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Functioning of the PPAR Gamma and its Effect on Cardiovascular and Metabolic Diseases

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INTRODUCTION

Peroxisome proliferator-activated receptors (**PPAR**) belong to nuclear super family of ligand-activated transcriptional factors. Name of this group of nuclear receptors was derived from their ability to stimulate proliferation of peroxisomes, which was described in rats in 1990 [1]. In humans, there are\we distinguish three isoforms of nuclear receptor PPAR: PPARα, PPARβ/δ a PPARγ. This isoforms differ by their distribution and specific function [2]. PPAR is nutrient sensor, which regulates a number of homeostatic functions, such as glucose and lipid metabolism. PPAR regulates uptake, utilization, oxidation and storage of fatty acids. Also, PPAR regulates apoptosis, growth and migration of cells, modulates oxidative stress and inflammatory disease in cardiovascular system and is particularly involved in vascular tonus regulation [3,4]. Disruptions this pathways can result in development of several diseases and pathological states, such as obesity, obesity-induced inflammation, atherosclerosis, diabetes mellitus, metabolic syndrome and hypertension [4-7].

PPAR activation leads to trans activation and trans-repression, which can result in induction and repression of gene expression [4]. Ligand-activated PPAR creates heterodimer with retinoic receptor, which is subsequently bound on sequence- specific target element in promoter region of target genes.

STRUCTURE OF PPAR

PPAR are orphan nuclear receptors. They consist from five or six structural regions, which create four function domains A/B, C, D and E/F. Similarly to other nuclear receptors, PPARs consist from C-terminal ligand-dependent domain with AF-2 motive (activation function motive 2) and N-terminal ligand-independent domain, which includes AF-1 motive [8] (Figure 1).

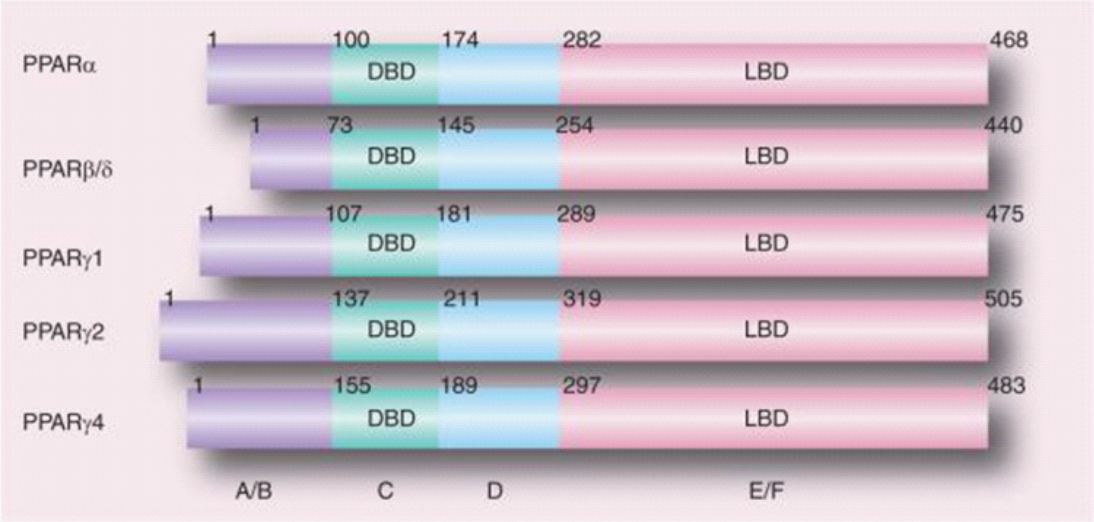

Figure1: Functional domains of human peroxisome proliferator-activated receptors PPARα, PPARδ/β, PPARγ1, PPARγ2 and PPARγ4 [9].

A/B domain is formed by variable NH2-terminal end, which is ligand-independent trans activation domain with AF-1 motive [10,11]. This function motive participates on phosphorylation [12] or SUMOylation [13] changes the ligand-binding affinity of the receptor.

C domain is 70 amino acid-long DNA binding domain (**DBD**). DBD contains of the two most conserved zinc finger motives. With the aid of DNA-binding domain, PPARs are targeted to a specific sequence of nucleotides within the regulatory regions of responsive genes. This sequence is denominated as PPAR response element (**PPRE**) [10,11].

D domain is flexible hinge region, which is needed for co-factors anchorage. D region permits the rotation of the DNA-binding domain, connects the DNA-binding domain to the preserved E/F region which contains the ligand-binding domain (**LBD**) as well as a co- activator/co-repressor-binding surface [14].

E/F domain is formed by C-terminal end, which is responsible for ligand specificity and ligand-dependent activation of PPARs. Moreover, this domain is involved in hetero dimerization with retinoid receptor X (**RXR**) and transcriptional co-activators via trans- AF-2 transactivation domain. It is also involved in the formation of binding with PPRE [10,11].

PPAR Isoforms

PPARs consist of functionally different isoforms, which are defined by their receptor/ligand binding affinity and also their gene expression and their activation, which is depend on metabolic pathways and tissues [4]. PPARs isoforms code genes localized on different chromosomes with highly conserved interspecies sequence [3,4,14-16]. It is interesting, while there is significant homology between PPAR isoforms, this proteins play different roles in energy metabolism [15].

PPARα was first identified PPAR isoform. PPARα gene is localized in human genome on chromosome 22q12.2-13.1. This gene was first discovered in 1990 in mouse and later in human too [17]. PPARα is expressed in tissue with high presence of fatty acid oxidation, such as liver, heart and skeletal muscle. In this tissues, PPARα performs task of major fatty acid homeostasis regulator [9,17-19]. Moreover, it is highly expressed in brawn adipose tissue (particularly in white adipose tissue too), kidneys, adrenals and in most type of cells, such as macrophages, vascular smooth muscle and endothelial cells [11,18,20]. PPARα is also primary regulator of mitochondrial and peroxisomal β-oxidation. Saturated and unsaturatedfatty acids (leukotriene derivate and VLDL) are endogenous ligand of PPARα with the greatest ligand/receptor binding affinity. PPARα activation inhibits expression of pro-inflammatory genes in VSMC (vascular smooth muscle cells) and decreases atherosclerosis development [21].

Other PPAR isoform is PPARβ/δ, which is in human genome localized on chromosome 6p21.1-21.2 [17] and which is expressed in adipose tissue, liver, heart and skeletal muscle, brain, kidneys, colon and in vessels [20,22]. As opposed to the PPARα and PPARγ, PPARβ/δ is ubiquitous expressed and therefore PPARβ/δ is not a target of any currently available drugs. Because of the lack of availability, PPARβ/δ- targeted drugs are coupled with its ubiquitous expression; the physiological function of PPARβ/δ is much less studied and understood [9].

Nevertheless, it is known, that PPARβ/δ activation increases lipid catabolism in adipose tissue, heart and skeletal muscle, improves HDL plasma level (high-density lipoprotein) and insulin sensitivity. Moreover, PPARβ/δ activation can induce cell proliferation and differentiation [23]. Also, it limits increase of body weight with anti-inflammatory effect in vessel wall, because PPARβ/δ activation inhibits gene expression of adhesive molecules such as VCAM-1 (vascular cell adhesion molecule 1) and MCP-1 (monocyte chemoattractant protein-1) [24].

The best studied PPAR isoform is PPARγ. Gen for PPARγ protein is localized in human genome on chromosome 3p25 [17]. It is synthesized in high range especially in adipose tissue. Multiple studies have shown that the PPAR-γ sequence is highly conserved between species [25,26].

PPARγ plays essential role in glucose metabolism regulation, adipocyte differentiation, lipid accumulation, because PPARγ can monitor transcriptional activity of a number of genes involved in these metabolic processes [11,27-30]. Some pivotal target genes of PPARγ transcriptional regulation are aP2 (fat-specific adipocyte protein 2), lipoprotein lipase, fatty acids translocase

FAT/CD36, transport protein for fatty acids, fatty acids binding protein, acetyl-CoA synthetase, glucokinase, GLUT4 (glucose transporter type 4), phosphoenolpyruvate carboxykinase, UCP1, 2 and 3 (uncoupling proteins 1,2 and 3) and LXR-α (liver X receptor-α) [11,28,29]. Further PPARγ regulates genes involved in insulin signaling pathway and expression of pro-inflammatory cytokines TNFα (tumor necrosis factor-α) [11,30].

Stability of PPARγ protein and his transcriptional activity is regulated by covalent modifications, involved phosphorylation, ubiquitination, O-glycosylation and SUMOylation [31,32].

Multiple and opposing functions of PPARγ suggest the existence of multiple PPAR-γ isoforms, each with unique ligand specificity and gene targets. The overall biological effect of PPAR-γ activation may be determined by abundance of individual transcript isoforms, their translational efficiencies, and the sum total of their regulation of various genes. Structural studies of the PPAR-γ gene and mRNA transcript support the existence of multiple PPAR-γ isoforms. PPARγ is expressed in three isoforms, which differ by their tissue distribution. They form mRNA transcript translation. These PPARγ isoforms and major features of their protein are on Figure 2.

Up to this day, seven diverse mRNA transcripts of PPARγ was found. mRNA transcripts of PPARγ are generated with different initiation types and alternative splicing of five exons sequences in 5'-terminal region (A1, A2, B, C a D) [33,34]. The open reading frame of the PPAR-γ gene consists of exons 1 to 6 [25,26,35,36]. Exons 2 and 3 encode the DBD, while exons 5 and 6 encode the LBD. The 5′-terminal region of the transcript is the most variable and is the determinant of the PPAR-γ isoform. Three exons had been identified in the 5′-terminal region. They are alternatively spliced with exons 1-6 of the open reading frame to generate three well established isoforms of PPAR-γ as shown in Figure 2 [35,36].

