

Review Article

PPAR Gamma and Angiogenesis: Endothelial Cells Perspective

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Received 15 July 2016; Revised 22 October 2016; Accepted 1 November 2016

Academic Editor: Andrea Flex

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We summarize the current knowledge concerning PPAR γ function in angiogenesis. We discuss the mechanisms of action for PPAR γ and its role in vasculature development and homeostasis, focusing on endothelial cells, endothelial progenitor cells, and bone marrow-derived proangiogenic cells.

1. Angiogenesis

In human embryos, the development of vasculature starts at day 21 after conception. The *de novo* formation of blood vessels occurs from cells called angioblasts, which form tubes in a process known as vasculogenesis. Angioblasts proliferate and generate the very first vascular plexus, which in turn grows and expands *via* angiogenesis [1]. Angiogenesis refers to the sprouting of endothelial cells from preexisting vessels as well as their migration and proliferation to create new tube-like structures [2]. During angiogenesis, one can distinguish between several well-characterized stages. After the activation of endothelial cells by angiogenic growth factors (e.g., vascular endothelial growth factor (VEGF) and basic fibroblast growth factor (bFGF)), ECs degrade the basement membrane and then proliferate and migrate to assemble into tubes. Finally, they deposit a new basement membrane, secrete cytokines (e.g., platelet derived growth factor (PDGF) and angiopoietins) to attract supporting cells, which in turn stabilize new vessels [3]. Such newly generated vessels can further grow or undergo remodeling *via* intussusception, which describes the generation of new capillaries by the splitting of preexisting ones (Figure 1). Angiogenesis is not restricted to embryonic development but also takes place in adults, where it is necessary for both physiological and pathological processes. Normal physiological processes that involve angiogenesis include the female reproductive cycle, wound healing, bone repair, postischemic repair, and hair

growth [1]. Importantly, excessive angiogenesis is a hallmark of diseases such as cancer, proliferative retinopathy, psoriasis, or rheumatoid arthritis [4, 5]. By contrast, insufficient blood vessel formation can lead to the development of nonhealing ulcers and myocardial or brain ischemia [5, 6].

For many years it was thought that the *de novo* formation of blood vessels from undifferentiated precursor cells occurs only during fetal development. It was believed that, in adults, the regeneration and formation of new blood vessels relied on the migration and differentiation of mature ECs. This widely accepted view has changed since the discovery by Asahara and coworkers of endothelial progenitor cells (EPCs) in the blood of adults that are capable of proliferation, migration, and incorporation into existing vessels. Isolated from the blood of adult volunteers, CD34⁺/VEGFR-2⁺ cells were grown *in vitro*, and after several days they began to express other endothelial-specific markers such as CD31, E-selectin, Tie-2, and eNOS [7]. Next, experiments carried out by the same group confirmed the participation of EPCs released from bone marrow in the formation of blood vessels, both under physiological (endometrial hyperplasia, blood supply to the corpus luteum, and wound healing) and pathological conditions (tumor growth, myocardial infarction, or ischemic hind limb) [8, 9]. However, despite very promising initial reports describing angiogenic properties for EPCs, researchers have failed to identify a specific antigen profile that uniquely characterizes such EPCs. As a result, a variety of protocols for EPC isolation, growth, and characterization

