The research progress of circular RNAs in hematological malignancies

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ABSTRACT

Objectives: Circular RNA (circRNA), a covalently closed loop structure lacking poly-adenylated tails, has attracted attention with the rapid development of its detection techniques such as bioinformatics and RNA sequencing. CircRNA plays important roles in several cell signaling pathways that are associated with cancer biogenesis, including acting as miRNA sponges, transcriptional regulators, protein adaptors and protein translators. The role of circRNA in hematological malignancy has been revealed recently. The purpose of this study was to explore the role of circRNA in hematological malignancy.

Methods: A comprehensive literature review was conducted through PubMed to summarize the published evidence on the circRNAs in hematological malignancies. English literature sources since 1976 were searched, using the terms circRNA, hematological malignancy.

Results: CircRNAs can regulate the gene expression of hematological malignancies mainly through adsorbing several miRNAs. Some circRNAs are potential biomarkers and therapeutic targets in hematological malignancies, such as acute myeloid leukemia (AML), chronic myeloid leukemia (CML), chronic lymphocytic leukemia (CLL), and acute lymphocytic leukemia (ALL). CircRNA served as miRNA sponges. CircRNA could competitively bind to miRNA, regulated miRNA-related gene activity, and competed with endogenous RNA to bind to miR-29b-2-5p and then decreased the expression of miR-29b-2-5p and then decreased the expression of P53 which could affect cell cycle progress and apoptosis, leading to the reduced radiosensitivity of P53.

Conclusion: CircRNAs play an important biological function and have great diagnostic and prognostic value in hematological malignancies.

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of bone marrow mesenchymal stem cells. (2) CircRNA served as transcriptional regulators. Intron-containing circRNA which was often located in the nucleus could promote maternal genes expression by regulating RNA polymerase II (RNA Pol II). For example, ci-ankrd52 was mainly accumulated in the nucleus, which could promote the transcription of ANKRD52 gene by RNA Pol II [19]. (3) CircRNA served as protein adaptors. CircRNA could be activated and played regulatory roles by binding to proteins. For example, circRNA interacted with RNA binding proteins (RBPs) which were involved in target gene transcription and translation and extracellular transport [20]. (4) CircRNA served as protein translators. Although circRNA was previously considered to be unable to be translated into proteins due to its lacking 5′-3′ end, there was potent evidence showing that circRNA had the function of encoding proteins. Researchers have demonstrated that circRNA with infinite reading frames could be used in the translation systems of prokaryotic and eukaryotic cells by simulating DNA rolling cycle amplification (RCA) [17]. For instance, circ-ZNF609, a functional circRNA that controlled the proliferation of myoblasts, contained an open reading frame that could be translated into proteins in a splice-dependent and cap-independent manner [21].

CircRNAs in AML

Increasing evidences suggested that circRNA were closely related with the pathogenesis, maintenance and progression of AML. CircRNA played important roles in the regulation of gene expression mainly by adsorbing various microRNA (miRNA). The dysfunction or silencing of miRNA was associated with the occurrence of leukemogenesis [22]. Furthermore, miRNA was involved in the post-transcriptional regulation of AML and activated the downstream signal cascade, indicating that circRNA might be the new targets for AML treatment. Some circRNAs associated with AML have been found, including circ-ANAPC7, circ-DLEU2, circ-PAN3, hsa_circ_0004277, hsa_circ_0075001 and circ-HIPK2, etc.

Circ-ANAPC7 was produced at the ANAPC7 gene site. The circ-ANAPC7–miR-181 axis was recently confirmed in AML. Chen et al. [7] explored circRNA expression profile in the bone marrow from AML patients. They found that 698 circRNAs were dysregulated in AML, indicating that circRNAs potentially participated in AML pathogenesis. Additionally, the circ-ANAPC7 expression level was significantly increased in AML, suggesting that it could be a carcinogenic circRNA. Circ-ANAPC7 was demonstrated to adsorb the miR-181 family miRNAs and then blocked their biological effects including regulating the development of immune cells [23]. The miR-181 could not only inhibit BCL2 and MCL1 [24,25] but also regulate the Akt and NFKB signaling pathways [26,27], promoting tumor cells death and inhibiting the generation of AML. Therefore, the pathogenesis and prognosis of AML were closely related to the dysregulation of miR-181 family miRNAs, and the circ-ANAPC7–miR-181 axis played an important role in regulating AML cell activity, which provided a novel therapeutic target for AML.

Circ-DLEU2 was originated from DLEU2 locus which was shown to induce adult cancers and leukemogenesis. Wu et al. [8] revealed that the expression of circ-DLEU2 in AML bone marrow cells was higher than healthy controls, but there was no significantly statistical difference of circ-DLEU2 expression among different risk stratifications of AML patients. Mechanistically, circ-DLEU2 acted as a miRNA sponge to competitively inhibit the activity of miR-496 which played an antagonistic role in PRKACB-related AML cell proliferation and apoptosis. The PRKACB, a kind of protein which was needed in the cell growth process, was highly expressed in AML [28]. The PRKACB expression in AML cells was negatively associated with miR-496 and promoted by circ-DLEU2, thus accelerating the development of AML. Circ-DLEU2 might act as a diagnostic marker and AML therapeutic target via inhibiting the miR-496/PRKACB axis.

