (2003). The Jun N-terminal kinase inhibitor SP600125 is a ligand and antagonist of the aryl hydrocarbon receptor. Drug Metab. Dispos. 31, 1279–1282. doi: 10.1124/dmd.31.11.1279

Kasai, S., and Kikuchi, H. (2010). The inhibitory mechanisms of the tyrosine kinase inhibitors herbimycin a, genistein, and tyrphostin B48 with regard to the function of the aryl hydrocarbon receptor in Caco-2 cells. Biosci. Biotechnol. Biochem. 74, 36–43. doi: 10.1271/bbb.90438

Kitamura, R., Asanoma, H., Nagayama, S., and Otagiri, M. (2008a). Identification of human liver cytochrome P450 isoforms involved in autoinduced metabolism of the antiangiogenic agent (Z)-5-[(1,2-dihydro-2-oxo-3H-indol-3-ylidene)methyl]-2,4-dimethyl-1H-pyrrole-3-pr opanoic acid (TSU-68). Drug Metab. Dispos. 36, 1003–1009. doi: 10.1124/dmd.107.019877

Kitamura, R., Matsuoka, K., Nagayama, S., and Otagiri, M. (2008b). Timedependent induction of rat hepatic CYP1A1 and CYP1A2 expression after single-dose administration of the anti-angiogenic agent TSU-68. Drug Metab. Pharmacokinet. 23, 421–427. doi: 10.2133/dmpk.23.421

Korashy, H. M., Anwar-Mohamed, A., Soshilov, A. A., Denison, M. S., and El-Kadi, A. O. (2011). The p38 MAPK inhibitor SB203580 induces cytochrome

P450 1A1 gene expression in murine and human hepatoma cell lines through ligand-dependent aryl hydrocarbon receptor activation. Chem. Res. Toxicol. 24, 1540–1548. doi: 10.1021/tx200141p

Maayah, Z. H., El Gendy, M. A., El-Kadi, A. O., and Korashy, H. M. (2013). Sunitinib, a tyrosine kinase inhibitor, induces cytochrome P450 1A1 gene in human breast cancer MCF7 cells through ligand-independent aryl hydrocarbon receptor activation. Arch. Toxicol. 87, 847–856. doi: 10.1007/s00204-012-0996-y

Madhukar, B. V., Brewster, D. W., and Matsumura, F. (1984). Effects of in vivo-administered 2,3,7,8-tetrachlorodibenzo-p-dioxin on receptor binding of epidermal growth factor in the hepatic plasma membrane of rat, guinea pig, mouse, and hamster. Proc. Natl. Acad. Sci. U.S.A. 81, 7407–7411. doi: 10.1073/pnas.81.23.7407

Matsuoka-Kawano, K., Yoshinari, K., Nagayama, S., and Yamazoe, Y. (2010). TSU-16, (Z)-3-[(2,4-dimethylpyrrol-5-yl)methylidenyl]-2-indolinone, is a potent activator of aryl hydrocarbon receptor and increases CYP1A1 and CYP1A2 expression in human hepatocytes. Chem. Biol. Interact. 185, 33–41. doi: 10.1016/j.cbi.2010.02.014

Mescher, M., and Haarmann-Stemmann, T. (2018). Modulation of CYP1A1 metabolism: from adverse health effects to chemoprevention and therapeutic options. Pharmacol. Ther. 187, 71–87. doi: 10.1016/j.pharmthera.2018.02.012

Meurer, S. K., and Weiskirchen, R. (2018). Usage of mitogen-activated protein kinase small molecule inhibitors: more than just inhibition! Front. Pharmacol. 9:98. doi: 10.3389/fphar.2018.00098

Mezrich, J. D., Nguyen, L. P., Kennedy, G., Nukaya, M., Fechner, J. H., Zhang, X., et al. (2012). SU5416, a VEGF receptor inhibitor and ligand of the AHR, represents a new alternative for immunomodulation. PLoS ONE 7:e44547.

doi: 10.1371/journal.pone.0044547

Monteiro, P., Gilot, D., Langouet, S., and Fardel, O. (2008). Activation of the aryl hydrocarbon receptor by the calcium/calmodulin-dependent protein kinase kinase inhibitor 7-oxo-7H-benzimidazo[2,1-a]benz[de]isoquinoline-3-carboxylic acid (STO-609). Drug Metab. Dispos. 36, 2556–2563. doi: 10.1124/dmd.108.023333

Munoz, L. (2017). Non-kinase targets of protein kinase inhibitors. Nat. Rev. Drug Discov. 16, 424–440. doi: 10.1038/nrd.2016.266

Murray, I. A., Patterson, A. D., and Perdew, G. H. (2014). Aryl hydrocarbon receptor ligands in cancer: friend and foe. Nat. Rev. Cancer 14, 801–814. doi: 10.1038/nrc3846

Park, S. J., Yoon,W. K., Kim, H. J., Son, H. Y., Cho, S.W., Jeong, K. S., et al. (2005). 2,3,7,8-Tetrachlorodibenzo-p-dioxin activates ERK and p38 mitogen-activated protein kinases in RAW264.7 cells. Anticancer Res. 25, 2831–2836.

Puga, A., Ma, C., and Marlowe, J. L. (2009). The aryl hydrocarbon receptor crosstalks with multiple signal transduction pathways. Biochem. Pharmacol. 77, 713–722. doi: 10.1016/j.bcp.2008.08.031

Reiners, J. J. Jr., Lee, J. Y., Clift, R. E., Dudley, D. T., and Myrand, S. P. (1998). PD98059 is an equipotent antagonist of the aryl hydrocarbon receptor and inhibitor of mitogen-activated protein kinase kinase. Mol. Pharmacol. 53, 438–445. doi: 10.1124/mol.53.3.438

Smutny, T., Bitman, M., Urban, M., Dubecka, M., Vrzal, R., Dvorak, Z., et al. (2014). U0126, a mitogen-activated protein kinase kinase 1 and 2 (MEK1 and 2) inhibitor, selectively up-regulates main isoforms of CYP3A subfamily via a pregnane X receptor (PXR) in HepG2 cells. Arch. Toxicol. 88, 2243–2259. doi: 10.1007/s00204-014-1254-2

Tian, Y. (2009). Ah receptor and NF-kappaB interplay on the stage of epigenome.

Biochem. Pharmacol. 77, 670–680. doi: 10.1016/j.bcp.2008.10.023

Watkins, R. E., Wisely, G. B., Moore, L. B., Collins, J. L., Lambert, M. H., Williams, S. P., et al. (2001). The human nuclear xenobiotic receptor PXR: structural determinants of directed promiscuity. Science 292, 2329–2333. doi: 10.1126/science.1060762

Weiss, C., Faust, D., Dürk, H., Kolluri, S. K., Pelzer, A., Schneider, S., et al. (2005). TCDD induces c-jun expression via a novel Ah (dioxin) receptor-mediated p38-MAPK-dependent pathway. Oncogene 24, 4975–4983. doi: 10.1038/sj.onc.1208679

Wu, P., Nielsen, T. E., and Clausen, M. H. (2015). FDA-approved smallmolecule kinase inhibitors. Trends Pharmacol. Sci. 36, 422–439. doi: 10.1016/j.tips.2015.04.005

Xie, G., Peng, Z., and Raufman, J. P. (2012). Src-mediated aryl hydrocarbon and epidermal growth factor receptor cross talk stimulates colon cancer cell proliferation. Am. J. Physiol. Gastrointest. Liver Physiol. 302, G1006–G1015. doi: 10.1152/ajpgi.00427.2011

Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

2.3 Vemurafenib acts as an aryl hydrocarbon receptor antagonist: Implications for inflammatory cutaneous adverse events

Journal:	Allergy
Impact factor:	6,0
Beitrag zur Publikation:	Allgemeine Zellkultur, Promotorstudien, Proteinfraktionierung, WesternBlot, ELISA Analysen
Status der Publikation:	Akzeptiert zur Veröffentlichung: 21. Mai 2019

Vemurafenib acts as an aryl hydrocarbon receptor antagonist: Implications for inflammatory cutaneous adverse events

Heike C. Hawerkamp[a*], Andreas Kislat[a*], Peter A. Gerber[a], Marius Pollet[b], Katharina M. Rolfes[b], Anatoly A. Soshilov[c], Michael S. Denison[c], Afaque A. Momin[d], Stefan T. Arold[d], Angeliki Datsi[a], Stephan A. Braun[a], Péter Oláh[a,e], Mario E. Lacouture[f], Jean Krutmann[b], Thomas Haarmann-Stemmann[b#], Bernhard Homey[a#], and Stephan Meller[a#]

[a]*Department of Dermatology, Medical Faculty, Heinrich-Heine-University, Duesseldorf, Germany*

[b]*Leibniz-Research Institute for Environmental Medicine, Duesseldorf, Germany*

[c]*Department of Environmental Toxicology, University of California, Davis, CA, USA*

[d]*King Abdullah University of Science and Technology (KAUST), Computational Bioscience Research Center (CBRC), Division of Biological and Environmental Sciences and Engineering (BESE), Thuwal, 23955-6900, Saudi Arabia*

[e]*Department of Dermatology, Venereology and Oncodermatology, University of Pécs, Pécs, Hungary*

[f]*Dermatology Service, Department of Medicine, Memorial Sloan-Kettering Cancer Center, New York, NY, USA*

*#Equal contribution

Corresponding author:
Stephan Meller, MD
Department of Dermatology, Medical Faculty, Heinrich-Heine-University
Duesseldorf
Moorenstr. 5
D-40225 Duesseldorf, Germany
Phone: + 49 211 811 7853
Fax: + 49 211 811 7316

e-mail: Stephan.Meller@uni-duesseldorf.de

Short title: Vemurafenib acts as aryl hydrocarbon receptor antagonist

Conflict of interest statement: The authors declare no potential conflicts of interest.

Keywords: aryl hydrocarbon receptor, drug eruption, lymphocyte activation test, melanoma, vemurafenib

Abbreviations:

ADR, adverse drug reaction

AhR, aryl hydrocarbon receptor

BRAFi, BRAF protein kinase inhibitor

EGFRi, EGFR inhibitor

h, hours

LAT, lymphocyte activation test

SI, stimulation index

VIR, vemurafenib-induced rashes

XRE, xenobiotic response element

Word count:

3.497 words (including author contributions and acknowledgements, not including abstract, figure legends and references)

Abstract

Background: In recent years, the BRAF-inhibitor vemurafenib has been successfully established in the therapy of advanced melanoma. Despite its superior efficacy, the use of vemurafenib is limited by frequent inflammatory cutaneous adverse events that affect patients' quality of life and may lead to dose reduction or even cessation of anti-tumor therapy. To date, the molecular and cellular mechanisms of vemurafenib-induced rashes have remained largely elusive.

Methods: In this study we deployed immunohistochemistry, RT-qPCR, flow cytometry, lymphocyte activation tests and different cell-free protein-interaction assays.

Results: We here demonstrate that vemurafenib inhibits the downstream signaling of the canonical pathway of aryl hydrocarbon receptor (AhR) *in vitro*, thereby inducing the expression of proinflammatory cytokines (e.g. *TNF*) and chemokines (e.g. *CCL5*). In line with these results we observed an impaired expression of AhR regulated genes (e.g. *CYP1A1*) and an upregulation of the corresponding proinflammatory genes *in vivo*. Moreover, results of lymphocyte activation tests showed the absence of drug-specific T cells in respective patients.

Conclusion: Taken together, we obtained no hint of an underlying sensitization against vemurafenib but found evidence suggesting that vemurafenib enhances proinflammatory responses by inhibition of canonical AhR signaling. Our findings contribute to our understanding of the central role of the AhR in skin inflammation and may point towards a potential role for topical AhR agonists in supportive cancer care.

1 Introduction

The introduction of immune checkpoint-inhibitors (e.g. ipilimumab, pembrolizumab, nivolumab), BRAF- (vemurafenib, dabrafenib) and MEK-inhibitors (cobimetinib, trametinib), has revolutionized the treatment of advanced melanoma. The first of its class BRAF protein kinase inhibitor (BRAFi) vemurafenib received FDA approval in August 2011 [1]. Although vemurafenib is generally well tolerated, cutaneous adverse events occur in up to 70% of treated patients [2] and include inflammatory maculo-papular rashes (up to 37%), photosensitivity (12%) or secondary skin tumors (20%) [1]. Cutaneous adverse effects represent a serious threat to patients´ therapy adherence and may lead to dose reduction or therapy cessation. Despite clinical relevance, molecular and cellular mechanisms of vemurafenib-associated rashes (VIR) have remained largely elusive. Interestingly, Chapman and coworkers reported that pre-treatment with the pro-inflammatory, anti-cytotoxic T lymphocyte-associated antigen blocking antibody ipilimumab was associated with a higher frequency (up to 70%) of VIRs [3]. The authors proposed that increased numbers of severe grade 3 rashes are the result of hypersensitivity drug reactions to vemurafenib driven by immune checkpoint inhibition. In patients who developed grade 3 rashes vemurafenib therapy was paused until the rash resolved and were successfully retreated with lower doses of vemurafenib [3,4]. These clinical observations point towards either drug hypersensitivity through allergic sensitization or a pharmacological mechanism enhancing cutaneous inflammations. Here, we set out to investigate the underlying molecular mechanisms of VIRs. To this end, we characterized the phenotype of the rashes, performed lymphocyte activation tests (LAT) and investigated potential interactions with the signaling of the aryl

hydrocarbon receptor (AhR) representing a well-known xenobiotic receptor and central regulator of inflammatory processes [5-7].

2 Material and Methods

2.1 Human samples

Punch biopsies were obtained from patients with VIRs after obtaining informed consent. For skin explants, punch biopsies were taken from healthy skin. Buffy coats from healthy donors were obtained from the Institute of Hemostasis and Transfusion Medicine, University Hospital Duesseldorf. The guidelines of the Declaration of Helsinki were deployed and the study was authorized by the local ethics committee (No: 1928).

2.2 Animal samples and preparation of cytosol

Hartley guinea pigs were obtained from Charles River (Wilmington, DE, USA). Guinea pig hepatic cytosol was prepared as described [8] and stored properly until use. All animal protocols were approved by the Institutional Animal Care and Use Committee of the University of California, Davis.

2.3 Culture of human cells and explants

The culture of *ex vivo* explants, keratinocytes and T cells is described in the supplemental methods.

The tested concentrations of vemurafenib (up to 100 µM) [9,10] and dabrafenib [10,11] correspond to published studies. Cell viability was confirmed using 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (not shown).

2.4 RNA extraction and qPCR

Biopsies were homogenized in TRIzol® using a POLYTRON PT2500E (KINEMATICA AG, Luzern, Switzerland). RNA was isolated using RNeasy Mini Kit (Qiagen, Hilden, Germany) following the manufacturer's instructions, reverse transcribed into cDNA and analyzed by quantitative real-time PCR (ABI PRISM® 7000 Sequence Detection System/ QuantStudio 6 Flex, Thermo Fisher Scientific) [12].

