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Basic Study

Effects of different concentrations of nicotinamide on hematopoietic stem cells cultured *in vitro*

Ren Y *et al.* Concentration of NAM affects the expansion of UCB-HSCs

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Abstract

BACKGROUND

In vitro expansion to increase numbers of hematopoietic stem cells (HSCs) in cord blood could improve clinical efficacy of this vital resource. Nicotinamide (NAM) can promote HSC expansion *ex vivo*, but its effect on functional subtypes of HSCs-short-term repopulating HSCs (ST-HSCs, CD34+CD38-CD45RA-CD49f+) and long-term repopulating HSCs (LT-HSCs, CD34+CD38-CD45RA-CD49f+CD90+) is not yet known. As a sirtuin 1 (SIRT1) inhibitor, NAM participates in regulating cell adhesion, polarity, migration, proliferation, and differentiation. However, SIRT1 exhibits dual effects by promoting or inhibiting differentiation in different tissues or cells. We propose that the concentration of NAM may influence proliferation, differentiation, and SIRT1 signaling of HSCs.

AIM

To evaluate the effects and underlying mechanisms of action of different concentrations of NAM on HSC proliferation and differentiation.

METHODS

CD34⁺ cells were purified from umbilical cord blood using MacsCD34 beads, and then cultured for 10-12 d in serum-free medium supplemented with cytokines, with different concentrations of NAM added according to experimental requirements. Flow cytometry was used to detect phenotype, cell cycle distribution, and apoptosis of the cultured cells. Real-time polymerase chain reaction was employed to detect the transcription levels of target genes encoding stemness-related factors, chemokines, components of hypoxia pathways, and antioxidant enzymes. DCFH-A probes were used to evaluate intracellular production of reactive oxygen species (ROS). Determination of the effect of different culture conditions on the balance of cytokine by cytometric bead array.

RESULTS

Compared with the control group, the proportion and expansion folds of hematopoietic stem and progenitor cells (CD34+CD38-) incubated with 5 mmol/L or 10 mmol/L of NAM were significantly increased (all $P < 0.05$). The ST-HSCs ratio and fold expansion of the 5 mmol/L NAM group were significantly higher than those of the control and 10 mmol/L NAM groups (all $P < 0.001$), whereas the LT-HSCs ratio and fold expansion of the 10 mmol/L NAM group were significantly higher than those of the other two groups (all $P < 0.05$). When the NAM concentration was greater than 10 mmol/L, cell viability significantly decreased. In addition, compared with the 5 mmol/L NAM group, the proportion of apoptotic cells in the 10 mmol/L NAM group increased and the proportion of cells in S and G2 phase decreased. Compared with the 5 mmol/L NAM group, the HSCs incubated with 10 mmol/L NAM exhibited significantly inhibited SIRT1 expression, increased intracellular ROS content, and downregulated expression of genes encoding antioxidant enzymes (superoxide dismutase 1, peroxiredoxin 1).

CONCLUSION

Low concentrations (5 mmol/L) of NAM can better regulate the balance between proliferation and differentiation, thereby promoting effective expansion of HSCs. These findings may allow adjustment of NAM concentrations according to expansion needs.

Key Words: Hematopoietic stem cells; Nicotinamide; Concentration; Proliferation; Differentiation; Sirtuin 1

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Core Tip: This study reveals the dominant subgroups of hematopoietic stem cells (HSCs) and molecular mechanisms underlying the effects of different nicotinamide (NAM) concentrations. Activation or inhibition of sirtuin 1 (SIRT1) is determined by the

concentration of NAM. High concentrations inhibit SIRT1 but are not conducive to self-renewal of HSCs, whereas low concentrations balance HSC proliferation and differentiation by regulating the SIRT1-HIF1A pathway and reactive oxygen species production, effectively promoting *in vitro* expansion of the stem cells. These findings could allow adjustment of NAM concentrations according to expansion needs and may help predict small molecules that synergistically promote expansion with NAM.

INTRODUCTION

Hematopoietic stem cells (HSCs) are cells that can ² self-renew and give rise to mature cells of all hematopoietic lineages following extensive proliferation and differentiation^[1,2]. Umbilical cord blood (UCB) is an important source of HSCs because it can be obtained through non-invasive means, has low requirements for the matching of human leukocyte antigens, and has a low incidence of chronic graft-*versus*-host disease. However, the limited cell dose in a single UCB can lead to delayed engraftment after transplantation^[3]. Obtaining a sufficient number of HSCs through *ex vivo* expansion has the potential to overcome such challenges.

