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POSTER ABSTRACTS

602.MYELOID ONCOGENESIS: BASIC

Phase Separation Mediates RUNX1- Mutation Leukemic Transformation

Xiuhua Su[1], *Tao Sun*[1], *Yuan Xia*[1], *Mingying Li*[1], *Dongmei Wang*[1], *Fei Lu, MD*[2], *Jingjing Ye*[1], *Chunyan Ji, MD PhD*[2]

[1]Department of Hematology, Qilu Hospital, Cheeloo College of Medicine, Shandong University, Jinan, China
[2]Department of Hematology, Qilu Hospital, Cheeloo College of Medicine, Shandong University, Jinan, China

B ackground :
The high degree of genetic heterogeneity in acute myeloid leukemia (AML) leads to refractory or relapse in some patients, so it is important to deepen the mechanistic studies and find specific drug targets.As a classical regulatory molecule associated with AML, the absence or down-regulation of RUNX1 can lead to the development of AML. Liquid-liquid phase separation is closely related to the occurrence and development of the disease, and it is not clear whether RUNX1 can participate in the pathogenesis of AML through phase separation. This study is the first to explore the involvement of RUNX1 in the pathogenesis of AML from the perspective of phase separation.

Methods:
The fluorescent expression of RUNX1 was detected by immunofluorescence. The extracellular phase separation of RUNX1 and the mutants were studied by prokaryotic expression of the target protein RUNX1. The intracellular phase separation of RUNX1 was studied by cellular transfection and live-cell imaging. The cellular differentiation ability after phase separation of AML cell lines was investigated by using Rachel Giemsa staining and flow cytometry. The specific mechanism of RUNX1 phase separation regulation of downstream target molecules was examined by Co-IP coupled with mass spectrometry and Western Blot. The effect of RUNX1 phase separation on the pathogenesis of AML was studied by using the AML mouse model.

Results:
In this study, we firstly confirmed that RUNX1 showed obvious spot aggregation during hematopoietic differentiation. We then confirmed that RUNX1 can perform liquid-liquid phase separation both in vitro and intracellularly. Furthermore, based on the structural domain of RUNX1 and the RUNX1 mutation sites in AML patients, it was confirmed that the IDR region of RUNX1 drove occurrence of phase separation, while the RHD region had an inhibitory effect on phase separation, and the RUNX1 point mutants associated with poor prognosis of AML had abnormal phase separation.The occurrence of phase separation of RUNX1 could regulate the differentiation function of AML cells, and the differentiation of AML cells in the phase separation-deficient type was inhibited. Transcriptome sequencing results showed that RUNX1 phase separation promoted the enrichment of signaling pathways such as cell differentiation, and it was also found that RUNX1 phase separation promoted downstream target molecules to participate in the regulation of AML cell function. In addition, we demonstrated that aberrant RUNX1 phase separation was involved in the pathogenesis of AML in the mouse model of AML.

Conclusion:
This study reveals for the first time that RUNX1 liquid-liquid phase separation is involved in the process of AML occurrence and development, and refines the functional regulation mechanism of RUNX1, providing new ideas for AML targeted therapy.

Disclosures No relevant conflicts of interest to declare.

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