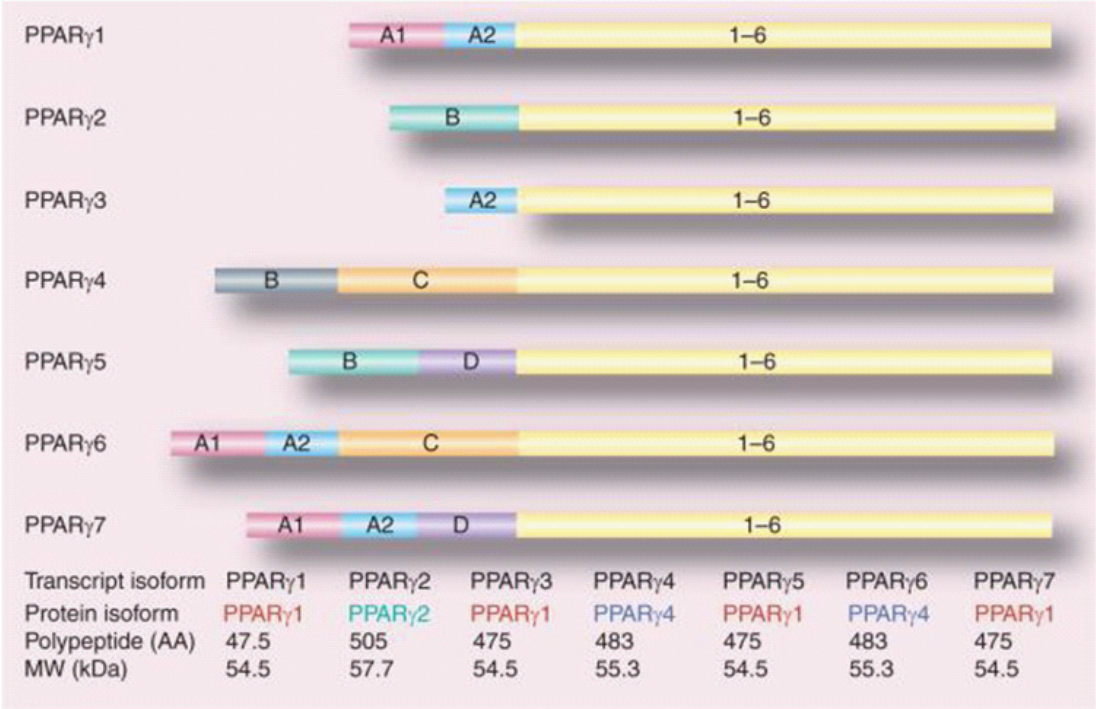

Figure 2: cDNA structures of seven PPARγ isoforms and major features of their protein products [9].

PPAR-γ1 consists of exon A1 and A2 spliced together with exons 1-6. PPARγ1 is expressed in heart and skeletal muscle, pancreatic β- cells in the islets of Langerhans, spleen, small intestine, kidneys, adrenals, vascular smooth muscle cells, endothelial cells and macrophages [33,37,38]. The mRNA for PPAR-γ2 consists of exon B and exons 1-6. PPARγ2 expression is primary delimited in adipose tissue, on the other hand PPARγ3 is freely represented in macrophages, colon and in adipocytes [33]. A third isoform, PPAR-γ3, identified in humans, consists of only exon A2 in its 5′-terminal region and exons 1-6.

Recently, we identified two novel exons in PPAR-γ cDNA from monkey macrophages [39]. Both of these exons combine with either exons A1-A2 or with exon B identified in the 5′-terminal region to form four novel PPAR-γ isoforms which we have called PPAR-γ4, -γ5, -γ6 and -γ7. Thus, the PPAR-γ gene encodes for at least seven unique transcript isoforms resulting from alternative splicing of the five exons at the 5′-terminal region. The open reading frame region encoded by exons 1-6 remains identical in all isoforms. There is a translation initiating ATG in exon B; however, in isoforms of PPAR-γ4 and PPAR-γ5, this site is inactivated by stop codons present in both exons C and D. Thus, exon B contributes 30 NH2- terminal amino acids PPAR-γ2 but not in PPAR-γ4 and PPAR-γ5. A translation initiating ATG is also present in exon C resulting in an additional eight amino acids at the NH2-terminus of PPAR-γ4 and PPAR-γ6. Thus, the seven transcripts encode for a total of 3 different PPAR-γ protein isoforms.

PPARγ activation plays essential role in regulation of eNOS expression in endothelial cells. Increased eNOS expression via PPARγ activation leads to increased bioavailability of NO [40]. Also it is known, that vascular PPARγ is periphery regulator of cardiovascular rhythm. PPARγ controls circadian variation in blood pressure and heart rate via BMAL-1 protein [41]. It seems, that PPARγ is major component of "vascular clock". Pioglitazone - PPARγ agonist modifies blood pressure circadian rhythm in patients with diabetes mellitus 2. Type from non-dipper (BP profile was changed in mean awake blood pressure to sleep blood pressure was less than 10%) to dipper (BP profile was changed in mean awake blood pressure to sleep blood pressure was 10% or higher) [42]. It seems that PPARγ plays important role in regulation of vascular homeostasis. Disruption of PPARγ function in this processes can participate on pathogenesis of vascular pathological states, such as atherosclerosis, rest enosis and last but not least hypertension too [31,37,38].

PPARS ACTIVATION

Heterodimer PPAR-RXR is formed after endogenous or synthetic ligand PPAR activation. RXR is an obligate heterodimer partner for all three PPAR isoforms. Heterodimer creation can result in conformational changes with subsequent translocation in nucleus [10,11]. PPAR-RXR is bounded on PPRE element in promoter region of target genes and subsequently dynamic of co-activator/co-repressor is changed. That modulates transcriptional apart of gene expression control, which subsequently affects transcription initiation (via up-regulation or down-regulation) [11,43-46].

Besides the presence of ligand and heterodimer partner - RXR, PPARs activation also depends on presence of co-activators and co-repressors [47].

PPARγ- RXR Complex

An important factor in determining PPAR responses is RXR activity, because PPARs are obligate RXR heterodimeric partners. The contact between a PPAR or RXR ligand and its cognate receptor's LBD induces a conformational change in the AF2 domain, facilitating the recruitment of co activators and release of co repressors. The assembly of these accessory molecules is a key determinant of a nuclear receptor's transcriptional activity [45].

Many aspects of RXR, including genetic variation, specific retinoid molecules modulated its activity, the enzymes involved in generating retinoid modulators, the binding proteins that handle retinoid transport and delivery may influence RXR responses and functional PPAR effects [48-50].

RXR occurs in three isoforms (RXRα, RXRβ and RXRγ). RXRα is broadly expressed with highest expression in the liver. RXRβ is also broadly expressed with relatively low expression in liver, while RXRγ has a more limited distribution with highest expression in striated muscle [50,51].

In the absence of ligand, RXR is maintained in transcription ally inactive tetramers that rapidly dissociate on ligand binding [52]. RXRα inactivation is embryonic ally lethal with hypo plastic cardiac ventricular development and ocular malformations.

RXRs are activated by metabolites of vitamin A, an essential fat soluble vitamin also known as retinol [53]. Natural retinoids derive mainly from dietary sources and because they cannot be generated *in vivo* de novo synthesis [54]. These are usually entered from dietary sources as retinyl esters, which subsequently undergo enzymatic cleavage. Retinol is metabolized to retinaldehyde through enzymatic action of alcohol dehydrogenises. Similarly, may convert retinol to retinaldehyde short-chain dehydrogenises/reductases. Retinaldehyde is metabolized to retinoic acid through the action of retinaldehyde dehydrogenises. Retinoic acid occurs in two forms: Trans (ATRA) or 9 cis retinoic acid (**9cRA**). ATRA has been demonstrated *in vivo* comparing to 9cRAit has never been demonstrated *in vivo*. 9cRA activates RXR *in vitro* [55,56]. ATRA, through RXR activation, represses adipogenesis, whereas 9cRA, promotes adipocyte differentiation, underscoring how specific retinoids direct distinct cellular responses. Moreover, retinoic acid may also activate PPARδ, with transport proteins helping determine which nuclear receptor is targeted [57].

Retinoids and the elaborate system controlling their generation, metabolism, transport, and subsequent nuclear receptor activation may influence atherosclerosis indirectly, for example through actions on adipogenesis and inflammation [58].

PPARγ Co activators

Transcriptional activation of PPAR is enhanced by co activators. Co activator complexes facilitate the liganded PPAR to achieve transcriptional activation of specific target genes [59]. Co activators possess one or more LXXLL motifs (L: leucine and X: any amino acid), some of which may make contact with a co activator-binding groove in the ligand-binding domain of nuclear receptor [60].

We recognized two major categories of PPAR co activators. Once co activators are recruited to a liganded nuclear receptor they remodel chromatin structure by the intrinsic histone acetyltransferase (**HAT**) or methyl transferees activities [59]. p160 steroid receptor co activator (**SRC**) family owns HAT activity. p160 recruits to the AF-2 domain and complex with the universal co activator CREB (cyclic-AMP responsive element binding protein)-binding protein (**CBP**) and its homologue p300 [61,62].

The second categories are co activators without known enzymatic functions. They participate in the formation of a multi subunit protein complex, variously called TRAP/DRIP/ARC/Mediator complex [44,59,63-66]. Mediator complex facilitates interaction with poll II of the basal transcription machinery [44,63]. Mediator complex play an important role in connecting CBP/p300 bound co activators with pol II containing preinitation complex [45].

A number of other co activators and co activator-associated proteins have been identified in the active PPAR transcriptional complex e.g. co activators possess enzymatic activities like methyltransferase (**CARM1**) [67], helicase (PRIC285, p68), and ATP dependent chromatin remodeling properties (PRIC320, SWI/SNF) [68,69] or those without any enzymatic activities such as PPAR coactivator-1α (**PGC-1α**), PGC- 1β, and BAFs [70,71]. Some other important co activators such as PRIP (peroxisome proliferator-activated receptor-interacting protein)/ASC2/AIB3/RAP250/NCoA6 [72-75] and PIMT (PRIP-interacting protein with methyl transferees domain/NCoA6IP) [76] are also identified, which serve as linkers between the initial HAT complex of CBP/p300 and p160 co activators and the downstream mediator complex [45].

PPARγ Corepressors

Corepressors such as NCoR (nuclear receptor co repressor) and SMRT (silencing mediator of retinoic acid and thyroid hormone receptor) associate with heterodimer ofnuclear receptors PPARγ and RXR in the absence of ligand to repress their transcriptional activity [77,78]. The physiological relevance of their implication in metabolic regulations has been demonstrated in the context of PPARγ-mediated adipogenesis, during which they promote a target-gene-specific repression of PPARγ activity [8]. NCoR and SMRT contain an NR interaction domain that interacts with an NR region between the DNA-binding domain and the ligand-binding domain. However, they can also affect chromatin conformation into an inactive state for transcription as their N-terminal domains can interact with other corepressors, such as Sin3 and histone deacetylases [79]. NcoR and SMRT and form multi-subunit co-repressor complex [78,80]. SMRT works as protein platform for HDAC (histone deacetylase) mobilization. HDAC is bounded in DNA through the use of specific interaction transcriptional factors RIP 140 (receptor interacting protein-140) and NRIP 1 (nuclear receptor interacting protein-1). Also, competition of HDAC and PPAR co-activators attenuates their activity [78, 80-82].

