Published in final edited form as:

Gene. 2020 October 20; 758: 144966. doi:10.1016/j.gene.2020.144966.

Cohesin subunit RAD21: From biology to disease

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Abstract

RAD21 (also known as KIAA0078, *NXP1, HR21*, Mcd1, Scc1, *and hereafter called RAD21)*, an essential gene, encodes a DNA double-strand break (DSB) repair protein that is evolutionarily conserved in all eukaryotes from budding yeast to humans. RAD21 protein is a structural component of the highly conserved cohesin complex consisting of RAD21, SMC1a, SMC3, and SCC3 [STAG1 (SA1) and STAG2 (SA2) in metazoans] proteins, involved in sister chromatid cohesion. This function is essential for proper chromosome segregation, post-replicative DNA repair, and prevention of inappropriate recombination between repetitive regions. In interphase, cohesin also functions in the control of gene expression by binding to numerous sites within the genome. In addition to playing roles in the normal cell cycle and DNA DSB repair, RAD21 is also linked to the apoptotic pathways. Germline heterozygous or homozygous missense mutations in *RAD21* have been associated with human genetic disorders, including developmental diseases such as Cornelia de Lange syndrome (CdLS) and chronic intestinal pseudo-obstruction (CIPO) called *Mungan syndrome*, respectively, and collectively termed as cohesinopathies. Somatic mutations and amplification of the *RAD21* have also been widely reported in both human solid and hematopoietic tumors. Considering the role of RAD21 in a broad range of cellular processes that are hot spots in neoplasm, it is not surprising that the deregulation of *RAD21* has been increasingly evident in human cancers. Herein, we review the biology of RAD21 and the cellular processes that this important protein regulates and discuss the significance of *RAD21* deregulation in cancer and cohesinopathies.

Keywords

RAD21; Cohesin; DNA Repair; CDLS; Cohesionpathy; Haematopoiesis

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Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.gene.2020.144966.

1. Introduction

Since the discovery of RAD21 at the end of the last century as a principal component of chromosomal cohesin complex, numerous other functions of this important molecule have been described. During the last two decades, more than 4500 manuscripts have been published on RAD21, describing not only its canonical functions in sister chromatids cohesion and DNA damage repair but also other functions such as regulation of gene transcription, maintenance of nuclear architecture, biogenesis of centrosomes, meiosis, apoptosis, and hematopoiesis. In view of these pleiotropic functions of RAD21 in a broad range of cellular processes, it is not surprising that the deregulation of *RAD21* has been increasingly evident in human diseases including developmental diseases, such as cohesinopathies, and cancer. How *RAD21* regulates such a diverse array of cellular functions and how *RAD21* mutations cause human diseases remain unclear. This review is an attempt to provide a broad view of RAD21 with a goal to synthesize a handbook of our current knowledge of RAD21, which we expect to serve as a link between the past and the future studies in this field.

2. Identification of *RAD21*

rad21 encoding a 628 amino acid (aa) protein was first cloned by Birkenbihl and Subramani in 1992 by complementing the radiation sensitivity of the *rad21–45* mutant fission yeast, *Schizosaccharomyces pombe*. They reported that *rad21* not only has a role in DNA double-strand break (DSB) repair but also is essential for the mitotic growth of *S. pombe*. By sequencing a human immature myeloid cell line-derived complementary DNA (cDNA) library, Nomura et al. (1994) identified a cDNA encoding a homolog of *S. pombe rad21* that they termed KIAA0078 that encodes a 631aa protein. The murine and human homologs of S. pombe rad21 were cloned by McKay et al. (1996). By probing a testis cDNA library with the mouse sequence, they obtained a cDNA encoding hRAD21, which they termed *HR21*. Sadano et al. (2000) cloned *RAD21* by immunoscreening a placenta cDNA expression library, which they designated *NXP1*. In 1993, by screening mutant budding yeast *Saccharomyces cerevisiae* strains with defective sister chromatid cohesion, Guacci et al. (1993) identified Mitotic Chromosome Determinant (Mcd1). By screening for mutation of the genes that result in loss of chromosomes in budding yeast as a function of an anaphase-promoting complex with a known role in the loss of sister chromatid cohesion, Michaelis et al. (1997) identified sister chromatid cohesion 1 (Scc1). Mcd1/Scc1 was found to be an ortholog of RAD21, a structural component of the chromosomal cohesin complex in the mitotic cell cycle that, in addition to RAD21 (Mcd1/Scc1), comprises SMC1, SMC3, and SCC3 subunits in yeast [also known as STAG1 (SA1) and STAG2 (SA2) in multicellular organisms].

The human *RAD21* (*hRAD21*) gene is located on the long (q) arm of chromosome 8 at position 24.11 (8q24.11) (Nomura et al., 1994; McKay et al., 1996), and its molecular locations spread across 28,933 bases from 116,845,934 to 116,874,776 on chromosome 8 (Homo sapiens Updated Annotation Release 109.20200228, GRCh38.p13) (NCBI). *hRAD21* consists of 28,933 bases, 13 protein-coding exons, and 12 introns with a transcript length of 3,660 bps and translation length of 631 amino acid (aa) residues.

Northern blot and RNA-seq analyses revealed ubiquitous expression of a 3.7-kb transcript in human tissues, with the highest expression in testis, thymus, bone marrow, and lymph node, and least expression in the pancreas. Northern blot analysis determined that expression increases during the S phase and peaks in the G2 phase in HeLa cells (McKay et al., 1996). No increase in expression was noted after ionizing radiation was applied. Expression analysis revealed ubiquitous expression of a 3.1-kb transcript in mouse tissues, with the highest expression in testis and thymus. Testis also expressed a 2.2-kb transcript in postmeiotic spermatids (McKay et al., 1996), which possibly encodes the meiotic version of Rad21.

3. Characteristics of RAD21 protein

3.1. RAD21 protein

RAD21 is a nuclear phospho-protein, exists in all eukaryotes, and ranges in size from 278aa in the house lizard (*Gekko Japonicus*) to 746aa in the killer whale (*Orcinus Orca*), with a median length of 631aa in most vertebrate species including humans. Immunofluorescence microscopy and Western blot analysis collectively revealed nuclear expression of a 120 kDa protein in human and mouse, which was higher than the predicted 68 kDa, most likely due to post-translational modifications, including hyper phosphorylation of RAD21. Sequence similarity comparison indicates hRAD21 is 96% and 25% identical to the mouse and yeast proteins, respectively (McKay et al., 1996). They are most conserved at the N-terminus (NT) and C-terminus (CT), which bind to SMC3 and SMC1, respectively. The STAG domain in the middle of RAD21, which binds to SCC3 (SA1/SA2), is also conserved (Fig. 1). These proteins have nuclear localization signals, an acidic-basic stretch and an acidic stretch (Fig. 1), which is consistent with a chromatin-binding role.

RAD21 belongs to a superfamily of eukaryotic and prokaryotic proteins called Kleisins (derived from the Greek word for closure: *kleisimo*) that include bacterial ScpA, eukaryotic RAD21, Rec8, and Barren (in fly) (Nasmyth and Haering, 2005). Based on the conserved sequence motifs, there are three categories of Kleisin proteins in the eukaryotic superfamily: Kleisin-α, β, and γ (Wildpaner et al., 2001; Schleiffer et al., 2003), and based on the homology, hRAD21 is a member of the α-Kleisin family (Nasmyth and Haering, 2005)

RAD21 binds to the V-shaped SMC1 and SMC3 heterodimer, forming a tripartite ring-like structure (Gligoris et al., 2014), and then recruits SCC3 (SA1/SA2). The 4 element-complex is called the cohesin complex (Fig. 2). SMC1 and SMC3 are ABC-like ATPases. The NT and CT of the SMC molecules fold back on themselves, forming anti-parallel intramolecular coiled coils (Haering et al., 2002). The conserved protein domains on the CT and NT of RAD21 bind to the ATPase heads of the SMC1 and SMC3 heterodimer, respectively, to thus form a triangular ring, and SCC3 (SA1/SA2) binds to STAG domain on RAD21 to reinforce the ring (Fig. 2A) (Gruber et al., 2003). The binding of ATP to the ATPase head of SMC1 is required for RAD21 association with the SMC1 and SMC3 heterodimer (Arumugam et al., 2003). Unlike budding yeast in which rad21 CT (269–566aa) but not the NT (1–180aa) physically interacts with Scc3 (Haering et al., 2002), in humans the RAD21 middle part (MP, 173–449aa) but not the RAD21 CT (451–631aa) interacts with SCC3 orthologs, SA1 and SA2 (Zhang et al., 2013b). Both the yeast rad21 (566aa) and the hRAD21 (631aa) have

two Separase cleavage sites. The cleavage sites of the yeast rad21 are at R180 and R268 (Uhlmann et al., 1999; Uhlmann et al., 2000). The sizes of the Separase-cleavage fragments of yeast rad21 NT (1–180aa) and hRAD21 NT (1–172aa) are similar, but the Separase-cleavage fragments of yeast rad21 CT (269–566aa) and hRAD21 CT (450–631aa) are remarkably different. Although hRAD21 CT (451–631aa) cannot immunoprecipitate SA1/2, its NT extended version of RAD21 (254–631aa) can. It is possible that the interaction of yeast Scc3 and rad21 CT is dependent of its long MP, just like the RAD21 MP in humans (Zhang et al., 2013b).

