



# Functional Genomics of Ovarian Cancer

[www.cambridgecancer.org.uk/jamesbrenton](http://www.cambridgecancer.org.uk/jamesbrenton)

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Our laboratory focuses on discovering improved treatments for epithelial ovarian cancer. Ovarian cancer has a high healthcare burden because of low cure rates and frequent recurrent disease that causes significant symptoms for patients. This is despite the fact that ovarian cancer is initially sensitive to systemic treatments and most patients are free of disease after completing initial surgery and chemotherapy. The fundamental problem we are addressing is to understand how ovarian cancer cells escape initial treatment and the molecular mechanisms by which they acquire resistance to further therapy. Using genomic and functional studies, we are identifying new biomarkers and treatment targets for testing in clinical trials.

### Genomic studies of chemotherapy response *in vivo*

To identify genes that are selected for drug resistance we are carrying out prospective clinical studies that collect cancer samples before and during neoadjuvant treatment. Our initial studies have focused on the drugs carboplatin and paclitaxel as these are the most important therapies in ovarian cancer. By using expression analysis and bioinformatics methods developed to model the acquisition of resistance, we have

identified clinically relevant biomarkers that overlap with independently identified genes from RNA interference screens (Swanton et al., *Cancer Cell* 2007; 11:498).

Our studies depend upon having homogeneous patient cohorts with similar clinical characteristics. However, response to treatment in tumour masses can be heterogeneous and a mixed response frequently occurs at different anatomical sites. For example, primary ovarian masses may respond better than peritoneal metastases. This differential response may be a result of variable blood supply and hypoxia that limits the delivery and efficacy of chemotherapy.

We have confirmed these observations using functional magnetic resonance imaging for perfusion (Figure 1) and diffusion and are now using imaging data to target the collection of tissues from responding and non-responding areas. This will allow us to calibrate genomic profiles much more precisely and to better identify the molecular determinants of resistance. High-throughput sequencing with Illumina technologies is being used to quantify expression changes and to identify novel fusion transcripts and mutations (Figure 2).

### Mechanisms of taxane resistance and the role of the extracellular matrix

Taxanes, such as paclitaxel, interfere with the dynamic growth of microtubules by directly binding to them, leading to mitotic

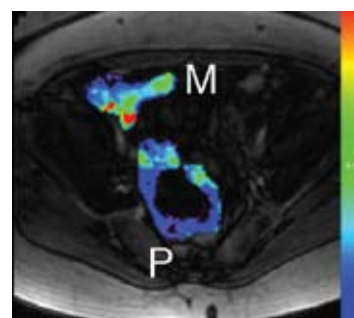


Figure 1. Dynamic contrast enhanced MRI showing  $K_{trans}$  maps from primary (P) ovarian cancer and peritoneal metastasis (M). There is differential perfusion within tumour masses with highest (red) values seen in the metastatic disease.

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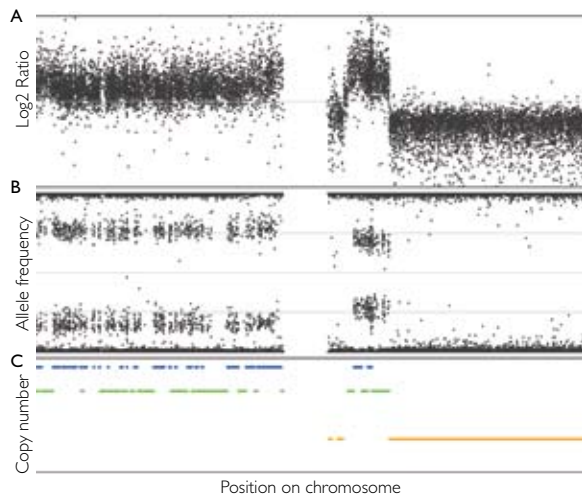


Figure 2. High-resolution array CGH analysis of ovarian tumours. The Illumina 1M SNP array gives (A) Copy number data and (B) SNP allele calls. Applying the QuantiSNP segmentation algorithm provides (C) copy number calls. Blue, 4 copies; Green, 3 copies; Yellow, 1 copy. We are using this along with high-throughput sequencing data to characterise ovarian tumour heterogeneity and evolution towards chemotherapy resistant disease.

arrest and apoptosis. Paclitaxel is widely used to treat ovarian and breast cancers but drug resistance limits its clinical usefulness to only half of the patients who receive it.

