



Study on the Mechanism of CD37 Regulating PI3K/AKT Signal Pathway in AML

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Abstract: Objective: To analyze the mechanism of CD37 in regulating the PI3K/AKT signaling pathway in acute myeloid leukemia (AML). Methods: AML mouse models were established with CD37 knockout (CD37^{-/-} group) and wild-type (CD37^{fl/fl} group). The function of leukemia stem cells (LSCs) was evaluated by flow cytometry, colony formation assay, and serial transplantation assay. Signaling pathway changes were analyzed by RNA-seq and Western blot. The interaction between CD37 and integrin was verified by co-immunoprecipitation. Results: Compared with the CD37^{fl/fl} group, the CD37^{-/-} group showed decreased GFP⁺ cells in peripheral blood, reduced bone marrow blasts, and prolonged median survival ($P < 0.05$). RNA-seq revealed that the PI3K/AKT pathway was the most significantly enriched pathway. The levels of p-FAK and p-PI3K were decreased in the CD37^{-/-} group. Co-immunoprecipitation demonstrated that CD37 directly bound to integrin $\alpha 4$ and $\beta 7$ subunits, and the expression of integrin $\alpha 4$ and $\beta 7$ was downregulated after CD37 deletion ($P < 0.05$). Conclusion: CD37 regulates the self-renewal of LSCs by activating the PI3K/AKT pathway through integrin $\alpha 4\beta 7$, and may serve as a potential safe target for AML therapy.

Keywords: CD37; PI3K; AKT; signaling pathway; AML; mechanism

1. Introduction

Acute Myeloid Leukemia (AML) is a malignant clonal disease characterized by excessive proliferation and abnormal differentiation of myeloid blasts, resulting in suppressed normal hematopoietic function. As the most common type of leukemia in adults, AML causes more than 80,000 deaths worldwide annually, and its incidence is on the rise.

CD37 is a member of the tetraspanin superfamily, mainly expressed on the surface of hematopoietic cells. Recent studies have revealed that CD37 is significantly highly expressed in AML leukemia stem cells (LSCs) but at a low level in normal hematopoietic stem cells (HSCs). This differential expression profile makes it a highly promising immunotherapeutic target [1]. Studies have shown that CD37-targeted antibody-drug conjugates (ADC) and CAR-T cells exert significant cytotoxicity against CD37-positive AML cells without toxicity to normal HSCs [2].

However, the biological function and molecular regulatory mechanism of CD37 in the initiation and progression of AML remain unclear. In this study, we established an AML model using CD37-knockout mice, combined with *in vitro* and *in vivo* functional assays and transcriptomic analysis, to systematically explore the molecular mechanism by which CD37 regulates the self-renewal of AML LSCs, with a focus on its relationship with integrin signaling and the PI3K/AKT pathway.

2. Materials and Methods

2.1 Experimental Animals

CD37-knockout mice (CD37^{-/-} group) and wildtype control mice (CD37^{fl/fl} group) were purchased from a biological research center, with a C57BL/6J background, aged 8–12 weeks, with equal numbers of males and females. All animal experiments were approved by the Animal Ethics Committee of the Second Affiliated Hospital of Qiqihar Medical University, Heilongjiang Province, and conducted in accordance with the Guide for the Care and Use of Laboratory Animals.

2.2 Methods

The AML model was established by infecting cKit⁺ cells with MLLAF9 retrovirus, followed by tailvein injection into sublethally irradiated recipient mice.

Cell functional assays: Flow cytometry was used to detect the proportion of LSCs (GFP⁺Lin⁻cKit⁺CD34⁺FcγR⁺), apoptosis (Annexin V/7AAD), and cell cycle (7AAD). Sorted LSCs were subjected to methylcellulose colonyforming assays (serial passages) and limiting dilution serial transplantation assays (secondary and tertiary transplantation) to evaluate self-renewal capacity.

