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NF-κB is a Key Regulator for Oxidative Stress, Cancer and Beyond

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Introduction

Nuclear factor-κB (NF-κB) pathway plays important roles in the control of cell growth, differentiation, apoptosis, inflammation, stress response, and many other physiological processes by regulating cellular signaling (1,2). The NF-κB family is composed of five proteins: RelA (p65), RelB, c-Rel, NF-κB1 (p50), and NF-κB2 (p52), each of which may form homo- or hetero-dimers (3). In almost all cell types, NF-κB is sequestered in the cytoplasm through tight association with its inhibitors: IκB which acts as NF-κB inhibitor, and p100 proteins which serves as both inhibitor and precursors of NF-κB DNA-binding subunits. The activation of NF-κB occurs through phosphorylation of IκB by IKKb and/or phosphorylation of p100 by IKKa, leading to degradation of IκB and/or the processing of p100 into small form (p52). This process allows two forms of NF-κB (p50-p65 and p52-RelB) to become free resulting in the translocation of active NF-κB into the nucleus for binding to NF-κB-specific DNA-binding sites and, in turn, regulating gene transcription (See Figure on the following page) (4,5). It has been reported that IKK-a also phosphorylates histone H3 and regulates the activation of NF-κB-directed gene expression (5-7). In this way, NF-κB controls the expression of many genes that are involved in the cellular physiological processes. The disorder of these physiological processes has been demonstrated to link with the onset of cancers and chronic diseases. Therefore, NF-κB has been described as a major culprit in cancer and a therapeutic target for the management of cancer and chronic inflammation (8,9).

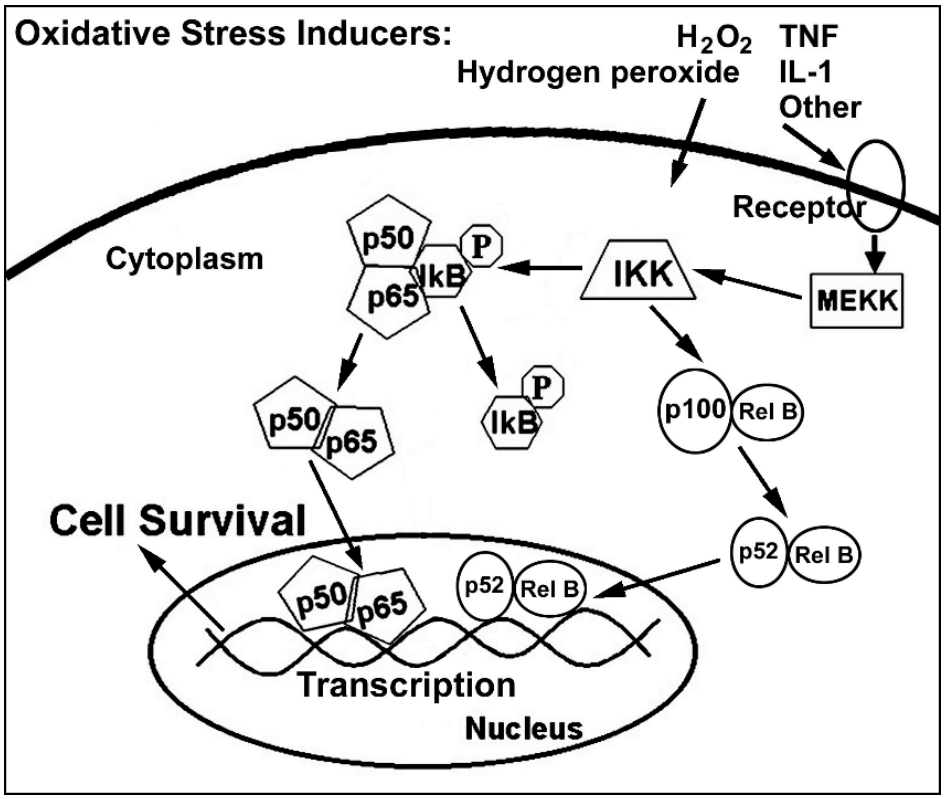

Figure 1. Oxidative stress and the NF-κB signaling pathway

Oxidative stress is defined as an increase in intracellular reactive oxygen species (ROS) such as H_2O_2, superoxide, hydroxyl radical, etc (10). ROS, whether produced endogenously as a consequence of normal cell functions or derived from external sources, pose a constant threat to cells as they can cause severe damage to DNA, protein, and lipid, and alter signal transduction pathways including NF-κB pathway (11). It has been found that direct addition of H_2O_2 to culture medium activates NF-κB in many types of cell lines (10). ROS in cells have been shown to be increased in response to agents that also activate NF-κB (10,12). These findings suggest that oxidative stress activates NF-κB activity in the cells.

