Off by a whisker

Much of what we know about cancer comes from studying mice, and potential therapies are tested in the animals. But the differences between the species can scupper the best laid plans of researchers and drug companies, reports **Carina Dennis**.

It was in 1991 that Bob Weinberg first realized he had a problem with mice. He and his postdoc Tyler Jacks were trying to develop a mouse model for retinoblastoma, a childhood cancer of the retina. It results from the loss of a gene called *Rb*, so the team genetically engineered mice to lack the same gene. But the mice didn't get retinoblastoma. Instead, they developed tumours in their pituitary glands. The finding shocked Weinberg. "Up until then, I had always believed that all mammals were biologically equivalent," he says. "This planted the seeds of doubt in my mind."

Weinberg, based at the Whitehead Institute for Biomedical Research in Cambridge, Massachusetts, is one of the pioneers of the molecular age of cancer research. He was involved in the early work on the first human cancer-causing and cancer-suppressing genes in the early 1980s. But when he saw that mutations in such genes didn't cause the same kind of cancer in mice and humans, he began to ask himself why. He became aware of other examples that challenged researchers' faith in how accurately mice could replicate human tumours, and has since sought to bring this to his colleagues' attention[1]. "There is a laundry list of problems with mouse models of cancer," he says.

Although some genetically engineered mice have come a long way since the early days, many researchers concede that other kinds of mouse model remain especially problematic. Of the potential anticancer drugs that give promising results in tests on mice with cancer, only about 11% are ever approved for use in people[2]. It is also possible that drugs that would have worked in people failed in preclinical mouse trials, although there is no way of knowing. "I'm not a naysayer who thinks we should discard valuable mouse models, thereby throwing the baby out with the bathwater," says Weinberg. "My rallying cry is that we need to be working on this problem." Now many researchers, Weinberg included, are doing just that, by making mouse models more faithful replicas of human cancer development.

Different strokes

Mice are still crucial for cancer research. After all, there is only so much one can learn from studying cells in a lab dish. Cancer is a complex, three-dimensional disease that changes, evolves and spreads through the body. Mice, easy to breed and genetically manipulate, and with a completed genome sequence, are the obvious choice for scientists wanting to pick apart these processes and test new drugs. And, as mammals, they share enough biology with us to make some useful comparisons possible.

But despite having broad similarities, mice have significant differences that can scupper cancer experiments (see graphic, overleaf). For one thing, most mouse tumours originate in different types of tissues from ours and, unlike us, their healthy cells can maintain the ends of their chromosomes, a key factor influencing which mutations tumour cells develop. One of the key mouse models, especially in drug testing, involves grafting human cancer cells into mice and seeing how the resulting tumours respond to treatment. But human cells are likely to behave differently in a mouse than in a human body, making results hard to interpret.

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Some of these problems are insoluble, arising as a result of fundamental differences between the two animals. But others can be fixed. In recent years, researchers have developed tricks to genetically engineer mice, or tinker with the human cells they graft into them, that more accurately reflect cancer in humans. And now both academia and industry are keen to put these mice to the test and see whether they can better predict how humans will respond to new drug compounds. "It took a while to make mouse models that accurately reflect human diseases but now that we have them, now is the time to use them," says David Tuveson of the University of Pennsylvania in Philadelphia.

Early attempts to genetically engineer mice to develop cancer were, in retrospect, simplistic: turning a cancer-associated gene on or off in every cell in every tissue of the mouse. These mice often developed tumours, but they rarely looked anything like the human cancer, as Weinberg discovered. What's more, the technology used to alter mouse genes took effect early in development, whereas most human tumours develop later in adult life, following a stepwise series of mutations that turn a normal cell into an invasive cancer.

Over the past decade, researchers have been able to recreate these steps more accurately. A key breakthrough was being able to control when and where the cancer-causing mutations occurred[3]. "We're getting the right combination of mutations at the right place at the right time," says Jacks, who now heads his own lab at the Massachusetts Institute of Technology in Cambridge.

Human touch

But recreating the sequence of genetic mishaps in a cluster of cells isn't necessarily enough to replicate human cancer. The physical differences between humans and mice can be a major obstacle, says Glenn Merlino of the National Cancer Institute (NCI) in Bethesda, Maryland. Merlino works on melanoma, which is induced by ultraviolet (UV) irradiation of the skin. Melanocytes, the pigment-producing cells that become cancerous in melanoma, are found in the outermost layer of human skin, but in mice, they are confined to hair follicles, making it harder to study the effects of UV radiation. What's more, the resulting tumours show a different cellular structure under the microscope.

