# Silencing the Killers: Paracrine Immune Suppression in Pancreatic Cancer

Adrienne D. Cox[1,*] and Kenneth P. Olive[2]
[1]Departments of Radiation Oncology and Pharmacology, Lineberger Comprehensive Cancer Center, University of North Carolina at Chapel Hill, Chapel Hill, NC 27599, USA
[2]Departments of Medicine and Pathology, Herbert Irving Comprehensive Cancer Center, Columbia University Medical Center, New York, NY 10032, USA
*Correspondence: adrienne_cox@med.unc.edu
DOI 10.1016/j.ccr.2012.05.029

**Pancreatic cancers are characterized by high levels of inflammatory cells and profound immune suppression. In this issue of *Cancer Cell*, Bayne et al. and Pylayeva-Gupta et al. show that KRAS-driven, tumor cell-secreted GM-CSF recruits myeloid-derived suppressor cells to the stroma to abrogate tumor cell immune clearance by killer T lymphocytes.**

Soldiers in the army of immune surveillance may fight on the side of the host or may be be co-opted to fight on the side of the tumor. Host immune surveillance is thought to be important to limit both cancer development and cancer progression (Schreiber et al., 2011), whereas failure may be due to a countervailing local immunosuppression mediated by the tumor.

Pancreatic ductal adenocarcinomas (PDA or PDAC) are among the deadliest cancers, notable for their aggressiveness, profound immunosuppression, and remarkable degree of desmoplasia surrounding the nests of ductal epithelial cells (Clark et al., 2007). Tumor cells are encased in a high-pressure, fibrous stromal mass composed of a dense extracellular matrix and of fibroblasts, pancreatic stellate cells, endothelial cells, nerve cells, and large numbers of inflammatory cells, especially of immature myeloid lineages. This intricate stromal remodeling in PDAC is also distinguished by the conspicuous absence of T lymphocytes.

Inflammation leading to PDAC desmoplasia depends on paracrine signals produced by neoplastic epithelial cells, a process largely driven by oncogenic *KRAS*, which is mutated in essentially all human PDAC (Jones et al., 2008). Indeed, acute loss of mutant *KRAS* in established pancreatic tumors results in rapid quiescence and involution of pancreatic tumor stroma (Collins et al., 2012). Hedgehog signaling is known to promote fibroblast proliferation in pancreatic tumors, but the signals for other cell types have not been well established. Two new studies provide some answers to this critical question.

In this issue of *Cancer Cell*, Bayne et al. (2012) sought to determine which signals lead to the accumulation in PDAC of myeloid-derived suppressor cells (MDSCs), the immature myeloid cells that are characterized by $Gr1^{+}CD11b^{+}$ markers and are thought to play a key immunosuppressive role in this tumor type (Ostrand-Rosenberg and Sinha, 2009). Pylayeva-Gupta et al. (2012), also in this issue of *Cancer Cell*, asked which early changes in pancreata harboring oncogenically mutated *KRAS* drive initiation of the desmoplastic stromal response. Both groups of researchers have applied neutralizing antibodies and short hairpin (sh) RNAs to systematically test the requirements for candidates in PDAC stromal responses, using cell culture, mouse models, and human PDAC samples. Their investigations led to the identification of a paracrine circuit in PDAC, based on the pro-inflammatory cytokine GM-CSF secreted by tumor cells, that engages stromal myeloid cells to exert an immunosuppressive effect on local killer T cells (Figure 1) (Bayne et al., 2012; Pylayeva-Gupta et al., 2012). Several themes emerged from these investigations: (1) a key role for KRAS in driving the inflammatory tumor microenvironment, beginning early in pancreatic intraepithelial neoplasia (PanIN) development and continuing through frank carcinoma; (2) the critical and surprisingly specific importance of GM-CSF; (3) the dependence of both emerging and established PDAC on GM-CSF-responsive MDSCs recruited to the pancreatic stroma; and (4) the failure of CD8 cytotoxic T cell immunity unless either GM-CSF or MDSCs was disrupted.

