HELQ as a DNA helicase: Its novel role in normal cell function and tumorigenesis (Review)

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Received February 22, 2023; Accepted August 8, 2023

DOI: 10.3892/or.2023.8657

Abstract. Helicase POLQ-like (HELQ or Hel308), is a highly conserved, 3'-5' superfamily II DNA helicase that contributes to diverse DNA processes, including DNA repair, unwinding, and strand annealing. HELQ deficiency leads to subfertility, due to its critical role in germ cell stability. In addition, the abnormal expression of HELQ has been observed in multiple tumors and a number of molecular pathways, including the nucleotide excision repair, checkpoint kinase 1-DNA repair protein RAD51 homolog 1 and ATM/ATR pathways, have been shown to be involved in HELQ. In the present review, the structure and characteristics of HELQ, as well as its major functions in DNA processing, were described. Molecular mechanisms involving HELQ in the context of tumorigenesis were also described. It was deduced that HELQ biology warrants investigation, and that its critical roles in the regulation of various DNA processes and participation in tumorigenesis are clinically relevant.

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Abbreviations: ATM, ataxia telangiectasia mutated protein; ATR, ataxia telangiectasia and Rad3-related protein; BLCA, bladder urothelial carcinoma; BRCA, breast invasive carcinoma; CESC, cervical squamous cell carcinoma and endocervical adenocarcinoma; CHK1, checkpoint kinase 1; CHOL, cholangiocarcinoma; COAD, colon adenocarcinoma; CPT, camptothecin; dsDNA, double-stranded DNA; DSBs, DNA double-stranded breaks; ESCA, esophageal carcinoma; ESS, endometrial stromal sarcoma; FA, Fanconi anemia; FANCD2, Fanconi anemia group D2; GBM, glioblastoma multiforme; GTEx, genotype-tissue expression; HELQ, helicase POLQ-like; HelicC, helicase C-terminal domain; HNSC, head and neck squamous cell carcinoma; HR, homologous recombination; HROB, homologous recombination factor with OB-fold; HTH, helix-turn-helix; ICL, inter-strand cross-link; KICH, kidney chromophobe; KIRC, kidney renal clear cell carcinoma; KIRP, kidney renal papillary cell carcinoma; LIHC, liver hepatocellular carcinoma; LUAD, lung adenocarcinoma; LUSC, lung squamous cell carcinoma; MCM, mini-chromosome maintenance; MMEJ, microhomology mediated end joining; NER, nucleotide excision repair; NHEJ, non-homologous end joining; OS, overall survival; OV, ovarian cancer; PAAD, pancreatic adenocarcinoma; PARP, poly ADP ribose polymerase; PCPG, pheochromocytoma and paraganglioma; PRAD, prostate adenocarcinoma; RAD51, RecA-like ATP-dependent recombinase 51; READ, rectum adenocarcinoma; RIPK3, receptor-interacting serine/threonine kinase 3; RPA, replication protein A; SF2, superfamily-2; SSA, single strand annealing; STAD, stomach adenocarcinoma; ssDNA, single-stranded DNA; TCGA, The Cancer Genome Atlas; THCA, thyroid carcinoma; UCEC, uterine corpus endometrial carcinoma

Key words: helicase POLQ-like, DNA repair, DNA unwinding, tumorigenesis, signaling pathway

Contents

1. Introduction

The Helicase POLQ-like (*HELQ*) gene, also referred to as *Hel308*/holliday junction migration protein, maps to human chromosome 4q21 and was first cloned by its homology to *Drosophila melanogaster (D.melanogaster)* mutagen-sensitive 308 (Mus308) (1). In humans, HELQ is typical superfamily-2 (SF2) ATPase-dependent 3' to 5' helicase as a functions to unwind DNA (2,3). As HELQ has ATPase and helicase functions, it has been classified as a member of the Ski2-like subfamily of SF2 helicases (4), which is a small subfamily (5,6)

involved in RNA processing, splicing and degradation pathways (7). Similar to other SF2 helicases, HELQ is a regulator of genome stability (2), DNA recombination (2), DNA binding (8), DNA unwinding (9), DNA replication (10), DNA strand annealing (9) and DNA repair (11,12).

HELQ is widely expressed in normal human tissues, particularly of the reproductive system, and plays an important role in maintaining germ cells by promoting DNA repair protein RecA-like ATP-dependent recombinase 51 (RAD51) homolog 1 (RAD51) paralog-dependent repair (11). Furthermore, HELQ is abnormally expressed in multiple tumors, and contributes to cell proliferation, metastasis and chemotherapy resistance through various molecular pathways, such as nucleotide excision repair (NER), as well as the checkpoint kinase 1 (CHK1)-RAD51 and ataxia telangiectasia mutated protein/ataxia telangiectasia and Rad3-related protein (ATM/ATR) signaling pathways (12,13). HELQ can also contribute to germ cell maintenance and tumorigenesis in mammals by regulating its target genes, which include *CHK1*, fanconi anemia group D2 (*FANCD2*), *RAD51* and replication protein A (*RPA*), among others (3,4,9,14). Of note, the low expression or loss of HELQ can have a significant impact on clinical features and prognosis in patients with cancer.

In the present review, the structural features, function, target genes, roles in reproduction, molecular mechanisms associated with tumorigenesis, and potential applications of HELQ were summarized. It was concluded that HELQ is an attractive future clinical target in conditions involving germ cell maintenance and in molecular targeted cancer therapies.

2. Structural features of HELQ

SF2 helicase family. The DNA helicases are classified into five different superfamilies (SF1-SF5) (15). SF1 and SF2 helicases encompass a large group of DNA and RNA helicases found in eubacteria, archaea, viruses and eukaryotes. They possess an ATP-dependent translocation module consisting of two RecA-fold domains responsible for nucleic acid and ATP binding (15,16). HELQ was first described by Marini in 2002, which belongs to the SF2 protein family (1). SF2 family proteins have a pair of RecA-like domains that provide motion associated with helicase activity (6,17-19) and play key roles in chromatin rearrangement, DNA repair and transcription (20-22) and RNA metabolism (17,23). SF2 family proteins are divided into Ski2-like, RecQ-like, RecG-like, RecA-like ATP-dependent recombinase 3 (Rad3)/XPD, type I restriction enzymes, DEAD-box and NS3/NPH-II subfamilies, Swi/Snf, DEAH/RNA helicase A, RIG-I-like, based on sequence homology (6,24,25). In archaea, in all Ski2-like helicases including Mtr4, Ski2, and Bad Response to Refrigeration 2 homolog, the molecular 'core' of them is a ring-like assembly of four domains comprising a ratchet domain, a winged helix domain and two RecA domains (25).

