Marta Chesi of the Mayo Clinic displays a transgenic mouse used to model multiple myeloma.

ANIMAL MODELS

Towards a myeloma mouse

Introducing a human disease into mice is helping researchers explore its nature and find potential therapies.

BY SARAH DEWEERDT

A few years ago, two scientists from the Magna Graecia University of Catanzaro in Italy found themselves riding together on a train to Rome. By the time the train reached its destination, the pair — oncologist Pierfrancesco Tassone and bioengineer Filippo Causa — had devised a new way of studying multiple myeloma in a mouse model¹.

First, Causa would create tiny, cylindrical scaffolding made of high-tech plastic, shot through with pores of various sizes that mimic the architecture of human bone. Tassone would then inject cells from human bone marrow into the artificial bone scaffolds and implant them under the skin of immune-deficient mice. He would later inject the scaffolds with purified myeloma

cells taken directly from patients with the disease.

Tassone compares the scaffolding to “an empty apartment, a house for cells”. Once the apartment and its bone-marrow furnishings are in place, the myeloma cells can move in, giving scientists the ability to study the interaction between a patient’s myeloma cells and his or her own bone marrow. They call the arrangement SCID-synth-hu, for the immune-deficient *SCID* mouse, synthetic bone and human bone marrow.

This might sound like a convoluted system, but that’s not unusual for animal models of multiple myeloma. “The myeloma cell depends on the microenvironment of the bone marrow,” says Nikhil Munshi, who studies myeloma at the Dana-Farber Cancer Institute in Boston, Massachusetts. “It requires human bone marrow to grow and survive.”

This fact makes studying the disease in the laboratory rather complex. But over the past 15 years, the models that have emerged, flawed though they may be, have yielded important insights into the biology of multiple myeloma.

MAYO CLINIC

MAKING A MODEL

Perhaps the simplest way to study cancer in an animal model is to inject cancer cells into an immune-deficient mouse and allow them to grow into tumours. This approach, called a xenograft model, is widely used to study many types of cancer, and in myeloma it can be a quick and inexpensive way to screen possible new drugs. A molecule that doesn’t affect xenograft tumours is unlikely to work in patients with multiple myeloma, for example.

But myeloma xenografts are generated from lab-grown cell lines, which don’t behave much like the primary multiple myeloma cells found in patients’ bone marrow. They are more like cells from the most advanced, aggressive stage of the disease, called plasma cell leukaemia. Researchers want to arrest the disease before it gets to that point, however, when it is still confined to the bone marrow and growing slowly. This is difficult to model because it is hard to grow human primary myeloma cells in mouse bones.

Even so, mice can get a form of multiple myeloma. In the late 1970s, a Dutch gerontology researcher, Jiri Radl, noticed that mice of a particular strain occasionally developed the disease late in life. When Radl isolated these myeloma cells and injected them into young mice of the same strain, the cells colonized the bone marrow and caused myeloma. Repeating this process over and over again led to a group of myeloma animal models collectively known as 5TMM mice.

Several different types of 5TMM mice exist, each bearing tumour cells from a different original ‘donor’ and having specific characteristics. For example, 5T2MM mice develop bone disease very similar to that of human myeloma, says Karin Vanderkerken, professor of haematology and immunology at Vrije Universiteit Brussel in Belgium. Vanderkerken has led extensive studies of 5TMM mice — primarily the 5T2MM and 5T33MM strains — in order to unravel the large variety of cells and molecules involved when myeloma cells travel to, enter and establish themselves in the bone marrow.

Vanderkerken and colleagues have found that this process, called homing, involves a high degree of redundancy. Blocking one pathway will not stop the process, she says. “But if you combine several different ones then you can block it.”

More recently, Vanderkerken’s studies have pointed in a new therapeutic direction. In 5T33MM mice, her team found that the bone marrow surrounding multiple myeloma cells is much lower in oxygen than the bone marrow around healthy cells². Low oxygen, or hypoxia, is known to play a role in other forms of cancer, but this was the first evidence of it in multiple myeloma. Further studies showed that TH-302, a drug that is activated by hypoxia and that is

being investigated in clinical trials for other types of cancer, caused the death of multiple myeloma cells in the bone marrow of 5T33MM mice.

TRANSGENIC MICE

Vanderkerken acknowledges that using 5TMM mice has its downsides. In humans, myeloma is highly variable in terms of both genetics and tumour behaviour. Because 5T2MM mice all have myeloma cells derived from the same original donor mouse, however, they fail to capture this variability. "One of the limitations is that we work with two models [5T2MM and 5T33MM], so we have two 'patients,'" Vanderkerken says. That simply isn't enough.

