

**EMER CASEY FELLOW REPORTS TO THE EMER CASEY FOUNDATION  
May 2010**

**Emer Casey Fellow 1 [ Mairead Murphy]**

**Title: Developing and validating diagnostic serum based biomarker panels in ovarian cancer**

As mentioned in my previous report, my work aims to identify biomarkers associated with ovarian cancer. A biomarker is a substance indicative of biologic state, i.e. a substance that can provide information about underlying processes within the body. To determine biomarkers of ovarian cancer I have profiled antibody patterns in the serum of ovarian cancer patients, and have compared the patterns to control/healthy volunteers using high content protein arrays.

The results of this work have yielded a pattern of some previously identified biomarkers (eg. p53, which is a very well known cancer marker) and also more novel cohorts of potential biomarkers. These identified markers may hold the potential to be used as biomarkers of ovarian cancer, ovarian disease and related diseases. To date in the lab, antibody profiling has been carried out on many different ovarian associated diseases. Antibody patterns have been identified in early and late ovarian cancer, benign ovarian disease, healthy/normal patients and also primary peritoneal cancer, which is often mistaken for ovarian cancer. To define the best possible treatment and care for ovarian cancer patients it is vitally important to distinguish between all these differing ovarian diseases.

The next step is to validate the results of the high content protein array screening using different scientific methods. Enzyme linked immunosorbent assay or ELISA is a gold standard method used in clinical practice to screen patient serum samples. Optimization work on this method for validation and reproducibility is ongoing. Further validation will also be undertaken by interrogating patient tissue for the gene expression profiles of the previously identified antigens. The tissue sections used are kindly donated by ovarian cancer patients and are part of the Discovery Bioresource.

Previously my work has focuses on profiling the immunoglobulin G (IgG) response of patients. IgG is the most abundant antibody and can persist in patient blood for ~ 10 years. This antibody response allows us to probe the immune repertoire of patients over a long period of time and hence provides much information on cancer progression. Immunoglobulin M (IgM) antibodies are another class of antibody that appear even earlier in exposure than IgG antibodies. For this reason my work aims to interrogate this immune response as IgM may provide an immediate antibody profile associated with cancer patients. Recent publications have also linked the importance of the IgM response in cancer cell recognition (Vollmers and Brandlein et al. 2009). The patient IgG profile coupled with the patient IgM profile should provide a greater insight into cancer progression and patient immune response.

## Emer Casey Fellow 2 [Lynda mcEvoy]

### Title of project: Developing Novel Therapeutic Approaches in Chemoresistant Ovarian Cancer Patients

Although most patients diagnosed with ovarian cancer respond well to chemotherapy initially, they often relapse, and are then resistant to treatment. It is vital to understand why this chemoresistance happens, in order to develop new treatments, and improve current treatments for patients. One reason which has been proposed to account for chemoresistance is tumour hypoxia (a reduced level of oxygen in the tumour).

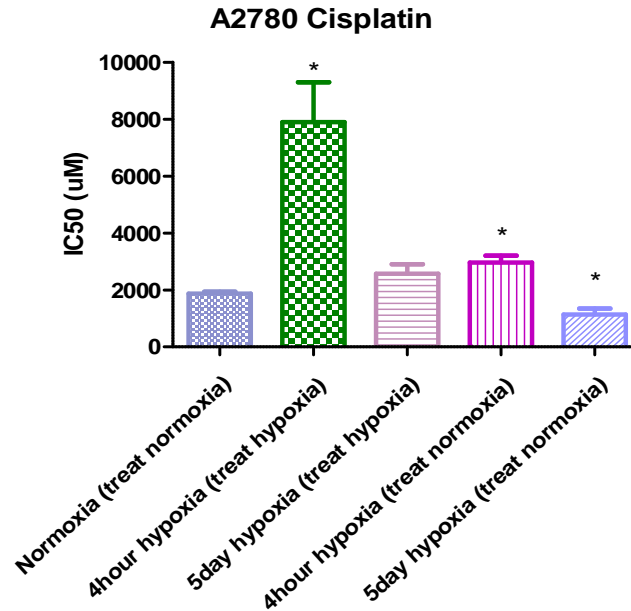
The standard treatment for ovarian cancer is a platinum-taxane combination. I have been looking at the effect of hypoxia on response to the platinum drug, cisplatin and the taxane, paclitaxel using two ovarian cancer cell line models, a cisplatin-sensitive cell line, A2780, and a cisplatin-resistant line, A2780cis. The cells are either kept in normal oxygen conditions (normoxia, 21% O<sub>2</sub>) or pre-exposed to hypoxia (0.5% O<sub>2</sub>), and then treated with the drug for 72 hours. Following drug treatment, the cells are assessed using a cell viability assay, to determine the effectiveness of the drug treatment. I have been looking at the effect of hypoxia using a design matrix:

1. Cells maintained in normoxia, treated in normoxia.
2. Acute (4 hour) exposure to hypoxia, followed by treatment in hypoxia
3. Acute exposure to hypoxia, followed by treatment in normoxia.
4. Chronic (5 day) exposure to hypoxia, followed by treatment in hypoxia.
5. Chronic exposure to hypoxia, followed by treatment in normoxia.
6. Cells maintained in normoxia, followed by treatment in hypoxia (for 24, 48 or 72 hours).

