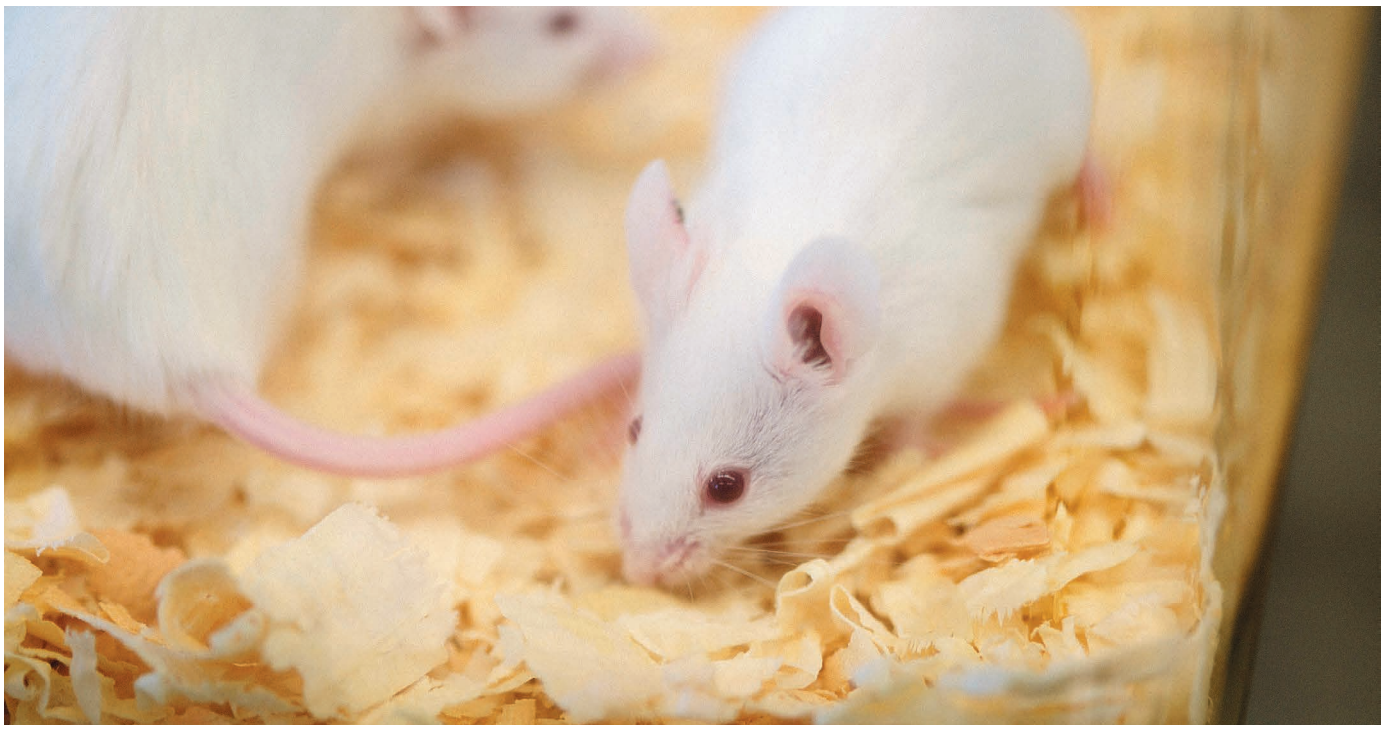
TECHNOLOGY FEATURE

MOUSE MODELS WITH A HUMAN TOUCH

Engineered mice are valuable for disease and drug research, but scientists hunger for cancer models that better mirror the condition in humans.



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Mice are commonly used to study cancer, but scientists are still working to improve modelling of the human disease.

BY MIKE MAY

In 1915, with the world at war, Japanese pathologist Katsusaburo Yamagiwa and his assistant Koichi Ichikawa were focused on a killer nearly as deadly as the battle raging on the Western Front. The duo, based at what was then the Imperial University of Tokyo, had spent more than 150 days painting coal tar on the ears of rabbits. Finally, they found that the rabbits had cancer.

