MUTUAL ANTAGONISM BETWEEN NFKAPPAB AND RETINOIC ACID RECEPTORS (RAR) IN THE MODULAR CONTROL OF TUMOR METASTASIS

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Introduction:
We previously ascribed a pivotal role to the transcription factor NFκB in tumor metastasis. Our arguments were based on the observation that NFκB reciprocally regulated a set of putative prometastatic factors (matrix metalloprotease 9- MMP9, urokinase like plasminogen activator – uPA) and their corresponding antimetastatic inhibitors (Tissue Inhibitor of matrix metalloprotease TIMP1 and Plasminogen Activator Inhibitor 2 – PAI 2), tipping the scale towards metastatic propensity such that inhibition of NFκB decreased in vivo tumor metastasis.

To extend these studies, we carried out a high throughput gene expression analysis of the murine lung alveolar carcinoma cell line (Line 1) and its non-metastatic variant, transduced with a dominant negative inhibitor of NFκB (mIκB). The results of these analyses, confirmed the reciprocal effect of NFκB transcriptional activity on metastatic gene expression over a broader set of genes, and identified retinoic acid receptor alpha (RARα) to be highly expressed (12 fold induction) in the non-metastatic cells.

Retinoids posses intrinsic antitumor, anti-inflammatory and immunomodulatory activities, establishing them as bona fide chemopreventive agents in human cancers. The wide range of biologic responses elicited by retinoids is explained by the multitude of retinoid receptors activated and the potential for cross-talk with other pathways. Given NFκB has been demonstrated to negatively interact (cross-couple) with members of the steroid/thyroid/retinoid superfamily of nuclear hormone receptors, we set out to evaluate the possibility that retinoid receptors may tip the scale towards malignant reversion by cross-coupling of NFκB mediated responses.

Materials and Methods:
We used the 19,000 microarray gene set (Affymetrix) to obtain the gene expression readout of WT and mIκB Line 1 tumor cells. To validate our microarray findings we performed RT-PCR, using RNA from metastatic and non-metastatic Line 1 tumor cells as template, and RAR subtype specific primers.

RAR reporter constructs were used to assess differential transactivity of RARs in WT and mIκB tumor cells and to establish the correlation between receptor expression and activity.

Co-immunoprecipitation studies, using agarose conjugated NFκB antibodies and oligonucleotides, were carried out to document retinoid receptor interactions with free or DNA bound NFκB proteins. The responsiveness of these interactions was evaluated by exposing cells to increasing concentrations of all-trans retinoic acid (0.1, 0.3 and 1μM) and the functional translation of these changes was assessed by looking at RAR and NFκB reporter activities. Under identical experimental conditions, we monitored changes in the expression of putative prometastatic factors (MMP9 and uPA) and their corresponding antimetastatic inhibitors (TIMP1 and PAI2 ) by real time PCR.

Results.
We observed enhanced expression of all RAR subtypes (α, β and γ) and a 7 fold increase in RAR reporter activity in the non-metastatic tumor cells. Co-immunoprecipitation studies, confirmed NFκB-RAR protein-protein and protein-protein-DNA interactions, and demonstrated responsiveness of these interactions to all trans-retinoic acid. We observed increased association of RARs to NFκB-DNA complexes, paralleled by a decrease in NFκB reporter activity and an increase in RAR reporter activity. In accordance with the aforementioned observations, dominant negative inhibitors of RAR and retinoid antagonists enhanced NFκB reporter activity. As expected, all trans retinoic acid repressed MMP 9 and uPA expression while enhancing TIMP 1 and PAI 2 expression in a dose dependent manner.

Discussion
Retinoids have been extensively investigated as chemopreventive agents in settings of a known predisposition to a given cancer type or with the intent of maintaining the remission state of a previously cured cancer. Our data suggests that the antitumor activity of retinoids is based on retinoids activating NFκB mediated responses and elicit retinoid mediated responses. We therefore propose that the stoichiometry of active NFκB and RAR proteins determines the metastatic phenotype. This proposition is supported by the reported decrease in RAR expression in progressive models of carcinogenesis, matched by an increase in NFκB signaling.

Conceivably, increased NFκB signaling activity in cancers (presumably due to the smoldering inflammatory process, germane to cancerous lesions), mops up RAR mediated responses, a primordial response being the autoinduction of RAR expression, decreased expression of prometastatic factors and enhanced expression of antimetastatic factors.

Reference:

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