Also other co repressors with distinct molecular actions also repress PPARγ activity. The transcriptional co activator with the PDZ-binding motif (TAZ) is a ligand-independent PPARγ corepressor. TAZ inhibits PPARγ activation through co activation of Runx2 [83]. The scaffold attachment factor B1 (**SAF-B1**), which represses PPARγ potentially represents a novel class of co regulators that regulate transcription by linking NRs to the nuclear architecture [84]. The unphosphorylated retinoblastoma protein, RB, is also a PPARγ co repressor. It inhibits PPARγ activation by recruiting the histone deacetylase 3, a protein mediating transcriptional inhibition through histone tail deacetylation [85]. Finally, it is noteworthy that certain co repressors interact with nuclear receptors only in the presence of ligand [86].

PPARγ and Kinase/Phosphatase Activation

Similar to other nuclear receptors, the PPARs are affected by cross-talk with kinases and phosphatases. Phosphorylation by the MAPK, PKA, PKC, AMPK and GSK3 affect their activity in a ligand-dependent or -independent manner. The effects of phosphorylation depend on the cellular

context, receptor subtype and residue metabolized which can be manifested at several steps in the PPAR activation sequence including ligand affinity, DNA binding, coactivator recruitment and proteasomal degradation [87].

PPARγ is phosphorylated by activators of MAPK. Insulin [88,89] and PDGF (platelet derived growth factor) [90] decrease transcriptional activity of PPARγ. *In vitro* assays demonstrate that ERK2 and JNK are able to phosphorylate PPARγ2 [91]. The MAPK phosphorylation site, which can be used by both ERK- and JNK-MAPK [92] was mapped at Ser82 of mouse PPARγ1, which corresponds to Ser112 of mouse PPARγ2 [93]. Human PPARγ1 phosphorylation at this site (Ser84) inhibits both its ligand-dependent and -independent transactivating function. The phosphorylation status of Ser112 plays a role in the conformation of the unliganded receptor which regulates the affinity of PPARγ for its ligands and affects co activator recruitment ability [93]. It has been proposed that phosphorylation- mediated inhibition of transcriptional activity of nuclear receptors is an important "off-switch" of ligand-induced activity [94]. Extracellular signals which activate intracellular phosphorylation pathways can influence the degradation process of PPARγ [32].

Activation of PKA increased the basal and ligand-induced activity of PPARγ [95], similarly PKA increases Activation of PPARα PPARβ/δ. Treatment with PKA stimulators markedly increased activity of PPARγ while MEK and PI3K overexpression resulted in a decrease in PPARγ activity [96]. PPARγ is also sensitive to AMPK activity. PPARγ phosphorylation by AMPK represses both the ligand-dependent and - independent Trans activating function of the receptor [97].

PPARγ Agonists

Up to this day number of ligands PPARs was identified [98]. PPARγ activation depends on presence of co-activators and co-repressors, RXR availability, presence of others PPAR isoforms, promotor region status of target genes and ligands presence [47]. The most important aspect is ligand specificity to differ PPAR isoforms. PPAR isoforms deviate their amino acid composition of ligand-binding domain, which forms base for selective PPAR isoforms activation. Binding site volume of ligand binding domain (1300°A) permits PPAR access of structural differently ligands [99]. PPAR binding capacity of ligands is three to four times higher than in other nuclear receptors. That is reason why PPARs are able to bound multiple set of synthetic (Thiazolidinediones) and nature lipophilic acids (docosahexaenoic acid, eicosapentaenoicacid, phytanic acid, 15-deoxy12,14-prostaglandin J2 etc.) [100].

For metabolic disorders treatment number of nature or synthetic PPARγ agonists are being used. These supplements have different features and specificity to individual PPAR isoforms. They deviate their absorption, volume of distribution and characteristic profile of gene expression regulatory mechanisms [27,101-103].

To the group of endogenous PPAR ligands belong already mentioned essential fatty acids e.g. eicosapentaenoic acid (**EPA**). EPA is applied preventive or therapeutic for cardiovascular

or metabolic disease treatment. Except essential fatty acids PPAR activates also leukotrieneB_4, which stimulates PPARα and prostaglandin PGJ_2activated PPARγ [101].

In clinical practice synthetic ligands are used too. For example, fibrates (PPARα ligand) are/is used for dyslipidemic pathologic status treatment (hypertriglyceridemia) and thiazolidinedione, which activates PPARγ. They are being used for insulin sensitivity improvement in patients with diabetes mellitus 2 type [104-106].

Endogenous PPARγ agonists

Production and distribution of endogenous agonists of nuclear receptor PPAR depends on physiological and pathophysiological conditions of organism.

Selective modulators of PPARγ are often named SPARM (selective peroxisome proliferator activated receptor modulators). The distinct actions of SPARMs depend on the cellular context and on different receptor conformations, resulting in diverse gene interactions [107]. However, selected fatty acids are considered natural modulators of PPARγ, their connection with the receptor does not always lead to PPAR activation and target gene transcription.

Activation of PPARγ via polyunsaturated fatty acid, such as docosahexaenoic acid, eicosapentaenoic acid, incites functional respond of receptor [108]. Alike, phytanic acid is endogenous ligand, which exhibits similar activity to omega-3 polyunsaturated fatty acids. Phytanic acid improves insulin sensitivity trough increased glucose uptake [109].

Endogenous produced 15d-PGJ2 (15-deoxy- -12,14-prostaglandin J2) can activate PPARγ, but for activation higher concentration of 15d-PGJ2 is needed comparing to 15d-PGJ2 level, which is synthesized in organism [110].

Other endogenous agonist is lipoic acid [111]. Biological actions of lipoic acid are mainly associated with antioxidant attribute [112], and also with anti- carcinogen and anti-mutagens attribute [113,114].

Exogenous-synthetic PPARγ agonists

Synthetic PPARγ ligands are tiazolidindionederivates (**TZDs**). They are selective agonists of nuclear receptor PPARγ, -α and -β isoform activate TZDs minimally [115]. To TZDs belong troglitazone, rosiglitazone, pioglitazone and tideglusib. Trogliatzone was approved in 1997, but it was withdrawn from the market for increased risk liver failure and extensive hepatotoxicity in 2000 [116].

Tideglusib belongs to TZDs too. Tideglusib is a potent, irreversible, non ATP- competitive glycogen syntheses kinase-3β (**GSK-3β**) inhibitor and also PPARγ agonist. It seems to be, that tideglusib is a suitable candidate for Alzheimer disease treatment or for treatment of stroke [117], but his application in cardiovascular disease treatment was not examined.

TZDs improve insulin sensitivity and glycemia. Because of this TZDs are often called insulin sensitizers. TZDs are used for diabetes mellitus 2 type treatment [118]. Pharmacological action of PPARγ activation through TZDs indirectly increases insulin- stimulated glucose uptake in adipocytes, hepatocytes and in skeletal muscle [119,120], decreases free fatty acids level and lipid storage in adipose tissue. TZDs influence also an ability of fat redistribution and increase adiponectin level and decrease gene expression of TNFα [120,121].

Pioglitazone (**PIO**) and rosiglitazone (**RSG**) belong to TZDs and they are used for diabetes mellitus type 2 treatment for already mentioned actions. On the other hand, their effects in treatment of cardiovascular disease are different. PRO active study points out to positive effect of PIO treatment in cardiovascular disease patients. Comparing to placebo, PIO reduced cardiovascular complications by 16 % [122]. In opposite to the PIO, RSG treatment was associated with increased incidence of myocardial Infarction and death due to the cardiovascular causes after relatively short-term exposure [123]. European Medicines Agency withdrew approval of this medication in 2010 due to these cardiovascular safety concerns [121].

Different action of PIO and RSG can be probably caused by their diverse effect on lipids sub-fractionation [120]. PIO raises HDL cholesterol in human, reduces TAG level and free fatty acids plasma level without influence on total cholesterol level in plasma and LDL cholesterol level. RSG increases in plasma HDL cholesterol [121,124,125], and total cholesterol and LDL cholesterol level [124].

To this different action of PIO and RSG indicate works of Dovinová et al. and Kvandová et al., in which PIO treatment to experimental hypertensive rats affects more significantly red ox regulation in young animals comparing to adult spontaneously hypertensive rats (**SHR**). In young SHR, PIO treatment slowed down blood pressure increment, improved lipid profile (decreased total cholesterol and VLDL) and also increased gene expression of SOD2 isoform in brain steam [126,127]. As opposed, Chan and collective suggest that RSG improves BP in both young and adult SHR and also up-regulates antioxidant response in rostral ventrolateral medulla [128].

Long-term TZDs treatment decreased glycemia and insulinemia, but also improves vascular dysfunction [129], improves BP [104], decreases inflammatory [130,131] and reduces angiogenesis. Short-term TZDs treatment in patient with diabetes mellitus 2.type raises NO-dependent vasodilatation of coronary arterioles due to reduction of vascular superoxide [132].

Despite many beneficial features of TZDs (metabolic and anti-arteriosclerotic activity), they also exhibit side effects. In patient with diabetes mellitus 2.type treated by TZDs in clinical trials, was this treatment attended weight gain, edema, bone fractures, heart failure and increased risk of myocardial infarctions, which have limited the use of these drugs in diabetic patients with high lipid levels. These side effects were also observed in animal experimental long-term studies [133].

PPARγ Antagonists

Likewise, to PPARγ agonists modulate PPARs activity also PPARγ antagonists. Between aantagonists of PPARγ belong GW9662, PPARγ Antagonist III-G3335, SR 202, BADGE etc.

GW 9662 is a selective, irreversible, and effective peroxisome proliferator activated receptor-γ (**PPARγ**) antagonist [134,135]. GW9662 inhibits PPARγ with an IC50 of 3.3 nM [134]. Mass spectrometric analysis of the PPAR gamma ligand binding domain treated with GW9662 established Cys^{285} as the site of covalent modification and does not affect transcription of full-length PPARα and PPARδ. GW 9662 has been shown to block RZG-mediated activation of PPARγ, and improve growth inhibition induced by Rosiglitazone in breast tumor cell research. Additionally, reports indicate that GW 9662 blocks the protective activity of lipopolysaccharide, LPS, and acts as an agonist for the farnesoid X receptor (**FXR**) and pregnane X receptor (**PXR**) nuclear receptors [134].