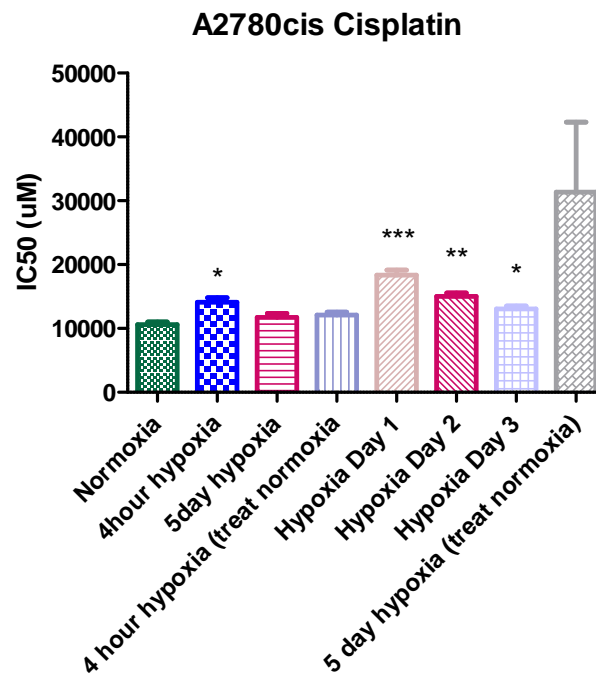
Overall, the results show that hypoxia has a significant effect on the cells' response to cisplatin and paclitaxel. Figures 1 and 2 show the responses of both cell lines to cisplatin under the various oxygen conditions. There is an inverse relationship between the responses – cells resistant to cisplatin are sensitive to paclitaxel and *vice versa*. This relationship can also be seen when the cells are exposed to hypoxia. Furthermore, cisplatin sensitive cell lines do not cope well when exposed to hypoxia during treatment without being exposed to hypoxia beforehand, whereas cisplatin resistant cell lines can respond similarly when pre-exposed and when not pre-exposed to hypoxia. The mechanisms underlying these different response patterns may provide novel targets for new therapeutic drugs.

Future work: Next, I plan to work with our collaborators in DCU to transfer these larger scale experiments to the small scale, using a lab-on-a-chip device. Hopefully, these devices could be used in the future to examine the response of cells taken from a patient's tumour to different concentrations of chemotherapy drugs and different oxygen concentrations in order to tailor a chemotherapy regimen that is suited to that patient's particular tumour. Also, I will be looking at the expression of various genes associated with resistance to cisplatin and paclitaxel such as MDR1 and BRCA in cells which have been

exposed to conditions corresponding to various points of the design matrix above. Understanding the mechanisms involved in chemoresistance related to hypoxia will be very important in the search for improved treatments for ovarian cancer patients.



**Figure 1.** The response of cisplatin sensitive cells, A2780 to treatment with cisplatin following various exposures to hypoxia. Acute exposure to hypoxia increases the cells' IC50 value (the amount of drug necessary to kill 50% of cells). \*  $p < 0.05$



**Figure 2.** The response of cisplatin resistant cells, A2780cis to treatment with cisplatin following various exposures to hypoxia. Acute exposure to hypoxia increases the cell's IC50. Treatment of cells with cisplatin in hypoxia without pre-exposure also increases IC50. \*  $p < 0.05$  \*\*  $p < 0.01$  \*\*\*  $p < 0.005$

## Emer Casey Fellow 3 [Brendan French]

### Title: Isolation, Characterisation and Silencing of Ovarian Cancer Stem Cells.

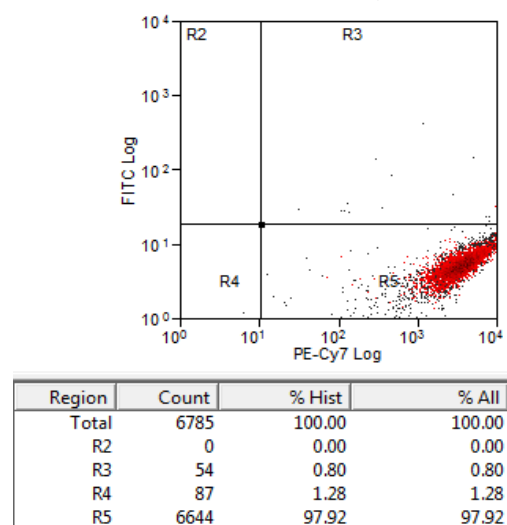
The cancer stem cell hypothesis suggests that as in normal tissue, growth and repair of cancerous tissue is carried out by stem cells (Cancer stem cells – CSCs). Stem cells have unlimited replicative potential and have the ability to differentiate into all the cells of their lineage. Cancer stem cells are thought to be resistant to therapeutic approaches and capable of replenishing tumours post-treatment.

