REFERENCES

Cozzio, A., Passegue, E., Ayton, P.M., Karsunky, H., Cleary, M.L., and Weissman, I.L. (2003). Genes Dev. *17*, 3029–3035.

Heath, V., Suh, H.C., Holman, M., Renn, K., Gooya, J.M., Parkin, S., Klarmann, K.D., Ortiz, M., Johnson, P., and Keller, J. (2004). Blood *104*, 1639–1647.

Huntly, B.J., Shigematsu, H., Deguchi, K., Lee, B.H., Mizuno, S., Duclos, N., Rowan, R., Amaral, S., Curley, D., Williams, I.R., et al. (2004). Cancer Cell *6*, 587–596.

Kirstetter, P., Schuster, M.B., Bereshchenko, O., Moore, S., Dvinge, H., Kurz, E., Theilgaard-Monch, K., Mansson, R., Pedersen, T.A., Pabst, T., et al. (2008). Cancer Cell, this issue.

Krivtsov, A.V., Twomey, D., Feng, Z., Stubbs, M.C., Wang, Y., Faber, J., Levine, J.E., Wang, J., Hahn, W.C., Gilliland, D.G., et al. (2006). Nature *442*, 818–822.

Nerlov, C. (2004). Nat. Rev. Cancer *4*, 394–400.

Passegue, E., Jamieson, C.H., Ailles, L.E., and Weissman, I.L. (2003). Proc. Natl. Acad. Sci. USA *100* (*Suppl 1*), 11842–11849.

Porse, B.T., Bryder, D., Theilgaard-Monch, K., Hasemann, M.S., Anderson, K., Damgaard, I., Jacobsen, S.E., and Nerlov, C. (2005). J. Exp. Med. *202*, 85–96.

Wagner, K., Zhang, P., Rosenbauer, F., Drescher, B., Kobayashi, S., Radomska, H.S., Kutok, J.L., Gilliland, D.G., Krauter, J., and Tenen, D.G. (2006). Proc. Natl. Acad. Sci. USA *103*, 6338–6343.

Wechsler, J., Greene, M., McDevitt, M.A., Anastasi, J., Karp, J.E., Le Beau, M.M., and Crispino, J.D. (2002). Nat. Genet. *32*, 148–152.

Zhang, P., Iwasaki-Arai, J., Iwasaki, H., Fenyus, M.L., Dayaram, T., Owens, B.M., Shigematsu, H., Levantini, E., Huettner, C.S., Lekstrom-Himes, J.A., et al. (2004). Immunity *21*, 853–863.

A Molecular View of Anti-ErbB Monoclonal Antibody Therapy

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Abnormal activation of the epidermal growth factor receptor (EGFR) and its homolog HER2 (Neu/ErbB2) has been associated with many human cancers, and monoclonal antibodies targeting EGFR and HER2 are effective anticancer therapies. Structural studies of these receptors and antibodies have revealed much about how they function. In this issue of *Cancer Cell*, Schmiedel et al. report structural and functional studies of the anti-EGFR monoclonal antibody Matuzumab. They show that Matuzumab binds and inhibits EGFR in a manner distinctive from that of other therapeutic anti-EGFR antibodies and suggest that combination therapies with Matuzumab and other antibodies may prove beneficial.

The epidermal growth factor receptor (EGFR/ErbB1/HER1) consists of an extracellular ligand binding region followed by a single membrane-spanning helix, a cytoplasmic tyrosine kinase domain, and a C-terminal tail of ~230 amino acids (Burgess et al., 2003). Ligand binding to the extracellular region promotes receptor dimerization, which in turn leads to activation of the cytoplasmic tyrosine kinase (Holbro and Hynes, 2004). When activated, the EGFR kinase phosphorylates several tyrosines in the EGFR C-terminal tail that then serve as docking sites for downstream signaling effectors that initiate signaling cascades and stimulate cell growth and differentiation (Holbro and Hynes, 2004). Three EGFR homologs, HER2 (Neu/ErbB2), HER3 (ErbB3), and HER4 (ErbB4) are found in humans and, together with EGFR, make up the EGFR/ErbB family of receptors. HER2 is an atypical member of this family in that it is not directly activated by ligand but rather serves as a universal heterodimeric partner for each of the other ErbB family members (Holbro and Hynes, 2004).