PPARγ Antagonist III, G3335 is a cell-permeable dipeptide (H-Trp-Glu-OH) that acts as a selective and reversible PPARγ antagonist (KD ~ 8 μM). The surface Plasmon resonance technique clearly showed that G3335 has a highly specific binding affinity towards PPARγ and was able to block RZGin its stimulation of the PPARγ - LBD-RXRa-LBD interaction in a dose- dependent manner (IC50 = 31.9 μM) in transfected COS-7 cells. In addition, molecular- modeling results provided a possible binding mode for the PPARγ-LBD-G3335 interaction at the atomic level. The importance of Cys^{285} for PPARγ-G3335 binding was further confirmed by a PPARγ point-mutation (PPARγ-LBD-Cys^{285}Ala) assay [136].

SR 202 is other PPARγ selective antagonist's antidiabetic and antiobesity agent. SR 202 attenuates troglitazone-induced PPARγ transcriptional activity. *In vitro*, SR 202 inhibits PPARγ-dependent adipocyte differentiation [137].

Bisphenol is a diglycidyl ether (BADGE) can inhibit the peroxisome proliferator-activated receptor (**PPARγ**) and adipocyte differentiation, and has been shown to induce apoptosis in tumor cells. Research shows that BADGE can suppress the tumor necrosis factor-α (**TNF-α**) and that it has the ability to induce F-actin depolymerisation [138].

PPARΓ AND MECHANISMS OF BLOOD PRESSURE REGULATION

TZDs treatment increases BP in diabetic patients [139] and also in non-diabetic hypertensive patients [140]. PPARγ activation can lead to decrease of BP, but also to decrease of cell growth at vascular remodeling in hypertension. Moreover, TZDs improve endothelial dysfunction in rat mesenteric resistance arteries after angiotensin II (**Ang-II**) infusions [141]. It was observed, that pleiotropic beneficial action of PPARγ on vasculature are glycemia-independent [142]. This effect can be mediate via several possible mechanisms, such as due to PPARγ mediated modulation of expression and/or phosphorylation of specific signaling molecules of insulin signaling pathway. This PPARγ dependent modulation of insulin receptor signaling can lead to reestablishment of

balance between PI3K/Akt/eNOS and MAPK/ET-1with subsequent increase of NO bioavailability, with vasodilatation action of vascular tone [38].

Further, PPARγ can directly modulate gene expression of antioxidant and prooxidant genes and maintain redox homeostasis via interaction between PPARγ and signaling pathways, which are involved in oxidative stress modulation. It is cooperation of signaling pathways, such as Nrf2 (Nuclear factor E2-related factor 2), NF-κB (nuclear factor kappa B), Wnt (Wingless- type MMTV integration site)/β-catenin and FOXO (Fork head box protein O) proteins [143]. PPARγ activation can decrease oxidative stress in rostral ventrolateral medulla, which plays a critical role in pathogenesis of hypertension [128]. This oxidation stress modulation can lead to slow-down of hypertension development in pre-hypertensive period [126].

Further PPARγ can inhibit gene expression of AT1 receptor and through Ang II induced ERK 1/2 activation, which is inhibited vascular remodeling [144]. Also, PPARγ activation has influence on renin-angiotensin system. PPARγ can inhibit this system and decrease BP [145].

PPARγ and PI3K/Akt/eNOS Signaling Pathway

Insulin resistance is defined as reduction of sensitivity and/or reaction on metabolic activation of insulin receptor. Insulin resistance is associated with series of metabolic disease and hemodynamic disruption. In animal experimental models and in human studies there is a reciprocal relationship between insulin resistance and endothelial dysfunction [146,147]. Already Reaven and Hoffman in 1987 assumed, that insulin resistance potentially contributes to pathogenesis of hypertension, but connection between insulin resistance and hypertension in human with genetic predisposition to hypertension remains till now unexplained [148].

Insulin has classically been considered a hormone that plays essential role in the control of glucose homeostasis on skeletal muscle, adipose tissue and the liver. Moreover, several studies indicate that insulin is also a vascular hormone that has an essential role in both regulating glucose homeostasis through influencing blood flow and in maintaining vascular health. Further insulin regulates several anabolic processes, such as glycogen and lipid synthesis, lipid storage in liver and adipose tissue, inhibition of fatty acids oxidation, glycogenolysis and gluconeogenesis.

The most important insulin effect on cardiovascular system is stimulation of nitric oxide (**NO**) production in vascular endothelium. Abnormally reduced insulin sensitivity affects vascular homeostasis that manifests as impaired endothelial function, vasodilatation, small vessel disease (i.e. retinopathy and nephropathy), and enhanced vascular inflammation and atherosclerotic lesion formation.

Insulin is synthesized in pancreatic β- cells in the islets of Langerhans as response to increased glycemia after a meal [149]. Insulin is synthesized as 110- amino acid precursor known as pre pro insulin, which contains a hydrophobic N-terminal signal peptide. This N-terminal peptide facilitates pre pro insulin through signal recognition particles into the lumen of the rough

endoplasmic reticulum. Subsequently is signal peptide from preproinsulin cleaved by a signal peptidase to proinsulin [150]. Proinuslin undergoes posttranslational modification. Proinsulin is folded in an oxidizing environment allowing for formation of three intra molecular disulfide bonds. for this processes a diverse range of endoplasmic reticulum chaperone proteins are needed [150]. The folded proinsulin is transported to the Golgi apparatus where proinsulin enters immature secretary vesicles and it is cleaved to yield insulin and C-peptide. Proinsulin is proteolyticallycleavedon insulin consisting of the B and A chains held together by disulfide bonds plus the C-peptide that is co-secreted with insulin and has distinct biological effects. This proteolytically cleavage is initialized by prohormone convertase 1 orprohormone convertase 2, and then C-terminal basic residues are removed by carboxy peptidase E.

Insulin signaling pathway is actuated after join of insulin on insulin receptor. Insulin receptor is localized on cell membrane. It is a cell-surface heterotetramereric transmembrane protein, which consists of two extracellular α subunits and two transmembrane β subunits joined by disulfide bonds. Insulin-α extracellular subunits of insulin receptor binding leads to conformational changes, that activate intrinsic tyrosine kinase of β trans membrane subunits. Activation of the receptor tyrosine kinase promotes trans-autophosphorylation of the β subunits aswell as the tyrosine phosphorylation of multiple docking proteins, including insulin receptor substrates 1 (**IRS-1**) and 2 (**IRS-2**). IRS-1 is necessary for insulin-stimulated NO production in the endothelium. Whereas, IRS-2 may also contribute to NO production, it is primarily implicated in delivery of insulin to theskeletal muscle interstitium [151]. IRS1 or IRS 2 phosphorylation leads to activation of two signaling pathways: PI3K/Akt/eNOS and MAPK/ET-1 (Figure 3).

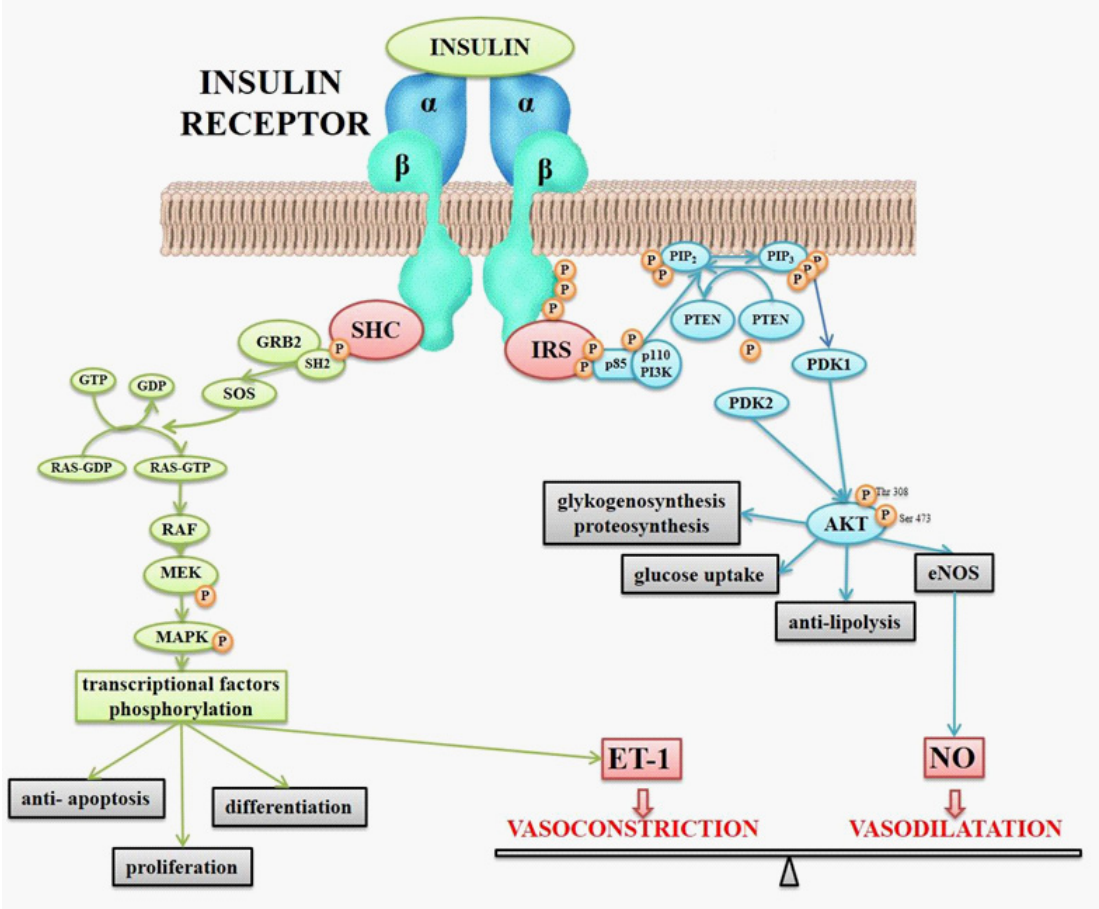

Figure 3: Insulin signaling pathway.

The tyrosine phosphorylated IRS-1 activates phosphoinosiditde 3-kinase (**PI3K**) through phoyphorylation of the src homology 2 (**SH2**) domain of the p85 regulatory subunit of PI3K. Activating catalytic p110 subunit of PI-3K cleaves catalytically the membrane phospholipid phosphatidylinositol 4,5-bisphosphate (**PIP2**) to phosphatidylinositol 3,4,5-trisphosphate (**PIP3**). PIP3 is needed for PIP3-dependent phosphorylation of PDK1 (3'-phosphoinositide-dependent kinase-1) and Akt kinase. Finally, Akt phosphorylates several effector proteins, including GSK-3β, FOXO and eNOS. GSK-3β activation is insulin-independent although it may be activated trough insulin signaling pathway too. GSK-3β phosphorylation through IRS1 activation and PKB inhibition is more present in basal conditions comparing to insulin receptor stimulation [152].

