



# Functional Genomics of Drug Resistance

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Current chemotherapy strategies for most cancers are empirical, and have had limited effects in curing patients because the molecular basis for drug response and resistance is not well understood. This inability to individualise care by choosing the best drug treatment for each patient remains the fundamental clinical problem in oncology. It is therefore essential to develop biomarkers that identify patients who will not respond to chemotherapy prior to, or very early after, initial treatment, so that alternative therapies can be evaluated.

Our work has focussed on ovarian cancer, a disease which has a high health care burden because of low cure rates. Recurrent abdominal disease is therefore common despite initial surgical and chemotherapy treatment. The clinical problem is to understand what the determinants of clinical drug resistance are. We have approached this by developing clinical studies which sequentially collect ovarian cancer tissues before and during therapy, and use molecular profiling to identify key determinants of response.

We have correlated clinical response to carboplatin and paclitaxel chemotherapy with expression profiles of cancer samples collected before and during treatment in the Cambridge Translational Cancer Research Ovarian 01

(CTCR-OV01) Study. To identify genes that are selected for resistance we have developed novel bioinformatic methods, including the use of statistical mixture models, to get maximum information from pre- and post-treatment samples. These analyses have discovered several new markers for carboplatin resistance which have been validated using functional assays in cell lines. One of the proteins is strongly associated with survival in a cohort of 195 patients treated with platinum-based chemotherapy (carboplatin and related compounds). Comparison of genes identified from the CTCR-OV01 study with those from RNAi screens carried out by Charles Swanton and Julian Downward (Cancer Research UK London Research Institute) has also revealed significant overlaps, specifically with the identification of the ceramide transporter COL4A3BP in our study as a determinant of paclitaxel response.

Taxanes, such as paclitaxel, interfere with the dynamic growth of microtubules by directly binding to them, leading to mitotic arrest and apoptosis. Paclitaxel has been used extensively to treat lung, ovarian and breast cancers but drug resistance limits the clinical usefulness of this drug to only about half of breast or ovarian cancer patients. It is clear that taxane resistance is associated with alterations in the ratio of unstable to stable microtubules and that microtubule stability can be influenced by signals from the extracellular matrix (ECM). However, the role for ECM proteins in the modulation of paclitaxel sensitivity has not been established. By studying cell line models and samples from the CTCR-OV01 study we have found that the ECM protein transforming growth factor beta induced (TGFB1), was significantly reduced in paclitaxel-resistant cells (Figure 1). Importantly, TGFB1 mediated sensitization to paclitaxel and loss of TGFB1 was sufficient to induce paclitaxel resistance. TGFB1 induces microtubule stabilisation that is dependent on integrin-mediated FAK and Rho signaling. Analysis of ovarian cancer samples taken after treatment with paclitaxel revealed

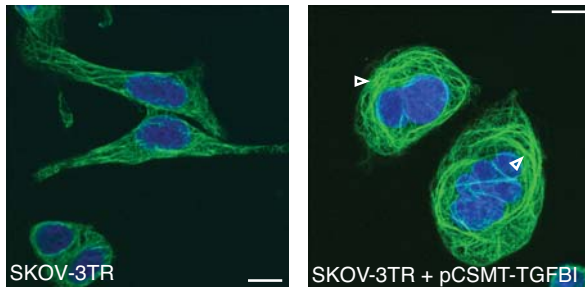


Figure 1. Overexpression of TGFBI in the paclitaxel-resistant ovarian cancer cell line SKOV-3TR sensitizes microtubules to the polymerizing effect of paclitaxel. Arrowheads indicate paclitaxel-induced bundles. Green, tubulin; blue, Dapi-stained DNA. Scale bars, 10  $\mu$  m.

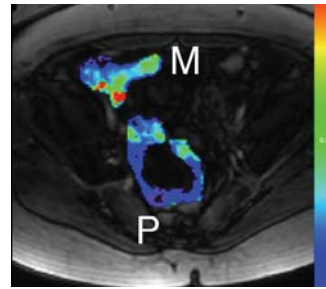


Figure 2. Dynamic contrast enhanced MRI showing  $K_{trans}$  maps from primary (P) ovarian cancer and peritoneal metastasis (M). There is differential perfusion within tumour masses with highest (red) values seen in the metastatic disease.

that paclitaxel-induced cell death was associated with high levels of TGFBI expression.

These results identify TGFBI as an ECM protein that induces microtubule stability and modulates sensitivity to paclitaxel in ovarian cell lines and in patients receiving paclitaxel therapy. On-going studies are defining further the signalling pathways that directly modulate microtubules downstream of FAK and Rho, the developmental role of TGFBI and the value of TGFBI as a predictive biomarker for taxane response.

We are now incorporating functional imaging into genomic clinical studies with measurement of perfusion and spectroscopy in ovarian tumour masses using magnetic resonance imaging (in collaboration with Dr Evis Sala from the University of Cambridge Department of Radiology and

Professor John Griffiths from the CRI). This is important as the microenvironment around cancer cells may also have important effects on drug resistance. Tumours have variable blood supply and poorly perfused regions of tumours are hypoxic, which limits delivery and efficacy of anticancer drugs. (Figure 2). Multiple sampling of tumour masses from areas of high and low perfusion is being carried out and combined with the analysis of longitudinal changes in imaging parameters. This will also allow genomic profiles to be more precisely correlated with areas of response and is likely to identify better molecular and imaging biomarkers for response.

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