mice[1,2]. Both studies show a crucial role of TRPA1 in nociceptor function, but no significant role in hearing. The actions of bradykinin and mustard oil on nociceptor activation and sensitization strongly depend on TRPA1, although the studies conflict over whether TRPA1 is the only receptor for mustard oil.

Kwan *et al.*[2] observed marked deficits in sensation of acute noxious cold and mechanical sensation in TRPA1 mutant mice, whereas these deficits were not detected by Bautista *et al.*[1]. Perhaps these discrepancies resulted from variations in experimental conditions. For example, only female mutant mice show a pronounced deficit in the noxious cold plate assay performed by Kwan *et al.* It is not clear whether Bautista *et al.* examined females in their analysis. Both groups delete the portion of the TRPA1 gene encoding the pore of the channel, so allelic differences are perhaps less likely to be a source of variation. These disparities highlight the benefits of creating and analyzing multiple knockout alleles of the same gene. Future in-depth analysis should resolve most of these issues.

Bautista *et al.* also show that acrolein (2-propenal), an environmental irritant present in vehicle exhaust and tobacco smoke, and a metabolized byproduct of chemotherapy agents, activates TRPA1 (ref. 1). The identification of acrolein as a TRPA1 activator may be the tip of the iceberg in terms of the types of chemical insults that exert their noxious effects through TRPA1. More studies must be performed to determine the *in vivo* specificity of compounds like acrolein, as such α,β unsaturated aldehydes are highly reactive and may have pleiotropic effects.

The acrolein experiments were performed in cultured sensory neurons. But this approach does not directly address whether the cytotoxicity of acrolein is mediated by activation of TRPA1 in sensory neurons; it should be possible to test this hypothesis in mice lacking TRPA1.

These studies have shown that TRPA1 is required for sensing disparate noxious stimuli and make TRPA1 an enticing drug target for the development of new analgesics (**Fig. 1**). TRPA1 is also a potential contributor to pain resulting from nerve injury: mRNA encoding TRPA1 is upregulated in rat sensory neurons after injury, producing cold hyperalgesia that can be abolished by treatment with antisense TRPA1 RNA[10]. Kwan *et al.*, however, find that mechanical hyperalgesia in response to nerve injury is not abolished in the TRPA1 mutant mice.

It is possible that TRPA1 inhibitors may benefit people with neuropathic pain, many of whom do not respond to traditional analgesics. Furthermore, if irritants like acrolein prove to be acting through TRPA1, then TRPA1 antagonists could be useful for treatment of pulmonary edema and respiratory irritation and may extend the effectiveness of chemotherapy by reducing dose-limiting toxicity.

Although the functional characterization of TRPA1 mutant mice has solidified the requirement of TRPA1 for nociception, many important questions still remain. How is TRPA1 activated by such varied stimuli? Are mechanical forces one of these stimuli, and do these stimuli act directly on TRPA1 or through intracellular signaling mechanisms? Ongoing research in this area should answer many of these questions

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Virus smuggling, tax evasion and tumor assassination

Kevin Harrington & Richard Vile

A new approach to killing tumors combines two methods that each alone have shortcomings. A tumor-killing virus is loaded into a cell type that homes to tumors, thereby evading the antiviral immune response.

The concept of using viruses to kill tumors is not new, but the field has suffered setbacks. The problem with using systemic virotherapy to treat disseminated cancer is that the immune system, quite frankly, does not approve of it. In fact, the immune system has many sophisticated methods to seek and destroy viruses and, unfortunately, cannot distinguish between the benevolent, engineered viruses of gene therapists and dangerous pathogens. After intravenous injection, only a tiny fraction of viruses finds its way in to systemic tumors, while the rest are either washed away to irrelevant normal tissues or captured by endothelium, circulating immune cells or antibodies (**Fig. 1a**).

It is the immune system that levies the greatest taxation on the virus, and this burden increases upon readministration after specific cellular and humoral responses are established. Therefore, however elegantly we endow viral vectors with the power to transduce, express or replicate selectively in tumor cells, we have to face the reality of paying crippling taxes to the immune system.