PI3K/Akt/eNOS signaling pathway is critical point for vascular tonus regulation, because eNOS catalyzes the conversion of L-arginine to L-citrulline and produces NO. On the other hand, MAPK cascade activation plays important role in insulin-regulated mitogen processes, such as cell proliferation, differentiation and cell survival [153].

Balance between vasodilatation and vasoconstriction effect due to insulin receptor activation is disrupted in vascular endothelium cells of insulin resistance animal models. PI3K/Akt/eNOS signaling pathway is suppressed while signaling pathway MAPK/ET-1 is preserved [154]. This balance disruption between via endothelial cells produced vasorelaxant and vasoconstrictor factors influences vascular tonus for the benefit of vasoconstriction. This imbalance is designated as endothelial dysfunction [155].

PPARγ activation due to tiazolidindiones improves insulin sensitivity in diabetic patient with presence of plasma level of insulin and glycemia reduction. PPARγ activation affects insulin signaling pathway by modulation of expression and/or phosphorylation of specific signaling molecules, which can lead to reestablishment of balance between vasodilatation and vasoconstriction effect due to insulin receptor activation.

Study of [156] shows, that in the young male spontaneously hypertensive rat (**SHR**) PI3K expression and phosphorylation of PKB-eNOS in vascular tissues is significantly decreased. Administration of rosiglitazone raises vascular PPARγ expression, resulting in restoration of PI3K/PKB/eNOS signaling activation, followed by improvement of endothelial function in the young SHR due to increase of NO release [156].

TZDs treatment can influent and change expression of signaling molecules of insulin signaling cascade (IRS, PI3K, Akt, eNOS, 5 ́-AMP kinase, GLUT4, etc.) because of improved insulin sensitivity and/or endothelial dysfunction.

IRS proteins: IRS proteins is group of proteins anchoring between insulin receptor and a complex of intracellular signaling molecules. Several studies suggest modulation effect of TZDs on IRS phosphorylation. Increased phosphorylation of serine residues of IRS1 was observed in adipose tissue of obese Zucker rats after TZDs treatment [157]. Administration of TZDs can reduce level of circulated free fatty acids, what can induce phosphorylation of IRS1 serine residues due to activation of PKCθ (protein kinase C) [158]. In Zucker rats can TZDs activation of PPARγ potentially raise phosphorylation of tyrosine residues of insulin receptor and so induce Akt activation [157].

PI3K/Akt signaling pathway: In subcutaneous adipose tissue of diabetic patient after troglitazone treatment insulin administration had beneficial effect of Akt kinase phosphorylation [159]. Administration of troglitazone raised insulin-stimulated IRS1 association with PI3K and activation of Akt kinase in patient with diabetes mellitus type 2 in skeletal muscle [160]. Moreover, this treatment increases Akt phosphorylation in skeletal muscle in patient with insulin resistance too [161]. However, influence of rosiglitazone on PI3K/Akt signaling differs in some studies. Miyazaki and collective points, that insulin sensitivity improvement after rosiglitazone treatment is attended with IRS1 phosphorylation improvement associated with incensement PI3K activity [162]. In contrast to Miyazakis work, Karlsson and collective didn't observe any changes from the

aforementioned parameters after rosiglitazone treatment in recently diagnosed diabetic patients [163]. Alike, was not observed any effect of insulin sensitizer treatment in combination with biguanid, concretely metformine, on activation of PI3K or Akt [160,164].

5'-AMP kinase: 5'-AMP kinase is protein kinase, which plays important role in regulation of glucose and lipid metabolism. Activation of 5'-AMP kinase increases oxidation of fatty acids in skeletal muscle due to increased malonyl-CoA concentration. TZDs, concretely pioglitazone [165] and biguanid - metformine [164] improve glucose tolerancy via 5'-AMP kinase. Metformin-activated 5'-AMP kinase decreased glycemia and plasma level of triacylglycerol because of glucose uptake and inhibition of gluconeogenesis in liver [166]. Pioglitazone- stimulated activation of 5'-AMP kinase and acetyl-CoA carboxylase in muscle of diabetic patients increased expression of genes involved in mitochondrial functions and fatty acids oxidation [165].

Glucose transporter: Activation of PPARγ can directly regulate expression of GLUT4 [167] and CAP (c-Cbl associating protein), which are involved in regulation of glucose transport due to insulin stimulation [168]. GLUT4 is insulin- dependent glucose transporter and plays important role in modulation of glucose disposition in adipose tissue and in skeletal muscle. TZDs increase expression of insulin-dependent glucose transporter-GLUT4. Expression of GLUT4is reduced in human obese patient with diabetes mellitus. Troglitazon administration re-establishes gene expression of GLUT4 on physiological level [169].

PPARγ and endothelial Nitric Oxide Synthase

The regulation of eNOS expression and its activation is influenced by various cellular events such as transcriptional regulation, protein–protein interaction, phosphorylation and dephosphorylation at different amino acid sequences of eNOS regulated by various kinases and phosphatises [170]. eNOS has a few well-described phosphorylation/dephosphorylation sites such as Ser^{1177} and Thr^{495}. Also some other sites have been identified which regulates NOS activity Ser^{633}, Ser^{114} and Ser^{615}, but their precise roles remain controversial [171]. This regulated phosphorylation/dephosphorylation sites of eNOS can be activated by multiple protein kinases, including PKA, PKB, PKC, AMP- activated protein kinase (**AMPK**), ERK and phosphatases such as protein phosphatase (**PP**)1 and PP2A in response to multiple stimuli via shear stress, growth factors, insulin, etc. [171-176]. Regulation of eNOS action by multiple protein kinases and phosphatases is summarized in Table 1.

Expression and activation of eNOS is regulated via several mechanisms. Besides phosphorylation and dephosphorylation of eNOS regulatory sites, eNOS also regulates oxidized low-density lipoprotein (**ox-LDL**). A low concentration of ox-LDL up-regulates gene expression of eNOS [177] and conversely, increasesox-LDL concentration decreases eNOS expression [178]. Further, excessive NO itself regulateseNOS expression of a negative feedback regulatory mechanism through cGMP- mediated activation [179].

Table 1: Regulation of eNOS action by multiple proteinkinases and phosphates, modified by [180].

Enzyme	*eNOS regulatorysite*	*Effect*	*References*
Pak	Ser1177 phosphorylation	activation	[173]
	Thr495 dephosphorylation		
PKB	Ser1177 phosphorylation	activation	[172]
PKC	Ser1177 dephosphorylation	inactivation	[173]
	Thr495phosphorylation		
AMPK	Ser1177 phosphorylation	activation	[175]
ERK1/2	Ser1177 phosphorylation	activation	[176]
PP1	Thr495dephosphorylation	inactivation	[173,174]
PP2A	Ser1177 dephosphorylation	inactivation	[171,173]

The protein-protein interaction between eNOS and caveolin-1 results in eNOS inactivation because of caveolin-1 hampers calmodulin binding to eNOS when cytosolic calcium levels are low [181]. Caveolintonically inhibits eNOS in quiescent cells both by impeding the signaling of caveolae-targeted receptors that transduce eNOS stimulatory signals as well as by inhibiting calmodul in binding to eNOS [182]. On the other hand, eNOS activity is positively regulated through the protein-protein interaction of heat shock protein 90 (**hsp90**) with eNOS. Heat shock protein 90 (**hsp90**) is a chaperone involved in protein trafficking and folding, modulates agonist-dependent eNOS activation. Interaction of hsp90-eNOS enhances eNOS activity by inducing calmodulin-stimulated displacement of eNOS from caveolin-1 (183). Further, this interaction enhances Akt-mediated activation of eNOS [184,185].

Recent studies have suggested a potential regulatory role of PPARγ- dependent eNOS expression and NO generation in the vascular endothelium. Several studies suggest that a direct transcriptional mechanism cna be involved in PPARγ-mediated release of NO in endothelial cells. Activation of PPARγ using 15d-PGJ2 or ciglitazone stimulates the NO release from the endothelium [186] Mechanisms of PPARγ activation-mediated increase in endothelial NO production are not yet understood [187] suggested that PPARγ-dependent eNOS activation is mediated via hsp90-dependent mechanism. Rosiglitazone and 15d-PGJ2 stimulated hsp90-eNOS interaction, resulting in eNOS activation (at Ser^{1177} phosphorylation). Conversely, ciglitazone did not have this effect, which suggests different effects of PPARγ agonists. It seems, that hsp90 plays the key role in this context because co-administration PPARγ agonist with geldanamycin (inhibitor of hsp90) attenuates eNOS activation and NO release from endothelial cells [187].

PPARγ activation also decreases in HUVEC membrane NADPH oxidase subunits such as Nox1, gp91phox (Nox2) and Nox4, which is accompanied by an enhanced expression of SOD resulting in reduction of oxidative stress. The elevated vascular oxidative stress reduces endothelial bioavailability of NO due to peroxynitrite generation. Hwang et al. suggest that, NO release from

the endothelium due to PPARγ activation could also enhance NO bioavailability by reducing endothelial superoxide anion generation and oxidative stress [188].

Endothelial dysfunction can be influenced with adiponectin too. This hormonal protein, secreted in adipose tissue can improve endothelial function via PPARγ activation- mediated activation of eNOS. Administration of rosiglitazone in the diabetic mouse due to stimulated adiponectin release activation of AMPK/eNOS and cAMP/PKA signaling pathways in the aorta, resulting in enhancement of NO bioavailability, leading to an improvement in endothelial function [189].

Rho-kinase plays important part in endothelial dysfunction development too. This serine threonine kinase inactivates eNOS and reduces NO bioavailability [190,191]. PPARγ activation inhibites up-regulation of protein tyrosine phosphatase-2 (**SHP-2**) Rho-kinase. Pioglitazone administration in angiotensin-II-treated rat cultured aortic smooth muscle cells up-regulates SHP-2 which subsequently dephosphorylates a GTP/GDP exchange factor of Rho-kinase Vav, resulting in inactivation of Rho-kinase.

