Abstract: P710

Title: MONOCYTES MEDIATE NK CELL FUNCTION EXHAUSTION IN MDS PATIENTS VIA CD200/CD200R PATHWAY

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Session Title: Myelodysplastic syndromes - Biology & Translational Research

Background:

The mechanisms leading to immune disorders in MDS patients remain elusive.So it is urgent to find predictive biomarkers. CD200 exerts its immunomodulatory effect through cells expressing CD200R. Thus,our study focuses on the role played by the CD200/CD200R pathway in the pathogenesis of MDS.

Aims:

To assess the expression of CD200 on MDS myeloid cells and the role it plays in MDS exhaustion.

Methods:

The study included 40 MDS patients, 40 AML patients, and 30 healthy controls who were diagnosed in the Department of Hematology, Tianjin Medical University General Hospital, Tianjin, China from July 2019 to July 2021. 1. We dected the expression of CD200 on the surface of monocytes as well as CD200R on the surface of NK cells using flow cytometry.2. We added CD200 mAb to the co-culture system of NK-92 with SHI-1.We detected the expression of activation receptors on the surface of NK cells and the apoptosis of SHI-1 before and after co-cultured by flow cytometry. 3.We used siRNA to knockdown the expression of CD200R in NK-92 cells. Using western blot to detect the expression of Erk and STAT3 of NK-92 cells.

Results:

The expression of CD200 on monocytes was significantly lower in healthy controls than in MDS patients and significantly lower than in AML patients (6.52%±0.55% vs 16.97%±2.13% vs 31.45%±3.94%, $p<0.05$)(Figure1 A).Indicating that CD200 is highly expressed on monocytes of MDS patients. The expression of CD200R on NK cells in the MDS group (31.49%±3.98%) was significantly higher than that in healthy controls (8.76%±1.11%) ($P<0.05$)(Figure B). This indicates that CD200R expression was increased on the surface of NK cells.

NK-92 cells are IL-2-dependent natural killer cells with cytotoxic activity against a wide range of malignant cells, while SHI-1 cells are human acute monocytic leukaemia cells. We found high expression of CD200R on the surface of NK-92 cells (Figure C)as well as high expression of CD200 on the surface of SHI-1 cells(Figure D).Therefore, we used a co-culture system between SHI-1 cells and NK-92 cells to simulate the interaction between monocytes and NK cells in their microenvironment.

