Cohesin Mutations in Cancer

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Cohesin is a large ring-shaped protein complex, conserved from yeast to human, which participates in most DNA transactions that take place in the nucleus. It mediates sister chromatid cohesion, which is essential for chromosome segregation and homologous recombination (HR)-mediated DNA repair. Together with architectural proteins and transcriptional regulators, such as CTCF and Mediator, respectively, it contributes to genome organization at different scales and thereby affects transcription, DNA replication, and locus rearrangement. Although cohesin is essential for cell viability, partial loss of function can affect these processes differently in distinct cell types. Mutations in genes encoding cohesin subunits and regulators of the complex have been identified in several cancers. Understanding the functional significance of these alterations may have relevant implications for patient classification, risk prediction, and choice of treatment. Moreover, identification of vulnerabilities in cancer cells harboring cohesin mutations may provide new therapeutic opportunities and guide the design of personalized treatments.

Cohesin is one of the three structural maintenance of chromosomes (SMC) complexes that exist in eukaryotic cells. The other two are condensin and the Smc5/6 complex. They are all composed of an SMC heterodimer and additional non-SMC subunits arranged in a characteristic domain architecture (Haering and Gruber 2016). Remarkably, bacteria and archaea also possess SMC complexes, although in this case the SMC proteins homodimerize. In all three kingdoms of life, the functions of SMC complexes are critical for genome organization, chromosome duplication, and segregation. In particular, cohesin was initially identified for its role in sister chromatid cohesion (Guacci et al. 1997; Michaelis et al. 1997; Losada et al. 1998), a requirement for proper chromosome segregation in mitosis and meiosis, as well as

for homologous recombination (HR)-mediated DNA repair (Nasmyth and Haering 2009). In addition, cohesin is currently recognized as a major player in higher-order chromatin structure together with the CCCTC-binding factor (CTCF) (Phillips-Cremins et al. 2013; Mizuguchi et al. 2014). How the same complex can perform all of these different functions is far from understood. Germline mutations in cohesin and its regulators are at the origin of human developmental syndromes collectively known as cohesinopathies, the most prevalent of which is Cornelia de Lange syndrome (CdLS) (Horsfield et al. 2012). Recent sequencing efforts of cancer genomes have revealed the presence of somatic mutations in cohesin in several cancer types. Understanding how cohesin works and how it is regulated will likely help us recognize the con-

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tribution of these mutations to tumor initiation and progression.

THE BASIC BIOLOGY OF COHESIN

Composition and Architecture

Cohesin is a ring-shaped complex that consists of Smc1, Smc3, Rad21, and SA (see Table 1 for nomenclature). SMCs are 1000–1500 amino-acid-long proteins that contain two coiled-coil stretches separated by a flexible globular domain called “hinge.” When folded at this domain, the amino and carboxyl termini of the protein are brought in proximity to create an ATPase head domain (hd; Fig. 1). Smc1 and Smc3 interact stably through their hinges and on the other end are bridged by the Rad21 subunit (Haering et al. 2002, 2004; Gligoris et al. 2014; Huis in ‘t Veld et al. 2014). The central region of Rad21 binds the fourth subunit of cohesin, SA (Haering et al. 2002; Orgil et al. 2015). SA is composed of many homologous huntingtin, elongation factor 3, A subunit, and TOR (HEAT) repeats and likely serves as an interaction platform for cohesin-interacting proteins (Hara et al. 2014). Among these are two regulatory subunits associated with chromatin-bound cohesin complexes throughout the cell cycle, Pds5 and Wapl (Sumara et al.

Figure 1. Cohesin composition and architecture. When the Smc1 and Smc3 proteins are folded at their flexible hinge domains, the NTP-binding motif and the DA box present at their amino- and carboxy-terminal globular domains come together to form a functional ATPase. Smc1 and Smc3 interact through their hinges, whereas the kleisin subunit Rad21 bridges their head domains and associates with SA. The outer diameter of the resulting ring-shaped complex is estimated at ~50 nm and could hold two 10-nm chromatin fibers.

2000; Losada et al. 2005; Gandhi et al. 2006; Kueng et al. 2006). There are also transient or position-specific interactors such as CTCF (Xiao et al. 2011) or the telomeric protein TRF1 (Canudas et al. 2007). In vertebrate somatic cells cohesin contains one of two SA sub-

Table 1. Nomenclature of cohesin subunits and regulators

Category	Yeast (<i>Saccharomyces cerevisiae</i>)	Mouse/human
Cohesin	Smc1	Smc1α (<i>SMC1A</i>) Smc1β (<i>SMC1B</i>)
	Smc3	Smc3 (<i>SMC3</i>)
	Scc1 Rec8	Rad21 (<i>RAD21</i>) Rad21L (<i>RAD21L1</i>) Rec8 (<i>REC8</i>)
	Scc3	SA1 (<i>STAG1</i>) SA2 (<i>STAG2</i>) SA3 (<i>STAG3</i>)
Associated factors	Pds5	Pds5A (<i>PDS5A</i>) Pds5B (<i>PDS5B</i>)
	Wapl/Rad61	Wapl (<i>WAPL</i>)
	–	Sororin (<i>CDCA5</i>)
Loader	Scc2	Nipbl (<i>NIPBL</i>)
	Scc4	Mau2 (<i>MAU2</i>)
CoAT	Eco1	Esco1 (<i>ESCO1</i>) Esco2 (<i>ESCO2</i>)
CoDAC	Hos1	Hdac8 (<i>HDAC8</i>)
Mitotic regulators	Sgo1	Sgo1 (<i>SGOL1</i>)
	Separase/Esp1	Separase (<i>ESPL1</i>)
	Securin/Pds1	Securin (<i>PTTG1</i>)

Meiosis-specific variants are shown in red. For mouse/human, the name of the gene appears in parentheses. CoAT, cohesin acetyl transferase; CoDAC, cohesin deacetylase.

units, SA1 or SA2 (Losada et al. 2000; Sumara et al. 2000; Remeseiro et al. 2012a). Additional meiosis-specific versions of all cohesin subunits exist except Smc3 (in red in Table 1).

