Molecular Mechanisms of the RECQ4 Pathogenic Mutations

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The human RECQ4 gene encodes an ATP-dependent DNA helicase that contains a conserved superfamily II helicase domain located at the center of the polypeptide. RECQ4 is one of the five RECQ homologs in human cells, and its helicase domain is flanked by the unique amino and carboxyl termini with sequences distinct from other members of the RECQ helicases. Since the identification of the RECQ4 gene in 1998, multiple RECQ4 mutations have been linked to the pathogenesis of three clinical diseases, which are Rothmund-Thomson syndrome, Baller-Gerold syndrome, and RAPADILINO. Patients with these diseases show various developmental abnormalities. In addition, a subset of RECQ4 mutations are associated with high cancer risks, especially for osteosarcoma and/or lymphoma at early ages. The discovery of clinically relevant RECQ4 mutations leads to intriguing questions: how is the RECQ4 helicase responsible for preventing multiple clinical syndromes? What are the mechanisms by which the RECQ4 disease mutations cause tissue abnormalities and drive cancer formation? Furthermore, RECQ4 is highly overexpressed in many cancer types, raising the question whether RECQ4 acts not only as a tumor suppressor but also an oncogene that can be a potential new therapeutic target. Defining the molecular dysfunctions of different RECQ4 disease mutations is imperative to improving our understanding of the complexity of RECQ4 clinical phenotypes and the dynamic roles of RECQ4 in cancer development and prevention. We will review recent progress in examining the molecular and biochemical properties of the different domains of the RECQ4 protein. We will shed light on how the dynamic roles of RECQ4 in human cells may contribute to the complexity of RECQ4 clinical phenotypes.

Keywords: RECQ helicase, cancer, aging, DNA replication, DNA repair, mitochondria

INTRODUCTION

The RECQ4 gene was first described in 1998 as one of the two last members of the RECQ helicase family identified based on their shared homology in the superfamily II (SFII) helicase domain (Kitao et al., 1998). Subsequently, mutations in the RECQ4 gene have been linked to the pathogenesis of three clinical diseases, which are Rothmund-Thomson syndrome (RTS), Baller-Gerold syndrome (BGS), and RAdial ray malformations, PAtellae hypo/aplasia and cleft or highly arched palate, DIarrhea and dislocated joints, Little size and limb malformation, NOse slender and normal intelligence (RAPADILINO) (Kitao et al., 1999a; Larizza et al., 2006; Siitonen et al., 2009). Premature aging, skeletal abnormalities, juvenile cataracts, skin hyperpigmentation and widened blood capillaries known as poikiloderma are the common clinical features associated with RTS. Immunodeficiency has also been reported in patients suffering RTS (De Somer et al., 2010; Smeets et al., 2014). RTS-associated abnormalities in bone development were recapitulated in the mouse models (Lu et al., 2015; Ng et al., 2015; Castillo-Tandazo et al., 2021).
| Mutation | Effect | Mutation location | Syndrome | Cancer type | References |
|----------|--------|------------------|----------|-------------|------------|
| c.84+6del16 | Missplicing | SLD2, MTS | RTS | — | Siitonen et al. (2009) |
| c.118 + 27del25 | Missplicing | SLD2, MTS | RTS | — | Broom et al. (2006) |
| c.119-1G > A | Missplicing | SLD2, MTS | RTS | — | Zhang et al. (2021) |
| c.160_161insGGGCC | p.Gly54X | SLD2 | RTS | — | Wang et al. (2018) |
| c.161A > G | p.Glu71Gly | SLD2, RTS | — | — | Suter et al. (2016) |
| c.161G > A | p.Glu231Ser | N-terminus | RTS | — | Suter et al. (2016) |
| c.164 > A | p.Trp269X | SLD2 | RTS | — | Sakuta et al. (2020) |
| c.358G > A | p.Gly120Arg | SLD2 | — | — | Zeng et al. (2021) |
| c.496C > T | p.Gln166X | BGS | — | — | Siitonen et al. (2009) |
| c.558_564dup | p.Gly189fsX | N-terminus | RTS | — | Suter et al. (2016) |
| c.691G > A | p.Gly231Ser | N-terminus | RTS | — | Gui et al. (2018) |
| c.806G > A | p.Trp269X | N-terminus | RTS | — | Siitonen et al. (2003) |
| c.853_854del | p.Pro285X | N-terminus | — | — | Sakuta et al. (2020) |
| c.866C > G | p.Ala289Gly | N-terminus | — | — | Sakuta et al. (2020) |
| c.910C > T | p.Gln304X | N-terminus | RTS | — | Wang et al. (2018) |
| c.978_979delTCinsG | p.Ser326fsX | N-terminus | RTS | — | Yadav et al. (2019) |
| c.1048_1049delAG | p.Arg350fsX | N-terminus | RTS | — | Wang et al. (2003), Suter et al. (2016), van Rij et al. (2017), Colombo et al. (2018) |
| c.1078C > T | p.Gln360X | N-terminus | RTS | — | Suter et al. (2016) |
| c.1132–2A > G | missplicing | N-terminus | RTS | — | Yadav et al. (2019) |
| c.1222C > T | p.Glu408X | ZnK | RTS | — | Suter et al. (2016) |
| c.1391–1G > A | Missplicing | N-terminus | RTS | — | Siitonen et al. (2003) |
| c.1397C > T | p.Pro466Leu | SF2 | RTS | — | Lindor et al. (2000), van Rij et al. (2017) |
| c.1483 + 25del11 | p.Trp269X | SF2 | RTS | — | Wang et al. (2003) |
| c.1568G > C_1573delT | Missplicing | SF2 | RTS | — | Colombo et al. (2018) |
| c.1573delG | p.Ser523fsX | ZnK | RTS | — | Cabral et al. (2008) |
| c.1649C > G | p.Ala550Gly | SF2 | RTS | — | Zeng et al. (2021) |
| c.1697T > C | p.Leu666Pro | SF2 | RTS | — | Wang et al. (2003) |
| c.1704 + 1G > A | Missplicing | SF2 | RTS | — | Wang et al. (2003) |
| c.1705–1G > A | Missplicing | SF2 | RTS | — | Sznajer et al. (2008) |
| c.1718delA | p.Gln573fsX | SF2 | RTS | — | Wang et al. (2003) |
| c.1724_1725delAC | p.His575fsX | SF2 | RTS | — | Wang et al. (2003) |
| c.1763delG | p.Gly588fsX | SF2 | RTS | — | Wang et al. (2003) |
| c.1770–1807del | p.Pro591fsX | SF2 | RTS | — | Wang et al. (2003) |
| c.1875_5 > A | Missplicing | SF2 | RTS | — | Wang et al. (2003) |
| c.1879 + 32_1879–27del24 | Missplicing | SF2 | RTS | — | Wang et al. (2003) |
| c.1878 + 3,1878 + 55del | Missplicing | SF2 | RTS | — | Wang et al. (2003) |
| c.1885del4 | p.Arg629fsX | SF2 | RAPADILINO | — | Suter et al. (2016) |
| c.1887del4 | p.Glu630fsX | SF2 | RAPADILINO | — | Wang et al. (2003) |
| c.1892G > T | p.Arg631Leu | SF2 | RTS | — | Wang et al. (2003) |
| c.1910T > C | p.Phe637Ser | SF2 | RAPADILINO | — | Wang et al. (2003) |
| c.1913T > C | p.Leu638Pro | SF2 | RTS | — | Wang et al. (2003) |
| c.1919_1924delTCACAG | p.Leu640_Ala642delinsP | SF2 | RAPADILINO | — | Wang et al. (2003) |
| c.1920_1925dup | p.Ala644_Thr645 dup | SF2 | RAPADILINO | — | Wang et al. (2003) |
| c.2059-1G > C | missplicing | SF2 | BGS | — | Cao et al. (2015) |

