Advances in Stem Cell Research Hold Promise for Disease Therapies

Over the course of the next year, hundreds of people will walk through the doors of the Yale Cancer Center, in New Haven, each of them hoping for a cure. Of those multitudes, about 100 fall into a special category. This group will range in age from 20 to over 70. They'll be suffering from a variety of the more serious cancers: leukemia, Hodgkin's lymphoma, multiple myeloma.

Their treatment will include stem cells.

Although these unique cells have captured the attention of the public only recently, as part of the ethical debate over cloning, they're hardly a new discovery: they've been studied and even used, in some form, for about fifty years.

Unique Properties Give Stem Cells A Crucial Role in Therapeutic Research

Many different types of cells comprise the family of stem cells. But they all share two critical properties: they can divide to produce more stem cells, like themselves, and they can become at least one other completely different type of cell. It's an unusual ability: it's as if there were a breed of cat that gave birth not only to kittens, but, occasionally, to puppies as well.

That's why stem cells are so important: researchers believe that if a part of someone's body has been destroyed, by, for instance, illness or old age, stem cells could be used to produce replacement parts—hearts, kidneys, livers, even new brain cells for those suffering from Alzheimer's or Parkinson's. And if a body part is genetically defective, as in cystic fibrosis, gene transfer techniques could be combined with stem cell treatments to grow replacements that are genetically normal.

Generally, when people think of stem cells, they think of the type known as "embryonic." These most generalized, or plastic, stem cells, can give rise to every cell in the body, and these are the kind that have generated debate, because, in theory, they are potentially capable of growing into an entire living being, as well as just a kidney.

The second, less familiar type are "adult" stem cells. These less plastic varieties specialize. Some produce different types of skin cells; some produce the various varieties of lung tissue. Some, like the ones from bone marrow used at the Yale Cancer Center, produce blood cells.

At Yale, A Startling Finding Leads to a New Paradigm

It had traditionally been assumed that adult stem cells were very limited in the kinds of cells they could generate, but, in a discovery that upset this long-held belief, Yale stem-cell biologist Diane Krause has found evidence that at least one type of adult stem cell possesses a plasticity far more extensive than anyone had anticipated.

Krause works with the same type of bone marrow stem cells used at the Cancer Center. "Up until about 1998," she explains, "I had been working predominantly on bone marrow as a source for blood stem cells." But then, a group of researchers found that bone-marrow derived cells can differentiate into skeletal muscle cells. That discovery, says Krause, opened up the paradigm. "We had thought that the bone marrow was where blood stem cells lived. But we had never thought of those as being stem cells for solid organs outside the blood."

Initially, Krause and liver pathologist Neil Theise, at New York University, looked at liver cells taken from female mice that had received bone marrow implants from male mice. "We looked for Y chromosomes in the two main cell types of the liver," she said. Those Y chromosomes would indicate that the cells, even though found in a female, had originally come from a male. In both cell types, they found what they were looking for: Y chromosomes. Those cells had to have originated in the males' bone marrow—and they had been transformed into liver cells.

"… it had been there for us to see for a long time."

That startling finding led Krause and her colleagues to look further. They examined slides from female mice into which just one single male bone marrow stem cell had been transplanted. The idea of looking at mice which had received just one single stem cell was appealing, explains Krause, because it allowed them to eliminate the possibility that bone marrow contained two types of stem cells, one that produced blood, and one that produced liver. It would demonstrate that one single adult stem cell did indeed have the ability to make both cell types.

"So that," says Krause, "is when we looked all along the GI tract—the esophagus, the stomach, the bowel—and we looked in the lungs, the trachea, the airways, and the alveoli, and we looked in the liver and in the skin, and we were able to find Y chromosome positive mature cells for each of those organ types." It was, she says, the first evidence of the extreme plasticity of bone marrow stem cells.

It's surprising, she says, that this plasticity had not been found earlier. "We didn't do anything unusual in our experiment—we did a normal bone marrow transplant, which had been done for years." What Krause did was look for something that no one had bothered to look for before. "I think that people were very surprised that the discovery was just being made," she recalls, "when in fact it had been there for us to see for a long time."

Krause's discovery has sparked much controversy. Some have even used her findings to claim that because adult stem cells seem to be so plastic, the more controversial embryonic stem cell research will not be necessary. But it would be a mistake, emphasizes Krause, to assume that, just because both cell types happen to be labeled "stem cells," they can do the same things.

"I think there are going to be certain therapeutic purposes where embryonic stem cells are the way to go, and there are going to be others where adult stem cells are the right way. We can't close off either route."

A valuable tool in the fight against cancer

At the Yale Cancer Center, medical oncologist Stuart Seropian uses adult stem cells to treat his patients. "For some cancers," he explains, "very high doses of chemotherapy are more effective than standard doses in putting people in remission and increasing the length of the remission."

The problem is, such potent doses not only eliminate the cancer, they kill off the patient's blood system as well. But bone marrow stem cells, transplanted into the patient, will recreate that blood system, so that patients can survive.

