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TGF-ß Signaling Pathway in Lung Adenocarcinoma Invasion

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Abstract

The histological distinction between bronchioloalveolar carcinoma (BAC) and other adenocarcinomas is tissue invasion. The clinical importance of lung adenocarcinoma invasion is supported by several recent studies indicating that the risk of death in non-mucinous BAC is significantly lower than that of pure invasive tumors and in tumors with greater than 0.6 cm of fibrosis or linear invasion. Using microarray gene expression profiling of human tumors, dysregulation of transforming growth factor-ß (TGF-ß) signaling was identified as an important mediator of tumor invasion. Subsequent studies showed that the CC chemokine RANTES (Regulated on Activation, Normal T-cell Expressed, and presumably Secreted) was upregulated in invasive tumors and was required for invasion in cells with repressed levels of the TGF-ß type II receptor. Taken together, these studies illustrate how information gained from global expression profiling of tumors can be used to identify key pathways and genes mediating tumor growth, invasion, and metastasis.

The World Health Organization subclassifies adenocarcinoma based upon predominant cell morphology and growth pattern.[1]. The histological distinction between bronchioloalveolar carcinoma (BAC) and other adenocarcinomas is tissue invasion. BAC tumor cells are cuboidal to columnar, with or without mucin, that grow in a noninvasive fashion along alveolar walls. Invasion, defined as tumor disruption of the alveolar basement membrane, is present in other subtypes of adenocarcinoma. Adenocarcinomas with mixed subtypes frequently contain regions of lepidic/noninvasive tumor at the periphery of invasive tumor.

Recent clinical reports suggest that the prognosis and radiographic appearance of BAC is unique and may support modifying the clinical approach to lung adenocarcinomas according to histological subtype. Metastases to lymph nodes and extrathoracic organs are unusual in nonmucinous BAC. The mean five year survival for Stage I BAC and other adenocarcinomas is 81% and 55%, respectively [2]. Recent reports suggest that for Stage IA BAC, limited resections rather than lobectomy, which is the current standard resection for Stage IA adenocarcinoma, may be curative[3]. Notably, low dose chest CT screening detected lung cancer is more likely to be adenocarcinoma than conventionally detected cancer (75% versus 40%)[4, 5]. In addition, 25% of screen detected cancers are BAC. As a result, the identification of invasion in screen detected malignancy may in the future guide a therapeutic decision of limited versus anatomic resection.

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Paralleling malignancies in other organs, such as breast and cervix, where tumors are defined as non-invasive (in-situ carcinoma), micro-invasive (microscopic invasion) or as invasive carcinomas, the extent of the invasive component seen in lung adenocarcinoma is associated with clinical outcomes. The clinical importance of lung adenocarcinoma invasion is supported by several recent studies [2, 6-9] indicating that the risk of death in non-mucinous BAC is significantly lower than that of pure invasive tumors and in tumors with greater than 0.6 cm of fibrosis or linear invasion. In 200 cases of small adenocarcinomas (diameter < 3 cm), Yokose reported no deaths among 66 BAC cases[10]. In 484 cases of BAC and adenocarcinoma, Terasaki reported that lymph node involvement was absent in all BAC and was present in 20% of adenocarcinomas that had an invasive area greater than 5 mm[11]. Similarly, among 178 patients with resected lung adenocarcinoma we found five year survival rates of 100% and 90% for patients with BAC or tumors with invasive length less than 6 mm, respectively[12]. Together, these studies suggest that non-invasive tumors are biologically indolent and that invasion increases the risk of metastatic disease and death in solitary mixed subtype tumors.

Invasion is the first step of carcinoma metastasis, in which epithelial cells lose cell-cell adhesion, gain motility and invade into adjacent stroma. Subsequent steps of metastasis include vascular intravasation and extravasation, establishment of a metastatic niche and angiogenesis.[13]. Tumor invasiveness, the morphologic characteristic that distinguishes BAC from adenocarcinoma, is determined by the interaction of tumor cells with the surrounding stroma [14, 15].

We [16] and others [17-19 20] have used microarray gene expression profiling of lung adenocarcinoma to identify signatures associated with histology and invasion. The results of unsupervised analyses, in which the specimens are sorted into groups in a dendogram based upon similarity of gene expression, show lung adenocarcinomas segregate into three major branches comprised predominantly of BAC, AC-Mixed subtype, and pure invasive tumors. These results provide biological plausibility to support the notion that these adenocarcinoma subtypes are distinct entities. Taken together with the clinical prognostic data, these studies have motivated efforts to reinforce the designation of purely noninvasive tumors and to create a designation for minimally invasive tumors in a revision of the WHO lung adenocarcinoma classification scheme.

