Modulation of epidermal growth factor receptor function by mutations within the actin -binding domain

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Abstract

The generation of site-directed mutants within the actin binding domain of the EGF receptor modulates receptor function in internalization and ligand binding. In addition, truncation of the EGFr at residue 996 results in a loss of high affinity ligand binding, inhibited internalization and reduced signaling capacity. Mutation of tyrosine 992 to phenylalanine (Y992F) and glutamate 991 to glutamine (E991Q) increases the rate at which receptors are internalized. The presence of a phenylalanine residue eliminates EGFr-mediated phosphorylation at Tyr992 while the E991Q mutation might also eliminate phosphorylation at this position due to a disruption of the kinase recognition motif. Thus, phosphorylation of Tyr992 appears to function in the regulation of receptor internalization. The mutation of tyrosine 992 to a glutamate residue (Y992E) causes a three-fold increase in receptor affinity for its ligand and demonstrates the existence of novel third and potentially fourth affinity states for the EGFr. A very high affinity EGFr state with a K$\sb{\rm d}$ of approximately 10 pM has been identified as has an intermediate state of 1.5 nM. The deletion of the C-terminal 190 amino acids of the EGFr causes a complete abolition of the previously observed high affinity state of the EGFr and also causes a significant reduction in the affinity of the low affinity state of the EGFr. Phorbol ester treatment of wild type and mutant EGFr causes a loss of the high affinity receptors, and also a decrease in the overall affinity of the receptor for its ligand which is similar to the loss seen in the deletion mutant. This suggests that control

of the affinity state of the EGFr is mediated through the C-terminal 190 amino acids of the receptor. In addition, the C-terminal 190 amino acids of the receptor have been identified as containing a domain which regulates the phorbol ester induced conversion of receptor affinity. The amino acid composition in the vicinity of tyrosine 992 has been shown to play a role in the internalization of the EGF receptor and in the regulation of receptor affinity for its ligand. ^

Subject Area

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