Looking at the transplanted mice, Krause and her colleagues found that when organs had been injured, stem cells seemed to flock to the site. They help heal the damage, and it's possible, the researchers suggest, that the cells are in some way "summoned" to the site of the injury by signals sent out by the damaged organ, in much the same way that inflammatory cells are summoned by infections.

This kind of healing, says Krause, may happen naturally on a regular basis. "We know," she says, "that the more damaged tissue is, the more marrow-derived cells differentiate into that cell type." Typically, she says, less than 1% of the cells found in any organ might be marrow derived. "But in a situation where there's a compromised ability of the organs to heal themselves,

then they might call on this 'extra-organ' stem cell source for help."

Stem cell transplants aren't the appropriate treatment for every cancer, says Stuart Seropian. But in the cases where they can be used, here is how effective they can be: In patients with multiple myeloma who initially responded to chemotherapy, a transplant to a patient who is in a first remission can double survival time, from about two and a half years from the time of diagnosis to about five years, in comparison to continuing standard, non-transplant therapy. For some relapsed lymphomas, he says, transplants increase survival rate from about 10% to over 50% over a five-year period.

And this benefit derives simply from using stem cells to create new blood cells—the task for which they had originally seemed designed.

Researchers are already trying to use these cells for more than just creating blood. They're looking, for example, at bone marrow derived stem cells as a way to heal damaged heart muscle. In Europe, says Krause, clinical trials are taking place in which bone marrow is directly injected into the patient's heart. "These cells seem to allow the heart to heal with some normal cardiac muscles and blood vessels, rather than healing with just scar tissue." And researchers here in the United State are developing a way to induce a patient's own stem cells to seek out and heal that kind of damage on their own.

To realize the extreme plasticity of these cells really makes you look differently at what's going on inside your body, says Krause. "It really changes our paradigms for how the body heals itself."

At UConn, advances in animal cloning with a focus on human therapies

For Xiangzhong "Jerry" Yang, who heads the Transgenic Animal Facility and the new Connecticut Center for Regenerative Biology at the University of Connecticut, (UConn) the focus is on cloning, and the therapeutic use of embryonic stem cells. Yang's wide-ranging work includes a multitude of advances, ranging from the development of transgenic rabbits that can produce pharmaceuticals in their milk, to a more efficient way to clone pigs. Yang was also, importantly, the first to clone mammals from skin cells.

"One of the major applications of cloning research," says Yang, "is human medicine therapy. We're using cloning as a tool to help cure patients."

At the University of Connecticut's Transgenic Animal Facility, Dr. Jerry Yang and his colleagues have shown that clones such as Daizy (shown above with her calf), cloned from aged cows, can have normal pregnancies and normal calves. (Photo: J. Yang)

That's one reason, he explains, that he's been working with older animals. His calf, Amy, was cloned from a 13-year-old cow, and he's also produced six calves from the skin cells of Kamitakafuku, a 17-year-old Japanese prize bull. Kamitakafuku, he says, was the equivalent of about a 90-year-old human being.

"We're focusing on older animals," he explains, "because it's older humans that tend to have organ problems, cell mutation problems, cancer, and Alzheimer's."

Ideally, he says, you'd want to clone replacement parts from the cells of the individual that needs the transplant, because then the transplant will be accepted more easily. But there's always been a concern that a clone produced from an elderly animal will be, in essence, an elderly animal itself: even if it looks like an infant it will have, for example, the life span of the animal from which it was taken.

"If you clone cells from an aged individual and get an aged cell, it will not help your disease, because many times when any organ fails, it's because some of the cells have stopped working, due to too much wear and tear."

Cloning, though, he says, is essentially a rejuvenating process -- it creates something young regardless of the age of the donor animal.

Telltale telomeres

Yang and his colleagues showed this by looking at telomeres, the short DNA sequences located at each end of a chromosome. Each time a cell divides, its telomeres get shorter, and eventually, if they get short enough, the cell cannot divide at all.

"In natural reproduction," says Yang, "the mom has short telomeres, the dad has short telomeres—but the offspring has normal telomeres, just through the normal reprogramming

process."

Yang and his colleagues compared the telomere lengths of 10 clones born at UConn with those of control calves born at the same time. "None of the clones had short telomeres. All of the clones' telomeres were as long as the control animals'. All of the clones' telomeres were way longer than those of the donor animals."

Normally, says Yang, this elongation takes place in the gonads: the ovaries and the testes. But, somehow, cloning also performs that function, bringing the telomeres back to their original length.

Successful cloning wipes the cellular slate clean

The process of successful cloning involves somehow erasing everything that's happened to that cell as it's aged, and returning it unscathed to the starting line. It means not only reversing the cell's aging, by lengthening telomeres, but also reversing its developmental path, by transforming it from a specialized, limited adult cell, to a young, undifferentiated cell that can still become anything.

"When we first started using skin cells," says Yang, "we talked about how much simpler and less invasive it was to obtain these cells [compared to the ovarian and mammary cells previously used]." But it turns out that skin cells have another advantage.