An important strength of these complementary reports is their use of genetically engineered mouse strains that express the oncogenic $KRAS^{G12D}$ from the endogenous *KRAS* locus specifically in the pancreas (Hingorani et al., 2005). Pylayeva-Gupta et al. (2012) isolated primary pancreatic ductal epithelial cells (PDECs) from such mice and compared the secretion of cytokines before and after the expression of $KRAS^{G12D}$ in vitro. They also generated orthotopic allografts by injecting $KRAS^{G12D}$ or wild-type PDECs into the pancreata of syngeneic hosts. This model is particularly suitable for studying the early pancreatic lesions known as PanINs, and this group used it to interrogate how initial immune responses to *KRAS* activation enable nascent tumors to proliferate and survive. In contrast, Bayne et al. (2012) allowed tumor formation to occur spontaneously in the "KPC" mouse model, in which both $KRAS^{G12D}$ and the *p53* mutant $Tp53^{R172H}$ were expressed (Hingorani et al., 2005). This model faithfully recapitulates the pathophysiological characteristics of different stages of human PDAC. Bayne et al. (2012) used this model to identify inflammatory cytokines upregulated during tumor progression and to determine the origin of MDSCs and their importance in negative regulation of local T cell immunity in established PDAC tumors. Crucially,

both approaches maintain an intact immune system, without which these studies would not have been possible.

Which inflammatory cytokines are secreted in KRAS-driven PDAC? Surprisingly, only GM-CSF was consistently upregulated in tumor cells and tumors from both *KRAS*-driven models, but not in PDEC that lacked $KRAS^{G12D}$ or in normal pancreatic duct cells. GM-CSF was also upregulated in conditioned medium from KPC-derived PDAC and in human tumor samples, where its expression was detected by immunohistochemistry in the vast majority of PanINs and PDAC. Lineage marking of the pancreatic epithelial compartment in KPC mice demonstrated conclusively that cells of epithelial but not stromal origin elaborated GM-CSF (Bayne et al., 2012). Near-complete abrogation of GM-CSF mRNA upon pharmacological inhibition of MEK or PI3K in KRAS-PDEC demonstrated that the Ras/MAPK and PI3K effector pathways regulate GM-CSF in these cells at the level of transcription (Pylayeva-Gupta et al., 2012).

How do we know that KRAS-mediated escape from T cell immunity is important? First, no CD8 T cells were present in nascent orthotopic tumors established from $KRAS^{G12D}$-PDECs, whereas tumors established from $shKRAS^{G12D}$-PDECs displayed a CD8 cell infiltrate and underwent apoptosis at 2 weeks after implant (Pylayeva-Gupta et al., 2012). These results suggest that CD8 cytotoxic T cells can recognize and clear incipient PDAC tumors, but that KRAS signaling is able to overcome that clearance. The extensive secretion of GM-CSF suggested that this cytokine plays a central role: disrupting GM-CSF secretion or neutralizing its activity inhibited tumor growth and maintenance. Conversely, depleting CD8 could rescue tumor growth impaired by loss of GM-CSF.

What are the origins of myeloid-derived immune suppressor cells in PDAC? The growth of KPC-derived PDAC was dependent upon $Gr1^+CD11b^+$ cells, which showed hallmarks of MDSCs, namely increased levels of arginase and iNOS (Bayne et al., 2012). iNOS played an important role in suppressing antigen-specific proliferation of T cells. The MDSCs were derived from c-$kit^+$ splenic precursors (Bayne et al., 2012), which proliferated and differentiated in response to conditioned medium from PDAC cells or to GM-CSF. Their maturation from bone marrow-derived $Gr1^-CD11b^-$ cells, recruitment, and immune-suppressing ability required GM-CSF. Collectively, these results support the existence and importance of a GM-CSF-driven paracrine immune suppression circuit in PDAC.

Many complexities remain to be unraveled. Which subcategory of $Gr1^+CD11b^+$ MDSCs are these? How do MDSCs block CD8+ cell activity? What antigen(s) do the successful CD8+ killer cells recognize on pancreatic precursor lesions and carcinomas? What dictates the selective upregulation and importance of GM-CSF seen here? GM-CSF is sufficient to elicit CD8 suppression by $Gr1^+CD11b^+$ MDSCs in a non-PDAC context (Bronte et al., 1999), yet KRAS induction of other important growth factors and inflammatory cytokines such as VEGF, IL-6, and IL-1beta also can regulate these cells. In what context does the GM-CSF axis interact with other KRAS-driven inflammatory pathways such as STAT3/MMP7 or PI3K/STAT3/SOCS? Should GM-CSF be used for KRAS vaccines in PDAC (Abou-Alfa et al., 2011)? How can KRAS-driven GM-CSF be downregulated? Are transplant recipients with chronic pancreatitis or cancer patients on chemotherapy at greater risk for PDAC if they also receive GM-CSF?