2.5 Statistics

Statistical significances were assessed with Mann-Whitney U tests or Kruskal-Wallis test with Dunn's post correction and calculated using GraphPad Prism 5.03 (GraphPad software, Inc., La Jolla, CA, USA). Statistical significances were depicted as follows: *$p<0.05$, **$p<0.01$ and ***$p<0.001$.

Additional methods are described in the supplemental methods.

3 Results

3.1 Vemurafenib-induced inflammatory rashes are characterized by a dense lymphohistiocytic infiltrate

Patients (n=5; 67-76 years) with VIRs and healthy controls (n=5; 52-74 years) were included in our analysis. Patients presented with a generalized maculopapular rash with small papules and macules without scaling (Figure 1A). Histopathologic evaluation of lesional skin biopsies demonstrated a superficial dermatitis without epidermal changes, with mild spongiosis or subtle vacuolar interface changes. Immunohistochemistry revealed a lymphohistiocytic infiltrate with equally distributed $CD4^+$ and $CD8^+$ T cells (Figure 1B). We did not observe any prominent infiltrates of eosinophils, neutrophils or mast cells.

3.2 Vemurafenib-induced inflammatory rashes are characterized by a predominant T_H1- signature

We next analyzed the expression of signature cytokines in lesional skin (VIR, n=4-5) compared to healthy controls (HS, n=5). Our analyses revealed a significant induction of T_H1-associated cytokine *IFNG*, IFN-γ-induced chemokines *CXCL9-11*, *CCL5* (Figure 1C), and a significant upregulation of homeostatic chemokines *CCL27* and *CXCL14* (Supplementary Figure S1B). Moreover, pro-inflammatory cytokines and chemokines such as *CCL2* and *IL1B* were found to be upregulated (Figure 1C, Supplementary Figure S1B). Although, we observed increased expression levels of the T_H2-associated chemokines *CCL1*, *CCL17*, *CCL18*, and *CCL22*, no relevant regulation was detected for *IL4.* Furthermore, *IL17A* or *IFNA* were not induced in lesional skin (Figure 1C, Supplementary

Figure S1B). Taken together, we observed a predominant upregulation of T_H1-associated chemokines.

3.3 Vemurafenib induces inflammatory cytokines and chemokines *in vitro*

Next, we analyzed the recruitment pathways directing leukocyte subsets to sites of vemurafenib-induced cutaneous inflammation. We performed comprehensive analyses of cytokine and chemokine expression in whole skin explants, primary human keratinocytes, and T cells treated with vemurafenib *in vitro*. In skin explants vemurafenib alone altered the expression of *CCL5* and *CCL18*, which tended to be upregulated, mimicking the expression profile of VIRs (Figure 2A). In keratinocytes vemurafenib induced a significant expression of *TNF* and *CCL5* (Figure 2A). In T cells, an early upregulation of *IFNG* after 6 h was observed, and after 24 h transcription and protein levels were increased (Figure 2B). Further, *IL17A* was upregulated after 6 h and 24 h of vemurafenib treatment, yet at overall low expression levels (Supplementary Figure S2A). *IL4* expression was induced by vemurafenib at negligible levels (Supplementary Figure S2B).

3.4 Absence of circulating drug-specific T cells in patients with vemurafenib-induced rashes

To discriminate between allergic or non-allergic pharmacologic effects, we performed LATs with leukocytes obtained from patients suffering from VIRs (n=4). To distinguish between allergic and non-allergic patients, Beeler *et al.* suggested a stimulation index (SI) cutoff value of 2 [13]. Using this threshold, we did not detect vemurafenib-specific T cells in any patient. Hence, VIRs are rather

a result of a non-allergic, pharmacologic mechanism than a result of a specific sensitization against the drug (Figure 2C and 2D).

3.5 The structure of vemurafenib is compatible with binding to AhR *in silico*

To identify non-allergic pharmacologic mechanisms as potential cause of VIRs we focused on the well-known xenobiotic receptor AhR that binds and regulates the metabolism of drugs and other xenobiotics. Recent findings indicate that the loss of the AhR results in cutaneous inflammation [14]. Several protein kinase inhibitors modulate AhR activity in an agonistic or antagonistic manner [15]. The AhR ligand indirubin also binds kinase domains in the same ATP-competitive way as does vemurafenib [16-19], suggesting some level of cross-reactivity between ligands for AhR and kinases. AhR binds to [^{3}H]TCCD and other aromatic hydrocarbon ligands (PAS-B, residues 277-393) through its second PAS domain [20]. The dimensions and molecular characteristics of vemurafenib are within the range of characteristics displayed by *bona fide* AhR ligands (i.e. ~14 x 12 x 5 Å planar, hydrophobic ring structures with some hydrophilic moieties [20]). To computationally probe vemurafenib binding to the AhR PAS-B domain, we inferred the atomic structure of PAS-B based on ~26% identical known PAS-B domain structures. The structural models of PAS-B showed a hydrophobic cavity, in shape and stereochemical character similar to the ligand binding pocket of the HIF-2α PAS domain (PDB 4xt2), suggesting that PAS-B binds ligands in the same way as does HIF-2α PAS. Using a conservative *in silico* docking approach that allowed ligand flexibility but kept the protein structure rigid, we observed that indirubin and vemurafenib can be accommodated in the PAS-B pocket without steric clashes (Figure 3A and 3B). The calculated binding energies of indirubin and vemurafenib were very similar (-8.29 and -7.91 kcal/mol, respectively,

corresponding to dissociation constants K_d of 1-3 µM) supporting that vemurafenib may be a relevant AhR binding partner.

3.6 Vemurafenib is an AhR antagonist

To test the hypothesis that vemurafenib is an AhR ligand, we used human HepG2 hepatoma cells, stably transfected with a luciferase reporter gene driven by two AhR binding-sites [21]. HepG2 cells were exposed to vemurafenib alone or in combination with the AhR agonist benzo[a]pyrene (BaP). Basal luciferase reporter gene activity was slightly reduced following incubation with 40 µM vemurafenib (data not shown). Similarly, incubation with 40 µM vemurafenib markedly reduced the level of AhR-dependent luciferase activity by BaP, suggesting that vemurafenib is acting as an AhR antagonist (Figure 3C). By contrast, luciferase assays did not show any inhibitory effect of dabrafenib or encorafenib on AhR activity in relevant concentrations (up to 1 µM) [10,22] (Supplementary Figure S3A/B). Dabrafenib and encorafenib even significantly increased AhR-dependent luciferase activity at low concentrations of 0.1 µM (in absence of BaP). The multikinase inhibitor sorafenib showed a slight trend towards AhR inhibition at pharmacological concentrations of 10 µM [23] in presence of BaP (Supplementary Figure S3C). A similar inhibitory trend was seen for the novel BRAF inhibitor PLX8394 (Supplementary Figure S3D).

To determine the mechanism by which vemurafenib reduces AhR-dependent gene expression, we examined its ability to directly bind to AhR. Hydroxyapatite ligand binding analysis using guinea pig hepatic cytosolic AhR revealed that vemurafenib competitively inhibits [^{3}H]TCDD, a high affinity AhR ligand, from binding to AhR. This inhibition occurs in a vemurafenib concentration-dependent manner; with maximum competitive inhibition observed at 40 µM and hence in

line with our micromolar affinities as calculated *in silico* (Figure 3D). While this analysis confirmed that vemurafenib is a direct ligand for AhR, it does not provide any information as to whether vemurafenib is binding as an AhR agonist or antagonist. To address this question, we utilized a gel retardation analysis to assess the ability of a chemical to stimulate AhR transformation and DNA binding, a characteristic of AhR agonists. Although incubation of guinea pig hepatic cytosol with vemurafenib did not stimulate AhR transformation or DNA binding, it did produce a concentration-dependent decrease in TCDD-stimulated AhR transformation and DNA binding (Figure 3E). Ligand and DNA binding analyses confirmed the ability of vemurafenib to act as an AhR antagonist (i.e. it exhibits no AhR agonist activity). Against the background of previous studies that have demonstrated species-specific differences in AhR ligand binding specificity, respective DNA binding analysis studies were repeated using *in vitro* synthesized human AhR and ARNT (the AhR dimerization partner required for AhR DNA binding) [5,24]. Similar to the results obtained with guinea pig hepatic cytosolic AhR, vemurafenib antagonized the ability of TCDD to stimulate transformation and DNA binding of the human AhR (Figure 3F). As expected, vemurafenib treatment inhibited basal as well as BaP-induced nuclear translocation of AHR in human keratinocytes (Figure 3G). Taken together, these results confirm the ability of vemurafenib to act as an AhR antagonist.

3.7 Vemurafenib impairs CYP1A1 expression *in vitro, ex vivo* and *in vivo*

In order to prove the AhR antagonism of vemurafenib *in vivo* and *in vitro*, we assessed the expression of AhR and AhR-related genes in lesional skin of VIRs compared to healthy controls as well as in skin explants, keratinocytes, and T

cells. As an additional control we analyzed papulopustular rashes of cancer patients treated with epidermal growth factor receptor (EGFR) inhibitors, which we have demonstrated to be caused by non-allergic, pharmacologic mechanisms [25]. *AHR* was significantly upregulated in VIRs (Supplementary Figure S4A). In contrast, no regulation of *AHR* was observed in EGFR-inhibitor (EGFRi) induced rashes or healthy controls (Supplementary Figure S4C). Next, we assessed a dysregulation of AhR signaling and analyzed the endpoints of the canonical and non-canonical pathway of AhR signaling with two representatives: Prostaglandin-endoperoxide synthase 2 (COX2), and Cytochrome P450 (CYP) 1A1. *COX2* was significantly upregulated in VIRs as compared to healthy controls (Supplementary Figure S4B). *CYP1A1* gene expression was significantly downregulated in VIRs compared to healthy controls (Figure 4A). In line with these results CYP1A1 protein expression was found to be reduced in lesional skin (Figure 4B). Furthermore, we observed a downregulation of *CYP1A1* after stimulation with vemurafenib in skin explants, keratinocytes, and T cells *in vitro* (Figure 4C). Vemurafenib did not only reduce the basal *CYP1A1* expression but also it decreases it when skin explants have been stimulated with the AhR agonist FICZ (Figure 4C).

Comparing vemurafenib to dabrafenib in terms of T cell stimulation, we found that dabrafenib did not induce IFN-γ and IL-17 at gene or protein level in the pharmacological relevant levels up to 1 µM (Figure 4F/G). Furthermore, dabrafenib did not affect *CYP1A1* gene expression and IL-22 protein expression in $CD4^+$ T cells (Figure 4F/G).

4 Discussion

Despite its clinical efficacy, vemurafenib therapy is associated with a wide range of adverse events including arthralgia, fatigue, and skin toxicities [26]. Cutaneous side effects are predominant and occur in up to 70% of the patients [2]. Four different cutaneous reaction patterns have been described: i) inflammatory reactions, ii) hair/ nail changes, iii) melanocytic disorders/ proliferations, and iv) keratinocytic proliferations [27]. Proliferative alterations have been systematically analyzed and are contributed to a pharmacologic, paradoxical activation of the RAS-RAF-MEK-ERK signaling pathway [28]. This concept is supported by clinical observations showing that BRAFi-induced proliferative lesions regress under concurrent administration of MEK-inhibitors (MEKi) [29,30]. By contrast, co-administration of MEKi results in a slight increase in incidence and severity of inflammatory rashes (35% for vemurafenib plus placebo versus 39% for vemurafenib plus cobimetinib) [29]. These observations support the hypothesis that inflammatory rashes and proliferative alterations are caused by different mechanisms.

From a clinical perspective, VIRs present as maculopapular drug exanthemas, pointing towards a T cell-mediated hypersensitivity reaction [31]. Indeed, immunohistochemical analyses of lesional skin revealed a lymphohistiocytic infiltrate. However, we did not observe prominent infiltrates of eosinophils. Our analyses of cytokines revealed a T_H1-signature with an induction of dendritic cell (DC)-associated as well as T cell-derived inflammatory mediators, such as *TNF*, *IL1B*, and *IFNG*, and the T_H1-associated chemokines *CXCL9-11*. In addition, the increased expression of *CCL27* and *CXCL14* facilitates the recruitment of T cells as well as macrophages [32]. The upregulation of *IL1B* indicates an activation of

the inflammasome in macrophages and/or other cells of the innate immune system. Conversely, we did not detect a relevant induction of T_H2-cytokines, *IFNA* or T_H17-associated cytokines. To test our hypothesis that VIRs are caused rather by pharmacologic than allergic mechanisms, we performed LATs of blood samples obtained from patients with VIRs. Whereas stimulated T cells expressed IFN-γ RNA and protein, LATs failed to detect vemurafenib-specific T cells in our collective. To conclude, the negligible numbers of eosinophils, the absence of an allergy-associated T_H2 signature, and the absence of drug-specific T cells, indicate that VIRs are caused by non-allergic pharmacological reactions rather than a drug-specific sensitization. This hypothesis is further supported by an observed induction of chemokines, including *CCL2, CCL5, CCL27* and *CXCL14*, which also play a central role for the pathogenesis of non-allergic, pharmacologic, papulopustular rashes induced by EGFRi [25]. In contrast, non-immediate allergic drug reactions are associated with a T_H1 chemokine signature [33,34]. To assess whether the induction of inflammation is caused by a direct pharmacological effect of vemurafenib on skin or by indirect, systemic effects, skin explants were stimulated with vemurafenib *ex vivo*. In line with the cytokine-chemokine signature observed in patient samples *in vivo*, we found a marked induction of *TNFA* and *CCL5*. Respective *in vitro* analyses in human keratinocytes and T cells showed similar results, indicating that structural cells and immune cells are targets of vemurafenib and central regulators of vemurafenib-induced inflammation in the skin.

Although, vemurafenib and dabrafenib are both potent inhibitors of the BRAF-kinase, reported incidences for inflammatory rashes are significantly higher for vemurafenib (41% vs. 30%), suggesting that these rashes are rather not caused

by a class-specific effect of BRAFi but by another, so far unknown vemurafenib-specific mechanism [35]. A putative target for vemurafenib is the xenobiotic receptor AhR, which has been shown to be modulated by several other protein kinase inhibitors in an either agonistic or antagonistic manner [7,15,36-38]. Interestingly, AhR is highly expressed in skin and other barrier organs [5]. Upon binding of an agonistic ligand, AhR stimulates expression of a battery of genes in a tissue- and cell-specific fashion [5,24]. Activation of the canonical signaling pathway involves ligand-dependent translocation of AhR into the nucleus, where it forms a dimer with the AhR nuclear translocator (ARNT) resulting in the binding to an upstream regulatory region of respective target genes [5]. In addition, AhR activation leads to stimulation of non-canonical signaling pathways, which may trigger pro-inflammatory responses [5,39,40]. As recently postulated, canonical and non-canonical AhR signaling pathways are tightly balanced in healthy skin thereby contributing to skin homeostasis [41]. In fact, our analysis revealed a decreased number of *CYP1A1* transcripts (as marker for canonical AhR signaling) in vemurafenib-induced rashes, which was paralleled by elevated mRNA levels of *COX2* (as marker for non-canonical AhR signaling). Of note, a recent study using an imiquimod mouse model of psoriasiform dermatitis indicates that loss of AhR results in exaggerated cutaneous inflammation [42]. Accordingly, treatment of wild-type mice with AhR agonists attenuated psoriasiform skin inflammation [42]. AhR activation by coal tar was shown to reduce pro-inflammatory responses and restore epidermal barrier functions in a 3D *in vitro* model of atopic dermatitis [43]. Furthermore, inflammatory responses in lipopolysaccharide-challenged AhR-deficient mice are characterized by induction

of TNF-α, IL-1β, IL-6, IL-18 and IFN-γ [44], a cytokine pattern closely resembling the one observed in VIRs.

