BREAST CANCER

Getting to know the neighbours

The behaviour of breast cancer cells is strongly influenced by stromal and other neighbouring cells. However, the details of how different components of the surrounding tissue contribute to this are not well understood. A recent *Cancer Cell* paper provides evidence that all cell types in mammary tissue undergo changes in gene expression during cancer progression, indicating that each of these components contributes to the development of breast cancer.

Kornelia Polyak and colleagues studied gene expression in cells from normal breast tissue, ductal carcinoma *in situ* (DCIS; a pre-invasive tumour type) and invasive breast carcinomas. They first separated the various cellular components — including epithelial cells, myofibroblasts and myoepithelial cells, and stromal cells — using magnetic beads bound to antibodies against cell-type-specific markers.

The authors then used the serial analysis of gene expression (SAGE) method to obtain transcriptional profiles for the separated pools of cells. For each population, a set of genes was defined that was expressed at a higher level than in any of the other cell types. Based on this data, a clustering algorithm was then used to determine how related the transcription profiles were between cells of the same type from different stages in tumour progression. This showed that for each cell type there was a progressive change in gene expression from normal tissue, through DCIS and finally to invasive carcinoma. This provides strong evidence that all the cell types in breast tissue — not just the epithelial cancer cells themselves — undergo molecular changes during tumour progression.

Many of the genes upregulated in the stromal and other neighbouring cell types in DCIS and invasive carcinomas were found to encode secreted molecules and receptor proteins. These included several proteases and protease inhibitors, consistent with the generally accepted role of these cell types in contributing to tumour progression mainly through processes such as matrix remodelling that promote migration and invasiveness. However, in support of recent studies indicating that these cells are also involved in signalling to cancer cells, a significant proportion of the upregulated genes encoded proteins with signalling functions, such as chemokines, interleukins and growth-factor receptors.

In light of this, the authors looked in more detail at the role of two signalling molecules — the chemokines CXCL12 and CXCL14 — that were upregulated in cancer tissue in myofibroblasts and myoepithelial cells, respectively. Both

HYPOXIA

Structural disruption

Effective disruption of the pathway that allows cancer cells to survive under hypoxic conditions for use as anticancer therapy has long been a goal of cancer researchers. Andrew L. Kung *et al.* have now discovered a small molecule that disrupts the structure of a key coactivator in the hypoxia-inducible factor (HIF) pathway and inhibits tumour growth *in vivo*.

Activation of the HIF1 pathway is linked with resistance of tumours to therapy, increased invasion and metastasis, and poor outcome. As oxygen concentrations decrease, HIF1α accumulates and dimerizes with HIF1β, and the coactivator p300/CREB binding protein (CBP) is recruited. This complex then binds to the hypoxia-response element to trigger transcription of genes that facilitate adaptive mechanisms.

Kung and colleagues developed a high-throughput screen to search for small molecules that could inhibit the crucial interaction of HIF1 with p300. They immobilized the 41-amino-acid polypeptide p300/CBP-binding domain of HIF1α (TADC) on multiwell plates and tested a library of over 600,000 compounds for the ability to disrupt binding of the 121-amino-acid HIF1α-binding domain of p300 (CH1) to the plates. After confirmatory *in vitro* and cell-based assays, a single specific inhibitor — chetomin — was found. In a luciferase reporter assay, the authors showed that while chetomin did not interfere with p300-dependent transcriptional activity of factors that bind to most domains in p300, it did prevent the activity of factors that bind specifically to the CH1 domain. NMR spectroscopy revealed that CH1 becomes less structured in the presence of chetomin, indicating that this is what prevents interaction with HIF1α.

So, what happens under hypoxic conditions *in vivo*? When chetomin was administered to mice bearing tumour xenografts, levels of vascular endothelial growth factor, which is induced by hypoxia, were attenuated in a dose-dependent manner. Serum levels of erythropoietin, a marker of physiological HIF1 function, were also decreased. To directly determine the effect of chetomin on the HIF1 pathway, the authors placed luciferase under the control of the erythropoietin enhancer — under hypoxic conditions the reporter activity increased more than 100-fold. On injection of chetomin, but not vehicle control, into mice bearing the hypoxia-reporter cell line in the right flank and a constitutively expressed luciferase cell line in the left flank, reporter activity in the right flank only was reduced, by about 50%. These results also indicate that chetomin specifically disrupts the TADC–CH1 protein–protein interaction within tumours.

Chetomin also significantly reduced colon and prostate tumour xenograft growth and led to substantial necrosis in the tumours. Local toxicity at the injection sites was seen, but the cause of this is unknown. Disruption of the tertiary structure of CH1 domain of p300 specifically inhibits binding to HIF1 and signalling through the HIF1 pathway, indicating a novel small-molecule approach of interfering with cancer growth.

