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REVIEW

Possible role of human ribonuclease dicer in the regulation of R loops

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R loops are three-stranded nucleic acid structures that form naturally in cells under various conditions, mainly as intermediates during replication or as by-products during transcription. R loops are involved in the regulation of many important cellular processes, including replication, transcription, centromere stabilization, protection of chromosome ends, or control of telomere length. Unscheduled R loops are linked to many diseases, including cancer, neurodegenerative, or inflammatory disorders. The list of cancer diseases linked to excessive R loop accumulation is growing rapidly. There is currently much debate about the understanding of abnormal R loop formation and its impact on genome instability and cancer development. In this review, we briefly describe the nature of R loops, their formation under physiological and pathological conditions, and the proteins involved in the regulation of R loops. In addition, we emphasize the possible role of the human ribonuclease Dicer, a multi-tasking protein mostly known for its important role in microRNA biogenesis, in the regulation of R loops. We also discuss the involvement of R loops in cancer development and their potential use as diagnostic biomarkers. Knowledge of the molecular mechanisms underlying R loop dysregulation may significantly improve our understanding of cancer biology and provide new directions for research.

R loops are three-stranded nucleic acid structures that consist of a DNA–RNA hybrid and a displaced single-stranded DNA (ssDNA) [1]. Among the physical properties of chromatin that promote the opening of the double-stranded DNA (dsDNA) and the formation of an R loop are negative DNA supercoiling, high GC content, and the breakage of chromosomal DNA. In addition, R loops can be stabilized by G-quadruplex structures (Fig. 1) [2]. R loops form naturally as intermediates during replication or as by-products during transcription. They also regulate telomere length and protect chromosome ends [3].

R loops as natural intermediates and regulators

R loops form during DNA replication initiation in mitochondrial DNA (mtDNA) [4], bacterial plasmids

Abbreviations
AID, activation-induced deaminase; ALL, acute lymphoblastic leukemia; ALT, alternative lengthening of telomere; AML, acute myeloid leukemia; CLL, chronic lymphocytic leukemia; CML, chronic myeloid leukemia; CSR, class-switch recombination; DDX5, DEAD-box helicase 5; diRNA, damage-induced small RNA; DNMT, DNA methyltransferase; DSBs, double-strand breaks; dsDNA, double-stranded DNA; dsRNA, double-stranded RNA; eRNA, enhancer RNA; hDicer, human Dicer; HOTTIP, HOXA transcript at the distal tip; HP1γ, heterochromatin protein 1γ; lncRNA, long non-coding RNA; LSD1, lysine-specific demethylase 1A; miRNA, microRNA; mtDNA, mitochondrial DNA; RAD51AP1, RAD51-associated protein 1; rDNA, ribosomal DNA; sEVs, small extracellular vesicles; siRNA, small interfering RNAs; ssDNA, single-stranded DNA; TAD, topologically associating domains; TERRA, telomeric repeat-containing RNA; THCA, thyroid cancer; Thrap3, thyroid hormone receptor-associated protein 3; TRF2, telomeric repeat-binding factor 2.