Small molecules represent novel modalities for *ex vivo* expansion of HSCs^[4-6], and the roles of different molecules in HSC transplantation are diverse. As a form of vitamin B-3, nicotinamide (NAM) can delay differentiation and maintain stemness, as well as promote neutrophil recovery in patients after transplantation^[7-9]. HSCs can be functionally divided into short-term repopulating HSCs (ST-HSCs, CD34+CD38-CD45RA-CD49f+) and long-term repopulating HSCs (LT-HSCs, CD34+CD38-CD45RA-CD49f+CD90+). ST-HSCs can maintain life-long hematopoiesis and are characterized by a delayed engraftment pattern upon transplantation, whereas LT-HSCs provide early and transient hematopoietic recovery^[10-12]. NAM promotes *in vitro* expansion of hematopoietic stem and progenitor cells (HSPCs), but its effects on ST-HSCs and LT-HSCs have yet to be elucidated. Emerging studies suggest that NAM may mediate the metabolic transition of aging stem cells by upregulating the expression of young genes such as sirtuin 1 (SIRT1), thereby maintaining stemness. This contradicts the viewpoint

that NAM acts as a SIRT1 inhibitor^[13]. Furthermore, SIRT1 exhibits a dual effect of promoting or inhibiting differentiation in different tissues or cells^[14]. Based on these observations, we propose that the proliferation, differentiation, and SIRT1 signaling pathway of HSCs may be influenced by the concentration of NAM.

In this study, we evaluated the effects of different concentrations of NAM on the proliferation and differentiation of HSCs during *in vitro* culture. Proliferation is reflected by fold expansion of total nucleated cells (TNC) and differentiation is reflected by phenotype analysis of HSPC, ST-HSC, and LT-HSC populations. Apoptosis ratio and cell cycle distribution were used to reveal the influencing factors of cell quantity differences; reactive oxygen species (ROS) production and cytokine levels were evaluated to reveal the relevant factors of differentiation differences; and real-time polymerase chain reaction (RT-PCR) detection of genes related to stemness, antioxidant enzymes, and chemotactic molecules were used to explore the molecular mechanism(s) underlying the actions of NAM. NAM at 5 mmol/L and 10 mmol/L could inhibit differentiation and maintain HSPCs, with the higher concentration being more conducive to the maintenance of LT-HSCs. However, owing to significant proapoptotic effects, this high concentration of 10 mmol/L of NAM reduced cell survival and self-renewal, whereas the lower concentration of 5 mmol/L of NAM could better regulate the balance between proliferation and differentiation, thereby promoting the effective expansion of HSCs. In general, the differential sensitivities of LT-HSCs and ST-HSCs to NAM may be used to improve the outcome of *ex vivo* expansion.

MATERIALS AND METHODS

Sample preparation and isolation of CD34⁺ cells from human UCB

A professional gynecologist collected UCB samples from consenting donors according to ethical procedures approved by the second hospital of Shanxi Medical University (Shanxi, China). UCB mononuclear cells (MNCs) were isolated by Ficoll (Tianjin Haoyang Biological Products Technology) and density-gradient centrifugation. Specifically, UCB depleted of erythrocytes (by adding hydroxyethyl starch) was slowly

pipetted on Ficoll at a ratio of 2:1 and centrifuged (2000 rpm, 20 min), followed by collection of the interface layer as MNCs. The MNCs were washed and suspended in column buffer [phosphate buffered saline (PBS) and 0.5% bovine serum albumin, Gibco], then CD34+ cells labeled with CD34 MicroBeads (Miltenyi Biotec) were separated out by loading the cell suspension onto LS Columns (Miltenyi Biotec) associated with a magnetic field.

Cell culture

Before being seeded into 24-well plates (Corning), CD34+ cells were resuspended in serum-free medium (5×10^4 /mL), which was composed of Iscove Modified Dulbecco Medium (IMDM, Gibco), supplemented with 10 ng/mL human stem cell factor (Miltenyi Biotec), 100 ng/mL thrombopoietin (Miltenyi Biotec), 1% Penicillin-Streptomycin-Glutamine (Gibco), and NAM at different concentrations. Based on the different concentrations of NAM, the cells were divided into control group (0 mmol/L), low concentration group (5 mmol/L), and high concentration group (10 mmol/L). Cells were incubated in a humidified incubator at 37 °C, 5% CO₂, and 5% O₂ for 10-12 d.