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TABLE 1 | (Continued) RECQ4 mutations with clinical implications. (del) deletion; (>.) nucleotide change from; (X) premature stop codon; (dup) duplication; (fs) frameshift; (ins) insertion.

| Mutation      | Effect | Mutation location | Syndrome | Cancer type                                                                 | References |
|---------------|--------|------------------|----------|----------------------------------------------------------------------------|------------|
| c.2085delA    |       | p.Leu695fsX      | SF2      | RTS                                                                       | Suter et al. (2016) |
| c.2091T > G   |       | p.Phe697Leu      | SF2      | RTS                                                                       | Siitonen et al. (2009) |
| c.2141_2142delAG |     | p.Glu714fsX      | SF2      | BGS                                                                       | Cao et al. (2015) |
| c.2149G > T   |       | p.Lys738fsX      | SF2      | —                                                                         | Esophageal squamous cell cancer Zeng et al. (2021) |
| c.2207ins1    |       | p.Gln757X        | SF2      | RTS                                                                       | Wang et al. (2003) |
| c.2269C > T   |       | p.Arg766GlyfsX   | SF2      | Ampullary carcinoma, lung adenocarcinoma, T cell lymphoma, chondroblastic osteosarcoma, renal cell carcinoma, neuroblastoma, hepatoblastoma, B-lymphoblastic leukemia/lymphoma, acute myeloid leukemia, optic nerve glioma Zeng et al. (2021) |
| c.2335del22   |       | p.Asn779fsX      | SF2      | BGS                                                                       | Sin et al. (2009) |
| c.2398C > T   |       | p.Gln800X        | SF2      | RTS                                                                       | Wang et al. (2003) |
| c.2419insS    |       | p.Glu807fsX      | SF2      | RTS                                                                       | Wang et al. (2003) |
| c.2421dupT    |       | p.Asp808fsX      | SF2      | Lynphoma                                                                  | Gu et al. (2018) |
| c.2428C > T   |       | p.Gln810Cys      | SF2      | —                                                                         | Wang et al. (2003) |
| c.2461C > T   |       | p.Gln821X        | C-terminus RTS | —                                                | Wang et al. (2003), Siitonen et al. (2009) |
| c.2476C > T   |       | p.Arg826X        | C-terminus RTS | —                                                | Wang et al. (2003), Siitonen et al. (2009) |
| c.2492_2493delAT |     | p.His831fsX      | C-terminus RTS | BGS | Osteosarcoma, lymphoma | Debeljak et al. (2009), Colombo et al. (2014), Gutierrez-Jimeno et al. (2020) |
| c.2506_2518del13 |     | p.Trp836fsX      | R4ZBD | BGS | Lymphoma | Debeljak et al. (2009) |
| c.2547–2548delGT |    | p.Phe850fsX      | R4ZBD | RTS | Osteosarcoma | Wang et al. (2003) |
| c.2552delC    |       | p.Arg851fsX      | R4ZBD | RTS | — | Suter et al. (2016) |
| c.2569_2574del |     | p.Cys857_T858dup | R4ZBD | R4ZBD | Prostate cancer | Colombo et al. (2018) |
| c.2572G > T   |       | p.Glu918X        | R4ZBD | RTS | — | Wang et al. (2003) |
| c.2576_2577delTT |    | p.Leu923fsX      | R4ZBD | RTS, R4ZBD | — | Wang et al. (2003), Siitonen et al. (2009) |
| c.2707T > G   |       | p.Leu927Arg      | R4ZBD | RTS | — | Cabr et al. (2008) |
| c.2788_2812del |     | p.His930_Leu937del | R4ZBD | RTS | — | Suter et al. (2016) |
| c.2868-1G > A |       | Missplicing      | R4ZBD | RTS | — | Zhang et al. (2021) |
| c.2930G > T   |       | Missplicing      | R4ZBD | RTS | — | Broom et al. (2006) |
| c.3014delG    |       | p.Arg1005fsX     | R4ZBD | BGS | Metachronous gastric cancer | Sakuta et al. (2020) |
| c.3014_3015AG |     | p.Arg1005Gln     | R4ZBD | — | Metachronous gastric cancer | Sakuta et al. (2020) |
| c.3016delIG   |       | p.Ala1006fsX     | R4ZBD | RTS | — | Sakuta et al. (2020) |
| c.3021_3022del |     | p.Cys1000fsX     | R4ZBD | R4ZBD | — | Colombo et al. (2018) |
| c.3056-2A > C |       | Missplicing      | R4ZBD | BGS | — | Van Maldergem et al. (2006) |
| c.3061C > T   |       | p.Arg1021Trp     | R4ZBD | RTS, BGS, R4ZBD | — | Fradin et al. (2013), Wang et al. (2018) |
| c.3062G > A   |       | p.Arg1021Gln     | R4ZBD | RTS | — | Wang et al. (2018) |
| c.3072_3073delAG |     | p.Val1026fsX6    | R4ZBD | RTS | Osteosarcoma | Wang et al. (2003) |
| c.3072delA    |       | p.Val1026fsX18   | R4ZBD | R4ZBD | R4ZBD | Wang et al. (2003) |
| c.3124_3127ACC |     | Missplicing      | R4ZBD | R4ZBD | — | Wang et al. (2003) |
| c.3151A > G   |       | p.Leu1065Val     | C-terminus BGS | — | B-lymphoblastic leukemia | Siitonen et al. (2009) |
| c.3214A > T   |       | p.Arg1072X       | C-terminus RTS | — | — | Siitonen et al. (2009) |
| c.3236G > T   |       | p.Ser1079le      | C-terminus RTS | — | — | Siitonen et al. (2009) |
| c.3270delG    |       | p.Glu1090fsX     | C-terminus RTS | — | — | Siitonen et al. (2009) |
| c.3271C > T   |       | p.Gln1091X       | C-terminus R4ZBD | — | — | Siitonen et al. (2009) |
| c.3276delG    |       | p.Asp1093fsX     | C-terminus RTS | R4ZBD | Osteosarcoma | Wang et al. (2003) |

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Some of the clinical features observed in the RTS patients are also common in the BGS patients (Mégarbané et al., 2000). Nonetheless, RTS and BGS remain as two separate syndromes, since BGS also displays craniosynostosis during embryonic development, which is not shared with RTS. Similarly, RAPADILINO patients also suffer clinical features partially overlapping with the RTS patients. Through these clinical overlaps, scientists were able to link mutations in the RECQ4 gene to RAPADILINO (Siitonen et al., 2003).