Cohesin Interaction with DNA

Evidence from a number of in vivo and in vitro studies supports a model in which cohesin entraps the chromatin fiber within its ring structure (Gruber et al. 2003; Haering et al. 2008). Cohesin loading occurs in G₁ and requires ATP hydrolysis and a heterodimeric complex composed of Nipbl and Mau2 (Fig. 2) (Arumugam et al. 2003; Weitzer et al. 2003; Gillespie and Hirano 2004; Watrin et al. 2006). In vitro, loading can occur in the absence of the loader, albeit very inefficiently (Murayama and Uhlmann 2014). After loading, cohesin binding to chromatin is dynamic and unloading mediated by Wapl occurs throughout the cell cycle (Gerlich et al. 2006; Bernard et al. 2008). Some evidence

supports the idea that DNA enters and exits the cohesin ring through different interfaces or “gates” (Nasmyth 2011; Buheitel and Stemmann 2013; Eichinger et al. 2013). The entry gate requires dissociation of the Smc1 and Smc3 hinges (Gruber et al. 2006), whereas the exit gate would be located in the interface formed by the coiled coil emerging from the Smc3 hd and two α helices in the amino terminus of Rad21 (Gligoris et al. 2014; Huis in ‘t Veld et al. 2014). The opening/closure of this second gate is regulated by the ATPase activity of the SMCs, by DNA sensing through two lysines present in the Smc3 hd (K105 and K106 in human Smc3), and by Pds5-Wapl (Murayama and Uhlmann 2015).

Cohesin Distribution

Chromatin immunoprecipitation (ChIP) studies provide a genome-wide view of cohesin distribution. In yeast, cohesin accumulates in a

Figure 2. Cohesin and its regulators throughout the cell cycle. Cohesin is loaded on chromatin by Nipbl-Mau2 throughout the cell cycle, starting in early G₁. Pds5 and Wapl associate with chromatin-bound cohesin and promote its unloading. Cohesin complexes may be encircling a single chromatin fiber or two fibers at the base of a chromatin loop that brings distal regions in proximity. During S phase, acetylation of Smc3 by CoATs Esco1/2 and Sororin recruitment, both facilitated by Pds5A/B (not depicted), results in cohesin establishment. A fraction of cohesin remains dynamic even after DNA replication (not depicted). Whether cohesin at the base of chromatin loops is also involved in tethering sister chromatids is not known. In prophase, most cohesin dissociates from chromatin in a process that requires Wapl and phosphorylation of cohesin and Sororin. Sgo1 and its partner PP2A prevent the dissociation of a population of cohesin, enriched at centromeres. This population is removed at the onset of anaphase when Securin (not depicted) is destroyed and the active Separase cleaves Rad21.

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50-kb region around centromeres and at sites of convergent transcription (Glynn et al. 2004; Lengronne et al. 2004). Because there is little colocalization of cohesin and its loader, it has been proposed that once topologically entrapping chromatin, the cohesin ring can slide away from the loading site (Hu et al. 2011; Ocampo-Hafalla and Uhlmann 2011). In contrast, cohesin and its loader do colocalize at sites of active transcription in *Drosophila* (Misulovin et al. 2008). Comparison of cohesin distribution in two different mouse tissues also reveals a correlation between active transcription and the presence of cohesin (Cuadrado et al. 2015). Importantly, cohesin accumulates at CTCF binding sites along the human and mouse genomes, but only when CTCF is present (Parelho et al. 2008; Rubio et al. 2008; Wendt et al. 2008; Remeseiro et al. 2012b). Whether cohesin is loaded at these CTCF sites or is loaded elsewhere and then slides to reach them is not known. The number of Nipbl positions identified by ChIP is 5–10 times lower than the number of cohesin or CTCF sites, and cohesin and its loader colocalize only at a subset of sites near active genes (Kagey et al. 2010) but not at CTCF sites (Zuin et al. 2014b). Considering the data from different model organisms, it is possible that cohesin is loaded mainly at sites of active transcription and then moves along the genome until it finds an obstacle, such as CTCF or another chromatin-binding protein with which it interacts. This ability of cohesin to slide along DNA may be consistent with “loop-extrusion” models recently proposed to explain how cohesin and CTCF contribute to the formation of chromatin loops (de Wit et al. 2015; Nichols and Corces 2015; Sanborn et al. 2015).

Cohesion Establishment and Dissolution

Cohesion establishment is coupled to DNA replication and requires acetylation of the two aforementioned lysine residues in the Smc3 hd by cohesin acetyltransferases (CoATs) and Sororin recruitment (Fig. 2) (Rolef Ben-Shahar et al. 2008; Unal et al. 2008; Zhang et al. 2008). The functional links between Smc3 acetylation, ATP hydrolysis, and DNA entrapment

are not clear yet (Heidinger-Pauli et al. 2010b; Ladurner et al. 2014; Camdere et al. 2015). There are two CoATs in mammalian cells, Esco1 and Esco2, with partially redundant functions (Hou and Zou 2005; Whelan et al. 2012; Minamino et al. 2015; Rahman et al. 2015). Pds5 proteins, which exist in two versions in vertebrate cells, Pds5A and Pds5B, are also required for cohesion establishment in yeast and mouse cells (Vaur et al. 2012; Carretero et al. 2013; Chan et al. 2013). As a result of establishment, Sororin displaces Wapl from its Pds5-interaction site and thereby counteracts its unloading activity (Nishiyama et al. 2010; Ouyang et al. 2016). In this way, a fraction of cohesin complexes tethering the two sister chromatids become stably bound to chromatin and maintain cohesion until mitosis (Gerlich et al. 2006; Schmitz et al. 2007). Whether a single complex embraces the two sister chromatids or two complexes are required, each one embracing a sister, is still a matter of debate (Eng et al. 2015).

