

Developing Novel Therapeutic Approaches in Chemoresistant Ovarian Cancer Patients

Lynda McEvoy Update January 2011

The aim of my project is to elucidate how hypoxia affects the response of ovarian cancer cells to chemotherapy. Hypoxia is a known factor in resistance to chemotherapy, and is a common feature in solid tumours such as ovarian cancer. 20% of all ovarian tumours are chemoresistant from the outset and of the other 80%, approximately two thirds will relapse and be chemoresistant. In order to investigate how hypoxia affects ovarian cancer cells, I have completed a comprehensive 'hypoxia matrix'. This matrix aims to replicate the various possible hypoxic conditions which may be present in a tumour: acute hypoxia, in which the tumour has only been exposed for a short period; chronic hypoxia, in which the tumour has been exposed for a long time; hypoxia during treatment, in which a tumour is not previously hypoxic, but during the course of chemotherapy it becomes hypoxic. All hypoxic conditions were compared to normal oxygen conditions. Two ovarian cancer cell line models were used, A2780 (cisplatin sensitive) and A2780cis (cisplatin resistant). The experiments were carried out in a 96-well format, and cell growth was measured using an MTT assay. Changes in resistance were measured by monitoring increases/decreases in IC_{50} (half maximal inhibitory concentration; the drug concentration necessary to inhibit growth in 50% of cells). Statistical analysis was carried out using Graph Pad Prism software. Student's t-tests and ANOVA were used, with significance taken at $p < 0.05$. These results have shown up several findings:

1. Acute hypoxia influences the resistance of ovarian cancer cell lines A2780 and A2780cis to the chemotherapeutic drugs cisplatin and paclitaxel. Acute hypoxia induced resistance to cisplatin in both cell lines. Acute hypoxia sensitized the A2780 cell line to paclitaxel. Conversely, acute hypoxia increased resistance to paclitaxel in A2780cis.
2. Chronic hypoxia also affects chemoresistance but has a less marked effect than acute hypoxia. Chronic hypoxia increased sensitivity to cisplatin and paclitaxel in A2780 cells. However, it did not affect the response of A2780cis to either drug.
3. Hypoxia influences the inverse relationship between cisplatin and paclitaxel resistance. There is an inverse relationship between the resistance profiles of A2780 and A2780cis to cisplatin and paclitaxel. A2780 are sensitive to cisplatin and resistant to paclitaxel; A2780cis are resistant to cisplatin and sensitive to paclitaxel. In the A2780 cell line, acute hypoxia increased resistance to cisplatin but increased sensitivity to paclitaxel. Acute hypoxia increased resistance to cisplatin in A2780cis, further enhancing the inverse resistance profiles of the two lines.

Currently I am optimizing scaled-up experiments based on these results. When I have scaled up these results, I will be able to extract RNA and protein from the cells. This will allow me to carry out whole transcriptome analysis using Affymetrix Human Gene Arrays to provide information on which genes are involved in the drug resistance patterns seen. I plan to examine some of the gene changes in patient tumour samples. Possible genes which may be involved include hypoxia inducible factors 1 and 2 (HIF 1, HIF 2), STAT3, TUBB3, BRCA1, BRCA2. Each of these genes has been linked to chemoresistance in the

literature. I will also be able to examine whether changes in genes are also seen at the protein level. I plan to look at cell death patterns using a technique called flow cytometry. In this, the cells are labelled with markers for DNA and passed through a specialized machine which can sort the cells depending on which markers they express. This will allow me to see how hypoxia influences cell death. Using these methods, I will be able to get a better picture of how hypoxia influences the cells – the effects on genes, proteins and cell death pathways. Hopefully these approaches will identify possible therapeutic targets for future research. Identification of new targets will hopefully lead to development of drugs which will improve the outcome for patients with ovarian cancer.

Developing and validating diagnostic serum based biomarker panels in ovarian cancer

Mairead Murphy Update January 2011

My work aims to identify biomarkers associated with ovarian cancer that may diagnose women using a blood test. To identify biomarkers of interest I have previously profiled antibody patterns in the serum of ovarian cancer patients, benign disease patients and healthy individuals. Antibodies (IgG) allow us to probe the immune repertoire of patients over a long period of time and hence may provide much information on cancer progression.

My work has verified previous publications, having identified autoantibodies to tumour protein 53 in 30% of late stage ovarian cancer. This protein is a previously validated cancer marker, which is very encouraging. There are also proteins of interest identified in early stage ovarian cancer, benign disease, and in primary peritoneal carcinoma.

For early ovarian cancer detection a protein has been identified that is present in higher proportions of early ovarian cancer patients compared to all other cohorts analysed. This protein is believed to be a transcription factor linked to generation of lymphocytes (white blood cells). Lymphocytes are critical to the immune response and this may indicate immune deregulation.

A protein of interest has also been identified in 30% of benign patients and was not present in the other cohorts. This protein has roles in protein trafficking and secretion. This process of protein transport from where it is made inside the cell to the external environment may shed light on the differences between benign growths and malignant tumours.

