The nuclear receptor factor peroxisome proliferator-activated receptor (PPARβ/δ) can regulate its target genes by transcriptional activation or repression through both ligand-dependent and independent mechanism as well as by interactions with other transcription factors. PPARβ/δ exerts essential regulatory functions in intermediary metabolism that have been elucidated in detail, but clearly also plays a role in inflammation, differentiation, apoptosis and other cancer-associated processes, which is, however, mechanistically only partly understood. Consistent with these functions clinical associations link the expression of PPARβ/δ and its target genes to an unfavorable outcome of several human cancers. However, the available data do not yield a clear picture of PPARβ/δ’s role in cancer-associated processes and are in fact partly controversial. This article provides an overview of this research area and discusses the role of PPARβ/δ in cancer in light of the complex mechanisms of its transcriptional regulation and its potential as a druggable anti-cancer target.

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[11–13], pointing to a context-dependent function of PPARβ/δ in immune regulation.

The role of PPARβ/δ in cancer and cancer-associated biological processes, including cell proliferation, differentiation and survival, is even less clear, and in some research areas published results are highly controversial. This applies in particular to the role of PPARβ/δ in colon carcinogenesis [2,4]. It is very likely that the impact of PPARβ/δ on tumor-associated functions depends on the cell type, its stage of differentiation, the cellular context and the microenvironment of soluble mediators, including PPARβ/δ ligands. The integration of these results in different mouse models, for instance, could easily lead to different outcomes resulting in a confusing overall picture. Furthermore, established cell lines and mouse models usually do not faithfully reflect the scenario in human cancer. Systematic studies addressing these issues are therefore urgently needed.

The present review attempts to analyze the role of PPARβ/δ in tumorigenesis from a different angle, i.e., by linking PPARβ/δ to the outcome of human cancers and discussing such associations in light of the known functions of PPARβ/δ. Another frequently neglected aspect is the fact that PPARβ/δ is not merely a ligand-controlled transcription factor, but can regulate its target genes in fundamentally different ways, including transcriptional repression and interactions with other transcriptional regulators. This complexity of the transcriptional PPARβ/δ network can make the interpretation of experimental data extremely difficult, in particular when it comes to complex biological questions, as its role in cancer. Different modes of transcriptional regulation by PPARβ/δ are therefore also covered in depth in this review and discussed in the context of clinical associations.

2. Modes of transcriptional regulation by PPARβ/δ

Like the other PPAR subtypes α and γ PPARβ/δ can regulate its target genes through binding to PPAR response elements (PPREs; also referred to as direct repeat elements, DREs), as heterodimers with members of the retinoid X receptor (RXR) family. Chromatin immunoprecipitation sequencing (ChIP-Seq) of PPARβ/δ, histone H3 trimethylated on lysine 4 and RNA polymerase II enrichment sites combined with transcriptional profiling of both WPMY-1 myofibroblasts [14] and MDA-MB-231 breast cancer cells (Figs. 1 and 2) [15] led to the identification of >100 bona fide direct PPARβ/δ target genes and three different modes of regulation:

- **Type I** genes harbor a *bona fide* PPRE that is constitutively bound by a PPARβ/δ-RXR complex acting as a repressor (hence upregulated by PPARβ siRNA). These genes are not inducible by agonists.

- **Type II** genes also harbor one or more *bona fide* PPREs bound by a PPARβ/δ-RXR repressor complex in the absence of exogenous agonist. These genes are upregulated by PPARβ siRNA like type I genes, but are inducible by agonists (canonical regulation).

- **Type III** genes are bound by a PPARβ/δ containing complex that interacts with PPRE-like motifs that are clearly different from DRE and acts as a transcriptional activator. These are downregulated by PPARβ siRNA and respond weakly, if at all, to ligands.

