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The Power of Daphnetin: Enhancing Nrf2 Signaling Pathway

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Abstract

A chronic inflammation and oxidative stress are involved in a range of diseases. The Nrf2 pathway is crucial in controlling cellular defenses against stresses. Daphnetin (DAP), a compound derived from plants such as *Daphne odora*, has shown promise to activate Nrf2, a protein with potential therapeutic uses. This review examines how DAP influences the Nrf2 pathway, increasing genes that promote antioxidant and anti-inflammatory effects. We present preclinical evidence showcasing the effectiveness of DAP in several illness models linked to inflammation and oxidative stress. In addition, we investigate recent progress in altering the structure of DAP to increase its effectiveness and improve its pharmacological characteristics. Lastly, we address prospective areas of future study, highlighting the importance of clinical translation studies in fully realizing DAP's therapeutic benefits. In summary, this study highlights the potential of DAP as a natural Nrf2 activator, which has important implications for preventing and managing diseases.

Keywords: Daphnetin, Nrf2 pathway, Anti-inflammatory, Antioxidant, Therapeutic potential

1. Introduction

Nuclear factor erythroid-derived 2 (NF-E2)-related factor 2 (Nrf2), a member of the basic leucine zipper (bZIP) transcription factor family encoded by the *NFE2L2* gene, plays a pivotal role in cellular defense mechanisms against oxidative stress and inflammation. Activation of Nrf2 triggers the expression of genes responsible for detoxification enzymes and antioxidants, thereby safeguarding cells from oxidative damage (1). One of the most basic mechanisms by which cells protect themselves from oxidative and electrophilic stresses is the network of phase II detoxifying and antioxidant enzymes. A cap'n'collar (Cnc) family member of essential leucine zipper transcription factors, Nrf2, may protect cells and tissues from oxidative damage by transcriptionally activating genes encoding detoxifying and antioxidant enzymes. Activation of this specific transcription factor enhances cellular antioxidant capacity, which blocks the onset of carcinogenesis. This property is shared by many chemoprotective or chemopreventive phytochemicals (2). Cap'n'collar (Cnc) transcription factors play critical roles in development and homeostasis and are conserved in metazoans. Cnc factors include the vertebrates Nrf1, Nrf2, Nrf3, the Caenorhabditis elegans SKN-1, and the *Drosophila* CncC. These factors mediate adaptive responses to cellular stress. Nrf2, which controls the transcriptional response of cells to oxidative stressors and electrophilic xenobiotics, is the most researched stress-activated Cnc factor (3). In normal tissues, Nrf2 controls the defensive reactions (4). As a result, activation of Nrf2 offers cytoprotection against various illnesses and conditions, such as metabolic disorders, autoimmune diseases, neurodegenerative diseases, and cancer (5). Nrf2 functions as both an antioxidant and a detoxifying activator and it also plays a crucial role in mitochondrial and intermediary metabolism as part of its cytoprotective function. Therefore, the activation of Nrf2 through the knock-down of Kelch-like ECH-associated protein 1 (Keap1) leads to an

enhancement in mitochondrial fatty acid oxidation (1), and is broken down quickly by the ubiquitin-proteasome system (6). Mitochondrial failure and neuroinflammation are common characteristics of various neurodegenerative disorders. Additionally, Nrf2 has been identified as a potential target for therapeutic intervention (7). Nrf2 is not necessary for the development of blood cells. Still it does play a role in activating a group of drug-metabolizing enzymes (DMEs), such as glutathione S-transferase (GST) and NAD(P)H:quinone oxidoreductase 1 (NQO1), in response to antioxidants and electrophiles. Induction necessitates the presence of a shared DNA sequence known as the antioxidant response element (ARE), which bears resemblance to the NFE2-binding motif (8). Several antioxidant and phase II drug-metabolizing enzymes are controlled at the transcriptional level by phenolic antioxidants and electrophilic substances. The encoding genes' promoters contain a cis-acting ARE, which mediates the response to these chemicals (9).

