Cancer Prevention Research

Commentary

See related article by Chaudhary et al., p. 186

Targeting Estrogen Receptor-β for the Prevention of Nonmelanoma Skin Cancer

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Abstract

The potential for targeting estrogen receptor (ER)-β in various cancer models has been gaining considerable attention in recent years. In this issue of the journal, Chaudhary and colleagues demonstrate markedly decreased ultraviolet B (UVB)-induced skin cancer in a mouse model using a highly specific ER-β agonist, ERB-041. The mechanisms that underlie this strong inhibitory effect are mediated by inhibition of proinflammatory signaling and epithelial–mesenchymal transition (EMT). The changes in EMT were due in part to modulation of WNT/β-catenin signaling. Collectively, the results from these studies provide important new insights into the mechanisms by which the ER-β agonist ERB-041 inhibits UVB-induced skin cancer and opens the door for future studies that could examine combinatorial approaches for UVB-dependent skin cancer chemoprevention. *Cancer Prev Res; 7(2); 182–5. ©2014 AACR.*

Since the original cloning and identification of the human estrogen receptor (ER)-α (1, 2) and numerous studies investigating the functional role of this receptor in cancer models, the targeting of ER-α–dependent signaling in breast cancer and other cancers has become a successful chemopreventive approach used in humans for years. The use of ER-α antagonists such as tamoxifen and raloxifene to inhibit ER-α signaling has become a common approach because of the essential role that ER-α plays in this disease, in particular, promoting cell proliferation. Moreover, in addition to drugs that specifically antagonize ER-α, other drugs that interfere with ER-α–dependent signaling, including aromatase inhibitors, retinoids, metformin, statins, and cyclooxygenase-2 inhibitors, have also shown promise in clinical trials (3), and in some cases, combining these drugs has proven even more efficacious. However, based on controversial results from studies examining the effects of estrogen and progestin in hormone replacement therapy in postmenopausal women, whether ER-α–dependent signaling is the sole target that will provide utility for breast cancer chemoprevention remains unclear (4). This is based on the fact that while there can be an increased risk of breast cancer associated with hormone replacement therapy suggesting that ER-α–dependent signaling mediates this effect, the evidence that ER-α mediates all of the effects of hormone replacement therapy, either positive or negative, is not known. Part of this confusion could be related to the fact that there are two distinctly different isoforms of the ER: ER-α and ER-β, both of which can be activated by estradiol.

The ER-α has been linked with the promotion of breast cancer due in part to its expression in this tissue and the resultant tumors where it is thought to promote cell proliferation. Successful targeting of the ER-α with antagonists prevents its signaling activities that result from normal circulating estrogens. Less well studied in carcinogenesis is ER-β, which was identified in 1996 in rat prostate and ovary (5). The discovery of ER-β led to a complete reevaluation of the role(s) of ERs in breast and many other cancers, due in part to the fact that ER-β could mediate effects similar to ER-α (6). Toward this goal, recent advances in drug discovery led to the identification of novel compounds such as ERB-041 that is a highly selective agonist for ER-β with little to no activity for ER-α (7, 8). Indeed, although activating ER-α with estradiol causes uterotrophic and mammotrophic effects, activation of ER-β with ERB-041 elicits no such effects in either the uterus or the mammary gland (9). Because of the development of specific agonists and antagonists for ER-β, coupled with the availability of *Era-* and *Erb*-null mouse models, there have been significant advances made in recent years in elucidating the role of ER-β in cancer and whether targeting this ER isoform with agonists or antagonists has comparable promise as that achieved with antagonists for ER-α for the treatment and prevention of breast and other cancers.