At the time of chromosome segregation, cohesin dissociates from DNA in two steps, in prophase and anaphase (Fig. 2) (Losada et al. 1998; Waizenegger et al. 2000). The prophase pathway requires SA phosphorylation by Plk1 and release of Sororin, after phosphorylation by Cdk1 and Aurora B, to restore Wapl unloading activity (Losada et al. 2002; Sumara et al. 2002; Dreier et al. 2011; Nishiyama et al. 2013). Shugoshin (Sgo1) and its partner, the protein phosphatase 2A (PP2A), prevent cohesin release around centromeres (McGuinness et al. 2005). Sgo1 outcompetes the binding of Wapl to SA-Rad21 (Hara et al. 2014), whereas PP2A counteracts Sororin dissociation (Liu et al. 2013b). Pericentromeric cohesin remains on chromatin and is essential to hold the sister chromatids together until all the chromosomes establish proper attachments to opposite spindle poles (Toyoda and Yanagida 2006; Liu et al. 2013a). Once this task is completed, activation of the anaphase-promoting complex (APC/C) leads to degradation of Securin and activation of Separase (Shindo et al. 2012). The protease cleaves the kleisin subunit of chromatin-bound cohesin and sister chromatid separation ensues (Hauf et al. 2001). In yeast, all chromatin-bound co-

hesin is released from chromatin in anaphase by this cleavage pathway (Uhlmann et al. 2000). Acetylated cohesin removed from chromosomes in prophase and anaphase is deacetylated by a cohesin deacetylase (CoDAC), Hdac8 in human cells and Hos1 in yeast (Beckouet et al. 2010; Borges et al. 2010; Deardorff et al. 2012).

Cohesin Functions

Our knowledge of cohesin functions comes from studies in many different experimental systems, most notably yeast and human cells, but also *Drosophila*, zebrafish, and *Xenopus* egg extracts. Mouse models carrying knockout alleles for genes encoding cohesin subunits or their regulators have also been generated and characterized to different extents (Table 2). As mentioned above, cohesin was first recognized as a mediator of sister chromatid cohesion (Fig. 3, left). During mitosis, cohesion contributes to the proper orientation of sister kinetochores (Sakuno et al. 2009) and prevents the premature separation of sister chromatids under the pulling forces of spindle microtubules, whereas chromosomes try to align at the metaphase plate (Daum et al. 2011). In the absence of cohesin, chromosome missegregation is commonly observed (Sonoda et al. 2001; Vass et al. 2003; Toyoda and Yanagida 2006; Barber et al. 2008; Solomon et al. 2013; Covo et al. 2014). Cohesin is also important for HR-driven DNA repair (Sjogren and Nasmyth 2001; Schmitz et al. 2007; Heidinger-Pauli et al. 2010a; Xu et al. 2010; Wu et al. 2012). Cohesin promotes usage of the sister chromatid as a template for faithful repair while preventing both damage-induced recombination between homologs (Covo et al. 2010) and end joining of distal double-strand breaks (DSB) (Gelot et al. 2015). A most intriguing function of cohesin is cohesion between mother and daughter centrioles, and its regulation bears similarities with that of sister chromatid cohesion (Wang et al. 2008; Beauchene et al. 2010; Schockel et al. 2011; Mohr et al. 2015).

Cohesin-SA1 and cohesin-SA2 coexist in vertebrate cells and mediate cohesion at telomeres and centromeres, respectively (Canudas

and Smith 2009; Remeseiro et al. 2012a). Telomeres are repeated regions prone to fork stalling (Sfeir et al. 2009). Cohesin-SA1 likely stabilizes stalled forks and facilitates their restart by HR, consistent with results in budding yeast (Tittel-Elmer et al. 2012). In SA1-null mouse embryo fibroblasts (MEFs), faulty telomere replication leads to chromosome missegregation (Remeseiro et al. 2012a). Cohesin-SA2 is preferentially recruited to laser-induced DNA damage sites in postreplicative human cells (Kong et al. 2014) but both complexes are loaded at double-strand breaks (DSBs) generated by a restriction enzyme (Caron et al. 2012). HR-mediated DNA repair in cells exposed to replication stress is also facilitated by both complexes (Remeseiro et al. 2012a).

Cohesin performs additional functions that do not require cohesion establishment (Fig. 3, right). These functions could be related to the ability of cohesin to tether chromatin fibers at the base of a chromatin loop to facilitate long-range interactions. Cohesin contributes to the spatial organization of the genome, together with CTCF. Recently developed chromosome conformation capture (3C)-related technologies together with improved microscopy and computational modeling offer the picture of a genome partitioned in “topological” domains that are conserved among cell types and even in evolution (Dixon et al. 2012). Within these domains, more local contacts allow or prevent communication between enhancers and promoters (Kagey et al. 2010; Phillips-Cremins et al. 2013; Downen et al. 2014; Tang et al. 2015). Down-regulation of cohesin leads to a loss of contacts and deregulation of gene expression (Hadjur et al. 2009; Mishiro et al. 2009; Nativio et al. 2009; Seitan et al. 2013; Sofueva et al. 2013; Zuin et al. 2014a). Importantly, different loci display very different sensitivities to loss of cohesin (Ing-Simmons et al. 2015; Viny et al. 2015). In the pancreata of SA1 heterozygous mice, for instance, a twofold decrease in SA1 protein levels is sufficient to alter the chromatin architecture and the expression of the *Reg* gene cluster. The resulting down-regulation of *Reg* proteins, involved in inflammation, may contribute to the increased incidence of pancreatic