The great Yogi Berra famously said, "When you come to a fork in the road, take it." The winning side of the war on pancreatic cancer may be determined in part by fork control: whether CD8 soldiers are battling for the host or are run off the battlefield by the tumor.

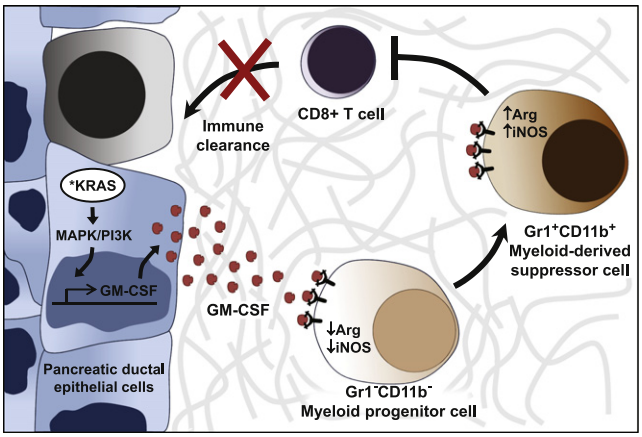


**Figure 1. Tumor Cell-Derived GM-CSF Drives Immune Suppression in Pancreatic Cancer**

Oncogenically activated KRAS (*KRAS) expressed in pancreatic ductal epithelial cells (PDECs) reprograms the tumor microenvironment by directing transcription of the inflammatory cytokine GM-CSF. Tumor-derived GM-CSF promotes recruitment of myeloid progenitor cells to the surrounding stroma and subsequent differentiation into myeloid-derived suppressor cells (MDSCs). MDSCs suppress the immune surveillance function of CD8+ killer T cells, preventing them from recognizing and clearing transformed PDECs. Arg, arginase; iNOS, inducible nitric oxide synthase. Both Arg and iNOS have been linked with immunosuppressive capabilities of MDSCs.

## REFERENCES

Abou-Alfa, G.K., Chapman, P.B., Feilchenfeldt, J., Brennan, M.F., Capanu, M., Gansukh, B., Jacobs, G., Levin, A., Neville, D., Kelsen, D.P., and O'Reilly, E.M. (2011). Am. J. Clin. Oncol. *34*, 321–325.

Bayne, L.J., Beatty, G.L., Jhala, N., Clark, C.E., Rhim, A.D., Stanger, B.Z., and Vonderheide, R.H. (2012). Cancer Cell *21*, this issue, 822–835.

Bronte, V., Chappell, D.B., Apolloni, E., Cabrelle, A., Wang, M., Hwu, P., and Restifo, N.P. (1999). J. Immunol. *162*, 5728–5737.

Clark, C.E., Hingorani, S.R., Mick, R., Combs, C., Tuveson, D.A., and Vonderheide, R.H. (2007). Cancer Res. *67*, 9518–9527.

Collins, M.A., Bednar, F., Zhang, Y., Brisset, J.C., Galbán, S., Galbán, C.J., Rakshit, S., Flannagan, K.S., Adsay, N.V., and Pasca di Magliano, M. (2012). J. Clin. Invest. *122*, 639–653.

Hingorani, S.R., Wang, L., Multani, A.S., Combs, C., Deramaudt, T.B., Hruban, R.H., Rustgi, A.K., Chang, S., and Tuveson, D.A. (2005). Cancer Cell *7*, 469–483.

Jones, S., Zhang, X., Parsons, D.W., Lin, J.C., Leary, R.J., Angenendt, P., Mankoo, P., Carter, H., Kamiyama, H., Jimeno, A., et al. (2008). Science *321*, 1801–1806.

Ostrand-Rosenberg, S., and Sinha, P. (2009). J. Immunol. *182*, 4499–4506.

Pylayeva-Gupta, Y., Lee, K.E., Hajdu, C.H., Miller, G., and Bar-Sagi, D. (2012). Cancer Cell *21*, this issue, 836–847.

Schreiber, R.D., Old, L.J., and Smyth, M.J. (2011). Science *331*, 1565–1570.