BLK Cross-linked Redox and Apoptosis Signaling Networks Promote the Survival of Transplanted BMSCs

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Disclosures: I have nothing to disclose.

INTRODUCTION: Stress-induced apoptosis of bone marrow mesenchymal stem cells (BMSCs) is a crucial factor that limits the ability of tissue-engineered bone to repair steroid-induced osteonecrosis of the femoral head (SONFH), and intervention strategies must be explored. The survival of BMSCs under oxidative stress involves cross-linking regulation of redox and apoptosis signaling networks. We previously confirmed that most BMSCs are sensitive to oxidative stress (S-BMSCs, S), while BMSCs surviving in the transplantation area have oxidative stress resistance (R-BMSCs, R). In previous studies, we used quantitative proteomics combined with phosphoproteomics analysis to identify a core factor, B lymphoid tyrosine kinase (BLK), that synergically regulates the redox (ERK1/2 and Nrf2) and apoptosis (NF-κB and STAT3) signaling networks in R-BMSCs. This study further revealed the role and regulatory mechanism of BLK in resisting stress-induced apoptosis of transplanted BMSCs.

METHODS: The human and animal experiments were approved by the Medical Science Ethics Committee of the Affiliated Hospital of Guizhou Medical University. In vitro: First, BLK was overexpressed or knocked down in BMSCs. Then, ERK, Nrf2, STAT, and NF-κB signaling pathways were blocked, and H_2O_2 was used to simulate oxidative stress to induce BMSC apoptosis to study the effect and regulatory mechanism of BLK on stress-induced apoptosis of BMSCs. In vivo: BLK-modified BMSCs were transplanted to repair SONFH models and evaluate the role of BLK in vivo. Statistics: The Shapiro–Wilk test was used to test the normality of the data, the two-tailed unpaired Student's t-test was used for comparison between two groups, and one-way ANOVA was used for comparison between multiple groups. If the data did not follow a normal distribution, the Kruskal–Wallis rank-sum test was used for comparison between groups. $P < 0.05$ was considered statistically significant.

RESULTS SECTION:

1. BLK knockdown aggravated stress-induced apoptosis and reduced the efficacy of BMSC transplantation.

In vitro, BLK knockdown weakened the oxidative stress resistance of R-BMSCs and aggravated stress-induced apoptosis (Fig. 1A). In vivo, BLK knockdown reduced the survival of R-BMSCs in osteonecrotic areas and decreased the transplantation efficacy of R-BMSCs on SONFH (Fig. 1B–D).

2. BLK overexpression inhibited stress-induced apoptosis and improved the efficacy of BMSC transplantation.

In vitro, the overexpression of BLK allowed S-BMSCs to resist oxidative stress and reduced stress-induced apoptosis (Fig. 2A). In vivo, BLK overexpression promoted the survival of S-BMSCs in osteonecrotic areas and improved the transplantation efficacy of S-BMSCs on SONFH (Fig. 2B–D).

3. BLK promoted resistance to stress-induced apoptosis of BMSCs by targeting the activation of ERK1/2, Nrf2, STAT3, and NF-κB signaling pathways.

BLK overexpression promoted nuclear translocations of p-ERK1/2, p-Nrf2, p-STAT3, and p-NF-κB and activated these pathways (Fig. 3A–D). Based on BLK overexpression, the above signaling pathways were blocked, which weakened the effect of BLK on resisting stress-induced apoptosis (Fig. 3E).

DISCUSSION: This study reports that BLK has a new function of resisting stress-induced apoptosis. From the therapeutic perspective, we determine that BLK can synergically regulate redox and apoptotic signaling networks to promote the survival of transplanted BMSCs in osteonecrotic areas, thereby improving the transplantation efficacy of BMSCs on SONFH. In addition, there is a shortcoming that it remains unclear whether specific surface markers exist in R-BMSCs. In future studies, we plan to use single-cell sequencing to identify specific surface markers to optimize cell screening protocols.

SIGNIFICANCE/CLINICAL RELEVANCE: This study validates the novel role of BLK in regulating stress-induced apoptosis in human BMSCs, which may be useful for clinical applications of BMSC transplantation therapy.