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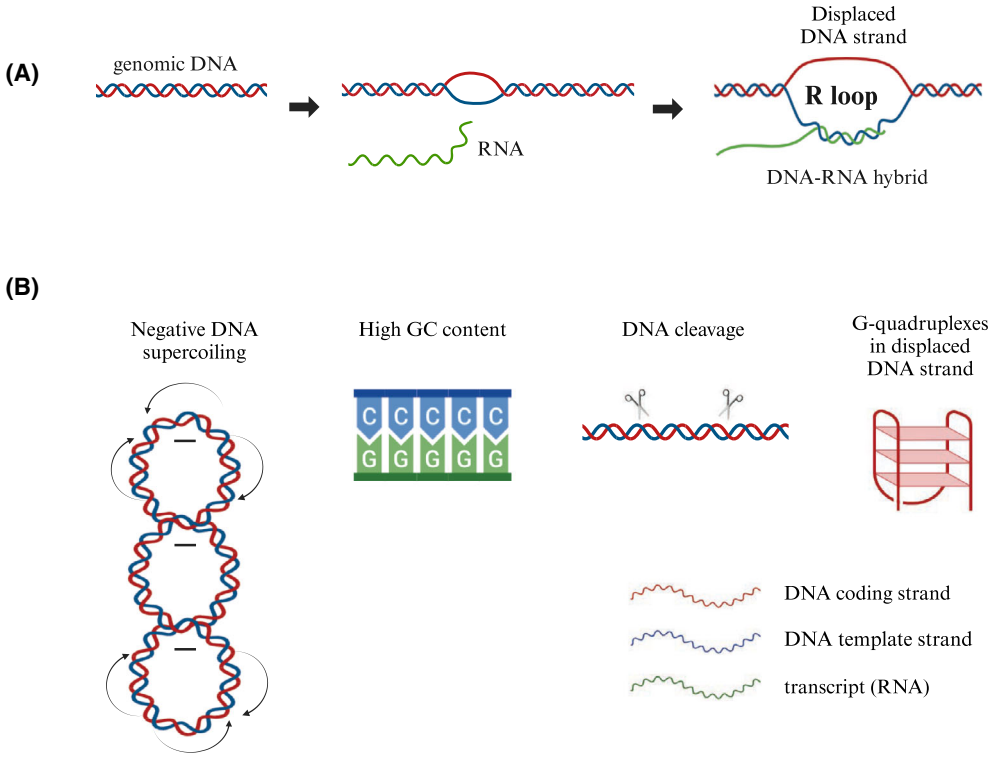

Fig. 1. Schematic representation of the R loop formation (A) and factors promoting R loop formation and stabilization (B). Figure generated in BioRender software.

[5], and some bacteriophages [6,7]. In these cases, RNA produced by the RNA polymerase serves as a primer for replication initiation. Interestingly, R loop formation has been found to regulate the copy number of mtDNA. It is known that a somatic cell contains 1000–5000 mitochondria, each with 5–10 copies of mtDNA [8]. In the case of gametes: spermatozoa contain less than one hundred mitochondria, each with a single mtDNA, and oocytes have about 100 000–400 000 mitochondria, also with a single mtDNA copy [9,10].

R loops are also involved in immunoglobulin class-switch recombination (CSR) [11,12]. CSR is important for the production of different classes of immunoglobulin by B lymphocytes after contact with a pathogen [13]. It is hypothesized that R loops boost mutation rates by generating long tracts of ssDNAs that serve as the substrate for activation-induced deaminase (AID), the enzyme that co-transcriptionally mutates ssDNA in so-called switch recombination sequences [14]. AID-induced mutations lead to DNA breakage and subsequent repair of two DNA sequences, which ultimately result in class-switch recombination [15].

R loops can also be formed by long non-coding RNAs (lncRNAs) [16]. A well-known example of such structures is a telomeric R loop mediated by a telomeric repeat-containing RNA (TERRA) [17–19]. TERRA, a lncRNA transcribed by RNA polymerase II from telomeric DNA sequences present at the ends of eukaryotic chromosomes [20,21], can invade telomeric DNA through direct base-pairing to form R loop structures. G-rich repeats (in humans, TTAGGG), single-strand breaks, and G-quadruplex structures in telomere sequences promote telomeric R loop formation. TERRA can regulate the telomeres [22–24]. The interaction between TERRA and G-quadruplexes at telomeres plays a critical role in maintaining telomere stability [25]. The shelterin complex, composed of six proteins: TRF1, TRF2, POT1, RAP1, TIN2, and TPP1, serves as the primary regulator of TERRA, playing a crucial role in maintaining telomere stability and protecting chromosome ends [18,19]. TRF1, TRF2, and POT1 bind directly to telomeric DNA sequences. The remaining three (RAP1, TIN2, and TPP1) are associated with telomeres via protein–protein interactions [18,19]. The shelterin complex is also involved in the regulation of the R loops [18,19,26]. Other examples of lncRNAs that can form R loops are enhancer RNAs (eRNAs) [27,28]. eRNAs are relatively long non-coding RNA molecules (50–2000 nucleotides) that are transcribed by RNA

polymerase II from the DNA sequence of enhancer regions. eRNAs hybridize with their corresponding DNAs to form R loop structures [29]. In this way, eRNAs may modulate the activity of the matching enhancer in target genes. It has been shown that increased level of eRNAs leads to unscheduled R loop formation and consequent genomic instability at enhancer regions [30], which is causally linked to tumorigenesis.

