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Cross-talk of Aryl Hydrocarbon Receptor (AHR)- and Vitamin D Receptor (VDR)-signaling in Human Keratinocytes

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Abstract. *Background/Aim: Vitamin D receptor (VDR), activated upon binding of 1,25(OH)$_2$D$_3$, was described as a tumor suppressor in the skin. New biological functions of non-classical vitamin D derivatives were recently identified, that are mediated via binding to alternate receptors, including the aryl hydrocarbon receptor (AHR) and that indicate functional interaction between AHR and VDR signaling in various human tissues. We aimed to gain further insights into the cross-talk of VDR and AHR signaling in skin photo-carcinogenesis. Materials and Methods: Using real-time quantitative PCR, we analyzed in vitro effects of the complete carcinogen UVB and of 1,25(OH)$_2$D$_3$ on the expression of members of the AHR and VDR pathways in human keratinocytes revealing characteristics of different stages of skin photo-carcinogenesis. Results: In precancerous HaCaT keratinocytes, induction of a target gene of AHR-mediated transcription (CYP1A1) was markedly stronger after treatment with UVB, as compared to treatment with 1,25(OH)$_2$D$_3$. In contrast, in SCL-1 cells (that reveal the complete phenotype of malignant transformation), expression of CYP1A1 was higher after treatment with 1,25(OH)$_2$D$_3$ as compared to treatment with UVB. The classical VDR target CYP24A1 was up-regulated by 1,25(OH)$_2$D$_3$, but not by UVB, in both cell lines. However, the combined treatment with UVB strongly enhanced the 1,25(OH)$_2$D$_3$-mediated up-regulation of CYP24A1 exclusively in SCL-1, but not in HaCaT cells. Conclusion: There is a differential regulation of VDR and AHR target genes by UVB and 1,25(OH)$_2$D$_3$ in HaCaT and SCL-1 cells, that points to a complex and highly orchestrated network of vitamin D derivatives (and other photoproducts) and its relevance for photo-carcinogenesis.*

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As the frontier of the human body to the environment, the human skin represents an important defense line against many different hazards, including infections, intoxications, and exposure to UV- and other types of radiation. It is well known that ultraviolet B radiation (UVB; wavelength range: 290-320 nm), found in solar radiation, is a potentially toxic and carcinogenic environmental factor. Whereas acute effects of skin exposure to UVB radiation are dose-dependent and include sunburns or immune modulation (1), long-term exposure is known to be a main risk factor for developing non-melanoma skin cancer including cutaneous squamous cell carcinoma (cSCC) (2, 3) and its precancerous skin lesion actinic keratosis (AK) (4, 5). The UVB-induced stress response in the human skin is called UV response (6-8). An important mechanism involved in this process is the activation of the Aryl hydrocarbon receptor (*AHR*) (9). This ligand-dependent receptor belongs to the basic helix-loop-helix/Per-Arnt-Sim (bHLH/PAS) family (10) and is located in the cell cytoplasm, bound to a chaperone complex (Hsp90/XAP2/p23) (11, 12). It is known for its role in the detoxification of harmful substances like 2,3,7,8-Tetrachlorodibenzo-p-dioxin (TCDD) (12), polycyclic aromatic hydrocarbons (PAHs) (13-16), and natural flavonoids (17, 18) and is highly expressed in barrier organs like the skin (19, 20). The *AHR* activation caused by UVB activates two signaling pathways, one located in the nucleus and one in the membrane

of the cell. In the nuclear pathway, UVB radiation triggers *AHR* translocation into the nucleus and causes induction of cytochrome P450 1A1 (*CYP1A1*) gene expression in a ligand-dependent manner (9). The xenobiotic metabolizing enzyme *CYP1A1* is one of the most widely studied *AHR* target genes (14, 21) and is linked to various cutaneous immunologic processes (19), chemical carcinogenesis (22-24), and the development of non-melanoma skin cancer (25-27). Moreover, the DNA is able to absorb UVB, which results in formation of DNA photoproducts (28-32). In the cell membrane pathway, *AHR* activation causes internalization of the EGFR and activation of its downstream target MAP kinases ERK1 and ERK2. As a result, cyclooxygenase-2 (*COX2*) mRNA expression is up-regulated (9), which is linked with photo-carcinogenesis and skin inflammation (33-39).

The hormonally active form of vitamin D_3 [$1,25(OH)_2D_3$; calcitriol] is produced in keratinocytes of the epidermis in a UVB-dependent, 3-step process (40). It modulates important physiological and pharmacological processes like immunomodulation and bone metabolism (41, 42). In the skin specifically, it plays a vital role in the epidermal proliferation and differentiation (43, 44), apoptosis (45, 46), and barrier function (47-49). It also exhibits a protective effect against inflammatory skin diseases including psoriasis (50) and atopic dermatitis (51) and inhibits the growth of skin cancer such as melanoma (52, 53), basal cell carcinoma (BCC) (54, 55), and squamous cell carcinoma (SCC) (56). $1,25(OH)_2D_3$ unfolds its biological function through binding to the vitamin D receptor (*VDR*; *NR1I1*) (57), a member of the nuclear receptor superfamily of transcription factors highly expressed in keratinocytes (58, 59). The *VDR* regulates gene expression by forming a heterodimeric complex with retinoid X receptors (RXRs), translocating to the nucleus and interacting with vitamin D responsive elements (VDREs) in the promoter of target genes (57, 60, 61). A major target gene of the *VDR* is cytochrome P450 24A1 (*CYP24A1*) (62, 63), which encodes for the 25-hydroxyvitamin D_3 24-hydroxylase. By this enzyme, inactivation of $1,25(OH)_2D_3$ through hydroxylation and termination of its biological activity is induced (64, 65). In some forms of cancer, it has been suggested to represent an oncogene (66).

Recent scientific findings indicate a functional interaction between *AHR* and *VDR* signaling in various human tissues. In this regard, new biological functions of non-classical vitamin D derivatives have recently been identified, that are at least in part mediated *via* binding to alternate receptors, including the *AHR*. In human naïve $CD4^+$ T-cells, a suppressive effect of $1,25(OH)_2D_3$ on *AHR* expression was found (67). In human oral keratinocytes (OKF6/TERT-2 cells) however, $1,25(OH)_2D_3$ increased LPS-induced *AHR* and *CYP1A1* expression (68). In human epidermal keratinocytes (HEKn and HaCaT cells), *AHR* was the major receptor target for vitamin D derivative $20,23(OH)_2D_3$, (with *VDR* being the second signaling pathway identified) whereas weaker *AHR* activation was observed by $1,25(OH)_2D_3$, $20(OH)D_3$ and $17,20,23(OH)_2D_3$ (69). Matsunawa *et al*. (70, 71) demonstrated that combined activation of *AHR* and *VDR* enhanced *CYP1A1* and *CYP24A1* expression in breast cancer (MCF-7) and macrophage-derived (THP-1) cells. In the present study, we aimed to gain further insights into the cross-talk of $1,25(OH)_2D_3$-induced *VDR* and UVB-induced *AHR* signaling in skin photo-carcinogenesis. We here found a differential regulation of *VDR* and *AHR* target genes by UVB and $1,25(OH)_2D_3$ in precancerous HaCaT and malignant SCL-1 cells, which points to a complex and highly orchestrated network of vitamin D derivatives (and other photoproducts) and its relevance for photo-carcinogenesis.

Materials and Methods

Cell culture. Spontaneous immortalized human HaCaT (Human adult low Calcium, high Temperature) keratinocytes were purchased from CLS Cell Lines Service GmbH (Eppelheim, Germany) and cultivated in Dulbecco's modified eagle's medium (DMEM) (Gibco, Thermo Fisher Scientific, Dreieich, Germany). They exhibit a p53 mutation (p53mut) and represent initiated keratinocytes that express elements of early stage non-melanoma skin carcinogenesis (72, 73). *In vivo* animal studies have shown that HaCaT cells exhibit characteristic features of precancerous skin lesions (*e.g.*, AK), including development of a stratified epithelium with dysplastic morphologic properties but no tendency to invasive or metastatic growth (74). SCC cell lines (SCL-1) were maintained in RPMI 1640 medium (Gibco). They represent malignant human keratinocytes that lack expression of the p53 protein (p53null) (75-79) and exhibit characteristic features of the non-melanoma skin cancer phenotype, including invasive and metastatic growth tendency (80). Both cell lines were supplemented with 1% L-glutamine (Thermo Fisher Scientific) and 10% fetal calf serum (Gibco). They were seeded in culture dishes (10 cm in diameter) and grown in a humidified atmosphere of 5% CO_2 at 37˚C. Cell culture medium was changed twice a week.

UVB irradiation. After the culture medium has been aspirated from the cell culture dishes, cells were washed with phosphate-buffered saline (PBS) and irradiated with UVB (50 J/cm^2, midrange wavelength 302 nm) using Crosslinker CL-1000M (Ultra-violet products Ltd, purchased by Analytik Jena, Jena, Germany). Following irradiation, cells were provided with fresh medium and treatment substances.

Cell treatment. Cells were treated with $1,25(OH)_2D_3$ (Sigma, Taufkirchen, Germany) in a final concentration of 10^{-7} M [5 μl of the $1,25(OH)_2D_3$ of (10^{-4} M) stock solution solved in ethanol (EtOH) were added per 5 ml medium per culture dish]. Bovine serum albumin (BSA, 1%) was added to the medium when treating cells with $1,25(OH)_2D_3$ to reduce unspecific binding of $1,25(OH)_2D_3$ to the culture dish. Control samples were treated with EtOH (5 μl EtOH per 5 ml medium per culture dish) and BSA (1%, Sigma). In preliminary experiments, we first demonstrated that EtOH had no effect on gene expression, because cells treated with BSA alone showed similar results as compared to cells treated with BSA and EtOH. Cells were treated with AHR-Antagonist CH223191 (stock solution 10^{-4} M, solved in EtOH; final concentration 10^{-7} M, Sigma)

and partly VDR-Inhibitor 25-Hydroxyvitamin D_3 ($25(OH)_2D_3$, stock solution 10^{-4} M, solved in EtOH; final concentration 10^{-7} M, Sigma). Previous studies (81-83) confirmed that CH223191 and $25(OH)D_3$ in a final concentration of 10^{-7} M effectively block their corresponding receptors, *AHR* and *VDR*, respectively.

Cell harvesting. HaCaT-keratinocytes and SCL-1 cells were harvested (6 h intervals over 24 h) after irradiation and/or substance treatment.

RNA isolation. RNA isolation was performed with RNeasy Kit and QIA shredder (Qiagen, Hilden, Germany) according to the manufacturers' manual.

Reverse transcription. Reverse transcription was performed with Omniscript RT Kit (Qiagen) according to the manufacturers' instructions. Oligo-dT-primers, RNase inhibitors, and 1 μg mRNA were used in every reaction as templates.

Quantitative real-time PCR (RTqPCR) and analysis. Expression of the target genes *AHR, CYP1A1, COX2, VDR, CYP24A1* was examined in 96-well plates using RTqPCR (120 cycles in StepOnePlus Real-Time PCR System, Thermo Fisher Scientific). The level of expression of each target gene was normalized against the mean of *GAPDH* and β-actin gene expression and shown as mean±standard deviation. Each sample was analyzed in duplicate. All gene-specific primers were purchased from Qiagen (Table I). The relative quantification method ($RQ=2^{-\Delta\Delta Ct}$) was used in order to calculate the relative fold gene expression of the target genes (84). First, the relative amount of the target gene to each reference gene was determined for each sample (ΔCt). Then, the target/reference ratio of the treated sample was divided by the target/reference ratio of the control sample (ΔΔCt). To find out the N-fold target gene expression in treated samples relative to the control sample (final values), we calculated 2 to the power of the negative ΔΔCt ($2^{-\Delta\Delta Ct}$).

Statistical analysis. All data are represented as a mean±standard deviation (SD) of three experiments per cell line. The two-tailed, unpaired Student's *t*-test was used to assess statistical significance and performed with the Microsoft Excel software (Microsoft Corporation, Redmond, WA, USA). Mean differences were considered to be significant when $p \leq 0.05$ (*), decisive (very significant) when $p<0.005$ (**) and conclusive (extremely significant) when $p<0.0005$ (***).

Results

Gene expression of AHR and CYP1A1 is elevated in untreated HaCaT and of COX2, VDR and CYP24A1 in SCL-1 cells. In untreated spontaneously immortalized HaCaT keratinocytes we observed higher *AHR* ($p<0.0005$) and *CYP1A1* mRNA levels as compared to those in untreated SCL-1 cells (Figure 1A-D). In contrast, in untreated cancerous SCL-1 cells, mRNA expression of *COX2*, *VDR*, and *CYP24A1* was higher than HaCaT (Figure 1E-J).

$1,25(OH)_2D_3$ and UVB radiation exert differential effects on the expression of key elements of the AHR signaling pathway in HaCaT-keratinocytes and SCL-1 cells. In non-malignant HaCaT keratinocytes, UVB radiation induced a strong increase in *CYP1A1* mRNA (7.7-fold increase), that was markedly stronger as compared to treatment with $1,25(OH)_2D_3$ (2.8-fold increase) (Figure 2C). Combined treatment increased *CYP1A1* mRNA stronger than treatment with $1,25(OH)_2D_3$ alone (3.7-fold increase, $p \leq 0.05$). In contrast, in malignant SCL-1 cells, expression of *CYP1A1* was markedly higher after treatment with $1,25(OH)_2D_3$ (6.9-fold increase, $p<0.0005$) as compared to treatment with UVB (2.4-fold increase, $p<0.005$) (Figure 2D). Combined treatment showed a synergistic effect, by conclusively up-regulating *CYP1A1* mRNA to the highest extent (9.8-fold increase) (Figure 2D). UVB radiation up-regulated *AHR* mRNA in both cell lines (HaCaT: 2.2-fold increase; SCL-1: 1.6-fold increase). In contrast, lower levels of *AHR* expression were observed after treatment with $1,25(OH)_2D_3$ (HaCaT: 17% decrease; SCL-1: 26% decrease). Combined treatment did not show any regulatory effect as compared to untreated controls (Figure 2A and B).

Table I. *Gene-specific primers used in RTqPCR*.

