REVIEW ARTICLE

PROGNOSTIC AND THERAPEUTIC ROLE OF NUCLEAR FACTOR-kappa B (NF-κB) IN BREAST CANCER

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Nuclear Factor- kappa B (NF-κB) is an essential transcription factor that not only modulates cellular responses to stress but also plays a pivotal role in inflammation, immunity, cell cycle growth and survival. NF-κB-regulated genes have been documented to be involved in cellular proliferation and invasion along with tumour related angiogenesis and lymphangiogenesis. Dysregulation of NF-κB associated pathways are seen in multiple malignancies. Its constitutive activation in the clinically aggressive and prognostically poor ER-negative, Her2-neu positive and inflammatory breast cancer could formulate the basis for its evolution as a potential prognostic and therapeutic target.

Keywords: Nuclear Factor kappa B, NF-κB, Breast Cancer, ER-negative

Nuclear Factor-kappa B (NF-κB)

Pathogenesis of disease process evolves around aberrant activation and expression of genes leading to generation of abnormal products culminating in initiation and progression of the disease process.[1–3] This genetic transformation is in part controlled by NF-κB[3] an essential transcription factor that not just only controls cellular responses to stress but also plays a pivotal role in inflammation, immunity, cell-cycle/growth, survival and apoptosis[4–8] by directly influencing the gene expression of the growth factors, chemokines, cell adhesion molecules and some acute phase proteins[2,3] involved in these processes.

The NF-κB family consists of five mammalian members p50, p52, p65(relA) c-rel and relB that exist as 'homo' or 'hetero' dimers, with the most abundant form being p50/p65 heterodimer.[9] The heterodimer of p65(rel A) and p50 is the predominant active NF-κB complex in epithelial cells.[10–15] This transcription factor was discovered in immune cells and believed to be involved primarily in the transmission of inflammatory signals by modulation of the expression of immune response genes.[10] Later, NF-κB was discovered to be present in most cell types in an inactive state, complexed with the inhibitory κB protein (IκB) in the cytoplasm.[11,16]

Activation of NF-κB

In the resting cells NF-κB is cytoplasmically sequestered in a latent, inactive form bound to family of molecules, the inhibitors of *κ*B or I*κ*B proteins.[9,15] Cellular stimulation by tumour necrosis factor alpha (TNFα) or its activation by a large spectrum of inducers comprising diverging molecules, such as cytokines, mitogens, growth factors, bacterial and viral gene, ultraviolet radiation and inhaled occupational particles[11] leads to activation of certain kinases, the inhibitory κB kinase (IκKs), which phosphorylate I*κ*Bs, selecting them for targeted degradation. The degraded I*κ*Bs then releases the sequestered NF-*κ*B dimers which are free to translocate into the nucleus. Once inside the nucleus these bind to specific DNA sequences in the promoter or enhancer regions of target genes and transactivate responsive genes, including those for I*κ*B and the zinc finger protein A20. The phosphorylated IκB is rapidly modified by ubiquitinylation and degraded in proteasomes.[10–14,17,18]

Newly synthesized I*κ*B translocates to the nucleus, attaches to NF-*κ*B dimers and eliminates them from the nucleus, while A20 protein stays in cytoplasm and suppresses the activity of TNFα receptors.[19] Thus the NF-*κ*B system comprises a minimum of two negative feedback loops, one involving cytoplasmic sequestration mediated by I*κ*B and another involving A20 protein.[15]

Functions of NF-κB

Activated NF-κB causes induction of multiple cellular functions comprising increased cell proliferation and decreased apoptosis10–14 altered intra cellular adhesions,[20–24] recruitment of inflammatory cells,[25,26] amplification of primary pathogenetic signals,[27] and commencement or acceleration of tumorigenesis.[28]

NF-κB and p-53

The expression of genes regulated by NF-*κ*B is tightly integrated and coordinated with the activities of many other signalling and transcription-factor pathways including the p53 signalling pathway.[29–33] The independent NF-*κ*B signalling pathway has been studied extensively, the existence and mechanisms of the interactions between the NF-*κ*B pathway and other signalling pathways are yet not completely deciphered.[15]

The tumour suppressor and transcription factor p53 is the major modulator of cellular stress responses, and its activation is preceded by cellular apoptosis in many cell types. A role of NF-*κ*B in p53 mediated apoptosis has been documented. Carsten *et al* evaluated role of NF-κB in p53-mediated neuron death. Exposure of neurons to fatal stress activates p53 and disrupts the cascade of NF-*κ*B mediated survival signalling. Inhibitors of p53 provide marked neuro-protective effects because they block p53-mediated induction of

cellular death and simultaneously augment NF-κB-induced survival signalling.[34]

NF-κB and apoptosis

NF-κB has been documented to have a protective role against apoptosis primarily by up-regulation of genes encoding anti-apoptotic products comprising interleukins such as IL-1, IL-2, IL-6 along with a wide array of colony stimulating factors, e.g., macrophage colony-stimulating factor (M-CSF), granulocyte colony stimulating factor (G-CSF), granulocyte-macrophage colony-stimulating factor (GM-CSF) and also superoxide dismutase and the zinc finger protein A20.[1–3] The inter relationship of NF-κB and other antiapoptotic genes yet remains to be elaborated.

