

Project Update 2003

Development of NF- κ B Directed Therapy:

Title: Activation of NF- κ B: A Potential Novel Therapeutic Target for ER-Negative EGFR Family Receptor-Overexpressing Breast Cancer Patients.

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Introduction:

Overexpression of Erb2 (Her2neu), one of the epidermal growth factor (EGF)-family receptors, is detected in about 30% of ER-negative (ER-) human breast cancers. The prognosis of this class of ER- patients is poor, although currently used therapies with the anti-Her2neu antibody Herceptin in combination with other chemotherapeutic agents show limited success. The primary goal of this proposal is to define a target directed therapy for this class of human breast cancer patients i) by influencing the interactions of different components of the nuclear factor kappa B (NF- κ B) that is activated by cell signaling pathway involving EGFR family receptors. ii) Consequence of activation of NF- κ B on cell proliferation and death of the ER- breast cancer cells will be monitored, iii) followed by establishment of its role in carcinogenesis of ER-/Erb2 (Her2neu) overexpressing human mammary epithelial cells iv) finally to identify low molecular weight compounds that block its function thereby qualifying this specific cellular molecule as a potential therapeutic target for this class of breast cancers.

Our previous results with ER-/Erb1+ (EGFR) human breast cancer cell lines (MDA-MB-231 and MDA-MB-468 and mouse mammary epithelial cell line CSMLO revealed that EGF/ErbB1-interaction-induced cell proliferation and death signals are transmitted at least partly via the activation of NF- κ B (Biswas et al., 2000, 2001, & 2003). The NF- κ B family of transcription factors are heterodimeric complexes of Rel proteins (Karin & Lin, 2002). The predominantly detected one is the p50/p65 complex. In most cell types NF- κ B is present in an inactive state being complexed with an inhibitory protein called I κ B. A variety of extracellular signals such as inflammatory cytokines, mitogens, growth factors, bacterial and viral infections, irradiation, oxidative stress, etc, activate NF- κ B by generating cellular signals leading to the phosphorylation of I κ B by a specific kinase (IKK) and its degradation by a proteasome dependent pathway. Release of I κ B allows the active p50/p65 complex to move into the nucleus where it transactivates NF- κ B responsive genes by interacting with specific DNA sequences in the promoter region of these genes.

The **rationale** for choosing NF- κ B activation as a potential therapeutic target for ER-human breast cancers is based on our results on its controlled overactivation in ER-human breast cancer cells in culture. Specific inhibition of NF- κ B activation by stable expression of dominant negative inhibitory kappa B kinase (dnIKK α) resulted in the loss of tumorigenic potential of mouse mammary epithelial adenocarcinoma cells (CSMLO). The PKC α / β inhibitor Go6976 that blocked NF- κ B activation inhibited the growth and regressed full grown tumors developed in mice by subcutaneous implantation of CSMLO cells. The NF- κ B-activation associated antiapoptotic phenotype of breast cancer cells is reversed by these inhibitors.

Research Accomplishments During the Period of the Report: In the light of the above described work with in vitro (cell culture) and in vivo (animal) systems, initially we examined the level of active NF- κ B in 31 human breast tumor biopsy specimens. These included four classes of human mammary tumors with respect to ER and EGFR family receptor levels, **7, Class 1 (ER-/Erb2+), 9, Class 2 (ER-/Erb2-), 8, Class 3 (ER+/Erb2+), and 7, Class 4 (ER+/Erb2-)**. Elevated level of NF- κ B activation was detected predominantly in ER-/Erb2+ (6 out of 7) biopsy specimens. Immunofluorescence examination of frozen sections of the same specimens identified active NF- κ B (nuclear p50/p65) complex in islands of epithelial (CK19+) cells in ER-/Erb2+ tissues, whereas this is detected mostly in stromal cells in other classes, specifically in ER-/Erb2- samples. We are currently examining Heregulin-Erb2 (Her2neu)-mediated NF- κ B activation in ER-/Erb2+ human breast cancer cell line SKBr3 (Her2neu overexpressing), an analogical in vitro working system similar to the ER-/Erb2+ class of human breast cancers. Preliminary results demonstrate that the level of active NF- κ B in SKBr3 is elevated within 2 hrs of Heregulin-treatment and persists as long as 18 hr. This could be blocked by anti-ErbB2-antibody Herceptin, and most importantly by the peptide NBD that specifically blocks the action of the regulatory subunit of the IKK complex that activates NF- κ B. The consequences of the specific inhibition of NF- κ B activation on proliferation and apoptosis in SKBr3 cells are being studied. An abstract with these results is submitted for presentation in the upcoming Keystone Symposium on “NF- κ B: Biology and Pathology”, to be held in Snowbird, Utah, January of 2004.

