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Effects of Selected Chemopreventive Phytochemicals on p38 MAPK- and ERK-mediated Activation of NF-kappa B and Subsequent Expression of Cyclooxygenase-2 in Mouse Skin and Cultured Human Breast Epithelial Cells

Young-Joon Suh

Kyung-Soo Chun, Jung-Hwan Kim, and Hyun-Ho Cha,

College of Pharmacy, Seoul National University, Seoul 151-742

There are multiple lines of compelling evidence supporting that COX-2 plays a role in the development of tumors. Thus, inappropriate up-regulation of COX-2 prolongs the survival of malignant or transformed cells and leads to phenotypic changes associated with metastatic potential. Cells genetically engineered to overexprss become resiatant to apoptosis. Conversely, selective COX-2 antagonists suppressed proliferation and induced apoptosis in certain cancer cells. Although most of these studies have addressed the association of anti-proliferative or apoptosis-inducing activities of NSAIDs with their suppression of COX-2, results from other studies indicate that certain COX antagonists inhibit cell proliferation by inducing apoptosis in a COX-2/PGE_2-independent manner. All these findings support a role of COX-2 in the pathogenesis of cancer and hence suggest that targeted inhibition of improper up-regulation of this particular enzyme could provide one of the most effective and promising approaches to cancer chemoprevention.

A growing body of evidence indicates that the ubiquitous eukaryotic transcription factor NF-kappa B plays a central role in general inflammatory as well as immune responses. In light of its roles as a coordinating regulator in expression of a variety of early-response genes involved in the inflammatory and immune reactions, NF-kappa B has drawn much interest as an attractive therapeutic target for novel anti-inflammatory and immunomodulatory drugs. The 5'-promoter region of COX-2 contains two putative NF-kB binding sites. In line with this notion, NF-kappa B has been shown to be a positive regulator of COX-2 expression in murine macrophages and human colon adenocarcinoma cell lines exposed to LPS. According to our study, topical pretreatment of pyroliodine carbamate (PDTC), a known NF-kappa B inhibitor, resulted in dose-related suppression of TPA-induced expression of COX-2 as well as

NF-kappa B binding activity in mouse skin.

One nuclear target of the signaling pathways responsible for induction of COX-2 expression is NF-kappa B. Accumulating evidence indicates that NF-kappa B activation is modulated by MAPK/ERK kinase kinase-1 (MEKK1), a kinase upstream of JNKs as well as p38 MAPK. MEKK1 induced site-specific phosphorylation of I-kappa B-alpha at Ser 32 and Ser 36 in HeLa cells and also directly activated the I-kappa B kinase (IKK) complex. MEKK1 has been shown to preferentially phosphorylate and thereby activates IKKb whereas the kinase activity of IKKa is apparently stimulated by NF-kappa B-inducing kinase (NIK). The resulting phosphorylation of serine residues of Ikappa B targets this inhibitory protein for degradation by the ubiquitin-proteosome pathway, resulting in the release of the active NF-kappa B dimer that translocates to nucleus.

Since inflammation is closely linked to tumor promotion, substances with potent anti-inflammatory activies are anticipated to exert chemopreventive effects on carcinogenesis, particularly in the promotion stage. An example is curcumin, a yellow pigment of turmeric (*Curcuma longa* L., Zingiberaceae), that strongly suppresses tumor promotion. Recent studies from this laboratory have demonstrated that some naturally occurring diarylhepatanoids have substantial anti-tumor promotional activities. Thus, yakuchinone A [1-(4'-hydroxy-3'-methoxyphenyl)-7-phenyl-3-heptanone] and yakuchinone B [1-(4'-hydroxy-3'-methoxyphenyl)-7-phenylhept-1-en-3-one] present in *Alpinia oxyphylla* Miquel (Zingiberacease) as well as curcumin attenuate phorbol ester-induced inflammation and skin tumor promotion in mice. These diarylheptanoids suppressed phorbol ester-induced activation of ornithine decarboxylase and its mRNA expression in mouse skin. Phorbol ester-induced expression of epidermal COX-2 and iNOS was similarly repressed by curcumin and yakuchinones, which appears to be mediated through inactivation of the eukaryotic transcription factor, NF-kappa B. Capsaicin, a major pungent ingredient of red pepper, also attenuated phorbol ester-stimulated activation of NF-kappa B. One of the plausible mechanisms underlying inhibition by aforementioned phytochemicals of phorbol ester-induced NF-kappa B activation involves repression of degradation of the inhibitory unit, I-kappa B, which hampers subsequent nuclear translocation of the functionally active p65 subunit of NF-kappa B. Curcumin exhibited inhibitory effects on expression of p38 MAPK in phorbol ester-treated mouse skin. Certain anti-inflammatory phytochemicals also exert inhibitory effects on phorbol ester-induced COX-2 expression and/or NF-kappa B activation in the immortalized human breast epithelial (MCF-10A) cell line. Treatment of these cells with p38 MAPK inhibitor (SB 203580) or ERK1/2 (PD 985059) or dominant negative mutation of these kinases abolished phorbol ester-induced activation of NF-kappa B and induction of COX-2.