EGF receptor mutations in lung cancer: From humans to mice and maybe back to humans

Deletions in exon 19 and nucleotide substitutions in exon 21 are the most common mutations of the EGFR (ErbB1) in NSCLC. These mutations endow the receptor with constitutive kinase activity. Most tumors expressing these mutants respond well to EGFR tyrosine kinase inhibitors, suggesting that they are dependent on mutant EGFR signaling. Two groups developed transgenic mice in which expression of these mutants is temporally induced in mouse lung. Mice expressing EGFR mutants develop bronchioloalveolar cancer and lung adenocarcinoma, which are highly sensitive to EGFR inhibitors. These mouse models provide important opportunities for studying the biology of NSCLC and the refinement of anti-EGFR therapies.

In 2004, three groups reported somatic mutations in the *EGFR* gene in non-small cell lung cancer (NSCLC) (Lynch et al., 2004; Paez et al., 2004; Pao et al., 2004). The mutations are either short, in-frame deletions or insertions or substitutions clustered around the region encoding the ATP binding pocket of the receptor's tyrosine kinase domain in exons 18–21. The majority of patients with these tumors exhibit durable clinical responses to the EGFR tyrosine kinase inhibitors (TKIs) gefitinib and erlotinib, suggesting that they are "gain-of-function" mutations that represent a functional marker of EGFR dependence in NSCLC. The two most common mutations are an in-frame deletion in exon 19, which eliminates a conserved LREA motif, and an L858R substitution in exon 21 (Shigematsu et al., 2005). Two groups have now reported results with mouse transgenic models expressing these mutations (Ji et al., 2006 [this issue of *Cancer Cell*]; Politi et al., 2006). Expression of the EGFR mutants was targeted to lung type II alveolar cells by crossing mice carrying Clara cell secretory protein (CCSP)-regulated reverse tetracycline transactivator (rtTA) transgene with mice carrying either L585R/EGFR or EGFR with deleted LREA (Del/EGFR) transgene regulated by tetracycline (tet)-responsive elements. This allowed for the generation of bitransgenic mice (CCSP/rtTA × tet-op/L858R hEGFR and CCSP/rtTA × tet-op/Del hEGFR) in which administration of doxycycline resulted in the expression of mutant human EGFR in lung pneumocytes.

Administration of doxycycline led to tissue-specific expression of the transgenes and, within a few weeks, the development of focal or diffuse bronchioloalveolar carcinoma (BAC), in some cases preceded by precancerous adenomatous lesions. Upon longer transgene induction (>4 weeks), mice developed invasive adenocarcinomas. Doxycycline withdrawal resulted in complete regression of lung cancers with no recurrences observed during the withdrawal period, although the follow-up was short. Treatment with the EGFR TKI erlotinib caused rapid regression of doxycycline-treated tumors as well as inhibition of EGFR phosphorylation and postreceptor signal transducers. It remains to be determined if tumors recur after prolonged deinduction or longer therapy with erlotinib and whether they remain EGFR dependent at the time of relapse. If erlotinib was not as effective in providing long-term tumor control compared to doxycycline withdrawal, this would suggest that the small molecule inhibitor did not achieve complete and prolonged inactivation of the mutant kinase. In the paper by Ji et al., treatment with the irreversible covalent inhibitor HKI-272 and more prolonged therapy (>2 weeks) with the EGFR monoclonal antibody cetuximab also resulted in tumor regression.

These results in genetically engineered mice confirm that L8558R/EGFR and Del/EGFR are dominant oncogenes that alone can catalyze the whole process of cancer development. The histological changes induced by expression of the mutant transgenes represent a progressive spectrum of lesions similar to the one observed in patients with NSCLC harboring these

