Efp as a new molecular target for breast cancer therapy

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Breast cancer is the most common type of cancer among women in Western countries. Breast cancer is commonly treated by various combinations of surgery, radiation therapy, chemotherapy and hormone therapy. Prognosis and selection of therapy may be influenced by the age and menopausal status of the patient, stage of the disease, histological and nuclear grade of the primary tumor, estrogen-receptor (ER) and progesterone-receptor (PR) status, HER2/*neu* status, and inherited mutations in genes such as *BRCA1* and *BRCA2*.

Among several types of treatment, hormone therapy is generally considered as adjuvant systemic therapy for a postoperative patient with localized breast cancer or as first-line treatment for a postmenopausal patient with newly diagnosed metastatic disease. Most often, a selective estrogen receptor modulator (SERM) tamoxifen has been clinically used for years. Another SERM raloxifene has been recently shown to be effective in reducing the risk of invasive breast cancer [1]. The benefit of SERMs is restricted only to women with ER/PR-positive breast tumors. Evaluation of hormone receptor status as well as histological study of the tumor specimen is clinically important for predicting response to hormone therapy. Patients with advanced stages of metastases who have received an antiestrogen within the past year are given a second-line hormone therapy. Selective aromatase inhibitors, such as anastrozole and letrozole, are used for those advanced patients, especially in the postmenopausal stage.

Antiestrogenic agents, however, are not basically effective in ER-negative tumors. Breast cancer that is initially responsive to an antiestrogenic agent sometimes acquires resistance against hormone therapy in advanced stage of the disease. Although tamoxifen is beneficial in ER/PR-positive tumors, there are, however, several critical side effects including development of endometrial cancer or an increased incidence of venous thrombosis and strokes. Venous thromboembolic events and vaginal bleeding events are lower in patients treated with aromatase inhibitors such as anastrozole [2]; however, osteoporosis due to estrogen deprivation is a significant side effect. Thus, new targeted therapy with minimal side effects must be developed for effective breast cancer treatment.

Why do tumors in advanced stages acquire resistance against hormone therapy? The molecular clue may lie in the mechanism of the estrogen actions in our bodies. We hypothesized that identification of new downstream molecules of the estrogen receptor signaling pathway may provide some answers. Using genomic-binding site cloning techniques, our group has identified several target genes of the estrogen receptor that include estrogen-responsive elements in their promotor-enhancer regions. Among the ER-downstream molecules, Efp, an estrogen-responsive RING finger protein of 630 amino acids, has the structure of the RING-finger B-box-coiled-coil (RBCC) motif including a RING finger, B-box-coiled-coil region and SPRY domain [3]. Interestingly, several other cancer-related RING finger proteins, including PML, as a responsible gene for acute pre-myelocytic leukemia when it fuses with RARα, and BRCA1, as a tumor suppressor gene for familial breast and ovarian cancers, have been identified. Efp is predominantly expressed in estrogen target tissues including mammary glands and uteri [4], and also in breast cancers [5]. Efp is essential for growth of female organs such as the uterus, since mice deficient in the Efp gene have an underdeveloped uterus [6].

The important question is whether Efp is a key molecule for breast tumor growth. The answer primarily came from experiments using nude mice inoculated with human breast cancer MCF7 cells [7]. When the tumor volume reached 300 mm^3, mice were treated with ovariectomy or with antisense or sense oligonucleotides. Efp antisense oligonucleotide efficiently reduced the size of tumor generated by MCF7 cells in those recipient mice. The growth of MCF7 cells was initially estrogen dependent; however, the cells overexpressing Efp could grow even in ovariectomized nude mice that have very low levels of circulating estrogen. These results implicate Efp as a critical determinant factor for progression of breast cancer of advanced stages, as increased expression levels of Efp could provide estrogen-independent tumor growth.

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If Efp could function as an accelerator for breast tumor growth, what is the mechanism of Efp function in tumor cell proliferation? Recent advances of molecular research have revealed that some RING finger proteins bind to ubiquitin-conjugating enzymes and form complexes called ubiquitin ligases. Ubiquitin ligases bring specific degradation signals to protein substrates, i.e. a multi-ubiquitin chain linked to the substrates. Polyubiquitinated substrate proteins are then recognized and subsequently destroyed in the proteosome. This proteasome-dependent proteolytic system is important to eliminate misfolded or abnormal proteins as well as to confer short half-lives on specific normal proteins such as mitotic cyclins whose critical concentrations must change promptly with alterations in the state of a cell. To investigate whether Efp plays a role as a RING finger type of ubiquitin ligase and has a particular substrate, we performed yeast two-hybrid screening from a mouse embryo cDNA library using Efp as a bait. These screens led to the identification of 14-3-3σ as an Efp interacting clone [7]. 14-3-3σ is transcribed in a p53-dependent manner and has been shown to arrest the cell cycle, especially at the G_2/M phase, by sequestrating cdc2 in the cytoplasm [8]. Although 14-3-3σ is expressed in mammary glands, reduced levels of 14-3-3σ seem to be related to breast malignancy, as down-regulation of the protein [9] or hypermethylation of its promoter region [10] is reported in breast cancer. Experimental evidence suggests Efp may provide unlimited proliferation of breast cancer cells by accelerated destruction of 14-3-3σ that functions as one of the cell cycle brakes. In that mechanism, estrogen may be the initial factor that up-regulates Efp levels; however, once Efp levels are constitutively increased, estrogen may no longer be required to promote cell cycle progression by regulating Efp expression. This estrogen-independent overexpression of Efp causes down-regulation of 14-3-3σ, allowing tumor cells to continue proliferation. Thus, overexpression of Efp may be one of the reasons for advanced tumor resistance to hormone therapy.

It remains to be determined whether Efp plays a similar critical role in human breast tumor progression. Nonetheless, based on our findings in the mouse model system, we anticipate Efp could be used as a potential molecular target for clinical application that provides a promising future direction for breast cancer treatment. For example, if and when selective inhibitors for Efp are developed and utilized, such agents have the potential to reduce breast tumor growth with minimal side effects—a significant problem observed in current antiestrogenic therapies. Tumors resistant to previous antiestrogenic therapies could be regulated by anti-Efp agents if the tumors express Efp. Finally, another interesting issue is whether Efp could promote tumor growth in other endocrine-related cancers such as endometrial cancer. Future research efforts will elucidate a more precise mechanism of action of Efp in endocrine-related cancers and develop more powerful antiestrogenic agents that selectively inhibit Efp function.

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