The crosstalk between acinar cells with \textit{Kras} mutations and M1-polarized macrophages leads to initiation of pancreatic precancerous lesions

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Keywords: acinar-to-ductal metaplasia, inflammation, \textit{Kras}, macrophages, pancreas, pancreatic cancer, PanIN, pancreatitis

Recent studies on the processes that lead to the development of pancreatic cancer indicate that inflammatory macrophages have key functions in the initiation of pre-neoplastic lesions. Specifically, acquisition of an activating \textit{Kras} mutation in pancreatic acinar cells leads to upregulation of intercellular adhesion molecule-1 (ICAM-1), which serves as a chemoattractant for M1-polarized macrophages. M1 macrophages then contribute to acinar cell metaplasia and development of precancerous lesions through inflammatory cytokines and secreted proteases.

**Introduction**

Metaplasia of pancreatic epithelial cells leads to a dedifferentiated, progenitor-like cell type that can develop to pancreatic intraepithelial neoplastic lesions (PanINs) or other types of pancreatic lesions.\textsuperscript{1,2} In genetic animal models for pancreatic cancer the metaplasia of pancreatic acinar cells and further progression to PanINs can be induced by introduction of a \textit{Kras} mutation,\textsuperscript{3} which is also found in over 95% of all human pancreatic cancers. Although in the above mentioned animal models \textit{Kras} mutations are expressed in all acinar cells, the occurrence of areas of acinar-to-ductal metaplasia (ADM) and PanINs is focal, indicating additional non-genetic factors involved in their formation.

Presence of pancreatic cancer goes along with desmoplasia and pancreatic inflammation (pancreatitis), and inducers of inflammatory responses such as obesity and smoking have been identified as environmental risk factors.\textsuperscript{4} Moreover, a synergistic connection between oncogenic \textit{Kras} mutations and presence of chronic pancreatitis was demonstrated in mice to abrogate the senescence barrier in low-grade PanINs and to accelerate the development of pancreatic ductal adenocarcinoma (PDA).\textsuperscript{5} Similarly, a high-fat diet accelerates development of PDA in mice expressing mutant \textit{Kras} by causing pancreatic inflammation and macrophage infiltration.\textsuperscript{6}

While in acinar cells \textit{Kras}-initiated signaling pathways that drive development of pancreatic cancer are relatively well defined,\textsuperscript{7} the crosstalk with immune cells that contribute to this process remains unclear. Our recent study for the first time reveals a direct cooperative mechanism between oncogenic \textit{Kras} mutations and the inflammatory environment to drive the initiation of pancreatic cancer. We demonstrate that \textit{Kras}-expressing pancreatic acinar cells initiate the chemotraction of M1 macrophages to induce local inflammation. Once present in areas of ADM, M1 macrophages expedite \textit{Kras}-driven transdifferentiation and formation of PanINs by providing enzymes that mediate extracellular matrix (ECM) degradation and cytokines that further drive the ADM process.\textsuperscript{8}

**Macrophages are Needed for Initiation and Progression of Pancreatic Cancer**

Precursor lesions to PDA often are associated with focal pancreatitis. By treating \textit{p48\textsuperscript{cre};LSL-Kras\textsuperscript{G12D}} mice with the macrophage toxin Gadolinium (III) chloride we found that macrophages contribute to formation and progression of mutant \textit{Kras}-caused PanIN lesions.\textsuperscript{8} Similar depletion of macrophages in a mouse model for acute pancreatitis completely protected acinar cells from undergoing metaplasia,\textsuperscript{9} indicating that inflammation alone may be sufficient to initiate the transdifferentiation process. While in absence of oncogenic \textit{Kras} this is reversible, in presence of mutant \textit{Kras} it can accelerate formation of pancreatic cancer. Mechanistic insight of how inflammation may contribute to changes in the pancreas microenvironment was provided by demonstrating that macrophages secrete proteases that modulate the ECM and cytokines that can drive ADM. For example we show increased activity of matrix metalloproteinases (MMPs), mainly MMP-9, in regions of ADM and PanINs and also increased presence of the pro-inflammatory cytokine tumor necrosis factor (TNF). This, combined with \textit{Kras}-caused changes in gene expression in acinar cells, can expedite the formation of precancerous lesions.

Kras-expressing Acinar Cells Upregulate ICAM-1 to Facilitate Microinflammation

By analyzing human patient tissue samples, we found that pancreatic regions

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Submitted: 01/07/2015; Accepted: 01/10/2015
http://dx.doi.org/10.1080/2162402X.2015.1008794
of ADM express the ICAM-1. Immuno-
histochemical analysis of pancreata from
p48
cre; LSL-Kras
G12D mice indicated that
ICAM-1 expression is due to expression of
mutant Kras. Additionally, transfection of
oncogenic mutant Kras into normal acinar
cells led to dramatic increase in ICAM-1
mRNA and protein expression. Moreover,
ICAM-1 expressed by acinar cells was
processed to a soluble version (sICAM-1),
possibly functioning as chemoatractant
that can give rise to pancreatic intraepithelial neoplastic (PanIN) lesions, which then can further progress to
pancreatic cancer (B).

Figure 1. Crosstalk of macrophages with mutant Kras-expressing pancreatic acinar cells. Acquisition of an
activating Kras mutation (Kras*) in acinar cells leads to expression of ICAM-1. A fraction of the ICAM-1 pro-
duced is shed and soluble (sICAM-1). Soluble ICAM-1 can act as a chemoattractant for M1-polarized macro-
phages (A). Macrophages then may directly interact with acinar cells via membrane ICAM-1. They provide
enzymes that allow degradation of extracellular matrix (ECM) and inflammatory cytokines and chemokines
that can drive transdifferentiation signaling. This leads to metaplasia of acinar cells to a duct-like phenotype
that can give rise to pancreatic intraepithelial neoplastic (PanIN) lesions, which then can further progress to
pancreatic cancer (B).

Conclusions

Two key problems prevent therapeutic
efforts in pancreatic cancer. First, pancre-
atic cancer usually is diagnosed late, when
it often is already metastatic. Sec-
ond, once diagnosed PDA is difficult
to treat because of its desmoplastic and inflammatory
environment, which blocks drugs from reaching tumor cells.10 The
key to solve both issues is to develop methods for an early
diagnosis and treatment. Moreover, at a certain age PanINs are
present in almost every individual; and an additional difficulty is
to determine which PanINs will progress to PDA. The characteri-
ization of the signaling events that
drive formation and progression
of PanINs will allow such develop-
ment of novel methods for early diagnosis and for early treat-
ment. Moreover, it will allow to
reliably distinguish between indi-
viduals with high-grade pre-neo-
plastic lesions and individuals
where it is “safe” to continue
surveillance.

Experimentally-induced pancreatic inflammation enhances
Kras-driven PanIN expansion and development of PDA in
mice.5 Our data now indicate that acquisi-
tion of a Kras mutation can also initiate
microinflammation, which then leads to
formation of PanINs.8 Accompanying
increased levels of sICAM-1 released by
acinar cells or cytokines (e.g. TNF)
produced by attracted macrophages, now
could serve as markers for progression of
disease. Both can be detected in pancreatic
cystic fluid of pancreatitis and cancer
patients. Moreover, we show that the use
of neutralizing antibodies (e.g., ICAM-1
NAB) can reduce the progression of
Kras
-driven PanINs
in vivo, by decreasing macro-
phage infiltration into the pancreas.
Thus, we provide a proof-of-principle
experiment for the use of such blocking
antibodies for clinical use in humans.

Disclosure of Potential Conflicts of Interest
No potential conflicts of interest were
disclosed.

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