In this project we aim to isolate CSCs from ovarian cancer. Other groups have identified markers for ovarian cancer stem cells. These markers are proteins that can be found on the surface of the cells. It has been found that certain proteins are found on only the cells in a tumour with stem cell properties. Using a panel of four such markers we aim isolate putative cancer stem cells from ovarian cancer cell lines.

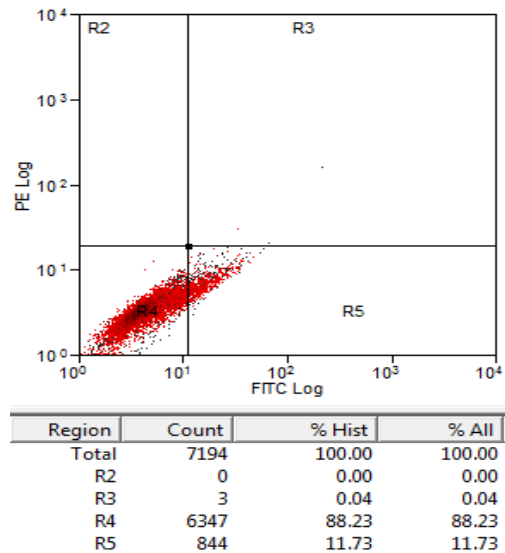
Once isolated we intend to validate the putative CSCs as CSCs through the various hallmarks of cancer stem cells e.g. chemoresistance, resistance to low oxygen conditions, increased tumourgenicity etc.

Once validated as stem cells we will then go on to see at a molecular level, what makes the cancer stem cells different to the differentiated bulk of cancer cells, with the intention of identification of targets to differentiate/kill these cancer stem cells – thus making treatment of ovarian cancer far more successful.

So far we have identified and isolated a population of putative cancer stem cells from one of the ovarian cancer cell lines we are working with. No validation was possible at this stage due to insufficient cell numbers. We will continue to optimise the isolation and culturing methods to improve this.



**Figure 1:** Each dot in this diagram represents a single cell. All the cells in the region labelled R5 are positive for one of the markers we are looking at.



**Figure 2:** Each dot in this diagram represents a single cell. All cells in the region labelled R5 are positive for a second marker that we were looking at. Cells in R4 are not of interest.

We isolated cells that were positive for both of these markers (data not shown), and these isolated markers were our putative cancer stem cell population.

We have also looked into single cell plating, and special media conditions that could allow for enrichment of CSCs prior to isolation, as well as aiding in the validation of stemness properties post-isolation.

We are also in the process of running chemoresistance assays on the cell lines to use as a base line chemoresistance for comparison to the sub-populations that are going to be isolated.

### **Additional work being carried out by Emer Casey Fellows:**

The Emer Casey fellows have worked hard to establish national and international collaborations based on their study work to date.

**Nationally:** the fellows are working with Amanda McCann PhD, UCD in defining a role for the protein MAD-2 in ovarian cancer. This protein may have an important role in the development of ovarian cancer particularly influencing how tumours cells undergo programmed cell death.

In addition, the fellows are working with Charles d'Adhemar, TCD examining the expression of toll-like receptor 4 [TLR 4] and its adaptor molecular MyD88. They have defined that cancer cells expressing TLR 4 and My D88 are more likely to be chemoresistant and will behave like cancer stem cells. This potentially provides a new marker for ovarian cancer patients and will aid in stratification of therapy for ovarian cancer patients.

The fellows based on their work on cancer stem cells are collaborating with the RCSI and DCU in examining the role of platelets in cancer cell metastasis. The process called 'platelet cloaking' is a major step forward in our

understanding of how tumour cells spread in the blood stream. It appears that the platelet forms a cloak on the circulating tumour cell, allowing it to evade the immune system. It also appears to protect the cell from the full effect of the chemotherapeutic agent. Together with colleagues in DCU and RCSI we are examining the mechanism of circulating tumour cell [CTC] interaction with platelets.

**International:** The Fellows are commencing a collaboration with colleagues at the Dana Faber Cancer Institute, Harvard, University, USA focussing on in depth analysis of the role of My D88 in tumour cell chemotherapeutic responses and to carry out live cell imaging of cancer cell platelet interactions. The link with Dana Faber is of huge importance to the foundation and to the fellows. It creates a dynamic two-way interaction between scientists on both sides of the Atlantic.