HER2/HER3 heterodimers in prostate cancer: Whither HER1/EGFR?

In this issue of *Cancer Cell*, Mellinghoff et al. (2004) demonstrate that a small molecule inhibitor of the EGF receptor (EGFR) and the HER2/ErbB2/c-Neu kinase blocks signaling to the androgen receptor by a mechanism that involves HER2/HER3 heterodimerization. Surprisingly, the EGFR is peripheral to this signaling mechanism. These results have implications for the design of targeted therapy for hormone-refractory prostate cancer.

The finding that the HER2 receptor tyrosine kinase (RTK) is involved in breast cancer was one of the first great bench-to-bedside discoveries that originated from the application of molecular biology techniques to human cancer (Slamon et al., 1989). Almost immediately, speculation in the prostate cancer field turned toward HER2 as a potential player in this solid tumor, which resembles breast cancer in its hormone dependence and in other physiological features. Despite many studies of model systems and clinical specimens spanning more than a decade, the role of HER2 in prostate cancer—or whether there is indeed any role at all—has remained unresolved. A paper in this issue of *Cancer Cell* from the Sawyers laboratory (Mellinghoff et al., 2004) may be a significant step toward understanding the precise role of HER2 in prostatic malignancy.

HER2 belongs to the ErbB class of RTKs, of which the epidermal growth factor receptor (EGFR/ErbB1) is the founding member. ErbB receptors form signaling complexes by homo- and heterodimerizing within members of this kinase family (Yarden and Sliwkowski, 2001). Although ErbB receptors are activated by EGF-like growth factors of several kinds, HER2 is a ligandless receptor that signals by avid association with other ErbB RTKs activated by conventional ligand binding. The promiscuous nature of HER2 as a receptor dimerization partner has been another major rationale for studying it, the reasoning being that if an ErbB receptor is processing a signal, and sufficient levels of HER2 are present, HER2 will be a participant in the circuit.

Suspected ErbB receptor involvement in prostate cancer originates partly from the realization that these proteins are capable of promoting the transcriptional activity of the androgen receptor (AR), a steroid hormone receptor responsible for regulating genes involved in masculinization and virilization. A hallmark of prostate cancer is its evolution from an androgen-dependent to an androgen-independent state. The AR is now known to be involved in this transition to hormone independence, despite the fact that androgen ablation therapy, which removes the physiologic stimulus for AR activation, is the mainstay of prostate cancer treatment (Isaacs and Isaacs, 2004). An example of this apparent contradiction is the rise in circulating levels of the androgen-dependent protein, prostate-specific antigen (PSA), with hormone treatment failure, a process that the AR is now believed to mediate. The freeing of the AR from the constraints imposed upon it by its normal relationship with testicular androgens and their derivatives allows the transcription factor to stimulate gene expression in a manner that promotes tumor cell proliferation and survival.

AR activation by EGF and other growth factors in a low-androgen environment was first reported 10 years ago (Culig et al., 1994) and has since been

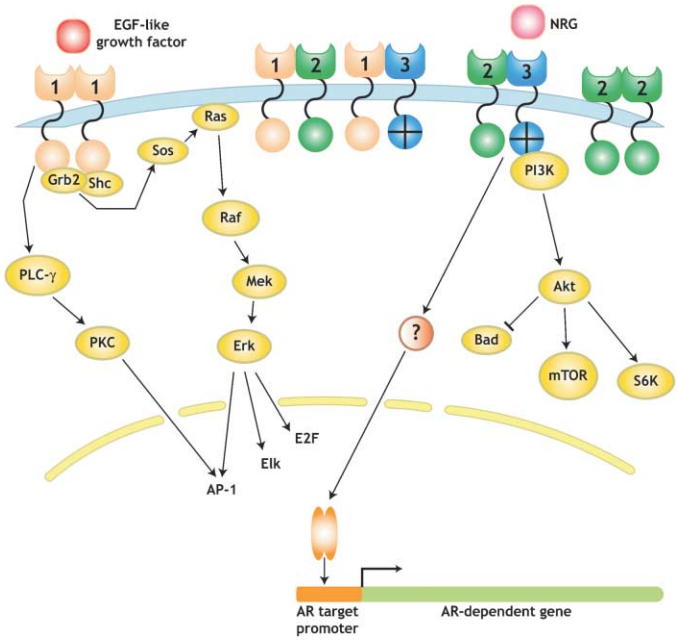

Figure 1. Activation of the androgen receptor (AR) as a result of HER2/HER3 dimerization

