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Mechanisms of resistance to ErbB-targeted cancer therapeutics

Qiang Wang, Mark I. Greene

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Commentary

The ErbB receptors, such as EGFR, have been intensely pursued as targets for cancer therapeutics. However, a large percentage of patients who are initially responsive to ErbB-targeted therapies experience tumor recurrence and become refractory to therapy. In this issue of the *JCI*, Guix et al. demonstrate that downregulation of IGF-binding protein 3 (IGFBP-3) and -4, the negative regulators of IGF-I receptor signaling, contributes to the resistance of human squamous cell carcinomas to the EGFR inhibitor gefitinib (see the related article beginning on page 2609). Understanding the mechanisms involved in the resistance of some tumors to ErbB-targeted molecules may provide guidelines for developing more efficient therapeutic approaches.

Perspectives

The study by Leonard et al. (3) and other recent work (12, 15, 16) have greatly advanced our understanding of the molecular bases of the pathology of measles. However, an important piece of information is still missing. What is the MV receptor on epithelial cells? Data obtained by Leonard et al. (3) and Tahara et al. (16) suggest that the receptor may be a molecule related to tight junctions. Use of cell junction molecules as receptors may be a common strategy for viruses to facilitate their transmission (18–20). Identification in future studies of the epithelial cell receptor for MV will likely reveal further details of the elegant strategy of infection (i.e., differential usage of two receptors during the course of infection) employed by this highly contagious virus.

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Address correspondence to: Makoto Takeda, Department of Virology, Faculty of Medicine, Kyushu University, Fukuoka 812-8582, Japan. Phone: 81-92-642-6138; Fax: 81-92-642-6140; E-mail: mtakeda@virology.med.kyushu-u.ac.jp.

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Mechanisms of resistance to ErbB-targeted cancer therapeutics

Qiang Wang and Mark I. Greene

Department of Pathology and Laboratory Medicine, University of Pennsylvania School of Medicine, and Abramson Family Cancer Research Institute, Philadelphia, Pennsylvania, USA.

The ErbB receptors, such as EGFR, have been intensely pursued as targets for cancer therapeutics. However, a large percentage of patients who are initially responsive to ErbB-targeted therapies experience tumor recurrence and become refractory to therapy. In this issue of the *JCI*, Guix et al. demonstrate that downregulation of IGF-binding protein 3 (IGFBP-3) and -4, the negative regulators of IGF-I receptor signaling, contributes to the resistance of human squamous cell carcinomas to the EGFR inhibitor gefitinib (see the related article beginning on page 2609). Understanding the mechanisms involved in the resistance of some tumors to ErbB-targeted molecules may provide guidelines for developing more efficient therapeutic approaches.

Nonstandard abbreviations used: GR, gefitinib resistant; IGFBP, IGF-binding protein; IGF-IR, IGF-1 receptor; mTOR, mammalian target of rapamycin; TKI, tyrosine kinase inhibitor.

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Members of the ErbB family of receptor tyrosine kinases, which include EGFR, ErbB2 (known as $p185^{c\text{-}neu}$ in rodents and HER2 in humans), ErbB3, and ErbB4, are overexpressed in a variety of human solid tumors (1). Activation of the ErbB molecules correlates strongly with the pathogenesis and poor prognosis of many forms of cancer. Ligands for EGFR, ErbB3, and ErbB4, such as EGF and the polypeptide heregulin, bind to the extracellular domain of the receptor, leading to receptor dimerization and autophosphorylation of the intracellular tyrosine kinase domain. These events subsequently upregulate downstream signaling cascades, including the MAPK, PI3K, and mammalian target of rapamycin (mTOR) pathways. As such, ErbB molecules modulate cell proliferation, survival, and mobility.

