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Transmembrane TNF-TNFR2 signaling as a critical immunoregulatory node in pancreatic cancer

Erin M. Dickey[a*], Anna Bianchi[a*], Haleh Amirian[a], Peter J. Hosein[b,c], Denise Faustman[d], Roberta Brambilla[e], and Jashodeep Datta[a,c]

[a]Division of Surgical Oncology, DeWitt Daughtry Department of Surgery, University of Miami Miller School of Medicine, Miami, FL, USA; [b]Department of Medicine, University of Miami Miller School of Medicine, Miami, FL, USA; [c]Sylvester Comprehensive Cancer Center, Miami, FL, USA; [d]Department of Medicine, Massachusetts General Hospital, Harvard Medical School, Boston, MA, USA; [e]The Miami Project to Cure Paralysis, Department of Neurological Surgery, University of Miami Miller School of Medicine, Miami, FL, USA

ABSTRACT

Pancreatic cancer is characterized by extreme therapeutic resistance. In pancreatic cancers harboring high-risk genomes, we describe that cancer cell-neutrophil signaling circuitry provokes neutrophil-derived transmembrane (tm)TNF-TNFR2 interactions that dictate inflammatory polarization in cancer-associated fibroblasts and T-cell dysfunction – two hallmarks of therapeutic resistance. Targeting tmTNF-TNFR2 signaling may sensitize pancreatic cancer to chemo±immunotherapy.

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Pancreatic ductal adenocarcinoma (PDAC) is a lethal malignancy and poses a significant therapeutic challenge due to intrinsic and acquired resistance to chemotherapy and/or immunotherapy. Emerging evidence implicates three dominant hallmarks of therapeutic resistance: (1) hostile genome, typified by cooperativity of oncogenic *KRAS* and *TP53* mutations[1]; (2) robust intratumoral trafficking of suppressive innate immune cells, particularly polymorphonuclear/neutrophilic myeloid-derived suppressor cells (PMN-MDSC) and macrophages; and (3) pro-inflammatory polarization of cancer-associated fibroblasts (CAF), which promote stromal inflammation via secreted factors (i.e., Cxcl1, IL-6) that sustain a myelo-enriched immune milieu. In our recent publication in *Cancer Discovery*, we connect the molecular "dots" between these distinct factors to identify a previously unrecognized cancer cell-PMN-MDSC signaling circuit that underlies the intersection between high-risk cancer genotypes, cell-autonomous transcriptional networks, suppressive immune contexts, and myeloid-CAF pro-inflammatory crosstalk to dictate therapeutic resistance in PDAC. Using *KRAS-TP53* genomic cooperativity as a model for high-risk biology, we identified Cxcl1 as a key cancer cell-intrinsic factor that not only governs the recruitment and suppressive behavior of PMN-MDSCs but also spatially constrains effector T-cells from their antitumor functions. Moreover, Cxcl1-CXCR2 engagement galvanized PMN-MDSCs into a dominant extracellular hub of tumor necrosis factor (TNF) signaling. Unexpectedly, PMN-MDSC-derived TNF – through a CXCR2-MAP kinase dependent mechanism – emerged as a central regulator of stromal inflammation and T-cell dysfunction via transmembrane TNF (tmTNF)-TNFR2 interactions. Proof-of-concept targeting of TNFR2 with etanercept (TNFR2-F_c decoy) disrupted this circuitry and improved sensitivity to chemotherapy *in vivo*, offering a novel strategy to improve outcomes in this deadly disease.[2] Taken together, our data provide a conceptual framework by which targeting context-dependent TNF signaling may overcome hallmarks of therapeutic resistance in PDAC (Figure 1).

TNF is primarily produced as a type II transmembrane protein arranged in stable homotrimers and can be cleaved into a soluble form (sTNF). sTNF and tmTNF mediate their biological activities predominantly through TNFR1 or TNFR2 receptor signaling, respectively, which have distinct downstream signaling due to subtle but meaningful differences in intracellular elements. TNFR1 induces death domain-mediated pro-apoptotic pathways, whereas TNFR2 has a TNF Receptor-Associated Factor (TRAF) binding site that interacts with signaling adaptors such as cIAP1/2 to generate proliferative and pro-survival signaling via activation of NF-κB, JNK, or STAT3/5 pathways.[3] While TNFR1 is expressed ubiquitously in various cell types (tumor cells, CAF, etc.), TNFR2 is constitutively expressed predominantly by immune and endothelial cells. Intriguingly, our data reveal that *cell-cell contact* between PMN-MDSCs and PDAC tumor cells or CAFs dynamically

CONTACT Jashodeep Datta jash.datta@med.miami.edu Division of Surgical Oncology , DeWitt Daughtry Department of Surgery, University of Miami Miller School of Medicine, Sylvester Comprehensive Cancer Center, 1120 NW 14th Street , Suite 410, Miami, FL 33136, USA
*contributed equally.