Phytochemicals are linked to enhanced human health and longer lifespan as a result of their antioxidant qualities, which lower oxidative stress (OS) and mitigate the harmful effects of illnesses, including cancer, cardiovascular diseases, and neurodegenerative disorders (NDs) (10). Phytochemicals are naturally occurring secondary metabolites found in plants. These substances are classified into several categories according to their chemical composition. Coumarin is a naturally produced compound belonging to the group of secondary metabolites derived from benzopyrone. This metabolite was among the earliest discovered in the 1930s and has been detected in several plant species (11).

Several years of studies have shown that coumarins and their derivatives can reduce inflammation and inflammatory reactions by interacting with different types of receptors, such as Toll-like receptors (TLR). They also affect various signaling pathways and molecules, including inflammasomes, Janus Kinase/Signal Transducer and Activator of Transcription (JAK/STAT), mitogen-activated protein kinase (MAPK), nuclear factor- κ-light-chain-enhancer of activated B cells (NF- κB), and transforming growth factor-β/small mothers against decapentaplegic (TGF-β/SMAD) pathways (12). Daphnetin (DAP), a coumarin derivative found in plants like *Daphne odora*, has demonstrated promising neuroprotective and anti-inflammatory effects by modulating NR2B-containing N-methyl-D-aspartate (NMDA) receptors and inhibiting pro-inflammatory cytokine production. There is promising evidence that DAP can halt cognitive decline and memory loss. The down-regulation of NMDA receptors and the subsequent calcium buildup that glutamate activates to excite neurons were both prevented by this compound. DAP protected neurons from damage by maintaining a steady *Bcl-2* and *Bax* expression level in mouse cortisol neurons and blocking NMDA-induced apoptosis (13). The administration of DAP effectively reduced the severity of polyarthritis by inhibiting the production of pro-inflammatory cytokines, including IL -1 and TNF-α (14). On the other hand, Heiss *et al*. showed that the activation of Nrf2, either by the use of the small chemical sulforaphane or by eliminating the Nrf2 inhibitor Keap1, results in enhanced cellular glucose absorption and an increased dependence on glucose in fibroblasts (15).

1.1. Overview of Nrf2 Signaling Pathway

An indispensable transcription factor in orchestrating cellular defense against oxidative stress and inflammation is Nrf2 (2, 16). Under normal conditions, Keap1, an inhibitor of Nrf2, sequesters Nrf2 in the cytoplasm, tagging it for proteasomal degradation [4]. However, in response to various stimuli such as oxidative stress, electrophiles, and certain phytochemicals, Nrf2 undergoes nuclear translocation (5, 17). In response to environmental stress, cells activate pathways that control energy metabolism, antioxidant defense, and cellular detoxification through the transcription factor Nrf2 and its repressor Keap1 (1).

Upon nuclear translocation, Nrf2 binds to AREs located in the promoter regions of its target genes, thereby initiating the transcription of genes involved in antioxidant enzyme production, phase II detoxification, and other cytoprotective proteins (18). Given its pivotal role in regulating inflammatory pathways and preserving cellular redox homeostasis, the transcriptional response mediated by Nrf2 presents an attractive therapeutic avenue for oxidative stress and chronic inflammation-related diseases (19). Interestingly, the activation of Nrf2 elicits distinct effects on the pentose phosphate pathway (PPP) flux in different cellular contexts, such as astroglial cells and neurons. While Nrf2 activation enhances PPP flux in astroglial cells, it reduces it in neurons, underscoring the diverse functions of Nrf2 in these cellular environments, warranting further exploration (1).

1.2. Importance of Nrf2 Activation in Cellular Defense Mechanisms

The Nrf2 signaling pathway is pivotal role in enabling cells to counter oxidative stress and inflammatory stimuli (20, 21). Nrf2 acts as a master transcriptional regulator, controlling the expression of various genes critical for cellular defense. These genes encode:

- Antioxidant enzymes: Superoxide dismutase, catalase, and glutathione peroxidase (8, 22). These enzymes neutralize reactive oxygen species (ROS), preventing oxidative damage (23).
- Phase II detoxification enzymes are glutathione S-transferases and NQO1 (8, 22). These enzymes facilitate the elimination of xenobiotics and detoxify harmful compounds.
- Anti-inflammatory substances: By regulating the expression of these molecules, Nrf2 modulates inflammatory signaling pathways (22).

