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Cohesin Mutations in Human Cancer

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Abstract

Cohesin is a highly-conserved protein complex that plays important roles in sister chromatid cohesion, chromatin structure, gene expression, and DNA repair. In humans, cohesin is a ubiquitously expressed, multi-subunit protein complex composed of core subunits SMC1A, SMC3, RAD21, STAG1/2 and regulatory subunits WAPL, PDS5A/B, CDCA5, NIPBL, and MAU2. Recent studies have demonstrated that genes encoding cohesin subunits are somatically mutated in a wide range of human cancers. STAG2 is the most commonly mutated subunit, and in a recent analysis was identified as one of only 12 genes that are significantly mutated in four or more cancer types. In this review we summarize the findings reported to date and comment on potential functional implications of cohesin mutation in the pathogenesis of human cancer.

Keywords

cohesin; cancer; STAG2; urothelial carcinoma; Ewing sarcoma; myeloid malignancy

1. Introduction

Cohesin is a ubiquitously expressed multi-protein complex best known for its involvement in sister chromatid cohesion (SCC), but which also plays important roles in the maintenance of chromatin structure, gene expression, and DNA repair [1,2]. In vertebrate somatic cells, the cohesin complex consists of four core subunits: SMC1A (structural maintenance of chromosomes protein 1A), SMC3 (structural maintenance of chromosomes protein 3), RAD21 (double-strand-break repair protein rad21 homolog), and either STAG1 or STAG2 (cohesin subunit SA1/2). STAG1 and STAG2 are mutually exclusive components of the complex - STAG1-cohesin is thought to primarily enforce telomeric cohesion, whereas STAG2-cohesin is primarily involved in centromeric cohesion [3]. Several additional components serve primarily to regulate the core cohesin complex, including NIPBL (Nipped-B-like protein) and MAU2 (MAU2 chromatid cohesion factor homolog) which are required for loading cohesin onto chromatin; WAPL (wings apart-like protein homolog), PDS5A, and PDS5B (sister chromatid cohesion protein PDS homolog A and B) which are required for unloading of cohesin from chromatin; and CDCA5 (sororin) which is involved

in the establishment of SCC. The core cohesin subunits are thought to form a ring-like structure that encircles chromatin to cohere sister chromatids and to bring together otherwise divergent regions of chromatin. The most widely accepted current model is depicted in Fig. 1 [4]. In brief, the SMC1A and SMC3 subunits dimerize via their hinge domains, forming a V shape that is connected at the bottom by so-called “kleisin subunit” RAD21 to form a tripartite ring. RAD21 then binds a kleisin interacting subunit, either STAG1 or STAG2. Regulatory factors then bind to the complex as, and when, required. The complex is highly conserved in both unicellular organisms and metazoans - the subunits were initially described in yeast as mutants displaying premature sister chromatid separation [5,6,7]. Mutations in genes encoding components of the cohesin complex cause developmental disorders and cancer in humans.

2. Cellular roles of the cohesin complex

Enforcing SCC is the “canonical” function of cohesin. To achieve efficient cohesion and precise segregation of sister chromatids into daughter cells, cohesin loading and unloading is tightly regulated during the cell cycle. Loading and unloading of cohesin occurs as a dynamic process beginning in the early stages of G1. Loading requires that the NIPBL/MAU2 heterodimer bind to the core cohesin complex, stimulating the ATPase activity of SMC1A/SMC3. This results in transient separation of the hinge region, allowing the complex to encircle chromatin [8]. Unloading of cohesin at this stage is dependent upon binding of WAPL and PDS5A/B [9]. In order to achieve cohesion during DNA replication, the chromatin bound fraction of cohesin is stabilized. Stabilization requires both the post-translational modification of SMC3 by cohesin acetyltransferases ESCO1 and ESCO2, and the displacement of WAPL by the binding of CDCA5 to PDS5A/B, which prevents WAPL and PDS5A/B-dependent unloading [9,10]. Once cells enter mitosis, cohesin is removed from chromosome arms via post-translational modifications of CDCA5 and STAG1/2, which promote the removal of CDCA5, enabling WAPL-dependent unloading. A small proportion of cohesin is protected from this process by SGO1 (Shugosin) and remains at the centromere [11,12,13,14]. At the onset of anaphase, centromeric cohesion is alleviated via the cleavage of RAD21 by the cysteine protease separase, enabling sister chromatids to separate into the two daughter cells [15,16,17].

In addition to SCC, cohesin also plays important roles in establishing and regulating genome organization at the level of chromatin structure. In post-mitotic cells, genomic sites bound by cohesin overlap with CCCTC-binding factor (CTCF) binding sites [18]. CTCF is involved in chromatin organization, particularly in relation to chromatin looping - structures important for intra- and inter-chromosomal contacts [19]. CTCF is particularly well known for providing insulator properties between enhancer and promoter elements. Evidence suggests that the cohesin complex is important for this insulator function, affecting the long-range chromosomal architecture and therefore transcriptional control of certain loci [18,20]. In mouse embryonic stem cells, cohesin has also been shown to interact with the mediator complex to bring about DNA looping, also with transcriptional consequences [21]. Of note, the mechanism behind Cornelia de Lange syndrome (CdLS; see below) has been proposed to be related to altered gene expression rather than via an effect on SCC [22].