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Table 2. Mouse models of cohesin subunits and regulators

Targeted gene	References	Phenotype
<i>SMC3</i>	White et al. 2013; Viny et al. 2015	Embryonic lethality (prior to E14.5). Heterozygous animals have reduced body weight and higher mortality rates, and a subset showed a distinct craniofacial morphology (reminiscent of Cornelia de Lange syndrome [CdLS]). Deletion in hematopoietic compartment in adult mice results in rapid lethality.
<i>RAD21</i>	Xu et al. 2010; Seitan et al. 2011	Embryonic lethality (prior to E8.5). Heterozygous mouse embryo fibroblasts (MEFs) are defective in homologous recombination (HR)-mediated DNA repair. Heterozygous animals show increased sensitivity to irradiation, particularly in the gastrointestinal tract and the hematopoietic system. Deletion in thymocytes results in reduced differentiation efficiency and impairs TCR α locus rearrangement.
<i>STAG1</i>	Remeseiro et al. 2012a,b	Embryonic lethality (from E12.5). Null MEFs show telomere cohesion defects leading to faulty replication and chromosome missegregation, altered transcription, and decreased colocalization of cohesin at CTCF sites and promoters. Heterozygous animals show increased incidence and earlier onset of cancer but are protected against acute carcinogenesis.
<i>WAPL</i>	Tedeschi et al. 2013	Embryonic lethality. Heterozygous animals healthy. Conditional elimination in MEFs leads to aberrant retention of cohesin on chromatin, altered transcription, defects in cell-cycle progression, and chromosome segregation.
<i>PDS5A</i>	Zhang et al. 2009; Carretero et al. 2013	Late embryonic lethality (from E12.5) or death soon after birth (depending on the allele) with cleft palate, skeletal patterning defects, growth retardation, congenital heart defects. Null MEFs proliferate slowly but show no chromosome missegregation.
<i>PDS5B</i>	Zhang et al. 2007; Carretero et al. 2013	Late embryonic lethality (from E12.5) or death soon after birth (depending on the allele) with multiple congenital anomalies, including heart defects, cleft palate, fusion of the ribs, short limbs. Null MEFs show centromere cohesion defects and delocalization of the chromosomal passenger complex (CPC), chromosome missegregation, and aneuploidy.
<i>NIPBL</i>	Kawauchi et al. 2009; Remeseiro et al. 2013; Smith et al. 2014	Embryonic lethality (prior to E9.5). Up to 80% of heterozygous animals die during the first weeks of life and display CdLS-like defects such as small size, craniofacial anomalies, heart defects, delayed bone maturation, and behavioral disturbances. Heterozygous MEFs show gene expression alterations but no cohesion defects.
<i>MAU2</i>	Smith et al. 2014	Embryonic lethality (prior to E9.5). Heterozygous animals are normal.
<i>ESCO2</i>	Whelan et al. 2012	Embryonic lethality (prior to E8). Conditional elimination in MEFs leads to defects in centromere cohesion and chromosome segregation.
<i>HDAC8</i>	Haberland et al. 2009	Homozygous mice show perinatal lethality with dramatic skull abnormalities.
<i>SGO1</i>	Yamada et al. 2012	Embryonic lethality. Heterozygous MEFs show chromosome missegregation and aneuploidy. Heterozygous animals viable but display increased susceptibility to colon and liver cancer induced by treatment with azoxymethane.

Continued

Table 2. Continued

Targeted gene	References	Phenotype
<i>ESPL1</i>	Kumada et al. 2006; Wirth et al. 2006	Embryonic lethality (E3.5). Conditional elimination in MEFs leads to proliferation defects and polyploidy, with multiple chromosomes connected at their centromeric regions. Depletion in bone marrow causes aplasia.
<i>PTTG1</i>	Mei et al. 2001; Kumada et al. 2006	Homozygous mice are viable but display testicular and splenic hypoplasia, thymic hyperplasia, and thrombocytopenia. Null MEFs grow slowly in culture and accumulate in G ₂ .

cancer observed in SA1 heterozygous mice (Remeseiro et al. 2012a).

Cohesin depletion also increases RNA polymerase II pausing at cohesin binding genes in *Drosophila*, suggesting that it regulates its tran-

sition to elongation (Schaaf et al. 2013). In human cells, cohesin-SA1 is specifically involved in interactions with the super elongation complex (SEC) involved in mobilization of the paused polymerase (Izumi et al. 2015). Transcriptional

Figure 3. Cohesin functions. Cohesin plays important roles in several cellular processes involving DNA. These roles rely on the ability of cohesin to hold two DNA strands in *trans* (the sister chromatids) or in *cis* (e.g., at the base of a chromatin loop). Accurate chromosome segregation in mitosis and meiosis, HR-mediated DNA repair, and restart and/or protection of stalled replication forks require sister chromatid cohesion (*left*). DNA looping mediated by cohesin in collaboration with CTCF, Mediator, or transcription factors, among others, likely provides a major organizational principle for the genome (*right*). This organization regulates transcription both globally, through generation of active/silent domains, and locally, facilitating interactions between enhancer and promoters required for gene activation or RNA Pol II pause release. It also facilitates coordinated origin firing at replication factories and recombination at loci such as IgH or TCR α . For simplicity, a single cohesin ring embracing the two DNA fibers is drawn, but alternative configurations are possible (Eng et al. 2015).