EGFR was the first cell-surface receptor to be associated with cancer, and abnormal EGFR or HER2 function has subsequently been found to contribute to the severity of many human tumors (Hynes and Lane, 2005). For this reason, agents targeting EGFR or HER2 have been actively pursued as cancer therapies. These agents fall into two general classes: monoclonal antibodies, which bind to receptor extracellular regions and will be discussed here, and small-molecule kinase inhibitors that target the cytoplasmic kinase activity. To date, two monoclonal antibodies against EGFR, Cetuximab (Erbitux) and Panitumumab (Vectibix), have been approved by the FDA for treatment of colorectal and/or head-and-neck cancer, and two EGFR kinase inhibitors, erlotinib (Tarceva) and gefitinib (Iressa), have been approved for the treatment of lung cancer. A monoclonal antibody targeting HER2, Trastuzumab (Herceptin), and a pan-ErbB kinase inhibitor, lapatinib (Tykerb), have also been approved for treatment of HER2-overexpressing breast cancers. Many other ErbB-targeted therapies are under development.

Beginning ~5 years ago, X-ray crystallographic studies of the extracellular regions of ErbB family members uncovered the basic mechanism by which ligand binding

regulates receptor dimerization and activity (Burgess et al., 2003) (Figure 1A). The extracellular regions of ErbB family members are composed of four subdomains. Domains I and III are homologous, and both contribute to ligand binding. Domains II and IV are homologous and form extended, cysteine-rich structures (Burgess et al., 2003). In the absence of ligand, an extended loop from domain II contacts a pocket at the C terminus of domain IV and constrains the extracellular region to a compact, "tethered" conformation in which domains I and III are held far apart (Figure 1A, left panel). To bind ligand with high affinity, a domain rearrangement occurs in which the domain II/IV contact is broken and domains I and II rotate as a pair to bring domains I and III into proximity and allow them to bind ligand simultaneously in a clamp-like interaction (Figure 1A, middle panel). In this ligand-bound, extended structure, the domain II loop that contacted domain IV in the absence of ligand becomes exposed and mediates receptor dimerization (Figure 1A, middle and right panels). This loop is, thus, frequently referred to as the "dimerization arm."

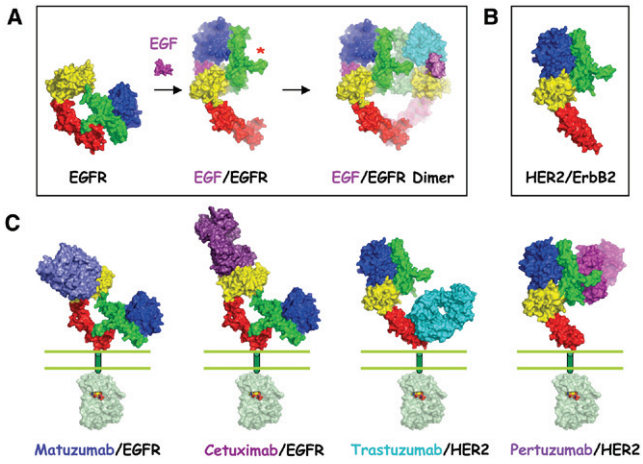

Figure 1. Surface Representations of EGFR and HER2 in Active, Inactive, and Antibody-Bound Conformations

(A) A surface representation of the extracellular region of EGFR in the absence of ligand is shown with domains I (blue), II (green), III (yellow), and IV (red) colored as indicated (left panel). Ligand (EGF, purple) binding stabilizes a domain rearrangement in which domains I and II rotate as a pair and break the domain II/IV contact, bringing domain I (blue) and III (yellow) into proximity to bind ligand. This rearrangement exposes the previously buried domain II dimerization arm, which is marked with a red asterisk (middle panel). The exposed dimerization arm then mediates receptor dimerization and activation (right panel).

(B) The HER2/ErbB2 extracellular region adopts a constitutively "active-like" structure in which domains I and III contact each other directly and the domain II dimerization arm is exposed.

(C) The Fab fragments of Matuzumab (slate blue) bound to EGFR (far left), Cetuximab (purple) bound to EGFR (second from left), Trastuzumab (cyan) bound to HER2 (second from right), and Pertuzumab (magenta) bound to HER2 (far right) are shown. The plasma membrane is indicated with two green lines, and a membrane-spanning region is represented with a green cylinder. A surface representation of the EGFR kinase is shown in light green with a space-filling representation of a bound nucleotide.

