Environmental estrogens and breast cancer therapeutics: Characterization of the diverse ligand binding properties of the estrogen receptor

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In vertebrates, natural estrogens play a critical role in regulating normal reproduction, development and growth via their interaction with the estrogen receptor (ER). But the ER, a member of the nuclear steroid receptor superfamily, also binds a remarkably diverse set of non-physiologic ligands including environmental estrogens, phytoestrogens, and antiestrogens. Environmental estrogens are compounds, such as pesticides, which act as estrogen mimics and alter the reproductive function of wildlife. The estrogenic behavior of these compounds has proven difficult to predict from their structures; many of these hormone mimics bear little structural resemblance to natural estrogens. Estrogenic compounds and natural estrogens bind to the ER and stimulate transcription at genes containing estrogen responsive elements (ERE). Antiestrogens also bind tightly to the ER but do not activate transcription. These compounds, such as tamoxifen, are currently used to treat breast cancer.

The available structural information on the ER *(1)(2)* shows that the structural conformation of the ER is ligand dependent. However, the details of the conformational changes that allow the ER to tightly bind such a diverse array of compounds, and tune the degree of gene activation, are not understood. The goal of this project is to develop a structural understanding of how the ER binds several environmental estrogens, estrogens and antiestrogens, and to use this information to develop a predictive assay. Because of the sheer number of ligands the ER binds, we are interested in developing a scanning technique that provides structural information.

In the context of the available structural data*(1)(2),* we are using multidimensional nuclear magnetic resonance spectroscopy (NMR) studies of complexes of these compounds with ligand binding domain (LBD) of the ER to investigate changes which occur on ligand binding. Our strategy of chemical shift mapping by NMR works as follows: the ER-LBD is prepared ^{15}N isotopically labeled by expression in *E. coli.* in media containing $(^{15}NH_4)_2SO_4$ as the sole nitrogen source, ^{15}N-^{1}H correlation spectra are taken of different ligand-ER-LBD complexes, partial chemical shift assignments are made, and ^{15}N-^{1}H chemical shift changes are monitored. Several techniques for NMR studies of large proteins have been implemented to address linewidth problems including pulse sequence strategies and deuteration.

Chemical shifts are exquisitely sensitive to changes in the local chemical environment and in conformation, and provide information on each amino acid in the ER-LBD. Monitoring chemical shift changes allows an assessment of the ligand dependency of conformational changes, and allows rapid analysis of many complexes. Our strategy will assist in identifying estrogenic activity in compounds before they are introduced into the environment, as well as in identifying compounds with potential therapeutic benefit for treating breast cancer.

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2. A. Shiau. *et al.*, Cell 95, 927-937, (1998).