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control at the level of elongation is key for a number of developmental genes (Smith and Shilatifard 2013) and has been linked to pathogenesis in some leukemias (Lin et al. 2010).

In addition to transcription regulation, several lines of evidence support the idea that chromatin loops stabilized by cohesin organize DNA replication factories to promote efficient origin firing (Guillou et al. 2010), and facilitate V(D)J recombination (Degner et al. 2011) and T-cell receptor α locus rearrangement (Seitan et al. 2011).

COHESIN MUTATIONS IN CANCER

Recent pan-cancer studies have placed cohesin and its regulators among the networks most frequently mutated in cancer (Kandoth et al. 2013; Lawrence et al. 2014; Leiserson et al. 2015). Mutations in genes encoding cohesin subunits had been first reported in colorectal cancer after targeted sequencing of genes essential for chromosome segregation in yeast (Barber et al. 2008). A few years later, mutations in the gene encoding SA2, *STAG2*, were found in glioblastoma, Ewing sarcoma, and melanoma (Solomon et al. 2011). These two studies pointed to chromosome missegregation as the main contribution of cohesin dysfunction to tumorigenesis. However, sequencing of acute myeloid leukemia (AML) samples revealed the presence of recurrent mutations in *STAG2*, *SMC3*, *RAD21*, and *SMC1A* that were not associated with cytogenetic abnormalities, implying alternative pathological pathways (Welch et al. 2012). The correlation between *STAG2* mutations and aneuploidy in bladder cancer was also unclear (Balbas-Martinez et al. 2013; Guo et al. 2013). In the next sections we will review these and other recent studies that provide evidence for the presence of cohesin mutations in cancer.

Cohesin Mutations in Myeloid Malignancies

Identification of Mutations in Cancer Cells

AML results from the aberrant proliferation and impaired differentiation of hematopoietic stem

and progenitor cells. Reports using next-generation sequencing in AML samples appeared by 2012 and identified mutations in cohesin genes (Ding et al. 2012; Dolnik et al. 2012; Walter et al. 2012; Welch et al. 2012). According to The Cancer Genome Atlas (TCGA) Research Network (2013), AML genomes have fewer mutations than most adult cancers and, among them, 13% correspond to cohesin-related genes (Fig. 4A). Thol et al. (2014) performed targeted sequencing of genes encoding the five cohesin core subunits in samples from 389 AML patients and identified mutations in all of them (collectively in 6% of the cases). Most patients carrying cohesin mutations had a normal karyotype, supporting the hypothesis that they do not affect genome integrity. Importantly, cohesin mutations were found in myeloid malignancies other than AML (Kon et al. 2013). *STAG2* and *RAD21* were the most mutated cohesin genes (Fig. 4B). An even higher frequency of cohesin mutations ($\sim 15\%$, most of them in *STAG2*) was found in MDS samples in another study (Haerlach et al. 2014). Even in the absence of cohesin mutations, low expression of cohesin components was detected in a significant fraction of myeloid malignancies (Thota et al. 2014). Mutations in additional components of the cohesin network including *PDS5B*, *NIPBL*, or *ESCO2* were also identified in some studies (Fig. 4A,B).

The prognostic impact of cohesin mutations in myeloid disorders is unclear, with studies reporting a positive (Kihara et al. 2014), negative (Thota et al. 2014), or no significant effect (Thol et al. 2014) on survival. The presence of these mutations in the major tumor populations points to their early origin during the neoplastic process (Kon et al. 2013; Thol et al. 2014). Interestingly, two patients analyzed by Kon et al. harbored each two independent subclones with different *STAG2* mutations, which suggests that loss of *STAG2* could confer a strong advantage to preexisting leukemic cells during clonal evolution. Analysis of clonal dynamics in another report revealed that cohesin mutations were not commonly present in the founder clone but rather promoted clonal expansion and transformation to more aggressive disease

Figure 4. Cohesin mutations in myeloid malignancies and bladder cancer. Mutation matrix of cohesin subunits, associated factors (*PDS5A*, *PDS5B*, *WAPL*, *CDC5A*), loaders (*NIPBL*, *MAU2*), CoAT/CoDAC (*ESCO1*, *ESCO2*, *HDAC8*), mitotic regulators (*SGOL1*, *ESPL1*, *PTTG1*), and *CTCF* in AML (A), other myeloid malignancies (B), and bladder cancer (C). Nonsense, frameshift, and splice site mutations are grouped as truncating mutations (red), and missense and inframe indels are labeled as missense mutations (blue). (Data from Ding et al. 2012; Jan et al. 2012; Welch et al. 2012; Balbas-Martinez et al. 2013; Cancer Genome Atlas Research Network 2013, 2014; Guo et al. 2013; Kon et al. 2013; Yoshida et al. 2013; Lohr et al. 2014; Pellagatti et al. 2014; Thota et al. 2014.)

(Thota et al. 2014). Cohesin mutations often co-occurred with other mutations, most often in nucleophosmin (*NPM1*), epigenetic regulators such as *ASXL1*, *TET2*, or *DNMT3A*, or transcription factors like *RUNX1* (Cancer Genome Atlas Research Network 2013; Walter et al. 2013; Kihara et al. 2014; Thol et al. 2014). Further support for a key role of cohesin mutations in malignant transformation came from the analysis of Down syndrome–related acute

megakaryocytic leukemia (DS-AMKL) (Yoshida et al. 2013). Children with Down syndrome often suffer transient abnormal myelopoiesis (TAM) that, in some cases, evolves to DS-AMKL. Genomic profiling of TAM, DS-AMKL, and non-DS-AMKL samples revealed that TAM is caused by a *GATA1* mutation and progression to DS-AMKL requires additional mutations. More than half of them were found in cohesin genes. Here again, most cases with mutated co-

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hesin had normal karyotypes, except for constitutive trisomy 21.

