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Review

Chemopreventive role of anthocyanins in atherosclerosis via activation of Nrf2–ARE as an indicator and modulator of redox

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ABSTRACT

Anthocyanins have been reported to induce the expression of enzymes involved in both cellular antioxidant defenses and attenuating inflammation-associated pathogenesis. Induction of such enzymes by edible anthocyanin largely accounts for their atherosclerosis chemo-protective activities. Nuclear factor erythroid 2-related factor 2 (Nrf2) plays an essential role in the coordinated induction of those genes encoding redox-responsive and cellular defense antioxidant enzyme termed antioxidant response element (ARE). Current studies have revealed that Nrf2–ARE signaling is involved in attenuating inflammation-associated pathogenesis such as atherosclerosis. Conversely, reduction in Nrf2 signaling leads to enhanced susceptibility to oxidative stress and inflammatory tissue injuries. The activation of Nrf2–ARE might inhibit the production of pro-inflammatory mediator including cyclooxygenase-2, chemokines, cytokines, cell adhesion molecules, and induction nitric oxide synthase. This review highlights the gene expression induced by dietary anthocyanin via Nrf2 signaling on redox-regulated transcription factor in atherosclerosis disorders.

1. Introduction

It is now well known that atherosclerosis is an inflammatory disease [1]. Initial atherosclerotic vascular lesions preferentially develop in regions with low fluid shear stress and non-laminar blood flow. Conversely, lesion development is declined in areas with high fluid shear stress and steady laminar flow [2]. In this connection, increased expression of antioxidant response element (ARE) mediated antioxidant genes such as quinone oxidoreductase (NQO1), and heme oxygenase-1 (HO-1) through nuclear factor erythroid 2-related factor-2 (Nrf2) dependent mechanism induced prolonged physiological levels of laminar shear stress for endothelial cells [3].

The inducible expression of several inflammatory genes such as vascular cell adhesion protein 1 (VCAM-1), and monocyte chemoattractant protein (MCP)-1 is known to be regulated through oxidation-redox-sensitive mechanisms which play a crucial role in the initiation and progression of atherosclerosis [1,4]. Oxidant stress contributes to the pathogenesis of atherosclerosis by stimulating inflammatory gene expression. The enhanced expression of the Nrf2/ARE regulated cytoprotective proteins may contribute to the atheroprotective and anti-inflammatory phenotype observed in areas of the vasculature exposed to laminar flow. Therefore, the activation of the Nrf2/ARE pathway may suppress redox-sensitive inflammatory gene expression and protect against oxidant-mediated injury in endothelial cells.

Nrf2 is a redox-sensitive transcription factor, which contains the basic leucine zipper region [5]. Nrf2 binds to ARE located in the promoter area of genes encoding many phase II detoxifying or antioxidant enzymes and related stress-responsive proteins which include glutathione S-transferase (GST), NQO1, HO-1, glutathione peroxidase (GPx), glutamate cysteine ligase (GCL), and peroxiredoxin I (Prx I). These enzymes play key roles in cellular defense by enhancing the removal of cytotoxic electrophiles or reactive oxygen species (ROS) [5]. Induction of these enzymes through the Nrf2/ARE signaling provide an effective means for achieving cellular protection against ROS and inflammatory disorders [6].

Anthocyanins, a category of phytochemicals are largest group of water-soluble red, purple, blue flavonoid natural pigments. These compounds have a complex structure of an aromatic three-ring molecular area, one or more attached sugar, and sometimes acyl groups attached to sugar (Fig. 1).

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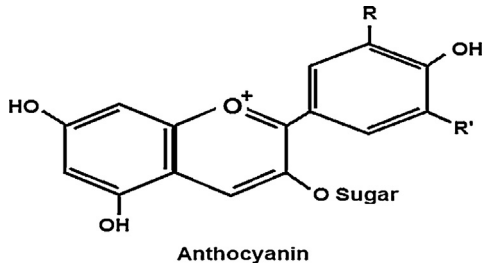

Fig. 1. Selected chemopreventive phytochemicals that can activate Nrf2–ARE signaling.

According to this structure, anthocyanynins are electrophiles, positively charged species that are attracted to an electron rich center [47]. The main dietary sources of anthocyanin are blue and purple fruit, such as berries and grapes [7]. It has been shown that anthocyanin-rich extract are effective antioxidant compounds able to reduce inflammation [8], lipid peroxidation, and the deleterious effects of ROS in vitro [9]. This anti-inflammatory effect may contribute to the overall benefits against atherosclerosis as an inflammatory disorder [10]. Anthocyanins have the ability to protect human endothelial cells via the activation of nuclear factor 2 (Nrf2) pathway, through the involvement of extracellular signal-regulation kinase (EPK 1,2) [11].

This review tried to focus mainly on the protective role of Nrf2 via stimulation by anthocyanin as potential antioxidant in inflammatory atherosclerosis disorders which has a crucial pro- and anti-inflammatory mediator and is modulated by Nrf2/ARE signaling.