The coordinated upregulation of these cytoprotective and detoxifying pathways by Nrf2 enhances the cell's capacity to:

- **Scavenge ROS:** This mitigates oxidative stress and protects cellular components from damage (24, 25).
- **Eliminate Xenobiotics:** Detoxification pathways effectively remove harmful foreign substances (26).
- **Modulate Inflammation:** Nrf2 helps maintain a balanced inflammatory response, preventing excessive tissue damage (27).

This orchestrated cellular defense system safeguards against various disease-related conditions by promoting cellular homeostasis (28, 29).

1.3. Role of Phytochemicals in Nrf2 Activation

The growing understanding of the Nrf2 pathway's role in cellular defense has spurred interest in its therapeutic potential [10, 30]. This has led researchers to explore naturally occurring phytochemicals as potential activators of Nrf2 signaling. Plant-derived compounds, including polyphenols, terpenoids, and alkaloids, offer a promising avenue for Nrf2 modulation due to their diverse mechanisms of action (7, 31). These phytochemicals can:

- **Modulate Nrf2-Keap1 interaction:** Some phytochemicals directly influence the interaction between Nrf2 and its negative regulator Keap1, preventing Nrf2 degradation and promoting nuclear translocation (32).
- **Indirect Nrf2 activation:** Other phytochemicals indirectly stimulate Nrf2 activation by influencing upstream signaling cascades (32).

Phytochemicals activate Nrf2 and upregulate cytoprotective genes, ultimately reducing oxidative stress and inflammation (7, 31).

Among the Nrf2-activating phytochemicals, daphnetin, a coumarin derivative found in plants like *D. odora*, has garnered significant attention [31]. DAP exhibits potent antioxidant, anti-inflammatory, and neuroprotective properties, primarily attributed to its ability to stimulate the Nrf2 pathway (32, 33). However, a deeper understanding of the mechanisms by which Daphnetin influences Nrf2 activation and its potential therapeutic applications in oxidative stress-related and inflammatory diseases remains to be elucidated.

This review paper seeks to offer a thorough examination of daphnetin's involvement in stimulating the Nrf2 signaling pathway and its potential use in treating different diseases. This review will enhance our understanding of the underlying processes and the existing data from preclinical and clinical trials about the therapeutic potential of daphnetin and other phytochemicals that target Nrf2.

2. Daphnetin: A Natural Compound

In recent years, DAP, a naturally occurring coumarin compound, has attracted considerable attention within the scientific community. Coumarins, characterized by their benzopyrone structure, constitute a diverse group of organic compounds found abundantly in the plant kingdom. Among them, 7,8-dihydroxycoumarin stands out, primarily sourced from plants of the genus *Daphne* (11).

Typically, DAP is a tasteless and odorless white or off-white powder that dissolves somewhat in water but readily in ethanol, methanol, and dimethyl sulfoxide. It melts at 262.0°C and has a molecular weight of 178.14 g/mole (11). Various varieties of *Daphne* are the sources of the DAP. The Thymelaeaceae family includes the 70–95 species of perennial and evergreen shrubs that comprise the genus, *Daphne*. These plants are initially from Africa, Europe, and India. The fragrant blooms and vibrantly colored fruit of these plants are well-known (11).

These plants' colorful fruit and fragrant blossoms

DAP-8-glucoside, which is synthesized from DAP-7-glucoside. Aside from *D. oleoides*, other sources of DAP include *D. mezereum*, *D. Koreana* Nakai, *D. tangutica*, *D. giraldii*, (synthesized from shoots), and *D. gnidium* (isolated from stems and leaves). One of the seventeen chemicals extracted from *D. oleoides* was DAP. *Dyckia pedunculata* stems and leaves (11).

DAP has been documented to display a diverse array of biological actions, encompassing anti-inflammatory (32), antioxidant (34, 35), anticoagulant [36], and neuroprotective characteristics (37). The pharmacological actions of DAP have prompted research into its potential as a therapeutic agent for treating various ailments, including cardiovascular disorders, neurological diseases, and cancer (38). Previous research has indicated that daphnetin may exert its effects through AMPK activation or NOX inhibition, suggesting its involvement in regulating cellular signaling pathways (38).

