

The Logic of the Response

Working at the molecular level, HHMI researchers study how wounds heal—and probe genetic links between that process and cancer.

BELOW _ RESEARCHING WOUND RESPONSE, MARK KRASNOW (LEFT) AND MICHAEL GALKO CAN WATCH IT AT THE CELLULAR LEVEL, STUDY IT IN LIVE ANIMALS, AND ANALYZE IT RAPIDLY.



TIMOTHY ARCHIBALD

WHILE STUDYING A HOMELY “KISSING bug,” British insect physiologist Sir Vincent Wigglesworth injured a spot on the creature’s epidermis. He observed an inflammatory response and then watched the epidermis grow back under the outermost protective cuticle layer and reseal itself.

Wigglesworth documented his findings about how a wound heals in 1937. But even through today, basic understanding of wound healing hasn’t progressed much beyond his work. Researchers, for example, still don’t know much about the key genes involved and their specific roles.

Wanting to study wound healing at the molecular level, HHMI investigator Mark A. Krasnow was inspired to repeat Wigglesworth’s experiment. Only this time, he and postdoctoral researcher Michael J. Galko were studying the tiny fruit fly (*Drosophila*)—and they were armed with the latest light and electron microscopes as well as techniques for creating mutants and analyzing gene expression. “Wigglesworth would have killed for these tools,” says Krasnow.

Their cutting-edge approach allowed the Stanford University researchers to develop a powerful new model for the study of wound healing. “We’ve begun to dissect out the logic of the response, which has proved very difficult to sort out in vertebrates,” says Krasnow. Because

the ability to heal wounds exists in even the simplest animals and must have evolved early, he and Galko believe the core molecular controls in fruit flies will be similar to those in higher animals, including humans.

Galko figured out how to poke a hole in *Drosophila*’s cuticle and epidermis without killing the insect. He identified a larval stage amenable to study—when the then-clear cuticle makes it easy to see fluorescent markers that signal gene expression. He also found a way to turn off a critical gene in the epidermis at this larval stage so that early development would remain normal and undisturbed. “We can now watch the wound response at the cellular level in transgenic larvae; study it in live animals, including mutants; and analyze it rapidly,” Galko says.

The Stanford researchers tested their model by knocking out two genes suspected of playing a role in wound healing: the transcription factor *lozenge* (which controls development of a specific kind of blood cell) and the gene that codes for Jun N-terminal kinase (an enzyme critical for programming development of epidermal sheets). Their experiments showed that the latter is required for epidermal closure, while the former is needed for scab formation. Although the two genes are involved in separate pathways, they clearly engage in some crosstalk. The

researchers published their findings in the August 2004 issue of *Public Library of Science Biology*.

This work is a real breakthrough, says Paul Martin, a developmental cell biologist at the University of Bristol in England. “Now we have an immediate route to get at the genetics of this process; we can trawl through 50 to 100 genes and see what’s important,” he says. Indeed, Krasnow and Galko are now busily knocking out other genes and repeating their experiments on the mutants.

They’re also getting help from a group down the hall at Stanford that is probing the link between wound healing and cancer. HHMI investigator Patrick O. Brown, Howard Y. Chang, and their colleagues took human fibroblasts—a kind of skin cell that plays a role both in normal tissues and in some tumors—and exposed them to serum derived from clotted blood. Then, using DNA microarray technology to see which genes were turned on, they identified a stereotypical pattern, or “wound-response signature,” that involves about 500 genes. This information is helping Krasnow and Galko select candidate genes for testing in their *Drosophila* model.

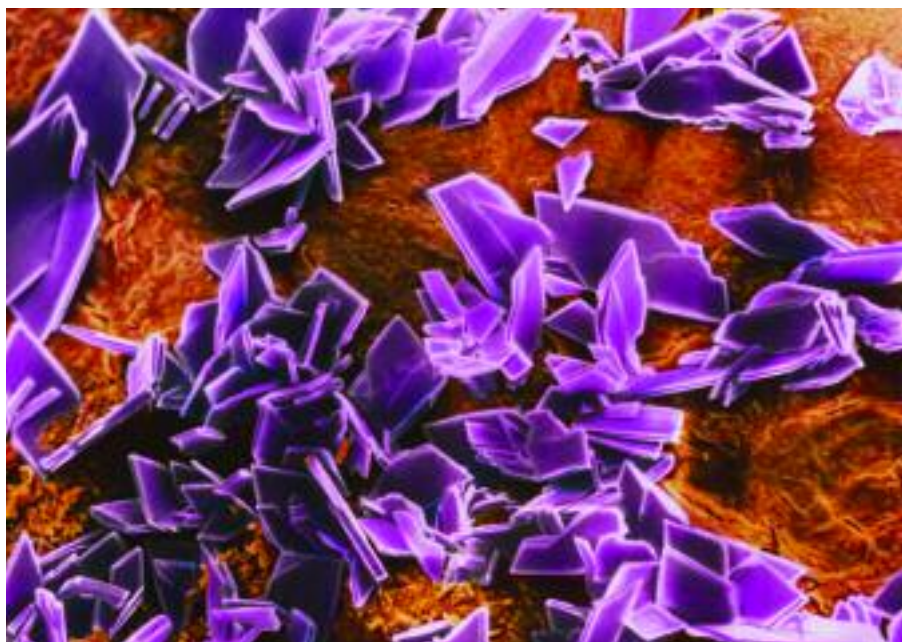
Equally important, Brown’s team confirmed that wound healing and cancer growth are truly related. For decades,

“We found a striking and consistent tendency of tumors, compared with normal tissue, to express signature wound-healing genes.”

PATRICK BROWN

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RIGHT _ WHEN THE SKIN IS CUT, BLOOD IS RELEASED ONTO THE EPIDERMIS (ORANGE). ALBUMIN PROTEINS IN THE BLOOD PLASMA HARDEN INTO CRYSTALS (PINK) OVER THE WOUND, JOINING WITH OTHER PROTEINS TO FORM A CLOT. ONE OF THE MANY MOLECULES INVOLVED IN HEALING, ALBUMIN HELPS MAINTAIN PROPER LEVELS OF HORMONES AND CALCIUM IN THE BLOOD, AND ALSO ASSISTS WATER FLOW BETWEEN TISSUES AND THE BLOODSTREAM.



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researchers have theorized that “wound healing gone awry” makes tumor cells migrate and proliferate. One piece of evidence is that people suffering from chronic inflammation, which involves cycles of damage and repair, also have high rates of cancer. Last year, Brown’s group finally found a link. “We found a striking and consistent tendency of tumors, compared with normal tissue, to express the signature wound-healing genes,” he says.

“This is not at all surprising,” says Martin, “but it’s fantastic to have real molecular evidence.” And, notes Brown, it raises the possibility that interventions targeting wound healing might also help treat cancer.

Already, the Brown lab’s efforts suggest one potential medical benefit: a test to identify early those cancer patients who will likely need follow-up treatment such as chemotherapy. The researchers found that tumors of the breast, lung,

and stomach were more likely to metastasize if they had a gene-expression signature suggestive of active wounds. They published the work in the March 8, 2005, issue of the *Proceedings of the National Academy of Sciences*. The team is now investigating ways to develop a simple “wound-response” test that would be useful in the cancer clinic. ■

-Karen F. Schmidt-

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MARK KRASNOW

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