To date, over 100 of clinically relevant mutations have been identified throughout the RECQ4 gene (Table 1). In addition to developmental abnormalities and premature aging, cancer predisposition is characteristic of the human diseases linked to several RECQ4 mutations (Liu, 2010). Defining the functions and regulation of human RECQ4 is critical for advancing our knowledge of the fundamental biology of development, aging, and cancer. This review will provide an overview on the efforts made during the past 2 decades to dissect the biochemistry, cellular functions, and regulations of the RECQ4 protein. We will discuss how the biochemical and cellular properties of RECQ4 may be affected by the clinical mutations.

**TABLE 1 |** (Continued) RECQ4 mutations with clinical implications. (del) deletion; (> ) nucleotide change from; (X) premature stop codon; (dup) duplication; (fs) frameshift; (ins) insertion.

| Mutation         | Effect          | Mutation location | Syndrome | Cancer type       | References          |
|------------------|-----------------|-------------------|----------|-------------------|---------------------|
| c.3330dupA       | p.Glu1111fsX    | Acidic patch      | RTS      | —                 | Suter et al. (2016) |
| c.3409G > A      | p.Asp1137Asn    | C-terminus        | —        | Metachronous gastric cancer | Sakuta et al. (2020) |
| c.3501-3502delCG | Missplicing     | C-terminus        | RTS      | —                 | Wang et al. (2003)  |
| c.3532C > T      | p.Gln1175X      | NES               | RTS      | —                 | Wang et al. (2003)  |
| c.3552dupG       | p.Arg1185fsX    | NES               | RTS      | —                 | Zhang et al. (2021) |
| c.3573C > G      | p.Ser1191Arg    | C-terminus        | RTS      | —                 | Suter et al. (2016) |
| c.35999_3600delCG| p.Thr1200fsX    | C-terminus        | RAPADILINO | — | Siitonen et al. (2009) |

**FIGURE 1 |** Schematic of the human RECQ4 protein domains, including the SLD2 (yellow) and conserved SF2 helicase domains (green). NTS: nuclear targeting signal. MTS: mitochondrial targeting signal. ZnK: zinc knuckle. MTE: mitochondrial exclusion; NES: nuclear export signal. R4ZBD: RECQ4 zinc binding domain. (**Top**) Protein-protein interactions domains. (**Middle**) RECQ4 cellular functions and the domains involved. (**Bottom**) The disease-associated mutations that have been implicated in the RECQ4 cellular functions, as indicated with red circles in the middle.

**RECQ4 IN DNA REPLICATION INITIATION**

Human RECQ4 is a 1,208 amino-acid (a.a.) long protein. In addition to the SFI helicase domain between residues 470 and 820 located at the center of the polypeptide (Figure 1), RECQ4 also contains unique amino (N) and carboxyl (C) termini that are
not shared by other members of the RECQ family in their sequences (Kitao et al., 1998; Liu, 2010; Ellis et al., 1995; Yu et al., 1996). Two mouse models highlight the essential role of the RECQ4 N-terminus in embryonic development. The first attempt to generate viable RECQ4 knockout mice was not successful, as the mice died at the embryonic stage between day 3.5 and 6.5 (Ickikawa et al., 2002). However, mice expressing intact N-terminal fragment survived (Mann et al., 2005). Similarly, chicken DT40 cells lacking full-length RECQ4 underwent apoptosis but were rescued by expressing the N-terminal fragment containing the first 496 residues (Abe et al., 2011). The molecular function of the RECQ4 N-terminus was uncovered when a yeast Sld2-like domain was identified within the first 200 a. a. of the RECQ4 protein (Figure 1) (Sangrithi et al., 2005; Matsuno et al., 2006). Consistent with the critical role of yeast Sld2 in DNA replication, it was first demonstrated that *xenopus* RECQ4 also functions in DNA replication initiation (Sangrithi et al., 2005; Matsuno et al., 2006). The involvement of RECQ4 in DNA replication initiation is also conserved in human cells (Im et al., 2009; Xu et al., 2009; Thangavel et al., 2010). The N-terminus of RECQ4 alone is sufficient in recruiting essential replication factors to the origin of replication to initiate DNA synthesis (Shin et al., 2019). Through the SLD2 domain, human RECQ4 forms cell cycle-dependent, chromatin-bound protein complexes containing core replisome factors MCM10, MCM2-7 helicase, CDC45 and GINS at replication origins (Xu et al., 2009). Additional studies revealed that the activation of MCM2-7 replicative helicase activity through the formation of a stable CDC45-MCM2-7-GINS (CMG) complex requires RECQ4 and MCM10 (Im et al., 2009). Furthermore, the formation and retention of the RECQ4-CMG complex on chromatin is restricted within the S-phase of the cell cycle by checkpoint protein TIMELESS (Xu et al., 2016). Given the important role of RECQ4 in DNA replication initiation, it is perhaps not a surprise that the association of RECQ4 and MCM10 with replication origins is subjected to negative regulation by the DNA damage checkpoint control to suppress S-phase entry in response to DNA damage (Im et al., 2015).

The dependency of cell growth on the RECQ4 SLD2 domain is expected to impose a selection pressure against mutations within this domain in the human population, as changes that abolish RECQ4 function in DNA replication initiation may be lethal. Replication defect may also explain why mutations in the RECQ4 N-terminus trigger replicative senescence (Lu et al., 2014). Moreover, additional functions of the N-terminal RECQ4 may be required for cell survival. For example, the N-terminus of RECQ4 is required for proper chromosome segregation to prevent G2/M cell cycle arrest and mitotic cell death (Fang et al., 2018). RECQ4 may promote proper chromosome segregation by stabilizing Aurora B Kinase important for mitotic spindle assembly through a direct protein-protein interaction or via its association with the microtubules to ensure correct chromosome alignment (Yokoyama et al., 2019). Nonetheless, in chicken DT40 cells, even though RECQ4-MCM10 interaction is important for efficient replication origin firing, defects in this interaction do not lead to cell death (Kliszczak et al., 2015). In addition, a significant number of the disease associated mutations have been found within the RECQ4 SLD2 motif (Table 1). It is possible that these mutations do not impair DNA replication or chromosome segregation. Alternatively, additional pathway(s) to mediate DNA replication initiation or chromosome segregation exists in chicken DT40 cells or is activated in patient cells to support cell viability.

