Journal of
Cancer Science & Therapy

Tsukahara et al., J Cancer Sci Ther 2013, 5.6
DOI: 10.4172/1948-5956.1000208

Review Article Open Access

New Insights into the PTB-Associated Splicing Factor (PSF) and Peroxisome Proliferator-Activated Receptor gamma (PPARγ) in Colorectal Carcinogenesis

Tamotsu Tsukahara[1], Yoshikazu Matsuda[2] and Hisao Haniu[3]*

[1]*Department of Molecular and Cellular Physiology, Shinshu University School of Medicine, 3-1-1 Asahi, Matsumoto, Nagano 390-8621, Japan*
[2]*Clinical Pharmacology Educational Center, Nihon Pharmaceutical University, Ina-machi, Saitama, 362-0806, Japan*
[3]*Department of Orthopaedic Surgery, Shinshu University School of Medicine, 3-1-1 Asahi, Matsumoto, Nagano 390-8621, Japan*

Abstract

PTB-associated Splicing Factor (PSF) is a multifunctional protein involved in transcription repression, pre-mRNA processing, and DNA repair. Transcriptional repression by PSF is mediated by its interaction with nuclear hormone receptors. However, the physiological context of PSF in regard to nuclear receptor signaling is still unclear. In this review, our recent study identified PSF as a novel PPARγ-interacting protein and demonstrated that PSF is involved in several important regulatory steps of colon cancer cell proliferation. Recently, the aberrant expression of PSF in tumor cells has been implicated in resistance to drugs that are commonly used in cancer therapy. Therefore, it might be possible to develop and to optimize drugs that inhibit PSF and correct abnormal splicing, resulting in enhanced tumor cell migration, invasion, and proliferation. In this review, we summarize current understanding of the mechanisms underlying the PSF-PPARγ axis and its role in the control of colorectal carcinogenesis.

An Overview of PSF and PPARγ

PTB-associated splicing factor (PSF), one of the major nuclear proteins [1] in eukaryotic cells, consists of a single polypeptide chain of 76 kDa (Figure 1A). PSF is highly basic because the amino terminus is rich in proline and glutamine residues [2]. Notably, the amino acid composition of the entire ORF comprises 16% proline and 7% glutamine residues. A recent study revealed that PSF belongs to a family of putative tumor-suppressor proteins [3] that contain an RNA-binding domain (RBD) and a DNA-binding domain (DBD). The NH_2-terminal region of PSF was shown to bind response sequences for Retinoid X Receptor (RXR) [4]. Members of the type II nuclear hormone receptor subfamily (including PPARγ) bind to their response sequences with or without ligand [5]. In the absence of ligand, the DNA-bound receptors repress gene expression to different degrees [6]. Thus, PSF is a highly complex protein that may be an important component of the transcriptional repression of many different genes via different mechanisms. Recently, Wang et al. reported that PSF plays a central role in the reversible regulation of mammalian cell proliferation and tumorigenesis [7]. Alteration in the expression of PSF and its binding partners therefore has potential as a therapeutic strategy against cancer [3,8].

PPARγ is a nuclear receptor that plays an essential role in lipid and glucose homeostasis [9,10]. PPARγ is comprises four functional parts (Figure 1B): the N-terminal A/B region, which contains a ligand-independent transcription-activating motif, AF-1; the C region binds response elements; the D region, which binds to various transcription cofactors; and the E/F region, which has an interface for dimerizing with retinoid X receptor α (RXRα), an AF-2 ligand-dependent transcription-activating motif, and a Ligand-Binding Domain (LBD) [11]. Recent reports indicate that PPARγ is over-expressed in many types of cancer, including colon, lung, breast, ovary, and stomach cancer, suggesting that regulation of PPARγ affects cancer pathogenesis [9,12-14]. While previous studies have demonstrated that some PPARγ agonists inhibit the growth of cancer cells, many reports show that PPARγ ligand-mediated growth inhibition varies depending on the cancer cell type. In colon cancer cells, the growth-suppressing effect of PPARγ ligands evident in *in vitro* studies was not clearly confirmed by *in vivo* studies. For example, PPARγ deficiency enhanced colon tumorigenesis in $APC^{+/Min}$ mice, while another study reported that a synthetic PPARγ agonist, rosiglitazone, induced colon carcinogenesis [15]. The discrepancy in results may be due, in part, to PPARγ-dependent and -independent pathways [16].