Di Meglio *et al.* identified that deficiency or antagonism of the non-hematopoietic skin compartment is necessary and sufficient for the development of skin inflammation [42]. Their results ruled out a central role for DCs or macrophages but did not exclude a possible involvement of T or B cells in AhR-mediated skin inflammation. In line with these results we did not observe significant effects of vemurafenib on DCs (not shown) but demonstrate that vemurafenib downregulates the AhR prototype target gene *CYP1A1* in keratinocytes and T cells *in vitro* as well as skin explants *ex vivo*. These findings are supported by the observation that *CYP1A1* gene expression was down-modulated in VIRs *in vivo*. By contrast, *CYP1A1* was not altered in EGFRi-associated rashes as compared to healthy donors. This indicates that VIRs are likely triggered by disturbance of canonical but not non-canonical AhR signaling that involves the c-Src/EGFR/ERK pathway [40]. Our hypothesis that vemurafenib acts as a potent AhR antagonist is further supported by reporter gene analyses, showing that vemurafenib treatment inhibited both basal as well as BaP-induced activity of the AhR-dependent luciferase construct. In fact, competitive ligand-binding assays and determination of DNA-bound AhR/ARNT protein provided evidence that vemurafenib is an antagonistic ligand of AhR. In contrast to a recent study on melanoma cells, reporting that vemurafenib treatment induces nuclear translocation of AhR (Corre et al. [45]), our data clearly show that the BRAF inhibitor retained AhR in the cytosol of solvent- as well as BaP-treated keratinocytes and thus behaves as a pure antagonist. The molecular mechanism responsible for this apparent cell-specific differences remains to be elucidated. As opposed to vemurafenib, dabrafenib did

not interfere with AhR and dabrafenib-treated patients develop cutaneous rashes at lower incidences [46,47]. Taking in account our hitherto results it is also not surprising that immunostimulatory immune-checkpoint inhibitors such as ipilimumab, nivolumab or pembrolizumab, can aggravate the severity of vemurafenib-induced rashes [3,4,48]. Up to 70% of patients treated with vemurafenib develop a drug eruption. The initial trigger remains unclear. Unknown factors such as infectious triggers, environmental /food related xenobiotics or an individual genetic predisposition may favor the spontaneous onset of the observed rashes.

Taken together, our results indicate that vemurafenib-induced inflammatory rashes are caused by a pharmacologic mechanism rather than a specific sensitization against the drug. Moreover, our results imply that this mechanism is not a class-specific, direct inhibition of BRAF, but rather a vemurafenib-specific inhibition of the AhR. Interestingly, Smith *et al.*, identified a naturally derived topical AhR agonist called tapinarof (GSK2894512) capable of inducing CYP1A1 expression together with an improvement of murine AhR-mediated imiquimod-induced psoriasiform skin lesions [49]. Against this background, we hypothesize that activation of AhR for instance via an AhR agonist-rich diet, (e.g. brassica vegetables containing indole-3-carbinol), or via topical application of non-toxic AhR ligands may represent a novel strategy to prevent or treat VIRs [50].

Acknowledgments

We thank Paul Chapman and Katja Schindler for providing clinical images and proofreading the manuscript and Katarina Gradin and Lorenz Poellinger for generously providing the XRE-HepG2 reporter cell line. The research by Stefan T. Arold and Afaque A. Momin was supported by funding from King Abdullah University of Science and Technology (KAUST). Mario E. Lacouture is funded in part through the NIH/NCI Cancer Center Support Grant P30 CA008748. Thomas Haarmann-Stemmann and Katharina M. Rolfes were supported by grants of the Deutsche Forschungsgemeinschaft (HA 7346/2-1) and the Jürgen Manchot Foundation.

Author contributions

H.C.H., A.K. and P.A.G. designed, performed and analyzed most of the experiments. M.P. helped in performing the luciferase assays and implemented the AhR translocation assay. K.M.R. conducted the EROD assays. A.A.S and M.S.D. designed and performed the AhR binding experiments. A.A.M and S.T.A. conducted the *in silico* computational modelling. A.D. performed flow cytometry experiments. S.A.B. did the immunohistochemistry experiments and helped with microscopy. P.O., M.E.L. and J.K. provided suggestions and discussions throughout the study. S.M. supervised the study and wrote the manuscript together with H.C.H., A.K., P.A.G., T.H. and B.H.

References

1. Chapman PB, Hauschild A, Robert C, et al. Improved survival with vemurafenib in melanoma with BRAF V600E mutation. *N Engl J Med.* 2011;364(26):2507-2516.
2. Manousaridis I, Mavridou S, Goerdt S, Leverkus M, Utikal J. Cutaneous side effects of inhibitors of the RAS/RAF/MEK/ERK signalling pathway and their management. *J Eur Acad Dermatol Venereol.* 2013;27(1):11-18.
3. Harding JJ, Pulitzer M, Chapman PB. Vemurafenib sensitivity skin reaction after ipilimumab. *N Engl J Med.* 2012;366(9):866-868.
4. Minor DR, Rodvien R, Kashani-Sabet M. Successful desensitization in a case of Stevens-Johnson syndrome due to vemurafenib. *Melanoma Res.* 2012;22(5):410-411.
5. Esser C, Rannug A. The aryl hydrocarbon receptor in barrier organ physiology, immunology, and toxicology. *Pharmacol Rev.* 2015;67(2):259-279.
6. Jin UH, Lee SO, Safe S. Aryl hydrocarbon receptor (AHR)-active pharmaceuticals are selective AHR modulators in MDA-MB-468 and BT474 breast cancer cells. *J Pharmacol Exp Ther.* 2012;343(2):333-341.
7. Zhao B, Degroot DE, Hayashi A, He G, Denison MS. CH223191 is a ligand-selective antagonist of the Ah (Dioxin) receptor. *Toxicol Sci.* 2010;117(2):393-403.
8. Denison MS, Rogers JM, Rushing SR, Jones CL, Tetangco SC, Heath-Pagliuso S. Analysis of the aryl hydrocarbon receptor (AhR) signal transduction pathway. *Curr Protoc Toxicol.* 2002;Chapter 4:Unit4 8.
9. da Rocha Dias S, Salmonson T, van Zwieten-Boot B, et al. The European Medicines Agency review of vemurafenib (Zelboraf(R)) for the treatment of adult patients with BRAF V600 mutation-positive unresectable or metastatic melanoma: summary of the scientific assessment of the Committee for Medicinal Products for Human Use. *Eur J Cancer.* 2013;49(7):1654-1661.
10. Yaktapour N, Meiss F, Mastroianni J, et al. BRAF inhibitor-associated ERK activation drives development of chronic lymphocytic leukemia. *J Clin Invest.* 2014;124(11):5074-5084.
11. Falchook GS, Long GV, Kurzrock R, et al. Dabrafenib in patients with melanoma, untreated brain metastases, and other solid tumours: a phase 1 dose-escalation trial. *Lancet (London, England).* 2012;379(9829):1893-1901.
12. Homey B, Wang W, Soto H, et al. Cutting edge: the orphan chemokine receptor G protein-coupled receptor-2 (GPR-2, CCR10) binds the skin-associated chemokine CCL27 (CTACK/ALP/ILC). *J Immunol.* 2000;164(7):3465-3470.
13. Beeler A, Zaccaria L, Kawabata T, Gerber BO, Pichler WJ. CD69 upregulation on T cells as an in vitro marker for delayed-type drug hypersensitivity. *Allergy.* 2008;63(2):181-188.
14. Abel J, Haarmann-Stemmann T. An introduction to the molecular basics of aryl hydrocarbon receptor biology. *Biological chemistry.* 2010;391(11):1235-1248.
15. Frauenstein K, Tigges J, Soshilov AA, et al. Activation of the aryl hydrocarbon receptor by the widely used Src family kinase inhibitor 4-amino-5-(4-chlorophenyl)-7-(dimethylethyl)pyrazolo[3,4-d]pyrimidine (PP2). *Archives of toxicology.* 2014.
16. Leclerc S, Garnier M, Hoessel R, et al. Indirubins inhibit glycogen synthase kinase-3 beta and CDK5/p25, two protein kinases involved in abnormal tau phosphorylation in Alzheimer's disease. A property common to most cyclin-dependent kinase inhibitors? *J Biol Chem.* 2001;276(1):251-260.
17. Marko D, Schatzle S, Friedel A, et al. Inhibition of cyclin-dependent kinase 1 (CDK1) by indirubin derivatives in human tumour cells. *Br J Cancer.* 2001;84(2):283-289.

18. Karoulia Z, Wu Y, Ahmed TA, et al. An Integrated Model of RAF Inhibitor Action Predicts Inhibitor Activity against Oncogenic BRAF Signaling. *Cancer Cell.* 2016;30(3):485-498.
19. Mapelli M, Massimiliano L, Crovace C, et al. Mechanism of CDK5/p25 binding by CDK inhibitors. *J Med Chem.* 2005;48(3):671-679.
20. Fujii-Kuriyama Y, Mimura J. Molecular mechanisms of AhR functions in the regulation of cytochrome P450 genes. *Biochem Biophys Res Commun.* 2005;338(1):311-317.
21. Gradin K, Wilhelmsson A, Poellinger L, Berghard A. Nonresponsiveness of normal human fibroblasts to dioxin correlates with the presence of a constitutive xenobiotic response element-binding factor. *J Biol Chem.* 1993;268(6):4061-4068.
22. Delord J-P, Robert C, Nyakas M, et al. Phase I Dose-Escalation and -Expansion Study of the BRAF Inhibitor Encorafenib (LGX818) in Metastatic BRAF-Mutant Melanoma. *Clinical Cancer Research.* 2017;23(18):5339.
23. Mai H, Huang J, Zhang Y, et al. In-vivo relation between plasma concentration of sorafenib and its safety in Chinese patients with metastatic renal cell carcinoma: a single-center clinical study. *Oncotarget.* 2017;8(26):43458-43469.
24. Denison MS, Soshilov AA, He G, DeGroot DE, Zhao B. Exactly the same but different: promiscuity and diversity in the molecular mechanisms of action of the aryl hydrocarbon (dioxin) receptor. *Toxicol Sci.* 2011;124(1):1-22.
25. Lichtenberger BM, Gerber PA, Holcmann M, et al. Epidermal EGFR controls cutaneous host defense and prevents inflammation. *Sci Transl Med.* 2013;5(199):199ra111.
26. Sosman JA, Kim KB, Schuchter L, et al. Survival in BRAF V600-mutant advanced melanoma treated with vemurafenib. *N Engl J Med.* 2012;366(8):707-714.
27. Rinderknecht JD, Goldinger SM, Rozati S, et al. RASopathic skin eruptions during vemurafenib therapy. *PLoS One.* 2013;8(3):e58721.
28. Hatzivassiliou G, Song K, Yen I, et al. RAF inhibitors prime wild-type RAF to activate the MAPK pathway and enhance growth. *Nature.* 2010;464(7287):431-435.
29. Larkin J, Ascierto PA, Dreno B, et al. Combined vemurafenib and cobimetinib in BRAF-mutated melanoma. *N Engl J Med.* 2014;371(20):1867-1876.
30. Chen FW, Tseng D, Reddy S, Daud AI, Swetter SM. Involution of eruptive melanocytic nevi on combination BRAF and MEK inhibitor therapy. *JAMA Dermatol.* 2014;150(11):1209-1212.
31. Hoetzenecker W, Nageli M, Mehra ET, et al. Adverse cutaneous drug eruptions: current understanding. *Semin Immunopathol.* 2016;38(1):75-86.
32. David BA, Kubes P. Exploring the complex role of chemokines and chemoattractants in vivo on leukocyte dynamics. *Immunological reviews.* 2019;289(1):9-30.
33. Fernandez TD, Mayorga C, Torres MJ, et al. Cytokine and chemokine expression in the skin from patients with maculopapular exanthema to drugs. *Allergy.* 2008;63(6):712-719.
34. Meller S, Gerber PA, Kislat A, et al. Allergic sensitization to pegylated interferon-alpha results in drug eruptions. *Allergy.* 2015;70(7):775-783.
35. Welsh SJ, Corrie PG. Management of BRAF and MEK inhibitor toxicities in patients with metastatic melanoma. *Ther Adv Med Oncol.* 2015;7(2):122-136.
36. Korashy HM, Anwar-Mohamed A, Soshilov AA, Denison MS, El-Kadi AO. The p38 MAPK inhibitor SB203580 induces cytochrome P450 1A1 gene expression in murine and human hepatoma cell lines through ligand-dependent aryl hydrocarbon receptor activation. *Chem Res Toxicol.* 2011;24(9):1540-1548.
37. Reiners JJ, Jr., Lee JY, Clift RE, Dudley DT, Myrand SP. PD98059 is an equipotent antagonist of the aryl hydrocarbon receptor and inhibitor of mitogen-activated protein kinase kinase. *Mol Pharmacol.* 1998;53(3):438-445.

