Abstract: P1385

Title: ELTD1 NEGATIVELY REGULATES DIFFERENTIATION OF HEMOGENIC ENDOTHELIAL CELLS FROM HUMAN EMBRYONIC STEM CELLS THROUGH WNT/HPIP PATHWAY

Abstract Type: Poster Presentation

Topic: Hematopoiesis, stem cells and microenvironment

Background:

Pluripotent stem cells (PSCs) serve as an attractive cell source for generating hematopoietic cells and hold great application potential in the clinic due to their unlimited proliferative capacity and pluripotency. However, the low efficiency of PSCs hematopoietic differentiation limits current attempts to generate large-scale blood cells to suffice clinical needs due to the lack of understanding of the regulatory network of human hematopoiesis. Therefore, exploring the underlying mechanisms of hematopoietic differentiation from PSCs is essential for the acquisition of hematopoietic cells *in vitro*.

Aims:

During embryonic hematopoiesis, hematopoietic stem/progenitor cells (HSPCs) develop from hemogenic endothelial cells (HECs) though endothelial to hematopoietic transition (EHT). However, little is known about how the generation of HEC is regulated during hematopoiesis. Here, we discovered that ELTD1 as a new regulator of HECs via bioinformatics, and validated that ELTD1 expression is significantly higher in HECs. The focus of this study was to assess the function and mechanism of ELTD1 on the generation of HECs during hematopoietic differentiation of human embryonic stem cells (hESCs).

Methods:

ELTD1 was identified as a potential regulator of HECs by genome-wide gene profiling. We deleted ELTD1 in hESCs using the iCRISPR/Cas9 technology and induced them to undergo hematopoietic differentiation. Flow cytometry, quantitative RT-PCR, immunofluorescence and lentiviral transduction were used for phenotype assessment. RNA Seq-based gene profiling was applied to analyze genes with significantly altered expression and altered signaling pathways. Finally, co-immunoprecipitation combined with mass spectrometry (CoIP/MS) and flow cytometry were utilized to explore molecular mechanism.

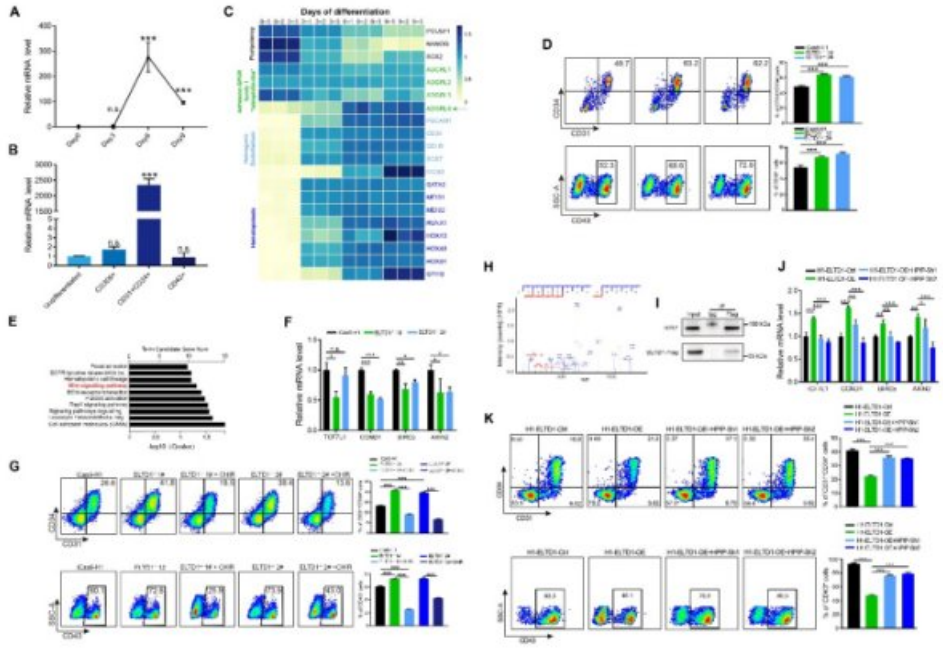
Results:

RT-PCR analysis and bioinformatic studies demonstrated that ELTD1 expression parallels hESCs hematopoietic differentiation and subsequently decreased as they differentiate into hematopoietic cells (Figure A, C). Stage-specific cell populations at different time points were sorted to document the cell-type specificity of ELTD1 and the results showed that ELTD1 was exclusively expressed in Day 6 CD31+CD34+ HECs (Figure B). Loss-of-function studies revealed that ELTD1 mediates hematopoietic differentiation of hESCs by specifically facilitating the generation of HECs, thereby promoting endothelial-to-hematopoietic transition to generate more hematopoietic cells (Figure D). To further explore the underlying molecular mechanisms, the differentiated cells at day 6 were used to perform the RNA-Seq and the differentially expressed genes were prominently enriched in Wnt signaling pathway (Figure E). RT- PCR results showed that Wnt target genes: TCF7L1, CCND1, BIRC5, and AXIN2 were decreased in ELTD1-deleted cells, which are in line with the results of RNA-seq (Figure F). Next, we treated ELTD1-KO1# and ELTD1-KO2# hESCs with the Wnt agonist CHIR99021 to further confirm the results. As expected, treatment with CHIR99021 inhibited the generation of CD31+CD34+ HECs compared to ELTD1 deleted alone (Figure G). To investigate how ELTD1 regulates Wnt signaling pathway, we used co-immunoprecipitation combined with mass spectrometry to identify its interaction partners. The results revealed that the HPIP physically interacted with ELTD1 (Figure H, I), suggesting that ELTD1 might mediate Wnt signaling through HPIP. We then examined whether HPIP is required for ELTD1-mediated expression of Wnt target genes. The results showed that ELTD1 overexpression increased expression of Wnt targets, whereas

knockdown of endogenous HPIP inhibited the expression of Wnt targets (Figure J) and reversed the reduction of HECs and hematopoietic cells caused by ELTD1 overexpression (Figure K), indicating that HPIP is a target in ELTD1 regulatory network of hESCs hematopoietic differentiation.

Summary/Conclusion:

We identified that ELTD1 mediates Wnt signaling pathway through interacting with HPIP during HECs production and provided new insights into understanding the mechanism of human hematopoiesis.



Keywords: Embryonic stem cells, Differentiation, Hematopoiesis