Currently, there are two major competing models of sister chromatid cohesion (Fig. 2B). The first one is the one-ring embrace model (Haering et al., 2002), and the second one is the dimeric handcuff-model (Zhang et al., 2008b; Zhang and Pati, 2009). The one-ring embrace model posits that a single cohesin ring traps two sister chromatids inside; however, biochemical and cell biology studies in mammalian cells (Zhang et al., 2008b; Zhang and Pati, 2009) and genetic studies in budding yeast (Eng et al., 2015) argue that two or more cohesin molecules work together to generate cohesion, and the model by Pati and colleagues supports a dimeric cohesion ring in handcuff configuration (Zhang et al., 2008b; Zhang and Pati, 2009). Based on the molecular associations of cohesin subunits, results of a fluorescence protein complement assay (PCA), protein–protein interaction along with other cell biology techniques (Zhang et al. (2008b) provide evidence for a handcuff model of the cohesin complex, which consists of two rings. Each ring has one set of RAD21, SMC1, and SMC3 molecules. The handcuff is established when two RAD21 molecules move into anti-parallel orientation that is enforced by either SA1 or SA2. Inhibition of SA1/SA2 leads to dissociation of the rings, resulting in the loss of cohesion. Cattoglio et al. (2019) recently reported that cohesin dimers occupy at least ~8% in mouse embryonic stem cells (mESCs). There is also recent evidence for dimerization or oligomerization states of cohesin subunits in budding yeast and human cells, and correlate this state with replication and cohesin acetylation (Guacci et al., 2019)hi et al 2020. Using chromatin immunoprecipitation and sequencing (ChIP-Seq) for SMC1a and SMC3 in *STAG2* WT and KO cells, Viny et al. (2019) recently showed lack of statistically significant differential loci and no differential occupancy in either STAG1/2-common or STAG1/2-unique binding sites on the chromatin, suggesting that absence of STAG1/2 may have no effect on the cohesin ring occupancy on the chromatin but may just lack the bridge/cohesion between the cohesin rings.

By using the single-molecule imaging, the DNA loop extrusion compacted by human cohesin has been visualized, and two cohesin molecules were most frequently contained in the loop-extruding complexes (Kim et al., 2019), suggesting cohesin dimerization. With the chromosome regions marked, the dynamics in mitotic chromosome resolution and compaction have been clarified (Eykelenboom et al., 2019). With more high-throughput chromosome conformation capture (Hi-C) experiments, there will be more observation of cohesin-regulated, high-order chromatin structures at kilobase resolution, which cannot only elucidate the cohesin-chromatin interaction but also will reveal the true nature of the cohesin rings.

Despite numerous attempts using both budding yeast and human proteins, the crystal structure of full-length RAD21 has not been solved. However, fragments of hRAD21 protein

(18–87aa, 295–420aa, 321–395aa) complexed with other cohesin subunits and associated proteins, including SMC1a, SMC3, STAG1, STAG2, PDS5, and CTCF, have been reported in the literature (Deardorff et al., 2012; Kon et al., 2013; Li et al., 2013; Zhang et al., 2013b; Gligoris et al., 2014; Gligoris and Lowe, 2016; Li et al., 2020) and PDB database. NT domain of yeast Rad21 contains two alpha-helices, forming a 4-helix bundle with the coiled-coil emerging from the adenosine triphosphatase (ATP) head of Smc3 (Gligoris et al., 2014). In contrast, the CT domain of yeast Rad21 contains three helices, followed by two ß strands (Haering et al., 2004), corresponding to the boundaries and secondary structure predictions for the CT domains of all Kleisins (Schleiffer et al., 2003). A crystal structure of budding yeast Smc1 nucleotide binding domain (NBD) bound to Rad21 CT domain shows that the Rad21 forms a winged-helix domain (WHD) that binds through extensive hydrophobic interactions to the two most CT ß strands of the Smc1 NBD (Haering et al., 2004). This interaction appears to alter the structure of Smc1 NBD in a manner that is essential for ATP binding and hydrolysis (Arumugam et al., 2006). Crystal structure of human STAG2 complexed with RAD21 showed multiple HEAT repeats of STAG2 form a dragon-shaped structure, and RAD21 makes extensive contacts with STAG2 (Hara et al., 2014). Crystal structure of STAG2-Rad21 in complex with CTCF at a resolution of 2.7 Å has recently been reported, revealing that the interaction of CTCF and STAG2-RAD21 complex is specifically required for CTCF-anchored loops and contributes to the positioning of cohesin at CTCF binding sites (Li et al., 2020).

3.2. Proteolytic cleavage of RAD21 during mitosis and apoptosis

During the metaphase to anaphase transition, RAD21 is proteolytically cleaved by the CD clan endopeptidase, Separase (aka Separin), encoded by the *ESPL1*, which is required for the dissociation of the cohesin complex for the orderly segregation of the sister chromatids and completion of cytokinesis (Michaelis et al., 1997; Nasmyth et al., 2000; Zhang and Pati, 2017). There are two mitotic cleavage sites for Separase on RAD21 (Fig. 1) reported in budding yeast, fission yeast, mouse, and human cells (Uhlmann et al., 1999; Uhlmann et al., 2000; Hauf et al., 2001). The RAD21 motif cleaved by Separase in yeast is (D/E)xxR, and that in vertebrates is ExxR. Separase cleaves the peptidyl bond after arginine residues of the core motif. Interestingly, human Separase cannot cleave yeast Rad21, and *vice versa* (Waizenegger et al., 2002). It is not clear what factors determine the specificity of Separase's cleavage of RAD21. According to one study, one of the determining factors is the adjacent amino acid residues before arginine (R) and the acidic amino acid residue aspartic acid/glutamic acid (D/E) of the motif (D/E)xxR (Sullivan et al., 2004; Winter et al., 2015). In a recent study, Rosen et al. (2019) identified an LPE motif on the RAD21 (Fig. 1), which is distinct from the Separase cleavage site and is required for rapid and specific cleavage of RAD21 by Separase. Securin (Pds1), an inhibitory chaperon of Separase, also contains a conserved LPE motif that blocks Separase engagement of the RAD21 LPE motif, suggesting that rapid cohesin cleavage by Separase requires a substrate docking interaction outside the active site (Rosen et al., 2019).

Calcium-dependent cysteine endopeptidase Calpain-1 also has been shown to be a RAD21-peptidase (Panigrahi et al., 2011). Calpain-1 cleaves hRAD21 at conserved L192 in a calcium-dependent manner (Fig. 1). RAD21 cleavage by Calpain-1 promotes the separation

of chromosome arms, which coincides with calcium-induced partial loss of cohesin at several chromosomal loci. Engineered cleavage of RAD21 at the Calpain-cleavable site without activation of Calpain-1 can lead to loss of sister chromatid cohesion.

In addition to the proteolytic cleavage of RAD21 during mitosis, hRAD21 is also cleaved during apoptosis (Chen et al., 2002; Pati et al., 2002), and the cleaved RAD21 is translocated from the nucleus to the cytoplasm much earlier than when chromatin condensation and nuclear fragmentation occur during apoptosis (Pati et al., 2002). Apoptotic cleavage site is mapped to the residue D279 of hRAD21, which is different from the mitotic cleavage sites required for chromosomal segregation (Hauf et al., 2001) (Fig. 1). *In vitro* cleavage assays indicate that Caspase-3 and −7 can cleave RAD21, but they (at least caspase-3) may not be essential because RAD21 can also be cleaved in MCF7 breast carcinoma cells that lack Caspase-3 activity (Kurokawa et al., 1999). In an *in vitro* cleavage assay, the use of apoptotic-induced Molt4 cell lysate resulted in 64 and 60 kDa hRad21 cleavage products (Pati et al., 2002). However, only the 64 kDa product was observed when caspase-3 and −7 were used (Pati et al., 2002). As RAD21 is a nuclear protein and the cleavage initially occurs in the nucleus, the protease that cleaves RAD21 may reside inside the nucleus. These findings suggest the presence of a novel caspase or caspase-like molecule in the nucleus that cleaves RAD21 early in apoptosis (Panigrahi and Pati, 2009). However, the physiological protease that cleaves RAD21 during apoptosis and the mechanisms by which the apoptotic signal is amplified remain to be identified.

