Ascending the PEAK1 toward targeting TGFβ during cancer progression: Recent advances and future perspectives

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Abstract

Cancer is the second leading cause of death in the United States. Mortality in patients with solid, epithelial-derived tumors strongly correlates with disease stage and the systemic metastatic load. In such cancers, notable morphological and molecular changes have been attributed to cells as they pass through a continuum of epithelial-mesenchymal transition (EMT) states and many of these changes are essential for metastasis. While cancer metastasis is a complex cascade that is regulated by cell-autonomous and microenvironmental influences, it is well-accepted that understanding and controlling metastatic disease is a viable method for increasing patient survival. In the past 5 years, the novel non-receptor tyrosine kinase PEAK1 has surfaced as a central regulator of tumor progression and metastasis in the context of solid, epithelial cancers. Here, we review this literature with a special focus on our recent work demonstrating that PEAK1 mediates non-canonical pro-tumorigenic TGFβ signaling and is an intracellular control point between tumor cells and their extracellular microenvironment. We conclude with a brief discussion of potential applications derived from our current understanding of PEAK1 biology.

Keywords

TGFβ; PEAK1 kinase; EMT; Metastasis; Tumor Suppression; eIF5A; Ciclopirox Olamine (CPX); Cancer Biomarker

Introduction

Cancer is a major public health problem not only in the United States but also in many parts of the world. Currently, cancer is the second leading cause of death in the United States [1, 2]. Estimates demonstrate that lung and bronchus cancer cause the highest percentage of cancer-related deaths (28% in males and 26% in females) in the US. The second leading cancer-related deaths in the US are prostrate in men and breast cancer in women (9% and 15%, respectively). Colon, rectal and pancreatic cancers encompass the next tier of cancers.
leading to mortality in the US [1, 2]. Major advances in cancer diagnosis and treatment over the past several decades suggest that cancer management is at the forefront of global scientific efforts [1, 2]. In some malignancies (e.g., breast cancer), these developments in screening, personalized therapies, biomarker identification and targeted therapy have improved disease prognosis [3]. However, other malignancies (e.g., pancreatic ductal adenocarcinoma, PDAC) remain extremely deadly. Such poor disease prognosis is usually attributable to challenges in early detection, early metastatic dissemination and/or therapeutic resistance [4]. When cancer cells acquire the ability to invade tissues and metastasize throughout the body, the chance of disease reoccurrence is significantly increased and thereby the patient prognosis for survival is reduced. Therefore, it is important to understand the molecular and cellular mechanisms that govern metastatic progression.

In human cancers, transforming growth factor beta (TGFβ) can act as either a tumor suppressor or pro-tumorigenic factor, which induces epithelial to mesenchymal transition (EMT) and metastasis [5]. Although EMT is fundamental and is strictly regulated during embryogenesis and tissue homeostasis [6], it is deregulated during the progression of epithelial cancers and correlates with the acquisition of metastatic behavior in cancer [7]. TGFβ has been previously reported to suppress tumor growth by inducing cytostasis, apoptosis, cell differentiation and immune responses [8, 9]. Many studies have also demonstrated that deregulation of both canonical and non-canonical TGFβ signaling pathways convert this protein hormone into a pro-progressive factor in epithelial tumors [10, 11].

We previously identified a novel non-receptor tyrosine kinase, PEAK1 (pseudopodium enriched atypical kinase 1, Sgk269) that is enriched in the pseudopodia of migrating cells [12]. Subsequently, we demonstrated that PEAK1 promotes tumor growth/metastasis and therapy resistance in pancreatic cancers through its regulation of the actin cytoskeleton and Src, KRas and ErbB2 signaling pathways [13]. Most recently, we demonstrated that PEAK1 is necessary and sufficient for TGFβ-induced migration, EMT, metastasis and proliferation in breast cancer [14]. In this regard, we reported that PEAK1 kinase mediates signaling cross talk between TGFβ receptors and the ITGB3/Src/Grb2/MAPK pathway and is essential for TGFβ-induced ZEB1 upregulation [15]. Herein, we review recent studies characterizing underlying mechanisms of TGFβ-induced metastasis and EMT in the context of PEAK1-mediated signaling in human cancer that emphasize further mechanistic studies that aim to identify novel therapeutic targets for blocking human cancer progression.