Yamagiwa's diseased rabbits are considered to have been the first animal model for cancer research[1]. Since then, scientists have used everything from cell lines to engineered mice to try to mimic human cancer. But finding the option best suited to answering a specific experimental question requires a lot of thought.

According to medical oncologist David Weinstock of the Dana–Farber Cancer Institute in Boston, Massachusetts, what makes for a good cancer model is "a very complex question, and the simplest answer is it must be able to give me insight — truly answer the question that I want to ask. If it can't do that, I'm wasting my time."

For Nancy Boudreau, a branch chief at the US National Cancer Institute (NCI) in Bethesda, Maryland, a model's fidelity to the course of human cancer is key. "The more it recapitulates the human disease and progression, the better," she says. An ideal cancer model should replicate many of the features that occur in human cancer, including how it develops and progresses when facing a human immune system; how it metastasizes, or spreads from its primary source to other parts of the body; and how it reacts to therapy. That requires scientists to know the pros and cons of each cancer model, because none will answer every research question.

Some evidence suggests that, despite many options, no existing model of cancer is good enough for developing therapeutics. According to a report co-authored by the international Biotechnology Innovation Organization that examined clinical trials from 2006 to 2015, cancer drugs fared the worst out of 15 disease groupings, progressing from phase I to approval only 5.1% of the time (see go.nature.com/2pxfn16). By contrast, success rates for haematology and infectious-disease therapeutics were 26.1% and 19.1% , respectively.

"If better preclinical models could improve the clinical translatability by just 10%, that would very much improve the quality of preclinical cancer research and translate into enormous savings for drug developers," says Hellmut Augustin, a specialist in vascular oncology at the German Cancer Research Center in Heidelberg.

Groups collaborate worldwide to improve these models. Scientists at the NCI, Cancer Research UK in London, the Wellcome Trust ▶

Sanger Institute in Hinxton, UK, and the not-for-profit Hubrecht Organoid Technology in Utrecht, the Netherlands, for instance, have teamed up on an effort called the Human Cancer Models Initiative. It launched in 2016 with the goal of developing 1,000 new cancer models in cell lines for use by researchers around the world. Such projects suggest that many scientists agree on the value of expanding the pool of models.

MODIFIED MOUSE GENOMES

For many questions, the humble cultured cell provides sufficient insight. But these cells are typically grown in unnatural 2D formats that lack the conditions in which human cancers grow — especially, an immune system. This makes cultured cells ill-suited for modelling many aspects of disease. Instead, says cancer-systems biologist Shannon Hughes of the NCI, a good starting point for many investigations is a genetically engineered mouse (GEM). "They are well characterized and well controlled," she says.

For years, engineering a mouse required complicated processes to generate desired DNA, transform cells in culture and inject them into an embryo to modify its genes. But the options for making a GEM today, like most other genetic-modification applications, changed with the discovery of the CRISPR gene-editing system. "CRISPR has enabled more-subtle manipulations that were extremely challenging with previous technologies," says cancer biologist Lukas Dow of the Weill Cornell Medical College in New York City.

"For instance," Dow says, "it is now relatively straightforward to induce large chromosome rearrangements — inversions, deletions and translocations" — associated with disease. With CRISPR, scientists can even change a single base in a rodent's DNA. Base-by-base resolution offers "the ability to accurately recreate the precise mutations observed in human cancer", he notes. "Such detail has been largely ignored in model development thus far, but it is increasingly apparent that the devil is in the detail."

Taeyoung Koo, a genome engineer at South Korea's Institute for Basic Science, based in Daejeon, and her colleagues used CRISPR to target a mutation in non-small-cell lung cancer (NSCLC)[2]. They report that of human cases of NSCLC, 15% involve a change to just one DNA base — known as a single-nucleotide mutation — in the epidermal growth-factor receptor (*EGFR*) gene. Current treatment consists of drugs, such as gefitinib, that target the mutated protein produced by that gene.

