Cancers of the head and neck are among the most common neoplasms worldwide, characterized by local tumor aggressiveness, high rate of early recurrence, development of metastasis and second primary tumors. Although disease management of head and neck cancer has improved significantly, overall survival-rates remained largely unchanged over the last decades. Thus, in addition to modern chemo-radiation treatment strategies combined with sophisticated surgery, there is still a need for molecular markers and key regulatory factors exploitable for chemoprevention and targeted therapies.

A critical event in carcinogenesis is the uncontrolled modulation of genetic programs, mediated by deregulated signaling cascades, together with downstream transcriptional modulators. Hence, nuclear receptors, belonging to a superfamily of transcription factors implicated in a broad spectrum of physiological and pathophysiological processes, have also been associated with HNC. Enhanced expression of several nuclear receptors has been shown in head and neck cancer cells, and strategies targeting these molecules have been developed and tested in the clinics. In particular, the effects of retinoids targeting nuclear receptors of the thyroid hormone receptor-like receptor subfamily have been vigorously examined in large clinical chemoprevention trials.

This review seeks to provide a general overview of nuclear receptors’ molecular functions and summarizes their prognostic/therapeutic relevance, as well as the (pre)clinical studies targeting nuclear receptors in HNC.

Key words: Chemoprevention, Chemotherapy, Hormone response element, PPAR, RAR, Retinoids, RXR, Squamous cell carcinoma, Tamoxifen, Transcriptional activation

Introduction

Squamous cell carcinoma is the primary tumor type in head and neck cancer, which is the sixth most common malignant neoplasm in humans worldwide (Lippert et al., 2007; Jemal et al., 2009). The majority of head and neck squamous cell carcinomas (HNSCC) are induced by chronic exposure to a surplus of carcinogens enclosed in all forms of tobacco, synergized by heavy alcohol consumption and/or are associated with oncogenic human papillomaviruses (HPV) (Forastiere et al., 2006; Lippert et al., 2007; Fakhry et al., 2008). About 5-10% of suspicious lesions arising in the mucous membranes of the mouth, pharynx and larynx seem to undergo malignant transformation triggered by these risk factors.

HNC is characterized by local tumor aggressiveness, high rate of early recurrence and development of second primary carcinomas (see Bernier, 2008; Forastiere, 2008). Loco-regional relapse after therapy is the major cause of death despite modern disease management strategies (Argiris et al., 2008; Fakhry et al., 2008; Specenier and Vermorken, 2009). Hence, long-term survival rates remain low (30-40%), especially for advanced HNSCC, and have not improved significantly over the last decades (Bernier, 2008; Forastiere, 2008). Currently, EGFR-targeting agents, such as antibodies or tyrosine kinase inhibitors have gained major clinical attention, in particular for the treatment of locally advanced cancer with the intent of preserving speech and swallowing (Forastiere et al., 2008; Rapidis et al., 2008). Despite encouraging developments, EGFR-directed therapies are effective only in a relatively small percentage of cancer patients, underlining the need for additional treatment options (Bonner et al., 2006; Bernier, 2008).

The model that HNSCC are the end product of a multistep carcinogenic process, which might be blocked or even reversed before cells and tissues reach the cancer end stage has been the driving force behind chemo-
prevention research (details in Khuri et al., 1997; Mao et al., 2004). These studies suggest that clonal genetic alterations occur at an early, or premalignant stage of carcinogenesis, since chromosomal aberrations have been detected not only in tumor cells, but also in histologically defined premalignant lesions, such as oral leukoplakias and nonmalignant epithelial tissue adjacent to tumors. In addition to multistep carcinogenesis, the concept of field cancerization provides major rationale for chemoprevention of epithelial cancers (details in Khuri et al., 1997; Mao et al., 2004). Current local and systemic anticancer treatments do not eliminate efficiently the major consequence of field carcinogenesis, so-called second primary tumors (SPTs). These tumors represent a major cause of failure and death in locally treated primary cases of head and neck cancer, underlining the need for improved chemoprevention and treatments.

Hence, in order to better tailor current treatments and to develop novel therapeutic strategies for a better clinical management of head and neck cancer, the identification of prognostic factors, together with an improved molecular understanding of therapy resistance and SPTs are needed (Lippert et al., 2007; Bernier, 2008; Fakhry et al., 2008; Forastiere, 2008).

**Nuclear receptors: Highly versatile transcriptional regulators**

Nuclear receptors (NRs) are transcription factors implicated in a broad and highly complex spectrum of physiological and pathophysiological processes, and thus are recently attracting major interest as therapeutic targets (Gronemeyer et al., 2004; Schweitzer et al., 2008). NRs belong to a large superfamily of transcription factors, and are currently classified into seven subfamilies based on sequence comparison (Table 1). The modulation of transcription by NRs is achieved by both transcriptional activation, as well as repression (Gronemeyer et al., 2004; Germain et al., 2006; Schweitzer et al., 2008). Transcriptional regulation can either be ligand-dependent or -independent, allowing NRs to mediate gene repression or its release, gene activation, or even gene trans-repression (Gronemeyer et al., 2004; Perissi and Rosenfeld, 2005; Schweitzer et al., 2008).