It came as a pleasing surprise when crystal structures of the HER2 extracellular region showed that it does not adopt the tethered conformation. Instead, HER2 is fixed in an active-like conformation characterized by an interaction between domains I and III and a constitutively exposed dimerization arm (Cho et al., 2003; Garrett et al., 2003) (Figure 1B). This domain I/III interaction occludes the canonical ErbB ligand-binding surface and appears to mimic the effects of ligand binding, which rationalizes the absence of a HER2 ligand and the role of HER2 as a universal partner for other ErbB family members.

Given the long time scale of clinical trials, many ErbB-targeted therapies entered development long before the molecular underpinnings of ErbB activation and HER2 behavior became apparent. It has, thus, been particularly satisfying that as structural and biochemical studies of therapeutic anti-ErbB antibodies progress, a consistent picture of ErbB function is emerging. For example, Trastuzumab (Herceptin) binds to the juxtamembrane region of HER2 (Figure 1C) at a site that would not obviously interfere with HER2 dimerization or activation (Cho et al., 2003). Indeed, biochemical studies show that Trastuzumab does not block either dimerization or activation of HER2 (Agus et al., 2002). Trastuzumab does block proteolytic cleavage of the HER2 ectodomain, however, which occurs adjacent to the cell membrane and leaves behind an active kinase, and this effect may contribute to its antiproliferative activity (Baselga et al., 2001). Antibody-dependent cellular cytotoxicity also appears to contribute significantly to Trastuzumab activity (Clynes et al., 2000). In contrast, the anti-HER2 antibody Pertuzumab, currently in phase III clinical trials for ovarian cancer, binds directly to the HER2 dimerization arm and blocks both dimerization and activation in response to stimulation of a HER2 partner (Agus et al., 2002) (Figure 1C). This difference appears to explain why Pertuzumab is more effective than Trastuzumab in cancers where HER2 is activated, but not overexpressed.

Unlike HER2, targeting the dimerization arm of EGFR does not appear to be an effective strategy as it is generally buried at either an intra- or intermolecular interface. Indeed, the first anti-EGFR antibody to be approved by the FDA for cancer therapy, Cetuximab (Erbitux), competes with ligand for binding to EGFR and was shown by Ferguson and colleagues to bind and block the ligand binding site on EGFR domain III (Li et al., 2005) (Figure 1C). These authors also noted that Cetuximab binding to EGFR would sterically prohibit EGFR adopting the extended, active-like conformation (Figure 1), providing a dual mechanism of EGFR inhibition. The humanized anti-EGFR antibody IMC-11F8 binds at this same site and also works by this dual mechanism (Li et al., 2008).

In this issue of *Cancer Cell*, Schmiedel et al. now show that a third anti-EGFR mAb, Matuzumab, binds at a nearby but distinct site on EGFR and displays a different constellation of biochemical and inhibitory properties (Schmiedel et al. 2008). Matuzumab, which is currently in phase II trials for treatment of lung and stomach cancer, is like Cetuximab in that it binds to domain III of EGFR (Figure 1C). Unlike Cetuximab, however, the Matuzumab binding site does not overlap with the EGF binding site, and Matuzumab does not completely compete with EGF for binding to EGFR. Matuzumab does reduce the apparent affinity of EGF for EGFR. How to explain this behavior? Schmiedel et al. point out that although Matuzumab and EGF could simultane-

ously bind to EGFR domain III, the binding of Matuzumab would interfere with formation of the active-like EGFR conformation (Figure 1A, middle panel). Thus, in the presence of Matuzumab, EGF could only contact domain III (or domain I), and its affinity for EGFR would be reduced—exactly what is observed. Schmiedel et al. also show that Cetuximab and Matuzumab do not compete for binding to EGFR, as predicted from comparison of crystal structures of their complexes with EGFR, and suggest that combination therapy with Cetuximab (or IMC-11F8) and Matuzumab may result in added clinical benefit.

It is clear that basic and clinical studies of the ErbB family of receptors have come a long way in the last few years. The results from each type of inquiry has informed the other, and together, they are leading to a deeper understanding of ErbB function and how to treat ErbB-involved diseases. It is also clear that much remains to be learned, and exciting times are ahead.

