Identifying clinically relevant receptors for antibody therapeutics

Fiona Lickiss[1,2], Sachin Kote[1] Sofian Al Shboul[2], Paul Brennan[2], Steven Pollard[3], Kathryn Ball[2] Theodore Hupp[1,2]

[1]ICCVS, University of Gdansk, Gdansk, Poland, EU, [2]IGMM, University of Edinburgh, Edinburgh, UK, [3]SCRM, University of Edinburgh, Edinburgh, UK

International Centre for Cancer Vaccine Science

Introduction

Glioblastoma multiforme (GBM), is the most frequent and aggressive malignant primary brain cancer, a combination of radiotherapy, chemotherapy and surgery increases median survival from 2 months to 12 months. GBM is clearly a cancer of unmet clinical need, many chemotherapeutic, immunotherapeutic and chemoimmunotherapy's make it to early phase clinical trials with some promising results but later prove disappointing later clinical trials[1].

Aim

1) Using kinomics analyze whether cell lines are a good model for tissue.

2) Identify clinically relevant membrane receptors, using cells and/or tissue for novel antibody therapeutics.

1) PamStation

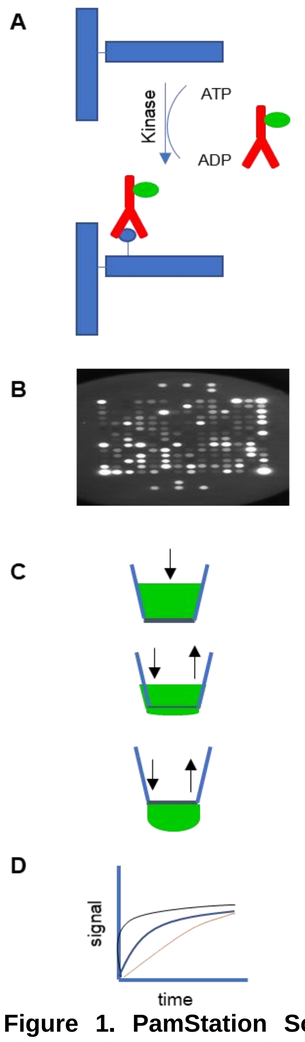

Figure 1. PamStation Schematic. A) Readout is based on kinetic measurement of phosphorylation occurring on the array using fluorescently labelled anti-phosphor antibodies. **B)** Microarrays consist of a porous ceramic membrane which allows flow through of sample, per array 144 peptide substrates are immobilised. **C)** The phosphorylation reaction occurs by up and down movement of the sample solution through the array giving kinases maximal opportunity to phosphorylate peptides on each array. **D)** Images taken of each array are used by software to generate kinetic data curves of each peptide[2].

2) Kinetic activity of GBM derived cells

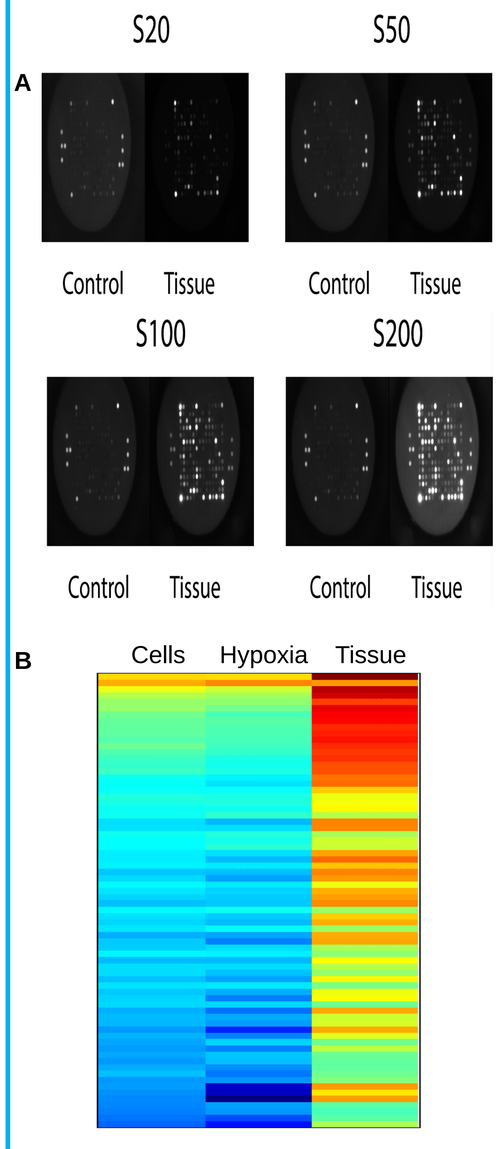

Figure 2. Spot arrays and heat map showing the kinetic activity of GBM derived cells and matched GBM tissue. A) Spot arrays (phosphor sites) for M cells (cells derived from GBM) in different time frames starting from S20 (20 milliseconds) to S200 (200 milliseconds). **B)** Heatmap compares kinase activity for control M cells, M cells under hypoxic conditions and M tissue. Red bands indicate phosphorylation, blue bands indicate absence of phosphorylation.