The mechanisms underlying these regulatory differences seem to depend, at least in part, on the sequence of the PPRE, which differs among type I, II and III genes [14]. Furthermore, the classification as a type I or II PPARβ/δ target gene is cell-type-dependent, since a given target gene can be inducible by agonists in one cell type but refractory to ligands in another. These results suggest that PPARβ/δ complexes are assembled in a promoter and cell type specific manner, pointing to a crucial influence of local structural features and the availability of coregulators.

PPARβ/δ can also regulate genes without making direct DNA contacts by directly interacting with specific transcription factors (Fig. 2), although the molecular mechanisms involved are poorly understood. For example, PPARβ/δ interacts with the p65 subunit of the NFκB dimer, impinging on TAK1-mediated signaling to NFκB and modulates NFκB signaling by ill-defined ligand-dependent mechanisms [11,16–18]. PPARβ/δ also interacts with BCL6 in macrophages in the absence of ligand, which prevents the repression of inflammatory genes by BCL6 in atherogenesis [19]. Deletion of PPARβ/δ in foam cells or the application of PPARβ/δ ligands abolishes this sequestration of BCL6, resulting in the repression of BCL6 target genes and an attenuation of atherogenesis-associated inflammation [19]. Whether these mechanisms also plays a role in cancer cells in currently unknown. However, genome-wide analyses strongly suggest that other PPRE-independent mechanism of PPARβ/δ-mediated transcriptional regulation exist, but this type of regulation remains largely enigmatic.

3. PPARβ/δ ligands

PPARβ/δ is activated by a large range of natural lipid ligands, including polyunsaturated fatty acids [20–22] and eicosanoid metabolites, such as prostacyclin [22–25] and 15-hydroxyeicosatetraenoic acid (15-HETE) [26]. Consistent with its regulation by these classes of compounds, the main functions of PPARβ/δ are the regulation of intermediary metabolism and inflammatory responses. This has led to the development of several highly selective and potent synthetic agonists that have been extensively used to address the biological functions of PPARβ/δ, including its role in tumorigenesis, as extensively reviewed in several recent publications [2,4,5]. The originally described function of all-trans retinoic acid (ATRA) as a PPARβ/δ agonist [27] could...
not be confirmed by other groups [28,29] and therefore remains questionable.

Besides agonistic ligands, several inhibitory inverse agonists have been developed and proved useful in analyzing the functions of PPAR\(\beta\)/\(\delta\) and the regulation of its target genes [30–37]. Inverse agonists are defined as ligands that, beyond antagonizing agonist binding, exert the opposite effect compared to an agonist. Thus, an agonist induces a conformation that favors the association of PPAR\(\beta\)/\(\delta\) with coactivators, while an inverse agonist triggers the recruitment of transcriptional corepressors. The latter results in the formation of a dominant repressor complex that also inhibits activation of the bound target gene by other transcription factors [15].

4. Association of PPAR\(\beta\)/\(\delta\) expression with human cancers

The meta-analysis by Gentles and colleagues [38] describes the association of transcriptome-derived gene expression data with the overall survival (OS) of 39 human cancers that can be accessed through the PRECOG database (https://precog.stanford.edu). For 26 of these entities, data from more than one study or for more than 100 patients were available. Analysis of this dataset revealed a strong association of PPAR\(\beta\) expression with poor survival (short OS) of three cancer types, i.e., breast cancer, lung adenocarcinoma and glioma, and a weak association with more favorable OS of prostate cancer (Fig. 3A). Different and partly opposite results were obtained for PPAR\(\alpha\) and PPAR\(\gamma\), including associations of PPAR\(\gamma\) and PPAR\(\alpha\) expression with a longer OS of glioma and breast cancer, respectively (Fig. 3A and B), indicating a specific role of each PPAR subtype in different cancer entities.

The online database KM plotter (http://kmplot.com/analysis/) [39] also contains large datasets for breast [40] and lung cancer [39], and can also be used to analyze associations with relapse-free survival (RFS) and specific features of a given entity. Breast cancer data are based on 3524 patients (2014 version), and lung adenocarcinoma data are derived from 451 patients (2015 version). Analysis of these datasets for RFS associations clearly confirmed the PRECOG data for OS. A higher PPAR\(\beta\) expression had a highly significant negative impact on the RFS (Fig. 3D) of breast cancer, in particular in HER2+ cases (KM plotter), and a striking association with the short time to relapse of lung adenocarcinoma (Fig. 3E).