Circ-PAN3 was derived from the PAN3 gene and recently raised wide concern mainly due to its close relation with the complex karyotypes of AML [29]. Jin et al. [9] found that the expression of 49 circRNAs

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**Table 1. CircRNAs in hematologic malignancies.**

| Disease | CircRNA | Expression level | Function | Mechanism | References |
|---------|---------|------------------|----------|-----------|------------|
| AML     | Circ-ANAPC7 | Upregulated | Unknown | To promote cell proliferation and inhibit cell apoptosis | Sponge miR-181 family | [7] |
|         | Circ-DLEU2 | Upregulated | Unknown | To promote cell proliferation and inhibit cell apoptosis | Sponge miR-496; upregulate PRKACB | [8] |
|         | Circ-PAN3 | Upregulated in R/R AML simples | To mediate chemo-resistance | Sponge miR-153-5p and miR-183-5p; upregulate XIAP | [9] |
|         | Hsa_circ_0004277 | Downregulated | Unknown | Unknown | Deregulate the TLR pathway | [10] |
|         | Hsa_circ_0075001 | Upregulated in total NPM1-expressed AML | To increase ATRA-induced differentiation of APL cells | Sponge miR-124-3p | [12] |
|         | Circ-HIPK2 | Deregulated in APL cells | To promote cell proliferation and inhibit cell apoptosis | Upregulated the ABL1 and BCR-ABL1 protein expression | Sponge miR-29b | [13] |
| CML     | Circ-BA9.3 | Upregulated in TKI-resistant cells | To promote cell proliferation and inhibit cell apoptosis | Sponge miR-607; upregulate FZD3; activate Wnt/β-catenin pathway | [14] |
| CLL     | Circ-CBFB | Upregulated | To promote cell proliferation and inhibit cell apoptosis | | [15] |

**CircRNAs in hematologic malignancies.**

CML Circ-BA9.3 Upregulated in TKI-resistant cells To promote cell proliferation and inhibit cell apoptosis Sponge miR-124-3p [12]

**References:**

[1] Chen et al. [7]
[2] Wu et al. [8]
[3] Jin et al. [9]
was significantly different between naive AML cells and doxorubicin-resistant cells by a high-throughput circRNA microarray, indicating circRNA was involved in AML drug-resistance. Circ-PAN3 ranked higher in these upregulated circRNAs, which was consistent with the verification results of bone marrow specimens from AML patients. Inhibiting circ-PAN3 by siRNA significantly improved the chemosensitivity of drug-resistant cells. Furthermore, Circ-PAN3 was identified to be negatively correlated with miR-153-3p and miR-183 and targeted XIAP by GO and KEGG analysis. MiR-153-3p inhibited leukemia cells reproduction, invasion and promoted cells apoptosis, while miR-183 enhanced leukemia cells transformation from G1 to S phase and prevented cells death [30,31]. XIAP, a class of protein involved in ribosomal biogenesis, apoptosis and cell proliferation, might function mainly via adsorbing miR-124-3p which had a close association with cells differentiation. Previous studies have found that miR-124a could act on the CEBPA through covalent bind and inhibit the protein expression [37]. Therefore, circ-HIPK2 could increase the CEBPA levels by adsorption of miR-124-3p to promote cells differentiation. Circ-HIPK2 was produced by the proto-oncogene HIPK2 which promoted the occurrence and development of AML [36]. Li et al. [12] found that the expression of circ-HIPK2 was much higher in healthy controls and other AML types than acute promyelocytic leukemia (APL) patients. Circ-HIPK2 could promote APL cell differentiation induced by ATRA, and its expression was positively correlated with total NPM1 expression in AML. The expression of hsa_circ_0004277 was much lower in newly-diagnosed and relapsed/refractory AML patients than the complete-remission(CR) AML patients and control groups. Hsa_circ_0004277 could act as a prognostic marker of AML. Furthermore, cytoscaper analysis found that some miRNAs and genes related to hsa_circ_0004277, such as SH3GL2 and PPARGC1A, which deserved further investigation. The date above suggested that hsa_circ_0004277 was closely related to the diagnosis and prognosis of AML patients and could be used as a biomarker and therapeutic target.

Hsa_circ_0075001 was a novel circRNA positively related to the total NPM1 expression in AML. The NPM1 not only encoded a multifunctional chaperone protein involved in ribosomal biogenesis, apoptosis and cell proliferation but also facilitated AML occurrence due to its impaired or enhanced function [33,34]. Hirsch et al [11] detected hsa_circ_0075001 expression in a cohort of NPM1 wild-type and mutated AML patients (n = 46). Hsa_circ_0075001 expression was positively correlated with total NPM1 expression and was closely associated with the lower expression of those genes related to the TLR signaling pathway, which would affect the survival of AML cells [35]. This was consistent with the results that high hsa_circ_0075001 expression was accompanied by low TLR gene expression as well as the more immature phenotype of AML blasts. Furthermore, the expression of various known miR-181 target genes was obviously reduced in hsa_circ_0075001 highly expressed patients. Since the NPM1 gene contained the miR-181 binding sites, circular NPM1 transcripts interacted with members of the miR-181 family and thus influenced the expression of genes involved in the TLR signaling pathway. Hsa_circ_0075001 expression was one of the decisive factors correlated with deregulated TLR signaling pathway and could serve as potential biomarkers for classification and risk stratification of AML.

