

# Macrophages in pancreatic cancer: Starting things off on the wrong track

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Chronic inflammation drives initiation and progression of many malignancies, including pancreatic cancer. In this issue, Liou et al. (2013. *J. Cell Biol.* <http://dx.doi.org/10.1083/jcb.201301001>) report that inflammatory macrophages are major players in the earliest stages of pancreatic cancer. They show that paracrine signals from the macrophages activate the nuclear factor  $\kappa$ B transcriptional program in normal pancreatic acinar cells, resulting in acinar–ductal metaplasia, a dedifferentiated state that is poised for oncogenic transformation.

Inflammation is fundamental to host defense, serving to eliminate pathogens and to heal damaged tissues. After tissue damage, macrophages act as sentinel cells that organize immune defenses and coordinate the tissue repair process via directing epithelial migration, angiogenesis, and matrix remodeling. This process is normally self-limiting because of a rapid production of antiinflammatory cytokines after the initial release of proinflammatory messengers. A failure of this resolution program leads to chronic inflammation characterized by an alteration in the immune cell types involved, including a marked increase in infiltrating macrophages (Medzhitov, 2008).

The pancreas is particularly prone to inflammatory injury, as the pancreatic acinar cells produce large amounts of proteolytic enzymes required for digestion. These enzymes can be prematurely activated in response to tissue damage, thereby causing cell lysis and further propagation of the injury. The link between inflammation and pancreatic ductal adenocarcinoma (PDA) pathogenesis is well established (Yadav and Lowenfels, 2013). For example, a greatly increased risk of developing PDA is observed in individuals with hereditary pancreatitis, a rare condition caused by germline mutations in the cationic trypsinogen gene (PRSS1), which results in autolysis of acinar cells and ongoing inflammation in the pancreas. More common cases of chronic pancreatitis arising from recurrent injuries to the pancreas as a result of smoking, alcohol abuse, unhealthy diet, or hereditary factors also correlate with an increased PDA risk.

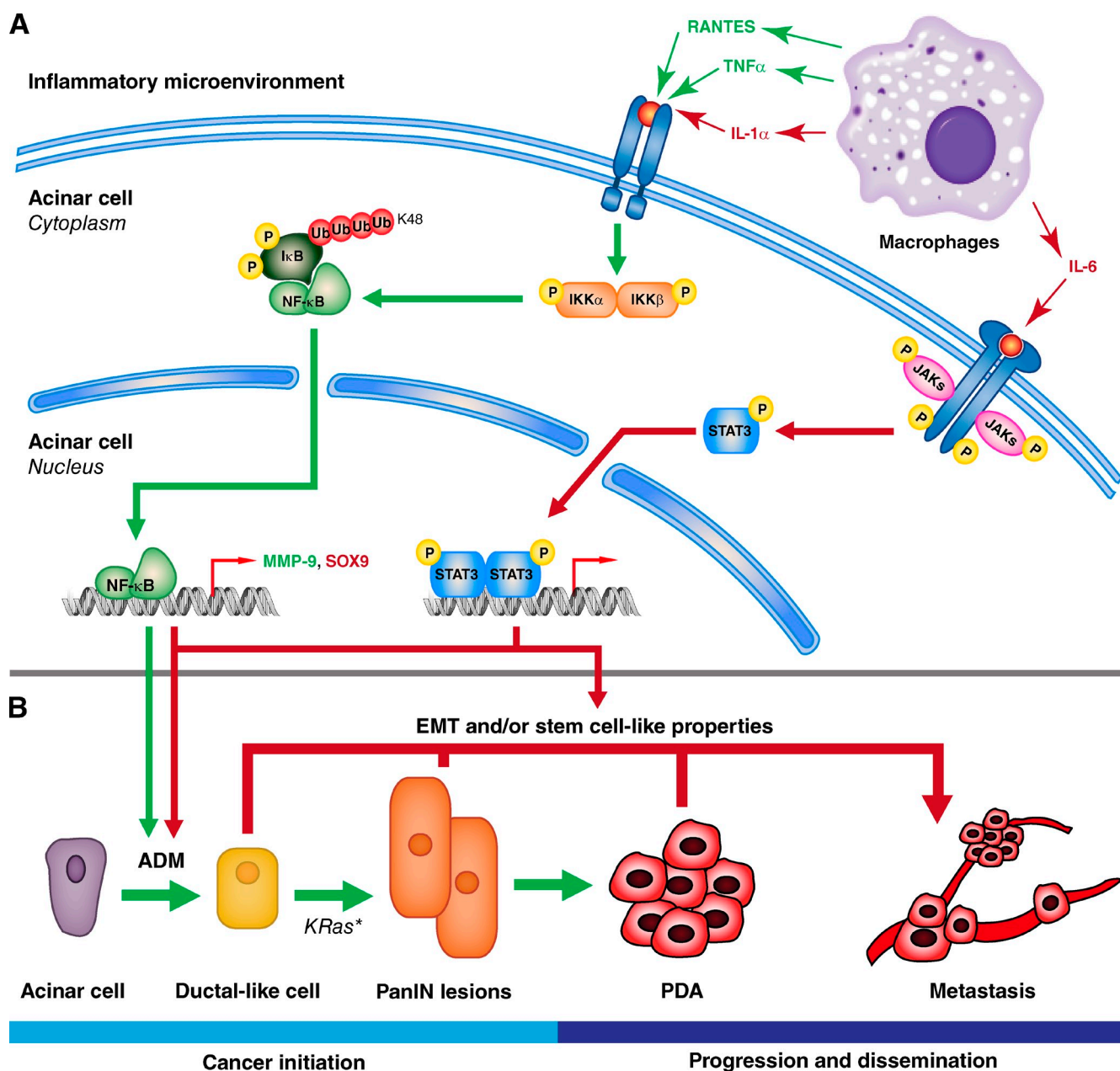
Experimental pancreatitis studies in genetically engineered mouse models provide further support for inflammation

