PEROXISOME PROLIFERATOR-ACTIVATED RECEPTORS (PPARS)

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Abstract

Peroxisome proliferator-activated receptors are a group (PPARs) of transcription factors whose differential distribution in different tissues, including adipocytes, hepatocytes, muscles and endothelial cells lead to different clinical outcomes. They are called lipid and insulin sensors due to the important role in lipid and glucose homeostasis. They are mainly of three types; 1) PPARα which influences fatty acid metabolism and its activation lowers lipid levels, 2) PPARβ causes fatty acid oxidation in skeletal and cardiac muscles, as well as regulates blood glucose and cholesterol levels and 3) PPARγ which is mostly involved in the regulation of the adipogenesis, energy balance, and lipid biosynthesis. The expression of these receptors is influenced by many natural and synthetic ligands. Realistic data on the expression and function of natural PPAR agonists on glucose and lipid metabolism are still missing, mostly because the same ligand influences several receptors and a number of reports have provided conflicting results. The current minireview focuses on the structure, functions, types and ligands of the PPAR.

Key words: peroxisome proliferator-activated receptors, adipocytes, ligand, glucose homeostasis

I. Introduction

PPARs

Peroxisomes are the cellular organelles present in all eukaryotic cell (Neels and Grimaldi 2014). These are involved in several cellular functions including the beta oxidation of fatty acids (Baker et al., 2015). Lack of peroxisomes in cell causes many human disorders and this is proved by many scientific discoveries (Shiomi et al., 2015). Peroxisomes have ability to proliferate in response to the certain cellular as well as synthetic chemicals (Neels and Grimaldi 2014). Cellular disparate chemicals are designated as the Peroxisomes proliferators (Keller and Wahli 1995; Berg et al., 2015). There is structural diversity in peroxisomes proliferators but they induce peroxisomal gene transcription through same mechanism (Mast et al., 2015). The isolation and characterization of one of the peroxisomes proliferators was the breakthrough in scientific knowledge and that was the Peroxisomes proliferators activated receptors (PPAR) (Lalwani et al., 1987).
PPARs actually belong to the super-family of transcriptional regulators (Murthi et al., 2013). In 1990, Isseman and Green reported the new member of super-family of nuclear receptors (also regulators) and these were the followings:

- Peroxisomes proliferator activated receptors
- Receptors for Steroid
- Receptors for Thyroid hormone
- Receptors for Retinoic acid  (Issemann and Green 1990)

**Evolution of the gene**

Pioneering work on the cloning of PPARs was start with the PPARs of mouse. At the same time independent cloning of three different PPARs also started including PPARs of *xenopus*, rat and of human (Dreyer et al., 1993). Dreyer et al. 1993 did the evolutionary analysis by comparison of the conserved portion of gene encoded for PPARs. They formed the phylogenetic tree of PPARs to observe its evolution throughout the past. According to the analysis there was correspondence of appearance of PPARs in early vertebrates. Moreover phylogenetic analysis strongly suggested the iso-forms of PPAR in mammalian species (Keller et al., 1993; Keshamouni et al., 2007).

**Modular structure of PPARs**

All the isoform of PPARs have same structural and functional features (Monsalve et al., 2013). There is contradiction between scientists regarding the domains of PPAR (Wurtz et al., 1996; Zhou et al., 2015; Diezko and Suske 2013). Some scientists agree on 4 domains while others believe on 6 domains. If we go with the four functional domains then these are:

- Domain A/B
- Domain C
- Domain D
- Domain E/F

The N-terminal A/B domain contains the ligand independent activation function and the domain plays very crucial role for functioning of PPAR and that is phosphorylation of PPAR (La Cour Poulsen et al., 2012). Domain C also known as DNA binding domain (DBD) and its function is to promote the attachment of PPAR at peroxisomes proliferator response element PPREs and this is present under the prompter region of target gene. Region C contains 66 amino acid that forms two zing fingers (Berger & Moller 2002).

These zing fingers functions as the core of this domain. These fingers have conserved amino acid sequences. The docking domain that is D domain is for the attachment of co-factor (La Cour Poulsen et al., 2012). Other name of Ligand binding domain is E domain that plays a very important role as it’s responsible for activation and specification of PPARs binding to the PPREs (Buzón et al., 2012). The attachment of co-factor on E domain increases the expression of target gene (Gearing et al., 1993). The other modular structure of PPARs contains the six functional domains; (Helsen et al., 2012).
- Domain A
- Domain B
- Domain C
- Domain D
- Domain E
- Domain F

Difference between these two modular structures is just of domains A, B, E and F (Lee et al., 1995). In first model domain A and B are the same while this is not case of second model, same is with the domains E and F (Fig 1) (Motojima 1993; Attianese and Desvergne 2015).

