Targeted Anticancer Therapies: Mouse Models Help Uncover the Mechanisms of Tumor Escape

Renée van Amerongen[1] and Anton Berns[1,*]
[1]Division of Molecular Genetics and Centre of Biomedical Genetics, Netherlands Cancer Institute, Plesmanlaan 121, 1066 CX Amsterdam, The Netherlands
*Correspondence: a.berns@nki.nl
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In contrast to conventional chemotherapeutic agents, modern anticancer therapies are aimed at attacking specific targets in a tumor. While these therapies show promising clinical effects, their success is limited by the development of resistance to the antitumor agent, a phenomenon that is well known in regular cancer therapies. As illustrated in a novel study by Debies and colleagues in *The Journal of Clinical Investigation*, mouse models for cancer serve as promising tools for advancing our understanding of the tumor response to targeted therapy. However, the experimental setup and selected model system may evoke unexpected escape mechanisms. Here, we discuss the promises and pitfalls of these approaches.

Conceptually, "targeted therapies" against aberrantly expressed or mutated cellular components that are crucial for tumor growth and survival seem promising. They are likely less toxic for normal cells in which these components are unaltered and present at physiological levels. Among the targeted anticancer therapies that have successfully been introduced in the clinic are trastuzumab (Herceptin) and imatinib (Gleevec), which are administered as adjuvant therapy to patients with HER2/NEU-positive breast cancer and as first-line therapy for patients with chronic myeloid leukemia (CML), respectively. While these treatments present a major advancement and often result in long-term patient survival, resistance frequently develops.

These targeted therapies are particularly appealing if they exploit a phenomenon known as "oncogene addiction," which refers to the fact that even advanced tumors remain dependent on initiating oncogenic lesions. While this was first demonstrated to be the case for MYC in hematological malignancies (Jain et al., 2002), it holds true for many tumors. Although inhibition of the initiating oncogenic activity often causes tumor regression, tumors frequently relapse. This can occur by "target reactivation," i.e., by acquiring novel mutations in the drug target rendering it refractory to treatment, as observed for the imatinib target BCR/ABL (Gorre et al., 2001) and the gefitinib/erlotinib target EGFR in lung cancer patients (Pao et al., 2005). Alternatively, tumors can activate compensatory signaling pathways, allowing them to bypass the effects of drug treatment (Stommel et al., 2007).

Current mouse models for cancer, carrying inducible oncogenic lesions, allow sporadic tumor formation. Resulting tumors, which often closely mimic human disease, offer the opportunity for preclinical testing and dissecting

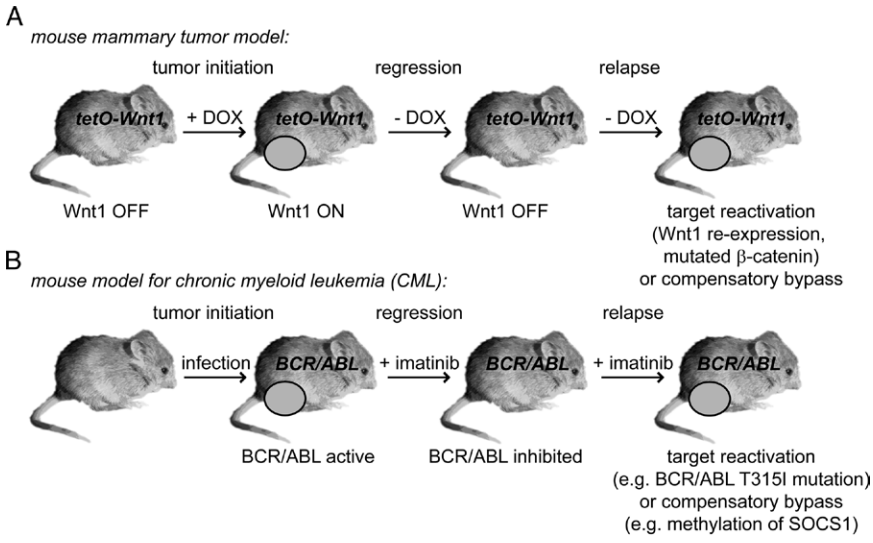

Figure 1. Mouse Models for Tumor Relapse
(A) Debies et al. studied tumor relapse in a mouse model for breast cancer. Mammary tumors are induced upon doxycyclin (DOX)-mediated activation of a *tetO-Wnt1* transgene. DOX withdrawal shuts off *Wnt1* transgene expression, causing tumors to regress. Potent escape mechanisms in the form of DOX-independent *Wnt1* transgene reactivation, oncogenic mutations in β-catenin, or compensatory bypass allow tumor relapse.
(B) This model mimics targeted therapy with novel anticancer agents such as the small-molecule BCR/ABL inhibitor imatinib. In BCR/ABL-driven CML tumor, relapse frequently occurs when cells acquire a T315I point mutation in BCR/ABL, rendering it insensitive to imatinib. Similarly, in lung cancer driven by EGFR hyperactivation, the acquirement of a T790M point mutation in EGFR desensitizes tumor cells to the EGFR inhibitors erlotinib or gefitinib. In addition, compensatory bypass mechanisms, such as methylation of the *SOCS1* gene promoter in BCR/ABL-driven CML, can also promote resistance to targeted therapies (Saudemont et al., 2007).