"Skin fibroblast cells are pretty much like a weed," says Yang. "You can grow them very very easily—a high school student could do it. But more importantly, skin fibroblast cells not only grow more easily, but they're much longer lasting in culture. We can cultivate them for a year, and that's a long time."

Cultivating cells for long periods is important, because, for one thing, it allows researchers time to perform the multistep process needed to correct defective genes.

But it's also important because of this: Yang has found that cells cultured for long periods of time are actually more efficient at producing healthy clones.

Cloning has a very low success rate, and, despite the field's dramatic advances, most clones die either before or soon after birth. Prior to Yang's work with Kamitakafuku, most successful cloning had been accomplished with cells that been cultured for short periods of time.

Long-Term Culture Key to Successful Cloning

But with this research, Yang cloned skin cells that had been first been cultivated for one, two and three months, and, startlingly, he found that embryos derived from skin cells that had been cultured longer developed as well as clones produced from the much younger fetal or embryonic cells. And, they developed better than clones produced from adult skin cells that had been cultured for a short time.

"We don't really know why long-term culture helps developmental competence," says Yang. But he believes that the answer may lie in what he calls "reprogramming phenomena."

Just as cloning, and normal reproduction, must lengthen telomeres, so, too, must they reprogram the genes inside the nucleus.

Every cell in your body contains exactly the same genes, explains Yang. But, for example, a skin cell is different than a nerve cell because in each one, a different set of genes is turned on. All the other genes are "locked." "Basically," says Yang, "the cloning process is the unlocking process of those locked genes." They must reverse these very specialized cells back to a non-differentiated cell type.

But, he says, suppose the cell is not completely reprogrammed—suppose that some of the genes are not unlocked properly, cannot be turned on, or turned off as they should be. Then, says Yang, the embryo will die.

Recently, Yang compared the behavior of X chromosomes in clones and in naturally reproduced animals. During the initial development of naturally reproduced female mammal embryos, both of the X chromosomes are active, while in later stages, one chromosome is turned off.

Yang and his colleagues found that in cattle cloned from skin cells, this crucial pattern often is disrupted. These clones, they point out in a recent paper, tend to start off with only one functioning X chromosome, because their genetic material was taken from an adult animal in which one of the two X chromosomes had already been turned off. The researchers found that in some of these clones, genes on both chromosomes were turned off—which means that certain proteins needed by the animal could not be produced.

When a clone is made from a skin cell, genetic material is plucked directly from an adult, fully differentiated cell, and inserted directly into an egg. The body's natural reprogramming process is bypassed. Whatever reprogramming takes place, must occur as the embryo grows.

"Perhaps," suggests Yang, speaking of his work with Kamitakafuku, "the long term culture of cells in the *in vitro* environment helps the reprogramming process along. Perhaps it makes that lock easier to open."

Bone Marrow Transplants: A Risky Procedure Becomes Routine

Back in the fifties and sixties, said Seropian, researchers only understood, in a kind of general way, that blood cells didn't live forever, and that therefore there must be some cells, which they knew to be in the bone marrow, that were replacing them. "Back in the sixties, they just knew that if you harvested enough bone marrow, you were going to have enough of those cells to support a transplant."

It was a difficult, painful procedure. "Back in the eighties, when we used bone marrow exclusively for autologous [patient-from-self] transplants, the risk of actually dying from the procedure was 10 to 15%." Patients could count on a month in the hospital, and the procedure wasn't even done in patients over 60. Now, he says, advances have made the procedure much more straightforward. The risk of dying from some sort of autologous transplant complication is less than 1% for most patients, and, says Seropian, at Yale, one-quarter of the patients never stay overnight in the hospital at all. Even allogenic transplants, a far more risky and complicated procedure in which the patient receives stem cells from some one else, and which does involve hospitalization, have become more possible.

Seropian describes a procedure that has become, over the years, fairly routine. For about ten days, patients give themselves injections of a natural hormone that increases the number of stem cells in the blood stream. Then, in a process much like dialysis, the stem cells are filtered from the blood stream, and frozen until they are needed.

Returning them to the blood is actually sort of anticlimactic, says Seropian. "Cause we kind of make a big deal about it, but it's just like a blood transfusion." It's an outpatient procedure that only takes about an hour or two. "Usually, it's pretty boring." Sometimes, he admits, people get a bad taste in their mouth from the chemicals that have been used to keep the frozen blood from crystallizing. "We give them root beer candy," he says.

Only a few years ago, says Yang, no one would ever have believed that a skin cell could generate a baby. Researchers just dreamed of the gene transfer techniques that are now commonly used.

And the plasticity of Diane Krause's adult stem cells had not not even been guessed at.

"The important thing," says Yang, "is that the technologies of tissue engineering and cloning and stem cells bring us much closer [to clinical applications] than just a few years ago. And here, we're beginning the research. We can't see the end yet. But we have a beginning."—***Karen Miller, science writer***

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