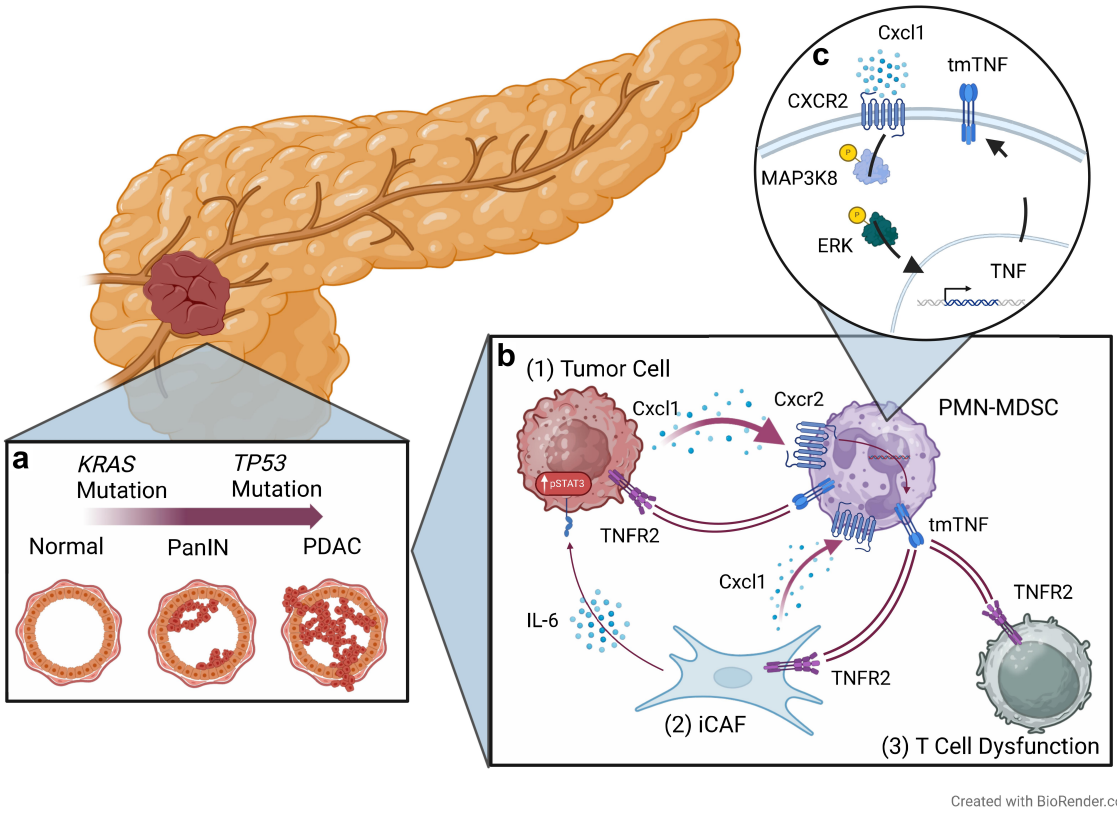

Figure 1. Transmembrane TNF-TNFR2 signaling as a Critical Immunoregulatory Node in pancreatic cancer. leveraging *KRAS-TP53* co-altered PDAC as a model for high-risk biology (*inset A*), our manuscript in *Cancer Discovery* reveals how unifying cancer cell-autonomous mechanisms, e.g., Creb activation, regulate pro-inflammatory chemokines such as Cxcl1 to dictate functional CXCR2^{+} neutrophilic myeloid-derived suppressor cell (PMN-MDSC) plasticity and enforce T-cell exclusion in the PDAC tumor microenvironment (*inset B*). Cxcl1-CXCR2 engagement regulates tumor necrosis factor (TNF) production via a MAP kinase-dependent mechanism in PMN-MDSCs (*inset C*). Via transmembrane (tm)TNF-TNFR2 interactions, MDSC-TNF instigates (a) feed-forward Cxcl1 production in *tumor cells*; (b) inflammatory *cancer-associated fibroblast* (CAF) polarization, which further drives chemoresistant IL6-STAT3 signaling in PDAC tumor cells; and (c) *T-cell* dysfunction. This signaling circuitry sustains an immunosuppressive myelo-enriched and T-cell excluded microenvironment to dictate therapeutic resistance.

induces TNFR2 expression in these cells. The ensuing PMN-MDSC-tmTNF and CAF-TNFR2 interactions amplify feed-forward CAF-IL-6 production and inflammatory CAF polarization, in turn inducing chemoresistant IL6-STAT3 signaling in tumor cells.[1] Our data uncover two other fascinating insights into TNF immunobiology: (1) PMN-MDSCs are the *dominant cellular source* of TNF in human and murine PDAC and (2) that PMN-MDSC-derived tmTNF-TNFR2 signaling has direct and indirect effects on T-cell dysfunction, leveraging findings from chronic inflammatory models where TNF has been implicated as a master regulator of T-cell exhaustion.[4] In this regard, while the majority of effects shown in our publication are antigen-*agnostic*, further investigation into whether PMN-MDSC-tmTNF mediates antigen-*restricted* CD8^{+} T-cell tolerance indirectly via TNFR2^{high} CD4^{+} T_{reg} interactions is warranted. Taken together, our data call for the development of thoughtful tmTNF-TNFR2 targeting strategies[5] to optimally unleash antitumor immunity and reprogram stromal inflammation in order to overcome therapeutic resistance in PDAC patients.