Primer	Sequence	Source
AHR	QuantiTect Primer Assay (QT00031437)	Qiagen
CYP1A1	QuantiTect Primer Assay (QT00012341)	Qiagen
PTGS2 (COX2)	QuantiTect Primer Assay (QT00040586)	Qiagen
VDR	QuantiTect Primer Assay (QT01010170)	Qiagen
CYP24A1	QuantiTect Primer Assay (QT00015428)	Qiagen
GAPDH	QuantiTect Primer Assay (QT00079247)	Qiagen
β-Actin	QuantiTect Primer Assay (QT00095431)	Qiagen

Induction of CYP1A1 mRNA expression by $1,25(OH)_2D_3$ depends on AHR. Treatment with AHR-antagonist CH223191 alone or in combination with $1,25(OH)_2D_3$ strongly suppressed expression of *CYP1A1* mRNA in HaCaT and SCL-1 cells (HaCaT after 6 h: CH223191: 94% decrease, $p<0.0005$, CH223191+$1,25(OH)_2D_3$: 84% decrease, $p<0.0005$; HaCaT after 24 h: CH223191: 95% decrease, $p<0.0005$, CH223191+$1,25(OH)_2D_3$: 85% decrease, $p<0.0005$; SCL-1 after 6h: CH223191: 82% decrease, $p<0.0005$, CH223191+$1,25(OH)_2D_3$: 62% decrease, $p<0.0005$; SCL-1 after 24 h: CH223191: 88% decrease, $p<0.0005$, CH223191+$1,25(OH)_2D_3$: 74% decrease, $p<0.0005$) (Figure 3). In $25(OH)D_3$-treated cells, *CYP1A1* expression was reduced (HaCaT after 6 h: 56% decrease, $p<0.0005$; HaCaT after 24 h: 19% decrease; SCL-1 after 6 h: 65% decrease, $p<0.0005$; SCL-1 after 24 h: 36% decrease, $p<0.05$). However, combined treatment with $1,25(OH)_2D_3$ and $25(OH)D_3$ had a stronger induction in *CYP1A1* mRNA compared to $1,25(OH)_2D_3$ alone (except in SCL-1 after 24 h)

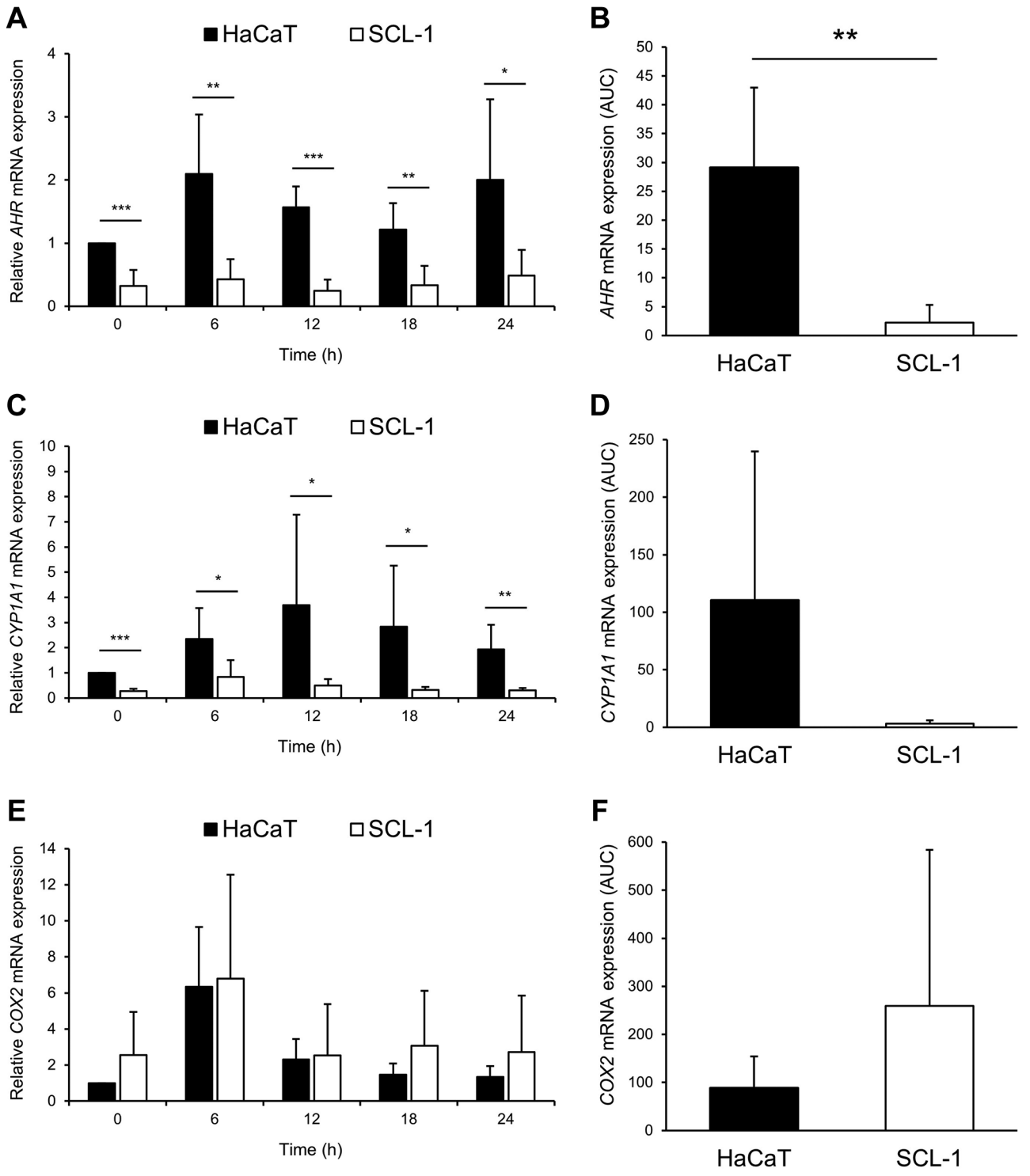

Figure 1. *Continued*

(HaCaT after 6 h: 1,25(OH)$_2$D$_3$+25(OH)D$_3$: 2.6-fold increase, $p<0.005$, 1,25(OH)$_2$D$_3$: 2.1-fold increase, $p<0.0005$; HaCaT after 24 h: 1,25(OH)$_2$D$_3$+25(OH)D$_3$: 1.23-fold increase, 1,25(OH)$_2$D$_3$: 1.17-fold increase; SCL-1 after 6 h: 1,25(OH)$_2$D$_3$+25(OH)D$_3$: 2.5-fold increase, $p<0.0005$, 1,25(OH)$_2$D$_3$: 2.4-fold increase, $p<0.0005$).

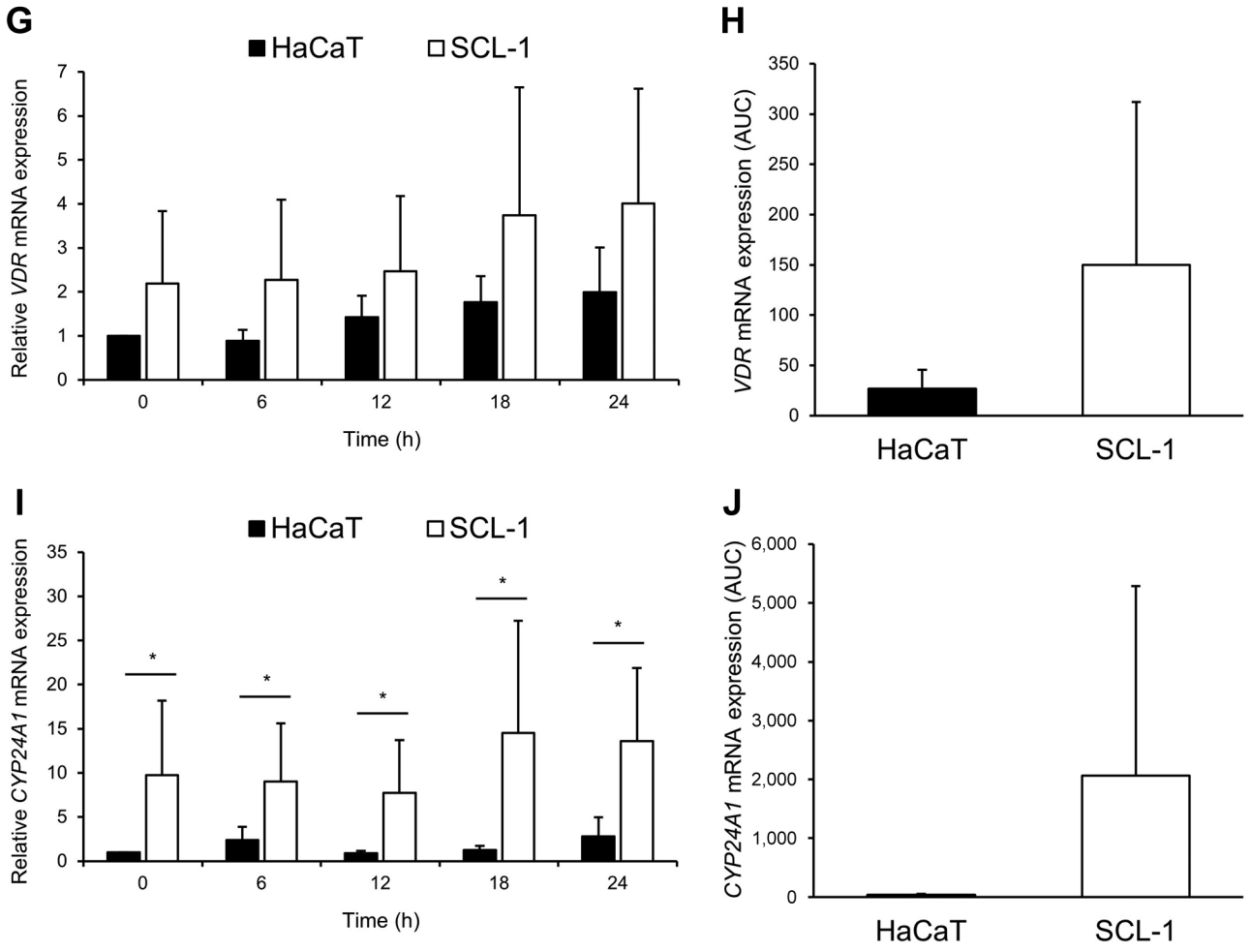

Figure 1. *Relative mRNA expression (A, C, E, G, I) and AUC (B, D, F, H, J) of AHR, CYP1A1, COX2, VDR, and CYP24A1 relative to the mean of GAPDH and β-actin in untreated HaCaT and SCL-1 cells (mean $2^{-\Delta\Delta Ct}$). Cells were treated only with culture medium and harvested in 6 h intervals over 24 h. The mRNA expression was measured with RTqPCR and the relative fold gene expression was calculated with the $2^{-\Delta\Delta Ct}$ method. HaCaT-cells harvested after 0 h were used as the internal control sample in the bar graphs (A, C, E, G, I). The "area under the curve" was calculated for each cell line from the respective time curve (data not shown). The values represent the means±SD of duplicate assays. The experiments were repeated thrice with similar results. *p≤0.05; **p<0.005; ***p<0.0005.*

1,25(OH)$_2$D$_3$ induces COX2 mRNA in HaCaT but not in SCL-1 cells. Treatment with 1,25(OH)$_2$D$_3$ strongly increased *COX2* mRNA expression in HaCaT cells (16.2-fold increase, $p \leq 0.05$), but barely altered it in SCL-1 cells (1.8-fold increase, $p \leq 0.05$) (Figure 2E and 2F). In both cell lines however, after treatment with 1,25(OH)$_2$D$_3$ and 25(OH)D$_3$, up-regulation of *COX2* gene expression was even stronger as compared to treatment with 1,25(OH)$_2$D$_3$ alone (HaCaT after 6 h: 1,25(OH)$_2$D$_3$+25(OH)D$_3$: 4.2-fold increase, $p<0.0005$, 1,25(OH)$_2$D$_3$: 2.8-fold increase, $p<0.0005$; HaCaT after 24 h: 1,25(OH)$_2$D$_3$+25(OH)D$_3$: 2.4-fold increase, $p<0.0005$, 1,25(OH)$_2$D$_3$: 2.3-fold increase, $p<0.005$; SCL-1 after 6 h: 1,25(OH)$_2$D$_3$+25(OH)D$_3$: 1.4-fold increase, $p<0.0005$, 1,25(OH)$_2$D$_3$: 1.1-fold increase; SCL-1 after 24 h: 1,25(OH)$_2$D$_3$+25(OH)D$_3$: 2.1-fold increase, $p<0.0005$, 1,25(OH)$_2$D$_3$: 1.4-fold increase, $p<0.0005$) (Figure 4). Treatment with UVB up-regulated *COX2* gene expression in both cell lines to a similar degree (HaCaT: 5.4-fold increase; SCL-1: 3.9-fold increase, $p \leq 0.05$). Combination treatment with UVB and 1,25(OH)$_2$D$_3$ exerted a synergistic effect only in HaCaT (38.3-fold increase, $p<0.05$) and not in SCL-1 cells (4.2-fold increase, $p \leq 0.05$) (Figure 2E and F).

1,25(OH)$_2$D$_3$, but not UVB, induces CYP24A1 mRNA in HaCaT and SCL-1 cells. *CYP24A1* mRNA expression was increased in HaCaT and SCL-1 cells, after treatment with 1,25(OH)$_2$D$_3$, but not after treatment with UVB (Figure 2I and J). Co-treatment with 1,25(OH)$_2$D$_3$ and 25(OH)D$_3$ induced *CYP24A1* mRNA in SCL-1 cells even stronger than