Studies investigating the mechanisms underlying the biological actions of DAP have revealed its ability to modulate various cellular signaling pathways and molecular targets. These include the inhibition of pro-inflammatory mediators, scavenging reactive oxygen species, and regulating apoptosis (39). DAP has shown low toxicity, further bolstering its potential as a therapeutic agent (40). Additionally, DAP has been found to alleviate endotoxin-induced death in animal models and inhibit the synthesis of inflammatory cytokines, offering promise for managing inflammatory diseases (41). In laboratory conditions, it was discovered that daphnetin inhibits the synthesis of inflammatory cytokines generated by LPS via decreasing the generation of ROS (42). Moreover, research has elucidated the molecular mechanisms underlying the anti-inflammatory effects of DAP, including its ability to increase the expression of TNF-α-induced protein 3 (TNFAIP3 or A20), a key regulator of NF-κB signaling (39, 43). Despite growing interest in daphnetin, ongoing studies aim to deepen our understanding of its pharmacological characteristics and mechanisms of action. This review seeks to provide a comprehensive overview of the current knowledge on the biological activities, pharmacological effects, and potential therapeutic applications of DAP, drawing upon the available scientific literature.

3. Nrf2 Signaling Pathway

As mentioned, Nrf2, also known as NF-E2-related factor 2, belongs to the Cnc transcription factor family and is essential in various cellular processes, including redox signaling, xenobiotic metabolism, and anti-inflammatory responses. Comprising 605 amino acids, the Nrf2 protein is structured into seven conserved functional domains, namely Neh1-Neh7 (44). Traditionally, Keap1 has been regarded as the primary redox sensor in the Nrf2 signaling pathway, orchestrating the release of Nrf2 in response to oxidative stress. This classical paradigm implies that oxidants induce the dissociation of Nrf2 from Keap1, allowing free Nrf2 to translocate into the nucleus to exert its transcriptional activities. However, recent experimental evidence has challenged this simplistic view, unveiling a more complex regulatory mechanism governing Keap1/Nrf2 interactions. The traditional assumption that oxidants solely induce the release of Nrf2 from Keap1 has been questioned, suggesting a more nuanced understanding of the process. Additionally, it has been proposed that the nuclear accumulation of free Nrf2 might only occur after its release from Keap1. Instead, recent findings suggest a dynamic and multifaceted interplay between Keap1 and Nrf2, revealing novel insights into the intricacies of cellular redox signaling pathways (45).

3.1. Molecular Mechanisms of Nrf2 Activation

Recent findings suggest a coordinated mechanism with multiple levels of regulation involved in Nrf2 activation. The binding of Keap1, a cytoskeleton-associated protein, is believed to inhibit Nrf2 function, and disruption of this interaction contributes to its activation (9).

Nrf2 comprises seven Nrf2-ECH homology (Neh) domains, with Neh1 containing the essential Cnc-bZIP domain for DNA binding. The DLG and ETGE motifs within Neh2 facilitate Nrf2 binding to Keap1, while lysine residues play a crucial role in Nrf2 ubiquitination by forming the Keap1-Cul3-Rbx1 complex, leading to Nrf2 degradation by the proteasome (30). Efforts have been made to identify chemicals capable of enhancing Nrf2 signaling. For instance, Rong *et al*. demonstrated that rosmarinic acid (RosA) mitigates Aβ -induced oxidative stress by stimulating the Nrf2 pathway.

Mechanistically, RosA increases nuclear Nrf2 concentration, activating the Nrf2/ARE defense mechanism via the protein kinase B/GSK3β pathway [46]. Nrf2 activation involves intricate molecular processes resulting in the transcription of genes responsible for antioxidant and cytoprotective functions (6). Under basal conditions, Nrf2 activity is maintained at low levels through ubiquitination and proteasomal degradation facilitated by Keap1, which binds to Nrf2 in the cytoplasm [9]. Keap1 facilitates the degradation of Nrf2 by ubiquitinating it and directing it toward a Cullin 3-based E3 ubiquitin ligase complex (6), as we will summarize in this paper.

