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631.MYELOPROLIFERATIVE SYNDROMES AND CHRONIC MYELOID LEUKEMIA: BASIC AND TRANSLATIONAL

Study on the TGF-β Ligand Trap Enhances Erythroid Differentiation in HEL Cell By Inhibiting Smad3 Phosphorylation

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Objective: Anemia is the most common clinical manifestation of MF, and its pathogenesis may be closely related to the over activation of TGF-β signaling pathway. This study aims to establish an erythroid differentiation model by inducing HEL cells with hemin and investigate the mechanism of the TGF-β ligand trap luspatercept enhances erythroid differentiation in HEL cell.

Methods:

1.ELISA was used to detect the levels of GDF11 in peripheral blood plasma in patients with MF and healthy people.
2.The erythroid differentiation model was established by inducing HEL cells with hemin. The differentiation and proliferation levels of cells were detected by benzidine staining and CCK8. The expression levels of mRNA and protein in the cells were detected by RNA-seq and TMT proteomic analysis, and verified by flow cytometry, RT-PCR and WB.
3.HEL cells were induced with 40 μM hemin as a model of erythroid differentiation. 100ng/mL GDF11 interfered with the over activation of TGF-β signal pathway in differentiated HEL cells. 5μg/mL luspatercept blocked the activation of TGF-β signaling pathway by GDF11. Divided them into three groups, named: hemin group , GDF11 group, and luspatercept group. WB was used to detect the protein levels of pSmad3 and Smad4 in nuclear. Benzidine staining and flow cytometry were used to detect the levels of cell differentiation. RT-PCR and WB were used to detect the expression levels of GATA-1 and KLF1. The changes of RNA expression were detected by RNA-seq and verified by WB.
4.All experimental data were analyzed by SPSS 22.0 statistical software.

Results:

1.The GDF11 levels of plasma of MF patients were higher than those of normal people (P=0.0411).
2.The erythroid differentiation model of HEL cells induced with 40 μM hemin was successfully constructed. The results of RNA-seq and TMT proteome analysis showed that compared with the control group, the expression of hematopoietic related genes such as GATA-1, KLF1 and EPOR in hemin group were up-regulated, and the intracellular proteins related to erythroid differentiation such as HBγ- 2, HB-α and CD235a were up-regulated. Flow cytometry indicated that the expression of CD235a in hemin group was significantly higher than that of the control group (33.83% *vs*81%, P<0.01). RT-PCR and WB experiment showed that the expression of GATA-1 and KLF1 in hemin group were higher than those in control group.
3.WB showed that compared with hemin group, the hyperphosphorylation of smad3 and Smad4 in nuclear levels were increased in GDF11 group. And luspatercept could inhibit the hyperphosphorylation of Smad3 and reduce Smad4 in nuclear levels of GDF11 group. Luspatercept could inhibit GDF11 mediated activation of TGF-β signaling pathway in HEL cells.
4.Compared with hemin group, the benzidine positivity rate of cells and the expression of CD235a on the cell surface in GDF11 group were significantly decreased (benzidine: 13.0% *vs* 9%, P<0.01; CD235a: 16.8% *vs* 33.83%, P<0.01), and the expression of GATA-1 and KLF1 were also decreased. GDF11 could inhibit the erythroid differentiation of HEL cells.
5.Compared with GDF11 group, the benzidine positivity rate of cells and the expression of CD235a on the cell surface in luspatercept group were significantly increased (benzidine: 29.0% *vs*0%, P<0.01; CD235a: 31.1% *vs* 16.8%, P<0.01), and the expression of GATA-1 and KLF1 were increased. Luspatercept could reverse the inhibition of GDF11 on the erythroid differentiation of HEL cells.
6.Compared with the hemin group, the expression of GATA-1, KLF1, EPOR and other genes related to hematopoiesis in GDF11 group were down-regulated, and the differentially expressed genes were related to the biological functions of ribosomal metabolism process, chromosome regulation, erythropoiesis, oxygen binding, oxidoreductase activity and so on, mainly

involving erythroid differentiation, oxidative phosphorylation, iron metabolism, TGF- β, JAK-STAT and other channels. Luspatercept could restore the expression of GATA-1, KLF1, EPOR and other genes by inhibiting the pathway over-activated by GDF11.

Conclusion:

Hemin could induce the erythroid differentiation of HEL cells. The TGF-β ligand trap luspatercept could inhibit GDF11-mediated hyperphosphorylation of Smad3 in HEL cells and promote the erythroid differentiation of HEL cells. Luspatercept could be a therapeutic tool for patients with myelofibrosis associated anemia.

Disclosures No relevant conflicts of interest to declare.

OffLabel Disclosure: Luspatercept (Acceleron Pharma, Cambridge, MA, USA) consists of the extracellular domain of the activin receptor II B fused to the Fc domain of human IgG1 and acts to trap activin and GDF11, which in turn inhibits Smad 2/3 signaling to promote differentiation of cells in the erythroid series.Luspatercept is currently licensed for the treatment of anemia in beta-thalassemia and lower risk myelodysplastic syndrome (MDS) with ring sideroblasts.

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