An Exciting ERα in Breast Cancer Treatment

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I. Abstract

According to the American Cancer Society, one in eight U.S. women will develop breast cancer in their lifetime. Strikingly, many of these women share a significant genetic commonality. It has been shown that many breast cancer patients test positive for high levels of Estrogen Receptor (ER_α), a protein that regulates the differentiation and maintenance of neural, skeletal, cardiovascular, and reproductive tissues in their cells. ER_α aids in the process of DNA transcription as a transcription factor. The activation of ER_α occurs when a ligand, estradiol, diffuses through the lipid membrane and binds to the active site at the Ligand Binding Domain (LBD) while ER_α is in the cytoplasm. Initially the LBD is inhibited by a chaperone protein, which immediately disjoins from ER_α to allow estradiol to bind. The LBD is located at amino acid residues 303 to 552 highlighted in the model designed by the Westosha Central High School SMART Team using 3D printing technology. Afterwards, the complex is transported into the nucleus where the DNA Binding Domain (DBD) of the ER_α protein binds to DNA and commences gene transcription. An over abundance of ER_α leads to excessive transcription which may cause breast cancer. Therefore, in the treatment of breast cancer, inhibiting or degrading ER_α is of immediate interest as a therapy.

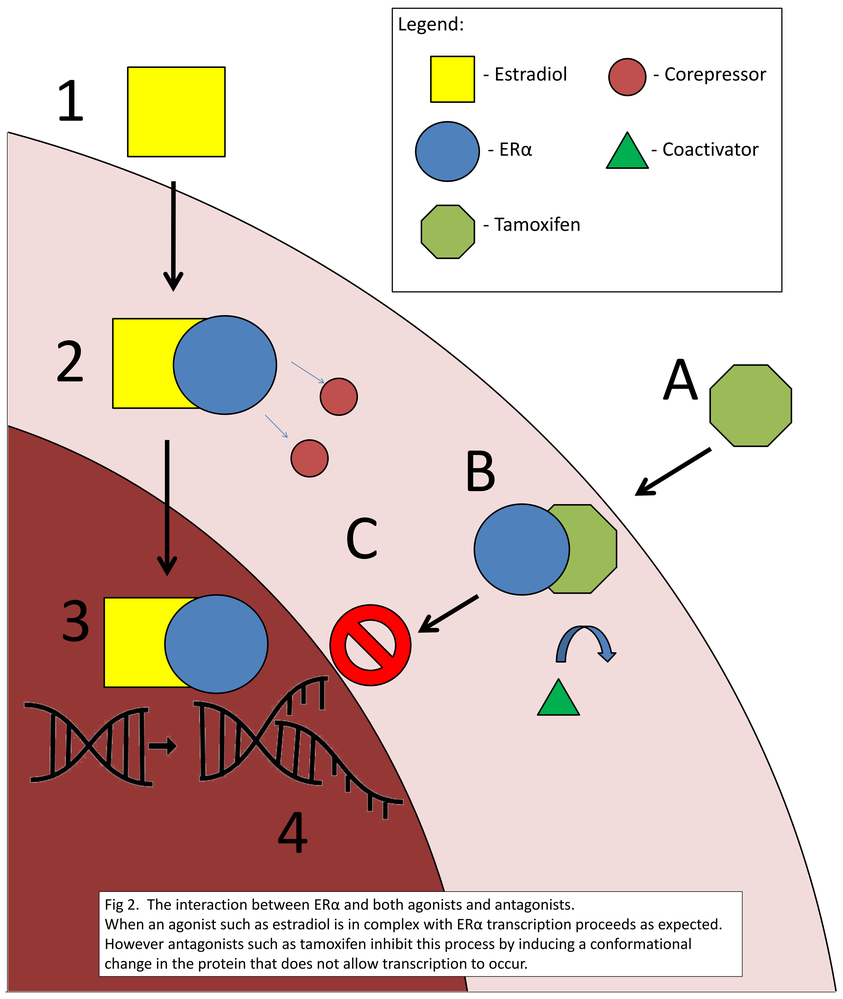

Fig 2. The interaction between ERα and both agonists and antagonists.
When an agonist such as estradiol is in complex with ERα transcription proceeds as expected. However antagonists such as tamoxifen inhibit this process by inducing a conformational change in the protein that does not allow transcription to occur.

II. Protein Structure

Amino Acids of Note

Lys362, Glu542 are amino acids that bind chaperones. These chaperones are disjoined when agonizing or antagonizing ligands bind to the protein. These amino acids are not featured in the model created using 3D printing technology because they are utilized in a previous stage of the protein's dynamic binding activity.

Arg394, Glu353, His524 define the binding site for ligands such as Tamoxifen or Estradiol in the so called "deep and shallow pockets". In the case of estradiol these amino acids interact with –OH groups found on the estradiol to anchor the ligand. This satisfies the hydrophobicity of the pockets, creating a conformational change in Helix 12.

