Oncogenic receptor tyrosine kinases: Implications for cancer progression and therapy

Growth factors, along with adhesion and other molecules, play critical roles in invasive cell growth taking place in the developing embryo. Invasive growth rarely occurs in adulthood, but malignancy often harnesses growth factors, or their downstream signaling pathways, to enhance tumor aggressiveness and metastasis. The keys for understanding growth factor action in cancer are their surface receptors: a group of transmembrane glycoproteins whose cytoplasmic tyrosine kinase function is stimulated upon growth factor binding to the extracellular receptor’s part, and induction of dimer formation. An example is provided by the ErbB family of receptor tyrosine kinases (RTKs), which bind a large family of growth factors sharing an epidermal growth factor- (EGF-) like domain. These receptors instigate a variety of intracellular pathways, which are schematically presented in Figure 1. In the case of ErbB-4, a portion of the cytoplasmic domain is cleaved by TACE and PS1 proteases upon stimulation, and directly translocates to the nucleus (see Figure 1). The generic pathway, however, entails a cascade of cytoplasmic proteins culminating in transcriptional regulation. Self-production of specific growth factors, expression of mutant forms of ErbB-1/EGFR or overexpression of either ErbB-1 or ErbB-2/HER2 characterize a large variety of tumors of epithelial and neural origin. Moreover, two classes of pharmacological drugs, namely: monoclonal anti-receptor antibodies and low molecular weight tyrosine kinase inhibitors, effectively intercept growth factor signaling in clinical settings.

ErbB-2/HER2 is one of the most potent oncoproteins, but unlike other family members it binds no soluble growth factor. Likewise, ErbB-3 binds several growth factors, but unlike its family members the intrinsic kinase domain of ErbB-3 is catalytically inactive. For these and other reasons, signaling by ErbB and other RTK families is best described in terms of highly interconnected, layered signaling networks. The fail-safe (robust) ability of the ErbB network to decode and integrate extracellular signals is attributed to its modular structure, as well as to a dense array of feedback regulatory loops, collectively establishing system control. Our research within the realm of system control has established over the past few years two general groups of regulatory mechanisms, along with a few examples, which are described below on their collapse in human cancer.

Transcription-independent regulatory mechanisms

By mobilizing pre-existing protein assemblies, the network launches a plethora of immediate restraining mechanisms. The major and most effective ones sort active receptors to internalization and degradation in lysosomes. We found that the underlying mechanism utilizes ubiquitin and Ned8 molecules, which label receptors destined for degradation. An E3 ubiquitin ligase called c-Cbl is recruited to tyrosine phosphorylated receptors and instigates receptor mono-Neddylation followed by conjugation of mono- or di-ubiquitins to multiple lysine residues within the intracellular kinase domain. A set of ubiquitin and Ned8 binding proteins located in clathrin-coated regions of the plasma membrane and in endosomes then transfer modified receptors to lysosomes. Our studies revealed that these mechanisms are defective in tumors: ErbB-2 only weakly couples to c-Cbl, hence enhances the default recycling pathway, and lung cancer mutants of ErbB-1/EGFR gain sustained signaling ability because they evade the degradative route. Focusing on the recycling pathways, our most recent research has addressed the identity of tyrosine phosphatases and de-ubiquitination enzymes that negate receptor sorting. Likewise, we studied in depth a novel endosomal protein called Lst-2, which regulates receptor desensitization.

Transcription-dependent regulatory mechanisms

An interesting pattern of gene expression that follows cell activation by EGF- family growth factors emerged from our collaborative studies with the laboratories of Prof. Eytan Domani (WIS) and Prof. Gideon Rechavi (Sheba). The first burst of newly synthesized mRNA molecules encodes primarily transcription factors, such as c-Fos and c-Jun. Slightly later, we observed several waves of mRNAs, many of which encode negative regulators of cell signaling, including transcriptional repressors, RNA-binding proteins and MAPK phosphatases. Also included in the delayed group of transcripts are mRNAs encoding regulators of Cbl-family E3 ligases, such as LRIG-1 and Sprouty. Another protein, RALT/Mig-6 inhibits the catalytic activity of ErbB proteins, similar to the function of Ack-1, a pre-existing tyrosine kinase we studied in depth. Interestingly, we found that the group of RNA- or DNA-binding proteins, which are induced in a delayed fashion is collectively down-regulated in a large variety of human malignancies. Furthermore, in collaboration with cancer pathologists we found that the levels of expression of the delayed genes correlate with time of survival of ovarian and prostate cancer patients. In the same vein, a new avenue of our studies addresses regulation of ErbB signaling by micro-RNA molecules.

The ability of ErbB-family receptors to spearhead a chemotactic response relevant to tumor metastasis is actively studied in our laboratory. Because the initiation of motility requires MAPK activation, as well as synthesis of a new set of RNA molecules, we have concentrated on the respective group of transcripts. Examples include Nav-3, a protein involved in axon navigation, and...
may drive metastasis of malignant mammary cells. Because clinical lines of evidence implicate ErbB-2/HER2 in breast cancer metastasis, we currently focus on the role played by this ligand-less receptor. These studies employ a three-dimensional culture system, which reliably mimics breast cancer progression and, hopefully, response to therapeutic agents.

The ErbB family is a well-established target for cancer therapy, and a system already ripe for next generation therapeutic approaches. Our own efforts address the therapeutic potential of targeting EGF-family ligands or the kinase-dead receptor, ErbB-3, in pancreatic and in prostate cancer. In addition, in collaboration with the group of Prof. Michael Sela, we seek ways to improve the clinical efficacy of anti-receptor monoclonal antibodies. We previously attributed the therapeutic effect of such antibodies to their ability to translocate ErbB proteins from the cell surface to lysosomes. Along this vein, we found that certain combinations of anti-receptor monoclonals can significantly accelerate receptor degradation, and the same combinations also synergistically inhibit tumor xenograft growth in animals. The molecular mechanism underlying antibody-induced receptor degradation will be addressed by our future studies.

Selected publications
of AMP-activated protein kinase by human EGF receptor 2/EGF receptor tyrosine kinase inhibitor protects cardiac cells. Proc Natl Acad Sci U S A 104: 10607-10612


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