38. Mezrich JD, Nguyen LP, Kennedy G, et al. SU5416, a VEGF receptor inhibitor and ligand of the AHR, represents a new alternative for immunomodulation. *PLoS One.* 2012;7(9):e44547.
39. Matsumura F. The significance of the nongenomic pathway in mediating inflammatory signaling of the dioxin-activated Ah receptor to cause toxic effects. *Biochem Pharmacol.* 2009;77(4):608-626.
40. Haarmann-Stemmann T, Bothe H, Abel J. Growth factors, cytokines and their receptors as downstream targets of arylhydrocarbon receptor (AhR) signaling pathways. *Biochem Pharmacol.* 2009;77(4):508-520.
41. Haarmann-Stemmann T, Esser C, Krutmann J. The Janus-Faced Role of Aryl Hydrocarbon Receptor Signaling in the Skin: Consequences for Prevention and Treatment of Skin Disorders. *J Invest Dermatol.* 2015;135(11):2572-2576.
42. Di Meglio P, Duarte JH, Ahlfors H, et al. Activation of the aryl hydrocarbon receptor dampens the severity of inflammatory skin conditions. *Immunity.* 2014;40(6):989-1001.
43. van den Bogaard EH, Bergboer JG, Vonk-Bergers M, et al. Coal tar induces AHR-dependent skin barrier repair in atopic dermatitis. *J Clin Invest.* 2013;123(2):917-927.
44. Sekine H, Mimura J, Oshima M, et al. Hypersensitivity of aryl hydrocarbon receptor-deficient mice to lipopolysaccharide-induced septic shock. *Molecular and cellular biology.* 2009;29(24):6391-6400.
45. Corre S, Tardif N, Mouchet N, et al. Sustained activation of the Aryl hydrocarbon Receptor transcription factor promotes resistance to BRAF-inhibitors in melanoma. *Nature communications.* 2018;9(1):4775.
46. Hauschild A, Grob JJ, Demidov LV, et al. Dabrafenib in BRAF-mutated metastatic melanoma: a multicentre, open-label, phase 3 randomised controlled trial. *Lancet.* 2012;380(9839):358-365.
47. Sanlorenzo M, Choudhry A, Vujic I, et al. Comparative profile of cutaneous adverse events: BRAF/MEK inhibitor combination therapy versus BRAF monotherapy in melanoma. *J Am Acad Dermatol.* 2014;71(6):1102-1109 e1101.
48. Imafuku K, Yoshino K, Ymaguchi K, Tsuboi S, Ohara K, Hata H. Nivolumab therapy before vemurafenib administration induces a severe skin rash. *J Eur Acad Dermatol Venereol.* 2016.
49. Smith SH, Jayawickreme C, Rickard DJ, et al. Tapinarof Is a Natural AhR Agonist that Resolves Skin Inflammation in Mice and Humans. *J Invest Dermatol.* 2017;137(10):2110-2119.
50. Benson JM, Shepherd DM. Dietary ligands of the aryl hydrocarbon receptor induce anti-inflammatory and immunoregulatory effects on murine dendritic cells. *Toxicol Sci.* 2011;124(2):327-338.

Figures

Figure 1

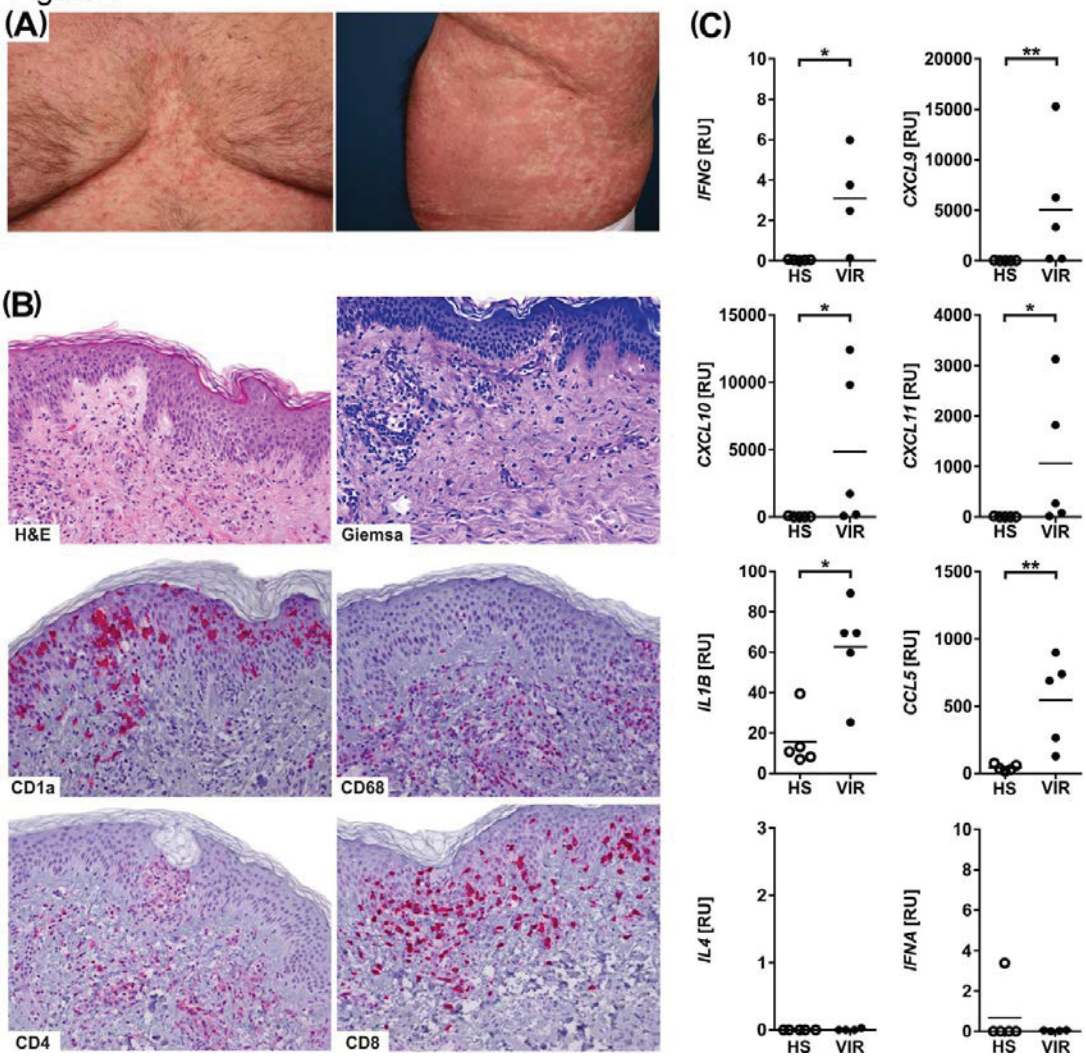

Figure 1: **Clinical, histologic and molecular characterization of vemurafenib-induced skin rashes.** (A), Representative patient with generalized maculopapular rash. (B), Hematoxylin and eosin (HE) stain, Giemsa stain and immunohistochemical analysis of CD1a, CD68, CD4 and CD8 in lesional skin of one representative patient. (C), semi-quantitative PCR analysis of cytokine and chemokine expression in healthy skin (HS, n=5) compared to lesional skin of vemurafenib-induced rashes (VIR, n=4-5). qPCR-values are shown as relative units compared to 18S rRNA expression. Data are presented as single values and median. Mann-Whitney U test was used to evaluate significant differences (*p<0.05, **p<0.01).

Figure 2

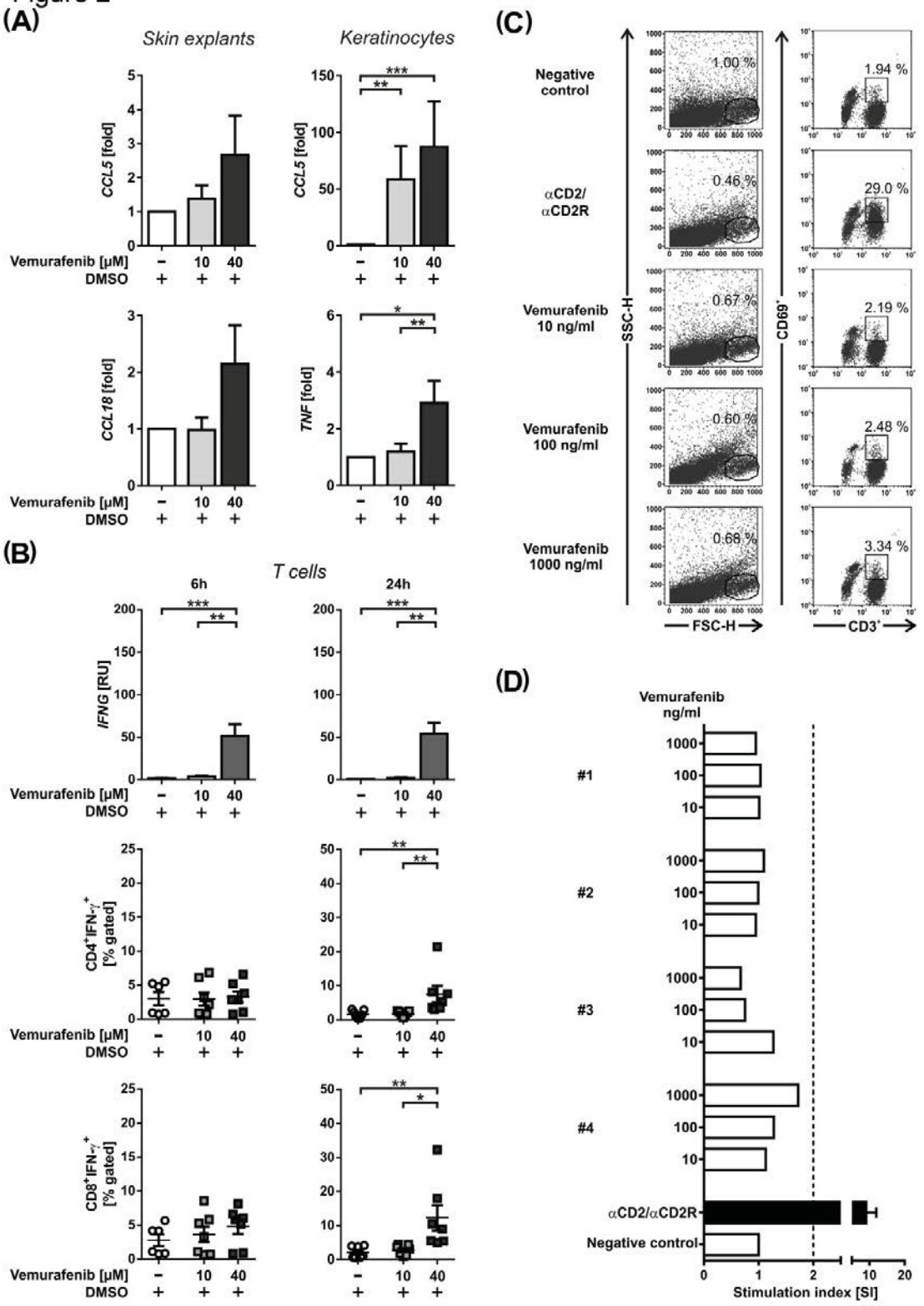
(A)
Skin explants
Keratinocytes
CCL5 [fold]
CCL18 [fold]
TNF [fold]
Vemurafenib [µM]
DMSO
(B)
T cells
6h
24h
IFNG [RU]
CD4+IFN-γ+ [% gated]
CD8+IFN-γ+ [% gated]
(C)
Negative control
αCD2/ αCD2R
Vemurafenib 10 ng/ml
Vemurafenib 100 ng/ml
Vemurafenib 1000 ng/ml
1.00 %
0.46 %
0.67 %
0.60 %
0.68 %
1.94 %
29.0 %
2.19 %
2.48 %
3.34 %
SSC-H
FSC-H
CD69+
CD3+
(D)
Vemurafenib ng/ml
#1
#2
#3
#4
αCD2/αCD2R
Negative control
Stimulation index [SI]

Figure 2: **Vemurafenib induces cytokines and chemokines in skin explants, keratinocytes and T cells, whereas it does not sensitize T cells.** (A, B), Skin explants (n=6), keratinocytes (n=14-15) and total T cells (n=9-14) were treated with vemurafenib [10; 40 µM]. qPCR-values are shown as mean + SEM of fold change normalized to 18S rRNA expression compared to DMSO. (B), IFN-γ expression of $CD4^+$/ $CD8^+$ T cells (n=7) after treatment, represented as single values and mean. (C, D), Analysis of $CD69^+CD3^+$ lymphocyte activation after incubation with vemurafenib (one representative patient). Stimulation indexes (SI) were calculated as fold-increase of the CD69 upregulation after vemurafenib stimulation of all four patients compared to control. Kruskal-Wallis test with Dunn's post correction was used to evaluate significances (*$p<0.05$, **$p<0.01$, ***$p<0.001$).

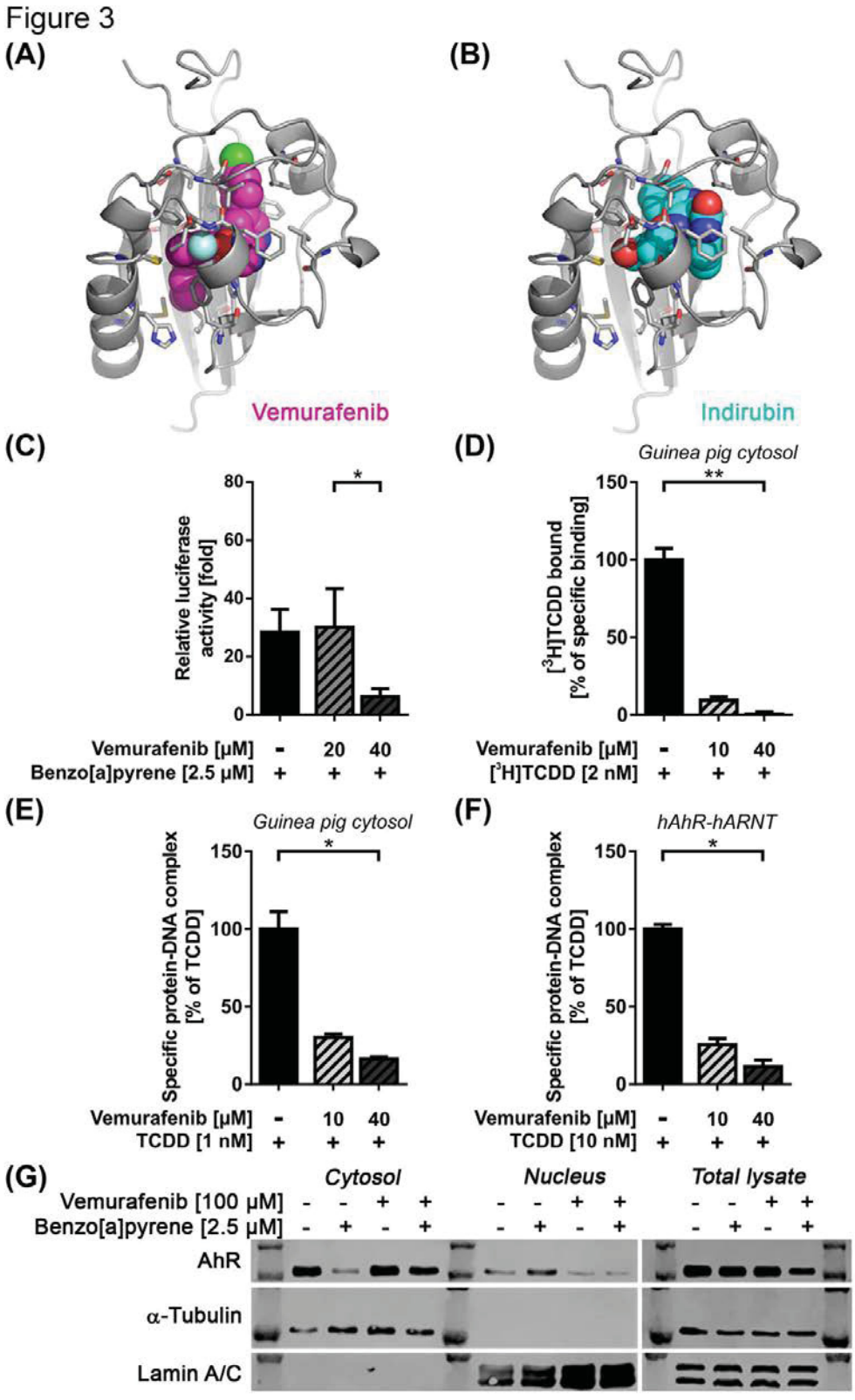

Figure 3: **Vemurafenib is an AhR antagonist.** (A, B), *In silico* docking of vemurafenib and indirubin to PAS-B homology models (grey). Best docking poses for are shown for vemurafenib (A, van-der-Waals sphere representation

with carbons colored in magenta) and indirubin (B, carbons in cyan). Residues of PAS-B forming the cavity are highlighted as stick models. (C), Reporter gene analyses using a XRE-HepG2 reporter cell line treated with vemurafenib [20; 40 µM] combined with benzo[a]pyrene [2.5 µM]. Luciferase activities were offset to protein concentration and normalized to DMSO. (D), Ligand binding was analyzed in guinea pig cytosol, using 2 nM [^{3}H]TCDD in combination with vemurafenib [10; 40 µM]. DNA binding was assayed in guinea pig cytosol (E) or using *in vitro* synthesized AhR/ARNT complexes (F) with TCDD combined with vemurafenib [10; 40 µM]. Values are normalized to [^{3}H]TCDD (D) or TCDD (E/F). Kruskal-Wallis test with Dunn's post correction was used to evaluate differences (*$p<0.05$, **$p<0.01$). (G), AhR translocation into the HaCaT cell nucleus after 2h treatment with vemurafenib [100 µM] or in combination with benzo[a]pyrene [2.5 µM]. One representative plot of two experiments is displayed showing cytosolic and nucleus fractions together with the total lysate and the controls α-tubulin and lamin A/C.

