

Roles of non-canonical phosphorylation in negative feedback regulation and intracellular trafficking of oncogenic EGFRvIII in glioblastoma cells

1. ERK-mediated negative feedback regulation of ErbB4

ErbB4 has four different isoforms classified by variants at extracellular juxtamembrane domain (JM-a and JM-b) and intracellular domain (CYT-1 and CYT-2). Here we used JM-b/CYT-1 isoform to investigate the roles of serine/threonine phosphorylation in MEK-ERK-dependent feedback control. TPA as an activator of ERK pathway exhibited strong induction of ErbB4 phosphorylation at Thr-674, the conserved common feedback site in the intracellular JM domain of ErbB receptors, which resulted in downregulation of tyrosine autophosphorylation. We also identified Ser-1026 as a novel ErbB4-specific ERK target site in the CYT-1 region. Moreover, double mutations (Thr-674/Ser-1026 to Ala) significantly upregulated ErbB4 activation, indicating that Thr-674 and Ser-1026 cooperatively regulate negative feedback regulation. Given the fact that ErbB4 mutation is one of the most commonly altered genes in melanoma cells, we demonstrated that a typical oncogenic ErbB4 mutant was resistant to the negative feedback regulation to maintain highly active status of tyrosine kinase activity. Together, these findings indicate that feedback mechanisms are key switching for oncogenic potentials of ErbB receptor kinases.

2. Negative feedback regulation of oncogenic EGFRvIII by ERK-mediated non-canonical phosphorylation in glioblastoma cells

EGFRvIII, the truncation mutant that lacks exon 2-7 in the extracellular domain, is the most common EGFR mutant in glioblastoma multiforme (GBM). EGFRvIII is a constitutively activated receptor that unable to bind ligand. The expression of this mutant is often correlated with a poor

patient prognosis due to its ability to extend the length of downstream signaling. To date, the role of negative feedback in an oncogenic EGFRvIII mutant remains unclear. In the present study, we showed that activation of the MEK-ERK pathway led to phosphorylation of Thr-402, a conserved negative feedback residue in the juxtamembrane domain corresponding to Thr-669 of wild type EGFR (EGFRwt), which resulted in rapid reduction of tyrosine phosphorylation of EGFRvIII in U87MG human glioblastoma and HEK293 cells. Moreover, in spite of incapability of EGFRvIII to bind ligand, EGF consequently caused downregulation of tyrosine phosphorylation of EGFRvIII via activation of endogenous EGFRwt-ERK pathway. These results demonstrated the conserved negative feedback mechanism in activation of EGFRvIII, which present a new aspect in functional interactions between EGFRvIII and EGFRwt in glioblastoma cells.