Phenotypic analysis

After incubation with NAM for 10-12 d, the cells were collected and washed with PBS. Next, the cells were stained in PBS supplemented with the following antibody and fluorophore combinations for 30 min at 4 °C: ECD-labeled anti-human CD34 (Beckman; IM2709U), FITC-labeled anti-human CD38 (Beckman; A07778), Pacific Blue-labeled anti-human CD45RA (Beckman; A82946), APC-CY7-labeled anti-human CD49f (Biolegend; 313628), and PerCP-CY5-labeled anti-human CD90 (Beckman; IM3703). After a washing step, the stained cells were analyzed by Navios flow cytometer (Beckman Coulter). For each sample, at least 30000 events were recorded. Isotype controls were also prepared for every experiment.

Apoptosis detection

The cultured cells were collected and washed twice with precooled PBS. Cells were then stained for 15 min at room temperature with PE-Annexin V Apoptosis Detection Kit (BD Biosciences). Specifically, 5 μ L of PE-Annexin V and 5 μ L of 7-aminoactinomycin D were added to each sample. Samples were then analyzed by Navios flow cytometer within 1 h.

Cell cycle analysis

The cultured cells were washed with precooled PBS, resuspended in 70% ethanol at 4 °C for 2-6 h, and then washed with PBS and incubated with 0.4 mL of propidium iodide staining solution (Biosciences) for 30 min at 37 °C in the dark. The 0.4 mL of propidium iodide staining solution was composed of 384 μ L staining buffer, 15 μ L of 25 \times propidium solution, and 1 μ L of RNase (10 mg/mL). A minimum of 40000 cells were collected at a low speed for each sample.

ROS detection

The cultured cells were resuspended at a density of 5×10^5 to 1×10^6 cells/mL, and the DCFH-DA probe was diluted with serum-free medium according to the manufacturer's instructions (Beyotime Biotechnology). The cells were resuspended in diluted DCFH-DA and incubated for 20 min in a 37 °C incubator. Mix it upside down every 3-5 min to ensure full contact between the probe and the cell. The cells were then washed with serum-free medium three times to fully remove DCFH-DA that had not entered the cells. The fluorescence intensity of ROS was measured within 2 h by Navios flow cytometer. The median level of mean fluorescence intensity of ROS was obtained in Kaluza software (Beckman) for each sample after adjusting samples to the same measuring cell number.

RT-PCR

Total RNA was extracted from the cells cultured with or without NAM by the Trizol method. For cDNA synthesis, total RNA was reverse transcribed with a cDNA

synthesis kit (Bio-Rad). PCR was performed using a SYBR Premix Ex Taq™II (Tli RNaseH Plus; TaKaRa) and the CFX96 RT-PCR detection system (Bio-Rad). Each reaction was repeated at least three times to demonstrate reproducibility, and data were analyzed using the CFX96 Real-Time System. Normalized values were obtained by subtracting the threshold cycle (Ct) of GAPDH from the Ct values of the target genes, yielding Δ Ct values, and the $\Delta\Delta$ Ct formula was used as an indication of the relative transcriptional level. The primer sequences used were: CD34 forward (5'-CTACAACACCTAGTACCCTTGGA-3') and reverse (5'-GGTGAACACTGTGCTGATTACA-3'); CD133 forward (5'-AGTCGGAAACTGGCAGATAGC-3') and reverse (5'-GGTAGTGTGTACTGGGCCAAT-3'); CXCR4 forward (5'-GGGCAATGGATTGGTCATCCT-3') and reverse (5'-TGCAGCCTGTACTTGTCCG-3'); SIRT1 forward (5'-CAAACCTTTGCTGTAACCCTGT-3') and reverse (5'-CAGCCACTGAAGTTCTTTCAT-3'); HIF1A forward (5'-GGTCTAGGAAACTCAAAACCTGA-3') and reverse (5'-TCCTCACACGCAAATAGCTGA-3'); superoxide dismutase (SOD)1 forward (5'-GGTGGGCCAAAGGATGAAGAG-3') and reverse (5'-CCACAAGCCAAACGACTTCC-3'); SOD2 forward (5'-GCTCCGGTTTTGGGTATCTG-3') and reverse (5'-GCGTTGATGTGAGGTTCCAG-3'); peroxiredoxin 1 (PRDX1) forward (5'-CCACGGAGATCATTGCTTTCA-3') and reverse (5'-AGGTGTATTGACCCATGCTAGAT-3'); β -actin forward (5'-AGAGCTACGAGCTGCCTGAC-3') and reverse (5'-AGCACTGTGTTGGCGTACAG-3').

