

ESTROGEN RECEPTOR AND BREAST CANCER

Resistance to tamoxifen treatment is observed in about half of the recurrences in breast cancer where the anti-estrogen tamoxifen acquires agonistic properties for transactivation of ER α . Tamoxifen resistant breast cancer often remains sensitive to other endocrine treatments and the tumors still express ER α . The clinical benefits of identifying pathways underlying resistance can therefore be profound, as a way to initiate personalized medicine where tamoxifen is only given to patients that are going to respond. We aim for molecular understanding of the mechanism of resistance to tamoxifen and other anti-estrogens and to develop tools to use as clinical decision markers in anti-estrogen therapy of breast cancer patients.

Target(s) of ER α phosphorylated at Serine 305 by Protein kinase A

The target(s) for ER α -S305P were identified from comparing the transcriptomes of U2OS containing a stably integrated ER α wt or the S305A mutant that cannot be phosphorylated by Protein Kinase A (PKA). The cells were treated with tamoxifen and PKA was activated for up to 24 hours. This resulted in 709 hits specific for ER α -S305P under tamoxifen conditions. Tumor relevant hits were further identified by comparison with expression profiles of 58 primary breast tumors, of which the metastases were treated with tamoxifen, and of which concurrent ER α -S305P staining was known. This resulted in 17 targets associated with presence of ER α -S305P and 6 targets associated with early recurrence in this tumor set. These numbers were reduced to respectively six and two by comparing these targets with expression profiles of tamoxifen-insensitive breast tumors in a meta-analysis study. The resulting eight hits that fulfilled all criteria are subject of further studies on the tumor relevant target(s) of ER α -S305P.

Properties of ER α phosphorylated at Serine 305 by Protein kinase A

Protein stability studies indicated that the phosphorylation of ER α at Serine 305 by PKA is rapidly lost during tamoxifen exposure as the result of dephosphorylation or degradation. We will study this ligand-specific and conformation-dependent loss of ER α -Serine 305P in more detail. In collaboration with Pamgene (Den Bosch), we observed that ER α phosphorylated at Serine 305 recruits cofactors more efficiently in the presence of tamoxifen than the non-phosphorylated form of ER α . This further underlines the significance of phosphorylation of ER α at Serine 305 as a mechanism of tamoxifen resistance.

Clinical relevance of phosphorylation of ER α In collaboration with Marleen Kok and Sabine Linn (Division of Molecular Biology) and Goran Landberg and colleagues (University of Lund), we found that phosphorylation of the Estrogen Receptor at Serine 305 predicted a poor response in a randomized trial that evaluated adjuvant tamoxifen treatment as well as in metastatic breast cancer patients. A combined measurement of two independent markers, phosphorylation of the Estrogen Receptor at Serine 305 by Protein kinase A and expression of the kinase PAK-1, identified approximately 60% of all tamoxifen resistant cases of ER-positive breast cancer. The opposite has been found for phosphorylation of Serine 118 in ER α , which is associated with an activated ER and with a benefit from treatment with tamoxifen.

We now studied two activated kinases, i.e. phospho-ERK1/2 and phospho-PKA in order to obtain a more detailed view of the *in vivo* association between ERK1/2, PKA, PAK1 and ER α S118-P and ER α S305-P as well as response to tamoxifen. Phospho-ERK1/2 (pERK1/2) was significantly linked to increased phosphorylation of ER α S118-P as well as ER α S305-P. There was an association between cytoplasmic pPKA and pERK1/2, suggesting a crosstalk between these two kinase pathways. Surprisingly, PAK1 did not correlate to any of the ER α phosphorylation sites or analysed kinases. In an analysis of subgroups, patients with high expression of ER α S118-P still had a better recurrence-free survival compared to control patients. Overall, our results indicate that resistance to tamoxifen is largely determined by two opposite effects of phosphorylation of ER α .



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Publications

Zwart W, de Leeuw R, Rondaij M, Neeffjes J, Mancini M and Michalides R. *The hinge region of the human Estrogen Receptor determines functional synergy between AF-1 and AF-2 in the quantitative response to estradiol and tamoxifen.* J.Cell Science 2010;123:1253-1261

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