Cohesin also plays a role in homologous recombination-mediated DNA repair. The RAD21 homologue Scc1 was originally identified in fission yeast as a mutant deficient in double strand break (DSB) repair [23]. The cohesin complex has since been shown to be recruited to sites of DSBs in yeast and initiate *de novo* cohesion [24]. Although the mechanisms remain unclear, the cohesin complex is also of significance for DSB repair in mammalian cells. In human cells, the interaction of cohesin and BRCA2 via PDS5A/B has been shown to be important for homologous recombination-mediated DNA repair and the normal response to DNA damage [25]. Heterozygous RAD21 deletion in mouse cells results in defective homologous recombination and increased sensitivity to ionizing radiation [26].

3. The cohesin complex and developmental disorders

Disruption of normal cohesin activity during human development can cause developmental disorders referred to as cohesinopathies. The most common of these is CdLS, which affects 1/10,000–1/30,000 live births. CdLS patients exhibit a large degree of phenotypic variation including craniofacial abnormalities, microcephaly, developmental delay, hirsutism, and upper limb abnormalities [27,28]. Heterozygous mutations in NIPBL are the most common cause of CdLS, accounting for ~65% of cases. NIPBL mutations range from nonsense/splice/frameshift mutations resulting in haploinsufficiency to missense mutations that are often associated with a milder phenotype. CdLS can also be caused by mutations in SMC1A, SMC3, HDAC8 and RAD21 [29,30,31,32]. Another similar, but extremely rare disorder, termed Roberts syndrome (RBS), is caused by recessive mutations within ESCO2, which acetylates SMC3 [33]. RBS patients exhibit similar phenotypes as CdLS patients. Most recently, microduplications involving STAG2 have also been associated with intellectual disability and behavioral problems [34,35].

4. Cohesin and cancer

The first somatic mutations of cohesin in cancer were reported in 2008 when Barber *et al.* identified heterozygous somatic missense mutations in the genes encoding SMC1A, SMC3, NIPBL, and STAG3 (a component of meiotic cohesin) in aneuploid colon cancers [36]. In 2010, individual deletions of RAD21 and STAG2 were reported in a chronic myelomonocytic leukemia (CML) and an acute myeloid leukemia (AML), respectively [37].

Then, in 2011 Solomon *et al.* reported mutations of STAG2 in cell lines and primary tumors from glioblastoma multiforme (GBM), Ewing sarcoma, melanoma, cervical carcinoma, and hematological cancers [38]. STAG2 is on the X chromosome and therefore requires only a single mutational event to completely inactivate in both males and females (due to X inactivation). Mutations were somatic, mostly truncating, and generally resulted in the complete absence of protein expression. Immunohistochemistry (IHC) analyses of primary tumor samples identified frequent STAG2 inactivation in GBM, Ewing sarcoma, and melanoma primary tumors. Solomon *et al.* then used AAV-mediated human somatic cell gene targeting to create several isogenic systems that differed only in their STAG2 mutational status. Using these cells they demonstrated roles for STAG2 mutation in sister chromatid cohesion, anaphase integrity and chromosomal stability.

These early manuscripts were followed by a host of additional studies reporting cohesin gene mutations in bladder cancer, Ewing sarcoma, myeloid leukemia, as well as other tumor types, as described in detail in the sections below [39]. A representative subset of the tumor-derived STAG2 mutations reported to date are depicted in Fig. 2. Tumor-derived mutations in STAG2 are considered loss-of-function since: (i) ~85% of the reported mutations are truncating, (ii) truncating mutations are present even in the earliest coding exons, resulting in a very short protein, and (iii) in many cases the truncating mutations lead to the absence of even a truncated STAG2 protein, likely due to nonsense-mediated decay of the mutant STAG2 mRNA [40]. As such, it is appropriate to classify STAG2 as a tumor suppressor gene.

5. Cohesin mutations in bladder cancer

In 2013, three studies published simultaneously in *Nature Genetics* reported frequent somatic mutation of STAG2 in urothelial carcinoma of the bladder. The results of these studies are described below and summarized in Table 1. Bladder cancer is the sixth most common cancer in the United States, with ~77,000 cases and ~17,000 deaths per year [41]. >90% of bladder cancers arise from the mucous membrane lining the bladder, referred to either as the 'urothelium' or 'transitional epithelium'. One of the most significant prognostic factors for bladder cancer is whether the tumor has invaded the muscle surrounding the bladder, since muscle-invasive tumors are much more likely to metastasize. Currently, it is very difficult to predict which nonmuscle-invasive tumors will eventually recur, invade, and metastasize.

Solomon *et al.* identified frequent STAG2 mutations in bladder cancer by performing STAG2 IHC on >2000 tumors representing virtually all common cancer types [42]. This study identified loss of STAG2 expression in 18% (52/295) of bladder tumors studied. DNA sequence analysis performed on a separate cohort identified STAG2 mutations in 21% (23/111) of the bladder tumors studied. Most of the mutations resulted in truncation of the encoded STAG2 protein. Strikingly, STAG2 mutations were much more common in nonmuscle-invasive tumors (36%) than in muscle-invasive tumors (16%). The authors also noted that TP53 mutation tended to co-occur with STAG2 mutation. Additionally, loss of STAG2 expression in nonmuscle-invasive cancers was found to be a good prognostic marker, predicting lack of recurrence ($p=0.05$). In contrast, loss of STAG2 in muscle-invasive tumors was associated with poor prognosis. Chromosomal copy number analysis of tumor samples provided little evidence for a link between STAG2 loss and aneuploidy in bladder cancer. However, depletion of wild-type STAG2 in a bladder cancer cell line led to alterations in modal chromosome number.

