

Effects of Natural Products on Nonalcoholic Fatty Liver Disease through Nrf2 Epigenetic Regulation

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Abstract: Non-alcoholic fatty liver disease has become one of the common illnesses among urban dwellers worldwide. Many consider natural products as better options for medicine and diet supplements. In recent years, researchers found that natural products can prevent human diseases by reactivating silencing genes through epigenetic regulation. They also discovered nuclear factor erythroid-2-related factor-2 (Nrf2) as an important factor in the response of anti-oxidation has a close relation to lipogenesis. Studies showed with the higher expression of DNA methyl-transferases, the higher methylation of Nrf2 promoter occurred. With the high methylation rate of the Nrf2 promoter, the expression of Nrf2 reduced thus enhanced lipid accumulation, which may cause NAFLD. Furthermore, with lower expression of Nrf2, the downstream anti-oxidant genes' expression decreased, which may cause oxidative stress to human body cells. Understanding the pathway of Nrf2 and NAFLD can shed a light on discovering new development of drugs. This paper reviews several natural products and their mechanisms of alleviating NAFLD and hopes to bring in new perspectives on the treatment and the development of new drugs on NAFLD.

1 Introduction

Non-alcoholic fatty liver disease (NAFLD) is a frequent cause of chronic liver diseases due to the prevalence of high-fat diet and obesity in modern society. It refers to a broad spectrum of liver pathology which varies from simple steatosis to non-alcoholic steatohepatitis (NASH) and even cirrhosis[1-3].

Nuclear factor erythroid-2-related factor-2 (Nrf2) is an crucial transcription factor in the response of cells to oxidative stress and a central key for cells to resist antioxidant damage, which control the composition of oxidative response element (ARE) dependent genes to direct the physiological reaction of free radical and antioxidant imbalance[4]. It exists in organs like liver and kidney and a variety of tissues and cells. Under normal physiological conditions, Nrf2 can bind to KEAP1. The dissociation of KEAP1 and Nrf2 is a result of response to oxidative and electrophilic stress because it phosphorylates and induces conformational changes of Nrf2. The dissociated Nrf2 is transferred to the nucleus, where it dimerizes with MAF family proteins and activates the ARE pathway, thereby improving transcription of various cell-protective genes, including HO-1, NQO1, SOD, GCLM, and MRP1. The anti-oxidative stress defense mechanism of the Nrf2-ARE pathway is a potential cancer prevention and treatment[5-7]. Recent research revealed that Nrf2 is related to NAFLD through multiple pathways[8].

Growing evidence indicates that epigenetic modifications control gene expression, which involves DNA methylation and histone modification. DNA

methyl-transferases (DNMTs) catalyze the methylation of CpG island cytosine in DNA, interfering with the binding of RNA polymerase and DNA to reduce transcription. Major DNMTs, including DNMTs 1, 3A, and 3B, transfer methyl groups from S-adenosylmethionine (SAM) to cytosine[9].

In recent studies, it is discovered that natural products have shown promises of preventing human diseases through epigenetic regulation by reactivating silencing genes. This paper reviews several natural products and their mechanisms of relieving NAFLD and hopes to provide new ideas on the treatment and the development of new drugs on NAFLD.

2 Mechanism of Nrf2 signaling pathway action in NAFLD

Under the basal condition, Keap1 protein promotes the proteasomal degradation of Nrf2 as its inhibitor, but it allows Nrf2 to stabilize and translocate into the nucleus to regulate transcription numerous genes under oxidative stress, which are vital in protection against oxidative stress[10-11](Fig. 1).

Nrf2 may also be related to the reduction in triglyceride level and decrease in the expression of lipogenesis-related genes. Nrf2 can protect cells from steatosis by inhibiting lipogenesis and promoting fatty acid oxidation because of the activation of ARE-containing transcription factors that adjust adipocyte differentiation and adipogenesis and by the protection against redox-dependent inactivation of metabolic

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enzymes[12]. Apart from enhancing the expression of antioxidant enzymes such as HO-1, Nrf2 can also participate in the anti-oxidation processes in the liver[13], which can reduce lipid growth by controlling lipogenesis.

