Exploring ErbB Membrane Organization by TEM & Agent-based Modeling

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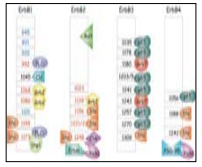
Abstract

We report the first topographical data comparing the native distributions of ErbB family members in SKBR3 breast cancer cells. These cells express ErbB2>>ErbB1>ErbB3. These highly transformed cells have few or no caveolae. We show that ErbB2 is highly phosphorylated in serum starved cells, strongly clustered on membrane sheets examined by immunoelectron microscopy, and catalytically active. ErbB2 activity and phosphorylation state is slightly enhanced by EGF addition, presumably via heterodimerization with ErbB1. Contrary to current dogma, we find ErbB3 has active kinase activity. ErbB3 activity is not due to co-precipitation with ErbB1 or ErbB2. Coclustering of ErbB2 with ErbB1 and ErbB3 is low and slightly increased by ligands for ErbB1 and 3. Consistent with ErbB2 activation in the absence of ligands for its hetero-dimerizing partners, some adaptors (ie, Shc) are pre-associated with the membrane; Shc is lost from the membrane after treatment with ErbB kinase inhibitors and further upregulated by EGF. We also present preliminary results of an agent-based, stochastic model designed to simulate receptor clustering, dimerization & signal propogation in a spatially realistic manner.



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ErbB expression levels in SKBR3 cells	
erbB1	4.34 x 10^4
erbB2	1.86 x 10^6
erbB3	1.55 x 10^4
erbB4	--

Fig 1. Kinetics of ErbB kinase activation & inhibitor sensitivity

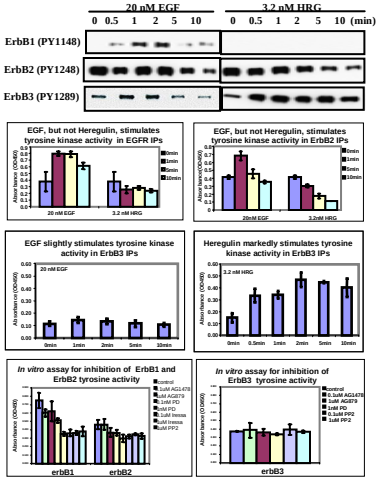


Fig 2. Translocation of adaptor proteins after ligand addition

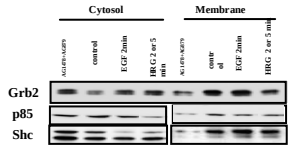
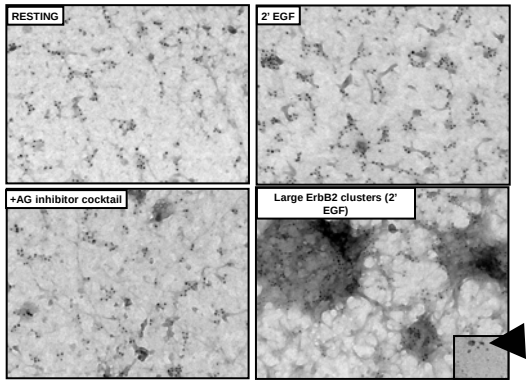
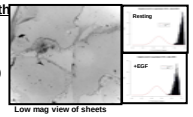


Fig 3. ErbB2 is preclustered in serum-starved SKBR3 cells. Clusters do not disperse after kinase inhibitor treatment; large clusters are slightly more prominent after EGF treatment.

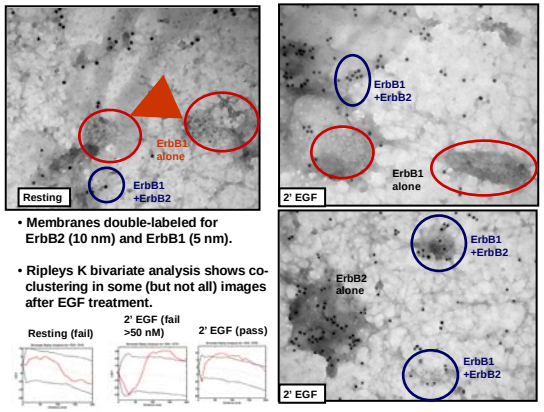


Here, treated and untreated cells were prefixed with 0.5 % PFA, ripped and then sheets were postfixed prior to labeling for ErbB2 cytoplasmic tails. Identical results were obtained under several different post-fixation protocols (ie, 2% PFA for 30 min *or* 4% PFA + 0.1 % glut for 15 min). Hopkins tests (right) confirm that the distribution is markedly non-random.



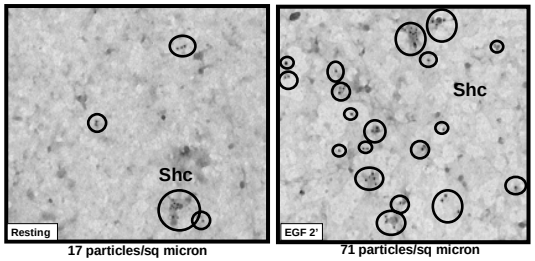
Low mag view of sheets

Fig 4. Coclustering of ErbB2 with ErbB1 (below) & ErbB3 (not shown) is only occasionally observed in resting cells. It is more common, but incomplete, in activated cells.



- Membranes double-labeled for ErbB2 (10 nm) and ErbB1 (5 nm).
- Ripleys K bivariate analysis shows co-clustering in some (but not all) images after EGF treatment.

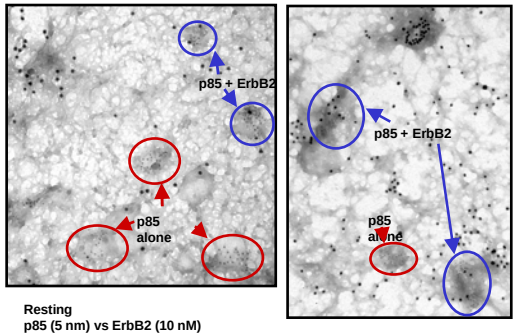
Fig 5. Consistent with blotting results, Shc is present on resting membranes & further recruited by EGF treatment



17 particles/sq micron

71 particles/sq micron

Fig 6. PI3-Kinase is clustered on resting & Heregulin-stimulated membranes. It is partially colocalized with ErbB2.

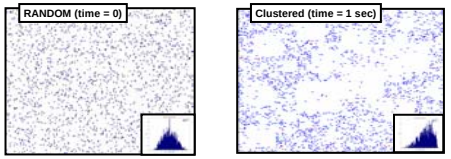


Resting
p85 (5 nm) vs ErbB2 (10 nm)

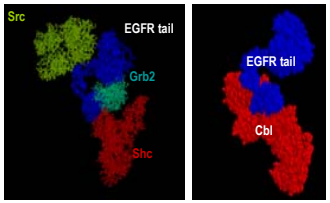
1 min Heregulin
p85 (5 nm) vs ErbB2 (10 nm)

Fig 7. Development of an Agent-based Platform for Spatio-Temporal Modeling

- Can handle species at low concentrations
- Molecules diffuse & react with nearby particles
- Traces details of each molecule at each time step.



Computer simulation of ErbB2 clustering using the agent based model.



Coarse Grain "Docking" of SH2-containing proteins to the tail of EGFR. Results suggest tail may be able to bind Src, Shc AND Grb2 at the same time. Once Cbl binds, other sites are likely to be blocked. These computer "docking" experiments will establish rules for our future simulations of the pathway.

References & Acknowledgements.

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