excellent model for the type of work necessary to advance our understanding of liver biology for the development of regenerative medicine.

COMPETING FINANCIAL INTERESTS
The author declares no competing financial interests.

1. Si-Tayeb, K., Lemaigre, F. P. & Duncan, S. A. *Dev. Cell* **18,** 175–189 (2010).
2. Gebhardt, R. *Pharmacol. Ther.* **53,** 275–354 (1992).
3. Jungermann, K. & Kietzmann, T. *Hepatology* **31,** 255–260 (2000).
4. Burke, Z.D & Tosh, D. *Bioessays* **28,** 1072–1077 (2006).
5. Spear, B. T., Jin, L., Ramasamy, S & Dobierzewska, A. *Cell Mol. Life Sci.* **63,** 2922–2938 (2006).
6. Planaz-Paz, L. *et al. Nat. Cell Biol.* **18,** 467–479 (2016).
7. Carmon, K. S., Gong, X., Lin, Q., Thomas, A. & Liu, Q. *Proc. Natl. Acad. Sci. USA* **108,** 11452–11457 (2011).
8. de Lau, W. *et al. Nature* **476,** 293–297 (2011).
9. Glinka, A. *et al. EMBO Rep.* **12,** 1055–1061 (2011).
10. Hao, H. X. *et al. Nature* **485,** 195–200 (2012).
11. Koo, B. K. *et al. Nature* **488,** 665–669 (2012).
12. Sekine, S., Lan, B. Y., Bedolli, M., Feng, S. & Hebrok, M. *Hepatology* **43,** 817–825 (2006).
13. Gerbal-Chaloin, S. *et al. Mol. Pharmacol.* **86,** 624–634 (2014).
14. Wang, B., Zhao, L., Fish, M., Logan, C. Y. & Nusse, R. *Nature* **524,** 180–185 (2015).
15. Font-Burgada, J. *et al. Cell* **162,** 766–779 (2015).

Fibroblasts form a hospitable metastatic niche in the liver

Neta Erez

The liver is the most common metastatic route of pancreatic cancer. Early recruitment of granulin-secreting inflammatory monocytes to the liver is now shown to reprogram hepatic stellate cells into myofibroblasts that modulate the liver microenvironment to support the growth of metastasizing tumour cells.

Pancreatic ductal adenocarcinoma (PDAC) is one of the leading causes of cancer-related mortality and is one of the most devastating human malignancies. Despite improvements in surgical and cytotoxic therapy approaches during the past decades, pancreatic cancer continues to have a dismal prognosis, with an average overall five-year survival of <6%[1]. The main reason for the low survival rate of pancreatic cancer is its aggressiveness. In the majority of cases, local invasion and metastatic spread of pancreatic tumours are evident already at diagnosis. In other cases, metastatic relapse occurs after tumour resection. The most common metastatic destination of PDAC is the liver — more than 60% of patients whose tumours were resected relapse with hepatic metastasis within two years after surgery[2]. Metastatic disease is currently incurable and existing therapies can only prolong life to a certain extent. Therefore, a better understanding of the mechanisms that facilitate PDAC metastasis is the key to developing therapeutic approaches that may prevent metastatic relapse and improve survival.

Although multiple studies have characterized the microenvironment of PDAC tumours[3], the mechanisms that govern PDAC metastasis remain largely unexplored. In this issue, Nielsen *et al.*[4] elucidate the interactions between macrophages and fibroblasts that facilitate the formation of a hospitable metastatic microenvironment in the liver.

The authors analysed human liver metastases of PDAC, and observed that they are surrounded by stromal deposition of connective tissue, activated fibroblasts and recruited immune cells, most prominently macrophages. To uncover the mechanisms underlying these clinical features of liver metastasis, they performed *in vivo* experiments using a model of PDAC experimental metastasis. In this model, pancreatic cancer cells isolated from a genetically engineered mouse model of PDAC (*Kras*^{G12D};*Trp53*^{R172H};*Pdx1–Cre* mice[5]) are injected into the spleen of mice, and colonize the liver via the portal circulation. Using adoptive bone marrow transplantations that allow tracking of cell origin, the authors demonstrated that metastasis-associated macrophages (MAMs) are recruited to liver micrometastases from the bone marrow. This recruitment precedes the accumulation of activated resident fibroblasts (hepatic stellate cells), which were only evident in established metastatic lesions. Using a model of mice that are genetically deficient in macrophage trafficking, the authors found that inhibition of macrophage recruitment from the bone marrow impairs pancreatic cancer metastasis to the liver and inhibits fibroblast activation, reflected by their expression of α-SMA and enhanced collagen deposition. Moreover, when macrophages were chemically depleted using clodronate liposomes, activation of fibroblasts was inhibited and progression of metastatic lesions was impaired, even after the initial colonization of the metastatic site by cancer cells, thus providing temporal insight on the cascade of events during liver metastasis.