Cytokine assay

This assay was conducted according to the Instruction Manual of BD™ Cytometric Bead Array Human Th1/Th2/Th17 Cytokine Kit (Catalog No. 560484). The fresh cytokine standards were prepared to run with each experiment. Add 50 μ L of sample and 50 μ L of the Human Th1/Th2/Th17 PE Detection Reagent to the sample tubes and

incubate the assay tubes for 3 h at room temperature, protected from light. Analyze data using FACS Array software.

Statistical analysis

The significant differences between each group were analyzed using SPSS 22.0 statistical software for all experimental data. The comparison was analyzed between two groups with an independent sample *t* test and among three groups with single-factor analysis of variance (ANOVA). The values were plotted as the mean \pm SD. Probability values $P < 0.05$ were considered statistically significant.

RESULTS

Effect of different concentrations of NAM on the quality of HSCs

We measured the proportion of CD34⁺CD38⁻ (HSPCs), CD34⁺CD38⁻CD45RA⁻CD49f⁺ (ST-HSCs), and CD34⁺CD38⁻CD45RA⁻CD49f⁺CD90⁺ (LT-HSCs) subsets in the control group (0 mmol/L) and different NAM groups (5, 10, and 15 mmol/L). The proportion of HSPCs in the 5 mmol/L and 10 mmol/L NAM groups was significantly higher than that in the control group (both $P < 0.05$), whereas there was no significant difference between the first two groups (Figure 1A). The proportion of ST-HSCs was highest in the 5 mmol/L NAM group, followed by the 10 mmol/L NAM group ($P < 0.001$), and both groups had significantly higher proportions of ST-HSCs than the control group ($P < 0.05$) (Figure 1B). Moreover, the proportion of LT-HSCs in the 10 mmol/L NAM group was significantly higher than that in the control group and the 5 mmol/L NAM group, and there was no significant difference between the latter two groups (Figure 1C). It is believed that the lower the differentiation of stem cells, the smaller their diameter, and the results of cell size testing in each group confirmed this statement (Figure 1D). Cell viability for the 15 mmol/L NAM group was poor and the cultures contained a lot of cell debris, which led to non-specific antibody binding that was unsuitable for phenotype analysis (Figure 1E). These results are consistent with other reports that NAM treatment can inhibit the differentiation of HSCs^[8].

Effect of different concentrations of NAM on the quantity of HSCs

Fold expansion of TNCs and the degree of cell differentiation are reliable indicators for evaluating the *ex vivo* expansion efficiency of HSCs. Under the microscope, small and round HSCs were visible, and the number of cells in the 15 mmol/L NAM group was significantly lower than for the other groups (Figure 2A). The 5 mmol/L NAM group had the highest fold expansion of TNCs, followed by the control group, both of which were significantly higher than the 10 mmol/L and 15 mmol/L NAM groups, with the 15 mmol/L NAM group having the lowest fold expansion of TNCs (Figure 2B). The fold expansions of HSPCs in the 5 mmol/L and 10 mmol/L NAM groups were significantly higher than that in the control group (Figure 2C). The fold expansion of ST-HSCs was highest in the 5 mmol/L NAM group and was significantly higher than in the control and 10 mmol/L NAM groups (Figure 2D). Meanwhile, the fold expansion of LT-HSCs was highest in the 10 mmol/L NAM group (Figure 2E).

Effect of different concentrations of NAM on HSC apoptosis and cell cycle distribution

To investigate whether NAM affects other biological behaviors of HSCs, we measured the proportion of apoptotic cells and cell cycle distribution in each group. The proportion of apoptotic cells in the 10 mmol/L NAM group was significantly higher than those in the control and 5 mmol/L NAM groups, with a significant decrease in the proportion in the control group compared with the 5 mmol/L NAM group (all $P < 0.001$) (Figures 3A and B). In addition, the proportion of cells in S and G₂ phase in the 5 mmol/L NAM group was significantly higher than those in the control and 10 mmol/L NAM groups, with a significant decrease in the proportion in the control group compared with the 10 mmol/L NAM group (Figure 3C). This indicated that the effect of NAM on HSC proliferation and apoptosis was concentration dependent.