R loop formation may also induce antisense transcription, leading to double-stranded RNA (dsRNA) generation, followed by H3K9me2 marks and recruitment of heterochromatin protein 1γ (HP1γ) [31]. These events lead to RNA polymerase II pausing and transcription termination [31]. In addition, R loop formation may protect CpG islands within the promoter regions from DNA methyltransferases (DNMTs), which in consequence affect methylation-dependent repression of transcription [32].

R loops as transcriptional by-products

R loops are primarily transcriptional by-products and are abundant in nucleolar regions, where RNA polymerase I drives the expression of ribosomal DNA (rDNA) [1]. These sites of R loop formation are particularly critical for genome stability, as RNA polymerase I accounts for more than 60% of total transcription [33].

R loop structures form also at centromeric regions [34]. Centromeres are chromosomal fragments responsible for the proper distribution of DNA during cell division. They consist of repetitive α-satellite sequences that are intensely transcribed and form DNA–RNA hybrids. R loop structures at centromeres are recognized by and associated with BRCA1, a well-known tumor suppressor preventing the accumulation of DNA–RNA hybrids [35]. In budding yeast, unscheduled R loops at centromeric regions have been found to contribute to defects in kinetochore biorientation and chromosomal instability [34].

One report has shown the importance of active transcription, the presence of a functional poly(A) signal, and termination G-rich pause elements in the formation of R loops [36]. It has been observed that depletion of senataxin, a known RNA/DNA helicase, leads to the accumulation of R loops specifically downstream of the poly(A) signal, emphasizing a senataxin's role in resolving these structures in transcription termination regions [36]. This finding suggests that while R loops may facilitate certain aspects of transcription termination, their persistence can be detrimental, highlighting the critical role of senataxin in clearing R loops to ensure proper gene expression and maintain genomic stability [36].

R loops as potentially harmful structures

R loops may impair replication fork progression and transcription; therefore, their removal is essential to preserve genome integrity [11,37]. Replication and transcription occur during the cell cycle. Since replication and transcription use the same DNA strand as a template, the cell cycle must be tightly regulated. Conflicts between these two events can result in errors in DNA replication and/or protein synthesis, both of which are essential for normal cell division [38]. R loop formation and removal is an important mechanism regulating these two processes. When replication and transcription proceed in opposite directions, RNA polymerase II cannot function due to a collision with DNA polymerase, and R loops accumulate, whereas co-directional collision does not result in R loop accumulation [38]. R loops have been found during several stages of cell division: G1, G2, M phases, and mostly in S phase. The S phase leads to DNA synthesis, which requires DNA replication and transcription to produce multiple proteins [39]. R loops can inhibit transcription and enable DNA replication, especially during meiosis [40,41]. Thus, precise processing of R loops is crucial to maintain the balance between replication and transcription.

Displaced ssDNAs in R loop structures are susceptible to damage. Unscheduled R loops often lead to replication stress, as they can stall replication forks, potentially causing double-strand breaks (DSBs) [42]. Moreover, unscheduled R loops may disturb the balance between replication and transcription, which may contribute to genomic instability. To counteract this, cells employ a variety of enzymes involved in R loop regulation and resolving, including topoisomerases, RNA/DNA helicases, chromatin modulators, RNA processing factors, and ribonucleases [43,44]. Examples of such proteins and their roles in the cell are summarized in Table 1.

Ribonuclease dicer as a multi-tasking protein

The Dicer ribonuclease is mostly known for its important role in the biogenesis of small regulatory RNAs, microRNAs (miRNAs) and small interfering RNAs (siRNAs) [45,46]. This canonical role of Dicer is associated with its cytoplasmic localization [47]. Dicer can also function in the nucleus. For example, it is already

Table 1. Exemplary enzymes involved in the regulation of R loops.

