Peroxisome proliferator activated receptor-γ and the ubiquitin-proteasome system in colorectal cancer

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Abstract

Peroxisome proliferator activated receptor-γ (PPARγ), a transcription factor of the nuclear receptor superfamily, plays a significant role in colorectal cancer pathogenesis. In most experimental systems PPARγ activation has tumor suppressing effects in the colon. PPARγ is regulated at multiple levels by the ubiquitin-proteasome system (UPS). At a first level, UPS regulates PPARγ transcription. This regulation involves both PPARγ transcription specific factors and the general transcription machinery. At a second level UPS regulates PPARγ and its co-factors themselves, as PPARγ and many co-factors are proteasome substrates. At a third level of regulation, transcription pathways working in parallel but also having inter-relations with PPARγ are regulated by the UPS, creating a network of regulation in the colorectal carcinogenesis-related pathways that are under UPS control. Activation of PPARγ transcription by direct pharmacologic activators and by stabilization of its molecule by proteasome inhibitors could be strategies to be exploited in colorectal cancer treatment.

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Key words: Peroxisome proliferator activated receptor-γ; Ubiquitin; Proteasome; Colorectal cancer; Carcinogenesis

INTRODUCTION

Peroxisome proliferator activated receptor γ (PPARγ) is a transcription factor of the nuclear hormone receptor superfamily. It has an important role in adipose tissue and in adipogenesis but is also expressed at high levels in colonic epithelium. PPARγ is involved in colorectal carcinogenesis and PPARγ-dependent transcription has anti-carcinogenic effects in many experimental colorectal cancer models while in some other instances PPARγ displays cancer promoting effects.

The ubiquitin proteasome system (UPS) is a cellular regulatory machinery that leads to the degradation of multiple proteins as a mode of permanent down-regulation. Virtually all cellular processes are regulated by the UPS including processes involved in carcinogenesis such as cell cycle, apoptosis, signal transduction and DNA transcription. Included in UPS-regulated processes are signal transduction effecting PPARγ activation and PPARγ-dependent transcription. PPARγ regulation by UPS as it pertains to colorectal cancer pathogenesis will be the subject of this editorial review.

PPARγ AND PPARγ-DEPENDENT TRANSCRIPTION

PPARγ together with two other PPARs (PPARα and PPARβ/δ) belongs to the orphan ligand sub-family of the nuclear hormone receptor super-family. Human PPARγ...
gene is located at chromosome 3p25[8]. Higher levels of protein expression are displayed in adipose tissue and colonic epithelium but many other tissues such as pancreatic β cells and vascular endothelium also express the transcription factor[8].

PPARγ molecule has a domain organization similar to all nuclear receptors. In the amino-terminal part of the protein there is a transcription regulation domain, followed by a DNA binding domain and a hinge region, while the carboxy-terminal part of the molecule is occupied by the ligand binding domain.

Various lipids such as prostaglandin 15d-PGJ2, arachidonic acid and eicosapentaenoic acid have been found to represent natural PPARγ ligands but the optimal ligand in different physiologic conditions in vivo is not well established[9,10]. Synthetic ligands of PPARγ have also been described, the most prominent of which are thiazaolinediones, a family of drugs that are used in the clinic as anti-diabetics[11]. After ligand binding, PPARγ undergoes a conformational modification, binds to co-activators and enters the nucleus to proliferosexiferator response elements (PPREs) on target gene promoters. With the aid of co-activators such as PGC-1α (PPARγ Co-activator-1α) and Hic-5 (Hydrogen peroxide induced clone 5, alternatively named ARA55- androgen receptor activator of 55 kDa), histone acetyltransferases are recruited and acetylate histones around the transcription initiation site and pave the way for RNA polymerase to join the complex[8,11]. Among genes induced by PPARγ are, in addition to lipid metabolism regulating genes, cell cycle inhibitors p18, p21 and p27 and phosphatase inhibitor of akt kinase, PTEN[12-14]. Furthermore PPARγ acts as a suppressor of cell cycle promoters such as cyclin D and phosphatase PP2A[15,16]. The addition of these transcriptional inductions and repressions implies that PPARγ activity would have an anti-proliferative and anti-carcinogenic effect in tissues that display significant PPARγ expression such as colorectal epithelium. Indeed such an effect has been observed in most, although not all, colorectal cancer experimental systems as will be discussed in a later section.