as a driver of PDA, with particularly important contributions of this process to tumor initiation. The cholecystokinin analogue, caerulein, is used to induce inflammatory injury in these experiments. In genetically engineered mouse models of PDA harboring an activating K-ras mutation, the earliest known genetic alteration in the human disease, caerulein treatment abrogates oncogene-induced senescence. The bypass of this putative tumor-suppressor mechanism correlates with accelerated development of preinvasive pancreatic intraepithelial neoplasias (PanINs) and subsequently of PDA (Guerra et al., 2011). Other observations suggest that inflammation promotes acinar-to-ductal metaplasia (ADM), a process of dedifferentiation of acinar cells to ductal cells with progenitor-like characteristics, which is thought to be an early event in PDA progression, preceding PanIN formation (Fig. 1; Guerra et al., 2007; Fukuda et al., 2011; Kopp et al., 2012). Macrophage infiltration occurs early and dominates the inflammatory microenvironment of the earliest preinvasive lesions (Clark et al., 2007). Moreover, macrophage-produced interleukin-6 (IL-6) has been reported to activate the Janus kinase (JAK)–STAT3 pathway (Lesina et al., 2011), which has an established positive role in inducing ADM and contributing to PDA (Miyatsuka et al., 2006; Fukuda et al., 2011; Lesina et al., 2011). It is important to note that in addition to the impact of these proinflammatory macrophages on ADM and PDA, subsets of alternatively activated macrophages have a contrasting antitumor surveillance function in PDA (Beatty et al., 2011).