To identify molecular pathways important for mediating the acquisition of invasion by lung adenocarcinoma, we performed supervised analysis of mRNA microarray data to identify genes differentially expressed in non-invasive BAC and in AC-mixed type tumors. Among the genes differentially expressed in the progression from BAC to invasive tumors was the transforming growth factor-ß (TGF-ß) type II receptor (*TβRII)*, which was less highly expressed by AC-Mixed and solid invasive tumors compared with BAC. This finding, which suggested that TβRII repression was required for lung adenocarcinoma invasion, is supported by genetic models combining targeted deletion of *TβRII* with other oncogenic events such as Adenomatosis polyposis coli (APC) mutation in colon tumors and KRAS mutations in pancreatic and oropharyngeal carcinomas [21-23]. The phenotypes of these TGF-β receptor cancer models clearly demonstrate the importance of TGF-β signaling in tumor invasion.

TGF-β, the ligand for the *TGF-β* type II receptor is a pleiotropic cytokine comprised of family members TGF-β 1, 2, 3 that regulate tissue homeostasis and prevent tumor initiation by inhibiting cellular proliferation, differentiation, and survival [24]. It is secreted as a latent molecule and is activated by cleavage by proteases and other molecules[25]. Signaling primarily occurs through SMAD protein dependent pathways whereby ligand binding to TBRII induces phosphorylation and activation of TGF-β type I receptor (TβRI*)*. After

interaction with TβRI, phosphorylated SMAD2 and SMAD3 dissociate to form a heterotrimeric complex with SMAD4 and translocate into the nucleus to regulate gene transcription (Figure 1A). TGF-β signaling may also proceed via less well understood SMAD independent pathways (Figure 1B). These "non-canonical" pathways involve various signaling cascades including Ras/ERK, Rho/ROCK, and TAK1/MAPK, and are likely to have important roles in mediating the pro-tumorigenic effects of TGF-β [26]. Depending upon context, TGF-β signaling may alternatively function to suppress tumor growth or to promote tumor cell invasion and metastasis [27-30].

TGF-β as a Tumor Suppressor

Although recent research has focused primarily on TGF-β receptor alterations, tumors may employ various mechanisms anywhere along the signaling cascade to circumvent the inhibitory effects of TGF-β[31-35]. Type II receptor genetic alterations are well characterized in gastrointestinal tumors, in which 25% of colorectal carcinomas have missense mutations associated with microsatellite instability. Animals with targeted deletion of TßRII in the colonic epithelium demonstrate increased tumor progression from adenomas to invasive carcinomas [36] similar to human colorectal tumors with loss of type II receptor [37]. In breast carcinoma models, mammary tumors in animals with targeted deletion of TßRII demonstrated increased progression and metastases [38]. A recent case control study in human breast tumors indicated that within breast hyperplasia specimens, the proportion of cells with decreased type II receptor immunostaining was associated with increased risk for the development of invasive breast cancer [39]. Multiple lung cancer cell lines, both small cell [40-42] and non-small cell [43-46], demonstrate reduced expression TGFβRII. This repression is accompanied by marked reductions in TGF-β mediated growth suppression which is rescued after restoration of the receptor. In human lung tumor specimens, type II receptor repression is evident in ~40% of lung adenocarcinomas overall and in up to 100% of poorly differentiated adenocarcinomas [47]. Mechanisms of repression include epigenetic silencing [48], microsatellite instability, and frameshift mutations involving the poly(A) tract [43]. For the *TGF-β* type I receptor, mRNA repression is detectable in non-small cell lung cancer (NSCLC) [49], and recent studies indicate that *TβRI* SNP variants are associated with an increased risk of lung cancer [50-52].

TGF-β as a Tumor Promoter

Several tumors, including those arising in the lung [53, 54, 55] express high levels of the TGF-β, which correlates with tumor progression and clinical prognosis [34, 56-60]. TGF-β signaling promotes epithelial to mesenchymal transition, a characteristic of invasive and metastatic cells [61, 62], with constitutive activation of TGF-β or TβRI leading to increased metastases in animal models of breast cancer [63-65]. Likewise, blockade of TGF-β signaling via either dominant negative expression of SMAD3 or defective TβRI leads to decreased lung metastases [66, 67]. Systemic inhibition of TGF-β has been shown to suppress metastasis [68-71] and TGF-β overexpression by NSCLC specimens was found by multivariate analysis to be an independent risk factor for pulmonary metastasis [72].

