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POSTER ABSTRACTS

621.LYMPHOMAS: TRANSLATIONAL-MOLECULAR AND GENETIC

NCAPD3 Prompts Diffuse Large B-Cell Lymphoma Progression through Modulating SIRT1 Expression in H3K9 Monomethylation-Dependent Manner

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Chromosomal instability, including number and structure abnormality, is the critical molecular basis in lymphoid oncogenesis. Condensin, a family of structural maintenance of chromosome complexes, has been shown to regulate chromosome compaction and segregation during mitosis. NCAPD3, a HEAT-repeat subunit of condensin II, plays a dominant role in condensin-mediated chromosome dynamics, whose abnormalities are related to tumorigenesis and developmental disorders but remain unexplored in lymphoma. This study aims to unravel the expression, molecular function and mechanism of NCAPD3 in diffuse large B-cell lymphoma (DLBCL).

We first elucidated the expression of NCAPD3 in DLBCL. Lymph node tissues from 70 DLBCL patients and 35 reactive hyperplasia cases were collected with informed consent approved by the Medical Ethical Committee of Shandong Provincial Hospital. Higher NCAPD3 expression was identified in DLBCL tissues and correlated with advanced Ann Arbor stage and higher IPI score. The survival analysis presented patients with higher NCAPD3 suffered shorter survival (P=0.045). Meanwhile, upregulation of NCAPD3 was validated in DLBCL cells compared with normal CD19+ B-cell.

We further explored the biological functions of NCAPD3 in DLBCL and constructed NCAPD3-knockdown, knockout and overexpression models. Deficiency of NCAPD3 impeded cell proliferation, induced apoptosis and increased the sensitivity to doxorubicin and ibrutinib. Instead, NCAPD3 overexpression facilitated cell proliferation and therapeutic resistance. In vivo experiments further indicated that xenograft DLBCL mice with deficient NCAPD3 presented delayed tumor growth. These suggested NCAPD3 promoted DLBCL progression.

Strikingly, we found knocking down NCAPD3 disturbed the mitosis, triggering the formation of aneuploidy, due to its function in chromatin structure and dynamics. Hence, we further portrayed the transformation of chromosome architecture under different NCAPD3 expression. Chromosome spreads exhibited chromosomes became slim and elongated upon NCAPD3 overexpression, instead, NCAPD3 knockout triggered short and highly-curled transformation with the global upregulation of H3K4me3, an indication of transcriptional activation and chromatin decompaction (Figure 1). This suggested NCAPD3 could modulate transcriptional activity.

Therewith, we investigated how NCAPD3 regulated gene transcription. We examined the interacting proteins of NCAPD3 by mass spectrometry and Clusters of Orthologous Groups analysis revealed they were mainly enriched in RNA processing and modification, chromatin dynamics and cell cycle control. Of them, transcription factor, GTF2I, may be responsible for NCAPD3-mediated transcription regulation. Coimmunoprecipitation (co-IP) verified their interaction and chromatin immunoprecipitation (ChIP) presented they were both located on the promoter of target gene, SIRT1. We further examined SIRT1 expression after regulating NCAPD3 expression, showing a significantly positive correlation. This suggested NCAPD3 could support SIRT1 transcription via being anchored by GTF2I on the promoter of SIRT1.

We further elucidated the potential mechanism how NCAPD3 regulated SIRT1 expression. Previous study found that NCAPD3 could interact with histone methylation markers in mammalian genome, thus, we explored whether NCAPD3 could modulate the SIRT1 expression via recognizing the histone methylation marker on SIRT1 promoter. Co-IP assay identified the interaction between NCAPD3 and monomethylation of lysine residue K9 of histone H3 (H3K9me1), an active promoter tag, in DLBCL and ChIP assay verified the location of H3k9me1 on SIRT1 promoter. When NCAPD3 downregulated, H3K9me1 on SIRT promoter diminished, ruining the transcription of SIRT. To clarify whether the oncogenic effects of NCAPD3 was mediated by SIRT1, we conducted functional reversion assays, showing regulating SIRT1 partially reversed the effects of NCAPD3. The above suggested NCAPD3 prompted DLBCL progression through modulating SIRT expression in H3k9me1-mediated manner.