NF-κB and malignancy

There is increasing evidence implicating a dysregulation of NF-κB-associated pathways in multiple malignancies, including breast cancer[14,35–40] where NF-κB-regulated genes are being proven to be involved in cellular proliferation and invasion along with tumour related angiogenesis and lymphangiogenesis. An element of inflammation is also an accompanying component.[37,41-42]

According to Miron *et al* the activated NF-κB dimers play a pivotal role in breast cancer and other malignancies by enhancing cellular proliferation and causing diminished apoptosis, but the basic trigger that initiated the activation of NF-κB is yet unclear. Alterations in genes encoding NF-κB/Rel/IkB proteins have been documented mostly in lymphoid neoplasms. These comprise chromosomal rearrangements, amplifications and mutations that in some cases lead to production of truncated abnormal proteins that localise to the nucleus and activate transcription. In breast cancer, however the precise mechanism of NF-κB activation still remains unclear, but its role in cancer progression has been delimited.[9]

NF-κB and breast cancer

Activation of NF-κB in human breast cancer is found mostly in the ER-negative subtype of cancers, specifically those that demonstrate members of the EGF family of receptors, including the EGFR (erb-1) and (HER-2/neu (erb-2). This activation is brought about by interaction of growth factor with their specific receptors in these cell types.[14,42]

NF-κB with ER negative and Her2-neu positive breast cancer

In oestrogen receptor-negative (ER-negative) breast cancer the main therapeutic impediment is absence of precise molecular target. Activated NF-κB could be that potential target in these sub set of cases as it is shows a stromal expression in ER-negative and Her2-neu tumours. This association suggests a significant role of activated NF-κB in modulating intercellular signalling between stromal and epithelial tumourous cells as these depend on NF-κB dependent cellular cascades and cycles for aberrant cell proliferation along with sustained cell survival by avoiding apoptosis.[43,44]

Singh *et al* demonstrated the of effect inhibition of NF-κB activation by the inducible expression of dominant-negative IKKbeta in ER negative and Her2-neu positive breast cancer. This resulted in blocking cellular proliferation, restoration of apoptosis, and significantly blocked xenograft tumour formation. In addition, they found the combination of trastuzumab (Herceptin), the humanized anti-erbB2 antibody and the specific IKK inhibitor NF-κB essential modifier-binding domain peptide effective in blocking NF-κB activation and the resultant cellular proliferation in addition to reinstating apoptosis in concentrations that were not effective when employed singly. These effects could pave a path for evolution of NF-κB transcription factor and its activation cascades as a potential therapeutic target for such breast cancers.[45]

NF-κB and Inflammatory Breast Cancer

Inflammatory breast cancer (IBC) has a poor prognosis and in spite of multimodal therapeutic regimens the patient prognostics are as dismal as metastatic breast cancer. Diagnosis is based on multiple well documented clinical parameters. The need to develop specific, precise prognostic factor prevails.[46,47] Two major lines of evidence demonstrate NF-Kb associated pathways to play a major contributory role in IBC. Firstly, the principal processes that are dysregulated and disturbed at the clinical and molecular levels, i.e., inflammation, cellular proliferation and invasiveness are basically controlled by NF-κB associated genes.[48] Secondly, recent studies documenting analysis of DNA microarrays in IBC have revealed abnormal expression of some NF-κB target genes.[49,50] Hence NF-κB may not only serve as a prognostic parameter but may also evolve as a novel therapeutic target in this context.[51]

NF-κB and tumour metastasis

Metastasis cancer cells includes a multistep complex mechanism comprising cellular invasion, angiogenesis with the cancer cells being carried through blood vessels, extravasations of malignant cells, organ-specific homing, and cellular growth. Matrix metalloproteinases, urokinase-type plasminogen activator, and cytokines play a pivotal role in the initial steps of invasion and angiogenesis. Chemokines such as stromal derived factor-1alpha (SDF-1alpha) and its associated receptors such as CXCR4 are determine the cellular motility, homing and aberrant proliferation. Helbig *et al* demonstrated upregulation of metalloproteinases, urokinase-type plasminogen activator, and cytokines by NF-κB in highly metastatic, aggressive breast cancer

cell lines. In addition it is also seen to increase motility of breast cancer cells by directly up-regulating the expression of CXCR4.[52]

As the multi-faceted dimensions of NF-κB are being unmasked, its role as a prognostic and therapeutic target is evolving specifically in aggressive breast cancer subtypes.

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