ROS, Aging and Chronic Diseases

For organisms living in an aerobic environment, exposure to ROS is continuous and unavoidable. Although a number of defense systems have evolved to combat the accumulation of ROS, these defense systems are not always adequate to counteract the productions of ROS, resulting in a state of oxidative stress. It is important to note that oxidative stress has been linked to the normal aging and a variety of chronic diseases including atherosclerosis, diabetes, pulmonary fibrosis, neurodegenerative diseases, and arthritis (13).

It has been well documented that the rate of production of superoxide anions and hydrogen peroxide in mitochondria is increased with age and that increase in hydrogen peroxide production under oxidative stress is related to the oxidative damage to mtDNA and membrane lipids of mitochondria (14). It has been also documented that an increase in the production of ROS with age may promote the induction of apoptosis (15). These will result in the increase in the mutation of mtDNA, the disfunction of mitochondria, and apoptosis with aging.

There are growing evidences implicating oxidative damage to DNA, protein, and lipid in the pathogenesis of various chronic diseases (13). It has been reported that ROS contribute significantly to tissue injury in rheumatoid arthritis, and that the control of inflammation in arthritic patients by antioxidants could become a relevant component of antirheumatic prevention and therapy (16). The increase in ROS generation has been related to a risk for cardiovascular diseases such as atherosclerosis, angina pectoris, and myocardial infarction

(17). Type I diabetes or insulin-dependent diabetes is characterized by the severe destruction of the insulin-producing b cells, and ROS have been believed to play a central role in b-cell death and disease progression (18). Alzheimer's disease is the most common neurodegenerative disease and shows progressive memory loss and dementia. Growing evidences show that there is a linkage between Alzheimer's disease and oxidative damage. Administration of vitamin E leads to a slowing of disease progression and the patients taking antioxidant vitamins and anti-inflammatory compounds have a lower incidence of Alzheimer's disease (19), suggesting the importance of ROS in the disease. Parkinson's disease is the second most common neurodegenerative disease and causes a progressive movement disorder. The oxidative damage to protein and the protein oxidation have been found in Parkinson's disease, suggesting the involvement of ROS in the disease (19).

ROS, generated by reduction-oxidation (redox) reactions, have been recognized as important chemical mediators that regulate cell signal transduction (15). At the cellular level, oxidative damage from ROS in aging and chronic disease elicits a wide spectrum of response from proliferation to growth arrest or to apoptotic cell death. Whatever the effect seen, it largely reflects the balance between varieties of cell signal pathways that are activated in response to the oxidative damage. Among them, the NF-κB pathway is an important cell signal transduction pathway because oxidative stress activates NF-κB activity and, in turn, induces the transcription of NF-κB-targeted genes related to immune function, inflammation, apoptosis, and cell proliferation. Because of its crucial role in the regulation of genes related to the pathogenesis of chronic diseases caused by oxidative damage, NF-κB has been believed to be a promising target for treatment of ROS-related chronic diseases.

ROS, NF-κB and Cancer

Because ROS can cause severe damage to DNA, and since DNA damage plays an important role in carcinogenesis, it is conceivable that oxidative stress could be carcinogenic (20). Thus imbalance between DNA damage and DNA repair is an important process in order to prevent mutagenesis. However, under oxidative stress, the repair of DNA damage can be inhibited by several redox-dependent metals, resulting in carcinogenesis. Moreover, the activation of NF-κB by ROS under oxidative stress has been known as a key event in carcinogenesis. NF-κB controls the expression of many genes that are involved in the control of cell growth and apoptosis (21). It has been reported that over-expression of NF-κB promotes cell growth and protects cells from apoptosis, while inhibition or absence of NF-κB induces apoptosis (22). An *in vivo* study showed that mice lacking NF-κB p65 died embryonically from extensive apoptosis in the liver, suggesting anti-apoptotic role of NF-κB (23). It has been demonstrated that the stimuli, which cause oxidative stress, can induce phosphorylation and subsequent degradation of IκB, thereby activating NF-κB which acts as an inhibitor of apoptosis (24). The up-regulated cell proliferation and/or inability of apoptotic cell death result in the development of cancer. Experimental studies have shown that NF-κB promotes cell growth and inhibits apoptotic processes in cancer cells (25). It has also been reported that NF-κB is constitutively activated in Hodgkin's tumor cells, whereas inhibition of NF-κB blocks Hodgkin's tumor cell growth (26).

