

I. Amit
J. Bao
A. Citri
L. Friedman
G. Gur
M. Katz
G. Kerber
B. Kochupurakkal

S. Lavi
M. Marmor
Y. Mosesson
S. Oved
C. Rubin
M. Shelly
K. Shtiegman
L. Yakir

Negative regulation of oncogenic signaling by receptor tyrosine kinases: implications for cancer therapy

Department of Biological Regulation

Tel. 972 8 934 3974

Fax. 972 8 934 2488

E-mail: yosef.yarden@weizmann.ac.il

Cell-to-cell interactions are essential for embryonic development and for a plethora of physiological processes in adulthood (e.g., wound healing). Along with hormones and neurotransmitters, growth factors are the major messengers of intercellular communication in mammals. Many growth factors bind trans-membrane receptors whose cytoplasmic domain initiates signaling by means of an intrinsic tyrosine kinase activity, and oncogenic processes often exploit growth factor signaling for malignant transformation. An example is provided by the ErbB family of receptors for the epidermal growth factor

(EGF) and neuregulins: self-production of ligands (autocrine loops), truncated ErbB-1 variants and over-expression of ErbB-2 are frequently associated with virulent tumors, such as carcinomas and glioblastomas. Our past studies concentrated on understanding the layered structure of the ErbB network of signaling and its positive regulators, a group of adaptors and enzymes (Fig. 1). Interestingly, a significant portion of the network is devoted to tuning of signals, a process accomplished by a fine balance between positive and negative signaling pathways. Genetic evidence derived from worms and flies

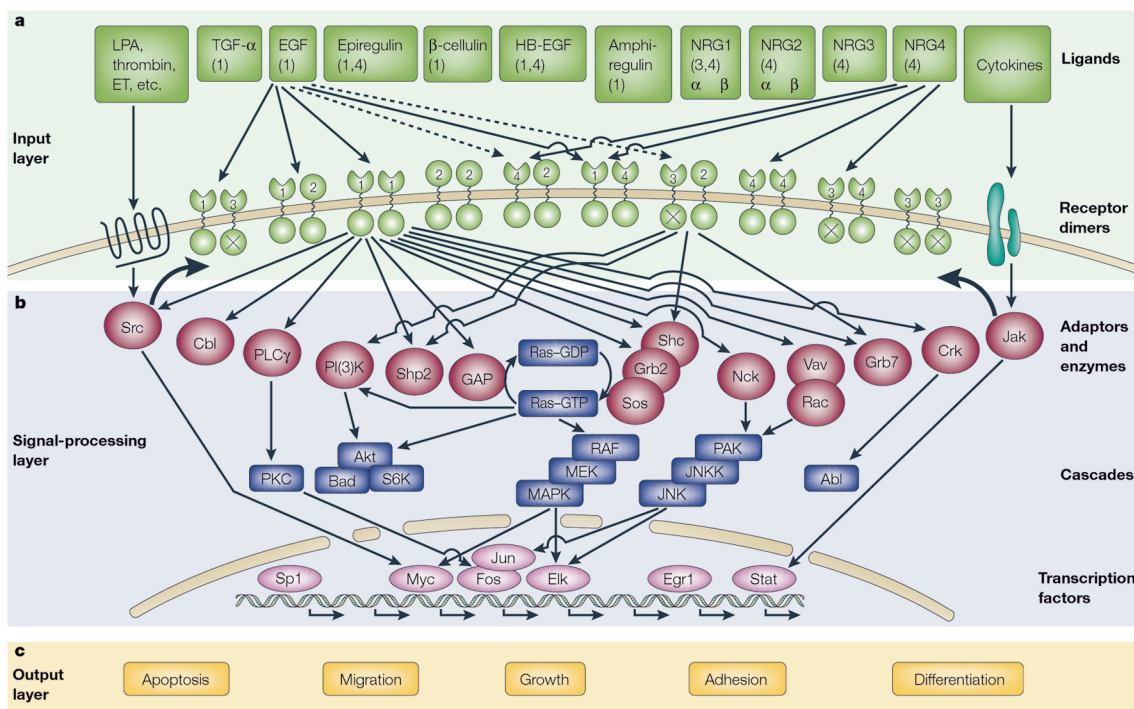


Fig. 1 The ErbB signalling network. a) Ligands and the ten dimeric receptor combinations comprise the input layer.

Numbers in each ligand block indicate the respective high-affinity ErbB receptors. For simplicity, specificities of receptor binding are shown only for epidermal growth factor (EGF) and neuregulin 4 (NRG4). ErbB2 binds no ligand with high affinity, and ErbB3 homodimers are catalytically inactive (crossed kinase domains). Trans-regulation by G-protein-coupled receptors (such as those for lysophosphatidic acid (LPA), thrombin and endothelin (ET)), and cytokine receptors is shown by wide arrows. b) Signalling to the adaptor/enzyme layer is shown only for two receptor dimers: the weakly mitogenic ErbB1 homodimer, and the relatively potent ErbB2-ErbB3 heterodimer. Only some of the pathways and transcription factors are represented in this layer. c) How they are translated to specific types of output is poorly understood at present.

suggests that negative circuits were added to the network relatively late in evolution, and they exhibit unexpected variation and complexity. Concentrating on negative mechanisms, we found that ligand-induced endocytosis and degradation of active receptors is a major regulatory pathway involving not only phosphorylation, but also ubiquitination of receptors and associated molecules. Alongside, constitutive endocytosis and chaperone-mediated stabilization of kinase's conformation are essential for network maintenance. In addition, because ErbB proteins are asymmetrically expressed on the surface of neuronal and epithelial cells, multi-molecular complexes regulating post-synthesis sorting are important for signaling. In-depth understanding of network's desensitization may facilitate development of new cancer therapies. For example, antibody-induced endocytic removal of ErbB proteins is already in clinical use and drugs interfering with kinase activity or chaperone's function are being tested on cancer patients. Identification of still unknown mechanisms that shut down oncogenic signal transduction will eventually expand the arsenal of therapeutic strategies.