Group	Enzyme	Role	Source
Topoisomerases	Topoisomerase I (TOP1)	Relaxes DNA supercoiling and prevents R loop formation	[118]
RNA/DNA helicases	DEAD-Box Helicase 1 (DDX1)	Plays a crucial role in the formation of R loops over immunoglobulin heavy-chain (IgH) switch regions by targeting AID (activation-induced cytidine deaminase) through a post-transcriptional mechanism. It binds to G-quadruplex structures within switch transcripts and facilitates their conversion into R loops in the switch recombination sequences (the S-regions)	[119]
	Senataxin (SETX)	Catalyzes the unwinding of DNA–RNA hybrid in R loops, promoting their resolution	[120]
Chromatin modulators	Metastasis-associated protein 2 (MTA2)	Regulates chromatin dynamics in regions where R loops are formed, promoting genome stability by controlling access to DNA	[121]
	DNA-dependent ADP-ribosyl transferase (PARP1)	Binds to R loops and initiates DNA repair processes, preventing genomic instability	[122]
	Breast cancer type 1 susceptibility protein (BRCA1)	Prevents accumulation of DNA–RNA hybrids	[35]
RNA processing factors	The conserved THO complex (THOC)	Regulates TERRA-associated R loops by binding to nucleoplasmic TERRA and reducing R loop accumulation at telomeres, thus maintaining telomere stability	[123]
Ribonucleases	RNase H1	Degrades the RNA portion of the R loops, returning the two DNA strands to dsDNA form	[124]
	RNase H2	Degrades RNA in DNA–RNA hybrids, including RNA primers and ribonucleotides erroneously incorporated into DNA. The activity of RNase H2 is mainly found during cell division, in the G2/M checkpoint. This phase of the cell cycle controls the quality of replicated DNA and allows or prevents cells from dividing	[43,125]
	RNA Exonuclease 5 (REXO5)	Plays a key role in the physiological control of R loops using its exonuclease domain	[126]
	Dicer	Cleaves the RNA strand of DNA–RNA hybrids within R loop structures	[57]
Recombinase	DNA repair protein (RAD51)	RAD51 interacts with TERRA and catalyzes R loop formation, a direct role in TERRA recruitment via strand invasion	[19,127]

known that nuclear Dicer can participate in chromatin structure remodeling [48–52], restrict the deleterious accumulation of endogenous dsRNAs [53] or contribute to DNA damage response activation by generating small non-coding RNAs, referred to as damage-induced small RNAs (diRNAs) or DDRNAs [54–56]. Interestingly, a recent study has shown that ribonuclease Dicer may be involved in nuclear R loop processing [57]. Under the *in vitro* conditions and in the cell, human Dicer (hDicer) cleaved RNA within harmful R loops, but not within the DNA–RNA hybrid without a loop, which suggested that hDicer activity was specific for the R loop structures [57]. Silencing of the human *DICER1* gene led to the accumulation of R loop structures in the nuclei, and even the overexpression of other ribonucleases involved in the R loop removal did not counteract the accumulation of R loops in the cells with downregulated *DICER1* expression [57]. It has been suggested that R loop removal by hDicer is supported by its annealing activity through facilitating DNA–RNA hybrid formation [57]. The annealing activity of hDicer was first revealed [55] and extensively characterized [56,58] in our laboratory. We demonstrated that hDicer can support hybridization between complementary sequences present in nucleic acids even when both of them are trapped within secondary structures [55,56]. Besides cleaving the RNA within the R loop structures, hDicer might influence R loops by involvement in DNA damage repair. Specifically, hDicer has been implicated in the DNA damage response and repair of DSBs in mammalian cells due to replication stress [59]. As mentioned above, during DSB stress, hDicer produces diRNAs that are essential for the repair of damaged DNA [60,61]. These small RNAs correspond to the sites of DSBs and are thought to serve as templates for efficient DNA repair [60]. In addition, hDicer can potentially interplay with other R loop-binding proteins, by direct interactions; e.g., helicase DHX9 [62–64], or indirectly;

