Published OnlineFirst September 22, 2011; DOI: 10.1158/1541-7786.MCR-11-0382

Molecular Cancer Research

Review

Gene Regulation by Cohesin in Cancer: Is the Ring an Unexpected Party to Proliferation?

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Abstract

Cohesin is a multisubunit protein complex that plays an integral role in sister chromatid cohesion, DNA repair, and meiosis. Of significance, both over- and underexpression of cohesin are associated with cancer. It is generally believed that cohesin dysregulation contributes to cancer by leading to aneuploidy or chromosome instability. For cancers with loss of cohesin function, this idea seems plausible. However, overexpression of cohesin in cancer appears to be more significant for prognosis than its loss. Increased levels of cohesin subunits correlate with poor prognosis and resistance to drug, hormone, and radiation therapies. However, if there is sufficient cohesin for sister chromatid cohesion, overexpression of cohesin subunits should not obligatorily lead to aneuploidy. This raises the possibility that excess cohesin promotes cancer by alternative mechanisms. Over the last decade, it has emerged that cohesin regulates gene transcription. Recent studies have shown that gene regulation by cohesin contributes to stem cell pluripotency and cell differentiation. Of importance, cohesin positively regulates the transcription of genes known to be dysregulated in cancer, such as *Runx1*, *Runx3*, and *Myc*. Furthermore, cohesin binds with estrogen receptor α throughout the genome in breast cancer cells, suggesting that it may be involved in the transcription of estrogen-responsive genes. Here, we will review evidence supporting the idea that the gene regulation function of cohesin represents a previously unrecognized mechanism for the development of cancer. *Mol Cancer Res; 9(12); 1587–607. ©2011 AACR.*

Introduction

Cohesin is a multisubunit complex that serves essential functions in chromosome biology, including mediating sister chromatid cohesion in meiosis and mitosis, and DNA double-strand break repair. Its importance in these roles indicates that disruption of normal cohesin function could contribute to aneuploidy and genome instability, features that are frequently associated with cancer (1, 2). Indeed, mutations in cohesin subunits, as well as cohesin over- and underexpression, have been associated with tumorigenesis (reviewed in refs. 3 and 4). However, in addition to its roles in the cell cycle and DNA repair, cohesin has a crucial function in transcriptional regulation. Control of transcription by cohesin is independent of the cell cycle and is thought to be key to the pathology of developmental syndromes (i.e., cohesinopathies) that result from insufficiency of proteins in the chromosome cohesion pathway. Over the past year, it has emerged that cohesin regulates genes that are crucial for cell proliferation and maintenance of pluripotency. This raises the possibility that the gene regulation function of cohesin could be just as central to neoplasia as are its cell-cycle and DNA repair roles. In this review, we highlight the potential for cohesin to contribute to cancer via its transcriptional role. In particular, we discuss cohesin's ability to positively regulate oncogenes and genes that maintain pluripotency, as well as its participation in hormone-dependent pathways underlying cancer.

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doi: 10.1158/1541-7786.MCR-11-0382

Cohesin: A Protein Complex That Is Crucial for Cell Division and DNA Repair

Cohesin is best known for its essential role in holding together sister chromatids from DNA replication in S-phase until chromosome separation occurs in anaphase (5, 6). The cohesin complex consists of 4 main protein subunits: structural maintenance of chromosomes (SMC) subunits Smc1 and Smc3, and 2 non-SMC subunits, Mcd1/Scc1/Rad21 and Scc3/Stromalin (SA; see Table 1 for cohesin subunit nomenclature). These proteins form a large ring-like structure, large enough to encircle DNA (5). In the leading model for sister chromatid cohesion, cohesin topologically entraps sister chromatids (7); however, alternative models have been proposed to explain how cohesin physically holds 2 molecules of DNA together (8, 9).

Several other proteins regulate the loading and unloading of cohesin onto DNA, its DNA binding stability, and its turnover and recycling (Table 1; reviewed in ref. 10).

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Table 1. Nomenclature and function of cohesin subunits

Generic name	Saccharomyces cerevisiae	Schizosaccharomyces pombe	Drosophila melanogaster	Homo sapiens	Function
Smc1	SMC1	psm1	SMC1	SMC1A	Core cohesin subunit
Smc1β				SMC1B	Cohesin subunit (meiosis)
Smc3	SMC3	psm3	Cap	SMC3/CSPG6/ Bamacan	Core cohesin subunit
Rad21	MCD1/SCC1	rad21	Rad21	RAD21	Core cohesin subunit
Rec8	REC8	rec8	c(2)M	REC8	Cohesin subunit (meiosis)
SA-1	IRR1	psc3	SA	STAG1	Cohesin subunit
SA-2	SCC3	psc3	SA2/Stromalin	STAG2/Stromalin	Cohesin subunit
SA-3		rec11	—	STAG3	Cohesin subunit (meiosis)
Nipbl	SCC2	mis4	Nipped-B	NIPBL	Cohesin loading
Scc4 (or MAU-2, *Caenorhabditis elegans*)	SCC4	ssl3		SCC4	Cohesin loading
Esco1/2	ECO1/CTF7	eso1	deco	ESCO1/2	Acetyltransferases, establishment of cohesion
Pds5A	PDS5	pds5	pds5	PDS5a	
Pds5B				PDS5b/APRIN/AS3	Cohesin dissociation
Wapl	RAD61/WPL1	wapl	wapl	WAPAL	Cohesin dissociation

Loading of cohesin onto chromosomes takes place in telophase in most organisms and is facilitated by a protein complex containing Scc2 (Nipped-B in *Drosophila* and NIPBL in human) and Scc4/MAU-2. Once loaded, cohesin exhibits highly variable residence times on chromosomes, indicating that it binds DNA with different modes of stability (11, 12). It is thought that the more stably bound fraction of cohesin has functions in addition to chromosome cohesion, including regulation of gene expression (11).

During S-phase, stably bound cohesin becomes cohesive by interaction with the DNA replication machinery (13–15), in association with the acetyltransferase Ctf7/Eco1 (yeast), or Esco1/2 (vertebrates; refs. 15–17). Esco1/2 acetylates cohesin subunit Smc3 to generate the cohesive form of cohesin that is necessary to hold together the sister chromatids through G_2–M-phase (18–20). However, in humans, it appears that ESCO2 is primarily required for cohesion in heterochromatic regions, and patients with Roberts syndrome who lack ESCO2 exhibit heterochromatin repulsion without chromosome segregation defects (21). In human cells (but not yeast), the sororin protein is additionally required to establish and maintain cohesion (22, 23).

After DNA replication is complete and before mitosis occurs, most cohesin is removed from chromosome arms by the prophase pathway. This process involves phosphorylation of cohesin subunit SA2 by Polo-like kinase (Plk) and Aurora B (24, 25) and interaction of a cohesion disestablishment complex containing Pds5 and Wapl with SA to unlock the cohesin ring (26–28). In the competing establishment activity, sororin and Esco2 function to antagonize the activity of the Pds5/Wapl complex (29–32), and the phosphatase Ssu72 appears to promote cohesion by countering the phosphorylation of SA2 (24, 33). During chromosome condensation, the prophase pathway prevails, and by metaphase, most cohesin has been removed from chromosome arms. The remaining, primarily centromeric cohesin, protected from removal by Shugoshin (34), is all that remains to hold the sisters together. Shugoshin binds to protein phosphatase 2A (PP2A), and this interaction is necessary for location of Shugoshin to centromeres in yeast and human cells (35, 36). Because depletion of Plk restores localization of Shugoshin to centromeres in PP2A-depleted cells (35), it is likely that the Shugoshin-PP2A complex protects sister chromatid cohesin by countering phosphorylation of cohesin by Plk.

At anaphase, APC-mediated degradation of a protein called securin (37) releases the protease separase, which becomes available to cleave the Rad21 subunit of cohesin (38–40). The remaining cohesin rings are opened, allowing chromosomes to separate (41). At the next cell cycle, the Smc3 subunit of cohesin can be recycled onto chromosomes, but deacetylation of Smc3 by class I histone deacetylase (HDAC) Hos1 (yeast) is required before this can happen (42–44). Figure 1 provides a simplified overview of cohesin function in the cell cycle.

