Members In the News *(Continued from page 4)*

One of these stem cells is not like the other" – KU Medical Center researcher Jay Vivian asks why

January 07, 2013
By Alissa Poh

When stem cells were first generated from human skin in the laboratory five years ago, scientists rejoiced. At long last, they could employ a highly-coveted characteristic of naturally-occurring stem cells — pluripotency, or the ability to become different cell types — in regenerative medicine, while bypassing a host of ethical issues associated with using embryonic stem cells. For their seminal research, some 40 years apart, that made these lab-created cells, called induced pluripotent stem (iPS) cells, possible, Sir
John B. Gurdon and Shinya Yamanaka shared this year's Nobel Prize in Physiology or Medicine.
Jau L. Vivian, PhD

It turns out, however, that for all their great therapeutic potential, iPS cells — and probably stem cells in general — have something in common with teenagers: they're often unpredictable.

"In a perfect world, we could direct a dish of pluripotent stem cells to differentiate, or turn into a specific kind of cell, and they'd do so in lockstep," says Jay Vivian, Ph.D., an assistant professor in the Department of Pathology and Laboratory Medicine at the University of Kansas Medical Center. "But nothing's perfect in science, and we usually end up with an ugly mess of stuff — some of the cells that we want, and a lot more that we don't." A member of KU's Institute for Reproductive Health and Regenerative Medicine, Vivian is trying to work out why these cells are more difficult to manipulate than previously imagined, and recently published a paper on this topic in the October 2012 issue of the journal Stem Cells.

The starting point, Vivian says, is overcoming the assumption that pluripotent stem cells are all alike. On the contrary, they don't behave in identical fashion and often don't even look the same. "They're heterogeneous," he says, which is science lingo for "not uniform." The cells are also dynamic, able to wander in and out of different states seemingly at will. And yet it isn't random, as he and his group explain in their research, which zeroes in on three of the molecular players responsible for driving these dynamic differences.

Meet Nanog, Nodal and a third character more broadly known by its initials: BMP.
Nanog is a master regulator and, Vivian says, "absolutely required" for stem cells to display pluripotency. Nodal and BMP both belong to a large group of signaling molecules called the TGF-beta superfamily. Each sets in motion a different chain of activities that ultimately regulates the overall process of transcribing individual genes into proteins.
"Here's the conundrum," Vivian says. "Nanog controls thousands of genes that keep cells pluripotent, or in an undifferentiated state. But pluripotent cells don't all have the same amount of Nanog. In fact, it's highly variable. We divvied them up into four groups - cells with very

Continued on page 6

Members In the News *(Continued from page 5)*

high, high, medium or low Nanog levels - and asked, 'Why these differences, given Nanog's importance?'"

In the laboratory, Vivian suppressed Nodal's signaling pathway in mouse embryonic stem cells and saw that their shape changed. "They became very round and tightly clumped," he says. "These mouse ES cells like sticking together anyway, but became even more cohesive when Nodal signaling was switched off."

In a separate experiment, Vivian tagged Nanog so it glowed fluorescent green and tracked its levels in mouse cells. He noted that cells with higher amounts of Nanog resembled cells in which Nodal signaling was suppressed: tight and round. "But Nanog-low cells were somewhat flatter, with poky little edges to them," he says. "We wondered if these two observations were related, and Nodal and Nanog were communicating with each other. Sure enough, that turned out to be the case."

By teasing apart the conversational tangles, Vivian and his group found that Nodal talks to Nanog through BMP. Normally, BMP oversees a group of proteins collectively named Id, but Nodal interacts with and lowers BMP's signaling activity. "Id proteins heavily influence a cell's decision to have high or low Nanog expression," Vivian explains. "If Nodal is blocked, BMP's activity increases, which in turn pushes cells to a Nanog-high state."

"We've defined the molecular hierarchy at play here," he adds, "all because of a simple observation under the microscope: 'Some of these cells look a little funny.'"

Vivian says heterogeneous pluripotent stem cells are sort of like a mixed bag of M&M's. "Think of cells with very high Nanog levels as green M&M's, the ones with medium levels as yellow M&M's, and Nanog-low cells as those brown M&M's most people don't want to eat," Vivian says. But because the differences are dynamic, the candy is really more like the magical sweets Harry Potter and his friends consumed at Hogwarts: brown M&M's can turn green, and vice versa.

"Functionally, Nanog-high cells are much better to work with, so we want to learn more about other factors that influence a cell's decision to exist in this particular state," Vivian says. "We want more green M&M's, basically."

Mouse embryonic stem cells were part of this research because, Vivian explains, "they've been studied for 25 years and we know a lot more about working with them, compared to human iPS cells." But he and his crew are using iPS cells more, focused on directing their differentiation into neural cells that could be used to treat spinal cord injuries.
"I believe what we've learned with our mouse work can be extrapolated," Vivian says, "because heterogeneity also exists in iPS cells, and I'm pretty sure Nodal and BMP, at least, are influencing factors."

Vivian emphasizes that the use of pluripotent stem cells in regenerative medicine is still very much in its infancy. "We don't yet fully understand just how complex these cells are, much less everything that influences them to differentiate or remain in their original state," he says. "Once we have a clearer picture of how heterogeneity is established and how it might be tinkered with, we can better direct these cells toward becoming the types we want, therapeutically."