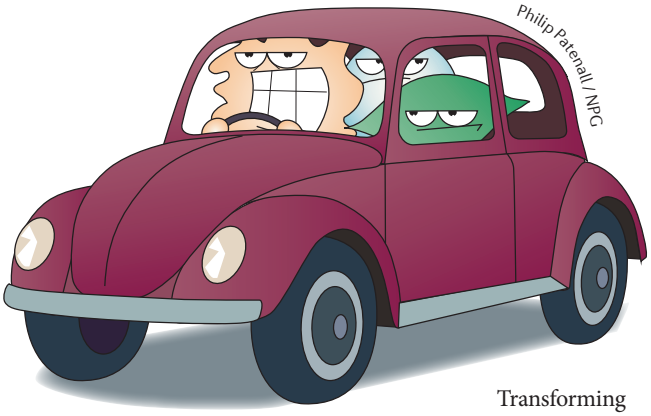
TUMOUR MICROENVIRONMENT

# Driving relapse




Colorectal cancer (CRC) can be stratified into molecular subtypes using various gene expression classifiers. Each of these classifiers identifies a poor-prognosis subgroup that is associated with mesenchymal cell markers. Two papers in *Nature Genetics* investigate the stromal involvement in poor-prognosis CRC.

Isella *et al.* used the classifiers to stratify 450 CRC samples and identified 3 major subgroups. The 'stem/serrated/mesenchymal' (SSM) subgroup was associated with genes expressed by mesenchymal cells. It is unclear whether these genes are expressed by CRC epithelial cells undergoing epithelial–mesenchymal transition (EMT) or by stromal cells within CRCs. SSM samples were composed of 54% tumour cells on average, which was significantly lower than non-SSM samples, indicating that mesenchymal genes were expressed by the stroma. Another gene expression data set revealed that SSM genes were upregulated particularly in cancer-associated fibroblasts (CAFs), as well as in leukocytes and endothelial cells.

To further investigate the contribution of the stroma, Isella *et al.* analysed the gene expression profiles of CRC patient-derived xenograft (PDX) models. They found that of the 270 SSM human genes expressed in the tumour samples, 199 of these genes were no longer detected in the corresponding PDX models. Tandem profiling of mouse and human gene expression in nine PDX models (from three CRC samples) revealed that the human genes that showed reduced expression were overexpressed in the mouse gene expression profiles. This indicates that the mouse stromal cells infiltrate the human PDX tumours. The authors confirmed that stromal cells expressed SSM genes using immunohistochemistry. In particular, ZEB1, MAP1B and TAGLN were all expressed in stromal cells and not in tumour epithelial cells. Finally, the authors generated gene expression signatures associated with CAFs, leukocytes and endothelial cells, and found that CRCs with a high CAF score had reduced disease-free survival.

In the second paper, Calon *et al.* analysed the CRC classifiers to identify genes that were significantly associated with reduced disease-free survival. To clarify the origin of these genes, they analysed the gene expression profiles of microdissected CRC samples and found that poor-prognosis genes were significantly upregulated in the tumour stroma (especially CAFs) compared with epithelial tumour cells. They also showed that poor-prognosis CRC subtypes are characterized by the increased expression of CAF-associated genes, and that 'good-prognosis' CRCs with a high risk of recurrence could be identified using these genes. Focusing on individual genes, the expression of three upregulated CAF markers (CALD1, FAP and IGFBP7) were independent prognostic factors that could be used to identify poor-prognosis CRCs.

Transforming growth factor-β (TGFβ) signalling is associated with poor prognosis in CRC, and the authors found that expression of *TGFB1* and *TGFB2* correlated with the poor-prognosis gene set. The expression of these genes, as well as CALD1, FAP and IGFBP7, was increased by treatment of normal colon fibroblasts with TGFβ. Co-implantation of HT29-M6$^{TGFβ}$ human CRC cells (which are engineered to be unresponsive to TGFβ and to secrete active TGFβ) with fibroblasts significantly increased the frequency of tumour-initiating cells by 200-fold compared with the parental line, although growth rates were unaffected. Tumour-initiating cells are thought to be responsible for relapse and metastasis, so this may explain the association of the stromal genes with poor prognosis. Furthermore, metastasis of cells from two CRC patient-derived organoids was inhibited by the TGFβR1 inhibitor LY2157299, which affected the stromal cells, as the tumour cells were insensitive to the effects of LY2157299.

Therefore, these two papers show the importance of the CRC tumour microenvironment in promoting relapse and metastasis.

*Gemma K. Alderton*

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**ORIGINAL RESEARCH PAPERS** Isella, C. *et al.* Stomal contribution to the colorectal cancer transcriptome. *Nature Genet.* http://dx.doi.org/10.1038/ng.3224 (2015) | Calon, A. *et al.* Stromal gene expression defines poor-prognosis subtypes in colorectal cancer. *Nature Genet.* http://dx.doi.org/10.1038/ng.3225 (2015)