

EGFR Induces Expression of the Proangiogenic Receptor EphA2 in Cancer Cells



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Background

- EGFR is involved in regulation of cell growth, proliferation, survival, and migration
- Overexpression of the epidermal growth factor receptor (EGFR) and/or expression of a constitutively active variant of EGFR (EGFRvIII) is frequently found in human cancer cells
- Our laboratory has identified the receptor tyrosine kinase EphA2 in a search for EGFR and EGFRvIII regulated genes¹
- EphA2 and its ligands have been associated with repulsion and attraction of neurons and endothelial cells during development
- EphA2 is frequently overexpressed in advanced cancers, and increasing evidence suggest that EphA2 contributes to tumour angiogenesis and metastasis

Aim

To investigate the regulation of EphA2 expression by EGFR and EGFRvIII

Materials and Methods

- Cell lines:** The murine fibroblast NR6, NR6wtEGFR (EGFR), NR6M (EGFRvIII), human head and neck carcinoma cell line HN5, and human skin carcinoma cell line A431
- Quantitative RT-PCR:** The real time PCR reaction was carried out using Trizol extracted RNA and SuperScript III Platinum Two-Step qRT-PCR Kit with SYBR Green (Invitrogen). The PCR cycling conditions: 95°C for 10m, 95°C for 20s, 56.7°C for 20s, and 72°C for 20 s, 40 cycles. The EphA2 products were normalized to RPL13A
- Immunoblot:** 5 µg whole-cell protein lysate was resolved by SDS-PAGE, electroblotted onto nitrocellulose membranes, and incubated with primary antibodies to EphA2, EGFR, EGFRvIII or Tubulin
- Promoter activity assay:** Cells were transiently transfected with a EphA2 promoter fragment cloned into a luciferase reported vector (-4030-EphA2-Luc)² or control plasmid and then stimulated with EGF and/or AG1478/Gefitinib. Luciferase activity was measured using the Luciferase Assay System (Promega)
- Immunohistochemistry:** 4 µm sections from formalin fixed, paraffin embedded material were used for confirming the diagnosis (HE). Slides were deparaffinized, blocked for endogenous peroxidase, and incubated with primary antibodies, followed by secondary antibodies to detect EGFR and EphA2

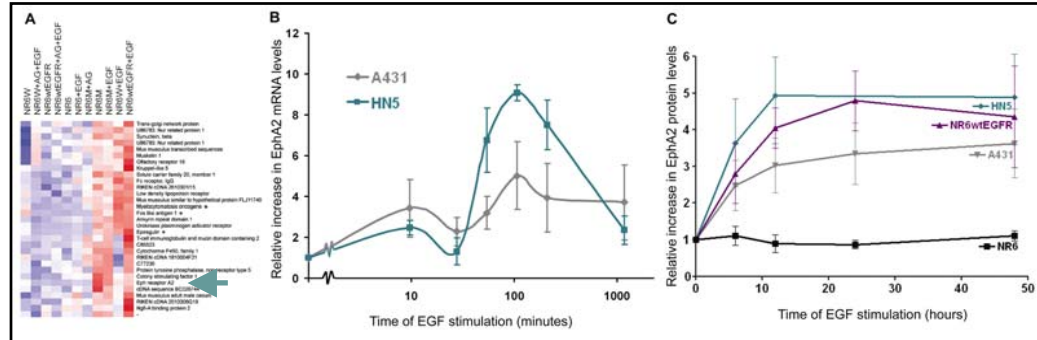


Figure 1: Activated EGFR/EGFRvIII induce the expression of EphA2. A) Cluster C2 from our Affymetrix GeneChip analysis of EGFR and EGFRvIII mediated gene expression B) Relative increase in EphA2 mRNA levels detected by quantitative RT-PCR in HN5 and A431 cells stimulated with 10 nM EGF. C) Quantitative analysis of EphA2 protein levels detected by Western blot analysis using EphA2 specific antibodies in five cell lines stimulated with 10 nM EGF (NR6, NR6wtEGFR, A431 and HN5) or released from AG1478 inhibition (NR6M).

