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POSTER ABSTRACTS

621.LYMPHOMAS: TRANSLATIONAL-MOLECULAR AND GENETIC

Extrachromosomal Circular DNA Drives the Progression of DLBCL through Activating the Sting Pathway

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Objective
Chromosome instability (CIN) as a hallmark of cancer is correlated with the prognosis of patients with diffuse large B cell lymphoma (DLBCL). However, little is known about how it drives the tumorigenesis and progression. DNA damage affects CIN and accumulated cytosolic DNA which activates the DNA sensing machinery cGAS-STING pathway in a sequence-independent manner. Extrachromosomal circular DNA (eccDNA), a type of circular DNA that derived from genomic DNA is independent of chromosomes. Existing widely in nature, eccDNA could be involved in tumorigenesis, heterogeneity and drug resistance. The purpose of this study is to reveal the expression profile of eccDNA in DLBCL, and to explore how eccDNA promotes the occurrence and development of the disease through the cGAS-independent STING pathway.
Methods
The expression profile of eccDNA in DLBCL was detected through multi-omics sequencing analysis including circular DNA sequencing (circle-seq), single-cell sequencing (scRNA-seq), whole exome sequencing (WES) and atomic force microscopy. CCK8, scRNA-seq, etc. were used to reveal the activation of eccDNA on the STING pathway to promote cell proliferation. In vivo and in vitro models were treated with chemotherapeutic drugs to verify the hypothesis that DNA damage induces the production of eccDNA, thereby activating the cGAS-independent STING pathway. GEO databases were applied to verify the prognosis of eccDNA-related gene sets, animal models to explore the anti-tumor effect of DNA damage therapy combined with STING inhibitors.
Results
Through the integration of multi-omics analysis results of 18 DLBCL cell lines, we divided DLBCL cells into two groups with high (H group) or low (L group) eccDNA abundance separately and confirmed that cells with high wGII were accompanied by abundant eccDNA. It suggested the potential roles of eccDNA in prognostic evaluation. Subsequent research revealed that eccDNA was upregulated in patients with DLBCL especially in R/R DLBCL. Pseudo-time analysis indicated that cells developed in two different differentiation directions. GSEA analysis demonstrated that high abundance of eccDNA contributed to the cell proliferative potential. To verify the role of eccDNA in cell proliferation, eccDNA extracted from H group cells were transfected into L group cells. As a result, cells transfected with eccDNA showed a significantly higher rate of propagation. These results confirmed that eccDNA had the promotional effects on proliferation DLBCL cells. GEO databases (GSE31312, GSE10846 and GSE87371) were applied to verify the prognosis of eccDNA-related gene sets. Gens that upregulated in H group was selected to construct a panel and overall survival analysis showed that patients with high levels of the panel had a worse prognosis.
DNA damage repair mechanism is a key component to maintain the stability of genome. Numerous studies have suggested that DNA damage repair is conducive to the generation of eccDNA. Here, we found that cells with serious DNA damage degree were accompanied with increased γH2AX levels. EccDNA was induced with the treatment of DNA damage agents

(cisplatin, doxorubicin, irinotecan and olaparib). Meanwhile, the levels of p-STING and downstream proteins were significantly upregulated. This phenomenon was also confirmed with immunodeficient and immunocompetent mice. H-151, the inhibitor of STING showed effective inhibitory effect on DLBCL through in vitro and in vivo assay. cells with high eccDNA abundance, and the results were further confirmed with scRNA-seq analysis. The combinational effects of STING inhibitor with DNA damage agents were observed. These results suggested that cells with high eccDNA abundance were more sensitive to the inhibition of STING pathway and the combined use of chemotherapy drugs and STING inhibitors can significantly enhance the anti-tumor effect.

Conclusion

DLBCL is a highly heterogeneous aggressive non-Hodgkin's lymphoma. STING plays a double-edged role in tumor development and immune regulation. However, its role in DLBCL and related mechanisms have not been reported so far. Our study reveals the expression, function and clinical significance of eccDNA in DLBCL for the first time, clarifies the mechanism of eccDNA-mediated STING pathway activation in disease progression and drug resistance, and provides a new theoretical basis for clinical treatment and prognosis evaluation of DLBCL.