REFERENCES

Agus, D.B., Akita, R.W., Fox, W.D., Lewis, G.D., Higgins, B., Pisacane, P.I., Lofgren, J.A., Tindell, C., Evans, D.P., Maiese, K., et al. (2002). Cancer Cell *2*, 127–137.

Baselga, J., Albanell, J., Molina, M.A., and Arribas, J. (2001). Semin. Oncol. *28*, 4–11.

Burgess, A.W., Cho, H.S., Eigenbrot, C., Ferguson, K.M., Garrett, T.P., Leahy, D.J., Lemmon, M.A., Sliwkowski, M.X., Ward, C.W., and Yokoyama, S. (2003). Mol. Cell *12*, 541–552.

Cho, H.S., Mason, K., Ramyar, K.X., Stanley, A.M., Gabelli, S.B., Denney, D.W., Jr., and Leahy, D.J. (2003). Nature *421*, 756–760.

Clynes, R.A., Towers, T.L., Presta, L.G., and Ravetch, J.V. (2000). Nat. Med. *6*, 443–446.

Garrett, T.P., McKern, N.M., Lou, M., Elleman, T.C., Adams, T.E., Lovrecz, G.O., Kofler, M., Jorissen, R.N., Nice, E.C., Burgess, A.W., and Ward, C.W. (2003). Mol. Cell *11*, 495–505.

Holbro, T., and Hynes, N.E. (2004). Annu. Rev. Pharmacol. Toxicol. *44*, 195–217.

Hynes, N.E., and Lane, H.A. (2005). Nat. Rev. Cancer *5*, 341–354.

Li, S., Kussie, P., and Ferguson, K.M. (2008). Structure *16*, 216–227.

Li, S., Schmitz, K.R., Jeffrey, P.D., Wiltzius, J.J., Kussie, P., and Ferguson, K.M. (2005). Cancer Cell *7*, 301–311.

Schmiedel, J., Blaukat, A., Li, S., Knoechel, T., and Ferguson, K.M. (2008). Cancer Cell, this issue.

RanBP2: A Tumor Suppressor with a New Twist on TopoII, SUMO, and Centromeres

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In vertebrate cells, the small ubiquitin-like modifier SUMO plays a poorly defined role in targeting DNA topoisomerase II (TopoII) to centromeres (*CEN*s) during mitosis, presumably to facilitate the untangling of sister chromatids as cells transition into anaphase. A new study by Dawlaty in the April 4 issue of *Cell* identifies the nucleoporin RanBP2 as a novel tumor suppressor that acts as a SUMO ligase for TopoII. Analysis of this interaction reveals TopoII recruitment to *CEN*s is likely to play an important role in preventing chromosome segregation errors that lead to cancer.

RanBP2 is a remarkably large (350 kD!) protein that contains, as its only enzymatic function, an unusual SUMO E3 ligase domain (Pichler et al., 2002). In the final step of SUMO modification, the E2 conjugating enzyme Ubc9 transfers activated SUMO moieties to lysines on substrate proteins. This reaction typically requires, or is greatly stimulated by, SUMO E3 ligases. The best understood SUMO E3s are the PIAS family of proteins, which contain a RING finger motif and promote sumolyation by recruiting substrates to the E2 enzyme (Jackson, 2001). The RanBP2 E3 domain, in contrast, fits tidily within a ~300 amino acid segment that is structurally unrelated to PIAS proteins. Rather than binding substrates, this E3 acts more like a cofactor for Ubc9, possibly serving to directly stimulate E2 catalysis (Reverter and Lima, 2005).

The cell biology of RanBP2 has also provided surprises. In addition to binding Ubc9, the RanBP2 E3 domain interacts specifically with SUMO-modified forms of RanGAP1. During interphase, this complex localizes to the cytoplasmic face of the nuclear pore. But once mitosis is underway, the entire RanBP2-SUMO~RanGAP1-Ubc9 complex partners with the nuclear export receptor Crm1 and moonlights as a component of the kinetochore (K; Arnaoutov et al., 2005). This is arguably even more important than RanBP2's day job, as RanBP2 depletion produces severe mitotic defects, including perturbations to K-microtubule (MT) attachment, mis-