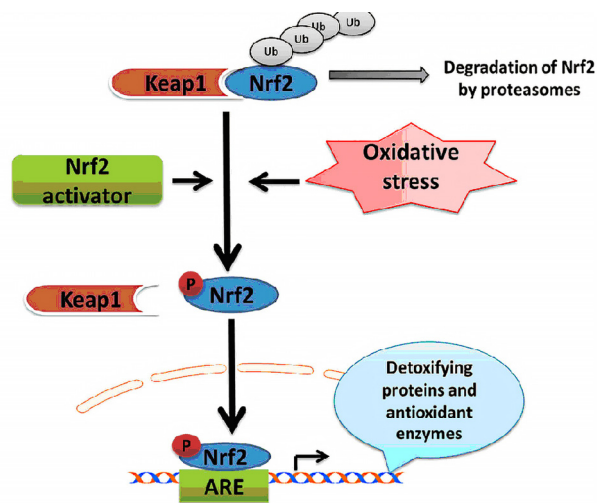


Fig. 1. Nrf2 signaling pathway

3 Effects of natural products on NAFLD

DNA methylation is one of the critical epigenetic mechanisms for controlling gene expression in eukaryotes. Based on several studies, the Nrf2 promoter is known to be highly methylation in NAFLD cells[17-19], however, the underlying mechanism is still not completely explored.

Resveratrol is a natural polyphenol and phytoalexin, which has abundant biological properties as anti-inflammatory, antioxidant, anti-tumor, anti-aging, cardiovascular protective and chemo-protective, anti-diabetes, etc[14-16]. Zhao et al. suggested that DNA demethylation may create a positive link for reactivating the Nrf2 signaling in Alzheimer's disease cellular model[20].

According to Hosseini et al., resveratrol could reduce the DNA methylation rate of the Nrf2 promoter in mice fed by a high-fat diet (Fig. 2)[21]. After feeding HFD, the level of Nrf2 methylation in those mice was significantly high as well as mRNA and the protein expression level of Nrf2. By adding resveratrol, the level of Nrf2 mRNA and the expression of Nrf2 protein increased, together with the expression of downstream antioxidant, sterol regulatory element-binding transcription factor 1c (SREBP-1c), and fatty acid synthase (FAS) genes.

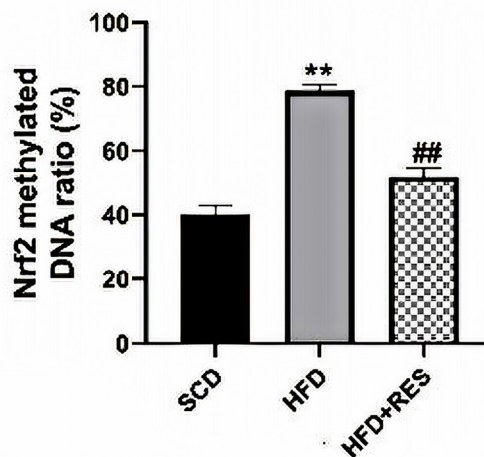


Fig. 2. Nrf2 methylated DNA ratio[21]

Aged citrus peel (Chenpi) is made from the dried peel of orange or tangerine belonging to the family Rutaceae[21]. Mainly composed of essential oils, pectin, phenolic acid, flavonoids, and polymethoxyflavonoids (PMFs), the citrus peel can play an effective role in liver health. condition According to Lin et al., Chenpi triggered the antioxidative pathway induced by Nrf2. Besides, Chenpi can limit the expression of DNA methylation transferase(DNMT) 1 and 3A, which are the main enzymes in transferring methyl-CpG sites(Fig. 3), to decrease the methylation level of the Nrf2 promoter. Taken together, Chenpi silences DNMT and decreases the rate of Nrf2 gene promoter methylation[23], resulting in demethylating the Nrf2 promoter.

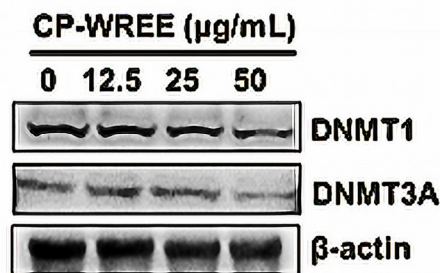


Fig. 3. Expression of DNMT1 and DNMT3A[23]

Sulforaphane, produced from cruciferous vegetable plants, has been proved to inhibit or retard tumor incidence and progression in models of breast, colon, stomach, and lung cancer[24]. According to Zhou et al., sulforaphane can create homeostatic protection of cells and tissues against carcinogens[25], by repressing DNMT1 protein expression and lessening DNMT1 activity to lead to the demethylation of the promoter region of Nrf2 and increased activation of Nrf2, inducing the transcription of the defensive enzymes glucuronyl transferase(UGTs).