In this issue, Liou et al. confirm and extend findings regarding the role of the inflammatory context in promoting ADM and tumor initiation (Fig. 1). They observed that specific pharmacologic depletion of macrophages significantly limited formation of ADM in mice treated with the cholecystokinin analogue, caerulein, an inducer of pancreatitis. Macrophage-conditioned media also induced ADM of explanted pancreatic acinar cells, suggesting that these effects are mediated by secreted factors rather than by direct cell–cell interactions. The authors identified macrophage-derived RANTES and TNF as paracrine regulators of ADM that act via activation of the nuclear factor  $\kappa$ B (NF- $\kappa$ B)

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**Figure 1. Macrophages in pancreatic cancer initiation and progression.** (A) Molecular mechanisms proposed for macrophage-derived PDA initiation. Liou et al. (2013) show that macrophages secrete RANTES and TNF during pancreatitis, thereby activating the NF- $\kappa$ B pathway in acinar cells. The latter induces the expression of MMP-9 to promote ADM (green arrows). Other mediators that may contribute to macrophage-induced ADM include IL-1 $\alpha$ , the IL-6–STAT3 axis, and other NF- $\kappa$ B target genes, including SOX9 (red arrows; Miyatsuka et al., 2006; Fukuda et al., 2011; Lesina et al., 2011; Maniati et al., 2011; Kopp et al., 2012; Ling et al., 2012; Prévot et al., 2012; Sun et al., 2013). (B) Cellular evolution in PDA initiation and progression. In addition to the role of NF- $\kappa$ B in driving ADM and then PDA initiation (green arrows), it is possible that this pathway contributes to additional types of cellular reprogramming during PDA progression and metastasis (red arrows). Ub, ubiquitin; P, phosphorylation.

transcription factor in acinar cells, a pathway whose activation is a hallmark of pancreatitis and PDA and that is required for PDA progression in mouse models (Maniati et al., 2011; Daniluk et al., 2012; Ling et al., 2012). MMP-9 (matrix metalloproteinase-9) was found to be an NF- $\kappa$ B target gene required for ADM induction. Additional NF- $\kappa$ B target genes also clearly contribute to the process with a plausible candidate being SOX9, a critical mediator of K-ras-induced ADM (Fig. 1 A; Kopp et al., 2012; Prévot et al., 2012), which is activated by NF- $\kappa$ B in PDA cells (Sun et al., 2013).

It is likely that additional macrophage-derived secreted factors and downstream signaling programs, beyond RANTES/TNF-mediated NF- $\kappa$ B induction, are involved in inducing ADM because conditioned media from activated macrophages were more effective at inducing ADM than either cytokine and because NF- $\kappa$ B inhibition abolished ADM in RANTES/TNF-treated cells but was less effective in cells treated with macrophage-conditioned medium (Fig. 1 A). Macrophage-derived IL-6 and resulting STAT3 activation is a plausible additional mechanism for ADM induction as discussed earlier. Overall, it

appears that macrophages act as a signaling amplifier because there is evidence that autocrine signaling pathways can induce STAT3 and NF- $\kappa$ B in the tumor cells, via epithelial cell-derived IL-6 and IL-1 $\alpha$  (or TNF), respectively (Fukuda et al., 2011; Maniati et al., 2011; Ling et al., 2012).

ADM represents a developmental reprogramming of acinar cells to an undifferentiated state that is highly sensitized to malignant transformation as compared with differentiated acinar cells or ductal cells (Kopp et al., 2012). The identification of direct functions of macrophages in this process raises the question of whether these inflammatory cells have a more general role in reprogramming cell differentiation states in other cancer contexts. In this regard, inflammation, secretion of TNF, activation of NF- $\kappa$ B, and MMP expression have each been shown to mediate epithelial-mesenchymal transition (EMT; Li et al., 2012; Rhim et al., 2012; Chen et al., 2013) and thereby promote metastasis (Maier et al., 2010; Fukuda et al., 2011). Notably, EMT and epithelial cell dissemination occur at very early stages during PDA initiation, before the formation of an identifiable tumor (Rhim et al., 2012). The NF- $\kappa$ B pathway may also contribute to the growth of a subpopulation of cells with stem cell-like characteristics in PDA (Sun et al., 2013). The potential role for macrophages in these different reprogramming events is depicted in Fig. 1 B. The functions of the NF- $\kappa$ B pathway in promoting ADM, and perhaps EMT, reinforce the interest in the therapeutic targeting of this pathway in PDA. Such strategies could help in the development of preventive therapies for those at high risk for PDA, a group that includes individuals prone to chronic pancreatitis.

