Final Report

Date: 30th June 2015
Author: Clinical Associate Professor Michael Buckland
Qualification: BSc (Med), MBBS, PhD, FRCPA
Institution: University of Sydney and Royal Prince Alfred Hospital

Title of Project: Glioblastoma stem cell-derived microvesicles

Summary:

Microvesicles are small membrane-bound vesicles released by many cells, and represent a previously underappreciated form of intercellular communication. Microvesicles release is increased in many cancer cell types, including glioblastoma (GBM), and these microvesicles contain distinct cargoes of protein, RNA and DNA.

In this project, we aimed to build upon our prior successful work in assessing the composition and effects of GBM-derived microvesicles. We also aimed to examine differences in microvesicles from normal GBM cells and GBM cells with stem cell-like properties (GBM stem cells). GBM stem cells represent a small but important component of normal GBM, and are thought to play a major role in driving tumour growth and tumour relapse following treatment.

Our proposed model system was based on inducing a stem cell-like phenotype in a well characterised GBM cell line, U251 MG. The model was based on contemporary reports of using hypoxia and glucose deprivation to induce stem cell-like properties in vitro, but we (and others) were unable to replicate the published methods. We therefore sourced several primary human GBM stem cell lines from our collaborators from QIMR Berghofer in Brisbane. We then were able to differentiate these stem cells into more typical GBM cells, and used these stem- and differentiated primary cell lines for microvesicle harvesting. The cell lines were characterised by flow cytometric analysis for CD133 (Figure 1) and showed distinct morphological differences between stem and differentiated conditions (Figure 2).

Microvesicles were been characterised by electron microscopy, nanocyte particle tracking analysis (Figure 3) and Western blotting. We have constructed small RNA libraries for deep sequencing of their RNA contents, and are currently preparing them for mass spectrometry.

Microvesicles (both stem and differentiated) have been added to primary human astrocyte cultures, and their uptake into the astrocytes has been tracked by DiL labelling and live cell fluorescence imaging (Figure 4). Following 24 hour incubation, significant protein expression changes were detected between astrocytes treated

with stem and differentiated microvesicles, as well as between untreated controls and astrocytes treated with their own microvesicles. Some of the most significant changes are related to cell invasion and invadopodia formation, and we have demonstrated that GBM stem cell-derived microvesicles have potent stimulatory effects on cell invasion through a gelatin degradation assay (Figure 5). We have also collected RNA from treated astrocytes and are currently assaying gene expression changes in treated astrocytes via Affymetrix microarrays.

Hypothesis vs Findings, Unanswered Questions

This project is ongoing. To date, our findings are in keeping with the hypothesis that GBM stem cells mediate some of their unique effects via distinct populations of secreted microvesicles. Several unanswered questions are currently being addressed: the effects of different microvesicles on astrocyte proliferation and cytokine release, and the molecular machinery that mediates these effects.

What these research outcomes mean

This Brain Foundation Grant has formed the foundation of an ever growing research focus on microvesicles in GBM, in the BMRI Molecular Neuropathology laboratories. It has allowed us to gain valuable experience in tumour stem cell cultures and microvesicle handling. The research is ongoing and we anticipate one manuscript to be submitted for publication this year, and at least one further manuscript in 2016.

We have shown, for the first time, that GBM stem cell-derived microvesicles are distinct from normal GBM-derived microvesicles and have more potent effects on normal brain astrocytes. Our understanding of their role in GBM growth and treatment resistance is still incomplete. They may offer very valuable and novel therapeutic targets in the future. Our work is ongoing and would not have been possible without the generous support of the Brain Foundation