To explore the genetics of multiple myeloma, researchers have turned to transgenic models, in which mice are genetically engineered to have changes in myeloma-related genes. For example, one transgenic mouse model expresses abnormally high levels of XBP-1s, a protein involved in the differentiation of B cells, from which myeloma is eventually derived. Many of these mice develop a precursor condition called MGUS (monoclonal gammopathy of undetermined significance) and some of the mice develop myeloma at around one year of age³.

In another transgenic model, researchers spliced the human *MYC* gene into mouse DNA near genes related to the production of antibodies⁴. As they ramp up antibody production, B cells undergo rapid DNA changes. In the mice, these changes can lead to the *MYC* gene being turned on and the onset of myeloma. Rearrangements, or translocations, of DNA are thought to give rise to myeloma in humans, says Leif Bergsagel, professor of medicine at the Mayo Clinic in Arizona, who led the development of the *MYC* mouse model. "This mouse reproduces that effect."

The *MYC* oncogene has a well-known role in other cancers, being involved in the rapid cell proliferation of many tumours. But because myeloma cells divide slowly, researchers had believed that *MYC* had only a minor role in the disease. "I think we give the cells just enough *MYC* to observe this oncogenic role without really becoming proliferative," says molecular biologist Marta Chesi, who is married to Bergsagel and works with him at the Mayo Clinic.

The researchers say the *MYC* model could be used to test potential myeloma drugs. Studies of existing cancer drugs have shown that "drugs that work in the clinic work in the mice, and drugs that do not work in the clinic do not work in the mice," Chesi says. "It's highly predictive."

MORE HUMAN

Transgenic mice and mice with myeloma are useful tools, but they are still mice, and some research questions need human cells. For these studies they have used a model known as SCID-hu, in which small pieces of human fetal bone are implanted under the skin of immune-deficient mice⁵. The SCID-synth-hu model developed by Tassone and colleagues is a variation on this.

The SCID-hu model was first developed in the

Hybridomas produced by fusing plasma cells with myeloma cells and used to test for antibodies.

Micro-CT scans reveal bone damage from myeloma in a 5T2MM-bearing mouse.

late 1980s for studying normal blood cell formation in bone marrow. Myeloma specialist Joshua Epstein at the University of Arkansas for Medical Sciences in Little Rock soon realized that this approach might also enable him to study abnormal events in the bone marrow, such as myeloma.

Epstein's goal was to answer one of the field's most contentious questions: what kind of cell gives rise to myeloma? At the time, scientists knew that myeloma was related to B cells, but didn't know if it arose from antibody-producing plasma cells or a precursor, such as a B cell. "No other cells in the bone marrow or the blood could cause myeloma other than these mature plasma cells in our model," Epstein says (see 'Testing ground for cancer stem cells', page S43). His team implanted two fetal bones in a single mouse and injected myeloma cells into just one of them. Eventually, the myeloma cells colonized the second bone — but couldn't be found anywhere else in the mouse. "So we had a model in which we could show that myeloma cells depend on the human microenvironment," Epstein says. These findings cemented the importance of the bone-marrow microenvironment in myeloma (see 'Neighbourhood watch', page S48).

Other groups also began working with SCID-hu. In one series of studies, researchers showed that the molecule DKK1, produced by myeloma cells, plays a crucial role in causing the disease's characteristic bone damage by inhibiting bone-forming cells called osteoblasts. In SCID-hu mice, an antibody to DKK1 mitigates the bone disease and inhibits myeloma cell growth⁶. "The perfect model to test this was the SCID-hu model," says Munshi, who led these studies.

But the interest in using SCID-hu led to competition for a limited supply of fetal bones. Tassone's model incorporating synthetic bone scaffolds provides a solution. When production of the synthetic bones is scaled up, Tassone says, more researchers will be able to conduct larger studies than is now possible. And because the synthetic bones are all identical, these studies will be more uniform than those based on fetal bones.

"If it all works out, this will be a great model," says Vanderkerken. But she and others caution that the SCID-synth-hu approach is still new and largely untested.

Even if the model does catch on, it will not necessarily render other animal models of multiple myeloma irrelevant. The SCID-hu model and its variations are good for studying the human microenvironment, for example, even though they can't capture the role of the immune system in responding to this form of cancer. As Epstein puts it: "Each model has its purpose." ■

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