We are currently also examining the effects of specific down regulation of members of the IKK complex on the inhibition of NF- κ B. We have taken two approaches **a)** transient down regulation of IKK α (IKK2), one of the catalytic subunits, and IKK β (Nemo) the regulatory subunit of IKK complex, using small inhibitory RNAs (siRNA) and **b)** stable expression of a dominant negative IKK α (dnIKK α) expression plasmid. We are in the early stages of these investigations, but preliminary results demonstrate that cellular IKK α protein levels can be reduced by greater than 90%, using siRNA oligos, in both MDA-MB-231 (ER-) and MCF7 (ER+) cells, we are currently investigating the consequences of this specific inhibition on NF- κ B activation, cell proliferation, apoptosis and gene transcription. Similarly, we have shown that IKK α levels can be reduced by approximately 95%. The influence of the down regulation of IKK on the down stream events of NF- κ B activation is being investigated. Using the second approach we have isolated a number of clones expressing the dnIKK α mutant protein. Using the electrophoretic mobility shift assay (EMSA) we have demonstrated a significant reduction in levels of active NF- κ B, as compared to the vector control; again the down stream effects are currently being studied.

Future Experimental Strategies to Accomplish the Projected Goals: A third ongoing experimental approach for establishing the role of NF- κ B activation in mammary epithelial cell carcinogenesis is to a) construct an inducible expression vector that stably produces dominant negative IKK (dnIKK) in a tet on/off system in ER- cells such as MDA-MB-231 (ER-/Erb1+) and in SKBr3 (ER-/Erb2+) cells in culture and b) then to study tumor formation in nude mice in the presence or absence of the inducer.

Construction of such a plasmid in MDA-MD-231 (ER-/Erb1+) is completed and that in SKBr3 (ER-/Erb1-/Erb2+) and MDA-MB-468 (ER-/Erb1+/Erb2-) are ongoing

Screening of Low Molecular Compounds that Block NF- κ B Activation: This will involve i) culture cell systems and ii) in vivo tumor formation system in nude mice. Initially we plan to examine the following few compounds, i) Go6976, a PKC α/β inhibitor that has been found to be a potent inhibitor of NF- κ B activation. This compound blocked tumor formation and tumor regression in mice without causing any toxic side effects in the treated animals (Biswas et.al. 2003). In addition we will use ii) **Velcade** and iii) **PS-1145** that are proteasome inhibitors. By blocking I κ B degradation these compounds block NF- κ B activation, and iv) **PS-341** is an inhibitor of I κ B phosphorylation (Hideshima et al, 2002). All of these compounds will be tested either singly or in combinations with Herceptin in cell culture systems with SKBr3 (ER-/Erb2+), MDA-MB-231 and MDA-MB-468 (ER-/Erb1+) cells and in vivo system. Tumors will be generated by implantation of these ER- cells in nude mice. Influence of systemic administration of these compounds individually or in combinations on tumor formation and tumor regression will be examined.

The **long term goal** of this project is to identify combinations of low molecular compounds that block NF- κ B activation thereby blocking cell proliferation and inducing apoptosis. As NF- κ B activation is a consequence of diseased conditions such as cancer, it is anticipated that the inhibited NF- κ B activation associated reduced cell proliferation and enhanced apoptosis will modulate only the phenotypes of cancer cells and not of the normal cells.

Refereneces:

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