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COMMENTARY

Anticancer Activities of PPAR γ in Breast Cancer Are Context-Dependent

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In this issue of *The American Journal of Pathology*, Nakles et al¹ used mouse mammary glands with null *Brca1* and haplo-insufficient *Trp53* as a tumor model to evaluate the effect of PPAR agonist efatutazone on mammary tumorigenesis. Mice were treated at age 4 months, so the findings could be more relevant to treatment rather than prevention. The authors found that, although efatutazone did not have a significant effect on the incidence of invasive tumors, it significantly reduced the incidence of non-invasive mammary tumor and promoted more differentiated histology in the tumors that formed. Efaturazone also reduced the prevalence of dense preneoplastic lesions including lobular growth and the number of hyperplastic alveolar nodules (HANs). Tumors from the efaturazone group had reduced expression of phosphor-AKT but not total AKT, and CDK6 was the only cell cycle regulator that was down-regulated. These findings not only provide insight but also help clarify several issues in an area that could lead to new therapy in the treatment of breast cancer.

Targeting PPAR γ

The peroxisome proliferator-activated receptor (PPAR) belongs to the nuclear receptor (NR) superfamily that includes estrogen receptors (ERs), retinoic acid receptors (RARs), retinoid X receptors (RXRs), and the vitamin D receptor (VDR). PPAR γ is one of the PPARs that are expressed in different tissues. On the binding of its ligand, PPAR γ heterodimerizes with RXR to form a complex that translocates into the nucleus, where the complex and its coactivators bind to gene promoters to regulate the transcription of PPAR γ target genes. Known coactivators and corepressors of PPAR γ include the histone acetyltransferase p300 (CBP), SRC-1, TEF2, Drip205 (Med220), and PGC-1. Binding of ligand to PPAR γ usually leads to its dissociation from corepressors and

association with coactivators.² PPAR γ regulates different biological processes including adipocyte differentiation, glucose homeostasis, cell proliferation and differentiation, inflammation, apoptosis, angiogenesis, development, and carcinogenesis.^{3,4} The diverse functions of PPAR γ are mediated by different group of genes that are known to regulate these processes, including those involved in cell cycle arrest, apoptosis, and DNA damage response.^{5,6}

After the widely used anti-diabetic thiazolidinedione (TZD) drugs were found to be ligands for PPAR γ ,⁷ some natural ligands for PPAR γ have been identified and used to elucidate the role of PPAR γ in cellular functions both *in vitro* and *in vivo*.⁸ Derivatives of TZD have also been developed with higher specificities and activities. The agonist used in this study, efaturazone (also called CS-7017 or RS5444), is a selective high-affinity TZD-class agonist of PPAR γ . Efaturazone induces PPAR γ -dependent transactivation but has no effect on the activation of either PPAR α or PPAR δ .

In human tissues, the *PPARG* gene is expressed in normal cells and can undergo mutations in some cancers. For example, *PPARG* mutation has been detected in colon cancer and chromosomal translocation that produces an oncogenic fusion protein involving PPAR γ ; ie, PAX8–PPAR γ , occurs in thyroid follicular carcinoma,⁹ which suggests that PPAR γ could be oncogenic. In breast cancer, however, the *PPARG* gene is not mutated. PPAR γ is expressed in both normal and malignant mammary tissues and its expression level is often higher in cancer tissues.¹⁰ However, an increased expression in cancer cells does not necessarily mean an oncogenic role in tumor development. Therefore, there is no genetic evidence for either a tumor suppressor or an oncogenic function of PPAR γ in breast cancer.

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PPAR γ as a Tumor Suppressor?

The key finding of Nakles et al¹ is that the activation of PPAR γ by its agonist reduced the incidence of non-invasive cancer and promoted more differentiated histology without affecting the incidence and histology of invasive cancers. These results clearly indicate a tumor suppressor function for PPAR γ in breast cancer and confirm the complexity of PPAR γ functions in carcinogenesis. Targeting the signaling pathways transduced by NRs is a valid approach for the treatment and prevention of different diseases including cancer. One well known example is the commonly prescribed tamoxifen, an ER α antagonist, for the treatment of ER α -positive breast cancers. As a crucial regulator of cell proliferation, differentiation, and apoptosis,¹¹ PPAR γ has been evaluated as a target for therapeutic development, and agonists for PPAR γ have been developed for the treatment of different diseases.²