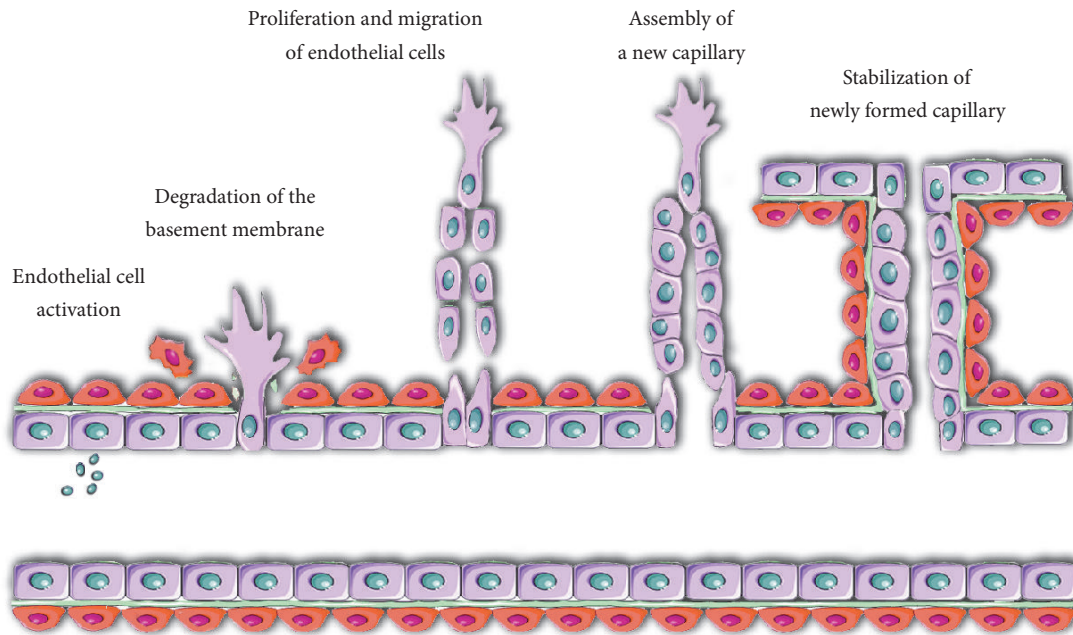


FIGURE 1: Angiogenesis. Development of a new capillary from preexisting blood vessel is called angiogenesis. Angiogenesis is a multistage process that requires activation of endothelial cells by angiogenic growth factors (e.g., vascular endothelial growth factor and basic fibroblast growth factor), followed by degradation of the basement membrane. Next, endothelial cells proliferate and migrate to assemble into tubes. Finally, they deposit a new basement membrane, secrete cytokines (e.g., platelet derived growth factor and angiopoietins) to attract supporting cells, which in turn stabilize new vessels.

are used [10]. Today, despite 19 years of studying EPCs, there is a growing number of contradictory conclusions with respect to their role in the cardiovascular system. These discrepancies are mainly due to the strong phenotypic overlap between EPCs and circulating proangiogenic cells from the hematopoietic lineage, a lack of universal data reporting, and—as reported before—differing definitions of the studied cell populations [11]. It was thought that EPCs are present in the bone marrow niche, from where, in response to injury or hypoxia, they are released into the blood and mobilized to the injured tissue [9, 12, 13]. It was originally postulated that, after entering a damaged or ischemic tissue, EPCs stimulate the formation of blood vessels by differentiating into mature endothelial cells (Figure 2). They could also directly incorporate into existing damaged vessel structures, filling gaps in the endothelial layer [7]. However, due to conflicting results concerning the participation of EPCs in already formed vessels (from 0% to 80% of endothelial cells), it is difficult to determine the true importance of this process [14–20]. Moreover, it is clear that the cell surface antigens and colony assays used to identify EPCs have significant overlap with those of cells from the hematopoietic lineage. Such hematopoietic subsets (e.g., $CD31^+ CD34^{\text{bright}} CD45^{\text{dim}} AC133^+$) also circulate in the blood and participate in vascular repair and regeneration [21].

It seems, however, that paracrine stimulation of blood vessel regeneration might be the key mechanism of EPCs (or bone marrow-derived proangiogenic cells) action. Proangiogenic cytokines that are produced at high levels by EPCs include interleukin-8 (IL-8), hepatocyte growth factor (HGF), VEGF, platelet derived growth factor-BB (PDGF),

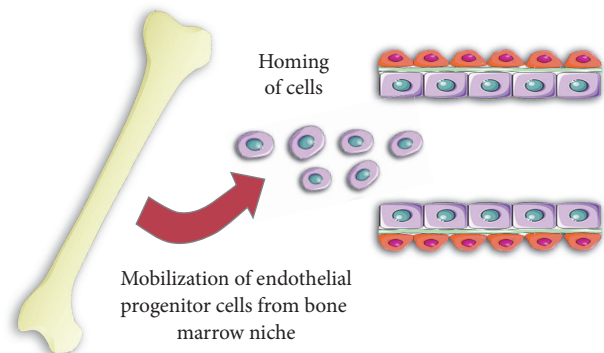


FIGURE 2: Endothelial progenitor cells and angiogenesis. EPCs were described as cells that are present in the bone marrow niche, from where, in response to injury or hypoxia, they are released into the blood and mobilized to the injured tissue. View of EPCs mode of action changes over time and today scientists postulate that their complex nature needs more studies; please see main text for more details.

bFGF, and monocyte chemoattractant protein-1 (MCP-1) [22]. Importantly, intramuscular administration of conditioned media collected from EPCs improved blood flow regeneration in an ischemic rat hind limb model. Our recent data also proved that conditioned media is more efficient than the injection of cells for restoring blood perfusion in a mouse ischemic limb model. It was also noteworthy that, in this model, approximately 70% of the injected cells were eluted from the site of injection within first 6 hours, and most