![Diagram of PPAR Isoforms](image)

**Figure 1:** Isoform of PPAR gene (PPARα, PPARβ and PPARγ) and basic overview of PPAR structure (Daynes and Jones 2002).

**Transcriptional machinery of PPARs**

PPARs can be activated by natural as well as synthetic ligands (Sakharkar et al., 2015). Natural ligand includes the retinoid X receptor (RXR), PPREs and certain co-factors that plays crucial role in achieving the desired transcriptional activity (Berger & Moller 2002). Transcriptional machinery of PPARs having;

- RXR and hetero-dimerization
- Peroxisomes proliferator response elements (PPREs)
- Cofactors (co-activator/ co-repressors) (Sauer 2015).

**RXR and heterodimerisation**

For the binding on PPREs, ligand binding domain (LBD) has to make dimer with the (RXR) retinoid X receptor (Osz et al., 2015). With the recruitment of co-factors on domain E, heterodimer of Ligand binding domain and retinoid X receptor (LBD and RXR) binds to the PPREs (Gearing et al., 1993; Osz et al., 2015).

**Peroxisomes proliferator response elements (PPREs)**

Structure of PPREs consist direct repeats (DR-1) elements and these contain two hexanucleotides sequences (AGGTCA) (Juge-Aubry et al., 1997). Spacer of Single nucleotide can separated both hexanucleotide. The direct
repeats pattern of DR-1 is specific for the homodimer of RXR and PPAR which makes it different from the other Directs repeats (DR3 and DR4) (Piskunov 2012).

**Co-factor (co-activator and co-repressor)**

To initiate or suppress the transcriptional process there are several proteins act as co-repressor or co-activator. These co-factors mediate the transcriptional process by the attachment on domain E of PPAR (Varga et al., 2011). They recruit on domain and interact in ligand dependent manner. The process of transcription basically controls by the ligand bound and unbound state of the PPAR (Chen and Evans 1995, Dharap et al., 2015).

In ligand unbound state heterodimer associates with the co-repressor, that contains the histone deacetylase activity such as thyroid hormone receptor and nuclear receptor co-repressor (Dharap et al., 2015). Transcription inhibited by the state of histone which is deacetylation (Murata et al., 2001). In alternative manner co-activator such as PPAR binding protein and steroid receptor co-activator contain histone acetylase activity that can initiate the series of events which induces the transcriptional process upon binding of ligand. So this is liganded state of PPAR (Qi et al., 2000; Tzeng et al., 2015).

**Isoforms of PPAR**

To date there are 3 different isoforms of PPARs, encoded by the separate gene.

- PPARα
- PPARβ
- PPARγ (Harris & Phipps 2012).

**a). PPARγ**

As described earlier PPAR has three iso-forms, the first one is PPARγ (Schild et al., 2002). On the short arm of chromosome 3 (3p25) the gene which is encoding for PPARγ (PPARG) is located. The entire gene is of 100 kilo bases approximately and nine exons encoded this specific gene (Ikezoe et al., 2001).

The PPARγ contains three different promoters that on transcription yields further three iso-forms of PPARγ, namely PPARγ1, PPARγ2 and PPARγ3 (Fajas et al., 1999; Kalonia et al., 2010). Transcript of PPARγ1 and PPARγ3 translate into identical protein. Expression of PPAR is basically tissue dependent. PPARγ1 is the iso-form (Banks et al., 2015). PPARγ3 express abundantly in white adipose tissue, in macrophages, and in large intestine (Singh et al., 2013; Mair and McGarvey 2008).

**PPARγ gene mediated transcription**

The transcription mechanism of all iso-forms of PPAR is identical (Banks et al., 2015). Transcriptional process begins when ligand bind to the PPARγ receptor, ligand can be exogenous or endogenous (Penumetcha and Santanam 2012). Ligand bound the heterodimer of PPAR and RXR, this heterodimer binds to the promoter region which is PPREs after the recruitment of co-activators (Penumetcha and Santanam 2012; 46 Hörlein, et al., 1995). These all events lead to enhance the transcription of number of genes that are involved in the biological process of body. The biological process
includes adipogenesis, lipid metabolism, and homeostasis of glucose. The major mechanism in which PPARγ is involved is the improvement of insulin resistance (Fig 2) (Shi et al., 2006).

