

# The function of normal and mutated epidermal growth factor receptors in Glioblastoma Multiforme - Establishment of an *in vivo* and *in vitro* model



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## Background

- The epidermal growth factor receptor (EGFR) is involved in regulation of cell growth, proliferation, survival, and migration
- Overexpression of EGFR and/or expression of a constitutively active variant of EGFR (EGFRvIII) is frequently found in human cancers<sup>1</sup>
- Glioblastoma Multiforme (GBM) is the most common and most malignant brain tumor in adults
- The tumors are divided into primary, arising *de novo*, and secondary, developing from low grade gliomas
- Primary GBMs often express EGFRvIII and overexpress EGFR<sup>2</sup>
- GBM cells grown *in vitro* lose their expression of EGFRvIII and overexpression of EGFR<sup>3</sup>
- GBM cell lines established during stem cell culture conditions more closely mimic the original patient tumor, than traditionally cultured cells in the presence of serum<sup>4</sup>

## Aim

To establish an experimental model for GBM expressing EGFR and EGFRvIII

## Conclusions

- The patient material was representative of the reported expression of EGFR and EGFRvIII in GBM<sup>2</sup> (Figure 1, Table 1)
- Xenografts retained the EGFR/EGFRvIII expression of the primary tumor (Figure 2)
- Following initial *in vivo* passages the xenografts grew with reduced and stabilized lag-period and growth rate (Figure 3)
- In vitro* cultures were positive for GFAP, verifying the astrocytic origin of the cells (Figure 4)
- Cell cultures established during stem cell conditions grew as spheres which is a characteristic of normal neural stem cells (Figure 5)

## Materials and Methods

- Patient material:** Tumor material was obtained during surgery at Copenhagen University Hospital, Denmark and was approved by the Scientific Ethical Committee for Copenhagen and Frederiksberg (KF 01-034/04). Tumors were diagnosed as GBM according to the WHO 2000 guidelines.
- Tumor growth analysis:** Tumor xenografts were generated by subcutaneous transplantation into the flanks of 6-week-old female NMRI nu Tac nude mice (Taconic, Ry, Denmark). Tumors were measured in two perpendicular dimensions (d1 and d2) and tumor area,  $A=d1 \times d2$  was calculated.
- Western blotting (WB):** Protein lysates (5µg) were separated on 3-8% NuPAGE TA gels (Invitrogen, Denmark), electroblotted onto nitrocellulose membranes, incubated with primary antibodies overnight at 4°C and visualized by ECL. Primary antibodies: goat anti-EGFR (Fitzgerald, USA), mouse anti-EGFRvIII (DH8.3, Novocastra, UK) and rabbit anti-GAPDH (Santa Cruz, USA).
- Immunohistochemistry (IHC):** 4µm sections from formalin fixed paraffin embedded material was used for confirming diagnosis (H+E), EGFR (Merck, De) and GFAP (DAKO, Denmark). For EGFRvIII (DH8.3, Novocastra, UK), frozen sections were used.
- Cell culture:** Cells were established from xenograft GBM tumor explants and maintained in Neurobasal+b27, N2, EGF, bFGF or DMEM + 10%FCS (Invitrogen, Denmark).

## Results

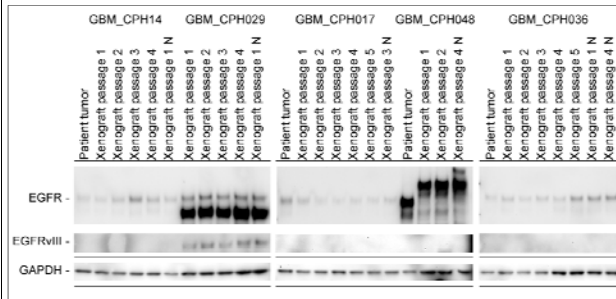
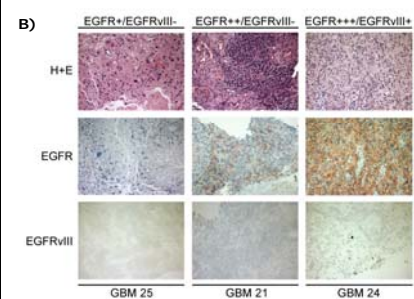
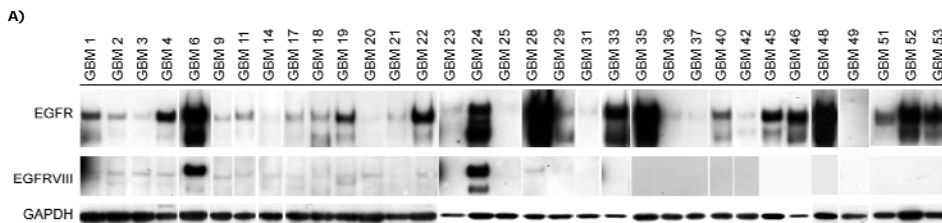


Figure 3: Mean tumor volume of a representative GBM xenografts. The tumor xenografts showed shortened and stabilized lag-time and growth rate following the initial *in vivo* passages in nude mice.

Expression	Number of Tumors	Percentage
EGFR Low	18	54%
EGFR High	15	46%
EGFRvIII pos	1	12%

Expression	Number of Tumors	Percentage
EGFR Low	8	53%
EGFR High	7	47%
EGFRvIII pos	5	33,3%

Table 1: Frequency of EGFR and EGFRvIII expression in A) patient material and B) tumor xenografts as detected by western blotting.

Figure 1: EGFR and EGFRvIII expression in the patient GBM tumors included in the study. A) western blotting (WB) B) immunohistochemistry (IHC) of representative tumors. H+E staining was used to confirm diagnosis.

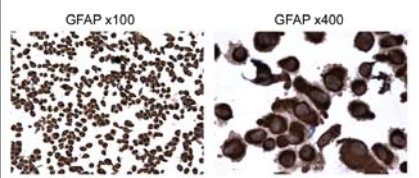


Figure 4. *In vitro* cultured cells were positive for the astrocyte marker glial fibrillary acidic protein (GFAP) as shown by IHC.

Figure 2: Xenografted tumors retained the EGFR and EGFRvIII expression of the primary tumor. WB showing EGFR and EGFRvIII expression in consecutive passages.

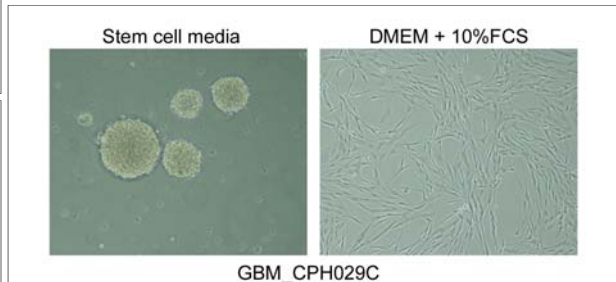


Figure 5: *In vitro* cell cultures of a GBM xenograft. The cells grew as spheres in stem cell media (neurobasal, b27, N2, EGF, bFGF) and adherently in DMEM with serum.

## Future Directions

Verify EGFR and EGFRvIII expression in the established cell lines and compare to original tumor tissue

Investigate *in vivo* tumorigenicity of the cell lines

Characterize possible tumors in relation to original patient tumor

### References:

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