Balbas-Martinez *et al.* also identified STAG2 as a commonly mutated gene in bladder cancer, in this case using exome sequencing [43]. 16% (12/77) of tumors studied had a mutation in STAG2. They also identified less frequent somatic mutations in genes encoding other cohesin subunits (Table 1). In agreement with Solomon *et al.*, STAG2 mutations were primarily truncating, and were more common in nonmuscle-invasive tumors (21%) than in muscle-invasive tumors (11%). Using IHC they identified loss of STAG2 expression in 29% of tumors studied (197/671). Also in agreement with the findings of Solomon *et al.*, STAG2

deficient nonmuscle-invasive tumors were less likely to recur or progress to muscle invasion than STAG2 expressing tumors. However, in disagreement with Solomon *et al.*, STAG2-deficiency also correlated with good clinical outcomes in muscle-invasive tumors. Furthermore, Balbas-Martinez *et al.* emphasized that since many STAG2 mutant tumors were euploid (not aneuploid), the role of STAG2 mutations in the pathogenesis of bladder cancer was unlikely to be via the initiation of chromosomal instability and aneuploidy.

In another large scale sequencing study, Guo *et al.* identified STAG2 as one of 13 novel significantly mutated genes in urothelial carcinoma of the bladder [44]. In this cohort, 11% of tumors harbored mutations in STAG2, the majority of which were truncating. Deletions of STAG2 were also observed in 5% of cases, and promoter hypermethylation in 23% of cases. In contrast to the findings of Solomon *et al.* and Balbas-Martinez *et al.*, Guo *et al.* found that STAG2 mutant tumors had more chromosomal copy number alterations than STAG2 wild-type tumors. In this cohort, both nonmuscle-invasive and muscle-invasive tumors harboring STAG2 mutations had worse clinical outcomes than tumors with wild-type STAG2. Guo *et al.* also identified occasional somatic mutations in the cohesin subunits NIPBL (6%), SMC1A (3%), and SMC3 (2%).

A subsequent STAG2 candidate gene sequencing study confirmed and extended the results from the three aforementioned studies. Taylor *et al.* identified mutations in 26% of primary tumors and 17% of cell lines [45]. Most of the mutations were truncating. In agreement with Solomon *et al.* and Balbas-Martinez *et al.*, STAG2 mutations were most commonly found in tumors of low stage and grade. In this cohort, positive correlations were identified between the presence of mutations in STAG2, FGFR3, and PI3KCA, and the presence of wild-type TP53. Taylor *et al.* identified no correlation between the presence of STAG2 mutations and increased frequencies of copy number alterations, further suggesting that STAG2 mutations may not cause aneuploidy in bladder cancer.

Taken together, these four studies indicate that the overall STAG2 mutation frequency in urothelial carcinoma of the bladder is ~15–20%. Mutations of STAG2 are more common in nonmuscle-invasive tumors (~30% mutation frequency) than in muscle invasive tumors (~10% mutation frequency). In agreement, in 2014 The Cancer Genome Atlas (TCGA) reported an 11% STAG2 mutation frequency in muscle-invasive bladder cancer (TCGA has not studied nonmuscle-invasive bladder cancer) [46]. These findings, when considered in light of the related observation that non-muscle invasive tumors harboring STAG2 mutations appear less likely to recur than STAG2 wild-type tumors, suggest that STAG2 expression could be useful as a biomarker for predicting whether non-muscle invasive bladder tumors will recur and invade. The observation that bladder tumors harboring STAG2 mutations tend to be less aggressive than tumors with wild-type STAG2 was recently confirmed by Qiao *et al.* [47]. There is some disagreement among these studies with regards to a potential role of STAG2 mutations in causing aneuploidy in bladder cancer. However, considering that STAG2 mutations are most common in the earliest stage tumors, many of which are euploid (ie. non-aneuploid), the role of STAG2 mutations in the pathogenesis of bladder cancer seems most likely due to phenotype(s) other than the initiation of chromosome segregation and aneuploidy.

6. Cohesin mutations in Ewing sarcoma

Ewing sarcoma is a pediatric bone and soft tissue cancer that affects ~200 children and adolescents in the United States each year. The 5-year survival rate is ~70% for patients with localized disease and ~40% for patients with metastatic disease [48]. Ewing sarcoma is defined pathologically by the presence of a translocation involving the EWSR1 gene on chromosome 22, most commonly with the FLI1 gene on chromosome 11 [49]. Substantial effort has gone into identifying other genes that are mutated in Ewing sarcoma tumors.

In the initial study identifying STAG2 mutations in multiple tumor types, frequent mutations of STAG2 were identified in Ewing sarcoma cell lines [38]. Subsequently, three comprehensive Ewing sarcoma sequencing studies were published, each of which identified somatic mutation of STAG2 as the most common mutational event in Ewing sarcoma other than the tumor-defining EWS-FLI1 translocation. Results of these three studies are discussed below and summarized in Table 1.

Brohl *et al.* identified STAG2 mutations in 22% of tumors (14/65) and 44% (16/36) of cell lines [50]. As expected, the majority of the mutations were truncating. Interestingly, 5 samples (4 tumors and 1 cell line) harbored the same nonsense mutation (R216X), indicating the presence of a minor mutational hotspot. There was also evidence of reduced STAG2 protein expression (with no identifiable mutation) in 7 additional samples. There was a statistically significant co-occurrence of STAG2 mutations and TP53 mutations in Ewing sarcoma cell lines; however, this correlation was less evident in primary tumor samples.