Validation of putative biomarkers is a necessity in biomarker discovery. Enzyme linked immunosorbent assay or ELISA is the preferred method used to test patient samples in the clinic. This method is now optimised and has been used to determine how differential methods of antigen identification affect the identified antigens of interest. The potential differences between these two methods are a vital part of developing a diagnostic assay as reproducibility is extremely important in a clinical setting. Western blotting has also been used to determine the antigens suitability for a diagnostic assay format.

Secondary validation is now being carried out to identify a subset of antigens that hold the most promise for future analysis. For this a miniaturised and high-throughout method will be utilised to maximise patient serum usage. Protoarrays are the newest technology in this field and will be used to cross reference previous identified antigens, in a post-translationally modified platform and it is anticipated that additional biomarkers will be identified which aid in the diagnosis of early and late stage ovarian cancer.

Isolation, Characterization and Silencing of Ovarian Cancer Stem Cells.

Brendan Ffrench Update January 2011

Introduction:

Cancer therapies have improved greatly in recent years, boosting the prognosis of many malignancies. Other cancers, ovarian cancer included, have maintained high mortality rates. Chemoresistance is a huge therapeutic obstacle, requiring new therapeutic approaches. One such approach would be to target cancer stem cells. Cancer stem cells are believed to be the driving force behind tumour initiation, development, recurrence and metastasis, making them an obvious therapeutic target. These cells have proved difficult to identify, isolate and analyse. In this project, ovarian cancer stem cells will be identified and isolated based on the presence or absence of certain proteins. These proteins are believed to mark populations of cancer stem cells in ovarian cancer. Once identified, these suspected cancer stem cells will be run through a series of tests and subsequently characterised at a molecular level. It is hoped that this characterisation may lead to the identification of molecular targets that can be exploited to remove the cancer causing capacity from these cancer stem cells.

Aims:

- i) To identify suspected cancer stem cells from ovarian cancer cell lines.
- ii) To isolate these cells making them available for analysis.
- iii) To analyse them cells on a biological and molecular level.
- iv) To compare the cancer stem cells to the non-cancer stem cells with the intention of identifying possible therapeutic targets.

Results:

The initial phase of this project was spent developing a model system that could be used to screen any sample for the presence of ovarian cancer stem cells (ovCSC). To a large extent this system is now up and running. The project is now entering the phase where multiple samples can be screened for the presence of ovCSC. This will allow us to get data on ovCSC from multiple sources, which will allow us to build up a precise picture of their role in ovarian cancer.

To date, two ovarian cancer cell lines have been screened, one of which has shown the presence of a suspected ovCSC population (Figure 1). Once this ovCSC population was identified it was isolated and passed through a series of tests to:

- a) confirm it as a ovCSC population.
- b) determine its biological properties relevant to the biology of ovarian cancer.

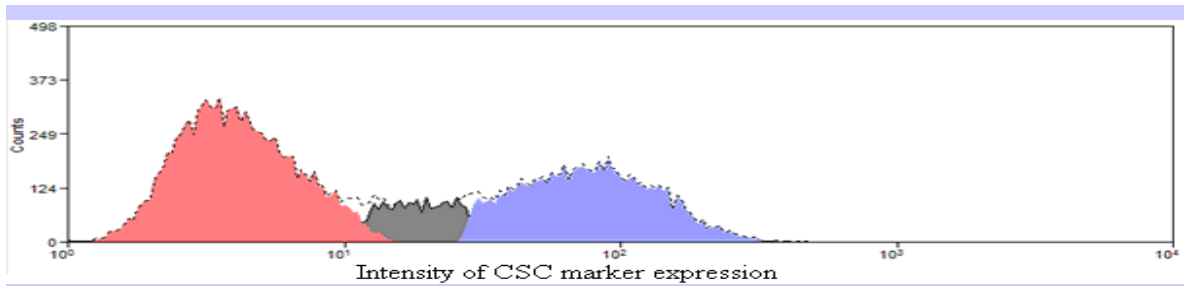


Figure 1: Suspected ovarian cancer stem cells (ovCSC) detected – This diagram shows the number of cells detected on the vertical axis. The horizontal axis allows us to discriminate ovCSCs from non-ovCSCs. Here the pink population represents the non-ovCSCs and the blue represents the ovCSCs.

a) Confirmation of ovCSC status:

The isolated suspected ovCSCs showed very good evidence that they are in fact ovarian cancer stem cells. They were able to rebuild the original cell line, while the non-ovCSCs were not (Figure 2).

