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Review

Peroxisome proliferator-activated receptor (PPAR) and vitamin D receptor (VDR) signaling pathways in melanoma cells: Promising new therapeutic targets?☆

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ABSTRACT

Peroxisome proliferator-activated receptor (PPAR) and vitamin D receptor (VDR) signaling pathways regulate a multitude of genes that are of importance for a multitude of cellular functions including cell proliferation, cell differentiation, immune responses and apoptosis. Ligands and other agents influencing the PPAR and VDR signaling pathways have been shown to reveal chemopreventive potential by mediating tumor suppressive activities in a variety of human cancers. Use of these compounds may represent a potential novel strategy to prevent melanoma pathogenesis and to inhibit melanoma progression. We recently showed that 1,25-dihydroxyvitamin D_3 and some of the investigated PPAR ligands inhibited proliferation of the human melanoma cell line MeWo. In addition to this, our results gave an indication of an interconnection of the PPAR and VDR signaling pathways at the level of cross-regulation of their respective transcription factor mRNA levels. The provided link between VDR and PPAR may play an important role in treatment and prevention of melanoma. This review summarizes the currently available data on the roles of the PPARs and the VDR in pathogenesis and progression of melanoma as well as their role as promising future therapeutic targets.

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Contents

1. Introduction

In the past decades the incidence of malignant melanoma has worldwide dramatically increased. Due to its high tendency to metastasise to other parts of the body, including the liver, lungs, and brain, malignant melanoma is recognized as one of the most aggressive cancers [1]. In addition to this, there is little effective treatment available. To the point dacarbazine has the best efficacy with a response rate ranging from 5 to 29% and a short 4-month median response duration [2]. The limited success of available treatments needs to develop new therapeutic and preventive approaches for melanoma, using synthetic or natural substances to prevent or reverse the transition of premalignant lesions into invasive cancer [3].

2. PPARs in cancer

Since their discovery in the early 1990s it has become clear that peroxisome proliferator-activated receptors (PPARs) are ligand-activated transcription factors that are involved in the genetic regulation of complex pathways of mammalian metabolism, including fatty acid oxidation and lipogenesis. PPARs are adopted

Abbreviations: COX2, cyclooxygenase-2; CYP24A1, vitamin D 24-hydroxylase; DHA, docosahexaenoic acid; mRNA, messenger ribonucleic acid; PPAR, peroxisome proliferator-activated receptor; PPRE, peroxisome proliferator response element; PCR, polymerase chain reaction; RXR, retinoid-X receptor; VDR, vitamin D receptor; VDRE, vitamin D response element.

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orphan nuclear hormone receptors [4,5] and belong to the nuclear receptor superfamily that includes receptors for steroids, thyroid hormone, vitamin D, and retinoids [6]. Three genetically and functionally distinct PPAR isoforms have been described: PPARα, PPARδ and PPARγ. All three PPARs exhibit distinct patterns of tissue distribution and differ in their ligand-binding domains [7]. In the last decade PPAR ligands and other agents influencing the PPAR signaling pathways have been shown to be also implicated in cellular proliferation, differentiation, tumor promotion, apoptosis, and immune reaction/inflammation [8]. Therefore modulating PPAR signaling pathways represents a potential novel strategy for inhibiting tumor carcinogenesis and progression. Besides natural ligands such as long-chain fatty acids, in particular, polyunsaturated fatty acids (including linoleic acid, linolenic acid and arachidonic acid) a large number of synthetic PPAR ligands have meanwhile been identified. Also clinically used drugs like the thiazolidinediones (troglitazone, rosiglitazone, pioglitazone), a class of insulinsensitizing agents and the fibrates (bezafibrate, clofibrate, fenofibrate), that are used as hypolipidaemic drugs, are binding to the PPARs [9–13].