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## References

- Beatty, G.L., E.G. Chiorean, M.P. Fishman, B. Saboury, U.R. Teitelbaum, W. Sun, R.D. Huhn, W. Song, D. Li, L.L. Sharp, et al. 2011. CD40 agonists alter tumor stroma and show efficacy against pancreatic carcinoma in mice and humans. *Science*. 331:1612–1616. <http://dx.doi.org/10.1126/science.1198443>
- Chen, Q.K., K. Lee, D.C. Radisky, and C.M. Nelson. 2013. Extracellular matrix proteins regulate epithelial-mesenchymal transition in mammary epithelial cells. *Differentiation*. <http://dx.doi.org/10.1016/j.diff.2013.03.003>
- Clark, C.E., S.R. Hingorani, R. Mick, C. Combs, D.A. Tuveson, and R.H. Vonderheide. 2007. Dynamics of the immune reaction to pancreatic cancer from inception to invasion. *Cancer Res.* 67:9518–9527. <http://dx.doi.org/10.1158/0008-5472.CAN-07-0175>
- Daniluk, J., Y. Liu, D. Deng, J. Chu, H. Huang, S. Gaiser, Z. Cruz-Monserrate, H. Wang, B. Ji, and C.D. Logsdon. 2012. An NF- $\kappa$ B pathway-mediated positive feedback loop amplifies Ras activity to pathological levels in mice. *J. Clin. Invest.* 122:1519–1528. <http://dx.doi.org/10.1172/JCI59743>
- Fukuda, A., S.C. Wang, J.P. Morris IV, A.E. Folias, A. Liou, G.E. Kim, S. Akira, K.M. Boucher, M.A. Firpo, S.J. Mulvihill, and M. Hebrok. 2011. Stat3 and MMP7 contribute to pancreatic ductal adenocarcinoma initiation and progression. *Cancer Cell*. 19:441–455. <http://dx.doi.org/10.1016/j.ccr.2011.03.002>
- Guerra, C., A.J. Schuhmacher, M. Cañamero, P.J. Grippo, L. Verdager, L. Pérez-Gallego, P. Dubus, E.P. Sandgren, and M. Barbacid. 2007. Chronic pancreatitis is essential for induction of pancreatic ductal adenocarcinoma by K-Ras oncogenes in adult mice. *Cancer Cell*. 11:291–302. <http://dx.doi.org/10.1016/j.ccr.2007.01.012>
- Guerra, C., M. Collado, C. Navas, A.J. Schuhmacher, I. Hernández-Porras, M. Cañamero, M. Rodríguez-Justo, M. Serrano, and M. Barbacid. 2011. Pancreatitis-induced inflammation contributes to pancreatic cancer by inhibiting oncogene-induced senescence. *Cancer Cell*. 19:728–739. <http://dx.doi.org/10.1016/j.ccr.2011.05.011>
- Kopp, J.L., G. von Figura, E. Mayes, F.F. Liu, C.L. Dubois, J.P. Morris IV, F.C. Pan, H. Akiyama, C.V. Wright, K. Jensen, et al. 2012. Identification of Sox9-dependent acinar-to-ductal reprogramming as the principal mechanism for initiation of pancreatic ductal adenocarcinoma. *Cancer Cell*. 22:737–750. <http://dx.doi.org/10.1016/j.ccr.2012.10.025>
- Lesina, M., M.U. Kurkowski, K. Ludes, S. Rose-John, M. Treiber, G. Klöppel, A. Yoshimura, W. Reindl, B. Sipos, S. Akira, et al. 2011. Stat3/Socs3 activation by IL-6 transsignaling promotes progression of pancreatic intraepithelial neoplasia and development of pancreatic cancer. *Cancer Cell*. 19:456–469. <http://dx.doi.org/10.1016/j.ccr.2011.03.009>