Structural and functional characteristics of HELQ. HELQ maps to chromosome 4q21 (1) and generates a full-length mRNA (NM_133636.5) comprising 3,543 base pairs and encoding a protein of 1,101 amino acids (aa) (1). A total of 7 transcribed splice isoforms of *HELQ* have been described, encoding six HELQ protein variants (26). HELQ is a conserved protein that possesses three main protein domains: The DEAD/DEAH box helicase domain, a helix-turn-helix (HTH_61) domain and a helicase C-terminal domain (Fig. 1). The domain of DEAD/DEAH box helicase contains a DEAH box and an ATP-binding region (putative ATP binding site), and is involved in unwinding nucleic acids. In addition, human HELQ has a disordered domain (212-261 aa), containing two compositionally biased regions, comprising basic and acidic (214-228 aa) and polar (229-253 aa) residues. A structural diagram of the human HELQ protein is presented in Fig. 1.

The DEAD/DEAH box helicase class of proteins, which share eight conserved motifs (I, Ia, Ib, II, III, IV, V and VI) (27). ATP is bound to motifs I and II, while RNA is bound to motifs Ia and Ib. The DEAD/DEAH box is named after the Walker B motif (motif III) consensus sequence (28) and functions in ATP hydrolysis. Motifs IV and V perform similar functions to those of motifs Ia and Ib, respectively, in RNA binding (27). DEAD/DEAH box helicases are found in various prokaryotes and eukaryotes (28), and are involved in several aspects of RNA metabolism, such as pre-mRNA splicing, RNA decay, nuclear transcription, editing, nucleocytoplasmic transport, ribosome biogenesis, translation and organellar gene expression (29-31).

The helicase C-terminal domain (HelicC) is present in various SF1 and SF2 helicases and helicase-related proteins. The HelicC domain does not fold autonomously, but rather as a component of the helicase, which participates in the ATP-dependent unwinding of DNA or RNA.

The α-helical protein domain family includes winged helix-turn-helix (HTH) domains that have characteristic folds, which function as sequence-specific DNA-binding domains (32). The HTH plays an important role in DNA binding and protein interactions (33,34) and consists of two α-helices (α-helix 22 and 23) and a β-sheet turn, where a double-stranded DNA (dsDNA) major groove can be recognized by α-helix 23 (14,2).

HELQ expression pattern. HELQ is highly conserved across a wide range of species from archaea through to mammals (https://www.ncbi.nlm.nih.gov/homologene/?term=HELQ). For example, *Homo sapiens* HELQ shares a >97, 78 and 60% DNA similarity with the homologous *Macaca mulatta*, *Mus musculus*, and *Danio rerio* genes, respectively (Table I). It is a conserved gene in eukaryotes that HELQ (HomoloGene ID: 14667) has important molecular functions (i.e., ATP binding, DNA binding, single-stranded 3'-5'DNA helicase activity) and cellular component classifications (i.e., nucleus, site of DNA damage), and acts in biological processes (i.e., DNA duplex unwinding, rRNA processing) (1,9,35-37) (https://www.alliancegenome.org/gene/HGNC:18536) (Table II). Since *HELQ* is expressed in a wide variety of species, it was likely present in a common ancestor of vertebrates.

3. Physiological functions of HELQ

DNA helicases are ubiquitous in living organisms, where they facilitate processes involved in DNA metabolism through unwinding the DNA double helix (3). It is essential for the maintenance of genome stability for DNA helicases to function in the replication and repair of DNA (38). HELQ

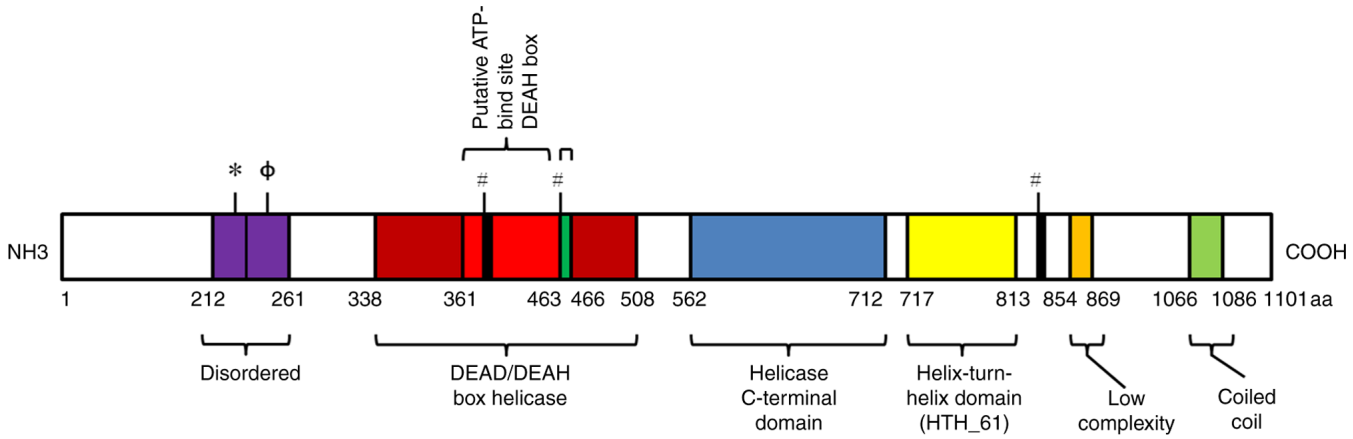

Figure 1. Structural diagram of HELQ protein. HELQ encompasses three domains, DEAD/DEAH box helicase (red color) containing the ATP-binding region and DEAH box, helicase C-terminal domain (blue color) and a helix-turn-helix (HTH_61) domain (yellow color). DEAD/DEAH box helicase (338-508 aa): A family of proteins involved in unwinding nucleic acids. This domain contains the ATP-binding region (361-463 aa, putative ATP binding site) and DEAH box (463-466 aa). Disordered (212-261 aa): Contains two compositionally biased regions. *basic and acidic residues (214-228 aa); ϕpolar residues (229-253 aa); #mutagenesis (365, 463, 818-819 aa). Low complexity (854-869 aa): No additional details recorded. Coiled-coil region (1066-1086 aa): No additional details recorded. HELQ, helicase POLQ-like.

is a single-stranded 3'-5' DNA helicase with critical roles in DNA repair, binding, unwinding, replication and strand annealing (2,8,9).