In conclusion, this study unraveled that dysregulation of NCAPD3 could disturb chromosome compaction and segregation, induce genome instability and alter the transcription of SIRT1 via modulating H3k9 monomethylation, which provided novel insights into NCAPD3-based targeting strategy for DLBCL.

Disclosures No relevant conflicts of interest to declare.

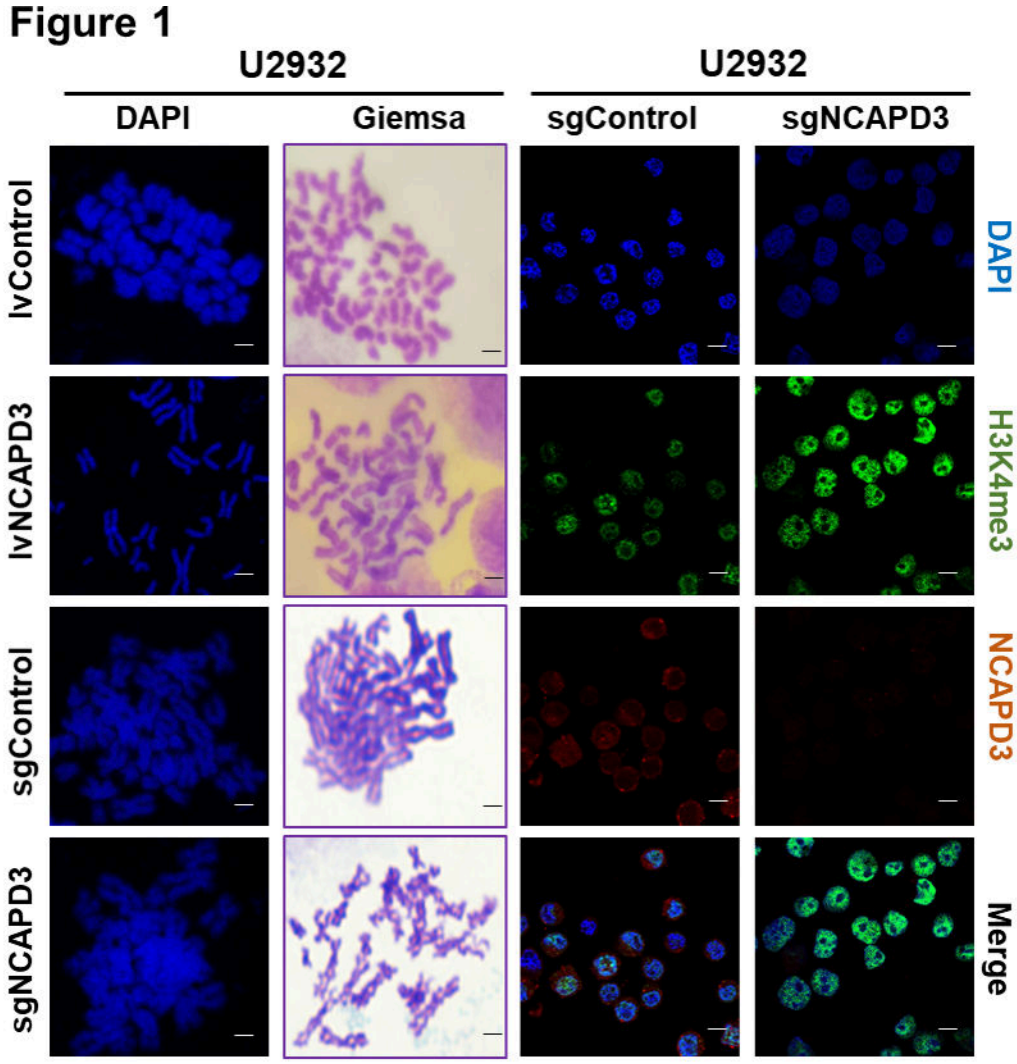

Figure 1

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