Crompton *et al.* also identified STAG2 mutation as a common event in Ewing sarcoma, albeit with a lower frequency of mutations (8%) than Brohl *et al.*, but with a higher frequency of loss of expression by IHC (15%) [51]. Interestingly, one cell line demonstrated a STAG2-MAP7D3 fusion event resulting in loss of STAG2 expression. There were also a small number of cases in which STAG2 protein expression was lost without evidence of mutation. In agreement with Brohl *et al.*, Crompton *et al.* also noted a statistically significant co-occurrence of mutations in STAG2 and TP53. Additionally, there was also evidence of convergent evolution of mutations in STAG2 and TP53. STAG2 loss was also associated with a greater incidence of metastatic disease at the time of diagnosis - 88% of patients whose tumors lacked STAG2 expression had metastatic disease, compared to only 27% of patients whose tumors expressed STAG2.

Tirode *et al.* reported a 15% overall frequency of STAG2 mutation in Ewing sarcoma tumors [52]. As expected, the majority of the mutations were truncating. This study also identified the R216X hotspot, which was mutated in 5 cell lines and 2 primary tumors. In agreement with Brohl *et al.* and Crompton *et al.*, Tirode *et al.* also identified co-occurrence of STAG2 mutations and TP53 mutations. In addition, mutual exclusivity was observed between STAG2 mutation and CDKN2A deletion in both tumors and cell lines of this cohort. In further agreement with Crompton *et al.*, the presence of STAG2 mutation was associated with a significantly lower probability of survival. Patients whose tumors harbored both STAG2 and TP53 mutations had the worst outcomes.

Taken together, these studies reported mutations in 15–20% of Ewing sarcoma tumors, identifying STAG2 as the most common somatic mutation in Ewing sarcoma other than the defining EWS-FLI translocation. Of note, unlike in bladder cancer and myeloid malignancies (see below), no cohesin genes other than STAG2 were found to be mutated in Ewing sarcoma at statistically significant frequencies. The finding of STAG2 mutation is particularly noteworthy since, like most pediatric cancers, Ewing sarcomas harbor very few somatic mutations [53]. In addition, STAG2 mutations appears to be a marker for worse clinical outcomes [51,52], and tend to co-occur with TP53 mutation [50,51,52].

7. Cohesin mutations in myeloid neoplasms

Myeloid malignancies are a group of related neoplasms with varying degrees of severity that arise in hematopoietic progenitor cells. The subtypes of myeloid leukemia that are known to harbor cohesin gene mutations are discussed individually below, and an overview is provided in Table 2.

7.1 Acute myeloid leukemia (AML)

AML is the most common form of adult acute leukemia in the United States, with ~20,000 cases and ~10,000 deaths per year [41]. AML is classified either as *de novo* AML (without evidence of a precursor chronic malignancy) or secondary AML (when it develops from a precursor chronic myeloid malignancy such as myelodysplastic syndrome). Several recent reports have identified cohesin gene mutations in both *de novo* and secondary AML.

The first report of cohesin mutations in AML identified somatic mutations in SMC3 at a frequency of 3% [54]. Subsequently, TCGA confirmed and extended this result, identifying a 3% mutation frequency in each of the four core cohesin subunits SMC1A, SMC3, STAG2 and RAD21 in adult *de novo* AML [55]. The mutations were mutually exclusive, resulting in a cumulative cohesin mutation frequency of 13% (26/200). Subsequently, Thol et al. evaluated the mutation frequency of STAG1, STAG2, SMC1A, SMC3 and RAD21 in a panel of 389 AML samples, and reported a cumulative, mutually-exclusive mutation frequency of 5% [56]. In disagreement with the TCGA study, the most frequently affected gene was STAG1, followed by STAG2, SMC3, RAD21, and SMC1A.

Kon *et al.* and Thota *et al.* assessed the mutation frequency of cohesin subunits in both *de novo* and secondary AML (as well as in other types of myeloid malignancies - see below). In both studies, AML harbored the most frequent cohesin mutations, with only the genes encoding core cohesin subunits mutated at an appreciable frequency [57,58]. Kon *et al.* identified cohesin mutations in 12% (19/157) of AML samples. The frequency of cohesin mutation was slightly higher in *de novo* AML (13%; 16/120 samples studied) than in secondary AML (8%; 3/37 samples studied). In contrast, Thota *et al.* identified a higher frequency of mutations in secondary AML samples (20%; 30/149) than in *de novo* AML (11%; 32/301). In both studies, STAG2 was the most frequently mutated cohesin gene, followed by RAD21 and SMC3. In support of the observation by Thota *et al.* that cohesin mutations are more frequent in secondary AML than *de novo* AML, a recent report has identified STAG2 as one of eight mutated genes specific for secondary AML compared with *de novo* AML [59].

There is some evidence that cohesin gene mutations may be an initiating event in a subset of AMLs. Welch *et al.*, compared genomes from AML samples with and without a known initiating event (PML-RARA), and identified a group of thirteen genes including SMC1A, SMC3 and STAG2 that were recurrently mutated only in genomes lacking a PML-RARA translocation [60]. Overall, the frequency of cohesin mutations in genomes without a known initiating event was 10%. In addition, a study by Walter *et al.* identified a STAG2 mutation in both a secondary AML and the precursor myelodysplastic syndrome from the same patient, implicating STAG2 mutation as an early event in the pathogenesis of this cancer [61].

