

Estrogen Receptor Phosphorylation Leads to Changes in Receptor Interactions Consistent with Enhanced Estrogen Agonism and Reduced Tamoxifen Activity

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Abstract

Posttranslational modifications of estrogen receptor (ER) are emerging as important regulatory elements of cross talk between different intracellular and extracellular signaling pathways. Interestingly, ER phosphorylation has been implicated in the ligand-independent effects of the receptor and in tamoxifen resistance of breast tumors. In our studies, Western analysis of endogenous ER phosphorylation at S118 and S167 reveal ligand-dependant and ligand-specific phosphorylation in parental MCF-7. Interestingly, this ligand dependence is lost in the tamoxifen resistant, MCF-7 Her2/neu cells. Further, using highly purified components and sensitive fluorescence techniques we show that ER phosphorylation events alter receptor action through unique mechanisms. Receptor phosphorylation by Src and PKA increases affinity for E2 but does not alter TOT binding. Surprisingly, ER phosphorylation by MAPK does not alter E2 binding, but decreases binding of TOT. Affinity of the receptor for the estrogen response element (A2 ERE) is also altered by phosphorylation in a ligand-specific manner, with a decrease in affinity of MAPK and Src phosphorylated ER in presence of TOT. In addition, ER phosphorylation by MAPK, Akt or PKA exhibit an increase in recruitment of SRC3 receptor interaction domain to the DNA-bound receptor in presence of E2; however, no recruitment was observed with vehicle or TOT. Taken together, these results suggest that ER phosphorylation should lead to an increase in E2 agonism and a decrease in TOT activity. Thus, through our studies, we have identified distinct differences in ER phosphorylation that are dependent on ligand and tamoxifen-sensitive or resistant cell state (Her2 status). Further, we demonstrate that phosphorylation alters receptor functions including ligand, DNA and coactivator binding, effecting changes that might enhance E2 agonism and contribute to tamoxifen resistance.

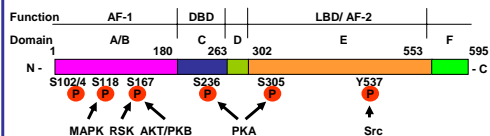
Aim

An *in vitro* model for consequences of ER phosphorylation on receptor function.

Approach

- Purified full length ER expressed using Baculovirus expression system.
- *In vitro* phosphorylation of ER verification by immuno assay.
- Fluorescence-based assay for Ligand, DNA and Coactivator binding.

ER Phosphorylation



Phosphorylation Status of Endogenous ER

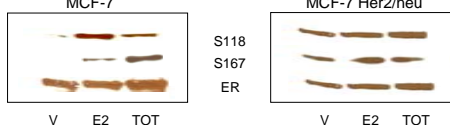
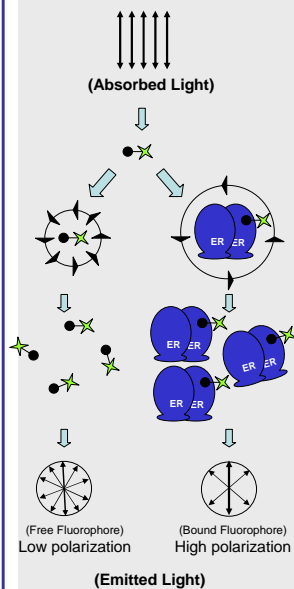


Figure 1. Panel A. Western analysis of endogenous ER from MCF-7 and MCF-7(HER2/neu) cells treated with vehicle or E2 or TOT.

Fluorescent Polarization



Ligand Binding

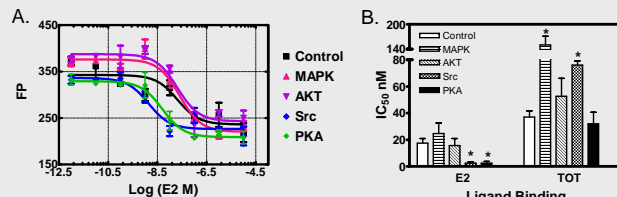


Figure 2. Panel A. Fluorescent polarization assays of E2 binding to ER (unphosphorylated or phosphorylated) and polarization of Fluoromone was measured with increasing E2 concentration. Panel B. Comparison of phosphorylation effects on IC50s for E2 and TOT.

