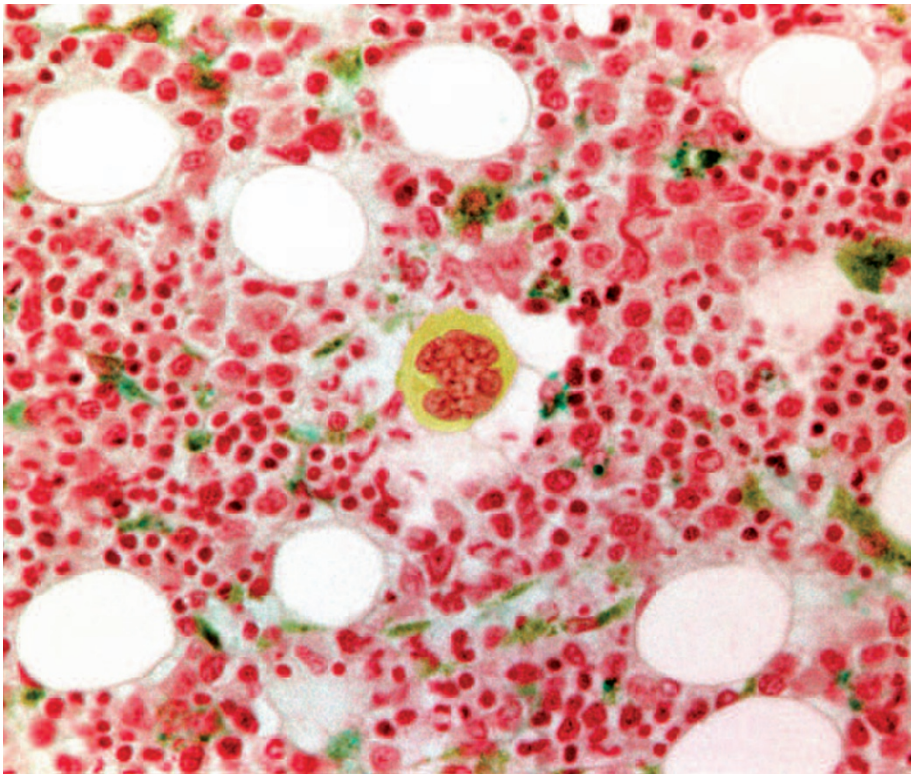

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Bone marrow contains a rich variety of cells and structures that might affect the growth of cancer.

MICROENVIRONMENT

Neighbourhood watch

In the fight against myeloma, researchers are investigating its interactions with molecular neighbours in the bone marrow.

BY VIRGINIA HUGHES

A tight budget in William Dalton's lab in the mid-1990s led to a major discovery. His group was investigating the drug resistance that inevitably occurred in patients with multiple myeloma, a cancer affecting blood plasma cells. When isolated in the lab, these cancer cells responded to treatment. So what happened in the body to make them drug resistant?

Dalton had a hunch that it was somehow connected with myeloma's home in the bone marrow, the spongy, bright-red tissue deep inside the bone that holds a motley mix of blood cells, chemicals, fats and proteins. The part he was most interested in was the extracellular matrix, a web of supportive proteins including collagen and fibronectin. "To be honest, fibronectin is cheaper than collagen, so we decided to study fibronectin," says Dalton, who is now chief executive of the Moffitt Cancer Center in Tampa, Florida. It turned out to be a good choice.

The researchers placed myeloma cells in liquid suspension, either in a clean flask or in one coated with fibronectin. Myeloma cells in the uncoated flask died when exposed to two common cancer drugs. In contrast, the cells in the coated flask attached to the fibronectin, as if they were homing into the bone marrow, and were able to survive the chemical onslaught.

This work[1], published in 1999, was a milestone in the study of myeloma's microenvironment. Until the late 1980s, myeloma researchers had studied cancer cells largely in isolation because the bone marrow's complexity is difficult to mimic in the lab. Since then, thanks to improved cellular and animal models, researchers have taken a closer look at the way myeloma exploits its many neighbours in the marrow, opening the way to new treatments.

"There's a growing recognition that cancer has no respect for its neighbourhood — it hijacks it," Dalton says. "We've got to create models that consider this microenvironment and then start looking at new drugs and targets using this more complex model."

