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Hormone-regulated transcriptomes: Lessons learned from estrogen signaling pathways in breast cancer cells

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ABSTRACT

Recent rapid advances in next generation sequencing technologies have expanded our understanding of steroid hormone signaling to a genome-wide level. In this review, we discuss the use of a novel genomic approach, global nuclear run-on coupled with massively parallel sequencing (GRO-seq), to explore new facets of the steroid hormone-regulated transcriptome, especially estrogen responses in breast cancer cells. GRO-seq is a high throughput sequencing method adapted from conventional nuclear run-on methodologies, which is used to obtain a map of the position and orientation of all transcriptionally engaged RNA polymerases across the genome with extremely high spatial resolution. GRO-seq, which is an excellent tool for examining transcriptional responses to extracellular stimuli, has been used to comprehensively assay the effects of estrogen signaling on the transcriptome of ERα-positive MCF-7 human breast cancer cells. These studies have revealed new details about estrogen-dependent transcriptional regulation, including effects on transcription by all three RNA polymerases, complex transcriptional dynamics in response to estrogen signaling, and identification novel, unannotated non-coding RNAs. Collectively, these studies have been useful in discerning the molecular logic of the estrogen-regulated mitogenic response.

1. Introduction

1.1. Estrogen signaling and estrogen receptors

Estrogens are a class of endogenous hormones that play critical roles in diverse aspects of human physiology in females and males, including sexual development, reproduction, cardiovascular and neuronal activity, as well as liver, fat, and bone metabolism. Dysregulation of estrogen signaling can lead to a variety of human diseases, such as breast and uterine cancers, osteoporosis, cardiovascular and neurodegenerative diseases, and insulin resistance (Deroo and Korach, 2006; Cadenas and Bolt, 2012). The actions of estrogens are mediated through estrogen receptor proteins (ERα and ERβ), which are members of the nuclear receptor superfamily. They function as ligand-regulated nuclear transcription factors, but also as key components of cytoplasmic membrane-initiated signaling cascades (Bjornstrom and Sjoberg, 2005; Levin, 2009; Welboren et al., 2009). The two ER isoforms are encoded from two separate genes in two different chromosomal locations. They share about 96% homology between their DNA binding domains (DBDs), but only 56% homology between their ligand binding domains (LBDs) and 28% homology between their amino-terminal activation functions 1 (AF-1s) (Warner et al., 1999). ERα and ERβ can homo- or hetero-dimerize (Bai and Gust, 2009; Cheung et al., 2003), indicating that the two isoforms can act together or separately. The two ER isoforms share overlapping functions due, in part, to the significant homology of their DNA binding domains. However, ERα and ERβ have different expression patterns in tissues, distinguishable differences in structure, and distinct biological functions (Harrington et al., 2003; Katzenellenbogen and Katzenellenbogen, 2000; Pearce and Jordan, 2004; Pfaffl et al., 2001). The signaling and transcriptional effects of ERs underlie the aforementioned physiological and pathological effects of the estrogen signaling pathways.

Abbreviations: ChIP, chromatin immunoprecipitation; E2, 17β-estradiol; ERα, estrogen receptor alpha; ERBS, estrogen receptor α binding site; eRNA, enhancer RNA; GRO-seq, global nuclear run-on and sequencing; HMM, hidden Markov model lncRNA, long non-coding RNAs; mRNA, messenger RNA; Pol (I/II/III), RNA polymerase (I, II, III); rRNA, ribosomal RNA; SERM, selective estrogen-receptor modulator; tRNA, transfer RNA.

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1.2. Estrogen signaling and breast cancer

Estrogen and ERs have been shown to play a mitogenic role in breast, uterine, and ovarian cancers (Deroo and Korach, 2006; Pearce and Jordan, 2004). The etiology of these hormone-responsive cancers has shown that estrogen stimulates the unregulated cellular proliferation in these target tissues, which can interfere with normal physiological processes and can drive tumor formation or progression (Pearce and Jordan, 2004; Platet et al., 2004; Prall et al., 1998; Rochefort et al., 1998). Many of the effects of estrogen signaling in breast cancers are driven by estrogen-dependent changes in the breast cancer cell transcriptome. Most studies have implicated ERα in these processes, but ERβ may play a role as well (Pearce and Jordan, 2004). Currently, ERα-positive breast cancers are treated with selective ER modulators (SERMs) (e.g., tamoxifen) and aromatase inhibitors (e.g., anastrozole) to block the estrogen signaling pathways in cancer cells (Osborne and Schiff, 2005; Cazzaniga and Bonanni, 2012), which ultimately alters ERα-mediate transcription programs and inhibits estrogen-dependent proliferation. Given that these targeted therapies focus on changing the availability of estrogen or the activity of ERα, it is important to understand the set of target genes of the estrogen signaling pathway and how they are regulated in target cells, which will provide insights for developing therapeutics with minimal side effects.