7.2 Myelodysplastic syndromes (MDS)

MDS is a group of diverse disorders in which the normal process by which the bone marrow produces blood cells becomes disrupted. Each year, ~10,000 cases are diagnosed in the United States; the three-year survival rate is ~35% [62]. MDS is often the precursor to secondary AML. As mentioned above, MDS samples from patients whose disease later progressed to secondary AML have been shown to harbor STAG2 mutations [61]. A subsequent study using a cohort of 150 MDS samples identified a 6% frequency (10/154) of STAG2 mutations [63]. Similarly, Kon *et al.* identified cohesin mutations in 8% (18/224) of MDS samples, the majority of which were in STAG2, with lower mutation frequencies in SMC3 and RAD21 [57]. Thota *et al.* studied cohorts of both high risk and low risk MDS, and identified cohesin mutations in 17% of high risk samples and 11% of low-risk samples [58].

7.3 Other myeloid malignancies

Cohesin mutations have also been identified in myelomonocytic leukemia (CMML), chronic myelogenous leukemia (CML), myeloproliferative neoplasms (MPN), and MDS/MPN (MPN and MDS overlap syndrome) at frequencies of 4–10% [57,58]. A remarkably high cumulative mutation frequency (53%) of the cohesin components STAG2, RAD21, SMC3, SMC1A, and NIPBL has also been identified in Down syndrome-associated acute megakaryoblastic leukemia [64].

The studies described above demonstrate that cohesin gene mutations are present in a substantial subset of myeloid malignancies. The most commonly mutated gene is STAG2, with mutations in genes encoding other core components also identified in a significant fraction of tumors. Mutations in cohesin genes are mutually exclusive. The presence of cohesin mutation in AML precursors and in AML genomes that do not contain well-characterized initiating genetic events suggests that cohesin mutations may function as an initiating event in a subset of myeloid malignancies. The generation of aneuploidy does not appear to be the primary role of cohesin mutations in myeloid malignancies, since many cohesin gene mutations have been identified in euploid samples [55,60,61]. Instead, as described in greater detail below, studies of mouse models have pointed to lineage skewing resulting from aberrant chromatin structure and transcription factor accessibility as a particularly important effect of cohesin mutations in myeloid precursor cells [65,66,67].

8. Cohesin mutations in other tumor types

Cohesin gene mutations have also been identified in several other tumor types. TCGA has reported STAG2 mutations in 7% of glioblastoma multiforme (GBM) tumors [68]. Further support for a role of cohesin gene mutations in gliomas has been recently provided by Ceccarelli *et al.*, who identified a 16% frequency of cohesin mutations and copy number alterations, including the first report of recurrent NIPBL mutations in gliomas [69]. Interestingly, Bailey *et al.* has suggested that GBM cells harboring STAG2 mutations may have enhanced susceptibility to PARP inhibitors [70]. Additionally, TCGA has identified mutations in STAG2 (9%) and NIPBL (10%) in endometrial carcinoma [71]. STAG2 was also identified as a significantly mutated gene in TCGA analysis of papillary renal-cell carcinoma [72]. Lower frequencies of cohesin gene mutations have also been identified in medulloblastoma, pancreatic ductal carcinoma, breast cancer and colorectal cancer [73,74,75,76]. In addition to mutations of cohesin itself, mutations of cohesin/CTCF genomic binding sites are commonly found in diverse human cancers [77]. It remains unclear why some tumor types such as Ewings sarcoma primarily harbor mutations in STAG2 (85% truncating), whereas other cancers such as myeloid leukemias harbor primarily heterozygous missense mutations in a wider range of cohesin genes. Remarkably, a recent analysis of TCGA data from 12 major cancer types identified STAG2 as one of only 12 genes that were significantly mutated in four or more cancer types [78].

9. Potential functional implications of cohesin mutations in cancer

There are many potential functional effects of cohesin mutations on human cells, including the initiation of genomic instability and aneuploidy, alterations in chromatin organization resulting in changes in gene expression and/or replication stress, and enhanced susceptibility to DNA damage [79]. However, at present the specific mechanism(s) through which cohesin gene mutations lead to cellular transformation remains unknown. In the following sections we will present the current state of knowledge regarding the specific effects of cancer-causing cohesin gene mutations on the structure and function of cohesin.

10. Effects of cohesin mutations on the protein composition of the cohesin complex

The most straightforward hypothesis regarding the biochemical loss-of-function caused by tumor-derived mutations in cohesin is that the mutations uniformly abrogate the ability of the encoded protein to interact with the cohesin complex. Two groups have now independently tested this hypothesis and have demonstrated that the hypothesis, while appealing, is false – in many cases the cohesin subunits encoded by genes harboring tumor-derived mutations retain their ability to interact with cohesin. To test this, Kim *et al.* created an epitope-tagged wild-type human STAG2 expression vector and derivatives with ~60 different tumor-derived mutations. After transfection of 293T cells, they performed epitope tag immunoprecipitation followed by Western blot with antibodies for other components of cohesin. These experiments demonstrated that STAG2 proteins encoded by genes with tumor-derived late truncating mutations and tumor-derived missense mutations both retained their ability to interact with the rest of the cohesin complex [40]. Mullenders *et al.* also

tested the ability of AML-derived mutations in RAD21, STAG2, and SMC3 to interact with cohesin, and found that proteins encoded by missense mutations and a subset of truncating mutations retained their ability to interact with cohesin [65]. Taken together, these studies demonstrate that the loss of the ability to interact with cohesin is not the only key property targeted by tumor-derived mutations in cohesin subunits.

