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

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Results

- Surgical biopsies was collected from 54 patients, 18 were excluded due non-GBM diagnosis, necrotic or normal tissue
- Of the 33 patient tumors analyzed by Western Blotting, 46% expressed high levels of EGFR and 12% expressed EGFRvIII (Fig 1, Table 1A)
- The frequency of EGFR expression was as expected, whereas EGFRvIII expression was lower than previously reported. However, some tumors were positive for EGFRvIII by immunohistochemistry and negative by western blotting, indicating low sensitivity by western blotting or intratumoral heterogeneity
- 16 of 36 histologically verified GBM-biopsies were established as xenografts in nude mice, i.e. the take rate was 44%
- 75% of the established xenografts express high levels of EGFR, and 38% express EGFRvIII, in accordance with reported EGFR and EGFRvIII frequencies (Table 1B)
- The xenografts maintained the EGFR/EGFRvIII expression of the primary tumor (Fig 2)

Conclusions

- Patient material included in the study was representative of the reported expression of EGFR and EGFRvIII in GBM
- Xenografts retained the EGFR/EGFRvIII expression of the primary tumor
- Following the initial in vivo passages in nude mice the tumor xenografts grew with reduced and stabilized lag-period and growth rate
- We have established an in vivo model for the development and testing of new treatments and for investigating molecular mechanisms in GBM

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
- Glioblastoma Multiforme (GBM) is the most common and most malignant brain tumor in adults and to which there is no cure
- The tumors can be divided into primary, arising de novo, and secondary, developing from low grade gliomas
- Primary GBMs often express EGFRvIII and overexpress EGFR
- GBM tumors grown in vitro loose their expression of EGFRvIII and overexpression of EGFR, thus there are at present no satisfactory in vivo or in vitro models for GBM

Aim

To establish an experimental model for GBM expressing EGFR and EGFRvIII

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 of tumor tissue into the flanks of 6-week-old female NMRI nu Tac nude mice (Tacnoic, Ry, Denmark). Tumors were measured in two perpendicular dimensions (d1 and d2) and tumor area, A=d1 x d2 was calculated and used to construct mean growth curves and to calculate tumor volume doubling time (TD) from best-fit Gompertz functions as previously described (1).
- Western blotting: Protein lysates (fsg) were separated on precast 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: 4μm sections from formalin fixed paraffin embedded material was used for confirming diagnosis (H+E) and for EGFR (Merck, De). For EGFRvIII (DH3, Novostra, UK), frozen sections were used.

Table 1: Frequency of EGFR and EGFRvIII expression in A) patient material and B) tumor xenografts as detected by western blotting. The frequency of EGFR overexpression is as reported in previous studies, however, the frequency of EGFRvIII expression in the patient material was lower than expected. This is most likely due to insensitivity of western blotting and intratumoral heterogeneity.

References:
1 Rygaard and Spang-Thomsen, 1997.