DNA repair. DNA damage is a common event that may have either endogenous or exogenous causes and can lead to mutations, cell/organ death and cancer (39,40). It is crucial to repair damaged DNA after it has been damaged, as this allows damaged DNA to regain its original structure and function normally (34,41). Various pathways recognize and repair different types of DNA lesions, including direct repair, NER, base excision repair, inter-strand cross-link (ICL) repair and double-strand break (DSB) repair (39). HELQ maintains genomic stability and avoids tumorigenesis through its involvement in different repair pathways, including NER, DSB repair and ICL repair (9,11-13).

A NER pathway is involved in the removal of DNA damage after certain types of DNA damage have occurred (13). The expression levels of NER pathway proteins [e.g., xeroderma pigmentosum complementation group A (XPA), XPC, replication protein A (RPA) and ERCC excision repair 1, endonuclease non-catalytic subunit] are important mediators for responses to platinum-based chemotherapy and influence DNA repair activity (13,42). HELQ is crucial for cellular responses to cisplatin through its role in regulating the expression of NER pathway proteins (13).

DSBs are major DNA lesions deleterious to cell survival and genetic stability (43). When DSBs are not repaired, they can result in chromosome loss and rearrangements and even carcinogenesis (44). Two major pathways can repair DSBs: Homologous recombination (HR) and non-homologous end joining (NHEJ) (45,46), as well as alternative pathways, such as microhomology-mediated end joining (MMEJ) and single strand annealing (SSA) (45,46). HR is essential for DSB repair in post-replicative chromatin following replication fork collapse (47,48), and requires the loading of the RAD51 recombinase onto single-stranded DNA (ssDNA) through DNA ends or at post-replicative ssDNA gaps (45). The function of HELQ in HR is to capture RPA-bound ssDNA and then to displace it to speed up the annealing of complementary DNA strands (9). HELQ immediately interacts with BCDX2, a paralog of RAD51, to accelerate effective HR in damaged replication forks (11). RFS-1 binds to HELQ and plays a complementary role in facilitating the breakdown of RAD51 dsDNA filaments in postsynaptic HR intermediates, which is required to complete meiotic DSB repair in *Caenorhabditis elegans* (47). In addition, the HR factor with OB-fold (HROB)-mini-chromosome maintenance 8 (MCM8)-MCM9 pathway functions redundantly with HELQ, supporting a postsynaptic step of HR in a parallel pathway (49). HELQ accelerates HR-efficiency at compromised replication forks by working in parallel with the FA pathway in $HELQ^{\Delta C/\Delta C}$ and $FANCD2^{-/-}$ double mutant mice (11). Furthermore, HELQ is essential for the function of the synthesis-dependent strand annealing modes of HR, MMEJ of G4-induced DSBs and SSA in genome stability and tumor avoidance (9).

ICLs are also deleterious DNA lesions caused by endogenous (malondialdehyde) or exogenous [mitomycin C (MMC); cis-platinum and psoralens] sources (50,51), which induce mutations and chromosomal rearrangements by inhibiting DNA replication and transcription (1,34). ICL repair is complex and proteins from the NER, translation synthesis and HR pathways are involved in ICL repair (40). With its association with RAD51 paralogs, HELQ prevents germ cell loss and reduces cancer susceptibility through ICL repair (11,52). HELQ deficiency leads to germ cell attrition and ICL repair sensitivity in mouse and human cells (11,12,53). The FA pathway plays a critical role in recruiting RAD51-mediated HR during ICL repair (40). In parallel with HELQ, HROB participates in ICL repair as epistatic with MCM8-MCM9 (49), which also directly facilitates the repair of ICL-induced damage downstream of FANCD2 ubiquitylation (49,54).

DNA binding and unwinding. The HELQ HTH domain is associated with DNA strand binding and protein interactions (2,34). The dissociation constant values of wild-type HELQ for

Table I. Conserved gene HELQ homology in Eukaryota (HomoloGene: 14667) [https://www.ncbi.nlm.nih.gov/homologene/?term=HELQ].

HomoloGene: 14667. Gene conserved in Eukaryota

Species	Symbol	Genetic location	Protein Acc.	Protein length	Identity (%)* protein	DNA
H. sapiens	HELQ	Chr4 q21.23	NP_598375.2	1101 aa		
P. troglodytes	HELQ	Chr4	XP_003310356.1	1101 aa	99.1	99.4
M. mulatta	HELQ	Chr5	XP_001104832.1	1101 aa	97.0	97.7
C. lupus	HELQ	Chr32	XP_544959.2	1072 aa	85.9	88.4
B. taurus	HELQ	Chr6	XP_002688448.3	1094 aa	83.7	86.3
M. musculus	Helq	Chr5 E4	NP_001074576.1	1069 aa	78.6	79.3
R. norvegicus	Helq	Chr14 p22	NP_001014156.2	1065 aa	79.1	79.0
G. gallus	HELQ	Chr4	XP_420565.1	1048 aa	68.8	69.7
X. tropicalis	helq	Chr1	XP_002939628.2	1000 aa	66.0	66.8
D. rerio	helq	Chr21	XP_691411.3	1010 aa	60.9	61.6
D. melanogaster	mus301	Chr3-22cM	NP_648178.1	1051 aa	45.8	50.5
A. gambiae	AgaP-AGAP012297	Chr3L	XP_551895.3	914 aa	44.4	48.3
C. elegans	helq-1	ChrIII	NP_001022911.1	923 aa	41.2	49.9

HELQ, helicase POLQ-like.

Table II. Function-Gene Ontology Annotations of HELQ (https://www.alliancegenome.org/gene/HGNC:18536).

Category	Classification term	Gene Ontology ID[a]	Reference
Molecular function	ATP binding	GO:0005524	GO_REF:0000043
	ATP hydrolysis activity	GO:0016887	GO_REF:0000116
	DNA binding	GO:0003677	GO_REF:0000043
	Single-stranded 3'-5' DNA helicase activity	GO:1990518	(1,9,35)
Cellular component	Nucleus	GO:0005634	(9,36)
	Site of DNA damage	GO:0090734	(9)
	DNA double-strand break processing involved in repair via single-strand annealing	GO:0010792	(9)
	DNA duplex unwinding	GO:0032508	GO_REF:0000108
	Double-strand break repair via alternative non-homologous end joining	GO:0097681	(9)
Biological process	Double-strand break repair via homologous recombination	GO:0000724	(37)
	Double-strand break repair via synthesis-dependent strand annealing	GO:0045003	(9)
	Positive regulation of double-strand break repair via Homologous recombination	GO:1905168	GO_REF:0000024
	rRNA processing	GO:0006364	(36)

[a]http://amigo.geneontology.org/amigo.

ssDNA and dsDNA were reported as 0.14 and 5.3 μM, respectively, indicating a strong preference for ssDNA binding and suggesting that the protein must track along, displacing the dsDNA strand (2). Furthermore, HELQ mutations reduce dsDNA binding, but ssDNA binding is not affected (55). The HELQ-ssDNA interaction is essential for the translocation mechanism. Mechanistically, HELQ interacts with RPA, and RPA coordinates the loading of HELQ onto ssDNA. HELQ helicase core is activated by ATP-Mg^{2+} binding and translocates along ssDNA as a dimer when loaded onto ssDNA (35).