Mechanistically, Nielsen *et al.* showed that MAM-derived granulin activated hepatic stellate cells into myofibroblasts, in agreement with granulin's previously shown pro-fibrogenic activity[6,7]. The central role of granulin in mediating fibroblast activation was supported by their finding that mice deficient in granulin had a lower burden of liver metastasis and attenuated fibroblast activation following injection of pancreatic cancer cells. To dissect the molecular mechanism by which macrophages activate pro-fibrogenic activity in fibroblasts, the authors analysed the secretome of fibroblasts incubated with macrophage-conditioned medium. Analysis revealed significant changes in proteins associated with remodelling of the extracellular matrix (ECM), in particular periostin, previously shown to be upregulated in the metastatic stroma of multiple cancers[8,9]. Importantly, the authors also found that periostin was upregulated in human spontaneous hepatic metastases of PDAC, implying that it has a role in human metastatic disease. Functional experiments *in vitro* indicated that

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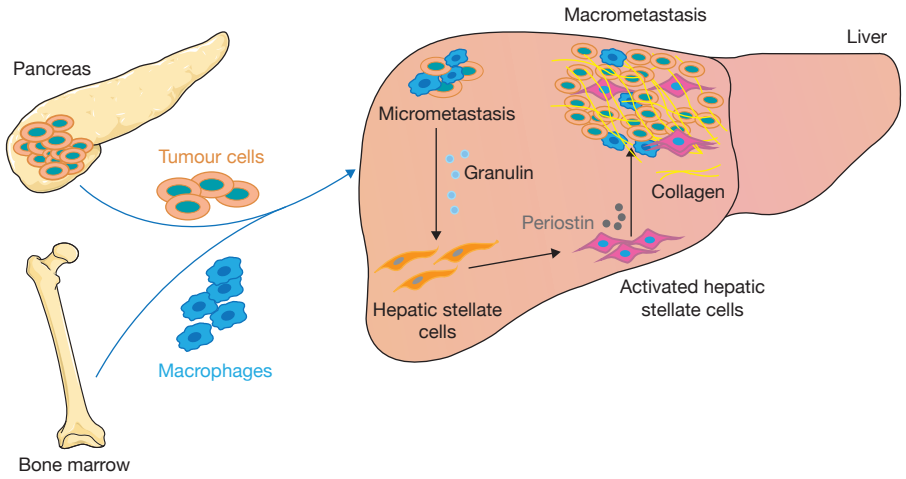

Figure 1 Metastasizing pancreatic cancer cells recruit monocytes from the bone marrow and colonize the liver to form micrometastases. Macrophages secrete granulin, which activates resident hepatic stellate cells to secrete periostin, thus promoting collagen deposition and facilitating metastatic growth.

periostin activity was required to promote pancreatic cancer cell survival and growth: secreted factors from hepatic stellate cells enhanced tumour cell growth, and inhibition of periostin by a neutralizing antibody attenuated these growth-promoting effects. Moreover, induction of periostin in fibroblasts was shown to depend on granulin: secreted factors from granulin$^{-/-}$ macrophages were unable to induce the expression of periostin in hepatic stellate cells. Finally, the authors performed a series of *in vivo* experiments that confirmed that fibroblast activation, enhanced collagen deposition and liver metastasis indeed depend on secretion of granulin from recruited MAMs. Interestingly, in PDAC patients as well as in mice with liver metastasis, granulin was upregulated in circulating inflammatory monocytes, suggesting that monocyte-derived granulin may have a role in the formation of a pre-metastatic niche.

The study by Nielsen *et al.* provides insights into the complex interactions between macrophages and resident fibroblasts in the liver. They dissect temporally and molecularly the sequence of events leading to fibroblast activation and ECM remodelling, which results in a permissive microenvironment and metastatic growth (Fig. 1).

The bidirectional interactions of cancer-associated fibroblasts with macrophages and other immune cells in the tumour microenvironment have been studied mostly in primary tumors[10]. This study offers a new and important dimension to the emerging landscape of the metastatic microenvironment.

