The role of EphA2 in EGF-induced cancer cell proliferation and motility

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Background

- The epidermal growth factor receptor (EGFR) is involved in regulation of cell growth, proliferation, survival, and migration
- Our laboratory has recently identified the receptor tyrosine kinase EphA2 as an EGFR transcriptional target[1,2]
- EphA2 is frequently overexpressed in advanced cancers, and increasing evidence suggest that EphA2 contributes to tumor angiogenesis and metastasis

Aim

To investigate the effect of EphA2 activation on EGF-induced cancer cell proliferation and motility

Materials and Methods

- **Cell lines:** The human head and neck carcinoma cell line HN5, the human skin carcinoma cell line A431, the human glioblastoma cell line SKMG3 and the human astrocytoma grade III cell line U373MG
- **Immunoprecipitation:** Cell lysates were precleared and 1000 µg protein was 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
- **Immunoblot:** 5 µg whole-cell protein lysate was resolved by SDS-PAGE, electroblotted onto nitrocellulose membranes, and incubated with primary antibodies to EphA2 (Santa Cruz Biotechnology), EGFR (Fitzgerald Ind.), phoshorylated ERK or total ERK (Cell Signaling Tech.)
- **MTT-assay:** Cells were seeded in 96-well plates in low serum medium, and stimulated with EGF and/or Ephrin-A1/Fc 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
- **Wound-healing assay:** Confluent cells were wounded, and the remaining cells treated with EGF and/or Ephrin-A1/Fc in low serum media. Migration of cells was observed at 0, 24 and 48 h. Random selected images were acquired with a phase contrast microscope (Nikon) and wound sizes were measured

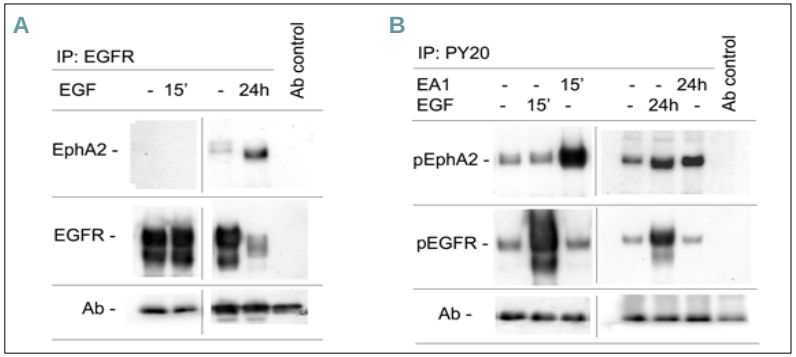

Figure 1: EGFR and EphA2 coimmunoprecipitation and transphosphorylarion. A) Serum-starved A431 cells stimulated with 10 nM EGF for 15 min or 24 h. Immunoprecipitation of EGFR and detection of EphA2 and EGFR by Western blot analysis. **B)** Serum-starved A431 cells stimulated with 10 nM EGF or 1 µg/ml Ephrin-A1/Fc (EA1) for 15 min or 24 h. Immunoprecipitation with anti-PY20 antibody and detection of EphA2 and EGFR by Western blot analysis.

