Research Highlights

Highlights from the latest articles in personalized breast cancer treatment

Coregulator recruitment determines ER- α -mediated gene regulation in response to ligands

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Evaluation of: Romano A, Adriaens M, Kuenen S *et al.*: Identification of novel ER- α target genes in breast cancer cells: gene- and cell-selective co-regulator recruitment at target promoters determines the response to 17 β -estradiol and tamoxifen. *Mol. Cell Endocrinol.* 314, 90–100 (2010).

Estrogen signaling can occur through a so-called classical pathway in which ligand-activated estrogen receptors (ERs) interact with estrogen-responsive elements associated with target genes and bring coactivators or corepressors to the ER complex to regulate these genes [1]. The ER- α antagonistic properties of tamoxifen in the breast forms the basis for the treatment of ER- α -positive breast cancer. Importantly, tamoxifen displays tissue-specific agonist/antagonist activity, which is thought to reflect, in part, the cellular availability of coregulators in different cell types [2]. For example, the agonistic activity in bone tissue is a desired therapeutic profile, while the agonistic activity on the endometrium is a undesired therapeutic profile. The interplay between ER and coregulators at the level of chromatin had not previously been studied at a global level.

Romano *et al.* used the combined technique of chromatin immunoprecipitation (ChIP) with promoter DNA array hybridization (ChIP-chip) to identify ER- α -binding regions in T47D breast cancer cells [3]. They identified 904 ER- α -interacting promoter regions at the whole-genome level. To correlate that 17 β -estradiol and tamoxifen

mediated up- or down-regulation of transcription with selective recruitment of different coregulators, they examined mRNA levels and recruitment of coactivators or corepressors for 10 genes derived from the ChIP-chip analysis. The recruitment of coactivators or corepressors were examined by ChIP using a pool of antibodies against the coactivators p300, CBP and SRC-1, or a pool of antibodies against the corepressors NcoR and SMRT, respectively. They demonstrated that selective recruitment of coactivators or corepressors determines the subsequent ER- α -mediated transcriptional activation or repression. In addition, they demonstrated that differential coregulator recruitment reflects the opposite regulation of genes by tamoxifen in breast and endometrial cancer cells. To further substantiate the association between transcriptional regulation and coregulator recruitment, they overexpressed either the coactivator SRC-1 or the corepressor SMRT. For a number of genes, the transcriptional response to tamoxifen in T47D breast cancer cells or the endometrial cancer cell line ECC1 could be modified or even inverted by overexpression of coregulators. In summary, these observations support the hypothesis that coregulators play key roles in the response to a given agonist or antagonist in ER- α -mediated signaling, providing potential targets for further research and therapeutic intervention. However, recent studies on global mapping of ER- α -binding regions have demonstrated that only a minority of ER- α -binding sites are located proximally to gene promoters [4,5]. Thus, additional studies on the interplay between ER and

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coregulators at nonpromoter proximal regions will be needed to gain further insight into whether the above findings can be translated into a general property of ER-mediated transcription.

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FKBPL modulates ER- α signaling and influences the response to endocrine therapy in breast cancer

Evaluation of: McKeen HD, Byrne C, Jithesh PV *et al.*: FKBPL regulates estrogen receptor signaling and determines response to endocrine therapy. *Cancer Res.* 70, 1090–1100 (2010).

Estrogen receptor (ER)- α expression is one of the indicators of good prognosis and the target for endocrine therapy in breast cancer. The antagonist tamoxifen is a standard adjuvant endocrine treatment as it blocks ER- α signaling in breast cancer cells. However, 30% of patients with ER- α -positive tumors do not respond to tamoxifen, and 30–40% of patients receiving adjuvant tamoxifen eventually relapse [1]. Thus, tamoxifen resistance is a significant clinical problem and there exists an unmet medical need for novel therapeutic strategies for breast cancer. Understanding the mechanisms of tamoxifen resistance will aid the development of improved therapeutic strategies including identification of novel therapeutic targets.

FKBPL, a novel immunophilin-like protein, has been demonstrated to inhibit cell proliferation of lymphoma cells [2] and associate with glucocorticoid receptor/Hsp90 chaperone complexes,

thus modulating glucocorticoid receptor signaling [3]. In this study by McKeen *et al.*, FKBPL was found to associate with ER- α in the Hsp90 chaperone complex [4]. The authors demonstrated that overexpression of FKBPL inhibits breast cancer cell growth and potentiates the effect of the antagonists tamoxifen and fulvestrant (the latter being a pure estrogen antagonist as opposed to tamoxifen, which is antagonistic in the breast but agonistic in, e.g., bone) with regard to inhibition of cell growth. Furthermore, FKBPL silencing leads to an increase in ER- α levels and a decrease in p21 levels, resulting in estrogen-independent signaling and resistance to endocrine therapy [5]. They demonstrated that higher FKBPL mRNA levels are linked to a better survival outcome and increased distant metastasis-free survival in clinical datasets, demonstrating that FKBPL could serve as a potential prognostic marker of breast cancer. This study identifies a novel role for FKBPL in regulating ER- α signaling and, ultimately, the response to endocrine therapy. Clearly, replication of the association between FKBPL expression and disease outcome is necessary, including studies of FKBPL protein levels, in large randomized trials is critical to establish whether FKBPL

expression can be used as a prognostic marker. Furthermore, the interplay between FKBPL and ER- α is complex, involving estrogen regulation of FKBPL as well as FKBPL regulation of ER- α signaling. Thus, further studies of the cross-talk between ER- α and FKBPL are necessary, including the effects of antagonist treatment on FKBPL levels.

