Mouse models of human cancer as tools in drug development

Histologically accurate mouse models of human cancers generated by somatic or germline genetic modification strategies recapitulate the genetic alterations found in human tumors. In this issue of *Cancer Cell*, Romer et al. (2004) test a novel signaling inhibitor in a genetically defined mouse medulloblastoma and show the utility of these models in drug development. They demonstrate that targeting signaling components downstream of tumor initiating mutations can be an effective therapeutic strategy for solid tumors.

The manuscript by Romer et al. (2004) describes a preclinical trial of a novel small molecule inhibitor of smoothened (Smo) in a genetically engineered mouse model for medulloblastoma. The authors have used the well-described Ptc1$^{+/-}$, p53$^{-/-}$ mice that develop histologically accurate medulloblastoma within 12 weeks (Wetmore et al., 2001). Cultured cells and allografts from medulloblastomas arising in the Ptc1$^{+/-}$, p53$^{-/-}$ mice have been used to test antitumor activity of the natural product cyclopamine (Berman et al., 2002). However, such systems have the potential to be misleading, as the molecular pathways important for tumor growth in situ may be altered in cultured tumor cells and xenografts. Here, Romer and colleagues used the tumor-bearing, genetically engineered mice themselves to test a novel compound HhAntag. This compound was identified in a cell-based screen as blocking Smo with tenfold higher affinity than cyclopamine. This work is particularly significant as it illustrates the use of genetically engineered mouse models of cancer in the process of drug development.

It is clear that developing rational therapeutic approaches for medulloblastoma is essential. Although many children with medulloblastomas are cured of their tumor by surgery, radiation, and chemotherapy, they are frequently devastated by the treatment. This tumor is an excellent example of how cancer can be an aberration of normal development (Raffel, 2004). Medulloblastomas arise from the precursors of the internal granule cells. Originally occupying the external germinal cell layer, these cells are driven to proliferate by sonic hedgehog (Shh) signaling. Under normal conditions, they then begin to differentiate, and then migrate inward to their final resting place, the internal granule cell layer (Ingham and McMahon, 2001). Several lines of evidence indicate that a subset of human medulloblastomas are caused by inappropriately continued SHH signaling and a derailment of normal differentiation. Mouse models of this disease have been particularly informative in this regard.

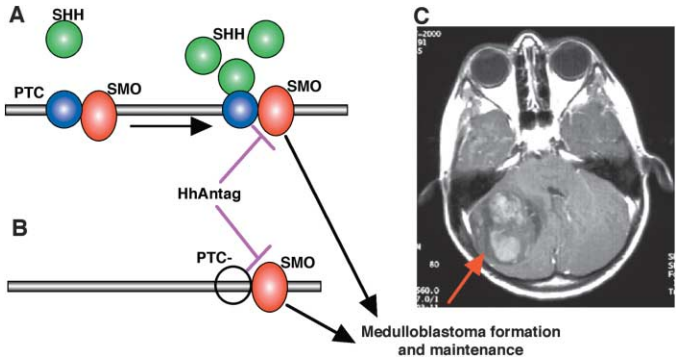

Figure 1. Aberrant SHH signaling and the development of medulloblastoma

Hyperactivation of the SHH signaling pathway leading to medulloblastoma formation can occur by either inappropriately elevated SHH ligand binding to PTC and inactivating its tonic repression of SMO (**A**) or loss of PTC (**B**). Both mechanisms lead to the formation of medulloblastomas in mice. Romer et al. have used the PTC loss mouse model for medulloblastoma formation to verify that the novel SMO inhibitor HhAntag could be therapeutically useful for medulloblastomas in humans (**C**) (red arrow). MRI courtesy of Dr. Mark Souweidane.

Either gain of Shh signaling or loss of Ptc1 cooperates with other alterations, leading to the formation of tumors with histological similarities to the human tumors (Figure 1), indicating that the SHH signaling pathway can be causally related to medulloblastoma genesis (Wetmore et al., 2001, Rao et al., 2004, Goodrich et al., 1997). Further, the gene expression profiles of many of the mouse models of medulloblastomas show a high degree of similarity to the expression profile of the cerebellum five days after birth, the point where the Shh-driven EGL proliferation is at its highest in normal development (Lee et al., 2003). Given all of this, it is a reasonable assumption that the SHH signaling pathway is central to medulloblastoma biology.