the molecular mechanisms underlying tumor escape. A recent study by Debies and colleagues made use of conditional *Wnt1*-transgenic mice (*tetO-Wnt1*) to explore tumor relapse (Figure 1) (Debies et al., 2007). Transgene expression in these mice can be switched on by doxycyclin administration, causing *tetO-Wnt1* mice to develop mammary tumors. Tumors regress upon doxycyclin withdrawal, illustrating their addiction to Wnt1 expression.

During a 1 year follow-up, 10 of 34 regressed tumors relapsed. Sixty percent of the relapses resulted from target reactivation, as measured by the expression of Wnt/β-catenin target genes. In half of these, *Wnt1* transgene expression was reactivated in a doxycyclin-independent manner. More strikingly, the other half expressed oncogenic forms of the downstream Wnt-signaling component β-catenin, underscoring the strong dependence of these tumors on the Wnt/β-catenin pathway. The remaining 40% of relapsed tumors did not express Wnt/β-catenin target genes, suggesting that compensatory bypass was responsible for tumor escape.

When the authors next studied tumor relapse of *tetO-Wnt1*-induced tumors in a $p53^{+/-}$ background, they observed a reduced incidence of Wnt/β-catenin target gene activation. Not only did fewer tumors display *Wnt1* transgene reactivation, none of these relapsed tumors carried oncogenic mutations in β-catenin. In contrast, 75% of the tumors that did display target reactivation had undergone *p53* LOH. The authors hypothesize that re-expression of Wnt-pathway target genes in these tumors results from loss of a p53-mediated signal antagonizing Wnt/β-catenin signaling.

To find out whether this effect might also be mediated by loss of other p53-pathway components (such as p19Arf) or other tumor suppressor pathways (such as the p16/Rb pathway) Debies et al. crossed their *tetO-Wnt1* mice onto *p16Ink4a/p19Arf-*, *p16Ink4a-*, or *p19Arf*-deficient backgrounds. Tumor regression was similarly impaired on a *p19Arf-* or *p53*-deficient background, while p16Ink4a loss did not affect either regression or relapse. In addition, p19Arf deficiency resembled Ink4a/Arf deficiency in promoting tumor escape. Although the nature of the compensatory bypass mechanisms in each of the genetic backgrounds remains unknown, this study does suggest that depending on the genetic make-up of the primary lesion, tumors may be more prone to relapse by either target reactivation or compensatory bypass. Moreover, the presence of oncogenic mutations in β-catenin in the wild-type background underscores that unexpected and powerful escape mechanisms may occur, which likely differ with tumor type and experimental setup.

In spite of the valuable information that can be obtained from mouse model studies as described above, the system does have its drawbacks. First, while the switching of conditional oncogenes allows direct control over the initiating oncogenic lesion, it is by no means identical to administering an anticancer drug. It therefore remains to be seen whether true therapeutic agents (such as small-molecule Wnt-pathway inhibitors) would illicit the same responses. Recent work by Rottenberg and colleagues has shown the power and feasibility of such studies by testing a number of conventional chemotherapeutic agents in a mouse mammary tumor model (Rottenberg et al., 2007).

Second, although Wnt-pathway hyperactivation is a powerful means of inducing mammary tumors in mice, lesions in the Wnt/β-catenin pathway are only rarely observed in human breast cancers, leaving the value of this mouse model for human breast cancer questionable.

Third, while the possibility to sample the role of distinct oncogenic lesions in tumor relapse is attractive, the use of mice carrying germline lesions is rather artificial. In mice, the engineered lesion in the presumed compensatory pathway is present from the outset in both stromal and tumor cells. This may affect tumor initiation and growth, and as such, it could skew tumor behavior during regression and relapse. For instance, p19Arf loss not only influences the response of CML to imatinib, but also enhances tumor oncogenicity, raising the question whether tumors in a *p19Arf*-proficient and -deficient background are similar or not (Williams et al., 2006). Likewise, in a $p53^{+/-}$ background, the system is greatly predisposed to *p53* LOH. In contrast, in humans, the lesions in compensatory pathways will occur only sporadically and provide a growth or survival advantage to rare tumor cells under the selective pressure of targeted therapy. In real clinical practice, other modes of resistance may be more important. Future studies should, therefore, attempt to mimic human disease more closely. This could, for instance, be achieved by including an inducible RNAi knockdown construct in the mouse model of choice, such that a given compensatory pathway can be (in)activated in tumor cells concomitant with annihilation of the initiating oncogenic lesion.

