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PRINCIPAL INVESTIGATOR: Mingfang Ao, M.D.

CONTRACTING ORGANIZATION: Vanderbilt University Medical Center
Nashville, TN 37203

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14. ABSTRACT There is strong evidence that inflammation and interactions with the surrounding stromal microenvironment are critical for cancer initiation and progression. As a major component of the stroma, fibroblasts are recognized as prominent modifiers of cancer progression. The contribution of carcinoma associated fibroblasts (CAF) to cancer has been approved and become accepted, research has been conducted to understand the mechanisms underlying this stromal-epithelial interaction. In this project we have demonstrated that relatively small changes in the expression levels of TGFβ and SDF1/CXCL12 in human prostate cancer stromal cells can drive carcinogenesis in human prostatic epithelium. In two publications we showed linkage between the two pathways in that TGFβ elevates CXCR4, the cognate receptor for SDF1, in the epithelial cells allowing activation of the SDF signaling pathway. This in turn activates Akt phosphorylation which is sufficient to suppress the growth inhibitory response to TGFβ. This link provides a mechanism for the switch in TGFβ activity from growth suppressive in normal tissue to growth promoting in cancer and suggests routes for therapeutic intervention.					
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Introduction

This project was terminated upon the graduation with a Ph.D. degree of the P.I. who moved on to a postdoctoral position in 2006. We were recently informed that the final report submitted in 2006 was not accepted and this amended report is therefore submitted to allow closure of this file.

Carcinoma arises from epithelium, however, there is growing evidence that inflammation and interactions with the surrounding stromal microenvironment are critical for cancer initiation and progression. Stromal alterations during tumorigenesis have been shown in prostate cancer and many other tumors. As a major component of the stroma, fibroblasts are recognized as prominent modifiers of cancer progression. The contribution of carcinoma associated fibroblasts (CAF) to cancer has been appreciated and become accepted, research has been conducted to understand the mechanisms underlying this stromal-epithelial interaction.

Transforming growth factor-beta (TGF- β) is a pleiotropic growth factor with actions that are dependent upon circumstances including dose, target cell type and context. TGF- β can elicit both growth promoting and suppressive activity. In normal tissue, TGF- β generally acts to restrict growth and maintain differentiation. However, during tumorigenesis, changes in TGF- β expression and cellular responses can promote tumorigenesis.

Body

In the initial stages of this project we examined the effects of TGF- β on the non-tumorigenic human prostatic epithelial cell line BPH1 and on three derivative tumorigenic sublines BPH1CAFTD-1, -3 and -5. The data (which were published in Cancer Research in 2006, see reference below) demonstrated that TGF- β has different effects on the non-tumorigenic and tumorigenic cells. The non-tumorigenic cells were growth inhibited by TGF- β . In contrast the tumorigenic sub-lines were not growth inhibited but instead underwent an epithelial to mesenchymal transformation (EMT) in response to TGF- β . The

tumorigenic lines showed constitutively elevated levels of phosphorylated Akt which modulated their response to TGF- β by blocking Smad3 and p21 nuclear translocation. Upon TGF- β stimulation of the tumorigenic sublines the activated Akt allowed the cells to escape cell cycle arrest. The PI3K/Akt pathway was also found to be involved in TGF- β induced EMT, defined here by induction of vimentin expression and enhanced cellular motility.

In vivo, tumorigenic cells with constitutively active TGF- β signaling showed increased invasion with EMTs, which expressed vimentin, located specifically at the invasive front of the tumor. These data indicated that following malignant transformation TGF- β can play a direct role in promoting prostatic cancer and further that these responses are context specific in vivo.

In final year of the project, we aimed to identify pathways which could elicit tumor-promoting paracrine effects and whose expression patterns correlated with those seen in human disease. This work was described in a paper published in Cancer Research in 2007, see reference below.