Sister chromatid cohesion is necessary for homologous recombination-mediated DNA double-strand break repair in yeast and vertebrate cells (reviewed in ref. 45). For double-strand breaks to be effectively repaired, the cohesive form of cohesin must be established at the location of the break (46). Stabilization of cohesin at double-strand breaks depends on acetylation of the Rad21 subunit by Esco2, plus antagonism of the disassociation complex containing Wapl (47). Cohesin is recruited *de novo* at double-strand breaks in G_2-phase

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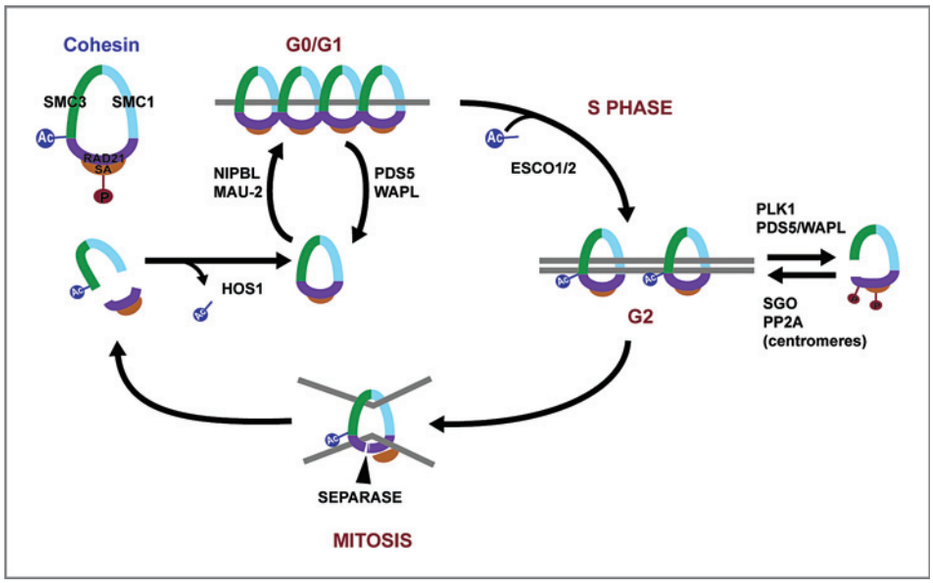

Figure 1. Cohesin's function in the cell cycle. Cohesin is loaded onto chromosomes before S-phase and holds sister chromatids together throughout G_2-phase until their separation at M-phase. Phosphorylation (P), acetylation (Ac), and the interaction of cohesin with numerous other proteins regulate the binding and dissociation of cohesin with chromatin throughout the cell cycle. See text for details.

(48), and in vertebrates, this association also involves another SMC complex, Smc5/6 (49, 50). Other molecular events contribute to cohesin function in double-strand break repair. In budding yeast, it was shown that the phosphorylation of Mcd1 (Rad21) through the ATR and Chk1 pathway is important for cohesion and double-strand break repair (51). In human cells, cohesive cohesin at double-strand breaks also depends on the pro-establishment activity of Sororin (23).

Mutations in sister chromatid cohesion proteins lead to chromosome segregation defects and impaired repair of DNA double-strand breaks. Chromosome instability (CIN) can result from the mis-segregation of chromosomes or defective repair of DNA double-strand breaks. The consequences of CIN are chromosomal rearrangements and aneuploidy, which can lead to loss of heterozygosity at tumor suppressor genes, causing neoplasia. Although many tumors are aneuploid, debate remains as to whether CIN drives the formation of tumors or is a consequence of their rapid proliferation (reviewed in ref. 1). Mathematical models have shown that it is theoretically possible for CIN to arise before other cancer-causing mutations and to form the initial driving cancer mutation (2, 52). In support of a driver hypothesis, more than 20% of colorectal cancers have mutations in the chromosome cohesion pathway (53). Somatic mutations in *SMC1*, *SMC3*, *STAG3*, and *NIPBL* were found in colorectal tumors at a statistically higher rate than in normal cells (53). However, for cells carrying chromosome cohesion defects to be viable, the spindle assembly checkpoint (SAC) would somehow need to be bypassed. The function of the SAC is to sense correct bipolar attachment of spindle fibers to kinetochores, together with the presence of tension across the kinetochores (54). Normally, sister chromatid cohesion defects lead to SAC activation and subsequent apoptosis; thus, additional mutations compromising SAC function are likely to be necessary if such cells are to survive and proliferate. Arguing against the idea that aneuploidy is an initial driver of tumorigenesis, studies in several organisms have shown that aneuploidy on its own has multiple harmful effects on growth and development (reviewed in ref. 55). In yeast (56) and mammalian cells (57), artificial generation of aneuploidy for single chromosomes was detrimental to cell proliferation and placed additional metabolic stress on cells. The disadvantages of aneuploidy are manifest in human tumor cells and may provide an opportunity for tumor therapy (58). In particular types of cancer, cohesin proteins are overexpressed rather than underexpressed or mutated (Table 2), making it more difficult to explain mechanistically how CIN and aneuploidy could result. It is possible that overexpression of a particular cohesin subunit could lead to sequestration of other subunits and decrease the amount of cohesin available for sister chromatid cohesion. However, because only a small proportion of cohesin actually represents the cohesive pool (12), stoichiometry would have to be severely disrupted before effects on sister chromatid cohesion became apparent.

Cohesin Regulates Tissue-Specific Gene Transcription

Over the last decade, a new role for cohesin in the regulation of gene expression has emerged. The first evidence for this role came from a forward genetic screen in *Drosophila* that identified the Scc2 ortholog *Nipped-B* as a modulator of enhancer–promoter interactions at the *cut* and *Ubx* genes (59). Depletion of cohesin subunits also affected expression of the *cut* gene but in the opposite direction from *Nipped-B* (60, 61). The best explanation so far for these divergent effects are that small changes in the dose of cohesin and *Nipped-B* can have variable or biphasic effects on gene expression, leading to opposite effects on transcription

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Table 2. Evidence for cohesin's involvement in cancer

Cancer	Cohesin subunit involved (context of study)	Description of study	Reference
Breast cancer	⇑ *RAD21* mRNA expression (cell lines)	Quantitative gene expression analysis revealed that *RAD21* mRNA expression is lower in normal and immortalized breast cancer cell lines compared with 9/11 tumorigenic breast cancer lines. siRNA knockdown of *RAD21* effectively inhibited proliferation of MCF-7 and T-47D cell lines. *RAD21* knockdown in MCF-7 cell line renders cells more sensitive to the DNA-damaging chemotherapeutic agents etoposide and bleomycin.	149
Breast cancer	⇑ *RAD21* mRNA expression (primary tumors)	cDNA microarray analysis of primary breast tumors from patients without tumor cells in local lymph nodes at diagnosis (lymph node–negative) identified a gene expression signature strongly predictive of a short interval to distant metastases (poor-prognosis signature). *RAD21* was found to be significantly upregulated in the poor-prognosis signature.	95
Breast cancer	⇑ *RAD21* mRNA expression (primary tumors)	cDNA microarrays were profiled to identify functional pathways that determine the outcome of breast cancer patients with supraclavicular lymph node metastases. Thirty-one breast cancer patients with supraclavicular lymph node metastasis without distant metastases were divided into poor, intermediate, or good-prognosis groups. Wnt signaling and mitochondrial apoptosis pathways emerged, with 6 genes (*DVL1, VDAC2, BIRC5, Stathmin1, PARP1,* and *RAD21*) found to be overexpressed in the poor-prognosis group compared with the good-prognosis group.	96
Breast cancer	⇑ *RAD21* mRNA expression in response to BRCA1 overexpression (cell lines)	Suppression subtractive hybridization was used to compare the expression profiles of control MCF7 cells with MCF7 cells ectopically expressing BRCA1, to identify genes whose expression is regulated by BRCA1. *RAD21* is upregulated following overexpression of BRCA1.	153
Breast cancer	⇑ *RAD21* protein and mRNA expression and *RAD21* gene amplification (primary tumors)	Immunohistochemistry was used to evaluate RAD21 expression in a cohort of *in situ* and invasive breast cancers. RAD21 levels were significantly lower in invasive cancers compared with *in situ* cancers. Levels of RAD21 correlated with larger tumor size and lymph node involvement but not with tumor grade, HER2 status, or ER status. Positive RAD21 protein expression was seen in 37% luminal, 24% basal, 22% HER2, and 18% null cancers, and significantly correlated with shorter relapse-free survival. RAD21 expression correlated with relapse in grade 3 but not in grade 1 or 2 tumors. Further analysis of grade 3 tumors according to subtype showed a significant correlation between RAD21 expression and shorter relapse-free survival in the luminal, basal, and HER2 cancers but not the null-type cancers.	97

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Table 2. Evidence for cohesin's involvement in cancer (Cont'd)

Cancer	Cohesin subunit involved (context of study)	Description of study	Reference
		In patients not treated with chemotherapy, there was no correlation between RAD21 expression and overall survival, whereas in patients treated with chemotherapy, there was a significantly shorter overall survival in patients whose tumors were RAD21-positive. Array comparative genomic hybridization and transcription data from 48 grade 3 invasive ductal carcinomas of luminal, basal-like, and HER2 subtypes were integrated to examine the association of *RAD21* mRNA expression with *RAD21* copy number. *RAD21* mRNA expression correlated with gene copy number in luminal, basal, and HER2 tumors, suggesting that the positive *RAD21* expression observed in a subset of grade 3 tumors may be due to gene amplification. shRNA-mediated gene silencing of *RAD21* in the MDA-MB-231 basal-like breast cancer cell line rendered the cells more sensitive to the chemotherapy drugs cyclophosphamide and 5-fluorouracil in a manner that directly correlated with the level of *RAD21* expression.	
Prostate cancer	⇑ *RAD21* mRNA expression and *RAD21* gene amplification (cell lines and primary tumors)	Sought to identify genes that are overexpressed, especially from gene amplification, in prostate cancer. Quantitative RT-PCR revealed that *RAD21* expression was increased in the PC-3 prostate cancer cell line. In tumors, *RAD21* was 1 of 7 genes that were overexpressed, mainly in samples found to contain amplification in the chromosomal regions harboring the genes. Expression of these 7 genes was examined by quantitative RT-PCR in cases of benign prostate hyperplasia, untreated prostate carcinoma, and hormone-refractory prostate carcinoma. *RAD21* expression was significantly higher in carcinomas than in benign prostate hyperplasia. FISH results showed that *RAD21* was amplified in PC-3 cells. Furthermore, in a screen of 10 xenografts and 12 hormone-refractory prostate carcinomas, *RAD21* showed high-level amplification in 32% of samples.	154
Oral squamous cell carcinoma	⇓ *RAD21* expression in invasive growth pattern vs expansive growth pattern (primary tumors)	Investigated the relevance of *RAD21* in invasion and metastases of squamous cell carcinoma. Laser microdissection and quantitative PCR revealed that *RAD21* expression was significantly decreased in areas of INF-γ invasion (associated with poorer prognosis) compared with areas that showed INF-α invasion.	98