HER/ErbB family receptor tyrosine kinases initiate intracellular signal transduction by dimerizing with each other or with other members of the receptor family. Signaling cascades originating from ErbB1/ErbB1 (1-1) and ErbB2/ErbB3 (2-3) dimers are shown, along with known protein intermediates. The Mellinghoff et al. (2004) study in this issue of *Cancer Cell* demonstrates the existence of a signal from the 2-3 dimer to the AR, resulting in enhancement of AR-dependent, hormone-independent signals. The paper presents evidence that the EGFR (participating in 1-1, 1-2, or 1-3 dimers) or the serine-threonine kinase Akt/protein kinase B, which lies downstream from the 2-3 dimer, are not involved in mediating this signaling mechanism. These findings indicate that targeting the 2-3 dimer specifically may be a promising means of therapeutic intervention in aggressive prostate cancer. The question mark indicates that the pathway from the 2-3 dimer to the AR was not identified. The ErbB3 icon has a cross in the cytoplasmic domain because the intrinsic kinase is inactive. NRG, neuregulin, a growth factor capable of activating the 2-3 dimer.

verified by a number of groups. If we accept the premise that ErbB receptors activate AR without a need for the conventional hormone, then we must ask which ErbB proteins are the key villains. This is more than academic, because the various dimeric combinations of receptors are believed to produce radically different downstream signals (Yarden and Sliwkowski, 2001). For example, the inclusion of HER3 in a dimerization complex should be a potent activator of the phosphoinositide-3-kinase (PI3K)→Akt pathway because of a plethora of binding sites for the PI3K p85 regulatory subunit in the HER3 cytoplasmic domain.

The approach taken by Mellinghoff and colleagues to the question of ErbB involvement in prostate cancer was to employ an EGFR/HER2 small molecule inhibitor, PKI-166 (Bruns et al., 2000), along with a series of pharmacologic and genetic approaches in combination with an array of in vitro and in vivo models. These studies have resulted in two surprising conclusions. First, the target for PKI-166 inhibition of ErbB signaling to the AR was found to be HER2, not EGFR. The active signaling complex inhibited by this drug was HER2/HER3, which was shown to be capable of AR activation in an androgen-depleted environment. HER2/HER3 effects on AR included effects on protein stability and stimulation of DNA binding to AR target genes. This is a novel insight that should allow the development of a more focused class of anti-prostate cancer agents, which are inhibitors of the HER2/HER3 dimerization complex specifically (see Figure 1). Another surprise was the finding that the serine-threonine kinase Akt/protein kinase B (PKB), which, in principle, should lie immediately downstream of the HER2/HER3 dimer, appears not to be involved in activation of AR. This is potentially a critical insight with respect to the molecular profiling of prostate cancer tissues.

Analysis of clinical specimens with antibodies directed against the phosphorylated activation residues of Akt has provided strong evidence for the involvement of this kinase family in prostate cancer progression (Kreisberg et al., 2004). Mechanisms known to activate Akt include inactivation of the PI3K/Akt pathway inhibitor, the lipid phosphatase PTEN, as well as stimulation of upstream signaling proteins, such as the ErbB proteins and other RTKs. If the HER2/HER3 dimer does not activate AR through Akt, then what is the consequence of activated Akt signaling in prostate cancer?

There are likely several answers to this last question. From numerous studies on cells that do not express AR, it is evident that Akt is quite capable of acting on its own to stimulate cell growth and survival mechanisms (Luo et al., 2003). Although AR is present in most aggressive prostate cancers, expression is often heterogeneous, and inactivating mutations may be common. Thus, Akt activation may reflect a situation where AR is bypassed rather than co-opted. Alternatively, Akt and AR pathway signals may intersect in nefarious ways to keep prostate cancer cells in the game in the face of proapoptotic stimuli, such as androgen withdrawal and chemotherapy. Protein complexes containing AR and Akt have been demonstrated by several groups, and AR was shown to be phosphorylated by Akt on at least two residues (Wen et al., 2000; Lin et al., 2001), a result that is now somewhat controversial for the endogenous protein (Gioeli et al., 2002).