In addition, HELQ is an ssDNA-activated ATPase, which is important for unwinding forked DNA (10,56). As well as unwinding ssDNA and dsDNA junctions, and HELQ is capable of unwinding 3' overhangs, 3' lagging strand forks,

Y-structures, and D-loops (3); however, HELQ cannot unwind using ATPγS or 5' overhang substrates (9). The HELQ unwinding of 3' overhang substrates is inhibited by RPA, whereas RAD51 stimulates the unwinding activity of its D-loops by forming a complex with HELQ (9).

DNA replication. HELQ is an ATP-dependent enzyme involved in the recovery of replication forks that have stalled or collapsed following DNA damage (3). HELQ can act on damaged replication forks where the leading strand template for DNA replication has stopped, causing the polymerase to uncouple and continue DNA synthesis from the lagging strand template (10). Following treatment with camptothecin (CPT), an agent that stalls and collapses replication forks, HELQ is recruited to stalled replication sites that are associated with replication resumption. HELQ can facilitate replication resumption, possibly through colocalizing with IdU incorporation sites and RPA foci, by unwinding the nascent lagging strand, or alternatively through HELQ co-localization with RAD51 and FANCD2 at sites of stalled replication (3,12). In addition, HELQ has an important role in rescuing stalled forks during the normal S phase, and the loss of HELQ results in increased stalled forks, a role which is not epistatic with that of FA complementation group C (53).

DNA strand annealing. Previous studies of HELQ have focused more on its DNA repair and DNA unwinding functions, while its role in DNA strand annealing has been underappreciated. Tafel *et al* (3) reported that HELQ does not exhibit a strong annealing activity, and that RPA can suppress separated strand reannealing by binding to unwound ssDNA generated by HELQ. By contrast, Anand *et al* (9) reported that RPA strongly accelerated the DNA strand annealing activity of HELQ. Mechanistically, in addition to capturing RPA-bound DNA strands, HELQ is capable of superseding RPA and stimulating complementary DNA strands. Furthermore, it was found that ATP binding and hydrolysis are essential for HELQ DNA annealing activity, whereas the RAD51 addition was unaffected by HELQ-dependent DNA annealing activity (9).

4. Gene functions regulated by HELQ

One important question is 'How are HELQ, its downstream mediators, and their mechanisms related in normal and cancer cells?' Further, there are various studies reporting that HELQ can contribute to both germ cell maintenance and tumorigenesis in mammals by regulating its target genes: *CHK1*, *FANCD2*, *RAD51* and *RPA* (3,4,9,14).

CHK1. CHK1 is a primary effector of DNA lesion and replication checkpoint responses, and its inhibition promotes DNA damage and reduces HR repair (57,58). CHK1 physically interacts with RAD51, while CHK1/RAD51 disruption or inactivation induces defective HR, aberrant replication dynamics, and chromosome instability (59). In osteosarcoma cells, CHK1 activation is promoted by HELQ, and HELQ colocalizes with RAD51 to participate in the repair of damaged forks by HR (3).

FANCD2. FA is a rare genetic disease, and patients with FA are hypersensitive to DNA ICL-inducing agents such as mitomycin C and cisplatin (60). FA signal transduction involves 22 proteins, which share a common pathway that is activated on DNA damage (61). FANCD2 is currently the focus of research into the FA pathway, and is crucial in cellular responses to DNA lesions (62). HR may be regulated by the FA pathway, where activated FANCD2 is translocated to chromatin-associated foci, where it colocalizes with HR proteins [RAD51 and breast invasive carcinoma (BRCA)2] (63). The strong negative effect of HELQ knockdown on single-stranded templated repair via the HR repair pathway may be explained by its physical interaction with FANCD2 and RAD51 (64). Following CPT treatment, HELQ is involved in fork repair and restart by localizing to replication forks, unwinding lagging strand structures, and co-localizing with RAD51 and FANCD2 (3,14). In addition, although the FA pathway, ICL sensitivities, and HELQ are additive, there is no epistasis of HELQ for downstream targets of FANCD2, and HELQ plays an independent role from FA pathway in ICL processing (12).

RAD51. RAD51 is a DNA-binding protein that can bind to both ssDNA and dsDNA and maintain genome stability in DNA replication (65,66). RAD51 is also an ATPase that forms nucleoprotein filaments on ssDNA and facilitates the search for homologous repair templates, such as homologous chromosomes, sister chromatids or ectopic homologous sequences (67). Hence, RAD51 is essential for regulating fork reversal by discovering and invading homologous DNA sequences during DSB repair by HR (65,67). In addition, five RAD51 paralogs [RAD51B/C/D and X-ray repair cross complementing (XRCC)2/3] that form two main complexes, BCDX2 (RAD51B/C/D-XRCC2) and CX3 (RAD51C-XRCC3), are involved in the HR repair of collapsed replication forks and maintenance of genome stability (68-70). The BCDX2 complex preferentially binds to ssDNA and accelerates HELQ ATPase activity (71), which plays a central role in recognizing damage and stimulating fork remodeling in response to fluctuating dNTP pools (72). Adelman *et al* (11) found that HELQ plays a critical role in replication-coupled HR by interacting with complexes of the RAD51 paralog, BCDX2, to avoid germ cell loss and tumorigenesis. Anand *et al* (9) also demonstrated that HELQ binds to BCDX2 complexes, and that the latter strongly stimulates the translocation of HELQ during DNA unwinding. Takata *et al* (12) reported that HELQ interacts with ATR and HR-related RAD51 paralogs and participates in DNA LCL resistance in human cells.

RPA. As a major ssDNA binding protein, RPA is abundant in eukaryotic cells (66,73). RPA comprises three subunits, RPA14, RPA32 and RPA70, which are first responders in the event of the disruption of DNA metabolism. RPA is essential for replication, recombination and repair by binding to ssDNA to maintain genome duplication and stability (74). RPA and HELQ physically interact to form a supershift complex with HELQ on ssDNA (4). Furthermore, RPA stimulates HELQ helicase activity by binding to unwound regions generated by HELQ and inhibiting reannealing (4). Similarly, in mammals, RPA can restrain DNA unwinding while promoting DNA strand annealing by HELQ. Mechanistically, HELQ is recruited to ssDNA by interacting with RPA, followed by RPA displacement to stimulate complementary DNA strand annealing (9).

