**Introduction**

- **Glioblastoma multiforme** (GBM) is the most common, and the most malignant tumour of the brain and CNS found in adults.
- Common distinguishing factors of GBM include determining IDH status and subgroup type; classical, neural, proneural and mesenchymal.
- The WHO as of 2016 recognise GBM as a grade IV astrocytoma [Louis et al., 2016].
- As shown below, the most predominant feature is regions of hypoxic tissue with cancer stem cell-like (CSC) features driven by EMT.

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**Methods**

**Sub-Culture**

Following quantification using the cell counter, a target of 0.3 X 10^5 cells was decided for growth. The normoxic cells were cultured for 24hrs, with sphere and hypoxic cells cultured for 6 days.

**RNA Extraction**

Upon collection of the cells, 300µl of lysing solution was added to the cells. RNA was then isolated by first removing the DNA, centrifuging and washing in stages.

**RT-qPCR**

A two step process was designed to first create cDNA by diluting RNA and deionised H2O (10µL) with RT master mix (10µL). The addition of specific EMT primers and the TaqMan™ gene expression assay (ΔΔ Ct analysis) then amplified the target DNA using qPCR.

**Results**

Following successful cultures shown in figure 6, RT-qPCR expression was determined using the normoxia cells and HART-1 primed results as controls.

Comparative ΔΔ Ct analysis of the data extracted provided quantifiable results of gene expression as shown below.

**Discussion**

- EMT ATF's are currently accepted to play a role in repressing expression of E-Cadherin via E-box binding. This is believed to be an essential step to induce epithelial disorganization and the EMT process in embryonic development and cancer metastasis [Dave et al., 2011].
- Significant increase in SNAIL2 expression in hypoxic cells support the theory that the protein is induced and upregulated in hypoxia to induce EMT; however, the low and/or insignificant expression of other ATF's across cell lines oppose this hypothesis.
- Variability of results has been a significant problem in hypoxic culture. This could indicate that hypoxia does not play a role in their activation.
- Expression in sphere cells using DEMM-F12 produced better results than DMEM, supporting the hypothesis that there is higher stability for GBM spheres in a serum free medium. Variability of expression between sphere cell lines support the "multiforme" phenotype.
- The significant expression in both sphere and hypoxic cells, particularly of SNAIL2 and TWIST1 U87MG clones, supports the hypothesis that hypoxia and EMT ATF's may play a pivotal role in maintaining CSC stemness.

**Conclusions**

- The difference in results between each cell line cultured strongly supports the "multiforme" phenotype known of GBM, displaying significant tumour heterogeneity.
- The results collected suggest that a combination of growth factor stimulation and hypoxia play a role in driving CSC action via the activation of the EMT pathway in GBM.