1.3. Transcriptional regulation by estrogen receptors

Many studies over the past three decades have elucidated the molecular mechanisms by which estrogen signaling and nuclear ERs regulate transcription and affect gene expression outcomes. Estrogens, such as the predominant naturally occurring endogenous estrogen 17β-estradiol (E2), are lipophilic and can diffuse freely into cells, where they initiate cytoplasmic and genomic signaling events that ultimately promote global changes in gene expression in the nucleus (Deroo and Korach, 2006; Cheung and Kraus, 2010; Hall et al., 2001; Hammes and Levin, 2007; Hammes and Levin, 2011). Cytoplasmic estrogen signaling is mediated by a small pool of cytoplasmic membrane-associated ERs, which stimulate kinase-mediated signaling pathways that lead to changes in the localization and activity of nuclear transcription factors (Hammes and Levin, 2007; Hammes and Levin, 2011). Nuclear estrogen signaling is mediated by nuclear ERs, which function as ligand-regulated transcription factors (Deroo and Korach, 2006; Hall et al., 2001). In the classical or "direct" nuclear pathway, E2 induces the dimerization of ER, which then binds directly to genomic DNA containing estrogen responsive elements (EREs) consisting of two AGGTCA half sites arranged palindromically around a three-basepair spacer (Berry et al., 1989; Kumar and Chambon, 1988) (Fig. 1, top left). In the non-classical or "indirect" nuclear pathway, ERs bind indirectly to genomic DNA through regulatory elements by "tethering" through other transcription factors, such as AP-1, Sp1, and NF-κB (Heldring et al., 2011; Kushner et al., 2000; Stender et al., 2010) (Fig. 1, bottom left). In either case, the binding of ER at enhancers promotes the recruitment of coregulators (e.g., histone modifying and remodeling enzymes, chromatin looping factors) that mediate posttranslational modifications of histones or other transcription factors, as well as chromatin remodeling and looping events (Kininis and Kraus, 2008) (Fig. 1). Liganded ERs and coregulators connect estrogen signaling to promoter-engaged RNA polymerase II (Pol II) and the basal transcriptional machinery through looping mechanisms that induce the changes in occupancy or activity of Pol II (Carroll et al., 2005; Hsu et al., 2010; Pan et al., 2008; Tan et al., 2011) (Fig. 1). This ultimately leads to profound changes in the transcriptome of cells.

How ERs bind to their cognate binding sites upon estrogen treatment and how the DNA sequences at those binding sites affect ER binding on a genomic scale have been studied extensively (Welboren et al., 2009; Cheung and Kraus, 2010; Kininis and Kraus, 2008; Carroll et al., 2006; Fullwood et al., 2009; Welboren et al., 2009). However, in order to understand how ERs regulate transcription, it is essential to understand the underlying mechanisms of how Pol II is regulated at estrogen target promoters. In a widely accepted view of estrogen-mediated transcriptional regulation, ERs and coactivators are recruited to target genes upon estrogen stimulation, which in turn modulates the recruitment of the Pol II machinery to their promoters (Bjornstrom and Sjoberg, 2005; Hall et al., 2001; Kininis and Kraus, 2008; Carroll and Brown, 2006). Under this view of estrogen-dependent transcriptional regulation, the recruitment of the Pol II pre-initiation complex is a rate-limiting step. Interestingly, recent gene specific- and genome-wide studies in flies and mammals have shown an alternative view of gene transcription by Pol II, where Pol II is widely distributed at the promoters of target genes prior to stimulation (Adelman and Lis, 2012), perhaps as a means of synchronizing transcriptional responses (Boettiger and Levine, 2009). Estrogen signaling through nuclear and membrane-initiated estrogen signaling may act at both levels, facilitating Pol II loading, as well as release of Pol II from pause sites (Welboren et al., 2009; Aiyar et al., 2004; Danko et al., 2013; Kininis et al., 2009). In spite of recent progress characterizing some of the mechanisms of estrogen-regulated transcription, many key questions remain to be answered about which transcripts comprise the set of primary or direct estrogen-regulated target genes and which specific molecular mechanisms control the expression of these genes.

1.4. Genomic studies of estrogen signaling and gene regulation: Approaches used to define primary estrogen target genes

Primary response genes, in the classical sense, are those that are regulated as an immediate response to cellular signaling pathways, without the need for protein synthesis or other secondary regulatory events (Herschman, 1991; Winkles, 1998). More recently, the concept of "direct" target genes has emerged, which typically refers to target genes whose promoters come into physical contact with, and are regulated by, a transcription factor bound at a proximal or distal enhancer (Carroll et al., 2005, 2006; Fullwood et al., 2009; Welboren et al., 2009). Primary/immediate response genes and direct target genes are two sides of the same coin. Although they are often incorrectly used synonymously, they focus on distinct aspects of the gene regulatory process. For the former, regulatory effects on Pol II are key; for the latter, binding and enhancer-promoter looping events are key. In the section below, we describe some of the approaches that have been used to identify primary/immediate response genes and direct target genes.

1.4.1. Expression microarrays and RNA-seq

Numerous gene expression microarray studies have been used to identify and define estrogen-regulated target genes in breast cancer cells, including MCF-7 cells, a widely used human breast adenocarcinoma cell line (Cheung and Kraus, 2010; Kininis and Kraus, 2008). These studies have been useful in characterizing the global effects of estrogen signaling and defining key concepts in estrogen-mediated gene regulation. However, the results from analyses using the same cell lines have shown variable results from experiment to experiment due to a variety of factors, including cell growth and estrogen treatment conditions, as well as the array platforms used (Cheung and Kraus, 2010; Kininis and Kraus, 2008; Carroll et al., 2006; Lin et al., 2004). Estimates of the number of target genes that are regulated upon estrogen stimulation based on expression microarrays varies from 100 to 1500 (Welboren

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