In summary, sequencing studies have revealed a high prevalence of mutations in the genes encoding cohesin components in AML and other myeloid cancers (Fig. 4A,B). As expected from proteins working as part of the same complex, cohesin mutations are mutually exclusive. Although mutation rates differ among these studies, they typically account collectively for less than 10% of the cases. Cohesin mutations are usually heterozygous, with the exception of those present in the X-linked genes *STAG2* and *SMC1A* in male patients. In female samples, mutations in *STAG2* and *SMC1A* often reside in the active chromosome. While *SMC1A*, *SMC3*, and *STAG1* mutations are often missense, those in *STAG2* and *RAD21* are usually truncating (i.e., frameshift, nonsense, or splice site mutations). No clear mutation hotspot has been identified in any of these genes, a characteristic of tumor suppressors. Importantly, no association of cohesin mutations and unstable karyotypes or aneuploidy has been reported. Thus, the contribution of cohesin dysfunction to development of myeloid malignancies is possibly not related to cohesion defects and genomic instability, and instead could be the result of altered transcription.

Functional Studies in Hematopoietic Cells

Cohesin mutations may reduce the levels of cohesin complexes in the cell or may alter their functionality. Understanding the contribution of these changes to tumorigenesis requires functional studies. Mazumdar et al. (2015) introduced a missense *SMC1A* mutant (*SMC1A* R711G) and a *RAD21* truncation mutant (*RAD21* Q592*), previously identified in AML (Cancer Genome Atlas Research Network 2013), in primary human hematopoietic stem and progenitor cells (HSPCs). No clear defects in proliferation or cell death were observed, but differentiation was impaired. The defects were restricted to the most immature populations of cord blood cells, consistent with the observation of cohesin mutations in the most

immature forms of AML (Welch et al. 2012). Increased chromatin accessibility was observed in regions enriched for DNA-binding motifs of ERG, GATA2, and RUNX1, all transcription factors (TFs) involved in maintenance of the stem-cell program in HSPCs (Wilson et al. 2010). Knockdown of any of these factors reversed the differentiation block of cohesin mutants.

Two additional studies have explored the consequences of cohesin knockdown (kd) in hematopoiesis. Transgenic mice carrying inducible shRNAs against SA2, *Smc1α*, and *Rad21* allowed ubiquitous and inducible cohesin kd in vivo in adult mice (Mullenders et al. 2015). Efficient reduction of cohesin levels, at least in the hematopoietic organs, was well tolerated, suggesting that a small fraction of cohesin complexes is sufficient to carry out its essential functions. Lineage skewing toward myeloid lineage commitment was observed in the spleen of cohesin-deficient mice, as well as in HSPCs, and gene expression changed accordingly. SA2 kd led to increased chromatin accessibility in regions enriched in the GATA motif. In the other study, mice carrying a conditional KO allele of *SMC3* were used (Viny et al. 2015). Complete ablation of *SMC3* in the hematopoietic compartment led to rapid lethality, whereas deletion of a single *SMC3* allele resulted in increased cell renewal capacity of HSPCs and reduced expression of transcription factors and other genes associated with lineage commitment. Hematopoietic progenitors of *Scm3* heterozygous animals displayed increased accessibility in regions harboring binding sites for yet another transcription factor, STAT5.

Taken all together, these studies suggest that decreased cohesin levels may promote transformation of HSPCs through delaying or skewing differentiation and instead enforcing stem-cell programs. They appear to do so through modulation of chromatin accessibility of TFs involved in stem-cell maintenance. An alternative possibility is that the observed changes are a consequence rather than the cause of the differentiation block. Cohesin has been proposed to regulate the expression of cell identity genes together with CTCF (Dowen et al. 2014), or

with tissue-specific TFs (Schmidt et al. 2010) through the control of chromosome structure. To extend the studies described here, it would be informative to actually compare cohesin binding to chromatin in cells carrying or not the cohesin mutants, or partially deficient in cohesin subunits. This comparison could be performed in bulk by using chromatin fractionation, at genome wide scale resolution by using ChIP-seq and at specific sites by ChIP-qPCR. It would also be of interest to look for changes in chromatin architecture near the promoters of genes required to promote or prevent terminal myeloid differentiation, including the above-mentioned TFs. For instance, cohesin-mediated contacts between *cis*-regulatory elements modulate tissue-specific RUNX1 expression in zebrafish embryos, and probably also in human hematopoietic cells (Horsfield et al. 2007; Marsman et al. 2014).

Importantly, *SMC3* haploinsufficiency by itself did not result in AML, but it enhanced tumorigenesis when combined with a *FLT3*-internal tandem duplication (ITD) mutation often found in AML (Viny et al. 2015). Aged cohesin knockdown mice in the study by Mullenders et al. (2015) developed phenotypes resembling myeloid neoplasias, but did not develop frank AML. These observations are consistent with the idea that cohesin mutations cooperate with additional mutations to promote myeloid malignancies.