However, PPAR γ appears to have contradicting functions in tumorigenesis, which is likely determined by the molecular contexts of cells. First of all, a number of studies have showed a suppressive function of PPAR γ in cultured breast cancer cells, including both ER-positive (eg, MCF-7) and ER-negative cells (eg, MDA-MB-231). The suppressive activities include inhibition of cell proliferation, induction of apoptosis, and promotion of differentiation.¹² The effects have been detected in both ER-positive and ER-negative breast cancer cells, and the findings among different studies are generally consistent. However, findings in PPAR γ functions from animal studies are less consistent and sometimes contradicting, which is somewhat parallel to the dual expression trends of PPAR γ in human tumors. On one hand, there are plenty of animal studies showing a tumor suppressor function of PPAR γ . For example, TZD suppresses the tumorigenesis of MCF-7 human breast cancer cells in nude mice,¹³ knockout of PPAR γ increases tumor incidence and metastasis in DMBA-induced tumorigenesis,¹⁴ and PPAR γ agonist shows both tumor suppressive and chemoprotective effects on tumorigenesis induced by the combined treatment of excess hormone and the DMBA carcinogen.¹⁵ In addition, expression of the dominant-negative *Pax8-PPARG* fusion gene in mouse mammary epithelial cells, although not tumorigenic itself, makes cells highly susceptible to DMBA-induced tumorigenesis.¹⁶ The paper by Nakles et al¹ adds to the list of studies.

On the other hand, an oncogenic role has also been detected for PPAR γ , although the mechanism for such an opposite function is not really understood. For example, overexpression of PPAR γ increases the risk of mammary tumor in mice that are prone to developing the disease.¹⁷ Using the MMTV-Her-2/neu transgenic mouse model of mammary tumorigenesis, Saez et al¹⁸ found that PPAR γ overexpression did not prevent, but instead greatly accelerated tumorigenesis, although bigenic tumors are more secretory and differentiated. Expression of a constitutively active form of PPAR γ also accelerated mammary tumorigenesis in the transgenic

MMTV-PyV mice, which are prone to mammary cancer development.¹⁷ Contradicting findings have also been reported in other types of cancers including colon cancer.¹⁷

Clinical studies have also been inconsistent for the effects of PPAR γ agonists on human breast cancer. Use of PPAR γ agonists TZD is associated with better survival for diabetics with HER2-positive breast cancer,¹⁹ but patients with metastatic breast cancer do not appear to show any clinical benefits of TZD use.²⁰ There are at least several possibilities for discrepancies in PPAR γ function in breast cancer among different studies, and Nakles et al¹ provide some insight. Better understanding of how PPAR γ functions and interacts with other signaling pathways should help clarify the issue, thereby improving the design and outcome of clinical trials. For example, if PPAR γ is only relevant to more differentiated ER-positive breast cancer as suggested by Nakles et al¹ and other studies, it would not make sense to use advanced breast cancers, which are often metastatic and ER-negative, in a clinical trial for PPAR γ agonists.

The Role of Other Pathways

One possibility for inconsistent functions of PPAR γ is that PPAR γ functions in a context-dependent manner. For example, certain molecular pathways need to be present or absent for PPAR γ to function. Nakles et al¹ used mice that undergo a mammary epithelial cell targeted deletion of *Brca1* in somatic cells coupled with germline *Trp53* haplo-insufficiency. These mice provide a model that is relevant to the human disease because both genes are functionally inactivated in some human breast cancer. The findings of a tumor suppressor function for PPAR γ thus suggest that breast cancers with abnormal *BRCA1* and *TP53* would respond to PPAR γ agonists. There are reported links between PPAR γ function and *BRCA1* and *TP53*. For *TP53*, TZD rosiglitazone has been shown to increase the expression of *TP53* and its effector p21 (*WAF1/Cip1*).²¹ In addition, mammary epithelial cells from mice with *TP53* insufficiency undergo more frequent spontaneous immortalization, and treatment with rosiglitazone, another agonist of PPAR γ , reduces the frequency of spontaneous immortalization.²² Taken together with Nakles et al,¹ it is likely that proper function of PPAR γ needs functional *TP53*. If that be the case, efatutazone treatment could increase *TP53* expression, which was not examined in the paper. A relevance of PPAR γ to *BRCA1* is also possible, as PPAR γ agonists induce the expression of *BRCA1* in both cultured cells and mammary tissues.²³