2. Anthocyanins (polyphenols) role in oxidative stress via Nrf2 pathway

Polyphenols include anthocyanin are abundant components of fruits and vegetables have an inverse association with the incidence of various degenerative diseases [12]. Mechanisms underlying its beneficial effects on the human health are being investigated worldwide. Varieties of studies have demonstrated that anthocyanins have modulatory effects in cells by interacting with a wide range of molecular targets central to the cell signaling to reduce oxidative damage [13]. Such oxidative damages can be prevented by bZIP domain family transcription factors through differentially regulating antioxidant and detoxification genes, which contain ARE and its homologues in their promoters [16]. Amongst, Nrf2 is a master regulator of expression of drug-metabolizing enzymes, and its activity is negatively regulated by Keap1. Regulatory networks indicate that anthocyanins trigger multiple signaling pathways to integrally activate cytoprotective genes against cytotoxic insults and oxidative stress.

3. Structure of Nrf2–Keap1 complex

Kelch-like ECH protein (Keap1) consists of 624 amino acid residues in five domains. The two protein–protein interaction motifs, the BTB domain and the Kelch domain, are separated by the intervening region (IVR). The BTB domain together with the IVR mediates homodimerization of Keap1 and binding with Cullin3 (Cul3). The Kelch domains mediate the interaction with ETGE and DLG in Neh2 motifs (Fig. 2). Keap1 is rich in cysteine residues that excellent targets of electrophiles and oxidants. The modification pattern of the cysteine residues by electrophiles is known as the cysteine code [14].

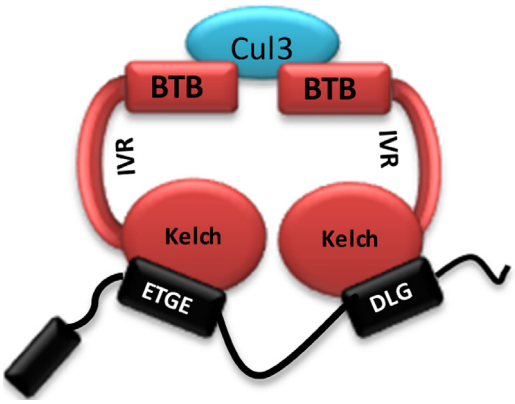

Fig. 2. A cytoskeleton protein structure of Nrf2 and Keap1. Nrf2 (black) interacts with two molecules of Keap1 (red chain) through its Neh2 ETGE and DLG motifs. Both ETGE and DLG bind to similar sites on the bottom surface of the Keap1Kelch. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

Nrf2 consists of 589 amino acids in six domains, Neh1–6 (Fig. 2). Neh1 contains a basic region-leucine zipper (L-Zip) structure, which is responsible for DNA recognition and it mediates dimerization with small Maf proteins. Neh6 functions as a degron to mediate degradation of Nrf2 in the nucleus. Neh4 and 5 are transactivation domains. Neh2 contains ETGE and DLG motifs, which are required for the interaction with Keap1 and a hydrophilic region of lysine residues. These mentioned motifs are essential for the Keap1-dependent polyubiquitination and degradation of Nrf2 [15,25].

4. Mechanisms underlying up-regulation of Nrf2/ARE-dependent genes

Nrf2 is associated with the cytoskeleton protein Keap1 in the cytoplasm under the basal resting condition (Fig. 3) [16]. Keap1 functions as a down regulator of Nrf2 by promoting ubiquitination and proteasomal degradation of Nrf2 [17,18]. When liberated from its repressor Keap1, Nrf2 translocates into the nucleus and forms a heterodimer with a small Maf protein [19]. The Nrf2–Maf dimer then binds to ARE, a cis-acting DNA regulatory element where localized in the promoter region of many genes whose products have a cellular defensive function [6].

Generate reactive species (ROS) or electrophiles that can interact with cysteine (thiol) residues present in the functionally critical motif of Keap-1 as a sensor for electrophilic compounds [5] as illustrated in Fig. 3. Upon stimulation of cells with ROS, the reactive cysteine residues within Keap1 undergo oxidation and form an intramolecular disulfide bond. A compound with high electron withdrawing potency can also function as an Nrf2 activator through modification of cysteines in Keap1 [20]. Over-expression of Nrf2 leads to an increased ARE transcriptional activity, thereby augmenting expression of several ARE-dependent antioxidant and cytoprotective enzymes including HO-1, GCL, GPx, and NQO1 [3,21–24].

It is quite likely that the sites of Keap1 modification may vary depending on the type of reactive chemicals and also the intracellular redox environment [25,27]. The selection of compounds which do not have cellular toxicity, but they have stimulation on modify Keap1 structure, may provide important clues for the progression of therapeutically applicable drugs targeting Nrf2.