![Figure 2: Modular structure of Gene transcription mechanisms of PPAR. (Kota et al., 2005)](image)

**Biological mechanism of PPARγ**

Biological mechanism of PPARγ includes;

- Insulin sensitization
- Adipocyte differentiation
- Cancer
- Inflammation and atherosclerosis
- Retinal disorders

**Insulin sensitization**

PPARγ is associated with a number of genes that are directly and indirectly effect insulin action (Banks et al., 2015). Tissue necrosis factor alpha which is cytokine in nature, has been linked to the resistance of insulin (Ferrante 2007). In-vivo experimentation proved that PPARγ agonists improve insulin resistance by inhibiting the TNF-α effect on adipocytes (Jin et al., 2014). For glucose transport there is a gene GLUT4 (Kitagawa et al., 2004). Expression of this gene by PPARγ agonists’ adipocytes is playing crucial role in the process of glucose uptake (Cohen et al., 2003).

Resistin is a hormone that is secreted by the adipocytes and its function is to elevate the glucose level in blood. Resistin can be inhibited by thiazolidinediones TZDs (Lee et al., 2003). TZDs are the exogenous ligands for PPARs. There are many studies depicts that TZDs also increases glucose disposal in skeletal muscles by the mechanism of increasing
membranous protein kinase activity and phosphatidylinositol 3-kinase activity (Savage et al., 2003; Cock et al., 2004). PPARγ expression is lesser in skeletal muscles as compare to adipocytes. There are certain adipocytes-derived factors such as adipocytes related complement protein and 11β-hydroxysteroid dehydrogenase 1, are influenced by the PPARγ activation in glucose homeostasis and in improving insulin resistance (Cock et al., 2004).

**Adipocyte differentiation**

Adipogenesis is the process refers to the differentiation of pre-adipocyte precursor’s cell into mature adipocyte that can perform various functions such as capable of lipid filling, expression of cytokines and hormones (Cipolletta et al., 2012). PPARγ is the important transcription factor that is involve in the adipocyte cell growth and stop of cell growth, followed by the progress of cells into phenotype which is fully differentiated (Wang et al., 2007). PPARγ activation also promotes the process of apoptosis in mature adipocytes which are lipid filled (Moerman et al., 2004).

In addition to that process ligand induced apoptosis of mature cells stimulate the adipogenesis of others pre adipocyte precursors, leads in the increase of small number of adipocytes which are insulin sensitive (Morrison & Farmer 2000).

**Cancer**

Cell differentiation and apoptosis are the properties of PPARγ. It is found that these properties of PPARγ make it very important in treatment of different types of human cancer that includes pancreatic, breast, prostate, Colon, gastric and pituitary (Giovannucci et al., 2007; Heaney et al., 2003; Sarraf et al., 1999; Rovito et al., 2013).

**Inflammation and atherosclerosis**

Macrophages and Smooth muscle cells (VSMCs), have relation with PPARγ for its functions with metabolism of lipid (Handschin and Spiegelman 2013). This fact prompt research on PPARγ properties which are anti-inflammatory. Similarly, role of PPARγ in arthritis and inflammatory bowel syndrome are also under study (Schmuth et al., 2014). Different animal studies were shown that PPARγ agonists have anti-atherosclerotic effects (Chawla et al., 2001). However, these all agonists were also showed many deleterious effects by the over expressing the oxidized LDL scavenger and by induction in foam cell (Pasceri et al., 2000).

There are many mechanisms reported in which counteracted the PPARγ pro-atherogenic activity. In endothelial cells, TZDs inhibited the site of the intercellular adhesion molecule (ICAM-1) and cell adhesion molecule (VCAM-1) expression, which results in reduction of arterial accumulation of monocyte (Marx et al., 1998).