In a recent issue of *Science*, Thorne *et al.*[1] show what we have long suspected: to achieve delivery of viral vectors to systemically distributed metastases in immunocompetent hosts, these vectors will have to be smuggled through the circulation and into the tumor bed[2,3]. The authors achieved this by loading a virus into a cell that would have free right of passage through the immune system and used it to ferry the virus to its destination—namely, the tumor[2].

Even though the immune system is clearly the problem for systemic viral delivery, Thorne *et al.* have recruited it to the cause of smuggling viruses. They exploited the tumor-homing properties of a class of $CD8^+$ natural killer T cells, cytokine-induced killer cells (CIKs)[1]. CIKs are easily isolated and expanded *in vitro* with cytokines and antibodies without prior knowledge of the tumor's antigenic repertoire[4,5]. Moreover, CIKs localize to tumors after intravenous injection by using the expression of specific stress-inducible ligands by the tumors as both homing and killing targets (**Fig. 1b**).

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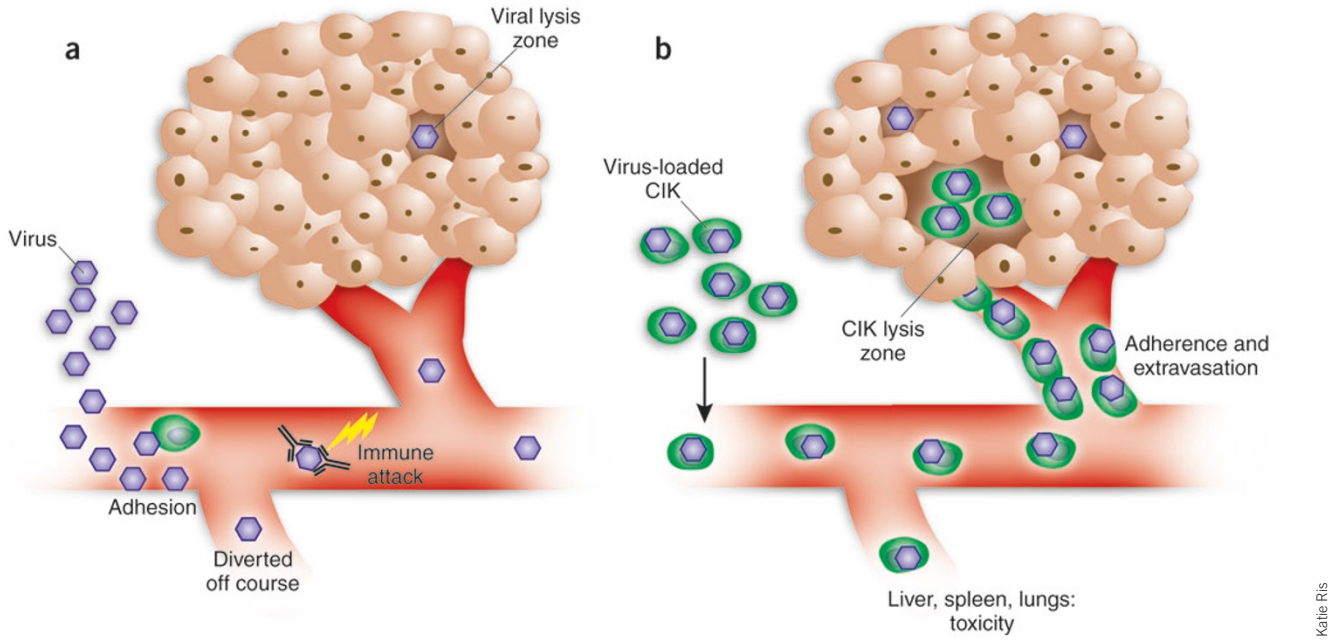

Katie Ris

Figure 1 Smuggling the assassins to the tumor. (**a**) Stocks of virus injected intravenously will have a perilous journey through the circulation to the tumor. Many will adhere nonselectively to endothelial walls or to circulating cells; others will be carried off course. If immunity to the virus exists, it could also be cleared. The few that make it near the tumor may be unable to stop and extravasate. Very few particles will survive the passage and infect the tumor—setting up a spreading infection and creating a zone of viral lysis that will probably have very limited therapeutic effect. (**b**) The journey is much less dangerous for autologous cells such as CIKs. These cells will not be neutralized by immune mechanisms, and if they go off course, they should be able to survive and recirculate. Moreover, CIKs will selectively adhere to vessel walls only at the site of specific cytokine and inflammatory signals, characteristic of activated endothelium in tumors. These cells will specifically extravasate into the tumor, where they will kill tumor cells expressing target ligands, generating a zone of CIK lysis. Thorne *et al.* loaded CIKs with an engineered vaccinia virus, enabling both viral and CIK-mediated tumor lysis.