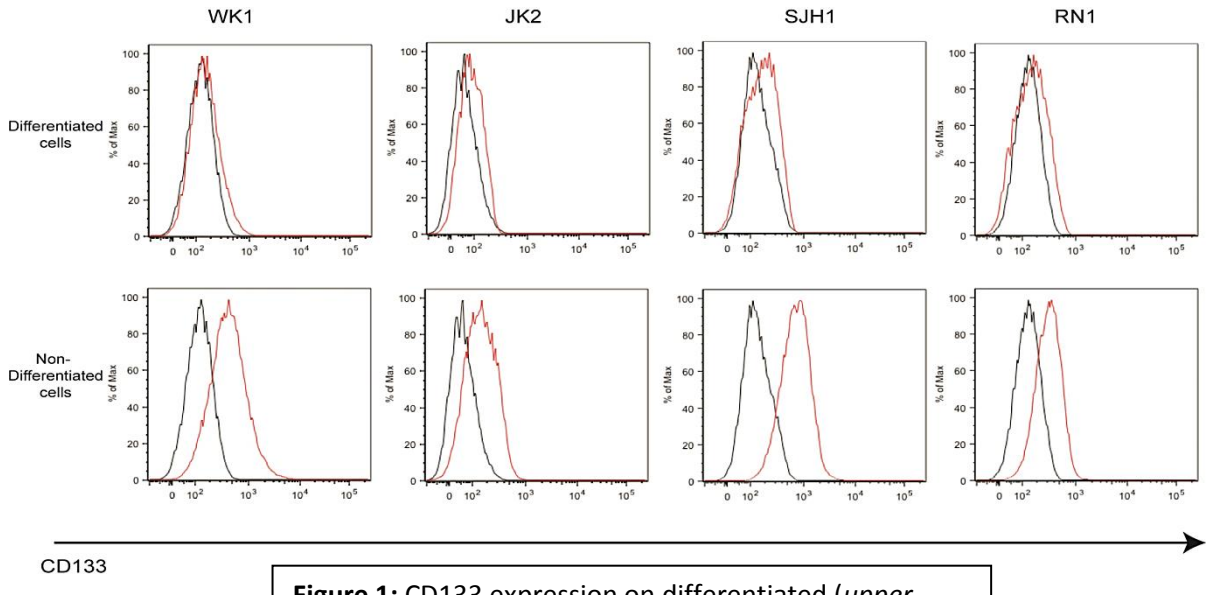

Figure 1: CD133 expression on differentiated (*upper panels*) and stem-like (*lower panels*) GBM cell lines

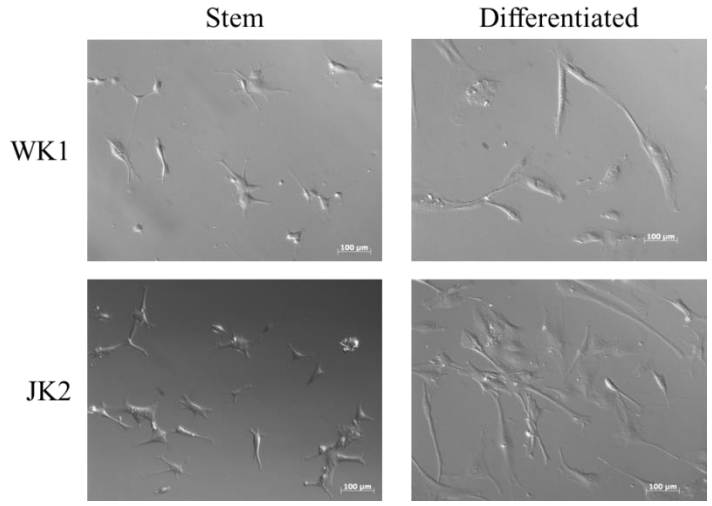

Figure 2: Distinct morphological differences between stem (*left panels*) and differentiated (*right panels*) GBM cell lines

