Cancer Prevention Research

Perspective
Perspective on Subbaramaiah et al., p. 1183

PPARγ Agonists Target Aromatase Via Both PGE_2 and BRCA1

Ofer Margalit[1], Dingzhi Wang[1], and Raymond N. DuBois[1,2]

Abstract

Obesity is a well-recognized risk factor for postmenopausal breast cancer. Although the underlying mechanisms are not clearly defined, aromatase is thought to play a pivotal role in connecting obesity-associated inflammation with postmenopausal breast cancer. It has been well established that both the proinflammatory prostaglandin E_2 (PGE_2) and the BRCA1 tumor-suppressor gene regulate aromatase expression. In this issue of the journal (beginning on p. 1183), Subbaramaiah and colleagues improve our understanding of the molecular mechanisms by which PPARγ inhibits aromatase expression. They found that pioglitazone, a PPARγ agonist, inhibited aromatase expression by inhibition of PGE_2 signaling and upregulation of BRCA1. Their findings provide potential targets for preventing or treating obesity-related breast cancer. *Cancer Prev Res; 5(10); 1169–72. ©2012 AACR.*

Multiple epidemiologic and animal studies have established the link between obesity and increased risk for postmenopausal breast cancer. Because obesity is referred to as the epidemic of the 21st century with around 36% of the adult United States women defined as obese and another one third as overweight (1), this disease has a dramatic effect on quality of life as well as on life expectancy in the United States. The estimated increased risk for breast cancer is around 1.3- to 2-fold for obese postmenopausal women as compared with normal-weight postmenopausal women (2, 3). Several mechanisms have been proposed as being responsible for this increased risk for breast cancer. Among those, a well-studied mechanism is altered estrogen biosynthesis, mainly involving increased aromatase expression (4). Aromatase catalyzes the last steps of estrogen biosynthesis from androgens. Estrogen executes its effect mostly by binding to estrogen receptors (ER). Nuclear ERs function as transcription factors and regulate gene transcription by binding to specific DNA sequences called estrogen response elements (ERE). In clinical practice, selective ER modulators and aromatase inhibitors are commonly and successfully used for breast cancer prevention and treatment. Aromatase is expressed mainly in undifferentiated adipose fibroblasts (also referred to as visceral preadipocytes) but not in mature adipocytes (5). Indeed, undifferentiated breast adipose fibroblasts have been shown to play a major role in aromatase expression leading to breast cancer development (6–8). Therefore, in obese or overweight women, a larger mass of breast adipose tissue with a correspondingly high number of fibroblasts may result in increased local production of aromatase.

PPARγ is a member of the PPAR family, including PPAR-α, β/δ, and γ, which belongs to the superfamily of nuclear hormone receptors. PPARγ is a ligand-activated transcription factor, which along with retinoid X receptors (RXR) forms heterodimers that activate transcription of its target genes by binding to PPAR response elements (PPRE) located in the promoter region. In addition to the role of PPARγ in glucose and lipid metabolism, PPARγ stimulated adipocyte differentiation (9, 10), and inhibition of PPARγ attenuated adipocyte differentiation induced by breast cancer epithelial cells (11). Furthermore, PPARγ ligand therapy was tested in many preclinical studies of solid tumors, including breast cancer, showing a beneficial effect (12, 13). For example, several *in vivo* studies showed that PPARγ inhibited breast cancer growth (14–16). The inhibitory effects of PPARγ on mammary tumorigenesis occur through inducing terminal differentiation, cell-cycle arrest, and apoptosis of human breast cancer cells (17–19) as well as inhibiting invasion (20) and angiogenesis *in vitro* and *in vivo* (21, 22). In this issue of the journal, Subbaramaiah and colleagues (23) report for the first time that pioglitazone, a PPARγ agonist, inhibits aromatase expression via inhibiting prostaglandin E_2 (PGE_2) signaling and upregulating BRCA1 *in vitro* and *in vivo*. Importantly, they show that pioglitazone inhibition of aromatase is PPARγ–dependent in human visceral preadipocytes.