Koo's team developed a CRISPR–Cas9 guide-RNA sequence that recognizes the most commonly mutated *EGFR* region, which accounts for more than 40% of *EGFR*-mutation-related NSCLC cases. They then implanted mice with human NSCLC tumours and targeted the mutation with CRISPR–Cas9 and a specific guide RNA. Their results showed that a properly designed guide RNA is sufficiently precise to break the diseased sequence, yielding a potential therapeutic strategy. The "mutant allele-specific Cas9 can efficiently distinguish the *EGFR* mutant allele from the wild-type allele, leading to targeted oncogene disruption and cancer cell death", they report[2].

Although CRISPR shows remarkable target specificity, the result of its activity can be highly variable. So, if the goal is to create consistent and uniform genetic changes across all cases, nucleases such as Cas9 are a poor choice, says Dow. "The random nature of DNA repair in traditional CRISPR systems means that you have to deal with a significant amount of heterogeneity in cell populations."

GEMs have limitations, too, especially concerning the timing and heterogeneity of disease. "Mouse tumours progress incredibly fast," Hughes explains. That speed enables researchers to accelerate their experiments, but they fail to replicate the pace of disease in humans. Plus, she says, the tumours tend to be too homogeneous to reflect human disease properly: a GEM usually includes just one or two genetic changes, whereas human tumours often have many.

To address the lack of heterogeneity and produce a more human-like model, biomedical scientist Lorenzo Federico and his colleagues, working in the laboratory of systems biologist Gordon Mills at the University of Texas MD Anderson Cancer Center in Houston, engineered a collection of transplantable grafts from primary breast tumors in transgenic mice[3]. The procedure yielded 12 new graft lines of mice — mouse models that can reliably produce specific types of cancer with a wide array of genetic changes. "Ideally, different primary tumours arising in different mice should be characterized by different molecular alterations to more closely reflect the genetics of human cancer," Federico says.

These models have already been used successfully as preclinical platforms for the assessment of targeted therapeutics, including inhibitors of molecular pathways involved in cancer. According to Federico, they are also well suited for studying the role of the immune system in tumorigenesis and therapeutics development. Yet, because these transplantable grafts were derived from engineered mouse tumours, he says, the results recorded from this approach "must be always taken with a grain of salt".

How cancer arises and progresses depends intimately on its interaction with the host immune system. Some of the most promising treatments, called immunotherapies, engineer a patient's immune system to attack a specific tumour. To study these therapies, scientists need mice with an intact immune system — better yet, a human one. That led to humanized mice.

Organoid options

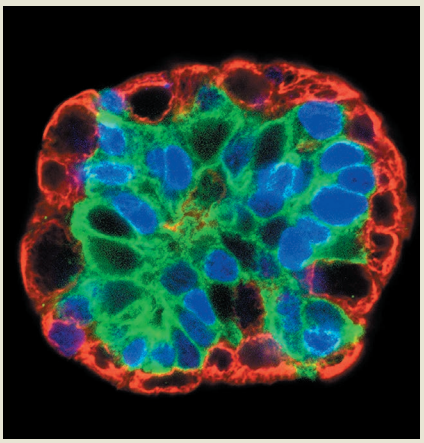
Mice aren't the only options researchers have for modelling cancer. A popular emerging alternative is the organoid — a 3D cell culture that mimics some of the micro-anatomy of an organ, such as its system of blood vessels.

"A tumour is a kind of organ, where tissues cooperate," says molecular biologist Claudine Kieda of the Centre for Molecular Biophysics in Orleans, France. "A 3D cell model takes into account the microenvironment, such as the level of oxygen around and in the tumour."

