Influence of estrogens and antiestrogens on the expression of selected hormone-responsive genes

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Abstract

Estrogen exerts a primary regulatory role on a wide variety of physiological processes in different tissues and organs. Agonistic ad antagonistic compounds are widely used in human health and, therefore, a deep understanding of their mechanisms of action at the molecular level is mandatory. The effect of 17β-estradiol and three antiestrogenic drugs, comprising two selective estrogen receptor modulator (SERM, 4-OH-tamoxifen, Raloxifene) and the pure antiestrogen ICI 182,780, on genome-wide gene expression levels was evaluated in breast carcinoma cell lines by DNA microarray analysis. Different clusters of genes, showing specific coregulation patterns, were found. First, several groups of genes displaying temporal-specific up- or down-regulation were characterized. Second, clusters of genes responding to different antiestrogenic drugs in either antagonistic or agonistic fashion, were found. Genes responding specifically to antiestrogens, but not to estrogen, were also identified. In addition, each individual compound exhibited a very specific gene regulation. Bioinformatic analysis was applied to the regulatory sequences of different groups of genes and confirmed that specific pathways and secondary responses are activated at each temporal point and in response to different compounds. Our results underline the complexity of genomic responses to estrogen in breast cancer cells and strongly suggest that the molecular characterization of estrogen agonists and antagonists used in human therapy should be carefully studied.

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1. Physiological role of endogenous estrogens

Estrogen regulates a wide variety of physiological processes that affect multiple tissues in the organism. Most relevant effects concern cell growth, differentiation and specific organ functions. Of course, estrogen
primary regulatory action is on reproductive tissues. However, important estrogen effects are known on other tissues outside the reproductive system, including bone, liver, the cardiovascular system and brain [1].

Most of the actions of estrogens are mediated by two intracellular estrogen receptor (ER) subtypes, ERα and ERβ, that are members of a large family of ligand-dependent, DNA-binding transcription factors, called collectively nuclear receptors. Consequently, the primary level of action of estrogen is the regulation of gene expression that, as detailed below, affects directly or indirectly hundreds to thousands of genes in each target cell type.

Estrogen displays differential effects in different tissues. In the uterus and mammary gland, estrogens are strong mitogens, inducing massive cell proliferation at puberty as well as during fertility cycling. In the bone estrogens exert a trophic effect, since they favour osteoblast proliferation rather than osteoclast, so that their action results in gain in bone mass and strength. In the cardiovascular system, estrogen may show protection against cardiovascular disease. In the brain, estrogen receptors act both during development, by determining formation of a number of dimorphic nuclei, and in the adult, favouring neuronal survival and stabilizing cognitive ability.

2. Exogenous estrogens, selective estrogen receptor modulators and endocrine-related cancers

Natural estrogens, as well as a number of estrogen receptor binding compounds, called collectively selective estrogen receptor modulators (SERM) are used in human therapy for a number of treatments. First, the most important application is represented by human breast cancer. Patients whose tumour tissues are diagnosed to contain ER are successfully treated with drugs that (at least on the breast) show anti-estrogenic action, such as tamoxifen, raloxifene and more recently fulvestrant (ICI 182,780), which is not a SERM in the strict sense, as it targets ER for rapid degradation [2]. Notably, SERM compounds show a marked tissue-specificity: for example, tamoxifen is antiestrogenic for breast carcinoma cells, but has estrogen-like activities in bone and brain, i.e. it behaves as an agonist – rather than antagonist – in these tissues. Second, post-menopausal women are treated with hormone replacement therapy (HRT), to avoid a number of possible risks connected with the loss of endogenous estrogen, like osteoporosis and neurodegenerative diseases and to alleviate menopausal symptoms. Treatment with estrogen or SERM, however, may increase the risk of breast and endometrial cancer, due to the proliferative effects of estrogen and some SERM on these tissues. Finally, estrogen-like compounds from environmental sources, such as phytoestrogens in vegetable foods, estrogens in meats and by-products of industrial manufacturing, such as the recently recognized bisphenol, which is present in plastic containers for beverages and foods [3] exert a variable influence on human well-being and cancer risk.

3. Estrogens and gene transcription regulation

Estrogens regulate gene transcription by several different mechanisms [4,5]. The first is called the “classical” genomic pathway; the estrogen receptors (ER) are activated by ligand binding, that allows ER to dissociate from hsp inhibitory complexes, dimerize and bind to their cognate DNA elements (ERE—estrogen response element) within regulatory regions of target genes. Here, receptors interact with a class of nuclear proteins, called coactivators, whose action is to activate transcription. A number of genes that are regulated by ER as an early response do not contain EREs. Protein–protein interaction of ER with other transcription factors, such as AP-1, Sp1 or NFkB, that has been widely documented, provides explanation. Either tethering of ER to other transcription factors bound to their elements, or “squelching” of these factors out of the interaction with DNA or cofactors, has been documented [6]. A third mechanism involves the rapid action of estrogen on a fraction of ER, localized to a juxtamembrane compartment, that activates the MAPK cascade, finally acting on gene transcription independently of nuclear ER effects [7]. The recent discovery of a G-linked seven-helix receptor, located in the endoplasmic reticulum membrane, that may represent an additional (and completely different from those known) estrogen receptor will possibly further complicate this matter in the near future [8].
4. Agonistic/antagonistic activity of SERMS on human tissues

The antagonistic action of SERM has been understood in detail. In breast cancer cells, tamoxifen- or raloxifene-bound ER binds to estrogen target gene regulatory sequences, but the peculiar conformation of the ligand binding domain, due to the chemical structure of the SERM in the binding pocket, lowers the affinity of ER interaction with coactivators. Instead, a high affinity interaction is seen with NCoR and SMRT corepressor complexes ([9,10] and refs. therein; [11–13]).