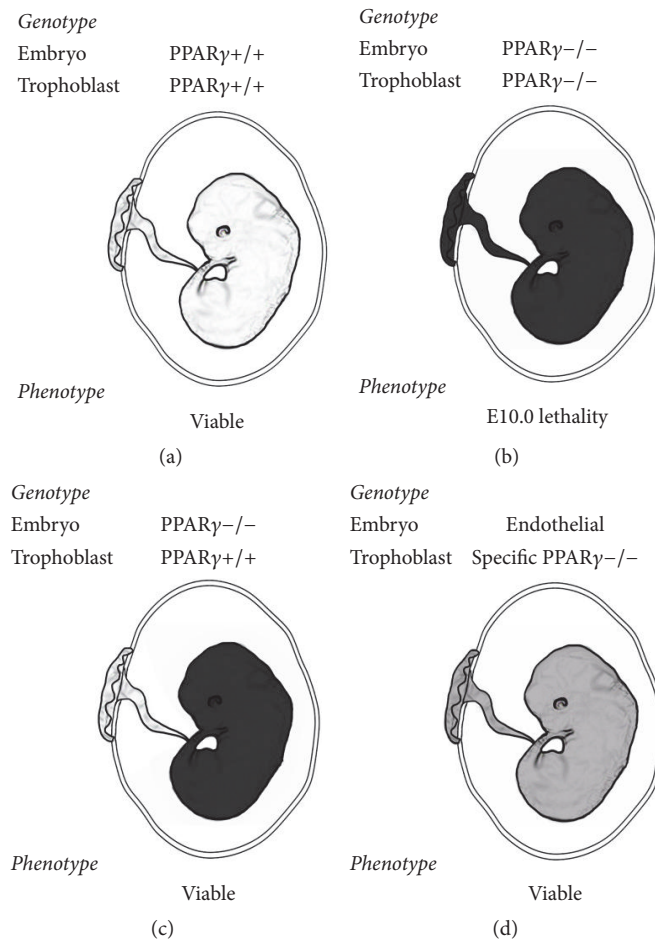


FIGURE 3: Role of PPAR γ in vascular development. (a) Expression of PPAR γ ($PPAR\gamma^{+/+}$) is necessary for proper angiogenesis during embryogenesis. (b) PPAR γ knockout ($PPAR\gamma^{-/-}$) mice are embryonically lethal by E10.0 due to the impaired placental vascularization. (c) Deletion of PPAR γ in the embryo, but not in the trophoblast, or (d) specifically in the endothelial cells does not disturb angiogenesis and leads to the generation of viable pups.

remaining cells died during first three days after injection [23, 24].

2. Role of PPAR γ in Vascular Development

PPAR γ was identified for the first time in murine adipose tissue, although in humans its cDNA was first isolated from hematopoietic cells [25, 26]. Today we know that it belongs to the nuclear receptor superfamily, which are ligand-activated transcription factors that drive specific gene expression programs upon stimulation with a ligand (for review about PPAR γ mechanism of action please see [27, 28]). PPAR γ is most abundantly expressed in adipose tissue, where it regulates adipocyte maturation [29, 30]. It is also involved in the regulation of lipid metabolism, as ligand-dependent activation leads to increased fatty acid uptake and storage. Furthermore, PPAR γ plays an important role in glucose homeostasis as an insulin-sensitizing agent, which is why agonists of PPAR γ are currently used to treat diabetes. Although PPAR γ was initially found to be critical for adipocyte differentiation and function, over time, it was also

found to play an important role in the cardiovascular system. Importantly, its activity has been demonstrated in the vessel wall, both in endothelial cells (ECs) and in vascular smooth muscle cells (VSMCs), suggesting its vital role in angiogenesis [31, 32].

The importance of PPAR γ in angiogenesis was demonstrated by the generation of knockout animals in 1999. As reported by Barak and coworkers, PPAR γ null mice are embryonically lethal by E10.0, due to placental dysfunction characterized by defective trophoblast differentiation and markedly impaired placental vascularization (Figures 3(a) and 3(b)) [33]. Moreover, supplementation of PPAR $\gamma^{-/-}$ embryos with wild-type placentas resulted in an apparently normal vascular system during further embryogenesis, although the pups died some days after birth due to combination of pathologies, including severe lipodystrophic changes and hemorrhages [33]. In a latter study, to rescue the embryonic lethality of global PPAR γ knockout embryos, floxed PPAR γ mice were crossed with Mox2-Cre mice to inactivate PPAR γ in the embryo but not in trophoblasts

