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ORAL ABSTRACTS

602.MYELOID ONCOGENESIS: BASIC

Trisomy 8 Remodels Chromatin and Activates Transcription of Runx1-Target Genes in Hematopoietic Stem Cell

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Myelodysplastic syndrome (MDS) is a poor prognosis cancer that predominantly affects the elderly, arising from hematopoietic stem cell (HSC), resulting in bone marrow failure and predisposition to acute myelogenous leukemia (AML). It has long been known that numerical chromosome anomalies such as trisomy 8 are critical for the development of MDS. Trisomy 8 is known to be associated with relatively poor prognosis, and is a criterion for the diagnosis of MDS in patients. Systemic inflammation has been implicated in the selective advantage for clonal hematopoietic stem cells and the progression of MDS, such as trisomy 8 MDS associated with Behcet's disease. Next generation sequencing studies revealed that trisomy 8 MDS cells highly mutated *RUNX1* transcriptional factor (TF) and *ASXL1*, an epigenetic modifier, both of which regulate HSC function and the differentiation, relative to MDS cells without trisomy 8, suggesting that those mutations may help trisomy 8 to drive the development of MDS. However, previous clinical studies failed to identify genes on the chromosome 8 commonly amplified among trisomy 8 MDS patients, which may account for the pathogenesis of MDS, the molecular mechanism of development of trisomy 8 MDS remains unclear. Thus, we hypothesized that trisomy 8 initiates the transformation by multiple malfunctions of epigenetic and transcriptional regulators in HSC other than a gene dosage effect due to the extra chromosome 8.

In order to determine how trisomy 8 affects hematopoiesis, we established a new trisomy 8 model by introducing a human chromosome 8 to mimic trisomy 8 in mouse ES cells. We succeeded in generating trisomy 8 chimeric mice and confirmed the mice showing emergence of hematopoiesis in fetal liver but significantly decreased the chimerism of trisomy 8 cells than the control mice. Wecompetitively transplanted trisomy 8 fetal liver cells at E14.5 into lethally-irradiated wild-type primary recipient mice, and moreover serial transplanted hematopoietic stem and progenitor cells (HSPCs) which isolated from the primary recipient mice. We found that trisomy 8 reduced the self-renewal capacity of HSPC and impeded the differentiation, but it was not sufficient to develop myeloid malignancies in mice.

To elucidate the molecular mechanism underlying the impaired hematopoiesis by trisomy 8, we performed RNA sequencing in HSCs isolated from the primary transplanted mice. Gene set enrichment analysis revealed that in comparison with the control HSCs, trisomy 8 HSCs had significantly positive enrichments in type I and type II interferon response genes, but also the canonical target genes of Runx1 and PU.1 TFs were positively enriched in trisomy 8 HSCs. In addition, ATAC-seq analysis revealed that trisomy 8 HSCs changed chromatin accessibility enriched with the binding motif of Runx TF, compared to control HSCs. Given the changes in chromatin accessibility in trisomy 8 HSC, we performed chromatin conformation capture sequencing (Hi-C) in HSPCs and found that trisomy 8 induced changes in chromatin structures such as topologically associating domain (TAD) and compartment A/B in the other chromosomes, which were partly associated with changes in transcription of genes.

Since the Runx1 gene is critical for HSC integrity and differentiation, and loss-of-function mutations of *RUNX1* were often found in human myeloid malignancies with trisomy 8, we attempted to determine whether *Runx1* deletion cancel the impaired self-renewal capacity of trisomy 8 HSC. We found that *Runx1* deletion partially canceled the impaired repopulating capacity of trisomy 8 cells in recipient mice, suggesting the selective advantage of loss of function mutation of the *RUNX1* gene in trisomy 8 mosaic people and patients with trisomy 8 MDS. Furthermore, as Janus kinase (JAK) and TANK-binding kinase 1 (TBK1) are critical for interferon signalling and activation of their downstream target genes, we revealed that the inhibition of either JAK or TBK function ameliorated trisomy 8-induced chromatin remodelling in HSCs. Overall, trisomy 8 changes chromatin structure and transcription of genes on other chromosomes in HSC accompanied with the malfunction of Runx1 TF, at least in part, due to the activation of interferon signaling, and results in the impaired hematopoiesis. We demonstrated that trisomy 8 creates the basis for transformation of MDS via remodeling chromatin structures in HSC.

Disclosures No relevant conflicts of interest to declare.

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