Nrf2 and NF-κB for Cancer

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Contributor: Wanda Baer-Dubowska

Nrf2 (nuclear factor erythroid 2-related factor 2) and NF-κB (nuclear factor–kappa B) signaling pathways play a central role in suppressing or inducing inflammation and angiogenesis processes. Therefore, they are involved in many steps of carcinogenesis through cooperation with multiple signaling molecules and pathways. Targeting both transcription factors simultaneously may be considered an equally important strategy for cancer chemoprevention and therapy.

Keywords: Nrf2 ; NF-κB ; inflammation ; naturally occurring compounds ; cancer chemoprevention ; cancer therapy ; polyphenols ; phytochemical combinations

1. Introduction

Moreover, although Nrf2 and NF-κB transcription factors are directly involved in many steps of carcinogenesis, cooperation with multiple other signaling molecules and pathways may ultimately affect cell differentiation and proliferation [1]. Since interference exists between these two pathways, their concomitant modulation, i.e., induction of Nrf2 and inhibition of NF-κB in normal cells and inhibition of both in cancer cells, may be considered the best strategy of cancer chemoprevention and therapy, respectively. Modulation of multiple signaling pathways is an element of the therapeutic approach named anakoinosis [2]. The concept of anakoinosis is an alternative to conventional chemotherapy, which is usually based on a single target or focused on a single area of the tumor and can equally be applied in chemoprevention.

Several hundreds of phytochemicals, mainly edible vegetables and fruits components, were shown to activate Nrf2 and mediate antioxidant response. One of the first in this group was naturally occurring and subsequently chemically modified triterpenoids [3]. Similar numbers of phytochemicals were shown to modulate the NF-κB pathway. In this group, curcumin (diferuloylmethane) was one of the first widely described as an inhibitor of NF-κB in cancer cells [4]. Among them, only a relatively small amount affected both pathways, and several of their modulators reached clinical trials level [5].

The activity of these phytochemicals was assessed mainly as single compounds but also in the natural food matrix. Recently, attempts were made to select the best combination of naturally occurring compounds for chemoprevention or therapeutic purposes, including modulation of Nrf2 and NF-κB pathways.

2. Overview of Nrf2 and NF-κB Signaling Pathways and Their Interconnections

Besides the canonical pathway of activation, Nrf2 can also be regulated by phosphorylation. Post-translational modification of Nrf2 by various protein kinases can affect the release of Nrf2 from the complex with Keap1, its nuclear translocation, and stability [6]. In one of the mechanisms of the non-canonical pathway of Nrf2 activation, the p62 (SQSTM1) protein is involved. This protein is an autophagy receptor for protein and mitochondria degradation. Similar to the interaction of Keap1 with Nrf2, p62 protein is able to interact with Keap1 by the KIR domain in p62. The interaction of Keap1 with p62 induces a dependent autophagy degradation of Keap1 and subsequent Nrf2 stabilization and activation in MEF and HEK293 cells [7].

The Nrf2 activation dependent on p62 increases the expression of NAD(P)H:quinone oxidoreductase (NQO1), glutathione -S-transferases (GSTs) and anti-apoptotic proteins such as Bcl-2 (B-cell lymphoma 2) and Bcl-xL (B-cell lymphoma- extra-large), decreasing ROS levels and protecting the cell against oxidative stress [8]. However, sustained Nrf2 activation by impairment of autophagy and an increase in p62 phosphorylation promotes cancer cell proliferation. Mutations in the KIR domain in p62, which prevents Keap1-p62 interaction, is associated with a ROS increase [8][9]. Epigenetic mechanisms, such as Nrf2 or Keap1 promoter methylation and microRNA have also been implicated in the complex regulation of Nrf2 pathway activity [6].