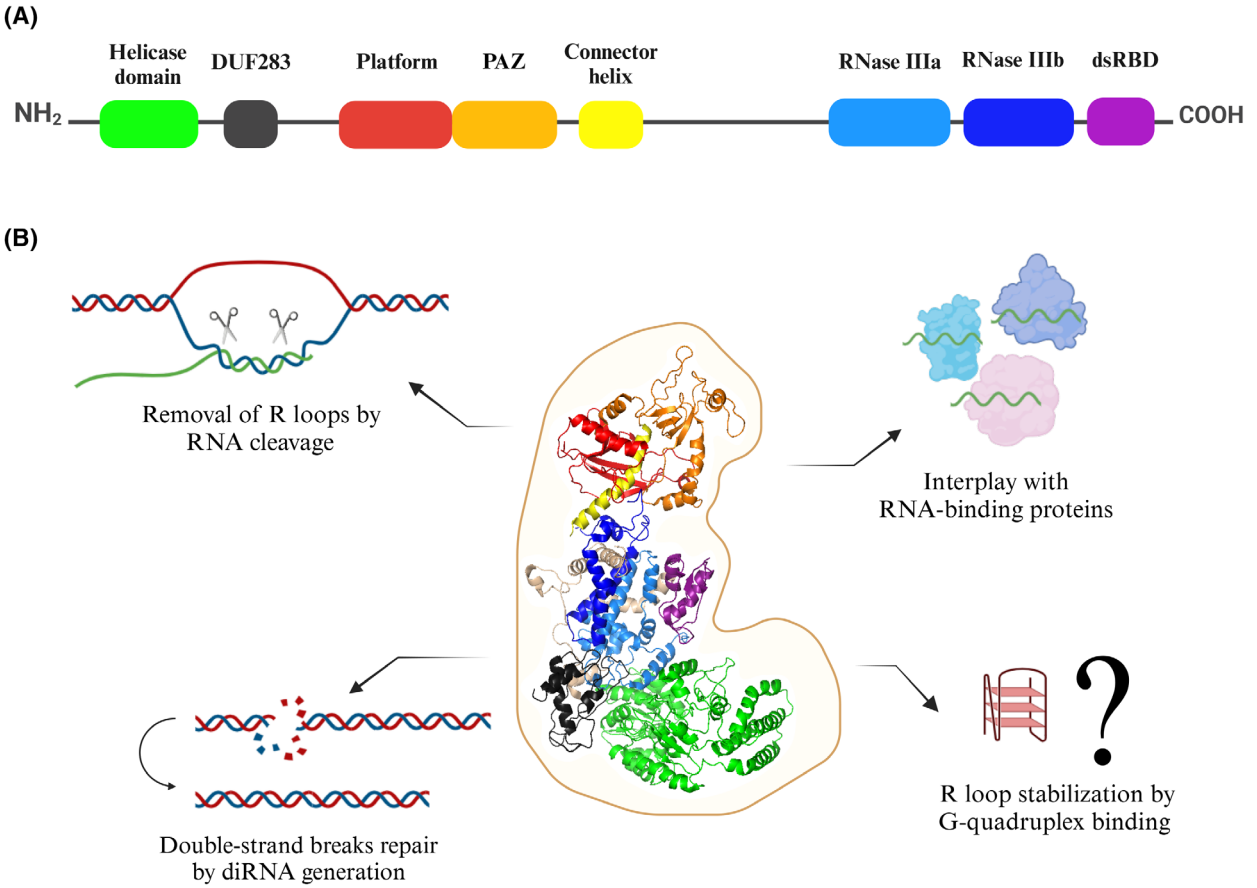

Fig. 2. Dicer as a multi-tasking protein. (A) Schematic showing the domain organization of hDicer. Domains are color-coded: helicase (green), DUF283 – a domain of unknown function 283 (dark gray), Platform (red), PAZ – Piwi-Argonaute-Zwille domain (orange), Connector helix (yellow), RNase IIIa (blue), RNase IIIb (dark blue) and dsRBD—a dsRNA-binding domain (purple). (B) A proposed Dicer's role in the R loop level regulation. The 3D structure of hDicer (PDB entry 5ZAL) [128] was visualized by PyMOL. The domains are color-coded as in (A). Figure generated in BioRender software.

e.g., BRCA2/BRCA1 [65,66], BRD4 [67,68], SETX [69,70]. Furthermore, hDicer can potentially stabilize R loops by binding to G-quadruplexes. Importantly, the results of our recent studies have indicated that hDicer binds both DNA and RNA G-quadruplexes, including TERRA, with high affinity [71]. The potential of hDicer to interact with G-quadruplexes and R loops, two structures with regulatory functions in the cell, strongly indicates that this ribonuclease is a multi-tasking protein not only involved in miRNA and siRNA biogenesis, but also in many other cellular pathways (summarized in Fig. 2).

