DISSECTING THE ESTROGEN RESPONSE OF BREAST CANCER CELLS BY MICROARRAY ANALYSIS AND PROMOTER MINING

Chin-Yo Lin

Genome Institute, Singapore

Estrogens belong to a family of hormones that binds to nuclear receptors that in turn interact with regulatory elements in the chromatin and the basal transcriptional machinery and affect gene expression in target tissues. In addition to their roles in human development and physiology, estrogens and estrogen receptors (ER's) have been shown to be important for the development, progression, and treatment of breast cancer, the most common form of malignant disease in women worldwide. To better understand the molecular mechanisms of cellular responses to estrogens, we used high density DNA microarrays to examine the gene expression profiles of ER+ T47D breast tumor cells following treatment with estradiol. As was previously shown by Ström *et al*., growth-arrested T47D cells re-entered the cell cycle upon hormone treatment. This was confirmed by distinct temporal expression profiles of cell proliferation and estrogen-responsive genes. Furthermore, the estrogen-responsive expression profiles were disrupted by the induction of a know repressor of estrogen response, HES-1. The hundreds of estrogen-responsive genes identified in the microarray experiments include those that function in cell cycle regulation and proliferation, signaling pathways, metabolism, transcriptional regulation, and cellular structures, as well as previously unknown and uncharacterized genes. By conventional methods, characterization of hundreds of genes to identify direct and relevant targets and pathways would indeed be a daunting task, both in terms of efficiency and economy. To overcome the limitations posed by traditional approaches, we have employed an informatics strategy to identify direct targets of estrogen receptors and elucidate downstream events and pathways. This computational approach is based on previous observations that many genes that have been shown to be responsive to estrogens contain conserved sequences, or so called estrogen response elements (ERE's), in their promoters that encode binding sites for the estrogen receptors. The general strategy, made possible by the availability of human genome sequences and informatics tools that have been developed, is to isolate promoter sequences from responsive genes and then query the sequences for the presence of EREs. Thus far, the combination of microarray-based gene expression analysis and subsequent promoter mining has yielded over one hundred early estrogen-responsive genes with putative ERE's. In contrast, the number of reported human genes with putative ERE's, discovered and characterized by standard biochemical and molecular methods during the past decade, is approximately forty. These results suggest that the strategy described here may be employed for comprehensive high-throughput identification and validation of estrogen responsive genes as well as targets of the nuclear hormone receptor family and other transcription factors. Such systemic approaches will not only extend our understanding of the complex molecular mechanisms and signaling networks of hormone responses, particularly in human

diseases, but also provide experimental platforms for clinical and drug discovery applications. More details can be found at
http://sdmc.krdl.org.sg/ERE/