**TGFβ signaling in cancer metastasis**

The transforming growth factor beta (TGFβ) superfamily contains more than 30 secreted, extracellular ligands in human and other mammalian species [16] – many of these ligands are also conserved to lower vertebrates. Protein hormones in this superfamily include Activin [17], Nodal-related [18], TGFβ [19], Growth and Differentiation Factor (GDF) and Bone Morphogenetic Protein (BMP) [20] families. These ligands regulate a vast number of cellular processes such as tissue homeostasis, cell proliferation/differentiation, embryonic development, immune system responses, angiogenesis, wound or tissue damage repair and endocrine function [17, 18, 20–24]. As such, it is not surprising that disruption of the proper
function of ligands within this superfamily contributes substantially to multiple disease states including fibrosis and cancer [25–31].

Canonical signaling of TGFβ occurs via its interaction with two types of transmembrane receptors (type I and type II) that contain intrinsic serine/threonine kinase activities, called receptor serine kinases (RSKs) [23]. Following the characterization of the first vertebrate RSK [32], a dozen RSKs have now been identified in humans [23]. Type I RSKs are referred to as ALK1–7, for Activin Receptor-Like Kinases [33–38]. The general mechanism of activation for TGFβ ligand-receptor complexes involves TGFβ first binding to the type II receptor. This leads to binding and activation of the type I TβRI receptor (ALK5) to the ligand-type II receptor complex [39] – this mechanism was first established for TGFβ, but similar receptor activation mechanisms have been reported for other ligands in this superfamily [40–42]. Once activated in a ligand-dependent manner, intracellular Smad proteins are the targets of the type I serine/threonine receptors [43–46]. The TGFβ superfamily can be divided into Smad2/3-(e.g., Activin, Nodal and TGFβ) and Smad1/5/8-activating (e.g., BMPs and GDFs) ligands [47]. Upon phosphorylation of Smad proteins, they transport from the cytoplasm to the nucleus in complex with co-Smad proteins and directly interact with DNA and other transcriptional activators/repressors in a cell type and context-dependent manner. Ultimately, this leads to the activation or repression of target genes [7, 9, 23, 48].

Non-canonical signaling mechanisms have also been identified for TGFβ and other superfamily ligands, involving a myriad of well-characterized Smad-independent signaling pathways including RhoGTPases, Wnt, MAPK, Src and PI3K/Akt [5, 49]. Notably, the majority of the non-canonical TGFβ signaling mechanisms that have been characterized to date are associated with dysregulated TGFβ responses and the deleterious effects of TGFβ that contribute substantially to human diseases [50, 51]. Current developments during the past two decades, attesting to the cellular/molecular complexity within tissues [52], have paved the way for researchers to identify novel factors within the heterogeneous extracellular environment that regulate TGFβ signaling responses. In many instances, these factors shift the signaling responses from canonical to non-canonical and are at the root of disease-specific TGFβ signaling mechanisms [5, 49, 53–55].

Context-dependent TGFβ signaling has paradoxical effects on various tissue types [9]. For example, Smad2/3 signaling in response to TGFβ can induce varied and/or opposite effects on epithelial-mesenchymal transition (EMT), mesenchymal-epithelial transition (MET), cellular proliferation, apoptosis and differentiation. Importantly, these responses often depend on cell type and context [56]. In regard to TGFβ’s Smad2/3-dependent tumor suppressor function, TGFβ has been reported to block proliferation, induce terminal differentiation or apoptosis in multiple cell types [29]. While these TGFβ functions are critical for normal embryonic development, tissue maintenance and tumor suppression, dysregulation of these pathways have been identified and characterized as regulators of human cancer initiation and/or progression, including breast and pancreatic cancer [26, 29, 31, 57]. Dysregulated canonical and elevated non-canonical TGFβ signaling cause tumor cells to become refractory to the antiproliferative effects of the Smad2/3 pathway. Under these circumstances, TGFβ signaling promotes cell invasion/motility/proliferation/
survival and EMT in tumor cells as well as within the tumor microenvironment \cite{30, 56, 58–60}. This shift in TGFβ outcome from tumor-suppressing to-promoting occurs in conjunction with quantitative and qualitative changes in the proteome, transcriptome and subcellular signaling landscapes within the tumor cells as well as in the associated stromal cells.