Interestingly, ER-β is expressed at a much higher level in human epidermis as compared with ER-α (10, 11), suggesting that ER-β could mediate the effects of natural ligands of this receptor in the skin. In addition, more recent studies indicated that papillomas from carcinogen-susceptible mice exhibit downregulation of ER-β and upregulation of ER-α (12). This suggests that during nonmelanoma skin cancer progression, ER-β could function as a tumor suppressor through an undefined mechanism. Indeed,

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evidence from several models supports the hypothesis that agonism of ER-β could prevent diseases, including nonmelanoma skin cancer, that are promoted by proinflammatory signaling. For example, in a human U2OS osteosarcoma cell line overexpressing ER-β, ERB-041 repressed TNF-α–induced expression of proinflammatory cytokines (13). Similar repression of lipopolysaccharide-induced proinflammatory cytokines was also observed in human peripheral blood mononuclear cells (13), a cell type that is known to express ER-β (14, 15). Furthermore, administration of ERB-041 inhibited inflammatory bowel disease and adjuvant-induced arthritis in rat models (9). Because ERB-041–induced inhibition of inflammatory bowel disease was reversed with an ER antagonist, this suggests that the effect was mediated by ER-β (9). In addition, administration of the ER-β agonist ERB-041 markedly increased survival in an experimentally induced sepsis mouse model, and this effect seemed to be mediated in part by inhibition of proinflammatory gene expression. Cho and colleagues performed a series of experiments suggesting a role for ER-β in UV-induced skin cancer. Expression of immunoprotective upregulation of UVA-induced epidermal expression of IFN-γ and interleukin (IL)-12 was inhibited in *Erb*-null mice as compared with controls (16). *Erb*-null mice exposed to UVB also exhibited enhanced expression of the immunosuppressive cytokine IL-10 (16). Using an ER-β antagonist or *Erb*-null mice, they also demonstrated that chemical antagonism of ER-β or genetic disruption of ER-β following irradiation with UV led to increased tumor growth that was due in part to altered immune function (17). Collectively, these studies provide strong support for the examination of ER-β agonists such as ERB-041 in diseases that are mediated, at least in part, by proinflammatory signaling such as skin cancer.

The study reported by Charudhary and colleagues in this issue of the journal is a logical extension that moves the field forward by not only demonstrating the feasibility of targeting ER-β for chemoprevention of UVB-induced skin cancer but also confirming and extending potential new targets for this nuclear receptor (18). The studies included both *in vivo* and *in vitro* analyses that were highly complementary. Agonism of ER-β delayed the onset of UVB-induced skin tumor formation and caused greater than 60% reduction in tumor multiplicity as compared with controls. The decrease in tumor volume was also reflected by a marked decrease in the multiplicity of squamous cell carcinomas (SCC) per mouse by 86%. Although the rationale for the concentration of the ER-β agonist ERB-041 used to elicit this strong chemopreventive effect was not provided, it was applied topically, and thus the issue of bioavailability is not as relevant for this approach. However, extrapolation to other models may need to consider this and dose–response studies might be warranted. Interestingly, ERB-041 actually increased expression of ER-β not only in the tumors but also in adjacent skin and in two human SCC cell lines. This is of interest to note because previous work by others showed that ER-β expression decreases during skin tumor progression while expression of ER-α increases (12). Again the issue of whether ER-β acts as a tumor suppressor gene is intriguing.

Charudhary and colleagues also showed that, similar to what has been reported in other models, ERB-041 inhibited proinflammatory signaling as noted by decreased infiltration of neutrophils, decreased myeloperoxidase activity, and decreased expression of proinflammatory cytokines as compared with controls. These changes were accompanied by decreased presence of $CD11b^{+}/GR1^{+}$ myeloid cells and $F4/80^{+}$ macrophages and reduced phosphorylated ERK1/2 and p38 mitogen-activated protein kinase (MAPK) compared with controls. The decreased proinflammatory response seemed to be mediated by reduced activation of NF-κB in response to ERB-041 treatment, which is consistent with the observed phenotype in ERB-041–treated, UVB-irradiated SKH mice compared with controls. Combined, these data support the view that activating ER-β by ERB-041 can inhibit UVB-induced skin cancer, at least in part, by inhibiting proinflammatory signaling. The finding that activation of ER-β with ERB-041 inhibited UVB-induced skin tumorigenesis by interfering with proinflammatory signaling is consistent with other studies. The mechanism underlying this effect was not definitively shown in this study but could be due to ER-β interfering with NF-κB–dependent signaling as suggested by others (19). This type of interaction has also been noted with other nuclear receptors such as peroxisome proliferator-activated receptors (20).