Merlino's team engineered mice to overexpress a cell signal that causes melanocytes to be found in the outer layers of the rodents' skin, as in humans. They went on to show that very young, but not adult mice, exposed to UV radiation develop malignant melanoma, giving strong evidence to back up previous epidemiological studies highlighting the potential dangers of sun exposure in children[4].

Humanized mice such as these could help researchers tackle some fundamental ques-

Vive la difference

While some scientists are bent on making the mouse more human-like, others are celebrating our differences. Comparing mouse cancers with human ones helps researchers cut through the complexity of cancer and discover new genes and processes key to the disease. "It can act as an evolutionary filter to sift through the noise," says Jeff Green, a molecular biologist at the National Cancer Institute in Bethesda, Maryland.

Comparing gene activity in mouse and human tumours can help reveal the biochemical pathways involved in the development of cancer that would otherwise be obscured by the complexity of the data obtained from humans alone. Green, for example, is using this method to uncover the biochemical pathways involved in oestrogen signalling that are present in both species.

The idea is that pathways that have been conserved through the millions of years of evolution that separate humans and mice are likely to play a crucial role in the basic cell biology of breast cells, and so might play a role in cancer.

Tyler Jacks, of the Massachusetts Institute of Technology in Cambridge, on the other hand, used this strategy to uncover new mutations and altered signalling in human lung cancer. The work revealed that a gene called *KRAS2* is often mutated — and the signalling pathway of which it is part perturbed— in a subset of lung cancers[9].

Seeing how the human and mouse genomes compare is also yielding new clues. Lynda Chin of the Dana-Farber Cancer Institute in Boston, Massachusetts, and her colleagues recently used a method that highlights differences between the two genomes to identify a gene that enhances the spread, or metastasis, of the skin cancer melanoma[10].

The team found that one region of the mouse genome had extra copies in those tumour cells with the potential to metastasize. The equivalent region in human melanoma cells also turned out to have extra copies. The team subsequently pinpointed a gene in the region called *NEDD9*, which was overactive in tumours and made cells more invasive.

Meanwhile, Scott Lowe of the Cold Spring Harbor Laboratory, New York, and his colleagues used a similar strategy to compare the genomes of human tumours and that of a mouse model of liver cancer.

They identified a region of the mouse genome that has extra copies present, found similar copies in human tumours, and showed that the overactivity of two genes within this copied region accelerates tumour growth[11].

Both Chin and Lowe's work show how the mouse can act as a filter to find new genes involved in cancer formation, maintenance and metastasis — and spotlighting potential new drug targets. "You can glean more information from looking at both animals, than either in isolation," says Jacks. **C.D.**

tions — such as where and how cancer begins. "The problem is we don't know much about the cell-of-origin in human cancers," says Jacks. Some suspect that it is the stem cells that repair and renew adult tissues (see 'The root of the problem', page 742). "Those are hard questions to get to in humans. This is an example of the value of mouse models — they are highly manipulable, for which there is no comparison in human," says Jacks.

The latest generation of mouse models mimics the human disease process more faithfully. But, in therapeutic terms, the real test is how well they reflect the human response to drugs. Early signs are encouraging[5]. "New mouse models hold a lot of promise. But now they have to show what they are worth," says Anton Berns, of the Netherlands Cancer Institute in Amsterdam.

Foreign bodies

The pharmaceutical industry has typically used 'xenograft' models to screen new drugs. In these, human cancer cells are injected under the skin of a mouse with a deficient immune system, to prevent rejection of the tumour. Although virtually every successful cancer drug on the market will have undergone xenograft testing, many more that show positive results in mice have had little or no effect on humans, possibly because the human tumours are growing in a foreign environment. "In many cases, the mouse is just a container for the human cells," says Roberto Weinmann, the director of oncology at pharmaceutical company Bristol-Myers Squibb in Princeton, New Jersey.

Many companies therefore are interested in the new genetically engineered mouse models. "We are in a transition," says Giulio Draetta, head of basic cancer research at drug company Merck, based in Boston. "We are now building a substantial operation using transgenic models and are investing in licensing genetic models that currently exist as well as building our own models," he says.

"We still rely very heavily on xenografts, as do most companies. But, as the models get better, we are watching the field very carefully," adds Richard Gaynor, vice-president of cancer research at Eli Lilly in Indianapolis.