Compared to cell surface receptors, such as the epidermal growth factor receptor (EGFR), which indirectly modulate genetic programs through complex intracellular signaling cascades, NRs are directly able to regulate transcription by binding to specific DNA-sequences, so-called hormone response elements (HREs). To execute their biological functions, NRs are composed of a N-terminal regulatory domain (activation function 1 = AF1), followed by a DNA-binding domain (DBD), a ligand binding (LBD) and a C-terminal domain (activation function 2 = AF2) (Fig. 1) (Gronemeyer et al., 2004; Schweitzer et al., 2008).

Despite their conserved structural organization, their functions are highly diverse. Nevertheless, two main

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**Fig. 1.** Domain organization and structural binding modes of NRs. **A.** NRs are composed of a N-terminal regulatory domain (activation function 1 = AF1), followed by a DNA-binding domain (DBD), a ligand-binding domain (LBD) and a C-terminal domain (activation function 2 = AF2). **B.** Certain NRs (e.g., steroid receptors) bind to half-site HRE inverted repeats as homodimers, others preferentially bind HRE direct repeats as heterodimers with retinoid X receptor-like receptor partners. Orphan receptors may bind to direct HRE repeats as homodimers or even to single site HREs in their monomeric form.
modes of action can be assigned according to their intracellular steady-state localization in the absence of ligand (Fig. 2). Cytoplasmic NRs are confined to the cytosol within multi-protein-complexes and actively enter the nucleus as a consequence of ligand binding, where they bind to their respective HREs as homo- or heterodimers (Gronemeyer et al., 2004; Schweitzer et al., 2008). Other receptors already reside in the nucleus in a complex with co-repressor proteins, while ligand binding triggers co-repressor dissociation and the recruitment of co-activators (Fig. 2) (Nettles and Greene, 2005; Michalik and Wahli, 2007). However, to fulfill multiple biological tasks, minor to major deviations from these two major modes of NR function exist (Gronemeyer et al., 2004; Schweitzer et al., 2008).

As NRs modulate various cellular processes, including cell proliferation, apoptosis, invasion and migration, all clearly representing hallmarks of cancer cells, several highly successful cancer drugs target this receptor superfamily. Since NRs are differentially expressed also in HNC cells, NRs are most likely to also contribute to cancer development and progression in this tumor entity. So far, the majority of reports and clinical studies in HNC have focused on two classes of the NR superfamily, the thyroid-hormone receptor-like and the estrogen receptor-like receptors (Table 1). Hence, this review will mainly summarize our current knowledge on members of these subfamilies. To limit the references to a reasonable number, several recent up-to-date reviews are included, sometimes in place of relevant articles. We apologize to our colleagues when, due to lack of space, a recent review article is mentioned instead of multiple original references.

**The thyroid hormone receptor-like receptors subfamily**

**Retinoid acid receptors (RARs)**

Within the thyroid hormone receptor-like receptor subfamily (Table 1), the retinoic acid receptor subtypes RARα, β and γ are all characterized by their activation through binding of retinoid acid (see Khuri et al., 2006; Altucci et al., 2007; Schweitzer et al., 2008). Upon activation, RARs can heterodimerize with retinoid X receptors (RXRs), belonging to the retinoid X receptor-like receptor subfamily and as such bind to specific HREs to regulate the transcription of target genes (Figs. 1, 4) (Altucci et al., 2007; Wrangle and Khuri, 2007; Schweitzer et al., 2008, and references within). A large number of co-activator and co-repressor proteins are involved in this process, thereby allowing transcriptional fine-tuning, ranging from repression to full activation of genetic programs (see Altucci et al., 2007; Wrangle and Khuri, 2007; Schweitzer et al., 2008). RAR activation often leads to differentiation, cell-cycle arrest or apoptosis, thus counteracting cancer cell proliferation and tumor progression (details in Khuri et al., 1997, 2006; Altucci et al., 2007; Wrangle and Khuri, 2007; Schweitzer et al., 2008). Hence, the ligand retinoid acid or natural and synthetic derivatives thereof have been intensely investigated for decades, in particular in head and neck cancer chemoprevention studies (Table 3) (see Khuri et al., 1997, 2006). The retinoid isotretinoin, also

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**Fig. 2.** Model illustrating the two major modes of NR activation. Natural or synthetic ligands diffuse through the cell membrane and bind to cytosolic or nuclear NRs. Ligand binding to cytoplasmic NRs triggers conformational changes resulting in dissociation of heat shock proteins (HSPs) and receptor dimerization, allowing active nuclear import and transactivation by binding to HREs. Other NRs are constitutively nuclear and complexed with corepressors in the absence of ligands. Ligand binding induces conformational changes resulting in the recruitment of coactivators to activate transcription of target genes.
known as 13-cis-retinoic acid, is converted to all-trans-retinoic acid, which preferentially targets RARs, whereas 9-cis-retinoic acid also activates RXRs (Figs. 3, 4). RXRs are promiscuous in that they can form heterodimers with different NRs, including thyroid hormone receptors, the vitamin D receptor, peroxisome proliferator-activated receptors (PPARs), as well as a number of orphan receptors, such as LXR, PXR and FXR (Table 1) (see Shulman and Mangelsdorf, 2005; Schweitzer et al., 2008). Hence, the various biological effects induced by retinoids may not only depend on the targeting of RARs but also on RXRs, as well as their interaction partners.