Dysregulation of hDicer in cancer

Many cases have been reported in which abnormal *DICER1* expression was correlated with the development of carcinogenesis; reviewed in [46]. In the context of a specific cancer, disturbances in *DICER1* expression should be considered at multiple levels, including mutations in the *DICER1* gene, the level of *DICER1* expression, as well as global miRNA expression. As mentioned above, disturbed levels of hDicer may also affect the R loop accumulation in cells [57]. On the one hand, *DICER1* overexpression can cause excessive R loop removal, either directly (by RNA cleavage) or indirectly (by impaired miRNA biogenesis). On the other hand, decreased levels of hDicer can cause ineffective removal of R loops in cells, which threaten genome integrity [57]. Here, we focus on aberrant *DICER1* expression in leukemia and thyroid cancer.

Leukemia is a hematologic malignancy originating in the bone marrow, characterized by the abnormal proliferation of blood cells. Four main types of leukemia can be distinguished: acute lymphoblastic leukemia (ALL), acute myeloid leukemia (AML),

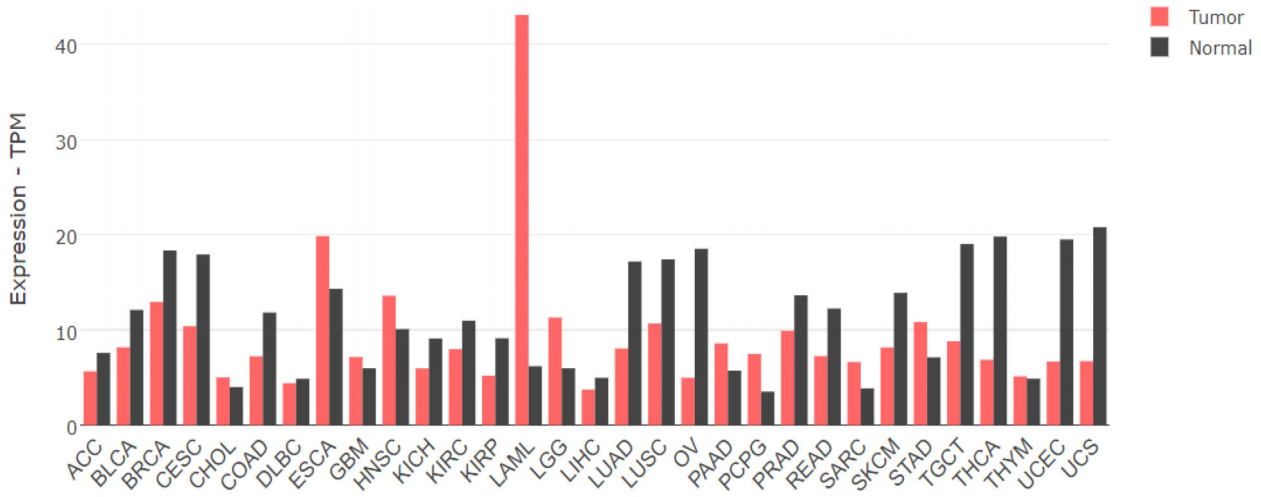

Fig. 3. Expression levels of *DICER1* in various cancers; based on the data obtained from the GEPIA2 database, which provides extensive resources for gene expression analysis derived from cancer and normal samples in the TCGA and GTEx databases [73]. ACC, adrenocortical carcinoma; BLCA, bladder urothelial carcinoma; BRCA, breast invasive carcinoma; CESC, cervical squamous cell carcinoma and endocervical adenocarcinoma; CHOL, cholangio carcinoma; COAD, colon adenocarcinoma; DLBC, lymphoid neoplasm diffuse large B-cell lymphoma; ESCA, esophageal carcinoma; GBM, glioblastoma multiforme; HNSC, head and neck squamous cell carcinoma; KICH, kidney chromophobe; KIRC, kidney renal clear cell carcinoma; KIRP, kidney renal papillary cell carcinoma; LAML, acute myeloid leukemia; LGG, brain lower grade glioma; LIHC, liver hepatocellular carcinoma; LUAD, lung adenocarcinoma; LUSC, lung squamous cell carcinoma; MESO, mesothelioma; OV, ovarian serous cystadenocarcinoma; PAAD, pancreatic adenocarcinoma; PCPG, pheochromocytoma and paraganglioma; PRAD, prostate adenocarcinoma; READ, rectum adenocarcinoma; SARC, sarcoma; SKCM, skin cutaneous melanoma; STAD, stomach adenocarcinoma; TGCT, testicular germ cell tumors; THCA, thyroid carcinoma; THYM, thymoma; UCEC, uterine corpus endometrial carcinoma; UCS, uterine carcinosarcoma; UVM, uveal melanoma.