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Table 2. Evidence for cohesin's involvement in cancer (Cont'd)

Cancer	Cohesin subunit involved (context of study)	Description of study	Reference
Colorectal cancer	Mutations in several subunits (primary tumors)	Systematically identified somatic mutations in potential CIN genes in colorectal cancers by determining the sequence of 102 human homologs of 96 yeast CIN genes known to function in various aspects of chromosome transmission fidelity. In a panel of 132 colorectal cancers, 11 somatic mutations, distributed among 5 genes, were identified. Ten of these mutations were found in the genes encoding the cohesin subunits SMC1L1, NIPBL, CSPG6, and STAG3. *RNAi* was used to reduce SMC1L1 and CSPG6 protein levels, and resulted in CIN and chromatid cohesion defects in human cells.	53
Colon carcinoma	⇑ *SMC3* mRNA (cell lines and primary tumors)	*SMC3* expression was increased in mouse colorectal carcinoma cells compared with a primary colon cell line. Similarly, *SMC3* expression was increased in colon carcinoma tissue compared with normal colon tissue, and 5 independent human colon carcinoma cell lines displayed the same degree of *SMC3* overexpression as the colon carcinoma sample. 70% of human colon carcinoma tissue samples ($n = 19$) displayed a significant increase in *SMC3* mRNA levels compared with matched normal colon tissue samples.	155
Myeloid leukemia	*RAD21* and *STAG2* gene deletions (leukemia cells)	To identify potential new genes involved in myeloid diseases, array comparative genomic hybridization was performed on 167 samples, including myelodysplastic syndrome, chronic myelomonocytic leukemia, and acute myeloid leukemia. In a case of chronic myelomonocytic leukemia diagnosed in 2007, a small heterozygous deletion at 8q24 was revealed. This region includes the *RAD21* gene. This chronic myelomonocytic leukemia transformed to M5 FAB acute myeloid leukemia in 2008, and array comparative genomic hybridization again revealed the *RAD21* loss but no other additional alteration. The patient died 6 months after transformation. In a case of M6 FAB acute myeloid leukemia diagnosed in 2005, a small deletion centered on the *STAG2* gene was identified. The patient died in 2007, 5 months after relapse. In both cases, the karyotype did not show any abnormality, and no other array comparative genomic hybridization alterations were noticed.	156

according to the severity of consequences for dose reduction (the consequences of halving the gene dose is more severe for *Nipped-B* than for cohesin subunits; refs. 62 and 63). Evidence that cohesin also has a gene regulatory role in vertebrates initially came from work showing that expression of the *runx1* and *runx3* genes was abolished in a tissue-specific manner in early zebrafish embryos mutant for cohesin subunit *rad21* (64).

Shortly after the findings in *Drosophila* emerged, mutations in genes encoding the NIPBL (human Scc2 ortholog),

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SMC1A, and SMC3 cohesin proteins were found to cause Cornelia de Lange syndrome (CdLS), a developmental disorder characterized by mental retardation, upper-limb abnormalities, growth delay, and facial dysmorphisms (65–68). Of interest, affected individuals were shown to have altered gene expression and developmental effects without overt defects in chromosome segregation, suggesting that the pathology of CdLS is independent of cohesin's role in sister chromatid cohesion (69). Animal models of CdLS also support the idea that the pathology is due to altered expression of numerous developmental genes (70, 71). An emergent, diverse spectrum of human developmental disorders resulting from mutations in various proteins responsible for sister chromatid cohesion led to coining of the term "cohesinopathies" to refer to such disorders (72).

The strongest support for a cell-cycle–independent role for cohesin in gene regulation arose from studies that examined cohesin expression and function in postmitotic tissues, where cells no longer require cohesin for proliferation. Cohesin can be expressed in postmitotic cells in a tissue-specific manner; for example, cohesin genes are expressed in nonproliferating cells in the developing zebrafish brain (73). In *Drosophila*, a function for cohesin was identified in the pruning of postmitotic neurons. An insertional mutagenesis screen identified the cohesin subunit Smc1 as being essential for pruning γ neurons in mushroom bodies (74). In an elegant strategy for cohesin ablation, the placement of a TEV-cleavage site into cohesin subunit Rad21 enabled artificial cleavage of cohesin to remove its function in postmitotic neurons of the *Drosophila* mushroom body. TEV-mediated cohesin cleavage abolished the developmentally controlled pruning of both axons and dendrites of γ neurons (75). Ablation of cohesin function in *Drosophila* salivary glands by the same technique confirmed that cohesin directly regulates the expression of a distinct set of genes, including the ecdysone receptor (EcR) and other genes that mediate the ecdysone response (76). The responsiveness of gene expression to cohesin depletion was sufficiently rapid to suggest that cohesin acts directly on genes to control their transcription.

Mechanisms of Gene Regulation by Cohesin

The notion that cohesin is a regulator of gene expression in metazoans is supported by evidence that cohesin and NIPBL bind to transcriptionally active regions of the genome in *Drosophila* (77) and mammals (78, 79). Genome-wide analyses of cohesin binding were among the first to shed light on potential mechanisms for cohesin-dependent transcription. In 2008, several studies revealed that much of cohesin colocalizes genome-wide with the CCCTC-binding factor (CTCF) insulator protein (78–81). CTCF has been well studied, and a recent comprehensive analysis of its role in three-dimensional chromatin organization showed that it is an integral organizer of chromosome conformation in the nucleus (82). A whole-genome investigation of CTCF-mediated interactions in the nucleus of mouse embryonic stem cells identified chromatin loops anchored by CTCF that demarcate distinct chromatin domains (82). The ability of CTCF to mediate the formation of chromatin loops may in some cases depend on cohesin action. For example, chromosome conformation capture revealed that cohesin is necessary for CTCF-dependent chromatin conformation at the imprinted H19-IGF2 locus (83). Consistent with cohesin-dependent chromatin conformation having a regulatory role, depletion of the Rad21 subunit of cohesin caused increased levels of IGF2 mRNA (83). Potentially in association with CTCF, cohesin has also been found to mediate chromatin looping at a number of other genes, including the interferon γ locus (84), the locus control region of the β-globin gene (85, 86), and the HoxA locus (87) in mice. At the β-globin locus, cohesin or Nipbl depletion interfered with transcriptional activation of globin genes and abrogated the formation of chromatin loops (85). These findings lend support to the increasingly popular theory that cohesin regulates gene expression by mediating long-range chromosome interactions.

Several recent studies suggest that cohesin need not always cooperate with CTCF to regulate chromatin interactions. It appears that cohesin localizes on chromosomes with a variety of transcriptional regulators in a tissue-specific manner. In MCF7 breast cancer cells, cohesin binding coincides with estrogen receptor α (ER), whereas in liver cells, binding of cohesin coincides with that of HNF4A (88). Furthermore, several genomic sites bound by both cohesin and ER in MCF7 cells were associated with ER-anchored chromatin loops (88, 89). Therefore, it is possible that cohesin is functionally involved in the formation of ER-anchored loops in a tissue-specific manner. Stage-specific recruitment of cohesin to immunoglobulin and β-globin loci in mice adds credence to the idea that cohesin is involved in forming tissue- and developmental-stage–specific chromatin structures (86, 90). Of significance, in mouse embryonic stem cells, cohesin and the Mediator complex have been shown to mediate long-range interactions between distal enhancers and the pluripotency genes *Oct4* and *Nanog* (91). In all of these cases, there is evidence that the contribution of cohesin to chromatin structure has diverse functional consequences ranging from the concatenation of immunoglobulin loci (90) to gene transcription (91).

Regulation of local chromatin structure is another potential mechanism by which cohesin might contribute to transcriptional control. Polycomb group (PcG) and trithorax group (TrxG) protein complexes maintain chromatin in silent and activated states, respectively, through modification of histone tails. Cohesin function has been implicated in both PcG and TrxG activity. A *Drosophila rad21* mutant called *verthandi* behaves like a TrxG mutation in that it can suppress PcG mutations in some tissues (92). Cohesin binding to *Drosophila* chromosomes is predominantly excluded from regions enriched in trimethylated lysine 27 on histone H3 (H3K27Me3), a signature of PcG repression (93). Of interest, some genes that respond the most to changes in cohesin or Nipped-B dose are enriched in both H3K27Me3 and cohesin (62), raising the possibility that PcG proteins sometimes cooperate with cohesin to regulate

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transcription. In support of this idea, an inducible biotinylation-tagging approach used to purify PcG-associated factors from *Drosophila* embryos identified an association of PcG protein complexes with cohesin (94). Furthermore, Polycomb-dependent silencing of a transgenic reporter was shown to depend on cohesin function (94).

