Final Report for BAOMS Research Grant

Awarded October 2016

Title of Project:

Prognostic value of oral cancer cell lines: investigation of amoeboid cells in oral squamous cell carcinoma

Supervisor:

Professor Ian Mackenzie, Professor of Stem Cell Science, QMUL

Site of project:

The Blizard Institute, Barts and The London School of Medicine and Dentistry, QMUL
4 Newark Street, London E1 2AT

Grant award:

£5980. Used towards the aims and objectives of the clinical translational aspect of a PhD, 'An investigation into the properties of the amoeboid cell in oral squamous cell carcinoma'. The thesis (63,000 words) has been submitted (within 48 months as per University of London requirements) and awaits examination.

Although survival outcomes for patients with oral squamous cell carcinoma (OSCC) have improved over the past 3 decades, improvements have been relatively modest particularly in those with more advanced disease. This might be explained by the activity of cancer stem cells (CSCs), a small sub-fraction of cells within a tumour that are more resistant to chemo- and radiotherapy. CSCs also possess another ability, cellular plasticity, which confers upon them the ability to undergo transformation from cohesive, epithelial carcinoma cells into individual migratory cells. The best described process of plasticity is epithelial-mesenchymal transition (EMT), whereby epithelial cells become elongated, invasive mesenchymal cells.

Whilst investigating EMT in OSCC cells, I identified and isolated another distinct cell type in oral cancer. This was the amoeboid cell, a cell that had not previously been described in OSCC but had been seen in melanoma and sarcoma cell lines. Although not isolated in these cancers, it was associated with greater invasive and metastatic potential than other cells and its presence with clinical poorer outcomes. The literature had relatively little information about

this cell type or the plasticity that gave rise to them, so my research aims moved to focus on the role of amoeboid cells in oral cancer.

Amoeboid cells were subsequently identified in all OSCC cell lines that the Mackenzie lab used. As the first group to isolate amoeboid cells from cancer lines, we were able to investigate their properties in detail. Whereas mesenchymal cells were elongated, spindle-shaped and slow moving, amoeboid cells were rounded and significantly faster. They were smaller than both epithelial and mesenchymal cells and were shown to share migratory and genetic characteristics with leukocytes. On this basis, amoeboid cells were very similar to their most aggressive counterparts in malignant melanoma. We demonstrated that the cells were more invasive in virtually all conditions compared to other cell types. Importantly, we were able to demonstrate that amoeboid cells were plastic: they were able to switch readily between both mesenchymal and epithelial phenotypes. Both mesenchymal-amoeboid (MAT) and epithelial-amoeboid transition (EAT) were identified.

Amoeboid isolation also allowed gene expression to be analysed and compared. Genes associated with stemness and EMT were up-regulated. In addition, and for the first time, a genome-wide gene expression analysis of amoeboid cells from any tumour was performed. This identified gene clusters significantly associated with angiogenesis, neurogenesis, therapy-resistance and apoptosis-resistance. It identified increased activity of several cancer-associated pathways. From this data, 17 amoeboid-specific genes (ASGs) were identified.

The grant monies were used to continue the investigation of amoeboid cells in oral cancer. One of the controversies of the role of EMT in the metastatic cascade is the absence of elongated individual mesenchymal cells in histopathological specimens. Of note, amoeboid cells expressed significantly lower levels of keratins commonly found in cells of epithelial origin potentially complicating histopathological tumour margin clearance. Using the ASGs, antibodies were purchased to investigate the presence of individual amoeboid cells in OSCC tumour sections. Using two candidate antibodies, we were able to show just that; individual cancer cells invading through tumours that had not been identified using standard histopathology techniques.

Using the ASGs, a Kaplan-Meier analysis of ovarian, gastric and breast cancers showed significantly poorer outcomes in patients with high levels of OSCC amoeboid genes. Drug response profiles showed that amoeboid cells were more *anoikis*-resistant, allowing them to survive in suspension, and occasionally increasing in number in response to certain chemotherapy agents. A more detailed investigation into cellular plasticity and mechanisms

was performed. Reagents, antibodies, kit and equipment time related to this work was purchased. Much of the work as whole depended upon isolation of the various cells from cell lines using flow cytometry techniques, also purchased from the grant.

In summary, the grant has enabled us to confirm that amoeboid cells are not a phenomenon of cell culture. They are a distinct cell type within OSCCs, found in tumour sections. They have the ability to switch between other phenotypes and move faster and invade further than them. Their presence is associated with poorer outcomes in tumours beyond OSCC. They share similarities with leukocytes and the most aggressive melanoma cells, supported by detailed genetic analysis, with obvious implications for metastasis.

EMT research is at the forefront of metastasis research. If EMT gives rise to invasive cells that contribute to the metastatic cascade and to poor outcomes, then the processes that produce amoeboid cells are equally important. There are clear clinical implications. The presence of amoeboid cells as yet another migratory cell type present in epithelial tumours establishes them as major interest both to pathological prognosis and to therapy.

The support of BAOMS, through the *Joint BAOMS-FSRF Research Fellowship* in 2013-15 and the Research Grant in 2016, has allowed significant progress into the investigation of amoeboid cell properties. Professor Mackenzie and I are currently planning the first of two publications that will describe the key findings of the project. It is hoped that this research will lay the foundations for further work that will improve the survival outcomes of patients with OSCC, and other cancers.