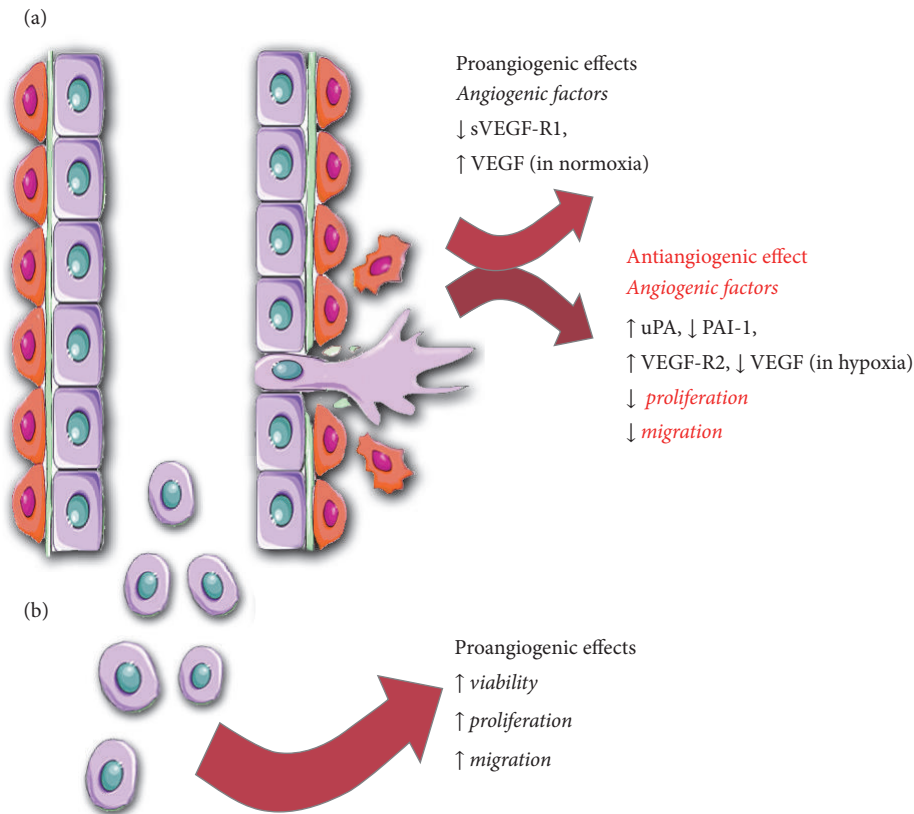


FIGURE 4: Effects of PPAR γ activation on angiogenesis. (a) Angiogenic properties of endothelium is decreased upon activation of PPAR γ via reduction of migratory properties and inhibited proliferation and by diminished production of angiogenic factors. (b) Stimulation of PPAR γ in diabetic endothelial progenitor cells induces angiogenesis. Detailed description of molecular mechanisms responsible for observed effects is in the main text.

(Figure 3(c)). Such an approach allowed for the generation of viable PPAR γ ^{-/-} animals that were characterized by lipodystrophy, insulin resistance, and hypotension [34]. These mice showed increased endothelium-dependent relaxation in response to acetylcholine, which was not associated with changes in eNOS expression or phosphorylation [34]. Mice in which PPAR γ function was selectively knocked out only in endothelial cells—based on the same Cre-Lox system and Tie2-Cre construct—were phenotypically indistinguishable from wild-type littermates (Figure 3(d)) [35]. However, when Tie2-Cre transgenic mice were fed high-fat diets, they had significantly elevated systolic blood pressure that was not observed after a normal diet or salt-loading [35]. This observation could be at least partially explained by data showing that intact aortic segments from endothelial-specific PPAR γ ^{-/-} mice released less nitric oxide than those from controls [36]. Importantly, disruption of endothelial PPAR γ contributes to endothelial dysfunction in vivo, as reduced nitric oxide production in PPAR γ ^{-/-} aortas was associated with increased oxidative stress and enhanced activation of NF κ B in aortic homogenates [36]. The results mentioned above indicate that tight regulation of PPAR γ expression is crucial for proper angiogenesis. Importantly, PPAR γ ^{-/-} mice displayed defects in vasculature structure as well as a lack of balance between pro- and antiangiogenic factors

[33, 37]. Consistent with this data, McCarthy and coworkers demonstrated that administration of a PPAR γ antagonist (T0070907) to pregnant rats led to endothelial dysfunction, reduced expression of VEGF, and increased levels of plasma soluble VEGF receptor-1 (sVEGFR-1), which acts as a VEGF scavenger [38]. Interestingly, the treatment of pregnant wild-type mice with rosiglitazone also resulted in a disorganization of placental microvasculature [37]. However, our recently published data indicate that angiogenesis in wound healing and hind limb ischemia models is not affected by an ~50% decrease in the expression of PPAR γ in normoglycemic PPAR γ ^{+/-} mice [24].

3. PPAR γ and Endothelial Cells

The first evidence of PPAR γ expression in ECs came from studies examining the influence of PPAR γ activation on angiogenic and apoptotic properties of ECs [31, 39]. Today, after many years of study, we know that PPAR γ is a very important regulator of EC biology that is involved in the regulation of angiogenesis at a variety of stages. PPAR γ activation was shown to influence the production of cytokines by ECs as well as their proliferation, migration, and ability to form capillaries, although certain studies showed variable results (Figure 4(a)).

4. Angiogenic Factors

Degradation of the extracellular matrix is a necessary step during early stages of angiogenesis. Among the various proteases produced by ECs that are involved in capillary growth are urokinase plasminogen activator (uPA) and the matrix metalloproteinases (MMPs). The primary physiological substrate of uPA is plasminogen, which is an inactive form of serine protease plasmin. The first evidence for modulation of the expression of uPA and its inhibitor (PAI-1, plasminogen activator inhibitor-1) by PPAR γ came from studies performed in 1999 by Xin and coworkers. They observed that treatment of HUVECs with 15d-PGJ2 reduced the mRNA levels of uPA and increased the levels of PAI-1 mRNA [31]. Subsequent experiments showed that treatment of HUVECs with PPAR α and PPAR γ activators (linolenic acid, linoleic acid, oleic acid, and PGJ2) augmented PAI-1 mRNA expression and protein secretion in a concentration-dependent manner [40]. Similarly, our data proved that activation of PPAR γ by 15d-PGJ2 could potentially inhibit the synthesis of uPA in HMEC-1 cells. Importantly, this effect was also reproduced by the treatment of cells with troglitazone, suggesting PPAR γ -dependent action [41].