Although Nrf2 pathway plays an essential role in maintaining cellular redox and electrophilic homeostasis, it was also demonstrated that Nrf2 is overexpressed in cancer cells and may contribute to increased proliferation, invasion, and chemoresistance [10]. Several mechanisms are involved in the prooncogenic activation of the Nrf2 pathway in cancer cells and include both genetic alterations and epigenetic changes [11]. Evidence exists that this pathway is associated with the proliferation of cancer cells through metabolic reprogramming [12].

Thus, induction of Nrf2 activation by phytochemicals in normal cells or at the early stages of carcinogenesis is an important strategy of chemoprevention. In cancer cells, the naturally occurring inhibitors of this pathway are desired.

3. Phytochemicals as Modulators of Nrf2 and NF-kB Signaling Pathway

The differences in the effect of xanthohumol and the other hop-derived prenylflavonoids in proliferating and differentiated colorectal cancer cells (CaCo-2 cells) were demonstrated. In the latter, expression of phase II enzymes also showed specificity toward their isozymes, namely GST [13].

However, often occurring in cancer cells, overexpression of Nrf2 due to both genetic and epigenetic mechanisms prompt the searching of naturally occurring compounds that inhibit its activity and expression to avoid chemo -or radiotherapy resistance.

The results of several studies in vitro, and to a lesser extent, in animal models indicate that combinations of phytochemicals may increase their chemopreventive and chemotherapeutic potential and can efficiently target the signaling pathways involved in cell proliferation and survival.

In most of the in vitro studies, equimolar concentrations of the tested phytochemicals were applied. Only in several cases did arbitrary selection occur. The in vivo studies in the context of this review are sparse thus far. Therefore, the examples shown in **Table 1** refer mostly to the inflammation model. Concerning the mode of interaction between the phytochemicals, only synergetic effects were observed. However, evaluation based on the Chou–Talalay method of the nature of interaction was rarely employed. While the combinations of phytochemicals may be more efficient, particularly in chemoprevention, naturally occurring compounds may have the potentially inhibitory and antagonistic effect on chemotherapeutics' activity when used in support of conventional therapy. Therefore, the identification of phytochemicals acting in this way can potentially explain causes for drug failure or resistance and propose the timing and use of naturally occurring compounds along with cancer chemotherapy [14]. In the context of Nrf2 interference with cancer chemotherapy, its status of anti- or pro-tumorigenic is defined by many different modalities, but mainly the loss of functional Keap1 or its mutation contribute to deregulation of Nrf2 in cancer cells. However, it was demonstrated using synthetic triterpenoid RTA 405 that pharmacological activation of Nrf2 may be distinct from genetic activation and does not provide a growth or survival advantage to certain cancer cells, including pancreatic cancer cells. Moreover, pre-treatment with RTA 405 did not protect cancer cells from doxorubicin- or cisplatin-mediated growth inhibition [15]. However, considering the complex mechanism of Nrf2 overexpression in cancer cells, the increased activation of this transcription factor by phytochemicals should be avoided as it may enhance chemoresistance.

Table 1. Modulation of Nrf2 and NF-κB pathways by selected combination phytochemicals in vitro and in vivo model.

Phytochemicals Combination	Phytochemicals Interactions	Experimental Model	Concentrations	Effect on NF-κB	Effect on Nrf2	Ref.
Resveratrol and Phenethyl isothiocyanate	Synergism	Human Pancreatic cancer cells (Mia-Pa-Ca-2 cells)	* Resveratrol 10 μM; Phenethyl isothiocyanate 10 μM		↑ expression of Nrf2 and binding Nrf2 to DNA, and expression of SOD, NQO1, GSTP	[87]
	Synergism	PANC-1 cells	Resveratrol 10 μM; Phenethyl isothiocyanate 10 μM	↓ binding NF-κBp65 to DNA and expression of NF-κBp65 and COX-2		[53]