Another proliferation-related pathway suppressed by PPAR γ agonist is Akt/PTEN, as TZDs increase PTEN expression in MCF-7 breast cancer cells and decrease Akt phosphorylation.²⁴ Consistently, interruption of PPAR γ by expressing the *PAX8/PPAR* fusion gene reduces Pten expression and increases AKT activation in both normal and cancer cells.¹⁶ Nakles et al¹ confirm that efatutazone decreased the expression of p-AKT without affecting the total level of

AKT expression, although Pten expression was not evaluated in the model. There is evidence for PPAR γ signaling to interact with other signaling pathways, including STAT5B, NF- κ B, TGF- β , and Ras/MAPK.¹⁶ Ligand activation of PPAR γ has been shown to potentiate Wnt signaling in an *in vivo* model of mouse mammary tumorigenesis.¹⁷ These pathways were not evaluated in the Nakles study,¹ so it is not clear whether the effect of efatutazone on tumorigenesis in the discussed paper is associated with any of these pathways. Improved understanding of molecular signatures associated with different effects of PPAR γ agonists should help us to develop PPAR γ -based therapies in the treatment and prevention of breast cancer.

The connection with ER is another important aspect in the function of PPAR γ signaling. In the Nakles study,¹ efatutazone treatment led to the formation of more differentiated ER-positive tumors, which suggests a positive association between PPAR γ function and ER expression. On the other hand, inhibition of PPAR γ function via expressing the dominant-negative Pax8-PPAR γ fusion gene made DMBA-induced mouse mammary tumors also ER-positive, and subsequently highly responsive to the ER antagonist fulvestrant in tumor prevention.¹⁶ Based on this study and Nakles et al,¹ it becomes interesting to know whether combined use of efatutazone and an ER antagonist has any additive or synergistic (or even neutralizing) effect on mammary tumorigenesis induced by Brca1 deficiency and Trp53 insufficiency.

A direct connection between PPAR γ function and ER transcription has also been reported, although in an opposite manner. In mouse mammary organ cultures, although DMBA-induced mammary alveolar lesions (MAL) were inhibited by either an agonist or an antagonist of PPAR γ , it is the antagonist and not the agonist that induced ER and PR expression in the presence of estrogen.²⁵ Further insights into the relationship between PPAR γ signaling and ER signaling could lead to better intervention of breast cancer using a combined use of PPAR γ agonists and ER antagonists.

Future Directions

An interesting observation by Nakles et al¹ is that efatutazone lacks an effect on invasive tumors. It is possible that this group of cancers originated from cells with distinct molecular alterations. It is also possible, as the authors discuss, that invasive cancers come from efatutazone-resistant cancer or cancer progenitor cells that are not clinically well characterized.

Although not addressed in the study, it would be interesting to compare invasive cancers to the non-invasive, more differentiated, and ER-positive cancers for the molecular pathways associated with PPAR γ signaling. Molecular differences between the two groups of cancers should help clarify what molecular pathways and signatures are required or altered by PPAR γ activation and what are specific to invasive cancers that are not responsive to PPAR γ activation.

Another outstanding question is how both enhanced and attenuated PPAR γ activities induce ER-positive tumors in

the mammary gland. As nuclear receptors, it is not impossible that PPAR γ and ER have direct interactions. A speculation is that the pro-differentiation function of PPAR γ restrains the pro-proliferative/tumorigenic function of ER via direct interaction, and the inactivation of PPAR γ could thus release or enhance the pro-proliferative/tumorigenic function of ER, leading to the transformation of luminal cells that are already ER-positive to tumor cells that remain ER-positive. This possibility is worth testing in future studies. It is also possible that progenitor cells, which are ER-negative, have acquired tumor-initiating events while differentiating into ER-positive cells under the influence of pro-differentiation function of PPAR γ , and eventually become fully transformed cells that are ER positive.

Finally, efatutazone was administered at four month of age, which leaves several questions unaddressed. For example, could efatutazone treatment modify some early cancer or progenitor cells toward a more differentiated phenotype? Could early administration of efatutazone prevent the occurrence of the more differentiated ER-positive cancer? Is the effect of efatutazone reversible?

Concluding Remarks

In summary, the results produced by Nakles et al¹ have several implications: i) PPAR γ activation indeed interferes with tumor development, ii) the tumor suppressor function of PPAR γ is more likely associated with its pro-differentiation function, and iii) PPAR γ activation is only effective in specific cells that could be at a specific stage of malignant transformation or have a distinct set of molecular alterations. Clarification of molecular basis for the different effects of efatutazone on tumor development and histology should help the development of PPAR γ agonists as therapeutic and/or preventive reagents in breast cancer and other types of cancers.

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