Oxidative stress and electrophilic insults trigger Nrf2 activation by altering Keap1 conformation and modifying cysteine residues, preventing Nrf2 ubiquitination (47). Following destabilization of the Nrf2-Keap1 complex, Nrf2 re-establishes contact with ARE in target gene promoter regions, initiating their transcription (9). Nrf2 activity is also regulated by phosphorylation mediated by protein kinase C (PKC), glycogen synthase kinase 3 beta (GSK-3β), and MAPKs. Phosphorylation enhances Nrf2 liberation from Keap1 or facilitates its nuclear translocation, augmenting its gene regulatory capacity (48).

Genetic and epigenetic factors play significant roles in Nrf2 control. Genetic alterations in *NFE2L2* or *KEAP1* may result in continuous Nrf2 activation, particularly in cancer, leading to chemotherapy resistance and poor prognosis. Aberrant Nrf2 activation in cancer cells is associated with transcriptional dysregulation and oncogene activation (47). Nrf2 activation interacts with other cellular pathways, including the PI3K/Akt pathway and autophagy, which may be relevant in oxidative stress- and inflammation-related disorders(48).

3.2 Regulation of Nrf2 Activity in Cellular Stress Response

Post-translational modifications and various signaling pathways intricately regulate Nrf2 function. For instance, nuclear translocation and transcriptional activity of Nrf2 can be enhanced through phosphorylation by kinases such as protein kinase C (PKC), MAPK, and phosphoinositide 3-kinase (PI3K) (49, 50). Additionally, acetylation, ubiquitination, and SUMOylation affect Nrf2's stability, subcellular localization, and DNA binding ability (50, 51). Moreover, stress-responsive transcription factors and epigenetic processes impact the Nrf2 signaling pathway. Notably, the crosstalk between Nrf2 and the inflammatory transcription factor NF-κB has been extensively studied, as they can either synergize or antagonize each other's actions depending on the cellular context (45). Furthermore, changes in DNA methylation patterns and histone modifications influence Nrf2 expression and transcriptional activity (52). Under oxidative and electrophilic stress, reactive cysteine residues on Keap1 undergo modification, leading to decreased E3 ligase activity, stabilization of Nrf2, and robust induction of cytoprotective genes. Structural studies have revealed that Keap1, in its intact homodimer state, forms a complex structure with one Nrf2 molecule interacting with two Keap1 molecules via two binding sites in Nrf2's Neh2 domain, which appears crucial for Nrf2 ubiquitination (53).

One promising strategy for reducing cancer-related oxidative/nitrosative stress and chronic inflammation is to increase the body's levels of antioxidant enzymes while simultaneously controlling Nrf2 activation. While antioxidant supplementation can elevate antioxidant levels, activation of Nrf2 through ROS/RNS -dependent and independent pathways is necessary to boost antioxidant enzyme levels (16). Understanding the intricate regulation of Nrf2 is imperative for developing therapeutic interventions targeting the Nrf2 pathway in various diseases, including cancer, neurological disorders, and metabolic syndromes (54).

Upon translocation to the nucleus and binding to AREs, Nrf2 forms heterodimers with unknown proteins, activating ARE-controlled genes. Nuclear factors such as Fra1, c-Fos, big Maf (c-Maf), small Mafs (MafG and MafK), and ARE act cooperatively to inhibit ARE-mediated gene expression. The intricate regulation of Nrf2 activity aims to maintain the expression of free radical-protective enzymes to keep cellular defenses active or rapidly restore enzyme levels to normal (55). Ongoing research continues to unravel the complex processes controlling Nrf2 activity and its significance in cellular stress response and disease pathogenesis.

3.3 Importance of Nrf2 in Redox Homeostasis and Detoxification

A master regulator of redox homeostasis, Nrf2 protects cells against electrophiles and oxidants by triggering the production of many phase II cytoprotective genes (47). When exposed to electrophilic chemicals or oxidative stress, the connection between Keap1 and Nrf2 disengages, allowing Nrf2 to translocate into the

Nrf2 disengages, allowing Nrf2 to translocate into the nucleus and bind to antioxidant response elements (AREs) in the promoters of its target genes (56). These target genes encode several cytoprotective proteins, including transporters that eliminate harmful chemicals, antioxidant enzymes, and phase II detoxification enzymes (57). Enzymes such as heme oxygenase-1 (HO-1), NQO1, glutathione peroxidases, and glutamate-cysteine ligase (GCL), activated by Nrf2, significantly influence cellular redox equilibrium, reduce oxidative damage, and scavenge ROS (55, 58). These enzymes significantly impact the cellular redox equilibrium, oxidative damage reduction, and ROS scavenging (Figure 1).

