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Genistein Inhibits NF-κB-dependent COX-2 Expression in Human Breast Epithelial Cells

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According to epidemiological studies, Asian women and men who consume a diet containing relatively large amounts of soy products have a low incidence of breast, colon or prostate cancer.[1)] Genistein, a soy derived isoflavone, has been reported to have substantial chemopreventive effects. The compound exerts protective effects against chemically-induced carcinogenesis in animals as well as malignant transformation in cultured cells. However, the molecular mechanism underlying chemopreventive or chemoprotective effects of genistein remains largely unresolved.

Prostaglandins are among the potent groups of autocrine and paracrine lipid mediators. These endogenous substances play pivotal roles in many physiological processes, such as inflammation, edema, platelet aggregation, fever, and hyperalgesia.[2~4)] There are two isoforms of cyclooxygenase (COX) that catalyze the formation of prostaglandins from arachidonic acid. COX-1 is a housekeeping enzyme that is expressed constitutively in most tissues.[4)] COX-2 is an immediate early response gene product that is highly inducible by mitogenic and inflammatory stimuli, including tumor promoters, growth factors, cytokines, reactive oxygen species and oncogene products.

Elevated levels of prostaglandins and enhanced COX-2 activities have been often observed in various cancers of epithelial origin[5,6)] and also in transformed cells,[7~9)] whereas levels of COX-1 remain essentially unchanged. It becomes increasingly evident that prostaglandins are implicated in the pathogenesis of cancer because they affect mitogenesis, cellular adhesion, immune surveillance and apoptosis. Overexpression of COX-2 blocked apoptosis and potentiated the invasiveness of malignant cells.[10,11)] Based on these findings, it is conceivable that targeted inhibition of COX-2 is a promising approach to prevent cancer.[12)]

NF-κB regulates the expression of many genes involved in immune and inflammatory responses.[13)] Nuclear factor-κB (NF-κB) is a ubiquitous, pleiotropic, multi-subunit eukaryotic transcription factor consisting of either homo- or heterodimers of various subunits of Rel family proteins referred to as p50, p52, p65 (Rel A), c-Rel, and Rel B.[14,15)] The conventional active form of NF-κB is a heterodimer, which normally consists of two proteins, p65 (Rel A) subunit and p50 subunits. NF-κB is activated by antigens, viruses, bacteria, inflammatory cytokines, phorbol esters, etc., and plays a crucial role in transcriptional initiation of a diverse set of genes whose products are important in mediating inflammatory responses.[13)]

In most cells, NF-κB exists as an inactive heterodimer, sequestered in the cytoplasm as a complex with an inhibitory protein, IκB.[16,17)] NF-κB activation is achieved through the signal-induced

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proteolytic degradation of IκB in the cytoplasm. Extracellular stimuli initiate a signaling cascade leading to activation of distinct IκB kinases (IKK-1, IKKα, IKK-2, IKKβ) which phosphorylate IκB at specific *N*-terminal serine residues (Ser32 and Ser36 for IκBα, Ser19 and Ser23 for IκBβ), ubiquitinated, and then degradaded by the 26S proteasome.[18,19] The free NF-κB complex is then translocated into the nucleus and binds to the DNA consensus sequence, thereby transactivating the target genes, such as COX-2. Recently, it has been reported that phosphorylated p65 subunit of NF-κB is essential for its transcriptional activation.[20~23] In addition, the association of the carboxyl terminus of p65 with basal transcription factors, such as transcription factor IIB (TFIIB) and TATA-binding protein (TBP), is known to be important for transcriptional regulation of NF-κB.[24]

In the present study, we found that pretreatment of cultured human breast epithelial (MCF10A) cells