Kim *et al.* also considered a related hypotheses - that mutations in STAG2 might alter the expression of other cohesin subunits and/or alter the subunit composition of the cohesin complex itself. These hypotheses were based in part based on previous studies showing altered levels of other cohesin subunits after depletion of individual core subunits [80,81]. Initially Kim *et al.* measured the levels of cohesin complex proteins in isogenic sets of STAG2 mutant and corrected GBM cell lines, and found that mutations in STAG2 resulted in a decrease in the levels of core cohesin subunits SMC1A, SMC3, and RAD21 [40]. Next, they purified cohesin via SMC immunoprecipitation from isogenic sets of STAG2 mutant and corrected GBM cells, then measured the abundance of individual cohesin subunits in the purified complexes via Western blot. They found that the presence of a tumor-derived mutation in STAG2 reduced the ability of regulatory subunits WAPL, PDS5A, and PDS5B to interact with the core cohesin ring [40]. This finding that the interaction of WAPL with cohesin is dependent in part on STAG2 is in agreement with recently published structural studies showing that STAG2 functions as a structural scaffold for the interaction of WAPL with the core cohesin ring [82].

11. Cohesin mutations and aneuploidy

In their initial study identifying STAG2 mutations in cancer, Solomon *et al.* proposed that the mutations caused chromosomal instability and aneuploidy [38]. This conclusion was based on the observations that (i) targeted correction of endogenous mutant STAG2 in aneuploid human cancer cells led to a reduction in the number and variability of chromosome counts, and (ii) introduction of a non-tumor derived STAG2 nonsense mutation into a chromosomally stable, near-diploid cell line resulted in alterations in chromosome counts. These findings were consistent with other observations in yeast, mice, and other model systems indicating that mutations in cohesin subunits lead to chromosomal non-disjunction and aneuploidy [83,84,85,86]. However, since this initial publication, other studies have reported little if any correlation between STAG2 mutational status and aneuploidy in human tumors, and have further shown that many STAG2 mutant tumors are, in fact, euploid [43,57]. In an effort to address these discrepancies, Kim *et al.* used human somatic cell gene targeting to introduce nine different tumor derived STAG2 mutations into HCT116 cells, a near-diploid human cancer cell line with wild-type cohesin genes and intact sister chromatid cohesion [40]. They then measured sister chromatid cohesion, anaphase integrity, and chromosome counts. To their surprise, while all nonsense mutations resulted in substantial defects in SCC, the missense mutations tested were wild-type for SCC. Furthermore, anaphase integrity was disrupted in only 3/9 cell lines. Finally, only one of the nine cell lines displayed alterations in chromosome counts. Kim *et al.* interpreted these findings as further calling into question whether the cancer-relevant phenotypes of STAG2 mutations are directly related to sister chromatid cohesion and aneuploidy.

12. Effects of cohesin mutations on the differentiation of myeloid stem/precursor cells

Three recent studies have focused on better understanding the role of cohesin inactivation in the differentiation of myeloid precursor cells. As described below, two groups have created and studied mice in which individual cohesin genes have been inactivated by either gene targeting or shRNA, and another group has used primarily tissue culture-based approaches [65,66,67].

Mullenders *et al.* used a recently described short-hairpin RNA (shRNA) based approach to generate inducible knockdown mouse models of RAD21, SMC1A and STAG2 [65]. Upon depletion of cohesin subunits animals exhibited altered hematopoiesis (particularly in relation to myeloid differentiation) and, when allowed to age, SMC1A knockdown mice exhibited features consistent with myeloproliferative neoplasms. Lineage skewing towards the myeloid lineage was observed in RAD21, SMC1A, and STAG2 knockdown animals and hematopoietic stem cells (HSCs) indicated that the differentiation bias was occurring in the early stages of hematopoiesis. At the molecular level, transcriptional changes were identified between knockdown and control purified HSCs which included a marked upregulation of genes involved in myeloid differentiation and downregulation of genes involved in lymphoid development. Furthermore, increased chromatin accessibility around upregulated genes and reduced chromatin accessibility around downregulated genes was also observed. Additionally, the GATA transcription factor motif was found to be the most enriched transcription factor motif at the most accessible sites.

Viny *et al.* generated SMC3 inducible knockout mice. Induction of knockout in the hematopoietic compartment of adult animals resulted in lethality in 11.5 days [66] with animals exhibiting numerous features that included an absence of myeloid elements in the peripheral blood. Upon heterozygous SMC3 knockout, animals were viable and bone marrow cells demonstrated enhanced self-renewal properties *in vitro* and *in vivo* that were especially pronounced when cells were also expressing the FLT3-ITD mutant (a mutation previously reported to co-occur with cohesin mutations in myeloid malignancies [55]). At the HSC level, heterozygous SMC3 knockout mice demonstrated an increase in short-term HSCs and multipotent progenitors but a reduction in long-term HSCs, the number of absolute multipotent progenitors was also increased. At the molecular level, hematopoietic progenitors from heterozygous SMC3 knockout animals demonstrated a global decrease in transcription, however, despite this global reduction, some genes remained relatively unchanged and some genes exhibited very pronounced levels of downregulation. Unchanged genes included those that are associated with stem cell maintenance whereas markedly downregulated genes included those that are associated with lineage-commitment. Furthermore, reduced chromatin accessibility was observed around the transcription start sites of downregulated genes compared with unchanged genes.

Mazumdar *et al.* generated inducible expression constructs of SMC1A, SMC3, RAD21 or STAG2 containing tumor-derived mutations [67]. Reduced cellular differentiation was observed upon expression of mutant cohesin subunits in cell lines and CD34-enriched primary human cord blood cells upon relevant stimulation. In addition, increased retention

of hematopoietic stem and progenitor cells (HSPCs) was observed when cohesin mutant expressing CD34-enriched cells were treated with stem cell retention-promoting cytokines. On the molecular level, differentially expressed genes in mutant cohesin expressing HSPCs were identified that included upregulation of genes important for HSCs and downregulation of genes required for myeloid differentiation. Furthermore, while cohesin mutant cells showed a global reduction in chromatin accessibility at transcription start sites, some individual sites showed an increased level of chromatin accessibility. These sites included those enriched for ERG, GATA2 and RUNX1 transcription factors. Global increased binding of these factors in RAD21 mutant cells was also observed. Interestingly, shRNA knockdown of ERG, GATA2 or RUNX1 along with expression of a cohesin mutant in CD34-enriched cord blood cells prevented any increase in CD34-enriched cell retention that would normally be seen with mutant cohesin expression alone.