3.3. Rad21 interactome

A total of 285 RAD21-interactants have been reported (https://www.ncbi.nlm.nih.gov/gene/5885). As a principal component of the cohesion complex, it is not surprising that RAD21 physically interacts with the other cohesion structural subunits including SMC3, SMC1, and STAG1/2 and cohesion complex associated proteins PDS5A, PDS5b, NIPBL, WAPL, and cohesin protease, Separase (Fig. 3). To understand how cohesin coordinates its diverse functions, Panigrahi et al. (2012) used a comprehensive approach to identify RAD21-interacting proteins that included a yeast 2-hybrid screen with *hRAD21* as the bait, an immunoprecipitation-coupled-mass spectrometry analysis for hRAD21-bound proteins, and a hRAD21-affinity pull-down assay. Their analyses revealed 112 novel protein interactors of RAD21 that function in different cellular processes, including mitosis, regulation of apoptosis, chromosome dynamics, chromosomal cohesion, replication, transcription regulation, RNA processing, DNA damage response, protein modification and degradation, and cytoskeleton and cell motility (Fig. 4). Identification of cohesin interactors provides a framework for explaining the various non-canonical functions of the cohesin complex.

Hakimi et al. (2002), using elaborate biochemical purification methods, reported the isolation of a human SNF2-containing chromatin remodeling complex that encompasses components of the cohesin and NURD complexes. They showed that the RAD21 subunit of the cohesin complex directly interacts with the ATPase subunit SNF2. Mapping of RAD21, SNF2, and Mi2 binding sites by chromatin immunoprecipitation experiments revealed the specific association of these three proteins with human DNA elements containing all sequences. They showed that the state of DNA methylation can regulate the association of

the cohesin complex with chromatin, and also presented evidence pointing to a role for the ATPase activity of SNF2 in the loading of RAD21 on chromatin.

4. RAD21 functions

RAD21 plays multiple physiological roles in diverse cellular functions (Fig. 5). The primary function of RAD21 is in the repair of DNA DSBs, as well as in sister chromatid cohesion during mitosis. As a subunit of the cohesin complex, RAD21 is involved in sister chromatid cohesion from the time of DNA replication in S phase to their segregation in mitosis, a function that is evolutionarily conserved and essential for proper chromosome segregation, chromosomal architecture, post-replicative DNA repair, and the prevention of inappropriate recombination between repetitive regions (Hauf et al., 2001; Zhang and Pati, 2014). RAD21 may also play a role in spindle pole assembly during mitosis (Gregson et al., 2001) and progression of apoptosis (Chen et al., 2002; Pati et al., 2002). In interphase, cohesin may function in the control of gene expression by binding to numerous sites within the genome. As a structural component of the cohesin complex, RAD21 also contributes to various chromatin-associated functions, including DNA replication (Takahashi et al., 2004; Ryu et al., 2006; Terret et al., 2009; Guillou et al., 2010; MacAlpine et al., 2010), DNA damage response (DDR) (Strom et al., 2004; Cortes-Ledesma and Aguilera, 2006; Watrin and Peters, 2006; Unal et al., 2007; Ball and Yokomori, 2008; Heidinger-Pauli et al., 2009; Watrin and Peters, 2009; Kim et al., 2010; Sjogren and Strom, 2010), and, most importantly, transcriptional regulation (Parelho et al., 2008; Wendt et al., 2008; Liu et al., 2009; Dorsett, 2010; Kagey et al., 2010; Pauli et al., 2010; Schmidt et al., 2010; Skibbens et al., 2010). Studies conducted during the past several years have demonstrated that cohesin affects: 1) allele-specific transcription by interacting with the boundary element CCCTC-binding factor (CTCF) (Parelho et al., 2008; Wendt et al., 2008; Schmidt et al., 2010; Skibbens et al., 2010; Degner et al., 2011; Guo et al., 2012), 2) tissue-specific transcription by interacting with tissue-specific transcription factors (Hadjur et al., 2009; Schmidt et al., 2010; Seitan et al., 2011; Faure et al., 2012; Yan et al., 2013; Zhang et al., 2013a), 3) general progression of transcription by communicating with the basal transcription machinery (Kagey et al., 2010; Fay et al., 2011; Schaaf et al., 2013; Yan et al., 2013), and 4) RAD21 co-localization with CTCF-independent pluripotency factors (Oct4, Nanog, Sox4, and KLF2). RAD21 cooperates with CTCF (Rubio et al., 2008), tissue-specific transcription factors, and basal transcription machinery to regulate transcription dynamically (Dorsett and Merkenschlager, 2013). Also, to effectuate proper transcription activation, cohesin loops chromatin to bring two distant regions together (Guo et al., 2012; Zhang et al., 2013a). Cohesin may also act as a transcription insulator to ensure repression (Wendt et al., 2008). Thus, RAD21 can affect both activation and repression of transcription. Enhancers that promote transcription and insulators that block transcription are located in conserved regulatory elements (CREs) on chromosomes, and cohesins are thought to physically connect distant CREs with gene promoters in a cell-type specific manner to modulate transcriptional outcome (Leeke et al., 2014). Therefore, alterations in RAD21 or other cohesin components could affect cohesin binding to CREs, thereby altering their interaction with promoters and, subsequently, gene activity. Although only a modest reduction in chromatin-bound cohesin is sufficient to cause

changes in expression of numerous genes, still little is known about how cohesin and RAD21 is recruited and removed from transcription sites to regulate transcription.

4.1. Role in sister chromatid cohesion and separation

Studies in yeast and higher eukaryotes including humans have indicated that RAD21 is required for appropriate arrangement of chromosomes during normal cell division (Guacci et al., 1997; Michaelis et al., 1997; Uhlmann and Nasmyth, 1998; Hartman et al., 2000; Nasmyth et al., 2000; Nasmyth, 2001; Nasmyth, 2002). Analyses of rad21 function in fission yeast, *S. pombe*, and Scc1/Mcd1 function in budding yeast, *S. cerevisiae*, demonstrate that this nuclear phosphoprotein is required for appropriate chromosomal cohesion during the mitotic cell cycle and DSB repair after DNA damage occurs (Biggins and Murray, 1999; Nasmyth et al., 2000). *RAD21* mRNA is cell-cycle regulated in human cells, increasing in the late S phase to a peak in the G2 phase (McKay et al., 1996). Biochemical analysis of cohesin indicates that RAD21 acts as a molecular glue, and human cohesin can promote intermolecular DNA catenation, a mechanism that links two sister chromatids together (Losada and Hirano, 2001). In budding yeast as well as in higher organisms including humans, loss of cohesion at the metaphase-anaphase transition is accompanied by proteolytic cleavage of the RAD21 protein (Uhlmann et al., 1999; Uhlmann et al., 2000; Waizenegger et al., 2000) followed by its dissociation from the chromatids (Nasmyth et al., 2000; Tomonaga et al., 2000; Waizenegger et al., 2000; Hauf et al., 2001). The cleavage depends on Separase (Ciosk et al., 1998; Uhlmann et al., 1999; Uhlmann et al., 2000), which is complexed with its inhibitor Securin prior to anaphase (Ciosk et al., 1998; Zou et al., 1999; Leismann et al., 2000). In metaphase, ubiquitin-mediated degradation of the Securin protein by APC/C-Cdc20 ubiquitin-ligase releases Separase protein, which proteolytically cleaves cohesin RAD21, thereby releasing the sister chromatids (Cohen-Fix et al., 1996; Funabiki et al., 1996; Ciosk et al., 1998; Jallepalli et al., 2001). In budding yeast, fission yeast, and human and mouse cells, RAD21 has two mitotic cleavage sites for Separase (Fig. 1) (Uhlmann et al., 1999; Uhlmann et al., 2000; Hauf et al., 2001), and cleavage by Separase appears to be essential for sister chromatid separation and the completion of cytokinesis (Hauf et al., 2001). In contrast to the simultaneous release of cohesin from the chromosome arms and centromere region in budding yeast by Separase cleavage, in metazoans, most cohesin is removed in early prophase from chromosome arms by a cleavage-independent mechanism (Waizenegger et al., 2000; Hauf et al., 2001). Only residual amounts of cohesin are cleaved at the onset of anaphase, coinciding with its disappearance from centromeres. Thus, RAD21 plays a critical role in the eukaryotic cell division cycle by regulating sister chromatid cohesion and separation at the metaphase-to-anaphase transition.

4.2. Role in centrosome cycles

Cohesin is required for the engagement of centrioles (Nakamura et al., 2009; Tsou et al., 2009; Liu et al., 2011; Schockel et al., 2011). Along with RAD21, cohesin core subunits (SMC1 and SMC3) have been found in centrosomes (Guan et al., 2008; Kong et al., 2009; Nakamura et al., 2009; Beauchene et al., 2010; Gimenez-Abian et al., 2010). RAD21 is recruited at the centrosomes by associating with AKI1 during mitosis to promote centriole cohesion to inhibit the premature centriole splitting in HeLa cells (Nakamura et al., 2009). Depletion of RAD21 not only causes the aberrant sister chromatid cohesion (Losada et al.,

2005) but also the formation of multipolar spindles (Losada et al., 2005; Nakamura et al., 2009), and, importantly, centriole splitting (Nakamura et al., 2009; Beauchene et al., 2010). RAD21 plays a vital role in the maintenance of centrosomes' integrity by preventing gamma-tubulin overexpression (Beauchene et al., 2010).