**Retinal disorders**

In retinal pigment epithelial (RPE) cells and vascular endothelial growth factor (VEGF)-induced choroidal angiogenesis can be inhibited by Troglitazone (Marx et al., 1998). In monkey and rats progression of choroidal neovascularization can also repressed by Troglitazone. These all effects suggested the application of PPARγ ligands in retinal disorders which are induced by diabetes and age-related retinal disorders (Aoun et al., 2003).
a). **PPARα**

PPARα is also a receptor like the PPARY for structurally different class of compounds that includes the hypolipidemic fibrates. In human and rodents, PPARα is expressed in many tissues including heart, liver, skeletal muscle, kidney and brown fat (Schmuth et al., 2014, Auboeuf et al., 1997). There is also expression of PPARα in a numerous vascular cells such as monocytes/macrophages and endothelial cells VSMCs (Fig 3) (Diep et al., 2000).

b). **PPARβ**

Despite extensive research on PPARα and PPARY, the functional characterization of PPARβ still unclear (Yao et al., 2013). Like other PPARS PPARβ is also expressed into variety of tissues and cells, but the expression level in the brain is high comparatively. Expression is also there in skin and adipose tissue (Fig 3) (Berger & Moller 2002).

![Figure 3: PPARs gene transcription mechanisms and their biologic effects in different organs (Kota et al., 2005).](image-url)
c). Role of PPARγ in diabetes

Obesity and dyslipidemias have strong correlation with diabetes (Bray and Popkin 2014). Elevated level of triglycerides and free fatty acids has been linked with the development of insulin resistant in muscle (Steneberg et al., 2015). Normalization of lipid storage by correcting the adipocyte function might be the cause to improve insulin sensitivity. A study on rats supported this hypothesis (Lefebvre et al., 1997). The inhibitory effect of TNF-α on insulin signaling was overcome by treated with TZD and other PPAR- RXR agonists, in 3T3-L1 adipocyte cell line. The effect of PPARγ stimulation is specific to the action of TNF-α on signaling (Murphy & Holder 2000). Another mechanism that could result to enhance insulin sensitivity is suggested by the study that showing the treatment to induce adipogenesis with PPARγ in rat model (Fasshauer & Paschke 2003). Insulin resistance in humans is also correlated with the abdominal obesity (Fig 3) (Ruderman et al., 2013).

Mutation resulting in loss of function of PPARγ caused the development of severe insulin resistance that leads to diabetes in two patients (Ruderman et al., 2013; Barroso et al., 1999; Purdel et al., 2014). Missense mutation Pro115Gln and Ser114 constitute the negative effects on activity of protein and cause insulin resistance. This observation was supported by the experiment on transgenic mice (Kubota et al., 1999). Higher expression of PPARγ observed in the muscle derived from obese and diabetic patients (Mayoral et al., 2015). In this view up-regulation PPARγ mRNA and protein expression following treatment with the insulin sensitizers in in-vitro study (Fig 4) (Murphy & Holder 2000).

![Figure 4: Role of Peroxisome-Proliferator–Activated Receptor (Ristow et al., 1998).](image)

**PPARγ and its mutations**

Up to date, there are 1 non coding sequence mutation and 17 coding sequences mutation of PPARγ gene have been reported (Agostini et al., 2006). Majority of these mutations are associated with FPLD3 (Monajemi et al., 2007;
Lüdtke et al., 2007). Out of these ten are mis-sense mutation, which is either located on LBD or DBD (Savage et al., 2003; Lüdtke et al., 2007; van Beekum et al., 2008). In addition two non-sense mutations (Monajemi et al., 2007), two frame-shift mutations and one mutation of promoter are also reported (Savage et al., 2002). Except for the mutation of promoter all other mutations can affect the protein function and cause the reduction of transcriptional activity of protein (Fig 5) (Francis et al., 2006).

Figure 5: Overview of PPARγ mutations. Distinct domains of the PPARγ2 protein (with PPARγ1 missing the first 30 amino acids) and the location of the different mutations. In addition, 1 non-coding sequence mutation in the PPARγ4 promoter has been reported and is depicted separately (Jeninga et al., 2009)

II. Conclusion
Peroxisome proliferator-activated receptors are a group (PPARs) of transcription factors also known as lipid and insulin sensors. PPARs actually belong to the super-family of transcriptional regulators. They are mainly of three types; PPARα, PPARβ and PPARγ. All the isoform of PPARs have same structural and functional features. PPARγ has more importance due to its important functions. Up to date there are 1 non coding sequence mutation and 17 coding sequences mutation of PPARγ gene have been reported. Mutation of gene all can affect the protein function and cause the reduction of transcriptional activity of protein.

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