UPS

Ubiquitination, that is the covalent attachment of the protein ubiquitin to a target protein, is a post-translational modification that can result in diverse outcomes. Ubiquitin is a 76 aminoacid protein that contains several lysine residues through which different types of chains can be formed[13]. A chain of at least four ubiquitin molecules attached to each other through a covalent link between lysine 48 of one molecule and the carboxyterminal glycine of the next molecule and finally attached to a target protein identifies this protein for proteasome degradation. Other types of ubiquitin attachments such as attachment of a single ubiquitin molecule to a target protein (mono-ubiquitination) or attachment of a ubiquitin chain to other lysine residues (e.g. Lys68) result in different outcomes and play roles in DNA repair, endocytosis, histone regulation and nuclear export. Attachment of ubiquitin to the target requires the action of three enzymes. Initially an enzyme called E1 or ubiquitin-activating enzyme binds ubiquitin and transfers it to a second enzyme called E2 or ubiquitin conjugating enzyme using energy from the degradation of ATP to ADP. Finally, a third enzyme called ubiquitin ligase or E3 transfers ubiquitin from E2 to the target protein[17].

After attachment of at least four ubiquitin molecules, the target protein is recognized by specific sub-units of 19S regulatory particle (RP) of the proteasome. 19S RP is a multi-protein structure that caps the two sides of the core particle (CP) of the proteasome, a cylinder shaped multi-unit structure with a hollow central chamber[18]. Inside this chamber takes place the enzymatic degradation of target proteins executed by three enzymatic activity-possessing subunits of the CP. CP consists of four seven-member rings that are stacked one on the other. The two peripheral rings are similar to each other and are called α rings and the two central rings are also similar to each other and are called β rings. Three of the seven sub-units of the β rings, β1, β2 and β5 possess the enzymatic activities of the proteasome; trypsin-like activity, chymotrypsin-like activity and post-glutamyl (caspase-like) activity respectively.

PPARγ IN COLORECTAL CARCINOGENESIS

Given the high expression level of PPARγ in colorectal epithelium, there is a particular interest in defining the role of PPARγ transcription in colorectal cancer. Activation of PPARγ after exposure of colorectal cancer cell lines to the natural ligand 15-S-hydroxy-eicosatetraenoic acid (15S-HETE) and to thiazolidinedione synthetic ligands leads to growth arrest and induction of apoptosis[19-22]. Re-induction of differentiation related genes suppressed in colorectal cancer, such as cytokeratins 18 and 19 and intestinal alkaline phosphatase, is also noticed after activation of PPARγ in colorectal cancer cells[20,23]. In contrast, genes that are induced in cancer such as polyamine metabolism enzyme ornithine decarboxylase and metastasis promoting protein laminin binding protein are repressed after PPARγ activation[24].

Mice bearing human colorectal cancer xenografts display decreased rate of tumor growth after feeding with PPARγ ligands[25]. Rats in which preneoplastic colon lesions, aberrant crypt foci, have been induced by exposure to the chemical azoxymethane, also display decreased lesion formation after PPARγ activation[26,27]. A third in vivo model of PPARγ activation using Min mice, a strain of mice with activation of adenomatous polyposis coli (APC) gene due to germline mutation, has given controversial results with some studies observing increased colonic polys formation after PPARγ activation[26,27] while others have observed decreased tumor formation[28]. Another mouse strain with APC inactivation, Apc mice, also displayed decreased poly formation after PPARγ activation[29]. Overall, in vivo models support a suppressive role of PPARγ in colorectal carcinogenesis[30]. Controversial
results with strains bearing disabled APC may relate to the fact that APC regulates the β-catenin transcription program and different residual β-catenin activity may have diverse roles in carcinogenesis\[9]. This residual activity is further modulated by increased PPARγ activity as will be discussed later.