One emerging paradigm for increased aromatase expression in breast cancer is associated with inflammation and higher levels of the potent proinflammatory PGE_2 (24–29). PGE_2 induced aromatase expression by enhancing interaction between p-CREB, p300, and the aromatase promoters I.3/II via the EP_2/EP_4-cAMP-protein kinase A (PKA)

Authors' Affiliations: Departments of [1]Cancer Biology and [2]Gastrointestinal Medical Oncology, The University of Texas MD Anderson Cancer Center, Houston, Texas

Corresponding Author: Raymond N. DuBois, Departments of Gastrointestinal Medical Oncology and Cancer Biology, The University of Texas MD Anderson Cancer Center, Unit 1492, 1515 Holcombe Blvd, Houston, TX 77030. Phone: 713-745-4495; Fax: 713-792-6375; E-mail: rdubois@mdanderson.org

doi: 10.1158/1940-6207.CAPR-12-0365

pathway (24, 25, 27). Another pathway that regulates aromatase expression in breast cancer is through the BRCA1 tumor-suppressor gene. Mutations in the *BRCA1* gene lead to breast and/or ovarian cancer with increased aromatase expression (30). Several studies have shown that BRCA1 inhibits aromatase expression in human adipose fibroblasts and malignant breast epithelial cells (31–33). Moreover, adipose-specific PPARγ knockout mice exhibited decreased BRCA1 expression in mammary stromal adipocytes (34). Interestingly, PGE_2 inhibited BRCA1 expression in human visceral preadipocytes and breast cancer cells (24), suggesting a link between these 2 pathways. Because PPARγ agonists were shown to inhibit PGE_2 (35, 36) and upregulate BRCA1 (37), it is conceivable that PPARγ agonists inhibit aromatase expression through both the PGE_2 and BRCA1 pathways.

In this issue of the journal, Subbaramaiah and colleagues elegantly address the complicated pathways that mediate pioglitazone inhibition of aromatase expression *in vitro* and *in vivo*. First, pioglitazone inhibits early growth response factor-1 (Egr-1), known to induce Snail, a repressive transcription factor that in turn inhibits the expression of 15-hydroxyprostaglandin dehydrogenase (15-PGDG), the enzyme responsible for PGE_2 degradation. Therefore, this derepression of 15-PGDH reduces PGE_2 levels. Moreover, by using silencing of 15-PGDH and overexpression of Snail, they show for the first time that both these factors mediate pioglitazone inhibition of aromatase. Notably, previous studies showed that PPARγ ligands reduce PGE_2 level by downregulating the PG synthase microsomal prostaglandin E synthase-1 (mPGES1) via inhibiting its activators Egr-1 and IL-1β (35, 38). Therefore, PPARγ ligands could affect PGE_2 level through both mPGES1-dependent biosynthesis and 15-PGDH–dependent degradation pathways. Subbamaraiah and colleagues also show for the first time that pioglitazone can inhibit aromatase *in vivo* in murine mammary glands, along with inducing 15-PGDH and BRCA1, further substantiating the link between pioglitazone, the PGE_2, and BRCA1 pathways in mediating aromatase inhibition *in vivo*.

Although aromatase inhibitors are indeed beneficial in breast cancer prevention and treatment, their systemic side effects include musculoskeletal symptoms, osteoporosis, and increased rate of bone fracture. One way to reduce these systemic side effects without interfering with treatment efficacy would be to specifically inhibit aromatase levels in breast cancer tissue. In normal adipose tissue, aromatase expression is driven by its promoter I.4. However, in breast cancer and adipose fibroblasts surrounding breast tumors, aromatase expression is driven by its promoters I.3 and II (39, 40). Indeed, both PGE_2 signaling and BRCA1 were shown to regulate aromatase expression through the I.3/II promoters (24, 27, 31, 32). Therefore, inhibition of aromatase expression via both inhibition of PGE_2 and upregulation of BRCA1 provides an opportunity for a highly tissue-specific endocrine therapy that targets the estrogen required for tumor growth, whereas sparing other important sites of estrogen action, such as bone. Hence, using PPAR-γ agonists to inhibit aromatase via the PGE_2 and BRCA1 pathways is expected to reduce aromatase-related systemic side effects.

PPARγ can also regulate the estrogen signaling through aromatase-independent pathways by inhibiting ER expression through either the PTEN pathway (15, 41) or inducing proteasome-dependent degradation of ER (42). Furthermore, the PPARγ/RXR heterodimer binds to the ERE, which inhibits ER transactivation (43). By the same token, ER was shown to bind PPREs and negatively interfere with PPARγ signaling in breast cancer cells (44, 45). This cross-talk further supports the possible benefit from combination therapy of ER antagonists or aromatase inhibitors along with PPARγ agonists.