Taken together, these studies have implicated a role for cohesin in the correct establishment of lineage development in the hematopoietic system and suggest that this may be occurring as a result of altered chromatin accessibility at specific loci.

13. Conclusions

Since the initial discovery of cohesin mutations in colon cancer in 2008, numerous studies have demonstrated that cohesin gene inactivation is a common and important event in the pathogenesis of diverse human cancers. At present it appears that the tumor types harboring most frequent cohesin mutations include bladder cancer, Ewing sarcoma, and myeloid malignancies. Cohesin gene mutations are found in GBM, endometrial carcinoma, and other tumor types. The most commonly mutated gene is STAG2, with other cohesin genes including RAD21, SMC1A, SMC3, NIPBL mutated in a smaller fraction of tumors, depending on tumor type. The location and types of mutations in STAG2 (~85% truncating, spread evenly throughout the X-linked gene including in the first exons) indicate that the mutations are inactivating, identifying STAG2 as a tumor suppressor gene. Initial studies suggested that the key role of cohesin inactivation was the initiation of aneuploidy, though more recent studies have called this conclusion into question. The most recent studies have pointed to alterations in progenitor/stem cell differentiation as an important phenotype of cohesin inactivation. Other possible effects of cohesin mutations include alterations in chromatin structure, transcriptional regulation, and DNA repair, though which (if any) of these is a key cancer-causing phenotype of cohesin inactivation remains unknown. In the coming years we are likely to see a dramatic improvement in our understanding of the key cancer-relevant biochemical effects and phenotype(s) of cohesin inactivation in the pathogenesis of cancer.

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Fig 1.
Model of cohesion complex.

Fig 2.

Diagram of the STAG2 protein with the locations of a representative subset of mutations that have been identified in human tumors. The mutations displayed are from refs. 38, 42–46, 50–52, 56, 57, 60, 61, 64. STAG2 stromal antigen domain. SCD, stromalin conserved domain.

Table 1

Summary of major publications documenting STAG2 mutations in bladder cancer and Ewing sarcoma.

Samples tested	Original detection approach	Frequency of samples harboring STAG2 mutations	Frequency of samples exhibiting STAG2 protein loss by IHC	Disease correlations	Genetic correlations with STAG2 mutation/loss	Other cohesin subunits affected by mutation	Reference
Bladder tumors of various stages and grades and cell lines	IHC tissue microarray	21% (n=111) tumors overall; 36% nonmuscle-invasive (n=25); 27% superficially invasive (n=22); 13% muscle-invasive (n=64); 16% cell lines(n=32)	18% (n=295)	Higher rate of STAG2 mutation/loss in nonmuscle-invasive cancers	TP53 mutation and overexpression (co- occurrence)	Not performed	Solomon <i>et al.</i> , 2013 [42]
				Loss of STAG2 expression associated with better prognosis in nonmuscle- invasive cancers and worse prognosis in muscle- invasive cancers	No significant association with aneuploidy in tumors but some evidence in cell line experiments		
Bladder tumors of various stages and grades (mostly nonmuscle-invasive)	WES	16% (n=77) overall; 21% non-aggressive (n=29); 11% aggressive (n=47)	29% (n=671)	Higher rate of STAG2 mutation/loss in nonmuscle-invasive tumors	No significant association with aneuploidy	STAG1 (8%); SMC1A (2%); SMC1B (3%); MAU2 (3%); RAD21 (2%); SMC3 (2%); NIPBL (2%); ESCO2 (2%); PDS5B (2%) (n=60)	Balbas-Martinez <i>et al.</i> , 2013 [43]
				Loss of STAG2 expression associated with better prognosis in both muscle- invasive and nonmuscle- invasive tumors			
Bladder tumors of various stages and grades	WES	11% (n=99)	Not performed	STAG2 mutation associated with worse prognosis	More aneuploidy in STAG2 mutant tumors	NIPBL (6%); SMC1A (3%); SMC3 (2%) (n=99)	Guo <i>et al.</i> , 2013 [44]
Bladder tumors of various stages and grades and cell lines	Targeted single gene mutation analysis	26% tumors (n=307); 17% cell lines (n=47)	Not performed	STAG2 mutation associated with low tumor grade and stage	Lower number of chromosomal copy number alterations in samples with STAG2 mutation	Not performed	Taylor <i>et al.</i> , 2014 [45]
Muscle-invasive bladder tumors	WES/WGS	11% (n=200)	Not performed	N/A	N/A	NIPBL (4%); RAD21 (3%); STAG1 (2%); SMC3 (2%); SMC1A (2%);	TCGA, 2014 [46]

