The Rebirth of a Phoenix: Ovarian Cancers Are Addicted to ErbB-3

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Several cancer drugs intercept the ErbB family receptors EGFR (ErbB-1) and HER2 (ErbB-2). However, the therapeutic value of targeting ErbB-3 has been less clear. A report in this issue of *Cancer Cell* by Sheng et al. renews hopes that intercepting ErbB-3-mediated autocrine loops bears potential for treatment of ovarian cancer.

Animal and other experimental models have provided ample evidence in support of a major role of the epidermal growth factor (EGF) receptor (EGFR) family (HER1, -2, -3, and -4, a.k.a. ErbB1, -2, -3, and -4) in differentiation of the epithelial lineage into diverse derivatives, such as the simple columnar epithelium decorating the outer surface of the ovary. A broad family of EGF-like growth factors that selectively bind discrete members of the EGFR family are secreted by stromal cells that underline the epithelium. These growth factors bind ErbB receptors expressed at the basolateral aspect of epithelial cells to enhance the tyrosine phosphorylation of these receptors and their subsequent coupling to an inositol lipid kinase (PI3K) and activation of the downstream AKT kinase pathway. The activated AKT pathway, together with the RAS-MAPK pathway, enables cell survival and proliferation. While studying tumor viruses in the late 1970s, George Todaro and Michael Sporn noted a general, virally induced secretion of growth factors, which enables repeated autostimulation of infected cells, a phenomenon they coined "autocrine stimulation" (Sporn and Todaro, 1980). Later studies documented countless examples of autocrine loops in human cancer (Burgess, 2008). However, whether autocrine loops can actively drive tumor initiation and progression, in analogy to driver mutations affecting oncogenes and tumor suppressor genes, remains an open issue that bears relevance for cancer treatment: intercepting growth factors using antibodies, soluble decoy receptors, and other means represents a validated approach for cancer therapy.

Qing Sheng and colleagues present, in this issue of *Cancer Cell*, compelling evidence identifying an essential autocrine loop, amenable for therapeutic intervention in ovarian cancer, the deadliest form of all gynecological cancers (Sheng et al., 2010). As a springboard, their study screened a library of short hairpin RNAs (shRNAs) targeting 89 human tyrosine kinases, a family of enzymes frequently involved in cancer due to gain-of-function mutations or gene amplification. On introduction into SKOV3 ovarian cancer cells, they observed reproducible inhibition of cell proliferation by shRNAs targeting EphA4, KDR, and ErbB-3. ErbB-3 not only scored the highest but also represented an interesting "catch" of the functional fishing expedition: unlike KDR and EphA4, it belongs to an enigmatic subgroup of tyrosine kinase family members called pseudokinases because their catalytic function is inactive. Aberrant expression or mutation of ErbB-3 in tumors, including ovarian cancer, is extremely rare, unlike the other three ErbB family members that are often mutated or overexpressed, e.g., ErbB-4 in melanoma (Prickett et al., 2009), ErbB-1/EGFR in lung cancer, and ErbB-2/HER2 in breast, ovarian, and gastric cancer (Slamon et al., 1987) (see Figure 1). To complicate the matter, the extracellular domain of ErbB-3 binds several growth factors, called neuregulins (NRGs), and upon transphosphorylation by several other tyrosine kinases, such as ErbB-2/HER2, the cytoplasmic domain of ErbB-3 robustly recruits PI3K.

SKOV3 cells carry complex genetic alterations, which include amplification of *ErbB2*, mutation of *PIK3CA*, deletion of *CDKN2A*, and mutation of *TP53*, and are near tetraploid, therefore, likely represent only a small subset of ovarian cancers. Candidates from the original screen using SKOV3 cells were validated using OVCAR8 cells, which have a mutation of *TP53* but do not have other genetic aberrations present in SKOV3 cells mentioned above. It is thus likely that additional tyrosine kinase screens in other genetic backgrounds relevant to ovarian cancer would identify additional dependencies and potential therapeutic targets.

Using OVCAR8, which expresses a constitutively phosphorylated form of ErbB-3, Sheng and collaborators applied an exhaustive set of tests that validated the ability of ErbB-3 to support cellular proliferation. Intriguingly, despite the role for the PI3K pathway as the main target for ErbB-3 in maintaining cell viability, they did not find evidence for an effect of knockdown of ErbB-3 on cellular viability in vitro. Next, they addressed the molecular basis of constitutive ErbB-3 phosphorylation in OVCAR8 cells. After excluding activation due to mutations or interactions with other ErbB family members, they examined a potential NRG autocrine loop. Indeed, PCR and immunoblotting detected NRG1 synthesis and secretion, and siRNAs specific to NRG1 (siNRG1) reduced ErbB-3 phosphorylation, as well as proliferation of OVCAR8 cells in vitro. Importantly, proliferation of ovarian cancer cells that express neither NRG1 nor a phosphorylated form of ErbB-3 was not affected by siNRG1, suggesting both an important mechanism and a potential biomarker for selection of patients likely to benefit from interruption of the NRG1/ErbB-3 autocrine loop.