In conclusion, Subbaramaiah and colleagues have meticulously delineated the pathways that mediate pioglitazone inhibition of aromatase expression. Their findings suggest that PPARγ agonists may hold promise as potent agents in breast cancer prevention and treatment, with possibly fewer aromatase-related systemic side effects. In addition, their work provides several novel drug targets, such as the transcription factor Snail, for the prevention and treatment of breast cancer. In the clinical setting, 2 small-scale and short-term clinical trials of PPARγ agonists in early (46) and metastatic (47) breast cancer have failed to show positive results. It would be interesting to see the results from a small-scale trial completed in August 2012, which was designed to determine whether the addition of the PPARγ agonist rosiglitazone, along with metformin, could improve the efficacy of the aromatase inhibitor exemstane in postmenopausal obese women with ER positive metastatic breast cancer (NCT00933309). Further large-scale and long-term clinical trials using PPARγ agonists in combination with ER antagonists or aromatase inhibitors may be considered.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

Authors' Contributions

Conception and design: O. Margalit, D. Wang
Writing, review, and/or revision of the manuscript: O. Margalit, D. Wang, R.N. DuBois
Study supervision: R.N. DuBois

Received August 23, 2012; revised August 27, 2012; accepted August 31, 2012; published online October 5, 2012.

References

1. Flegal KM, Carroll MD, Ogden CL, Curtin LR. Prevalence and trends in obesity among US adults, 1999–2008. JAMA 2010; 303:235–41.

2. Eliassen AH, Colditz GA, Rosner B, Willett WC, Hankinson SE. Adult weight change and risk of postmenopausal breast cancer. JAMA 2006;296:193–201.