Two cohesin regulatory enzymes, Plk1 and RAD21-protease Separase, also have been found to play a role in the centrosome cycle. Recent studies report that at the late G2 and early M phases (before the onset of anaphase) Plk1 regulates mitotic licensing of centriole duplication in the following S phase (Tsou et al., 2009). Plk1 also promotes Separase-dependent centriole disengagement by phosphorylating RAD21, which is proteolytically cleaved by Separase in the late M phase (Schockel et al., 2011). That Separase inhibitors, Securin and Cyclin B (Tsou and Stearns, 2006), and the depletion of Separase itself (Thein et al., 2007) inhibit centriole disengagement underscores the importance of Separase and cleavage of its substrate, RAD21, in the centrosome cycle. The function and regulation of cohesin in the centrosome cycle appear to mirror those in the chromosome cycle. However, the mechanism that governs the function and regulation of cohesin in the centrosome cycle is less understood compared to that of the chromosome cycle.

4.3. Role in DNA double strand break (DSB) repair

RAD21 plays an essential role in DNA DSB repair, which was first reported in the fission yeast *S. pombe* (Birkenbihl and Subramani, 1992), and later in *C. elegans* and humans (McKay et al., 1996). The requirement of RAD21 in DSB repair is conserved from yeast to humans. As indicated earlier, rad21 was cloned originally by complementing the radiation sensitivity in fission yeast with a function in DNA-DSB repair, before its role in sister chromatid cohesion was identified. The mutant rad21 in fission yeast exhibited hypersensitivity to radiation owing to its impaired DNA DSB repair (Birkenbihl and Subramani, 1992).

A number of more recent studies implicate cohesin in the DNA damage response and repair in eukaryotic cells (Darwiche et al., 1999; Dorsett, 2011; Deardorff et al., 2012; Ding et al., 2012). In addition to the sister chromatid cohesion generated in the S phase during DNA replication, additional cohesins must be recruited to a DSB, and a new cohesion is created de novo in response to the damage for repair (Kim et al., 2010). This newly created cohesion is called *damage-induced cohesion* (DI-cohesion). DSB in the G2 phase causes genome-wide DI-cohesion in both yeast and human cells (Strom et al., 2007; Unal et al., 2007; Kim et al., 2010). Besides cohesin itself, factors that are required to load cohesin onto chromatin, to establish cohesion, and to maintain cohesion are needed for repair of the damaged DNA (Sjogren and Nasmyth, 2001; Strom et al., 2004; Unal et al., 2004; Schmitz et al., 2007; Strom et al., 2007; Unal et al., 2007; Unal et al., 2008). How does DSB cause de novo cohesion establishment? DSB has been shown to activate Chk1 that phosphorylates Rad21 at the conserved serine residue (S83) in yeast (Heidinger-Pauli et al., 2008). S83 phosphorylation facilitates the acetylation of K84 and K210 residues in Rad21 by Eco1, which in turn antagonizes Wpl1/Rad21 to establish DI-cohesion (Heidinger-Pauli et al., 2009). DI-cohesion is different from the sister chromatid cohesion generated during the S phase, in which Smc3 is acetylated by Eco1 to counteract the anti-establishment activity of

Wpl1 (Rolef Ben-Shahar et al., 2008; Unal et al., 2008; Zhang et al., 2008a; Rowland et al., 2009; Sutani et al., 2009). Interestingly, the DNA damage-induced phosphorylation and acetylation on RAD21 in human cells have not been observed. Instead, the DNA damage-induced phosphorylation and acetylation on SMC3 were found to be important for the genome-wide DI-cohesion and DNA DSB repair (Kim et al., 2010).

4.4. Role in gene expression and chromatin architecture

The evidence for a role of RAD21 and cohesin complex in gene expression first came from the studies in zebrafish, in which Horsfield et al. (2007) demonstrated that monoallelic loss of rad21 resulted in a reduction in the transcription of runx1 and the proneural genes ascl1a and ascl1b, indicating that downstream genes are sensitive to rad21 dose. In fruit fly mutations in Rad21 and Nipped B, a subunit of cohesin loading complex, suppressed polycomb-group genes and hedgehog gene (Hallson et al., 2008). Fay et al. (2011) showed that Drosophila Rad21 interfered with the transition of paused RNA polymerase to elongation to repress the gene expression. A number of independent studies have shown that global gene expression is more sensitive to cohesin changes than to their effect on sister chromatid cohesion and DNA repair (Krantz et al., 2004; Tonkin et al., 2004; Schaaf et al., 2009; Heidinger-Pauli et al., 2010).

Cohesin-regulated gene expression is independent of its role in cell division because it can influence gene expression in non-dividing cells (Pauli et al., 2008; Schuldiner et al., 2008; Seitan et al., 2011). The expression of cohesin-regulated genes can be affected by a change of the cohesin level within a few hours (Liu et al., 2009; Schaaf et al., 2009; Kagey et al., 2010; Pauli et al., 2010), suggesting that cohesin regulates gene expression directly and rapidly. Although binding of RAD21 and other cohesin subunits to genes seems different among organisms, the common point that cohesins associate with transcriptionally active genes indicate a conserved cohesin-mediated, gene expression mechanism. In zebrafish, rad21-regulated genes include proto-oncogene myca (c-Myc in human), tumor suppressor p53, and mdm2 (Rhodes et al., 2010). rad21 is found at transcription start sites of p53 and mdm2, expression of which is enhanced by the depletion of either rad21 or CTCF. In contrast, loss of rad21 decreases myca expression. Positive transcriptional regulation of the c-Myc gene by cohesin is evolutionally conserved as loss of Rad21 or Nipped-B in Drosophila decreases the expression of both myc and its target genes (Rhodes et al., 2010). RAD21 also binds to and represses the apolipoprotein B (APOB) gene promoter (Bonora et al., 2015). Mutations of Rad21 in patients with chronic intestinal pseudo-obstruction (CIPO) interrupt the ability of Rad21 to regulate genes such as *RUNX1* and *APOB*. Reduced expression of rad21 in zebrafish and dysregulation of *RAD21* target genes, including APOB, disrupt intestinal transit and the development of enteric neurons (Bonora et al., 2015).

Although it is evident that cohesins are involved in gene transcription, how they are regulated during this process remains unclear. Do cohesins have only a passive role as a component of transcriptional factors or an active role in recruiting other factors and remodeling chromatin structure? Do cohesins associate with chromatin in the same fashion in gene expression regulation and sister chromatid cohesion? It seems that cohesins are loaded to specific sites by cohesin-loading complex in order to function. However, it is not

known whether cohesins are required to be unloaded after their missions are accomplished, and if so, how cohesins are removed.

Cohesin-mediated chromatin organization plays an important role in the formation and stabilization of chromosome architecture and gene regulation. Cohesin RAD21 interacts with CTCF and other cohesin-associated proteins to maintain and stabilize multidimensional organizations of topologically associating domains (TADS) and chromatin loops by entrapping two segments of chromatin in cis. Depletion of CTCF, RAD21, or cohesin-associated proteins was shown to affect the majority of domains and loops in a manner that is consistent with a model of DNA folding through the extrusion of chromatin loops (Rhodes et al., 2010). Degradation of CTCF or cohesin resulted in a genome-wide loss of loops at individual loci (Rao et al., 2017; Wutz et al., 2017). The removal of CTCF resulted in a substantial loss of insulation between many neighboring TADs (Nora et al., 2017). Many TADs were also lost upon removal of cohesin (Rao et al., 2017; Wutz et al., 2017). Loops and TADs were reestablished after the restoration of CTCF (Nora et al., 2017) or cohesin (Rao et al., 2017). However, the mechanisms by which cohesin shapes chromosomes and regulates gene expression remains unclear and an area of active research.

4.5. Role in hematopoiesis

In 2012, Panigrahi and Pati suggested that cohesin and its associated proteins may play a central role in the orchestration of hematopoiesis and may serve as a master transcriptional regulator of hematopoietic genes. As indicated above, Rad21 regulates the expression of hematopoiesis regulator, Runx1, during zebrafish development (Horsfield et al., 2007; Rhodes et al., 2010). In this model, loss of cohesin *rad21* represses r*unx1*, and the bone marrow cells fail to develop differentiated blood cells (Horsfield et al., 2007). In mice, haploinsufficiency in *Rad21* causes impaired clonogenic regeneration of the bone marrow stem cells (Xu et al., 2010), and RAD21 plays a critical role in T-cell-receptor rearrangement and thymocyte differentiation (Seitan et al., 2011). Numerous recent functional and genomic studies have implicated chromosomal cohesin proteins as critical regulators of hematopoiesis (Mazumdar et al., 2015; Mullenders et al., 2015; Viny et al., 2015; Fisher et al., 2017a; Rao, 2019).