Reduced RARβ mRNA levels have been observed in malignant tumors of the head and neck, but appear to be prominent early in pre-malignant oral lesions (see Khuri et al., 1997, 2006; Freemantle et al., 2006; Ralhan et al., 2006). Recently, these results were confirmed on the protein level by immunohistochemistry showing the different RARα/β/γ expression levels during oral SCC.

Table 1. Categorization of the NR superfamily into subfamilies according to sequence homology. Subfamilies are divided into NR and orphan NR. Trivial abbreviations are given in brackets. NRs implicated in head and neck tumorigenesis are highlighted.

<table>
<thead>
<tr>
<th>Subfamily</th>
<th>Thyroid hormone receptor-like</th>
<th>Retinoid X receptor-like</th>
<th>Estrogen receptor-like</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nuclear receptors</td>
<td>Retinoic acid receptor (RAR)</td>
<td>Retinoic acid receptor (RAR)</td>
<td>Retinoic acid receptor (RAR)</td>
</tr>
<tr>
<td></td>
<td>Peroxisome proliferator-activated receptor (PPAR)</td>
<td>Retinoid X receptor (RXR)</td>
<td>Retinoid X receptor (RXR)</td>
</tr>
<tr>
<td></td>
<td>Vitamin D receptor (VDR)</td>
<td>Hepatocyte nuclear factor (HNF4)</td>
<td>Hepatocyte nuclear factor (HNF4)</td>
</tr>
<tr>
<td></td>
<td>Thyroid hormone receptor (TR)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Farnesoid X receptor (FXR)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Retinoid X receptor (ROR)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Constitutive androstane receptor (CAR)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Orphan receptors</td>
<td>Liver X receptor (LXR)</td>
<td>Chicken ovalbumin upstream promoter-transcription factor (COUP-TF)</td>
<td>Chicken ovalbumin upstream promoter-transcription factor (COUP-TF)</td>
</tr>
<tr>
<td></td>
<td>Rev-Erbα</td>
<td>Testicular receptor 2 and 4 (TR)</td>
<td>Testicular receptor 2 and 4 (TR)</td>
</tr>
<tr>
<td></td>
<td>Estrogen receptor (ER)</td>
<td>Estrogen related receptor 1 and 2 (ERR)</td>
<td>Estrogen related receptor 1 and 2 (ERR)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Subfamily</th>
<th>Nerve growth factor IB-like</th>
<th>Steroidogenic factor-like</th>
<th>Germ cell nuclear factor-like</th>
<th>Miscellaneous</th>
</tr>
</thead>
<tbody>
<tr>
<td>Orphan receptors</td>
<td>Nerve growth factor IB (NGFIB)</td>
<td>Steroidogenic factor 1 (SF-1)</td>
<td>Germ cell nuclear factor (GCNF)</td>
<td>Small heterodimer partner (SHP)</td>
</tr>
<tr>
<td></td>
<td>Nuclear receptor related 1 (NURR1)</td>
<td>Liver receptor homolog 1 (LHR-1)</td>
<td></td>
<td>Dosage-sensitive sex reversal, adrenal hypoplasia critical region (DAX)</td>
</tr>
<tr>
<td></td>
<td>Neuron-derived orphan receptor 1 (NOR1)</td>
<td></td>
<td></td>
<td></td>
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</tbody>
</table>

Fig. 3. Model illustrating the proposed channeling of RAR heterodimerization with RXR or PPAR. All-trans retinol is bound by the cellular retinol binding protein CRBP1. It is enzymatically converted to retinal by the retinol dehydrogenase (RoDH), whereas oxidation of retinal to all-trans RA is mediated by the retinal dehydrogenase (RALDH). RA can either bind to the cytoplasmic RA transporter CRABP2 (cellular retinoic acid binding protein) or the fatty acid binding protein FABP5, depending on their cellular abundance. RA-isomerase can further convert all-trans into 9-cis RA, thus activating RXR and leading to RAR/RXR heterodimerization and activation of RARE-dependent genes. Mobilization of the transporter protein FABP5 by unsaturated fatty acids may also lead to PPAR activation, RAR/PPAR heterodimer formation, and thus binding to PPRE-containing promoter regions.
development and progression (Ralhan et al., 2006). These studies revealed a significant association between the decrease in RARß levels and the patient’s response to isotretinoin (see Khuri et al., 1997, 2006; Ralhan et al., 2006). The comparison of non-exposed normal oral mucosa with histologically normal oral tissues from patients with oral lesions not only showed p53 accumulation, but also significant loss of RARß, as well as of the cell cycle regulator p21 (Ralhan et al., 2006). Multivariate analysis further indicated that this RARß-p21 phenotype was indeed associated with shorter disease-free survival (Ralhan et al., 2006). Thus, RARß seems to contribute to the suppression of the pre-malignant phenotype, whereas its loss promotes
malignancy (see Khuri et al., 1997, 2006; Freemantle et al., 2006; Ralhan et al., 2006). The exact molecular mechanisms leading to downregulation or loss of RARβ are still poorly understood. Besides genetic modulation of NR expression, epigenetic silencing caused by aberrant hypermethylation of CpG islands in the RARβ promoter was linked to its downregulation (Youssef et al., 2004; Petty et al., 2005; Freemantle et al., 2006). Moreover, it was suggested that RARβ expression also depends on the intracellular level of retinoids, as decreased RARβ levels were observed during vitamin A deficiency, and its expression was re-stimulated by retinoid acid treatment (see Khuri et al., 1997, 2006; Freemantle et al., 2006; Ralhan et al., 2006).