Role of TGFβ in promoting EMT

The role of TGFβ in promoting EMT during normal and pathophysiological processes has been extensively characterized \cite{5, 8, 21, 22, 61–66}. EMT is a morphologic and phenotypic shift in cells that is associated with specific changes in gene expression, protein translation and post-translational modification. EMT is essential and strictly regulated during embryogenesis and tissue homeostasis \cite{6, 67}; however, it is dysregulated during wound healing and the progression of epithelial cancers to promote fibrosis and metastasis, respectively \cite{68}. During EMT, cells gradually lose their epithelial nature leading to decreased apical-basal polarity, ability to attach to the basement membrane and assembly of protein complexes that mediate tight cell-cell contacts. These changes are also associated with down regulation of epithelial genes (e.g., CDH1 or MUC1) and increased expression of mesenchymal genes (e.g., ZEB1 and FN1) - the resulting cells tend to migrate and invade more extensively and adopt a more spread, fibroblast-like morphology \cite{67}. While it is well accepted that TGFβ signaling is modified to enable a pro-EMT outcome, the molecular mechanisms that govern TGFβ’s ability to switch between its paradoxical growth suppressing and EMT promoting functions remain to be fully elucidated \cite{9, 69}. Notably, TGFβ has been shown to cooperate with extracellular matrix (ECM) and growth factor pathways to promote EMT, migration, invasion and metastasis of breast cancer cells \cite{54, 70–73}. In this regard, previous reports have demonstrated that specific ECM proteins which activate integrin beta 3 (ITGB3) shift TGFβ signaling away from the Smad2/3 pathway and toward the Src/TβRII/Grb2/MAPK pathway to promote EMT in normal and malignant epithelial cells \cite{70, 71}.

Biology and Mechanism of PEAK1 Kinase Function

Kinases play central roles in most cellular processes such as cell-cycle regulation, cell motility, tissue regeneration, differentiation and the development/progression of cancers. Therefore, understanding kinase functions and their mechanisms of action will illuminate new therapeutic strategies for combating cancer \cite{74–76}.

In order to examine, characterize and reveal the functions of known and novel kinases, the tyrosine phosphoproteome (pY) of subcellular pseudopodia in migrating cells was previously interrogated by immuno-precipitating pY proteins and identifying differentially enriched pY proteins in the pseudopodium via mass spectrometry-based proteomics \cite{12}. Pseudopodia (or invadopodia during cancer cell invasion) are highly specialized, actin- and signaling molecule-rich structures protruding from the cell’s body. Along with predicted molecular components of the pseudopodia, the initial findings revealed that PEAK1 kinase (Pseudopodium-Enriched Atypical Kinase, alternatively named KIAA2002 or SGK269) was tyrosine phosphorylated, enriched in the pseudopodium and associated with the actin cytoskeleton and BCAR1/Crk complex \cite{12}. As a pseudopodium-enriched kinase, it was...
further hypothesized that PEAK1 may promote cancer metastasis. Subsequently, a key role for PEAK1 was established in PDAC (an aggressive malignancy with no early detection biomarkers and few targeted therapies) – finding that PEAK1 was upregulated in metastatic lesions from patients and required for cell metastasis in a nu/nu orthotopic xenograft model. This body of work also highlighted the role of PEAK1 in therapy resistance – driving resistance to both gemcitabine (the common PDAC chemotherapeutic) and trastuzumab (Herceptin, Genentech) \[13\]. In agreement with our work, other phosphoproteomic screens have identified PEAK1 as a relevant kinase target in lung cancers and sarcomas \[77, 78\]. Additionally, other groups have reported that PEAK1 overexpression in non-malignant mammary epithelial cells induces EMT and can mediate EGF signaling by binding to Grb2/Shc1 complexes and activating Lyn kinase (a Src family member) \[79, 80\]. More recently, our collaborators reported a unique role for hypusination of the translation factor, eIF5A, in driving PEAK1 translation and PEAK1-dependent PDAC progression \[81\].