Several groups (Mazumdar et al., 2015; Mullenders et al., 2015; Viny et al., 2015; Fisher et al., 2017a) reported the phenotype induced by cohesin haploinsufficiency with an agreement that loss of cohesin enhances hematopoietic stem and progenitor cell (HSPC) self-renewal, a critical first step in the development myeloid malignancies. These studies also revealed that altered chromatin accessibility (Mazumdar et al., 2015; Mullenders et al., 2015; Viny et al., 2015) and/or elevated expression of the transcription factor HOXA9 (Fisher et al., 2017b) were key drivers of this abnormal HSPC self-renewal. However, in a recent study, Sasca et al. (2019) has identified a specific defect in erythroid lineage commitment as a potential consequence of cohesin mutations in myeloid leukemia. Depletion of cohesin severely impairs erythroid differentiation, particularly at Etv6-prebound loci, but augments self-renewal programs. In cohesin haploinsufficient cells, cohesin levels cannot increase during erythroid commitment, which prevents the eviction of Etv6 and induction of genes required for erythroid differentiation.

Using conditional knockout (cKO) mouse models to target cohesin subunit *Rad21* alleles in hematopoietic stem and progenitor cells (HSPC), we have examined the physiological consequences of cohesin-*Rad21* perturbation on normal hematopoiesis. Although there is an absolute requirement for cohesin in hematopoietic stem cell (HSC) function, *Rad21* haploinsufficiency has distinct hematopoietic phenotypes contrasting other cohesin subunits cKO models (e.g. Smc3) (Kumar et al, unpublished). Overall, our results demonstrate that Rad21 haploinsufficiency leads to impaired hematopoietic differentiation and increased HSC self-renewal. It has also been suggested that *Rad21* acts as a negative regulator of hematopoietic self-renewal through epigenetic repression of *HoxA7* and *HoxA9*, indicating its possible implication in leukemogenesis (Fisher et al., 2017b).

4.6. Role in apoptosis

In addition to playing roles in the normal cell cycle and DNA DSB repair, human RAD21 is also linked to the apoptotic pathways, a surprising finding demonstrated by our and other laboratories (Chen et al., 2002; Pati et al., 2002). Cleavage of RAD21 can be induced in a number of leukemia cells such as Molt4 and Jurkat, by a broad spectrum of apoptotic stimuli (Pati et al., 2002). The apoptotic cleavage site is at residue D279, which is different from the mitotic cleavage sites required for chromosomal segregation (Hauf et al., 2001) (Fig. 1). In an *in vitro* cleavage assay, use of apoptotic-induced cell lysates resulted in 64 and 60 kDa RAD21 cleavage products (Pati et al., 2002). Although Caspase-3 and −7 can cleave RAD21 *in vitro*, the physiological protease that cleaves RAD21 during apoptosis and the mechanisms by which the apoptotic signal is amplified remain to be identified (Panigrahi and Pati, 2009).

Transfection experiments indicate that CT RAD21 (280–631aa) can induce apoptosis in many cell lines that are sensitive or resistant to apoptosis, but full-length RAD21 and NT RAD21 (1–279aa) have little or no apoptotic effect (Chen et al., 2002; Pati et al., 2002). Apoptosis induced by CT RAD21 and the tumor necrosis factor (TNF) receptor superfamily may share part of a common pathway. Blast search indicates that a region of 104 amino acid residues in CT RAD21 has high consensus (26% identities, 43% positives) with the sequence upstream of the death domain (DD) of several apoptosis-related proteins (Panigrahi and Pati, 2009). TNF receptor superfamily members have DDs, and their involvement in apoptosis requires TNF signaling from outside of the cell. CT RAD21 does not have a DD. It is currently not known whether CT RAD21-induced apoptosis requires extracellular signals, such as those in the TNF superfamily. Interestingly, as mentioned earlier, cleavage of cohesin RAD21 is carried out by a Separase in mitosis and by a caspase-like molecule in apoptosis at different sites in the protein. Both of these proteases belong to the distantly related CD-clan protease family (Uhlmann et al., 2000), suggesting an evolutionarily conserved mechanism shared by the mitotic and apoptotic machinery. RAD21 may serve as the link between the two key cellular processes of mitosis and apoptosis (Panigrahi and Pati, 2009).

4.7. Role in meiosis

Meiosis occurs in two sequential cell divisions and produces four haploid cells. Most of the events that differentiate meiosis from mitosis occur in prophase I, when homologous

chromosomes form bivalents (or tetrads) and cross over/recombine between non-sister chromatids. Cohesin complexes specific to meiosis are required to mediate homologous chromosome pairing, synapsis, recombination, and segregation (Ishiguro et al., 2011; Lee and Hirano, 2011; Llano et al., 2012; Fukuda et al., 2014; Ishiguro et al., 2014; Llano et al., 2014; Winters et al., 2014; Ward et al., 2016).

In most organisms, the Rad21 cohesin subunit is replaced by a meiotic-specific isoform, called Rec8, during meiosis. There are two paralogs of Rad21 – Rec8 and Rad21L – in vertebrates, which are expressed in cells undergoing meiosis and form a complex with the other meiosis-specific cohesin subunits (McKay et al., 1996; Parisi et al., 1999; Ishiguro et al., 2011; Lee and Hirano, 2011; Ishiguro, 2019). In mouse, both Rec8 and Rad21L appear on chromosomes at pre-meiotic S-phase (Ishiguro et al., 2011; Lee and Hirano, 2011), and they are critical for the formation of chromosomal axes during the meiotic prophase (Ward et al., 2016). Rec8/Rad21L double mutants show an earlier “leptotene-like” arrest with complete absence of STAG3 on chromosomes. Both Stag3/Rad21L and Stag3/Rec8 double mutants can progress further into prophase I than can the Rec8/Rad21L double mutant (Ward et al., 2016), suggesting Rec8 and Rad21L cohesin complexes can partially compensate each other. Rad21L, but not Rec8 or Rad21, was found to interact biochemically with the synaptonemal-complex protein SYCP1 (Lee and Hirano, 2011). Interestingly, Rad21L disappears from chromosomes once recombination is complete, whereas homologues remain juxtaposed by the synaptonemal complex, and Rec8 persists along chromosome axes. The early dissociation of Rad21L complexes from chromosomes, promoting by Polo kinase (Ishiguro et al., 2011), is possible to facilitate synaptonemal-complex disassembly. It also suggests that the major role of Rad21L cohesin complex is in homologue pairing and synapsis, not in sister chromatid cohesion, whereas Rec8 most likely functions in sister chromatid cohesion. Intriguingly, concomitantly with the disappearance of RAD21L, Rad21 appears on the chromosomes in late pachytene and mostly dissociates after diplotene onward (Ishiguro et al., 2011; Lee and Hirano, 2011). The function of Rad21 cohesin that transiently appears in late prophase I is unclear.

5. *RAD21* animal models

Mutant mouse and zebrafish models of *Rad21* have been reported (Seitan et al., 2011; Bonora et al., 2015). Biallelic deletion of cohesin subunits results in cell death (Guacci et al., 1997; Michaelis et al., 1997; Heo et al., 1998). As in yeast, the homozygous deletion of *Rad21* in mice is embryonically lethal, but heterozygous animals are viable with no significant phenotypes. Using a tissue-specific Cre-recombinase (CD4-Cre), Seitan et al. (2011) have generated a thymocyte-specific deletion of the *Rad21* locus in mouse at a time in development when these cells stop cycling and rearranging their T-cell receptor alpha locus (TCRA). CD4–Cre-mediated deletion of *Rad21* generates thymocytes that die when forced to divide yet have an average lifespan as non-dividing cells *in vivo*. This feature allows the interrogation of cohesin functions in interphase, independent of essential cohesin functions during cell division. *Rad21*-deficient thymocytes had an average life span and retained the ability to differentiate, but with reduced efficiency. The loss of *Rad21* in this model led to defective chromatin architecture at the *Tcra* locus, which has now been confirmed using Hi-C, a method to study the three-dimensional architecture of genomes

(Seitan et al., 2013). A distinct role of *Rad21* in hematopoiesis has been studied using this conditional knockout model of *Rad21* (Kumar et al., in revision).

Mutant *rad21* zebrafish provides a model for Mungan syndrome. Injecting zebrafish embryos with a *rad21a* splice-blocking morpholino to suppress the expression of rad21, Bonora et al. (2015) observed a chronic intestinal pseudo-obstruction phenotype often seen in patients with Mungan syndrome. The mutants showed delayed food transit compared to wildtype zebrafish, and quantitative analysis of the zebrafish gut revealed marked depletion of enteric neurons at 4 and 5 days post fertilization in the mutants compared to controls, suggesting a neurogenic cause of the observed motility defects, and a role of Rad21 in this process.