Selected Publications

- Alroy, I., Soussan, L., Seger, R. and Yarden, Y. (1999) Neu differentiation factor stimulates phosphorylation and activation of the Sp1 transcription factor. *Mol. Cell. Biol.* 19, 1961-1972.
- Harari, D., Tzahar, E., Romano, J., Shelly, M., Pierce, J.H., Andrews, G.C. and Yarden, Y. (1999) Neuregulin-4: a novel growth factor that acts through the ErbB-4 receptor tyrosine kinase. *Oncogene* 18, 2681-2689.
- Klapper, L.N., Glathe, S., Vaisman, N., Hynes, N.E., Andrews, G.C., Sela, M. and Yarden, Y. (1999) The ErbB-2/HER2 oncoprotein of human carcinomas may function solely as a shared coreceptor for multiple stroma-derived growth factors. *Proc. Natl. Acad. Sci. USA* 96, 4995-5000.
- Levkowitz, G., Waterman, H., Ettenberg, S.A., Katz, M., Tsygankov, A.Y., Alroy, I., Lavi, S., Iwai, K., Reiss, Y., Ciechanover, A., Lipkowitz, S. and Yarden, Y. (1999) Ubiquitin ligase activity and tyrosine phosphorylation underlie suppression of growth factor signaling by c-Cbl/Sli-1. *Mol. Cell.* 4, 1029-1040.
- Waterman, H., Alroy, I., Strano, S., Seger, R. and Yarden, Y. (1999a) The C-terminus of the kinase-defective neuregulin receptor ErbB-3 confers mitogenic superiority and dictates endocytic routing. *EMBO J.* 18, 3348-3358.
- Waterman, H., Levkowitz, G., Alroy, I. and Yarden, Y. (1999b) The RING finger of c-Cbl mediates desensitization of the epidermal growth factor receptor. *J. Biol. Chem.* 274, 22151-22154.
- Bao, J., Alroy, I., Waterman, H., Schejter, E.D., Brodie, C., Gruenberg, J. and Yarden, Y. (2000) Threonine phosphorylation diverts internalized epidermal growth factor receptors from a degradative pathway to the recycling endosome. *J. Biol. Chem.* 275, 26178-26186.
- Harari, D. and Yarden, Y. (2000) Molecular mechanisms underlying ErbB2/HER2 action in breast cancer. *Oncogene* 19, 6102-6114.
- Hurwitz, E., Klapper, L.N., Wilchek, M., Yarden, Y. and Sela, M. (2000) Inhibition of tumor growth by poly(ethylene glycol) derivatives of anti-ErbB2 antibodies. *Cancer Immunol Immunother.* 49, 226-234.
- Klapper, L.N., Kirschbaum, M.H., Sela, M. and Yarden, Y. (2000a) Biochemical and clinical implications of the ErbB/HER signaling network of growth factor receptors. *Adv. Cancer Res.* 77, 25-79.
- Klapper, L.N., Waterman, H., Sela, M. and Yarden, Y. (2000b) Tumor-inhibitory antibodies to HER-2/ErbB-2 may act by recruiting c-Cbl and enhancing ubiquitination of HER-2. *Cancer Res.* 60, 3384-3388.
- Levkowitz, G., Oved, S., Klapper, L.N., Harari, D., Lavi, S., Sela, M. and Yarden, Y. (2000) c-Cbl is a suppressor of the neu oncogene. *J. Biol. Chem.* 275, 35532-35539.
- Vaisman, N., Nissim, A., Klapper, L.N., Tirosh, B., Yarden, Y. and Sela, M. (2000) Specific inhibition of the reaction between a tumor-inhibitory antibody and the ErbB-2 receptor by a mimotope derived from a phage display library. *Immunol. Lett.* 75, 61-67.
- Rubin, I. and Yarden, Y. (2001) The basic biology of HER2. *Ann. Oncol.* 12, S3-S8.
- Waterman, H. and Yarden, Y. (2001) Molecular mechanisms underlying endocytosis and sorting of ErbB receptor tyrosine kinases. *FEBS Lett.* 490, 142-152.
- Xu, W., Mimnaugh, E., Rosser, M.F., Nicchitta, C., Marcu, M., Yarden, Y. and Neckers, L. (2001) Sensitivity of mature ErbB2 to geldanamycin is conferred by its kinase domain and is mediated by the chaperone protein Hsp90. *J. Biol. Chem.* 276, 3702-3708.
- Yarden, Y. (2001) The EGFR family and its ligands in human cancer: signalling mechanisms and therapeutic opportunities. *Eur. J. Cancer* 37, 3-8.
- Yarden, Y. and Sliwkowski, M.X. (2001) Untangling the ErbB signalling network. *Nat. Rev. Mol. Cell Biol.* 2, 127-137.

Acknowledgements

Our laboratory is supported by grants from the National Cancer Institute (NIH), the European Community, the U.S. Army, The Israel Science Fund, Genentech Inc., Willner Family Center for Vascular Biology and a special donation by Dr. Marvin Klein. Y.Y. is the incumbent of the Harold and Zelda Goldenberg Professional Chair in Molecular Cell Biology.

This work is in collaboration with Prof. Michael Sela's group.