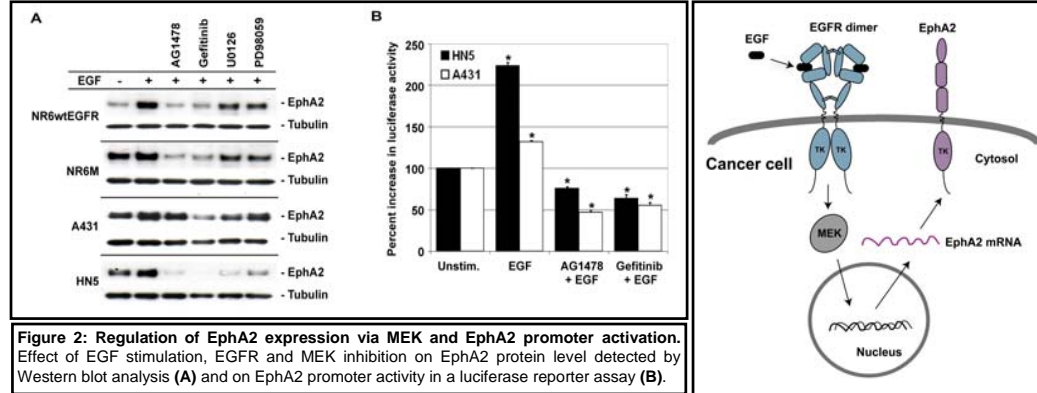


Figure 2: Regulation of EphA2 expression via MEK and EphA2 promoter activation. Effect of EGF stimulation, EGFR and MEK inhibition on EphA2 protein level detected by Western blot analysis (A) and on EphA2 promoter activity in a luciferase reporter assay (B).

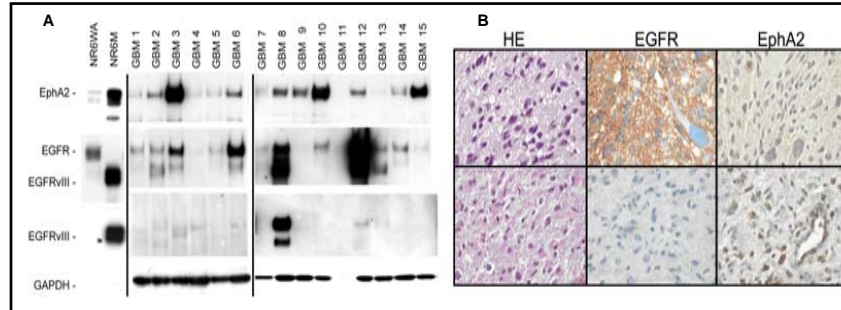


Figure 3: EGFR and EphA2 co-expression in vivo. A) Detection of EGFR/EGFRvIII and EphA2 levels in 15 primary GBM tissues by Western blot analysis using EGFR/EGFRvIII and EphA2 specific antibodies. GAPDH levels used as protein loading controls. B) Immunohistochemistry analysis of two primary GBM.

Results

- In a global search for EGFR and EGFRvIII regulated genes using Affymetrix oligonucleotide arrays the receptor tyrosine kinase EphA2 was identified (Figure 1A)
- EGF stimulation of EGFR expressing cells was found to induce EphA2 mRNA (Figure 1B) and protein levels (Figure 1C)
- Similarly, releasing EGFRvIII expressing cells (NR6M), from inhibition with AG1478 (EGFR/EGFRvIII tyrosine kinase inhibitor) induces EphA2 protein levels (Figure 1C)
- Using a panel of molecule inhibitors the EGFR induced expression of EphA2 was found to be dependent on EGFR tyrosine kinase activity and partially on MEK activity (Figure 2)
- EGF stimulation of A431 and HN5 cells increases EphA2 promoter activity in a luciferase reporter assay (Figure 3)
- Co-expression of EGFR and EphA2 is detected in GBM tissues (Figure 4), indicating that activation of EGFR could contribute to the observed overexpression of EphA2 in GMB

Conclusions

- Activated EGFR and EGFRvIII induce the expression of EphA2
- The regulation is dependent on EGFR tyrosine kinase activity, on downstream activation of MEK and is a direct effect on the EphA2 promoter
- EGFR and EphA2 are expressed in primary GBM tissues
- EphA2 could play a significant role in EGFR mediated tumorigenesis

References:

- Pedersen M.W. *et al.* J Cell Biochem. 2005;96:412-427
- Xu H. *et al.* Am. J. Physiol Renal Physiol. 2004;188:855-866