ErbB-targeted cancer therapeutics

For the past twenty years, targeting the ErbB receptors has been intensely pursued as an important cancer therapeutic strategy (1). Immunological approaches have

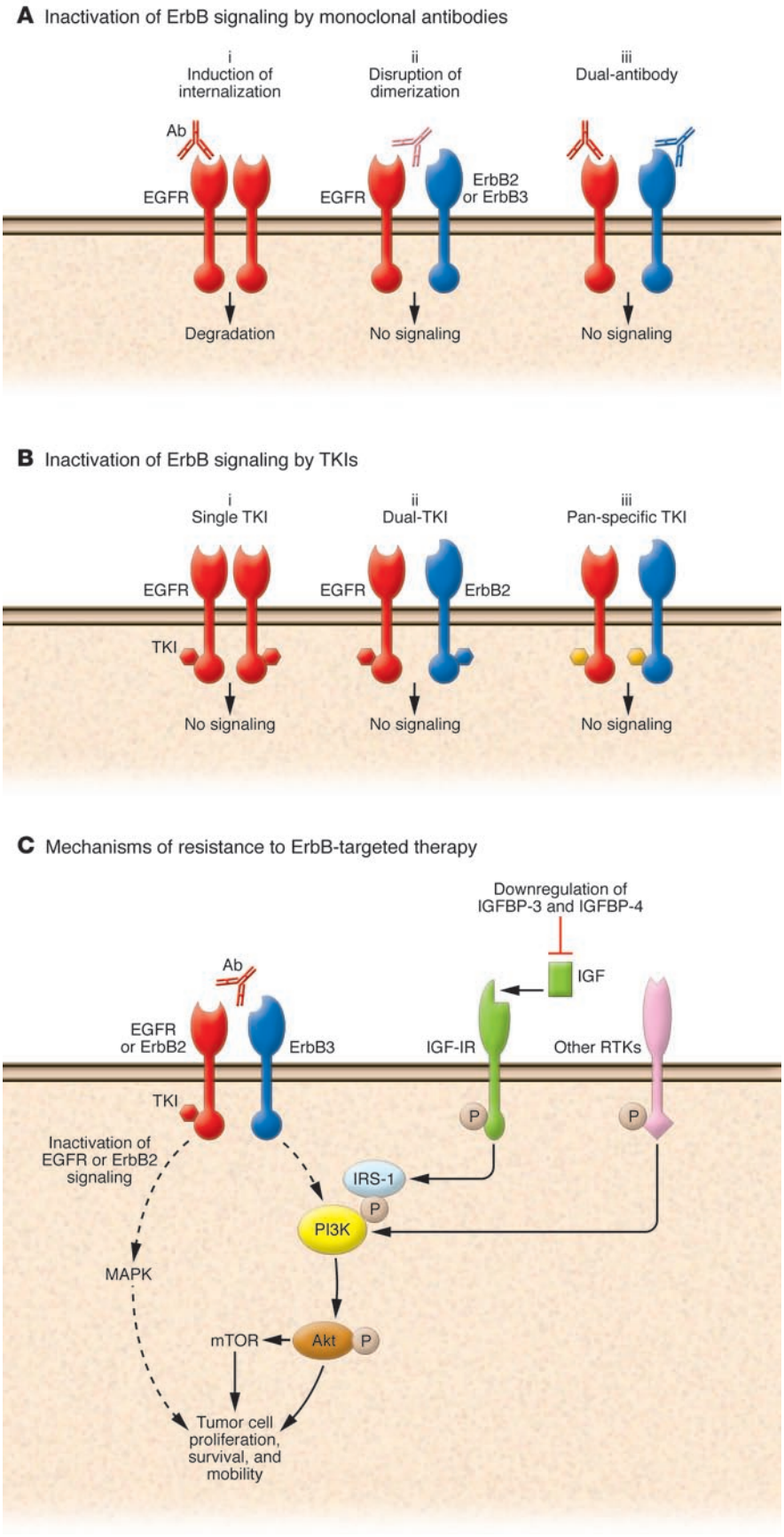

Figure 1
ErbB-targeted therapeutics and mechanisms of resistance. (**A**) Inactivation of ErbB signaling by monoclonal antibodies. Binding of antibody to EGFR may cause receptor internalization and degradation (i). An ErbB-specific antibody may sterically block receptor dimerization (ii). The use of two anti-ErbB antibodies may produce a synergistic effect to inhibit ErbB-mediated signaling and transformation in a more complete manner (iii). (**B**) The ErbB-specific TKIs target the ATP-binding site of the tyrosine kinase domain of the receptor and directly inhibit ErbB receptor kinase activity. These small molecules can be used either as a single agent (i) or in combination (ii). The pan-specific ErbB-targeted TKI can simultaneously target multiple ErbB receptors (iii). (**C**) Mechanisms of resistance to ErbB-targeted therapy. ErbB-targeted therapeutics cause downregulation of the MAPK, mTOR, and PI3K signaling pathways (dashed lines indicate reduction of signaling). Resistance may arise in tumor cells through allelic and adaptive changes, leading to activation of PI3K through other receptor tyrosine kinases (RTKs). In this issue of the *JCI*, Guix et al. (10) demonstrate that downregulation of IGFBP-3 and -4, negative regulators of IGF-IR signaling, causes activation of IGF-IR and the PI3K-Akt pathway and contributes to the resistance of human squamous carcinoma cells to the EGFR inhibitor gefitinib. The heterotetramer of IGF-IR has been simplified in this schematic representation.