These associations support the hypothesis that PPAR\(\beta\)/\(\delta\) plays a role in a subset of human cancers. However, to be able to draw more definitive conclusions it will be necessary to analyze the level, the subcellular localization and the transcriptional activity of PPAR\(\beta\)/\(\delta\) in patient-derived samples and to correlated these data with the clinical course of the disease. Such analyses are of particular interest in view of Human Protein Atlas data (http://www.proteinatlas.org/ENSG00000112033-PPAR\(\beta\)/cancer), which show an increased expression of nuclear PPAR\(\beta\)/\(\delta\) protein in subset of human tumors, including breast cancer, lung adenocarcinoma and glioma. Another potential caveat of the survival association studies described above is the fact that it cannot be ruled out that PPAR\(\beta\) induction reflects the activation of other signaling pathways, for example AP-1 [41], rather than representing a causative event. This highly relevant question must be addressed by future work through bioinformatic tools and functional studies.

5. Cancer-related functions of PPAR\(\beta\)/\(\delta\) target genes

The functions of a number of PPAR\(\beta\)/\(\delta\) target genes have been linked to various aspects of tumorigenesis (Fig. 2). The best studies PPAR\(\beta\)/\(\delta\) target genes are probably ANGPTL4 and PDK4. Both genes are regulated by the canonical mechanism and generally show a particularly strong induction by agonists due to the presence of multiple PPREs [14,42–44]. Importantly, the expression of both genes is associated with the OS of multiple cancer types (Fig. 2F and G).
ANGPTL4 is a secreted protein that is proteolytically cleaved in the blood, yielding N-terminal (nANGPTL4) and C-terminal (cANGPTL4) fragments [45]. Besides the metabolic function of nANGPTL4 in the inhibition of lipoprotein lipase [42], cANGPTL4 figures in multiple tumorigenesis-associated processes [46]. For example, ANGPTL4 released by tumor cells in response to TGF-β into the circulation increases the permeability of lung capillaries and facilitates the extravasation of disseminated breast cancer cells in a mouse model [47,48]. ANGPTL4 can also promote cell migration [47,49–51] and angiogenesis [52,53]. ANGPTL4 inhibits anoikis, which is essential for the survival of circulating tumor cells [54]. In cardiac cells ANGPTL4 is induced by dietary fatty acids to protect the heart against fatty acid-induced oxidative stress [55]. Moreover, ANGPTL4 expression is increased in human clear-cell renal carcinoma [53,56] and correlates with vascular invasion of human gastric and colon carcinoma cells [57,58]. ANGPTL4 is also part of gene expression signatures associated with distant metastasis of human breast cancer [39,60].

Consistent with these protumorigenic functions of ANGPTL4, several oncogenic signaling pathways have been shown to converge on its transcriptional enhancers, including HIF-1 [61], AP1 [43] and TGF-β-induced SMAD factors [43,47], PPARβ/δ-specific inverse agonists prevent the induction of ANGPTL4 by these oncogenic signaling pathways, including TGF-β-signaling [15]. This effect is attributable to the ability of these inverse agonists to trigger the establishment of a transcriptional repressor complex, which in turn blocks transcription initiation and thereby presumably also the effects of transcriptional enhancers. As a consequence, the TGFβ or serum-induced matrigel invasion by MDA-MB-231 breast cancer cells, which is dependent on ANGPTL4, is blocked by these ligands.

In summary, a large body of published data points to a function for ANGPTL4 in promoting cancer progression.