In addition to the changes observed in proinflammatory signaling, Charudhary and colleagues also examined markers and potential mechanisms that modulate cell proliferation, epithelial–mesenchymal transition (EMT), and angiogenesis. Activation of ER-β with ERB-041 decreased UVB-induced proliferating cell nuclear antigen, cyclin D1, and Ki67 compared with controls, indicating that ER-β can inhibit cell proliferation of UVB-induced skin tumors. In contrast, terminal deoxynucleotidyl transferase–mediated dUTP nick end labeling (TUNEL)-positive cells in UVB-induced skin tumors were increased by treatment with the ER-β agonist ERB-041 compared with controls, indicating that ER-β also facilitates an increase in apoptotic signaling. This was consistent with the increase in expression of BAX (a proapoptotic protein) and the decrease in expression of Bcl2 (an antiapoptotic protein) in UVB-induced skin tumors treated with ERB-041 as compared with controls. Expression of CD31 and vascular endothelial growth factor, markers of angiogenesis, was markedly reduced in UVB-induced skin tumors as compared with controls, indicating that activation of ER-β with ERB-041 can inhibit angiogenesis. This is of interest to note because there can be an overlap in the signaling pathways that regulate EMT and angiogenesis (21). Thus, it is not surprising that ERB-041 increased expression of the epithelial marker E-cadherin and reduced the expression of mesenchymal markers Snail, Slug, Twist, and N-cadherin as compared with controls. These changes in proteins associated with EMT were consistent with the decrease in the differentiation status of the UVB-induced SCC in the ERB-041–treated samples as

compared with control. To confirm the human relevance of these findings, the authors confirmed that the activation of ER-β with ERB-041 inhibited cell migration of both A431 and SCC133, two human SCC cell lines. Moreover, it is also of interest to note that activation of ER-β with ERB-041 caused reduced PI3K activity and a decrease in phosphorylated AKT (p-AKT) compared with controls, because this signaling can contribute to modulation of cell survival, EMT, and angiogenesis as observed in the present studies.

Although inhibition of PI3K and p-AKT can influence cell survival, Charudhary and colleagues also showed that activation of ER-β with ERB-041 caused G_1 arrest and reduced clonogenicity of A431 and SCC13 cells as compared with controls. This provides more support for extrapolation from the mouse models to human models and is highly consistent with many of the changes observed in this study, including the inhibition of cell proliferation and the alteration in EMT. The most interesting finding from these studies were the results demonstrating that activation of ER-β with ERB-041 caused reduced expression of WNT7B, β-catenin, and phosphorylated glycogen synthase kinase-3β (p-GSK3β), and that these changes were accompanied by diminished nuclear localization of β-catenin and reduced expression of c-myc and cyclin D1. This suggests the direct involvement of WNT/β-catenin signaling, which has not been shown to date to occur in response to activation of ER-β. Indeed, inhibition of WNT signaling with XAV939 in A431 and SCC13 cells reduced WNT-dependent signaling, including expression of WNT3A, WNT7B, FZD1, β-catenin, GSK3β, and cyclin D1. Thus, these findings directly link WNT/β-catenin signaling with modulating the effects of activation of ER-β with ERB-041 in UVB-induced skin cancer and human SCC cell lines.

The two most novel findings from these studies are (i) the fact that activation of ER-β with ERB-041 caused an increase in expression of ER-β in skin and skin tumors, and (ii) the chemopreventive effects of activating ER-β with ERB-041 seem to be mediated at least in part by WNT/β-catenin signaling. The former is of interest because expression of ER-β has been noted to be downregulated during skin tumor progression (12). Whether this feature can be used for chemoprevention in UVB-induced skin cancer or other cancer models is unclear. For this reason, the mechanism underlying this effect deserves more experimentation. In addition, the mechanism by which ER-β modulates WNT/β-catenin signaling is exciting and should be examined in greater detail. Altogether, the fact that activating ER-β was so effective for chemoprevention of UVB-induced skin cancer raises the possibility that targeting this receptor in conjunction with other molecular targets may prove useful for this and other cancers.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

Authors' Contributions

Writing, review, and/or revision of the manuscript: P.-L. Yao, F.J. Gonzalez, J.M. Peters

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