Ezzie Hutchinson

References and links

ORIGINAL RESEARCH PAPER Kung, A. L. *et al*. Small molecule blockade of transcriptional coactivation of the hypoxia-inducible factor pathway. *Cancer Cell* **6**, 33–43 (2004)

FURTHER READING Semenza, G. L. Targeting HIF-1 for cancer therapy. *Nature Rev. Cancer* **3**, 721–732 (2003)

WEB SITE

Andrew Kung's lab: http://www.dana-farber.org/abo/danafarber/detail.asp?PersonID=122&RD=Tr

of these chemokines stimulate the growth, migration and invasiveness of breast cancer cells *in vitro*. Polyak and colleagues found that the receptors for both chemokines were expressed in epithelial cells at higher levels in invasive tumours than in DCIS or normal tissue. In addition, they showed that epithelial cells adjacent to the myoepithelial layer show an increased rate of proliferation *in vivo*. So, myoepithelial cells and myofibroblasts provide paracrine signals that might be important for several stages of tumorigenesis.

Further investigation of the genes that are differentially expressed in cell types surrounding cancer cells should provide more clues to the molecular changes that drive breast cancer development. Identifying these changes might also provide opportunities to develop therapeutic strategies that target stromal and mesenchymal cells, as well as cancer cells themselves.

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References and links

ORIGINAL RESEARCH PAPER Allinen, M. *et al*. Molecular characterization of the tumor microenvironment in breast cancer. *Cancer Cell* **6**, 17–32 (2004)

FURTHER READING Bissell, M. J. & Radisky, D. Putting tumours in context. *Nature Rev. Cancer* **1**, 46–54 (2001)

WEB SITE

Kornelia Polyak's lab:
http://research.dfci.harvard.edu/polyaklab

SIGNAL TRANSDUCTION

Time for a 'signal-o-some'?

Many techniques have been developed to determine expression profiles of cancer cells, but these cannot tell us which signalling pathways are actually active. Garry Nolan and colleagues have therefore developed a way to measure activation of phosphoprotein-driven signalling, correlating the activity of different networks with response of acute myeloid leukaemia (AML) cells to therapy.

The STAT and RAS–MAPK signalling pathways are commonly activated in AML precursor cells. However, there is not a strong association between activating mutations in signalling proteins, increased phosphorylation levels of their targets, and patient prognosis. As the levels of the signalling proteins themselves cannot be used to determine if a certain pathway is active in an AML cell, Nolan and colleagues attempted to quantify the phosphorylation status, and therefore the activation level, of members of these signalling pathways in cells from different patients. By labelling AML blast cells from 30 patients with antibodies against phosphorylated forms of STAT1, STAT3, STAT5, STAT6, p38 and ERK1/ERK2, they were able to determine the basal activation level of these different proteins in different cell types, as well as their response to exposure to various cytokines, using multiparameter flow cytometry.

The authors observed that although the responses of normal lymphocytes to cytokine stimulation did not differ between donors, there were great variations in activation of different signalling molecules among the AML patient samples. For example, although some patients' cells responded to treatment of the cytokines GM-CSF and G-CSF by phosphorylation of STAT5, others did not. All patients but one underwent phosphorylation of STAT1 following interferon-γ (IFNγ) treatment. In total, the authors report that 7 of the 30 cytokine response states measured displayed significant variation across AML patient samples. Importantly, even the basal phosphorylation status of the signalling proteins varied significantly between patient samples.

So can the signal-transduction pathways that are active in a cancer cell determine its response to therapy? Using unsupervised clustering based on 'signalling profiles', Nolan and colleagues identified four main groups of patients, determined by basal phosphorlylation levels of the signalling proteins and the ability by these proteins to become activated in response to cytokine exposure. The authors found that resistance to one course of chemotherapy was indeed correlated with a specific signalling profile — cells that had high levels of STAT3 and STAT5 phosphorylation in the absence of STAT1 phosphorylation, following IFNγ exposure. These cells were characterized by the ability to respond to one or more upstream cytokine activators. The authors also correlated specific signalling profiles with certain genetic features, such as mutations in *FLT3* or chromosome translocations.

Nolan's group proposes that signalling profiles can not only be used to gain better insight into mechanisms of malignant progression, but also in diagnosis and prognosis. They are also trying to link these signalling pathways with apoptotic responses that might underlie drug response.

Kristine Novak

References and links

ORIGINAL RESEARCH PAPER Irish, J. M. *et al.* Single cell profiling of potentiated phospho-protein networks in cancer cells. *Cell* **118**, 217–228 (2004)