Thus, it is possible that cohesin contributes to gene regulation by modifying chromatin at both local and global levels.

Altered Cohesin Expression Is Associated with Distinct Cancer Phenotypes

Of significance, both over- and underexpression of cohesin are associated with cancer. The expression of cohesin is dysregulated in a number of cancers, including breast, prostate, and oral squamous cell carcinoma, and alterations in genes encoding cohesin proteins are found in colorectal cancer and myeloid malignancies (Table 2).

The level of the RAD21 subunit of cohesin is frequently elevated in cancer (Table 2). *RAD21* overexpression in breast cancer is associated with more aggressive cancers and results in a poorer prognosis for the patient (95–97). For example, *RAD21* was identified as part of a gene expression signature that conferred a short interval to distant metastases in breast cancer patients, with *RAD21* found to be significantly upregulated in the poorer prognosis group (95). Similarly, *RAD21* overexpression was associated with a poor prognosis in breast cancer patients with supraclavicular lymph node metastases (96).

There is some evidence to indicate that raised levels of cohesin may be associated with tumor proliferation, at the expense of tumor metastasis (97, 98). In breast cancer, *RAD21* expression was shown to be significantly lower in invasive tumors compared with their *in situ* counterparts (97). Although *RAD21* overexpression did correlate with larger tumor size, perhaps indicative of increased proliferation, there was no correlation with tumor grade. However, raised *RAD21* expression significantly correlated with shorter relapse-free survival in grade 3 tumors compared with grade 1 and 2 tumors (97). Grade 3 tumors are characterized by a high proliferative index, and it is possible that *RAD21* contributes to the proliferative potential of the cancer. Conversely, Yamamoto and colleagues (98) showed that in oral squamous cell carcinoma, *RAD21* expression was downregulated in tumors that displayed an invasive growth pattern compared with tumors that grew more expansively, consistent with a proliferative role for cohesin in tumor progression. Of interest, the authors speculated that hypoxic conditions, which are known to downregulate *RAD21* in many human tumor cells, may lead to an invasive potential (98). These observations raise the possibility that changes in *RAD21* expression are associated with switching between tumor invasiveness and tumor proliferation.

A variety of studies have provided strong evidence that cohesin has an important role in cancer (Table 2). Cohesin dysregulation can lead to aneuploidy, which is usually assumed to be the pathological driver in cancers with cohesin mutations. However, the potential effects on transcription resulting from altered cohesin function should also be considered. It is interesting to note that the cancers in which cohesin involvement is associated with a worse prognosis are those in which cohesin is overexpressed. There is likely to be ample cohesin available for intact mitosis in such cancers, and therefore, alternative cause-and-effect relationships between cohesin and cancer could be in play.

Individuals with CdLS have reduced cohesin function and thus may be informative regarding a role for cohesin in cancer. There is little conclusive evidence that CdLS patients have overt defects in sister chromatid cohesion, even though these individuals are compromised for cohesin loading or function (99). Anecdotally, cancer is, if anything, underrepresented in CdLS cohorts. A higher than normal incidence of Barrett's esophagus leading to carcinoma is likely to be linked to the prevalent gastrointestinal reflux found in individuals with CdLS (100). It is interesting to speculate that the prominent cohesin insufficiency in CdLS patients could actually protect against cancer, perhaps because cancer genes that are positively regulated by cohesin are downregulated. For example, expression of the *c-MYC* oncogene is downregulated in cells from CdLS patients (ref. 101 and see below).

The overexpression of cohesin in many cancers raises the possibility that cohesin contributes to cancer via transcriptional regulation. Below, we describe 2 means by which cohesin-mediated gene transcription could contribute to cancer, namely, regulation of oncogenes and genes that maintain pluripotency, and cohesin's participation in hormone-dependent pathways underlying cancer.

Links between the Expression of Pluripotency Factors and Cancer

Embryonic development relies on pluripotent stem cells that derive from the inner cell mass of the early stage blastocyst and have the unique ability to self-renew and differentiate into all of the cell lineages present in the embryo and adult. This cellular paradigm of embryonic development is the basis of the cancer stem-cell model of carcinogenesis that is currently at the forefront of the ongoing debate surrounding the initiation and progression of cancer (102). The cancer stem-cell model suggests that cancer propagation is usually driven by subpopulations of cancer cells with stem-cell properties, including self-renewal and multilineage differentiation (103). Cancer stem cells are predicted to give rise to a heterogeneous population of tumor cells comprising more cancer stem cells, progenitor cells with limited proliferative potential, and aberrantly differentiated cells with no proliferative potential (103). If this model is correct, understanding normal stem-cell self-renewal and differentiation is fundamental to understanding cancer.

The cancer stem-cell model of carcinogenesis posits that tumor cells hijack properties of normal stem cells, including the capacity for self-renewal. Therefore, it is not surprising that the expression of pluripotency transcription factors is

Published OnlineFirst September 22, 2011; DOI: 10.1158/1541-7786.MCR-11-0382

dysregulated in many cancers, including breast, prostate, and colorectal cancers (Table 3). For example, OCT4, NANOG, MYC, and SOX2 are important transcription factors that are critical for the establishment and maintenance of pluripotency and that show altered expression in cancer. Of interest, expression of pluripotency transcription factors is frequently upregulated in cancers that also have elevated cohesin levels. Furthermore, overexpression of pluripotency transcription

Table 3. Evidence for pluripotency factor involvement in cancer

Cancer	Pluripotency factor involved (context of study)	Description of study	Reference
Breast cancer	⇑ *OCT4* and *NANOG* mRNA expression (primary tumors and cell lines)	Quantitative RT-PCR on a stage 3 breast carcinoma sample showed increased *NANOG* and *OCT4* expression compared with nondetectable levels in normal breast tissue. Immunohistochemistry revealed NANOG protein in breast carcinoma sample but not in normal breast tissue. The MCF7 breast carcinoma cell line was found to express *OCT4* and *NANOG.*	157
Breast cancer	⇑ SOX2 protein levels (primary tumors)	Immunohistochemistry was used to analyze SOX2 protein levels in a cohort of 95 patients with sporadic, postmenopausal, early breast cancer. Four expression scores were defined to distinguish SOX2-negative and -positive samples (score 0 = no SOX2-positive cells; score 1 = >0 and <10% SOX2-positive cells; score 2 = ≥10 and <50% SOX2-positive cells; score 3 = ≥50% SOX2-positive cells. SOX2 was expressed in 24/86 invasive breast carcinoma samples and 4/9 DCIS samples. Tumors expressing ≥50% SOX2-positive cells were significantly larger and significantly associated with lymph-node metastases. FISH of selected samples revealed that increased SOX2 protein levels were not due to *SOX2* gene amplification, suggesting that the aberrant gene expression is driven by other mechanisms.	104
Breast cancer	⇑ *NANOG, OCT4, SOX2* mRNA expression (mammospheres) ⇑ *NANOG* and *SOX2* mRNA expression (primary tumors)	Investigated the effects of estrogen on the stem/progenitor cell population in normal breast and breast cancer tissues. *NANOG, OCT4,* and *SOX2* expression was used to monitor the differentiation status of breast stem cells in the presence of either estrogen or tamoxifen. Expression levels of *NANOG, OCT4,* and *SOX2* were determined in freshly isolated organoids from reduction mammoplasties, breast epithelial cells derived from the organoids and grown as adherent cells in the presence of serum, and mammospheres originating from single breast epithelial cells. Compared with freshly isolated organoids, *NANOG*, *OCT4*, and *SOX2* mRNA expression was lower in differentiated (adherent) cells and significantly higher in the mammospheres. Estrogen treatment significantly reduced *NANOG, OCT4,* and *SOX2* expression in mammospheres. Furthermore, estrogen treatment reduced the percentage of stem/progenitor cells in mammospheres, whereas tamoxifen increased the percentage. *NANOG* and *SOX2* mRNA expression levels were determined in breast tumor samples and compared with levels in normal adjacent tissue. Increased expression of *NANOG* and *SOX2* was seen in the breast tumor samples.	158

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Published OnlineFirst September 22, 2011; DOI: 10.1158/1541-7786.MCR-11-0382

Table 3. Evidence for pluripotency factor involvement in cancer (Cont'd)