Phase II detoxifying enzymes, coordinated by Nrf2, effectively catalyze the removal of xenobiotics and endogenous electrophiles. These enzymes encompass UDP-glucuronosyltransferases, glutathione S-transferases, and sulfotransferases (53). Nrf2-mediated upregulation of these detoxification enzymes enhances cells' ability to eliminate potentially harmful chemicals and protect against chemical-induced toxicity.

Under normal physiological conditions, Nrf2 remains inactive and associates with endogenous Keap1, which acts as an E3 ubiquitin ligase. Keap1 continuously targets Nrf2 for ubiquitination and subsequent degradation by Cul3-Rbx1 in the proteasome. However, during oxidative stress, Nrf2 dissociates from Keap1 and translocates to the nucleus, forming heterodimers with Maf proteins. These heterodimers then recognize and bind to AREs, influencing the expression of various genes, including antioxidant enzymes (e.g., HMOX-1, γ-GCS, PRDX1, GR, TXNRD1, SRXN), drug metabolizing and detoxification enzymes (e.g., NQO1, UGT, GST), as well as metabolic enzymes and regulators (e.g., PGC1-β, G6PDH, transketolase, RXRα, malic enzyme) (58).

Beyond its role in antioxidant defense and detoxification, the Nrf2 pathway modulates cellular energy metabolism, mitochondrial biogenesis, and gene expression in proteostasis ([59), among other functions, including antioxidant defense and detoxification.

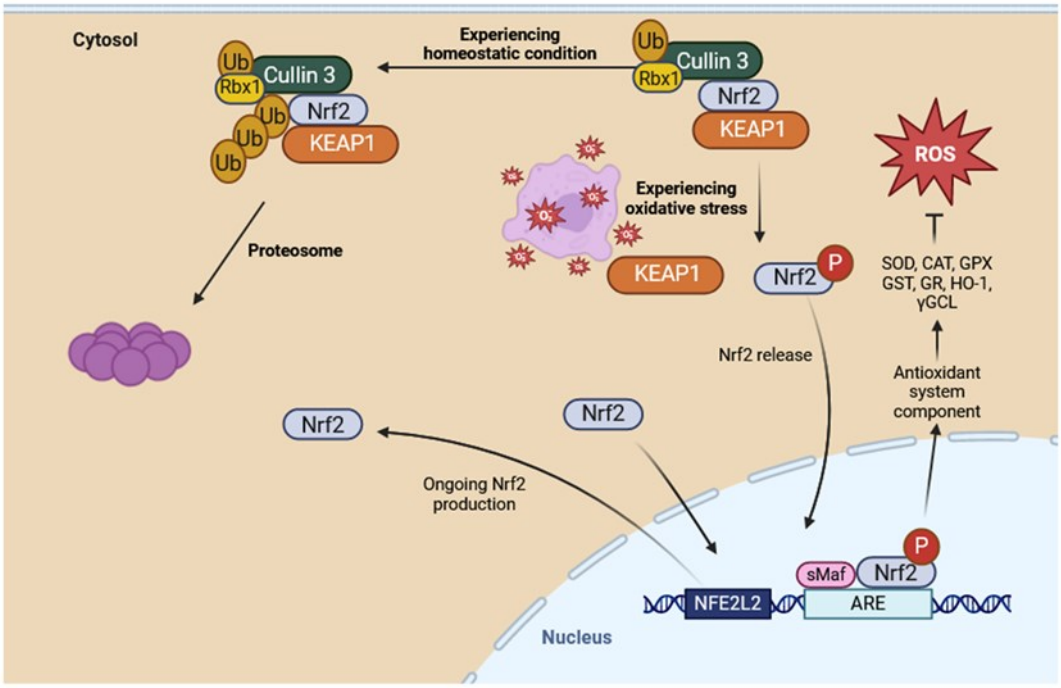

Figure 1. Keap1-Nrf2 signaling pathway activation mechanism (adapted from Qin *et al*. (2022) (58)). Nrf2 is linked to the endogenous protein Keap1, an E3 ubiquitin ligase in a homeostatic state. Keap1 continuously ubiquitinates Nrf2 with Cul3-Rbx1, and proteasomal destruction occurs. Several genes, including those encoding antioxidant enzymes and cytokines, are impacted by Nrf2's detachment from Keap1 and subsequent nuclear translocation and heterodimerization with Maf in response to oxidative stress.