We found that human prostatic carcinoma-associated fibroblasts (CAF) induce tumorigenesis in initiated but non-malignant human prostatic epithelial cells (BPH-1) using a combination of mild overexpression of chemokines and cytokines. CAF express elevated levels of both transforming growth factor-beta1 (TGF- β 1) and stromal cell-derived factor-1 (SDF-1/CXCL12). TGF- β inhibits the growth of BPH-1 cells in vitro but was found to be necessary for the tumorigenic response to CAF. This counterintuitive result suggested that the TGF- β signaling system was involved in other processes relating to tumorigenesis. The SDF-1 receptor, CXCR4, is expressed at low levels in benign prostate tissue and in BPH-1 cells in culture. However CXCR4 levels increase during prostate cancer progression. CXCR4 was found to be induced and localized to the cell membrane in BPH1 cells by CAF conditioned medium and by CAF cells in tissue recombinants. TGF- β was found to be both necessary and sufficient to allow detection of membrane localized CXCR4 in BPH1 cells. Suppression of epithelial cell CXCR4 expression abrogated the tumorigenic response to CAF. SDF-1, secreted by CAF, acts via the TGF- β -regulated CXCR4 to activate Akt in the epithelial cells. This mechanism elicits tumorigenesis and obviates the growth inhibitory effects of TGF- β . Thus tumor stroma can contribute to carcinogenesis through synergism between TGF- β , SDF1, and CXCR4. These experiments suggest mechanisms by which TGF- β can shift its role from an inhibitor to a promoter of proliferation during tumor progression. Both the TGF- β and SDF1 pathways are targets of drug discovery efforts, these data suggest potential benefits in co-targeting of these pathways.

The data presented in these two papers led us to generate a model of stromally-driven tumor progression which is summarized in figure 1.