How do we reconcile these findings with those suggesting TGF-β is a tumor suppressor? Context dependency in terms of cell type, tumor stage, and mode of inhibition of TGF-β signaling are important. Other important issues are the degree of repression of TGF-β receptor levels and the stromal response to TGF-β signaling inhibition. Rojas and colleagues have shown that different levels of repression of the TGF-β receptor are associated with differences in the activation of the SMAD and MAPK pathways such that at lower levels of TGF-β receptor activation, the pro-tumorigenic non-SMAD signaling pathways dominate [73]. Yang and colleagues showed that targeted deletion of TβRII in the mammary epithelium promoted breast cancer metastases through the CXCL5/CXCR2 chemokine axis mediated

recruitment of Gr-1$^+$/CD11b$^+$ myeloid derived suppressor cells. Increased stromal TGF-β levels at the invasive front of tumors was shown to be important for tumor progression and for inhibition of tumor immunosurveillance [74].

Chemokine Signaling in Human Tumors with Repressed TβRII expression- CCL5

Our results in lung adenocarcinoma and in *in vitro* systems indicate that repression of the *TGF-β* type II receptor increases invasiveness. We have shown that activation of SMAD2 and Akt are lower in *TβRII* knock-down cells while p38 activation is slightly increased [16]. We expect that TGF-β signaling in cells with moderately reduced type II receptor levels persists in the invasive tumors and in the knock-down cells and that SMAD independent pathways modulate this effect [73, 75]. We used a tumor cell invasion system and microarray analysis to identify and characterize downstream mediators of TGF-ß signaling important for lung adenocarcinoma invasion [16]. Among potential mediators identified was the CC (or *β*-chemokine) family member CCL5 (RANTES), which was upregulated by invasive tumors and *TßRII* knockdown cells. RANTES is involved in immunoregulatory and inflammatory processes and is secreted by T cells and other inflammatory cells, stromal cells, as well as tumor cells and normal bronchial epithelium. RANTES is a ligand for chemokine receptors CCR1, CCR3, CCR4, and CCR5, which are expressed on epithelial cells, macrophages, lymphocytes, dendritic cells and stromal cells [76-79]. Inhibition of RANTES signaling significantly abrogates tumor invasion, suggesting that RANTES is required for invasion in *TGF-β* type II receptor repressed lung adenocarcinoma cells (Figure 1C). The clinical significance of this pathway is further supported by the finding that tumor expression of RANTES and CCR5 in lung adenocarcinoma is associated with patient survival[80]. Small molecule inhibitors of CCR5 may have the potential to treat and prevent lung adenocarcinoma. Taken together, these studies illustrate how information gained from global expression profiling of tumors can be used to identify key pathways and genes mediating tumor growth, invasion, and metastasis.

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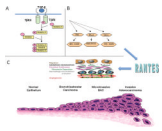

Figure 1.

TGF-β signaling occurs primarily via SMAD dependent pathways. A. Ligand binding to the TGF-β type II receptor (TβRII) induces phosphorylation and activation of type I receptor (TβRI), which phosphorylates and activates the receptor complex SMAD2 and SMAD3. Dissociated SMAD2/3 forms a heterotrimeric complex with SMAD4 that translocates into the nucleus to regulate gene transcription.

B. TGF-β signaling may also proceed via SMAD-independent pathways that involve various signaling cascades including Ras/ERK, Rho/ROCK, and TAK1/MAPK. These "non-canonical" pathways are likely to have important roles in mediating the pro-tumorigenic effects of TGF-β.

C. The histological distinction between bronchioloalveolar carcinoma (BAC) and other adenocarcinomas is tissue invasion. Invasion requires loss of cell-cell adhesion, migration, membrane degradation with vascular intravasation and extravasation and establishment of the metastatic niche angiogenesis and recruitment of stromal elements (top panel). We have shown that repression of TGF-β type II receptor in lung adenocarcinoma cells increases invasiveness and have used microarray analyses and inhibitor studies to identify the CC chemokine RANTES as an important mediator of lung adenocarcinoma invasion in TβRII deficient tumors. Figure reprinted[81] with permission from the American Thoracic Society