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Cohesin modulates transcription of estrogen-responsive genes

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ABSTRACT

The cohesin complex has essential roles in cell division, DNA damage repair and gene transcription. The transcriptional function of cohesin is thought to derive from its ability to connect distant regulatory elements with gene promoters. Genome-wide binding of cohesin in breast cancer cells frequently coincides with estrogen receptor alpha (ER), leading to the hypothesis that cohesin facilitates estrogen-dependent gene transcription. We found that cohesin modulates the expression of only a subset of genes in the ER transcription program, either activating or repressing transcription depending on the gene target. Estrogen-responsive genes most significantly influenced by cohesin were enriched in pathways associated with breast cancer progression such as PI3K and ErbB1. In MCF7 breast cancer cells, cohesin depletion enhanced transcription of *TFF1* and *TFF2*, and was associated with increased ER binding and increased interaction between *TFF1* and its distal enhancer situated within *TMPRSS3*. In contrast, cohesin depletion reduced *c-MYC* mRNA and was accompanied by reduced interaction between a distal enhancer of *c-MYC* and its promoters. Our data indicates that cohesin is not a universal facilitator of ER-induced transcription and can even restrict enhancer–promoter communication. We propose that cohesin modulates transcription of estrogen-dependent genes to achieve appropriate directionality and amplitude of expression.

1. Introduction

The control of gene transcription operates at multiple levels, including the recruitment of transcription factors, transcriptional co-factors and RNA polymerase. Transcription is also accompanied by changes in chromatin conformation and is informed by epigenetic modifications of chromatin and spatial organization of the genome [1,2].

Genome-scale chromosome capture assays have shown that mammalian chromosomes are partitioned into discrete topologically associated domains (TADs) [3,4]. TADs can range in size from several hundred kilobases to megabases; they represent compartments within which genes interact frequently, and thus can be regulated by a common set of factors [3–5]. TADs could therefore represent the framework for interactions between non-protein-coding gene regulatory elements and gene promoters, thereby influencing gene transcription [3–5]. Compartmentalization of genes into TADs could contribute to transcription control by restricting the number and types of regulatory elements that can interact with genes [3–6].

The cohesin complex is best characterized for its role in mediating sister chromatid cohesion during mitosis, however it also has important roles in DNA damage repair, ribosome biogenesis and gene regulation [7–9]. Cohesin consists of four core subunits, SMC1A, SMC3, RAD21 and STAG1/2/Stromalin [10]. Mutation of the cohesin loading factor NIPBL is responsible for around 65% cases of Cornelia de Lange Syndrome (CdLS [MIM 122470]), while mutations in subunits account for 5–10% cases [11], indicating that intact cohesin function is important for normal human development. The gene regulatory function of cohesin is independent of its role in cell cycle [12–15] and can be remarkably tissue-specific [16–19], indicating that it has an important but as yet poorly understood role in cell type-specific transcription. Although the exact mechanism by which cohesin regulates gene expression is unclear, it has been shown to facilitate long-range interactions between DNA elements, including interactions between

Abbreviations: ER, estrogen receptor alpha; ERE, estrogen response element; TAD, topologically associated domain; CdLS, Cornelia de Lange Syndrome; FOXA1, Forkhead Box A1; GATA3, GATA Binding Protein 3; ChIP, chromatin immunoprecipitation; ErbB, epidermal growth factor receptor; mTOR, mammalian target of rapamycin; PI3K, phosphoinositide 3-kinase; GAPDH, glyceraldehyde 3-phosphate dehydrogenase; RPL13A, ribosomal protein L13a; SOX4, sex determining region Y-box 4; TFF, Trefoil factor; BMPR2, bone morphogenetic protein receptor Type II; IL-20, interleukin-20; DKK1, Dickkopf 1 homolog; THBS1, thrombospondin 1; PIM2, Pim-2 oncogene; IRS2, insulin receptor substrate 2; CXCL12, chemokine (C-X-C) motif ligand 12; BAG1, BCL2-associated athanogene; CCNG2, cyclin G2; TMPRSS3, transmembrane protease, serine 3; RNAPII, RNA polymerase II; S5P, serine 5 phosphorylated

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enhancers and gene promoters [19–28]. Recently, cohesin was shown to be important for chromatin interactions within TADs [26,27], and to contribute to the formation of insulated neighborhoods [29], thereby potentiating its contribution to transcription via global genome organization.

