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

Marie-Thérése Stockhausen[1], Mette Villingshøj[1], Mogens Spang-Thomsen[2], Helle Broholm[3], Michael Kosteljanetz[4] and Hans S. Poulsen[1]

[1]Department of Radiation Biology, Section 6321, Finsen Center, Copenhagen University Hospital, Copenhagen, Denmark
[2]Department of Biomedical Sciences, Panum Institute, Copenhagen University, Copenhagen, Denmark
[3]Department of Neuropathology, Copenhagen University Hospital, Copenhagen, Denmark
[4]Department of Neurosurgery, Copenhagen University Hospital, Copenhagen, Denmark

email:marie.stockhausen@rh.regionh.dk
www.radiationbiology.dk

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[1]
- 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[2]
- GBM cells grown *in vitro* lose their expression of EGFRvIII and overexpression of EGFR[3]
- 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[4]

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[2] (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 x 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 4ºC 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

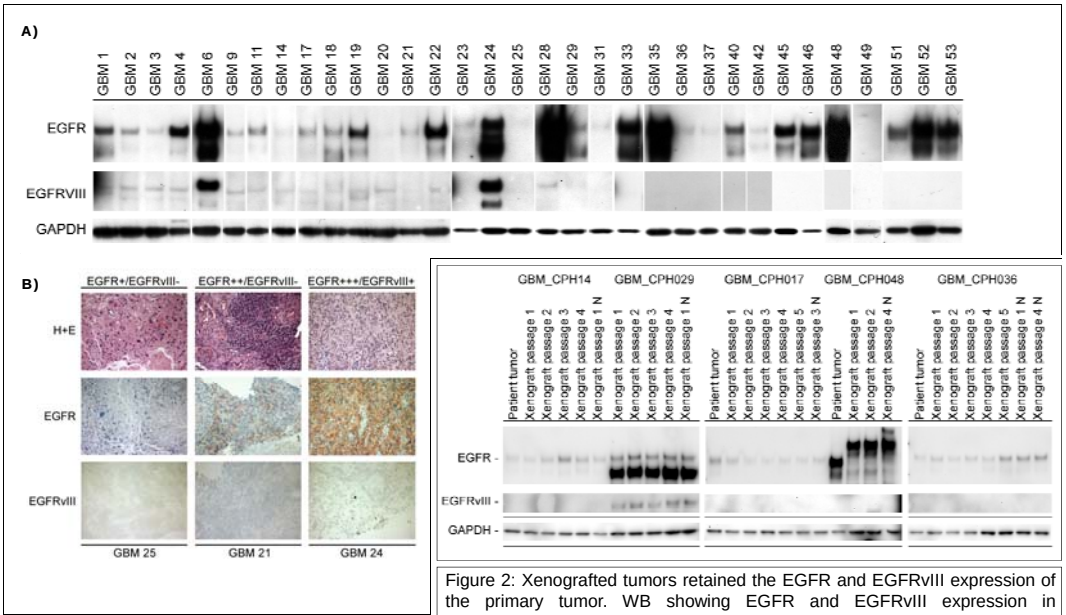

Figure 1: EGFR and EGFRvIII expression in the patient GBM tumors included in the study. A) western blotting (WB) B) immunohistochemistry (IHC) of representative tumors. H+E staining was used to confirm diagnosis.

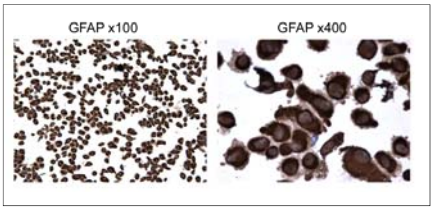

Figure 4. *In vitro* cultured cells were positive for the astrocyte marker glial fibrillary acidic protein (GFAP) as shown by IHC.

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

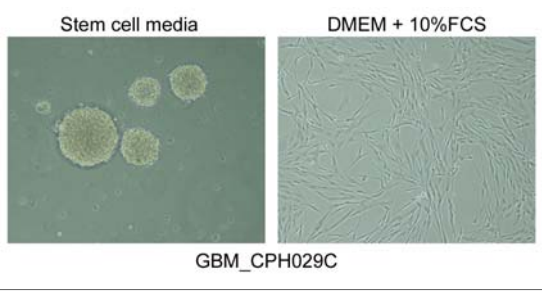

Figure 5: *In vitro* cell cultures of a GBM xenograft. The cells grew as spheres in stem cell media (neurobasal, b27, N2, EGF, bFGF) and adherently in DMEM with serum.

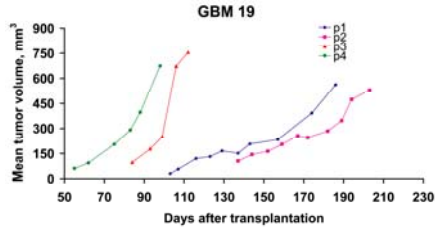

Figure 3: Mean tumor volume of a representative GBM xenografts. The tumor xenografts showed shortened and stabilized lag-time and growth rate following the initial *in vivo* passages in nude mice.

A)

Expression	*Number of Tumors*	*Percentage*
EGFR Low	18	54%
EGFR High	15	46%
EGFRvIII pos	4	12%

B)

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.

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:

1. Pedersen,M.W. *et al. Ann. Oncol.* **12**, 745-760 (2001).
2. Heimberger,A.B. *et al. J. Transl. Med.* **3**, 38 (2005).
3. Pandita,A. *et al. Genes Chromosomes. Cancer* **39**, 29-36 (2004).
4. Lee,J. *et al. Cancer Cell* **9**, 391-403 (2006).