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Monomeric Proanthocyanidins Increase Intracellular NAD+ Content by Enhancing NAMPT Transcription

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Abstract

Proanthocyanidins (PCs), a group of polyphenolic compounds that have been studied for almost 80 years, have a high antioxidant capacity and considerable benefits to human health. These compounds are effective at scavenging free radicals in organisms and have anti-inflammatory and antiaging effects. The content of nicotinamide adenine dinucleotide (NAD+) decreases with age. Exogenous supplementation with NAD+ plays a vital role in impeding aging and prolonging lifespan; thus, NAD+ is a potential target for antiaging research. There is evidence that PCs can affect the content of NAD+, but the effects of monomeric proanthocyanidins (MPCs) and oligomeric proanthocyanidins (OPCs) on NAD+ levels are still unclear. In our study, normal human diploid skin fibroblast BJ cells were treated with MPCs and OPCs, and changes in intracellular NAD+ content were examined. Real-time qPCR was used to detect changes in the transcription of enzymes associated with NAD+ synthesis. The results showed that both MPCs and OPCs could increase NAD+ content. In low-passage BJ cells, the effects of MPCs were greater than those of OPCs. Moreover, the qPCR results showed that the mRNA expression levels of the rate-limiting enzyme NAMPT were significantly upregulated in PC-treated low-passage and senescent BJ cells, suggesting that the effect of PCs on NAD+ content may be achieved by regulating NAMPT transcription, which may provide a theoretical basis for the effect of PCs on intracellular NAD+ content.

Keywords

Proanthocyanidins; NAMPT; NAD+.

1. Introduction

Nicotinamide adenine dinucleotide (NAD+), commonly known as coenzyme I, is a small coenzyme that has been researched for more than 100 years and that plays an important role in redox reactions [1,2]. Many studies have reported that NAD+ content gradually decreases with age, which is closely related to the occurrence of age-related diseases and metabolic diseases [3,4]. Under normal conditions, NAD+ consumption and synthesis maintain the NAD+ balance in the body, and the NAD+ biosynthesis pathway plays a particularly important role [5,6]. The Preiss-Handler pathway, de novo biosynthesis pathway, and salvage pathway are the three classical pathways of NAD+

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synthesis in mammalian cells [6]. Among them, the salvage pathway is the main pathway for the synthesis of NAD+, in which nicotinamide phosphoribosyl transferase (NAMPT) is the rate-limiting enzyme; NAMPT can catalyze the conversion of nicotinamide (NAM) to nicotinamide mononucleotide (NMN), the direct precursor of NAD+ [7,8]. In addition, nicotinamide riboside (NR) can also be phosphorylated by nicotinamide riboside kinase (NRK) to form NMN, which can then be converted to NAD+ by nicotinamide mononucleotide adenylyl transferases (NMNATs), which are localized and active in different tissues and cells [6].

Proanthocyanidins (PCs), a class of polyphenolic compounds widely present in plants, are natural antioxidants that can effectively scavenge free radicals in the human body [9,10]. More than 70 years ago, PCs were first discovered and extracted, and French scientist Jacques Masquelier was later the first to confirm their antioxidant function [11,12]. Currently, the main source of PCs is grape seeds [13,14]. PCs are characterized as three forms based on the degree of polymerization: monomeric proanthocyanidins (MPCs) with a degree of polymerization of 1, oligomeric proanthocyanidins (OPCs) with a degree of polymerization of 2-4, and polymer proanthocyanidins (PPCs) with a degree of polymerization greater than or equal to 5 [10,15,16]. Numerous studies have reported that OPCs have stronger antioxidant activity than do MPCs [9,17,18]. The antioxidant capacity of OPCs is dozens of times higher than that of vitamin C and vitamin E [19-22]; therefore, OPCs play important roles in antitumor activity, immune regulation, DNA damage repair, etc. [23].

Previous studies have indicated that OPCs have superior antioxidant capacity to MPCs [9,17,18,24]. Although several studies have suggested that PCs can regulate NAD+ content [7,25,26], the role of MPCs or OPCs in regulating NAD+ content has not been reported. In this article, we investigated the effect of MPCs and OPCs on NAD+ content and the underlying mechanism, aiming to provide a theoretical basis for the regulation of intracellular NAD+ content by PCs.

2. Materials and Methods

2.1 Reagents

PCs were purchased from Beijing Solaibao Technology Co.; NMN was a gift from Kingdomway Co., Ltd.

2.2 Cell Culture and Treaments

The human normal diploid skin fibroblast BJ cell line was preserved in our laboratory. BJ cells were grown in Dulbecco's modified Eagle’s medium/F-12 (SH30023.01, Gibco) supplemented with 10% fetal bovine serum (FSP500, ExCell Biology) and 1% penicillin and streptomycin (Q6532-100mL, Macklin) in a cell culture incubator at 37 °C with 5% CO2. The medium was changed every three days, and the cells were passaged at 80-90% confluence.