Cancer	Pluripotency factor involved (context of study)	Description of study	Reference
Breast cancer	Possession of embryonic stem cell expression signature correlateswith aggressive tumor behavior	Gene set expression analysis methods were used to assess whetherthe expression signatures and regulatory networks that define human embryonic stem-cell identity are also active in human tumors. Thirteen partially overlapping gene sets were compiled that represent the core expression signature of embryonic stem cells and reflect the activity of the regulatory pathways associated with their identity. These gene sets fall into 1 of 4 groups: embryonic stem-expressed genes; Nanog, Oct4, and Sox2 (NOS) targets; Polycomb targets; and Myc targets. Expression profiles from 6 published breast cancer studies, comprising a total of 1,211 tumors, were collected and analyzed. Grade 3 tumors showed an enrichment pattern resembling that observed in embryonic stem cells, including underexpression of Polycomb target gene sets and overexpression of embryonic stem cell–expressed sets, Myc-target gene sets, and some of the NOS-target gene sets. ER-positive tumors showed an embryonic stem cell–like enrichment pattern compared with ER-negative tumors. Tumors of larger size at the time of diagnosis were more likely to possess the embryonic stem-cell signature compared with smaller tumors, even within a given grade.	105
Colorectal cancer	⇑ NANOG protein levels (primary tumors)	Western blot was used to analyze NANOG levels in 175 fresh colorectal cancer samples. NANOG protein levels were higher in most of the colorectal cancer samples compared with paired normal mucosal tissue. Immunohistochemistry was used to analyze NANOG localization in paraffin-embedded colorectal cancer tissue. NANOG was mainly localized to the cytoplasm of cancer cells. Nuclear accumulation of NANOG was only observed in a small fraction of cancer cells. NANOG expression positively correlated with lymph node status and Dukes classification of patients. High NANOG expression correlated with a shorter survival or recurrence free survival. Colorectal cancer cells were studied for the effects of NANOG overexpression on proliferation, invasion, and motility. Overexpression resulted in increased proliferation, colony formation, and invasive ability.	106
Rectal cancer	⇑ *CD133, OCT4, SOX2* mRNA expression associated with distant recurrences (primary tumors)	*CD133, OCT4,* and *SOX2* levels were analyzed before and after chemoradiotherapy to clarify the association between expression of stem-cell markers and chemoradiotherapy resistance in rectal cancer. Thirty-three patients. Quantitative RT-PCR on pre-chemoradiotherapy endoscopic tumor samples revealed a positive correlation between *OCT4* and *SOX2* but not between *CD133* and *OCT4* or *SOX2*.	108

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Published OnlineFirst September 22, 2011; DOI: 10.1158/1541-7786.MCR-11-0382

Table 3. Evidence for pluripotency factor involvement in cancer (Cont'd)

Cancer	Pluripotency factor involved (context of study)	Description of study	Reference
		Analysis of formalin-fixed, paraffin-embedded, post-chemoradiotherapy residual cancer samples showed significant positive correlations among *CD133*, *OCT4,* and *SOX2*. Patients who developed distant recurrences had significantly higher post-chemoradiotherapy levels of *CD133*, *OCT4,* and *SOX2* compared with patients without recurrences. Of the 33 patients, 28 received a low dose of radiation and 5 received a high dose of radiation. Post-chemoradiotherapy *OCT4* levels were significantly higher in the high-dose radiation group compared with the low-dose radiation group. *CD133* and *SOX2* levels were also higher, but this difference did not reach statistical significance. Immunohistochemistry was used to confirm protein expression in residual cancer cells after chemoradiotherapy. CD133 was observed diffusely in the cytoplasm of residual cancer cells and at the apical/endoluminal surface of residual cancer cells with the formation of lumina and ducts. OCT4 and SOX2 were observed diffusely in the cytoplasm of residual cancer cells.	
Prostate cancer	⇑ OCT4 and SOX2 protein expression (primary tumors)	*OCT4, SOX2, NANOG, c-MYC,* and *Klf4* mRNA levels were increased in 28/55 prostate cancer samples. All possible combinations of transcription factors showed that significance was achieved only between *OCT4* and *SOX2*, suggesting a possible functional link between *OCT4* and *SOX2* in prostate cancer cases. Immunohistochemistry was used to evaluate OCT4 and SOX2 levels in normal prostate, benign prostate hyperplasia, and prostate cancer samples. Staining was categorized into 4 groups: (i) negative; (ii) low, <5%; (iii) intermediate, 5–25%; and (iv) high, 26–50%. The numbers of OCT4- or SOX2-expressing cells were significantly lower in normal prostate and benign prostate hyperplasia samples than in prostate tumor tissues. In prostate tumor samples, an increasing number of OCT4- and SOX2-expressing cells were evident with increasing Gleason score.	107
Bladder cancer	⇑ *OCT4* mRNA expression (primary tumors)	Investigated *OCT4* expression in bladder cancer. Semiquantitative RT-PCR showed *OCT4* expression in almost all (96%) of the examined bladder cancer samples. Expression was also detected in 23% of nontumor marginal tissues from thesame patients and 33% of nontumor bladder tissues obtained from patients with no obvious signs of bladder cancer. Densitometric evaluation of the semiquantitative RT-PCR results revealed that the intensity of *OCT4* expression was significantly higher in neoplastic tissues compared with nonneoplastic samples. Protein levels were also examined by immunohistochemistry. OCT4 was primarily localized to the nuclei of tumor cells, with no immunoreactivity in normal cells adjacent to the tumors.	159

Published OnlineFirst September 22, 2011; DOI: 10.1158/1541-7786.MCR-11-0382

factors in cancer leads to similar outcomes as cohesin overexpression, namely, poorer prognosis (104–107) and resistance to therapy (108). In breast cancer, tumors expressing >50% SOX2-positive cells were shown to be larger and associated with lymph node metastases (104). A meta-analysis of gene expression profiles from grade 3 breast tumors showed enrichment resembling that observed in embryonic stem cells, including underexpression of PcG target genes and overexpression of embryonic stem-cell–expressed genes, MYC target genes, and some NANOG/OCT4/SOX2 target genes (105). In colorectal cancer, NANOG protein levels were increased compared with normal adjacent mucosal tissue, and higher levels correlated with a shorter overall survival (106). A significantly greater number of OCT4/SOX2-expressing cells were observed in prostate tumors compared with normal and benign prostate hyperplasia (107). Furthermore, increased numbers of OCT4- and SOX2-expressing cells were associated with more-aggressive tumors that have a worse prognosis (107). Increased expression of pluripotency genes in rectal cancer contributes to chemo/radiotherapy resistance, and patients who relapsed had significantly higher posttherapeutic levels of OCT4 and SOX2 compared with patients without relapse (108).

Cohesin-Mediated Transcription of Pluripotency and Proliferation Genes

Maintenance of the pluripotency of embryonic stem cells depends on the correct regulation of a network of transcription factors, PcG repressor complexes, and microRNAs that are responsible for the transcriptional and epigenetic regulation of key stem-cell genes (102). Genome-wide binding analyses suggest that OCT4, SOX2, and NANOG contribute to pluripotency and self-renewal by activating their own transcription and that of other genes that are important during early development. OCT4, SOX2, and NANOG activate genes encoding components of the TGF-β and WNT signaling pathways, and they repress genes involved in differentiation processes (109). Although the function of these early pluripotency factors is relatively well understood, the upstream pathways that regulate their transcription remain enigmatic. Evidence that cohesin binds and positively regulates the expression of *Oct4*, *Nanog*, and *Sox2* in embryonic stem cells from human and mouse (91, 110) and *Myc* in fish, flies, and mammals (111) suggests that cohesin is an upstream regulator of pluripotency factors.

A short hairpin RNA (shRNA) screen for pluripotency factors identified subunits of the Mediator and cohesin complexes as proteins required to maintain the mouse embryonic stem-cell state (91). The Mediator complex is thought to bridge interactions between transcription factors at enhancers and the transcription initiation apparatus at core promoters (112). Reducing the levels of Mediator, cohesin, and Nipbl had the same effect on the embryonic stem-cell state as did loss of Oct4 itself, suggesting that they are important for maintaining expression of the key pluripotency transcription factors. ChIP followed by high-throughput sequencing identified a CTCF-independent subset of cohesin-binding sites that occupied the enhancer and core promoter sites bound by Mediator. Although these sites were associated with RNA polymerase II (RNAPII, indicating transcription), the cohesin-CTCF–bound sites were not.

The co-occupancy of Mediator, cohesin, and Nipbl at the promoter regions of *Oct4* and other active embryonic stem-cell genes indicates that these proteins may all contribute to the control of their transcription. Approximately one quarter of the genes co-occupied by Mediator, cohesin, Nipbl, and RNAPII showed significant gene expression changes when Mediator, cohesin, and Nipbl were each knocked down, suggesting that these actively transcribed genes depend on each of those factors for normal expression (91). This normal expression is likely to occur through looping between enhancers and the core promoters of the active genes, given that chromosome conformation capture identified physical contact between enhancers and the promoters of *Nanog, Phc1, Oct4*, and *Lefty1* in embryonic stem cells (91). These interactions were not detected in differentiated fibroblasts in which these genes are silent. Depletion of cohesin or Nipbl abolished enhancer–promoter interactions and decreased *Oct4* and *Nanog* expression (91).