The paradoxical effect that in some cases retinoid acid promoted rather than inhibited tumor cell survival appears to be due to the promiscuous nature of NRs (Freemantle et al., 2006; Schug et al., 2007). It was suggested that RARs heterodimerize with peroxisome proliferator-activated receptors (PPARs) (Schug et al., 2007). As a consequence, RAR/PPAR heterodimers may trigger the expression of prosurvival factors, such as components of the PDK-1/Akt pathway or survivin (Lippert et al., 2007; Schug et al., 2007). For the channeling of retinoid acid to these two different NR heterodimers (RAR/RXR versus RAR/PPAR) and thus, for the biological consequences of retinoid acid treatment, expression of the retinoid acid transporters CRABP2 and FABP5 appears to be crucial (Fig. 3) (Schug et al., 2007). Overexpression of CRABP2 may lead to preferential RAR/RXR activation and hence, inhibition of tumorigenesis. In contrast, elevation of FABP5 levels favor RAR/PPAR stimulation and activate carcinogenic genetic programs (Schug et al., 2007). Both proteins have been shown to be differentially expressed in metastatic and HPV-associated HNSCC (Martinez et al., 2007; Uma et al., 2007). However, the in vivo relevance of such systems for ligand guidance to the appropriate receptor, as well as their impact on the outcome of retinoid-based clinical trials in HNC remain to be proven (Freemantle et al., 2006; Khuri et al., 2006).

In summary, the rationale for clinical cancer chemoprevention is largely based on preclinical and early clinical studies in which retinoids suppressed epithelial carcinogenesis (see Khuri et al., 1997, 2006; Freemantle et al., 2006). However, the results of phase III trials showed that isotretinoin was not effective in mediating chemoprevention in patients with early-stage HNSCC, as well as with stage I non-small-cell lung cancer (details in Freemantle et al., 2006; Khuri et al., 2006). The possible reasons for this lack of isotretinoin clinical chemoprevention activity range from its unfavorable pharmacokinetics over inherent tumor resistance to the lack of intact retinoic acid-signaling. Unfortunately, the expression of RARs, as well as their modulation during isotretinoin treatment was not analyzed in these studies. Current and future trials (Table 3) aim to resolve these controversies by recruiting appropriate study populations and the use of receptor specific drugs, as well as by multi-targeted combinations (see Gronemeyer et al., 2004; Khuri et al., 2006; de Lera et al., 2007; Wrangle and Khuri, 2007). Clearly, sustained efforts are required to design clinically predictive and mechanistic trials with validated biomarker endpoints to verify drug activity. Although biomarkers besides RARs (Khuri et al., 1997; Higuchi et al., 2003), such as the insulin-like growth factor or the cell cycle regulators cyclin D1 and p21 (Dragnev et al., 2004; Wu et al., 2004), have been suggested, an in depth understanding of the molecular mechanisms and pharmacology of retinoids in HNC is required in order to fully exploit the therapeutic potential of RARs targeting.

**Vitamin D receptor (VDR)**

The VDR has traditionally been associated with calcemic activities, namely calcium and phosphorus homeostasis and maintenance of bone content. However, the observation that the VDR is also present in cells other than those of the intestine, bone and kidney, led to the recognition of noncalcemic actions of VDR ligands.

