The return of Dr Jekyll in cancer metastasis

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Metastasis, the process whereby tumour cells disseminate and colonize distant organs, is the primary cause of cancer mortality. Diverse models have been proposed to explain how tumour cells acquire metastatic competency. Calon *et al* (2012) now provide insight into the molecular underpinnings of metastasis by describing a key stromal, non-cell autonomous role for Transforming Growth Factor-beta (TGF β) in promoting the initiation of colonization in otherwise TGF β -resistant colorectal cancer (CRC) cells.

Growing tumour cells are surrounded by stroma, a heterogeneous population of cells that includes fibroblasts, endothelial precursors and cells of the immune system (Sethi and Kang, 2011; Valastyan and Weinberg, 2011). This stroma engages in an active dialogue with the tumour cells to create a unique microenvironment that is conducive to the survival and progression of a growing tumour. In late stage tumours, productive metastases arise when the tumour cells leave the primary site to disseminate throughout the body and seed new secondary tumours in distant organs. How tumour cells leave behind their primary microenvironment to

establish and successfully colonize secondary sites that might harbour tumour-hostile environments has been the subject of extensive research and speculation. A recent study by Calon *et al* (2012) provides new insights into this long-standing question with the discovery that Transforming Growth Factor-beta (TGF β) produced by tumour cells critically promotes colorectal cancer (CRC) cell colonization through its actions on the stroma (Figure 1).

The secreted factor, TGF β has been called the ‘Dr Jekyll and Mr Hyde’ of cancer (Bierie and Moses, 2006) due to paradoxical function as both a tumour suppressor and a tumour promoter. For instance, TGF β inhibits the proliferation of epithelial cells, an activity that most tumour cells must learn to overcome during cancer progression. However, TGF β also promotes the metastatic phenotype by enhancing tumour cell migration and promoting epithelial-to-mesenchymal transition (EMT).

In human CRC, the majority of tumour cells display constitutive Wnt signalling, typically because of mutations in either the adenomas polyposis gene (APC) or β catenin.

Figure 1 TGF β acting on stromal cells in the primary tumour promotes metastasis. Colorectal cancer cells (CRCs) are frequently insensitive to TGF β as a result of mutations in pathway components, including the TGF β receptors (TBRs) and Smads. At the primary tumour site, CRCs that secrete high level of TGF β induce expression of IL11 in the cancer-associated fibroblasts (CAFs) found in the adjacent stroma. The CRCs then respond to the IL11 via GP130 and this promotes tumour colonization of secondary sites.

However, mutations in TGF β signalling pathway components, including the cell-surface receptors or the intracellular Smad mediator proteins, also play a prominent role, consistent with a tumour suppressive function of TGF β . Nevertheless, high levels of TGF β are found in CRC patients and correlates with poor clinical outcome (Tsushima *et al*, 2001). This raises the question of how TGF β might promote poor clinical outcome in cancers that have acquired insensitivity to TGF β . To explore this, Calon *et al* (2012) examined TGF β expression levels in a large cohort of CRC patients and noted a strong positive association between the level of TGF β expression and the risk of cancer recurrence. Indeed, TGF β expression level outperformed the American Joint Cancer Committee (AJCC) staging system in predictive power. Consistent with the frequent loss of TGF β pathway mediators in CRC, staining of tumour sections for active TGF β signalling showed much higher levels in stromal cells as compared to the epithelial CRC cells. Expression profiling for TGF β -responsive gene signatures (TBRS) using isolated stromal cell populations corroborated this observation, with high levels of TGF β signalling evident in all stromal cell types tested, including fibroblasts, endothelial and immune cells. However, analysis of *in vivo* gene expression patterns revealed that it was the TBRS associated with cancer-associated fibroblasts (CAFs) that was the main predictor of poor outcome after therapy.

To provide direct evidence of a connection between stromal TGF β signalling and disease progression, Calon *et al* (2012) first conducted an elegant series of *in vivo* experiments in mice, using several colorectal cell lines that have inactivated TGF β signalling. Subcutaneous injection of variants of these lines engineered to overexpress TGF β led to activation of TGF β signalling in adjacent stroma. Calon *et al* (2012) next turned their attention to examining whether stromal TGF β signalling might influence metastasis. Inoculation of the engineered CRC cells in the caecum or spleen enhanced the rate of metastasis to the lung and/or liver that was particularly pronounced within the first 24 h post inoculation and most notably was abolished by administration of LY2157299, a TGF β receptor-selective inhibitor. Similarly, liver metastasis arising through intra-splenic injection of colon cancer stem cells isolated from a patient with TGF β receptor mutations was abolished by systemic TGF β receptor-inhibitor treatment. Thus, high levels of TGF β act to enhance the colonization capability of CRC cells at the initial phase of metastasis.

In breast cancer cells, which retain an intact TGF β signalling pathway, a cell-autonomous role for secreted TGF β in mediating organ-specific metastatic colonization has been delineated (Kang *et al*, 2003; Massague, 2008; Padua *et al*, 2008). However, since the CRC cells employed by Calon *et al* (2012) were insensitive to TGF β , the authors focused on the question of how stromal TGF β signalling might confer a metastatic phenotype to the tumour cells. They went on to show that the IL11, which is secreted by TGF β -stimulated CAFs, acting through the GP130/STAT3 pathway in the CRC cells, was required for colonization, most likely by suppressing tumour cell apoptosis (Figure 1). This likely allows the tumour cells to survive in the relatively hostile metastatic environments they encounter on their way to distant sites. Indeed, CRC cells engineered to produce their own IL11 effectively colonized liver, lungs, distant lymph nodes and brain.

The process of metastasis is extremely inefficient, so how a cell might undergo the genetic and/or epigenetic changes required to leave the primary site and colonize a different organ with a distinct microenvironment is a critical question. Calon *et al* (2012) now provide new insights into the process by showing that TGF β signalling in CAFs directed by the tumour cells feeds back on the cancer cell in the primary site to fuel metastasis. These studies thus provide support for the notion that tumour cells acquire the necessary changes to adapt to a new environment while still residing in the primary tumour site. However, it is important to remember that the activity of TGF β on immune cells is also a potent mechanism that regulates the tumour microenvironment to promote cancer progression (Yang *et al*, 2010). Although, the TBRS in CAFs was shown to be the most relevant for recurrence rates, the finding that all stromal cell types displayed TGF β -induced changes raises additional questions for future investigations that explore the extent of cellular interplay in contributing to the tumour-promoting role of TGF β . Understanding the ongoing dialogue between tumour cells and their microenvironment continues to yield a rich resource of new interventional targets that limit not only primary tumour growth, but also metastasis, the primary cause of cancer death.

Conflict of interest

The authors declare that they have no conflict of interest.

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