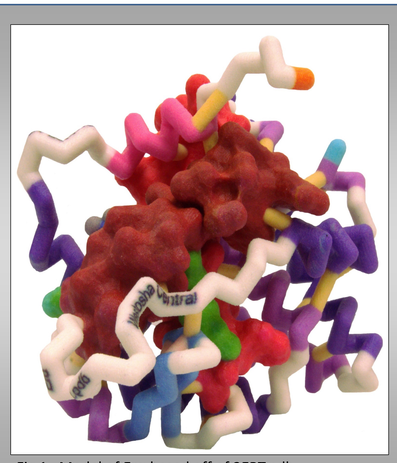

Fig 1. Model of Erα based off of 3ERT.pdb

Helix 12

The conformation of Helix 12 determines whether the protein is an agonist to transcription or an antagonist. Helix 12 binds to the cleft formed between helices 3 and 5 using the LXXLL Motif . This recognition sequence of Helix 12 interacts with one of the coactivator binding sites and prevents coactivators from binding. Residues 536 through 540 on ER_α are LYDLL. The LXXLL motif is conserved among multiple coactivators that directly interact with ER_α. By modulating the conformation of Helix 12, it is possible to inhibit or degrade ER_α, thus arresting progression of the disease. By binding certain ligands to the LBD, the protein can be further stabilized or be degraded depending on the dynamics of the protein-ligand complex.

III. Experimental Data

In this experiment, a fluorescent, hydrophobic dye that binds to the hydrophobic patches typically located on the interior of the protein is added to a solution containing soluble ERa. The more fluorescent dye bound to the protein, the greater the RFU will be. As the temperature rises, the protein gradually denatures and unfolds, exposing the interior hydrophobic area. Therefore, the point on the graph at which the plotted lines suddenly increase greatly marks the approximate temperature at which ER is thermally denatured. The graph plots three lines showing the effect of temperature on RFUs of DMSO, and Tamoxifen and Estradiol each bound to ER. DMSO is the solvent in which Estradiol and Tamoxifen are dissolved and act as the control. Estradiol is the natural ligand of ER and acts as an agonist. Tamoxifen is one of the first generation therapies used, and acts as an antagonist. In the absence of a ligand (DMSO control), hydrophobic patches on the protein are exposed, as is evidenced by high RFU values even at low temperatures, suggesting a highly dynamic and less stable protein conformation. Tamoxifen is similar to the Estradiol in its ability to stabilize ER, however the right shift between Tamoxifen and Estradiol, of about 5°C, implies when Estradiol is bound there is either better binding or more stable conformation . Through this graph it is shown how dynamic an enzyme ER is based on how much its shape changes due to the binding of a ligand.

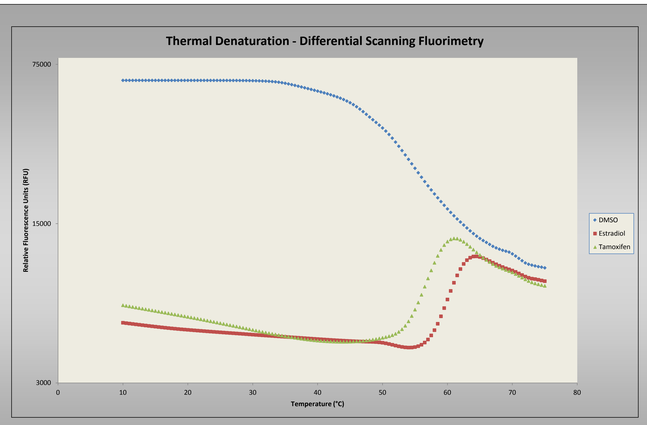

Fig 3[1]. Thermal Denaturation data exhibits the stabilizing ability of both estradiol and tamoxifen in complex in ERα.

IV. Mechanism of Action

Normal Pathway(with Estradiol)
1) The ligand, estradiol, diffuses through the lipid membrane and binds to the active site at the Ligand Binding Domain (LBD) while ER_α is in the cytoplasm.
2) As estradiol interacts with ER, the protein undergoes conformational changes which allow the corepressors to disjoin from ER and for the coactivators to interact with ER_α.
3) ER_α-Estradiol complex enters the nucleus and its DBD binds to DNA acting as a transcription factor.
4) The gene activated by ER is transcribed and eventually translated into the respective protein.
Synthesized Pathway(with Tamoxifen)
A)Tamoxifen, a first generation breast cancer drug that acts as an antagonist for ER_α, enters the cell.
B)As it binds to ER, it induces an antagonist conformation of the complex.
Tamoxifen causes Helix 12 to move into the cleft between Helix 3 and Helix 5, preventing coactivators from binding.
C)Because the coactivators can not interact with ER_α, transcription of the target gene is not initiated.
*There is a lively debate now in occurrence on whether or not ER enters the nucleus after Tamoxifen binds to it.

V. Biological Significance

The overarching goal of research into ER_α is the possibility of limiting its overexpression which results in breast cancer. Several options have been proposed as to accomplish this goal, however currently the best method is still in deliberation. The plausible options are to inhibit ER_α before it enters the nucleus or to degrade estradiol, ( ) before it even has a chance to bind, which would stop ER_α from allowing for transcription. For the developing stages of research it has been shown that ER_α can be inhibited. To further research efforts, the understanding of its structure and its relationship to functionality are necessary to discern next. The importance of this research is to advance therapies for breast cancer patients experiencing an overexpression of ER_α.

VI. Citation

1. Bordsky, O. et al (2014) [Thermal Denaturaion of ERa] Unpublished Raw Data

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