Alterations in the ratio of tubulin isoforms or mutations in tubulin can alter microtubule stability and sensitivity to taxane drugs. By studying cell line models of taxane resistance and clinical samples we have recently shown that loss of the ECM protein, transforming growth factor beta induced (TGFB1), was sufficient to induce paclitaxel resistance in cells and ovarian cancer tissues (Ahmed et al., *Cancer Cell* 2007; 12:514). We have also shown that TGFB1 induces microtubule stabilisation that is dependent upon integrin-mediated FAK and RHO signalling pathways. Extracellular matrix proteins have been implicated in the acquisition of drug resistance in ovarian cancer although the mechanism by which this is achieved is unclear. Loss of TGFB1 induces resistance by altering microtubules which are the direct pharmacodynamic target of paclitaxel. This work shows that the effects of ECM proteins on drug resistance may be very specific to particular cytotoxic treatments. As 30% of ovarian cancers do not express TGFB1, it may be an important biomarker for paclitaxel response.

Current projects are characterising how TGFB1 interacts with integrins and other cell surface receptors and how this may be modulated therapeutically (Figure 3). It is now clear that TGFB1 primarily exerts its effects in a beta-3 integrin dependent manner but is also co-regulated and interacts with other ECM proteins implicated in drug resistance. To

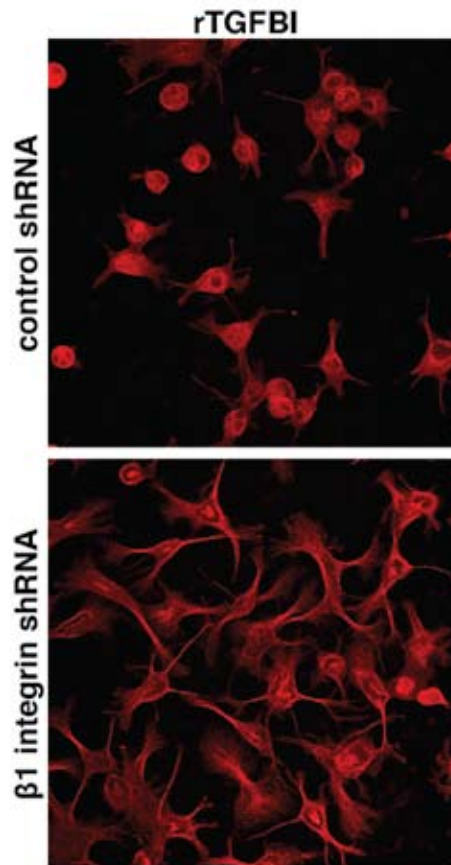


Figure 3. Suppression of  $\beta 1$  integrin expression increases ovarian cancer cell adhesion and spreading on rTGFB1. Ovarian cancer cells stably expressing either short hairpin non-target control RNA or short hairpin RNA against  $\beta 1$  integrin were respread on recombinant TGFB1 for 1 hour. The microtubule cytoskeleton was visualized by confocal immunofluorescence microscopy following staining with an antibody against alpha-tubulin.

identify the downstream pathways from FAK and RHO that alter microtubule stability, we have generated knock-out somatic cell lines using homologous recombination. These knock-out models have provided a powerful system to identify microtubule associated proteins responsible for effects on paclitaxel resistance. As TGFB1 has complex roles in organising interactions between cells and ECM, we have studied its function in early development in *Xenopus* to identify how it may affect cell migration. Both loss and gain of function experiments have shown that TGFB1 is required for somite development in *Xenopus*.

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