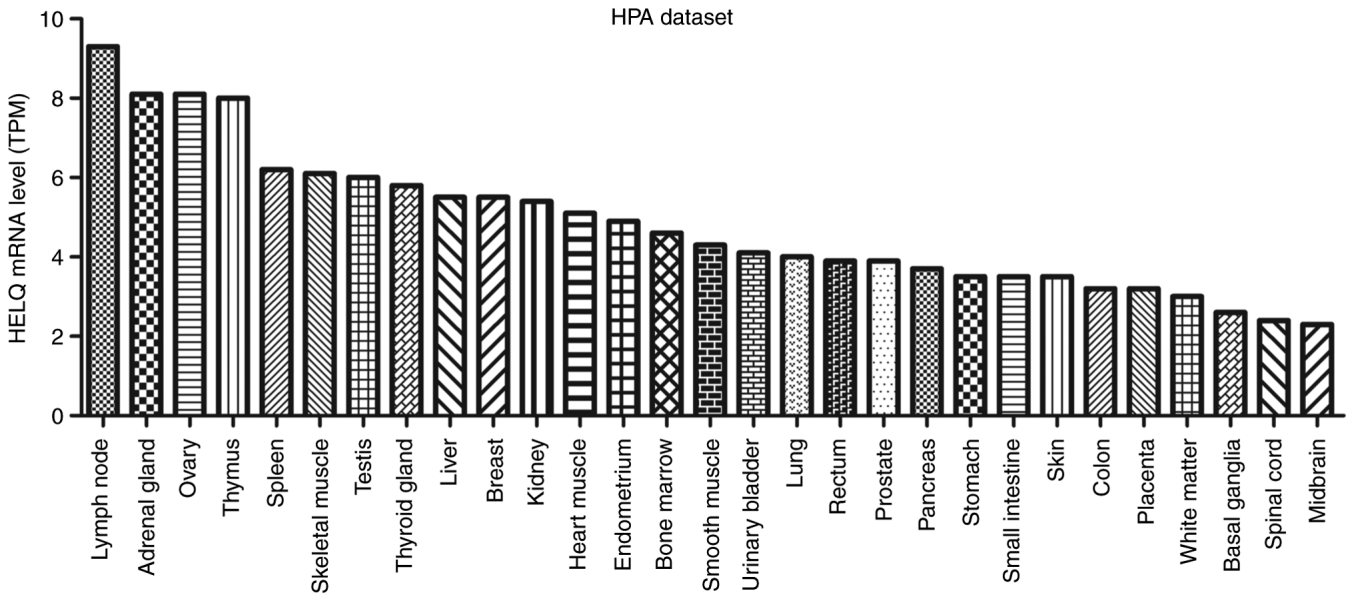

Figure 2. HELQ mRNA expression overview of tissue category from HPA dataset. HELQ is expressed at different levels in various tissues, including the testis, ovary, skeletal muscle and heart. HELQ, helicase POLQ-like; HPA, Human Protein Atlas; TPM, Transcript Per Million.

5. The roles of HELQ in reproduction

HELQ was first identified in the mouse and human genomes through its homology to *D. melanogaster* Mus308 (1). HELQ is expressed at different levels in various tissues, including the testes, ovaries, skeletal muscle and heart (75) in humans, and has also been detected in several other tissues (https://www.proteinatlas.org/ENSG00000163312-HELQ/tissue) (Fig. 2).

HELQ plays a crucial role in germ cell maintenance, and its loss results in subfertility. A high level of HELQ expression is found in the reproductive system, including the ovaries, testes, cervix, breast, epididymis, endometrium and prostate. In HELQ-deficient mice, HELQ facilitates RAD51 paralog-dependent repair, thereby preventing germ cell attrition (11). It is disputed whether mutant HELQ has an adverse effect on the male reproductive system. $HELQ^{gt/gt}$ male mice have significantly smaller testes, suffer from seminiferous tubule atrophy, and lack spermatocytes and spermatogonia, as compared with wild-type male mice, indicating that HELQ deficiency leads to a mild form of hypogonadism (53); however, two heterozygous mutant mouse models ($HELQ^{+}$/M5 and $HELQ^{+}$/M6) did not show any spermatogenic defects, indicating that heterozygous HELQ variants alone do not cause development of the sertoli cell-only syndrome phenotype in mice (76). Furthermore, Wang *et al* (77) found no evidence that HELQ mutations are associated with premature ovarian failure in Chinese women.

6. Potential roles of HELQ in tumorigenesis and underlying mechanisms

In addition to its physiological functions, HELQ is often implicated in tumorigenesis. Genetic alterations, such as deletions and mismatches, are frequently associated with tumorigenesis. Single-nucleotide polymorphism variants of *HELQ* have been associated with an increased risk of various cancers, including upper aerodigestive tract cancers (78,79), esophageal squamous cell carcinoma (80,81), head and neck cancers (82), gastric adenocarcinoma (81), and breast and ovarian cancer (OV) (83-85) through genome-wide association studies (Table III). Furthermore, there is accumulating evidence indicating that HELQ may act as a tumor suppressor for several cancers, such as osteosarcoma (86), OV (12,13,34,77,87), chronic lymphocytic leukemia (CLL) (88), non-small cell lung cancer (NSCLC) (89) and endometrial stromal sarcoma (ESS) (Table IV) (90). HELQ is an important regulator of cancer proliferation, invasion, migration and can contribute to poor patient prognosis and platinum resistance through several mechanisms (13,86,87) in different types of tumors (Fig. 3).

Osteosarcoma. HELQ plays a critical role in tumor suppression in mammals through interacting with the BCDX2 complex (11). HELQ overexpression inhibited osteosarcoma cell proliferation, migration, invasion and DNA damage repair (86). In addition, the antitumor activity of HELQ may be associated with the upregulation of the DNA damage-related proteins RAD51 and CHK1expression, and HELQ modulates an anti-invasive phenotype and DNA damage repair in osteosarcoma cells by activation of the CHK1-RAD51 signaling pathway (86).

NSCLC. HELQ is downregulated in NSCLC tissues and cells, while the malignancy of lung cancer cells was enhanced by HELQ depletion. HELQ overexpression inhibits cell proliferation and migration by suppressing DNA damage repair, and promotes cell death by inducing necrosis through its interaction with receptor-interacting serine/threonine kinase 3 (RIPK3) (89). HELQ is a favorable prognostic factor for patients with NSCLC, and low HELQ levels in patients with NSCLC were associated with a reduced overall survival (OS) through a Kaplan-Meier plotter.

Table III. Associations between SNPs within HELQ and cancer risk.