Several studies suggest that PPARγ plays a distinctive role in regulating the physiology and expression of eNOS in the endothelium, resulting in enhanced generation of vascular nitric oxide. The PPARγ activation mediates vascular anti-inflammatory and antioxidant response and regulates endothelial function, therefore, is beneficial in improving the vascular function in patients with atherosclerosis and hypertension with or without diabetes mellitus. Moreover, the therapeutic opportunities of agents that activate PPARγ in preventing vascular endothelial dysfunction and cardiovascular disorders associated with endothelial dysfunction are discussed.

PPARγ and Renin-angiotensin System

One mechanism, which can influence hypertension development through PPARγ activation is attuned AT1 receptor gene expression [141,142,144]. This gene suppression of AT1 receptor is result of protein - protein interaction between nuclear receptor PPARγ and Sp1 protein. Sp1 belong to class of zinc-finger motifs presented in transcription factors, which can join to GC rich promoters regions. PPARγ either down-regulates expression of AT1 receptor or inhibits trough Ang II mediated signaling pathways, such as PI3K and MAPK. PI3K and MAPK suppress ultimately activation of renin angiotensin system (**RAS**) cascade [144,192] and remodeling of vascular wall [144].

RAS is endocrine system which playes essential role in cardiovascular and renal physiology. RAS regulates BP and sodium ions concentration [193]. Further it influences sympathetic nervous system functions and due to aldosterone regulates extracellular fluid volume [194]. Dysregulation of RAS participates on pathogenesis of hypertension, atherosclerosis, ischemic heart disease and heart failure. Physiologically effect of RAS is mediated through Ang II, which is generated enzyme cascade and released to systemic circulation [195].

When BP in renal arteries decreases, cells of the juxtaglomerular apparatus produces and secerns protease-renin (43 kD). Renin is endopeptidase and cleaves angiotensinogen to decapeptide Ang I (angiotensin I). Angiotensinogen is synthesized in liver and consist of 14 amino acid residues. Elevated serum levels of angiotensinogen are often recognized as the cause of hypertension, because higher angiotensinogen concentration leads to supernormal Ang II production [196]. Especially in lungs circulation is Ang I converted on octapeptide Ang II (angiotensin II). This reaction is catalyzed by angiotensin converting enzyme 1(**ACE1**). ACE is metalloproteinase, which is presented in two forms- somatic and testicular. ACE is expressed in endothelial, epithelial and neuropithelial cells [197].It is also expressed in heart and on luminal surface of endothelial cell of vascular wall [198]. ACE1 also participates on bradykinin degradation.

Ang II is major stimulator of synthesis and secretion of aldosterone and activates sympathetic nervous system [199]. It is mediator of oxidative stress, stimulates cytokines production, synthesis of adhesive molecules and also stimulates vascular remodeling. Ang II can induce arteriolar constriction and consequently elevate systemic vascular tone and blood pressure [196]. Ang II is produced local in several tissues too. Their production in heart and vascular system significantly participates on cardiovascular diseases pathogenesis.

Except for Ang II, Ang 1-7 (angiotensin 1-7) can be produced from Ang I trough, alternative RAS axis". This alternative Ang 1-7 production is catalyzed by angiotensin converting enzyme 2 (**ACE2**) [200].

Ang II is joined to member receptors AT1 or AT2. They are associated with GPCR (G-protein-coupled receptors. Similarly it is associated with GPCR Mass receptor too. Ang 1-7 is bounded to Mas receptor. Activation of these receptors results in phosphorylation of differently signaling pathways, which often leads to opposite cellular roles.

ACE/Ang II/AT1 cascade mediates major effects of Ang II. Activation of AT1 receptors stimulates L-type of calcium channels through G protein. Ang II inhibits the voltage and Ca^{2+} activated K^{+} (BK) channels through a PKC-independent mechanism [201] while in renal arterial myocytes Ang II can inhibit voltage dependent potassium channels [202] via a signal transduction pathway involving PKC activation [203]. The voltage dependent potassium channels consist of amino acid sequences, which reveal sites for PKC phosphorylation in their amino acid sequences [204].

Activation of ACE/Ang II/AT2 cascade plays role especially in prenatal period, when this cascade activation acts contra regulatory to AT1 receptor. Activation of AT2 receptors results in anti-proliferator effect and also leads to vasodilatation [205].

The activation of the RAS and consequent generation of Ang II and activation of AT1 receptors is an important cause of vasoconstriction and vascular remodeling during essential hypertension. An increased expression of various components of the RAS during hypertension has been described [206].

Several kinases are activated through Ang II activated AT1 receptor for example MAPK, p70S3 kinase, etc. This kinase incensement is associated with BP increase and with hypertension development. AT1 receptor activation stimulates NADPH oxides in vascular wall, which is major source of reactive oxygen species [207].

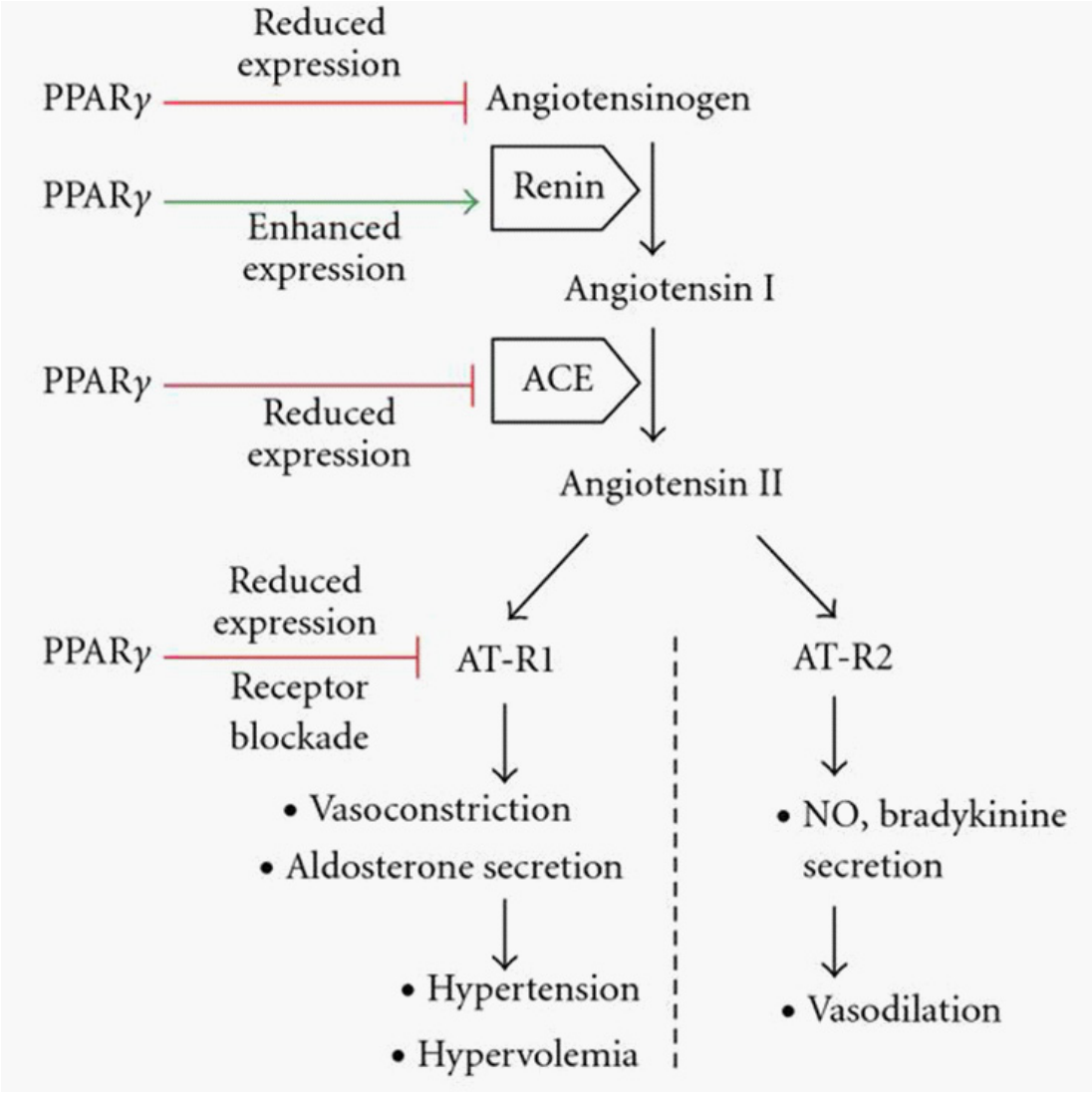

Figure 4: Role of PPAR in the modulation of the RAS cascade [145].

Gene expression of RAS molecules is modulated by PPAR (Figure 4). PPARγ is expressed in the juxtaglomerular apparatus cells. Endogenous and pharmacological PPARγ ligands have been shown to stimulate renin gene expression [145].

Alike the human angiotensinogen promoter is activated by PPAR/RXR heterodimers. Angiotensinogen promoter activity can be enhanced by nuclear receptor signaling via PPAR and RXR [145].

Ligands of PPAR and PPAR can suppress the gene expression of ACE in vascular tissues [208-210]. In streptozotocin-induced diabetes in rats, bezafibrate and pioglitazone can equally protect against the streptozotocin-induced up regulation of ACE in the aortic wall. Similarly, ACE gene expression in obese Zucker rats is reduced by chronic treatment with rosiglitazone [210]. Alike clinical studies have demonstrated that telmisartan inhibits ACE and blocks AT1 receptor, resulting in the vascular protection conferred to hypertensive type 2 diabetic patients by the anti-inflammatory and anti-atherogenic consequences of PPAR activation [211].

Two Angiotensin II receptor blockers, telmisartan and irbesartan, act as selective PPAR modulators [212-214]. Due to its partial PPAR agonist effect, telmisartan inhibits vascular ACE activity [209], AT1 receptor expression [215,216] and increases endothelial NO synthesis [217], preventing oxidative stress and endothelial dysfunction more effectively than non PPAR-agonist Angiotensin II receptor blockers.

PPAR also blocks the action of Ang II by transcription ally repressing AT1 receptor gene expression in VSMCs [212,214,216,218]. In addition to its role as a regulator of vascular tone, AT1 receptor activation contributes to vascular lesions and atherogenesis by promoting VSMC proliferation [219,220]. Therefore, a suppressed Ang II response can potentially slow the progression of atherosclerosis.

As already mentioned, Ang II plays important role in regulation of vascular tone, extracellular fluid volume, Na+ concentration and participates on vascular remodeling. Activation of vascular remodeling involves mechanisms, which are stimulated through AT1 receptor. These mechanisms involve pathological adaptation of vascular wall. This pathological adaptation exhibits changes in contractility, migration, growth and apoptosis of vascular smooth muscle cells and also changes in extracellular matrix storage and inflammatory [207,221].