**RECQ4 IN MITOCHONDRIAL BIOGENESIS**

RECQ4 not only localizes in the nucleus to initiate DNA replication but is also present in the cytoplasm (Ding and Liu, 2015; Croteau et al., 2012a; Wang et al., 2014; De et al., 2012). RECQ4 contains two nuclear targeting signals (NTS; Figure 1), one of which overlaps with residues missing in the highly cancer prone RECQ4 del(Ala420-Ala463) mutant protein associated with RAPADILINO and is important in negatively regulating RECQ4 cytoplasmic localization (Burks et al., 2007). As a consequence, the cancer mutant protein missing residues between Ala420 and Ala463 is cytoplasmic (Burks et al., 2007). Several lysine residues located immediately upstream of Ala420-Ala463 residues are modified by the p300 acetyltransferase, and their acetylation also promotes cytoplasmic localization (Dietschy et al., 2009). Further studies revealed that the increasing amount of the cytoplasmic del(Ala420-Ala463) mutant proteins is partly due to their accumulation in the mitochondria (Wang et al., 2014). Specifically, the missing residues in the del(Ala420-Ala463) mutant are involved in binding to hyaluronan binding protein HABP1/p32, which acts as a negative regulator of RECQ4 mitochondrial localization (Wang et al., 2014). Hence, the residues between Ala420 and Ala463 also serve as a mitochondrial exclusion (MTE) signal. Interestingly, while missing NTS/MTE enhances RECQ4 mitochondrial localization (Wang et al., 2014), deletion of two nuclear export signals (NES; Figure 1) found near the C-terminal end of the protein reduces mitochondrial localization (Chi et al., 2012). In addition, the first 84 residues of the RECQ4 protein contain a mitochondrial targeting signal (MTS; Figure 1) that also plays a positive role in targeting RECQ4 mitochondrial localization (De et al., 2012). It is clear that RECQ4 contains multiple regulatory motifs to balance the levels of RECQ4 in the nucleus and mitochondria.

Studies using RECQ4 disease mutations reveal a role of RECQ4 in mitochondrial biogenesis. For example, RECQ4 is involved in mitochondrial DNA (mtDNA) synthesis and maintenance, and abnormal mtDNA levels were observed in the RECQ4 mutant cells (Croteau et al., 2012a; De et al., 2012; Wang et al., 2014; Chang et al., 2020). The changes in mtDNA copy numbers and their contents correlate with the mitochondrial dysfunction phenotypes in human cells (Croteau et al., 2012a; De et al., 2012; Wang et al., 2014; Chang et al., 2020). RECQ4 interacts with mitochondrial replication factors, including TWINKLE/PEO1, TFAM and DNA polymerase γ (Croteau et al., 2012a; Wang et al., 2014). The interaction of RECQ4 with TWINKLE is enhanced by the
RECQ4 IN DNA REPAIR

In addition to participating in DNA synthesis in both the nucleus and the mitochondria, studies also reported that RECQ4 participates in DNA damage response and repair. For example, RECQ4 is recruited to DNA damage sites, and this re-localization requires residues between 363 and 492 (Singh et al., 2010). RECQ4 has been implicated in non-homologous end joining via its N-terminal interaction with Ku70 during G1 phase (Figure 1) (Lu et al., 2017; Shamaanna et al., 2014). During S/G2 phase, CDK-mediated phosphorylation of the RECQ4 N-terminus at the S89 and S251 residues and DDB1-CUL4A mediated ubiquitination after ionizing radiation switch RECQ4 interaction in favor of MRE11 for homologous recombination (Lu et al., 2017). RECQ4 N-terminus also binds to CtIP to recruit MRE11-RAD50-NBS1 complex to the DNA break site to facilitate homologous recombination (Figure 1) (Lu et al., 2016). Residues between 363 and 492 important for the RECQ4 recruitment to DNA damage sites also interact with Bloom’s syndrome helicase BLM (Figure 1), a RECQ homolog, in a DNA damage-dependent manner (Singh et al., 2012). BLM is not required for the recruitment but for the retention of RECQ4 at the DNA damage site (Singh et al., 2010; Singh et al., 2012). RECQ4 interaction also stimulates BLM helicase activity in vitro (Singh et al., 2012). However, the extent to which the two helicases function in the same repair pathway remain to be determined, as different sensitivities to DNA damaging agents have been observed between pre-B lymphocyte cells depleted with RECQ4 and BLM (Kohzaki et al., 2012). Both BLM-deficient mice and RECQ4 conditional mutant mice exhibit bone marrow failure, but the hematopoietic defects can be rescued by p53 deletion only in the BLM-deficient mice but not in RECQ4-deficient mice (Smeets et al., 2014). Given that RECQL4 depletion has additive effects on proliferation and sister chromatid exchange, these two RECQ proteins may function in non-overlapping pathways in DNA damage response and repair (Singh et al., 2012). Analysis of the point mutations in either the BLM or RECQ4 genes that specifically abolishes the interaction between these two proteins may provide insights into the function of this interaction.

In addition to the N-terminus, the conserved SF2 and the C-terminal domains are also central to the regulation of DNA damage response and repair. Cells isolated from a RTS patient compound heterozygous of RECQ4 mutations missing the SF2 and the C-terminal of RECQ4 showed defects in ATM activation after DNA damage (Park et al., 2019). In addition, RECQ4 depleted cells complemented with only the N-terminal fragment were viable, but these cells exhibited increased sensitivity to DNA damaging agents (Abe et al., 2011; Kohzaki et al., 2012). Even though the residues required for RECQ4 recruitment to DNA damage sites have been identified (Singh et al., 2010), it is yet to be determined if these residues are involved in direct DNA damage recognition or RECQ4 re-localization to the damage site via protein-protein interaction(s). Once bound to the DNA damage, RECQ4 requires RNF8-dependent ubiquitination at its C-terminus to dissociate from the site of DNA damage, allowing downstream DNA repair factors to bind to the damage site (Tan et al., 2021). While studies show that RECQ4 positively regulates homologous recombination and non-homologous end joining, RECQ4 suppresses the repair of DNA breaks via RAD52-mediated single-strand annealing pathway via its SF2 and/or the C-terminal domains (Kohzaki et al., 2020). Upon oxidative stress, a fraction of the RECQ4 proteins re-localize to the nucleolus, and the nucleolar localization is dependent on PARP1, which poly(ADP-ribosyl)ates the C-terminus of RECQ4 in vitro (Woo et al., 2006). RECQ4 may also participate in base excision repair in response to oxidative stress (Schorum et al., 2009).

RECQ4 BIOCHEMICAL PROPERTIES

*In vitro,* the N-terminus of RECQ4 binds to various DNA substrates, including double-stranded, single-stranded and splayed-arm DNA (Xu and Liu, 2009; Keller et al., 2014). Multiple regions within the N-terminal fragment participate in nucleic acid binding. For example, the first 54 residues of the RECQ4 protein exhibit affinity toward DNA (Ohlenschläger et al., 2012). RECQ4 also contains a conserved zinc knuckle motif between residues 397 and 421 that preferentially binds to RNA over DNA (Marino et al., 2016). Structural analysis located a second RECQ4 zinc-binding domain (R4ZBD; Figure 1) at the
The C-terminus of RECQ4 between residues 836 and 1,044, but R4ZBD does not play a significant role in DNA binding or ATPase activities (Kaiser et al., 2017). The nucleic acid binding by the N-terminal fragment of RECQ4 contributes to its strand annealing and strand exchange activities (Xu and Liu, 2009; Keller et al., 2014), providing an explanation for why pathogenic mutations within the RECQ4 N-terminus affect its annealing and strand exchange activities (Chang et al., 2020). RECQ4 N-terminus also shows high affinities toward unusual DNA structures, such as guanine quadruplex and RNA:DNA hybrids (Keller et al., 2014; Chang et al., 2020), supporting additional roles of RECQ4 N-terminus in nucleic acid metabolism, which is altered by the pathogenic RECQ4 Pro466Leu mutation. Specifically, Pro466Leu mutation increases RECQ4 affinities to both DNA and RNA in vitro and in mitochondria, and this enhanced affinity correlates with the elevated strand annealing activity between DNA and RNA (Chang et al., 2020). As a consequence, Pro466Leu mutant cells accumulate RNA:DNA hybrids on the mtDNA that block the completion of mtDNA synthesis (Chang et al., 2020). These enhanced activities were not observed in cells expressing the del(Ala420-Ala463) mutant protein, providing an explanation for why cells expressing Pro466Leu RECQ4 mutant do not contain high levels of mtDNA compared to those expressing the del(Ala420-Ala463) mutant (Croteau et al., 2012b; Wang et al., 2014; Chang et al., 2020). It would be a great interest to determine if the enhanced nucleic acid affinity by the Pro466Leu mutation may retain RECQ4 at DNA damage sites, hindering the binding and access of downstream DNA repair factors (Tan et al., 2021). Nonetheless, another report showed that Pro466Leu, Phe637Ser and Phe697Leu disease mutations decrease RECQ4 DNA binding (Jensen et al., 2012).