3. van den Brandt PA, Spiegelman D, Yaun SS, Adami HO, Beeson L, Folsom AR, et al. Pooled analysis of prospective cohort studies on height, weight, and breast cancer risk. Am J Epidemiol 2000;152: 514–27.
4. Bulun SE, Chen D, Moy I, Brooks DC, Zhao H. Aromatase, breast cancer and obesity: a complex interaction. Trends Endocrinol Metab 2012;23:83–9.
5. Cleland WH, Mendelson CR, Simpson ER. Aromatase activity of membrane fractions of human adipose tissue stromal cells and adipocytes. Endocrinology 1983;113:2155–60.
6. Bulun SE, Price TM, Aitken J, Mahendroo MS, Simpson ER. A link between breast cancer and local estrogen biosynthesis suggested by quantification of breast adipose tissue aromatase cytochrome P450 transcripts using competitive polymerase chain reaction after reverse transcription. J Clin Endocrinol Metab 1993;77: 1622–8.
7. Bulun SE, Sharda G, Rink J, Sharma S, Simpson ER. Distribution of aromatase P450 transcripts and adipose fibroblasts in the human breast. J Clin Endocrinol Metab 1996;81:1273–7.
8. O'Neill JS, Elton RA, Miller WR. Aromatase activity in adipose tissue from breast quadrants: a link with tumour site. Br Med J (Clin Res Ed) 1988;296:741–3.
9. Tontonoz P, Hu E, Spiegelman BM. Stimulation of adipogenesis in fibroblasts by PPAR gamma 2, a lipid-activated transcription factor. Cell 1994;79:1147–56.
10. Hu E, Tontonoz P, Spiegelman BM. Transdifferentiation of myoblasts by the adipogenic transcription factors PPAR gamma and C/EBP alpha. Proc Natl Acad Sci U S A 1995;92:9856–60.
11. Meng L, Zhou J, Sasano H, Suzuki T, Zeitoun KM, Bulun SE. Tumor necrosis factor alpha and interleukin 11 secreted by malignant breast epithelial cells inhibit adipocyte differentiation by selectively down-regulating CCAAT/enhancer binding protein alpha and peroxisome proliferator-activated receptor gamma: mechanism of desmoplastic reaction. Cancer Res 2001;61:2250–5.
12. Fenner MH, Elstner E. Peroxisome proliferator-activated receptor-gamma ligands for the treatment of breast cancer. Expert Opin Investig Drugs 2005;14:557–68.
13. Hatton JL, Yee LD. Clinical use of PPARgamma ligands in cancer. PPAR Res 2008;2008:159415.
14. Nicol CJ, Yoon M, Ward JM, Yamashita M, Fukamachi K, Peters JM, et al. PPARgamma influences susceptibility to DMBA-induced mammary, ovarian and skin carcinogenesis. Carcinogenesis 2004;25: 1747–55.
15. Yin Y, Yuan H, Zeng X, Kopelovich L, Glazer RI. Inhibition of peroxisome proliferator-activated receptor gamma increases estrogen receptor-dependent tumor specification. Cancer Res 2009;69: 687–94.
16. Suh N, Wang Y, Williams CR, Risingsong R, Gilmer T, Willson TM, et al. A new ligand for the peroxisome proliferator-activated receptor-gamma (PPAR-gamma), GW7845, inhibits rat mammary carcinogenesis. Cancer Res 1999;59:5671–3.
17. Mueller E, Sarraf P, Tontonoz P, Evans RM, Martin KJ, Zhang M, et al. Terminal differentiation of human breast cancer through PPAR gamma. Mol Cell 1998;1:465–70.
18. Wang C, Fu M, D'Amico M, Albanese C, Zhou JN, Brownlee M, et al. Inhibition of cellular proliferation through IkappaB kinase-independent and peroxisome proliferator-activated receptor gamma-dependent repression of cyclin D1. Mol Cell Biol 2001;21: 3057–70.
19. Elstner E, Muller C, Koshizuka K, Williamson EA, Park D, Asou H, et al. Ligands for peroxisome proliferator-activated receptorgamma and retinoic acid receptor inhibit growth and induce apoptosis of human breast cancer cells *in vitro* and in BNX mice. Proc Natl Acad Sci U S A 1998;95:8806–11.
20. Liu H, Zang C, Fenner MH, Possinger K, Elstner E. PPARgamma ligands and ATRA inhibit the invasion of human breast cancer cells *in vitro*. Breast Cancer Res Treat 2003;79:63–74.
21. Xin X, Yang S, Kowalski J, Gerritsen ME. Peroxisome proliferator-activated receptor gamma ligands are potent inhibitors of angiogenesis *in vitro* and *in vivo*. J Biol Chem 1999;274:9116–21.
22. Panigrahy D, Singer S, Shen LQ, Butterfield CE, Freedman DA, Chen EJ, et al. PPARgamma ligands inhibit primary tumor growth and metastasis by inhibiting angiogenesis. J Clin Invest 2002; 110:923–32.
23. Subbaramaiah K, Howe LR, Zhou XK, Yang P, Hudis CA, Kopelovich L, et al. Pioglitazone, a PPARγ agonist, suppresses CYP19 transcription: evidence for involvement of 15-hydroxyprostaglandin dehydrogenase and BRCA1. Cancer Prev Res 2012;5:1183–94.
24. Subbaramaiah K, Hudis C, Chang SH, Hla T, Dannenberg AJ. EP2 and EP4 receptors regulate aromatase expression in human adipocytes and breast cancer cells. Evidence of a BRCA1 and p300 exchange. J Biol Chem 2008;283:3433–44.
25. Subbaramaiah K, Morris PG, Zhou XK, Morrow M, Du B, Giri D, et al. Increased levels of COX-2 and prostaglandin E2 contribute to elevated aromatase expression in inflamed breast tissue of obese women. Cancer Discov 2012;2:356–65.
26. Chen D, Reierstad S, Lin Z, Lu M, Brooks C, Li N, et al. Prostaglandin E (2) induces breast cancer related aromatase promoters via activation of p38 and c-Jun NH(2)-terminal kinase in adipose fibroblasts. Cancer Res 2007;67:8914–22.
27. Zhao Y, Agarwal VR, Mendelson CR, Simpson ER. Estrogen biosynthesis proximal to a breast tumor is stimulated by PGE2 via cyclic AMP, leading to activation of promoter II of the CYP19 (aromatase) gene. Endocrinology 1996;137:5739–42.
28. Subbaramaiah K, Howe LR, Bhardwaj P, Du B, Gravaghi C, Yantiss RK, et al. Obesity is associated with inflammation and elevated aromatase expression in the mouse mammary gland. Cancer Prev Res (Phila) 2011;4:329–46.
29. Morris PG, Hudis CA, Giri D, Morrow M, Falcone DJ, Zhou XK, et al. Inflammation and increased aromatase expression occur in the breast tissue of obese women with breast cancer. Cancer Prev Res (Phila) 2011;4:1021–9.
30. Chand AL, Simpson ER, Clyne CD. Aromatase expression is increased in BRCA1 mutation carriers. BMC Cancer 2009;9:148.
31. Ghosh S, Lu Y, Katz A, Hu Y, Li R. Tumor suppressor BRCA1 inhibits a breast cancer-associated promoter of the aromatase gene (CYP19) in human adipose stromal cells. Am J Physiol Endocrinol Metab 2007;292:E246–52.
32. Lu M, Chen D, Lin Z, Reierstad S, Trauernicht AM, Boyer TG, et al. BRCA1 negatively regulates the cancer-associated aromatase promoters I.3 and II in breast adipose fibroblasts and malignant epithelial cells. J Clin Endocrinol Metab 2006;91:4514–9.
33. Hu Y, Ghosh S, Amleh A, Yue W, Lu Y, Katz A, et al. Modulation of aromatase expression by BRCA1: a possible link to tissue-specific tumor suppression. Oncogene 2005;24:8343–8.
34. Skelhorne-Gross G, Reid A, Apostoli A, Di Lena M, Rubino R, Peterson N, et al. Stromal adipocyte PPARgamma protects against breast tumorigenesis. Carcinogenesis 2012;33:1412–20.
35. Cheng S, Afif H, Martel-Pelletier J, Pelletier JP, Li X, Farrajota K, et al. Activation of peroxisome proliferator-activated receptor gamma inhibits interleukin-1β-induced membrane-associated prostaglandin E2 synthase-1 expression in human synovial fibroblasts by interfering with Egr-1. J Biol Chem 2004;279:22057–65.
36. Hazra S, Batra RK, Tai HH, Sharma S, Cui X, Dubinett SM. Pioglitazone and rosiglitazone decrease prostaglandin E2 in non–small-cell lung cancer cells by up-regulating 15-hydroxyprostaglandin dehydrogenase. Mol Pharmacol 2007;71:1715–20.
37. Pignatelli M, Cocca C, Santos A, Perez-Castillo A. Enhancement of BRCA1 gene expression by the peroxisome proliferator-activated receptor gamma in the MCF-7 breast cancer cell line. Oncogene 2003;22:5446–50.
38. Subbaramaiah K, Yoshimatsu K, Scherl E, Das KM, Glazier KD, Golijanin D, et al. Microsomal prostaglandin E synthase-1 is over-expressed in inflammatory bowel disease. Evidence for involvement of the transcription factor Egr-1. J Biol Chem 2004;279: 12647–58.
39. Agarwal VR, Bulun SE, Leitch M, Rohrich R, Simpson ER. Use of alternative promoters to express the aromatase cytochrome P450 (CYP19) gene in breast adipose tissues of cancer-free and breast cancer patients. J Clin Endocrinol Metab 1996;81:3843–9.