Granulin secreted by bone-marrow-derived cells was previously shown to induce pro-fibrogenic activation of fibroblasts that instigated the growth of indolent tumours, suggesting that in the context of metastasis, activated fibroblasts may be operative in overcoming metastatic dormancy[6]. The current study expands these observations, identifies bone-marrow-derived macrophages as the major source of granulin, and suggests that fibroblast activation supports the metastatic growth of disseminated cancer cells. In light of these findings, it would be interesting to investigate whether signalling from the primary tumour or from circulating monocytes affects stromal cells in the liver. The study by Nielsen *et al.* is limited by the use of a model of experimental metastasis, which does not allow investigation of the pre-metastatic niche in the liver. Therefore, it remains to be investigated whether fibroblast activation and enhanced collagen deposition precedes the formation of liver metastases. Future experiments using models of spontaneous metastasis will better uncover the dynamic molecular interactions and the timing of macrophage arrival at the metastatic site during the earliest stages of liver colonization. Are macrophages recruited systemically by signalling from the primary tumour, as previously demonstrated[6], or by early-disseminated cells in the liver, or both?

Another question that remains open is the mechanism by which fibroblast-derived periostin supports metastatic growth. The *in vitro* data indicated that periostin facilitates tumour cell survival and growth, whereas *in vivo* instigation of periostin was associated with enhanced fibrosis. Future studies could further elucidate the mechanism and link these observations.

Enhanced collagen deposition and crosslinking were previously shown to support the formation of lung metastasis in breast cancer[11]. The current study expands these findings to other metastatic niches (in this case, the liver), and suggests that enhanced fibrosis may be a more general pro-metastatic mechanism.

Cancer-associated fibroblasts are well established as central players in all stages of tumorigenesis and tumour progression[12], but their role in facilitating metastasis is only beginning to be revealed. Resident fibroblasts operate as sentinels of tissue homeostasis and integrity. A major physiological role of fibroblasts is their ability to sense tissue damage and activate a wound-healing program, which includes enhanced collagen deposition[13]. The study by Nielsen *et al.* suggests that this physiological function is hijacked during the formation of liver metastasis to drive a fibrotic microenvironment that facilitates organ colonization. These findings implicate fibroblasts as an attractive therapeutic target: preventing activation of resident liver fibroblasts may result in inhibition of hepatic metastatic relapse. However, recent studies suggest that the outcomes of targeting cancer-associated fibroblasts may be pro- or anti-tumorigenic, depending on the targeted molecule in fibroblasts and the tissue context, and therefore should be performed with extreme caution[14,15]. Therefore, deepening our insight on the early molecular interactions during organ-specific metastatic growth will form the basis for better design of targeted therapeutic approaches that may inhibit early metastatic spread, or even prevent metastasis altogether.

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1. Ying, H. *et al. Genes Dev.* **30,** 355–385 (2016).
2. Sperti, C., Pasquali, C., Piccoli, A. & Pedrazzoli, S. *World J. Surg.* **21,** 195–200 (1997).
3. Feig, C. *et al. Clin. Cancer Res.* **18,** 4266–4276 (2012).
4. Nielsen, S.R. *et al. Nat. Cell Biol.* **18,** 549–560 (2016).
5. Hingorani, S. R. *et al. Cancer Cell* **7,** 469–483 (2005).
6. Elkabets, M. *et al. J. Clin. Invest.* **121,** 784–799 (2011).
7. He, Z., Ong, C. H., Halper, J. & Bateman, A. *Nat. Med.* **9,** 225–229 (2003).
8. Erez, N. *Nature* **500,** 37–38 (2013).
9. Malanchi, I. *et al. Nature* **481,** 85–89 (2012).
10. Servais, C. & Erez, N. *J. Pathol.* **229,** 198–207 (2013).
11. Cox, T. R. *et al. Cancer Res.* **73,** 1721–1732 (2013).
12. Ostman, A. & Augsten, M. *Curr. Opin. Genet. Dev.* **19,** 67–73 (2009).
13. Ohlund, D., Elyada, E. & Tuveson, D. *J. Experiment. Med.* **211,** 1503–1523 (2014).
14. Ozdemir, B. C. *et al. Cancer Cell* **25,** 719–734 (2014).
15. Wagner, E. F. *Nature* **530,** 42–43 (2016).