Figure 4

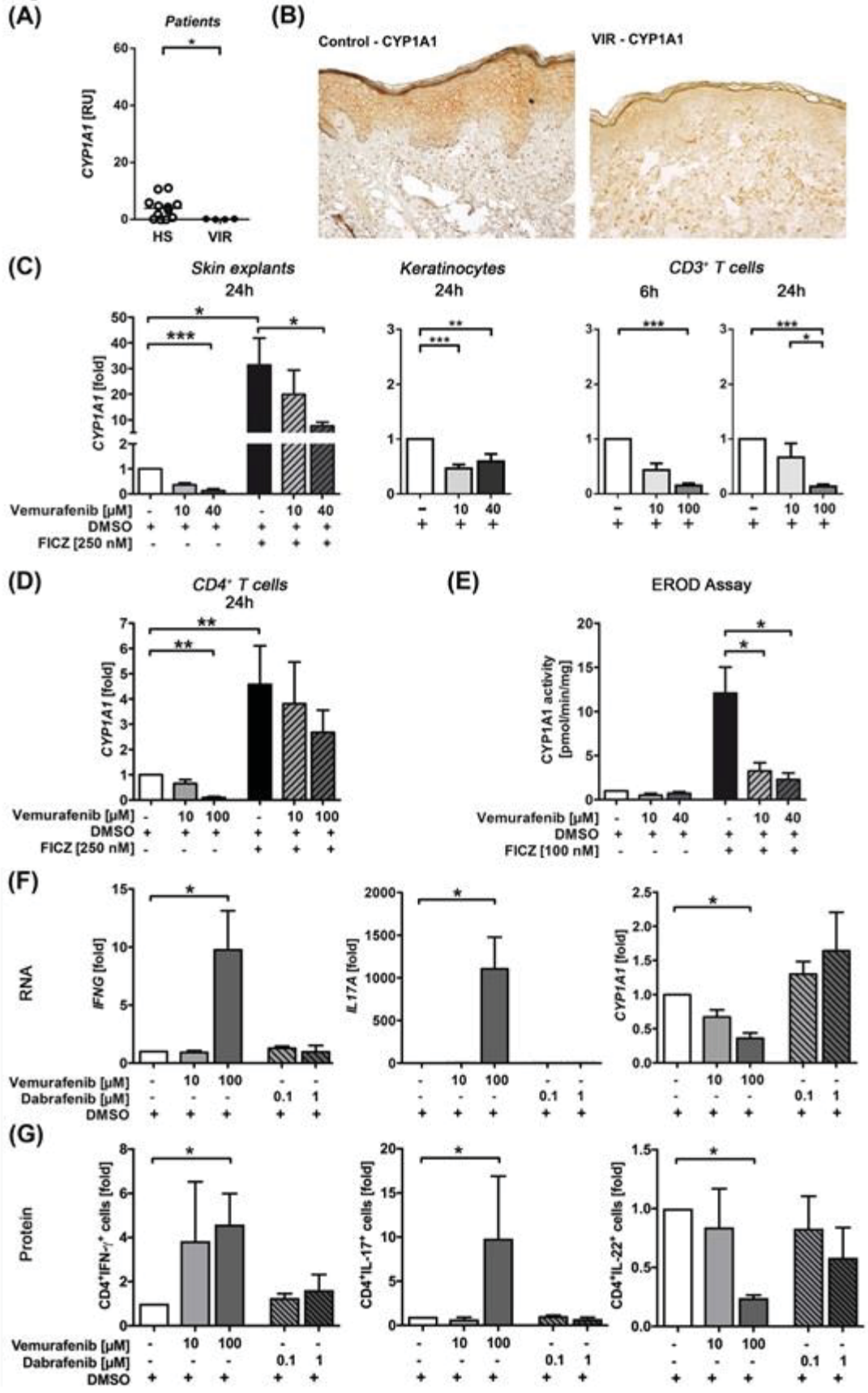
(A)
Patients
CYP1A1 [RU]
HS
VIR
(B)
Control - CYP1A1
VIR - CYP1A1
(C)
Skin explants
24h
Keratinocytes
24h
CD3+ T cells
6h
24h
CYP1A1 [fold]
Vemurafenib [µM]
DMSO
FICZ [250 nM]
(D)
CD4+ T cells
24h
CYP1A1 [fold]
Vemurafenib [µM]
DMSO
FICZ [250 nM]
(E)
EROD Assay
CYP1A1 activity [pmol/min/mg]
Vemurafenib [µM]
DMSO
FICZ [100 nM]
(F)
RNA
IFNG [fold]
IL17A [fold]
CYP1A1 [fold]
Vemurafenib [µM]
Dabrafenib [µM]
DMSO
(G)
Protein
CD4+IFN-γ+ cells [fold]
CD4+IL-17+ cells [fold]
CD4+IL-22+ cells [fold]
Vemurafenib [µM]
Dabrafenib [µM]
DMSO

Figure 4: **CYP1A1 expression is impaired by vemurafenib *in vitro* and *in vivo*.** Expression of CYP1A1 mRNA in lesional skin (VIR, n=4-5) compared to healthy controls (HS, n=11) (A), representative immunohistochemistry analysis of CYP1A1 protein in lesional skin versus control (B) and *CYP1A1* mRNA expression (C) in primary keratinocytes (n=14-15), skin explants (n=6), and $CD3^+$ T cells (n=9-15). (D) *CYP1A1* expression in purified $CD4^+$ T cells treated with vemurafenib alone or in combination with FICZ for 24h (n=5). qPCR-values are shown as fold change relative to 18S rRNA expression and compared to normalized DMSO control. (E) Ethoxyresorufin-O-deethylase (EROD) Assay on primary keratinocytes to detect CYP1A1 activity in presence of vemurafenib (n=4). CYP1A1 activity has been adjusted to protein concentration and normalized to DMSO. (F), $CD4^+$ T cells (n=4) were treated with vemurafenib [10; 100 µM] or dabrafenib [0.1; 1 µM]. *IFNG*, *IL17A* and *CYP1A1* gene expression values are shown as fold change normalized to 18S rRNA expression compared to DMSO. (G), IFN-γ, IL-17 and IL-22 protein expression of $CD4^+$ T cells (n=4) as fold change to DMSO after treatment with vemurafenib or dabrafenib. Data is presented as mean + SEM. Kruskal-Wallis test with Dunn's post correction and Mann-Whitney U test was used to evaluate significant differences (*$p<0.05$, **$p<0.01$, ***$p<0.001$).

3 Diskussion

Mehr als fünf Millionen Fälle von Hautkrebs im Jahr 2012 in den USA (Rogers et al. 2015) und eine Erhöhung der hautkrebsbedingten Krankenhausaufenthalte in Deutschland um 17% (Statistisches Bundesamt 2017) rücken das Bewusstsein für eine verbesserte Vorsorge und Behandlung von karzinogenen Hauterkrankungen, immer stärker in den Fokus. Expertenprognosen gehen von weiter steigenden Fallzahlen aus. Gründe hierfür sind beispielsweise kostengünstige Reisen in Regionen mit einem durchschnittlich höheren UV-Index sowie die Nutzung von artifizieller Strahlung in Solarien. Aber auch der Klimawandel wird in den kommenden Jahren einen Beitrag zu erhöhten Hautkrebsinzidenzen leisten. Durch intensivere Sommer mit einer wesentlich geringeren Wolkendecke steigt der UV-Index auch in vormals gemäßigten Breitengraden und damit das Risiko an Hautkrebs zu erkranken (Matthews-Trigg et al. 2019).

Dabei wird formal zwischen weißem Hautkrebs, der aus Zellen des Epithelgewebes resultiert, und schwarzem Hautkrebs, der seinen Ursprung in entarteten Melanozyten hat, unterschieden (Board, PDQ Adult Treatment Editorial 2018). Diese Dissertation hat das Ziel AHR abhängige Entstehung und Entwicklung von kutanen Plattenepithelkarzinomen (SCCs) genauer zu beschreiben, um einen Beitrag zur verbesserten Prävention und Therapie zu leisten. Des Weiteren wurden hautassoziierte Nebenwirkungen bei der Behandlung des malignen Melanoms mit Vemurafenib auf Einfluss des AHR untersucht. Dabei legen unsere Studien nahe, dass die Stimulation des AHR einen Beitrag zur nebenwirkungsärmeren Therapie dieser Erkrankung leisten kann.

Die in dieser Arbeit dargestellten Ergebnisse zeigen, dass der AHR eine destabilisierende Wirkung auf das Tumorsuppressorprotein p27 in humanen Keratinozyten und der Haut von SKH1-Mäusen hat. Eine chemische Inhibition des AHR im Anschluss an eine UVB-Bestrahlung von KC verstärkt die Reparatur von CPDs um ca. 30%. Diese DNA Läsionen sind der Hauptgrund für die Mutation von sonnenlicht-geschädigten Hautzellen.

Die ebenfalls durch UV-Strahlung induzierten 6-4 Photoprodukte entstehen in einem geringeren Maße als CPDs und können von der Zelle schneller repariert werden (Meador et al. 2000). Mäuse mit einem ausgeschalteten AHR weisen 48h nach der Bestrahlung

50% weniger CPDs und wiesen in unserer chronischen UVB-Bestrahlungsstudie 50% weniger Hauttumore auf.

Chemische Inhibition der Signalkaskade zwischen AHR und Proteinkinase-B / AKT vermittelter p27 Phosphorylierung zeigte einen kongruenten Effekt auf die CPD Reparatur in KC wie der AHR-Inhibitor MNF. Wir konnten zudem zeigen, dass es sich bei dem vom AHR beeinflussten Reparaturmechanismus um die Globale Genom Reparatur (GGR) handelt. Die Proteinmenge von CSB, XPC oder XPA wurde mittels siRNA in den Keratinozyten herabgesetzt. Im Gegensatz zu XPC hatte die Inhibierung von CSB keinen Einfluss auf die MNF vermittelte verbesserte Reparatur von CPDs, was für eine Modulation der GGR, nicht aber der TCR (Transkriptions gekoppelte Reparatur) spricht. Diese Befunde werden durch Forschungsdaten gestärkt, wonach XP Patienten mit defizienter GGR an erhöhter Bildung von SCCs leiden (DiGiovanna und Kraemer 2012). Patienten mit Cockayne Syndrom und einer geschädigten TCR zeigen hingegen keine Agglomeration von Mutationen und keine verstärkte Bildung von Hauttumoren (Reid-Bayliss et al. 2016). Es ist denkbar, dass die GGR in diesen Zellen auch die geschädigten Bereiche des zu transkribierenden Genoms repariert und so die Bildung von SCCs verhindert.

Unsere Daten bekräftigen darüber hinaus die Bedeutung des Proteinlevels von p27 in der Zelle. Die genaue Wechselwirkung zwischen p27 und der GGR konnte indes nicht aufgezeigt werden. Allerdings legen unsere Ergebnisse dar, dass der Tumorsuppressor zum Zeitpunkt der gesteigerten Reparatur keinen Einfluss auf den Zellzyklus hat. Die Interaktion von p27 mit CDK2 scheint somit keine Relevanz für die verbesserte NER zu haben.

Interessanterweise führt die Inhibierung von CDK7 (Cyklin anhängige Kinase 7) zur identischen Einflussnahme auf die NER wie die Überexpression von p27. CDK7 ist Teil des CAK (Cyclin aktivierender Komplex), der sich nach Bestrahlung von TFIIH löst, was einen entscheidenden Vorgang für die DNA-Reparatur darstellt. Es konnte gezeigt werden, dass eine chemische Inhibierung von CDK7 zu einer verbesserten Reparatur in Fibroblasten führt (Coin et al. 2008).

Kommende Studien müssen aufschlüsseln, ob es zu einer Interaktion von p27 und CDK7 in Keratinozyten nach UV-Exposition kommt. Diese scheint zellspezifisch zu sein. So konnten Kato et al. 1994 keine Interaktion der beiden Proteine in Makrophagen zeigen, Studien in Krebszellen der Lunge wiesen jedoch eine Bindung von p27 und CDK7 nach (Haines et al. 2018).

Unsere Daten demonstrieren zudem, dass eine reduzierte AHR-Aktivität 24 Stunden nach UVB Exposition zu mehr Doppelstrangbrüchen (DSB) und einer erhöhten Eliminierung von UV-geschädigten Keratinozyten mittels Apoptose führt. Dass eine verbesserte Reparatur von CPDs mit einer reduzierten Apoptoserate einhergeht, wie 1999 von Kulms et al. beschrieben, konnten wir somit nicht zeigen. Die Inhibierung des AHR und damit einhergehende Reduktion der Checkpoint Kinase 1 (CHK1) scheint der Grund hierfür zu sein (Frauenstein et al. 2013). CHK1 ist ein wichtiges Protein zur Initiation des Zellzyklusarrests nach einer Schädigung der DNA und um diese über die Aktivierung der Homologen Rekombinationsreparatur (HRR) zu beseitigen (Sørensen et al. 2005). Gelingt dies aufgrund eines verringerten Proteinlevels an CHK1 nicht, kann ein Verbleib von CPDs in der DNA zu Problemen bei der Replikation führen. Nach dem Entwinden des Doppelstrangs wird dieser in zwei Einzelstränge mittels des Enzyms Helikase gespalten. CPDs stören diesen Prozess und können zu einem Bruch des Doppelstrangs (DSB) führen (Ikehata und Ono 2011). DSB führen zur Aktivierung der Serin-Proteinkinase ATM welche über das Tumorsuppressorprotein p53 den intrinsischen apoptotischen Signalweg (siehe Abbildung 4 auf Seite 10) induzieren (Roos und Kaina 2006). Zudem zeigen vorherige Arbeiten, dass der AHR einen positiven Einfluss auf die HRR besitzt (Chan et al. 2004). Weitere Untersuchungen müssen zeigen, ob eine Inhibierung des AHR einen negativen Einfluss auf die HRR zur Folge hat.