- Li, C.W., W. Xia, L. Huo, S.O. Lim, Y. Wu, J.L. Hsu, C.H. Chao, H. Yamaguchi, N.K. Yang, Q. Ding, et al. 2012. Epithelial-mesenchymal transition induced by TNF- $\alpha$  requires NF- $\kappa$ B-mediated transcriptional upregulation of Twist1. *Cancer Res.* 72:1290–1300. <http://dx.doi.org/10.1158/0008-5472.CAN-11-3123>
- Ling, J., Y. Kang, R. Zhao, Q. Xia, D.F. Lee, Z. Chang, J. Li, B. Peng, J.B. Fleming, H. Wang, et al. 2012. KrasG12D-induced IKK2/ $\beta$ /NF- $\kappa$ B activation by IL-1 $\alpha$  and p62 feedforward loops is required for development of pancreatic ductal adenocarcinoma. *Cancer Cell*. 21:105–120. <http://dx.doi.org/10.1016/j.ccr.2011.12.006>
- Liou, G.-Y., H. Döppler, B. Necela, M. Krishna, H.C. Crawford, M. Raimondo, and P. Storz. 2013. Macrophage-secreted cytokines drive pancreatic acinar-to-ductal metaplasia through NF- $\kappa$ B and MMPs. *J. Cell Biol.* 202:563–577.
- Maier, H.J., U. Schmidt-Strassburger, M.A. Huber, E.M. Wiedemann, H. Beug, and T. Wirth. 2010. NF- $\kappa$ B promotes epithelial-mesenchymal transition, migration and invasion of pancreatic carcinoma cells. *Cancer Lett.* 295:214–228. <http://dx.doi.org/10.1016/j.canlet.2010.03.003>
- Maniati, E., M. Bossard, N. Cook, J.B. Candido, N. Emami-Shahri, S.A. Nedospasov, F.R. Balkwill, D.A. Tuveson, and T. Hagemann. 2011. Crosstalk between the canonical NF- $\kappa$ B and Notch signaling pathways inhibits Ppyr expression and promotes pancreatic cancer progression in mice. *J. Clin. Invest.* 121:4685–4699. <http://dx.doi.org/10.1172/JCI45797>
- Medzhitov, R. 2008. Origin and physiological roles of inflammation. *Nature*. 454:428–435. <http://dx.doi.org/10.1038/nature07201>
- Miyasaka, T., H. Kaneto, T. Shiraiwa, T.A. Matsuoaka, K. Yamamoto, K. Kato, Y. Nakamura, S. Akira, K. Takeda, Y. Kajimoto, et al. 2006. Persistent expression of PDX-1 in the pancreas causes acinar-to-ductal metaplasia through Stat3 activation. *Genes Dev.* 20:1435–1440. <http://dx.doi.org/10.1101/gad.1412806>
- Prévot, P.P., A. Simion, A. Grimont, M. Colletti, A. Khalaileh, G. Van den Steen, C. Sempoux, X. Xu, V. Roelants, J. Hald, et al. 2012. Role of the ductal transcription factors HNF6 and Sox9 in pancreatic acinar-to-ductal metaplasia. *Gut*. 61:1723–1732. <http://dx.doi.org/10.1136/gutjnl-2011-300266>
- Rhim, A.D., E.T. Mirek, N.M. Aiello, A. Maitra, J.M. Bailey, F. McAllister, M. Reichert, G.L. Beatty, A.K. Rustgi, R.H. Vonderheide, et al. 2012. EMT and dissemination precede pancreatic tumor formation. *Cell*. 148:349–361. <http://dx.doi.org/10.1016/j.cell.2011.11.025>
- Sun, L., L.A. Mathews, S.M. Cabarcas, X. Zhang, A. Yang, Y. Zhang, M.R. Young, K.D. Klarmann, J.R. Keller, and W.L. Farrar. 2013. Epigenetic regulation of SOX9 by the NF- $\kappa$ B signaling pathway in pancreatic cancer stem cells. *Stem Cells*. <http://dx.doi.org/10.1002/stem.1394>
- Yadav, D., and A.B. Lowenfels. 2013. The epidemiology of pancreatitis and pancreatic cancer. *Gastroenterology*. 144:1252–1261. <http://dx.doi.org/10.1053/j.gastro.2013.01.068>