**Table 2. Tissue distribution of PPAR isofrom expression and examples for their endogenous and exogenous ligands.**

<table>
<thead>
<tr>
<th>PPAR Subtype</th>
<th>Tissue distribution</th>
<th>Endogenous ligands</th>
<th>Exogenous ligands</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>PPARα</strong></td>
<td>brown adipose tissue, skeletal muscle, heart, liver, kidney, endothelium, vascular smooth muscle cells, monocytes, macrophages</td>
<td>naturally occurring saturated and unsaturated long chain fatty acids (arachidonic acid, linoleic acid, linolenic acid, docosahexanoic acid, eicosapentaenoic acid, elaidic acid, oleic acid, pretroselinic acid) and their intermediate metabolites (e.g., leukotrienes, prostaglandins)</td>
<td>hypolipidemic drugs (bezafibrate, ciprocibrate, clofibrate, ETYA, fenofibrate, gemfibrozil, GW2331, GW7647, GW9578, LY518674, WY14463, WY14643)</td>
</tr>
<tr>
<td><strong>PPARβ/δ</strong></td>
<td>ubiquitous, but markedly in brain, adipose tissue and skin</td>
<td>naturally occurring saturated and unsaturated long chain fatty acids (eicosapentaenoic acid, linoleic acid) and their intermediate metabolites (e.g., prostacyclin), retinoic acid</td>
<td>hypolipidemic drugs (bezafibrate, ETYA, L165041, GW0742, GW501516)</td>
</tr>
<tr>
<td><strong>PPARγ</strong> (3 splice variants)</td>
<td>white and brown adipose tissue, muscle, colon, liver, macrophages</td>
<td>naturally occurring saturated and unsaturated long chain fatty acids (arachidonic acid, eicosapentaenoic acid, linoleic acid, linolenic acid) and their intermediate metabolites (e.g., eicosanoids, prostaglandins)</td>
<td>hypolipidemic drugs (ciprocibrate, GW1929, GW2331, GW7845, JTT-501, L165041) thiazolidinediones/TZDs (ciglitazone, KRP-297, pioglitazone, rosiglitazone, troglitazone)</td>
</tr>
</tbody>
</table>
(Nagpal et al., 2005; Campbell and Adorini, 2006, and references within). The VDR, as well as its ligands, are clearly involved in cell proliferation, differentiation and apoptosis (Nagpal et al., 2005; Thorne and Campbell, 2008). Also, several epithelial cancer types, including HNSCC, exhibit increased expression of VDR in tumor cells when compared with their non-malignant counterparts (see Nagpal et al., 2005; Thorne and Campbell, 2008). Numerous studies have shown that the VDR ligand, 1,25-dihydroxyvitamin D$_3$/calcitriol as the active form of the prohormone vitamin D$_3$, inhibits proliferation of tumor cells derived from various cancer types (references in Takahashi and Morikawa, 2006; Slattery, 2007; Lu et al., 2008). Hence, testing of the therapeutic effects of VDR ligands is pursued in cell culture and animal cancer models, as well as in a limited number of open clinical trials, including head and neck cancer (Table 3) (references in Takahashi and Morikawa, 2006; Slattery, 2007; Lu et al., 2008). Since a barrier to the clinical use of calcitriol has been its hypercalcemic effects, analogues such as EB1089/seocalcitol, characterized by potent anti-tumor and significantly reduced hypercalcemic activity, are currently intensely studied in preclinical models, as well as clinical trials (see Takahashi and Morikawa, 2006; Slattery, 2007; Lu et al., 2008).

At the molecular level, 1,25-(OH)$_2$D$_3$ and its synthetic analogs modulate gene expression via a heterodimer formed between VDR and RXR (Campbell and Adorini, 2006; Thorne and Campbell, 2008). In the absence of ligand, most of the VDR is cytoplasmic, whereas ligand binding induces RXR-VDR heterodimerization and nuclear translocation (Figs. 2, 4) (Campbell and Adorini, 2006; Thorne and Campbell, 2008). The RXR-VDR heterodimer binds to vitamin D response elements (VDREs) present in the promoter regions of responsive genes, resulting in their activation, repression or even trans-repression (details in Nagpal et al., 2005; Schweitzer et al., 2008). Mechanistically, the anti-proliferative activity of VDR ligands involves the induction of cell cycle arrest by enhancing the expression of the cell cycle inhibitors p21 and p27, as well as of cyclin-dependent kinase inhibitors (CDKI) such as p57 (Hager et al., 2001; Lin et al., 2003; Lu et al., 2008).

### Table 3. Summary of clinical trials in the field of head and neck cancer targeting NR.

<table>
<thead>
<tr>
<th>NR</th>
<th>Clinical trial</th>
<th>Drug</th>
<th>Phase</th>
<th>Cancer type</th>
</tr>
</thead>
<tbody>
<tr>
<td>RAR</td>
<td>Chemoprevention study of oral cavity squamous cell carcinoma (NCT00201279)</td>
<td>13-cis Retinoc acid</td>
<td>Phase III completed</td>
<td>Oral cavity squamous cell carcinoma</td>
</tr>
<tr>
<td></td>
<td>Isotretinoin plus interferon in treating patients with recurrent cancer (NCT00002506)</td>
<td>Isotretinoin (combined with interferon $\alpha$)</td>
<td>Phase II ongoing, but not recruiting participants</td>
<td>Head and neck cancer, esophageal cancer, cervical cancer, lung cancer, non-melanomatous skin cancer, penile cancer</td>
</tr>
<tr>
<td></td>
<td>Isotretinoin, interferon alpha, and vitamin E in treating patients with stage III or stage IV head and neck cancer (NCT00054561)</td>
<td>Isotretinoin (combined with interferon $\alpha$ and vitamin E)</td>
<td>Phase III completed</td>
<td>Head and neck cancer</td>
</tr>
<tr>
<td>PPARγ</td>
<td>Pioglitazone in preventing head and neck cancer in patients with oral leukoplakia (NCT00099021)</td>
<td>Pioglitazone</td>
<td>Phase II ongoing, not recruiting participants</td>
<td>Head and neck cancer, precancerous/nonmalignant condition</td>
</tr>
<tr>
<td></td>
<td>Rosiglitazone in preventing oral cancer in patients with oral leukoplakia (NCT00369174)</td>
<td>Rosiglitazone</td>
<td>Phase II completed</td>
<td>Head and neck cancer, precancerous/nonmalignant condition</td>
</tr>
<tr>
<td></td>
<td>A phase 1/2 dose finding study of an experimental new drug CS7017, an oral PPAR$\gamma$ agonist taken by mouth daily in combination with paclitaxel chemotherapy (NCT00603941)</td>
<td>CS7017</td>
<td>Phase I/II currently recruiting participants</td>
<td>Anaplastic thyroid cancer</td>
</tr>
<tr>
<td></td>
<td>A pilot clinical trial for poorly differentiated thyroid cancer - correlation to retinoid and peroxisome-proliferator-activated receptor (PPAR$\gamma$) expression (NCT00718770)</td>
<td>Bexarotene</td>
<td>Phase I/II currently recruiting participants</td>
<td>Thyroid cancer</td>
</tr>
<tr>
<td>VDR</td>
<td>Vitamin D - celecoxib therapy (NCT00953849)</td>
<td>Vitamin D$_3$ (combined with celecoxib)</td>
<td>Phase II not recruiting participants</td>
<td>Mouth neoplasms</td>
</tr>
<tr>
<td>ER</td>
<td>Combination chemotherapy and tamoxifen in treating patients with solid tumors (NCT00002800)</td>
<td>Tamoxifen (combined with cisplatin and doxorubicin)</td>
<td>Phase II completed</td>
<td>Head and neck cancer, adrenocortical carcinoma, brain/central nervous system tumors, liver cancer, malignant mesothelioma, pheochromocytoma, sarcoma</td>
</tr>
</tbody>
</table>