5.2. Pyruvate dehydrogenase kinase 4 (PDK4)

The pyruvate dehydrogenase (PDH) complex is an enzyme that is localized in the mitochondrial matrix and plays a pivotal role in the oxidation of glucose by catalyzing the conversion of pyruvate to acetyl-CoA. PDH kinases (PDKs) inhibit the PDH complex by phosphorylating one of its subunits. PDK4 is increased in conditions associated with the switch from glucose to fatty acids utilization for oxidative phosphorylation, for instance during fasting. Transcription of the PDK4 gene is induced by multiple stimuli, including insulin, glucocorticoids and fatty acids acting as PPAR agonists [62]. PDK4 is also believed to play an essential role in tumor metabolism by promoting aerobic glycolysis at the expense of oxidative phosphorylation. This has been studied in detail in mammary epithelial cells [63], where PDK4 is induced by detachment from the extracellular matrix, resulting in a decreased PDH flux. However, Overexpression of ERBB2 (HER2) rescues PDH flux in an ERK-dependent manner, concomitantly with enhanced de novo lipogenesis and cell proliferation.

In lung cancer cells, PDK4 mRNA is targeted by miR-182, resulting in an enhanced pyruvate flux into acetyl-CoA for de novo lipogenesis and the promotion of tumorigenesis [64]. As miR-182 expression is inversely correlated with PDK4 expression in human lung adenocarcinomas, the miR-182 → PDK4 axis may be a clinically relevant regulator of lung cancer cell metabolism.

Consistent with these observations, analysis of publicly available transcriptomic data revealed that PDK4 expression is commonly...
down-regulated in tumors compared with the corresponding normal tissues [62,65], supporting the classification of PDK4 as a potential tumor suppressor gene in cancer cells dependent on de novo lipogenesis or oxidative phosphorylation for ATP synthesis from glucose. Lipogenesis is of particular relevance to tumor cells due to their high need for fatty acids for membrane biogenesis and protein modifications, and that essential for survival [66,67]. Furthermore, contrary to the prevailing opinion that tumor cells favor glycolysis for energy production (Warburg effect), cancer progression can be dependent on respiration under certain conditions, as shown, for example, in a recent study by Tan and colleagues [68].

Of note, several studies have described functions for PDK4 beyond its role as a gatekeeper of the TCA cycle that may be relevant in the context of tumorigenesis. For example, in human lung cancer cells PDK4 was identified as a critical regulator of EMT and resistance against the EGFR tyrosine kinase inhibitor erlotinib [65]. Thus, ectopic PDK4 expression inhibited TGFβ-induced EMT, and conversely, RNAi-mediated inhibition of PDK4 expression promoted both EMT and erlotinib resistance. Mechanistically, erlotinib resistance may involve a direct interaction between PDK4 and the mitochondrial apoptosis-inducing factor (AIF). Furthermore, contrary to the prevailing opinion that tumor cells favor glycolysis for energy production (Warburg effect), cancer progression can be dependent on respiration under certain conditions, as shown, for example, in a recent study by Tan and colleagues [68].

5.3. Other target genes

A substantial number of other PPARβ/δ target genes also have been linked to processes associated with tumorigenesis (Fig. 2), for example fatty acid oxidation (CPT1A, SLC25A20, ACA2, MLYCD, FABP4), fatty acid synthesis (FASN, LIPA), tricarboxylic acid (TCA) cycle (ACO2, SDHB; EEF genes for electron transfer; ISCA1 and CD51 for iron sulfur cluster assembly), alternative NADH generation in fumarase defective tumor cells (IMPA2, JAKMIP2, KLF10, KLF11, LRP5, PDE6G, RUNX1, SRC, TGFβ1) [13–15,71–76]. The frequently observed effect of PPARβ/δ on proliferation in a PPARβ/δ-dependent manner was demonstrated in MCF-7 cell lines where it mediates the ability of EGFR to induce cell proliferation in a PPARβ/δ-dependent fashion [78].