A further recent study in human embryonic stem cells identified CTCF-independent RAD21-binding sites that coincided with binding of the transcription factors OCT4, NANOG, SOX2, KLF4, and ESRRB (110). Analysis of global gene expression changes following the knockdown of RAD21 in embryonic stem cells revealed the downregulation of stem-cell maintenance genes, including *Oct4, Nanog, Tbx3, Essrb,* and *Klf4*, and the upregulation of a large number of developmental genes. Changes in gene expression were similar to those reported following depletion of pluripotency transcription factors, in particular NANOG-depleted cells. Of interest, many CTCF-independent RAD21-binding sites were not present in differentiated embryoid bodies, indicating that the colocalization of RAD21 with pluripotency transcription factors is specific for embryonic stem cells.

Studies in zebrafish were among the first to reveal a role for cohesin in the transcriptional regulation of *Myc.* Depletion of Rad21 and Smc3 in zebrafish resulted in a reduction in *myca* (zebrafish *Myc*) transcription in early embryos (111). This regulation is likely direct, because cohesin (Rad21) binds at the transcriptional start site and the Myc insulator element (MINE) just upstream of *myca.* Positive regulation of *Myc* by cohesin is also apparent in *Drosophila*, mouse (71), and human (101), suggesting this regulation is conserved through evolution. While the exact mechanism by which cohesin regulates *Myc* remains elusive, studies in zebrafish rule out involvement of the CTCF insulator protein (111). Depletion of Ctcf in zebrafish embryos had no effect on *myca* expression, and Rad21 was still able to bind the transcriptional start site and Myc insulator element of *myca* in the absence of Ctcf. In fact, there was a statistically significant increase in Rad21 binding at the Myc insulator element in Ctcf-depleted embryos (111). It is possible that cohesin regulates *myca* expression by modulating local chromatin

Published OnlineFirst September 22, 2011; DOI: 10.1158/1541-7786.MCR-11-0382

structure. In Rad21-depleted zebrafish embryos, elevated H3K27me3 and reduced H3K9Ac (histone H3 lysine 9 acetylation) were present at the *myca* locus, with a predominant peak around the transcriptional start site. Reduction of the H3K9Ac modification indicates a less open chromatin status, as does an increase in H3K27Me3, reflecting repression of *myca* gene expression. The latter modification is mediated by PcG activity, which could involve cohesin function (discussed above).

Stem-cell factors appear to modulate cohesin binding to feed back on their own regulation and to modulate transcriptional control of other developmental genes by cohesin. Some stem-cell transcription factors influence cohesin binding to gene targets. In embryonic stem cells, NANOG facilitates the placement of cohesin at CTCF-independent transcription factor binding sites through an interaction with the core cohesin protein STAG1 and the cohesin-associated protein WAPL (110). At the mammalian *HoxA* locus, the Oct4 protein antagonizes cohesin binding to a CTCF-bound chromatin barrier, which regulates chromatin looping of the *HoxA* gene (87).

Cancers that exhibit high levels of cohesin and/or pluripotency factors share similar genetic profiles and prognostic outcomes. Because cohesin modulates the transcription of key pluripotency factors, there is a strong possibility that cohesin-mediated gene transcription underlies certain types of cancer upstream of pluripotency factors. Cohesin could participate in reprogramming cells to have stem-cell–like characteristics, conferring a selective advantage, including self-renewal, to the tumor. Furthermore, because cohesin also regulates genes involved in cell fate determination [e.g., *Runx1* and *Runx3* (64)], it is tempting to view cohesin as a gatekeeper of cell fate, maintaining the balance between stem- and differentiated-cell populations through its gene regulation role (Fig. 2).

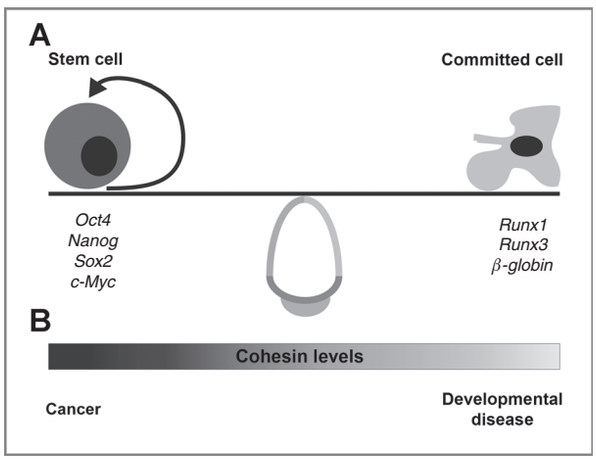

Figure 2. Cohesin as a gatekeeper of cell fate. A, during normal development, cohesin regulates the transcription of critical genes involved in pluripotency (e.g., *Oct4*, *Nanog*, *Sox2*, and *c-Myc*) and differentiation (e.g., *Runx1*, *Runx3*, and β-*globin*), thereby maintaining the balance between stem-cell and differentiated-cell populations. B, increased levels of cohesin may contribute to cancer by directly regulating the transcription of pluripotency genes, subsequently reprogramming cells to have stem-cell–like characteristics that maintain tumor growth. Conversely, reduced cohesin levels cause developmental disorders, including CdLS, the pathology of which is due to altered expression of a number of developmental genes.

Role of Cohesin in Hormone-Mediated Gene Regulation

Hormones induce dramatic and dynamic alterations in gene transcription. A small proportion of the transcriptional response to hormones is direct, via hormone receptors (HR) binding to promoters to activate transcription (113). However, a more significant component of hormone-dependent transcriptional response is accounted for by chromatin rearrangements and epigenetic modifications. A growing body of evidence suggests that cohesin modulates the regulation of genes by nuclear HRs and that this role is conserved through evolution (Table 4).

The first example of cohesin's role in hormone response was observed in *Drosophila,* where cohesin was found to be necessary for EcR-dependent axon pruning (74, 75). Cohesin's role in the response to ecdysone involves regulating transcription of the EcR gene (74–76) and directly regulating ecdysone-responsive gene transcription (76, 77). Remarkably, in humans, cohesin also appears to play an important role in the transcriptional response to androgen and estrogen (88, 114–116).

PDS5 is a HEAT-repeat–containing protein that is required for sister chromatid cohesion and that regulates the removal of cohesin from chromatin. Vertebrates possess 2 homologs of PDS5: PDS5A and PDS5B (Table 1). PDS5A/B-deficient mice exhibit CdLS-like developmental defects, including genital defects, without alterations in sister chromatin cohesion (70, 117). Of interest, PDS5B has been identified as an androgen-proliferation shutoff gene in prostate cancer cells, because it is essential for androgen-dependent growth inhibition both *in vitro* (115) and *in vivo* (116). In cells of the prostate, androgens initially increase proliferation but then induce quiescence. Regulation of gene transcription by androgens occurs through several epigenetic mechanisms, including histone modification, nucleosome remodeling, and chromatin looping (reviewed in ref. 118). It has been suggested that PDS5B regulates the androgen response through a chromatin modification role (116). Growth-inhibitory levels of androgens induce transcription of PDS5B, and PDS5B is also induced by the active metabolite of vitamin D, $1,25(OH)_2D_3$, another inhibitor of prostate cancer cell growth (114).

Estrogen-bound ER modulates genes that are required for reentry into the cell cycle, driving proliferation (119). In estrogen-dependent breast cancer cells, ligand-bound ER is recruited to thousands of specific sites throughout the genome (113, 120–122). Most ER-binding sites are cell-type specific and correlate with estrogen-regulated gene expression (120). Recently, a genome-wide binding study revealed tissue-specific, inducible cohesin binding in breast cancer cells in response to estrogen (88). This study showed that genes regulated by estrogen exhibit enriched binding of

Published OnlineFirst September 22, 2011; DOI: 10.1158/1541-7786.MCR-11-0382

Table 4. Role of cohesin in steroid hormone response pathways

Organism	Steroid hormone	Evidence of role for cohesin	Reference
Drosophila	Ecdysone	Cohesin is necessary for EcR-dependent axon pruning in the mushroom body.	74
		Ecdysone-responsive genes have high levels of cohesin binding.	76, 77
		Cohesin regulates EcR at the transcriptional level.	75–77
		Cleavage of cohesin affects expression of ecdysone-dependent genes. Changes in ecdysone-responsive genes occur before EcR levels can be affected, indicating that cohesin's role in ecdysone gene response is more than solely regulating EcR levels.	75
Human	Estrogen	Cohesin is required for estrogen-mediated reentry into the cell cycle (G_0/G_1–S-phase transition).	88
		Global analysis of cohesin binding to chromatin shows that cohesin colocalizes with the ER at many sites throughout the genome.	88
		In ER-positive MCF7 breast cancer cells, cohesin binding is positively correlated with estrogen-regulated genes.	88
		Genome-wide analysis of chromatin interactions throughout the genome identified ER-anchored chromatin loops in estrogen-responsive genes.	89
		Cohesin binding is enriched at sites involved in ER-mediated chromatin interactions, suggesting that cohesin may stabilize these loops.	88, 89
		An extensive shRNA screen revealed that downregulation of individual subunits of cohesin increased survival of MCF-7 breast cancer cells exposed to tamoxifen over an extended period.	136
		Clones of ER-positive ZR-751 breast cancer cells expressing ectopic RAD21 exhibit reduced sensitivity to the antiestrogen ICI 182780 or to tamoxifen in an agar growth assay.	139
		MCF7 cells with acquired resistance to tamoxifen have 1.6-fold higher expression of RAD21 and 3-fold lower expression of PDS5B than tamoxifen-sensitive cells.	138
	Androgen	PDS5b is required for androgen-mediated proliferative arrest in G_0/G_1.	116
		AR mediates looping of androgen-regulated genes.	123, 125
		PDS5b is induced by levels of androgens that inhibit cell growth.	114
		Rad21 levels are higher and PDS5b levels are lower in androgen-independent vs. androgen-dependent prostate cancer cell lines.	160

cohesin together with ER. Although the functional significance of cohesin binding is not known, it has been shown that cohesin depletion prevents the estrogen-responsive G_0/G_1–S transition in breast cancer cells, indicating that cohesin influences the physiological estrogen response (88).