NCI protocol ID are given in brackets (see: http://www.cancer.gov/CLINICALTRIALS). Cancer types other than head and neck are marked in gray.
al., 2008). In addition, a number of genes involved in differentiation and cell growth inhibition pathways have been identified by expression profiling of EB1089-treated cells (Lu et al., 2008), which, subsequent to the validation of their biological relevance, may be exploited as biomarkers for clinical trials.

Furthermore, an association of VDR gene polymorphisms with cancer risk has been reported, albeit not without controversies (Raimondi et al., 2009). In a comprehensive hospital-based case-control study, Liu et al. reported that certain VDR alleles and their genotypes were associated with a decreasing risk of HNSCC in a variant allele dose-dependent manner, particularly for the combined genotypes (Liu et al., 2005). Hence, polymorphisms in the VDR gene may have to be considered when designing and evaluating VDR targeting chemoprevention and chemotherapy trials.

Taken together, an enhanced understanding of the vitamin D signaling pathway has paved the way for its therapeutic exploitation. A major hurdle facing the translation of basic research into therapeutic VDR ligands is still hypercalcemia/hypercalciuria, although less calcemic analogs are currently tested with promising results (see Liu et al., 2005; Takahashi and Morikawa, 2006; Slattery, 2007; Lu et al., 2008). Besides an increased understanding of the mechanism of VDR action, the combination of VDR modulators with other differentiation inducers, such as histone deacetylase inhibitors (Kramer et al., 2008) or chemotherapeutic regimens may offer additive or synergistic benefits for the prevention and treatment of this tumor entity.

**Peroxisome proliferator-activated receptors (PPARs)**

Another group of the thyroid hormone receptor-like receptor subfamily relevant for cancers of the head and neck (Table 1) are the PPARs. So far, three isoforms of the PPAR (α, β/δ, and γ) and several splice variants have been identified, all able to form heterodimers with retinoid X receptors (Lehrke and Lazar, 2005; Schweitzer et al., 2008; Yasui et al., 2008). PPARs are expressed in different cell types (Table 2) controlling the transcription of genes involved in various biological processes, such as lipid metabolism and insulin sensitivity (Lehrke and Lazar, 2005; Yasui et al., 2008). Furthermore, a role in limiting inflammation has also been reported (Hsueh and Bruemmer, 2004; Lehrke and Lazar, 2005). As tumor cell metabolism and inflammation appear to be critical for tumorigenesis and clinical outcome, PPARs may thus directly or indirectly contribute to malignancies (Altucci et al., 2007; Kroemer and Pouyssegur, 2008). In the absence of ligand, PPARs are complexed with co-repressor proteins, acting as transcriptional repressors. Ligand binding induces conformational changes, which in turn allow heterodimerization with RXR, leading to the attraction of transcriptional co-activators (Figs. 3, 4) (Lehrke and Lazar, 2005; Nettles and Greene, 2005). Natural and synthetic ligands for PPARs include lipophilic molecules such as fatty acids, as well as thiazolidinedione (TZD) drugs and derivates thereof (Table 2) (Gronemeyer et al., 2004; Lehrke and Lazar, 2005; Schweitzer et al., 2008).