chronic lymphocytic leukemia (CLL), and chronic myeloid leukemia (CML) [72]. Disturbances in the hDicer level have been implicated in distinct leukemia subtypes. The extremely high overexpression of *DICER1* was found in AML (in TCGA database referred to as LAML) [73–75] (Fig. 3), and reduced levels of hDicer were detected in CLL, CML, and ALL [76–79]. Interestingly, in ALL, overexpression of *DICER1* has also been reported [80], which indicates that, depending on the cancer stage, the *DICER1* expression may be affected differently.

Several reports have shown the elevated levels of *DICER1* expression in samples from the bone marrow of AML patients [74,75] and leukemia cell lines [74], confirmed on both mRNA [74,75] and protein [74] levels. However, since no difference in overall survival of AML patients with increased *versus* decreased expression of *DICER1* in bone marrow was found [75], it was suggested that the influence of *DICER1* expression on clinical outcomes may depend on the tissue of origin [75]. Moreover, it was shown that in AML bone marrow samples, the *DICER1* gene expression was upregulated by the hematopoietic transcription factor, GATA1 [74], whose mutations are highly significant in AML patients [81]. In addition, upregulation of hDicer was found in various myelomas [82,83] and T-cell lymphoma [84]. Abnormal expression of the *DICER1* gene can affect the course of diseases [85–88].

While the precise cause of leukemia remains unclear, genetic mutations and environmental factors, such as smoking, ionizing radiation, viral infections, and exposure to harmful chemicals, are believed to play a role in its development and progression. These factors contribute to DNA damage [72]. As mentioned above, hDicer is one of the proteins involved in damaged DNA repair [89,90]. Knockdown of *DICER1* has been found to reduce DSB repair [91] and increase the accumulation of unscheduled R loops [57]. It is also important to mention that loss of a single allele of *DICER1* enhances tumorigenesis (e.g., by increased amounts of oncogenic *versus* tumor suppressive miRNAs) [46], while its complete loss leads to the accumulation of DNA damage and cell death [90]. Indeed, silencing of the *DICER1* gene in AML cells inhibited cell proliferation and promoted apoptosis [74].

Reduced levels of *DICER1* expression were observed in chemotherapy-resistant CML cells [79]. In this leukemia, the rs13078 *DICER1* variant (a single nucleotide polymorphism located in the 3′-UTR of the

DICER1 gene) was extensively examined [92]. Both the minor allele frequency and the minor homozygote genotype of rs13078 were significantly more prevalent in CML patients than in healthy controls. This variant is thought to alter the interaction of miRNAs or RNA-binding proteins with *DICER1* transcripts, potentially affecting the hDicer protein level and global miRNA expression, which may drive CML pathogenesis [92].

Reduced hDicer levels have also been reported in CLL [76,77]. The *DICER1* expression analysis revealed statistically significant differences between ill and healthy samples (based on RNA isolated from 30 patients and 29 controls), where the *DICER1* gene was downregulated in the patients with CLL compared to the controls [76]. Another study has demonstrated that reduced levels of hDicer in CLL are strongly associated with shorter overall survival and reduced treatment-free survival [77]. In this context, it is also important to remember that the reduced hDicer level may impact DSB repair and R loop regulation.

A significantly reduced *DICER1* level was also observed in thyroid cancer (THCA) [93] (Fig. 3). Thyroid cancer originates from thyroid parenchymal cells and has shown a steady rise in incidence globally, although mortality rates have remained stable in recent years [72,94]. hDicer is crucial for normal thyroid gland development, and mutations in the *DICER1* gene are associated with various thyroid abnormalities [95]. A reduced level of Dicer in thyroid cancer could exacerbate the accumulation of R loops. In addition, a study from 2021 showed that thyroid hormone receptor-associated protein 3 (Thrap3) plays a critical role in regulating R loop resolution by interacting with DEAD-box helicase 5 (DDX5) [96]. Since both DDX5 and hDicer can regulate R loops, it would be of great importance to understand the connections among proteins involved in R loop regulation.