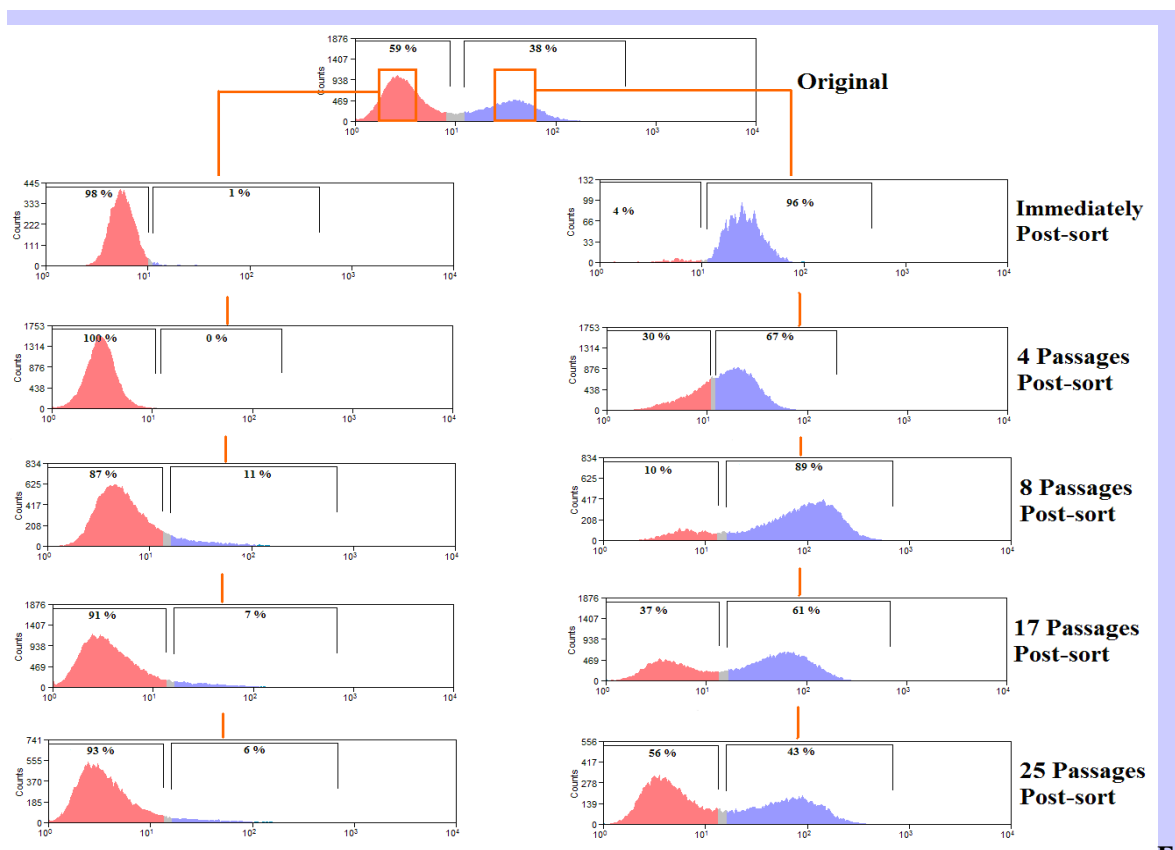


Figure 2: Only the suspected ovCSCs can rebuild the original cell line – The isolated ovCSC and non-CSC were allowed to grow for approximately 1 ½ months (Passages is a unit used to measure how long a cell line has been growing for). As expected only the ovCSCs could rebuild the original cell line.

b) Biological properties of the ovCSCs:

Unexpectedly we found that the ovCSCs were not more chemoresistant than the non-CSC. Cancer stem cells (CSCs) are thought to be a cause of chemoresistance in cancer, however, in this case both sets of cells are very chemoresistant.

Low oxygen (hypoxic) conditions are often encountered in tumours. CSCs are often associated with hypoxic regions of tumours. We investigated how well the ovCSCs and non-ovCSC grow in hypoxic conditions. Again producing another unexpected result, the non-ovCSCs were better able to grow in these conditions than the ovCSCs.

Initial work has also indicated that the ovCSCs have a better ability to repair damage better than the non-ovCSCs.

On top of these findings we have also noted that the two types of cell look distinctly different although they came from the same cell line.

Conclusions:

These results have helped to indicate that a two tier model of ovCSCs in ovarian cancer might be an over simplification. There may be many layers of ovarian cancer stem cells, similar to that found within the blood system. Through testing more sources of ovarian cancer for ovCSCs it is hoped that light can be shed on a multi-tiered model of ovarian cancer stem cells.

Future work:

- i) Using the one cell line described in the work above, I have been able to develop a model system through which multiple samples can be screened for ovarian cancer stem cells. I am about to start work on the next cell line, next week.
- ii) In my earlier work one of the methods I was using to identify ovCSCs was not working correctly (this is a method separate to the one shown in the above work). I have now worked out most of the chinks and this method will be up and running in the coming weeks.
- iii) There are two more tests that need to be developed to confirm that isolated cells are indeed cancer stem cells. I am currently working on one of these tests and it should be up and running within weeks. The other should be online within the coming months.
- iv) We also aim to be able to develop conditions that will allow isolated ovCSCs to grow stably in culture. Such conditions have not been published for ovCSCs to date.
- v) Through examination of multiple samples we hope to build up a complete of the role of ovCSC in ovarian cancer. Once this picture has been mapped out we aim to compare and contrast the different types of ovCSC and non-ovCSCs on a molecular level using SOLiD Next Generation Sequencing. Molecular analysis should allow us to identify novel targets to be used for the development of new therapies that are not susceptible to chemoresistance, recurrence or metastasis.