Increased phosphorylation of signaling pathways participating on growth and differentiation of cells are activated through Ang II, which results in activation of PI3K and ERK 1/2 kinase and kinase cascades [207].

PI3K activation via Ang II starts with phosphorylation of regulatory subunit 85α of PI3K. Subsequently lipide phosphatase SHIP2 (Src homology 2 domain containing inositol 5-phosphatase 2) is activated. PI3K generates phosphatidylinositol (3,4,5)-triphosphate (PIP3). SHIP2 phosphatase hydrolyzes PIP3 on primary lipids, which are needed for activation of Akt kinase [222-224]. Akt kinase is involved on mechanisms of cell survive, growth and vascular remodeling. Gingras et al. and also Kitamura et al. suggest that, although is Akt kinase only kinase of ,,cell survive", because of their antiapoptic effect [225,226], activation of PI3K/Akt cascade play important role in protein synthesis involved in vascular remodeling and hypertrophy

[227,228]. Mechanisms of trough AT1 receptor mediated PI3K- dependent activation of Akt is not yet absolutely understood, but it seems to be important role played redox sensitive signaling pathways and c-Src [229,230]. c-Src is cytoplasmic tyrosine kinase, which is activated especially via NADPH oxidase generated superoxide anions.

Except for PI3K activation Ang II can activate MAPK too. Mitogen-activated protein kinase (**MAPK**) cascades are universal signal transduction modules. In this group, which can be activated through Ang II belong ERK 1/2 (extracellular signalregulatedkinase), JNK (c-Jun N-terminal kinase), p38 etc. [231].

ERK 1/2 participates on regulation of expression of growth and differentially factors. Mechanism of activation of ERK is not yet completely explained. Activation starts through β and γ subunits of G protein and through receptor associated with tyrosine kinase Shc (Srchomologydomain) [232-234] with tyrosine kinase associated receptor stimulates via phosphorylation cascade joined p21ras receptor. This receptor includes adaptor proteins with guanine nucleotide exchange factor. GDP/GTP exchange of p21 ras protein is stimulated through Shc-Grb2-Sos (son-of- seven less) complex. Grb2 (growth factor receptor-bound protein 2) is stimulated by autophosphorylation of tyrosine kinase receptor. Serine c-Raf kinase is linked to plasmatic membrane with p21 ras protein. Serine c-Raf kinase phosphorylates MEK (MAPK/ERKkinase). MEK dually phosphorylates threonine and tyrosine residues in Thr-Glu-Tyr motive, resulting in ERK 1/2 activation (ERK 1: 44-kDa MAPK isoform; ERK 2: 42-kDa MAPK isoform) [235]. ERK 1/2 phosphorylation cascade plays role in regulation of expression of growth and differentially factors. *In vitro* studies suggest, that PPARγ activation can inhibit due to angiotensin induced activation ERK 1/2 [141,218,236,237].

PPARγ and Oxidative Stress

Oxidative stress is defined as disruption of redox homeostasis for benefit of prooxidants, which exceed antioxidant capacity of cell, resulting in potential damage [238]. Reactive oxygen species (**ROS**) are an integral part of several intracellular signaling pathways. ROS produce organism by controlled processes in low concentration [239]. ROS are formed in oxygen metabolism and their major sources are mitochondria and NADPH oxidase (**Nox**). ROS are produced almost every types of cell of vascular system. One of the most dominant producer of ROS in vascular wall are Nox, mitochondrial respiratory chain, uncoupling eNOS, xanthine oxidase, myeloperoxidase, lipoxygenase, cyclooxygenase, cytochrome P450 and heme oxygenase [239].

Members of ROS group are atom, molecule or ions, which consist of one or more unpaired electron and become highly reactive, allowing to attack bio macromolecules such as DNA [240], RNA, proteins, lipids etc. ROS can change gene expression due to nuclear receptors modulation and signaling protein functions modulation through interaction between ROS and cysteine residue or ionized thiolate forms. This change can influence their next protein interactions, allowing to modulate cell functions such as proliferation, differentiation and apoptosis [194].

Cause of oxidative stress is also decrease of ability to eliminate ROS. Decrease of bioavailability of antioxidant mechanisms intensifies oxidative stress and also is involved in oxidative damage associated with pathophysiology of hypertension [241]. Organisms have developed protective antioxidant and detoxifying systems employed in ROS elimination and oxidative stress decrease aim to survive.

Antioxidants are substances, which in low concentration significantly defer or avoid to oxidation of oxidizable substrate. Vascular system contains of several antioxidant systems, including superoxide dismutase (**SOD**), catalase (**CAT**), glutathione system, thioredoxin, peroxiredoxin, ROS scavengers such as vitamin A, C and E [242].

SOD is major antioxidant mechanism, which protects cells against superoxide- mediated cytotoxicity, such as mitochondrial proteins inactivation consist Fe-S center (aconitase, fumarase). SOD catalyzes superoxide anion dismutation on hydrogen peroxide and oxygen. Expression and activation of SOD have great impact on vascular wall response on acute ort chronically oxidative stress [243].

Major cells antioxidant signaling mechanism provides antioxidant system activation, resulting in ROS elimination is signaling pathway Keap1/Nrf2/ARE. This signaling cascade activation reduces ROS and protects cells against via oxidative damage mediated apoptosis. Keap1/Nrf2/ARE activates antioxidant and detoxifying proteins, which consist of antioxidant responsible element (**ARE**) in promoter region of target genes.

Nrf2 (Nuclear factor E2-related factor 2) is transcriptional factor regulated gene expression of antioxidant systems. Nrf2 is member of the cap 'n' collar-basic leucine zipper family of transcription factors. It plays important role in regulation of cytoprotective enzymes. Nrf2 consists of six high conservative protein regions Neh (Nrf2-ECH domains). Leucine zipper is situated on C-terminal end and it is needed for dimerization with small Maf protein (musculoaponeuroticfibrosarcoma virus) and for binding of Nrf2 with ARE. N-terminal end of Nrf2 consists of Neh2 domain and also domains Neh3-5, which are important for transcriptional activity of Nrf2. Neh6 domain has function of degrone. In oxidative stress absence is activity of Nrf2 signalization suppresses by sequestration and degradation of Nrf2 in proteasome cytoplasm after ubiquitination [244].

Keap1 (Kelch-like ECH associated protein) is negatively regulatory protein of Nrf2 activity, which is rich with cysteine residue. Keap1 controls cellular level of Nrf2 protein their activity in presence or absence of oxidative stress. In absence of ROS increase, interacts Nrf2 with Keap1 and subsequently is Nrf2 degraded due to quick induction by proteasome. Keap1 also negatively modulates Nrf2 function with their fixed sub cellular localization of cytoskeleton and avoids to translocation of nrf2 in nucleus in absence of oxidative stress.

In oxidative stress condition Keap1/Nrf2/ARE signalization starts by phosphorylation and/or red ox modification of Nrf2-Keap1 heterodimer complex, allowing their disorganization and

translocation in nucleus [245]. In nucleus Nrf2 binds on cis-activated element ARE and induces expression of protective antioxidant and detoxifying genes consisted ARE motif motive [194,246].

Genes regulated via ARE are directly involved in production of antioxidants (SOD, CAT, HO-1) detoxifying of xenobiotics due to production of detoxifying enzymes of II. Detoxifying phase (GST, NADPH: quinon oxidoreductase 1) and regulation of glutathione production (e.g. glutamate-cysteine ligase) [247]. Keap1/Nrf2/ARE is complex system maintaining redox homeostasis in normal or stress conditions [248].

Disruption of redox homeostasis and pathophysiological ROS level leads to series of damages effects participate on cardiovascular diseases development e.g. hypertension, hart failure, atherosclerosis etc.

Hypertension is multi factorial, complex disease, which involves damage of several organ systems. Factors participating on hypertension development involve SNS activation, RAS up-regulation, changed with G protein associated signalization and inflammatory [240,249]. A common factor of these processes is currently oxidative stress. In kidneys ROS increases production and secretion of vaso active agents, including Ang II and aldosterone, thereby affecting the function of heart, vessels and kidneys. ROS affect processes participate on vascular remodeling, endothelial dysfunction and increase vascular contractility, growth and proliferation of vascular smooth muscle cells, monocyte invasion, lipid per oxidation, increase protein storage in extracellular matrix, fibrosis and inflammatory, what are the signs of hypertension. Moreover, ROS increase levels in heart was associated with increasing of vascular contractility, fibrosis and with heart remodeling, manifested by cardiac output increase and BP increase [250].

With hypertension is associated in CNS (central nervous system) increased ROS production leads to a release of neurotransmitters, affected vascular reactivity and BP rise. Overproduction of ROS in CNS increases activation of sympathetic nervous system (**SNS)** in periphery vessels, thus are ROS involved in pathogenesis of hypertension [40,251]. Several studies suggest, that through Nox produced ROS affect Ang II mediated influx of Ca^{2+} in NTS (nucleus tractussolitaries) [252]. Further, up-regulation of AT1 receptor expression, almost in RVLM (rostral ventrolateral medulla) can modulate excitatory or inhibitory neurons and their postsynaptic activity. AT1 receptors are localized on glutamatergic neurons in RVLM [253]. Presynaptic activation of AT1 receptors in NTS can facilitate glutamatergic transmission [254]. Ang II-induced depolarization of sympathetic promoter neurons in RVLM persists also after synaptic transmission blocking [255]. AT1 receptors mediate excitatory response through postsynaptic receptors, allowing increased activity of SNS and also allowing increased BP [256].

Moreover, overproduction of ROS in kidneys causes increased production and secretion of Ang II. Gene expression of angiotensinogen in proximal tubules can be mediated trough ROS-dependent activation of p38 MAPK [257,258]. Also, overproduction of ROS increases production of Ang II and Ang II-mediated renal vasoconstriction (259), and renin release [260], renal nervous

activity, contraction of afferent arterioles, increases renal perfuse pressure and activation of receptors for Ang II II [261], ET-1 or thromboxane [252] with these vasoactive substances can be subsequently influent functions of heart, vascular system and kidneys.

TZD treatment decreases ROS production in vascular smooth muscle cells [262,263] and endothelial cells [262,264] allowing BP decrease [128]. Suggests that rosiglitazone administration can have central antihypertensive effect. With respect to the central role of oxidative stress in neural mechanisms of hypertension development [251,265] antihypertensive effect of RSG treatment can be mediated PPARγ-dependent transcriptional up regulation of antioxidant mechanisms in RVLM [128].