The diverse biology of TNF signaling is reflected in its often-paradoxical role in cancer initiation, maintenance, and progression. In parallel with studies of *soluble* TNF as anti-tumor therapy, evidence began to emerge that aspects of TNF-derived signaling could in fact be tumor-promoting.[6] Our findings add to this latter body of literature by conceptually challenging the dated model of exclusively antitumor TNF signaling in which global TNF silencing incapacitates T-cell-mediated immunologic control of tumors and illustrates a *context-dependent* influence of tmTNF-TNR2 signaling in mediating pro-tumorigenic immunosuppressive networks in PDAC.[7] To understand this signaling axis on a deeper mechanistic level, we are undertaking a comprehensive approach encompassing innovative preclinical modeling, pharmacologic TNFR2-selective targeting strategies, and a TNFR2 signaling-focused tissue and circulating biomarker portfolio that promises to unlock new dimensions in understanding the complexities of tmTNF-TNFR2 signaling. For example, we have developed PDAC tumor-bearing engineered murine models in which the endogenous TNF allele is replaced by knocking-in the Δ1–9;K11E TNF transgene, resulting in complete loss of TACE-mediated cleavage of tmTNF.[8] In a corollary approach to specifically dissect the immunoregulatory contributions of PMN-MDSC-*intrinsic* TNF, we have generated a neutrophil lineage-restricted TNF inactivation model by crossing knock-in *Tnf*$^{flox/flox}$ mice with mice bearing Cre-recombinase under control of MRP8/S100a8 PMN promoter. In early data reported at the 2023 AACR-Pancreatic Cancer meeting, we showed that orthotopically injected PDAC tumor cell-CAF spheroids underwent striking growth arrest in MRP8$^{Cre/+}$*Tnf*$^{flox/}$

^{flox} mice. In collaboration with the Brambilla group, we are generating diverse conditional TNFR2-ablation models to elucidate the specific contributions of CAF- and T-cell-restricted TNFR2 signaling to chemo±immunotherapy resistance. In addition, using imaging mass cytometry, we are investigating if spatially resolved ecosystems of TNF-TNFR2 expressing cellular dyads correlate with treatment outcomes and/or prognosis in PDAC patients. Finally, in collaboration with the Faustman group, we are testing if a highly selective anti-TNFR2 antibody TY-101[5] can overcome therapeutic resistance by remodeling the PDAC TME. Collectively, these efforts will provide robust and complementary insight into the precise cellular sources, spatiomechanistic details, and therapeutic vulnerabilities that underpin tmTNF-TNFR2 signaling crosstalk in PDAC.

The ultimate aspiration for these scientific efforts is to impact PDAC patients in the clinic. The lack of recognition of the aforementioned biologic nuances of context-specific tumor-*permissive* TNF signaling has undercut development of TNF-directed oncotherapeutics. To overcome this therapeutic impasse, we are developing an investigator-initiated phase 1b/2 clinical trial combining highly *selective* anti-TNFR2 antagonistic antibodies with standard-of-care chemotherapy in patients with treatment-naïve metastatic PDAC. The rationale for this trial is bolstered by two remarkable findings: (1) *upregulated* TNF signaling is the only tissue biomarker that predicted significantly worse survival in advanced PDAC patients treated with gemcitabine, *nab*-paclitaxel, and nivolumab chemoimmunotherapy in the PRINCE trial[9]; and (2) TNFR2 signaling is enriched in tumor transcriptomes of advanced PDAC patients enrolled in the COMPASS trial[10] demonstrating chemo-resistant compared with chemo-sensitive disease. Building on these data, and by leveraging our preclinical mechanistic insights, we will utilize our upcoming trial platform to develop a compendium of correlative studies to accelerate discovery of intratumoral and circulatory biomarkers of response and/or resistance. For instance, dynamics of circulating soluble TNFR2 ectodomain levels in plasma – evidence of overactive tmTNF-TNFR2 ligation – could serve as a predictive biomarker of response or provide an early window into disease progression. In a disease like PDAC, where one size does not fit all, insights gained from these upcoming scientific and clinical efforts promise to offer a personalized approach that optimally exploits the tmTNF-TNFR2 axis for therapeutic gain in select patients while facilitating informed decision-making in treatment selection and clinical trial design.

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ORCID

Jashodeep Datta http://orcid.org/0000-0003-2869-1571

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