The fatty acid binding protein 5 (FABP5) is a crucial mediator of ligand responses by PPARβ/δ by shuttling fatty acid ligands from the cytosol to the nucleus, thereby activating the transcriptional activity of the receptor [77]. An epidermal growth factor (EGF) receptor-driven pathways has been reported to induce FABP5 in MCF-7 cells where it mediates the ability of EGF to induce cell proliferation in a PPARβ/δ-dependent fashion [78].

Wang et al. showed that the ectopic expression of PPARβ/δ in MCF-7 cells increased their migration in vitro, enhanced their resistance to doxorubicin (DNA-damaging agent) [79]. Western blot analysis showed that PPARβ/δ promotes the adaptation of breast cancer cells to harsh microenvironmental conditions.

7. Role of PPARβ/δ in human lung cancer

The clinical course of lung adenocarcinoma shows the strongest association with PPARβ/δ expression (Fig. 3C). However, much less is known about the potential role of PPARβ/δ in lung cancer compared to breast cancer. Two studies reported an increased expression of PPARβ/δ in human non-small cell lung cancer (NSCLC) compared to normal lung [83,84]. In NSCLC cells lines PPARβ/δ activation promoted proliferation and viability, while reduced PPARβ/δ expression increased apoptosis. Furthermore, PPARβ/δ ligands induced the PTGS2 (COX-2) and VEGF genes in a PPARβ/δ-dependent, and chromatin immunoprecipitation identified VEGF as a direct PPARβ/δ target gene [83]. These observations would be consistent with a role for PPARβ/δ in promoting growth, apoptotic resistance, inflammation and angiogenesis in human lung adenocarcinoma. Consistent with this data, the irreversible PPARβ/δ antagonist SR13904 showed anti-proliferative activity in human NSCLC cells, even though the direct targets were not identified in this study [31]. However, neither the growth promoting effects nor activation of AKT by PPARβ/δ agonists reported in the former two studies [83,84] were observed by He et al. [85], indicating that...
further work is need to clarify the role of PPARβ/δ in lung cancer.

8. PPARβ/δ in skin cancer

Studies addressing a potential association of PPARβ/δ with clinical parameters of non-melanoma skin cancers are not available to date. However, the study by Montagner and colleagues suggests that PPARβ/δ promotes UV-induced squamous cell carcinoma (SCC) and its precursor lesion actinic keratosis [76]. These authors showed that UV exposure increases PPARβ/δ protein levels and activity in a mouse model, which induced Src as a direct PPARβ/δ target gene, SRC protein expression and SRC activity, which in turn boosted EGF receptor - ERK1 signaling and expression of EMT markers. In human SCC samples, PPARβ/δ expression was found to correlate with SRC, MMP19 and SNAI1, supporting the hypothesis that PPARβ/δ promotes EMT initiation. Interestingly, a meta-analysis of PPARβ/δ expression in different epithelial tumors revealed a positive correlation of PPARβ/δ with its targets SRC and TGFβ1 not only in human SCC, but also in lung, colon and ovarian cancer; consistent with the high SRC activity in these tumors. Collectively, these findings point to a regulatory role for a PPARβ/δ - SRC/TGFβ1 axis in different human epithelial cancers.

9. Relevance PPARβ/δ for other human cancers: role of natural agonistic ligands

While very little is known about the potential functions of PPARβ/δ in human glioma, its role in colon carcinogenesis has been extensively studied. However, the data are not conclusive due to opposing results in different experimental systems, as discussed in detail in other reviews [2,4]. A very recent study has shed new light on this issue by showing that a high fat diet augments the numbers and function of Lgr5 (+) intestinal stem cells through the activation of PPARβ/δ [86]. Furthermore, treatment of intestinal organoid cultures with fatty acid ligands enhanced their self-renewal potential in a PPARβ/δ-dependent fashion. Intriguingly, enforced PPARβ/δ signaling endowed intestinal progenitor cells with the ability to form tumors in mice lacking the tumor suppressor gene Apc. These data are in agreement with studies reporting an increased susceptibility to carcinogen-induced intestinal cancer in transgenic mice with colon epithelial PPARβ/δ overexpression [87] and, vice versa, an inhibition of colon tumorigenesis in mice with a targeted disruption of Ppard [88]. Although these findings provide compelling evidence for a tumor-promoting role of PPARβ/δ in colon carcinogenesis, they appear inconsistent with an earlier report describing an increased predisposition to intestinal tumorigenesis in Ppard null relative to Ppard wildtype mice in a background of inactivated Apc [89]. However, as the relevant target genes and their precise mode of regulation are not known, it is difficult to interpret these seemingly discrepant results. Therefore, the function of PPARβ/δ in models of intestinal cancer needs further clarification and any findings derived from these experiments require verification in human cells.