PPARγ can directly modulate expression of antioxidant and prooxidant genes and so modulate cellular response on oxidative stress [126]. Expression of CAT, which consists of PPRE in promoter region is trancriptionaly regulated through PPARγ [266]. PPARγ also increases expression of mitochondrial isoform of SOD **(SOD2**) [267], Gpx3 [268] and conversely decreases expression of cyclooxygenase 2 [269,270]. PPARγ protects from due to oxidative stress induced apoptosis of cardiomyocytes and glia cells by induction of transcription of Bcl-2 (B-Cell CLL/Lymphoma 2) [271].

In humans' vessels after TZD administration is induced expression of HO-1 in presence of oxidative stress and high glucose level [272]. Polvani et al. in their study points out, that nuclear receptor PPARγ maintains redox homeostasis by means of interaction with other signaling pathways, such as nuclear factor Nrf2, NF-κB, Wnt/β-catenin, FOXO proteins etc [143] (Figure 5).

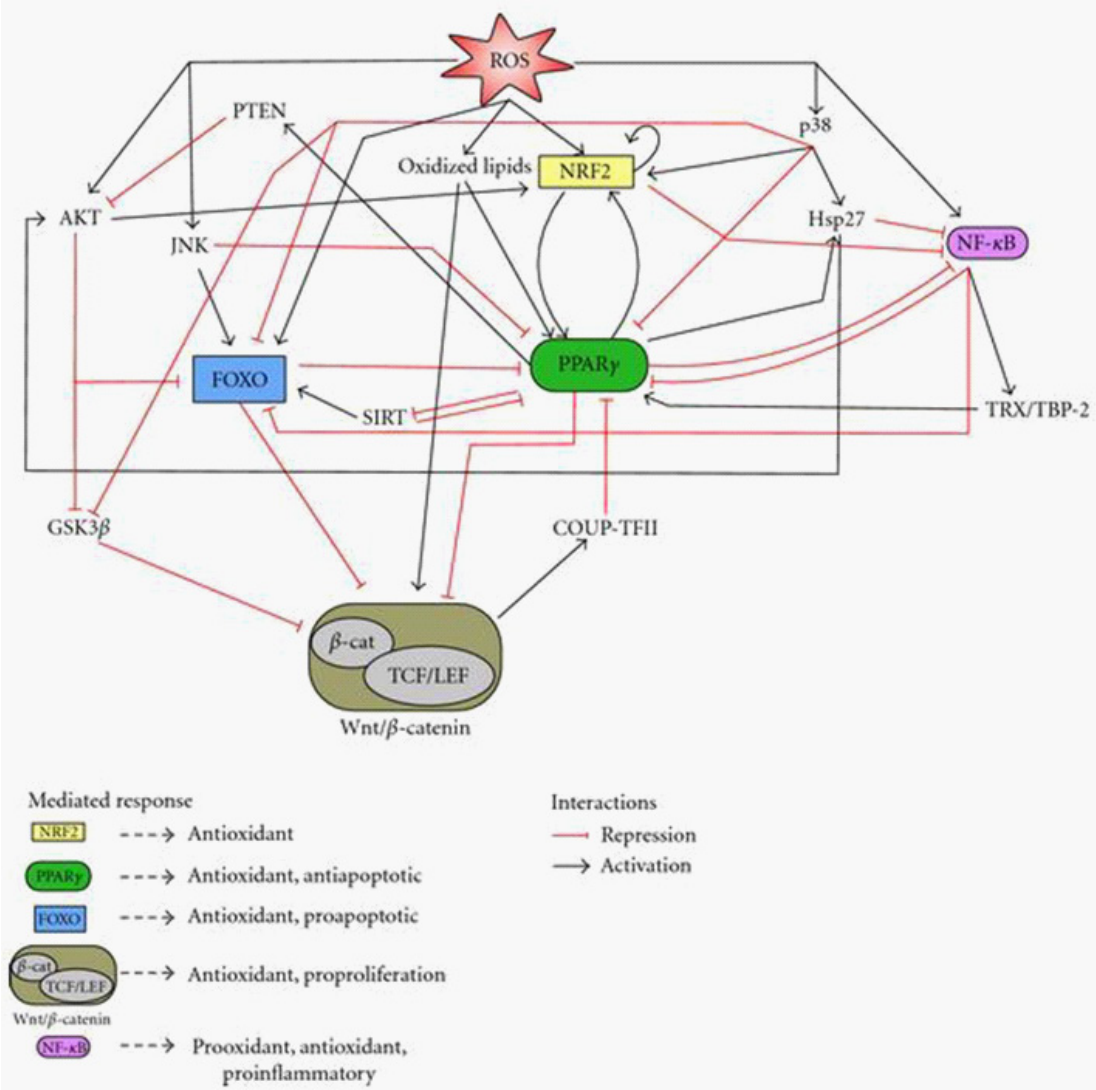

Figure 5: Crosstalk of PPARγ with other signaling pathways, such as nuclear factor Nrf2, NF-κB, Wnt/β-catenin, FOXO proteins in oxidative stress response [143].

Reactive oxygen species (**ROS**) are main contributors on oxidative stress in cells during physiological and pathological processes. These free radicals (eg. superoxide anions, hydroxyl radical, hydrogen peroxide and other) are capable react with other molecules and lead to oxidative damage (oxidative stress) of cellular and sub cellular components such as lipids, proteins and DNA [273]. Several mechanisms or pathways are responsible for the production of free radicals in cells. The main enzyme ROS sources include mitochondrial respiratory chain, nicotine amide phosphate adeninedinucleotide (**NADPH**) oxidases, xanthine oxidase and uncoupled NO syntheses [273-275].

Nrf2-ARE signaling pathway represents major mechanism of cellular protection against oxidative and electrophilic stress. This pathway controls gene expression, which protein products induce detoxification and eliminate reactive oxidants and electrophilic products through conjugation reactions and by increasing the antioxidant capacity of cells [248]. Nrf2 is ubiquitous transcription factor and regulator of homeostasis intracellular stress [276]. Nrf2 combined with Maf proteins creates heterodimers and trans activate antioxidant response elements (**ARE**). ARE is present within regulatory areas of several cytoprotective target genes. The genes regulated through the ARE are involved in the production of antioxidants (e.g. heme oxigenase-1) and production of glutathione (e.g. γ- glutamatecystein ligase), directly inactivate ROS (e.g. catalase, superoxide dismutase) or detoxify toxic xenobiotics (e.g. glutathione-S-transferase, NAD(P)H: quinone oxidoreductase 1) [277,278]. In various cells, Nrf2 is present in low concentrations due to continuous Nrf2 degradation through proteasome pathway [279]. Activity of Nrf2 is negatively regulated by protein Keap1 containing a large number of cysteine residues in its primary structure. Keap1 has two important functions related to the regulation of Nrf2. Keap1 isolate Nrf2 in cytoplasm [280] and ensures ubiquitin conjugation and subsequent degradation Nrf2 in proteasome [281-283]. During oxidative stress, oxidized molecules modify several cysteine residues on Keap1 and Nrf2 can be released from connection with Keap1. After the release, Nrf2 is translocated to the nucleus where induce transcription of genes by ARE [284,285]. Mechanisms for switching off Nrf2 activity follow-up after activation of Nrf2. GSK3β ("glycogen synthase kinase 3β") phosphorylates Fyn leading to the relocation of Fyn into the nucleus [286]. Fyn phosphorylates Nrf2 resulting export transcription factor from the nucleus and binding to Keap1 with subsequent degradation of Nrf2 [287]. Activation Nrf2 can be mediated also by different proteikinase pathways through phosphorylation [248]. These proteinkinase pathways represent: protein kinase C (**PKC**), phosphatidylinositol3 kinase/Akt (**PI3/Akt**), MAP kinase (ERK, p38 MAPK) and casein kinase-2 [288-290]. The activity of these kinases regulates the ability and localization of Nrf2 protein through phosphorylation serine or threonine residues depending on the cell type [278,291].

There exists also interaction between PPAR gamma and Nrf2 transcription factor in cells and can involved more mechanisms. The Peroxisome-proliferator activator receptors (**PPARs**) are ligand-activated nuclear receptor transcription factors that regulate the function and expression of complex gene networks. PPARγ creates heterodimer with the retinoid X reecptor (**RXR**) and together regulate gene transcription [14,292]. As dimers, PPARγ: RXR bind to PPAR response elements (**PPREs**) located in the promoter region of target genes. PPARγ may directly modulate the expression of several antioxidant and prooxidant genes in response to oxidative stress. It was observed, that ligand-activated PPARγ promotes the expression of manganese SOD (**MnSOD**) [293], GPx3 [268], the scavenger receptor CD36 [294], HO-1 [295], and the mitochondrial uncoupling protein 2 (**UCP2**) [128], whereas it down regulates COX- 2 and iNOS [143,270,296].

One mechanism of action PPAR gamma represents coexistence of PPREs and ARE in the same genes. The expression of scavenger receptor CD36 (mediates the recognition and internalization of oxidized lipids (61)), antioxidant enzymes catalase, HO-1 and mitochondrial MnSOD are transcriptional regulated through PPREs [266,293-295].

Reciprocal transcriptional regulation between genes of Nrf2 and PPAR gamma is another possible mechanism of interaction both genes. Nrf2 gene contain putative PPREs and counter PPAR gamma gene contain ARE [297,298]. Experiments with Nrf2 null mice show that expression of PPAR gamma is reduced due to direct effect of the lack of Nrf2. Nrf2 induces PPAR gamma expression binding to at least two ARE sequences in the upstream promoter region of the nuclear receptor [298,299]. Furthermore, Nrf2 expression is weakened in mice with decreased PPAR gamma [297]. There probably exist positive feedback loop among PPAR gamma and Nrf2, where PPAR gamma may act directly or through upstream pathway for Nrf2 activation [143]. In addition, nuclear receptor PPAR gamma and transcription factor Nrf2 may also act synergic ally in the activation of antioxidant genes [294,297]. ARE and PPRE response element coexist in GSTA2 promoter. GSTA2 gene encodes detoxification enzyme glutathione S-transferees A2, where ARE site is important in the trans activation of the GSTA2 gene by PPARγ and RXR ligands for the full gene trans activation [297]. Similar expression of CD36 is dependent on both proteins, but transcription of CD36 may be induced independent by Nrf2 or PPAR gamma, depending on the cellular context [294].

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