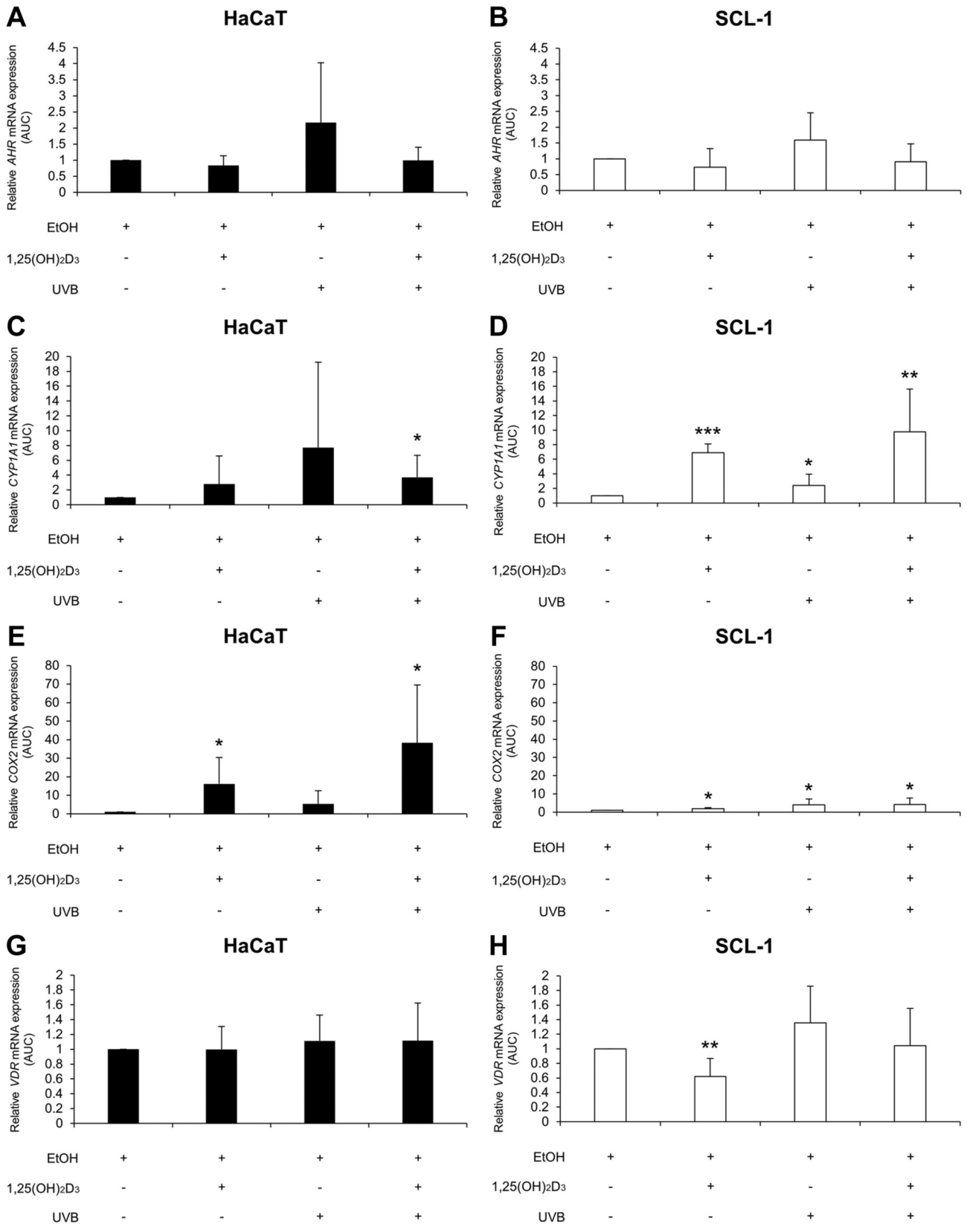

Figure 2. *Continued*

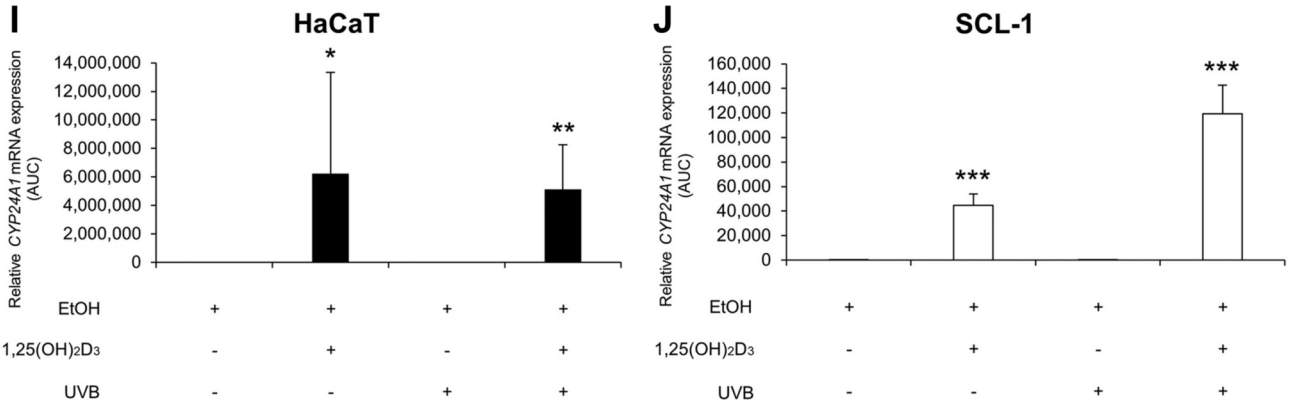

Figure 2. *Relative mRNA expression (AUC) of AHR (A, B), CYP1A1 (C, D), COX2 (E, F), VDR (G, H), and CYP24A1 (I, J) relative to the mean of GAPDH and β-actin in treated HaCaT and SCL-1 cells (mean $2^{-\Delta\Delta Ct}$). After treatment, cells were harvested in 6 h intervals over 24 h. The mRNA expression was measured with RTqPCR and the relative fold gene expression was calculated with the $2^{-\Delta\Delta Ct}$ method. Cells harvested after 0 h were used as the internal control sample for each time curve (data not shown). The "area under the curve" of every treatment condition was measured and set relative to the "area under the curve" of the EtOH-treated cells (vehicle). The values represent the means±SD of duplicate assays. The experiments were repeated thrice with similar results. All p-values are relative to cells treated with EtOH alone. *p≤0.05; **p<0.005; ***p<0.0005.*

$1,25(OH)_2D_3$ alone (after 6 h: $1,25(OH)_2D_3$+$25(OH)D_3$: 133.1-fold increase, $p<0.0005$, $1,25(OH)_2D_3$: 82.7-fold increase, $p<0.0005$; after 24 h: $1,25(OH)_2D_3$+$25(OH)D_3$: 123.5-fold increase, $p<0.0005$, $1,25(OH)_2D_3$: 94.9-fold increase, $p<0.0005$) (Figure 5). In contrast, expression of *VDR* was only altered in SCL-1 cells treated with $1,25(OH)_2D_3$ (38% decrease, $p<0.005$), while other treatments with $1,25(OH)_2D_3$ and/or UVB had only marginal effects in HaCaT or SCL-1 (Figure 2G and H). Combined treatment with UVB further enhanced the $1,25(OH)_2D_3$-induced increase in *CYP24A1* mRNA exclusively in SCL-1 [$1,25(OH)_2D_3$: 44,703.5-fold increase, $p<0.0005$, $1,25(OH)_2D_3$+UVB: 119,233.4-fold increase, $p<0.0005$], but not in HaCaT cells [$1,25(OH)_2D_3$: 6,233,471-fold increase, $p<0.05$, $1,25(OH)_2D_3$ +UVB: 5,127,778.3-fold increase, $p<0.005$].

Discussion

During the last decades, a continuously growing body of evidence has convincingly shown an important role of vitamin D in carcinogenesis and the progression of many malignancies (85-87). It can be speculated that during the next years, these new scientific findings in the vitamin D field, which include the identification of *AHR*, RORs, and LXR as alternative receptors for vitamin D compounds (69, 88, 89), will have a great impact on the prevention and therapy of cancer. It was the aim of this study to understand the role of the vitamin D endocrine system in the multistep process of skin photo-carcinogenesis (90), that shows characteristic early (*e.g.,* initiated cells) and late (*e.g.*, cells that express the complete malignant phenotype) stages (72-79). In particular, we investigated the molecular interaction of two different nuclear receptor pathways for vitamin D, which are activated either by binding of the classical biologically active vitamin D metabolite, $1,25(OH)_2D_3$, to the *VDR*, or by binding of non-classical vitamin D hydroxyderivatives [*e.g.,* $20,23(OH)_2D_3$] to the *AHR*.

By analyzing the expression of *AHR*, *CYP1A1*, and *COX2* as well as of *VDR* and *CYP24A1*, we showed that the expression of genes encoding for key elements of both *VDR* and *AHR* pathways are differentially expressed and regulated during different stages of skin carcinogenesis. For example, expression of *AHR* and *CYP1A1* was much stronger in untreated HaCaT as compared to untreated SCL-1 cells, while in contrast, expression of *VDR*, *CYP24A1*, and *COX2* was stronger in untreated SCL-1 as compared to HaCaT cells. It remains to be investigated in future studies, whether stage-dependent differences in the expression of key elements of these different nuclear signaling pathways for vitamin D compounds contribute to the carcinogenesis of non-melanoma skin cancer. It may be speculated that these findings are caused by functional changes associated to the p53 status in HaCaT (p53 mutation, p53mut) and SCL-1 (no p53 protein present, p53null) cells, as previous studies have reported a p53-mediated tissue-dependent regulation of *AHR* (91-93) and *VDR* (94, 95) signaling. Moreover, it can be speculated whether low basal levels of *AHR* and *CYP1A1* in SCL-1, and of *VDR*, *CYP24A1*, and *COX2* in HaCaT cells may point at a functional defect of *AHR* signaling in SCL-1 and of *VDR* signaling in HaCaT cells.

To further investigate the interaction between *AHR* and *VDR* signaling, we treated cells with UVB, and/or the VDR-ligand $1,25(OH)_2D_3$, its precursor $25(OH)D_3$ [that has been described as a partial VDR-antagonist (83)], and the AHR-

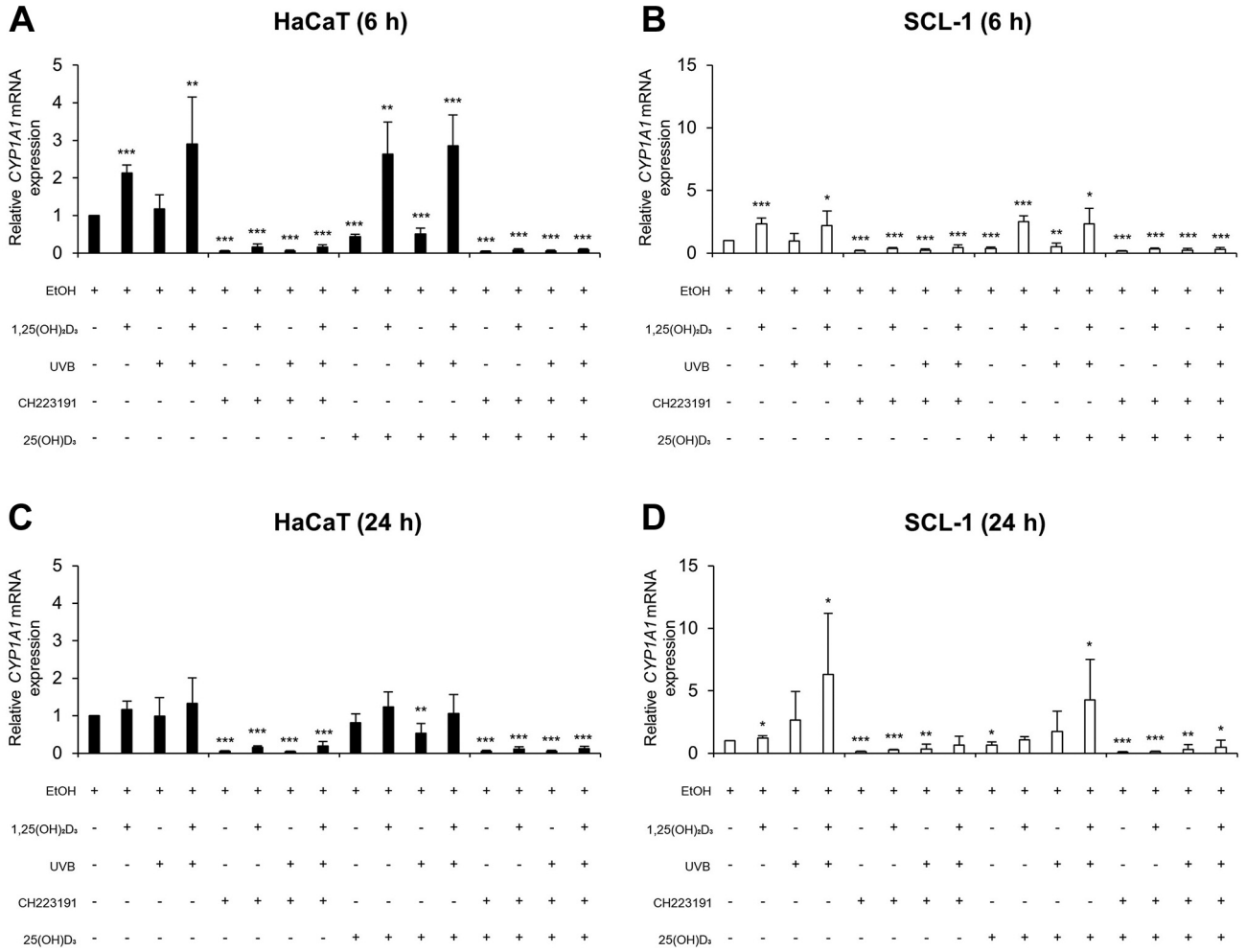

Figure 3. *Relative mRNA expression of CYP1A1 in HaCaT and SCL-1 cells 6 h (A, B) and 24 h (C, D) after treatment relative to the mean of GAPDH and β-actin (mean $2^{-\Delta\Delta Ct}$). Cells were harvested 6 and 24 h after treatment. The mRNA expression was measured with RTqPCR and the relative fold gene expression was calculated with the $2^{-\Delta\Delta Ct}$ method. Cells treated with solvent vehicle alone (EtOH) were used as the internal control. The values represent the means±SD of duplicate assays. The experiments were repeated thrice with similar results. All p-values are relative to cells treated with EtOH alone. *p≤0.05; **p<0.005; ***p<0.0005.*

antagonist CH223191 (81, 82). It has to be noted that we did not succeed in obtaining the non-classical hydroxyderivatives [*e.g.*, 20,23$(OH)_2D_3$] of vitamin D that were recently described as AHR-ligands.

This study also examined whether effects of UVB on expression of *AHR* target genes may be mediated *via* the UVB-induced cutaneous synthesis of 1,25$(OH)_2D_3$ or 25$(OH)D_3$, indicating that oral supplementation with vitamin D could compensate for the effects of UVB both on *AHR* and *VDR* signaling pathways. Until now, only a few studies have analyzed the effects of 1,25$(OH)_2D_3$ on the expression of *AHR* target genes; however, studies in cutaneous SCC cells are lacking (68, 69). We here show that the complete carcinogen UVB and the anti-carcinogenic agent 1,25$(OH)_2D_3$ exert different effects on the expression of key elements of the *VDR* and *AHR* pathways. Although the results of our investigation do not allow definite conclusions, these findings do not support the assumption that effects of UVB on *CYP1A1* expression are mediated *via* UVB-induced cutaneous production of 1,25$(OH)_2D_3$.