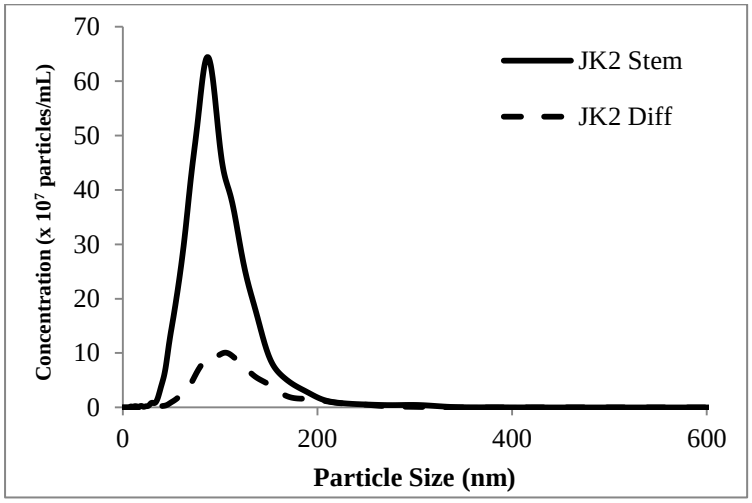

Figure 3: Nanocyte particle tracking analysis confirms the relatively distinct size profile of GBM microvesicles from stem (*left panels*) and differentiated (*right panels*) GBM cell line JK2.

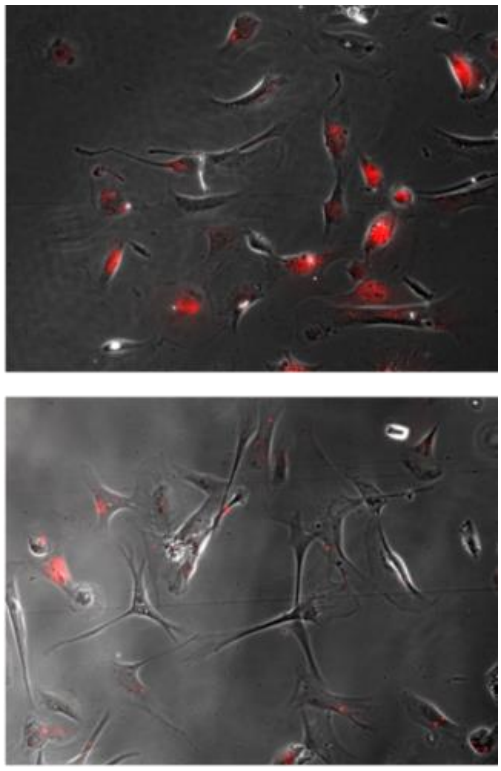

Figure 4: Dil labelling of microvesicles demonstrates uptake by normal human fetal astrocytes in culture.

