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Workshop 2.5

Use of gene expression profiling to understand the transcriptional program associated with estrogen-induced uterine growth*

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Abstract: The use of gene expression data in predictive and mechanistic toxicology is hindered by a lack of information on the relationships between transcriptional events and physiological and pathological changes. We discuss the analysis of these relationships using the rodent uterotrophic response as a model experimental system for estrogen-induced uterine growth.

INTRODUCTION

The successful survival and growth of an organism depends upon highly regulated cellular and molecular interactions that are orchestrated by coordinated patterns of, and changes in, gene expression. Not unexpectedly therefore, the adverse health effects that may result from exposure to xenobiotics are invariably associated, either directly or indirectly, with alterations in gene expression. There are now available a variety of technology platforms that permit measurement of simultaneous changes in the expression of many hundreds or many thousands of genes, and in the context of toxicology the application of such methods for multiple transcript profiling is termed "toxicogenomics". The judicious application of such approaches will facilitate the more detailed interrogation of the mechanisms through which adverse effects are induced by chemicals and drugs, with the promise in the future of informing hazard identification and risk assessment paradigms.

With the advent of new genomic technologies, it is now possible to identify, rapidly and holistically, the molecular alterations associated with exposure to toxicants. However, the increase in the rate at which these data can be generated has not been matched by corresponding advances in the ability to interpret them into biologically meaningful information. The use of gene expression data in mechanistic and predictive toxicology is hindered by a lack of information on the relationships between transcriptional events and physiological and pathological changes. We have begun to analyze these relationships in studies designed to improve our understanding of how estrogenic chemicals induce uterine growth and cellular differentiation. Using the rodent uterotrophic response to estrogenic compounds as a model experimental system [1], we are applying transcript profiling to elucidate the molecular events that lead to uterine growth induced by a reference estrogen (17β-estradiol).

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MOLECULAR MECHANISMS OF ESTROGEN SIGNALING

The cellular effects of estrogenic compounds are mediated by two subtypes of estrogen receptor, ERα and ERβ. These receptors belong to a superfamily of nuclear receptors that act as ligand-activated transcription factors [2–4] and show tissue-specific distribution patterns, with both ER subtypes being expressed in the rodent uterus. In the classical model of estrogen receptor action, estrogenic compounds diffuse across the plasma membrane and bind to ER, inducing the receptor to dimerize and bind to specific estrogen response elements (EREs) in the promoter regions of target genes to regulate gene expression. However, it is now established that the mode of action of ER is much more complex than this classical model.

Both ER subtypes possess two transactivation domains, termed AF-1 and AF-2. In addition to ligand-dependent activation via the AF-2 region, ER may influence gene expression in a ligand-independent manner via the AF-1 domain. Activation of AF-1 is due, at least in part, to phosphorylation of conserved serine residues. As AF-1 and AF-2 may interact in a synergistic fashion to influence promoter activity [5], there is considerable potential for interplay between ER ligand binding and the activation of kinase signaling cascades to influence gene expression. ER modulates gene expression in the nucleus by forming protein complexes with cofactors [6,7]. These ER-associated cofactors facilitate and augment the recruitment of the basal transcriptional machinery, and thus mediate ER-dependent transcription. The tissue-specific expression of these cofactor proteins may provide another level at which specificity in the effects of estrogens in different tissues can be achieved. Indeed, it has been shown that tissue-determined differences in the action of selective estrogen receptor modulators (SERMs), such as tamoxifen, which is used to treat hormone-dependent breast cancer, may be due to differences in the tissue-specific expression profile of ER cofactors [8]. In addition to ERE-bound ER forming complexes with cofactors, ER may itself be capable of exhibiting cofactor activity, as it has been shown to interact with DNA-bound AP-1 transcription factor and determine transcriptional responses in a manner that does not depend on its own direct interaction with DNA [9,10].

In addition to the nuclear activity of ER, rapid extranuclear actions have been demonstrated, including activation of mitogen-activated protein kinases that have pleiotropic cellular effects at both genomic and nongenomic levels [11,12]. The realization that ERs employ diverse molecular mechanisms for regulating gene expression has generated a need for more holistic studies of alterations in gene expression in response to estrogenic chemicals, for whereas a relatively small number of genes have been described as being directly ER-responsive, estrogens may yet influence the expression of many more genes via the activation of alternative signaling cascades.