We showed that the expression of genes encoding for proteins that contribute to *AHR* signaling is regulated differentially by UVB in HaCaT and SCL-1 cells, representing keratinocytes that reveal phenotype characteristics for early and late stages of skin carcinogenesis, respectively. *CYP1A1* mRNA was regulated differentially by 1,25$(OH)_2D_3$ and UVB in HaCaT and SCL-1 cells. In SCL-1, induction of *CYP1A1* mRNA was stronger after treatment with 1,25$(OH)_2D_3$ (6.9-fold induction compared to control) as compared to treatment

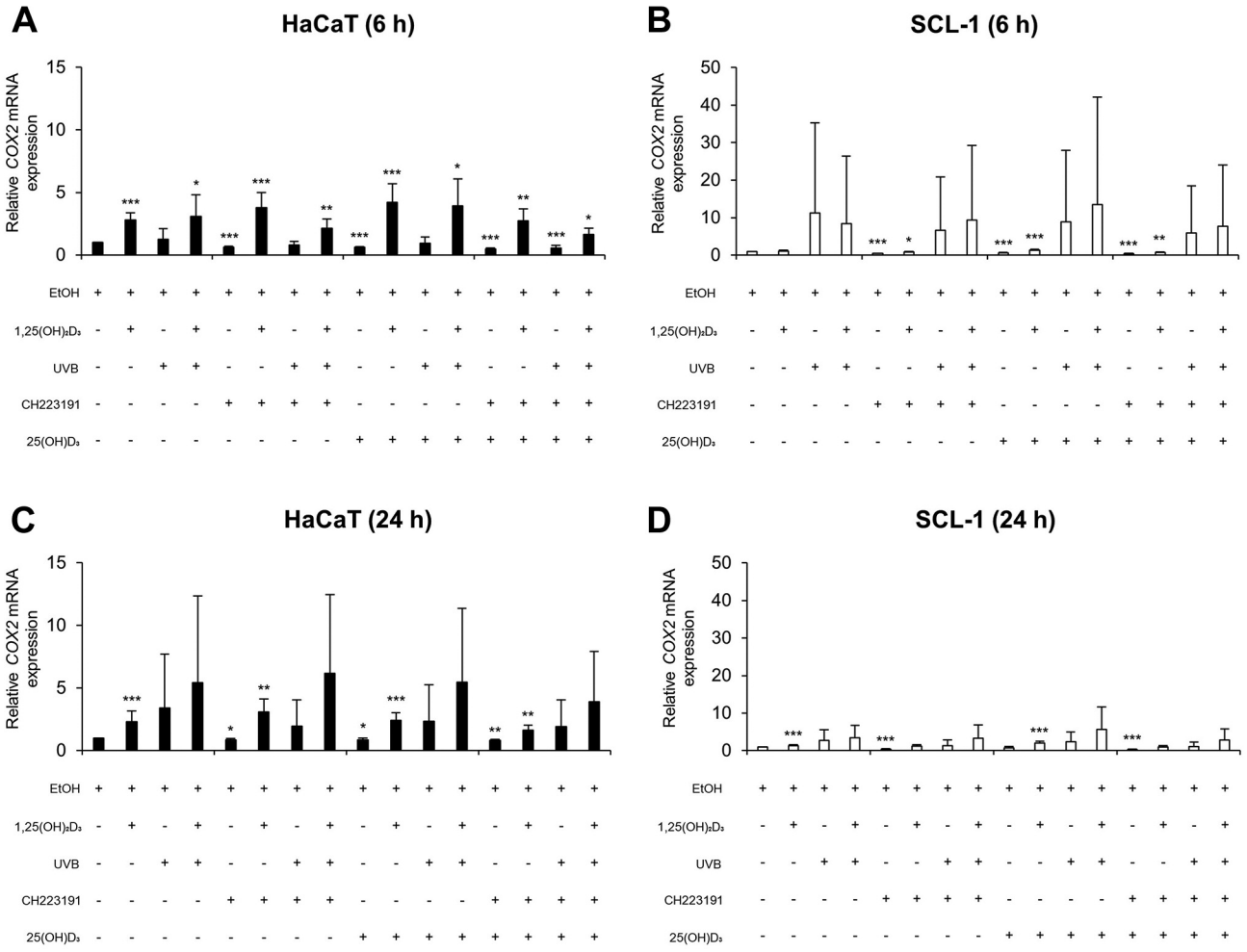

Figure 4. *Relative mRNA expression of COX2 in HaCaT and SCL-1 cells 6 h (A, B) and 24 h (C, D) after treatment relative to the mean of GAPDH and β-actin (mean $2^{-\Delta\Delta Ct}$). Cells were harvested 6 and 24 h after treatment. The mRNA expression was measured with RTqPCR and the relative fold gene expression was calculated with the $2^{-\Delta\Delta Ct}$ method. Cells treated with solvent vehicle alone (EtOH) were used as the internal control. The values represent the means±SD of duplicate assays. The experiments were repeated thrice with similar results. All p-values are relative to cells treated with EtOH alone. *p≤0.05; **p<0.005; ***p<0.0005.*

with UVB (2.4-fold induction compared to control); however, opposite effects were seen in HaCaT cells. In these cells, induction of *CYP1A1* mRNA was stronger after treatment with UVB (7.7-fold induction compared to control) as compared to that after treatment with $1,25(OH)_2D_3$ (2.8-fold induction compared to control).

It has been reported that *CYP1A1* induction can be mediated *via* several independent mechanisms that include elevation of intracellular calcium and subsequent cell differentiation (96-98), or involve other nuclear receptors (NR). It is well established that $1,25(OH)_2D_3$ plays a crucial role in calcium homeostasis (99-101) and promotes differentiation in cultured skin cells (102) and cancer cells like human colon cancer, CAFs, and CSCs (103). Thus, $1,25(OH)_2D_3$ could regulate the *CYP1A1* mRNA activity through its pro-differentiating effect. Interestingly, combination treatment with $1,25(OH)_2D_3$ and UVB increased *CYP1A1* mRNA activity even further. *In vivo* studies report, that even minimal UVB radiation levels of 18 mJ/cm^2 are enough to activate $1,25(OH)_2D_3$ synthesis in the skin (104). An enhancing effect of UVB radiation on $1,25(OH)_2D_3$-induced *CYP1A1* mRNA expression through additional endogenous $1,25(OH)_2D_3$ production could therefore be considered. However, our results do not exclude the possibility that induction of *CYP1A1* may be induced *AHR* independently *via* other mechanisms that may include the activation and involvement of other NR pathways. The Pregnane X receptor (PXR), a member of the nuclear

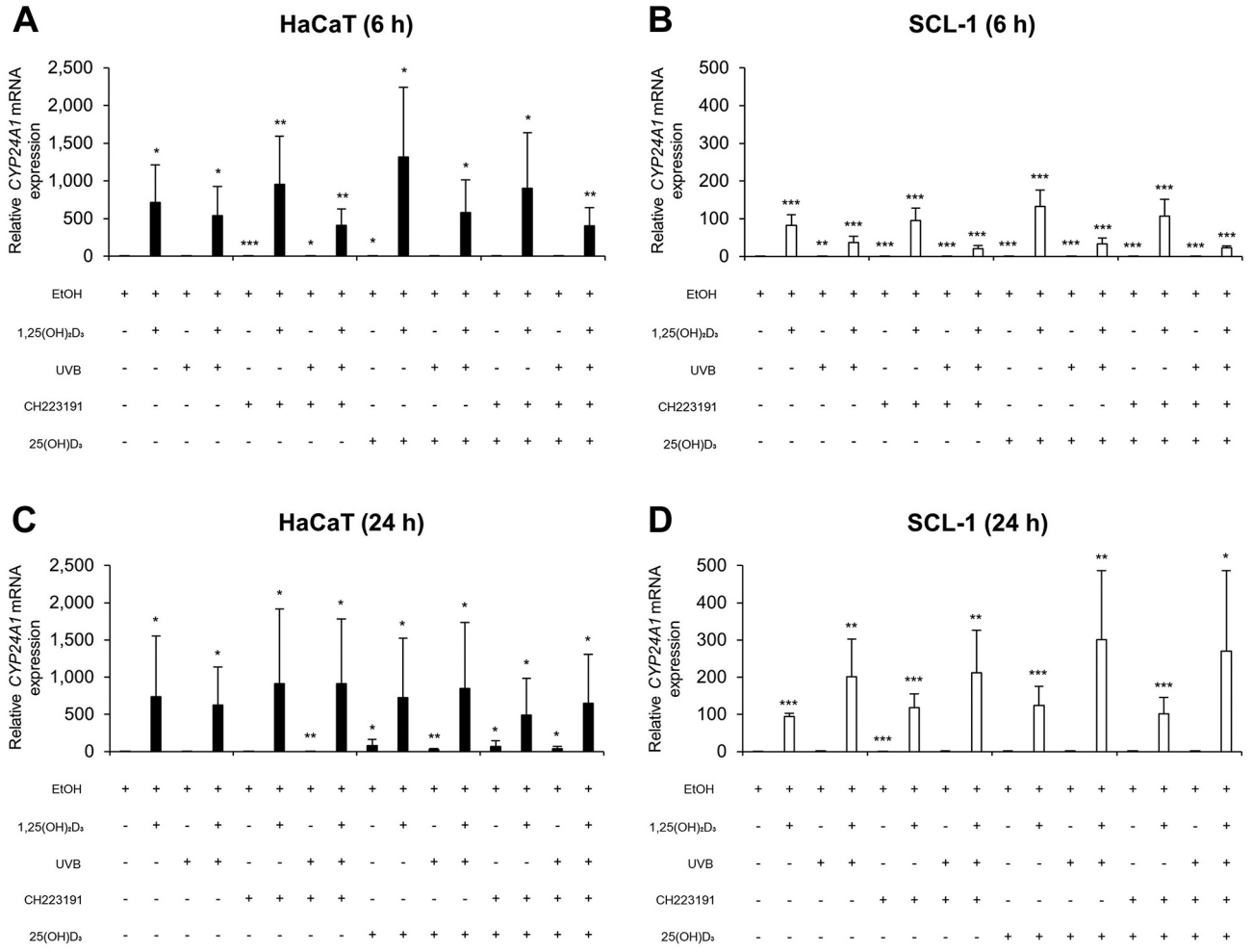

Figure 5. *Relative mRNA expression of CYP24A1 in HaCaT and SCL-1 cells 6 h (A, B) and 24 h (C,D) after treatment relative to the mean of GAPDH and β-actin (mean $2^{-\Delta\Delta Ct}$). Cells were harvested 6 and 24 h after treatment. The mRNA expression was measured with RTqPCR and the relative fold gene expression was calculated with the $2^{-\Delta\Delta Ct}$ method. Cells treated with solvent vehicle alone (EtOH) were used as the internal control. The values represent the means±SD of duplicate assays. The experiments were repeated thrice with similar results. All p-values are relative to cells treated with EtOH alone. *p≤0.05; **p<0.005; ***p<0.0005.*

hormone receptor family and regulator of xenobiotic and drug metabolism (105-107), that was linked to the development of SCC (108), was found to have similarities to both the *AHR* (109, 110) and *VDR* (111, 112), and was shown to regulate expression of *CYP1A1* (113).

Interestingly, Wilkens *et al*. (114) were able to demonstrate that intravenous administration of $1,25(OH)_2D_3$ up-regulates the mRNA expression of PXR in sheep renal tissue. Other CYP1A1-regulating NRs (115) including the Glucocorticoid receptor (GR), Estrogen receptor (ER) and Retinoid acid receptor (RAR) have also shown interactions with $1,25(OH)_2D_3$ (69, 116, 117).

In agreement with previously published reports (82), exposure of HaCaT cells to a single dose UVB (50 J/cm^2) induced expression of *AHR* and *CYP1A1* mRNA, indicating that UVB-induced regulation of *AHR* signaling functions correctly in these cells.

$1,25(OH)_2D_3$ in a dose of 10^{-7} M had no conspicuous regulating effect on *AHR* expression in HaCaT and SCL-1 cells. These findings are in agreement with the results of Slominski *et al*. (69), who reported in epidermal keratinocytes a dose-dependent, $1,25(OH)_2D_3$-induced *AHR* expression, that was detected after treatment with $1,25(OH)_2D_3$ at a dose of 10^{-6} M, but not at a dose of 10^{-7} M.

In SCL-1 cells, UVB (50 J/cm^2) induced expression of *AHR* and *CYP1A1*. *CYP1A1* mRNA induction after treatment with $1,25(OH)_2D_3$ was almost 7 times higher than that of the control group and 2.5 times stronger than that after UVB

treatment. Until now, only a few studies have reported an effect of $1,25(OH)_2D_3$ on the *AHR* target gene; however, none of them was carried out in cutaneous SCC (68, 69).

To analyze whether the induction of *CYP1A1* mRNA in HaCaT and SCL-1 cells was AHR-dependent, we used CH223191 (AHR-antagonist). Treatment with CH223191 suppressed *CYP1A1* mRNA expression both in HaCaT and SCL-1 cells under all experimental conditions.

It is well established that $1,25(OH)_2D_3$ exerts its cancer-inhibiting activity in many cell types through various direct (*e.g.,* regulation of the cell cycle, induction of apoptosis, inhibition of angiogenesis and tumor-invasiveness, -metastasis and -proliferation) and indirect (*e.g.,* regulation of immuno-modulation, effect on tumor microenvironment) mechanisms (95, 118). Although AHR-activated *CYP1A1* is associated with pro-carcinogen transformation and cancer development, some studies documented a contribution to cancer prevention (27). Therefore, key elements of *AHR* signaling may at least in part contribute to $1,25(OH)_2D_3$-mediated anti-cancer mechanisms.

Notably, it has been shown that $1,25(OH)_2D_3$ modulates the cell cycle through checkpoint regulation (118). Binding to the promoter region of genes encoding p21 and p27 results in cyclin dependent kinase (CDK) inhibition and cell cycle arrest in the G_1 phase *via* decreased cyclin D1 expression (95, 118). Interestingly, ligand-dependent *AHR* activation was also found to increase p21 and p27 expression in addition to *CYP1A1*, resulting in G_1 phase cell cycle arrest (119, 120). Another mechanism that may be involved in $1,25(OH)_2D_3$-induced cell cycle regulation is executed through activation of distinct molecular pathways including intracellular kinase pathways (*e.g.,* ERK, PI3K), pathways of transforming growth factor β (TGF-β) and of insulin-like growth factor-binding proteins (IGF-BP), which are found to interact with *AHR* signaling (121-127). Moreover, $1,25(OH)_2D_3$ induces protein kinase C (PKC) activation, which plays an important role in the regulation of gene expression, cell differentiation, mobility, and metastasis. The subsequently induced mitogen-activated kinases 1 and 2 (MAPK1 and MAPK2) are regulators of cell growth (95) and of transcription factors as well as co-regulatory and chromatin proteins in malignant melanoma (128). Recent studies demonstrated, that PKC activity is required for classical AHR-mediated signaling in a tissue-dependent manner (129). MAPKs induced by TCDD were also found to be important for the induction of AHR-dependent gene transcription and *CYP1A1* expression (130).

Inhibition of angiogenesis represents another anti-tumor mechanism exerted by $1,25(OH)_2D_3$ (95). Through interaction with nuclear factor kappaB (NF-κB), inhibition of Interleukin-8 (IL-8) transcription is achieved. Suppression of growth factors like vascular endothelial growth factor (VEGF) or platelet-derived growth factor (PDGF) and of hypoxia inducible factor 1 alpha (HIF1α) also seems to be an important part of this process (131). Tight interactions between NF-κB and *AHR* signaling have been studied in various immune cells contributing to xenobiotic metabolism and carcinogenesis. *AHR* has been found to modulate peptidoglycan (PGN)-induced expression of IL-8 in human sebocytes involving the NF-κB pathway (132). Another finding showed that after UVB irradiation, NF-κB preliminary suppressed *CYP1A1* expression, indicating a role of NF-κB in UVB-dependent *AHR* signaling and potentially in a photo-protective cellular response (133).

The Hedgehog (Hh) signaling pathway, whose inappropriate activation is associated with cancer stimulation and progression (134, 135), represents another target of the cancer-inhibitory function of $1,25(OH)_2D_3$. Different types of human cancer, including skin BCC, have been linked to deregulation of Hh signaling caused by gene mutations or uncontrolled Hh ligand production (135, 136). $1,25(OH)_2D_3$ inhibits Hh-induced proliferation and signaling through modulation of Hh target gene GLI1 (137). Contrary to its previous described cancer-promoting properties, *AHR* signaling was found to inhibit the Hh pathway *in vivo* in medulloblastoma and was identified as a potent tumour suppressor (138). It remains to be clarified how *CYP1A1* is involved in the regulation of Hh signaling and whether it participates in the execution of tumor-suppressive functions.