DNA Binding

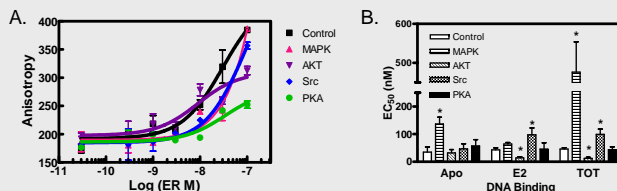
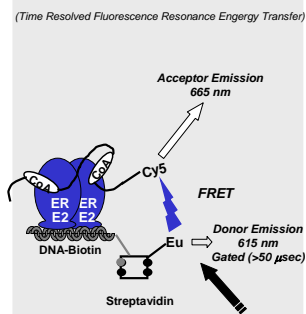


Figure 3. Panel A. Fluorescent polarization assays for E2-ER binding to fluorescein-labeled ERE. Anisotropy values were plotted against log of ER concentration. Panel B. Comparison of EC50s for different phosphorylation states in absence of ligand and in the presence of E2 and TOT.

TR-FRET



Coactivator Binding

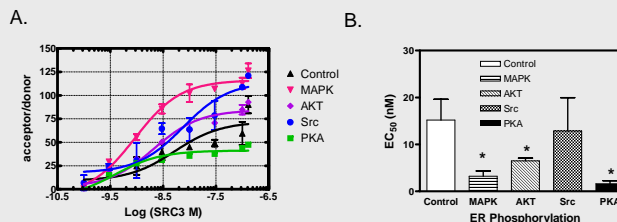
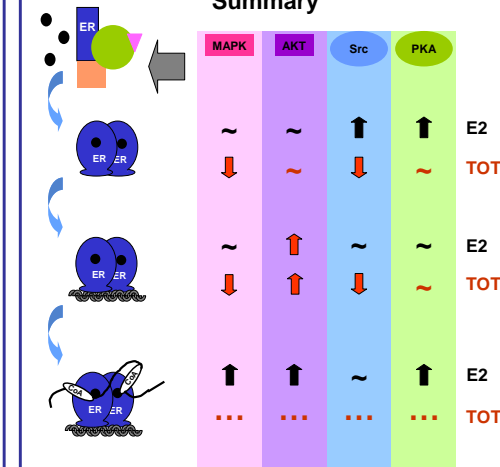


Figure 4. Panel A. Time Resolved Fluorescent Resonance Energy Transfer assay for coactivator binding. Increasing concentrations of Cy5-labeled SRC1 RID were incubated with ER, E2, biotinylated ERE and Europium-labeled streptavidin. Donor and acceptor emissions were measured and expressed as acceptor to donor ratio against SRC3 concentrations. Panel B. Comparison of EC50s for SRC1 RID binding to ER with different phosphorylation states in presence of E2.

Summary



Discussion

ER phosphorylation by each kinase has a characteristic and distinct effect on the three ER functions [See Summary, above]

- MAPK – decreases TOT affinity, ER-DNA affinity in presence of TOT but increases binding of SRC3 to ER-E2
- AKT – effect is more modest, enhancing SRC1 binding to ER-E2 and further increases ER-DNA interaction in presence of E2 and TOT
- Src – enhances E2 binding and reduces ER-TOT binding to DNA
- PKA – enhances E2 binding and ER-E2 binding of SRC3
- Phosphorylation differentially affect the magnitude as well as the affinity of coactivator binding, indicating differences in complex conformations [see Summary]

Thus, different kinase signaling pathways could enhance agonistic action of E2 through unique modifications of ER. In addition, ER phosphorylation by MAPK could decrease TOT action.

Taken together, our studies indicate that the phosphorylation status of endogenous ER and alteration of ER function by these modifications may dictate physiological outcome.

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