Cohesin Mutations in Bladder Cancer

Urothelial bladder cancer (UBC) is a heterogeneous disease. Tumors are classified according to the stage of invasion (Tis-T4), and graded based on their cellular characteristics. At diagnosis, around 60% of bladder cancers are non-muscle-invasive (NMIBC) papillary tumors of low grade. Stage T1 tumors, which have penetrated the epithelial basement membrane but have not invaded the muscle, are mostly of high grade. Also aggressive are the muscle-invasive bladder cancers (MIBCs). Sequencing of 99 low-grade tumors revealed mutations in *STAG2* (16%), *NIPBL* (4%), *SMC1A* (3%), and *SMC3* (2%), as well as in the gene-encoding separase,

ESPL1 (6%) (Guo et al. 2013). Individuals with *STAG2* mutations had worse prognosis and increased number of copy number variations (CNVs), indicative of increased genomic instability. Soon afterward, a discovery exome sequencing screen ($n = 17$), followed by a prevalence screen ($n = 60$), identified mutations in *STAG2* (16%) and some other cohesin subunits in UBC (Balbas-Martinez et al. 2013). *STAG2* was mutated mainly in tumors of low stage or grade, commonly genomically stable, and unlike the previous study, its loss was associated with improved outcome. Moreover, chromosome number changes were not associated with *STAG2* deficiency. Another analysis of aggressive MIBCs identified *STAG2* mutations in 14 out of 131 tumors (11%), often co-occurring with mutations in epigenetic regulators (Cancer Genome Atlas Research Network 2014). Tumors with mutations in other cohesin subunits (9%) and cohesin regulators (12%) were also identified (Fig. 4C).

Solomon et al. (2013) reported higher mutation frequencies after sequencing *STAG2* in 111 tumors of different stages/grades. Around 36% and 27% of *STAG2* mutations were found in pTa and pT1 NMIBCs, respectively, and 16% in MIBCs. In low-grade NMIBCs, loss of *STAG2* expression was significantly associated with increased disease-free survival, whereas the opposite was observed in MIBCs. Chromosomal copy number aberrations were found in many tumor samples, but even in the presence of wild-type *STAG2*. Another study sequencing *STAG2* in 307 bladder tumors confirmed higher mutation frequencies in NMIBC noninvasive tumors (33%) or superficially invasive tumors (21%), and lower in MIBCs (13%) (Taylor et al. 2013). No significant association was found with disease recurrence in either NMIBC or MIBCs. Whole chromosome copy number alterations measured by aCGH showed an inverse relationship to *STAG2* mutation. No association between *STAG2* mutation and outcome was found in another report analyzing 109 high grade UBCs (16% *STAG2* mutation rate) (Kim et al. 2014).

Taken all together, we can conclude that in UBC: (1) *STAG2* mutation frequencies are high-

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er than for genes encoding other cohesin subunits, and also higher than in other cancers; (2) most mutations in *STAG2* are truncating, whereas other cohesin genes harbor missense mutations, similar to what was observed in myeloid syndromes; (3) cohesin mutations appear more frequently in lower grade/stage UBCs; and (4) there is no clear correlation with prognosis or aneuploidy.

Functional Studies in Bladder Cancer Cell Lines

Very limited functional experiments in bladder cells have been reported to date. SA2 kd in UBC cell lines with normal SA2 expression did not consistently alter chromosome number in one study (Balbas-Martinez et al. 2013) but it did in another (Solomon et al. 2013). Similarly, reintroduction of *STAG2* cDNA in UBC cell lines with truncating *STAG2* mutations led to a significant decrease in colony formation in one study (Balbas-Martinez et al. 2013) but did not affect proliferation in vitro or in xenografts in the other (Solomon et al. 2013). To interpret these conflicting observations, it would be important to quantify the functional cohesin complexes remaining in the cell under each experimental condition. Given the role of cohesin-SA2 in centromeric cohesion, one would expect chromosome segregation defects when *STAG2* expression is lost (Canudas and Smith 2009). Indeed, SA2 kd led to chromosome missegregation in some human cell lines (Barber et al. 2008; Solomon et al. 2011; Kleyman et al. 2014). In contrast, and similar to UBCs, premature sister chromatid separation was not observed after SA2 kd in mouse hematopoietic progenitor cells, and was detected in only a small percentage of cells after efficient kd of Smc1 α , Smc3, or Rad21 (Mullenders et al. 2015). Arm cohesion mediated by cohesin-SA1 may compensate for the loss of centromeric cohesion in the absence of cohesin-SA2, and the relative levels of both complexes may differ among cell types. In addition, a low amount of cohesin may be sufficient to maintain cohesion in mitosis. In yeast, cohesin levels must be reduced below 13% to result in detectable co-

hesion and segregation defects (Heidinger-Pauli et al. 2010a).

Genes involved in chromatin regulation are more frequently mutated in urothelial carcinoma than in any other common cancer studied so far. Cohesin belongs to this category. These mutations likely modulate the activity levels of various TFs and pathways implicated in cancer (Cancer Genome Atlas Research Network 2014). Mutations in *STAG2* may be more frequent because the gene is located in the X chromosome and because in the absence of cohesin-SA2, cohesin-SA1 may be sufficient to perform essential cohesin functions. It is also possible that transcriptional dysregulation of key genes involved in tumorigenesis depends on cohesin-SA2, not on cohesin-SA1. So far the functional specificities of cohesin-SA1 and cohesin-SA2 in terms of chromatin regulation are poorly understood. In MEFs, genome-wide distribution of both complexes is similar and overlaps with the distribution of CTCF. Upon ablation of *STAG1*, however, cohesin could be detected at additional sites that showed less overlap with promoters and CTCF (Remeseiro et al. 2012b). It was then proposed that cohesin-SA1 could be more important than cohesin-SA2 for transcriptional regulation (Cuadrado et al. 2012). Consistent with this possibility, the transcriptomes of paired human glioblastoma cell lines with and without *STAG2* expression did not differ significantly (Solomon et al. 2011). However, in one of the studies described in the previous sections, bone marrow cells treated with shRNAs against SA2 did display significant alterations in gene expression. Moreover, these alterations were similar to those observed on kd of Smc1 α , suggesting that cohesin-mediated transcriptional regulation in HPSCs relies specifically on cohesin-SA2 (Mullenders et al. 2015). The reasons underlying this specificity are unknown. Even the relative abundance of cohesin-SA1 versus cohesin-SA2 in different cell types could be different. It will be of great interest to compare gene expression profiles before and after SA2 kd in bladder cell lines, as well as other cell types, to better understand how the cohesin variants contribute to cell proliferation and gene expression in a tissue-specific manner.