Indirect anti-cancer effects of $1,25(OH)_2D_3$ mainly concern the tumor microenvironment. They include modulation of immune mediators [*e.g.,* DNA methylation of CpG regions, production of Interleukin-1β (IL-1β) and tumor necrosis factor-α (TNF-α)], cancer-associated metabolic cascades (*e.g.,* Inhibition of estrogenic signaling through down-regulation of CYP19A1, suppression of 27-hydroxycholesterol (27HC) and CYP27A1), and homeostatic processes in surrounding tissues (*e.g.,* down-regulation of pyruvate carboxylase, up-regulation of CYP3A4, reduction of AMP hydrolysis and adenosine production) (118). Interestingly, some of these effects may also be related to key players of *AHR* signaling. In human salivary cells, TNF-α significantly induced *AHR* along with *CYP1A1* expression, whereas IL-1β did not affect *AHR* or *CYP1A1* mRNA levels (139). Additionally, *AHR* inhibits ER activity in human breast cancer cells, rodent uterus, and mammary tumors (140). *AHR* activators PAHs and TCDD increased CYP3A4 mRNA expression in HepG2 cells (141). CYP3A4 induction by xenobiotics largely depends on PXR, which tightly regulates CYP3A4 expression (142). As there is ample evidence for an interaction between PXR and *AHR* (143-145), a possible role for *AHR* signaling in the regulation of the *CYP3A4* gene is conceivable.

Additionally, cells were treated with $25(OH)D_3$ (partly VDR-inhibitor) to determine whether the *CYP1A1* mRNA induction was VDR-dependent. Co-treatment with $25(OH)D_3$ induced and suppressed *CYP1A1* gene expression in

$1,25(OH)_2D_3$- and in EtOH-treated cells, respectively. In conclusion, these findings indicate that $25(OH)D_3$ does not act as a partly *VDR* inhibitor, at least in HaCaT cells. As $25(OH)D_3$ is converted in epidermal keratinocytes to $1,25(OH)_2D_3$ by the 1-alpha-hydroxylase (*CYP27B1*) (146-148), it could exert agonist activity and intensify the effect of $1,25(OH)_2D_3$ on *CYP1A1*. Moreover, the mechanisms by which $25(OH)D_3$ suppresses *CYP1A1* mRNA expression in these cells remain to be elucidated.

We here show that transcriptional activity of *CYP24A1* is significantly up-regulated after $1,25(OH)_2D_3$ treatment, while *VDR* mRNA expression is only marginally altered (HaCaT) or even down-regulated (SCL-1) when compared to the control group. It was reported that *CYP24A1*, the major metabolizing enzyme of $1,25(OH)_2D_3$, is elevated in many human tumor tissues (149, 150) including non-melanoma skin cancer (151) and is associated with poor prognosis in various cancer types (152). Thus, *CYP24A1* has been considered a possible oncogene (66). Consistent with these findings, our study demonstrated that mRNA expression of *CYP24A1* was stronger in untreated SCL-1 cells, revealing the complete phenotype of malignant transformation, as compared to precancerous HaCaT cells. Increased levels of *CYP24A1* mRNA could lead to rapid inactivation of $1,25(OH)_2D_3$, resulting in abolition of its antiproliferative effects against cancer. It would therefore be conceivable, that the high transcriptional level of *CYP24A1* is not due to up-regulation by the physiological $1,25(OH)_2D_3$/VDR signaling pathway, but rather to over-expression of the gene. Identically to *CYP1A1*, the combination of $1,25(OH)_2D_3$ and UVB increased *CYP24A1* transcriptional activity in SCL-1 cells even more.

Notably, $1,25(OH)_2D_3$ induced *CYP24A1* expression 100 times stronger in HaCaT as compared to SCL-1 cells. The p53 status of HaCaT (p53mut) and SCL-1 (p53null) keratinocytes and the crosstalk of p53 with *VDR* may contribute to this finding. Under physiological conditions, the p53 protein protects cells from DNA damage by several mechanisms (*e.g.*, apoptosis induction, cell cycle progression halting, cellular aging) (153). In several tumor types, the cancer-associated and mutated p53 (p53mut) has been found to exert new mechanisms that have been termed gain-of-function (GOF), enabling it to act at the molecular level in a similar way to $1,25(OH)_2D_3$ (95). It has been shown that p53mut is able to interact with the *VDR*, modulate the expression of VDR-regulated genes and enhance the nuclear *VDR* translocation and accumulation (154). As these effects were even more prominent after $1,25(OH)_2D_3$ supplementation, a mechanism leading to stronger *VDR* target gene expression in p53 mutated cells might be plausible. Importantly, p53mut reversed the impact of $1,25(OH)_2D_3$ on cell death and converted it from a pro-apoptotic to an anti-apoptotic agent. Thus, p53 status may alter the biological function of $1,25(OH)_2D_3$ in precancerous and cancerous skin cells and deregulate the anti-cancer effects of the *VDR* pathway. As p53 also modulates *AHR* target genes like *CYP1A1* (93) and *COX2* (155-157), it could be speculated that the *AHR* pathway may exert a similar effect in cooperation with $1,25(OH)_2D_3$. However, this assumption could only be confirmed for *COX2*, as its mRNA expression after $1,25(OH)_2D_3$ treatment was 9 times stronger in HaCaT cells than in SCL-1 cells.

In addition to its anti-apoptotic activity also induced by TNF-α, TNF-related apoptosis-inducing ligand (TRAIL) and Fas ligand (FasL), $1,25(OH)_2D_3$ has been described to increase cell survival after UV damage and protect some cancer cell lines against cytotoxic drugs (154, 158). However, several other studies have shown contradictory results regarding the association between $1,25(OH)_2D_3$ and skin tumorigenesis (95, 118), questioning the exclusivity of the anti-cancer properties of $1,25(OH)_2D_3$ in the skin and speculating about possibly harmful and cancer-promoting effects.

In summary, we here show differential regulation of AHR- and VDR-mediated signaling in HaCaT as compared with SCL-1 cells and after treatment with UVB as compared with $1,25(OH)_2D_3$. In conclusion, our data indicate that the complex network of AHR- and VDR-mediated signaling may contribute to the photo-carcinogenesis of non-melanoma skin cancer. Treatment of keratinocytes with UVB exerts additional biological effects in human skin cells as compared to treatment with $1,25(OH)_2D_3$. These findings imply that oral uptake of vitamin D (*e.g.*, by food or supplements) cannot compensate for all effects of UVB on human health, that include effects of non-classical, AHR-activating vitamin D derivatives. However, the exact mechanisms behind this are yet not fully understood. Further investigations are required to demonstrate the underlying pathophysiological relevance of our results. Advanced detection and assay methods, other malignant and non-malignant skin cell lines and CYP1A1-related signaling pathways, extended examination time points as well as multiple $1,25(OH)_2D_3$ concentrations and UVB doses should be considered, in order to eventually open new perspectives regarding the prevention and treatment of skin cancer.

Conflicts of Interest

Prof. Reichrath is member of the Arnold Rikli-Award Jury of the Jörg Wolff Foundation. The Saarland University, together with Prof. Reichrath as one of several responsible group leaders, has received a research grant from the Jörg Wolff Foundation, Stuttgart, Germany.

Authors' Contributions

Christoforos Christofi: Study design, literature search, experimental implementation, data analysis, manuscript preparation; Leandros Lamnis: Study design, literature search, experimental implementation; Alexandra Stark: Experimental implementation,

data analysis; Heike Palm: data analysis; Klaus Römer: data analysis; Thomas Vogt: Study design; Jörg Reichrath: Study design, literature search, manuscript preparation.

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References

1 Tilgen W, Rass K and Reichrath J: 30 Jahre dermatologische Onkologie. Aktuelle Dermatologie *31(3)*: 79-88, 2017. DOI: 10.1055/s-2004-826050

2 Elmets CA and Athar M: Milestones in photocarcinogenesis. J Invest Dermatol *133(E1)*: E13-E17, 2013. PMID: 23820719. DOI: 10.1038/skinbio.2013.179

3 Ratushny V, Gober MD, Hick R, Ridky TW and Seykora JT: From keratinocyte to cancer: the pathogenesis and modeling of cutaneous squamous cell carcinoma. J Clin Invest *122(2)*: 464-472, 2012. PMID: 22293185. DOI: 10.1172/JCI57415

4 Marks R: An overview of skin cancers. Incidence and causation. Cancer *75(2 Suppl)*: 607-612, 1995. PMID: 7804986. DOI: 10.1002/1097-0142(19950115)75:2+<607::aid-cncr2820751402>3.0.co;2-8

5 Salasche SJ: Epidemiology of actinic keratoses and squamous cell carcinoma. J Am Acad Dermatol *42(1 Pt 2)*: 4-7, 2000. PMID: 10607349. DOI: 10.1067/mjd.2000.103342

6 Devary Y, Rosette C, DiDonato JA and Karin M: NF-kappa B activation by ultraviolet light not dependent on a nuclear signal. Science *261(5127)*: 1442-1445, 1993. PMID: 8367725. DOI: 10.1126/science.8367725

7 Rosette C and Karin M: Ultraviolet light and osmotic stress: activation of the JNK cascade through multiple growth factor and cytokine receptors. Science *274(5290)*: 1194-1197, 1996. PMID: 8895468. DOI: 10.1126/science.274.5290.1194

8 Herrlich P, Ponta H and Rahmsdorf HJ: DNA damage-induced gene expression: signal transduction and relation to growth factor signaling. Rev Physiol Biochem Pharmacol *119*: 187-223, 1992. PMID: 1604153. DOI: 10.1007/3540551921_7

9 Fritsche E, Schäfer C, Calles C, Bernsmann T, Bernshausen T, Wurm M, Hübenthal U, Cline JE, Hajimiragha H, Schroeder P, Klotz LO, Rannug A, Fürst P, Hanenberg H, Abel J and Krutmann J: Lightening up the UV response by identification of the arylhydrocarbon receptor as a cytoplasmatic target for ultraviolet B radiation. Proc Natl Acad Sci U.S.A. *104(21)*: 8851-8856, 2007. PMID: 17502624. DOI: 10.1073/pnas.0701764104

10 Kewley R, Whitelaw M and Chapman-smith A: The mammalian basic helix-loop-helix/PAS family of transcriptional regulators. The International Journal of Biochemistry & Cell Biology *36(2)*: 189-204, 2021. DOI: 10.1016/S1357-2725(03)00211-5

11 Nebert DW and Gonzalez FJ: P450 genes: structure, evolution, and regulation. Annu Rev Biochem *56*: 945-993, 1987. PMID: 3304150. DOI: 10.1146/annurev.bi.56.070187.004501

12 Mimura J and Fujii-kuriyama Y: Functional role of AhR in the expression of toxic effects by TCDD. Biochimica et Biophysica Acta (BBA) - General Subjects *1619(3)*: 263-268, 2022. DOI: 10.1016/S0304-4165(02)00485-3

13 Denison MS and Heath-Pagliuso S: The Ah receptor: a regulator of the biochemical and toxicological actions of structurally diverse chemicals. Bull Environ Contam Toxicol *61(5)*: 557-568, 1998. PMID: 9841714. DOI: 10.1007/pl00002973

14 Poland A and Knutson JC: 2,3,7,8-tetrachlorodibenzo-p-dioxin and related halogenated aromatic hydrocarbons: examination of the mechanism of toxicity. Annu Rev Pharmacol Toxicol *22*: 517-554, 1982. PMID: 6282188. DOI: 10.1146/annurev.pa.22.040182.002505

15 Safe S: Polychlorinated biphenyls (PCBs), dibenzo-p-dioxins (PCDDs), dibenzofurans (PCDFs), and related compounds: environmental and mechanistic considerations which support the development of toxic equivalency factors (TEFs). Crit Rev Toxicol *21(1)*: 51-88, 1990. PMID: 2124811. DOI: 10.3109/10408449009089873

16 Denison M, Seidel S, Rogers W, Ziccardi M, Winter G and Heath-Pagliuso S: Natural and synthetic ligands for the Ah receptor. In: Puga A, Wallace KB (eds). Molecular Biology Approaches to Toxicology. Philadelphia, PA, Taylor & Francis, pp. 393-410, 1998.

17 Zhang S, Qin C and Safe SH: Flavonoids as aryl hydrocarbon receptor agonists/antagonists: effects of structure and cell context. Environ Health Perspect *111(16)*: 1877-1882, 2003. PMID: 14644660. DOI: 10.1289/ehp.6322

18 Ciolino HP, Daschner PJ and Yeh GC: Dietary flavonols quercetin and kaempferol are ligands of the aryl hydrocarbon receptor that affect CYP1A1 transcription differentially. Biochem J *340 (Pt 3)*: 715-722, 1999. PMID: 10359656.

19 Esser C, Bargen I, Weighardt H, Haarmann-Stemmann T and Krutmann J: Functions of the aryl hydrocarbon receptor in the skin. Semin Immunopathol *35(6)*: 677-691, 2013. PMID: 23949496. DOI: 10.1007/s00281-013-0394-4

20 Haas K, Weighardt H, Deenen R, Köhrer K, Clausen B, Zahner S, Boukamp P, Bloch W, Krutmann J and Esser C: Aryl hydrocarbon receptor in keratinocytes is essential for murine skin barrier integrity. J Invest Dermatol *136(11)*: 2260-2269, 2016. PMID: 27430407. DOI: 10.1016/j.jid.2016.06.627

21 Whitlock JP Jr: Induction of cytochrome P4501A1. Annu Rev Pharmacol Toxicol *39*: 103-125, 1999. PMID: 10331078. DOI: 10.1146/annurev.pharmtox.39.1.103

22 Rao MS, Subbarao V, Prasad JD and Scarpelli DG: Carcinogenicity of 2,3,7,8-tetrachlorodibenzo-p-dioxin in the Syrian golden hamster. Carcinogenesis *9(9)*: 1677-1679, 1988. PMID: 3409472. DOI: 10.1093/carcin/9.9.1677

23 Guengerich FP and Shimada T: Oxidation of toxic and carcinogenic chemicals by human cytochrome P-450 enzymes. Chem Res Toxicol *4(4)*: 391-407, 1991. PMID: 1912325. DOI: 10.1021/tx00022a001

24 Shimizu Y, Nakatsuru Y, Ichinose M, Takahashi Y, Kume H, Mimura J, Fujii-Kuriyama Y and Ishikawa T: Benzo[a]pyrene carcinogenicity is lost in mice lacking the aryl hydrocarbon receptor. Proc Natl Acad Sci U.S.A. *97(2)*: 779-782, 2000. PMID: 10639156. DOI: 10.1073/pnas.97.2.779

25 Lira MG, Provezza L, Malerba G, Naldi L, Remuzzi G, Boschiero L, Forni A, Rugiu C, Piaserico S, Alaibac M, Turco A, Girolomoni G and Tessari G: Glutathione S-transferase and CYP1A1 gene polymorphisms and non-melanoma skin cancer

risk in Italian transplanted patients. Exp Dermatol *15(12)*: 958-965, 2006. PMID: 17083362. DOI: 10.1111/j.1600-0625.2006.00500.x