In preserving cellular integrity dysregulation of Nrf2 signaling has been implicated in the pathogenesis of various diseases, including cancer, neurological disorders, and metabolic diseases [3). Consequently, there is growing interest in investigating the Nrf2 pathway and its downstream targets for developing therapeutic interventions targeting this system.

4. Therapeutic Potential of Daphnetin-Nrf2 Axis

The therapeutic potential of DAP, a naturally occurring coumarin derivative, lies in its ability to modulate cellular stress responses, particularly those associated with oxidative stress and inflammation. Daphnetin has been shown to activate the Nrf2 signaling pathway, a key regulator of the cellular antioxidant response (60). This activation unlocks a multifaceted approach to combating cellular stress. Daphnetin employs various pathways to activate Nrf2. One crucial mechanism involves inhibiting the Nrf2-Keap1 complex. This inhibition prevents Nrf2 degradation, allowing it to accumulate and translocate to the nucleus, where it acts as a transcription factor to upregulate the expression of antioxidant genes like HO-1 and NQO1 (60). These antioxidant enzymes bolster cellular defenses against oxidative damage and inflammation.

DAP's protective effects extend beyond Nrf2 activation. It can directly modulate mitochondrial function, a critical cellular process for energy production and redox balance. Studies suggest that DAP protects cells from oxidative damage by reducing ROS production and affecting the mitochondrial electron transport chain (33). The Nrf2 pathway also plays a role in DAP's anti-inflammatory properties. By activating Nrf2, DAP may downregulate pro-inflammatory cytokines and influence immune responses, potentially offering therapeutic benefits in chronic inflammatory diseases (11). The DAP-Nrf2 axis holds promise for treating inflammation and oxidative stress disorders. For instance, daphnetin-mediated Nrf2 activation may shield neurons from oxidative damage and inflammation in neurodegenerative diseases, potentially slowing disease progression (60). Similarly, it might alleviate oxidative stress and inflammation in the cardiovascular system, contributing to overall cardiovascular health. DAP may lower oxidative stress and inflammation in the cardiovascular system while treating cardiovascular illnesses, hence enhancing cardiovascular health in general. The antioxidant and cytoprotective effects of daphnetin, a naturally occurring coumarin derivative found in many plant species, have been the subject of much research. These effects are believed to be mediated, at least in part, via the Nrf2 signaling pathway (1,2). Numerous *in vitro* and *in vivo* investigations have indicated that daphnetin activates Nrf2, a master regulator of the cellular antioxidant response.

Table 1. Several studies of daphnetin via activation of Nrf2 signaling pathway

Pharmacological activity	Type of study	Organ target	Characteristics	Ref.
Anti-inflammatory	*in vivo*	Kidney	DAP suppressed the harmful effects on the kidneys caused by cisplatin by blocking the activity of NF-κB and promoting the activation of Nrf2 signaling pathways. Also, it suppressed the generation of TNF-α, IL-1ß, ROS, and MDA.	(31)
	in vivo	Mitochondria and spinal glial	In order to alleviate pain, DAP activates the Nrf2/HO-1 pathway in the spinal cord. The NF-κB pathway in the spinal cord is negatively regulated by DAP.	(61)
Antioxidant	*in vitro* and *in vivo*	Mitochondria	DAP dramatically stimulated JNK and ERK phosphorylation, although ERK and JNK inhibitor pretreatment significantly reduced Nrf2 nuclear translocation. DAP also reduced t-BHP-induced cytotoxicity and ROS overproduction in Nrf2 deletion RAW 264.7 cells and peritoneal macrophages.	(60)
Anticancer	*in vivo*	Mammary carcinoma	DAP prevents the development of breast cancer in a number of ways. Effects of DAP on the Nrf-2-Keap1 pathway and NF-κB expressions in a dual manner	(32)
Premature ovarian failure	*in vivo*	Ovarian	Daphnetin's ability to activate Nrf2 and enhance antioxidant defense may provide protection against early ovarian failure. While Nrf2 and the antioxidants GCLC, HO-1, and NQO1 decreased, TXNIP and NLRP3 rose considerably.	(62)

