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Role of peroxisome proliferator-activated receptor-γ in pathogenesis of diseases mediated by inflammation, in malignancy, and in lipid and glucose metabolism disorders

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ABSTRACT

Lipid mediators can exert their effects by interactions with well-characterized cell surface G-protein-linked receptors. Recently, a group of intracellular receptors have been identified that are activated by a large variety of lipid-derived mediators. Amongst these novel targets, the peroxisome proliferator-activated receptors (PPARs), a family of three (PPAR α, β/δ and γ) nuclear receptor/transcription factors has become a major area for investigation. PPARs are found throughout the body, where they have diverse roles regulating lipid homeostasis, cellular differentiation, proliferation and the immune response. There is a great interest, therefore, in the roles of PPARs in a variety of pathological conditions, including diabetes, atherosclerosis, cancer and chronic inflammation. Although, a number of naturally occurring compounds can activate PPARs, it has been difficult, as yet, to characterize any of these mediators as truly endogenous ligands. These findings have lead to the suggestion that PPARs may act just as general lipid sensors. Acting as lipid sensors, PPARs may take changes in lipid/fatty acid balance in the diet or local metabolism and translate them to tissue-specific ligands, exerting tissue-specific effects.

FUNCTIONS OF PEROXISOME PROLIFERATOR - ACTIVATED RECEPTORS

Lipid mediators from fatty acids to eicosanoids and related products have important roles in physiology and pathophysiology. These lipid mediators, signal through at least two distinct mechanisms, either by linking to classical cell surface G-protein-linked receptors, or has been recently described through a variety of intracellular protein targets (1-5). Amongst these intracellular targets, of great interest has been the family of nuclear receptor transcription factors, the peroxisome proliferator-activated receptors (PPARs). The PPARs are a family of three receptors: α (NR1C1), β/δ (also referred to as NUC1; NR1C2) and γ (NR1C3), which heterodimerise with 9-cis-retinoic acid retinoid X receptors (RXRs).

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PPARs have a number of potentially important roles in lipid homeostasis, cellular differentiation, vascular biology, cancer and inflammation (6-8). The naming of PPARs, like many receptor and mediators, is purely historical and only applies to PPARa-mediated toxic responses in rodents. Important to note is that none of the PPARs cause peroxisome proliferation in man. The heterodimer-binding partner RXRs can form homodimers, and are also common binding partners for a number of other nuclear receptors, including the thyroid hormone receptor, Vitamin D receptor and the all trans-retinoic acid receptor. Addition of RXR ligands although often additive or synergistic in mediating effects are not required for PPAR-mediated effects.

The peroxisome proliferator-activated receptor -γ (PPAR-γ) is a nuclear receptor that controls expression of many genes relevant to the regulation of lipid metabolism and insulin sensitization and to the processes of carcinogenesis and inflammation. Originally discovered as an orphan receptor, and subsequently known as the receptor of the antidiabetic thiazolidinedione drugs, PPAR-γ is linked to various major chronic human diseases, such as atherosclerosis, rheumatoid arthritis, Alzheimer's disease, breast and colon cancers, and inflammatory digestive diseases.

Nuclear receptors constitute a group of about 50 transcription factors implicated in many different biological processes (panel), which are important targets in development of new drugs. For activation, PPAR-γ must form a heterodimer with another nuclear receptor, such as the retinoid X receptor (RXR). The PPAR-γ RXR heterodimer is widely expressed in the intestinal tract, pancreas, and liver, and has anti-inflammatory effects by its inhibition of transcription of inflammatory cytokines. Thus, activators of PPAR-γ, RXR, or both represent a potential new treatment for inflammatory diseases.

The human PPAR-γ gene is localized on chromosome 3p25. By contrast with mice, in which two isoforms of PPAR-γ mRNA have been identified, three isoforms have been described in man: PPAR-v 1, PPAR-γ 2, and PPAR-γ 3.In most types of tissue, PPAR-γ 1 is expressed more frequently than PPAR-γ 2. Only adipose tissue expresses high concentrations of PPAR-? 2, representing about 20% of PPAR-γ total mRNA in this tissue type. Expression of PPAR-γ is restricted to colon epithelium and adipose tissue.

PPAR-γ is highly expressed by adipose tissue, in which it has a role in adipocyte differentiation and in regulation of insulin responses (2,3). The other major tissue expressing PPAR-γ is the colon, in which epithelial cells and macrophages are thought to be the main cellular sources. The stomach, small intestine, liver, pancreas, and other tissues such as cartilage, skin, glial cells, airway epithelial cells, and kidneys contain low concentrations of PPAR-γ, whereas this gene is almost undetectable in muscles.