6. Rad21 and human disease

6.1. Cohesinopathies

Cohesinopathies are a variety of rare genetic human diseases triggered by the mutations in the core subunits of cohesin complex or regulators that participate in cohesin complex dynamics. Cornelia de Lange syndrome (CdLS, OMIM 122470, 300590, 610759, 614701, 300882) is one of the best-known cohesinopathies (Barbero, 2013). CdLS is a rare, clinically variable and genetically heterogeneous disorder, with an estimated occurrence in 0.5–10 every 100,000 births (Barisic et al., 2008; Kline et al., 2018). It is characterized by mental retardation, facial dysmorphism, upper limb abnormalities, growth delay, and numerous other signs and symptoms (Jackson et al., 1993; Boyle et al., 2015; Kline et al., 2018).

CdLS is caused by variants in any one of seven genes, *NIPBL, SMC1A, SMC3, RAD21, HDAC8, BRD4, and ANKRD11*, all of which have a structural or regulatory function in the cohesin complex. Mutations in NIPBL can be identified in approximately 70% of CdLS cases (Kline et al., 2018). *RAD21* variants cause a small percentage of CdLS cases, and the phenotype of those CdLS cases is non-classic (Kline et al., 2018). To date, 49 patients from 33 families with 31 different *RAD21* variants have been reported (Wuyts et al., 2002; McBrien et al., 2008; Deardorff et al., 2012; Ansari et al., 2014; Minor et al., 2014; Pereza et al., 2015; Boyle et al., 2017; Martinez et al., 2017; Gudmundsson et al., 2019; Dorval et al., 2020; Krab et al., 2020). Seven of the 31 variants are unique copy number variations (CNVs) that *RAD21* is deleted (six of which included other genes in addition to *RAD21*). Twenty-four of the 31 variants are intragenic sequence variants. Of the 24 different sequence variants, 13 are truncated (2 nonsense, 2 splice site, and 9 frameshift variants), 3 are in-frame deletions (2 of which affect a single amino acid, whereas the 665 bp deletion includes the whole exon 13), and 8 are missense mutations. Two of the 31 variants [p.C585R (reference SNP (rs) 387907213) and p.R586* (no rs# available)] were recurrent, and each was found in two families. A relatively large proportion of the cases (9 of 21) are familial. Interestingly, the truncated variants are scattered throughout the gene, suggesting that the protein either is not made or not functional. In contrast, the variants of in-frame deletion and missense mutation are mainly clustered on the functional domains of the RAD21 (Fig. 2) (i.e., N-terminal SMC3 interacting domain, middle STAG domain, and C-terminal SMC1 binding domain) (Krab et al., 2020). Structural and functional analyses indicate that most of the missense mutations and in-frame deletions interrupt the interaction between RAD21 and

SMC1A, SMC3, or STAG1/2, implying the pathogenicity of *RAD21* variants (Krab et al., 2020).

Because a small number of CdLS cases are caused by *RAD21* mutations, it is difficult to link the phenotype to a genotype or compare phenotypes caused by different genotypes (microdeletions *vs*. intragenic variants, truncating *vs*. non-truncating sequence variants). It is hard even to compare the phenotypes (especially in cognition and behavior) in patients with an intrafamilial variation. Several families have patients with intellectual disabilities and those with apparently normal cognitive functioning (Krab et al., 2020).

RAD21 variants have also been associated with other diseases, such as sclerocornea (Zhang et al., 2019a; Zhang et al., 2019b) and Mungan syndrome (Chronic Intestinal Pseudo-obstruction (CIPO); OMIM #611376) (Mungan et al., 2003; Bonora et al., 2015). Sclerocornea is a rare congenital disorder characterized by the opacification of the cornea. Six patients with peripheral sclerocornea in one family spanning across three generations were identified, and the disease was found to be inherited in an autosomal dominant manner (Zhang et al., 2019a). A *RAD21* variant (c.C1348T, p.R450C) (rs1301282588) was identified cosegregating with the peripheral sclerocornea in those patients. Although this variant abolishes the Separase cleavage site at ^{447}EPDR^{450}, no mitosis and ploidy defects were found in cells from peripheral sclerocornea-affected family members (Zhang et al., 2019a), suggesting that the Separase cleavage site at ^{169}EIMR^{172} on RAD21 (Fig. 1) is sufficient for Separase to remove cohesins from sister chromatids at mitosis, while the function other than sister chromatid cohesion might be affected. Expression of a RAD21 (R450C) variant in *X. laevis* led to disrupted eye development with disorganized corneal stroma and decreased diameters of collagen fibrils. These eye defects can be rescued by overexpression of the wildtype rad21 (Zhang et al., 2019b), supporting that the RAD21 (R450C) variant is the cause of peripheral sclerocornea.

Mungan syndrome was identified from a large consanguineous Turkish family (Mungan et al., 2003). It is an autosomal recessively inherited disorder characterized by gastrointestinal hypomotility related to visceral neuromyopathy, which causes CIPO. The patients with Mungan syndrome were found to have biallelic *RAD21* p.A622T variants (rs775266057), and the pathogenic effect of variant p.A622T is supported by studies showing decreased bowel transit and loss of enteric neurons in zebrafish with p.A622T variants (Bonora et al., 2015).

Besides the non-classic CdLS features, patients with loss-of-function variants in cohesin genes, including *RAD21*, were found to have holoprosencephaly, a cephalic disorder in which the prosencephalon (the forebrain of the embryo) fails to develop into two hemispheres. (Kruszka et al., 2019).

6.2. Rad21 and cancer

According to the COSMIC database (https://cancer.sanger.ac.uk/cosmic/search?q=rad21), 673 (~1.3%) of 53,383 human tumor specimens tested carry somatic mutations in the *RAD21* coding region. These mutations are primarily in hematological malignancies compared to solid tumors. According to the TCGA PanCancer atlas studies, 7% of all

queried patients had alterations in *RAD21* (https://www.cbioportal.org/). Most of these alterations include gene amplifications, particularly in ovarian and breast cancers, accounting for 20% and 13%, respectively. *RAD21* is also overexpressed in other cancers, including prostate, melanoma, bladder, and liver tumors. *RAD21* mutations are found to be mutually exclusive with other cohesin component genes, particularly *SMC3* and *STAG2*, and is not associated with aneuploidy.

Although *RAD21* mutations are rare occurrences in human solid tumors, expression levels of *RAD21* have been associated with prognosis and metastatic behavior (Mintzas and Heuser, 2019). Overexpression of RAD21 has been linked with epithelial breast cancer and was correlated with poor disease outcome and resistance to chemotherapy (van ‘t Veer et al., 2002; Xu et al., 2011), whereas low RAD21 expression characterized metastatic breast and oral squamous cancers (Yamamoto et al., 2006). In a large study of colorectal cancer, 50% of patients had positive RAD21 expression in the nucleus, which was correlated with metastasis and reduced disease-specific survival (Deb et al., 2014). Overexpression of RAD21 was also observed in cases of primary and hormone-refractory prostate carcinomas compared to benign prostate hyperplasia (Porkka et al., 2004) and in gastric tumors, for which 60% of patients had elevated levels of RAD21 (Yun et al., 2016).

RAD21 variants in cancer patients exhibiting acute radiation toxicity suggested an association between RAD21 gene variants and normal tissue protection that may be defective in some radiation-sensitive cancer patients (Severin et al., 2001). Using a *Rad21*$^{+/-}$ mouse model, McKay and colleagues have shown that *Rad21* haploinsufficiency impedes DNA repair and enhances gastrointestinal radiosensitivity in mice (Xu et al., 2010) [dummy_incomplete para]

Although initial studies using cell lines from solid tumors suggested that the key role of RAD21 and other cohesin subunit inactivation was the initiation of aneuploidy, more recent studies have questioned this suggestion, pointing to alterations in progenitor/stem cell differentiation as an important phenotype of cohesin inactivation (Fisher et al., 2017b). Moreover, it has been found that mutant cohesins that impair HSPC differentiation by controlling chromatin accessibility and transcription factor activity possibly contribute to leukemic disease (Mazumdar et al., 2015). Recent studies have reported that *RAD21* is somatically mutated in a wide range of hematological malignancies including acute myeloid leukemia (AML) and myelodysplastic syndrome (MDS) (Solomon et al., 2014; Thota et al., 2014; Mullenders et al., 2015; Hill et al., 2016). *RAD21* mutations are considered early events in leukemogenesis (Corces-Zimmerman et al., 2014) and cohesin appears to act as a tumor suppressor (Mullenders et al., 2015). Sequencing studies have shown that *RAD21* mutations are mainly heterozygous nonsense mutations that lead to premature truncation and a loss-of-function protein.