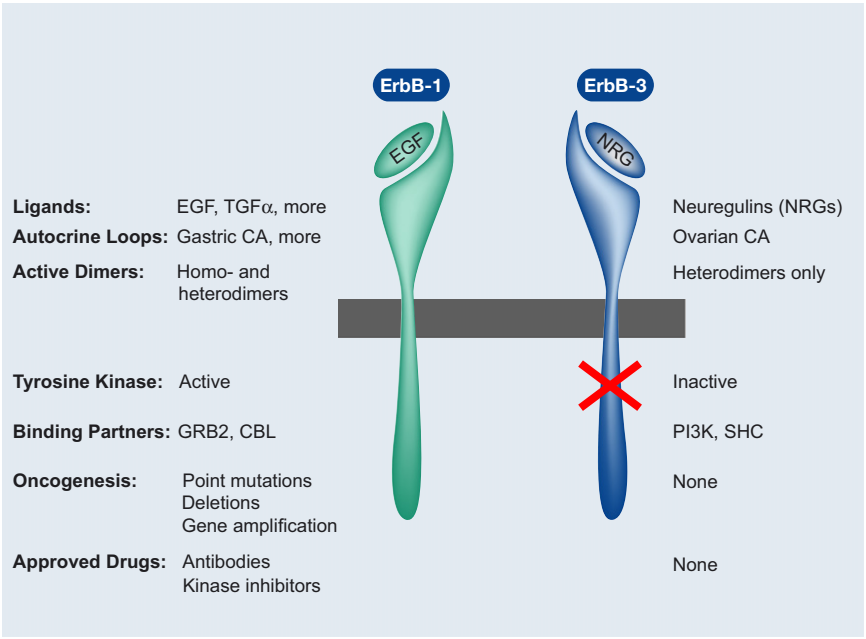

Figure 1. Functional and Pathological Differences between ErbB-1 and ErbB-3

Several monoclonal antibodies able to inhibit EGF binding to EGFR/ErbB-1 have been approved for treatment of human cancers (e.g., Bonner et al., 2006). Likewise, although no growth factor directly binds with HER2/ErbB-2, trastuzumb, a monoclonal antibody that recognizes the extracellular domain of this receptor, has improved outcomes for breast cancer patients. A similar, previously developed human monoclonal antibody able to displace NRG1 from its binding site on ErbB-3 (Schoeberl et al., 2009), was utilized by Sheng et al. to test the relevance of the autocrine loop they discovered to treatment of ovarian tumors. Consistent with the ability of the anti-ErbB-3 antibody to reduce tyrosine phosphorylation of ErbB-3 in cultured OVCAR8 cells, when injected into animals bearing small OVCAR8 xenografts, the antibody decreased, but did not abrogate, tumorigenic growth. In addition, inducible knockdown of ErbB-3 expression in a similar xenograft model lent support to their hypothesis that the NRG1/ErbB-3 autocrine loop drives growth of ovarian tumors in vivo. Indeed, one of the inducible shRNA constructs decreased tumor volume in vivo, compatible with the possibility that complete interruption of the NRG1/ErbB-3 autocrine loop, which only decreased cell growth in vitro, might result in death of ovarian cancer cells in the in vivo environment. This suggests that targeting ErbB-3 may demonstrate activity as a single agent; however, as with many other targeted therapies, the optimal efficacy is likely to be realized through combinatorial therapy with the potential that blocking NRG1 binding to ErbB-3 will sensitize ovarian tumors to the toxic effects of platinum-based or other chemotherapy regimens.

Beyond offering a novel target for therapy, as well as a potential treatment approach to ovarian cancer, which remains a devastating disease, the authors propose an attractive biomarker for patient selection, namely the level of ErbB-3 phosphorylation in tumors. Moreover, their analyses of cell lines and 20 fresh preparations of cells isolated from patients' ascites fluid, estimate that approximately a quarter of advanced ovarian cancer patients may present an operational NRG1/ErbB-3 autocrine loop, and hence benefit from treatment with anti-ErbB-3 targeted agents.

Three ErbB-3 targeted approaches are currently in clinical trials (clinicaltrials.gov): AMG888 (Amgen) and MM-121 (Merrimack Pharmaceuticals) are antibodies that prevent binding of ligands to ErbB-3 and may modestly downregulate receptor expression or membrane localization. MM-111 (Merrimack Pharmaceuticals), a bispecific antibody designed to target both ErbB-2 (HER2) and ErbB-3, is in trials for tumors overexpressing ErbB-2. However, none of these trials use the presence phosphorylated ErbB-3 or the presence of an HRG1 ErbB-3 autocrine loop to select patients likely to benefit and, hence, may miss the most appropriate patient population to determine the utility of ErbB-3-targeted therapy. A number of preclinical studies targeting ErbB-3 are underway. One of these, EZN-3920 (Enzon Pharmaceuticals), a high-affinity, locked nucleic acid (LNA) antisense molecule that silences ErbB-3 and demonstrates efficacy in animal models, is nearing clinical testing. In summary, together with recent studies identifying the kinase-dead receptor, ErbB-3, as the culprit behind patient resistance to kinase inhibitors (Engelman et al., 2007; Sergina et al., 2007), the new discovery of an ErbB-3-mediated addiction of a subset of ovarian cancers to growth factors zooms a major clinical spotlight on this rather neglected brother of an oncogenic receptor family.

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