EGFRvIII Mutation Initiates Gliomagenesis from the Subventricular Zone and Spinal Cord in Mice

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Introduction

Gliomas are the commonest intrinsic tumor of the brain; the malignant form, glioblastoma, has a poor prognosis. The EGFRvIII mutation is found in 30-40% of primary glioblastomas. It is unclear whether this mutation occurs early or late in glioma formation and from where these mutated tumors originate.

Methods

We generated transgenic mice expressing the human EGFRvIII mutation under control of the nestin-cre recombinase (with early expression throughout the CNS). 50 mice were assessed for signs of neurological disease and their brains and spinal cords were analysed for histology, immunohistochemistry, cytogenetics and in vitro growth properties.

Results

EGFRvIII-Nes-cre mice displayed neurological signs from 4 months, with severity of signs necessitating culling at a median age of 8 months. Examination of the brains and spinal cords of these mice revealed multiple, often large, tumors that showed pathological features of glioma on H&E staining. These mice generated gliomas with 100% incidence, of which 10% were high grade gliomas.

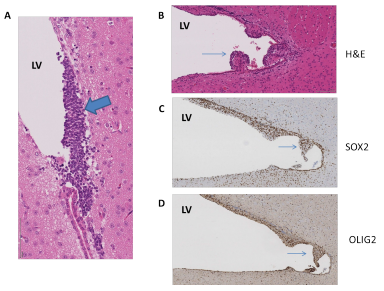
Conclusions

This is the first study to demonstrate EGFRvIII is sufficient to initiate gliomagenesis from the subventricular zone and spinal cord in vivo, with early leptomeningeal spread. Importantly, identification of this mutation as a driver of spinal gliomas suggests these intractable tumours may be amenable to treatment with EGFR inhibitors.

Results (Continued)

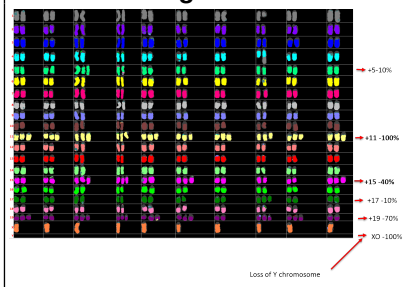
Immunohistochemistry for human glioma markers revealed these tumors were positive for stem cell markers (SOX2, OLIG2, nestin), proliferation (Ki67), and caspase 3 (an apoptotic marker). Analysis of tumors prior to onset of signs revealed the earliest changes were hyperproliferation and tumour precursor lesions (microneoplasias) in the subventricular zone, followed by early leptomeningeal metastasis. Cytogenetic analysis of high grade brain gliomas demonstrated amplification of chromosome 11 (containing EGFRvIII transgene) in 65% of tumours, and of chromosome 15 (containing c-myc) in 40%. Primary cultures of these gliomas were highly sensitive to afatinib (an EGFR inhibitor).

EGFRvIII drives glioma formation from the subventricular zone



Analysis of the subventricular zone as a site of glioma origin of EGFRvIII conditional mice. A - H&E staining showing a hyperproliferative lesion within the SVZ. B -H&E staining shows multiple glioma precursor lesions (microneoplasias) within one SVZ. C and D demonstrate these microneoplasias are positive for neural stem cell immunohistochemical markers (SOX2, OLIG2), reflecting human gliomas. LV = lateral ventricle.

Clonal amplification of Chromosome 11 in an EGFRvIII -mouse glioblastoma



FISH cytogenetic analysis of 10 cells from a single mouse EGFRvIII-driven GBM. All 10 cells contain an extra copy of chromosome 11, suggesting this amplification occurred early in gliomagenesis. Chromosome 11 contains the EGFRvIII transgene, supporting the notion that EGFRvIII is a critical initiator of glioma formation.

Learning Objectives

-Understand the role of EGFRviii in gliomas.

-Discuss the site of origin of gliomas.

-Understand how targeted therapeutics may help treat brain and spinal tumors.

References

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