2.3 Cell Viability Assay

The cells were plated into 96-well plates at 80-90% confluence. CCK8 (Cell Counting Kit-8; C0038, Beyotime) solution (10 μL) was added to each well on the second day after the cells reached confluence. Then, the absorbance value of each well was measured at 450 nm in a microplate reader (BioTek).

2.4 RNA Extraction and Real-time qPCR

Total cellular RNA was extracted by using RNAiso Plus reagent (9109, Takara) according to the manufacturer's instructions. RNA concentration and purity were detected using a NanoDrop 8000 Spectrophotometer (Thermo Fisher Scientific). A total of 1 μg of purified RNA was reverse transcribed into cDNA using HiScript III RT SuperMix for qPCR (+gDNA wiper) (R323, Vazyme). Real-time quantitative PCR was performed with SYBR Green Master Mix using a QuantStudio6 Flex real-time PCR system (Thermo Fisher Scientific). The relative mRNA levels of the selected genes were calculated with the $2^{-\Delta\Delta Ct}$ method and normalized to those of β-actin. The primer sequences are listed in Table 1.

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Table 1. Primer sequences for qPCR

Gene	Species	Forward (5'-3')	Reverse (5'-3')
β-actin	human	CATGTACGTTGCTATCCAGGC	CTCCTTAATGTCACGCACGAT
NAMPT	human	AGGGTTACAAGTTGCTGCCACC	CTCCACCAGAACCGAAGGCAAT
NMNAT1	human	GTGGAAAGAGACTCTGAAGGTGC	CTTGTGTTTCAGTCCACTTCCTC
NMNAT2	human	GTAGTGACCTGCTGGAGTCCTT	ATGATTCGGTCTGTGTCGGCTG
NMNAT3	human	GGATGGAGACAGTGAAGGTGCT	GTCGAGAAGAGTGCCTTGCCAT

2.5 NAD+ Content Assay

The NAD+ content was measured with a commercial kit (S0175, Beyotime). All experimental procedures were conducted according to the manufacturer's instructions.

3. Results

3.1 Pcs Can Increase Intracellular NAD+ Content

First, to investigate the effects of an MPC and an OPC on cell viability, we conducted CCK8 assays. The results showed that both the MPC and OPC promoted cell proliferation at low concentrations (10 μg/mL and 20 μg/mL). At higher concentrations, both compounds inhibited cell proliferation, and the inhibitory effect of the MPC was more pronounced. To study the effect of PCs on the NAD+ content of low-passage and senescent BJ cells, a concentration of 20 μg/mL was chosen, as there was no difference in the effects of the MPC or OPC on cell proliferation at this concentration. Using a kit to detect intracellular NAD+ content, we found that both the MPC and OPC increased NAD+ content in low-passage and senescent BJ cells. Interestingly, the MPC was more effective than the OPC at increasing NAD+ content in low-passage BJ cells but not in senescent BJ cells.

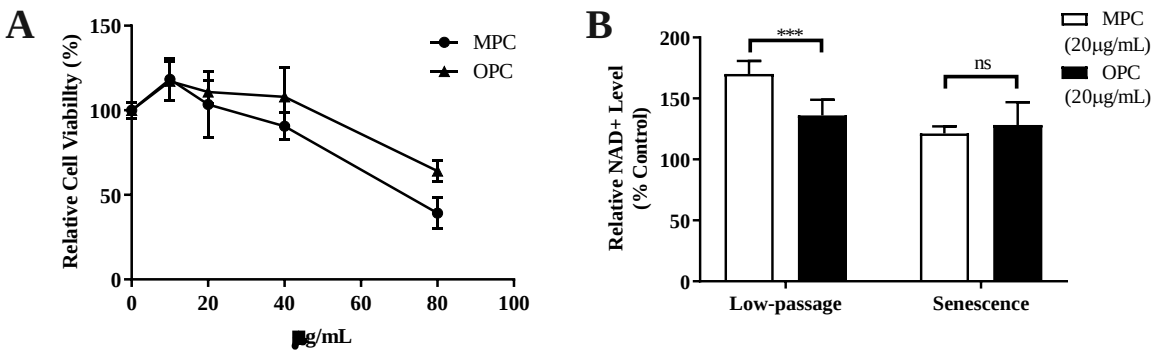

Figure 1. The MPC and OPC increased intracellular NAD+ content

A, Effect of different concentrations of the MPC and OPC on cell viability. B, Effect of 20 μg/mL MPC and OPC on NAD+ content in low-passage and senescent BJ cells. n≥3, ***P<0.001.

3.2 The MPC and NMN Equivalently Increased Intracellular NAD+ Content

NMN is a direct precursor of NAD+, and many studies have reported that NAD+ content can be increased by exogenous NMN supplementation [7,27]. We studied the effect of different concentrations of NMN on BJ cell proliferation. The results showed that treatment with 0-1 mM NMN promoted cell proliferation, but high concentrations (2 mM and 4 mM) of NMN did not affect cell viability. The NAD+ content in the 20 μg/mL MPC-treated group was equivalent to that in the 1 mM NMN-treated group.