PPAR-γ can be activated by a number of ligands including docosahexaenoic acid (DA), linoleic acid, the anti-diabetic thiazolidinediones (TZDs or glitazones), and a number of lipids, including oxidized LDL, azoyle-PAF and eicosanoids, such as 5,8,11,14-eicosatetraynoic acid and the prostanoids PGA1, PGA2 and PGD2. Of clinical importance, the TZD insulin-sensitising compounds, currently being used as a new therapy for type II diabetes are potent PPAR-γ ligands. This family includes the compounds rosiglitazone, troglitazone, pioglitazone and ciglitazone. These TZDs have a binding affinity on PPAR-γ in the nanomolar range in vitro, which is interesting considering that the majority of proposed endogenous ligands have binding affinities in the micromolar range.

Like PPAR-α and β/δ, PPAR-γ has important roles in lipid metabolism, cell proliferation and inflammation. *In vitro* and *in vivo* PPAR-γ ligands inhibit vascular cell proliferation, migration and inflammatory gene expression, as well as inhibiting atherosclerosis and restenosis after balloon injury in animal models. Anti-inflammatory/vascular-protective effects of PPAR-γ are often similar to those of PPAR-α, though the relative contribution of each of the receptors to an overall anti-inflammatory effect described does vary from group to group. Though in terms of metabolism, PPARα's effects would seem to be via the liver, while PPAR-γ via fat storage/insulin-sensitive metabolic tissue.

In a variety of cancer cells, PPAR-γ ligands induce cell death via apoptosis or in susceptible cells terminal differentiation (8). This idea has also had clinical

success with the effective treatment of liposarcoma in a small trial using synthetic PPAR-γ ligands. One area of controversy is that of colon cancer, where PPAR-γ ligands effectively inhibited xenograft tumour formation but potentiated polyp formation in the intestine and colon of *APC* genetic mouse models. One explanation may be an anti-angiogenic effect of PPAR-γ ligands, likely to be required to a higher degree for tumour growth in the xenograft model. Indeed, PPAR-γ ligands can inhibit the growth of PPAR-γ tumors by targeting host PPAR-γ and neo-vascular growth (5).

PPAR-γ ligands have been shown to be effective in a number of inflammatory models from ulcerative colitis, rheumatoid arthritis, allergic encephalomyelitis, Parkinson's disease, Alzheimer's disease and myocardial infarction. Initial studies in macrophages strongly suggested that PPAR-γ was directly anti-inflammatory, being able to trans-suppress a variety of pro-inflammatory signaling pathways (AP-1, NFkB and Stat1). Anti-inflammatory/anti-proliferative effects of PPAR-γ ligands can be reproduced not only in other inflammatory cells (e.g. TH1 and B lymphocytes), but also local stromal cells involved at the inflammatory site. Although a number of PPAR-γ targets in this area have been identified (nuclear factor for activated T-cells, cyclin-dependent kinase inhibitor p21, PTEN), subsequent findings in cells from mice indicate that PPAR-γ ligands can have anti-inflammatory and anti-proliferative effects independent of PPAR-γ.

PPAR-y mice have been produced and demonstrate two independent lethal phases. Within the developing embryo, PPAR-γ deficiency inhibits trophoblast differentiation and placental vascularisation, resulting in severe myocardial thinning and death. Experiments producing PPAR-γ with wild-type placentas correct the cardiac defect, though animals surviving to term exhibit severe lipodystrophy and multiple hemorrhages. In man, mutations of PPAR-γ occur, of particular interest are P467L and V290M, which act in a dominant negative manner and are associated with patients suffering a severe insulin resistance and metabolic syndrome X-like condition.

MECHANISM OF ACTION

When combined as a PPAR-RXR heterodimer, PPAR ligands and RXR ligands (*9-cis*-retinoic acid) can act synergistically to induce PPAR responses. The specificity of these dimers in inducing different gene transcription is due in part to subtle differences in the 'response element' promoter regions they bind. These response elements for nuclear hormone receptors have a general pattern of either direct repeats, palindromic or inverse palindromic sequences separated by one or more nucleotides.

Protein interactions play an important role in the actions of PPARs. In the inactivated state nuclear receptors, such as PPARs, are considered complexes bound with co-repressor proteins. Upon ligand activation, PPARs dissociate from co-repressors and recruit co-activators, many of which with chromatin remodeling functions as well as RNA polymerase. Apart from trans-activating genes, PPARs can trans-suppress inflammatory gene activation. PPAR-γ directly binding to a transcription factor, NFAT or CBP, may account for the ability of PPAR-γ ligands to inhibit inflammatory gene expression. Further possible interactions may also govern the activity of PPAR-γ. As RXR is a common nuclear receptor-binding partner, activation of other nuclear receptor pathways may act as a sink for RXR, limiting the amount that can be used by PPAR- γ for its actions. Specificity of ligands and receptors are, therefore, likely to result from a combination of ligand, receptor, cell-specific co-interacting proteins and cell-specific targets. Even on the same receptor different ligands can have different effects. The novel non-TZD PPAR-γ ligand, N-(9-fluorenylmethyloxycarbonyl)-L-Leu, is a potent insulin sensitizer, but has a low adipogenic activity. This pharmacological difference is the results of a different active receptor conformation causing differential co-factor recruitment.

