INTRODUCTION

Dysregulation of receptor tyrosine kinase signaling is a major contributor to cancer, including gliomas.1 High level expression of epidermal growth factor receptor (EGFR) is frequently observed in glioma, usually in combination with wild-type and/or mutant EGFR gene amplification that has been associated with reduced survival.2,3 The most common of the rearrangements leads to the deletion of exons 2-7 in the EGFR mRNA, causing an in-frame deletion of 801 bp of sequence coding for a portion of the extracellular domain.4-6 The resulting protein, deleted-(2-7) EGFR (also known as EGFRvIII, EGFR* and AEGFR, which are referred to here as AEGFR), is expressed primarily in glioblastoma/astrocytoma grade IV.5,6,7 AEGFR, which can transform NIH3T3 cells,5,6,7 confers enhanced tumorigenicity to glioma cells in vivo, enhances tumorigenicity and growth rate while reducing apoptosis.5,6,7 AEGFR is phosphorylated in a ligand-independent fashion in the absence of significant internalization and down regulation.5,6,7 Therefore, AEGFR is a potent glioma oncogene understanding its aberrant signaling is important.

We are using a novel proteomics approach, the ProteomLab™ PF2D from Beckman Coulter, to identify molecular differences between gliomas cells bearing amplified EGFR and amplified AEGFR in human gliomas xenografts as one approach to understanding AEGFR. One advantage of this approach is that alterations in the proteome due to changes in signaling pathways, such as phosphorylation levels, are more easily detected by proteomics than genomics.

MATERIALS & METHODS

Preparation of Xenografts

- Two glioblastoma xenografts were established in the flank of male mice.
- Tumor #6 over expresses AEGFR while Tumor #12 expresses wild-type EGF.
- Tumors were harvested and approximately 10 mg of wet tissue mass was lyzed according to the protocol provided with the ProteomLab PF2D chemistry kit.

1st Dimension Separation, Chromatofocusing

- HPC chromatofocusing column
- Solvents: Start Buffer (pH 8.5), Elution Buffer (pH 4.0), 1M NaCl
- Flow rate = 0.2 mL/min
- Fraction collection = pH or time
- Detection: pH and absorbance at 280 nm

2nd Dimension Separation, Reversed Phase

- HPR reversed phase column
- Solvents: A=0.10% TFA - Water, B=0.08% TFA - Acetonitrile
- Gradient = 0%-100% B in 90 minutes
- Flow rate = 0.75 mL/min
- Temperature = 50ºC
- Detection: Absorbance at 214 nm

UV/vis map of glioblastoma #6 xenograft proteins.

Glioblastoma, which carries a AEGFR amplification, is detected in the ProteoVue+ application. This application displays the full scans as lane and the 2nd dimension separation time as vertical position in individual lanes. The intensity of the bands at the time points in the right hand sample is twice as intense as the left hand sample. The peak that is shown in the left hand column is from sample #6 that has a pH range of 4.0-4.2.

UV/vis map of glioblastoma #12 xenograft proteins.

Glioblastoma #12, which carries a wild type EGF amplification, is shown in the ProteoVue+ application. As to the above figure, the separation time between 6 and 26 minutes are shown for the 2nd dimension where most protein peaks are found. The peak times shown in the left hand panel is Lane #1, which have a pH range of 6.8-4.9, and the right hand panel is Lane #2 which have a pH range of 4 and 9.2 where a major peak at around 17 minutes, but other small peaks differ.

The ProteomLab PF 2D System

SUMMARY

Proteomic analysis of tumor samples with the ProteomLab PF2D system allows rapid and high sensitivity resolution of proteins, thereby facilitating the identification of molecular differences and accelerating their exploitation by downstream technologies.