Approximately 12% patients with AML harbor mutations in one of the cohesin subunit genes, with *RAD21* in 3% of patients (Cancer Genome Atlas Research et al., 2013; Kon et al., 2013; Thol et al., 2014; Thota et al., 2014; Lindsley et al., 2015; Tsai et al., 2017; Eisfeld et al., 2018; Weinberg et al., 2018). Core binding factor AML (CBF-AML), a distinct genetic subset of AML, follows the same pattern as other AML groups, with 9% of patients

carrying a cohesin mutation, with slightly increased RAD21 mutation frequency. Interestingly, the mutations were found exclusively in patients with translocation t(8;21) and not in the subset of patients with inversion of chromosome 16 (Duployez et al., 2016). Cohesin mutations are observed more frequently in therapy-associated AML and secondary AML that has evolved from myelodysplastic syndromes. Approximately 15% of therapy associated-AML patients carry a cohesin mutation, with *RAD21* and *STAG2* mutations being the most frequent (Lindsley et al., 2015). Cohesin mutations may also play a major role in the evolution of transient myeloproliferative disorder (TMD) to acute megakaryoblastic leukemia (AMKL) in infants with Down syndrome (DS). TMD, which arises from a single GATA1 mutation in trisomy 21, may evolve into AMKL with the acquisition of subsequent mutations. In 53% of the cases, a cohesin mutation was present in the DS-AMKL clones, with *RAD21* and *STAG2* mutations being most frequent (22 and 18%, respectively) (Yoshida et al., 2013; Leeke et al., 2014; Solomon et al., 2014).

In addition to mutations in cohesin genes, the regulation of cohesin expression also plays a role in cancer. Methylation status of the *RAD21* gene in patients with chronic lymphocytic leukemia (CLL) provides evidence for a possible pathogenetic role of *RAD21* promoter methylation in the development of CLL, probably via self-renewal of CLL cells and not the formation of chromosomal abnormalities (Ioannidou et al., 2018).

7. Concluding statement

To conclude, RAD21, an important component of the cohesin complex, is an evolutionarily conserved protein. It is highly similar to the gene product of *S. pombe* rad21, a gene involved in the repair of DNA DSBs, as well as in chromatid cohesion during mitosis. In addition to playing roles in maintaining the chromatin architecture during the normal cell cycle and DNA DSB repair, RAD21 is also linked to an array of other functions, including apoptosis and hematopoiesis. Germline heterozygous or homozygous missense mutations in *RAD21* and other cohesin component genes have been associated with human genetic disorders and developmental abnormalities collectively termed as cohesinopathies (Krantz et al., 2004; Tonkin et al., 2004; Deardorff et al., 2007; Deardorff et al., 2012; Lehalle et al., 2017). Somatic mutations and amplification of the *RAD21* have also been widely reported in both human solid and hematopoietic tumors. As a subunit of cohesin complex that functions as a suppressor of tumorigenesis, deregulation of Rad21 in human tumors is not that unexpected. Targeting RAD21 and other cohesin component proteins is an underexplored area of drug development. The high frequency of cohesin mutations in multiple cancers and mutual exclusivity of cohesin component genes in any particular tumor suggest that specific targeting strategies such as synthetic lethal interactions could potentially be efficacious. Although *RAD21* is amplified up to 20% of human tumors, very little is known on the causes and consequences of *RAD21* overexpression in tumorigenesis, and inhibition of RAD21 has not yet been considered to target *RAD21* overexpressed tumors. Therefore, exploiting experimental strategies that correct dysfunctional *RAD21* and coupling them with current therapeutic strategies can provide novel, innovative, and more effective treatment regimens. In this regard, a study finding BET inhibitor, JQ1 as a potential RAD21 inhibitor in Kaposi's sarcoma cells is notable, and it would be interesting to explore the effect of JQ1 in RAD21 overexpressing tumors (Baltz et al., 2016; Carrà et al., 2017). However, inhibition

of RAD21 or any cohesin subunit proteins for therapy should be considered carefully in the context of the diverse physiological roles these molecules have in normal cell biology.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgements

This review and the corresponding Gene Wiki article are written as part of the Gene Wiki Review series–a series resulting from a collaboration between the journal GENE and the Gene Wiki Initiative. The Gene Wiki Initiative is supported by National Institutes of Health (GM089820). Additional support for Gene Wiki Reviews is provided by Elsevier, the publisher of GENE. This work was supported by the Cancer Prevention and Research Institute of Texas Grant # DP150064, and RP190002. We also thank Dr. B. Lee Ligon of the Department of Pediatrics, Baylor College of Medicine, for editorial assistance.

The corresponding Gene Wiki entry for this review can be found here: < https://en.wikipedia.org/wiki/RAD21 >

Abbreviations:

AMKL	Acute Megakaryoblastic Leukemia
AML	Acute Myeloid Leukemia
CBF-AML	Core Binding Factor Acute Myeloid Leukemia
CDLS	Cornelia de Lange Syndrome
ChIP-Seq	Chromatin Immunoprecipitation and Sequencing
CIIP	Chronic idiopathic Intestinal Pseudo-obstruction
CKO	Conditional KnockOut
CLL	Chronic Lymphocytic Leukemia
CRE	Conserved Regulatory Elements
CT	Carboxyl Terminus
DSB	Double Strand Break
HI-C	High-throughput Chromosome Conformation Capture
HR21	human *RAD21*
hRAD21	human RAD21
MCD1	Mitotic Chromosome Determinant 1
MDS	Myelodysplastic Syndrome
NBD	Nucleotide Binding Domain
NT	Amino Terminus

PCA	protein complement assay (PCA)
SA1	STAG1
SA2	STAG2
SCC1	Sister Chromatid Cohesion 1
TAD	Topologically Associating Domains
TCRA	T-Cell Receptor Alpha locus
TMD	Transient Myeloproliferative Disorder

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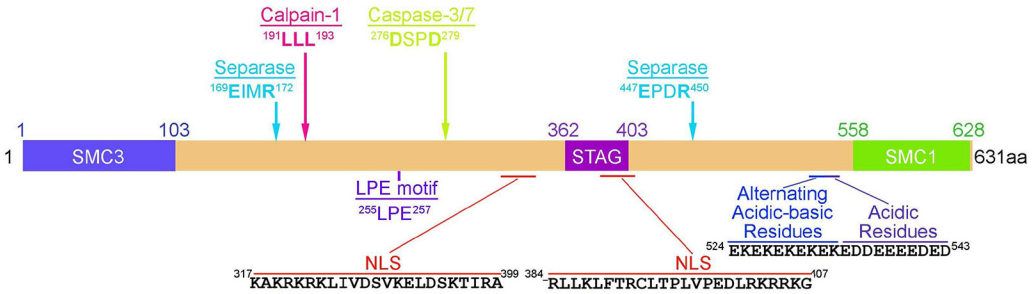

Fig. 1. Characteristics on human RAD21.

RAD21 has three binding domains that interact with corresponding protein: SMC3 (1–103aa), STAG1/2 (362–403aa) and SMC1A (558–628aa); a LPE motif (255–257aa): required for rapid and specific cleavage of RAD21 by Separase; two bipartite nuclear localization signals (NLS) (317–399aa and 384–407aa) predicted by cNLS Mapper (http://nls-mapper.iab.keio.ac.jp/); one alternating acidic-basic residues stretch (524–533aa); one acidic residues stretch (534–543aa); four cleavage sites: two Separase cleavage sites (ExxR), one Calpain-1 cleavage site (LLL) and one Caspase-3/7 site (DxxD). The numbers indicate the location of amino acid residue on human RAD21. The arrow shows the site where it is cleaved.