A cell's transcriptional response to hormone signaling is complex. Entire cohorts of genes are turned on or off at different times poststimulation, both directly via canonical mechanisms of gene activation and less directly by altering the chromatin milieu. How does cohesin influence gene regulation by hormones? We suggest 3 possible ways in which cohesin could modulate hormone-mediated gene transcription:

- Altering the levels or binding of HRs.
- Stabilizing chromatin interactions required for gene activation/repression.
- Modulating histone modifications in response to hormones.

It is possible that any one of these 3 options, or a combination thereof, could be in play at any one time (Fig. 3). Below, we discuss each possibility.

Does cohesin alter levels or binding of nuclear HRs?

Rather than modulating the transcriptional activity of HRs, cohesin could act farther upstream by influencing HR levels. In support of this idea, it was shown that cohesin directly regulates EcR transcription in *Drosophila* salivary glands (75, 76). However, ablation of cohesin function affects the transcription of some ecdysone-regulated genes hours before EcR levels are reduced, as shown by chromosome puffs associated with ecdysone-regulated genes (76). Therefore, although cohesin appears to directly regulate

Published OnlineFirst September 22, 2011; DOI: 10.1158/1541-7786.MCR-11-0382

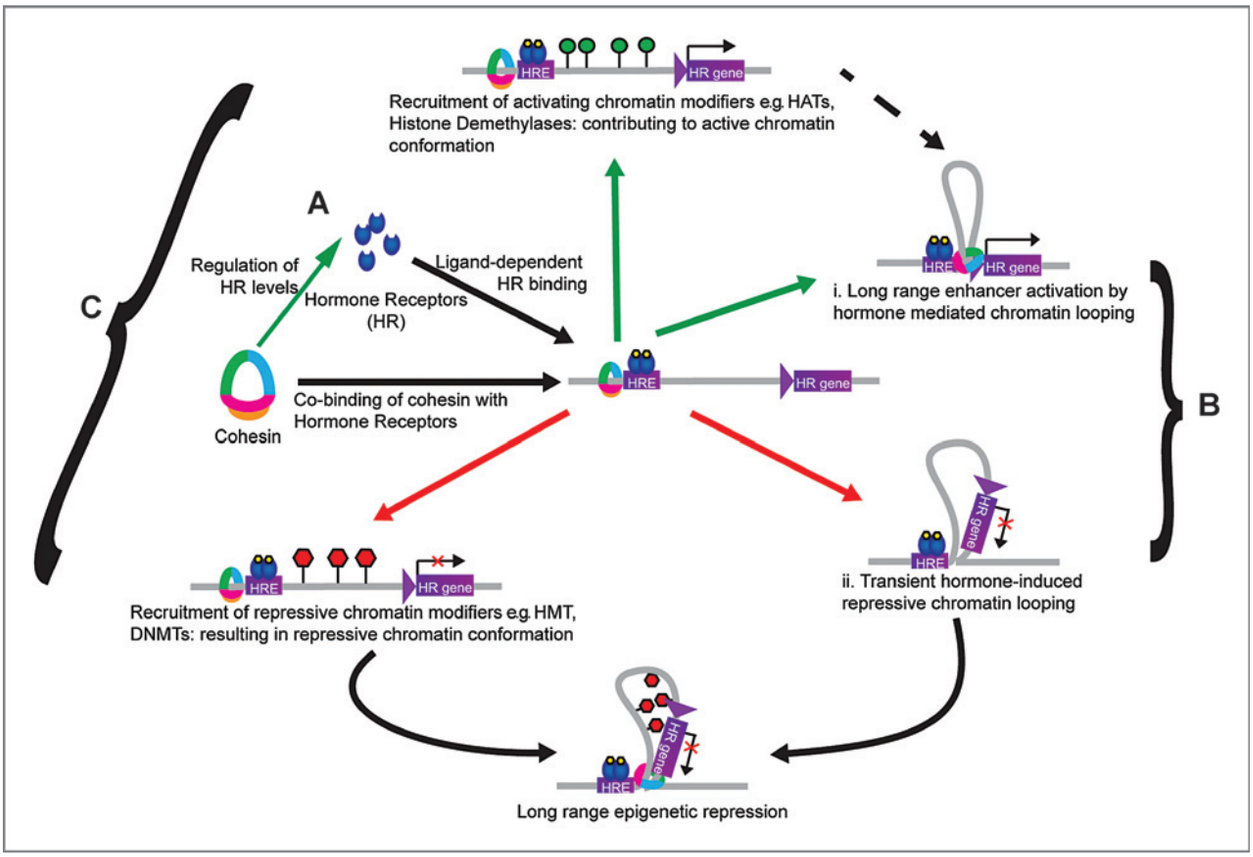

Figure 3. Modulation of hormone-mediated gene regulation by cohesin. Evidence suggests that cohesin is involved in several layers of hormone-mediated gene regulation. A, levels and binding of HRs. There is direct evidence that cohesin can modulate the level of EcR in *Drosophila* (76); however, this does not appear to be the case for other HRs. Cohesin cobinds with HR at HR elements of hormone-responsive genes (76, 77, 88). B, hormone-induced chromatin looping. HR elements are often located several kilobases from the promoter of hormone-responsive genes. (i) Activating loops that bring HR-bound HR elements into contact with PolII bound at the promoter of hormone-responsive genes have been shown in breast and prostate cancer cells (89, 123, 125). Cohesin binding is enriched at these sites of HR-anchored chromatin interaction in breast cancer cells (88). (ii) Transient repressive chromatin loops form in normal breast cells in response to estrogen; however, these loops are stabilized in breast cancer cells, resulting in long-range epigenetic silencing (126). Cohesin may contribute to stabilization of these loops. C, hormone-induced epigenetic modifications. HRs and cohesin have been shown to both recruit and have their activity modulated by histone-modifying complexes that contribute to (i) activation or (ii) repression of hormone-responsive genes.

EcR transcription, this does not account for all of the ecdysone-regulated transcriptional response that also occurs downstream of cohesin.

In contrast to EcR in *Drosophila*, there is little evidence to suggest that cohesin regulates the levels of steroid HRs in vertebrates. In human breast cancer cells, depletion of RAD21 blocked estrogen-mediated reentry into the cell cycle without altering ER protein levels (88). Furthermore, microarray analysis of zebrafish depleted for Nipbl and a variety of cohesin subunits showed no alteration in the transcript levels of ERs (ref. 111; M. Mönnich and J. Horsfield, unpublished results). These results argue against the idea that cohesin contributes to steroid hormone response by modulating transcription of HRs, at least in vertebrates. It is not known whether receptor stability is influenced by cohesin.

Cohesin may affect the ability of HRs to bind to chromatin or affect where HRs bind by modulating histone marks or even direct interactions with the receptors; however, there is no experimental evidence for this as yet.

Cohesin mediates chromatin interactions in response to nuclear HR binding

HR-binding sites can be located at some distance from the promoter of hormone-regulated genes (113, 118). For example, loops that bridge a distal androgen receptor (AR) site to the proximal promoter have been identified by chromosome conformation capture in several androgen-responsive genes, including prostate-specific antigen (123), FKBP5 (124), and TMPRSS2 (125). Fullwood and colleagues (89) identified loops anchored by ER in human breast cancer cells using a genome-wide technique to analyze chromatin interactions (chromatin interaction analysis–paired end tag, ChIA-PET). These chromatin interactions are enriched in estrogen-responsive genes,

Published OnlineFirst September 22, 2011; DOI: 10.1158/1541-7786.MCR-11-0382

suggesting that the loops play a functional role in gene regulation.

The looping of regulatory elements to promoters of hormone-responsive genes suggests that cohesin, which has a function in the formation of chromatin loop structures, could contribute to a hormone response by this mechanism. In support of this idea, it was shown that cohesin colocalizes with ER at many sites in ER-positive breast cancer cells (88). Several of these sites have been shown to act as intrachromosomal loop anchors, suggesting that cohesin may play a role in stabilizing these loops (88, 89). It is possible that cohesin also stabilizes AR-mediated chromatin interactions; however, there is no evidence for this as yet.