3) Biotinylation of membrane proteins

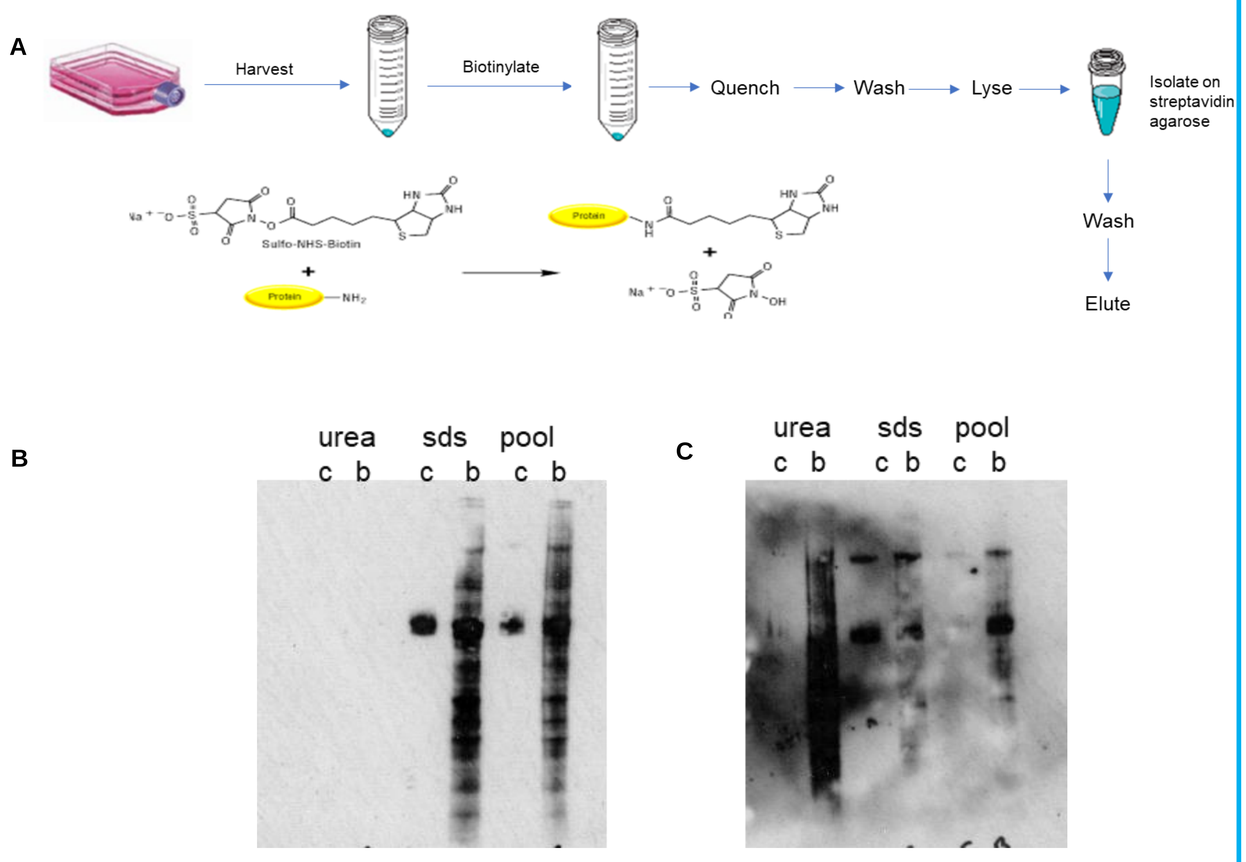

Figure 3. Biotinylation and elution of M cell membrane proteins. A) Schematic showing biotinylation method. M cells are harvested from flask before biotinylation, once biotinylation reaction is quenched the biotinylated proteins are isolated on streptavidin agarose. **B)** Western blots showing elution methods of biotinylated proteins from M cells. Using Urea as an elution buffer is preferable as it is compatible with mass spectrometry. The western above shows that urea elution buffer does not elute biotinylated membrane proteins, isolated from M cells, from the agarose. SDS buffer successfully elutes the biotinylated proteins. This means an extra FASP step is needed before Mass spec. **C)** Western blot showing elution methods of biotinylated proteins from SH-SY5Y cells. Conversely urea elution buffer can elute biotinylated membrane proteins, isolated from SH-SY5Y cells, from the agarose.

References

1) Azizul Haque, Naren L. Banik, Swapan K. Ray, Molecular alterations in glioblastoma: potential targets for immunotherapy, Prog Mol, Biol, Transl, Sci, 2011, 98: 187-234

2) www.pamgene.com/en/pamstation-technology.htm

Conclusion

These preliminary results show a marked difference in kinase activity of cells lines compared to tissue. This is not entirely unexpected due the heterogeneity of tumour tissue and the selection process involved in the generation of stable cell lines, though it does cause us to ask, 1) Do we use cell lines or tissue for target discovery? 2) Can we develop methods to capture receptors on tissue and cells for identification using proteomics? We will compare GBM tumour tissue and their derived cell lines to look for novel receptor targets for the development of antibody therapeutics.

European Funds Smart Growth

FNP Foundation for Polish Science

European Union European Regional Development Fund

Fiona.Lickiss@igmm.ed.ac.uk
sachin.kote@ug.edu.pl