PTEN-Mediated Resistance to Epidermal Growth Factor Receptor Kinase Inhibitors

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Abstract  Molecularly targeted therapies are transforming the treatment of cancer. Elucidating the dynamic signaling networks that underlie sensitivity and resistance to these inhibitors is critical for successful clinical application. There is considerable evidence to suggest that constitutively activating mutations in kinases that regulate cellular growth may result in tumor cell “addiction” and favorable response to targeted inhibition. However, there is emerging evidence to suggest that clinical response may also be determined by other changes in the molecular circuitry of cancer cells, such as loss of key tumor-suppressor proteins. Here, we will discuss resistance to epidermal growth factor receptor tyrosine kinase inhibitors in glioblastoma patients that is mediated by loss of the PTEN tumor-suppressor protein.

Background

Recent advances in molecular oncology have generated great optimism that the empirical use of anticancer agents will soon be transformed into a practice of “personalized” cancer medicine where the molecular profile of the patient’s tumor, rather than tissue type and population-based trials, will guide the choice of therapeutic interventions (1). This enthusiasm seems justified by the remarkable success of selected kinase inhibitors in the clinic (2) and our increasing ability to interrogate the cancer genome (3), transcriptome (4), and proteome (5) in clinical tumor samples. There is compelling evidence that activating mutations in signaling pathways can result in tumor cell “addiction” to this pathway (6) and predict clinical responses to pathway inhibition (7). It has also become clear, however, that tumor cell responses are further determined by the molecular circuitry, in which these activating mutations occur. This concept of context-dependent oncogene addiction has important implications for the design of molecularly targeted combination therapies. In this review, we will discuss one example for this type of drug resistance [i.e., modulation of epidermal growth factor receptor (EGFR) kinase inhibitor response by the phosphatase and tensin homologue deleted on chromosome 10 (PTEN)].

Clinical Translational Implications

The EGFR as therapeutic target in cancer. The EGFR is a receptor tyrosine kinase that regulates fundamental processes of cell growth and differentiation. Overexpression of EGFR and its ligands, particularly transforming growth factor-α, was reported for various epithelial tumours in the 1980s (8) and generated interest in EGFR as a potential target for cancer therapy (9, 10). These efforts have been rewarded in recent years as ATP site-directed EGFR tyrosine kinase inhibitors, and EGFR-specific monoclonal antibodies have shown antitumor activity in subsets of patients with non–small cell lung cancer (11, 12), colorectal carcinoma (13), glioblastoma (14, 15), squamous cell carcinomas of the head and neck (16), and selected other malignancies (17). The identification of oncogenic EGFR kinase domain mutations in lung tumors that responded to EGFR kinase inhibitor therapy (18–20) provided gratifying clinical proof for the concept of oncogene addiction, which now has further been validated in genetically defined model systems (21–23). EGFR kinase domain mutations, however, have not been found in every tumor that subsequently responded to anti-EGFR agents. This observation suggests that tumor cell addiction to EGFR can result from other activating events in the EGFR receptor family network (24), including expression of the oncogenic EGFRvIII deletion mutant (15, 25), missense mutations in the EGFR extracellular domain (26) EGFR amplification (27, 28), or alterations in EGFR coreceptors (29–32). In this context, it is worth remembering that EGFR plays an important physiologic role in the proliferation and survival of normal epithelial cells, likely mediated through autocrine and paracrine mechanisms. This lineage-specific EGFR “dependence” might contribute to the clinical activity of anti-EGFR antibodies in certain cancer types where kinase domain mutations or amplifications seem to be rare (squamous cell carcinomas of the head and neck and colon cancer).

Patterns of resistance to EGFR kinase inhibitors. Failure to respond to EGFR kinase inhibitor therapy can be explained by several mechanisms and, from a clinical point of view, be categorized as either “upfront” resistance or “acquired” resistance. Acquired resistance to EGFR kinase inhibitors in non–small cell lung cancer (similar to the development of acquired resistance to BCR-ABL kinase inhibitors in chronic myeloid leukemia) seems to involve selection for kinase inhibitor–resistant mutant clones (33, 34). Because tumors remain EGFR dependent, drug resistance might be overcome with second-generation kinase inhibitors that can block enzymatic activity through alternative mechanisms of action (35). Upfront
resistance to EGFR kinase inhibitors, on the other hand, is likely to be encountered in tumors that have arisen as a result of EGFR-independent genetic alterations (e.g., KRAS; ref. 36) and do not require EGFR for maintenance of the malignant phenotype. Upfront resistance, however, may also occur in EGFR-driven tumors if additional genetic alterations render the EGFR signal dispensable for tumor cell survival. The following paragraph will show this concept of context-dependent oncogene addiction in more detail.

PTEN loss as resistance factor in EGFR kinase inhibitor therapy. Glioblastoma is the most aggressive human brain tumor (37) and a particularly attractive indication for anti-EGFR strategies because ~40% of glioblastomas show amplification of the EGFR gene locus (38) and about half of these tumors express a mutant receptor (EGFRvIII) that is constitutively active due to an in-frame deletion of exons 2 to 7 within the extracellular ligand-binding domain (39–41). Based on recent clinical trials, 10% to 20% of unselected glioblastoma patients respond to small-molecule EGFR kinase inhibitors as determined by tumor regression on magnetic resonance imaging (14,15). To understand the molecular basis for drug response, we conducted a retrospective analysis of tumor tissue from patients who responded or did not respond to EGFR kinase inhibitor therapy. None of the tumors that responded to EGFR kinase inhibitor therapy harbored a mutation in the ligand-binding domain, consistent with previous reports that EGFR kinase domain mutations are indeed very rare (i.e., <1%) in this disease (42–44). In contrast, the majority of responding tumors expressed the oncogenic mutant EGFRvIII. Because EGFRvIII expression was also found in drug resistant tumors, we further examined all tumors for molecular aberrations that might render oncogenic EGFR signals dispensable for tumor cell survival. Loss of the tumor suppressor PTEN was highly correlated with treatment failure. In fact, coexpression of EGFRvIII and PTEN strikingly predicted treatment responses (15). These data, which were also validated in isogenic cell systems, support the model that EGFRvIII, much like EGFR kinase domain mutations in lung cancer, sensitize tumor cells to EGFR kinase inhibitor therapy, but this addiction can be overcome by simultaneous loss of PTEN.