Disclosures No relevant conflicts of interest to declare.

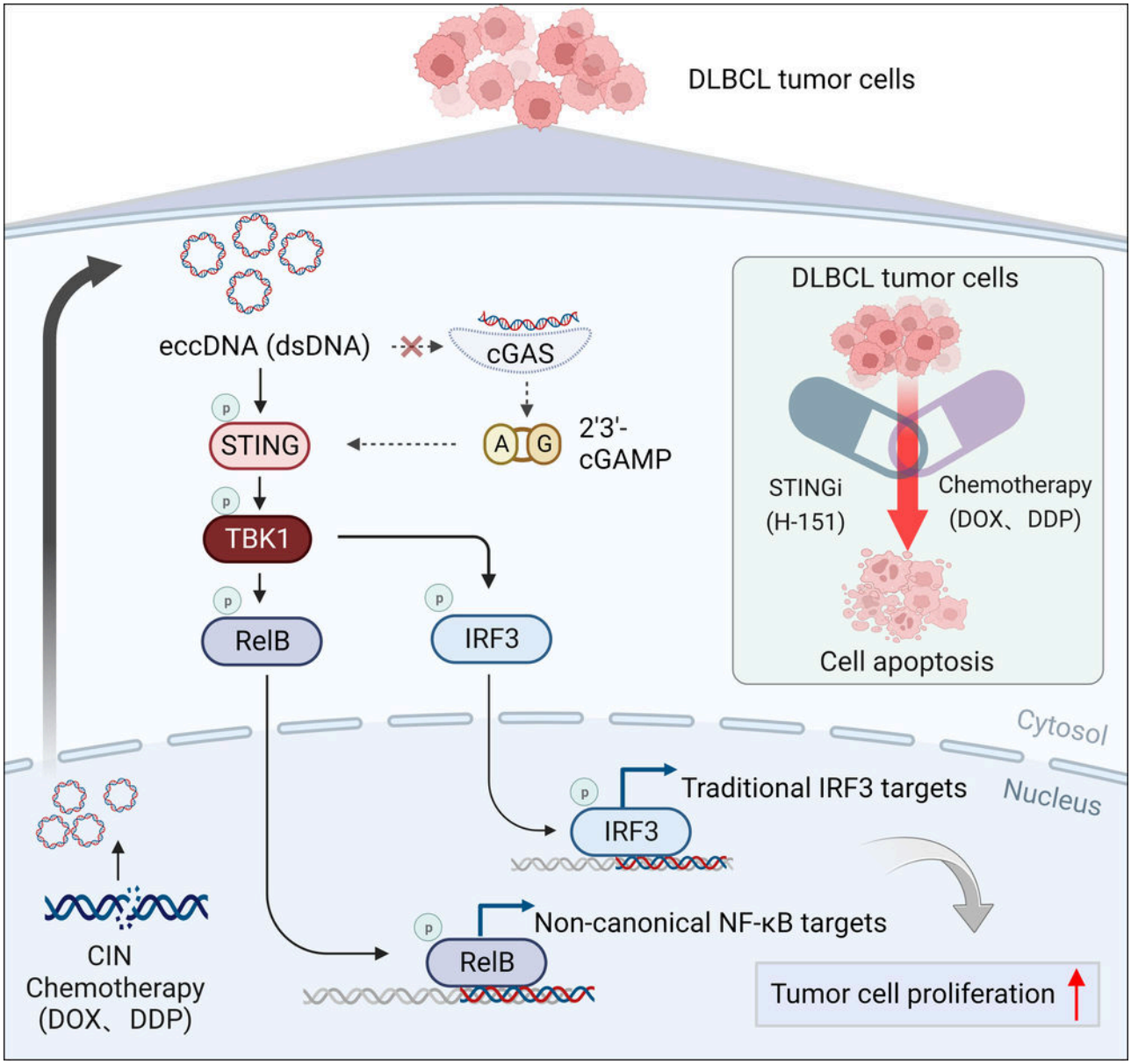

Figure 1

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