SNP	Genotype	Alleles		Reason for replication attempt	Cancer type	Risk factors	Significant association	(Refs.)
		Major	Minor					
rs1494961	/	T	C	Non-synonymous	Upper aero-digestive tract (UADT) cancers	Age and sex	Yes, $P=1x10^{-8}$	(78)
rs1494961	/	/	C	Missense mutation V306I		Smoking and/or alcohol	Yes, $P=2.65x10^{-4}$	(79)
rs1494961	C/C + C/T and T/T	/	/	/	Head and neck squamous cell carcinoma	Alcohol consumption and smoking pack-years	Yes, P<0.001	(82)
rs1494961	TT, TC, CC	T	C	/	ESCC	Age, Smoking, Drinking	Yes, P=0.032	(80)
rs13115704	/	T	C	/	ESCC	/	Yes, $P=8.07x10^{-3}$	(81)
rs1494961	/	T	C	/	Gastric cancer (GC)	/	Yes, P=0.035	
rs13141136 rs7665103 rs141700135 rs138939487 rs1494961	/	/	/	Synonymous and non-synonymous missense variants	Breast and ovarian cancer	/	No, P>0.05	(83)
Rs11099601		A	G	/	Breast cancer	/	Yes, $P=5.62x10^{-6}$	(84)
Rs4693089	GG GA/AA		G	/	Breast cancer	/	Yes, $P=2.38x10^{-19}$	(85)

ESCC, esophageal squamous cell carcinoma.

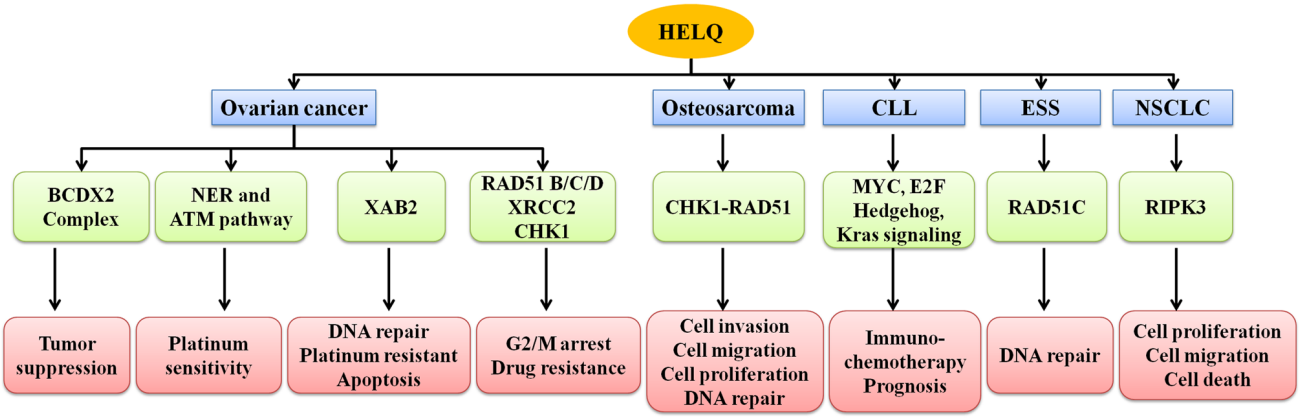

Figure 3. Mechanisms of HELQ in tumorigenesis. HELQ inhibits DNA repair and drug resistance, induces G2/M arrest and apoptosis through XAB2, NER/ATM, BCDX2 pathway in ovarian cancer. HELQ inhibits osteosarcoma cell proliferation, migration, invasion and DNA repair by CHK1-RAD51 pathway. HELQ was associated with improved immuno-chemotherapy response in patients with CLL associated with activation of MYC signaling, E2F signaling, and suppression of Hedgehog and Kras signaling. DNA repair involving HELQ and RAD51C may participate in ESS occurrence and development. HELQ inhibits NSCLC cell proliferation and migration through suppressing DNA damage repair, and promotes cell death through inducing necrosis by interacting with RIPK3. HELQ, helicase POLQ-like; NER, nucleotide excision repair; ATM, ataxia telangiectasia mutated protein; CHK1, checkpoint kinase 1; RAD51, RecA-like ATP-dependent recombinase 51; CLL, chronic lymphocytic leukemia; ESS, endometrial stromal sarcoma; NSCLC, non-small cell lung cancer.

ESS. HELQ and RAD51C expression levels are decreased in ESS compared with normal endometrial tissues. HELQ expression was found to be correlated with the size and type of ESS. Neither HELQ nor RAD51C expression were correlated with age, FIGO stage or lymph node metastasis status. The occurrence and development of ESS may be affected by DNA repair involving HELQ and RAD51C (90).

Table IV. Summary of the current literature on HELQ dysregulation in different cancers.

Cancer	Deregulation	Downstream targets	Phenotypic effect	Studies	(Refs.)
Osteosarcoma	Low	CHK1↓, RAD51↓	DNA repair↓, cell invasion↑, cell migration↑, cell proliferation↑	*In vitro*	(86)
CLL	Low (CLL vs. normal B cells)	MYC↓, E2F1↓, DNA repair pathway↓, Hedgehog signaling↑, Kras signaling pathway↑	response to immuno-chemotherapy ↓, Richter transformation↓, unfavorable OS	GEO dataset	(88)
Ovarian cancer	Loss/mutation	RAD51B/C/D↓, XRCC2↓, CHK1↓	G2/M arrest↓, cellular sensitivity↑, chromosome radial formation↑	*In vitro*	(12)
High-grade serous ovarian cancer	High in platinum resistance	XAB2↑	Poor prognosis, DNA damage repair↑, platinum resistance↑, apoptosis↓	*In vitro* and TCGA database	(87)
Cisplatin-resistant ovarian cancer	High in cisplatin-resistant	NER pathway proteins (RPA32, RAP70, XPA, XPC, ERCC1)↑, ATM/ATR pathway protein↑	The cellular resistance to cisplation↑, DNA repair activity↑, Nucleotide excision repair pathway↑	*In vitro*	(13)
Non-small cell lung cancer	Low	RIPK3↓	Cell proliferation↑, cell migration↑, chemotactic response↑, DNA damage repair↓, cell necrosis↓, cell death↓	*In vitro* and *in vivo*	(89)
Endometrial stromal sarcoma	Low	RAD51C↓	DNA repair↓	Clinical tissues	(90)

CLL, chronic lymphocytic leukemia.