Enhanced expression has been demonstrated for PPARβ and PPARγ in HNSCC (Lehrke and Lazar, 2005; Masuda et al., 2005; Hamakawa et al., 2008). Also, PPARγ was detected in pleomorphic adenomas and adenoid cystic carcinomas of the salivary gland, but not in the corresponding normal tissues (Hamakawa et al., 2008). Agonist binding to PPARγ can induce cell differentiation, growth arrest and apoptosis of cancer cells (Masuda et al., 2005; Hamakawa et al., 2008; Schweitzer et al., 2008). Thus, synthetic PPARγ ligands were tested as anti-cancer and chemopreventive agents in various tumor types, including head and neck cancer (Masuda et al., 2005; Hamakawa et al., 2008). However, the exact role of PPARγ in carcinogenesis as well as the effects of PPAR-targeting compounds is still not resolved (Lehrke and Lazar, 2005; Masuda et al., 2005; Hamakawa et al., 2008). For example, PPARγ was found overexpressed in OSCCs, but PPARγ inhibitors and not ligands interfered with adhesion and metastasis in vitro (Masuda et al., 2005; Hamakawa et al., 2008). Such pivotal effects may be explained by the fact that PPARs directly and/or indirectly modulate crucial cancer-relevant pathways, such as Wnt- and NFκB-signaling and the activity of cell-cycle regulators, which are subjected to additional stringent control mechanisms (Lehrke and Lazar, 2005; Yasui et al., 2008). Alternatively, the growth-inhibiting effects of synthetic PPAR ligands in HNSCC may be mediated by cross-talk with other NRs such as the RARs (Masuda et al., 2005; Altucci et al., 2007; Hamakawa et al., 2008). Such a scenario is supported by the observation that the ligand-induced biological effects were not only dependent on PPARγ levels, but also on the type of agonist/antagonist and their concentration, as well as on the tumor cell type (Lehrke and Lazar, 2005; Hamakawa et al., 2008; Yasui et al., 2008). In addition, it is likely that the tumor inhibiting or promoting effects of PPARγ ligands are mediated indirectly by modulating the vitality of components of the tumor microenvironment, such as cancer-associated fibroblasts or tumor endothelial cells (Whiteside, 2008). In fact, PPARγ ligands have been shown to affect endothelial cell proliferation and migration, thereby regulating angiogenesis (Wang, 2008). Also, hypoxia-induced angiogenesis can be affected by PPARγ ligands in cancer therapy, even if the precise mechanisms still remain unclear (Kim and Surh, 2008). As angiogenesis is a crucial aspect for tumor development, therapy resistance and metastasis, modulation of angiogenesis by PPARγ ligands may thus have contributed to at least some of the clinical benefits observed.

The therapeutic potential of PPARγ ligands for HNC was investigated in several clinical trials, but outcomes proved to be rather diverse. Whereas some trials revealed up to 40% partial response rates, others could not demonstrate significant beneficial effects (Kebebew
et al., 2006; Baetz et al., 2007). The results of ongoing or recently completed trials (Table 3) will hopefully contribute to evaluate the potential clinical benefit of PPARγ ligands as an additional treatment modality for HNC. Notably, PPARs seem to be also targeted indirectly by various novel treatment strategies. As an example, COX-2 inhibitors, actively tested for HNC chemoprevention and treatment, also appear to affect PPARγ, thereby generating a potential autocrine loop (Eibl, 2008; Michalik and Wahli, 2008).

In summary, PPARγ ligands may be considered as useful agents for the treatment of head and neck cancer. Increasing knowledge about the mode of action, specificity and dosage dependence of PPAR agonistic and antagonistic ligands will hopefully allow a better modeling of PPAR receptor function, and thus may lead to a more effective design of combinatorial application schemes for cancer prevention and treatment.

**The estrogen receptor-like receptors subfamily**

It is accepted that hormonal stimulation is critically involved in the etiology of carcinomas of the reproductive tract. Hence, the subfamily of estrogen receptor-like receptors, containing the estrogen receptors ERα and β, as well as the androgen and the progesterone receptor (Table 1) are of prognostic and therapeutic value in breast, ovarian and prostate cancer (Osborne and Schiff, 2005; Dahlman-Wright et al., 2006; Lu et al., 2006; Schweitzer et al., 2008).

Expression of these sex hormone receptors has also been demonstrated for various head and neck cancer subtypes (Lukits et al., 2007; Hamakawa et al., 2008). Both ER isoforms, as well as the progesterone receptor, were detectable in cancer cells of the oral cavity, the salivary gland and in laryngeal/hypopharyngeal cancers, whereas the tumor stroma was mostly negative (Lukits et al., 2007; Hamakawa et al., 2008). Expression of ERα inversely correlated with that of ERβ in esophageal carcinomas, and a correlation of ERβ levels with tumor de-differentiation and staging was suggested (Nozoe et al., 2007; Kalayarasan et al., 2008). Steroidal anti-estrogens such as tamoxifen inhibited proliferation and invasion of HNSCC cell lines and triggered apoptosis, which was further enhanced upon combination with chemotherapeutics (Ishida et al., 2007; Ku and Crowe, 2007). In salivary gland cancer, expression of progesterone appears to be associated with tumor progression, although progesterone treatment inhibited proliferation of salivary gland cancer cells in vitro (reviewed in Hamakawa et al., 2008). Also, the androgen receptor was reported to be present in salivary gland cancer, such as carcinomas and pleomorphic adenomas, salivary duct carcinomas and basal cell adenocarcinomas (see Hamakawa et al., 2008). However, the data reporting protein expression of sex steroid hormone receptors in clinical samples of HNCs need to be interpreted with caution, since these patterns, as well as their correlation with clinico-pathological parameters, vary substantially between the studies (Lukits et al., 2007; Hamakawa et al., 2008). Clearly, standardized operating procedures covering immunohistochemistry protocols are urgently required to allow the comparison and interpretation of future studies.