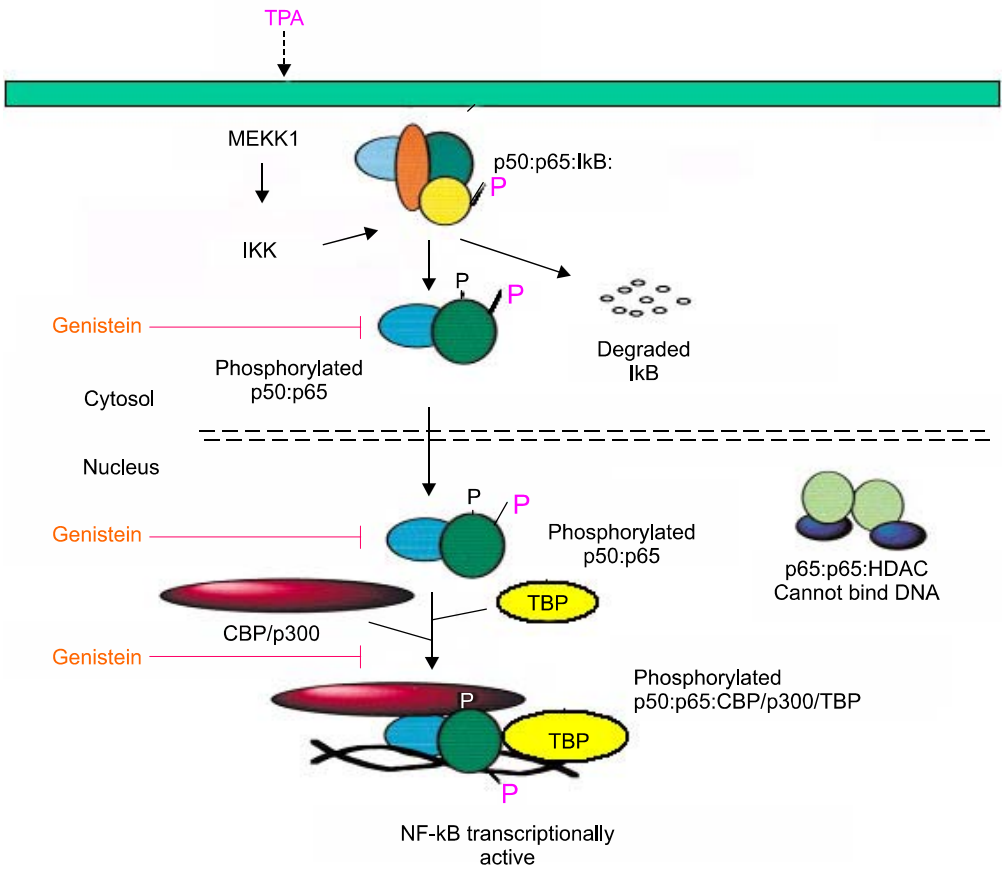

Fig. 1. Postulated mechanism by which genistein inhibits transcriptional activation of the NF-κB. Cells are stimulated by TPA initiate signaling pathways that lead to transcriptional activation at NF-κB target sequences. Genistein inhibits NF-κB transcriptional activation at a step after nuclear translocation of p65 and specific binding of the p50-p65 heterodimer to DNA. The phosphorylated p65 of NF-κB regulates transactivation of NF-κB which is a potential target of genistein inhibition. Other potential sites of genistein inhibition of transcriptional activation in the nucleus include recruitment of transcriptional coactivators cAMP response element-binding protein or p300 to p65 or interaction between p65 and TBP.

with genistein reduced the expression of COX-2 and production of PGE_2 induced by 12-*O*-tetradecanoylphorbol-13-acetate (TPA). Inhibition of PGE_2 production by genistein appeared to be attributable to its suppression of both catalytic activity and expression of COX-2. There are multiple lines of evidence supporting that the induction of COX-2 is regulated by the eukaryotic transcription factor NF-κB. In agreement with this notion, pyrrolidine dithiocarbamate and N-α-*p*-tosyl-L-lysine chloromethylketone that are inhibitors of NF-κB significantly attenuated TPA-induced expression of COX-2 in MCF10A cells. Genistein failed to inhibit TPA-induced DNA binding of NF-κB, but blocked its transcriptional activity induced by TPA. Immunofluorescence staining also demonstrated that increased nuclear translocation of the functionally active active p65 subunit in TPA-stimulated MCF10A cells was not abolished by genistein. More importantly, TPA-induced phosphorylation of p65 faciliated interaction of this subunit with CBP and formation of p65-TBP complex. Genistein inhibited phosphorylation of p65 and its association with CBP and subsequent interaction with TBP. Above findings, taken together, suggest that genistein inhibits COX-2 expression and PGE_2 production in MCF10A cells by down-regulating NF-κB-dependent transcriptional activation by interfering with NF-κB/TBP association, as shematically represented in Fig. 1.

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