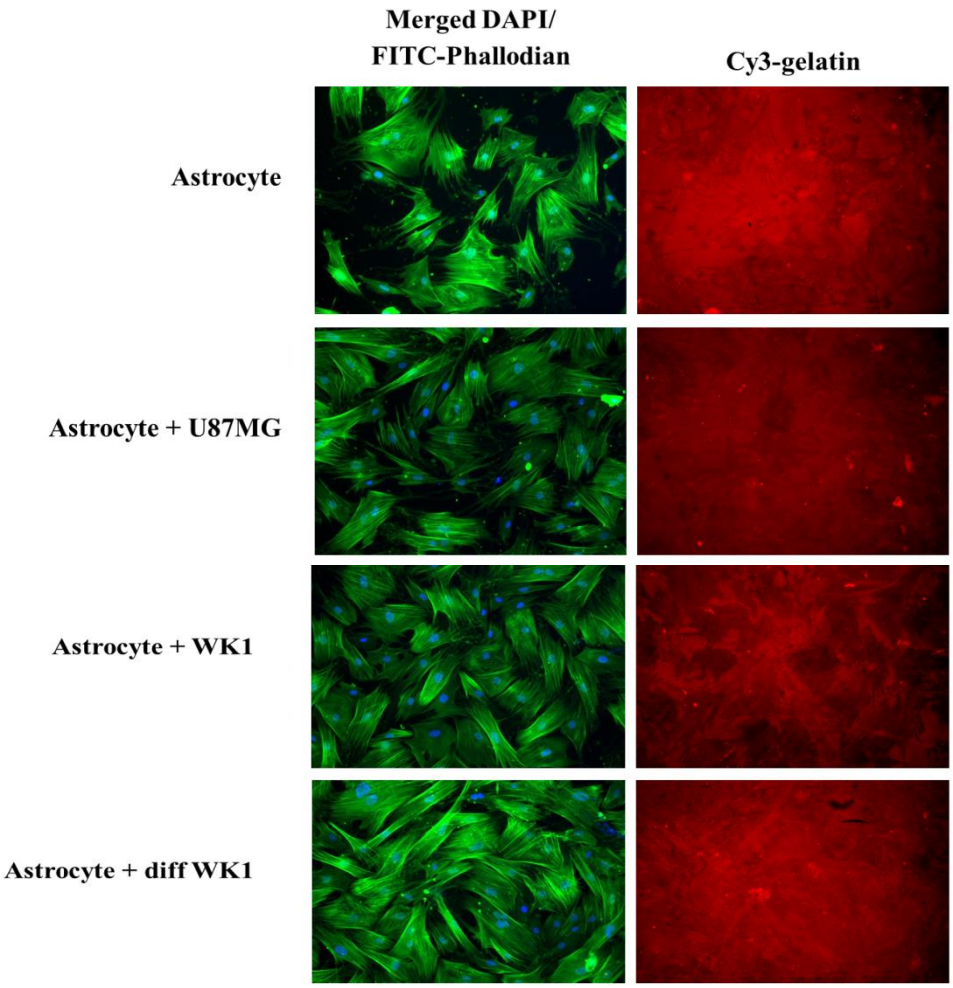

Figure 5a: Examples of gelatin degradation/invasion assays of normal human fetal astrocytes treated with various microvesicles.

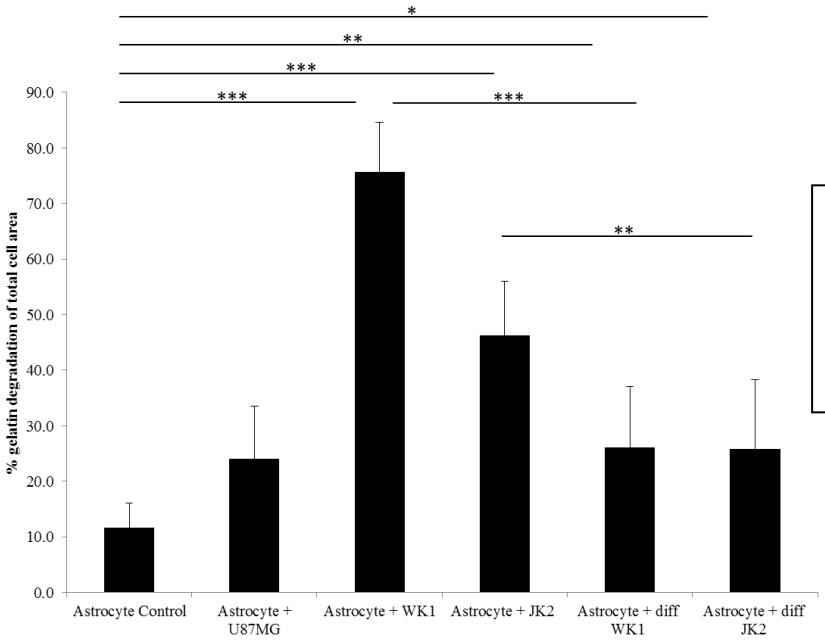

Figure 5b: GBM stem cell-derived microvesicles induce significantly increased invasion in treated astrocytes compared to their differentiated counterparts and controls.