Cohesin genes are frequently altered in cancer [30,31], although strikingly, not always in the same way. In leukemia [32–34] and in colorectal cancer [35] genes encoding subunits of the cohesin complex are frequently mutated, whereas in ovarian and breast cancer, these genes are more commonly upregulated or amplified (particularly RAD21 near 8q24) [31,36,37]. Although cohesin gene alterations could lead to aneuploidy and genome instability [38], it is not clear that these anomalies are inevitably responsible for the development of cancer. It may also be possible that dysregulation of downstream genes resulting from cohesin alteration underlies the development of cancers that harbor cohesin alterations [30,31,38].

Estrogen receptor alpha (ER)-associated transcription is the output of a ligand-dependent signaling pathway that drives gene expression and tumor growth in majority of breast cancers [39]. ER-mediated gene transcription involves many layers of regulation, including the binding of ER to estrogen response elements (EREs), and recruitment of co-factors such as *FOXA1* and *GATA3* [39,40]. In recent years, the formation of chromatin loops that juxtapose regulatory elements and promoters has emerged as an essential feature of ER-dependent transcription [40–42]. Many estrogen-responsive genes interact with spatially distant EREs via long-range interactions [40–45].

Genome-wide binding analysis in estrogen-responsive MCF7 breast cancer cells revealed estrogen-induced cohesin binding of chromatin frequently coincides with sites bound by ER [25]. This concordance in cohesin and ER binding was particularly striking at estrogen-regulated genes, and also at regions that are involved in ER-associated chromatin interactions [25,41]. These findings raise the possibility that cohesin is involved in regulating ER-dependent transcription.

Previously, we demonstrated that cohesin is required for the estrogen-induced transcription of the oncogene c-*MYC* (subsequently referred to as *MYC*) in MCF7 cells [46]. Cohesin binds at the *MYC* gene promoters and at enhancers upstream of *MYC* in an estrogen-dependent manner [46]. Moreover, depletion of cohesin hampered ER binding at the *MYC* gene enhancers and promoters [46]. *Myc* is also regulated by cohesin in zebrafish and Drosophila [47] and is downregulated in lymphoblastoid cells from CdLS patients [48]. Despite the clear relationship between cohesin and ER in the regulation of *MYC* gene expression, it remains unknown whether there is a general transcriptional dependence for cohesin at estrogen-responsive genes.

Here we aimed to identify other ER-regulated breast cancer genes that are dependent upon cohesin for transcriptional activity. We found that cohesin only influences the estrogen response of a subset of genes and is not a universal facilitator of ER associated transcription. Cohesin depletion in MCF7 cells elicited a range of responses, including up- and downregulation of estrogen responsive genes and gain in estrogen sensitivity. Within the TFF gene cluster, cohesin depletion, enhanced expression of *TFF1*, *TFF2* and *TMPRSS3* genes, was accompanied by increased ER recruitment, and increased interaction between estrogen-induced cohesin binding sites at the *TMPRSS3* enhancer and the *TFF1* promoter. By contrast, decreased *MYC* transcription upon cohesin depletion was associated with diminished enhancer–promoter interaction at the *MYC* locus. Together our results suggest that cohesin can both positively and negatively influence ER-mediated transcription in a gene-dependent manner.