Induction of ed3 inhibitors p21, p18 and p27 by PPARγ is involved in cell cycle arrest following PPARγ activation\[12,32] while interaction of PPARγ with Rb further promotes cell cycle arrest by recruiting histone deacetylase 3 and silencing transcription\[33]. Phosphatase PTEF is a target of the PPARγ transcription program and its induction inhibits akt kinase activation, promoting apoptosis\[34].

Expression of PPARγ has been shown in a significant percentage of tumor specimens from colorectal cancer patients\[35,36] and has been co-related with improved prognosis in these patients\[37]. Compared with adjacent normal colonic epithelium, PPARγ in tumor tissues displays decreased expression, further supporting a role of PPARγ suppression in colorectal carcinogenesis\[37]. In contrast, another study reported that PPARγ mRNA levels were increased in colorectal tumors compared with adjacent normal colon\[38]. This discrepancy may imply that PPARγ down-regulation in colon cancer is effected at the post-translational level.

**PPARγ REGULATION BY THE UPS**

Post-translational regulation of PPARγ involves its degradation by the UPS (Table 1)\[39]. This is an event that follows ligand binding and nuclear receptor transcription activation and represents a negative feed-back regulation, a common theme in most nuclear receptors. This mechanism has been shown to regulate PPARγ heterodimeric partner RXR as well as retinoic acid receptor, estrogen receptor, progesterone receptor and androgen receptor\[40-42]. Interestingly the two other PPAR family members PPARα and PPARβ/δ, although structurally similar to PPARγ, undergo a reverse regulation upon ligand binding and display delayed ubiquitination and proteasome degradation\[43,44]. PPARγ degradation is dependent on ligand binding but independent of transcription per se.

PPARγ co-activator PGC-1α is also regulated by the UPS\[45]. PGC-1α ubiquitination through a domain at the C-terminal part of the molecule leads to proteasome degradation. This helps in maintaining an optimal level of the co-activator for facilitation of transcription of PPARγ and also of other transcription factors for which PGC-1α functions as a co-activator such as PPARα, PPARβ/δ and estrogen related receptor α (ERRα)\[46]. Proteasome inhibition leads to ubiquitinated PGC-1α accumulation and formation of non-functional aggregates. Thus, the UPS function maintains the optimal levels of PGC-1α in order to perform its co-activator function\[47]. PGC-1α is additionally regulated at the transcriptional level by both PPARγ and ERRα, creating a regulation loop\[48,49].

PPARγ transcriptional activity is regulated through phosphorylation by MAP kinases ERK and JNK\[49,50]. Phosphorylation suppresses PPARγ activity in most cases but there are instances, such as the insulin-induced mitogen activated protein kinase (MAPK) phosphorylation of PPARγ, where this phosphorylation induces PPARγ transcriptional activity\[51,52]. Given that MAP kinases and other proteins upstream in their activation cascade, such as EGFR and akt kinase, are proteasome substrates\[53-56], this represents an additional point of regulation of PPARγ transcriptional activity by UPS.

**PPARγ INTERACTION WITH OTHER SIGNALLING PATHWAYS AND REGULATION BY THE UPS**

PPARγ transcription factor interacts with several other factors and pathways in colorectal cancer and the final output is defined by a network of interactions in which the ubiquitin-proteasome system participates at multiple levels. Some of these interactions are outlined in the following paragraphs.

