EGFR expression and activation in bovine cumulus cells and EGFR intramolecular regulation through interactions between tyrosines

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Abstract

The epidermal growth factor receptor (EGFR) plays important roles in the control of many fundamental cellular processes including cell cycle, cell migration, cell metabolism and survival, cell proliferation and differentiation, as well as regulation of oocyte maturation and embryonic development. In the first part of this work I studied the EGFR expression and activation in cumulus cells (CCs). CCs are special cells immediately surrounding the oocyte. It has been shown that CCs in the isolated cumulus cell/oocyte complexes (COCs) exhibit both a slow rise in intracellular calcium concentration ($[Ca^{2+}]_i$) and plasma membrane permeabilization in response to epidermal growth factor (EGF) stimulation. But cultured individual bovine CCs rarely showed a $[Ca^{2+}]_i$ increase. The lack of response was confirmed to be due to a decrease of expression of endogenous EGFRs after dissociation. After CCs were reconstituted EGFR expression they showed robust, prolonged, EGF-stimulated $[Ca^{2+}]_i$ elevations characteristic of CC responses in intact COCs followed by CC permeabilization and death. These responses were also confirmed being mediated by the IP_3 signaling pathway. This EGFR activated Ca^{2+} response in CCs followed by cell death may play an important role in the regulation of oocyte maturation. ^ In the second part of this work, I identified an EGFR intramolecular regulation mechanism through study of the tyrosine phosphorylation in the EGFR regulatory domain (RD). EGFR signaling is partly controlled by tyrosine phosphorylation on the RD. There are 5 major tyrosine phosphorylation residues (992, 1068, 1086, 1148 and 1173) whose phosphorylation functions as the main platform for recruitment of downstream components. In order to understand the effect of intramolecular interactions among EGFR RD tyrosine residues, we

constructed a series of single site mutant RDs. Each one replaces one major tyrosine phosphorylation residue with phenylalanine. After *in vitro* phosphorylation, the phosphorylation degree of each major tyrosine residue was quantitatively compared between mutant RDs and wild type RD using LC-ESI Ion Trap mass spectrometry. Our results indicate that Y1068 increases the phosphorylation of Y1148 and Y1173, Y1086 inhibits the phosphorylation of Y1068, Y1148 inhibits the phosphorylation of Y992 and Y1086, and Y1173 inhibits the phosphorylation of Y1068. Thus the EGFR exhibits extensively intramolecular interaction among its major tyrosine phosphorylation sites. Such intramolecular interaction may increase the complexity of EGFR signal transduction as well as modulate its efficacy. ^

Subject Area

Biology, Molecular|Biology, Cell|Chemistry, Biochemistry

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