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IMAGES AND TABLES:

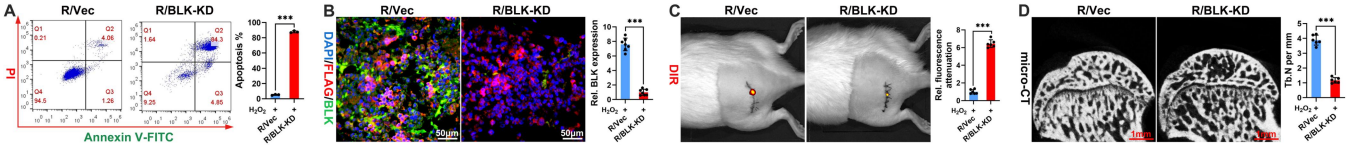

Fig. 1 The role of BLK in R-BMSCs. A. Analysis of the stress-induced apoptosis of R-BMSCs in vitro (n = 3); B. Verification of the BLK expression in R-BMSCs in vivo (n = 7); C. Detection of the survival of transplanted R-BMSCs (n = 6); D. Evaluation of bone necrosis repair (n = 6).

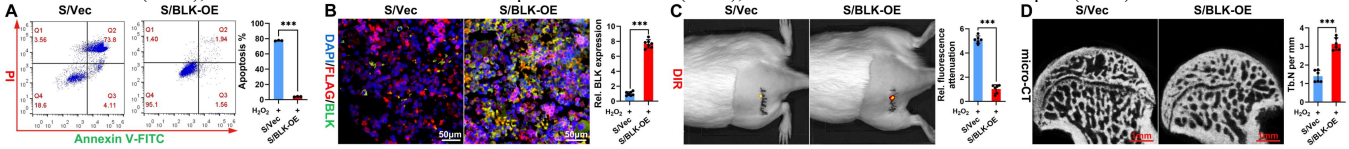

Fig. 2 The role of BLK in S-BMSCs. A. Analysis of the stress-induced apoptosis of S-BMSCs in vitro (n = 3); B. Verification of the BLK expression in S-BMSCs in vivo (n = 7); C. Detection of the survival of transplanted S-BMSCs (n = 6); D. Evaluation of bone necrosis repair (n = 6).

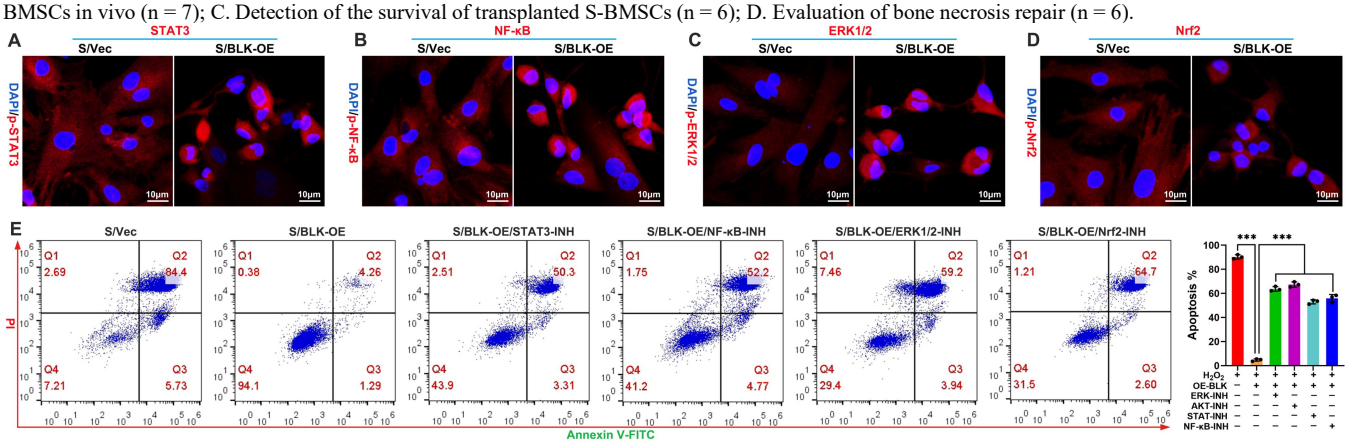

Fig. 3 The mechanism of BLK regulating stress-induced apoptosis. A–D. Nuclear translocations of STAT3, NF-κB, ERK1/2, and Nrf2 were detected after BLK was overexpressed in S-BMSCs (n = 3); E. Based on BLK overexpression, the above signaling pathways were blocked, and the stress-induced apoptosis of S-BMSCs was detected (n = 3).