Natural ligands for PPAR-γ are: Polyunsaturated fatty acid (omega3) linolenic acid, eicosapentaenoic acid and docosahexaenoic acid and prostaglandins J_2 (PGJ_2)

Crystal structure analysis revealing PPARs have large binding pockets, linked with their high promiscuity for ligands (all apart from the synthetic ligands at a relatively low potency) have led to the suggestion that PPARs act as a general lipid sensing receptors. PPAR activators are characterized into three main categories: 1.) lipid mediators that activate PPAR, but do not bind, and are, therefore, most likely acting as substrates for further metabolic pathways; 2.) the enzymic pathways that produce known PPAR ligands; and 3. non-enzymic/non-specific pathways that result in active mediators that directly bind PPARs. It is important to directly compare different ligand activation and functional assays.

Exogenous linoleic acid activates PPARα and γ responses. Levels of linoleic acid could provide a link between the diet and the metabolic effects of the PPARs. Linoleic acid does not directly bind to PPARs and is, therefore, most likely acting as a substrate for further metabolic pathways. The use of enzyme inhibitors suggests that linoleic acid either through 15-lipoxygenase (15-LO) or cyclooxygenase (COX) pathways can activate PPAR-α or γ, respectively. Linoleic acid is broken down via the β-oxidation pathway in mitochondria. Inhibitors of this linoleic acid metabolism also increase PPARα activation, providing good indirect *in vitro* evidence for linoleic acid as an endogenous substrate for PPARs.

Arachidonic acid (AA) is abundant in all cells and has extracellular concentrations similar to linoliec acid. Its release from the phospholipids stores within the cell is tightly controlled (mainly) by phospholipase A2 (PLA2), present in various isoforms for different functions but whose activity is almost ubiquitously expressed. AA has a similar activation profile on PPARs compared with linoleic acid. There is a small degree of activation of transfected PPARs but this is small compared with its potential downstream metabolites. Similar to linoleic acid, inhibition of its metabolic β-oxidation pathway increases the ability of AA to activate PPAR-γ. Aside from inducing apoptosis in tumor cells, 15-d-PG J_2 and PPAR-γ ligands can also inhibit tumorigenesis by inducing the differentiation of tumor cells. The anti-tumor activity of 15-d-PGJ_2 is, however, not restricted to the direct inhibition of tumor cells. 15-d-PG J_2 acts also on surrounding cells, such as endothelial cells, inhibiting their expression of the vascular endothelial growth factor receptor (VEGFR), which results in the inhibition of angiogenesis *in vivo.* Also, 15-d-PGJ_2 can down-regulate the expression of MMP-9 in vascular smooth muscle cells, which results in the inhibition of tissue destruction and a subsequent inhibitory effect on the migration of cells. Therefore, 15-d-PGJ_2, in contrast to PGE2, inhibits tumor formation by inducing the apoptosis of tumor cells and inhibiting tumor-cell migration and angiogenesis.

The roles of PGE2 and 15-d-PG J_2 in the immune system are diverse and complex. Both mediators have profound, and opposing, effects on tumorigenesis and are key regulators of inflammation. Owing to the fact that these two lipids are produced from the same precursor (arachidonic acid) by the same cyclooxygenase enzymes, additional means to regulate the production of one or the other prostaglandin must exist. Prime candidates are the prostaglandin synthases, which synthesize specific prostaglandins from the PGH2 precursor. Therefore, future study into the function of these synthases and regulation of the synthase-encoding genes will lead probably to new approaches for the treatment and, perhaps, prevention of immune disorders, ranging from inflammation to cancer.

CONCLUSION

New treatments for inflammatory, metabolic and malignant diseases are needed. Assessment is needed of PPAR-γ/RXR activators in diseases associated with upregulation of expression of TNF α and the NF-kB signaling pathway as new anti-inflammatory drugs in human inflammatory diseases. Better knowledge of co-activators of PPAR-γ/, which affect activity of certain PPAR-γ/activators, good understanding and use of natural and synergistic anti-inflammatory effects of PPAR-γ/ and RXR agonists, and development of new selective modulators of PPAR-γ/RXR are required. It will be necessary to

reduce adverse events of these compounds and to enhance development of drugs able to target more specifically the inflammatory metabolic or malignant cascade.

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