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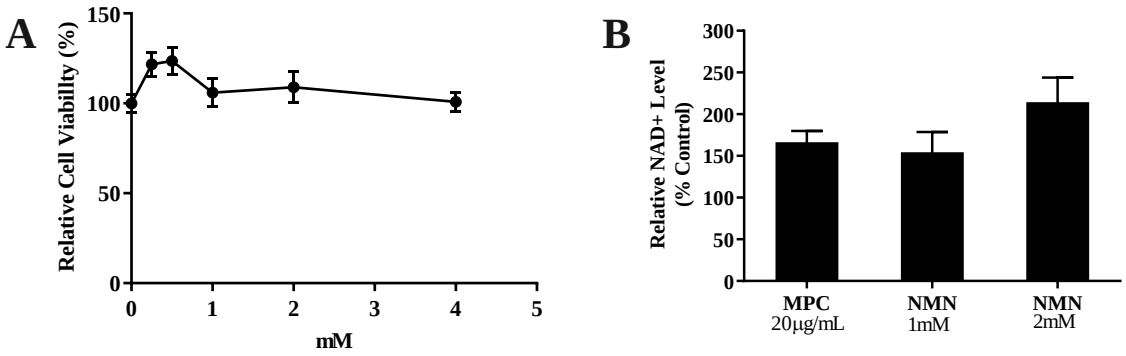

Figure 2. The MPC and NMN equivalently increased the intracellular NAD+ content

A, Effect of different concentrations of NMN on cell viability. B, Effect of MPC and NMN on NAD+ content in low-passage BJ cells at the indicated concentrations.

3.3 PCs Increased Intracellular NAMPT Transcript Levels

To investigate whether the MPC and OPC increased cellular NAD+ content through NAMPT, we examined NAMPT expression levels by qPCR assays. The results showed that the mRNA levels of NAMPT were significantly elevated in low-passage and senescent BJ cells in the MPC- and OPC-treated groups compared to the control group. Moreover, NAMPT expression levels were significantly higher in MPC-treated cells than in OPC-treated cells.

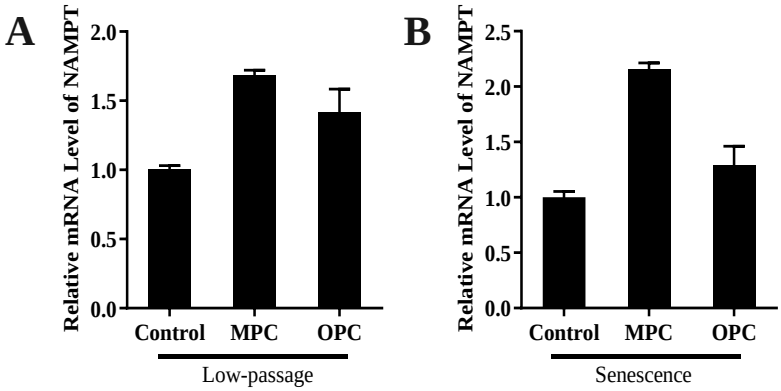

Figure 3. The MPC and OPC increased NAMPT transcript levels in BJ cells

A, NAMPT transcript levels in low-passage BJ cells treated with 20 μg/mL MPC or OPC. B, NAMPT transcript levels in senescent BJ cells treated with 20 μg/mL MPC or OPC.

4. Conclusion

At present, many studies have suggested that PCs have not only extraordinary antioxidant capacity but also anti-inflammatory and antiaging effects. PCs have been shown to increase NAD+ content in the rat liver, and higher concentrations of PCs have more pronounced effects, which are mainly achieved by regulating the concentration of NAD+ precursors in the liver and the mRNA levels of genes encoding enzymes related to NAD+ metabolism. Wencui Wan et al. demonstrated that PCs increased NAD+ content by increasing NAMPT transcription levels and mitigated retinal cellular senescence in aging mice.

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In this study, we found that PCs were responsible for enhancing the NAD+ content in both low-passage and senescent BJ cells and that the MPC was more effective than the OPC in low-passage BJ cells. Moreover, 20 μg/mL MPC was comparable to 1 mM NMN at boosting NAD+ content. The qPCR results demonstrated that the expression of NAMPT, the rate-limiting enzyme in the NAD+ salvage pathway, was significantly elevated in BJ cells after treatment with the MPC or OPC. Similarly, we found that the MPC was more effective than the OPC in both low-passage and senescent BJ cells. These results suggest that PC probably increases NAD+ content through NAMPT. Taken together, the data generated in this study shows that MPCs are more effective than OPCs at increasing NAD+ content in BJ cells, and this effect is likely achieved by increasing NAMPT transcript levels.

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