Circ-HIPK2 was produced by the proto-oncogene HIPK2 which promoted the occurrence and development of AML [36]. Li et al. [12] found that the expression of circ-HIPK2 was much higher in healthy controls and other AML types than acute promyelocytic leukemia (APL) patients. Circ-HIPK2 could promote APL cell differentiation induced by ATRA, and its expression was positively correlated with total NPM1 expression in AML. The expression of hsa_circ_0004277 was much lower in newly-diagnosed and relapsed/refractory AML patients than the complete-remission(CR) AML patients and control groups. Hsa_circ_0004277 could act as a prognostic marker of AML. Furthermore, cytoscaper analysis found that some miRNAs and genes related to hsa_circ_0004277, such as SH3GL2 and PPARGC1A, which deserved further investigation. The date above suggested that hsa_circ_0004277 was closely related to the diagnosis and prognosis of AML patients and could be used as a biomarker and therapeutic target.

CircRNA microarray analysis revealed that several miRNAs and genes were dysregulated in AML. Chen et al [38] detected the expression of these circRNAs in AML and indicated that differentially expressed circRNAs were significantly dysregulated in AML with EMI. Exons-derived circRNAs accounted for 84.77% of those detected differentially expressed circRNAs, of which 17 circRNAs were related to the activated biological processes of EMI including infiltrating other sites through cells migration. The study also found that several miRNAs and 9 target genes corresponding to the 17 circRNAs were associated with AML prognosis. The special function of circRNAs made it an early, quick and accurate diagnostic biomarker of AML.

CircRNAs in CML

Chronic myeloid leukemia (CML) was characterized by the BCR-ABL1 fusion gene [40], which has been demonstrated to be related to circ-BA9.3. Additionally, hsa_circ_0080145 was confirmed to be involved in the CML abnormal hematopoiesis [41].

Circ-BA9.3, an fusion circRNA which contributed to oncogenic transformation, was derived from the BCR-ABL1 fusion gene [13]. The mutual translocation of chromosomes 9 and 22 resulted in the BCR-ABL1...
oncogene, which was a primarily diagnostic marker for CML [42]. CircBA9.3, as one of the transcriptional attachments of BCR-ABL1, might vest CML cells with stronger carcinogenicity and TKIs resistance. Pan et al. [13] have confirmed that circBA9.3 could effectively promote CML cells proliferation and inhibit its apoptosis by raising c-ABL1 or BCR-ABL1 protein levels. Additionally, circBA9.3 expression was increased in some TKI resistant CML patients, which had a positive correlation with BCR-ABL1 expression level. CircBA9.3 also activated the tyrosine kinase, and promoted the occurrences of TKI resistance. Therefore, circBA9.3 was likely to be a targeted option for TKI-resistant CML patients.

Hsa_circ_0080145, common in fetal fibroblasts, was the most differently expressed circRNA in CML [43]. Liu et al. [14] found that hsa_circ_0080145 was significantly upregulated in CML patients. Hsa_circ_0080145 knockdown by siRNA significantly inhibited the proliferation of CML cells in vitro, which could be rescued by suppressing miR-29b. They demonstrated that hsa_circ_0080145 acted as a miR-29b sponge to accelerate the development of CML. In conclusion, these results verified that hsa_circ_0080145 might be a valid prognostic marker of CML patients and provided a new therapeutic research direction.

CircRNAs in CLL

Circ-CBFB, an oncogene in CLL, was generated by the CBFB gene. Xia et al. [15] found that the circ-CBFB expression was obviously higher in CLL groups than control groups, which corresponded to a significantly short survival time, indicating that circ-CBFB was of great value in the CLL diagnosis and prognosis. Functionally, circ-CBFB facilitated CLL cells reproduction and inhibited cells death, which could be weakened to block cells in G0/G1 phase by knocking out circ-CBFB. Mechanistically, circ-CBFB could enhance the expression level of FZD3 by adsorbing miR-607. The Wnt/β-catenin pathway was then activated, which had a positive relation with CLL progression [44]. Taken together, the circ-CBFB related signaling pathway played a vital role in CLL cells progression and made it a potential therapeutic target.

Conclusions

CircRNA was a closed circular non-coding RNA formed by reverse splicing of the precursor RNA, which was involved in various biological processes by serving as miRNA sponges, transcriptional regulators, protein adaptors and protein translators. Studies have shown that several circRNAs regulated the gene expression of hematologic malignancies mainly by adsorbing miRNAs. CircRNA research has become a hotspot and has been involved in CML, AML and CLL as regarding to the field of hematologic tumors. This tip of the iceberg has made circRNA a promising candidate not only as valuable biomarkers for hematological malignancies but also as potential therapeutic targets.

Disclosure statement

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