**β-catenin**

Abundance of β-catenin, a transcription factor activated in most human colorectal cancers, is regulated by at least three parallel pathways that culminate in its ubiquitination and proteasome degradation. The first pathway involves phosphorylation by glycogen synthase kinase 3β (GSK3β), facilitated by a complex in which APC takes part and results in ubiquitination by ubiquitin ligase TrCP\[57,58]. In the second pathway, ubiquitination of β-catenin is performed by ubiquitin ligase Siah-1 which is induced by p53 activation\[59,60]. In a third pathway, a currently unknown ubiquitin ligase mediates PPARγ-activated ubiquitination of β-catenin\[61]. Tumorigenic β-catenin displays enhanced transcription activity due to mutation of serine to alanine at position 37 (S37A) rendering it resistant to GSK3β/ APC-mediated phosphorylation and ubiquitination/ proteasome degradation. This molecule can interact with PPARγ and be ubiquitinated through an alternative mechanism in order to be degraded by the proteasome\[62]. In this way, PPARγ suppresses β-catenin activity through enhanced proteasome-mediated degradation which possibly is a result of induction of molecules involved in the ubiquitination process\[63]. Conversely when stabilized, β-catenin inhibits PPARγ activity, an effect requiring direct interaction of the two molecules. This interaction involves the TCF binding domain of β-catenin and a catenin binding domain in

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**Table 1**  PPARγ regulation by the ubiquitin-proteasome system

| Degradation of PPARγ itself | Degradation of co-activators | Degradation of inhibiting kinases | Regulation of antagonistic transcription factors (β-catenin, NFκB) | Degradation of calcineurin inhibitor DSCR1 (RCAN1) |
|----------------------------|-----------------------------|---------------------------------|---------------------------------------------------------------|----------------------------------------------------|

PPARγ: Peroxisome proliferator activated receptor-γ.  

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PPARγ that is close to the ligand binding domain of the molecule[63]. Another study, although confirming the interaction of β-catenin with PPARγ in colorectal cancer cells, found transactivation and not repression of PPARγ activity in putative PPREs[63]. Nevertheless this study used a highly artificial system in which both genes were transfected in colon cancer cell lines before co-immunoprecipitation and reporter gene experiments[81]. Their physiologic relevance is debatable in view of the already mentioned evidence for a reverse interaction. Furthermore, in adipocytes there is a mutual antagonistic relationship between the wnt/β-catenin signalling pathway and PPARγ[84-86], strongly supporting an analogous relationship in colorectal tissue. A role of the UPS in this interaction is implied by the fact that both transcription factors are regulated by the system, although the exact regulation mechanism of the interaction must be complex.

NF-κB
Another pathway interacting with both PPARγ and with β-catenin signalling and that is regulated by the UPS is the one culminating in the activation of transcription factors of the NF-κB family. NF-κB is activated in colorectal cancer as it is down-stream of activated K-ras. NF-κB has proliferative and anti-apoptotic effects and is activated in many cancers. Its main regulation is effectuated by phosphorylation and ubiquitination of its inhibitor I-κB. This is then degraded in the proteasome, releasing NF-κB in order to enter the nucleus and begin its transcription program. Additional regulation of NF-κB results from direct interactions with PPARγ and β-catenin which both result in inhibition of NF-κB transcription[67-69]. PPARγ also trans-represses NF-κB-regulated genes indirectly through binding to promoters and recruiting co-repressors. For this action, SUMOylation [i.e. Small ubiquitin-related modifier (SUMO) binding] of PPARγ is required[87]. Metastasis mediating chemokine receptor CXCR4 is regulated at the transcription level by NF-κB and PPARγ in a reciprocal way. Thus, the interaction has the potential to regulate metastasis of colorectal cancer cells, NF-κB being a promoter while PPARγ being a suppressor of colorectal cancer metastatic process[88].

Given that all three transcription factors play significant roles in colorectal cancer and all three are regulated by the UPS, it is evident that a complex interaction with UPS is central in determining the final proliferation outcome of the neoplastic cell. NF-κB is also of paramount importance in inflammation and inflammation-induced carcinogenesis. Nuclear receptors in general and PPARγ in particular can antagonize this action, having anti-inflammatory effects[79].