Anthocyanidins, abundant in many fleshy fruits such as berries, red grapes, etc. are important health-promoting phytochemicals[27]. Anthocyanidins give the intense color to many fruits and vegetables. Among all types of anthocyanidins, delphinidin is reported to be

one of the most efficient compounds. Delphinidin has been reported to specifically inhibit inflammatory signals[28]. According to Kuo et al., delphinidin could reduce the rate of CpG methylation in the Nrf2 promoter region. And decreased the expression of DNMT1 and DNMT3A(Fig. 4)[29]. The decreased methylated ratio of Nrf2 gene promoter causes the reactivate of Nrf2 signaling pathway which may decrease the risk of NAFLD.

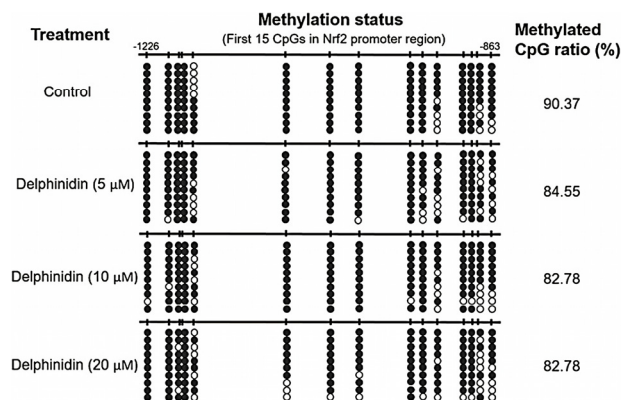


Fig. 4. Methylated CpG ratio[29].

4 Conclusion

This paper presents an overview of the up-to-date knowledge regarding the epigenetics of Nrf2 gene promoter of NAFLD. The main mechanism of these natural products is to silence the expression of DNMTs which can lower the methylation rate of Nrf2 and lead to the enhancement of Nrf2 and downstream antioxidant enzymes gene expression. These natural products provide new options on the treatment of NAFLD (Fig. 5).

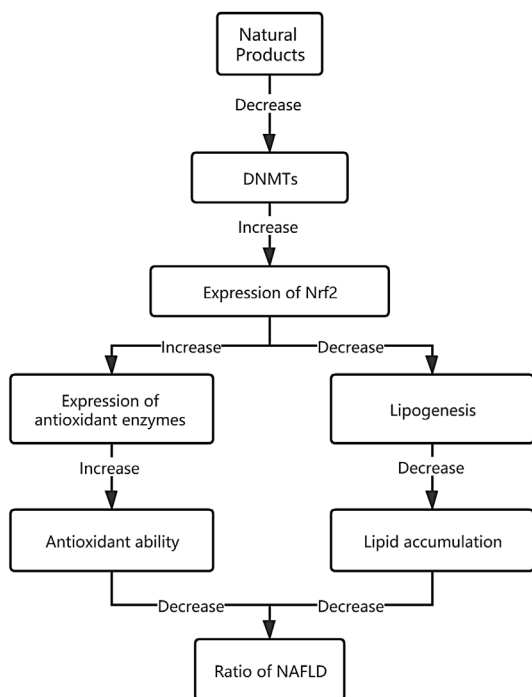


Fig. 5. Natural products decrease ratio of NAFLD

5 Prospect

The main clinical treatment for NAFLD is surgery. However, most patients concern the risk of surgical treatment. Patients now have more options, like specific drugs to balance lipid metabolism for NAFLD treatments, instead of the traditional operation. The active compound from the natural products has gathered more and more attention for the past few years. As for the invention of drugs on NAFLD, mainly consists of phenolic acids, alkaloids, flavonoids, etc. These compounds have different chemical structures but with similar effects and results. Hence, the epigenetic mechanism plays a vital role in the diagnosis and treatment of NAFLD. So by understanding the basic effect of these natural products, it can provide more clues on solving the underlying pathway of NAFLD. Fatty liver disease is known to relate to oxidation. Nrf2 served as the major antioxidant signaling pathway, so by learning the pathway of Nrf2 with NAFLD, we hope to discover more suitable drug supplement or dietary plan for NAFLD patients or susceptible population.

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