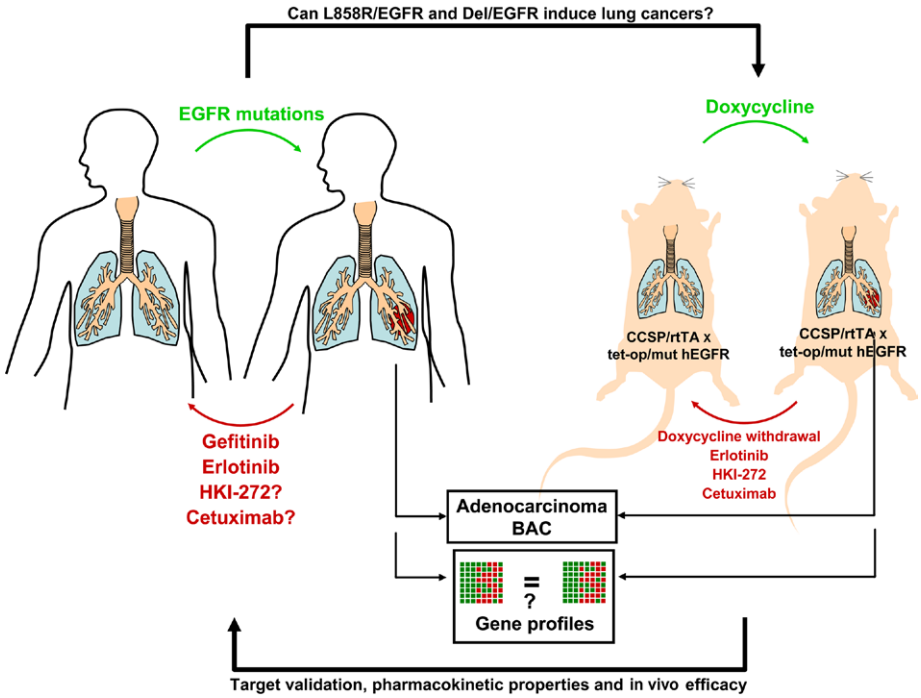

Figure 1. Transgenic mice expressing L858R/EGFR and Del/EGFR develop bronchioloalveolar cancer and lung adenocarcinoma

These histological types of tumors are identical to those observed in patients with NSCLC harboring these same receptor mutants. Mouse lung tumors responded dramatically to treatment with erlotinib, HKI-272, or cetuximab. Clinical activity of HKI-272 and cetuximab against human NSCLC expressing EGFR mutations remains to be formally tested. A comparative of gene profiles between mouse and human lung cancers expressing EGFR mutations has not been reported yet.

mutations. Interestingly, in most primary tumors, BAC is associated with a component of invasive adenocarcinoma similar to the tumor histologies observed by Ji et al. and Politi et al. Further, adenocarcinomas with BAC features are more responsive to the EGFR TKI gefitinib and may have a higher frequency of EGFR mutations than pure adenocarcinomas or pure BAC (Miller et al., 2004, 2006).

The marked reduction and/or elimination of lung cancers upon either doxycycline removal or treatment with the EGFR TKIs clearly suggests that lung tumorigenesis is specific to the EGFR mutations. The high efficacy of the covalent inhibitor HKI-272, which has activity against erlotinib- and gefitinib-insensitive EGFR mutants (Kwak et al., 2005), supports the possibility of using it in combination with erlotinib as first line therapy in order to reduce the likelihood of therapeutic resistance. The effect of cetuximab, an antibody that binds a conserved region in the ectodomain of human EGFR but not mouse EGFR (Goldstein et al., 1995) and thus is unlikely to exert off-target effects in mice, also supports transgene specificity. Interestingly, the effects of the antibody appeared to be independent of blockade of ligand binding, suggesting an alternative mechanism of action that perhaps can be exploited in combination with EGFR TKIs. Considering that the mice in this study are fully immunocompetent, antibody-dependent, cell-mediated cytotoxicity (ADCC) (Modjtahedi et al., 1994; Sampson et al., 2000) is a possible mechanism of cetuximab action.

Additional benefits from the temporally regulated models generated by Politi et al. and Ji et al. can be derived from their integration into the preclinical drug development process. For example, they can be used as "filters" where new EGFR inhibitors, either alone or in combination with other drugs, are tested for target validation (as was done in these papers with P-EGFR and postreceptor transducers), pharmacokinetic properties, and in vivo efficacy (Figure 1). Obviously, lack of molecular and/or clinical activity over erlotinib or HKI-272 in the long run would suggest reexamination of the development of such agents or combinations. This is probably an unlikely scenario for single drugs in light of the agents already available for these types of NSCLC. Another use of these models could be the elucidation (and timing) of biochemical or molecular readouts predictive of tumor response. A pertinent example was recently contributed by Majumder et al. in a transgenic mouse model in which prostate-specific expression of an activated allele of Akt resulted in prostatic intraepithelial neoplasia (PIN). Administration of a TOR inhibitor to these mice eradicated the PIN lesions and downregulated mRNAs regulated by HIF-1α, among these genes encoding enzymes regulatory of the glycolytic pathway (Majumder et al., 2004). Since these enzymes are responsible for uptake and retention of the positron emission tomography (PET) tracer [^{18}F]2-fluoro-2-deoxy-d-glucose (FDG), scanning with FDG-PET was proposed as a readout of TOR activation in tumors that can be used to monitor the efficacy of anti-TOR therapies with noninvasive methods. This question is being prospectively addressed in investigational clinical trials with TOR inhibitors.

