

EGFR AND ErbB2 PHOSPHORYLATION IS STRONGLY DEPENDENT ON GANGLIOSIDES

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ErbB2 and EGF receptors are two members of the ErbB receptor superfamily of tyrosine-kinases. Among the different tissues, they are of particular importance in the mammary gland during growth, differentiation, and suckling. Moreover the overexpression of ErbB2 and EGFR is main feature of poor prognosis mammary adenocarcinomas.

Gangliosides are important glycosphingolipids involved in many physio-pathological cell events, because of their ability to mediate cell function through modulation of growth factor receptors, like EGFR and ErbB2.

To point out the role of gangliosides in modulating ErbB2 and EGFR activation in HC11 mammary epithelial cell line, we analysed receptor activation in control and ganglioside-depleted cells. Ganglioside depletion was obtained by treatment with 30 μ M 1-phenyl-2-decanoylamino-3-morpholino-1-propanol (PDMP) for 5 days at 37°C, followed by stimulation of receptors with 10 nM EGF for 15 min at 37°C.

Western blot analyses of total cell lysates revealed that phosphorylated EGFR was visible as three bands, having an Mr range of 170-190 kDa, contrarily to EGFR in control cells that was identified as a single 170 kDa band. Moreover, EGFR was phosphorylated in ganglioside-depleted cells also in the absence of the proper stimuli. ErbB2 maintained the same phosphorylation profile in control and in PDMP-treated cells. Analyses of the immunoprecipitates, obtained with specific antibodies, indicated that only the band of EGFR with higher molecular mass immunoprecipitates with anti-ErbB2 antibody. To further investigate EGFR activation, we evaluated it by digestion with calf intestine alkaline phosphatase (CIAP) by adding 5 or 10 units CIAP for 30 min or 1 hour. Results clearly indicated that EGFR underwent sensitive variations after CIAP treatment. Indeed, EGFR displayed two co-migrating bands in PDMP-treated control and EGF-stimulated cells, further confirming the main role of gangliosides in EGFR activation and stimulation.

- 1) Milani S. et al. 2007 Ganglioside GM3 is stably associated to tyrosine-phosphorylated ErbB2/EGFR receptor complexes and EGFR monomers, but not to ErbB2. *Biochim Biophys Acta*, 1771, 873-8.