In this regard, recent studies on mouse models for CML are exemplary. This tumor model has been well characterized, and tumors are readily induced following infection of bone marrow stem cells with a BCR/ABL-expressing retrovirus. Moreover, BCR/ABL-driven leukemia in mice is susceptible to treatment with imatinib. This has allowed researchers to elucidate resistance mechanisms, as illustrated by a recent study which revealed that cytokines in the tumor microenvironment were able to mediate CML resistance to imatinib in a *p19Arf*-deficient background (Williams et al., 2007).

Can we point to a common denominator facilitating tumor relapse? Interestingly, a number of studies have shown an important role for antiapoptotic pathways acting through p53, Bcl2, and Akt in promoting tumor escape in a range of different settings (Berns et al., 2007; Martins et al., 2006; Ventura et al., 2007). These survival pathways may mediate resistance to a wide variety of targeted therapies.

A future challenge is to identify the genes that can evoke tumor relapse. As indicated above, these will likely differ with tumor type, targeted therapy, and genetic make up of the primary lesion. While genome-wide approaches such as transcriptome and proteome profiling will be a major help in pinning down these events (Stommel et al., 2007), experimental approaches that combine mouse models for tumor escape with gene-hunting techniques may also pay off. A nice example of the latter was recently reported by Miething and colleagues, who combined imatinib treatment in a BCR/ABL-driven mouse model for CML with retroviral insertional mutagenesis

(Miething et al., 2007). This led to the identification of *Runx3* as a proviral target that facilitated tumor relapse upon targeted therapy with imatinib. These new mouse models and genomic techniques can provide us with unique reagents to uncover new resistance mechanisms, thereby allowing us to design strategies to overcome resistance that can subsequently be tested in cancer patients.

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Autocrine IL-6 Signaling: A Key Event in Tumorigenesis?

Sergei Grivennikov[1] and Michael Karin[1,*]
[1]Laboratory of Gene Regulation and Signal Transduction, Department of Pharmacology, School of Medicine, University of California, San Diego, 9500 Gilman Drive, La Jolla, CA, 92093, USA
*Correspondence: karinoffice@ucsd.edu
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Tumorigenesis is a multistep process that requires constitutive cell division, growth, and survival. One strategy used by cancer cells to upregulate growth and survival pathways is through autocrine production of growth and survival factors. Two recent papers by Gao et al. and Sansone et al. published in *The Journal of Clinical Investigation* outline the importance of autocrine interleukin 6 (IL-6) in lung and breast cancers and implicate IL-6 as an important activator of oncogenic STAT3 in lung adenocarcinomas and of Jagged-1/Notch signaling in breast tumor mammospheres.

IL-6 is a multifunctional cytokine that is important for immune responses, cell survival, apoptosis, and proliferation (Kishimoto, 2005). IL-6 signals via a heterodimeric IL-6R/gp130 complex, whose engagement triggers activation of Janus (JAK) kinases, and the downstream effectors STAT3, SHP-2/Ras, and PI3K/Akt (Kishimoto, 2005). Early studies implicated IL-6 and its major effector STAT3 as protumorigenic agents in many cancers, including breast, lung, colon, prostate, ovarian, and hematological cancers as well as melanoma; and IL-6 levels are significantly elevated in lung and breast cancer patients, associated with poor prognosis (Hodge et al., 2005).

Activating mutations in epidermal growth factor receptor (EGFR) were found to result in constitutive STAT3 activation in lung cancer (Gao et al., 2007). Although EGFR can also signal to STAT3 (Quesnelle et al., 2007), pharmacological inhibition of its tyrosine kinase activity did not prevent STAT3 phosphorylation in lung cancer cells, while it significantly inhibited Akt and ERK activation (Gao et al., 2007). By contrast, complete blockade of STAT3 phosphorylation was found upon treatment with pan-JAK inhibitor (Gao et al., 2007). The knockdown of STAT3 or inhibition of its phosphorylation delayed cell growth in culture and tumor growth in a xenograft model, underscoring the critical role of phospho-STAT3 in lung cancer driven by EGFR. Next the authors searched for the signal responsible for JAK and STAT3 activation in their system. Cancer cell lines carrying EGFR mutations were found to produce high amounts of IL-6, and introduction of mutated EGFR into breast epithelial cells in vitro rapidly induced IL-6 production along with cellular transforma-