In addition to strand annealing and strand exchange, both of which are intrinsic activities of the RECQ4 N-terminal fragment, the conserved SF2 domain contain active ATPase and helicase activities (Rossi et al., 2016; Suzuki et al., 2009). Surprisingly, the conserved SF2 domain contain active ATPase and helicase activities which are intrinsic activities of the RECQ4 N-terminal fragment, including aneuploidy and increasing cancer incidents and bone marrow failure, suggesting that the RECQ4 helicase activity is required for disease prevention (Mann et al., 2005; Castillo-Tandazo et al., 2019). However, in contrast to the observations reported using cell culture and in mice carrying the SF2 truncation mutation mentioned above, mice with only a Walker A point mutation to abolish the ATPase and helicase activities went through normal development and showed no defects in hematopoiesis or increasing sensitivity to DNA damage agents (Castillo-Tandazo et al., 2019). Most likely, other biochemical properties of the RECQ4 SF2 domain independent of the helicase activity are important for disease prevention and normal development.

RECQ4 is a highly interactive protein in cells, and protein-protein interactions impact RECQ4 catalytic activities. For example, ribosomal protein S3 binds to the first 320 amino acids of RECQ4 and this interaction is enhanced by oxidative stress (Patil and Hsieh, 2017). However, this interaction inhibits RECQ4 DNA binding and helicase activities (Patil and Hsieh, 2017). Similarly, MCM10 interaction with RECQ4 promotes DNA replication initiation, but the association with MCM10 also suppresses RECQ4 strand exchange activity in vitro (Xu et al., 2009). Interestingly, RECQ4 N-terminus promotes mitochondrial DNA polymerase γ binding to mtDNA (Gupta et al., 2014), but the interaction with DNA polymerase γ negatively regulates RECQ4 helicase activity (Croteau et al., 2012a). The functional significance of these inhibitions on RECQ4 catalytic activities via protein-protein interactions remains unclear.

**DISCUSSION**

Extensive literature provides insights into the molecular functions of RECQ4 in DNA replication, mitochondrial biogenesis, and DNA damage response and repair. These findings are critical to our understanding of the etiology of the RECQ4 disease mutations. The complex functions of RECQ4 emphasize the importance of not treating all RECQ4 patient cell lines the same. For an example, both del(Ala420-Ala463) and Pro466Leu mutations are associated with RAPADILINO. However, while del(Ala420-Ala463) mutation is associated with high lymphoma incidents, which occurred in approximately 40% of the individuals homozygous or compound heterozygous for the mutation, the adjacent Pro466Leu clinical mutation has not been linked to cancer risk (Siitonen et al., 2003; Siitonen et al., 2009). Analysis of the mutant cell lines showed that mutations in this region impact RECQ4 mitochondrial localizations in both mutant cells, but the mutations affect mtDNA synthesis efficiency differently (Chang et al., 2020). Changes in the mtDNA copy numbers have been linked to lymphomagenesis (Kopinski et al., 2021). It remains to be determined if the difference in mtDNA synthesis efficiencies contributes to high incidents of lymphoma in patients carrying del(Ala420-Ala463) mutation, but not in those with Pro466Leu mutation. Alternatively, defects in DNA repair factors contribute to lymphoma (Knittel et al., 2018), and it is possible...
that the failure in recruiting RECQ4 to DNA damage sites and facilitating DNA damage repair due to the del (Ala420_Ala463) mutation contributes to lymphomagenesis (Singh et al., 2010; Singh et al., 2012). If so, it is necessary to demonstrate the Pro466Leu mutant cells are proficient in RECQ4 recruitment to DNA damage sites.

In addition to the del (Ala420_Ala463) mutation at the N-terminus of RECQ4, high cancer risks have also been reported in patients carrying the C-terminal truncation mutation Q757X (Siitonen et al., 2009), highlighting the importance of multiple RECQ4 domains in cancer prevention. Interestingly, a splicing mutation that produces a C-terminal truncated RECQ4 protein (R766X) similar to that of Q757X mutation has been reported as a recurrent hotspot in the tumor registry and considered oncoenic (cBioPortal cBioPortal. Av). Expression of the Q757X mutation also reprograms fibroblast to induced pluripotent stem cells (iPSCs) that can undergo cellular differentiation (Gatinois et al., 2020). Since increasing DNA replication competency contributes to iPSC reprogramming (Mouery et al., 2020), it is reasonable to speculate that the Q757X mutation promotes iPSC reprogramming through a change in DNA synthesis efficiency. While erroneous DNA replication due to loss-of-function mutations in factors important for this process drives genomic instability and cellular transformation, increasing replication rate as a consequence of gain-of-function mutations or up-regulation of replication factors may also contribute to genomic instability and tumorigenesis via enhanced cell proliferation and replication stress (Wang et al., 2018). It would be of great interest to determine if RECQ4 mutations such as Q757X/R766X play an oncogenic role as gain-of-function mutations in accelerating DNA synthesis to enhance cell growth and increase replication stress.

RECQ4 is overexpressed in multiple cancers, including pancreatic cancer, melanoma, prostate and ovarian cancers, and its expression is directly proportional to tumor grades (cBioPortal cBioPortal. Av; Su et al., 2010; Maire et al., 2009). Most likely, the high expression of RECQ4 is needed to support rapid cancer cell growth, further supporting a potential oncogenic role of RECQ4. Because of its high expression in cancerous cells, RECQ4 may be considered as a potential target for cancer therapy similar to other DNA replication and repair factors (Guha, 2011; Seo and Kang, 2018). Indeed, transient down regulation of RECQ4 blocks cell growth and induces PARP1-dependent apoptosis in metastatic prostate cancer cells (Su et al., 2010). In breast cancers, RECQ4 suppression not only impairs DNA synthesis but also increases cellular sensitivity to chemotherapeutic drugs possibly through reduced efficiency in DNA damage response (Arora et al., 2016). In gastric cancer cells, ectopic expression of RECQ4 also correlates with increasing resistance to DNA damage agents (Mo et al., 2016), further suggesting the potential of inhibiting RECQ4 to sensitize cells to the existing chemotherapies. Interestingly, chemoresistance due to RECQ4 overexpression may also be a consequence of deregulated transcriptional regulations. Specifically, RECQ4 was found to interact with the transcriptional factor YB1 to promote AKT-mediated phosphorylation of YB1 and YB1-dependent gene expressions including the multidrug resistance gene MDR1 (Mo et al., 2016). In addition, since the SF2 domain and the C-terminus of RECQ4 are involved in suppressing RAD52-mediated single-stranded annealing for repairing DNA breaks, cells lacking these regions of the RECQ4 protein increase sensitivity to DNA damaging agents in the presence of RAD52 inhibitors (Kohzaki et al., 2020). Therefore, identification of pharmacological inhibitors against the multifaceted roles of RECQ4 in supporting cancer cell growth and chemoresistance may provide new therapeutic strategies.