GENOMIC APPROACHES FOR UNDERSTANDING THE MOLECULAR MECHANISMS OF ESTROGEN ACTION

Transcript profiling represents a powerful tool for measuring the expression levels of thousands of genes in response to given toxicants [13]. It may thus be used to implicate certain genes in the mode of action of a toxicant, and these leads can be further studied to link them to more classical toxicity endpoints. Gene expression profiles identified using established toxicants may subsequently be compared to those seen with novel compounds to formulate predictive hypotheses on the mode of action of the novel compound. For example, the expression profile in response to estrogen may be readily compared to that obtained with other estrogenic (or potentially estrogenic) compounds which may differ in their relative affinities for ER and thus differ in potency and potential to induce toxicity. These approaches may be further enhanced by combining transcript profiling with transgenic technologies, using either transgenic animals or adapted cell lines. By applying transcript profiling to animal models in which a component of a pathway (e.g., ERα and/or ERβ) relevant to a given mode of action is perturbed, the direct target genes can be separated from those whose regulation is not directly governed by that pathway. Cell lines facilitate advanced mechanistic studies, as they may be engineered to knock in (or out) a par-

ticular pathway of interest in isolation, which can be more readily studied in a homogeneous cell type in which interference from other pathways may be minimized.

GENOMIC ANALYSIS OF THE TRANSCRIPTIONAL PROGRAM ASSOCIATED WITH ESTROGEN-INDUCED UTERINE GROWTH

One consequence of ER being capable of mediating transcription of its target genes in a variety of ways is that estrogenic compounds may induce a broad spectrum of cellular responses. However, the molecular mechanisms leading to pleiotropic estrogen-induced cellular responses in vivo have not yet been well described. Recent studies employing microarray analyses of gene expression changes at a single time point in the rodent uterus have revealed a range of novel genes that are responsive to estrogenic chemicals [14,15]. These genes encode proteins involved in a wide range of functions and biological pathways. Additional kinetic studies, in which the temporal pattern of gene expression in response to estrogen is compared to gravimetric and histopathological measurements of uterine growth, have great potential for enhancing our understanding of the relationships between estrogen-induced transcriptional networks and physiological responses. This type of approach is exemplified by the elucidation of the transcriptional program associated with the response of human fibroblasts to serum [16]. Hierarchical clustering of genes into groups on the basis of their temporal patterns of expression provided a detailed view of the molecular events involved in the control of the transition from G_0 to a proliferating state. This study also revealed an unexpected relationship between many components of the transcriptional program and the physiology of wound repair. A similar kinetic approach was used to reveal the co-expression of DNA replication fork genes during estrogen-induced mitogenesis in a human breast cancer cell line [17]. It is noteworthy that transcript profiling experiments can generate enormous data sets, the interpretation of which relies heavily on computational methods for classifying and displaying gene expression patterns [18]. Identification of common molecular or biological functions amongst large numbers of temporally coregulated genes requires the use of additional bioinformatic tools, such as functional annotation databases (e.g., Affymetrix NetAffx™ Analysis Center [19]; <http://www.affymetrix.com/analysis/index.affx>) and biological pathway maps (e.g., KEGG Encyclopedia; <http://www.genome.ad.jp/kegg/kegg2.html>). One additional consideration in designing experiments for a genomic analysis of the transcriptional program associated with estrogen-induced uterine growth is the choice of dosing regime for a given estrogenic chemical. Use of a single dose of estrogen may be necessary in order to avoid the complexity inherent with overlapping waves of gene expression that may be activated following the three successive daily administrations of E_2 that are usually employed using immature animals in the standard rodent uterotrophic assay [1]. Additional controls, comparing changes in gene expression occurring naturally during increased estrogen production at the onset of puberty during normal mouse development with the transcriptional programs associated with uterine-growth in immature mice exposed to an exogenous estrogen (i.e., a chemically induced uterotrophic response), should facilitate our understanding of the consequences of short- vs. long-term changes in hormonally regulated gene expression.

SUMMARY AND PERSPECTIVES

The genomic analysis of transcriptional programs associated with estrogen-induced uterine growth has the potential to reveal a wealth of novel information on the molecular events initiated by exposure of the rodent uterus to exogenous estrogen. This approach should also allow a comprehensive analysis of the molecular mechanisms of xenoestrogen (e.g., genistein and diethylstilbesterol) action. Furthermore, the identification of coregulated clusters of estrogen-responsive genes offers the possibility of gaining novel insights into the molecular mechanisms that regulate their expression. This could be achieved through extensive bioinformatic analyses of their regulatory regions [13]. The recent completion of the first draft rodent genome sequence [20] should greatly facilitate this phase of analysis. Characterizing

the biological signaling pathways that regulate transcriptional responses to estrogenic compounds will lead to a better understanding of their molecular mechanisms of action.

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