PSF Directly Interacts with PPARγ

Many biological functions involve the formation of protein-protein complexes. Our recent study using the HT-29 colon cancer cell line showed that PSF directly interacts with PPARγ [17]. Following affinity purification, tagged PPARγ was separated using SDS-PAGE. The identities of the proteins in the various bands were then determined using matrix-assisted laser desorption/ionization (MALDI)-time-of-flight (TOF) MS. This strategy identified several candidate proteins that might interact functionally with PPARγ [17]. One of the candidates, PSF, is most likely a PPARγ-interacting protein. Furthermore, we demonstrated that the mammalian two-hybrid assay is a very powerful technique with which to study pair-wise interactions between proteins [18]. The assay utilizes two GAL4-based transcription activation domains of *Saccharomyces cerevisiae*: the DNA-binding domain and the transcription activation domain of the herpes simplex virus VP16 protein [19]. We demonstrated the use of a PSF-PPARγ mammalian two-hybrid system to study protein-protein interactions. The interaction of chimeric Gal4-PSF deletion mutants with VP16-PPARγ was assessed using a mammalian two-hybrid reporter gene assay. Loss

***Corresponding author:** Tamotsu Tsukahara, Department of Molecular and Cellular Physiology, Shinshu University School of Medicine, 3-1-1 Asahi, Matsumoto, Nagano 390-8621, Japan, E-mail: ttamotsu@shinshu-u.ac.jp

Received May 02, 2013; **Accepted** May 22, 2013; **Published** May 25, 2013

Citation: Tsukahara T, Matsuda Y, Haniu H (2013) New Insights into the PTB-Associated Splicing Factor (PSF) and Peroxisome Proliferator-Activated Receptor gamma (PPARγ) in Colorectal Carcinogenesis. J Cancer Sci Ther 5: 209-211. doi:10.4172/1948-5956.1000208

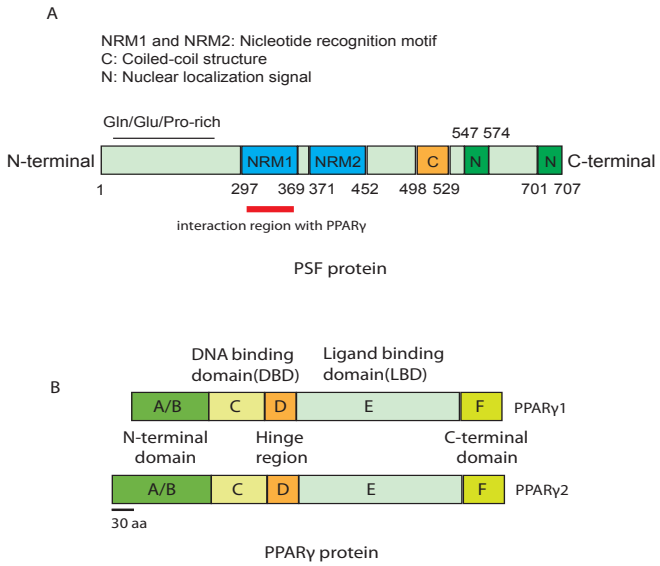

Figure 1: A, Domain structures of PSF. The PSF protein (707 amino acids) contains two predicted RNA binding domains (RRM1 and RRM2), a proline-rich and glutamine-rich amino terminal domain, and two carboxy terminal nuclear localization signals. The first central region of the nucleotide-binding domain (NRM1) is required for the interaction with PPARγ. B, Structure of PPARγ$_1$ and PPAR γ$_2$. Human PPARγ$_1$ and PPAR γ$_2$ proteins are 53 and 57 kDa, respectively, based on the deduced amino acid sequences. The two PPARγ isoforms differ only 30 amino acids at the N-terminal end. These added amino acids on PPARγ$_2$ result in increased adipose-selectivity.

of amino acids 1–290 of PSF had no effect on the interaction. Thus, the N-terminal domain is not essential for the interaction between these proteins. Loss of amino acids 291–370 of PSF disrupted the interaction between PSF and PPARγ. Deletion of amino acids 371–450, 451–662, and 452–707 of PSF also disrupted the interaction with PPARγ. Thus, our results identified the first nucleotide-binding domain (amino acids 291–370) as an important molecular site for PPARγ binding [17].