Calcium signaling
PPARγ activation has also been found to inhibit proliferation and migration of colorectal cancer cell lines by interference with calcium signalling[80]. PPARγ induces calcineurin inhibitor DSCR1 (Down syndrome candidate region 1, also known as RCAN1- regulator of calcineurin 1 and calcipressin). This induction of the endogenous inhibitor of phosphatase calcineurin results in the maintenance of transcription factor NFAT (Nuclear factor of activated T cells) in a phosphorylated and inactive form[74]. DSCR1 was initially recognized as a protein playing a role in the pathogenesis of Down syndrome[75] and is now recognized as playing a role in other pathologies such as Alzheimer’s disease, stroke and cardiac hypertrophy[76]. More recently its role in carcinogenesis has been described[76]. DSCR1 is a proteasome substrate and its proteasome degradation is enhanced through the action of protein CREB (c-AMP response element-binding protein)[83]. PPARγ may decrease this enhanced degradation by interfering with CREB, RNA-mediated knockdown of DSCR1 results in inability of PPARγ to inhibit colorectal cancer cell proliferation and invasion[89], indicating the importance of this protein and of inhibiting calcium signalling in mediating PPARγ anti-neoplastic effects. PPARγ involvement in calcium signalling regulation is of particular relevance in colorectal cancer given the extensive cross-talk of calcium with the K-ras oncogene[78].

Transforming growth factor β
Transforming growth factor β (TGF-β) is a ligand that initiates signal transduction after ligation of its cell surface receptors TβR I and TβR II. This ligation leads to activation of intracellular mediators Smad2 and Smad3 which heterodimerize with partner protein Smad4 [also called deleted in pancreatic cancer 4 (DPC4)] and enter the nucleus to activate transcription in collaboration with various other transcription factors[70,71]. TGF-β signalling is regulated by the UPS, as Smads are proteasome substrates for degradation after ubiquitination[71-73]. TGF-β signalling can have pro-carcinogenic or anti-carcinogenic effect depending on which other pathways are activated in a particular cell environment[86]. In particular, MAPK activation downstream of activated K-ras oncogene can direct TGF-β signalling towards cancer-promotion. This is of great importance in colorectal cancers as they bear an activating K-ras mutation in up to half the cases. PPARγ suppresses TGF-β1 production through a mechanism involving inhibition of kinase p70 Ribosomal S6 kinase-1. This inhibition keeps transcription factor Zine finger 9 in an inactive form that prevents it from transcribing TGF-β1 gene[86]. As a result TGF-β1-dependent induction of chemokines Interleukin-8 and monocyte chemoattractant protein 1 is prevented[86]. An additional point where PPARγ and TGF-β signal transduction inter-connect is their common induction of transcription factor TSC-22 (Transforming growth factor-stimulated clone 22). This is a zinc finger transcription factor that causes growth inhibition in colonocytes through induction of CDK inhibitor p21[85].

CONCLUSION
Colorectal carcinogenesis involves the acquisition of genetic lesions over time, leading to progression from hyperplasia to adenoma to carcinoma. Most common genetic lesions include APC mutations, activating β-catenin transcription, K-ras mutations and Smad4 (DPC4) mutations. All these
pathways and others involved in colorectal carcinogenesis are regulated by the UPS.

PPARγ is a nuclear receptor transcription factor with important roles in colorectal carcinogenesis. As is the case with several transcription factors of the nuclear receptor super-family, it is regulated by the ubiquitin-proteasome system. This system modulates PPARγ action not only by directly degrading the transcription factor itself but also through other transcription factors and other proteins working in parallel as a network. These multiple levels of regulation will have to be taken into account, using the new tools of molecular biology such as whole genome interrogations, when designing new rational targeted therapies and combinations. Both PPARγ and the UPS have been manipulated pharmacologically in the clinic, the former with the use (for the treatment of diabetes) of activators thiazolidinediones and the latter with the use of the proteasome inhibitor bortezomib. A combined use in order to activate the transcription factor in at least two levels could be attractive for the development, of drugs for the treatment of colorectal cancer. Determination of subsets of colorectal cancers that, due to specific molecular lesions, could be particularly sensitive to PPARγ activation would be of importance in this development.

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