By contrast, the opposite effect was also reported in control and TNF-stimulated ECs. TZDs decreased basal and TNF-stimulated PAI-1 secretion and mRNA expression in HUVECs in a dose-dependent fashion [42, 43]. As shown by Liu and coworkers, TZDs inhibited the induction of PAI-1 by TNF, although the specific PPAR γ inhibitor SR-202 failed to modulate this effect. Moreover, ECs transfected with a dominant-negative PPAR γ construct exhibited the same phenotype [43].

The most commonly used classification of MMPs is based on their substrate specificity and cellular localization, dividing them into collagenases, gelatinases, stromelysins, and membrane-type MMPs. We found that treatment of HMEC-1 with 15d-PGJ2 increased the synthesis of MMP-1 protein but that TZDs (ciglitazone and troglitazone) did not influence MMP-1 production, arguing against the involvement of PPAR γ . Importantly, stimulatory effects were reversed by NAC treatment, suggesting that 15d-PGJ2 upregulates MMP-1 expression in HMEC-1 cells through the induction of oxidative stress [44]. As shown by Park and coworkers, the antiangiogenic activity of troglitazone in ECs is correlated with the suppression of VEGF-induced MMP-2 and membrane-type 1-MMP expression and is also linked to ROS generation. Effects of troglitazone on VEGF-induced MMP-2 and MT1-MMP expression were abolished after addition of the NADPH oxidase inhibitor diphenylene iodium or the ERK inhibitor PD98056 [45]. In human brain microvascular endothelial cells, Huang and coworkers showed that the addition of exogenous PPAR γ agonists resulted in downregulation of MMP-2 and MMP-9 expression as well as their proteasome activities [46]. In addition, activation of PPAR γ with ciglitazone in mouse aortic vascular endothelial cells reduced MMP-9 activation [47].

Vascular endothelial growth factor (VEGF) is a crucial inducer of blood vessel formation during embryogenesis and in postnatal life. VEGF acts as a specific survival factor for

ECs, regulating many endothelial functions, such as proliferation, migration, morphogenesis, vascular permeability, and the production of vasoactive molecules [48]. The first report concerning the potential influence of PPAR γ ligands on VEGF action came from a study performed in 1999, where it was reported that treatment of HUVECs with 15d-PGJ2 reduced the mRNA levels of vascular endothelial cell growth factor receptors 1 and 2 [31]. We and others confirmed this finding, showing that PPAR γ activation results in reduced expression of VEGF-R2 and soluble VEGF-R1 [49, 50]. Consistent with these results, inhibition of PPAR γ in pulmonary arterial endothelial cells resulted in upregulation of VEGF-R2 [51]. By contrast, in one study, VEGF-R2 expression was found to be enhanced in response to PPAR γ activation by troglitazone and attenuated by GW9662, a specific antagonist of PPAR gamma [52].

Transcriptional upregulation of VEGF was reported for the first time in rat and human vascular smooth muscle cells stimulated *in vitro* with ciglitazone or rosiglitazone [53, 54]. In ECs, activation of PPAR γ was also shown to influence VEGF production. We have shown that treatment of HMEC-1 cells with 15d-PGJ2 significantly and dose-dependently increased VEGF promoter activity, mRNA expression, and protein secretion. By contrast, addition of ciglitazone caused a much weaker induction, suggesting a primarily PPAR γ -independent action. Cells treated with 15d-PGJ2 were characterized by augmented expression of heme oxygenase-1 (HO-1) protein. As inhibition of the HO-1 pathway significantly reduced the stimulatory effects of 15d-PGJ2 on VEGF synthesis, we postulated that the upregulation of VEGF expression in response to 15d-PGJ2 in HMEC-1 is mediated by activation of HO-1 [55]. Later experiments showed that the proangiogenic activity of HO-1 in ECs can be mimicked by the addition of carbon monoxide releasing molecules [41]. Also of note, we demonstrated that the regulation of VEGF by PPAR γ ligands is dependent on oxygen concentration. Under hypoxia, in contrast to normoxia, induction of PPAR γ by 15d-PGJ2 decreases VEGF synthesis through inhibition of the HIF-1 pathway [56]. More recently, Biscetti and coworkers showed that activation of PPAR α and PPAR γ in ECs leads to enhanced tube formation, which was associated with increased production of VEGF [57].

Further confirmation of PPAR γ -independent activation of VEGF by PPAR γ ligands came from *in vivo* studies. Using a hind limb ischemia murine model, Biscetti and coworkers found that pioglitazone enhanced the restoration of blood flow and capillary density in ischemic muscles and that this process is associated with increased expression of VEGF. However, direct activation of PPAR γ by GW1929 did not restore blood flow recovery, in contrast to combined treatment with pioglitazone and GW9662 (the selective PPAR γ inhibitor) suggesting a PPAR γ -independent action. Importantly, these beneficial effects were abrogated upon endogenous Akt inhibition [58].