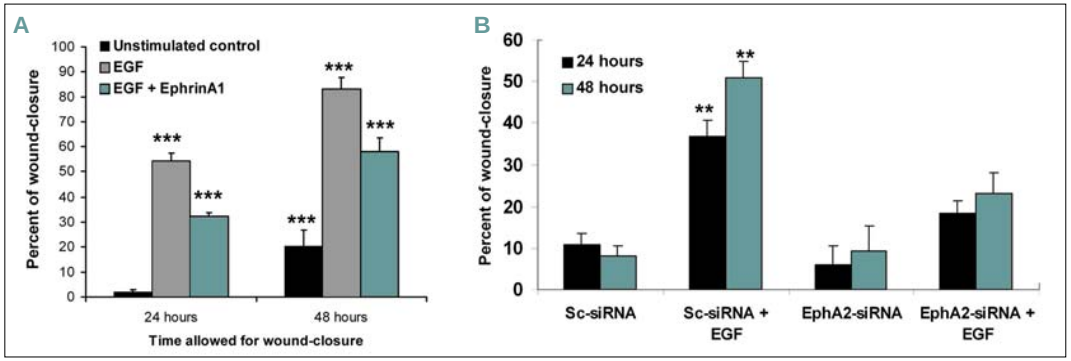

Figure 2: **Effect of EphrinA1 stimulation and EphA2 downregulation on EGF-induced cancer cell motility. A)** Wound-healing assay using serum-starved HN5 cells stimulated with 1 µg/ml Ephrin-A1/Fc and/or EGF. **B)** Wound-healing assay using HN5 cells transfected with EphA2 siRNA or negative control siRNA incubated with or without EGF stimulation for 24 or 48 h. *, P<0.05; **, P<0.001; ***, P<0.0001 compared with the zero time point.

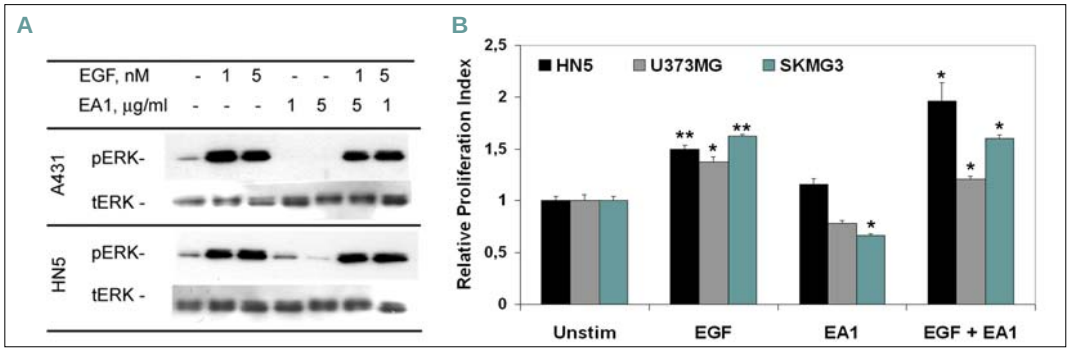

Figure 3: Effect of EphrinA1 stimulation on EGF-induced ERK activation and cancer cell proliferation. A) Western blot analysis showing protein levels of phophorylated ERK (pERK) and total ERK (tERK) of serum-starved A431 and HN5 cells stimulated with EGF and/or Ephrin-A1/Fc (EA1) for 15 min. **B)** MTT-assay with serum-starved HN5, U373MG and SKMG3 cells stimulated with 1.0/0.1 nM EGF and/or 5 µg/ml Ephrin-A1/Fc (EA1) for 72 h. *, P<0.05; **, P<0.001 compared with unstimulated cells

Results

- EphA2 and EGFR coimmuno-precipitatation is detected after EGF stimulation for 24 hours indicating an association between activated EGFR and EphA2 (Fig. 1A)
- EGF stimulation for 24 hours induce tyrosine phosphorylation of EphA2. This suggests that association of EGFR and EphA2 results in transphorpshorylation of EphA2 (Fig. 1B)
- Ligand stimulation of EphA2 induce EphA2 downregulation[2] and inhibits EGF-induced cancer cell motility (Fig. 2A). Similarly, EphA2 downregulation by siRNA inhibits EGF-induced cell motility (Fig. 2B). These results suggest that EGF-induced motility is dependent on EphA2 expression
- EphrinA1 stimulation inhibits ERK phosphorylation and cell proliferation in absence of EGF stimulation (Fig. 3A and 3B) and indicates that the role of EphA2 in regulation cell proliferation is insignificant when EGFR is activated

Conclusions

- **Ligand activated EGFR induce trans-phosphorylation of EphA2**
- **EphA2 downregulation inhibits EGF-induced cancer cell motility**
- **Ligand stimulation of EphA2 have no significant effect on EGF-induced cancer cell proliferation**

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,