MAKING CONTACT

The bone marrow is a colourful neighbourhood, with residents young and old, fast and slow, transient and permanent. It holds stem cells, in all stages of differentiation, and their many descendants, including blood cells and bone cells. When one particular long-lived blood cell, a plasma cell, acquires a certain combination of genetic mutations, it leads to the unchecked growth of myeloma. The cancer cells and their healthy counterparts occupy the same niche in the bone marrow, along with signalling molecules of the immune system called cytokines, supportive proteins such as fibronectin and collagen, and other blood cells.

"It appears to be a cluster of cells that for some reason are drawn together," says Kelvin Lee, chair of immunology at the Roswell Park Cancer Institute in Buffalo, New York. "You have all these things going on at the same time, in that niche, and so then that raises the question: do all those cells talk to each other?"

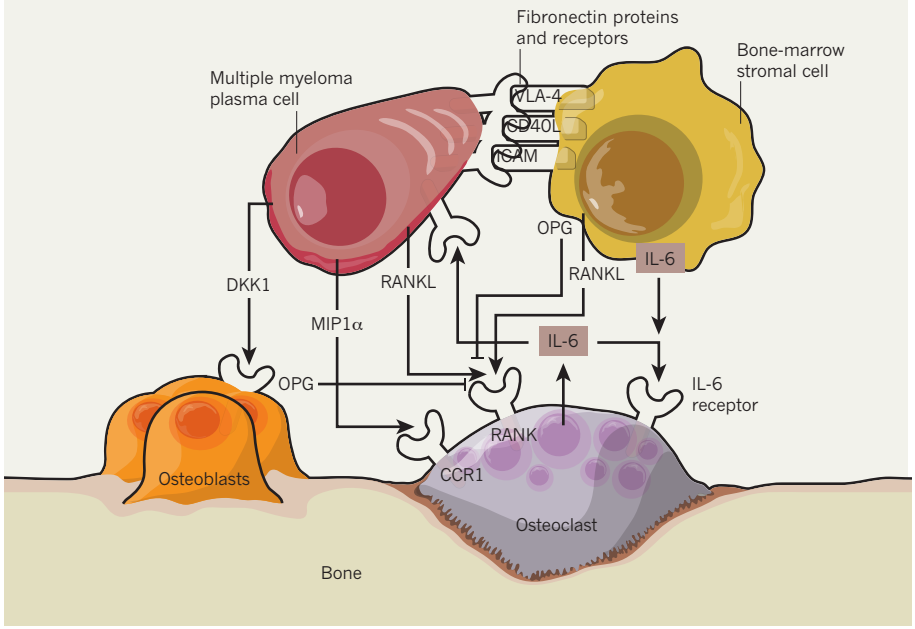
In the first models of myeloma's microenvironment, researchers studied a cancer cell's interaction with one neighbour at a time. This was the strategy that led Dalton to his discovery that myeloma can resist attack by sticking to fibronectin. Using a similar approach, several studies in the 1990s and early 2000s showed that when myeloma binds to various neighbours, it spurs them to produce growth factors that benefit the cancer cell. In one of the most studied examples, myeloma binds to the microenvironment's stroma (a catch-all term for many types of blood cell), causing the stroma cells to secrete interleukin-6 (IL-6), which in turn stimulates the cancer cells to proliferate[2].

More recently, with the help of genetic screening techniques, researchers have started to unravel exactly how contact with the microenvironment changes the cancer cell's genetic program. They have found, for example, that attachment to cells in the stroma can activate biochemical pathways that result in cancer proliferation and migration, blood-vessel growth, further adhesion to microenvironment cells, and the breakdown of bone. A recent study suggests that myeloma can trigger cells in the microenvironment to produce an enzyme that suppresses the activation of T cells — soldier cells of the immune system that would otherwise help the body to fight the cancer. "The myeloma cells are inducing the microenvironment to generate this immunosuppressive force field around them," says Lee, who led the study.

Intriguingly, it seems that these interactions with the microenvironment are the same in myeloma's healthy counterparts, the plasma cells. The difference is that myeloma has a mysterious way of expanding into more supportive niches, allowing it to grow unchecked. "Normal

COLOURFUL NEIGHBOURHOOD

Myeloma cells live in the bone marrow, the spongy, bright-red tissue deep inside the bone that holds a motley mix of blood cells, immune molecules, fats and proteins.



plasma cells take up about 5% of your bone marrow, whereas myeloma takes over the entirety of the marrow," Lee says. "It's just a numbers game."

CLOSE TO THE BONE

Around the same time that Dalton was uncovering the microenvironment's role in drug resistance, another group was revealing its role in the cancer's survival and proliferation.

