Abstract for PhD thesis – Elza Friedländer, MD

Molecular interactions of ErbB receptor tyrosine kinases – with an outlook on their therapeutic targeting

ErbB1 (EGFR) and ErbB2 belong to the ErbB family of class I growth factor receptor tyrosine kinases and are frequently implicated in human cancers, thus becoming targets of molecular therapies. As such, their interactions with each other and various cell surface molecules during activation as well as upon targeting with therapeutic antibodies (such as trastuzumab against ErbB2) are of special interest. Our aim was to contribute to the understanding of mechanisms behind ErbB1 and ErbB2 activation and transactivation, and compare, in this respect, trastuzumab resistant and sensitive breast tumor cell lines. We have used fluorescence correlation spectroscopy and derivatized paramagnetic microspheres in conjunction with immunofluorescence in confocal microscopy and digital image processing. Our results can be summarized as follows:

- ErbB1-eGFP chimeric molecules are functionally intact, and can serve as a good model system for mobility measurements when stably expressed in CHO cells. Fluorescence correlation spectroscopy (FCS) could resolve a slow, $1.17\pm0.51\times 10^{-9}$ cm^2/s intramembrane diffusion component of the fused receptor in addition to fast cytoplasmic diffusion and photochemical processes in the eGFP tag. FCS measurements were selective for diffusible molecular species as not or very slowly diffusing eGFP tags needed to be photobleached for average fluorescence to stabilize.
- Stimulation with EGF resulted in a reduction by 50% of ErbB1 membrane diffusion and by 25% of the concentration of diffusing particles, coherent with the idea of ligand induced aggregation. However, this would also cause an increase of the relative fluorescence per particle, but this parameter did not change. Consequently, it is likely that pre-formed ErbB1 dimers are present on the cell surface, that upon EGF binding get conformationally activated and associate with less mobile ErbB1 clusters that have already been photobleached, or interact with the cytoskeleton yielding slower average diffusion.
- FCS disclosed ErbB1 diffusion rates an order of magnitude faster than FRAP measurements for the same ErbB1-eGFP chimera. Also, diffusion resolved by FCS exhibited no anomaly (spatial hindrance) within the small sub-micron detection spot, while FRAP showed that influx of fluorescent species from distal areas into the bleached spot is a result of anomalous diffusion. Thus FCS and FRAP are complementary both in terms of assessing local and larger range diffusion, as well as being selective to faster and slower fluctuations, respectively.
- In various cell lines expressing ErbB1 and ErbB2 at different quantities, EGF and trastuzumab coated paramagnetic microspheres proved to be functional and stimulated local phosphorylation of the targeted ErbB1 or ErbB2 receptors. The signaling events remained laterally localized, and activated the internalization machinery.
- EGF coated microspheres transactivated also ErbB2, while trastuzumab coated ones did not transactivate ErbB1, indicating that trastuzumab binding favors ErbB2 homodimers. The extent of ErbB2 transactivation by EGF beads was directly proportional to increasing surface density of ErbB1, implying that in the presence of activated ErbB1, formation of ErbB1-ErbB2 heterodimers is favorable.
- Trastuzumab binds less on the surface of JIMT-1 cells, its dissociation constant is a magnitude higher than on SKBR-3. ErbB2 phosphorylation is low on JIMT-1 and is not enhanced substantially by trastuzumab. However, ErbB2 on these cells is functional, since high ligand density on trastuzumab-coated microspheres could evoke local ErbB2 activation. The steric effect likely exerted by the beads also hints at the possibility of hindered trastuzumab binding on JIMT-1.
- The mucosialoprotein MUC4 was found to be overexpressed on JIMT-1 cells and its expression was negatively correlated with trastuzumab binding. Given the large size and abundance of MUC4, it could well play a role in sterically hindering trastuzumab binding, and as such, might be a useful predictor of the success of ErbB2-directed antibody therapies.

Key words: ErbB1, EGFR, ErbB2, trastuzumab, trastuzumab resistance, RTK activation, RTK transactivation, fluorescence correlation spectroscopy, derivatized paramagnetic microspheres, immunocytochemistry, confocal microscopy, digital image processing