5. Proliferation

Fukunaga and coworkers evaluated basal proliferation in endothelial cells isolated from different human vascular beds

(aorta, carotid artery, and umbilical vein) as well as from the bovine carotid artery. When these cultured endothelial cells were treated daily with troglitazone or pioglitazone for 5 days at a 10 nmol/L dose, both compounds induced the proliferation of ECs. By contrast, activation of PPAR γ with higher concentrations (10 μ mol/L) significantly suppressed DNA synthesis [59]. The inhibition of EC proliferation was also observed in HUVECs overexpressing PPAR γ or wild-type cells stimulated with troglitazone alone. A combination of PPAR γ overexpression and troglitazone treatment resulted in a further decrease in the thymidine uptake by HUVECs [60]. VEGF-induced EC proliferation was also inhibited by other PPAR γ agonists, including pioglitazone, ciglitazone, troglitazone, and 15d-PGJ2. Although all tested compounds exerted the same effect, 15d-PGJ2 reduced EC proliferation much more potently than the TZDs [49, 61]. Activation of PPAR γ by 15d-PGJ2 was also shown to reduce only partially and transiently the expression of VEGFR-1 and VEGFR-2 in HUVEC, which was insufficient to fully explain the observed results. Additionally, stimulation with 15d-PGJ2 decreased the activities of c-Jun and c-Myc, and overexpression of c-Myc attenuated its antiproliferative effects in HUVEC [49]. In a recently published paper, a novel PPAR γ agonist KR-62980 also inhibited VEGF-induced HUVEC proliferation. KR-62980 downregulated VEGF-induced VEGFR-2 expression but increased the expression of phosphatase and tensin homolog deleted on chromosome 10 (PTEN) in parallel with reduced phosphorylation of extracellular signal-related kinase 1/2 (ERK1/2) and p38 MAPK [50]. Another explanation of the antiproliferative effects of PPAR γ activation came from studies performed by Sheu and coworkers, who demonstrated endothelial arrest at G1 phase after treatment with rosiglitazone. Rosiglitazone inhibited endothelial proliferation in a dose-dependent manner by decreasing the production and activity of several key cell cycle regulators (cyclin D1, A, E, cdk2, and cdk4, hypophosphorylation of Rb) that control G1/S progression [62]. Finally, another group confirmed that troglitazone significantly inhibits serum-induced proliferation of HUVECs in a concentration-dependent manner through the suppression of casein kinase 2 [63].

6. Migration

PPAR γ ligands are also involved in inhibiting EC migration. VEGF-induced migration of HUVECs is inhibited by troglitazone and ciglitazone through the inhibition of Akt phosphorylation [64]. By contrast, induction of EC migration by leptin was shown to depend on the PI3K/Akt and ERK1/2 MAPK pathways [65]. The authors demonstrated that the antimigratory effects of PPAR γ activation by troglitazone or ciglitazone were due to the inhibition of Akt but not the ERK 1/2 kinases. The inhibition of Akt phosphorylation by TZDs was accompanied by upregulation of PTEN, a phosphatase that functions as a negative regulator of PI3K/Akt signaling [65, 66]. In another study, using scrape-wound and chemotactic assays, researchers found that troglitazone dose-dependently inhibited the migration and proliferation of cultured macrovascular endothelial cells in low-glucose (5 mmol/L) and high-glucose (25 mmol/L)

media [67]. VEGF-induced EC migration is also inhibited by ciglitazone, 15d-PGJ2, and a novel PPAR γ ligand, KR-62980 [49, 50, 68]. As shown by Aljada and coworkers, EC migration through an 8- μ m pore filter to a feeder layer containing vitronectin as a chemoattractant was significantly blunted by pioglitazone or rosiglitazone treatment [68]. As described earlier, rosiglitazone markedly decreased VEGF-induced tube formation and endothelial cell migration in a wound-healing migration assay, which could have been due to disorganization of the actin cytoskeleton [62]. Similarly, troglitazone significantly suppressed VEGF-induced cell proliferation and invasion of HUVECs into the Matrigel basement membrane, which was not reversed by GW9662 [45].

7. Angiogenesis Tests

The most popular *in vitro* angiogenesis tests rely on the ability of ECs to form tube-like structures when plated on top of a reconstituted basement membrane extracellular matrix (e.g., Matrigel). Such a differentiation process involves steps that are similar to what occurs *in vivo* during blood vessel formation, including cell adhesion, migration, alignment, protease secretion, and tubule formation [69]. As shown for the first time in 1999, activation of PPAR γ by specific ligands, such as 15d-PGJ2, BRL49653, or ciglitazone, dose-dependently suppressed HUVEC differentiation into tube-like structures [70]. Consistent with this data, Murata and coworkers reported that incubation of bovine choroidal endothelial cells with rosiglitazone or troglitazone significantly inhibited not only VEGF-induced tube formation but also migration and proliferation [71]. In addition, our experiments proved that PPAR γ activation (by rosiglitazone or 15d-PGJ2) diminished the angiogenic potential of ECs plated not only on Matrigel but also in a three dimensional spheroid test [49]. We used ECs embedded in collagen gel because they can generate radial capillaries that more closely resemble angiogenesis *in vivo*. More recently, Aljada and coworkers used a chick chorioallantoic membrane (CAM) model to evaluate the efficacy of pioglitazone and rosiglitazone on VEGF and bFGF-induced angiogenesis. The TZDs used in that study significantly inhibited the proangiogenic effects of bFGF and VEGF in the CAM model [68]. As demonstrated by Park and coworkers, the antiangiogenic actions of TZDs in the CAM model were PPAR γ independent, as the observed phenotype was not reversed by treatment with PPAR γ antagonists, GW9662, or bisphenol A diglycidyl ether. Additionally, troglitazone blocked VEGF-induced ROS production and ERK phosphorylation, and again this inhibitory effect was not reversed by GW9662. NADPH oxidase or ERK inhibition mimicked effects obtained with troglitazone, suggesting that inhibition of angiogenesis by troglitazone is mediated by ROS production and ERK phosphorylation [45].