Kieda's lab combines melanoma and endothelial cells in a matrix composed of collagen, growth factors and a 3D scaffold called Matrigel. This mixture allows the cells to form a structure that resembles a tumour and its surroundings, especially in terms of oxygenation[7]. "Everyone is working in conditions that are like an incubator, where the partial pressure of oxygen is much higher than in the body," Kieda says.

Among other uses, organoids are valuable for drug development. For instance, Meritxell Huch, a tissue-repair biologist at the University of Cambridge, UK, and her colleagues created liver-cancer organoids for drug screening[8]. This type of tumour can be grown in mice only about 20% of the time, but Huch achieved a success rate of nearly 80% — and the process worked about twice as quickly as with a patient-derived xenograft. "Speed is the main advantage," Huch says. Using these organoids, Huch's team identified an inhibitor of a signalling pathway that represents a potential target for treating primary liver cancer.

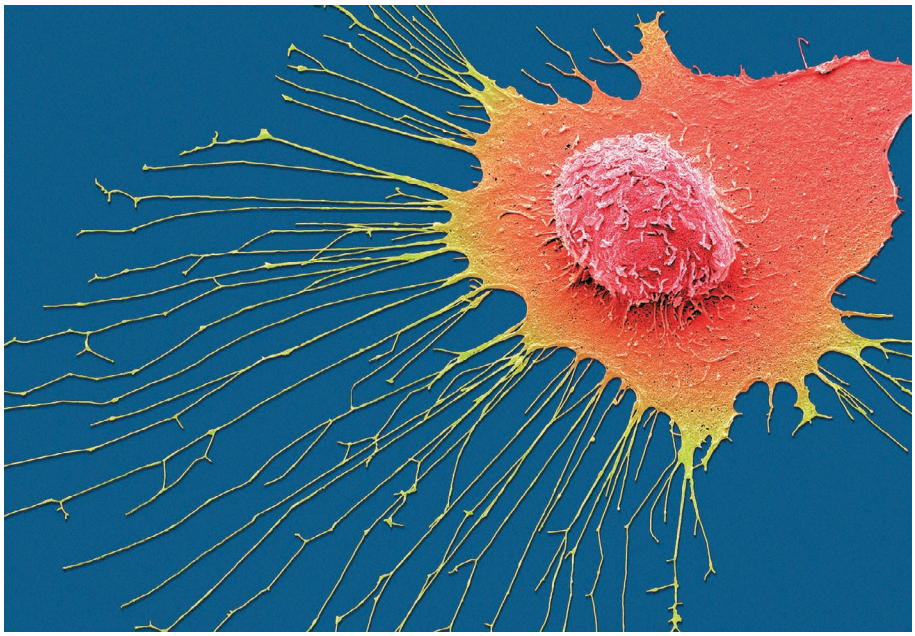
As with other cancer models, a good organoid replicates the human disease as faithfully as possible. Organoids, says Nancy Boudreau, a metastasis researcher at the US National Cancer Institute in Bethesda, Maryland, "are more biological than cells in regular culture". And they are less expensive than mice. M.M.



SALK INSTITUTE/NCI/SPL

Organoids are an increasingly popular model.

STEVE GSCHMEISSNER/SPL



A breast-cancer cell migrates under the microscope.

For these GEMs, human hematopoietic stem cells — precursors to an array of blood cell types — are implanted into an immune-deficient mouse. This process recreates certain aspects of the human immune system, such as white blood cells called T cells, which attack foreign cells. Then, a sample of a human tumour — called a patient-derived xenograft (PDX) — can be transplanted into the mouse, creating a more realistic model of human disease.

According to Augustin, PDX models are increasingly popular among drug developers, who use them as test beds for drug testing. PDX models are also moving into basic-research labs, and are commercially available. Working with more than 20 cancer clinics, the Jackson Laboratory in Bar Harbor, Maine, has created more than 450 of these mouse models, including ones for acute myeloid leukaemia and bladder, breast, lung, ovarian and pancreatic cancer. They usually cost about three times as much as standard immune-deficient mice, the non-profit says.