The abovementioned cases highlight the importance of understanding the molecular mechanisms underlying Dicer dysregulation and its contributions to cancer biology, particularly regarding R loop dynamics and their implications for genomic stability.

R loops, lncRNAs and cancer

The understanding of abnormal R loop formation and its impact on genome instability and cancer development is extensively discussed. The list of cancer diseases linked to the excessive R loop accumulation is growing rapidly [43]. Here, we focus on unscheduled R loops formed by lncRNAs.

It has been shown that a lncRNA HOTTIP (the HOXA transcript at the distal tip) regulates oncogene expression through topologically associating domains (TAD) formation via R loops, without causing DNA damage [72]. Mammalian chromosomes are organized within the nucleus into TADs, which support gene regulation, DNA replication, and repair [97]. TADs are dynamic structures formed by actively extruding loops that are restricted by TAD boundaries, enhancing intra-domain interactions [98–100]. HOTTIP lncRNA is abundantly expressed in AML. The HOTTIP-mediated TAD formation via R loops drives aberrant oncogene transcription and leukemia development [101].

Abnormal accumulation of TERRA-mediated R loops can interfere with telomere function, potentially leading to telomere-related dysfunction, promoting genomic instability in diseases like cancer [3]. It has also been reported that TERRA forms R loops to promote homology-directed DNA synthesis in the alternative lengthening of telomeres (ALT) pathway [102–104]. For example, TERRA contributes to ALT by recruiting the lysine-specific demethylase 1A (LSD1) to telomeres. The interaction between TERRA and LSD1 has been shown to promote R loop formation and enhance the activity of R loop regulatory molecules, supporting telomere maintenance in ALT pathways [102]. ALT can also be promoted by RAD51-associated protein 1 (RAD51AP1) [103,104]. It has been shown that TERRA R loops mediated by RAD51AP1 regulate repressive chromatin at telomeres [104]. Moreover, it has been reported that in multiple myeloma, stabilization of TERRA G-quadruplexes induced dissociation of telomeric repeat-binding factor 2 (TRF2) from telomeres, leading to the activation of the DNA damage response, cell cycle arrest, proliferation block, and apoptotic death [105].

Perspectives

There are reports suggesting that R loops can serve as diagnostic biomarkers. Their unscheduled accumulation has emerged as a promising diagnostic biomarker, with the potential to stratify patients and monitor disease severity across various conditions, including immune deficiencies like Wiskott–Aldrich syndrome [106], leukemia [72], embryonal tumors with correlation to loss of *DICER1* function [107], and glioblastomas [108], also in correlation with *DICER1* expression [109–111].

Landmark studies over the past decade have shown that small extracellular vesicles (sEVs, also referred to as "exosomes" [112]) from human blood contain genomic DNA fragments reflecting the host cell genome [111]. Notably, sEV-DNA from cancer patients

accurately mirrors the mutational status of the original tumor cells, highlighting its potential as a liquid biopsy biomarker for cancer detection and monitoring metastasis [111]. In addition, recent studies have shown the existence of microvesicles containing miRNAs and lncRNAs, which may activate multiple pathways involved in tumor development when transferred to the target cells [113–115]. The microvesicle-derived lncRNAs might as well contribute to unscheduled R loop formation. Elevated R loop levels are linked to genomic instability and tumor heterogeneity, and their presence offers prognostic value, guiding therapeutic approaches and predicting outcomes in disorders such as multiple myeloma [116] and uterine fibroids [117], particularly when analyzed alongside extracellular vesicle-derived genetic materials [111]. This could lead to a breakthrough in diagnosing diseases by utilizing easily accessible biological samples, such as blood, for rapid and accurate testing.

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Conflict of interest

The authors declare no conflict of interest.

Author contributions

AK-K conceptualized the work, KW and PK wrote the draft of the manuscript. KW prepared all figures and Table 1. AK-K revised and edited the manuscript and was responsible for its final form. All authors have read and agreed to the published version of the manuscript.

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