26 Lear JT, Heagerty AH, Smith A, Bowers B, Payne CR, Smith CA, Jones PW, Gilford J, Yengi L, Alldersea J, Fryer AA and Strange RC: Multiple cutaneous basal cell carcinomas: glutathione S-transferase (GSTM1, GSTT1) and cytochrome P450 (CYP2D6, CYP1A1) polymorphisms influence tumour numbers and accrual. Carcinogenesis *17(9)*: 1891-1896, 1996. PMID: 8824510. DOI: 10.1093/carcin/17.9.1891

27 Androutsopoulos VP, Tsatsakis AM and Spandidos DA: Cytochrome P450 CYP1A1: wider roles in cancer progression and prevention. BMC Cancer *9*: 187, 2009. PMID: 19531241. DOI: 10.1186/1471-2407-9-187

28 Bender K, Blattner C, Knebel A, Iordanov M, Herrlich P and Rahmsdorf HJ: UV-induced signal transduction. J Photochem Photobiol B *37(1-2)*: 1-17, 1997. PMID: 9043093. DOI: 10.1016/s1011-1344(96)07459-3

29 Kulms D, Pöppelmann B, Yarosh D, Luger TA, Krutmann J and 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 U.S.A. *96(14)*: 7974-7979, 1999. PMID: 10393932. DOI: 10.1073/pnas.96.14.7974

30 Tyrrell RM: Activation of mammalian gene expression by the UV component of sunlight—from models to reality. Bioessays *18(2)*: 139-148, 1996. PMID: 8851047. DOI: 10.1002/bies.950180210

31 Tyrrell RM: UV activation of mammalian stress proteins. EXS *77*: 255-271, 1996. PMID: 8856979.

32 Stege H, Roza L, Vink AA, Grewe M, Ruzicka T, Grether-Beck S and Krutmann J: Enzyme plus light therapy to repair DNA damage in ultraviolet-B-irradiated human skin. Proc Natl Acad Sci U.S.A. *97(4)*: 1790-1795, 2000. PMID: 10660687. DOI: 10.1073/pnas.030528897

33 El-Abaseri TB, Fuhrman J, Trempus C, Shendrik I, Tennant RW and Hansen LA: Chemoprevention of UV light-induced skin tumorigenesis by inhibition of the epidermal growth factor receptor. Cancer Res *65(9)*: 3958-3965, 2005. PMID: 15867397. DOI: 10.1158/0008-5472.CAN-04-2204

34 Bourcier C, Jacquel A, Hess J, Peyrottes I, Angel P, Hofman P, Auberger P, Pouysségur J and Pagès G: p44 mitogen-activated protein kinase (extracellular signal-regulated kinase 1)-dependent signaling contributes to epithelial skin carcinogenesis. Cancer Res *66(5)*: 2700-2707, 2006. PMID: 16510590. DOI: 10.1158/0008-5472.CAN-05-3129

35 Bode AM and Dong Z: Mitogen-activated protein kinase activation in UV-induced signal transduction. Sci STKE *2003(167)*: RE2, 2003. PMID: 12554854. DOI: 10.1126/stke.2003.167.re2

36 Katsanakis KD, Owen C and Zoumpourlis V: JNK and ERK signaling pathways in multistage mouse carcinogenesis: studies in the inhibition of signaling cascades as a means to understand their *in vivo* biological role. Anticancer Res *22(2A)*: 755-759, 2002. PMID: 12014647.

37 Katsanakis KD, Gorgoulis V, Papavassiliou AG and Zoumpourlis VK: The progression in the mouse skin carcinogenesis model correlates with ERK1/2 signaling. Mol Med *8(10)*: 624-637, 2002. PMID: 12477973.

38 Subbaramaiah K and Dannenberg AJ: Cyclooxygenase 2: a molecular target for cancer prevention and treatment. Trends Pharmacol Sci *24(2)*: 96-102, 2003. PMID: 12559775. DOI: 10.1016/S0165-6147(02)00043-3

39 Pentland AP, Schoggins JW, Scott GA, Khan KN and Han R: Reduction of UV-induced skin tumors in hairless mice by selective COX-2 inhibition. Carcinogenesis *20(10)*: 1939-1944, 1999. PMID: 10506108. DOI: 10.1093/carcin/20.10.1939

40 Bikle DD: Vitamin D and the skin: Physiology and pathophysiology. Rev Endocr Metab Disord *13(1)*: 3-19, 2012. PMID: 21845365. DOI: 10.1007/s11154-011-9194-0

41 Zittermann A and Gummert JF: Nonclassical vitamin D action. Nutrients *2(4)*: 408-425, 2010. PMID: 22254030. DOI: 10.3390/nu2040408

42 Bikle D: Nonclassic actions of vitamin D. J Clin Endocrinol Metab *94(1)*: 26-34, 2009. PMID: 18854395. DOI: 10.1210/jc.2008-1454

43 Itin PH, Pittelkow MR and Kumar R: Effects of vitamin D metabolites on proliferation and differentiation of cultured human epidermal keratinocytes grown in serum-free or defined culture medium. Endocrinology *135(5)*: 1793-1798, 1994. PMID: 7956903. DOI: 10.1210/endo.135.5.7956903

44 Gniadecki R: Stimulation *versus* inhibition of keratinocyte growth by 1,25-Dihydroxyvitamin D3: dependence on cell culture conditions. J Invest Dermatol *106(3)*: 510-516, 1996. PMID: 8648185. DOI: 10.1111/1523-1747.ep12343866

45 Manggau M, Kim DS, Ruwisch L, Vogler R, Korting HC, Schäfer-Korting M and Kleuser B: 1Alpha,25-dihydroxyvitamin D3 protects human keratinocytes from apoptosis by the formation of sphingosine-1-phosphate. J Invest Dermatol *117(5)*: 1241-1249, 2001. PMID: 11710939. DOI: 10.1046/j.0022-202x.2001.01496.x

46 De Haes P, Garmyn M, Carmeliet G, Degreef H, Vantieghem K, Bouillon R and Segaert S: Molecular pathways involved in the anti-apoptotic effect of 1,25-dihydroxyvitamin D3 in primary human keratinocytes. J Cell Biochem *93(5)*: 951-967, 2004. PMID: 15389877. DOI: 10.1002/jcb.20227

47 Effendy I, Kwangsukstith C, Chiappe M and Maibach HI: Effects of calcipotriol on stratum corneum barrier function, hydration and cell renewal in humans. Br J Dermatol *135(4)*: 545-549, 1996. PMID: 8915143.

48 Hong SP, Oh Y, Jung M, Lee S, Jeon H, Cho MY, Lee SH and Choi EH: Topical calcitriol restores the impairment of epidermal permeability and antimicrobial barriers induced by corticosteroids. Br J Dermatol *162(6)*: 1251-1260, 2010. PMID: 20302580. DOI: 10.1111/j.1365-2133.2010.09760.x

49 Oda Y, Uchida Y, Moradian S, Crumrine D, Elias PM and Bikle DD: Vitamin D receptor and coactivators SRC2 and 3 regulate epidermis-specific sphingolipid production and permeability barrier formation. J Invest Dermatol *129(6)*: 1367-1378, 2009. PMID: 19052561. DOI: 10.1038/jid.2008.380

50 Reichrath J and Holick M: Clinical utility of 1,25-Dihydroxyvitamin D3 and its analogues for the treatment of psoriasis. Vitamin D: 1043-1060, 2021. DOI: 10.1007/978-1-60327-303-9_57

51 Amestejani M, Salehi BS, Vasigh M, Sobhkhiz A, Karami M, Alinia H, Kamrava SK, Shamspour N, Ghalehbaghi B and Behzadi AH: Vitamin D supplementation in the treatment of atopic dermatitis: a clinical trial study. J Drugs Dermatol *11(3)*: 327-330, 2012. PMID: 22395583.

52 Danielsson C, Fehsel K, Polly P and Carlberg C: Differential apoptotic response of human melanoma cells to 1 alpha,25-

dihydroxyvitamin D3 and its analogues. Cell Death Differ *5(11)*: 946-952, 1998. PMID: 9846181. DOI: 10.1038/sj.cdd.4400437

53 Danielsson C, Törmä H, Vahlquist A and Carlberg C: Positive and negative interaction of 1,25-dihydroxyvitamin D3 and the retinoid CD437 in the induction of human melanoma cell apoptosis. Int J Cancer *81(3)*: 467-470, 1999. PMID: 10209963. DOI: 10.1002/(sici)1097-0215(19990505)81:3<467::aid-ijc22>3.0.co;2-2

54 Bijlsma MF, Spek CA, Zivkovic D, van de Water S, Rezaee F and Peppelenbosch MP: Repression of smoothened by patched-dependent (pro-)vitamin D3 secretion. PLoS Biol *4(8)*: e232, 2006. PMID: 16895439. DOI: 10.1371/journal.pbio.0040232

55 Tang JY, So PL and Epstein EH Jr: Novel Hedgehog pathway targets against basal cell carcinoma. Toxicol Appl Pharmacol *224(3)*: 257-264, 2007. PMID: 17276471. DOI: 10.1016/j.taap.2006.12.011

56 Hershberger PA, Modzelewski RA, Shurin ZR, Rueger RM, Trump DL and Johnson CS: 1,25-Dihydroxycholecalciferol (1,25-D3) inhibits the growth of squamous cell carcinoma and down-modulates p21(Waf1/Cip1) *in vitro* and *in vivo*. Cancer Res *59(11)*: 2644-2649, 1999. PMID: 10363987.

57 Haussler MR, Whitfield GK, Haussler CA, Hsieh JC, Thompson PD, Selznick SH, Dominguez CE and Jurutka PW: The nuclear vitamin D receptor: biological and molecular regulatory properties revealed. J Bone Miner Res *13(3)*: 325-349, 1998. PMID: 9525333. DOI: 10.1359/jbmr.1998.13.3.325

58 Bikle DD and Pillai S: Vitamin D, calcium, and epidermal differentiation. Endocr Rev *14(1)*: 3-19, 1993. PMID: 8491153. DOI: 10.1210/edrv-14-1-3

59 Reichrath J, Collins ED, Epple S, Kerber A, Norman AW and Bahmer FA: Immunohistochemical detection of 1,25-dihydroxyvitamin D3 receptors (VDR) in human skin. A comparison of five antibodies. Pathol Res Pract *192(3)*: 281-289, 1996. PMID: 8739475. DOI: 10.1016/S0344-0338(96)80231-7

60 Darwish H and DeLuca HF: Vitamin D-regulated gene expression. Crit Rev Eukaryot Gene Expr *3(2)*: 89-116, 1993. PMID: 8391882.

61 Ebihara K, Masuhiro Y, Kitamoto T, Suzawa M, Uematsu Y, Yoshizawa T, Ono T, Harada H, Matsuda K, Hasegawa T, Masushige S and Kato S: Intron retention generates a novel isoform of the murine vitamin D receptor that acts in a dominant negative way on the vitamin D signaling pathway. Mol Cell Biol *16(7)*: 3393-3400, 1996. PMID: 8668154. DOI: 10.1128/MCB.16.7.3393

62 Horváth HC, Lakatos P, Kósa JP, Bácsi K, Borka K, Bises G, Nittke T, Hershberger PA, Speer G and Kállay E: The candidate oncogene CYP24A1: A potential biomarker for colorectal tumorigenesis. J Histochem Cytochem *58(3)*: 277-285, 2010. PMID: 19901270. DOI: 10.1369/jhc.2009.954339

63 Jones G, Strugnell SA and DeLuca HF: Current understanding of the molecular actions of vitamin D. Physiol Rev *78(4)*: 1193-1231, 1998. PMID: 9790574. DOI: 10.1152/physrev.1998.78.4.1193

64 Knutson JC and DeLuca HF: 25-Hydroxyvitamin D3-24-hydroxylase. Subcellular location and properties. Biochemistry *13(7)*: 1543-1548, 1974. PMID: 4150615. DOI: 10.1021/bi00704a034

65 Prosser DE and Jones G: Enzymes involved in the activation and inactivation of vitamin D. Trends Biochem Sci *29(12)*: 664-673, 2004. PMID: 15544953. DOI: 10.1016/j.tibs.2004.10.005

66 Albertson DG, Ylstra B, Segraves R, Collins C, Dairkee SH, Kowbel D, Kuo WL, Gray JW and Pinkel D: Quantitative mapping of amplicon structure by array CGH identifies CYP24 as a candidate oncogene. Nat Genet *25(2)*: 144-146, 2000. PMID: 10835626. DOI: 10.1038/75985

67 Takami M, Fujimaki K, Nishimura MI and Iwashima M: Cutting Edge: AhR Is a Molecular Target of Calcitriol in Human T Cells. J Immunol *195(6)*: 2520-2523, 2015. PMID: 26276877. DOI: 10.4049/jimmunol.1500344

68 Li H, Li W and Wang Q: 1,25-dihydroxyvitamin D_3 suppresses lipopolysaccharide-induced interleukin-6 production through aryl hydrocarbon receptor/nuclear factor-κB signaling in oral epithelial cells. BMC Oral Health *19(1)*: 236, 2019. PMID: 31684930. DOI: 10.1186/s12903-019-0935-x

69 Slominski AT, Kim TK, Janjetovic Z, Brożyna AA, Żmijewski MA, Xu H, Sutter TR, Tuckey RC, Jetten AM and Crossman DK: Differential and overlapping effects of $20,23(OH)_2D_3$ and $1,25(OH)_2D_3$ on gene expression in human epidermal keratinocytes: Identification of AhR as an alternative receptor for $20,23(OH)_2D_3$. Int J Mol Sci *19(10)*: 3072, 2018. PMID: 30297679. DOI: 10.3390/ijms19103072

70 Matsunawa M, Amano Y, Endo K, Uno S, Sakaki T, Yamada S and Makishima M: The aryl hydrocarbon receptor activator benzo[a]pyrene enhances vitamin D3 catabolism in macrophages. Toxicol Sci *109(1)*: 50-58, 2009. PMID: 19244278. DOI: 10.1093/toxsci/kfp044

71 Matsunawa M, Akagi D, Uno S, Endo-Umeda K, Yamada S, Ikeda K and Makishima M: Vitamin D receptor activation enhances benzo[a]pyrene metabolism *via* CYP1A1 expression in macrophages. Drug Metab Dispos *40(11)*: 2059-2066, 2012. PMID: 22837390. DOI: 10.1124/dmd.112.046839

72 Lehman TA, Modali R, Boukamp P, Stanek J, Bennett WP, Welsh JA, Metcalf RA, Stampfer MR, Fusenig N and Rogan EM: p53 mutations in human immortalized epithelial cell lines. Carcinogenesis *14(5)*: 833-839, 1993. PMID: 8504475. DOI: 10.1093/carcin/14.5.833

73 Nakazawa H, English D, Randell PL, Nakazawa K, Martel N, Armstrong BK and Yamasaki H: UV and skin cancer: specific p53 gene mutation in normal skin as a biologically relevant exposure measurement. Proc Natl Acad Sci U.S.A. *91(1)*: 360-364, 1994. PMID: 8278394. DOI: 10.1073/pnas.91.1.360