Phytochemicals Combination	Phytochemicals Interactions	Experimental Model	Concentrations	Effect on NF-κB	Effect on Nrf2	Ref.
Xanthohumol and Phenethyl isothiocyanate	Synergism	PANC-1 cells	Xanthohumol 10 μM; Phenethyl isothiocyanate 10 μM		↑nuclear translocation of Nrf2, and binding Nrf2 to DNA, and expression of Nrf2, SOD, NQO1, GSTP	[53]
	Synergism	PANC-1 cells	Xanthohumol 10 μM; Phenethyl isothiocyanate 10 μM	↓ nuclear translocation NF-κB, and binding NF-κBp65 and NF-κBp50 to DNA, and expression of NF-κB and COX-2		[53]
Curcumin and Arctigenin	Synergism	Human prostate adenocarcinoma cells (LNCaP cells); MCF-7 cells	Curcumin 5 μM, Arctigenin 1μM	↓ phosphorylation of NF-κB; and p-IκB levels		[88]
Curcumin and Epigallocatechin gallate	Synergism	LNCaPcells; MCF-7 cells	Curcumin 5 μM; EGCG 40 μM	↓ phosphorylation NF-κB; ↓ p-IκB levels		[88]
3,3′-Diindolylmethane and Sulforaphane	Additive	Human liver hepatoma cells (HepG2-C8 cells)	3,3′-diindolylmethane 6.25μM; Sulforaphane 1μM		↑ expression of Nrf2 and SOD	[89]
Sulforaphane and Curcumin	Synergism	RAW264.7 cells	Sulforaphane 0.4 μM; Curcumin 2 μM	↓ expression of iNOS; COX-2; PGE2		[90]
	Synergism	RAW264.7 cells	Sulforaphane 0.4 μM; Curcumin 2 μM		↑ expression of Nrf2 and NQO1, HO-1	[90]
Sulforaphane and Phenethyl isothiocyanate	Synergism	RAW264.7 cells	Sulforaphane 0.4 μM; Phenethyl isothiocyanate 2 μM	↓ expression of iNOS; COX-2; PGE2		[90]
	Synergism	RAW264.7 cells	Sulforaphane 0.4 μM; Phenethyl isothiocyanate 2 μM		↑ expression of Nrf2 and NQO1, HO-1	[90]
Curcumin and Resveratrol	Synergism	Human hypopharyngeal carcinoma cells (Fadu cells) Human oral adenosquamous carcinoma cells (Cal-27 cells)	Curcumin 25 μM; Resveratrol 25 μM	↓ nuclear translocation of NF-κB		[91]
	Synergism	Xenografts SCID mouse Spinal cord injury model	** Curcumin 500 mg/kg; Resveratrol 150 mg/kg gavage	↓ NF-κB binding to DNA		[92]
Curcumin and Piperine	Lack of Synergism	Holtzman rats Periodontitis model	Curcumin 400 mg/kg; Piperine 20 mg/kg gavage	↓ phosphorylation and activation of NF-κB		[93]

* Concentrations of phytochemicals in in vitro studies are quoted in μM; ** Concentrations of phytochemicals in vivo studies are quoted in mg/kg.

4. Conclusions and Perspectives

As Nrf2 activation in normal cells or at early stages of carcinogenesis may protect against DNA insult and angiogenesis through the enhanced expression of 250 genes under the control of Nrf2, and protects against cancer development in cancer cells may contribute to resistance to chemotherapy.

Therefore, for cancer prophylaxis, naturally occurring activators or inducers of the Nrf2 pathway and inhibitors of NF-κB are required. In contrast, for cancer treatment, inhibitors of both of these pathways are investigated.

Therefore, the application of phytochemical combinations as modulators of Nrf2 and NF-κB and ultimately cancer prevention or therapy seems to be an attractive approach. However, several problems must be solved before the specific phytochemical combinations can be applied for this purpose.

Equally important is the evaluation of the type of interactions. Similarly, as in the case of single phytochemicals, their bioavailability in combinations must be assessed.

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