PSF Knockdown and its Phenotype

Adenocarcinomas are the most common type of gastrointestinal cancer, but there are many other forms [20,21]. Cell lines established from human colorectal carcinomas may provide useful tools with which to study the biology of gastrointestinal cancer and to develop and test new therapeutic strategies [22-24]. Our resent study showed that the proliferation of DLD-1 and Caco-2 cells, which express a low level of PPARγ, was significantly inhibited by knockdown of PSF, whereas the proliferation of HT-29 and LOVO cells, which express a higher level of PPARγ, was inhibited weakly or not at all. Interestingly, in DLD-1 cells, but not in HT-29 cells, PSF knockdown also induced morphological changes associated with apoptosis, i.e., cell shrinkage and condensation of nuclear chromatin [17]. Our recent work demonstrated that knockdown of PSF induced dramatic cytoplasmic vacuolization 48 and 72 h after siRNA transfection in 30 and 40% of cells in culture, respectively. Cell vacuolation increased in frequency and size, occupying increasingly larger areas of the cytoplasm, in a time-dependent manner. The reason why cytoplasmic vacuoles were present in only DLD-1 cells remains unclear. In electron micrographs, smaller vacuoles in some cancer cells appeared to be distended mitochondria [25]. However, it is unclear if this is a step of apoptosis or necrosis or a distinct form of colon cancer cell death. Our recent work suggested that cytoplasmic vacuolation and apoptotic changes correlated with the dysfunction of mitochondria [17]. We identified candidate proteins potentially involved in the PPARγ-PSF interaction and in apoptosis. As expected from a previous study [17], many of these proteins play a role in apoptosis (annexin and VDAC2) and cytoskeletal interactions (tubulin, MYH9, and filamin A) and act as molecular chaperones (HSP90 and T complex protein) (summarized in Figure 2). Interestingly, in DLD-1 cells, voltage-dependent anion-selective channel protein 2 (VDAC2) was upregulated. VDAC2 forms a channel through the mitochondrial outer membrane that allows the diffusion of small hydrophilic molecules [26,27]. VDAC2 has also been implicated in apoptosis, forming an open pore that allows the release of cytochrome *c* from the inner mitochondrial membrane [28]. We investigated VDAC2 mRNA and protein expression in DLD-1 cells after PSF knockdown. Real-time PCR and western blot analysis confirmed that VDAC2 and Bax were upregulated under PSF knockdown conditions in DLD-1 cells [17]. Furthermore, Sharathchandra et al. recently reported that annexin A2 and PSF interact with tumor suppressor protein p53 [29]. p53 levels were reduced after knockdown of annexin A2 and PSF. Annexin A2 is a calcium- and phospholipid-binding protein that is found on various cell types [30,31] and is upregulated in various cancers. It plays multiple roles in apoptosis, proliferation, migration, and invasion [32,33]. Annexin A2 might be useful as a biomarker for the diagnosis and treatment of colon cancer. Together, these studies indicate that VDAC2 and annexin A2 have potential as therapeutic targets to inhibit colon cancer progression.

Conclusions

Targeting specific molecules has become important for developing new cancer treatments. Herein, we reviewed our current understanding of the cellular and molecular interactions between PPARγ and it target in colon cancer. We provided a general overview of the broad therapies directed at PPARγ expression or its binding partners that may lead to novel approaches to treat colon cancer. The effect of PSF on PPARγ targets and the contributions of these targets to PSF-mediated cellular processes require further investigation. This study supports the potential use of PSF and its targets in the development of drugs targeting colon cancer and possibly other types of cancer. Such studies may open up new avenues for colon cancer research and therapeutics.