As a result, estrogen target genes are repressed and, in the case of breast cancer cells, proliferation stops. The balance of the affinity of ER for the different coactivator proteins versus corepressor proteins present in a given cell depends exquisitely on the conformational change that any particular ligand induces into the ER ligand binding domain. On this basis, different agonistic/antagonistic balance of any SERM in different tissues may be explained by relative availability of coactivator and corepressor proteins [14].

Different behaviour of SERM can be observed also at the level of different gene regulation in the same cell, i.e. SERM may show antagonistic action on some genes but agonist on other. Clearly, the presence of other transcription factor at a given promoter may favour the interaction of ER with either coactivator or corepressor.

5. Gene expression regulation by estrogens and SERMS in breast cancer cells

In order to obtain a genomic view of gene regulation by estrogen and antiestrogen in breast cancer, we have used gene expression profiling with microarrays of human breast carcinoma cell lines treated with estradiol and SERM. Using ZR57.1 cells, the kinetics of gene response to 17β-estradiol was followed, in two-hours steps, up to 32 h using a 10 K cdNA microarray platform [15]. Significant regulation, either up- or down-regulation, was found in approximately 4% of the genes examined, with different time kinetics. Early, early-delayed and delayed genes could be clustered from these studies, where each group can be drawn back to a specific mechanisms of action, as reviewed above. Interestingly, sets of estrogen-regulated genes can identify estrogen receptor-positive cases in microarray data from breast cancer tissues [16].

In the same model, the effects of treatment with three antagonistic (in the breast) compounds (tamoxifen, raloxifene and ICI 182,780) was assayed either alone or in combination with 17β-estradiol [17]. In this case, 24- and 72-h of treatment were studied. Out of the 601 genes found to be differentially expressed in the presence of estrogen, half were found to respond antagonistically to at least one compound. However, 30 genes responded agonistically to at least one compound and 176 genes did not show response to antiestrogens.

In addition, there were 52 genes showing regulation by antiestrogenic compounds, but not by estrogen (Fig. 1).

The compounds studied showed a marked individuality of behaviour, in terms of gene regulation. For example, only 133 of the 291 “antagonistic” genes did so in response to all three compounds and of the 52 antiestrogen-specific genes, only 8 were in common to all three drugs.

These data confirm that gene-specific actions of ER could be modulated by ligands, and have been confirmed further by independent studies of other groups [18,19]. A bioinformatic analysis of the regulatory sequences of the genes found here brought to the quite unexpected result that only 86 (14%) of these show evidence of an estrogen-responsive element (ERE). Differential enrichment of other transcription factor binding sites was observed in different group of genes showing specific patterns of regulation by antiestrogens.

6. Functional gene classification in response to estrogen/antiestrogen

Functional classification of the gene sets identified in response to antiestrogen was attempted, according to “Gene Ontology” classification. A significant number of genes belonging to the antagonist cluster is involved in cell cycle, cell proliferation, protein homeostasis, cell communication and signal transduction, cellular transport, transcription and RNA processing. Instead, the “agonistic” group included genes involved in signal transduction. In the group of estrogen-independent genes also were significantly overrepresented genes belonging to the classes of transcriptional regulation and development. This analysis confirmed that there is
a specific logic for the different behaviour of genes in response to antiestrogen, that probably (since antiestrogen are not natural ligands) reflects different modes of response to the natural estrogenic ligands [20].

One of the most intriguing group of genes observed in our studies, is represented by genes showing quite rapid down-regulation by estrogen. Curiously, many of these were genes that behaved agonistically in response to antiestrogen. Recently, evidences were provided that some gene exists where estrogen-activated ER recruits NCoR corepressor complexes, instead of coactivators [21]. We are currently employing a number of bioinformatic algorithms to understand whether the 5'-regulatory region of these genes can explain how differential regulation is attained.

7. Conclusions

Our genome-wide exploration of estrogen and antiestrogen effects on breast cancer cells has given a view of the complexity of genomic responses evoked by these molecules. It is possible that many different mechanisms are acting together to determine the final pattern of gene regulation observed. Primary regulation by direct ERE-ER interaction, or protein–protein interaction with different transcription factors as well as responses due to “nongenomic” MAPK activation, are possibly cooperating in short-term genomic regulation. Secondary responses, through the regulation of other transcription factor synthesis or stability, will superimpose to primary regulation, making the picture extremely complex. Nonetheless, by expanding the studies to other breast and nonbreast cell lines, and by using wider microarrays platform and more potent bioinformatic tools, we can predict that the different pathways operating in response to estrogen and SERM will be solved.

This biological knowledge will be essential in order to develop new, biologically targeted SERM compounds with desired behaviour in different tissues and organs and to characterize the response of cells and tissues to drugs during therapy (Fig. 2). Clinical data are accumulating in support of the differential sensitivity of ER positive tumours to endocrine treatments based on their biological characteristics. For instance, it is now accepted that ER positive tumours overexpressing the oncogene HER-2 derive lower benefit from
tamoxifen as compared to HER-2 negative tumors [22], and the same phenomenon has been suggested also for ER positive/progesterone receptor (PR) negative as compared to ER positive/PR positive tumors [23]. Interestingly, no difference based on HER-2 and PR status has been demonstrated with third generation aromatase inhibitors [24,25], suggesting that these new drugs may overcome tamoxifen resistance in this subset of breast cancer patients.

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References