Another tumor entity where the increased concentrations of PPARβ/δ ligands rather than increased PPARβ/δ protein expression might play a pro-tumorigenic role is ovarian carcinoma. In macrophages from ovarian cancer-associated ascites most direct PPARβ/δ target genes (including ANGPTL4 and PDK4) are upregulated, refractory to synthetic agonists, but repressed by inhibitory ligands [22]. High concentrations of polyunsaturated fatty acids, notably linoleic acid, acting as potent PPARβ/δ agonists were found as the cause of this deregulation of PPARβ/δ target genes. A similar scenario can be expected to exist in cancer cells exposed to the same microenvironment, suggesting a pro-tumorigenic role for lipid ligands in ovarian cancer, mediated by the activation of PPARβ/δ. However, this hypothesis still need to be tested experimentally. Apart from PUFAs, eicosanoid metabolites with immune regulatory functions can act as endogenous PPARβ/δ agonists [90], including 15-HETE and prostacyclin (see also section 3). However, very little is known about their role as regulators of PPARβ/δ activity in cancer cells. Furthermore, other endogenous PPARβ/δ ligands are not known, and it is unclear if high affinity agonists occur. Likewise, it is currently unknown if endogenous inhibitory PPARβ/δ ligands exist. However, in view of the close connection of tumorigenesis and inflammatory signaling in the tumor microenvironment, these open questions are highly relevant, in particular for cancers frequently linked to chronic inflammation, such as lung adenocarcinoma. Therefore a systematic search for inflammatory lipids acting as PPARβ/δ ligands, the identification of their cellular sources and target cells within the tumor microenvironment and their molecular and biological effects on tumor cells and tumor-associated host cells is urgently needed to elucidate the intercellular PPARβ/δ signaling network and the role of PPARβ/δ in human cancers.

10. Association of PPARβ/δ target genes with the outcome of human cancers

The significance of the observed clinical correlations shown in Fig. 3 was further assessed by analyzing potential associations of the expression of direct PPARβ/δ target genes with survival. Analysis of PRECOG data with a set of experimentally verified direct PPARβ/δ target genes (see legend to Fig. 4) revealed a highly significant association of a large fraction of these genes with the clinical outcome of those cancer also showing a similar link to PPARβ/δ expression levels (Fig. 3A), i.e., breast cancer, glioma and lung adenocarcinoma (28.5%, 39.7% and 22.1%, respectively, with p values determined by hypergeometric test of $3 \times 10^{-11}$, $5 \times 10^{-14}$ and $6 \times 10^{-7}$, respectively; Fig. 4A and B). This result is illustrated in more detail for individual target genes that are associated with a short or long OS of breast cancer in Fig. 4C and D. Consistent with the discussion in section 9, a clear link of survival to PPARβ/δ target gene expression was also found for ovarian cancer with a fraction of 8.9% OS-associated genes ($p = 0.004$; Fig. 4A and B).