In an effort to identify a potential mechanism by which PEAK1 might drive EMT and metastasis in breast cancer, our group returned to the pseudopodial phosphoproteome \[12\]. Using Cytoscape, we generated a literature-based interactome in which PEAK1 (SGK269) is centrally located (Figure 1A). A closer look at the PEAK1-focused network revealed that Grb2 is the most common shared interactor between PEAK1 and the other subnetwork members (Figure 1B). We then evaluated the expression levels of these PEAK1/Grb2 co-interactors in breast cancer samples that express elevated levels of PEAK1 (Figure 1C) \[82\]. These data led us to previous work demonstrating that the ITGB3/Src/T\(\beta\)RII/Grb2/MAPK signaling cascade can mediate TGF\(\beta\)-induced EMT and metastasis \[49, 64, 69, 83\]. Subsequently, we have demonstrated that PEAK1 kinase mediates signaling cross talk between TGF\(\beta\) receptors and the ITGB3/Src/Grb2/MAPK pathway. In this regard, our results suggested that PEAK1 is essential for TGF\(\beta\)-induced ZEB1 upregulation, proliferation, migration, EMT and metastasis in breast cancer \[14, 15\]. These findings are summarized in the Figure 2 schematic.

**PEAK1 in the context of cancer biomarkers and targeted therapies**

Reliable biomarkers can be used to differentiate patient diagnosis, predict cancer prognosis and determine the best therapeutic interventions. Notably, our findings have two primary clinical implications. First, we propose that the identification of subsets of breast cancer in which PEAK1 levels are elevated may indicate good candidates for anti-TGF\(\beta\) therapy. Since TGF\(\beta\) can function as a tumor suppressor, it is critical to ensure that therapeutic inhibition of TGF\(\beta\) is not administered in a clinical context when it is eliciting anti-proliferative or pro-apoptotic effects \[8, 9\]. Second, we propose that direct or indirect targeting of PEAK1 may abrogate the pro-tumorigenic signaling functions of TGF\(\beta\). In this regard, and as referenced above, we were previously involved in a collaboration identifying the eukaryotic initiation factor 5A (eIF5A) as a novel regulator of PEAK1 translation. Notably, this work demonstrated that hypusination of eIF5A (a post-translational modification required for its activity) mediated increased PEAK1 protein levels and pathogenesis in pancreatic cancer \[81\], eIF5A inhibition via pharmacological targeting of the enzymes deoxyhypusine synthase (DHPS) and deoxyhypusine hydroxylase (DOHH) that are required for hypusination/activation of eIF5A has also been previously demonstrated to

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block progression of breast, pancreatic and hematologic malignancies [81, 84–88]. Thus, we suggest that pharmacological inhibition of eIF5A may be an important means toward blocking the pro-tumorigenic effects of TGFβ (Figure 3).

**Future Perspectives**

Despite the seminal discoveries that have been made over the past 30 years addressing the functions and mechanisms of action for TGFβ-induced signaling and EMT, significant hurdles remain toward which future research efforts must be directed [8, 9]. Our work to date and that of others suggests that future efforts should address the following two major challenges in the field: first, the identification and characterization of new non-transcriptional control mechanisms of TGFβ-induced EMT and/or cancer progression; and second, to qualitatively and quantitatively analyze important context-dependent spatiotemporal regulation of TGFβ response regulators. Importantly, these studies will shed light on the development of novel methods for modulating TGFβ and PEAK1 kinase signaling in order to improve cancer survival.

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Figure 1.
Panel A: Cytoscape-derived literature-based interactome of phosphotyrosine proteins and common interactors derived from the cell pseudopodium of migrating cells. Panel B: PEAK1-focused subnetwork from the larger interactome in panel A. Panel C: IHC data from the Human Protein Atlas.
Figure 2. PEAK1 can bind and facilitate the recruitment of Src kinase to integrins and TgRII/Grb2 complexes to promote non-canonical TGFβ-induced MAPK signaling in the presence of extracellular matrix proteins that activate ITGB3. Additionally, PEAK1 can promote Smad2/3 activation in the presence of fibronectin while potentiating TGFβ-induced Smad2/3 signaling in the absence of ECM protein. Finally, PEAK1 is required for ZEB1 upregulation downstream of TGFβ. The net result is that PEAK1 converts TGFβ signaling from an anti-proliferative growth factor to a pro-tumorigenic one, leading to EMT and metastasis.
Figure 3. Schematic of eIF5A hypusination and eIF5A-dependent PEAK1 translation and tumor progression

Briefly, deoxyhypusine synthase (DHPS) catalyzes the addition of spermidine to lysine 50 on eIF5a. Subsequently, deoxyhypusine hydroxylase (DOHH) hydroxylates the spermidine-modification. Once activated via this hypusination mechanism, eIF5A drives PEAK1 translation and tumor progression. Both GC7 and CPX have been reported to inhibit eIF5A hypusination/activation, PEAK1 translation and tumorigenesis.