OV. In addition, HELQ plays a role in cellular resistance in response to ICLs and G2/M arrest, through its association with the RAD51 paralogs RAD51B/C/D and XRCC2, and promotes the activation of the ATR substrate, CHK1 (12). HELQ and XPA binding protein 2 are associated with platinum resistance, poor prognosis, decreased apoptosis and increased DNA damage repair in ascites from high-grade serous OV cells (87). In addition, HELQ decreases cisplatin sensitivity in OV cells by activating the NER and ATM/ATR pathways (13). OV patients with HELQ-low expression had improved overall and disease-free survival than those with a high HELQ level. There is evidence that HELQ can be used independently as a prognostic marker to predict survival in patients with OV (13).

CLL. CLL patients with low HELQ levels exhibited a significantly unfavorable OS compared with patients with high HELQ levels. HELQ may be useful in predicting patients at high risk for CLL based on the Richter transformation. Higher HELQ expression was also associated with an improved response to immuno-chemotherapy in patients with CLL. HELQ represented a prognostic marker for CLL associated with the activation of MYC signaling, E2 factor signaling and DNA repair pathways, as well as the suppression of Hedgehog and Kras signaling (88).

7. Pan-cancer analysis of HELQ

Pan-cancer analysis of HELQ was conducted using The Cancer Genome Atlas (TCGA) (https://tcga-data.nci.nih.gov/tcga/) and Genotype-Tissue Expression (GTEx) project (https://gtex-portal.org/) datasets. In TCGA dataset, HELQ expression was significantly lower in bladder urothelial carcinoma (BLCA), BRCA, cervical squamous cell carcinoma and endocervical adenocarcinoma (CESC), glioblastoma multiforme (GBM), colon adenocarcinoma (COAD), kidney chromophobe (KICH), kidney renal clear cell carcinoma (KIRC), kidney renal papillary cell carcinoma (KIRP), lung squamous cell carcinoma (LUSC), lung adenocarcinoma (LUAD), pancreatic adenocarcinoma (PAAD), pheochromocytoma and paraganglioma (PCPG), rectum adenocarcinoma (READ), prostate adenocarcinoma (PRAD), stomach adenocarcinoma (STAD), thyroid carcinoma (THCA), and uterine corpus endometrial carcinoma (UCEC) than that in normal tissues, while HELQ expression in cholangiocarcinoma (CHOL), esophageal carcinoma (ESCA), head and neck squamous cell carcinoma (HNSC) and liver hepatocellular carcinoma (LIHC) were significantly higher (Fig. 4A). In combined TCGA and GTEx datasets, HELQ expression levels in adrenocortical carcinoma, BRCA, BLCA, COAD, CESC, ESCA, KIRC, KICH, KIRP, LUSC, LUAD, PAAD, OV, PRAD, PCPG, READ, THCA, testicular germ cell tumors, uterine carcinosarcoma and UCEC were significantly lower than those in normal tissues, while in CHOL, GBM, lymphoid neoplasm diffuse large B-cell lymphoma, HNSC, LIHC, brain lower grade glioma, PAAD, STAD, skin cutaneous melanoma and thymoma were significantly higher (Fig. 4B).

8. Targeting HELQ for potential treatment

HELQ, a superfamily II DNA helicase, is a tumor suppressor that can mediate tumor-inhibiting activity. Furthermore,

Table V. Description of HELQ as therapeutic target at present.

Compound	Drugs/Inhibitors	Target HELQ	Mechanism	(Refs.)
Ulixertinib	A Kras signaling inhibitor	HELQ-low CLL	Inhibit the Kras signaling, negatively associated HELQ	(88)
Topotecan	A topoisomerase I inhibitor	HELQ mutants	induce one-ended DNA DSBs	(92)
Cam833	A RAD51 inhibitor	HELQ deficiency	Prevent RAD51-mediated homologous recombination	(93)
PARPi	A PARP inhibitor	HELQ deficiency	NA	(11)
Mitomycin C	ICL agent	HELQ deficiency	Inhibit replication	(11)
Camptothecin	Replication blocking agent	HELQ deficiency	Inhibit replication	(11)

HELQ, helicase POLQ-like.