Inhibition of NF-κB activation is now widely recognized as a valid strategy to combat inflammatory diseases (11,27). However, it has become obvious that inhibition of NF-κB activity is not only desirable for the treatment of inflammation but also for the treatment of cancer (28). Examination of the inflammatory microenvironment of neoplastic tissues has supported the hypothesis that inflammation is a cofactor in oncogenesis for a variety of cancers (9). Many anti-inflammation drugs inhibit NF-κB activity and induce apoptosis, therefore they may also be desirable in the treatment of cancer (9,29). Experimental studies have demonstrated that NF-κB regulates growth and survival of multiple myeloma and that NF-κB is a novel therapeutics target in multiple myeloma (30). Our studies also showed that natural compounds including I3C, DIM, and anti-oxidant genistein inhibited the activity of NF-κB and the growth of cancer cells, and induced apoptosis in cancer cells (31,32), suggesting that NF-κB is a target for cancer prevention as well as for treatment. More importantly, we have recently found that genistein can potentiate the anti-tumor activity of chemotherapeutic agents through regulation of NF-κB. It has been reported that some chemotherapeutic agents such as cisplatin and docetaxel induce the activation of NF-κB in cancer cells and this may be responsible for drug resistance in cancer cells (33-35). By *in vitro* and *in vivo* studies, we have shown that pre-treatment with genistein followed by treatment with lower doses of docetaxel or cisplatin elicited significantly greater inhibition of cell growth and induction of apoptosis compared to either agent alone (36-38). In both *in vitro* and *in vivo* studies, we found that NF-κB activity was significantly increased by docetaxel or cisplatin treatment, and the NF-κB inducing activity of these agents was completely abrogated in cells pre-treated with genistein. Our results clearly suggest that genistein pre-treatment, which inactivates NF-κB activity, may

contribute to increased cell growth inhibition and apoptosis with non-toxic doses of docetaxel or cisplatin, and that NF-κB is an important target for cancer therapy.

Measurement of NF-κB

NF-kB plays important roles in oxidative stress and carcinogenesis, and it is an excellent target for prevention or treatment of cancers and chronic diseases caused by ROS. Therefore, it is important to accurately measure the level of activated NF-κB in cells. The conventional method for detecting the DNA-binding activity of NF-kB in nuclear proteins is by electrophoretic mobility shift assay (EMSA). The assay is based on the observation that complexes of nuclear NF-κB protein and DNA migrate through a non-denaturing polyacrylamide gel more slowly than free DNA fragments. The gel shift assay is performed by incubating purified nuclear protein with ^{32}P end-labeled DNA fragment containing NF-κB binding site. The reaction products are then separated on a non-denaturing polyacrylamide gel and exposed to X-ray film. The results can be analyzed by densitometric analysis. Instead of ^{32}P end-labeled DNA fragment, biotin-labeled or fluorescence end-labeled DNA fragment have now been routinely used for NF-kB EMSA. An antibody against activated NF-κB has also been used for the detection of NF-kB activity by Western blot analysis. However, these methods for measurement of NF-κB are time-consuming, less-sensitive, and non-quantitative.

The new method for accurate measurement of activated NF-κB is chemiluminescence based sandwich type ELISA, which was developed by Oxford Biomedical Research, Inc. This method employs an oligonucleotide, containing NF-κB consensus sequence, bound to a 96-well ELISA

plate. NF-κB present in the sample binds specifically to the oligonucleotide coated on the plate. The DNA bound NF-κB is selectively recognized by the primary antibody, which in turn is picked up by the secondary alkaline phosphatase-conjugated antibody. The Relative Light Units (RLU) measured by a chemiluminescence detector, after addition of alkaline phosphatase substrate, offer greater sensitivity than absorbance detection. This method allows precise and quantitative measurement of NF-κB in samples using a standard curve. It allows successful quantitation (pg NF-κB/μg protein) of both the activated form of NF-κB and the total amount of NF-κB. It provides quantitative measurement of NF-κB and greater sensitivity than EMSA, with shorter time and no radioactive chemicals used.

Conclusion

NF-κB is an important transcription factor, which plays central roles in oxidative stress and carcinogenesis. It regulates the expression of genes critically involved in cancers and chronic diseases caused by ROS and may serve as a marker of oxidative stress. Targeting NF-kB may be a novel and important preventive or therapeutic strategy against human cancers and chronic diseases resulting from oxidative stress. Thus, accurate measurement of activated NF-κB is important for estimation of oxidative stress status, which could be important for the prevention and also for the treatment of cancers and chronic diseases caused by oxidative stress.

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