How does PTEN confer resistance to EGFR kinase inhibitors? PTEN serves as negative regulator of the phosphatidylinositol 3-kinase (PI3K) pathway by removing the third phosphate from the inositol ring of the second messenger PIP3. PTEN inactivation results in accumulation of PIP3 levels and persistent signaling through the serine/threonine kinase Akt/protein kinase B (Fig. 1A). PTEN loss could thus promote resistance to EGFR kinase inhibitors by dissociating EGFR/EGFRvIII inhibition from downstream inhibition of the PI3K signaling pathway. Consistent with this, increased levels of Akt/protein kinase B phosphorylation have been associated with resistance to erlotinib in malignant glioma patients (14). Does PTEN loss promote resistance to EGFR kinase inhibitors in other cancer types? Several model-based studies suggest that this is the case. Ectopic expression of PTEN in a PTEN null breast cancer cell line harboring EGFR amplification (MDA468) confers sensitivity to the proapoptotic effects of EGFR kinase inhibitors, and EGFR kinase inhibition resulted in Akt inhibition only in PTEN-reconstituted cells but not in parental cells (45). In PTEN-positive A431 cells, which harbor EGFR amplification and are exquisitely sensitive to EGFR kinase inhibition, however, overexpression of a constitutively Akt allele did not confer resistance to EGFR kinase inhibitors (15), suggesting that Akt activation may be required but not sufficient for EGFR kinase inhibitor resistance. More recent studies in the MDA468 model system indeed suggest an interplay between Akt and other EGFR effector pathways in the regulation of individual cell death effector proteins (46).

Future Challenges

The PI3K pathway is a critical effector of growth, proliferation, and survival pathways. Abrupt activation of this pathway occurs in many human malignancies as a result of activating mutations or gene copy alterations involving PTEN, PIK3CA, Akt, BRAF, K-ras, H-ras, N-ras, and neurofibromin-1 (47). It will be important to determine whether the relationship between PTEN loss and EGFR kinase inhibitor sensitivity in glioblastoma extends to other malignancies, which harbor activating mutations...
in the PI3K pathway and are currently targeted with ErbB signaling network inhibitors. In a small panel of ERBB2-overexpressing primary breast tumors, for example, it was shown that the expression level of PTEN was positively correlated with the clinical efficacy of the HER2 antibody trastuzumab (48).

The current data from retrospectively analyzed clinical trials and preclinical models (15, 45, 46) suggest that monotherapy with EGFR kinase inhibitors is unlikely to be effective in PTEN-deficient tumors, even if they harbor activating EGFR mutations. This could potentially result in upfront resistance to EGFR kinase inhibitors in highly PTEN-deficient tumors or to acquired resistance in molecularly heterogenous tumors, in which PTEN-deficient cells develop a selective growth advantage during treatment. This conclusion, if confirmed in prospective clinical trials, raises the question how PTEN status should best be ascertained in clinical samples. PTEN inactivation can result from gene deletion or mutation, but reductions in PTEN protein levels are often seen in the absence of genomic mutations (49).

In our clinical trial, we thus determined PTEN status by immunohistochemistry using PTEN staining of adjacent normal brain tissue and tumor vasculature as an internal positive control. This assay should be combined with some determination of PTEN function, either by staining for downstream PIP3 effector molecules (such as pAkt or pPRAS40) or by sequencing of PTEN to identify loss-of-function mutations.

One conclusion from our model of context-dependent EGFR addiction is that EGFR kinase inhibitor sensitivity should be restored in PTEN null tumors by inhibition of the PIP3 excess generated as a result of PTEN loss. Inhibitors of the PI3K, which catalyzes the production of PIP3, are thus particularly promising agents for this indication and are currently in clinical development (50). Because a major output of PIP3 is the Akt/protein kinase B, downstream substrates of Akt, such as the mammalian target of rapamycin, also present attractive therapeutic targets to overcome PTEN-related resistance to EGFR kinase inhibitors. Wang et al (51) and others (52) have indeed observed synergistic effects of EGFR kinase inhibitors and the mammalian target of rapamycin inhibitor sirolimus in glioblastoma cells and this combination regimen is currently being explored in clinical trials (53, 54).
As shown in this review for the interplay between PTEN and EGFR in glioblastoma, tumor responses to signal transduction inhibitors may ultimately be determined by the pathway circuitry in a tumor cell or tissue type (Fig. 1B). Further, positive and negative feedback loops within the Ras, PI3K, and mammalian target of rapamycin signaling network (55) may explain at least some cases of such context-dependent oncogene addiction (56, 57). Understanding the genetic basis of such regulatory loops currently seems daunting but may guide the development of multitarget kinase inhibitors (58) and bring us one step further toward the optimal deployment of signal transduction inhibitors for cancer.

References
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