3. VDR in cancer

Besides PPAR ligands also vitamin D analogs have been shown to be implicated in tumor progression and cellular differentiation. It is well known that 1,25-dihydroxyvitamin D_3, the biologically most active natural vitamin D metabolite, acts *via* binding to its corresponding intranuclear receptor (VDR), present in target tissue cells [14]. In various cell types, including normal and malignant human melanocytes, the effects of 1,25-dihydroxyvitamin D_3 and VDR mediated genomic pathways include the regulation of cell growth and differentiation [15–18].

4. Cross-talk between the VDR and the PPAR signaling pathways

After activation, PPARs and VDR form heterodimers with the retinoid-X receptor (RXR). These heterodimers preferentially bind to specific response elements, that are situated in enhancer sites of regulated target genes and are named peroxisome proliferator response elements (PPREs) or vitamin D response elements (VDREs), respectively. VDREs have been reported in the proximal promoter of a number of vitamin D-responding genes including VDR and the human vitamin D 24-hydroxylase (CYP24A1), the most responsive primary 1,25-dihydroxyvitamin D_3 target gene [19,20].

Using real-time PCR, we recently showed that treatment with 1,25-dihydroxyvitamin D_3 increased in the melanoma cell line MeWo not only the mRNA expression of the known target genes VDR and CYP24A1, but also of PPARα and PPARδ [21]. With the identification of a putative DR3-type VDRE in the proximal PPARδ promoter, the PPARδ gene was identified as primary target of 1,25-dihydroxyvitamin D_3 [22]. The increase of the PPARα expression could not be explained by the presence of a VDRE in the PPARα promotor, but may be induced by a multitude of coactivators and corepressors or on other unknown mechanisms [23]. Hints for a link between the signaling pathways of VDR and PPARα could also be found in other melanoma cell lines (SK-Mel-5, SK-Mel-25, SK-Mel-28), in the cutaneous squamous cell carcinoma cell line SCL-1, in the immortalized sebocyte cell line SZ95, but also in cell lines not deriving from skin (LNCaP = prostate cancer cells, MCF-7 = breast cancer cells) [24].

Moreover we recently showed that PPAR ligands induced in MeWo besides the PPAR expression also the VDR expression. These findings underline that the provided link between the PPARs and the VDR is bidirectional with either side being able to influence each other's activity [21]. This cross-talk involves a competition for the same heterodimerisation partner, RXR and the presence of VDREs and PPREs. However, the complete mechanisms of this cross-talk between the VDR and PPAR signaling pathways are not yet known. Further investigations are required to evaluate the physiological and pathophysiological relevance of this cross-talk. Some of the analysed ligands (WY14643 and GW501516) not only modulated the gene expression, but also had mild antiproliferative effects, which correlated with the increase in VDR expression afforded by these compounds at the same time-point (Fig. 1). It is therefore tempting to speculate that these compounds augment the VDR signaling system to achieve their antiproliferative action. In contrast to 1,25-dihydroxyvitamin D_3, the used PPAR ligands led to an upregulation of the VDR expression without an increase of the CYP24A1 expression. A possible explanation may be that the VDR protein itself exerts an antiproliferative effect since it was unregulated by the hormone wherever an antiproliferative effect was observed. The signaling pathways of PPARs and VDR regulate a multitude of genes that are of importance for various cellular functions including cell proliferation, cell differentiation, immune responses and apoptosis. Our findings therefore support the hypothesis that PPAR ligands open new perspectives for treatment and prevention of melanoma.

5. Future outlook: PPAR ligands in melanoma treatment and prevention

In the past few years the antiproliferative effects of PPAR ligands could be demonstrated in different cell lines *in vitro* [21,24–26].