Even though the majority of NSCLCs expressing EGFR mutations in the kinase domain respond to EGFR TKIs, insertions in exon 20 exhibit resistance to gefitinib and erlotinib (Greulich et al., 2005). In addition, the majority of EGFR mutant NSCLCs that initially respond to EGFR inhibitors eventually escape therapy. A secondary mutation in exon 20 (T790M) was discovered in patients with a primary mutation that progressed after an initial response to gefitinib or erlotinib (Kobayashi et al., 2005; Pao et al., 2005a). Because of the presence of the bulkier methionine side chain in the ATP binding pocket, T790M sterically hinders binding erlotinib and gefitinib, explaining drug resistance (Kobayashi et al., 2005). Furthermore, mutations in the *K-Ras* gene are mutually exclusive with *EGFR* mutations (Kosaka et al., 2004; Shigematsu et al., 2005) and predict for lack of sensitivity to EGFR inhibitors (Pao et al., 2005b). In most patients with NSCLC-containing *EGFR* mutations, the mechanisms of de novo or acquired resistance to EGFR TKIs are unknown. The emergence of therapeutic resistance, especially after therapy with a single antioncogene drug, is not surprising though if we consider that potent oncogenes, such as L858R/EGFR and Del/EGFR, induce sufficient cell expansion where additional compensatory or complementary mutations occur. Moreover, intrinsic pharmacological limitations (e.g., short half-life) may affect the ability of a single drug to completely disable amplified oncogenic networks, especially if the tumor burden is high.

Several reports suggest the possibility that conditional transgenic models may generate insights about secondary genetic alterations that eliminate the dependence on the original oncogene and thus potentially generate drug resistance. For example, regulated overexpression of c-Myc in the mouse mammary gland results in invasive cancers. These tumors regress upon deinduction of c-Myc. Eventually, after a few cycles of c-Myc induction and deinduction, a large proportion of tumors did not regress when c-Myc was downregulated with the majority of these Myc-independent cancers expressing K-*ras* or N-*ras* mutations (D'Cruz et al., 2001). In another study, transgenic mice expressing tetracycline-regulated Neu oncogene in the mammary gland developed mammary cancers upon induction of Neu. Deinduced tumors eventually escaped Neu dependence, upregulated the transcriptional repressor *Snail*, and exhibited evidence of an epithelial-to-mesenchymal transition (EMT). Snail was sufficient to induce mammary tumor recurrence in vivo and EMT, and its overexpression in primary human tumors predicted poor patient outcome in patients with breast cancer (Moody et al., 2005). In this example using a conditional transgenic mouse model of breast cancer, the acquisition of a secondary genetic alteration upon escape from Neu dependence provided information about a molecular pathway that may identify tumors resistant to anti-Neu therapies and/or with a poor prognosis.

These elegant observations in genetically engineered mice have to be followed and validated in patients. It can also be argued that there is not a good correlation between mouse and human cancer, and therefore, the examples chosen represent spurious leads. However, the papers by Ji et al. and Politi et al. suggest that that may not be the case, as regulated induction of either L858R/EGFR or Del/EGFR in mouse lung pneumocytes was able to induce syndromes that phenocopy to a significant degree those observed in humans. Further, recent studies using hierarchical clustering analysis of RNA signatures in mouse and human tumors clearly indicate patterns of gene expression and genes that can be interrogated in preclinical studies (Ellwood-Yen et al., 2003; Lee et al., 2004; Sweet-Cordero et al., 2005). Similar comparisons between the mouse lung tumors and primary human cancers expressing EGFR mutations are now doable using the animal models discussed in this preview. At the end of the day, however, how we exploit mouse models for the study of cancer biologyas well as within a streamlined process of drug development will depend on how open we are to exploring their utility and how inquisitive we want to be.

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