Finally, in addition to RECQ4, mutations in ANAPC1, a subunit of the anaphase promoting complex/cyclosome (APC/C), were recently identified as the second genetic risk factor for RTS (Ajewung et al., 2019). APC/C is an ubiquitin E3 ligase with crucial roles in regulating cell cycle progression for DNA synthesis and chromosome segregation (Barford, 2020). More research is needed to examine the potential functional interactions between RECQ4 and APC/C in DNA synthesis and chromosome segregation in normal development and RTS prevention.

AUTHOR CONTRIBUTIONS

XX, C-WC, ML, CL, and YL contributed to literature search, formulation of the research summaries and discussions. XX, C-WC, ML, CL, and YL contributed to the existing chemotherapies. Interestingly, chemoresistance due to RECQ4 overexpression may also be a consequence of deregulated transcriptional regulations. Specifically, RECQ4 was found to interact with the transcriptional factor YB1 to promote AKT-mediated phosphorylation of YB1 and YB1-dependent gene expressions including the multidrug resistance gene MDR1 (Mo et al., 2016). In addition, since the SF2 domain and the C-terminus of RECQ4 are involved in suppressing RAD52-mediated single-stranded annealing for repairing DNA breaks, cells lacking these regions of the RECQ4 protein increase sensitivity to DNA damaging agents in the presence of RAD52 inhibitors (Kohzaki et al., 2020). Therefore, identification of pharmacological inhibitors against the multifaceted roles of RECQ4 in supporting cancer cell growth and chemoresistance may provide new therapeutic strategies.

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AUTHOR CONTRIBUTIONS

XX, C-WC, ML, CL, and YL contributed to literature search, formulation of the research summaries and discussions. XX assisted in completing Table 1. YL wrote the manuscript. We thank Melody Wang for her assistance in proofreading the manuscript.

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REFERENCES

Abe, T., Yoshimura, A., Hosono, Y., Tada, S., Seki, M., and Enomoto, T. (2011). The N-Terminal Region of RECQL4 Lacking the Helicase Domain Is Both Essential and Sufficient for the Viability of Vertebrate Cells. Biochim. Biophys. Acta (Bba) - Mol. Cel Res. 1813 (3), 473–479. doi:10.1016/j.bbamcr.2011.01.001

Ajewung, N. F., Nguyen, T. T. M., Lu, L., Kucharski, T. J., Rousseau, J., Molderez, S., et al. (2019). Mutations in ANAPC1, Encoding a Scaffold Subunit of the Anaphase-Promoting Complex, Cause Rothmund-Thomson Syndrome Type 1. Am. J. Hum. Genet. 105 (3), 625–630. doi:10.1016/ajhg.2019.06.011

Abe, T., Yoshimura, A., Hosono, Y., Tada, S., Seki, M., and Enomoto, T. (2011). The N-Terminal Region of RECQL4 Lacking the Helicase Domain Is Both Essential and Sufficient for the Viability of Vertebrate Cells. Biochim. Biophys. Acta (Bba) - Mol. Cel Res. 1813 (3), 473–479. doi:10.1016/j.bbamcr.2011.01.001

Ajewung, N. F., Nguyen, T. T. M., Lu, L., Kucharski, T. J., Rousseau, J., Molderez, S., et al. (2019). Mutations in ANAPC1, Encoding a Scaffold Subunit of the Anaphase-Promoting Complex, Cause Rothmund-Thomson Syndrome Type 1. Am. J. Hum. Genet. 105 (3), 625–630. doi:10.1016/ajhg.2019.06.011
Croteau, D. L., Rossi, M. L., Canugovi, C., Tian, J., Sykora, P., Ramamoorthy, M., De Somer, L., Wouters, C., Morren, M.-A., De Vos, R., Van Den Oord, J., Xu et al. RECQ4 Disease Mutations

cBioPortal. Available at: https://cancerdiscovery.aacrjournals.org/

Castillo-Tandazo, W., Frazier, A. E., Sims, N. A., Smeets, M. F., and Walkley, C. R. (2021). Rothmund-Thomson Syndrome-like RECQL4 Truncating Mutations Cause a Haplosufficient Low-Bone-Mass Phenotype in Mice. Mol. Cell Biol 41 (3). doi:10.1128/mcb.00359-20

Chai, Z., Nie, L., Peng, Z., Yang, Q., Yang, K., Tao, J., et al. (2012). RecQL4 Cytoplasmic Localization: Implications in Mitochondrial DNA Oxidative Damage Repair. Int. J. Biochem. Cell Biol. 44 (11), 1942–1951. doi:10.1016/j.biocel.2012.07.016

Colombo, E. A., Fontana, L., Roversi, G., Negri, G., Castiglia, D., Paradisi, M., et al. (2014). Novel Physiological Recq4 Alternative Transcript Disclosed by Molecular Characterisation of Rothmund-Thomson Syndrome Sibs with Mild Phenotype. Eur. J. Hum. Genet. 22 (11), 1298–1304. doi:10.1038/ejhg.2014.18

Colombo, E., Locatelli, A., Cubelli Sánchez, L., Romeo, S., Elioglu, N., Maystdi, I., et al. (2018). Rothmund-Thomson Syndrome: Insights from New Patients on the Genetic Variability Underpinning Clinical Presentation and Cancer Outcome. Jmjts 19 (4), 1103. doi:10.1038/jmjts.201901103

Croteau, D. L., Rossi, M. L., Canugovi, C., Tian, J., Sykora, P., Ramamoorthy, M., et al. (2012). RecQL4 Localizes to Mitochondria and Preserves Mitochondrial DNA Integrity. Aging Cell 11 (3), 456–466. doi:10.1111/j.1474-9976.2012.00803.x

Croteau, D. L., Rossi, M. L., Ross, J., Dawut, L., Dunn, C., Kulikowicz, T., et al. (2012). RAPADILINO RecQL4 Mutant Protein Lacks Helicase and ATPase Activity. Biochim. Biophys. Acta (Bba) - Mol. Basis Dis. 1822 (11), 1727–1734. doi:10.1016/j.bbadis.2012.07.014

De S., Kumar, J., Madgal, R., Modi, P., Gupta, S., Futami, K., et al. (2012). RecQL4 Is Essential for the Transport of P53 to Mitochondria in normal Human Cells in the Absence of Exogenous Stress. J. Cel Sci 125 (Pt 10), 2509–2522. doi:10.1242/jcs.101501