74 Boukamp P, Petrussevska RT, Breitkreutz D, Hornung J, Markham A and Fusenig NE: Normal keratinization in a spontaneously immortalized aneuploid human keratinocyte cell line. J Cell Biol *106(3)*: 761-771, 1988. PMID: 2450098. DOI: 10.1083/jcb.106.3.761

75 Inman GJ, Wang J, Nagano A, Alexandrov LB, Purdie KJ, Taylor RG, Sherwood V, Thomson J, Hogan S, Spender LC, South AP, Stratton M, Chelala C, Harwood CA, Proby CM and Leigh IM: The genomic landscape of cutaneous SCC reveals drivers and a novel azathioprine associated mutational signature. Nat Commun *9(1)*: 3667, 2018. PMID: 30202019. DOI: 10.1038/s41467-018-06027-1

76 South AP, Purdie KJ, Watt SA, Haldenby S, den Breems N, Dimon M, Arron ST, Kluk MJ, Aster JC, McHugh A, Xue DJ, Dayal JH, Robinson KS, Rizvi SH, Proby CM, Harwood CA and Leigh IM: NOTCH1 mutations occur early during cutaneous squamous cell carcinogenesis. J Invest Dermatol

134(10): 2630-2638, 2014. PMID: 24662767. DOI: 10.1038/jid.2014.154

77 Li YY, Hanna GJ, Laga AC, Haddad RI, Lorch JH and Hammerman PS: Genomic analysis of metastatic cutaneous squamous cell carcinoma. Clin Cancer Res *21(6)*: 1447-1456, 2015. PMID: 25589618. DOI: 10.1158/1078-0432.CCR-14-1773

78 Pickering CR, Zhou JH, Lee JJ, Drummond JA, Peng SA, Saade RE, Tsai KY, Curry JL, Tetzlaff MT, Lai SY, Yu J, Muzny DM, Doddapaneni H, Shinbrot E, Covington KR, Zhang J, Seth S, Caulin C, Clayman GL, El-Naggar AK, Gibbs RA, Weber RS, Myers JN, Wheeler DA and Frederick MJ: Mutational landscape of aggressive cutaneous squamous cell carcinoma. Clin Cancer Res *20(24)*: 6582-6592, 2014. PMID: 25303977. DOI: 10.1158/1078-0432.CCR-14-1768

79 Cho RJ, Alexandrov LB, den Breems NY, Atanasova VS, Farshchian M, Purdom E, Nguyen TN, Coarfa C, Rajapakshe K, Prisco M, Sahu J, Tassone P, Greenawalt EJ, Collisson EA, Wu W, Yao H, Su X, Guttmann-Gruber C, Hofbauer JP, Hashmi R, Fuentes I, Benz SC, Golovato J, Ehli EA, Davis CM, Davies GE, Covington KR, Murrell DF, Salas-Alanis JC, Palisson F, Bruckner AL, Robinson W, Has C, Bruckner-Tuderman L, Titeux M, Jonkman MF, Rashidghamat E, Lwin SM, Mellerio JE, McGrath JA, Bauer JW, Hovnanian A, Tsai KY and South AP: APOBEC mutation drives early-onset squamous cell carcinomas in recessive dystrophic epidermolysis bullosa. Sci Transl Med *10(455)*: eaas9668, 2018. PMID: 30135250. DOI: 10.1126/scitranslmed.aas9668

80 Boukamp P, Tilgen W, Dzarlieva RT, Breitkreutz D, Haag D, Riehl RK, Bohnert A and Fusenig NE: Phenotypic and genotypic characteristics of a cell line from a squamous cell carcinoma of human skin. J Natl Cancer Inst *68(3)*: 415-427, 1982. PMID: 6950169.

81 Attignon EA, Distel E, Le-Grand B, Leblanc AF, Barouki R, de Oliveira E, Aggerbeck M and Blanc EB: Down-regulation of the expression of alcohol dehydrogenase 4 and CYP2E1 by the combination of α-endosulfan and dioxin in HepaRG human cells. Toxicol In Vitro *45(Pt 3)*: 309-317, 2017. PMID: 28673560. DOI: 10.1016/j.tiv.2017.06.029

82 Memari B, Nguyen-Yamamoto L, Salehi-Tabar R, Zago M, Fritz JH, Baglole CJ, Goltzman D and White JH: Endocrine aryl hydrocarbon receptor signaling is induced by moderate cutaneous exposure to ultraviolet light. Sci Rep *9(1)*: 8486, 2019. PMID: 31186463. DOI: 10.1038/s41598-019-44862-4

83 Zheng W, Duan B, Zhang Q, Ouyang L, Peng W, Qian F, Wang Y and Huang S: Vitamin D-induced vitamin D receptor expression induces tamoxifen sensitivity in MCF-7 stem cells *via* suppression of Wnt/β-catenin signaling. Biosci Rep *38(6)*: BSR20180595, 2018. PMID: 30314996. DOI: 10.1042/BSR20180595

84 Livak KJ and Schmittgen TD: Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)) Method. Methods *25(4)*: 402-408, 2001. PMID: 11846609. DOI: 10.1006/meth.2001.1262

85 Garland CF, Garland FC, Gorham ED, Lipkin M, Newmark H, Mohr SB and Holick MF: The role of vitamin D in cancer prevention. Am J Public Health *96(2)*: 252-261, 2006. PMID: 16380576. DOI: 10.2105/AJPH.2004.045260

86 Feldman D, Krishnan AV, Swami S, Giovannucci E and Feldman BJ: The role of vitamin D in reducing cancer risk and progression. Nat Rev Cancer *14(5)*: 342-357, 2014. PMID: 24705652. DOI: 10.1038/nrc3691

87 Holick MF: Vitamin D: its role in cancer prevention and treatment. Prog Biophys Mol Biol *92(1)*: 49-59, 2006. PMID: 16566961. DOI: 10.1016/j.pbiomolbio.2006.02.014

88 Slominski AT, Kim TK, Hobrath JV, Oak ASW, Tang EKY, Tieu EW, Li W, Tuckey RC and Jetten AM: Endogenously produced nonclassical vitamin D hydroxy-metabolites act as "biased" agonists on VDR and inverse agonists on RORα and RORγ. J Steroid Biochem Mol Biol *173*: 42-56, 2017. PMID: 27693422. DOI: 10.1016/j.jsbmb.2016.09.024

89 Slominski AT, Kim TK, Takeda Y, Janjetovic Z, Brozyna AA, Skobowiat C, Wang J, Postlethwaite A, Li W, Tuckey RC and Jetten AM: RORα and ROR γ are expressed in human skin and serve as receptors for endogenously produced noncalcemic 20-hydroxy- and 20,23-dihydroxyvitamin D. FASEB J *28(7)*: 2775-2789, 2014. PMID: 24668754. DOI: 10.1096/fj.13-242040

90 Reichrath J, Saternus R and Vogt T: Endocrine actions of vitamin D in skin: Relevance for photocarcinogenesis of non-melanoma skin cancer, and beyond. Mol Cell Endocrinol *453*: 96-102, 2017. PMID: 28526240. DOI: 10.1016/j.mce.2017.05.001

91 Li ZD, Wang K, Yang XW, Zhuang ZG, Wang JJ and Tong XW: Expression of aryl hydrocarbon receptor in relation to p53 status and clinicopathological parameters in breast cancer. Int J Clin Exp Pathol *7(11)*: 7931-7937, 2014. PMID: 25550834.

92 Panchanathan R, Liu H and Choubey D: Activation of p53 in human and murine cells by DNA-damaging agents differentially regulates aryl hydrocarbon receptor levels. Int J Toxicol *34(3)*: 242-249, 2015. PMID: 25878193. DOI: 10.1177/1091581815578013

93 Wohak LE, Krais AM, Kucab JE, Stertmann J, Øvrebø S, Seidel A, Phillips DH and Arlt VM: Carcinogenic polycyclic aromatic hydrocarbons induce CYP1A1 in human cells *via* a p53-dependent mechanism. Arch Toxicol *90(2)*: 291-304, 2016. PMID: 25398514. DOI: 10.1007/s00204-014-1409-1

94 Maruyama R, Toyota M, Suzuki H, Sasaki Y, Aoki F, Shinomura Y, Imai K and Tokino T: The functional relation of vitamin D receptor and p53 in cancer cells. Cancer Epidemiol Biomarkers Prev 15: B133, 2006.

95 Skrajnowska D and Bobrowska-Korczak B: Potential molecular mechanisms of the anti-cancer activity of vitamin D. Anticancer Res *39(7)*: 3353-3363, 2019. PMID: 31262856. DOI: 10.21873/anticanres.13478

96 Filvaroff E, Calautti E, Reiss M and Dotto GP: Functional evidence for an extracellular calcium receptor mechanism triggering tyrosine kinase activation associated with mouse keratinocyte differentiation. J Biol Chem *269(34)*: 21735-21740, 1994. PMID: 8063817.

97 Gradin K, Whitelaw ML, Toftgård R, Poellinger L and Berghard A: A tyrosine kinase-dependent pathway regulates ligand-dependent activation of the dioxin receptor in human keratinocytes. J Biol Chem *269(38)*: 23800-23807, 1994. PMID: 8089152.

98 Wanner R, Brömmer S, Czarnetzki BM and Rosenbach T: The differentiation-related upregulation of aryl hydrocarbon receptor transcript levels is suppressed by retinoic acid. Biochem Biophys Res Commun *209(2)*: 706-711, 1995. PMID: 7733941. DOI: 10.1006/bbrc.1995.1556

99 Christakos S, Dhawan P, Verstuyf A, Verlinden L and Carmeliet G: Vitamin D: Metabolism, molecular mechanism of action, and pleiotropic effects. Physiol Rev *96(1)*: 365-408, 2016. PMID: 26681795. DOI: 10.1152/physrev.00014.2015

100 Schwetz V, Trummer C, Pandis M, Grübler MR, Verheyen N, Gaksch M, Zittermann A, März W, Aberer F, Lang A, Treiber G, Friedl C, Obermayer-Pietsch B, Pieber TR, Tomaschitz A and Pilz S: Effects of vitamin D supplementation on bone turnover markers: a randomized controlled trial. Nutrients *9(5)*: 432, 2017. PMID: 28448457. DOI: 10.3390/nu9050432

101 Gallieni M, Cozzolino M, Fallabrino G, Pasho S, Olivi L and Brancaccio D: Vitamin D: physiology and pathophysiology. Int J Artif Organs *32(2)*: 87-94, 2009. PMID: 19363780. DOI: 10.1177/039139880903200205

102 Smith EL, Walworth NC and Holick MF: Effect of 1 alpha,25-dihydroxyvitamin D3 on the morphologic and biochemical differentiation of cultured human epidermal keratinocytes grown in serum-free conditions. J Invest Dermatol *86(6)*: 709-714, 1986. PMID: 2423618. DOI: 10.1111/1523-1747.ep12276343

103 Fernández-Barral A, Bustamante-Madrid P, Ferrer-Mayorga G, Barbáchano A, Larriba MJ and Muñoz A: Vitamin D effects on cell differentiation and stemness in cancer. Cancers (Basel) *12(9)*: 2413, 2020. PMID: 32854355. DOI: 10.3390/cancers12092413

104 Matsuoka LY, Wortsman J, Haddad JG and Hollis BW: *In vivo* threshold for cutaneous synthesis of vitamin D3. J Lab Clin Med *114(3)*: 301-305, 1989. PMID: 2549141.

105 Giguère V: Orphan nuclear receptors: from gene to function. Endocr Rev *20(5)*: 689-725, 1999. PMID: 10529899. DOI: 10.1210/edrv.20.5.0378

106 Kliewer SA, Goodwin B and Willson TM: The nuclear pregnane X receptor: a key regulator of xenobiotic metabolism. Endocr Rev *23(5)*: 687-702, 2002. PMID: 12372848. DOI: 10.1210/er.2001-0038

107 Mangelsdorf DJ, Thummel C, Beato M, Herrlich P, Schütz G, Umesono K, Blumberg B, Kastner P, Mark M, Chambon P and Evans RM: The nuclear receptor superfamily: the second decade. Cell *83(6)*: 835-839, 1995. PMID: 8521507. DOI: 10.1016/0092-8674(95)90199-x

108 Nebert DW, Shi Z, Gálvez-Peralta M, Uno S and Dragin N: Oral benzo[a]pyrene: understanding pharmacokinetics, detoxication, and consequences—Cyp1 knockout mouse lines as a paradigm. Mol Pharmacol *84(3)*: 304-313, 2013. PMID: 23761301. DOI: 10.1124/mol.113.086637

109 Tanaka Y, Uchi H, Ito T and Furue M: Indirubin-pregnane X receptor-JNK axis accelerates skin wound healing. Sci Rep *9(1)*: 18174, 2019. PMID: 31796845. DOI: 10.1038/s41598-019-54754-2

110 Stepankova M, Pastorkova B, Bachleda P and Dvorak Z: Itraconazole cis-diastereoisomers activate aryl hydrocarbon receptor AhR and pregnane X receptor PXR and induce CYP1A1 in human cell lines and human hepatocytes. Toxicology *383*: 40-49, 2017. PMID: 28390928. DOI: 10.1016/j.tox.2017.04.002

111 Pascussi JM, Robert A, Nguyen M, Walrant-Debray O, Garabedian M, Martin P, Pineau T, Saric J, Navarro F, Maurel P and Vilarem MJ: Possible involvement of pregnane X receptor-enhanced CYP24 expression in drug-induced osteomalacia. J Clin Invest *115(1)*: 177-186, 2005. PMID: 15630458. DOI: 10.1172/JCI21867

112 Reschly EJ and Krasowski MD: Evolution and function of the NR1I nuclear hormone receptor subfamily (VDR, PXR, and CAR) with respect to metabolism of xenobiotics and endogenous compounds. Curr Drug Metab *7(4)*: 349-365, 2006. PMID: 16724925. DOI: 10.2174/138920006776873526

113 Swanson HI: Cytochrome P450 expression in human keratinocytes: an aryl hydrocarbon receptor perspective. Chem Biol Interact *149(2-3)*: 69-79, 2004. PMID: 15501429. DOI: 10.1016/j.cbi.2004.08.006

114 Wilkens MR, Maté LM, Schnepel N, Klinger S, Muscher-Banse AS, Ballent M, Virkel G and Lifschitz AL: Influence of 25-hydroxyvitamin D_3 and 1,25-dihydroxyvitamin D_3 on expression of P-glycoprotein and cytochrome P450 3A in sheep. J Steroid Biochem Mol Biol *164*: 271-276, 2016. PMID: 26319202. DOI: 10.1016/j.jsbmb.2015.08.019

115 Honkakoski P and Negishi M: Regulation of cytochrome P450 (CYP) genes by nuclear receptors. Biochem J *347(Pt 2)*: 321-337, 2000. PMID: 10749660. DOI: 10.1042/0264-6021:3470321

116 Hemida MA, AbdElmoneim NA, Hewala TI, Rashad MM and Abdaallah S: Vitamin D receptor in breast cancer tissues and its relation to estrogen receptor Alpha (ER-α) gene expression and serum 25-hydroxyvitamin D levels in Egyptian breast cancer patients: a case-control study. Clin Breast Cancer *19(3)*: e407-e414, 2019. PMID: 30833174. DOI: 10.1016/j.clbc.2018.12.019