The problem of SHH signaling in medulloblastomas is essentially one step removed from BCR/ABL in CML or Kit in GIST, where a single disregulated signaling pathway is causally related to the formation of the tumor. In the case of CML and GIST, the presence of the translocation or mutation identifies it as the inciting event, and as the tumors evolve, they maintain dependence on that abnormal signaling activity. In these tumors, small molecule blockade of the mutated gene products is therapeutically effective. The general concept of a tumor's dependence on the product of the original genetic event is supported by several examples of experimentally induced mouse tumors (Chin et al., 1999, Felsher and Bishop, 1999). In the case of most solid human tumors, however, the inciting event is not known, and therefore, small molecule inhibitors of the actual mutated gene product are unavailable. This is the case in a large subset of medulloblastomas. Although there are mutations found in PTC1 in approximately 10% of the tumors, the elevated SHH signaling seen in a larger subset of medulloblastomas is frequently not explained by mutation, and the pharmacologic intervention available does not directly attack the mutated gene product. However, mutations in different components of the Shh pathway, which lead to inappropriate activity of the pathway, have been identified and may account for most of the medulloblastomas with high GLI1 expression. Therefore, Shh signaling

likely plays a central role in these tumors, which makes pharmacological blockade of the pathway an extremely attractive strategy. The data from this manuscript, in concert with other publications, strongly suggests that such a strategy may well work in a subset of medulloblastoma patients, and in other tumors that depend on SMO activity for growth

Perhaps more importantly, these studies highlight the evolution of thought regarding the use of mice in the understanding of cancer and development of novel therapies. The majority of preclinical work has used xenografts, cell lines derived from human tumors that are maintained in culture and implanted into the flank or orthotopic site of an immunocompromised mouse. The goal of such studies has been to determine if the drug in question shrinks the grafted tumors or extends the lifespan of the mice harboring them. Although these models are standardized and generate reproducible tumors, they have been less predictive of human response to drugs than one would like. Romer et al. provide an explanation for why such an approach may ultimately fail for those developing compounds that target the Shh pathway in medulloblastoma. They found that the pathway is promptly downregulated when cells are placed in culture. Further, they demonstrate that the antiproliferative effects of cyclopamine reported previously reflect a nonspecific toxicity of high compound concentrations as opposed to the specific effect of blocking the Shh pathway.

The new genetically defined mouse models of human cancer have major potential advantages over xenografts in preclinical trials (Holland, 2004). First, genetic models by definition recapitulate genetic events that are causally related to the formation of the tumor, and offer a proof-of-principle test of critical targets for therapy. The SHH signaling pathway has now been validated as a therapeutic target in medulloblastoma using this new approach. Second, genetically induced tumors arise in situ and frequently have histologies very similar to or indistinguishable from the human counterparts, as in the case of the medulloblastomas reported here. By contrast, xenografts rarely have histological similarity to the tumors from which they were originally derived. Finally, and most importantly for this work, the requirement for specific biologic activities in the context of defined inciting events can only be addressed in systems where the causal genetic events are known.

These genetic mouse models of cancer may be most useful in defining general rules for the dependence of tumors on individual and combined biologic pathways. The simplistic view of a response to therapy being tumor shrinkage or prolonged survival of a cohort of mice may be replaced with more specific questions, such as molecular efficacy, cell cycle progression, regional cell death, and morphologic conversion as response to therapy. Preclinical trials of this nature may also identify surrogate markers for effective intervention that could be used in human trials.

The differences in drug metabolism between mouse and human suggest that mice are not likely to identify the best drug for human use from a group that all attack the same target with similar affinity. However, mouse models may very well tell us that a particular target or pathway is important to address therapeutically in a particular molecular subset of human tumors. Further, such preclinical trials will likely be able to determine whether novel drugs can hit their targets and achieve biologic effects in vivo. In the case of medulloblastomas and SHH signaling, we can guess that blockade of the pathway by some small molecule may be effective in the subset of human medulloblastomas that are driven by SHH signaling. The best drug for that purpose in humans may not yet be identified, although the efficacy of HhAntag in vivo presented here is quite encouraging. The substantial power of mouse modeling has been developed by the collective scientific community over the last 20 years primarily for the purpose of understanding biology. Hopefully, studies such as these are now opening the door for uses of this technology to be applied directly toward improvement of the human condition.

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