Cross-talk between EphA2 and EGFR in cancer cells

Alice Bjerregaard Larsen, Marie-Thérése Stockhausen & Hans Skovgaard Poulsen

Department of Radiation Biology, Section 6321, Finsen Center, Copenhagen University Hospital, Copenhagen, Denmark

E-mail:alice.bjerregaard@rh.regionh.dk, www.radiationbiology.dk

Background

- **The epidermal growth factor receptor (EGFR) regulates cell growth, survival and migration**
- **The receptor tyrosine kinases EGFR and EphA2 are frequently overexpressed in advanced cancers and contributes to tumor angiogenesis and metastasis**
- **EphA2 is an EGFR transcriptional target in cancer cells (1;2) that contributes to EGF-induced cancer cell migration (2)**
- **EGF stimulation overrules the inhibitory effects of ephrinA1 stimulation on cell viability (3)**

Materials and Methods

- **Cell lines:** A431 (skin carcinoma), HN5 (head and neck carcinoma), MDA231 (breast carcinoma), U373MG and SKMG3 (glioblastoma)
- **Immunoprecipitation:** Serum starved cells were left umstimulated or stimulated with EGF, EA1 (Ephrin-A1/Fc) and/or gefitinib (Iressa, AstraZeneca). Cell lysates were precleared and immunoprecipitated with 5 µg Ab-1 mouse anti-EGFR (Calbiochem) or 5 µg mouse PY-20 anti-phosphotyrosine (Zymed Lab.). Immunocomplexes were precipitated with protein G agarose beads and analyzed by immunoblot.
- **Immunoblot:** 5 mg lysate was resolved by SDS-PAGE, electroblotted onto nitrocellulose membranes, and incubated with antibodies as indicated.
- **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 to detect EGFR and EphA2, followed by secondary antibodies
- **MTT-assay:** Cells were seeded in 96-well plates in low serum medium, and stimulated with EGF for 72 h. The plates were incubated for 4 h with MTT (Sigma), after which solubilization (10% SDS, 0,03 M HCl) was done over night and absorbance was measured at 570 nm
- **siRNA transfection:** Cells were transfected with either EphA2-siRNA (Qiagen) or RNAi negative control using LipofectAMINE 2000 reagent in OptiMEM I reduced Serum Medium (Invitrogen). After 4 h, the transfection mixture was replaced by low serum medium

Results

EphA2 and EGFR expression in glioblastoma multiforme

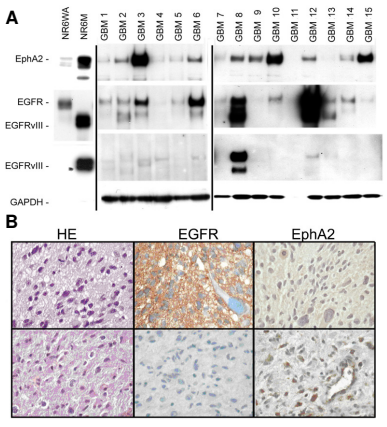


Table 1: EphA2 and EGFR/EGFRvIII detected by Western blot analysis in 46 GBM specimens

Antal GBM tumorer, N	46
EphA2 positive	33 (72%)
EGFR positive	24 (52%)
EGFRvIII positive	3 (6,5%)

Figure 1: A) EphA2 and EGFR/EGFRvIII protein levels detected by Western blot analysis in 15 GBM specimens. **B)** Detection of EphA2 and EGFR by Immunohistochemsitry in two selected GBM samples

EphA2 and EGFR co-localization

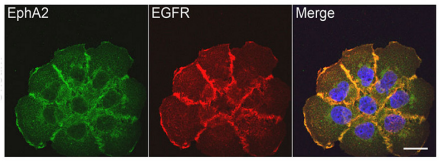


Figure 2: EphA2 and EGFR localization detected by confocal microscopy.

Larsen *et al.* Mol Cancer Res. 2007;5(3):283-293

Aim

To investigate the interaction between EphA2 and EGFR in cancer cells

Results

Effect of EphA2 and EGFR interaction on receptor phosphorylation and downstream signalling

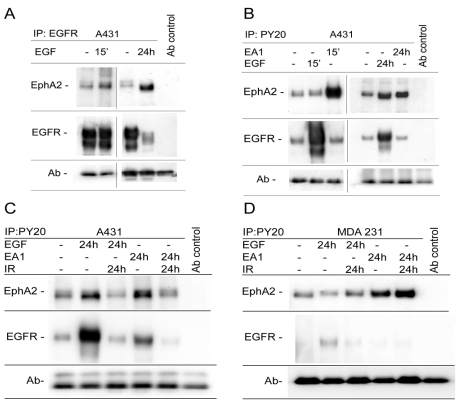


Figure 3: A) EphA2 immunoprecipitates (IP) with EGFR in A431 cells. **B)** EGF-stimulation induce EphA2 phosphorylation. **C)** EphA2 phosphorylation is dependent on EGFR tyrosine kinase (TK) activity **D)** Effect of EGF and EGFR TK inhibition on EphA2 phosphorylation is dependent on high EGFR expression level. Detected by Western blot analysis. Ab: antibody, EA1: ephrinA1/Fc, IR: Iressa/gefitinib

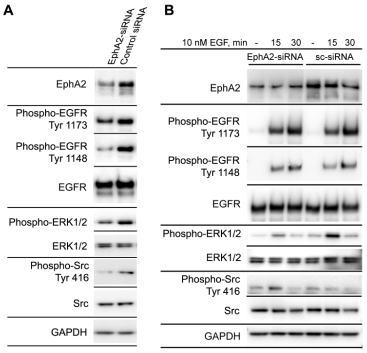
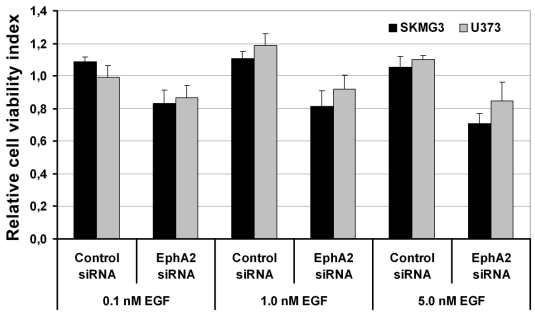


Figure 4: A) Inhibition of EphA2 expression in HN5 cells by siRNA inhibits EGFR, ERK and SRC phosphorylation. **B)** EphA2 downregulation inhibits EGF-induced ERK activation. Detected by Western blot analysis. GAPDH is used as protein loading control.

EphA2 downregulation inhibits EGF-induced cell viability



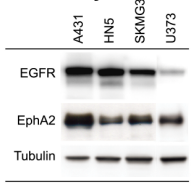


Figure 5: A) EphA2 downregulation by siRNA inhibits EGF-induced cell viability. Analysed by MTT-assay. **B)** Protein expression of EGFR and EphA2 in selected cell lines. Western blot analysis. Tubulin is used as protein loading control.

Conclusion

EGFR and EphA2 is expressed in glioblastoma multiforme and co-localize to the cell surface (Fig. 1 & 2). EphA2 phosphorylation is dependent on EGFR tyrosine kinase activity and EphA2 downregulation inhibits EGFR phosphorylation, downstream signalling and EGF-induced cell viability (Fig. 3, 4 & 5).

References: 1) Pedersen MW *et al.* J Cell Biochem. 2005;96:412-427, 2) Larsen AB *et al.* Mol Cancer Res. 2007;5(3):283-293, 3) Larsen AB *et al.* Cell. Signal. 2010;22(4): 636-44.