De Somer, L., Wouters, C., Morren, M.-A., De Vos, R., Van Den Oord, J., Devriendt, K., et al. (2010). Granulomatous Skin Lesions Complicating Varicella Infection in a Patient with Rothmund-Thomson Syndrome and Immune Deficiency: Case Report. Orphanet J. Rare Dis. 5, 37. doi:10.1186/1750-1172-5-37

Debeljak, M., Zver, A., and Jablac, J. (2009). A Patient with Baller-Gerold Syndrome and Midline NK/T Lymphoma. Am. J. Med. Genet. 149a (4), 755–759. doi:10.1002/ajmg.a.32736

Ding, L., and Liu, Y. (2015). Borrowing Nuclear DNA Helicases to Protect Mitochondrial DNA. Jmjts 16 (5), 10870–10887. doi:10.3390/jmjts160510870

Duan, Y., and Fang, H. (2016). RecQL4 Regulates Autophagy and Apoptosis in U2OS Cells. Biochem. Cell Biol. 94 (6), 551–559. doi:10.1139/bcb-2016-0005

Ellis, N. A., Groden, J., Ye, T.-Z., Straughen, L., Lennon, D. J., Ciocci, S., et al. (1995). The Bloom’s Syndrome Gene Product Is Homologous to RecQ Helicases. Cell 83 (4), 655–666. doi:10.1016/0092-8674(95)90105-1

Fang, H., Niu, K., Mo, D., Zhi, Y., Tan, Q., Wei, D., et al. (2018). RecQL4 Aurora B Kinase Axis Is Essential for Cellular Proliferation, Cell Cycle Progression, and Mitotic Integrity. Oncogenesis 7 (9), 68. doi:10.3389/fonc.2018.00880-4

Ferrarelli, L. K., Popuri, V., Ghosh, A. K., Tadokoro, T., Canugovi, C., Hsu, J. K., et al. (2013). The RecQL4 Protein, Deficient in Rothmund-Thomson Syndrome Is Active on Telomeric D-Loops Containing DNA Metabolism Blocking Lesions. DNA Repair 12 (7), 518–528. doi:10.1016/j.dnarep.2013.04.005

Fradin, M., Merklen-Djafari, C., Perriguard, C., Aral, B., Muller, J., Stoetzel, C., et al. (2013). Long-term Follow-Up and Molecular Characterization of a Patient with a RecQL4 Mutation Spectrum Disorder. Dermatology 226 (4), 353–357. doi:10.1159/000351311

Gatinov, I., Desprat, R., Pichard, L., Becker, F., Goldenberg, A., Balguerie, X., et al. (2020). IPSC Reprogramming of Fibroblasts from a Patient with a Rothmund-Thomson Syndrome RTS. Stem Cell Res. 45, 101807. doi:10.1016/j.scr.2020.101807

Guha, M. (2011). PARP Inhibitors Stumble in Breast Cancer. Nat. Biotechnol. 29 (5), 373–374. doi:10.1038/nbt0511-373

Gui, B., Song, Y., Hu, X., Li, H., Qin, Z., Su, J., et al. (2018). Novel Pathogenic RecQL4 Variants in Chinese Patients with Rothmund-Thomson Syndrome. Gene 654, 110–115. doi:10.1016/j.gene.2018.02.047

Gupta, S., De, S., Srivastava, V., Hussain, M., Kumar, J., Muniyappa, K., et al. (2014). RecQL4 and P53 Potentiate the Activity of Polymerase y and Maintain the Integrity of the Human Mitochondrial Genome. Carcin 35 (1), 34–45. doi:10.1093/carcin/bgt315

Gutiérrez-Jimeno, M., Panizo-Morgado, E., Tamayo, I., San Julián, M., Catalan-Lambán, A., Alonso, M. M., et al. (2020). Somatic and Germline Analysis of a Familial Rothmund-Thomson Syndrome in Two Siblings with Osteosarcoma. Npj Genom. Med. 5, 51. doi:10.1038/s41525-020-00166-x

Ikikawa, K., Noda, T., and Furuchi, Y. (2002). Preparation of the Gene Targeted Knockout Mice for Human Premature Aging Diseases, Werner Syndrome, and Rothmund-Thomson Syndrome Caused by the Mutation of DNA Helicases. Nippon Yakurigaku Zasshi 119, 219–226.

Im, J.-S., Kim, S.-H., Farina, A., Jung, D.-S., Hurwitz, J., and Lee, J.-K. (2009). Assembly of the Cdc45-Mcm2-7-GINS Complex in Human Cells Requires the Ctf4/And-1, RecQL4, and Mcm10 Proteins. Pnas 106 (37), 15628–15632. doi:10.1073/pnas.0908309106

Im, J.-S., Park, S.-Y., Cho, W.-H., Bae, S.-H., Hurwitz, J., and Lee, J.-K. (2015). RecQL4 Is Required for the Association of Mcm10 and Ctf4 with Replication Origins in Human Cells. Cell Cycle 14 (7), 1001–1009. doi:10.1080/15384101.2015.1057001

I Jensen, M. B., Dunn, C. A., Keijzers, G., Kulikowicz, T., Rasmussen, J. L., Croteau, D. L., et al. (2012). The Helicase and ATPase Activities of RecQL4 Are Compromised by Mutations Reported in Three Human Patients. Aging 4 (11), 790–802. doi:10.18632/aging.100506

Kaiser, S., Sauer, F., and Kisker, C. (2017). The Structural and Functional Characterization of Human Recq4 Reveals Insights into its Helicase Mechanism. Nat. Commun. 8, 15907. doi:10.1038/ncomms15907

Keller, H., Kiose, K., Sachsenweger, J., Haumann, S., Ohlenschläger, O., Nuutinen, T., et al. (2014). The Intrinsically Disordered Amino-Terminal Region of Human Recq4: Multiple DNA-Binding Domains Confer Anneling, Strand Exchange and G4 DNA Binding. Nucleic Acids Res. 42 (20), 12614–12627. doi:10.1093/nar/gku993

Kellermayer, R., Sittenen, H. A., Hadzisievi, K., Kestlía, M., and Kosztolányi, G. (2005). A Patient with Rothmund-Thomson Syndrome and All Features of RAPADILINO. Arch. Dermatol. 141 (5), 617–620. doi:10.1001/archderm.141.5.617
Kitao, S., Lindor, N. M., Shiratori, M., Furuchi, Y., and Shimamoto, A. (1999). Rothmund-thomson syndrome Responsible Gene, RECQL4: Genomic Structure and Products. Genomics 61 (3), 268–276. doi:10.1006/geno.1999.595988-7543(99)59595-1

Kitao, S., Oh sugi, I., Ichikawa, K., Goto, M., Furuchi, Y., and Shimamoto, A. (1998). Cloning of Two New Human Helicase Genes of the RecQ Family: Biological Significance of Multiple Species in Higher Eukaryotes. Genomics 54 (3), 443–452. doi:10.1006/geno.1998.5595