Although the antiangiogenic activity of PPAR γ agonists has been described in many papers, there is also some evidence for opposing effects. As mentioned above, 15d-PGJ2 induced expression of VEGF and IL-8 in ECs, but this action was PPAR γ independent [55, 56, 72]. Recently, Fujii and coworkers demonstrated that, in HUVECs, VEGFR-2

expression was enhanced in response to PPAR γ activation by troglitazone and attenuated by GW9662, a specific inhibitor. In the same cells, endothelial morphogenesis measured in a tube formation assay was also stimulated by troglitazone and inhibited by GW9662, indicating that PPAR γ activation positively mediates angiogenesis [52]. EC proliferation was also induced with low concentrations of TZDs [59]. The reasons for these discrepancies are not clear. It is possible that the concentrations of PPAR γ ligands used in some experiments were extremely high and therefore proapoptotic [45, 50]. It is also possible that PPAR γ ligands exert different effects on ECs from different vascular beds due to the heterogeneous expression of PPAR γ -regulated genes. CD36 is one such example of a PPAR γ -dependent gene that is upregulated by PPAR γ agonists. CD36 encodes a scavenger receptor that among many ligands binds antiangiogenic thrombospondin-1 [73]. CD36 receptor is mainly expressed in microvascular endothelial cells and at lower level in the venous endothelium [74]. What is more, even within the microvasculature, the expression of CD36 is organ specific, with the highest levels in the heart, muscles, and lungs and the lowest levels in the bone marrow [75]. Therefore, it is possible that such differences in expression could modulate the angiogenic response to PPAR γ agonists.

8. PPAR γ and Endothelial Progenitor Cells

As mentioned before, vascular repair and angiogenesis depend both on mature endothelial cells and endothelial progenitors [76, 77]. However, the phenotypic characterization of EPCs is still the subject of lively debate and controversy. There is also no commonly accepted standardized method for EPC isolation and culturing, which complicates the interpretation of results. Indeed, rigorous comparisons of recently described methods confirmed that the term “EPCs” as it is used today does not precisely define one cell population [10, 20]. In addition, characterizing EPCs is made difficult by the lack of specific antigens, as the markers used for their immunophenotyping are also expressed on surface of ECs and HSCs. Finally, the use of different isolation protocols, different culture conditions, and even different markers for EPC characterization makes it difficult to compare results obtained by different research groups. For these reasons, in our studies, we use term EPCs only for cells analyzed by flow cytometry with strictly a defined phenotype (CD45 $^-$ /Sca-1 $^+$ /KDR $^+$), whereas we refer to cells isolated and expanded in vitro as a bone marrow-derived proangiogenic cells (PACs) [24, 78].

Despite the lack of standardized definitions, analyses have consistently indicated that the angiogenic properties of EPCs/PACs are impaired in type 2 diabetes, including decreased cell numbers, decreased mobilization, and decreased angiogenic potential [12, 79–82]. Importantly, such properties could be improved using PPAR γ activation (Figure 4(b)) [13, 83–86]. Experiments evaluating the impact of TZDs on EPC function were first described by Pistrosch et al. in 2005. The authors described results of a three-month therapy program involving rosiglitazone in patients with diabetes resulting in the normalization of impaired EPC

migration and numbers, and beneficial effects persisted up to 9 weeks after treatment [83]. Similar results were obtained in patients enrolled in a combined antidiabetic therapy with pioglitazone and metformin. Both the direct and indirect effects of pioglitazone on EPCs increased their number and proliferation status and improved their migration and adhesion to fibronectin or collagen [84]. Activation of PPAR γ with rosiglitazone also attenuated the negative effects of advanced glycation end products (AGEs), and the stimulation of EPCs decreased inhibition of proliferation, migration, and activation of Akt and eNOS phosphorylation induced by AGEs [85, 87].

Intensive studies analyzing impact of TZDs on EPC biology has revealed some of the molecular mechanisms responsible for this phenomenon. As shown by Gensch and coworkers, administration of pioglitazone to mice not only increased the number of EPCs but also activated telomerase in endothelial cells via induced expression of telomere binding agent, type 2 (telomere repeat-binding factor 2, tert-2) [88]. Similar results were reported for human cells, as the activation of telomerase by pioglitazone in cultivated EPCs was prevented by Akt inhibitors [89]. It was also found that treatment with rosiglitazone facilitated reendothelialization in diabetic patients through decreased ROS generation and improved bioavailability of nitric oxide (NO) in endothelial cells [90].

