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Development of a stably transfected cell line to screen for potential endocrine disrupting chemicals

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Intro Estrogens are steroid hormones that diffuse across cell membranes to interact with estrogen receptors (ER) in the nucleus. An estrogen-bound ER acts as a transcription factor to regulate gene transcription in a cell. Synthetic chemicals with estrogenic activity (called xenoestrogens) also interact with the estrogen receptor (ER), interfering with the body's delicate balance of hormones. There is a great need to characterize these chemicals as well as screen for until now unknown xenoestrogens. Methods We developed a stably transfected MCF-7 human breast cancer cell line that, in combination with reporter gene assays, will aid in screening for and characterization of these chemicals. MCF-7 cells were transfected with DNA containing two copies of an estrogen response element (ERE) from the promoter of the vitellogenin gene. The ERE was linked to either LacZ or the firefly luciferase gene (both reporter genes). When a chemical interacts with the ER, the ER will associate with the ERE to begin the transcription of the reporter gene that will encodes for a quantifiable protein. Cells were co-transfected with a plasmid containing the neomycin gene, conferring resistance to the antibiotic G418 to allow for selection of stable transformants. Following transfection, cells were treated with G418 and grown for 18-25 days until colonies formed. Colonies were selected and a dose response of estradiol, the body's most common estrogen, was performed on the clones. Those populations showing the greatest sensitivity to estrogen were chosen for future use. Ongoing Studies Future experiments will include using reporter gene assays to screen common chemicals, such as the pesticide carbaryl, for estrogenic activity both alone and in environmentally relevant combinations of other xenoestrogens. Secondly, bioassays on clinical samples of peritoneal fluid from women with an estrogen-dependent disease will be performed and compared to control samples to determine total estrogenic activity.