Exploring the molecular mechanism of NAM affecting the proliferation and differentiation of HSCs

In vivo, primitive HSCs can be distinguished by their low mitochondrial activity, as they primarily rely on glycolytic metabolism as their energy source to maintain a dormant state. *Ex vivo* culture conditions cannot completely mimic the niche of HSCs, but do drive metabolic transitions toward oxidative phosphorylation and activation of inflammatory pathways, leading to an increase in ROS and inflammatory factors, which results in rapid differentiation^[15-17]. In this study, compared with freshly isolated CD34+ cells, the control group and the NAM groups exhibited a significant increase in intracellular ROS levels, with the control group having the highest ROS level and the NAM groups having a significantly lower ROS content than the control group (Figure 4A). Additionally, the concentration of interleukin-6 in the cell supernatant was significantly increased, with that of the 5 mmol/L and 10 mmol/L NAM groups not exceeding 3 ng/mL and being significantly lower than that of the control group (Figure 4B).

To further explore the molecular mechanisms by which NAM affects the proliferation and differentiation of HSCs, we compared the expression of genes encoding stemness-related factors, chemokines, components of hypoxia pathways, and antioxidant enzymes in each group of cells. The hypoxia pathway SIRT1-HIF1A was significantly upregulated in the 5 mmol/L NAM group compared with the other two groups. Compared with the control group, the 5 mmol/L NAM group showed significant upregulation of antioxidant enzymes PRDX1 and SOD1, consistent with the results of the ROS detection mentioned above. In addition, compared with the control and 5 mmol/L NAM groups, the 10 mmol/L NAM group had significantly inhibited SIRT1 expression. The expression of the chemokine CXCR4 was significantly increased in the 5 mmol/L and 10 mmol/L NAM groups compared with the control group.

DISCUSSION

The basic HSCs *in vitro* culture system consists of serum-free medium supplemented with cytokines, and the addition of small molecule substances can significantly enhance amplification efficiency^[18,19]. For example, NAM can significantly increase the

proportion of CD34⁺CD38⁻ cells (HSPCs). However, the CD34⁺CD38⁻ phenotype is an unpredictable surface marker for *in vivo* repopulating cell cultures, resulting in separation of phenotype from *in vivo* hematopoietic reconstitution ability^[20,21]. According to reconstruction dynamics in primary and secondary recipients after transplantation, HSCs can be divided into LT-HSCs and ST-HSCs. LT-HSCs are believed to be located at the top of the hematopoietic system and can differentiate downstream to produce ST-HSCs, pluripotent progenitor cells, lineage-specific progenitor cells, and terminally differentiated hematopoietic cells. LT-HSCs can provide delayed but persistent hematopoiesis, whereas ST-HSCs contribute to early and temporary hematopoiesis recovery^[22,23]. Therefore, compared with HSPCs, these two subpopulations can more accurately evaluate the *in vivo* hematopoietic reconstitution ability and the expansion efficiency of NAM on HSCs.

In this study, we evaluated the amplification efficiency of different concentrations of NAM on HSPCs, ST-HSCs, and LT-HSCs, and found that 5 mmol/L and 10 mmol/L NAM are beneficial for maintaining HSPCs. The lower concentration (5 mmol/L) of NAM was more conducive to expansion of ST-HSCs, whereas the higher concentration (10 mmol/L) had a more significant effect on promoting expansion of LT-HSCs. Based on these findings, the working concentration of NAM can be adjusted according to the expansion requirements.

The key to promoting self-renewal of HSCs lies in regulating the balance between proliferation and differentiation, which requires activating dormant stem cells to enter the proliferative cell cycle while also preventing excessive oxidative phosphorylation and ROS production. For example, the HSC self-renewal agonist ³UM171 stimulates *ex vivo* HSC expansion by establishing a critical balance between proinflammatory and anti-inflammatory mediators^[24].

In our research, low concentrations of NAM caused HSCs to exit G0 phase and enter the proliferative cell cycle, leading to an increase in cell numbers. In addition, the low concentrations of NAM inhibited differentiation by reducing ROS production. This indicates that low concentrations of NAM can better regulate the balance between

proliferation and differentiation, thereby promoting effective expansion of HSCs. When the concentration of NAM exceeds 10 mmol/L, apoptosis and cell necrosis increase, which is not conducive to the survival and self-renewal of HSCs. The high intracellular ROS levels detected in our 10 mmol/L NAM group contradict the fact that high concentrations of NAM are beneficial for maintaining LT-HSCs. This may be due the cytotoxicity of high concentrations of NAM, and this was confirmed by the significant increase in apoptotic cells and significant decrease in cell viability in the 10 mmol/L and 15 mmol/L NAM groups.