So how can CIKs be converted into effective virus smugglers? Having spent years rigorously excluding replication-competent contaminants from recombinant viral stocks, many investigators now crave the potential cytotoxic efficiency of delivering aggressively replicating viruses to tumors[6,7]. The trick lies in optimizing replication in tumor cells and preventing it in normal cells. This has been approached using wild-type viruses, which appear to replicate preferentially (and somewhat counterintuitively for their own evolutionary good) in tumor cells, or by modifying wild-type viruses to allow for tumor-selective transduction or gene expression[6,7].

Along these lines, Thorne *et al.* delivered a fully replicating vaccinia virus (VV), but used a variant (vvDD) modified to allow for preferential tumor-cell replication. This tumor selectivity is achieved two ways. The viral thymidine kinase is deleted, so that only highly replicating tumor cells, which have levels of endogenous thymidine kinase, can support viral replication. The gene encoding the viral VEGF-like growth factor is also deleted, preventing the virus from stimulating growth of target cells[1,8].

The cunning strategic observation that suggested the feasibility of this approach was that vvDD readily infects CIKs but, critically, is in no hurry to replicate in them. Thus, although VV typically causes rapid lytic replication in most cells, Thorne *et al.* found that in CIKs, vvDD produced negligible virus for the first 48 hours before undergoing an explosive burst of replication over the next 24 hours.

This lag phase of viral production conveniently coincides with the timeframe during which CIKs accumulate within tumors after adoptive transfer. These data suggested that CIKs infected with vvDD could smuggle virus past the circulating sentinels of the immune system while the virus works itself up into a replicative frenzy, ready for its release into the tumor (**Fig. 1**). Thus, viral release could be used to enhance the basal efficacy of adoptive CIK cell transfer alone.

Using an immunocompetent mouse model, the authors found that intravenous administration of CIKs preinfected with a vvDD encoding luciferase gave strong images of virus localization only in the tumor at 48 hours. This translated into impressive tumor regressions, with up to 75% of mice cured of established tumors after a single intravenous injection of virus-loaded cells, even in the presence of a fully functional immune system. In contrast, treatments with CIKs, or virus alone, had some beneficial effects on survival times but few cures.

For many years, the rallying call of gene therapy skeptics has been a demand to see effective systemic gene delivery to metastatic disease in meaningful (immunocompetent) models. Although the report by Thorne *et al.* provides an important step toward those demands, it falls short of demonstrating that the delivery problem for cancer gene therapy has been solved. Although a single dose of vvDD-loaded CIKs yielded therapeutic cures in mice, we should be under no illusions that translation into humans will require repeated administrations.

The biggest unanswered question from the current work is exactly what degree of immune privilege is conferred upon the vvDD particles by the CIK smugglers—and, conversely, whether carrying contraband exposes the CIKs to the risk of immune attack in later treatment cycles.

The authors provide some data to show that CIKs loaded with virus *ex vivo* do not present

VV antigens. However, the *in vivo* experiments use just a single shot of virus-loaded cells in mice that were not preimmune to VV.

Thus, several intriguing questions remain. Do preinfected CIKs display viral proteins on their surface—either during the initial 49-hour lag phase or thereafter—which might raise antibody responses against VV *in vivo*? If so, will repeat administrations of adoptively transferred, virus-loaded CIKs survive in the circulation? Does preimmunization of mice with VV affect direct, or virus delivery–mediated, cytotoxicity of CIKs against tumor? These experiments will indicate whether CIKs are good only for a single smuggling run, or whether they can continue to evade detection on repeat journeys into humans with large tumors.

