

pluripotency. Whether this reflects the developmental status of the ICM at the time of ESC derivation or differential signaling requirements for the maintenance of pluripotency (Roode et al., 2011) should be further explored. In the meantime, extrapolation from mouse pluripotent stem cell data to inform primate stem cell biology is likely to be inadequate. For this reason, it is important to encourage human stem cell biologists to avoid false expectations from other species such as the mouse. However, if primate pluripotent stem cells can be returned to the naive state of pluripotency, their unequivocal value may be

further enhanced, and mouse models may become more informative for human medicine.

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Understanding Metastasis in Pancreatic Cancer: A Call for New Clinical Approaches

David A. Tuveson^{1,2,*} and John P. Neoptolemos^{3,*}

¹Li Ka Shing Centre, Cancer Research UK Cambridge Research Institute, and the Cambridge Pancreatic Cancer Centre, Robinson Way, Cambridge CB2 0RE, UK

²The Lustgarten Foundation Pancreatic Cancer Laboratory at Cold Spring Harbor, 1 Bungtown Road, Cold Spring Harbor, NY 11724, USA

³Pancreas Biomedical Research Unit and the Liverpool Cancer Centre, National Institute for Health Research, Liverpool L69 3GA, UK

*Correspondence: david.tuveson@cancer.org.uk (D.A.T.), j.p.neoptolemos@liverpool.ac.uk (J.P.N.)

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Although metastasis is a major cause of morbidity and mortality in patients with pancreatic cancer, the requisite events are currently unknown. In this issue of *Cell*, Haeno et al. and Rhim et al. propose that metastasis occurs much earlier than previously anticipated, with clear implications for improving patient care.

Pancreatic cancer is the most lethal common malignancy, despite standardization of surgical techniques and advances in systemic treatments. Most pancreatic cancer patients present with inoperable disease and rapidly succumb from a devastating illness characterized by tumor spread and vital organ dysfunction, intractable pain, galloping cachexia, and coagulopathy. Surgical resection offers the only hope for cure in the ~20% of patients who qualify, yet few survive longer than 5 or 10 years, and the distinguishing features of this subgroup of patients are unknown. Systemic chemotherapy provides temporary benefits in

advanced disease, whereas it prolongs survival measurably in the adjuvant setting presumably by targeting microscopic foci of local and distant disease. Recent intriguing genomic analyses of pancreatic tumors proposed that the initial primary tumor proliferates for several years before producing metastatic clones (Yachida et al., 2010); however, patients with very small or clinically undetectable primary tumors still have a high risk of developing metastases. Therefore, understanding the mechanistic details and temporal pattern of pancreatic cancer metastasis is critical for designing effective interventions, as explored in this issue of *Cell* in

two articles by Rhim et al. (2012) and Haeno et al. (2012).

A traditional view of cancer metastasis seeks to identify the “seed and soil” factors that may promote this process. Along these lines, it is pertinent to consider the genetic rap sheet of the most common form of pancreatic cancer (pancreatic ductal adenocarcinoma or PDAC), as the four hallmark mutations of PDAC (*KRAS* [>90%], *p16^{INK4A}* [>90%], *TP53* [~70%], and *SMAD4* [55%]) have all previously been implicated in the metastatic process in human samples and genetically engineered mouse models. Indeed, oncogenic *KRAS* is known to

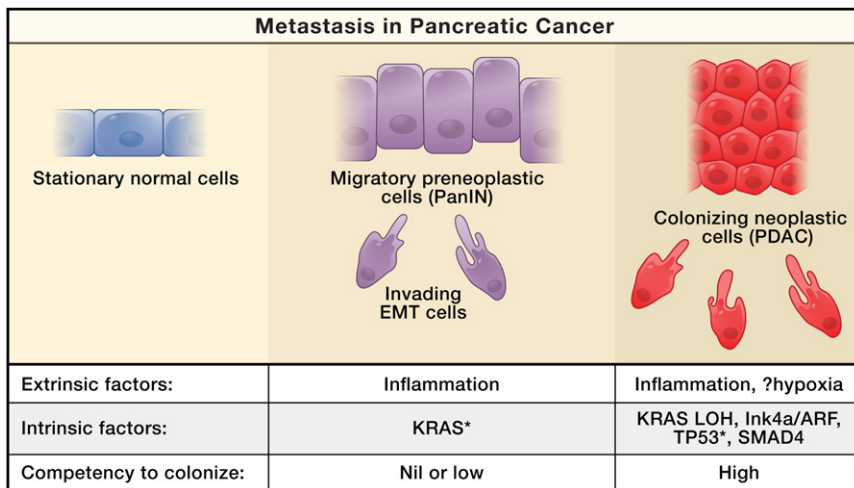


Figure 1. Metastasis in Pancreatic Cancer

Normal pancreatic ductal cells do not show invasion into stroma or extravasation, whereas PanIN cells and PDAC cells are capable of this behavior. The capacity to colonize effectively is restricted to transformed cells (PDAC).