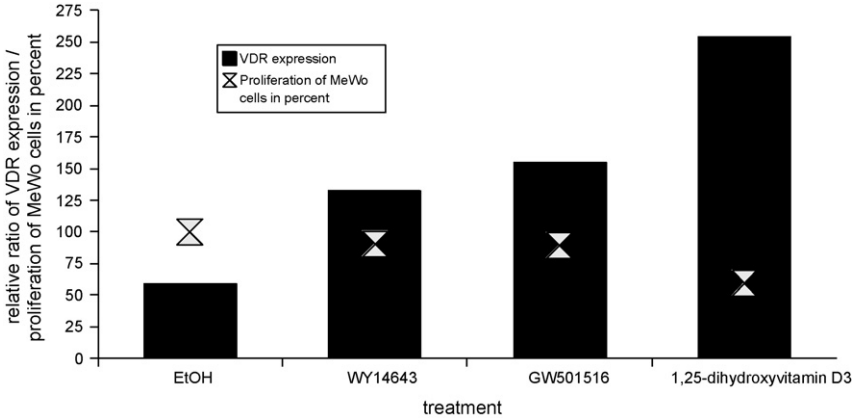

Fig. 1. Inverse correlation between the VDR expression after 120 h of treatment with different ligands compared to EtOH (black columns; measured by real-time PCR) and the proliferation (in percent) of the MeWo cells at this time-point (grey arrows; measured by crystal violet dye staining).

In addition to this, PPARα activation by corresponding ligands (fenofibrate) decreases the metastatic potential both in B16F10 mouse melanoma cells and in human SkMel188 cells *in vitro via* down-regulation of Akt signaling [3]. This is consistent with their previous report that Bomirski hamsters with melanoma subcutaneous tumors developed significantly fewer metastatic foci in the lungs after oral administration of fenofibrate, as compared to the control group. However, primary tumor growth remained unaltered [13]. Interestingly, in clinical trials with lipid-lowering drugs, significantly fewer patients treated with such a derivative, gemfibrozil, were diagnosed with melanoma as compared to these in the control group [11]. Nevertheless a recently published meta-analysis could not validate the possibility that statins or fibrates prevent melanoma [27]. Further studies have to show if fibrates or other PPAR ligands open new perspectives as agents decreasing metastatic potential of melanoma.

Evidence has been accumulated that thiazolidinediones, an other group of PPAR ligands, currently used to treat diabetes, inhibits the proliferation of cancer cells. The antiproliferative effects were mediated by cell-cycle arrest through a PPARγ-dependent pathway at low ciglitazone concentrations. At higher ciglitazone concentrations apoptosis was induced independently of PPARγ [28]. Moreover, the combination of PPAR agonists and cyclooxygenase-2 (COX2) inhibitors may have synergistic effects [29] and may increase the susceptibility of malignant cells to pulsatile chemotherapy by upregulating proapoptotic cellular mechanisms [30]. In a randomized multi-institutional phase II trial, administration of pioglitazone (PPAR ligand), rofecoxib (COX2 inhibitor) and trofosfamide was compared to treatment with trofosfamide alone in patients with treatment-resistant melanoma [31]. The combined treatment let to an improvement of the progression-free survival which was associated by an C-reactive protein response.

An other strategy to improve chemotherapeutic regimes is the creation of drug conjugates. A conjugate of the PPAR ligand docosahexaenoic acid (DHA) and doxorubicin was significantly more efficacious than free doxorubicin *in vitro* and in experimental animal tumor models [32]. A recently published phase I study analysed the maximum tolerated dose, dose-limiting toxicity and pharmacokinetics of weekly DHA–paclitaxel in resistant solid tumor malignancies [33]. DHA–paclitaxel administered weekly to a maximum dose of 600 mg/m^2 was well-tolerated and provided stable disease for 16 weeks in a patient with melanoma. In addition to this, the combined conjugates DHA–doxorubicin and DHA–paclitaxel were much less toxic than the chemotherapeutic agents alone [32–34].

Further investigations have to show the benefit of PPAR ligands compared to conventional chemotherapeutic regimens. The provided link between the PPAR and VDR signaling pathways may help guide molecular-based treatment strategies and allow the synthesis of new agents for melanoma treatment.

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