NEWS AND VIEWS

Towards comprehensive characterization of HER2 overexpression

Alan Berezov and Mark I Greene

Department of Pathology and Laboratory Medicine, University of Pennsylvania School of Medicine and Abramson Family Cancer Research Institute, Philadelphia, PA, USA

Molecular Systems Biology 3 October 2006; doi:10.1038/msb4100097

The past decade witnessed a revolution in molecular targeted therapy against cancer. Development of drugs for specific biological pathways with increased specificity and reduced toxicity has validated the long-held belief in the cancer research community that a precise molecular understanding of cancer can result in cancer therapy. One of the most successful recent examples of cancer-specific drugs is development of Herceptin, a monoclonal antibody against the HER2 receptor for breast cancer therapy. HER2 is an important target in cancer because its overexpression increases tumor cell proliferation, vessel formation and invasiveness and predicts poor prognosis. Disabling the Her2/neu receptor kinase complex with ecto-domain binding monoclonal antibodies (e.g., Herceptin) leads to a reversal of the malignant phenotype of tumor cells and has therapeutic and preventative applications for human malignancy. Despite the important role of HER2 overexpression in tumor progression for a variety of human cancers, the mechanism by which the overexpression dysregulates different signaling networks in cells is poorly understood.

In a study currently published in *Molecular Systems Biology*, Wolf-Yadlin *et al* (2006) make an important contribution to elucidation of this mechanism by studying the effects of HER2 overexpression on EGF- and HRG-mediated signaling of erbB receptors. To that end, they used quantitative mass spectrometry to study the phosphorylation kinetics of specific phosphorylation sites in a variety of proteins involved in the erbB receptor signaling network and compared the results obtained in human mammary epithelial cells with low and high expression levels of HER2. In addition to establishing links between erbB receptor dimerization and activation of specific phosphorylation sites that regulate proliferation and migration, the study enabled identification of novel proteins and phosphorylation sites involved in the erbB signaling pathway.

To quantify the effects of HER2 overexpression in a human mammary epithelial cell (HMEC) line 184A1, the authors compared the parental cell line containing approximately 20 000 HER2 receptors per cell with a stable retrovirally transduced clone that expresses approximately 600 000 HER2 receptors per cell and a comparable number of EGFR and HER3. Both parental and HER2-overexpressing cells were stimulated with either EGF or HRG and the temporal dynamics of tyrosine phosphorylation was studied by mass spectrometry after 0, 5, 10 and 30 min of ligand stimulation. The study identified 332 phosphorylated peptides from 175 proteins. A total of 20 phosphorylation sites were identified on EGFR, HER2 and

HER3 including three novel sites (Y1114 on EGFR and Y1005 and Y1127 on HER2) that have not been previously described in literature. In addition, phosphorylation sites on 15 different proteins in the EGFR canonical signaling pathway and on 16 proteins in the cell adhesion/migration pathway have been quantitatively analyzed. The study makes an original contribution to the field by identifying 122 novel phosphorylation sites that have not been previously described in literature. Using computational methods, including self-organizing map clustering and partial least-squares regression analysis, it also identifies specific combinations of phosphorylation sites that correlate with cell proliferation and migration and that can potentially represent targets for therapeutic intervention.

The study showed that overexpression of HER2 promotes increased cell migration, but does not significantly effect cell proliferation. Notable differences have been observed in cell migration between EGF- and HRG-stimulated cells that over-express HER2. EGF stimulation of HER2-overexpressing cells promoted migration by phosphorylation of proteins that belong to different pathways, including PI3K, MAPK, catenins and FAK. In contrast, HRG stimulation led to activation of a more specific subset of proteins, in particular FAK, Src, paxillin and p130Cas. Cell proliferation was not affected by HER2 overexpression and was primarily driven by EGF stimulation.

Unfortunately, owing to sensitivity limitations, only 68 out of 322 phosphorylation sites could be analyzed kinetically using all stimulation conditions and therefore the study does not provide a comprehensive analysis of the multitude of effects produced by HER2 overexpression. It does, however, mark an important breakthrough in characterization of the erbB receptor signaling network in tumors. Advances in the understanding of how tumor cells differ from normal tissue have made possible the development of a new class of targeted cancer therapies that interrupt processes important to tumor survival and progression.

However, in spite of recent advances in molecular biology leading to the introduction of clinically active novel agents, therapy of the most common epithelial tumors remains limited. The resistance to monotherapy can be at least partly explained by the diversity of molecular abnormalities in the tumors, suggesting that the efficacy of cancer therapy could be improved by using combinations of anticancer agents that target complementary signaling pathways. Concerted inhibition of multiple pathways required for tumor growth and progression could have a synergistic effect, resulting in a more

effective inhibition of tumor growth. Recently, a number of studies described important links of the EGFR signaling pathway with proteins that belong to other pathways including survivin (Wang and Greene, 2005), TRAIL (Dubska *et al*, 2005), VEGFR (Ciardiello *et al*, 2006), GPCRs (Ohtsu *et al*, 2006), among others. Two papers used proteomics and microarray approaches to study protein interactions with tyrosine phosphorylation sites of erbB receptors (Schulze *et al*, 2005; Jones *et al*, 2006; Uetz and Stagljar, 2006). The study by Wolf-Yadlin *et al* opens the way to a more thorough characterization of the EGFR signaling network in tumor cells by identifying proteins and phosphorylation sites activated at overexpressed levels of HER2, a condition known to be vital for the progression of numerous tumors. The identified sites can now be further characterized and the nature of their relationship with the EGFR pathway as well as significance as therapeutic targets can be studied in more detail.

References

- Ciardiello F, Troiani T, Bianco R, Orditura M, Morgillo F, Martinelli E, Morelli MP, Cascone T, Tortora G (2006) Interaction between the epidermal growth factor receptor (EGFR) and the vascular endothelial growth factor (VEGF) pathways: a rational approach for multi-target anticancer therapy. *Ann Oncol* **17**: vii109–vii114
- Dubska L, Andera L, Sheard MA (2005) HER2 signaling downregulation by trastuzumab and suppression of the PI3K/Akt pathway: an unexpected effect on TRAIL-induced apoptosis. *FEBS Lett* **579**: 4149–4158
- Jones RB, Gordus A, Krall JA, MacBeath G (2006) A quantitative protein interaction network for the ErbB receptors using protein microarrays. *Nature* **439**: 168–174
- Ohtsu H, Dempsey PJ, Eguchi S (2006) ADAMs as mediators of EGF receptor transactivation by G protein-coupled receptors. *Am J Physiol Cell Physiol* **291**: C1–C10
- Schulze WX, Deng L, Mann M (2005) Phosphotyrosine interactome of the ErbB-receptor kinase family. *Mol Syst Biol* **1**: 42–54
- Uetz P, Stagljar I (2006) The interactome of human EGF/ErbB receptors. *Mol Syst Biol*, Pubmed Identification Number: 2006.0006; doi:10.1038/msb4100048
- Wang Q, Greene MI (2005) EGFR enhances Survivin expression through the phosphoinositide 3 (PI-3) kinase signaling pathway. *Exp Mol Pathol* **79**: 100–107
- Wolf-Yadlin A, Kumar N, Zhang Y, Hautaniemi S, Zaman M, Hyung-Do K, Grantcharova V, Lauffenburger DA, White FM (2006) HER2-overexpression effects on cell signaling networks governing proliferation and migration. *Mol Syst Biol*, Pubmed Identification Number: 54; doi:10.1038/msb4100094