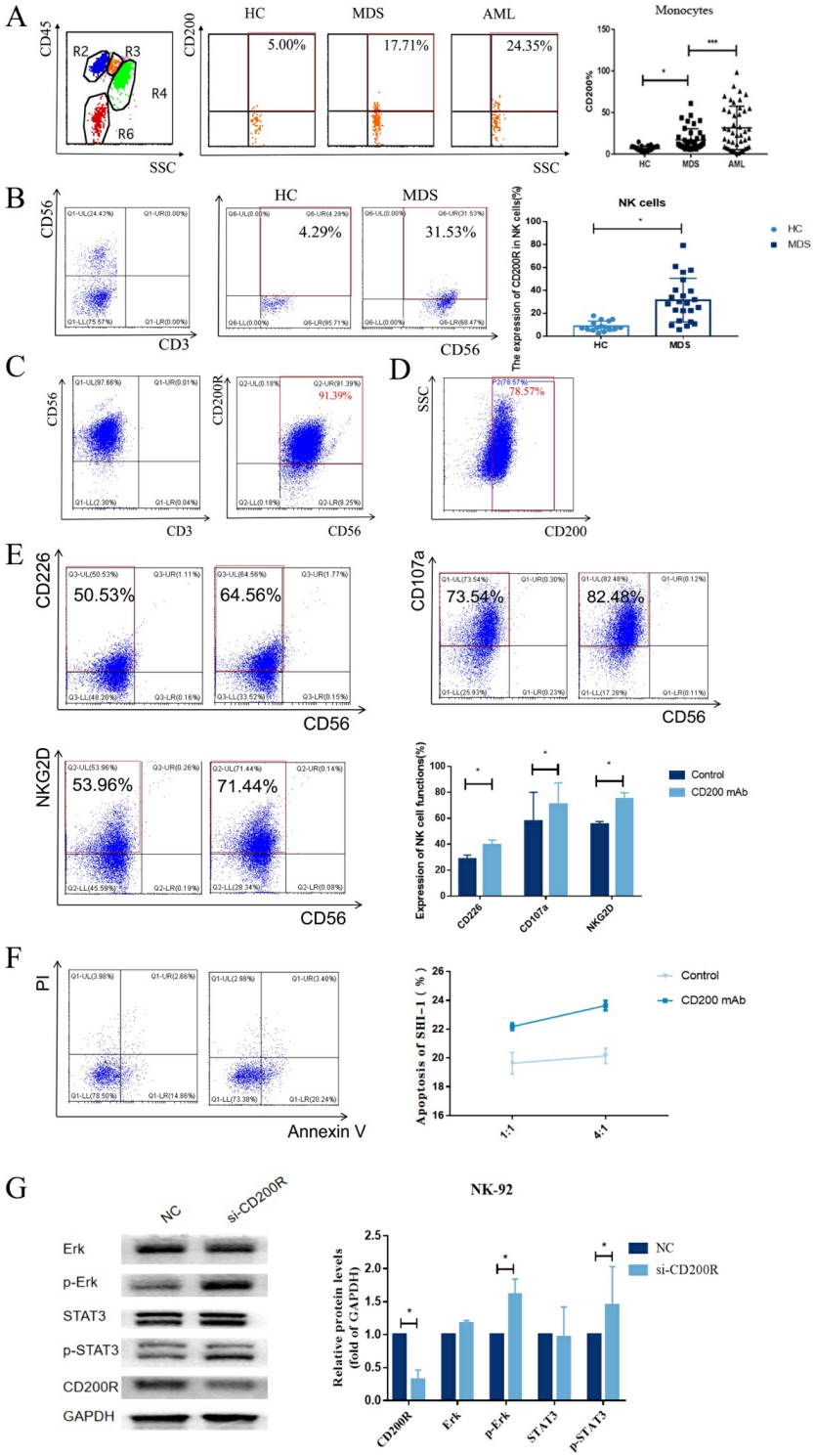
To further explore how monocytes affect NK cells, We added CD200 mAb to the co-culture system.Then we detected the activation receptors of NK cells before and after co-culture. The results showed that the expression of CD107a was significantly higher in the CD200 mAb group than control group (70.99% ± 16.25% VS 57.84% ± 22.20%, $P < 0.05$). The expression of CD226(39.73% ± 3.51% VS 28.54% ± 3.11% , $P < 0.05$) and NKG2D(74.83% ± 4.80% VS 55.40% ± 2.03%, $P < 0.05$) were both higher in the CD200 mAb group than in the control group(Figure E).We also detected the apoptosis of SHI-1 cells in the co-culture system, and the results showed that the apoptosis of SHI-1 in the CD200 mAb group was significantly higher than that in the control group (23.64% ± 0.34% vs 20.15% ± 0.53%) (4:1 for E: T)(Figure F).

We used siRNA to knock down CD200R expression in NK-92 cells.The results showed that the phosphorylation levels of Erk and STAT3 tyrosine sites were significantly increased in the CD200R knockdown group compared with the control group. Erk and STAT3 were not significantly different between the groups. This result illustrates that blocking CD200R enhances Erk and STAT3 phosphorylation(Figure G).This suggests that monocytes may

interact with NK cells through the CD200/CD200R pathway by inhibiting Erk and STAT3 phosphorylation.

Summary/Conclusion:

Monocytes mediate NK cell function exhaustion in MDS patients via CD200/CD200R pathway.



Keywords: MDS, NK cell, Monocyte