40. Harada N, Utsumi T, Takagi Y. Tissue-specific expression of the human aromatase cytochrome P-450 gene by alternative use of multiple exons 1 and promoters, and switching of tissue-specific exons 1 in carcinogenesis. Proc Natl Acad Sci U S A 1993;90: 11312–6.
41. Yuan H, Kopelovich L, Yin Y, Lu J, Glazer RI. Drug-targeted inhibition of peroxisome proliferator-activated receptor-gamma enhances the chemopreventive effect of anti-estrogen therapy. Oncotarget 2012;3: 345–56.
42. Qin C, Burghardt R, Smith R, Wormke M, Stewart J, Safe S. Peroxisome proliferator-activated receptor gamma agonists induce proteasome-dependent degradation of cyclin D1 and estrogen receptor alpha in MCF-7 breast cancer cells. Cancer Res 2003;63:958–64.
43. Keller H, Givel F, Perroud M, Wahli W. Signaling cross-talk between peroxisome proliferator-activated receptor/retinoid X receptor and estrogen receptor through estrogen response elements. Mol Endocrinol 1995;9:794–804.
44. Bonofiglio D, Gabriele S, Aquila S, Catalano S, Gentile M, Middea E, et al. Estrogen receptor alpha binds to peroxisome proliferator-activated receptor response element and negatively interferes with peroxisome proliferator-activated receptor gamma signaling in breast cancer cells. Clin Cancer Res 2005;11:6139–47.
45. Wang X, Kilgore MW. Signal cross-talk between estrogen receptor alpha and beta and the peroxisome proliferator-activated receptor gamma1 in MDA-MB-231 and MCF-7 breast cancer cells. Mol Cell Endocrinol 2002;194:123–33.
46. Yee LD, Williams N, Wen P, Young DC, Lester J, Johnson MV, et al. Pilot study of rosiglitazone therapy in women with breast cancer: effects of short-term therapy on tumor tissue and serum markers. Clin Cancer Res 2007;13:246–52.
47. Burstein HJ, Demetri GD, Mueller E, Sarraf P, Spiegelman BM, Winer EP. Use of the peroxisome proliferator-activated receptor (PPAR) gamma ligand troglitazone as treatment for refractory breast cancer: a phase II study. Breast Cancer Res Treat 2003;79:391–7.