Des Weiteren muss gezeigt werden, ob eine erhöhte Apoptose die Mutageneserate in bestrahlten KC herabsetzt und einen Schutzmechanismus zur Folge hat. Eine erhöhte epidermale Apoptose durch die topische Behandlung mit Koffein oder Resveratrol sind indes schon positiv auf die Prävention von Hauttumore in Mäusen getestet worden (Aziz et al. 2005; Conney et al. 2013).

Ein weiterer wichtiger Punkt ist der genaue Mechanismus zwischen einer Aktivierung des AHR und dem EGFR. Eine Aktivierung des EGFR durch Phosphorylierung über die im AHR-Multiproteinkomplex gebundene Tyrosinkinase c-Src gilt als wahrscheinlich (Enan und Matsumura 1996). Eine Stimulation des EGFR über die Aktivierung des AHR konnte auch von Xie et al. 2012 in Epithelzellen des Darms nachgewiesen werden. Sie zeigen, dass Src nach der Stimulation der Zellen mit TCDD phosphoryliert wird und MMP7 aktiviert. MMP7 katalysiert die Aktivierung von HBEGF, einem Liganden des EGFR. Auch die Expression der beiden EGFR-Liganden Amphiregulin (AREG) und Epiregulin (EREG) ist AHR abhängig (John et al. 2014). So führt die Aktivierung des AHR mittels TCDD zu erhöhtem Expressionslevel der mRNA. Die Sekretion beider

Liganden kann mit dem AHR Inhibitor CH223191 reduziert werden. Diese Daten prognostizieren einen anderen möglichen Signalweg zur Aktivierung des EGFR. Zur zielgerichteten Beeinflussung der Signalkaskade muss dieser noch genauer analysiert werden.

Ein wichtiger zu diskutierender Punkt ist der biologische Nutzen einer Inhibition des AHR. Vorangegangene Studien zeigen, dass durch UV-Strahlung induzierte Immunsuppression akute Schübe von Hauterkrankungen wie atopischer Dermatitis (AD) gelindert werden können (Valkova und Velkova 2004). Eine identische Wirkung zeigt die Behandlung von AD Patienten mit AHR-ligandenreichem Kohlenteer (van den Bogaard et al. 2013). Die immunsuppressive Wirkung von UV-Strahlung ist in der Haut von C57BL/6 Mäusen zu einem Großteil AHR vermittelt (Navid et al. 2013). Eine Therapie mit UV-Strahlung birgt immer das Risiko der Induktion von DNA Schäden, eine gezielte Aktivierung des AHR mittels Liganden könnte hierbei eine risikoärmere Therapie von Patienten mit atopischer Dermatitis darstellen (Bruhs et al. 2015). Solche positiven Effekte des AHR sollten bei kommenden Arbeiten nicht außer Acht gelassen werden.

Des Weiteren sollten künftige Arbeiten auf Bestrahlungen mit dem gesamten Spektrum der Sonne basieren. Erste Studien zeigen, dass das AHR Zielgen CYP1A1 für den Abbau des Photoprodukts FICZ von entscheidender Bedeutung ist und eine Akkumulation des Stoffs bei einer Bestrahlung mit UVA zu gesteigertem oxidativem Stress in der Zelle führt (Park et al. 2015). Wie vorrangig beschrieben führt oxidativer Stress zur Schädigung der Zelle und resultiert in Veränderungen der DNA und Apoptose. Eine Inhibierung des AHR könnte also negative Folgen bei einer Bestrahlung im UVA-Wellenlängenbereich haben.

Auch das sichtbare Licht der Sonne (380 – 780 nm) führt nachweislich zu einer gesteigerten Entstehung von reaktiven Sauerstoffspezies (Lawrence et al. 2018) und sollte bei der Interpretation der aktuellen Daten berücksichtigt werden. Die Kombination aus sichtbarem Licht und naher Infrarot-Strahlung resultiert in einer erhöhten Expression von Matrix Metalloproteasen 1 und 9 (MMP), die einen entscheidenden Einfluss auf den Alterungsprozess der Haut haben (Cho et al. 2008).

Eine verstärkte Expression von MMP-1 konnte auch in Hautproben detektiert werden, die nur mit IR-A Strahlen exponiert wurden (Grether-Beck et al. 2015). Diese Daten machen deutlich, dass die Betrachtung des gesamten polychromatischen Spektrums der Sonne in zukünftigen Projekten entscheidend, auf dem Weg zu einer gesteigerten biologischen Relevanz der Ergebnisse, ist.

Einen ebenso wichtigen Faktor stellt die Verwendung chemischer Inhibitoren strahlungsabhängiger Signalkaskaden dar. Ungeachtet dessen, ob diese in der Therapie oder der Forschung zum Einsatz kommen. Meurer und Weiskirchen zeigen in ihrer Arbeit auf, dass die Verwendung von MAPK-Inhibitoren zu unerwünschten Nebenwirkungen führen kann. Als *spezifisch* bezeichnete MAPK-Inhibitoren wirken als Aktivatoren auf andere Vertreter der MAPK Signalkaskade. Dies hat fundamentale Auswirkungen auf die Interpretation von physiologischen Endpunkten. So führt die Blockade von p38 durch den vermeintlich spezifischen Inhibitor SB203580 zur Phosphorylierung von ERK1/2 und JNK (Meurer und Weiskirchen 2018).

In ihrer Veröffentlichung bezeichnen die beiden Wissenschaftler diesen Effekt als *„Aktivierung durch Inhibierung“*. Diese Beschreibung passt ebenfalls, um den Einfluss einiger Proteinkinase-Inhibitoren auf den AHR zu veranschaulichen. Die von Meurer und Weiskirchen getesteten MAPK-Inhibitoren SB203580 (Korashy et al. 2011), U0126 (Andrieux et al. 2004), PD98059 (Reiners 1999) und SP600125 (Dvorak et al. 2008) modulieren die Aktivität des AHR und die AHR-abhängige Genexpression. Wie beschrieben ist der AHR nicht nur als Transkriptionsfaktor wichtig, sondern wird auch mit der Aktivierung des EGFR und der NF-ĸB Signalkaskade in Verbindung gebracht (Tian et al. 1999; Vogel et al. 2013), Signalwege die Zellproliferation, Apoptose oder auch die Immunreaktionen der Zelle lenken. Unser Kommentar zu Meurer und Weiskirchens Veröffentlichung beschreibt den zusätzlichen Einfluss verschiedener PKIs auf den AHR, um ein Bewusstsein für die mögliche Problematik bei der Interpretation von Forschungsergebnissen zu schaffen, die mit diesen chemischen Komponenten generiert wurden.

Ein in der Therapie von inoperablem malignem Melanom eingesetzter BRAF-Inhibitor Vemurafenib steht ebenfalls im Verdacht die AHR-Aktivität modulieren zu können. Das 2011 zugelassene Arzneimittel führt zu einer signifikant höheren Lebenserwartung von Patienten mit malignen Melanom als der zuvor eingesetzte Wirkstoff Dacarbazine (Chapman et al. 2011). Allerdings zeigen behandelte Patienten in 93% der Fälle hautassoziierten Nebenwirkungen. Eine Therapie mit Vemurafenib geht meist mit inflammatorischen Reaktionen einher. Zudem bilden ca. 18% der Patienten kutane Plattenepithelkarzinome aus (Chapman et al. 2011).

Anschließende Studien konnten zeigen, dass Vemurafenib zu einer erhöhten Aktivität des RAS Proteins führt (Yadav et al. 2012). Patienten, die mit dem BRAF Inhibitor behandelt wurden, wiesen verstärkte RAS Mutationen im Gewebe von SCCs auf (Su et

al. 2012). Um die Bildung von SCCs zu verringern und die Lebenserwartung von Patienten weiter zu erhöhen, wird Vemurafenib in Kombination mit dem MEK Inhibitor Cobimetinib verabreicht (Larkin et al. 2014).

Dementgegen konnten keine größeren Erfolge bei der Reduktion der Vemurafenib induzierten Hautausschläge (VIR) verzeichnet werden. Bei einer zu starken inflammatorische Reaktion der Haut muss die Medikamentendosis reduziert oder die Behandlung vollständig ausgesetzt werden. Harding et al. zeigten 2012, dass eine Vorbehandlung mit dem pro-inflammatorischen und ebenfalls zur Therapie von Melanomen eingesetzten Wirkstoff Ipilimumab zu einer erhöhten Ausbildung von VIR führt. In unserer Veröffentlichung zeigen wir, dass Vemurafenib die Aktivität des AHR in Keratinozyten als auch in T-Zellen moduliert. Die Expression des AHR Zielgens CYP1A1 war in beiden Zelltypen sowie in der Haut von Patienten, die mit Vemurafenib behandelt wurden, reduziert. Reportergenanalysen in HepG2 Zellen mit einem stabil transfizierten Luziferasegen und einer XRE reichen Promotorregion zeigten eine verringerte Luziferaseaktivität bei einer Ko-Behandlung von BaP und Vemurafenib verglichen mit der BaP behandelten Kontrolle. Diese Befunde konnten im AHR Liganden und DNA-Bindungsassay bestätigt werden. Unsere Daten zeigen deutlich, dass Vemurafenib den kanonischen Weg des AHR inhibieren kann.

Interessanterweise konnten wir erhöhte COX-2 Expression in Patientengewebe mit VIR als auch in Vemurafenib behandelten Keratinozyten nachweisen. Die Aktivierung des nicht kanonischen Signalwegs des AHR führt zu einer erhöhten COX-2 Expression (Köhle et al. 1999). Es kann nicht ausgeschlossen werden, dass Vemurafenib lediglich den kanonischen Signalweg selektiv inhibiert. Eine Vielzahl von Studien legen dar, dass ein gestörtes Gleichgewicht zwischen kanonischem und nicht-kanonischem AHR Signalweg zu Problemen der Homöostase der Haut führt (Haarmann-Stemmann et al. 2015). Eine temporäre Inhibierung des AHR reduziert die Schäden bei Exposition mit Umwelttoxinen wie PAHs oder UV-Strahlung. Eine Aktivierung des AHR wirkt sich wie zuvor beschrieben positiv auf die Behandlung von atopischer Dermatitis und Psoriasis aus (van den Bogaard et al. 2013; Di Meglio et al. 2014).

In unserer Veröffentlichung zeigen immunhistochemische Färbungen in Geweben mit VIR keine gesteigerte Invasion von Eosinophilen, Neutrophilen oder Mastzellen. Dies deutet darauf hin, dass keine allergische Reaktion der Haut vorliegt, die Hautausschläge also auf pharmakologische Reaktionen zurückzuführen sind. So konnten wir eine erhöhte Expression von IL-1β, TNF-α und INF-γ in der Haut mit VIR detektieren. Ein

Expressionsmuster, das sich auch in AHR-defizienten Mäusen finden lässt, wenn diese mit Lipopolysacchariden behandelt werden (Sekine et al. 2009).

Kürzlich veröffentlichte Daten bestätigen diesen Befund. Vemurafenib behandelte MCF7 Zellen weisen ebenfalls ein verändertes Expressionsmuster auf. Corre et al. gehen jedoch davon aus, dass Vemurafenib den AHR aktiviert und dieser als Transkriptionsfaktor im Zellkern aktiv ist. Dabei scheint es sich um einen zellspezifischen Effekt zu handeln. Von uns durchgeführte Proteinfraktionierungsversuche mit Vemurafenib behandelten Keratinozyten konnten diesen Befund nicht reproduzieren, sondern zeigten deutlich, dass Vemurafenib den AHR an einer Translokation in den Zellkern hindert. Unsere Daten legen nahe, dass Vemurafenib-induzierte Hautausschläge nicht auf eine allergische Reaktion des Körpers, sondern eine Inhibierung des AHR und damit einhergehende gestörte Homöostase der Haut zurückzuführen sind. Eine Behandlung von Patienten mit nicht toxischen AHR Liganden könnte eine derartige Reaktion der Haut unterdrücken und zu einer nebenwirkungsärmeren Therapie von Patienten mit malignen Melanom beitragen.

4 Literaturverzeichnis

Abdelrahim, Maen; Smith, Roger; Safe, Stephen (2003): Aryl hydrocarbon receptor gene silencing with small inhibitory RNA differentially modulates Ah-responsiveness in MCF-7 and HepG2 cancer cells. In: *Molecular pharmacology* 63 (6), S. 1373–1381. DOI: 10.1124/mol.63.6.1373.

Abel, Josef; Haarmann-Stemmann, Thomas (2010): An introduction to the molecular basics of aryl hydrocarbon receptor biology. In: *Biological chemistry* 391 (11), S. 1235–1248. DOI: 10.1515/BC.2010.128.

Andrieux, Lise; Langouet, Sophie; Fautrel, Alain; Ezan, Frederic; Krauser, Joel A.; Savouret, Jean F. et al. (2004): Aryl hydrocarbon receptor activation and cytochrome P450 1A induction by the mitogen-activated protein kinase inhibitor U0126 in hepatocytes. In: *Molecular pharmacology* 65 (4), S. 934–943. DOI: 10.1124/mol.65.4.934.

Assefa, Zerihun; van Laethem, An; Garmyn, Marjan; Agostinis, Patrizia (2005): Ultraviolet radiation-induced apoptosis in keratinocytes: on the role of cytosolic factors. In: *Biochimica et biophysica acta* 1755 (2), S. 90–106. DOI: 10.1016/j.bbcan.2005.04.001.

Aziz, Moammir Hasan; Reagan-Shaw, Shannon; Wu, Jianqiang; Longley, B. Jack; Ahmad, Nihal (2005): Chemoprevention of skin cancer by grape constituent resveratrol: relevance to human disease? In: *FASEB journal : official publication of the Federation of American Societies for Experimental Biology* 19 (9), S. 1193–1195. DOI: 10.1096/fj.04-3582fje.

Board, PDQ Adult Treatment Editorial (2018): Skin Cancer Treatment (PDQ®): National Cancer Institute (US).

Bode, Ann M.; Dong, Zigang (2003): Mitogen-activated protein kinase activation in UV-induced signal transduction. In: *Science's STKE : signal transduction knowledge environment* 2003 (167), RE2. DOI: 10.1126/stke.2003.167.re2.

Boehm, Manfred; Yoshimoto, Takanobu; Crook, Martin F.; Nallamshetty, Shriram; True, Andrea; Nabel, Gary J.; Nabel, Elizabeth G. (2002): A growth factor-dependent nuclear kinase phosphorylates p27(Kip1) and regulates cell cycle progression. In: *The EMBO journal* 21 (13), S. 3390–3401. DOI: 10.1093/emboj/cdf343.