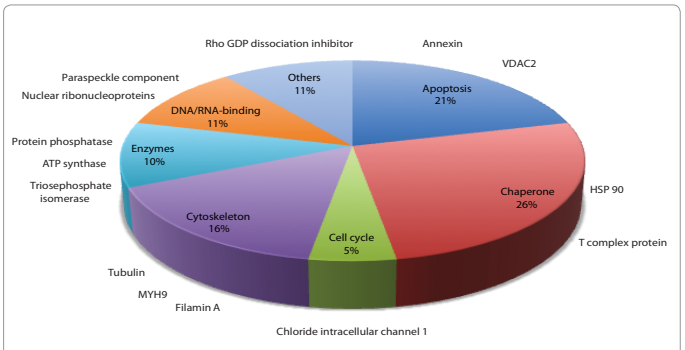

Figure 2: Upregulated proteins in DLD-1 cells after siRNA knockdown of PSF. DLD-1 cells were transiently transfected with control siRNA or siRNA targeting PSF. MS/MS analyses were performed using the AB SCIEX TOF/TOF™ 5800 System (AB SCIEX, Foster City, CA, USA). Protein identification was performed through ProteinPilot™ software (AB Sciex, Framingham, MA, USA) using the UniProt database. We identified 25 distinct proteins whose levels were significantly altered following PSF knockdown in DLD-1 cells [34]. On the basis of these results, we suggest that PSF activity may influence protein translation, the cell cycle, apoptosis, and cytoskeleton dynamics.

Acknowledgments

This work was supported by research grants from the Astellas Foundation for Research on Metabolic Disorders (to Tamotsu Tsukahara), Grant-in-Aid for Takeda Science Foundation (to Tamotsu Tsukahara), and Grants-in-Aid for Scientific Research (C) 22591482 (to Tamotsu Tsukahara) from the Japan Society for the Promotion of Science (JSPS).