Considering the impressive benefit of endocrine therapy in breast cancer, targeting sex steroid hormone receptor as a potential therapeutic strategy is also discussed for HNC (Table 3) (see Hamakawa et al., 2008; Vattemi et al., 2008). However, convincing evidence underlining the relevance of sex steroid

### Table 4. NR target genes, which are differentially expressed and are functionally involved in head and neck cancer development and progression.

<table>
<thead>
<tr>
<th>NR/Target gene</th>
<th>Function</th>
<th>Reference - Target gene</th>
<th>Reference - Head and neck</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>PPAR</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>G0S2</td>
<td>cell cycle</td>
<td>Zandbergen et al. (2005)</td>
<td>Tokumaru et al. (2004)</td>
</tr>
<tr>
<td>PDK1</td>
<td>energy homeostasis</td>
<td>Degenhardt et al. (2007)</td>
<td>Wigfield et al. (2008)</td>
</tr>
<tr>
<td><strong>RAR</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>p21WAF1/CIP1</td>
<td>cell cycle</td>
<td>Liu et al. (1996)</td>
<td>Kapranos et al. (2001)</td>
</tr>
<tr>
<td>C/EBPα</td>
<td>transcription factor</td>
<td>Schwarz et al. (1997)</td>
<td>Bennett et al. (2007)</td>
</tr>
<tr>
<td>cyclins, CDK</td>
<td>cell cycle</td>
<td>Bour et al. (2007)</td>
<td>Jeannon et al. (1998)</td>
</tr>
<tr>
<td><strong>VDR</strong></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>p21WAF1/CIP1</td>
<td>cell cycle</td>
<td>Liu et al. (1996)</td>
<td>Kapranos et al. (2001)</td>
</tr>
<tr>
<td>cyclins</td>
<td>cell cycle</td>
<td>Sirkkonen et al. (2005)</td>
<td>Jeannon et al. (1998)</td>
</tr>
<tr>
<td><strong>ER</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CRABP</td>
<td>carrier protein</td>
<td>Li et al. (2003)</td>
<td>Vo et al. (1998)</td>
</tr>
<tr>
<td>CXCL12/SDF-1</td>
<td>chemokine/ligand</td>
<td>Hall et al. (2003)</td>
<td>Rehman et al. (2008)</td>
</tr>
</tbody>
</table>
hormone receptors for the onset and progression of head and neck cancer, as well as their prognostic and therapeutic impact, is currently rather limited. Also, as hormonal therapies may affect diverse physiological processes, the potential therapeutic benefits of targeting this NR family has to outweigh potential adverse side effects.

Conclusions and outlook

NRs clearly play critical roles in regulating genetic programs under physiological and pathophysiological conditions, and as such may contribute to establishing the “hallmarks of cancer cells” (Vogelstein and Kinzler, 2004). Hence, target genes modulated by NRs have been identified as key elements in the molecular circuits also involved in head and neck cancer development and progression (Table 4) (Mao et al., 2004). For this tumor entity, mainly members of the thyroid hormone receptor-like receptors family, particularly the retinoid acid receptors, have been intensely investigated for over 20 years (see Khuri et al., 1997, 2006). Unfortunately, this chapter on translational cancer chemoprevention in HNSCC closes with the statement that low-dose retinoid monotherapy was not effective in reducing the incidence of second primary tumors or in increasing overall disease-free survival (see Khuri et al., 1997, 2006). Although the outcomes of current clinical trials (Table 3) will contribute to evaluating the prognostic and therapeutic potential of NR modulators for HNC, these results underline the need for a more systematic and interdisciplinary exploitation of the existing knowledge with respect, the development of novel compounds with improved activity and receptor specificity is currently being intensively pursued in the area of metabolic diseases (Gronemeyer et al., 2004; Schweitzer et al., 2008). Also, as the biological functions of NRs and hence clinical outcome of NR targeting approaches are highly dependent on a complex network of accessory proteins, such as transcriptional corepressors and co-activators, such studies should not be restricted to monitoring NR expression levels alone (Gronemeyer et al., 2004; Schweitzer et al., 2008).

To achieve a more comprehensive understanding of NR biology and its relevance for carcinogenesis, an interdisciplinary exploitation of the existing knowledge on the role of NRs in other diseases is important. In this respect, the development of novel compounds with improved activity and receptor specificity is currently being pursued in the area of metabolic diseases (Gronemeyer et al., 2004; Biggins and Koh, 2007; Schweitzer et al., 2008). Since cancer cell metabolism is now beginning to be considered as “cancer’s Achilles’ heel”, it may be conceivable to speculate that such novel classes of NR modulators may show efficacy also for HNC, thus opening up novel treatment options in the future (Hsu and Sabatini, 2008; Kroemer and Pouyssegur, 2008).

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Nuclear receptors in head and neck cancer

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