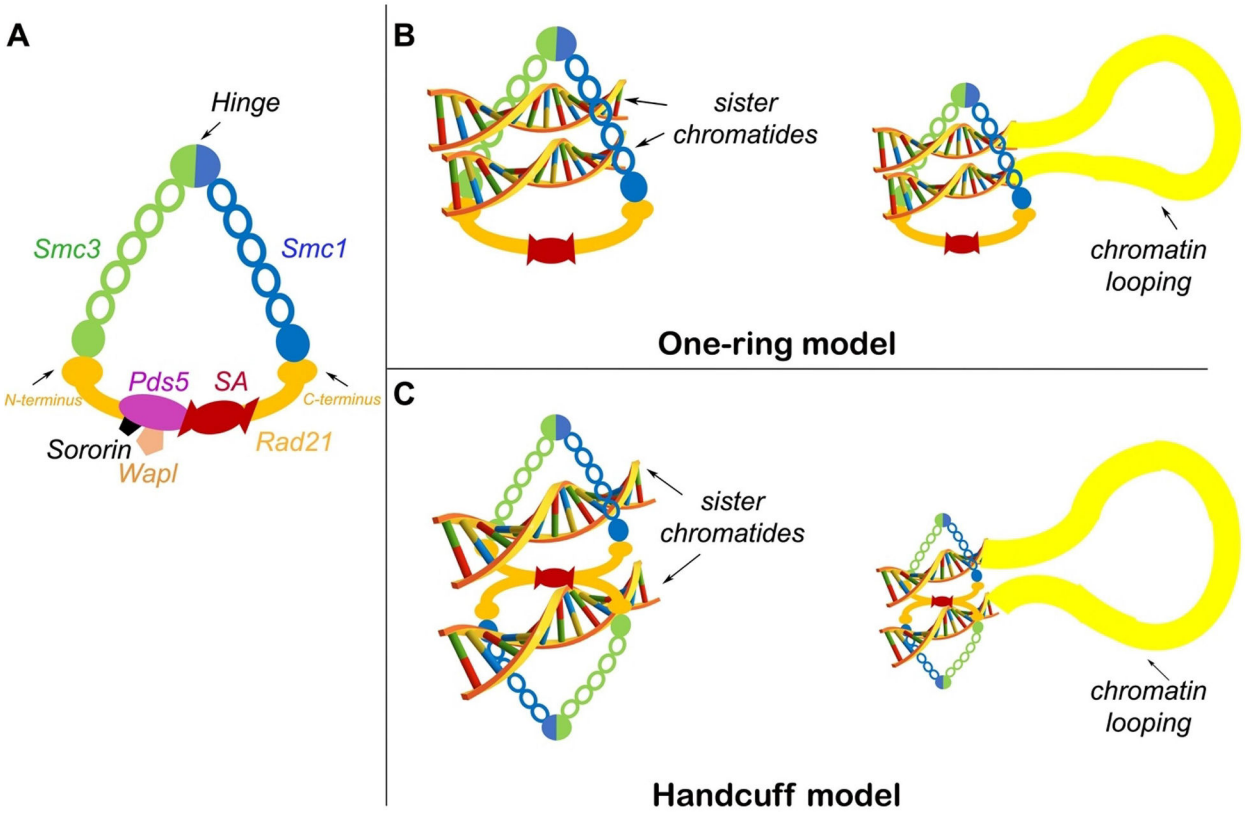

Fig. 2. Cohesin complex and models.

A. Cohesin is comprised of four core structural subunits: RAD21, SMC1, SMC3, and a SA protein (SA1 or SA2). PDS5, WAPL, and Sororin are cohesin-associate proteins. Sororin has not been found in yeast (Nishiyama et al., 2010; Zhang and Pati, 2012). B. One-ring model. C. Handcuff model. Figure adapted with modifications from Zhang and Pati (2014).

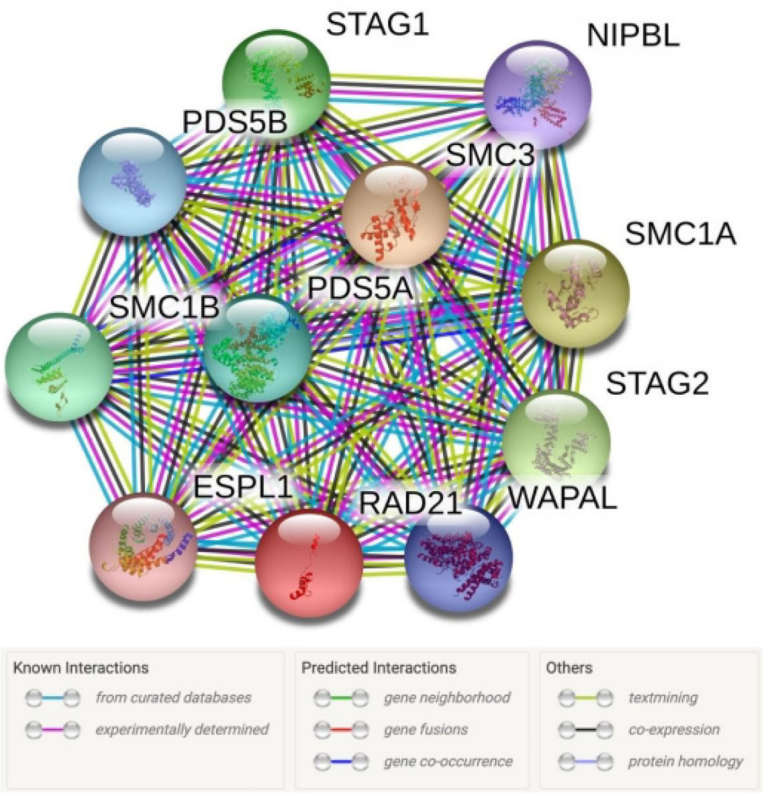

Fig. 3. Rad21 interaction with cohesin subunits.

Network nodes represent proteins with 3D structure known or predicted. Edges represent protein–protein associations. Figure adapted with modifications from String (https://string-db.org/).

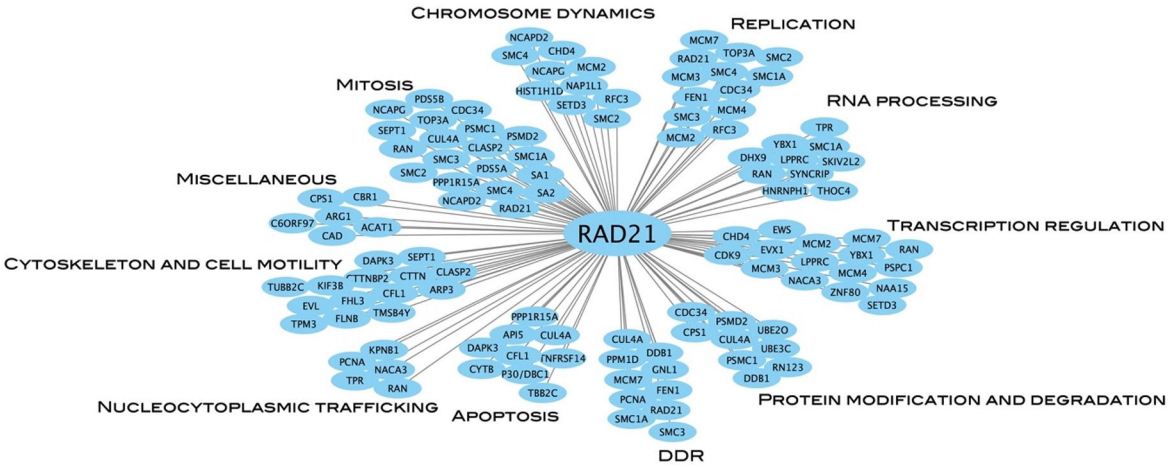

Fig. 4.

Functional classification of RAD21 interactors. Figure output by Cytoscape with the data retrieved from Panigrahi et al. (2012). Network nodes represent proteins. Edges represent protein–protein associations, clustered in different cellular processes.

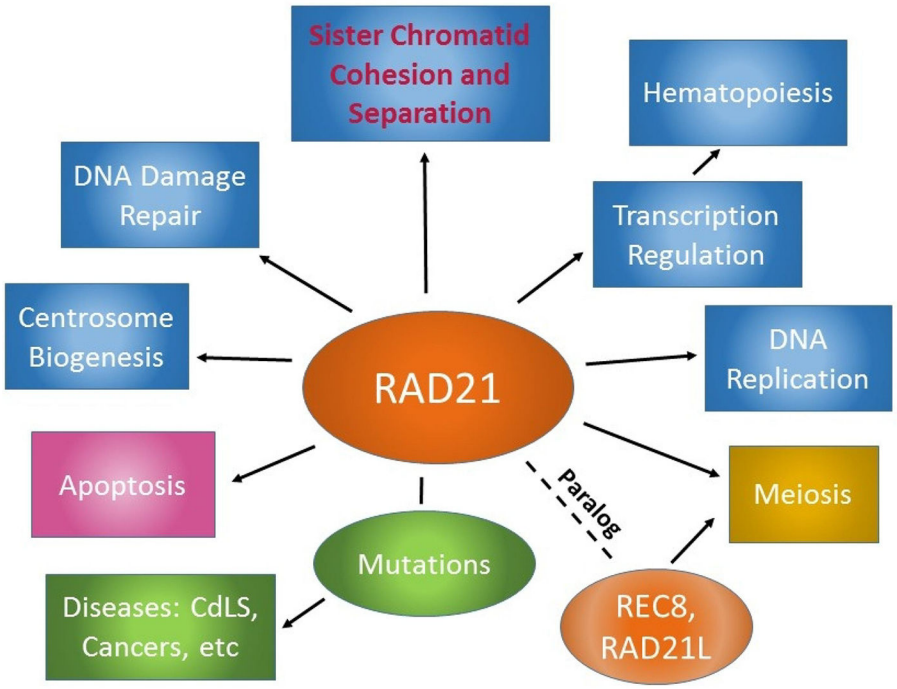

Fig. 5. RAD21 Functions in various cellular processes.

RAD21 forms cohesin complex with SMC1, SMC3 and STAG1/2 to function in various normal cellular processes (shown in blue). The canonical role of Rad21 is sister chromatid cohesion and separation. Other roles include DNA damage repair, transcription regulation, DNA replication, and centrosome biogenesis, etc. Diseases rise when mutations in RAD21 disrupt its function (in green). Caspase-cleaved Rad21 fragment promotes apoptosis (in purple). REC8 and RAD21L are paralogs of RAD21 in vertebrate, which function specifically in meiosis (in brown).