117 Somjen D, Grafi-Cohen M, Posner GH, Sharon O, Kraiem Z and Stern N: Vitamin D less-calcemic analog modulates the expression of estrogen receptors, vitamin D receptor and 1α-hydroxylase 25-hydroxy vitamin D in human thyroid cancer cell lines. J Steroid Biochem Mol Biol *136*: 80-82, 2013. PMID: 23059472. DOI: 10.1016/j.jsbmb.2012.09.015

118 Bilani N, Elson L, Szuchan C, Elimimian E, Saleh M and Nahleh Z: Newly-identified pathways relating vitamin D to carcinogenesis: a review. In Vivo *35(3)*: 1345-1354, 2021. PMID: 33910812. DOI: 10.21873/invivo.12387

119 Levine-Fridman A, Chen L and Elferink CJ: Cytochrome P4501A1 promotes G1 phase cell cycle progression by controlling aryl hydrocarbon receptor activity. Mol Pharmacol *65(2)*: 461-469, 2004. PMID: 14742689. DOI: 10.1124/mol.65.2.461

120 Han Z, Miwa Y, Obikane H, Mitsumata M, Takahashi-yanaga F, Morimoto S and Sasaguri T: Han Z, Miwa Y, Obikane H, Mitsumata M, Takahashi-Yanaga F, Morimoto S and Sasaguri T: Aryl hydrocarbon receptor mediates laminar fluid shear stress-induced CYP1A1 activation and cell cycle arrest in vascular endothelial cells. Cardiovasc Res *77(4)*: 809-818, 2008. PMID: 18065768. DOI: 10.1093/cvr/cvm095

121 Vázquez-Gómez G, Rocha-Zavaleta L, Rodríguez-Sosa M, Petrosyan P and Rubio-Lightbourn J: Benzo[a]pyrene activates an AhR/Src/ERK axis that contributes to CYP1A1 induction and stable DNA adducts formation in lung cells. Toxicol Lett *289*: 54-62, 2018. PMID: 29545172. DOI: 10.1016/j.toxlet.2018.03.012

122 Tsai CF, Hsieh TH, Lee JN, Hsu CY, Wang YC, Lai FJ, Kuo KK, Wu HL, Tsai EM and Kuo PL: Benzyl butyl phthalate induces migration, invasion, and angiogenesis of Huh7 hepatocellular carcinoma cells through nongenomic AhR/G-protein signaling. BMC Cancer *14*: 556, 2014. PMID: 25081364. DOI: 10.1186/1471-2407-14-556

123 Staršíchová A, Hrubá E, Slabáková E, Pernicová Z, Procházková J, Pěnčíková K, Seda V, Kabátková M, Vondráček J, Kozubík A, Machala M and Souček K: TGF-β1 signaling plays a dominant role in the crosstalk between TGF-β1 and the aryl hydrocarbon receptor ligand in prostate epithelial cells. Cell Signal *24(8)*: 1665-1676, 2012. PMID: 22560882. DOI: 10.1016/j.cellsig.2012.04.008

124 Nakano N, Sakata N, Katsu Y, Nochise D, Sato E, Takahashi Y, Yamaguchi S, Haga Y, Ikeno S, Motizuki M, Sano K, Yamasaki K, Miyazawa K and Itoh S: Dissociation of the AhR/ARNT complex by TGF-β/Smad signaling represses *CYP1A1* gene expression and inhibits benze[a]pyrene-mediated cytotoxicity. J Biol Chem *295(27)*: 9033-9051, 2020. PMID: 32409577. DOI: 10.1074/jbc.RA120.013596

125 Lee YC, Jogie-Brahim S, Lee DY, Han J, Harada A, Murphy LJ and Oh Y: Insulin-like growth factor-binding protein-3 (IGFBP-3) blocks the effects of asthma by negatively regulating NF-κB signaling through IGFBP-3R-mediated activation of caspases. J Biol Chem *286(20)*: 17898-17909, 2011. PMID: 21383009. DOI: 10.1074/jbc.M111.231035

126 Lee H, Kim SR, Oh Y, Cho SH, Schleimer RP and Lee YC: Targeting insulin-like growth factor-I and insulin-like growth factor-binding protein-3 signaling pathways. A novel therapeutic approach for asthma. Am J Respir Cell Mol Biol *50(4)*: 667-677, 2014. PMID: 24219511. DOI: 10.1165/rcmb.2013-0397TR

127 Chen S, Operaña T, Bonzo J, Nguyen N and Tukey RH: ERK kinase inhibition stabilizes the aryl hydrocarbon receptor: implications for transcriptional activation and protein degradation. J Biol Chem *280(6)*: 4350-4359, 2005. PMID: 15572374. DOI: 10.1074/jbc.M411554200

128 Hutchinson PE, Halsall JA, Popovici S, Papadogeorgakis E, Osborne JE, Powley IR, Dasari D, Saldanha G and Pringle JH: Compromised vitamin D receptor signalling in malignant melanoma is associated with tumour progression and mitogen-activated protein kinase activity. Melanoma Res *28(5)*: 410-422, 2018. PMID: 30004989. DOI: 10.1097/CMR.0000000000000475

129 Delescluse C, Lemaire G, de Sousa G and Rahmani R: Is CYP1A1 induction always related to AHR signaling pathway? Toxicology *153(1-3)*: 73-82, 2000. PMID: 11090948. DOI: 10.1016/s0300-483x(00)00305-x

130 Tan Z, Chang X, Puga A and Xia Y: Activation of mitogen-activated protein kinases (MAPKs) by aromatic hydrocarbons: role in the regulation of aryl hydrocarbon receptor (AHR) function. Biochem Pharmacol *64(5-6)*: 771-780, 2002. PMID: 12213569. DOI: 10.1016/s0006-2952(02)01138-3

131 Nabeshima Y and Imura H: alpha-Klotho: a regulator that integrates calcium homeostasis. Am J Nephrol *28(3)*: 455-464, 2008. PMID: 18160815. DOI: 10.1159/000112824

132 Hou XX, Chen G, Hossini AM, Hu T, Wang L, Pan Z, Lu L, Cao K, Ma Y, Zouboulis CC, Xia L and Ju Q: Aryl hydrocarbon receptor modulates the expression of TNF-α and IL-8 in human sebocytes via the MyD88-p65NF-κB/p38MAPK signaling pathways. J Innate Immun *11(1)*: 41-51, 2019. PMID: 30056444. DOI: 10.1159/000491029

133 Luecke S, Wincent E, Backlund M, Rannug U and Rannug A: Cytochrome P450 1A1 gene regulation by UVB involves crosstalk between the aryl hydrocarbon receptor and nuclear factor kappaB. Chem Biol Interact *184(3)*: 466-473, 2010. PMID: 20132803. DOI: 10.1016/j.cbi.2010.01.038

134 Jiang J and Hui CC: Hedgehog signaling in development and cancer. Dev Cell *15(6)*: 801-812, 2008. PMID: 19081070. DOI: 10.1016/j.devcel.2008.11.010

135 Nolan-Stevaux O, Lau J, Truitt ML, Chu GC, Hebrok M, Fernández-Zapico ME and Hanahan D: GLI1 is regulated through Smoothened-independent mechanisms in neoplastic pancreatic ducts and mediates PDAC cell survival and transformation. Genes Dev *23(1)*: 24-36, 2009. PMID: 19136624. DOI: 10.1101/gad.1753809

136 Gu D and Xie J: Non-canonical Hh signaling in cancer-current understanding and future directions. Cancers (Basel) *7(3)*: 1684-1698, 2015. PMID: 26343727. DOI: 10.3390/cancers7030857

137 Tang JY, Xiao TZ, Oda Y, Chang KS, Shpall E, Wu A, So PL, Hebert J, Bikle D and Epstein EH Jr: Vitamin D3 inhibits hedgehog signaling and proliferation in murine Basal cell carcinomas. Cancer Prev Res (Phila) *4(5)*: 744-751, 2011. PMID: 21436386. DOI: 10.1158/1940-6207.CAPR-10-0285

138 Sarić N, Selby M, Ramaswamy V, Kool M, Stockinger B, Hogstrand C, Williamson D, Marino S, Taylor MD, Clifford SC and Basson MA: The AHR pathway represses TGFβ-SMAD3 signalling and has a potent tumour suppressive role in SHH medulloblastoma. Sci Rep *10(1)*: 148, 2020. PMID: 31924815. DOI: 10.1038/s41598-019-56876-z

139 Drozdzik A, Dziedziejko V and Kurzawski M: IL-1 and TNF-α regulation of aryl hydrocarbon receptor (AhR) expression in HSY human salivary cells. Arch Oral Biol *59(4)*: 434-439, 2014. PMID: 24565903. DOI: 10.1016/j.archoralbio.2014.02.003

140 Matthews J and Gustafsson JA: Estrogen receptor and aryl hydrocarbon receptor signaling pathways. Nucl Recept Signal *4*: e016, 2006. PMID: 16862222. DOI: 10.1621/nrs.04016

141 Kumagai T, Suzuki H, Sasaki T, Sakaguchi S, Miyairi S, Yamazoe Y and Nagata K: Polycyclic aromatic hydrocarbons activate CYP3A4 gene transcription through human pregnane X receptor. Drug Metab Pharmacokinet *27(2)*: 200-206, 2012. PMID: 22076448. DOI: 10.2133/dmpk.dmpk-11-rg-094

142 Istrate MA, Nussler AK, Eichelbaum M and Burk O: Regulation of CYP3A4 by pregnane X receptor: The role of nuclear receptors competing for response element binding. Biochem Biophys Res Commun *393(4)*: 688-693, 2010. PMID: 20171174. DOI: 10.1016/j.bbrc.2010.02.058

143 Gerbal-Chaloin S, Pichard-Garcia L, Fabre JM, Sa-Cunha A, Poellinger L, Maurel P and Daujat-Chavanieu M: Role of CYP3A4 in the regulation of the aryl hydrocarbon receptor by omeprazole sulphide. Cell Signal *18(5)*: 740-750, 2006. PMID: 16109480. DOI: 10.1016/j.cellsig.2005.07.007

144 Rasmussen MK, Daujat-Chavanieu M and Gerbal-Chaloin S: Activation of the aryl hydrocarbon receptor decreases rifampicin-induced CYP3A4 expression in primary human hepatocytes and HepaRG. Toxicol Lett *277*: 1-8, 2017. PMID: 28571685. DOI: 10.1016/j.toxlet.2017.05.029

145 Kubota A, Goldstone JV, Lemaire B, Takata M, Woodin BR and Stegeman JJ: Role of pregnane X receptor and aryl hydrocarbon receptor in transcriptional regulation of pxr, CYP2, and CYP3 genes in developing zebrafish. Toxicol Sci *143(2)*: 398-407, 2015. PMID: 25424564. DOI: 10.1093/toxsci/kfu240

146 Bikle DD: Vitamin D metabolism, mechanism of action, and clinical applications. Chem Biol *21(3)*: 319-329, 2014. PMID: 24529992. DOI: 10.1016/j.chembiol.2013.12.016

147 Lehmann B, Pietzsch J, Kämpf A and Meurer M: Human keratinocyte line HaCaT metabolizes 1alpha-hydroxyvitamin D3 and vitamin D3 to 1alpha,25-dihydroxyvitamin D3 (calcitriol). J Dermatol Sci *18(2)*: 118-127, 1998. PMID: 9833978. DOI: 10.1016/s0923-1811(98)00033-4

148 Bikle DD, Nemanic MK, Gee E and Elias P: 1,25-Dihydroxyvitamin D3 production by human keratinocytes. Kinetics and regulation. J Clin Invest *78(2)*: 557-566, 1986. PMID: 2426308. DOI: 10.1172/JCI112609

149 Shabahang M, Buras RR, Davoodi F, Schumaker LM, Nauta RJ and Evans SR: 1,25-Dihydroxyvitamin D3 receptor as a marker of human colon carcinoma cell line differentiation and growth inhibition. Cancer Res *53(16)*: 3712-3718, 1993. PMID: 8393379.

150 Bortman P, Folgueira MA, Katayama ML, Snitcovsky IM and Brentani MM: Antiproliferative effects of 1,25-dihydroxyvitamin D3 on breast cells: a mini review. Braz J Med Biol Res *35(1)*: 1-9, 2002. PMID: 11743608. DOI: 10.1590/s0100-879x2002000100001

151 Kaukinen A, Siiskonen H, Pelkonen J and Harvima IT: Immunoreactivity to CYP24A1, but not vitamin D receptor, is increased in mast cells of keratinocyte skin cancers. Eur J Dermatol *27(6)*: 590-598, 2017. PMID: 29165303. DOI: 10.1684/ejd.2017.3132

152 Mimori K, Tanaka Y, Yoshinaga K, Masuda T, Yamashita K, Okamoto M, Inoue H and Mori M: Clinical significance of the overexpression of the candidate oncogene CYP24 in esophageal cancer. Ann Oncol *15(2)*: 236-241, 2004. PMID: 14760115. DOI: 10.1093/annonc/mdh056

153 Meulmeester E and Jochemsen AG: p53: a guide to apoptosis. Curr Cancer Drug Targets *8(2)*: 87-97, 2008. PMID: 18336191. DOI: 10.2174/156800908783769337

154 Stambolsky P, Tabach Y, Fontemaggi G, Weisz L, Maor-Aloni R, Siegfried Z, Shiff I, Kogan I, Shay M, Kalo E, Blandino G, Simon I, Oren M and Rotter V: Modulation of the vitamin D3 response by cancer-associated mutant p53. Cancer Cell *17(3)*: 273-285, 2010. PMID: 20227041. DOI: 10.1016/j.ccr.2009.11.025

155 Han JA, Kim JI, Ongusaha PP, Hwang DH, Ballou LR, Mahale A, Aaronson SA and Lee SW: P53-mediated induction of Cox-2 counteracts p53- or genotoxic stress-induced apoptosis. EMBO J *21(21)*: 5635-5644, 2002. PMID: 12411481. DOI: 10.1093/emboj/cdf591

156 Chen J, Wu F, Pei HL, Gu WD, Ning ZH, Shao YJ and Huang J: Analysis of the correlation between P53 and Cox-2 expression and prognosis in esophageal cancer. Oncol Lett *10(4)*: 2197-2203, 2015. PMID: 26622818. DOI: 10.3892/ol.2015.3624

157 Enache AO, Stepan AE, Mărgăritescu C, Pătraşcu V, Ciurea RN, Simionescu CE and Camen A: Immunoexpression of p53 and COX-2 in basal cell carcinoma. Rom J Morphol Embryol *59(4)*: 1115-1120, 2018. PMID: 30845292.

158 Duque G, El Abdaimi K, Henderson JE, Lomri A and Kremer R: Vitamin D inhibits Fas ligand-induced apoptosis in human osteoblasts by regulating components of both the mitochondrial and Fas-related pathways. Bone *35(1)*: 57-64, 2004. PMID: 15207741. DOI: 10.1016/j.bone.2004.03.005

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