Brash, Douglas E. (1988): UV MUTAGENIC PHOTOPRODUCTS IN Escherichia coli AND HUMAN CELLS: A MOLECULAR GENETICS PERSPECTIVE ON HUMAN SKIN CANCER*. In: *Photochemistry and photobiology* 48 (1), S. 59–66. DOI: 10.1111/j.1751-1097.1988.tb02786.x.

Bruhs, Anika; Haarmann-Stemmann, Thomas; Frauenstein, Katrin; Krutmann, Jean; Schwarz, Thomas; Schwarz, Agatha (2015): Activation of the arylhydrocarbon receptor causes immunosuppression primarily by modulating dendritic cells. In: *The Journal of investigative dermatology* 135 (2), S. 435–444. DOI: 10.1038/jid.2014.419.

Cadet, Jean; Douki, Thierry (2011): Oxidatively generated damage to DNA by UVA radiation in cells and human skin. In: *The Journal of investigative dermatology* 131 (5), S. 1005–1007. DOI: 10.1038/jid.2011.51.

Chan, Clara Y. Y.; Kim, Perry M.; Winn, Louise M. (2004): TCDD affects DNA double strand-break repair. In: *Toxicological sciences : an official journal of the Society of Toxicology* 81 (1), S. 133–138. DOI: 10.1093/toxsci/kfh200.

Chapman, Paul B.; Hauschild, Axel; Robert, Caroline; Haanen, John B.; Ascierto, Paolo; Larkin, James et al. (2011): Improved survival with vemurafenib in melanoma with BRAF V600E mutation. In: *The New England journal of medicine* 364 (26), S. 2507–2516. DOI: 10.1056/NEJMoa1103782.

Chen, Jun; Xia, Dan; Luo, Jin-Dan; Wang, Ping (2009): Exogenous p27KIP1 expression induces anti-tumour effects and inhibits the EGFR/PI3K/Akt signalling pathway in PC3 cells. In: *Asian journal of andrology* 11 (6), S. 669–677. DOI: 10.1038/aja.2009.51.

Cho, Soyun; Lee, Min Jung; Kim, Mi Sun; Lee, Serah; Kim, Yoon Kyung; Lee, Dong Hun et al. (2008): Infrared plus visible light and heat from natural sunlight participate in the expression of MMPs and type I procollagen as well as infiltration of inflammatory cell in human skin in vivo. In: *Journal of dermatological science* 50 (2), S. 123–133. DOI: 10.1016/j.jdermsci.2007.11.009.

Chomiczewska, Dorota; Trznadel-Budźko, Ewa; Kaczorowska, Anna; Rotsztejn, Helena (2009): Znaczenie komórek Langerhansa w układzie immunologicznym skóry. In: *Polski merkuriusz lekarski : organ Polskiego Towarzystwa Lekarskiego* 26 (153), S. 173–177.

Chu, Isabel M.; Hengst, Ludger; Slingerland, Joyce M. (2008): The Cdk inhibitor p27 in human cancer: prognostic potential and relevance to anticancer therapy. In: *Nature reviews. Cancer* 8 (4), S. 253–267. DOI: 10.1038/nrc2347.

Coin, Frédéric; Oksenych, Valentyn; Mocquet, Vincent; Groh, Stefanie; Blattner, Christine; Egly, Jean Marc (2008): Nucleotide Excision Repair Driven by the Dissociation of CAK from TFIIH. In: *Molecular Cell* 31 (1), S. 9–20. DOI: 10.1016/j.molcel.2008.04.024.

Conney, Allan H.; Lu, Yao-Ping; Lou, You-Rong; Kawasumi, Masaoki; Nghiem, Paul (2013): Mechanisms of Caffeine-Induced Inhibition of UVB Carcinogenesis. In: *Frontiers in oncology* 3, S. 144. DOI: 10.3389/fonc.2013.00144.

Corre, Sébastien; Tardif, Nina; Mouchet, Nicolas; Leclair, Héloïse M.; Boussemart, Lise; Gautron, Arthur et al. (2018): Sustained activation of the Aryl hydrocarbon Receptor transcription factor promotes resistance to BRAF-inhibitors in melanoma. In: *Nature communications* 9 (1), S. 4775. DOI: 10.1038/s41467-018-06951-2.

Davies, Helen; Bignell, Graham R.; Cox, Charles; Stephens, Philip; Edkins, Sarah; Clegg, Sheila et al. (2002): Mutations of the BRAF gene in human cancer. In: *Nature* 417 (6892), S. 949–954. DOI: 10.1038/nature00766.

Devary, Yoram; Gottlieb, Roberta A.; Smeal, Tod; Karin, Michael (1992): The mammalian ultraviolet response is triggered by activation of src tyrosine kinases. In: *Cell* 71 (7), S. 1081–1091. DOI: 10.1016/S0092-8674(05)80058-3.

Dever, Daniel P.; Opanashuk, Lisa A. (2012): The aryl hydrocarbon receptor contributes to the proliferation of human medulloblastoma cells. In: *Molecular pharmacology* 81 (5), S. 669–678. DOI: 10.1124/mol.111.077305.

Di Meglio, Paola; Duarte, João H.; Ahlfors, Helena; Owens, Nick D.L.; Li, Ying; Villanova, Federica et al. (2014): Activation of the Aryl Hydrocarbon Receptor Dampens the Severity of Inflammatory Skin Conditions. In: *Immunity* 40 (6), S. 989–1001. DOI: 10.1016/j.immuni.2014.04.019.

DiGiovanna, John J.; Kraemer, Kenneth H. (2012): Shining a light on xeroderma pigmentosum. In: *The Journal of investigative dermatology* 132 (3 Pt 2), S. 785–796. DOI: 10.1038/jid.2011.426.

D'Orazio, John; Jarrett, Stuart; Amaro-Ortiz, Alexandra; Scott, Timothy (2013): UV radiation and the skin. In: *International journal of molecular sciences* 14 (6), S. 12222–12248. DOI: 10.3390/ijms140612222.

Dvorak, Zdenek; Vrzal, Radim; Henklova, Pavla; Jancova, Petra; Anzenbacherova, Eva; Maurel, Patrick et al. (2008): JNK inhibitor SP600125 is a partial agonist of human aryl hydrocarbon receptor and induces CYP1A1 and CYP1A2 genes in primary human hepatocytes. In: *Biochemical Pharmacology* 75 (2), S. 580–588. DOI: 10.1016/j.bcp.2007.09.013.

Enan, Essam; Matsumura, Fumio (1996): Identification of c-Src as the integral component of the cytosolic Ah receptor complex, transducing the signal of 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) through the protein phosphorylation pathway. In: *Biochemical Pharmacology* 52 (10), S. 1599–1612. DOI: 10.1016/S0006-2952(96)00566-7.

Evans, Brad R.; Karchner, Sibel I.; Allan, Lenka L.; Pollenz, Richard S.; Tanguay, Robert L.; Jenny, Matthew J. et al. (2008): Repression of aryl hydrocarbon receptor (AHR) signaling by AHR repressor: role of DNA binding and competition for AHR nuclear translocator. In: *Molecular pharmacology* 73 (2), S. 387–398. DOI: 10.1124/mol.107.040204.

Frauenstein, K.; Sydlik, U.; Tigges, J.; Majora, M.; Wiek, C.; Hanenberg, H. et al. (2013): Evidence for a novel anti-apoptotic pathway in human keratinocytes involving the aryl hydrocarbon receptor, E2F1, and checkpoint kinase 1. In: *Cell Death Differ* 20 (10), S. 1425–1434. DOI: 10.1038/cdd.2013.102.

Friedberg, Errol C.; McDaniel, Lisa D.; Schultz, Roger A. (2004): The role of endogenous and exogenous DNA damage and mutagenesis. In: *Current opinion in genetics & development* 14 (1), S. 5–10. DOI: 10.1016/j.gde.2003.11.001.

Fritsch, Peter (1998): Dermatologie und Venerologie. Berlin, Heidelberg: Springer Berlin Heidelberg.

Fritsche, Ellen; Schäfer, Claudia; Calles, Christian; Bernsmann, Thorsten; Bernshausen, Thorsten; Wurm, Melanie et al. (2007): Lightening up the UV response by identification of the arylhydrocarbon receptor as a cytoplasmatic target for ultraviolet B radiation. In: *Proceedings of the National Academy of Sciences of the United States of America* 104 (21), S. 8851–8856. DOI: 10.1073/pnas.0701764104.

Goodsell, D. S. (2001): The Molecular Perspective: Ultraviolet Light and Pyrimidine Dimers. In: *The Oncologist* 6 (3), S. 298–299. DOI: 10.1634/theoncologist.6-3-298.

Grether-Beck, Susanne; Marini, Alessandra; Jaenicke, Thomas; Krutmann, Jean (2015): Effective photoprotection of human skin against infrared A radiation by topically

applied antioxidants: results from a vehicle controlled, double-blind, randomized study. In: *Photochemistry and photobiology* 91 (1), S. 248–250. DOI: 10.1111/php.12375.

Grimmler, Matthias; Wang, Yuefeng; Mund, Thomas; Cilensek, Zoran; Keidel, Eva-Maria; Waddell, M. Brett et al. (2007): Cdk-inhibitory activity and stability of p27Kip1 are directly regulated by oncogenic tyrosine kinases. In: *Cell* 128 (2), S. 269–280. DOI: 10.1016/j.cell.2006.11.047.

Haarmann-Stemmann, Thomas; Esser, Charlotte; Krutmann, Jean (2015): The Janus-Faced Role of Aryl Hydrocarbon Receptor Signaling in the Skin: Consequences for Prevention and Treatment of Skin Disorders. In: *The Journal of investigative dermatology* 135 (11), S. 2572–2576. DOI: 10.1038/jid.2015.285.

Haines, Eric; Chen, Ting; Kommajosyula, Naveen; Chen, Zhao; Herter-Sprie, Grit S.; Cornell, Liam et al. (2018): Palbociclib resistance confers dependence on an FGFR-MAP kinase-mTOR-driven pathway in KRAS-mutant non-small cell lung cancer. In: *Oncotarget* 9 (60), S. 31572–31589. DOI: 10.18632/oncotarget.25803.

Hara, T.; Kamura, T.; Nakayama, K.; Oshikawa, K.; Hatakeyama, S. (2001): Degradation of p27(Kip1) at the G(0)-G(1) transition mediated by a Skp2-independent ubiquitination pathway. In: *The Journal of biological chemistry* 276 (52), S. 48937–48943. DOI: 10.1074/jbc.M107274200.

Harding, James J.; Pulitzer, Melissa; Chapman, Paul B. (2012): Vemurafenib sensitivity skin reaction after ipilimumab. In: *The New England journal of medicine* 366 (9), S. 866–868. DOI: 10.1056/NEJMc1114329.

Herrlich, Peter; Karin, Michael; Weiss, Carsten (2008): Supreme EnLIGHTenment: damage recognition and signaling in the mammalian UV response. In: *Molecular cell* 29 (3), S. 279–290. DOI: 10.1016/j.molcel.2008.01.001.

IARC (2012): A Review of human carcinogens. Part D : Radiation. Lyon, France: International Agency for Research on Cancer (IARC monographs on the evaluation of carcinogenic risks to humans, v. 100D).

Ikehata, Hironobu; Ono, Tetsuya (2011): The mechanisms of UV mutagenesis. In: *Journal of radiation research* 52 (2), S. 115–125.

John, Kaarthik; Lahoti, Tejas S.; Wagner, Kelly; Hughes, Jarod M.; Perdew, Gary H. (2014): The Ah receptor regulates growth factor expression in head and neck squamous cell carcinoma cell lines. In: *Molecular carcinogenesis* 53 (10), S. 765–776. DOI: 10.1002/mc.22032.

Kato, J. Y.; Matsuoka, M.; Polyak, K.; Massagué, J.; Sherr, C. J. (1994): Cyclic AMP-induced G1 phase arrest mediated by an inhibitor (p27Kip1) of cyclin-dependent kinase 4 activation. In: *Cell* 79 (3), S. 487–496.

Kaya, G.; Saxer-Sekulic, N.; Kaya, A.; Sorg, O.; Boespflug, A.; Thomas, L.; Saurat, J. H. (2018): RASopathic comedone-like or cystic lesions induced by vemurafenib: a model of skin lesions similar but not identical to those induced by dioxins MADISH. In: *J Eur Acad Dermatol Venereol* 32 (8), S. 1368–1372. DOI: 10.1111/jdv.14945.

Kim, Arianna L.; Athar, Mohammad; Bickers, David R.; Gautier, Jean (2002): Ultraviolet-B-induced G1 arrest is mediated by downregulation of cyclin-dependent kinase 4 in transformed keratinocytes lacking functional p53. In: *The Journal of investigative dermatology* 118 (5), S. 818–824. DOI: 10.1046/j.1523-1747.2002.01734.x.

Kiyokawa, H.; Kineman, R. D.; Manova-Todorova, K. O.; Soares, V. C.; Hoffman, E. S.; Ono, M. et al. (1996): Enhanced growth of mice lacking the cyclin-dependent kinase inhibitor function of p27(Kip1). In: *Cell* 85 (5), S. 721–732.

Köhle, C.; Gschaidmeier, H.; Lauth, D.; Topell, S.; Zitzer, H.; Bock, K. W. (1999): 2,3,7,8-Tetrachlorodibenzo-p-dioxin (TCDD)-mediated membrane translocation of c-Src protein kinase in liver WB-F344 cells. In: *Archives of toxicology* 73 (3), S. 152–158.

Kolluri, S. K.; Weiss, C.; Koff, A.; Göttlicher, M. (1999): p27(Kip1) induction and inhibition of proliferation by the intracellular Ah receptor in developing thymus and hepatoma cells. In: *Genes & development* 13 (13), S. 1742–1753.

Korashy, Hesham M.; Anwar-Mohamed, Anwar; Soshilov, Anatoly A.; Denison, Michael S.; El-Kadi, Ayman O.S. (2011): The p38 MAPK Inhibitor SB203580 Induces Cytochrome P450 1A1 Gene Expression in Murine and Human Hepatoma Cell Lines through Ligand-Dependent Aryl Hydrocarbon Receptor Activation. In: *Chem. Res. Toxicol.* 24 (9), S. 1540–1548. DOI: 10.1021/tx200141p.

Krutmann, Jean; Morita, Akimichi; Chung, Jin Ho (2012): Sun exposure: what molecular photodermatology tells us about its good and bad sides. In: *The Journal of investigative dermatology* 132 (3 Pt 2), S. 976–984. DOI: 10.1038/jid.2011.394.

Kulms, D.; Pöppelmann, B.; Yarosh, D.; Luger, T. A.; Krutmann, J.; Schwarz, T. (1999): Nuclear and cell membrane effects contribute independently to the induction of apoptosis in human cells exposed to UVB radiation. In: *Proceedings of the National Academy of Sciences* 96 (14), S. 7974–7979.