Multiple preclinical studies support the link between daphnetin and Nrf2 activation. Zhang *et al*. demonstrated that DAP mitigated cisplatin-induced nephrotoxicity by inhibiting NF-κB activity and promoting Nrf2 signaling while also suppressing the production of pro-inflammatory cytokines (TNF-α, IL-1β) and markers of oxidative stress (ROS, MDA) (31). Kumar *et al*. further revealed the multifaceted effects of DAP in inhibiting mammary carcinogenesis, highlighting its dual action on the Nrf2-Keap1 pathway and NF-κB expression (32). These and other relevant studies exploring the Daphnetin-Nrf2 axis are summarized in Table 1.

DAP activates the Nrf2 signaling pathway, which provides a versatile method for treating cellular stress. It strengthens cellular defenses against oxidative damage and inflammation by increasing the expression of antioxidant genes such as HO-1 and NQO1. In addition, DAP's protective effects go beyond activating Nrf2. It also directly regulates mitochondrial activity and impacts immunological responses, providing therapeutic advantages in chronic inflammatory conditions. The DAP-Nrf2 axis has the potential for the treatment of several oxidative stress-related ailments, such as neurodegenerative diseases and cardiovascular illnesses. Preclinical investigations have shown convincing data that supports the effectiveness of DAP in stimulating Nrf2 and reducing oxidative stress and inflammation. Continuing to investigate the therapeutic mechanisms and clinical uses of DAP and its interaction with the Nrf2 signaling pathway is necessary. This study might lead to the creation of new therapeutic approaches.

Future Perspectives

The therapeutic potential of daphnetin in inhibiting the Nrf2 signaling pathway offers promise for the therapy of numerous oxidative stress-related and inflammatory illnesses. However, further study is necessary to thoroughly understand the underlying processes and enhance the pharmacological effects of daphnetin. Future research should prioritize investigating the specific molecular interactions between daphnetin and the essential elements of the Nrf2 pathway, including Keap1 and the downstream transcriptional targets. By employing advanced techniques such as structural biology and proteomics, comprehensive mechanistic investigations may yield novel insights into the mechanism by which daphnetin modulates Nrf2 activation.

In addition, enhancing the therapeutic effectiveness of daphnetin might be achieved by developing derivatives or analogs that have enhanced bioavailability, stability, and target specificity. Strategies in medicinal chemistry focused on improving the structure of compounds may result in creating more potent and specific Nrf2 activators using the daphnetin framework. Thorough preclinical assessments in different illness models, from neurological disorders to metabolic syndromes and cancer, will be essential to determine the extensive therapeutic capabilities of the daphnetin-Nrf2 axis. It is crucial to conduct well-designed clinical studies to confirm the therapeutic effectiveness of using daphnetin or its derivatives to target the Nrf2 pathway based on these encouraging preclinical findings.

Conclusion

In conclusion, daphnetin, a natural coumarin derivative, has attracted considerable interest because of its capacity to regulate the Nrf2 signaling pathway. This system is a central controller of cellular defense mechanisms against oxidative stress and inflammation. The evidence evaluation emphasizes how daphnetin stimulates the Nrf2 pathway, resulting in the increased expression of genes involved in antioxidant activity, detoxification, and cellular protection. As a result, this provides immunity against various disease-related diseases, such as neurodegenerative disorders, metabolic syndromes, and cancer. Further inquiry is needed to explore the therapeutic potential of the daphnetin-Nrf2 axis. This should involve advanced studies to understand the underlying mechanisms, optimize the structure, and conduct thorough preclinical and clinical investigations. To fully harness the therapeutic potential of the natural chemical daphnetin in treating oxidative stress-related and inflammatory illnesses, researchers aim to clarify the specific molecular interactions involved and create more powerful and targeted Nrf2 activators using the daphnetin scaffold.

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