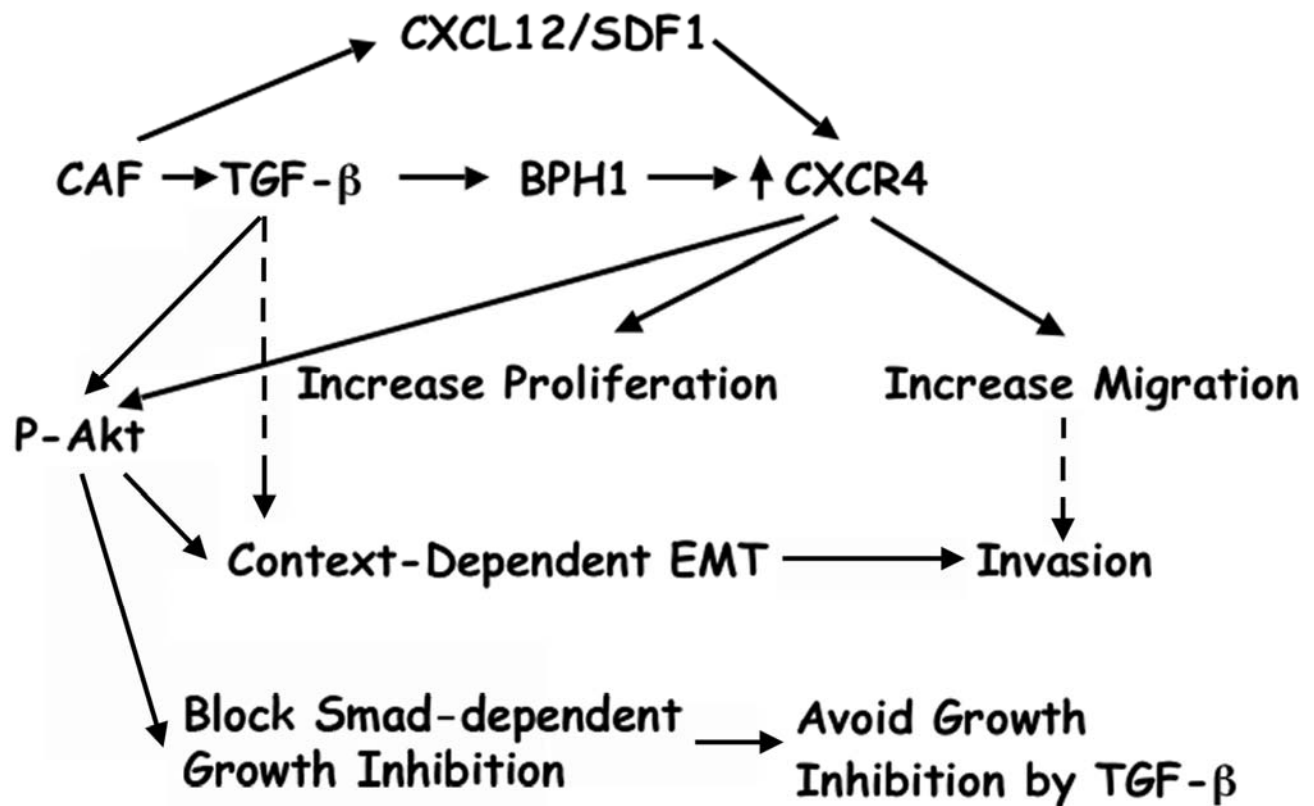


Figure 1. Summary of the mechanism by which CAF can contribute to carcinogenesis based upon data generated in this project. Briefly TGFβ expressed by carcinoma-associated fibroblasts (CAF) activates expression of CXCR4 in target epithelial cells. This allows activation of the SDF1/CXCL12 pathways with consequent activation of Akt and associated proliferation of epithelial cells. Subsequent to Akt activation Smad-dependent growth inhibition is suppressed allowing cells to escape the growth inhibitory response to TGFβ while retaining the pro-invasive responses such as activation of EMT.

Personnel Changes

The P.I. graduated with a Ph.D. degree and the project was terminated.

Key Research Accomplishments

The most important aim of pre-doctoral awards is to provide research training to fellows. This aim was clearly accomplished. The research aims of the project were largely fulfilled, major outcomes being the finding of interacting paracrine pathways that promote prostatic carcinogenesis and can offer the

possibility for medically-based therapies with less side effects than current options.

Reportable Outcomes.

Two papers were published describing the work performed.

Ao, M., Franco, O.E., Park, D., Raman, D., Williams, K., and Hayward, S. W. (2007). Cross-talk between paracrine-acting cytokine and chemokine pathways promotes malignancy in benign human prostatic epithelium. *Cancer research* 67, 4244-4253.

Ao, M., Williams, K., Bhowmick, N.A., and Hayward, S.W. (2006). Transforming growth factor-beta promotes invasion in tumorigenic but not in nontumorigenic human prostatic epithelial cells. *Cancer research* 66, 8007-8016.

Conclusions.

This project generated a set of data, summarized in two papers in *Cancer Research*, which demonstrate the potential of relatively mild changes in tumor stroma to contribute to the progression of prostate cancer. The use of in vivo models and human cells clearly enhances the clinical relevance. The fact that the imbalances on chemokine and cytokine expression were not huge is important as this underlines the consequences of small combinatorial effects of signals resulting in profound consequences. The work also strongly suggests that approaches to address these changes clinically might well not require total suppression of signaling but rather selective partial suppression of key pathways. The extracellular nature of the molecules provides further confidence that these can be tackled clinically. Inhibitory antibodies against TGF β are already in clinical trials, however there have been some adverse consequences reported due to high levels of pathway suppression. We would suggest that lower doses of such agents combined with partial suppression of SDF1/CXCL12 signaling might well provide a clinical benefit with significantly reduced negative patient impact.

The second major contribution of this work was to demonstrate a clear mechanism by which TGF β signaling can change in tumor progression from a tumor suppressive to a tumor promoting factor. This also suggests that suppression of Akt activation, beyond its obvious anti-proliferative consequences might also allow the pro-differentiative effects of TGF β to re-manifest themselves, providing a version of a differentiation therapy.

A third important outcome was the further demonstration of the importance of context in tumor responses to specific molecular changes, this is specifically demonstrated by the data presented in the final figure of the 2007 paper in which EMT occurs specifically at the invasive front of the tumor and not within the tumor mass, even though the putative activating mutation is present in all tumor cells.