Quantitative description of the constitutive and ligand-induced associations of ErbB receptors

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We presented a statistically reliable, flow cytometric homo-fluorescence resonance energy transfer (homo-FRET) method for quantitative characterization of the homoassociation of ErbB1 and ErbB2. ErbB1 and ErbB2 receptors were labeled by a mixture of unlabeled and fluorescent antibodies and homo-FRET was measured between two spectroscopically identical fluorophores. The excitation energy is distributed in the ensemble of molecules by homo-FRET. The only manifestation of homo-FRET is decreased fluorescence anisotropy which depends on the concentration of fluorophores. The decrease in the concentration dependent anisotropy shows whether the molecule is monomeric, dimeric, trimeric or forms higher order oligomers.

In quiescent A431 cells most ErbB1 receptors are monomeric and stimulation with EGF or serum leads to an increase in the cluster size of ErbB1 due to ligand-induced homodimerization of ErbB1. On the contrary, most of the ErbB2 proteins are inactivated and present in large homoclusters in unstimulated SKBR-3 cells whose size decreases upon EGF, heregulin or serum stimulation. We attribute this phenomenon to the recruitment of ErbB2 to heterodimers with ligand-activated ErbB1 and ErbB3 resulting in the removal of ErbB2 from homoclusters.

We developed the FRET-sensitized acceptor bleaching (FSAB) technique to quantitate the ratio of ErbB1 and ErbB2 in their heteroclusters by confocal microscopy. Briefly, a photostable donor excites a photolabile acceptor by FRET, and the acceptors within FRET distance to the donor will get photobleached and the fraction of acceptor molecules in the vicinity of donors can be determined. AlexaFluor546 and Cy5 fluorophores were used as a photostable donor and a photolabile acceptor, respectively.

In unstimulated SKBR-3 cells almost half of ErbB1 receptors form heteroclusters with ErbB2 and after EGF treatment, the fraction of heteroclustered ErbB1 did not change significantly, because EGF induces the formation of both ErbB1 homodimers and ErbB1-ErbB2 heterodimers. On the contrary, only 10% of ErbB2 is in heteroclusters with ErbB1 in quiescent SKBR-3 cells and this fraction doubles upon EGF stimulation, because ligand-activated ErbB1 recruits ErbB2 proteins from the large ErbB2 homoclusters.

The large-scale clusters described by us are the place of signal transduction mediated by ErbB receptors where rearrangements take place upon ligand binding. The large ErbB2 homoassociations present in quiescent cells ensure that ErbB2 proteins are easily accessible for ligand-activated ErbB1 and ErbB3 to form heteroclusters. The receptors in large clusters are present in high local concentration which is favorable for signal transduction activated by growth factors. In this way the clusters are the places of physiological and pathological signal transductions. A better understanding of these clusters may shed light on the mechanisms of malignant cell proliferation and its inhibition.

Keywords:
ErbB proteins, homo-FRET, anisotropy, FRET-sensitized acceptor bleaching (FSAB)

Kulcsszavak:
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