Scientists can also develop their own PDX mice. Oncologist Elizabeth Stewart of St. Jude Children's Research Hospital in Memphis, Tennessee, and her colleagues used samples of surgically removed paediatric solid tumours, representing brain, bone and other cancers, to generate 67 PDX mouse models covering a dozen tumour types[4].

Stewart and her colleagues' aimed to create models for studying treatment efficacy against different tumour types — an approach that requires the model to represent the original disease accurately. Stewart's team decided to compare the PDX and source tumours at the nucleic-acid level using whole-genome and whole-exome DNA sequencing. Overall, they found, the PDX sequences largely matched the genomic features of the source tumours, although new mutations also emerged. The PDXs "retained the molecular and cellular features of the patient tumour and the epigenetic landscape of their developmental origins", the researchers concluded.

That's not to say that PDXs are static. Todd Golub, director of the cancer programme at the Broad Institute of Harvard and MIT in Cambridge, Massachusetts, and his team studied genomic rearrangements called copy-number variations (CNVs) in 543 PDX models representing 24 classes of cancer[5]. They found that expansive regions of CNVs constituting more than 5 million bases had been introduced into 60% of the PDXs after 1 passage from the original mouse to its offspring, and 88% of PDXs after 4 passages. The results show that PDXs that initially mimic human disease can evolve into forms that do not. When that happens, the PDX loses its faithfulness to the target cancer.

Boudreau describes engrafting human PDX-model tissues into humanized mice as one of the most intriguing new cancer models to emerge, but says it's "not quite there yet" because researchers have yet to learn the ins and outs of the technology. That said, she adds, the technology could prove useful for one hot facet of therapeutics development: "Humanized mice will be pretty critical with the interest in immunotherapy and how human tumours respond," she says.

BACK TO THE BEGINNING

Rather than relying on genetic techniques to produce a better model of cancer, some scientists are going old school — using Yamagiwa's approach. This chemical-carcinogenesis method uses ordinary lab mice, and the results can create more-realistic cancer models.

"You treat a mouse with a carcinogen, like an environmental agent, to cause a specific type of damage and to get specific types of tumours, such as tumors in the skin," explains tumour biologist Melissa Reeves at the University of California, San Francisco. "This does a good job of recapitulating tumours in humans exposed to specific environments, because it models the natural evolution of a tumour caused by a wide array of genetic damage."

"The more it recapitulates the human disease and progression, the better."

Chemical carcinogens can damage DNA at hundreds of sites, and their impact can be followed over time. Reeves and her colleagues took this approach, using topical applications of known carcinogens called DMBA and TPA to induce skin cancer in mice, to study how tumours move from a primary site to a secondary one[6]. Her findings suggest that skin cancer does not travel serially from site to site — from skin to lymph nodes to lungs, for instance — but rather, "by parallel dissemination, going to the lymph nodes and lungs at the same time".

This finding, Reeves says, provides experimental validation of a well-documented clinical finding: that removing the lymph nodes around breast cancer doesn't always increase survival, an observation that led researchers to speculate about the possibility of parallel transmission.

Although chemical carcinogenesis creates diseases that, compared with GEMs and humanized mice, might better resemble the heterogeneity of human cancer, actually using these models has significant downsides. It can take 18 months to create a primary tumour through chemical means, remove it and study the course of metastasis. "Plus, every tumour is going to be a little bit different," Reeves says.

Likewise, every cancer model differs, and mice aren't always the best choice (see 'Organoid options'). Mice are expensive to maintain and pose ethical concerns, which will always make cell lines an option to consider.

For now, researchers must choose a model — despite its shortcomings — that they think will best answer their specific question. At the same time, scientists will keep advancing existing models and developing new ones. As Augustin notes, "It is well-invested money to develop and employ mouse tumour models with better translational relevance and impact." Otherwise, the performance of cancer drugs in clinical trials might never improve. ■

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