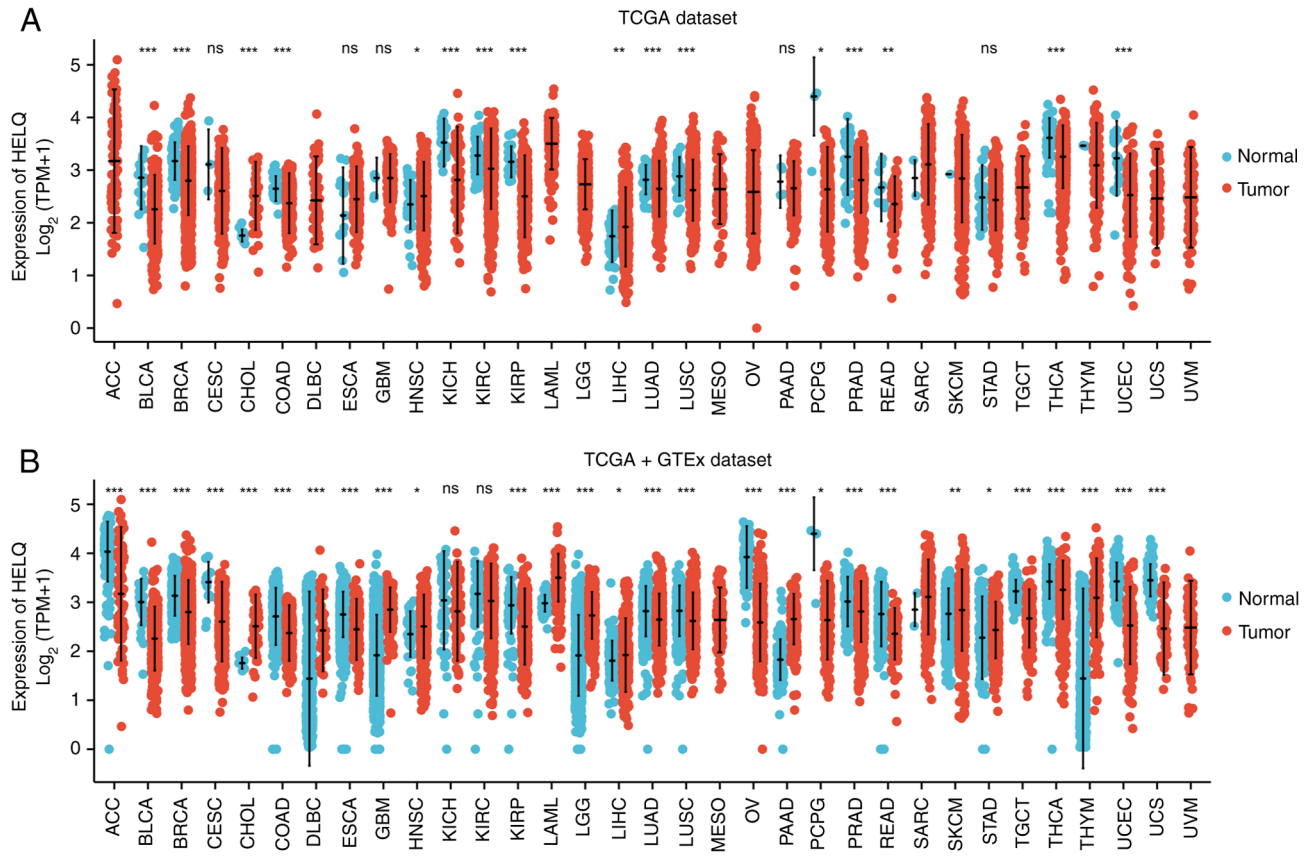

Figure 4. Pan-cancer analysis of HELQ expression in (A) TCGA dataset and (B) TCGA + GTEx combined dataset. (A) The expression of HELQ in BLCA, BRCA, CESC, GBM, COAD, KICH, KIRC, KIRP, LUSC, LUAD, PAAD, PCPG, READ, PRAD, STAD, THCA and UCEC cancer types was significantly lower than that in normal tissues, while levels in CHOL, ESCA, HNSC and LIHC were significantly higher in TCGA dataset. (B) HELQ expression levels in ACC, BRCA, BLCA, COAD, CESC, ESCA, KIRC, KICH, KIRP, LUSC, LUAD, PAAD, OV, PRAD, PCPG, READ, THCA, TGCT, UCS and UCEC were significantly lower than those in normal tissues, while levels in CHOL, GBM, DLBC, HNSC, LIHC, LGG, PAAD, STAD, SKCM and THYM were significantly higher in combined TCGA and GTEx datasets. HELQ, helicase POLQ-like; TCGA, The Cancer Genome Atlas; GTEx, BLCA, bladder urothelial carcinoma; BRCA: breast invasive carcinoma; CESC, cervical squamous cell carcinoma and endocervical adenocarcinoma; GBM, glioblastoma multiforme; COAD, colon adenocarcinoma; KICH, kidney chromophobe; KIRC, kidney renal clear cell carcinoma; KIRP, kidney renal papillary cell carcinoma; LUSC, lung squamous cell carcinoma; LUAD, lung adenocarcinoma; PAAD, pancreatic adenocarcinoma; PCPG, pheochromocytoma and paraganglioma; READ, rectum adenocarcinoma; PRAD: prostate adenocarcinoma; STAD, stomach adenocarcinoma; THCA: thyroid carcinoma; UCEC, uterine carcinosarcoma; CHOL: cholangiocarcinoma; ESCA, esophageal carcinoma; HNSC, head and neck squamous cell carcinoma; LIHC, liver hepatocellular carcinoma; OV, ovarian serous cystadenocarcinoma; TGCT, testicular germ cell tumors; DLBC, lymphoid neoplasm diffuse large B-cell lymphoma; SKCM: skin cutaneous melanoma; THYM, thymoma. *P<0.05, **P<0.01, ***P<0.001.

HELQ is pivotal in tumor proliferation, metastasis, platinum resistance, cell-cycle checkpoint regulation and DNA damage response. These important roles of HELQ in tumorigenesis highlight its potential as a target for the development of novel cancer therapeutics. Nevertheless, a few small compounds or drugs that target HELQ have been identified for potential

treatment (Table V). Kras signaling is negatively associated with HELQ expression in patients with CLL, and ulixertinib, a Kras signaling inhibitor, may offer a new therapeutic option for patients with HELQ-low CLL (88,91). Furthermore, topotecan, a topoisomerase I inhibitor, is sensitive in HELQ mutants that induce single-ended DNA DSBs in replicating cells (92). In additionally, a RAD51 inhibitor, Cam833, which disrupts the interaction between RAD51 and BRCA2, synergizes with the poly (ADP-ribose) polymerase (PARP) inhibitors (93). HR efficacy was reduced by 50-60% in HELQ knockout mice with increased sensitivity to PARP inhibitors (11). It was hypothesized that PARP inhibitors may also play a role in patients with HELQ deficiency, but further studies are required to test this hypothesis. HELQ-deficient mice and cells show hypersensitivity to the ICL agent MMC through more chromatid breaks and radial chromosomes (11). HELQ-deficient cells were also hypersensitive to CPT, a replication inhibiting agent (11).

9. Conclusions

HELQ is a DNA helicase with multiple biological functions under normal and pathological conditions. The diverse functions of HELQ, including DNA binding, DNA unwinding and DNA repair, among others, involve three HELQ protein domains: a DEAD/DEAH box helicase domain, a helicase C-terminal domain, and a HTH (HTH_61) domain. The DEAD/DEAH box helicase domain is responsible for ATP binding-mediated RNA metabolism, the helicase C-terminal domain is involved in ATP-dependent DNA or RNA unwinding, and the HTH domain is important for DNA strand binding and protein interactions. Under normal conditions, HELQ is abundantly expressed in healthy human tissues, especially in the reproductive system, where it plays an important role in maintaining germ cell viability through RAD51 paralog-dependent repair. In addition, recent studies have reported that HELQ is abnormally expressed in various cancers (86-90). HELQ is important for cell proliferation, metastasis and chemotherapy resistance by regulating various molecular signaling pathways (for example, the NER, CHK1-RAD51 and ATM/ATR pathways). HELQ deficiency is also associated with the clinical characteristics and prognosis of patients with cancer, and is considered a novel prognostic biomarker and potentially critical target in cancer therapy.

Of note, multiple biological processes involving HELQ are associated with tumorigenesis, and the protein plays a particular role in the reproductive system. Therefore, further studies focusing on the physiological roles of HELQ, as well as the detailed mechanisms underlying the relationship between HELQ and tumor occurrence under different pathological conditions, are warranted.

Acknowledgements

Not applicable.

Funding

The present study was supported by the National Natural Science Foundation of China (grant no. 81902802), the National Science Foundation of Guangdong (grant no. 2019A1515010633), the Guangzhou Science and Technology Project (grant nos. 202102010060, 202102020766 and 202201010020), and the Guangzhou Science and Technology Project of Traditional Chinese Medicine and Integrated Traditional and Western Medicine (grant no. 20232A011007).

Availability of data and materials

Not applicable.

Authors' contributions

XX and NT wrote original draft preparation. NT and WW prepared figures and tables; ZL and YW wrote review and revision; NT, XX, ZL and YW were responsible for conceptual design; YW carried out supervision and management. All authors have read and approved the final version of the manuscript. Data authentication is not applicable.

Ethics approval and consent to participate

Not applicable.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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