Kitao, S., Shimamoto, A., Goto, M., Miller, R. W., Smithson, W. A., Lindor, N. M., et al. (1999). Mutations in RECQL4 Cause a Subset of Cases of Rothmund-Thomson Syndrome. Nat. Genet. 22 (1), 82–84. doi:10.1038/78788

Kislczak, M., Sedlackova, H., Pichaci, G. P., Streicher, W. W., Krejci, L., and Hickson, I. D. (2015). Interaction of RECVQ and MCM10 Is Important for Efficient DNA Replication Origin Firing in Human Cells. Oncotarget 6 (38), 40464–40479. doi:10.18632/oncotarget.6342

Knittel, G., Rehkämper, T.,Nieper, P., Schmitt, A., Flümann, R., and Reinhardt, H. C. (2018). DNA Damage Pathways and B-Cell Lymphomagenesis. Curr. Opin. Hematol. 25 (4), 315–322. doi:10.1097/MOH.0000000000000433

Kohzaki, M., Chiourea, M., Versini, G., Adachi, N., Takeda, S., Gagos, S., et al. (2012). The Helicase Domain and C-Terminus of Human RECVQ4 Facilitate Replication Elongation on DNA Templates Damaged by Ionizing Radiation. Carcinogenesis 33 (6), 1203–1210. doi:10.1093/carcin/bgs147

Kohzaki, M., Ootsuyama, A., Sun, J., Mortika, T., and Okazaki, R. (2020). Human RECVQ Represses the RADS2-mediated Single-strand annealing Pathway after Ionizing Radiation or Cisplatin Treatment. Int. J. Cancer 146 (11), 3098–3113. doi:10.1002/ijc.32670

Kopinski, P. K., Singh, L. N., Zhang, S., Lott, M. T., and Wallace, D. C. (2021). Lu, H., Shamanna, R. A., de Freitas, J. K., Okur, M., Khadka, P., Kulikowicz, T., et al. (2014). Lu, H., Fang, E. F., Sykora, P., Kulikowicz, T., Becker, K. G., et al. (2018). Targeted Next Generation Sequencing Identifies Functionally Deleterious Germline Mutations in Novel Genes in Early-Onset/familial Prostate Cancer. Plos Genet. 14 (4), e1007355. doi:10.1371/journal.pgen.1007355

Predisposition in a Mouse Model of Type II Rothmund-Thomson Syndrome. Hum. Mol. Genet. 19 (4), 813–825. doi:10.1093/hmg/ddt075

Martino, F., Mojumdar, A., Zuccelli, C., Bhardwaj, A., Buratti, E., Vindigni, A., et al. (2016). Structural and Biochemical Characterization of an RNA/DNA Binding Motif in the N-Terminal Domain of RecQ4 Helicases. Sci. Rep. 6, 21501. doi:10.1038/srep21501

Matsumo, K., Kumanio, M., Kubota, Y., Hashimoto, Y., and Takisawa, H. (2006). The N-Terminal Noncatalytic Region of Xenopus RecQ4 Is Required for Chromatin Binding of DNA Polymerase a in the Initiation of DNA Replication. Mol. Cell. Biol. 26 (13), 4843–4852. doi:10.1128/mcb.02267-05

Mégarbané, A., Mélki, J., Souray, N., Gerbaka, J., Ghouzzi, V. E., Bonaventure, J., et al. (2000). Overlap between Baller-Gerold and Rothmund-Thomson Syndrome. Cl. Dysmorphol. 9 (4), 303–305. doi:10.1080/00195650-200009400-000118

Mo, D., Fang, H., Niu, K., Liu, J., Wu, M., Li, S., et al. (2016). Human Helicase RECVQ4 Drives Cisplatin Resistance in Gastric Cancer by Activating an AKT-YB1-MDRI Signaling Pathway. Cancer Res. 76 (10), 3057–3066. doi:10.1158/0008-5472.CAN-15-2361

Mourery, B. L., Mei, L., and Cook, J. G. (2020). Programming Pluripotent Stem Cells: Can’t Teach an Old Cell New DNA Replication Tricks. J. Cell Biol. 219 (9), e202008014. doi:10.1083/jcb.202008014

Ng, A. J. M., Walia, K. M., Smeets, M. F., Metsaars, A. J., Sims, N. A., Purton, L. E., et al. (2015). The DNA Helicase RecQ4 Is Required for normal Osteoblast Expansion and Osteosarcoma Formation. Plos Genet. 11 (4), e1005160. doi:10.1371/journal.pgen.1005160

Ohlenschläger, O., Kuhnert, A., Schneider, A., Haumann, S., Bellstedt, P., Keller, H., et al. (2012). The N-Terminal of the Human RecQ Helicase Is a Homeodomain-like DNA Interaction Motif. Nucleic Acids Res. 40 (17), 8309–8324. doi:10.1093/nar/gks591

Park, S.-Y., Kim, H., Im, J.-S., and Lee, J.-K. (2019). ATM Activation Is Impaired in Human Cells Defective in RecQ4 Helicase Activity. Biochem. Biophysical Res. Commun. 509 (2), 379–383. doi:10.1016/j.bbrc.2018.12.151

Patil, A. V., and Hsieh, T.-S. (2017). Ribosomal Protein S3 Negatively Regulates Unwinding Activity of RecQ-like Helicase 4 through Their Physical Interaction. J. Biol. Chem. 292 (10), 4313–4325. doi:10.1074/jbc.M116.764324

Pauio, P., Maia, S., Pinto, C., Pinto, P., Monteiro, A., Peixoto, A., et al. (2018). Somatic Alterations and Mutational burden Are Potential Predictive Factors for Metachronous Development of Early Gastric Cancer. Sci. Rep. 10 (1), 22071. doi:10.1038/s41598-020-79195-0

Salih, A., Inoue, S., and Onwuzurike, N. (2018). Rothmund-Thomson Syndrome (RTS) with Osteosarcoma Due toRECQL4 mutation. BMJ Case Rep. 2017, bcr-2017-222384

Sangrithi, M. N., Bernal, J. A., Madine, M., Philpott, A., Lee, J., Dunphy, W. G., et al. (2005). Initiation of DNA Replication Requires the RECQL4 Protein Mutated in Rothmund-Thomson Syndrome. Cell 121 (6), 887–898. doi:10.1016/j.cell.2005.05.015

Schurman, S. H., Hedayati, M., Wang, Z., Singh, D. K., Spina, E., Zhang, Y., et al. (2009). Direct and Indirect Roles of RECQL4 in Modulating Base Excision Repair Capacity. Hum. Mol. Genet. 18 (18), 3470–3483. doi:10.1093/hmg/ddp291

Seo, Y.-S., and Kang, Y.-H. (2018). The Human Replicative Helicase, the CMG Complex, as a Target for Anti-cancer Therapy. Front. Mol. Biosci. 5 (26). doi:10.3389/fmbio.2018.00026

Shahi, R. B., De Brakeleer, S., Caljon, B., Pauwels, I., Bonduelle, M., Joris, S., et al. (2019). Identification of Candidate Cancer Predisposing Variants by Performing Whole-Exome Sequencing on index Patients from BRCA1 and BRCA2-Negative Breast Cancer Families. BMC Cancer 19 (1), 313. doi:10.1186/s12885-019-5494-7