Our recently published results showed that PACs derived from db/db mice displayed impaired migration and showed the formation of cord-like structures on Matrigel and capillary outgrowth from spheroids. Only the proliferation rate was not significantly affected [24]. Our data are in accordance with other studies showing decreased angiogenic potential in diabetic EPCs isolated from both humans and rodents [12, 79–82]. A paper published by Liang et al. demonstrated that AGEs, which are present in diabetes, induce EPC apoptosis and impair SDF-1 and NO production [85]. Transcriptome analysis suggested that the impaired migration of PACs can be associated with upregulation of integrins and the concomitant downregulation of genes involved in filopodia formation, such as efxin or CCT2 [24, 91]. Furthermore, PACs isolated from db/db mice showed decreased paracrine potential, which could be related to decreased expression of VEGF-C, VEGF-D, FGF7, or angiogenin. Incubation of PACs with rosiglitazone upregulated VEGF and KC protein levels in wild-type and db/db PACs but had no effect on VEGF-R1 or VEGF-R2 expression [24].

Importantly, we proved a direct effect for PPAR γ activation on the angiogenic potential of PACs, as the incubation of cells with rosiglitazone in vitro enhanced migration, the formation of cord-like structures and capillary outgrowth. These effects were PPAR γ -dependent, as demonstrated *via* preincubation of cells with GW9662, an PPAR γ antagonist [24]. A similar increase in the migratory capacities of cultured bone marrow-derived cells was observed earlier after treatment with pioglitazone or troglitazone [87, 88], which could be mediated by reduced expression of ICAM-1 and VCAM-1 adhesion molecules upon PPAR γ activation [86].

Beneficial effects of TZDs on EPCs have also been shown in patients with normal glucose tolerance but who are

suffering from ischemic heart disease. Treatment for 30 days with pioglitazone increased not only the number of CD34+/KDR+ cells but also their clonogenic potential and SDF-1-induced migration in a PPAR γ -dependent manner [87]. Importantly, PPAR γ agonists improved endothelial functions and angiogenic capacities independently of insulin sensitization in normoglycemic patients [92]. Normoglycemic humans were treated for 6 weeks with rosiglitazone (8 mg once daily) or placebo and then adipose tissue vascularization was evaluated, with the authors reporting increased capillary density and angiogenic potential in patients receiving TZD [92].

PPAR γ activation was also reported to be beneficial in EPCs isolated from the bone marrow of normoglycemic rats. Pioglitazone prevented apoptosis *via* the PI3K/Akt signaling pathway [13]. Another study proved that a dual PPAR α / γ agonist, aleglitazar, administered to normoglycemic mice at a 10 mg/kg/day dose increased the number of Sca-1/VEGFR2 double-positive cells in bone marrow and peripheral blood. Aleglitazar also improved cell migration and enhanced neoangiogenesis. Importantly, cells isolated from healthy donors treated *in vitro* with aleglitazar were characterized by reduced oxidative stress-induced apoptosis and p53 expression, whereas the phosphorylation of eNOS and Akt was elevated [93].

9. Conclusions and Future Perspectives

PPAR γ originally described as a transcription factor regulating expression of genes involved in carbohydrate and lipid metabolism has been more recently studied in the context of cardiovascular system. Of great importance, its activity has been reported in vessel wall, in both endothelial cells and vascular smooth muscle cells. Because endothelium is a master regulator of angiogenesis, we attempted to summarize PPAR γ roles in this process focusing on endothelial cells.

Activation of PPAR γ shows predominantly antiangiogenic properties. There is also some evidence for the opposite effects, but 15d-PGJ2 mediated induction of VEGF and IL-8 expression in ECs turned out to be PPAR γ independent. Besides decreasing expression of angiogenic factors, PPAR γ activation leads to the reduced migration and proliferation of ECs. Additionally, an inhibitory net effect of PPAR γ induction was demonstrated in various angiogenic tests employing endothelial cells. During the past decade, accumulating evidence about circulating proangiogenic progenitors has considerably improved our understanding of PPAR γ actions. In contrast to mature ECs, activation of PPAR γ improves angiogenic potential of EPCs. These effects are described predominantly for cells isolated from diabetic patients; therefore it might be difficult to distinguish the direct effect of PPAR γ activation from the indirect results of improved glycaemic control. Finally, while studying PPAR γ activation by its ligands, one should not forget to differentiate between PPAR γ dependent and PPAR γ -independent actions, for example by using both PPAR γ agonists and antagonists.

Competing Interests

The authors declare that they do not have any conflict of interests.

Acknowledgments

This work was supported by European Union Framework Program POIG 01.02.00-069/09 and Opus and Sonata projects funded by the National Science Center 2012/07/B/NZ1/02881 and 2015/19/D/NZ5/00254. The authors used also the equipment obtained from EU Framework Programs POIG 02.01.00-12-064/08 and 02.02.00-00-014/08 to Faculty of Biochemistry, Biophysics and Biotechnology.

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