NEWS AND VIEWS

The interactome of human EGF/ErbB receptors

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Molecular Systems Biology 31 January 2006; doi:10.1038/msb4100048

When you search PubMed for 'EGF receptor' (EGFR), the database returns more than 5000 hits. This is not entirely surprising given the receptor's eminent role in cancer biology. However, it is truly surprising that two recent papers published by the groups of Gavin MacBeath and Matthias Mann add a whole new dimension to the problem of EGFR signaling. They do that by using protein microarray and quantitative proteomics technologies to comprehensively and quantitatively identify proteins associated with the cytosolic domains of the human EGFR family (Jones *et al.*, 2005; Schulze *et al.*, 2005).

This family of membrane receptors consists of four different proteins called EGFR/ErbB1/HER1, ErbB2/Neu/HER2, ErbB3/HER3, and ErbB4/HER4. Under normal physiological conditions, the ErbB receptors play crucial roles in propagating signals regulating cell proliferation, differentiation, motility, and apoptosis (Holbro and Hynes, 2004). They are activated by ligand binding, which leads to homo- or heterodimerization followed by trans-phosphorylation of specific tyrosine residues. These phosphorylated tyrosines, in turn, provide recognition sites for cytoplasmic proteins, which link ErbB receptors to downstream signaling transduction cascades such as the MAP kinase pathway. Among all ErbB family members, EGFR and ErbB4 are fully functional receptor tyrosine kinases, whereas ErbB2 does not bind any known ligand and ErbB3 has no intrinsic kinase activity (however, they still affect the activity of their partners by heterodimerization). As overexpression of EGFR and ErbB2 receptors is often found in several human tumors such as breast, lung, head, and neck (Marmor *et al.*, 2004), their precise role in the cell is of particular biological and pharmacological importance.

In the first large-scale ErbB-interactome study, Schulze *et al.* (2005) used a quantitative proteomic approach for identifying proteins associated with phosphotyrosine motifs of the ErbB-receptor members. To this end, they generated 89 different 'bait' peptides in phosphorylated and unphosphorylated forms covering all intracellular tyrosine residues of the ErbB-family members. In the next step, these peptides bearing phosphorylated and unphosphorylated tyrosine residues were incubated with HeLa cell lysates and pulldown experiments were performed to enrich specific binding partners for the phosphorylated bait peptides. Lastly, these proteins were identified and quantified by mass spectrometry. Surprisingly, only 40 out of the 89 examined tyrosine residues did have an interacting protein in their phosphorylated form. Most of the tyrosine residues that interacted with specific partners accumulated at

the C-terminal regions of the receptors. This study also indicated that the distribution of interacting partners of the different ErbB members shows clear differences between individual receptors, but also a significant overlap. For example, both EGFR and ErbB4 have multiple binding sites for the adaptor proteins Shc and Grb2, but only EGFR binds the ubiquitin ligase Cbl, whereas ErbB4 is unique in binding Nck. Altogether, Schulze *et al.* found 10 ErbB interactors, all of which have either an SH2 or PTB domain, thus emphasizing the specificity of these signal transduction modules (Pawson and Nash, 2003).

In a related project, Jones *et al.* used protein microarrays to identify ErbB interactors. Since the phosphotyrosines in ErbB receptors are primarily bound by SH2 and PTB domains, Jones *et al.* successfully expressed and purified 106 (out of 109) SH2 domains and 41 (out of 44) PTB domains encoded in the human genome. These domains were then spotted onto glass slides and subsequently probed with ErbB peptides that contained phosphorylated and nonphosphorylated tyrosines, respectively. While Schulze *et al.* used all possible 89 tyrosine-containing ErbB peptides for their pulldowns, Jones *et al.* concentrated on 29 peptides that were known to be phosphorylated in EGFR, ErbB2 and ErbB3, and four peptides that were predicted to be phosphorylated in ErbB4. These experiments revealed that each phosphotyrosine on EGFR binds seven different proteins on average, whereas those on ErbB2, ErbB3 and ErbB4 bind 17, 9 and 2 proteins on average, respectively. This adds up to many interactions, namely 54 with EGFR, 59 with ErbB2, 37 with ErbB3 and 8 with ErbB4, most of which were new. The sobering fact is that the mass spec study by Schulze *et al.* found quite different, and, in fact, much smaller numbers, namely nine, four, four, and eight different interacting proteins for the four receptors, respectively. While at least the number of identified ErbB4 interactors by both approaches appears to be identical, only one protein (Shc) is actually common to both ErbB4 data sets.

Where do these dramatic differences come from? Well, simply from the fact that the two groups looked at very different things: while Schulze *et al.* pulled down proteins that most likely bind to ErbB receptors in HeLa cells, Jones *et al.* looked at the whole SH2/PTB interaction space of ErbB receptors *in vitro*. That is, Jones *et al.* told us which SH2 and PTB domains *may* bind to which receptor if both are present in a cell. In contrast, Schulze *et al.* told us which SH2 and PTB proteins bind to ErbB receptors *specifically in HeLa cells*.

Unfortunately, we have to wait until further studies reveal which proteins can be pulled down in the other 200 + human cell types or, alternatively, which of the ErbB receptors and SH2/PTB proteins are expressed together in those cells. At least we know that the proteins detected in Schulze are coexpressed in HeLa; some differences (e.g., Grb2 interacts with all four ErbB in Schulze but only with ErbB2 in Jones; STAT5 interaction is missed altogether in Jones) might also be due to possible methodological differences. Actually, Jones *et al* reminded us that the task will become even more daunting when thermodynamic aspects come into play. In fact, the MacBeath group measured affinity constants for all their interactions too, but this is another story that will eventually require data about *in vivo* concentrations of all those proteins (in all human cell types!) for meaningful interpretation. Systems biologists need exactly this kind of information after all, and some, such as Oda *et al* (2005), may argue that systems biology only becomes feasible once we have this information in computer-readable form. Never mind the 5000 + papers, at

the end of the day two papers were enough to convince us that we are still at the beginning of systems biology!

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