2. Material and methods

2.1. Cell culture and siRNA transfections

MCF7 cells (ATCC) were cultured in Dulbecco's Modified Eagle Medium (DMEM, Life Technologies or Sigma) supplemented with 10% fetal bovine serum (FBS) in a 37 °C humidified incubator at 5% CO_2. T47D cells (ATCC) were cultured in RPMI-1640 media containing insulin (5 μg/ml) and 10% FBS in a 37 °C incubator at 10% CO_2. For hormone depletion, cells were cultured for 3 days in phenol-free media supplemented with 10% charcoal dextran (Sigma)-treated FBS. Hormone-depleted cells were treated with 17-β-estradiol (Sigma) at a final concentration of 100 nM for various time periods. Estradiol was dissolved in absolute ethanol, and as controls, hormone depleted cells were also treated with the same volume of ethanol (vehicle) for the same time periods. To deplete cohesin in MCF7 cells, we used the ON-TARGET plus siRNA DHA-J-006832-06 (GE Dharmacon) that targets the cohesin subunit, RAD21. The non-targeting control siRNA D-001810-01 (GE Dharmacon) was used as a negative control. RAD21 and control siRNAs were used at final concentrations of 5 nM for microarray experiments and 10 nM for chromatin immunoprecipitation (ChIP) and chromosome conformation capture (3C) experiments. Cells were reverse-transfected using Lipofectamine RNAiMAX (Life technologies) following 24 h of hormone depletion. After transfection, cells were cultured in hormone-depleted conditions for a further 48 h prior to estradiol stimulation.

2.2. RNA isolation, cDNA synthesis and gene expression analysis

Total RNA was isolated using the Machery Nagel Nucleospin RNA Isolation kit according to manufacturer's guidelines (Machery Nagel). 0.5 μg of total RNA was reverse-transcribed into first strand cDNA using the Superscript III first strand synthesis system (Life Technologies). Quantitative PCR (qPCR) was performed in technical duplicates with either Platinum SYBR Green qPCR SuperMix-UDG with Rox (Life Technologies) or TaKaRa SYBR Premix Ex Taq™ (Clontech) on an ABI 7300 (Applied Biosystems) or on the LightCycler 480 (Roche Diagnostics). Each primer was first validated for efficiency with an external standard curve generated by serial dilutions of the cDNA. Gene expression analyses were carried out on qBase Plus (Biogazelle) and were normalized relative to the mean of reference genes glyceraldehyde 3-phosphate dehydrogenase (*GAPDH*), *cyclophilin* and ribosomal protein L13a (*RPL13A*). Primer details are included in Supplementary Table S3.

2.3. Antibodies and immunodetection

Cells were lysed in RIPA extraction buffer containing protease inhibitors and proteins were quantified using the Bicinchoninic Acid assay kit (Pierce). For immunoblots, 30–60 μg of protein lysates was resolved on a 10% SDS-PAGE gel and transferred onto a nitrocellulose membrane. The Odyssey infrared detection (LI-COR Biosciences) system was used for protein detection and quantitation. Antibodies used were rabbit anti-RAD21 antibody (1:1000 dilution, Ab992, Abcam) and mouse anti-γ-Tubulin antibody (1:5000 dilution, T5326, Sigma) and were detected with IRDye 680-labeled goat-anti-rabbit IgG or IRDye 800-labeled goat-anti-mouse IgG (LI-COR Biosciences) at 1:15,000 dilution. Detected bands were quantified using the Image Studio Lite software (LI-COR Biosciences).

2.4. Microarray and transcript profiling

MCF7 cells growing in hormone-depleted conditions for 24 h were transfected with RAD21 or control siRNA and cultured in hormone-depleted conditions for a further 48 h. Cells were subsequently treated with 100 nM 17-β-estradiol or vehicle for 3 and 6 h. Cells were harvested for RNA and protein at both time points. Knockdown of RAD21 mRNA and protein were verified by qPCR and immunoblot respectively. RNA from three independent biological replicates for each time point was analyzed for quality and integrity by using the RNA 6000 Nano kit (Agilent Technology) run on a 2100 Bioanalyzer system (Agilent Technology) as per the manufacturer's instructions. Microarray expression