Larkin, James; Ascierto, Paolo A.; Dréno, Brigitte; Atkinson, Victoria; Liszkay, Gabriella; Maio, Michele et al. (2014): Combined vemurafenib and cobimetinib in BRAF-mutated melanoma. In: *The New England journal of medicine* 371 (20), S. 1867–1876. DOI: 10.1056/NEJMoa1408868.

Lawrence, Karl P.; Douki, Thierry; Sarkany, Robert P. E.; Acker, Stephanie; Herzog, Bernd; Young, Antony R. (2018): The UV/Visible Radiation Boundary Region (385–405 nm) Damages Skin Cells and Induces “dark” Cyclobutane Pyrimidine Dimers in Human Skin in vivo. In: *Scientific reports* 8 (1), S. 12722. DOI: 10.1038/s41598-018-30738-6.

Liu, Feifei; Yang, Xiaotong; Geng, Meiyu; Huang, Min (2018): Targeting ERK, an Achilles' Heel of the MAPK pathway, in cancer therapy. In: *Acta pharmaceutica Sinica. B* 8 (4), S. 552–562. DOI: 10.1016/j.apsb.2018.01.008.

Liu, Kangdong; Yu, Donghoon; Cho, Yong-Yeon; Bode, Ann M.; Ma, Weiya; Yao, Ke et al. (2013): Sunlight UV-induced skin cancer relies upon activation of the p38α signaling pathway. In: *Cancer research* 73 (7), S. 2181–2188. DOI: 10.1158/0008-5472.CAN-12-3408.

Lloyd, Ricardo V.; Erickson, Lori A.; Jin, Long; Kulig, Elzbieta; Qian, Xiang; Cheville, John C.; Scheithauer, Bernd W. (1999): p27kip1: A Multifunctional Cyclin-Dependent Kinase Inhibitor with Prognostic Significance in Human Cancers. In: *The American Journal of Pathology* 154 (2), S. 313–323. DOI: 10.1016/S0002-9440(10)65277-7.

Matthews-Trigg, Nathaniel T.; Vanos, Jennifer; Ebi, Kristie L. (2019): Climate Change and Cancer. In: Eric H. Bernicker (Hg.): CANCER AND SOCIETY. A multidisciplinary assessment and strategies for action, Bd. 113. [Place of publication not identified]: SPRINGER NATURE, S. 11–25.

Meador, Jarah A.; Walter, Ronald B.; Mitchell, David L. (2000): Induction, Distribution and Repair of UV Photodamage in the Platyfish, Xiphophorus signum¶. In: *Photochemistry and photobiology* 72 (2), S. 260–266. DOI: 10.1562/0031-8655(2000)0720260IDAROU2.0.CO2.

Meurer, Steffen K.; Weiskirchen, Ralf (2018): Usage of Mitogen-Activated Protein Kinase Small Molecule Inhibitors: More Than Just Inhibition! In: *Frontiers in pharmacology* 9, S. 98. DOI: 10.3389/fphar.2018.00098.

Mimura, Junsei; Ema, Masatsugu; Sogawa, Kazuhiro; Fujii-Kuriyama, Yoshiaki (1999): Identification of a novel mechanism of regulation of Ah (dioxin) receptor function. In: *Genes & development* 13 (1), S. 20–25.

Moennikes, Oliver; Loeppen, Sandra; Buchmann, Albrecht; Andersson, Patrik; Ittrich, Carina; Poellinger, Lorenz; Schwarz, Michael (2004): A constitutively active dioxin/aryl hydrocarbon receptor promotes hepatocarcinogenesis in mice. In: *Cancer research* 64 (14), S. 4707–4710. DOI: 10.1158/0008-5472.CAN-03-0875.

Moseley, Harry (1988): Non-ionising radiation. Microwaves, ultraviolet and laser radiation. Bristol: Hilger (Medical physics handbooks, 18). Online verfügbar unter http://www.loc.gov/catdir/enhancements/fy0745/87021271-d.html.

Navid, Fatemeh; Bruhs, Anika; Schuller, Winfried; Fritsche, Ellen; Krutmann, Jean; Schwarz, Thomas; Schwarz, Agatha (2013): The Aryl hydrocarbon receptor is involved in UVR-induced immunosuppression. In: *The Journal of investigative dermatology* 133 (12), S. 2763–2770. DOI: 10.1038/jid.2013.221.

Park, Sophia L.; Justiniano, Rebecca; Williams, Joshua D.; Cabello, Christopher M.; Qiao, Shuxi; Wondrak, Georg T. (2015): The Tryptophan-Derived Endogenous Aryl Hydrocarbon Receptor Ligand 6-Formylindolo3,2-bCarbazole Is a Nanomolar UVA Photosensitizer in Epidermal Keratinocytes. In: *The Journal of investigative dermatology* 135 (6), S. 1649–1658. DOI: 10.1038/jid.2014.503.

Pollet, Marius; Shaik, Siraz; Mescher, Melina; Frauenstein, Katrin; Tigges, Julia; Braun, Stephan A. et al. (2018): The AHR represses nucleotide excision repair and apoptosis and contributes to UV-induced skin carcinogenesis. In: *Cell Death Differ*. DOI: 10.1038/s41418-018-0160-1.

Rassner, Gernot (2013): Dermatologie. Lehrbuch und Atlas. 9. Aufl. München: Urban & Fischer Verlag/Elsevier GmbH.

Reid-Bayliss, Kate S.; Arron, Sarah T.; Loeb, Lawrence A.; Bezrookove, Vladimir; Cleaver, James E. (2016): Why Cockayne syndrome patients do not get cancer despite their DNA repair deficiency. In: *Proceedings of the National Academy of Sciences of the United States of America* 113 (36), S. 10151–10156. DOI: 10.1073/pnas.1610020113.

Reiners, J. J. (1999): Suppression of cell cycle progression by flavonoids: dependence on the aryl hydrocarbon receptor. In: *Carcinogenesis* 20 (8), S. 1561–1566. DOI: 10.1093/carcin/20.8.1561.

Rogers, Howard W.; Weinstock, Martin A.; Feldman, Steven R.; Coldiron, Brett M. (2015): Incidence Estimate of Nonmelanoma Skin Cancer (Keratinocyte Carcinomas)

in the U.S. Population, 2012. In: *JAMA dermatology* 151 (10), S. 1081–1086. DOI: 10.1001/jamadermatol.2015.1187.

Roos, Wynand P.; Kaina, Bernd (2006): DNA damage-induced cell death by apoptosis. In: *Trends in molecular medicine* 12 (9), S. 440–450. DOI: 10.1016/j.molmed.2006.07.007.

Schärer, Orlando D. (2013): Nucleotide Excision Repair in Eukaryotes. In: *Cold Spring Harbor Perspectives in Biology* 5 (10). DOI: 10.1101/cshperspect.a012609.

Sekine, Hiroki; Mimura, Junsei; Oshima, Motohiko; Okawa, Hiromi; Kanno, Jun; Igarashi, Katsuhide et al. (2009): Hypersensitivity of aryl hydrocarbon receptor-deficient mice to lipopolysaccharide-induced septic shock. In: *Molecular and cellular biology* 29 (24), S. 6391–6400. DOI: 10.1128/MCB.00337-09.

Shimizu, Y.; Nakatsuru, Y.; Ichinose, M.; Takahashi, Y.; Kume, H.; Mimura, J. et al. (2000): Benzo[a]pyrene carcinogenicity is lost in mice lacking the aryl hydrocarbon receptor. In: *Proceedings of the National Academy of Sciences* 97 (2), S. 779–782. DOI: 10.1073/pnas.97.2.779.

Sørensen, Claus Storgaard; Hansen, Lasse Tengbjerg; Dziegielewski, Jaroslaw; Syljuåsen, Randi G.; Lundin, Cecilia; Bartek, Jiri; Helleday, Thomas (2005): The cell-cycle checkpoint kinase Chk1 is required for mammalian homologous recombination repair. In: *Nature cell biology* 7 (2), S. 195–201. DOI: 10.1038/ncb1212.

Statistisches Bundesamt (2017): 17 % mehr stationäre Hautkrebsbehandlungen innerhalb von 5 Jahren, zuletzt geprüft am 10.01.2018.

Su, Fei; Viros, Amaya; Milagre, Carla; Trunzer, Kerstin; Bollag, Gideon; Spleiss, Olivia et al. (2012): RAS mutations in cutaneous squamous-cell carcinomas in patients treated with BRAF inhibitors. In: *The New England journal of medicine* 366 (3), S. 207–215. DOI: 10.1056/NEJMoa1105358.

Tauchi, Masafumi; Hida, Azumi; Negishi, Takaaki; Katsuoka, Fumiki; Noda, Shuhei; Mimura, Junsei et al. (2005): Constitutive expression of aryl hydrocarbon receptor in keratinocytes causes inflammatory skin lesions. In: *Molecular and cellular biology* 25 (21), S. 9360–9368. DOI: 10.1128/MCB.25.21.9360-9368.2005.

Tian, Yanan; Ke, Sui; Denison, Michael. S.; Rabson, Arnold B.; Gallo, Michael A. (1999): Ah Receptor and NF-κB Interactions, a Potential Mechanism for Dioxin Toxicity. In: *J. Biol. Chem.* 274 (1), S. 510–515. DOI: 10.1074/jbc.274.1.510.

Valkova, S.; Velkova, A. (2004): UVA/UVB phototherapy for atopic dermatitis revisited. In: *The Journal of dermatological treatment* 15 (4), S. 239–244. DOI: 10.1080/09546630410035338.

van den Bogaard, Ellen H.; Bergboer, Judith G. M.; Vonk-Bergers, Mieke; van Vlijmen-Willems, Ivonne M. J. J.; Hato, Stanleyson V.; van der Valk, Pieter G. M. et al. (2013): Coal tar induces AHR-dependent skin barrier repair in atopic dermatitis. In: *The Journal of clinical investigation* 123 (2), S. 917–927. DOI: 10.1172/JCI65642.

Viglietto, Giuseppe; Motti, Maria Letizia; Bruni, Paola; Melillo, Rosa Marina; D'Alessio, Amelia; Califano, Daniela et al. (2002): Cytoplasmic relocalization and inhibition of the cyclin-dependent kinase inhibitor p27(Kip1) by PKB/Akt-mediated phosphorylation in breast cancer. In: *Nature medicine* 8 (10), S. 1136–1144. DOI: 10.1038/nm762.

Vogel, Christoph F. A.; Wu, Dalei; Goth, Samuel R.; Baek, Jaeeun; Lollies, Anna; Domhardt, Rowena et al. (2013): Aryl hydrocarbon receptor signaling regulates NF-κB RelB activation during dendritic-cell differentiation. In: *Immunology and cell biology* 91 (9), S. 568–575. DOI: 10.1038/icb.2013.43.

Wang, X.; Gorospe, M.; Huang, Y.; Holbrook, N. J. (1997): p27Kip1 overexpression causes apoptotic death of mammalian cells. In: *Oncogene* 15 (24), S. 2991–2997. DOI: 10.1038/sj.onc.1201450.

Wincent, Emma; Bengtsson, Johanna; Mohammadi Bardbori, Afshin; Alsberg, Tomas; Luecke, Sandra; Rannug, Ulf; Rannug, Agneta (2012): Inhibition of cytochrome P4501-dependent clearance of the endogenous agonist FICZ as a mechanism for activation of the aryl hydrocarbon receptor. In: *Proceedings of the National Academy of Sciences of the United States of America* 109 (12), S. 4479–4484. DOI: 10.1073/pnas.1118467109.

Xie, Guofeng; Peng, Zhongsheng; Raufman, Jean-Pierre (2012): Src-mediated aryl hydrocarbon and epidermal growth factor receptor cross talk stimulates colon cancer cell proliferation. In: *American Journal of Physiology - Gastrointestinal and Liver Physiology* 302 (9), G1006-15. DOI: 10.1152/ajpgi.00427.2011.

Yadav, Vipin; Zhang, Xiaoyi; Liu, Jiangang; Estrem, Shawn; Li, Shuyu; Gong, Xue-Qian et al. (2012): Reactivation of mitogen-activated protein kinase (MAPK) pathway by FGF receptor 3 (FGFR3)/Ras mediates resistance to vemurafenib in human B-RAF V600E mutant melanoma. In: *The Journal of biological chemistry* 287 (33), S. 28087–28098. DOI: 10.1074/jbc.M112.377218.

5 Abbildungsverzeichnis

6 Danksagung

Ich danke Prof. Dr. Dr. Proksch und Prof. Dr. med. Krutmann für die jederzeit einwandfreie Betreuung und wissenschaftlichen Input.

Dr. Haarmann-Stemmann danke ich für die Überlassung des Themas, die allzeit guten und lösungsorientierten Gespräche und der motivierenden Arbeitsatmosphäre gerade in den ersten beiden Jahren der Dissertation.

Dem DAAD danke ich für das Stipendium um Auslandserfahrung an der UC Davis zu sammeln und damit auch herzlichsten Dank an Prof. Dr. Chris Vogel für eine wunderbare Zeit in deiner Arbeitsgruppe.

Danke an die vielzähligen Arbeitskollegen die die Arbeit im Labor aber natürlich auch die Pausen bereichert haben. Danke an Katrin, Fischi, Siraz, Motoki, Diane, Laura, Katha, Ragnhild, Mana, Selina, Chris, Maren, Marc, Ingo, Sonja, Judith, Sabine und an alle die ich hier leider vergessen habe aufzuzählen.

Natürlich auch danke an alle Freunde für die immer gleiche motivierende Frage „Wann bist du fertig?“ und den nötigen Ausgleich in der Freizeit.

Danke an meine Freundin für aufmunternde und motivierende Worte. Den Einblick in andere Bereiche der Arbeitswelt in denen es auch mal chaotisch zugehen kann, für die Unterstützung in der Schlussphase der Promotion und Verständnis für den Spruch, dass Zellkulturen keine Wochenenden und Feiertage kennen ☺

Meiner Familie möchte ich danken, vor allem meiner Mutter und meinem Bruder die, egal ob während der Ausbildung, des Studiums oder der Doktorarbeit, immer ein Stück mehr an mich geglaubt haben als ich selbst.

Eidesstattliche Erklärung / Declaration

Hiermit versichere ich an Eides statt, dass ich die vorliegende Arbeit „Einfluss des Arylhydrocarbon Rezeptors auf Plattenepithelkarzinome“ selbständig verfasst und ausschließlich die von mir angegebenen Hilfsmittel verwendet habe. Die Dissertation wurde in der vorgelegten oder einer ähnlichen Form noch bei keiner anderen Institution eingereicht. Ich habe bisher keine erfolglosen Promotionsversuche unternommen.

I declare that I have developed and written the enclosed Thesis “Einfluss des Arylhydrocarbon Rezeptors auf Plattenepithelkarzinome” completely by myself, and have not used sources or means without declaration in the text. Any thoughts from others or literal quotations are clearly marked. The Thesis was not used in the same or in a similar version to achieve an academic grading elsewhere.

Marius Pollet

Düsseldorf, Mai 2019