been widely exploited to treat ErbB-mediated cancer since the first studies in animal models showed that specific anti-p185$^{c\text{-}neu}$ antibodies can reverse the malignant phenotype of cells transformed by the *neu* oncogene in vitro and inhibit in vivo tumor growth of oncogenic, *neu*-transformed cells (2). In addition, p185$^{c\text{-}neu}$ ectodomain–specific antibodies also reverse the phenotype of cells transformed through the combined action of normal but modestly overexpressed p185$^{c\text{-}neu}$ and EGFR proteins (3). Moreover, combinations of p185$^{c\text{-}neu}$ ectodomain–binding antibodies specific for distinct p185$^{c\text{-}neu}$ epitopes that are relevant to dimer formation lead to a more complete inhibition of the transformed phenotype and can cause total tumor eradication in vivo in model systems (4). A number of humanized anti-ErbB2/HER2 monoclonal antibodies, such as trastuzumab (Herceptin) and pertuzumab (also known as 2C4 or Omnitarg), have been developed and Herceptin has already been approved for the treatment of human cancers. Similarly, humanized anti-EGFR antibodies, such as cetuximab (IMC-225; Erbitux), have been

developed to target cancers associated with overexpression of EGFR (5). Binding of the anti-ErbB antibodies generally leads to disruption of the normal dimeric state of the transforming receptor complex, dramatically inhibiting the kinase-complex activity as well as causing rapid downmodulation of the expression of the receptor on the cell surface (Figure 1A). The nonproteinaceous tyrosine kinase inhibitors (TKIs) represent a second major class of ErbB-targeted agents (Figure 1B). These small-molecule therapeutics are designed to bind to the ATP-binding site of the tyrosine kinase domain, preempting the binding of ATP and directly inhibiting the kinase activity of ErbB receptors such as EGFR or ErbB2 (1). For example, a number of TKIs for EGFR have been developed, including gefitinib (Iressa) and erlotinib (Tarceva) (1). In addition, TKIs that simultaneously target multiple ErbB species, such as CI-1033 (PD183805) and lapatinib (GW572016/Tykerb), have also been created (1).

Although both of these two ErbB-targeted approaches have shown clinical promise, an increasing body of evidence indicates that patients initially responsive to ErbB-targeted therapies may suffer from recurrence and develop tumors refractory to the original treatment (1, 6–9). Moreover, a large percentage of ErbB-positive cancers demonstrate a predisposition to resistance to ErbB-targeted therapeutics. It is conceivable that such cancer cells may have undergone allelic and adaptive changes that make the cells more resilient to the therapeutic effect of the antibodies or TKIs. Improved understanding of the mechanisms involved in the resistance of tumor cells to ErbB-targeted molecules may provide insights into developing more efficient ErbB-targeted therapeutic approaches as well as predicting the outcomes of the treatment.

Loss of IGFBPs and resistance to the EGFR-targeted TKI, gefitinib

In this issue of *JCI*, Guix et al. (10) examined some of the mechanisms involved in acquired resistance to the EGFR-targeted TKI, gefitinib. The authors isolated gefitinib-resistant (GR) human squamous carcinoma A431 cells by prolonged incubation of A431 cells with an increasing amount of the inhibitor. In the GR cells, the inhibitor reduced the phosphorylation levels of EGFR, ErbB3, and Erk, but not those of Akt. This adaptive change was accompanied by activation of the signaling events mediated by the IGF-1 receptor (IGF-IR), such as phosphorylation of IRS-1 and the interaction of IRS-1 with PI3K. The authors went on to show that inhibition of IGF-IR disrupted the association of IRS-1 with PI3K and restored the ability of gefitinib to reduce Akt phosphorylation and to inhibit cell growth. Furthermore, Guix et al. found that the expression levels of IGF-binding protein 3 (IGFBP-3) and -4, two of the negative regulators of IGF-IR signaling, were reduced in the GR cells (Figure 1C). Incubation of the GR cells with recombinant IGFBP-3 enabled gefitinib to reduce Akt signaling and inhibit cell proliferation. Moreover, combined treatment using gefitinib and an anti–IGF-IR antibody was found effective in preventing tumor growth in nude mice. The study demonstrates that loss of IGFBPs and activation of IGF-IR signaling may contribute to resistance to EGFR-targeted TKIs and that simultaneous inhibition of EGFR and IGF-IR may effectively prevent recurrence in human cancers characterized by overexpression of EGFR.