stimulate cellular migration and permit survival in limiting nutrients, effects that may be accentuated when the wild-type *KRAS* allele is lost as observed in metastases (Qiu et al., 2011). Deletion of the *Ink4a/ARF* locus in *Kras* mutant pancreatic cells promotes Notch and NF- κ B signaling and metastasis in mouse models (reviewed in Mazur and Siveke, 2011); likewise, point mutant *Trp53* alleles possess neomorphic properties that promote tissue invasion and metastasis by stimulating integrin/EGFR signaling (Muller et al., 2009). Finally, *Smad4* loss promotes PDAC metastasis in mice (reviewed in Mazur and Siveke, 2011), and *SMAD4* loss correlates with high metastatic burden clinically (Iacobuzio-Donahue et al., 2009). The “soil” counterpart of pancreatic cancer has also been implicated whereby the tumor fibroblasts/activated pancreatic stellate cells comigrate with PDAC cells to promote metastasis in a transplantation model system (Xu et al., 2010). Also, the uniquely hypovascular nature of PDAC (reviewed in Mazur and Siveke, 2011) could conceivably promote hypoxia and hence metastatic behavior. Therefore, and without precluding the possibility of additional cooperating events (both genetic and non-), all PDAC cells are in principal destined to metastasize.

Metastasis describes a continuum beginning with the invasion by carcinoma

cells through the basement membrane into the surrounding stroma, followed by extravasation into the circulatory systems (blood, lymph), and finally by the reestablishment of colonies of neoplastic cells at distant sites. This process is not thought to be operant in preneoplasms—the common putative precursor lesions that share many histological and molecular features with advanced carcinomas excepting breach of the basement membrane. Indeed, preneoplasms are oftentimes termed preinvasive neoplasms for this reason, with pancreatic intraepithelial neoplasms (PanINs) denoting the most common premalignant precursors for PDAC. Contrary to this prevailing dogma, Rhim et al. report here that even low-grade PanINs that harbor only oncogenic *Kras* mutations show evidence of cells that have “delaminated” and migrated away from the glandular preneoplasm into the surrounding tissue and circulatory system. The authors used clever gene reporter approaches in several genetically engineered mouse models of PanIN to determine that the “preneoplastic” cells that had migrated into the stroma had the appearance of mesenchymal cells as opposed to the PanIN cells that exhibited an epithelial morphology. As such, they may represent an *in vivo* example of the long-sought “epithelial-to-mesenchymal transition” (EMT) cells that have been contentiously

debated in the biomedical community. These mesenchymal PanIN cells coexpressed proteins characteristically associated with EMT, including Zeb1, slug, and snail. Importantly, they could be independently identified in both murine and some human PanIN specimens by utilizing the transcription factor Pdx1 as a pancreatic epithelial lineage marker. The circulating PanIN cells also expressed the “cancer-initiating cell” surface proteins CD24 and CD44 (Li et al., 2007), reminiscent of the association between EMT and cellular plasticity proposed by Robert Weinberg and colleagues (Mani et al., 2008). Rhim et al. found that inflammation with the secretagogue cerulean or following pancreatic duct ligation increases the number of circulating pancreatic preneoplastic cells, reinforcing the link between inflammation and PDAC as previously demonstrated in familial chronic pancreatitis patients and mouse models (reviewed in Mazur and Siveke, 2011). Furthermore, treatment with anti-inflammatory agents decreases the number of circulating PanIN cells and decreases the abundance of PanINs in tissue. Interestingly, the circulating PanIN cells isolated from *Kras* mutant mice are not competent to form colonies in culture and thus are likely unable to establish proliferating metastases prior to acquiring mutations in additional genes such as *Trp53* or *p16^{Ink4a/Arf}*.

As *Kras* mutant PanIN cells in circulation may be incapable of distant site colonization, the critical issue becomes the timing of clinically relevant metastasis. Using a mathematical modeling approach with radiological and pathological data on pancreatic cancer patients who underwent autopsy, Haeno et al. propose that PDAC grows at an exponential rate, and that cells with high metastatic competency were generated during tumor expansion (on the order of 1 in a million PDAC cells). They then predict that even very small primary tumors have frequently undergone microscopic metastasis prior to surgical removal and propose that upfront systemic chemotherapy may provide an improved outcome for patients who present with such “early stage” disease. The autopsy series also revealed that a tiny subset of patients (14/101) died with only locally advanced disease, suggesting that there may be some patients

who lack factors to promote metastases, carry traits that suppress metastases, or have exaggerated responses to systemic therapies.

Both of these studies transform the way that we consider pancreatic cancer evolution and provide the opportunity to refocus and prioritize our efforts toward improving the outcomes of our patients (Figure 1). Indeed, investigational clinical trials that compare neoadjuvant cytotoxic and antimetastatic therapies to upfront surgery should be considered immediately for all patients. Also, patients at risk for developing pancreatic cancer could be considered for enrolment in studies that evaluate anti-inflammatory and anti-metastatic strategies, as the bartering process between seed and soil begins during the PanIN stage. Furthermore, in addition to comparing the molecular details between primary tumors and paired metastases, we now have a greater impetus to understand the genetic, epigenetic, and nongenetic factors that may explain our long-term survivors and those who never develop metastases. However, these studies are a stark reminder that

our current methods for initially detecting pancreatic cancer are insufficient and must change. Mouse pancreatic cancer models have served as a fruitful companion system for pancreatic cancer research, and they should continue to be exploited to accomplish these goals. Of course, the findings here may be generally applicable to other carcinomas, and lineage markers that attempt to distinguish epithelial cells from stromal fibroblasts could be useful to search for “EMT cells” in this regard. Finally, although these new findings in pancreatic cancer may evoke dismay, they actually offer a different perspective that we may wield against this silent killer, to its undoing.

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