Molecular mechanism studies: RNAseq was performed to analyze the transcriptome of LSCs in both groups (GEO: GSE292186), followed by KEGG pathway enrichment analysis. Western blot was used to detect the phosphorylation levels

of proteins in the PI3K/AKT pathway. Coimmunoprecipitation was applied to verify the interaction between CD37 and integrins.

2.3 Observation Indicators

- (1) The effect of CD37 knockout on leukemia burden and survival in AML mice.
- (2) The effect of CD37 knockout on the PI3K/AKT pathway and integrin expression.

2.4 Statistical Analysis

Data were processed using SPSS 26.0. Enumeration data [n, (%)] were analyzed by the chisquare test, and measurement data (mean ± standard deviation) were analyzed by the ttest after confirming normal distribution. A value of $P < 0.05$ was considered statistically significant.

3. Results

3.1 Effect of CD37 Knockout on Leukemia Burden and Survival in AML Mice

Compared with the CD37^{fl/fl} group, the CD37^{-/-} group showed decreased GFP⁺ cells in peripheral blood, reduced bone marrow blasts, and prolonged median survival ($P < 0.05$), as shown in Table 1.

Table 1. Effects of CD37 knockout on leukemia burden and survival in AML mice

Group	n	Peripheral blood GFP ⁺ cells (6 weeks,%)	Myeloid cells (%)	Median survival time (d)
CD37 ^{-/-} -group	10	32.56±4.82	28.64±5.36	45.26±4.26
CD37 ^{fl/fl} group	10	72.26±6.73	68.42±7.26	28.56±3.28
<i>t</i>		15.166	13.940	9.823
<i>P</i>		<0.001	<0.001	<0.001

3.2 Effect of CD37 Knockout on the PI3K/AKT Pathway and Integrin Expression

RNA-seq showed that the PI3K/AKT pathway was the most significantly enriched signaling pathway. The levels of p-FAK, p-PI3K, and p-AKT were decreased in the CD37^{-/-} group. Co-immunoprecipitation demonstrated that CD37 directly bound to the integrin $\alpha 4$ and $\beta 7$ subunits, and the expression of integrin $\alpha 4$ and $\beta 7$ was downregulated after CD37 deletion ($P < 0.05$), as shown in Table 2.

Table 2. Effects of CD37 knockout on PI3K/AKT pathway and integrin expression

Group	n	p-PI3K/PI3K	p-AKT/AKT	p-FAK/FAK	integrin $\alpha 4$	integrin $\beta 7$
CD37 ^{-/-} -group	10	0.42±0.08	0.35±0.06	0.48±0.09	0.63±0.07	0.58±0.06
CD37 ^{fl/fl} group	10	1.05±0.13	1.02±0.11	1.03±0.11	1.01±0.08	1.03±0.09
<i>t</i>		13.052	16.909	12.237	11.304	13.156
<i>P</i>		<0.001	<0.001	<0.001	<0.001	<0.001

4. Conclusion

In this study, using a geneknockout mouse model combined with transcriptomic analysis, we revealed the important role and molecular mechanism of CD37 in the occurrence and development of AML. The main findings are that CD37 knockout significantly reduces leukemia burden and prolongs survival in AML mice. Mechanistically, CD37 activates the PI3K/AKT signaling pathway by interacting with integrin $\alpha 4\beta 7$.

Members of the tetraspanin superfamily usually act as scaffold proteins and recruit signaling molecules such as integrins and receptor tyrosine kinases by forming tetraspaninenriched microdomains (TEMs). We confirmed that CD37 directly interacts with integrin $\alpha 4\beta 7$, and CD37 deletion leads to decreased phosphorylation levels of FAK and PI3K/AKT [3]. Integrinmediated extracellular matrix adhesion signaling is an important microenvironmental factor for LSCs to maintain their selfrenewal capacity.

In summary, CD37 regulates the selfrenewal of LSCs through the integrin $\alpha 4\beta 7$ mediated PI3K/AKT signaling pathway. This molecular mechanism provides a new theoretical basis and potential target for targeted therapy of AML.

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