Activating SIRT1 can protect cells from oxidative stress damage and regulate cell proliferation by upregulating the SIRT1-HIF1A pathway^[25], whereas inhibiting SIRT1 may be related to delaying differentiation^[26]. NAM is considered a SIRT1 inhibitor to delay stem cell differentiation. We measured the transcription levels of SIRT1 in each group of cells and found that compared with the control group, SIRT1 was significantly upregulated in the 5 mmol/L NAM group and significantly downregulated in the 10 mmol/L NAM group. This indicates that NAM can only inhibit SIRT1 expression at high concentrations, whereas low concentrations of NAM not only cannot inhibit SIRT1 but also may upregulate transcription of this crucial regulator. Activation of SIRT1 may promote cell proliferation by upregulating the SIRT1-HIF1A pathway, which is consistent with the higher proportion of S phase cells and fold expansion of TNCs observed in the 5 mmol/L NAM group in our above results.

CONCLUSION

In conclusion, different concentrations of NAM have distinct effects on proliferation and differentiation of HSCs. Although a high concentration of NAM is more conducive to the expansion of LT-HSCs, the cells are affected by oxidative stress and apoptosis, which may negatively impact hematopoietic reconstitution after transplantation. Low concentrations of NAM can better regulate the balance between proliferation and differentiation, thereby promoting effective expansion of HSCs. In addition, the transcription level of SIRT1 is correlated with the concentration of NAM. Upregulation

of SIRT1-HIF1A can promote proliferation, whereas inhibition of SIRT1 may achieve a delayed differentiation effect.

ARTICLE HIGHLIGHTS

Research background

The *in vitro* expansion strategy of increasing the number of hematopoietic stem cells (HSCs) in cord blood is expected to improve its clinical efficacy. Nicotinamide (NAM) is one of the small molecules that can promote the expansion of hematopoietic stem and progenitor cells (HSPCs) *ex vivo*, but its mechanism of action and its effects on short-term repopulating HSCs (ST-HSCs) and long-term repopulating HSCs (LT-HSCs) have not yet been revealed. Revealing the dominant subgroups of NAM and elucidating the relevant mechanisms not only helps to recommend the working concentration of NAM according to demand, but also helps to reveal the signaling pathways involved in the regulation of HSC proliferation and differentiation.

Research motivation

The effects of different concentrations of NAM on proliferation and differentiation of HSCs, as well as whether it affects sirtuin 1 (SIRT1) transcription levels, have not been reported yet. This is crucial for selecting the working concentration of NAM and predicting small molecules that have a synergistic effect with NAM.

Research objectives

Evaluate the effects and mechanisms of different concentrations of NAM on HSC proliferation and differentiation.

Research methods

In this study, we added different concentrations of NAM to serum-free culture medium inoculated with CD34+ cells. We then measure the number, molecular phenotype, cycle distribution, and apoptosis of each group of cells and explore the mechanism by

detecting the levels of reactive oxygen species (ROS), inflammatory factors and related gene transcription.

Research results

In this study, we evaluated the expansion efficiency of different concentrations of NAM on HSPCs, ST-HSCs as well as LT-HSCs, and the results showed that both 5 mmol/L and 10 mmol/L NAM are beneficial for maintaining HSPCs, with low concentrations (5 mmol/L) of NAM being more conducive to ST-HSCs expansion, while high concentrations (10 mmol/L) of NAM have a more significant effect on promoting LT-HSCs expansion. In general, low concentrations of NAM can better regulate the balance between proliferation and differentiation, thereby promoting effective expansion of HSCs.

Research conclusions

Low concentration NAM did not inhibit but upregulated the transcription of SIRT1, promoting cell proliferation by activating the SIRT1-HIF1A pathway, delaying stem cell differentiation by increasing ROS clearance, and ultimately promoting effective expansion of HSCs.

Research perspectives

Our findings could allow adjustment of nicotinamide concentrations according to expansion needs and may help predict small molecules that synergistically promote expansion with nicotinamide.

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