Cohesin Mutations in Other Cancers

A look at TCGA database reveals the presence of cohesin mutations in many additional cancers (Fig. 5). Bladder cancer is the one in which alteration of the cohesin network components is more common, followed by melanoma, colorectal, and lung cancers. Other than bladder cancer, *STAG2* mutations are most frequent in Ewing sarcoma (EWS). This a pediatric tumor of the bone and soft tissues characterized genetically by the presence of translocations involving ETS family transcription factors such as EWS-FLI (Brohl et al. 2014; Crompton et al. 2014; Tirode et al. 2014; Agelopoulos et al. 2015). In one study looking for secondary genetic lesions, somatic mutations were detected in *STAG2* (17%), *CDKN2A* (12%), and *TP53* (7%). Although mutations in *STAG2* and *CDKN2A* were mutually exclusive, *STAG2* and *TP53* mutations co-occurred particularly in aggressive tumors (Tirode et al. 2014). Mutation rates ranged from 8% to 21% in the other studies. In some cases, loss of expression was detected without mutation, suggesting another mech-

anism of *STAG2* inactivation (Crompton et al. 2014). EWS is among the most genetically stable cancers. No association with aneuploidy was detected although tumors without *STAG2* showed an increased number of somatic CNVs. However, it was not clear if this was because of the association with *TP53* mutations (Crompton et al. 2014; Tirode et al. 2014).

One intriguing observation is the high frequency of mutations in meiosis-specific cohesin genes (labeled in yellow in Fig. 5). Whether these mutations have actual consequences (e.g., the genes are expressed in the tumor cells and act as dominant negative mutant proteins), or are just passenger or silent mutations, remains to be addressed. Similarly, it is also unclear whether meiotic versions of cohesin subunits become expressed in tumors with mutations in their somatic counterparts to compensate for their loss. For instance, truncating mutations in the X-linked *SMC1A* gene have been described in tumor samples from male patients. Maybe Smc1 β is expressed in these cells and forms functional complexes with Rad21 and SA1/2 (Mannini et al. 2015).

Figure 5. Mutations in cohesin complex and related genes in cancer. Bar graph showing mutations in the indicated cancer types for cohesin subunits and cohesin-related genes (grouped as in Fig. 4). (Data obtained from the cBioPortal for Cancer Genomics [Cerami et al. 2012; Gao et al. 2013] and from studies mentioned in Fig. 4.)

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In addition to mutations, CNVs encompassing cohesin genes have also been observed in several cancer types. Of all cohesin genes, *RAD21* is the most frequently amplified (e.g., 20% in invasive breast cancer) (Ciriello et al. 2015). This could be because of its proximity to the *MYC* locus, also present in 8q24. Abnormal levels of cohesin may also contribute to tumorigenesis. Finally, whole-genome sequencing data from more than 200 samples of colorectal cancer (CRC) patients together with ChIP-seq analyses in a CRC cell line revealed a high incidence of mutations in cohesin/CTCF binding sites in the noncoding genome (Katainen et al. 2015). A fraction of these mutations are predicted to affect CTCF binding affinity to *cis*-regulatory elements and could therefore contribute to tumorigenesis through aberrant expression of their target genes. Another epigenetic mechanism recently described in gliomas involves disruption of boundary elements through hypermethylation of CTCF/cohesin binding sites leading to oncogene activation (Flavahan et al. 2016). Thus, there are multiple ways in which cohesin dysfunction may contribute to tumorigenesis.

CONCLUSIONS AND PERSPECTIVES

Mutations in genes encoding cohesin subunits and regulators have been now identified in many cancer genomes, but their relevance for tumor initiation and progression is unknown. It is also unclear how they affect the functionality of the complex, particularly in the case of missense mutations. Because most mutations are heterozygous, they may either reduce the amount of fully functional cohesin complexes in the cell or even have a dominant negative effect. The diverse tasks accomplished by cohesin require different amounts of the complex and may rely on a particular variant. In the majority of tumors or cancer cell lines analyzed, there is no clear correlation between the presence of cohesin mutations and aneuploidy. Thus, cohesion is unlikely to be the function impaired in these tumor cells. Moreover, the recent functional studies performed in hematopoietic cells point to changes in chromatin ac-

cessibility and transcription as the most striking consequences of cohesin dysfunction. Future studies will tell if the same is true in other cell types. Identification of vulnerabilities in cancer cells harboring cohesin mutations will be an important step toward targeted therapies. A study in yeast and *Caenorhabditis elegans* identified a strong synthetic lethality between mutations in cohesin genes and genes involved in replication fork progression and stability, including Poly (ADP-ribose) polymerase or PARP (McLellan et al. 2012). Consistent with this observation, SMC1 down-regulation sensitized triple-negative breast cancer cells to PARP inhibition (Yadav et al. 2013). *STAG2*-mutated glioblastoma cell lines also displayed increased sensitivity to PARP inhibitors, especially when used in combination with DNA-damaging agents (Bailey et al. 2014). A better understanding of how cohesin works and how it contributes to proliferation, cell-identity determination, and homeostasis will hopefully guide improvements in diagnosis and treatment of cancer and other diseases related with cohesin dysfunction.

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