Samples tested	Original detection approach ^φ	Frequency of samples harboring STAG2 mutations	Frequency of samples exhibiting STAG2 protein loss by IHC	Disease correlations	Genetic correlations with STAG2 mutation/loss	Other cohesin subunits affected by mutation	Reference
Ewing sarcoma tumors and cell lines	WGS	22% tumors (n=65); 44% cell lines (n=36)	14% (n=210)	STAG2 mutation associated with metastatic disease	TP53 mutation (co-occurrence - cell lines only)	SMC1B (2%); CDCA5 (2%); WAPL (2%); STAG3 (2%) (n=200)	Brohl <i>et al.</i> , 2014 [50]
Ewing sarcoma tumors and cell lines	WES	8% tumors (n=96); 36% cell lines (n=11)	15% (n=73)	STAG2 mutation associated with metastatic disease	TP53 mutation (co-occurrence)	RAD21 (2%); PDS5B (2%); SMC1A (1%); NIPBL (1%); REC8 (1%); STAG3 (1%) (n=96)	Crompton <i>et al.</i> , 2014 [51]
Ewing sarcoma tumors and cell lines	WGS	15% tumors (n=411) 47% cell lines (n=19)	Not performed	N/A	TP53 mutation (co-occurrence) CDKN2A deletion (mutual exclusivity)	STAG1 (<1%); SMC1A (<1%) (n=112)	Tirode <i>et al.</i> , 2014 [52]

This table provides an overview of the major findings regarding STAG2 mutation and expression loss in bladder cancer and Ewing sarcoma. Frequencies of other cohesin gene mutations are also shown.

^φThis column describes the initial detection used to identify STAG2 mutations in each study.

Additional methods may have been used for prevalence screens. WES (whole exome sequencing); WGS (whole genome sequencing); IHC (immunohistochemistry).

Where additional cohesion genes were not discussed in the manuscript, available WES/WGS data or prevalence screen data was mined for mutations in the following genes: STAG1, STAG2, STAG3, RAD21, SMC3, SMC1A, SMC1B, NIPBL, CDCA5, WAPL, MAU2, ESCO1, ESCO2, PDS5A, PDS5B and REC8.

Table 2

Summary of major publications documenting cohesin mutations in myeloid malignancies.

Samples tested	Detection approach ^ϕ	Cumulative cohesin gene mutation frequency ^Σ	Breakdown of mutations across core cohesin subunits and regulatory factors	Reference
AML (de novo)	WES/WGS	~13% (n=200)	STAG2 (4%); SMC3 (4%); SMC1A (4%); RAD21 (3%); regulatory (2%)	TCGA, 2013 [55]
AML (de novo)	Targeted	~6% (n=348)	STAG2 (1%); STAG1 (2%); SMC3 (1%); SMC1A(1%); RAD21 (1%)	Thol <i>et al.</i> , 2014 [56]
AML (secondary)		~2% (n=41)	STAG1 (2%)	
AML (de novo)	WES/targeted	~14% (n=120)	STAG2 (7%); SMC3(1%); SMC1A (2%); RAD21 (5%); regulatory (1%)	Kon <i>et al.</i> , 2013 [57]
AML (secondary)		~11% (n=37)	STAG2 (5%); RAD21 (3%); regulatory (3%)	
CML		~8% (n=64)	STAG2 (3%); SMC1A (3%); RAD21 (2%); regulatory (2%)	
CMML		~14% (n=88)	STAG2 (10%); STAG1 (1%); regulatory (2%)	
MDS		~9% (n=224)	STAG2 (6%); SMC3 (1%); RAD21 (1%)	
MPN		~3% (n=77)	STAG2 (1%); regulatory (1%)	
AML (de novo)		~11% (n=301)	STAG2 (~2%); RAD21 (2%); SMC3 (3%)	
AML (secondary)	WES/targeted	~20% (n=149)	STAG2 (~12%); RAD21 (4%); SMC3 (3%)	Thota <i>et al.</i> , 2014 [58]
MDS low risk		~11% (n=237)	STAG2 (~5%); RAD21 (<2%); SMC3 (<2%)	
MDS high risk		~17% (n=149)	STAG2 (~15%); RAD21 (<2%)	
MPNs		~7% (n=55)	STAG2 (~5%)	
MDS/MPNs		~4% (n=169)	STAG2 (~2%); RAD21 (<1%); SMC3 (<1%)	
overall		~12% (n=1060)	STAG2 (~6%); RAD21 (2%); SMC3 (2%); SMC1A (<1%); STAG1 (<1%); regulatory (<1%)	
AML (with no known initiating event)	WGS/targeted	~11% (n=65)	STAG2 (5%); SMC3 (3%); SMC1A (3%); regulatory (8%; n=12)	Welch <i>et al.</i> , 2012 [60]
AML (secondary)	WGS	~29% (n=7)	SMC3 (14%); STAG2 (14%)	Walter <i>et al.</i> , 2012 [61]
MDS	WGS/targeted	~6% (n=150)	STAG2 (~6%)	Walter <i>et al.</i> , 2013 [63]
acute megakaryoblastic leukemia (Down syndrome associated)	WES/targeted	~53% (n=49)	STAG2 (18%); SMC3 (2%); SMC1A (4%); RAD21 (22%); regulatory (6%)	Yoshida <i>et al.</i> , 2013 [64]
acute megakaryoblastic leukemia (sporadic)	WES/targeted	~11% (n=19)	STAG2 (11%)	

This table provides an overview of the major findings regarding cohesin mutations in myeloid malignancies.

^ϕThis column describes the detection methods used to identify and screen cohesin genes for mutations. WES (whole exome sequencing); WGS (whole genome sequencing), targeted (targeted gene sequencing).

^ΣFrequencies are given as approximate percentages for mutations in any cohesin gene analyzed. When using targeted approaches the genes analyzed may vary between studies.

Any core cohesin member exhibiting mutations are shown with the frequency of samples affected. Mutations in regulatory factors have been grouped as 'regulatory'.

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