References

1. Meissner M, Dechat T, Gerner C, Grimm R, Foisner R, et al. (2000) Differential nuclear localization and nuclear matrix association of the splicing factors PSF and PTB. J Cell Biochem 76: 559-566.
2. Patton JG, Porro EB, Galceran J, Tempst P, Nadal-Ginard B (1993) Cloning and characterization of PSF, a novel pre-mRNA splicing factor. Genes Dev 7: 393-406.
3. Song X, Sun Y, Garen A (2005) Roles of PSF protein and VL30 RNA in reversible gene regulation. Proc Natl Acad Sci U S A 102: 12189-12193.
4. Mathur M, Tucker PW, Samuels HH (2001) PSF is a novel corepressor that mediates its effect through Sin3A and the DNA binding domain of nuclear hormone receptors. Mol Cell Biol 21: 2298-2311.
5. Tsukahara T (2012) The Role of PPARÎ³ in the Transcriptional Control by Agonists and Antagonists. PPAR Res 2012: 362361.
6. Aranda A, Pascual A (2001) Nuclear hormone receptors and gene expression. Physiol Rev 81: 1269-1304.
7. Wang G, Cui Y, Zhang G, Garen A, Song X (2009) Regulation of proto-oncogene transcription, cell proliferation, and tumorigenesis in mice by PSF protein and a VL30 noncoding RNA. Proc Natl Acad Sci U S A 106: 16794-16798.
8. Miyamoto K, Sakurai H, Sugiura T (2008) Proteomic identification of a PSF/p54nrb heterodimer as RNF43 oncoprotein-interacting proteins. Proteomics 8: 2907-2910.
9. Robbins GT, Nie D (2012) PPAR gamma, bioactive lipids, and cancer progression. Front Biosci 17: 1816-1834.
10. Tontonoz P, Hu E, Devine J, Beale EG, Spiegelman BM (1995) PPAR gamma 2 regulates adipose expression of the phosphoenolpyruvate carboxykinase gene. Mol Cell Biol 15: 351-357.
11. Tsukahara T (2013) PPAR Î³ Networks in Cell Signaling: Update and Impact of Cyclic Phosphatidic Acid. J Lipids 2013: 246597.
12. Kotta-Loizou I, Giaginis C, Theocharis S (2012) The role of peroxisome proliferator-activated receptor-Î³ in breast cancer. Anticancer Agents Med Chem 12: 1025-1044.
13. Zhou P, Cheng SW, Yang R, Wang B, Liu J (2012) Combination chemoprevention: future direction of colorectal cancer prevention. Eur J Cancer Prev 21: 231-240.
14. Yokoyama Y, Mizunuma H (2010) Peroxisome proliferator-activated receptor and epithelial ovarian cancer. Eur J Gynaecol Oncol 31: 612-615.
15. McAlpine CA, Barak Y, Matise I, Cormier RT (2006) Intestinal-specific PPARgamma deficiency enhances tumorigenesis in ApcMin/+ mice. Int J Cancer 119: 2339-2346.
16. Xu HE, Li Y (2008) Ligand-dependent and -independent regulation of PPAR gamma and orphan nuclear receptors. Sci Signal 1: pe52.
17. Tsukahara T, Haniu H, Matsuda Y (2013) PTB-associated splicing factor (PSF) is a PPARÎ³-binding protein and growth regulator of colon cancer cells. PLoS One 8: e58749.
18. Luo Y, Batalao A, Zhou H, Zhu L (1997) Mammalian two-hybrid system: a complementary approach to the yeast two-hybrid system. Biotechniques 22: 350-352.
19. He R, Li X (2008) Mammalian two-hybrid assay for detecting protein-protein interactions in vivo. Methods Mol Biol 439: 327-337.
20. Muto T, Bussey HJ, Morson BC (1975) The evolution of cancer of the colon and rectum. Cancer 36: 2251-2270.
21. Potter JD (1999) Colorectal cancer: molecules and populations. J Natl Cancer Inst 91: 916-932.
22. Leibovitz A, Stinson JC, McCombs WB 3rd, McCoy CE, Mazur KC, et al. (1976) Classification of human colorectal adenocarcinoma cell lines. Cancer Res 36: 4562-4569.
23. McBain JA, Weese JL, Meisner LF, Wolberg WH, Willson JK (1984) Establishment and characterization of human colorectal cancer cell lines. Cancer Res 44: 5813-5821.
24. Park JG, Oie HK, Sugarbaker PH, Henslee JG, Chen TR, et al. (1987) Characteristics of cell lines established from human colorectal carcinoma. Cancer Res 47: 6710-6718.
25. Albores-Saavedra J, Simpson KW, Bilello SJ (2006) The clear cell variant of solid pseudopapillary tumor of the pancreas: a previously unrecognized pancreatic neoplasm. Am J Surg Pathol 30: 1237-1242.
26. Schein SJ, Colombini M, Finkelstein A (1976) Reconstitution in planar lipid bilayers of a voltage-dependent anion-selective channel obtained from paramecium mitochondria. J Membr Biol 30: 99-120.
27. Kroemer G, Galluzzi L, Brenner C (2007) Mitochondrial membrane permeabilization in cell death. Physiol Rev 87: 99-163.
28. Roy SS, Ehrlich AM, Craigen WJ, Hajnóczky G (2009) VDAC2 is required for truncated BID-induced mitochondrial apoptosis by recruiting BAK to the mitochondria. EMBO Rep 10: 1341-1347.
29. Sharathchandra A, Lal R, Khan D, Das S (2012) Annexin A2 and PSF proteins interact with p53 IRES and regulate translation of p53 mRNA. RNA Biol 9: 1429-1439.
30. Hajjar KA, Guevara CA, Lev E, Dowling K, Chacko J (1996) Interaction of the fibrinolytic receptor, annexin II, with the endothelial cell surface. Essential role of endonexin repeat 2. J Biol Chem 271: 21652-21659.
31. Mai J, Waisman DM, Sloane BF (2000) Cell surface complex of cathepsin B/annexin II tetramer in malignant progression. Biochim Biophys Acta 1477: 215-230.
32. Sharma MR, Koltowski L, Ownbey RT, Tuszynski GP, Sharma MC (2006) Angiogenesis-associated protein annexin II in breast cancer: selective expression in invasive breast cancer and contribution to tumor invasion and progression. Exp Mol Pathol 81: 146-156.
33. Shiozawa Y, Havens AM, Jung Y, Ziegler AM, Pedersen EA, et al. (2008) Annexin II/annexin II receptor axis regulates adhesion, migration, homing, and growth of prostate cancer. J Cell Biochem 105: 370-380.
34. Tsukahara T, Haniu H, Matsuda Y (2013) PTB-associated splicing factor (PSF) is a PPARÎ³-binding protein and growth regulator of colon cancer cells. PLoS One 8: e58749.