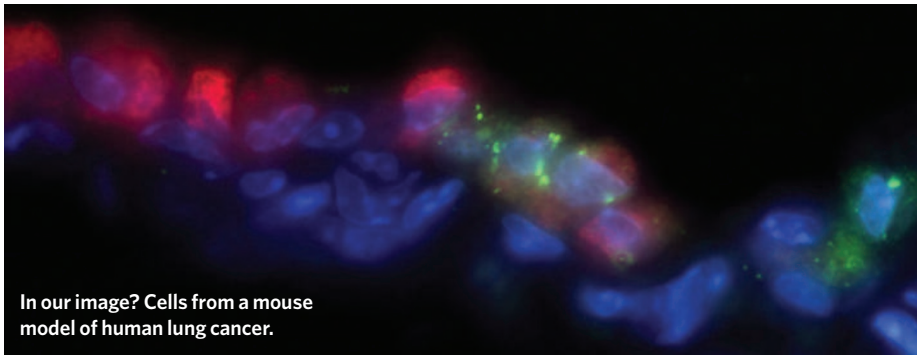
Some argue that a major factor preventing industry fully embracing transgenic mice are the patents associated with certain kinds of mice engineered to develop cancer, in particular the OncoMouse patents owned by chemical company DuPont, which constrains the commercial development of such mice[5]. Some think the patents have deterred drug companies from embracing transgenic mouse models. The licensing arrangements that commercial groups need to negotiate with DuPont have made using such models "more arduous and expensive", says Gaynor, although academics and non-profit groups can use the technology freely.

Intellectual-property issues aside, xenografts will remain many companies' model of choice for the foreseeable future. They can be ready within weeks, whereas transgenic mouse models can take months to develop tumours and are often unpredictable in where and when they develop them. As hundreds of mice are required for screening, xenografts are quicker and cheaper.

Making connections

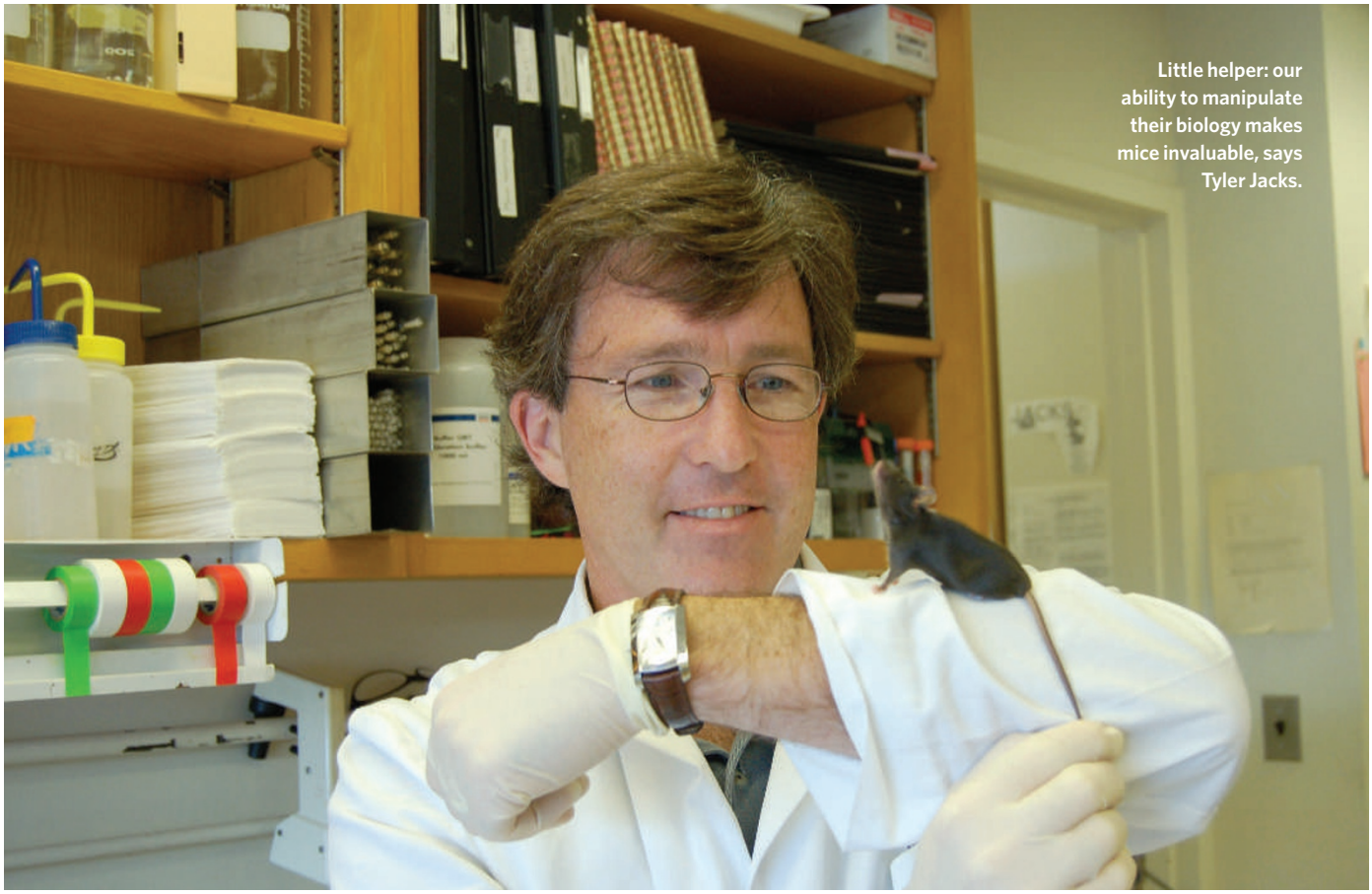
One possible way to improve xenografts is to manipulate the cellular environment into which cancer cells are injected. Connective tissue cells, or stromal cells, play an important role in the development of a tumour, often supplying it with the cell signals and nutrients it needs. But interactions between tumour cells and their neighbours are often lost in xenografts, because proteins from one species can't interact with their counterparts in the host. "The consequence is that the tumour often looks nothing like that seen in the patients," says Weinberg.