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FOXM1 is a target gene for ER- α and contributes to endocrine resistance in breast cancer

Evaluation of: Millour J, Constantinidou D, Stavropoulou AV *et al.*: *FOXM1* is a transcriptional target of ER α and has a critical role in breast cancer endocrine sensitivity and resistance. *Oncogene* 29(20), 2983–2995 (2010).

Breast cancer is one of the most common malignancies in women, and is the second leading cause of cancer death for women in the USA, exceeded only by lung cancer. Despite substantial improvements in the treatment of breast cancer, resistance to therapy remains a major clinical problem [1].

Most breast tumors are, at least initially, dependent on estrogen for growth, and the introduction of tamoxifen and other ER antagonists for the treatment of breast cancer represents a milestone for the treatment of the disease. However, resistance to this endocrine therapy represents a significant problem and identification of the factors that contribute to, or alleviate, endocrine resistance may potentially facilitate prognosis, stratify therapy and aid the development of novel therapeutic agents.

FOXM1 is a member of the forkhead box (Fox) family of transcription factors [2]. Evidence has emerged that FOXM1 is overexpressed in breast cancer as well as in carcinomas of other origins [3]. Millour *et al.* report a novel role of *FOXM1* as an estrogen/ER- α target gene and a mediator of endocrine therapy resistance [4]. Their results show that FOXM1 mRNA and protein levels are upregulated by estrogen and downregulated by the ER-antagonists tamoxifen and fulvestrant in ER- α -positive breast cancer cells, demonstrating that *FOXM1* is a target of ER- α . They identified an estrogen-response element-like site within the *FOXM1* promoter to confer E2 action and demonstrate binding of ER- α to this site, providing evidence that *FOXM1* is a direct target of ER- α . It is demonstrated that silencing of FOXM1 by RNAi overcomes acquired tamoxifen resistance, and that ectopic expression of FOXM1 can abrogate the antiproliferative effects of tamoxifen, establishing FOXM1 as a mediator of anti-estrogen resistance. Moreover, tamoxifen repressed FOXM1 expression in endocrine-sensitive but not -resistant breast carcinoma cell lines, suggesting that uncoupling FOXM1 from ER control is one mechanism to achieve endocrine resistance.

This study validates FOXM1 as a potential drug target in breast cancer, particularly in combination with endocrine therapy. Characterizing FOXM1 in relation to breast cancer could aid the development of personalized treatments for breast cancer patients. Further studies to determine whether FOXM1 overexpression is associated with poor prognosis in ER- α -positive tumors treated with tamoxifen as sole adjuvant therapy would further validate FOXM1 as a drug target in breast cancer.

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Identification of genes conferring anti-estrogen resistance in breast cancer

Evaluation of: van Agthoven T, Sieuwerts AM, Meijer D *et al.*: Selective recruitment of breast cancer anti-estrogen resistance genes and relevance for breast cancer progression and tamoxifen therapy response. *Endocr. Relat. Cancer* 17, 215–230 (2010).

Acquired resistance is a major problem limiting the clinical benefit of endocrine therapy for estrogen receptor (ER)- α -positive breast cancer. Several mechanisms have been proposed to account for the observed resistance, including changes in the expression of ER- α or ER- β , altered levels of coregulatory proteins, and the influences of cellular

kinase signal transduction pathways [1]. The majority of identified mechanisms have been derived from studies in anti-estrogen-resistant breast cancer cell lines rather than from patients with anti-estrogen-resistant cancer [2,3], emphasizing the need for confirmation that these pathways are determinants of tamoxifen resistance in clinical samples.

van Agthoven *et al.* used a functional genetic screening approach to identify individual genes that contribute to, or are responsible for, resistance to anti-estrogens [4]. In the study, cDNA expression libraries are introduced into estrogen-dependent human breast cancer ZR-75-1 cells by means of retroviral infection. Cells that acquire a gene product enabling growth in the presence of anti-estrogens can be recovered and the gene conferring resistance can be identified through DNA sequencing after appropriate amplification. According to their set criteria, they identified a total of 15 breast cancer anti-estrogen resistance (*BCAR*) genes individually yielding resistance in ZR-75-1 breast cancer cells. They demonstrated that overexpression of four identified genes (*HRAS*, *RAF1*, *PDGFRA* and *PDGFRB*) in ZR-75-1 cells leads to an increase in cell proliferation in

the presence of anti-estrogens. For several novel *BCAR* genes, they further investigated the association of *BCAR* genes with tamoxifen resistance in patients. They included 289 tumors from patients who received tamoxifen monotherapy as first-line treatment for advanced disease and tumor specimens of 518 lymph node-negative patients who had not received adjuvant systemic therapy in the study. They show that *HRAS* and *PDGFRA* mRNA levels are associated with tumor aggressiveness, there is no association of *RAF1*, *PDGFRB* or *NRG1* mRNA levels with tamoxifen resistance, and that low *EIF1* mRNA levels in breast tumors are associated with poor clinical outcome following tamoxifen treatment. Hence, the results derived from this cell model cannot be fully translated to the clinical setting. Clearly, further studies, including large numbers of breast cancer patient samples, are needed to further validate the *BCAR* genes identified in the applied cell model. It will be important to consider that there may be multiple resistance pathways, with some being relevant in some individuals but not relevant

in others. In addition, detailed studies of the molecular mechanisms of the *BCAR* gene products in relation to estrogen signaling will contribute to our understanding of resistance to endocrine therapy in breast cancer.

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