The study reported by Guix et al. has focused on two human cancer cell lines, namely A431 squamous carcinoma cells and HN11 head and neck carcinoma cells (10). It would be of interest to investigate how prevalent this mechanism of acquisition of resistance to gefitinib is in physiologic situations. Interestingly, the GR cells were cross-resistant to the EGFR-targeted TKI erlotinib and the EGFR-targeted monoclonal antibody cetuximab, suggesting that loss of IGFBPs is involved in resistance to other ErbB-targeting TKIs and antibodies. The current findings are consistent with earlier reports that activation of the PI3K pathway, which has been shown to be dominant in transformation-related signaling events caused by ErbB kinase complexes (11, 12), also contributes to resistance to ErbB-targeted therapeutics (13–15). Indeed, inhibition of IGF-IR signaling can enhance the efficacy of gefitinib to inhibit growth and induce apoptosis in a variety of human cancer cells (16–18).

Signaling pathways involved in resistance to ErbB-targeted therapy

The EGFR inhibitors gefitinib and erlotinib are somewhat effective in the treatment of non–small cell lung cancers that have activating mutations in the kinase domain of EGFR (19–21). These mutations are either substitutions or short, in-frame deletions or insertions located adjacent to the ATP-binding pocket of the kinase domain (19–21). These structural alterations appear to increase the affinity of gefitinib or erlotinib for the ATP-binding sites. However, following clinical treatment, even patients possessing these activating EGFR mutations may experience relapses. Recent studies indicate that development of resistance to EGFR-targeted TKIs can arise through different mechanisms. In many cases, resistance to the TKIs is associated with the subsequent acquisition of the T790M mutation in the EGFR kinase domain (6, 7). The threonine-to-methionine substitution at position 790 creates a steric hindrance that limits the binding of the TKIs, while preserving the kinase activity. In addition, other recent studies demonstrate that amplification of the *MET* proto-oncogene can also contribute to resistance to gefitinib (8). Moreover, persistent activation of the PI3K signaling pathway through ErbB3 has also been associated with gefitinib resistance (9). Conversely, loss of inhibitory elements of the PI3K pathway, such as the tumor suppressor PTEN and the signal-regulatory protein SIRP1, may play a role in resistance (11, 13–15). In addition to the PI3K pathway, signaling networks that act via mTOR can lead to refractory states in response to EGFR-targeted therapies. In this regard, blocking the mTOR pathway can overcome resistance to EGFR-targeted TKIs and can act cooperatively to inhibit tumor growth in vivo (22).

It is clear that the resistance phenotype can arise from diverse adaptive and genetic changes within transformed cells. A complete understanding of the mechanisms involved in gefitinib resistance will provide a framework to develop and optimize therapeutic strategies. Information regarding the genetic signatures of drug resistance can be used to predict clinical outcomes. Clearly, the evolution of cancer therapeutics (biologics, chemotherapeutics, and radiation) will involve regimens designed to simultaneously target multiple signaling pathways, thereby minimizing the risk of emergence of a resistant phenotype. In addition, medications targeting cancer metabolic phenotypes could be used in conjunction with ErbB-targeted therapeutics to achieve maximal therapeutic effects. The use of combination therapies to disable both the metabolic and the transformed phenotypes may be the most logical strategy for cancer treatment.

Address correspondence to: Mark I. Greene, Department of Pathology and Laboratory Medicine, University of Pennsylvania School of Medicine, Abramson Family

Cancer Research Institute, 252 John Morgan Building, 3620 Hamilton Walk, Philadelphia, Pennsylvania 19104, USA. Phone: (215) 898-2870; Fax: (215) 898-2401; E-mail: greene@reo.med.upenn.edu.

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