Weinberg is among a number of scientists who have addressed this problem by putting human connective tissue cells into mice along with the cancer cells. The resulting tumours have a more human-like structure and metastasize more like human cancers do[6,7]. "It gets us closest to the human situation," says Ronald DePinho of the Dana-Farber Cancer Institute



In our image? Cells from a mouse model of human lung cancer.

KIM *ET AL. CELL* **121**, 823–835 (2005)/ELSEVIER



A. DECONINCK

Little helper: our ability to manipulate their biology makes mice invaluable, says Tyler Jacks.

in Boston, Massachusetts, who has co-founded the company AVEO Pharmaceuticals to develop tissue-specific cancer models, including breast cancer.

Adding human cells into the mix is one approach. Another way to make mice respond to drugs in a more human-like fashion is to give the mouse human genes. The driving force behind this is the considerable differences in the way that mice and humans metabolize drugs. For instance, two human enzymes CYP2D6 and CYP3A4, which together metabolize more than 70% of drugs on the market, have markedly different activities compared with their rodent equivalents[8]. The consequence is that mouse models may be of limited value in predicting the effectiveness or toxicity of drugs in humans.

Mine of information

Researchers have tackled this problem by engineering genes encoding some of the human enzymes into the mouse[8]. Studies of these 'humanized' mice indicate they can process some drugs in a more human-like manner. Combining these humanized mice with revamped mouse cancer models could be a powerful way to test drug candidates.

In the future, researchers may broaden the types of human genes they add in to the mouse. "My hope is that we will be able to create mouse cells and tissues that may closely resemble human tissues," he says.

But there is a limit to how far scientists can get mice to be like humans. So other researchers are mining human clinical data for insights into the cancer process, then using this to inform mouse studies. In the past, clinical data have often been collected on an ad hoc basis. But now researchers are tackling the process in a systematic and comprehensive fashion. Assisted by the Internet and better bioinformatics tools, there are large-scale efforts to collect and catalogue data on a wide range of human tumours.

Howard Fine of the NCI is leading one such initiative. The aim is to perform genetic and molecular analyses of samples of human brain tumours sent to the NCI. Data are correlated with each patient's clinical course. The study is a pilot for the NCI's larger cancer Biomedical Informatics Grid, which will provide a global network for researchers to input information and access bioinformatics tools for mining cancer data. The study, which currently has data for 700 tumours and will eventually contain information for 2,000 tumours, is already yielding results, says Fine, who hopes to publish the findings shortly.

Researchers are increasingly shuttling between human and mouse, using information from human data to refine mouse models and using insight from the mouse to uncover new disease processes and test predictions for clinical responses. But industry and the scientific community shouldn't expect too much from the humble rodent. "Mice are valuable but they are, after all, still mice," says Fine. "The best study subject will always be the human." ■

Carina Dennis is *Nature*'s Australasian correspondent.

HOW MICE DIFFER FROM HUMANS

- Cancers tend to form in different types of tissue
- Tumours have fewer chromosomal abnormalities
- Ends of chromosomes (telomeres) are longer
- Telomere-repairing enzyme (telomerase) active in cells
- Short lifespan
- Fewer cell divisions (10^{11}) during life than humans (10^{16})
- Metabolic rate seven times higher than humans
- Lab mice highly inbred and genetically similar

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