Surprisingly, no significant association of PPARβ/δ target gene expression and clinical outcome was found for colon cancer. This may be attributable to different reasons. A likely explanation could be the fact that different PPARβ/δ target genes may be relevant for different cancer types, and these genes may not be appropriately represented in the query datasets used to establish clinical associations. It is also possible that in some entities the number of relevant target genes is too low to obtain statistically significant results. Furthermore, it is possible that the associations of PPARβ/δ target gene expression with survival of a given tumor type (e.g., colon cancer) is weaker compared to other tumor types, such as breast or lung cancer, leading to insignificant results after multiple hypothesis testing. This clearly applies to colon cancer, where 4.5% of all PPARβ/δ target genes are associated with OS. Even though these associations are not significant after Benjamini-Hochberg correction, this does not imply that the respective target genes are functionally irrelevant. Therefore, the lack of clinical correlations is not conclusive unless the relevant target genes have been identified in functional assays and are used for analyzing clinical associations.

Of note, survival-associated PPARβ/δ target genes can be linked to either a poor or a favorable clinical outcome. For lung adenocarcinoma, the number of genes associated with a short or long OS was n = 14 and n = 7, for glioma n = 25 and n = 15, respectively. In breast cancer approximately half of these genes are linked to a favorable clinical course (n = 16 and n = 17, respectively; Fig. 4E). This observation may be confusing at first glance, but in view of the
different modes of regulation exerted by PPARβ/δ it is conceivable that different genes respond to variations in the level of PPARβ/δ in a distinct fashion. This is illustrated in Fig. 5 that simulates the effects of increasing PPARβ/δ on different types of target genes and shows that opposite effects on individual genes can be expected under identical conditions. Since PPARβ/δ target genes can be associated with either tumor-promoting or tumor-suppressing functions, these findings potentially make sense. For example, ANGPTL4 is associated with a short RFS (Fig. 4D) and a protumorigenic function in breast cancer, while the opposite applies to PDK4, both in terms of a favorable clinical outcome (Fig. 4E) and its role as a potential tumor suppressor in most cancer types, as discussed in detail in section 5.2.

However, the scenario is more complex. ANGPTL4 and PDK4 are both type II genes that should respond to variations in level of PPARβ/δ or ligands in a similar manner, yet both exert opposing functions in tumorigenesis. This clearly suggests that PPARβ/δ is not a key regulator of at least one of these genes in cancer cells. Furthermore, PPARβ/δ in the absence of synthetic agonists represses rather than activates canonical (type II) target genes, at least in cultured cell lines, which is inconsistent with ANGPTL4 being a key target activated by PPARβ/δ in cancers with a clinically unfavorable outcome. On the other hand, repression of tumor suppressor genes in prognostically unfavorable cancers, such as PDK4 in breast cancer, by high PPARβ/δ levels would be consistent with the hypothesis that these genes are relevant targets of PPARβ/δ. Thus, while PPARβ/δ target genes are strongly associated with breast cancer, glioma and lung adenocarcinoma, their regulation by PPARβ/δ and contribution to distinct cancer-associated functions remains an open question. This will have to be addressed for each individual gene and tumor entity to be able to draw a conclusive picture.

11. Conclusions and perspectives

The clinical associations discussed in detail in this review clearly

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**Fig. 4.** Association of PPARβ/δ target gene expression with the clinical outcome of human cancers. A-B: PPARβ/δ target genes showing a significant association with the OS of human cancer types irrespective of the hazard ratio (p < 0.05 by hypergeometric test; n>5% of all PPARβ/δ target genes analyzed). A list of 540 PPARβ/δ target genes was compiled form a meta-analysis of experimentally verified target genes [72] (http://ppargene.org) and agonist-inducible target genes identified in human WPMY-1 myoblasts [14], human MDA-MB-231 breast cancer cells [15] or human monocyte-derived macrophages [13]. Clinical associations were derived from the PRECOG database [38]. A: percentage of all target genes showing a significant association (p value < 0.05 by hypergeometric test); B: corresponding p values. C-D: Associations of individual genes with poor OS (C) or favorable OS of breast cancer (D). Only genes with a statistically highly significant association are shown (PRECOG, p < 0.005 after Benjamini-Hochberg adjustment).