The current study also does not address whether cured mice become resistant to further tumor challenge. It is possible that the killing of tumor cells by the virus may induce the immune system to prime protective antitumor responses, which could mop up residual disease not cured by viral delivery alone. Whether the antiviral immune response primed by local, tumor-associated viral replication and cytotoxicity will swamp the antitumor immune response remains to be seen, but will be readily testable in the model established in this work.

In addition, even if virus-smuggling CIKs genuinely do not prime responses against VV during their voyage to the tumor, viral release, replication and cytotoxicity certainly will when the cargo is unloaded. This response will, in turn, affect the ability of preinfected CIKs to slip past the immune radar upon repeat administration.

Pragmatic questions will also need to be answered if this strategy is to find a place in clinical medicine. Human CIKs have been used in clinical trials[5], but not yet to smuggle viruses. The kinetics of their tumor trafficking must mirror that reported in mice to prevent them from exposing themselves to the immune system prematurely by releasing virus before they reach their targets. As mouse cells support viral replication poorly, levels of inadvertent viral release and off-target replication may be higher in humans than mice. Nonetheless, these questions are exactly those that should be easily addressed in clinical trials.

The significance of this work is that it continues to build the conviction that systemic delivery of immunogenic viral vectors will be possible. The investigators have used a population of immune cells, which are easy to isolate and require no tumor-specific characterization, to smuggle immunogenic, replication-competent viral particles directly to tumors within the context of an intact immune system. By so doing, they continue the unification of diverse disciplines, including adoptive cell transfer[9], virotherapy[7], gene therapy and immunotherapy.

For the future, there is a relatively long list of other viral contraband that could be shipped in CIKs[6]; in addition, the use of replication-competent, tumor-selective viral vectors can also be tested with other potential cell carriers[2] including T cells[3,10], endothelial progenitor cells[11–14] and macrophages[15]. It would seem that the business of smuggling viruses to tumors is only just beginning.

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Pressure on for Marfan syndrome

A commonly prescribed high blood pressure drug, losartan, may be useful in preventing aortic aneurysms in people with Marfan syndrome, suggests a study in mice (*Science* **312**, 117–121).

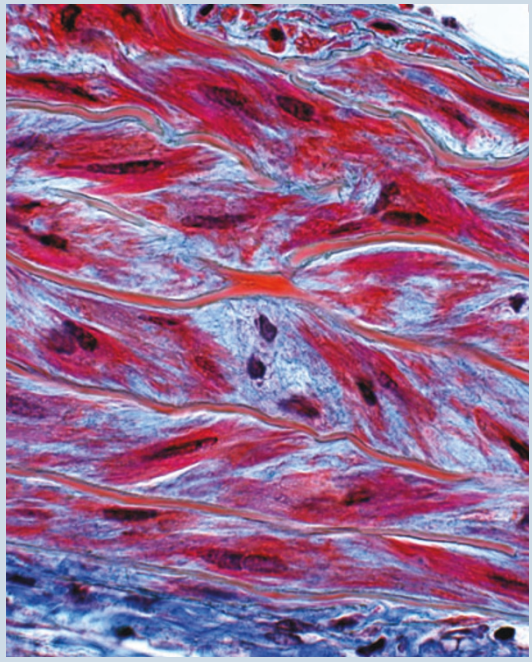
People with this disorder inherit mutations in the gene encoding fibrillin-1, a component of the extracellular matrix. Their abnormal connective tissue contributes to a range of symptoms, from tallness to a deadly predisposition to aortic aneurysms. Fibrillin-1 also seems to affect the cytokine TGF-β, and TGF-β–neutralizing antibodies have corrected some defects in a mouse model of the disease. Shown is the aortic wall in a mouse model of the disease. Elastic fibers are disrupted (red linear structures) and collagen deposition is increased (blue).

Jennifer P. Habashi *et al.* became interested in losartan, an antagonist of the angiotensin II type 1 receptor, because the drug also seems to antagonize TGF-β in some animal models. That seems to be the case in Marfan syndrome as well.

The researchers found that aortic aneurysms in the mouse model of the disease were associated with increased TGF-β signaling and could be prevented with losartan. Other manifestations of the disease also partially responded to the drug.

Clinical trials in people with Marfan syndrome are being planned.

Charlotte Schubert



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