One of the difficulties of working with myeloma is that the cells by themselves will not proliferate in cell culture. In 1998, Joshua Epstein's group at the University of Arkansas for Medical Sciences in Little Rock discovered how to make them grow inside a mouse — albeit an odd one[3].

The researchers used a newly developed mouse model called SCID-hu, in which a piece of human bone is implanted under the skin of immune-deficient mice (see 'Towards a myeloma mouse', page S38). Epstein injected these animals with fresh bone-marrow cells from patients with multiple myeloma. "Lo and behold, the bones developed myeloma," says Epstein. "It became very clear to us that the tumour cells depend on the human bone-marrow microenvironment, because they wouldn't grow anywhere else."

The SCID-hu model allowed Epstein to investigate which part of the microenvironment was helping the cells to thrive. He first focused on the cells that were already known to change with myeloma growth in patients: bone cells. Myeloma degrades bone by interfering with the crucial process of bone remodelling. Normally, cells called osteoclasts clear away old bone tissue while others called osteoblasts lay down new bone. In 1991, French researchers reported that patients in the very early stages of multiple myeloma have elevated numbers of both osteoclasts and osteoblasts, whereas in later stages of the disease they exhibited eroded bone surfaces and a sharp drop in osteoblast activity.

Epstein's group decided to investigate osteoclast activity in SCID-hu mouse models of myeloma. They showed that osteoclast-blocking drugs curbed bone destruction and tumour growth in the animals. They later found similar improvements by injecting the mice with osteoblast progenitor cells.

This research highlights what Epstein calls the "dangerous tango" of bone cells and cancer cells. "This is evidence that all the changes in bone metabolism that myeloma induces are not a simple manifestation or by-product, but rather an integral part of the disease," he says.

TREATMENT IMPLICATIONS

The growing interest in the myeloma microenvironment has led researchers to take a new approach to treatments. Rather than targeting the myeloma cells — whose genetic instability allows them to mutate quickly to evade drugs — why not go after their cellular neighbours?

Several groups of researchers are studying immune cells in the stroma called dendritic cells, for example. In 2007, Lee found that when myeloma cells encounter dendritic cells, the myeloma cells produce CD28, a signalling molecule in the immune system. CD28 then protects the myeloma from cancer drugs.

This was an exciting discovery, Lee says, because CD28 and similar molecules have long-established roles in the immune system and have already been targets of drugs used to treat rheumatoid arthritis and organ transplant rejection. "I suspect there is a whole bucketload of drugs that the rheumatologists and organ-transplant folks have been using that will be active in multiple myeloma," he says.

Kenneth Anderson, director of the Jerome Lipper Multiple Myeloma Center at the Dana-Farber Cancer Institute in Boston, Massachusetts, has taken an interest in another resident of the stroma: the plasmacytoid dendritic cell. In 2009, Anderson's team reported that in myeloma, plasmacytoid dendritic cells are immature and fail to trigger the host's immune response, allowing myeloma to thrive. Targeting these dendritic cells with pieces of synthetic DNA called CpG oligodeoxynucleotides can restore their development and dampen myeloma's ability to acquire drug resistance. The CpG oligodeoxynucleotides "don't have any direct action on the tumour itself", says Anderson. "It's a really good example of targeting only the microenvironment and having an effect on the tumour."

But because the microenvironment has so many influential characters, approaches that target only one aren't likely to have much effect. For example, after the early discoveries that IL-6 stimulates the growth of myeloma, Dalton and others tested methods of suppressing IL-6. Unfortunately, this approach "hasn't panned out to be, by itself, a very successful target therapeutically", says Ken Shain, one of Dalton's colleagues at the Moffitt Cancer Center.

So researchers have expanded the number of neighbours they study at the same time. Anderson, working with Dana-Farber colleague Constantine Mitsiades, last year published a method for screening drug candidates against myeloma cells in the presence of stromal cells. "The spectrum of potential therapeutic targets is vastly expanded by virtue of having the ability to study the tumour cell–host interaction," he says.

Dalton has also moved away from studying individual neighbours — such as fibronectin — to looking at the entire street. Dalton and Shain showed in 2009 that myeloma cells in the presence of both fibronectin and IL-6 activate a slew of pathways that are not turned on when either neighbour is there alone. They are now working with bioinformatics experts to create mathematical models that can account for multiple factors at the same time and so potentially predict how the various neighbours interact.

"It's exciting because it's starting to give us clues about how we would eventually combine therapies to interfere with the microenvironment's influence," Dalton says. "As my mathematician colleagues tell me, we've got to embrace the complexity and not run away from it." ■

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