**Fig. 5.** Simulations of the effects of varying PPARβ/δ levels on the expression of different types of target genes (as defined in Fig. 1).
point to a link of PPARβ/δ expression with a poor clinical outcome of breast cancer, glioma and lung adenocarcinoma. Furthermore, high levels of fatty acids in the ovarian cancer microenvironment acting as PPARβ/δ agonists suggest that the constitutively activated receptor might play a role in this cancer entity. However, it remains currently unclear, whether these associations are functionally important with respect to tumor growth and progression, and potential underlying molecular mechanisms remain obscure, although numerous studies described functions of PPARβ/δ in tumorigenesis-associated processes, including cell proliferation, survival, differentiation, inflammation and angiogenesis.

This lack of clarity is due to large extent to discrepant reports regarding the function of PPARβ/δ as a tumor-promoting or a tumor-suppressive protein, including its role in breast and lung cancer. This in turn presumably results from the use of experimental systems that do not reflect the clinical scenario and frequently do not adhere to the required standards, e.g. verification of cell lines or background of genetically altered mice. The major problem in this regard is the use of established cell lines that usually are inappropriate experimental systems to address clinical problems, since in most cases they do not phenocopy the tumor of origin, have undergone genetic alterations in the course of immortalization or adaptation [95–97]. On the other hand, artificial media that are highly divergent from the tumor microenvironment in vivo. In the case of PPARβ/δ, the latter aspect is highly relevant, as the presence or absence of ligands, e.g. fatty acids, directly affects transcriptional outcome and may determine whether PPARβ/δ acts as a repressor or activator. Even though mouse models are invaluable tools to address basic questions of tumor biology and have enabled significant advances in the field, there are serious limitations [93]. These include differences in tumor pathology, clonal evolution and the microenvironment, in particular in xenografted tumors. As PPARβ/δ seems to play a cancer-promoting role in the tumor microenvironment in syngeneic mouse models [94–96], the latter aspect is of particular relevance. In humans, this issue has not been addressed, apart from the deregulation of PPARβ/δ in ovarian cancer TAMs [22] alluded to above.

These considerations make it obvious that alternative and well defined model systems are required to obtain conclusive data regarding the function of PPARβ/δ in human cancer and to be able to assess its potential as a therapeutic target. A promising tool along these lines are primary tumor cells obtained from malignant effusions, which frequently occur in patients with advanced lung and breast cancer as pleural effusions and patients with gastrointestinal tumors or ovarian cancer as peritoneal effusions (ascites). Recent technological advances make it possible to grow primary cancer cells from effusions without crisis, for apparently unlimited population doublings and without decrease in growth rate [97,98]. Importantly, these cells retain the genomic, histopathological, molecular features and drug responses of the original tumors. Such experimental system, which is well established for breast [97], lung [99] and ovarian cancer [98], is likely to solve many of the problems of conventional cell lines discussed above. Furthermore, malignant effusions contain tumor-associated host cells that can be analyzed ex vivo as purified cells or in co-cultures with tumor cells, which will enable clinically relevant studies of tumor – host cells interactions.

Improved experimental systems will likely prove useful to solve many of the open questions discussed above, in particular (i) to clarify the role of PPARβ/δ in the growth, progression and therapy resistance of different human cancer entities, (ii) to identify relevant target genes, (iii) to investigate the relevant modes of regulation of these genes by PPARβ/δ (activation, repression, ligand dependence), (iv) to identify the tumor-relevant biological processes affected by these genes, (v) to clarify the spectrum of endogenous PPARβ/δ ligands and unravel their functions within the signaling networks of the tumor microenvironment and (vi) to determine whether PPARβ/δ might represent a potentially useful pharmacological target. The latter point is of great interest in view of the availability of both agonists and inhibitory ligands that are suitable for in vivo application. Inverse agonists deserve particular attention in this context, since they can trigger the establishment of dominant PPARβ/δ repressor complexes at PPREs that can block transcriptional activation of these genes by other oncogenic signaling pathways [15]. The metastasis-promoting ANGPTL4 gene is an excellent example in this context.

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