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Targeting estrogen receptor-β (ERβ) for the prevention of non-melanoma skin cancer

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Abstract

The potential for targeting estrogen receptor-β (ERβ) 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 ERβ 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 ERβ 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.

Since the original cloning and identification of the human ERα (1, 2) and numerous studies investigating the functional role of this receptor in cancer models, the targeting of ERα-dependent signaling in breast cancer and other cancers has become a successful chemopreventive approach used in humans for years. The use of ERα antagonists such as tamoxifen and raloxifene to inhibit ERα signaling has become a common approach because of the essential role that ERα plays in this disease, in particular, promoting cell proliferation. Moreover, in addition to drugs that specifically antagonize ERα, other drugs that interfere with ERα-dependent signaling, including aromatase inhibitors, retinoids, metformin, statins and cyclooxygenase-2 (COX2) 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 ERα-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

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associated with hormone replacement therapy suggesting that ERα-dependent signaling mediates this effect, the evidence that ERα 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: ERα and ERβ – both of which can be activated by estradiol.

The ERα 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 ERα with antagonists prevents its signaling activities that result from normal circulating estrogens. Less well studied in carcinogenesis is ERβ, which was identified in 1996 in rat prostate and ovary (5). The discovery of ERβ led to a complete re-evaluation of the role(s) of ERs in breast and many other cancers, due in part to the fact that ERβ could mediate effects similar to ERα (6). Towards 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 ERβ with little to no activity for ERα (7, 8). Indeed, while activating ERα with estradiol causes uterotrophic and mammotrophic effects, activation of ERβ with ERB-041 elicits no such effects in either the uterus or mammary gland (9). Due to the development of specific agonists and antagonists for ERβ, 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 ERβ in cancer and whether targeting this ER isoform with agonists or antagonists has comparable promise as that achieved with antagonists for ERα for the treatment and prevention of breast and other cancers.

Interestingly, ERβ is expressed at a much higher level in human epidermis as compared to ERα (10, 11) suggesting that ERβ could mediate the effects of natural ligands of this receptor in the skin. Additionally, more recent studies indicated that papillomas from carcinogen-susceptible mice exhibit downregulation of ERβ and upregulation of ERα (12). This suggests that during non-melanoma skin cancer progression, ERβ could function as a tumor suppressor through an undefined mechanism. Indeed, evidence from several models supports the hypothesis that agonism of ERβ could prevent diseases, including non-melanoma skin cancer, that are promoted by proinflammatory signaling. For example, in a human U2OS osteosarcoma cell line overexpressing ERβ, ERB-041 repressed tumor necrosis factor-α-induced expression of proinflammatory cytokines (13). Similar repression of lipopolysaccharide-induced proinflammatory cytokines was also observed in human peripheral blood mononuclear cells (PBMCs; ref. 13), a cell type that is known to express ERβ (14, 15). Further, administration of ERB-041 inhibited inflammatory bowel disease and adjuvant-induced arthritis in rat models (9). Since ERB-041-induced inhibition of inflammatory bowel disease was reversed with an ER antagonist, this suggests that the effect was mediated by ERβ (9). Additionally, administration of the ERβ agonist ERB-041 markedly increased survival in an experimentally-induced sepsis mouse model, and this effect appeared to be mediated in part by inhibition of proinflammatory gene expression. Cho and colleagues performed a series of experiments suggesting a role for ERβ in UV-induced skin cancer. Expression of immunoprotective up-regulation of UVA-induced epidermal expression of interferon-γ and interleukin-12 (IL12) was inhibited in *Erb*-null mice as compared to controls (16). *Erb*-null mice exposed to UVB also exhibited enhanced

expression of the immunosuppressive cytokine IL10 (16). Using an ERβ antagonist or *Erb*-null mice, they also demonstrated that chemical antagonism of ERβ or genetic disruption of ERβ 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 ERβ agonists such as ERB-041 in diseases that are mediated, at least in part, by pro-inflammatory signaling such as skin cancer.

The study reported by Chaudhary 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 ERβ 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 ERβ delayed the onset of UVB-induced skin tumor formation and caused greater than 60% reduction in tumor multiplicity as compared to 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%. While the rationale for the concentration of the ERβ 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 ERβ 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 ERβ expression decreases during skin tumor progression while expression of ERα increases (12). Again the issue of whether ERβ acts as a tumor suppressor gene is intriguing.

Chaudhary et al. 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 to controls. These changes were accompanied by decreased presence of CD11b+/GR1+-myeloid cells and F4/80+-macrophages and reduced phosphorylated ERK1/2 and p38 MAPK compared to controls. The decreased proinflammatory response appeared 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 to controls. Combined, these data support the view that activating ERβ by ERB-041 can inhibit UVB-induced skin cancer, at least in part, by inhibiting proinflammatory signaling. The finding that activation of ERβ 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 ERβ 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, Chaudhary and colleagues also examined markers and potential mechanisms that modulate cell proliferation, EMT and angiogenesis. Activation of ERβ with ERB-041 decreased UVB-induced proliferating cell nuclear antigen, Cyclin D1 and Ki67 compared to controls

indicating that ERβ can inhibit cell proliferation of UVB-induced skin tumors. In contrast, TUNEL-positive cells in UVB-induced skin tumors were increased by treatment with the ERβ agonist ERB-041 compared to controls indicating that ERβ also facilitates an increase in apoptotic signaling. This was consistent with the increase in expression of BAX (a pro-apoptotic protein) and the decrease in expression of Bcl2 (an anti-apoptotic protein) in UVB-induced skin tumors treated with ERB-041 as compared to controls. Expression of CD31 and vascular endothelial growth factor, markers of angiogenesis were markedly reduced in UVB-induced skin tumors as compared to controls indicating that activation of ERβ with ERB-041 can inhibit angiogenesis. This is of interest to note because there can be 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 to 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 to control. To confirm the human relevance of these findings, the authors confirmed that activation of ERβ 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 ERβ with ERB-041 caused reduced PI3K activity and a decrease in phosphorylated AKT (p-AKT) compared to controls, because this signaling can contribute to modulation of cell survival, EMT and angiogenesis as observed in the present studies.

While inhibition of PI3K and p-AKT can influence cell survival, Chaudhary and colleagues also showed that activation of ERβ with ERB-041 caused G1 arrest and reduced clonogenicity of A431 and SCC13 cells as compared to 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 ERβ 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 ERβ. 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 ERβ with ERB-041 in UVB-induced skin cancer and human SCC cell lines.

The two most novel findings from these studies are: 1) the fact that activation of ERβ with ERB-041 caused an increase in expression of ERβ in skin and skin tumors, and 2) the chemopreventive effects of activating ERβ with ERB-041 appear to be mediated at least in part by WNT/β-catenin signaling. The former is of interest because expression of ERβ has been noted to be downregulated during skin tumor progression (12). Whether this feature can be utilized for chemoprevention in UVB-induced skin cancer or other cancer models is unclear. For this reason, the mechanism underlying this effect deserves more experimentation. Additionally, the mechanism by which ERβ modulates WNT/β-catenin

signaling is exciting and should be examined in greater detail. Combined, the fact that activating ERβ 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.

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