The Mellinghoff et al. study leaves for another day the question of how we must now visualize the role of the EGFR in prostate cancer. The granddaddy of all of the RTKs may have just been put out to pasture in this disease. On the other hand, the paper from the Sawyers group is consistent with recent reports suggesting that our appreciation of the role of this protein in prostate cancer is simply maturing. Chronic EGFR activation can lead to AR downmodulation and functional attenuation under conditions of robust tumor growth in a castrate environment (Adam et al., 2002), suggesting that the EGFR may be able to suppress and consequently bypass AR while supporting conditions that maintain the growth potential of the tumor cell. Consistent with this view, recent studies have suggested that, while activation of Akt may correlate with disease progression in prostate cancer, Erk MAPK activation may inversely correlate with aggressive disease (Kreisberg et al., 2004). Significantly, both pathways lie downstream of the various ErbB dimers. These observations suggest that RTKs at the plasma membrane, as predicted for many years, are critical sites for the diversification of downstream signaling in prostatic malignancy. The Mellinghoff et al. study emphasizes the point that investigators interested in developing means of tumor profiling for directed therapy or moving kinase inhibitors into the clinical setting must take into account the discrete mechanisms of upstream activation, not just the downstream signaling players, such as the MAPKs, Akt, and other kinase pathways. This is because not all ErbBs, and probably not all RTKs, are cast from the same mold.

Michael R. Freeman*

Urological Diseases Research Center
Children's Hospital Boston
Harvard Medical School
Enders Research Laboratories, Suite 1161
300 Longwood Avenue
Boston, Massachusetts 02115
*E-mail: michael.freeman@childrens.harvard.edu

Selected reading

Adam, R.M., Kim, J., Lin, J., Orsola, A., Zhuang, L., Rice, D.C., and Freeman, M.R. (2002). Endocrinology *143*, 4599–4608.

Bruns, C.J., Solorzano, C.C., Harbison, M.T., Ozawa, S., Tsan, R., Fan, D., Abbruzzese, J., Traxler, P., Buchdunger, E., Radinsky, R., and Fidler, I.J. (2000). Cancer Res. *60*, 2926–2935.

Culig, Z., Hobisch, A., Cronauer, M.V., Radmayr, C., Trapman, J., Hittmair, A., Bartsch, G., and Klocker, H. (1994). Cancer Res. *54*, 5474–5478.

Gioeli, D., Ficarro, S.B., Kwiek, J.J., Aaronson, D., Hancock, M., Catling, A.D., White, F.M., Christian, R.E., Settlage, R.E., Shabanowitz, J., et al. (2002). J. Biol. Chem. *277*, 29304–29314.

Isaacs, J.T., and Isaacs, W.B. (2004). Nat. Med. *10*, 26–27.

Kreisberg, J.I., Malik, S.N., Prihoda, T.J., Bedolla, R.G., Troyer, D.A., Kreisberg, S., and Ghosh, P.M. (2004). Cancer Res. *64*, 5232–5236.

Lin, H.K., Yeh, S., Kang, H.Y., and Chang, C. (2001). Proc. Natl. Acad. Sci. USA *98*, 7200–7205.

Luo, J., Manning, B.D., and Cantley, L.C. (2003). Cancer Cell *4*, 257–262.

Mellinghoff, I.K., Vivanco, I., Kwon, A., Tran, C., Wongvipat, J., and Sawyers, C.L. (2004). Cancer Cell *6*, this issue.

Slamon, D.J., Godolphin, W., Jones, L.A., Holt, J.A., Wong, S.G., Keith, D.E., Levin, W.J., Stuart, S.G., Udove, J., and Ullrich, A. (1989). Science *244*, 707–712.

Wen, Y., Hu, M.C., Makino, K., Spohn, B., Bartholomeusz, G., Yan, D.H., and Hung, M.C. (2000). Cancer Res. *60*, 6841–6845.

Yarden, Y., and Sliwkowski, M.X. (2001). Nat. Rev. Mol. Cell Biol. *2*, 127–137.