Although cohesin may activate transcription of hormone-responsive genes by mediating enhancer–promoter communication, some cohesin-mediated interactions may also be repressive. Normal breast epithelial cells exhibit transient repressive DNA looping in response to estrogen. In breast cancer cells, the repressive loops are stabilized, resulting in long-range epigenetic silencing (126). *RAD21* mRNA levels are elevated in breast cancers relative to normal breast tissue (Table 2), raising the possibility that cohesin may also stabilize repressive loops and/or interactions that activate genes in response to estrogens.

Cohesin may modify chromatin in response to steroid hormones

Estrogen-responsive gene transcription results in 3 cycles of transcription (1 unproductive cycle followed by 2 productive cycles of transcription within 180 minutes) characterized by waves of chromatin modification and transcription-factor binding (reviewed in ref. 127). In each cycle, binding of ligand-bound ER is immediately followed by recruitment of the SWI-SNF chromatin remodeling complex and subsequently histone methyltransferase and histone acetyltransferases, leading to an open chromatin conformation that facilitates gene transcription (127). Of significance, ER functionally interacts with chromatin-modifying enzymes. For example, ER copurifies with the histone demethylase JMJD2B, the depletion of which inhibits estrogen-induced entry into the cell cycle (128). ER also interacts with the PHD-bromodomain chromatin reader TRIM24 to activate estrogen-responsive genes (129). AR also recruits histone acetyltransferases and HDAC to androgen-regulated genes, and these enzymes modify not only the chromatin conformation but also AR itself (reviewed in ref. 130). AR is coactivated by TIP60, a histone acetyltransferase, whereas HDAC1 directly interacts with and represses AR activity (131). In addition, AR interacts with several histone demethylases (132).

The cohesin subunit RAD21 was identified in an siRNA screen to identify epigenetic silencing factors in human cells, alongside known epigenetic factors such as DNMT3a, HDAC1, and TRIM24 (133). As described above, experiments in *Drosophila* have shown that cohesin influences the activity of chromatin modifiers; for example, the *Drosophila verthandi* (*rad21*) mutation genetically behaves like a TrxG member (92), and cohesin physically interacts with PcG proteins (94). TrxG and PcG proteins have a diverse range of roles in transcriptional activation and repression, including regulation of histone methylation and ATP-dependent nucleosome remodeling complexes (reviewed in ref. 134). In humans, cohesin was shown to be part of an ISWI-containing chromatin remodeling complex; indeed, the RAD21 subunit directly interacts with the ATPase subunit SNF2h of this complex (135). Thus, cohesin interacts with TrxG, PcG, and chromatin remodeling complexes, and it likely modulates their function. This raises the possibility that the chromatin remodeling complexes recruited by ER and AR also interact with cohesin, with functional consequences for hormone-dependent cancers.

Of significance, cohesin also appears to influence the function of selective ER modulators and estrogen antagonists. In a recent study, whole-genome shRNA screening was used to identify genes that confer resistance or sensitivity to the selective ER modulator tamoxifen in the invasive ductal breast cancer cell line MCF7 (136). Silencing of several individual components of cohesin (NIPBL, SMC3, and RAD21) led to reduced sensitivity to tamoxifen in MCF7 cells (136). This is in contrast to the observation by Van Agthoven and colleagues (137) that increased expression of the cohesin subunit Rad21 resulted in resistance to tamoxifen or the antiestrogen compound ICI 182780 in a semi-solid growth assay. The authors transduced ZR-75-1 ductal breast cancer cells with cDNA expression libraries from which they identified clones with increased resistance to various antiestrogens. The screen identified 15 genes, including RAD21, that when overexpressed led to antiestrogen resistance. A study that compared the gene expression profiles of MCF7 cells with acquired resistance to tamoxifen showed a significant increase in RAD21 expression (and a 3-fold decrease in PDS5B expression) in tamoxifen-resistant versus tamoxifen-sensitive cells (138). These studies are consistent with the idea that increased cohesin activity leads to resistance to selective ER modulators. How cohesin levels affect the response of ER-positive breast cancer cells to selective ER modulators is not yet clear. The evidence to date suggests that under- or overexpression of cohesin could result in a dysregulated transcriptional response to estrogen (88, 136, 137, 139). How cohesin levels affect ER binding, transactivation, and interaction with antiestrogens remains to be determined.

The *MYC* proto-oncogene is estrogen responsive (140) and regulates more than half of the genes in the estrogen proliferation pathway (141). Elevated levels of MYC can lead to endocrine resistance in breast tumors (142–144). ER-binding sites are found at the *MYC* promoter and at enhancers both upstream and downstream of the *MYC* locus (88, 89). Cohesin binds at the *MYC* gene and at several putative enhancers in an estrogen-dependent manner (88). Some enhancers are >300 kb away from the *MYC* gene, yet they interact long-range with the *MYC* promoters (145–147). Single-nucleotide polymorphisms associated with breast, colon, and prostate cancer reside within these

Published OnlineFirst September 22, 2011; DOI: 10.1158/1541-7786.MCR-11-0382

enhancers, and in at least one case, the single-nucleotide polymorphism genotype influenced *MYC* transcription (146). Binding of cohesin to distant enhancers for *MYC* raises the tantalizing possibility that cohesin participates in their long-range interaction with the *MYC* gene. However, evidence that cohesin is involved in these long-range interactions has not yet emerged.

Given the positive regulation of stem-cell and proliferation genes by cohesin, it may be possible that elevated cohesin levels can bypass the hormone dependency of tumors. Hormone-independent, ER-negative breast cancer cells are able to proliferate in the absence of a mitogenic estrogen signal. A recent meta-analysis and gene-set enrichment analysis revealed that the basal subgroup of ER-negative breast cancer mimics the ER-positive transcriptional response to estrogen, with increased levels of MYC (148). As discussed above, we have shown an evolutionarily conserved role for cohesin in the direct, positive regulation of *MYC* expression (111). Of significance, we found that cohesin is necessary for *MYC* expression in several human cancer cell lines, as well as for estrogen induction of *MYC* in ER-positive breast cancer cell lines (McEwan and Horsfield, unpublished data). It is tempting to speculate that elevated cohesin facilitates *MYC* transcription, which could lead to bypass of hormone dependency. It is not clear whether distant enhancers for *MYC* are involved in its cohesin-dependent regulation. However, because the location of *Myc* relative to its regulatory elements differs vastly between zebrafish and human (Rhodes and Horsfield, unpublished observations), we suspect that alternative, as yet unknown, mechanisms are also involved.

Cohesin: A New Therapeutic Target in Cancer?

Evidence suggests that a disruption of normal cohesin function could have positive outcomes for cancer. It is known that chemotherapy responses are compromised in patients whose tumors overexpress *RAD21* (97). Knocking down *RAD21* expression in MCF-7 breast cancer cells sensitized the cells to chemotherapeutic agents, including etoposide and bleomycin (149). Silencing of *RAD21* in a basal-like breast cancer cell line, MDA-MB-231, rendered the cells more sensitive to the chemotherapy drugs cyclophosphamide and 5-fluorouracil in a manner that directly correlated with the level of *RAD21* expression (97). In breast cancer patients not treated with chemotherapy, no correlation was found between *RAD21* expression and overall survival, whereas in patients treated with chemotherapy, a significantly shorter overall survival was observed in patients whose tumors were *RAD21*-positive (97). Mice heterozygous for a null mutation in *Rad21* are more sensitive to ionizing radiation (150), raising the possibility that chemicals targeting cohesin could be effectively used in combination with radiotherapy in patients. These studies suggest that elevated cohesin is associated with resistance to therapeutics and that targeting cohesin as part of a combination therapy may produce better patient outcomes.

How might cohesin be targeted? Like many other proteins, cohesin subunits are posttranslationally modified at different stages of the cell cycle, with a range of functional consequences. For example, forward/reverse phosphorylation and acetylation events regulate cell cycle stage–specific binding of cohesin to chromatin (Fig. 1). Designing drugs to interfere with enzymes that mediate cohesin modifications could pave the way to new combination therapies and overcome problems with tumors that are refractory to current therapies. For example, deacetylation of SMC3 by a class I HDAC is required for cohesin recycling onto chromosomes after cell division (42–44). Clinically available HDAC inhibitors slow the growth of breast tumors and reverse hormone therapy resistance in ER-positive breast cancers (151, 152). It is not yet known whether this class of compounds affects cohesin function.

In conclusion, genetic alterations that lead to an imbalance of cohesin levels may result in cancer through mechanisms other than aneuploidy and genome instability. Regulation of gene expression by cohesin, through mechanisms that affect chromatin structure and three-dimensional organization, may play a significant role in tumorigenesis. In support of this concept, multiple lines of evidence show that cohesin maintains the expression of pluripotency and cell proliferation factors and that it modulates hormone-dependent gene transcription in cancer cells.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

Acknowledgments

The authors thank Dale Dorsett for a critical reading of the manuscript.

Grant Support

Health Research Council of New Zealand (No. 10-873), Cancer Society of New Zealand, Genesis Oncology Trust, and Breast Cancer Research Trust.

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Received August 11, 2011; accepted September 1, 2011; published OnlineFirst September 20, 2011.

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Published OnlineFirst September 22, 2011; DOI: 10.1158/1541-7786.MCR-11-0382

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Mol Cancer Res 2011;9:1587-1607. Published OnlineFirst September 22, 2011.