Stress May Naturally Make Stem Cells of Mature Cells

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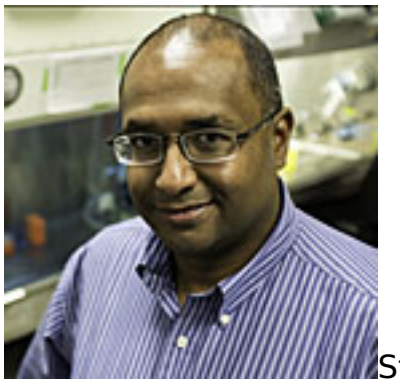
 Stress or injury may naturally prompt mammals to make “extraordinary” stem cells out of certain “ordinary” mature cells via dedifferentiation.

Three strong papers suggesting this were quietly published within weeks of the controversial *Nature* [1] “acid bath” work, where a team from Japan’s Riken Institute, and the US’s Harvard University, reported dedifferentiating neonatal mouse spleen cells into stem cells by stressing them, artificially in a dish, with coffee-mild acid.

Confidence in the “acid” work has hit a new low. It has not been repeated. A Riken investigation found lead author Haruko Obokata committed two acts of falsification and fabrication that may have crippled [2] the papers—and may prompt retraction. (See *Bioscience Technology* [3] stories [2] on “acid bath” developments.)

But the other three studies – conducted by unrelated teams on mammalian stomach, trachea, and kidney—have generated no controversy. Two drew raves from the *Faculty of 1000*.

The other three are “important and fascinating,” said University of Pennsylvania developmental immunologist Avinash Bhandoola in an email. Bhandooli, not involved in the papers, studies T cell regeneration. University of Pittsburgh oncologist Michael Lotze, who studies cellular stress, was also uninvolved in the papers. He said in an interview: “The three papers are amazing in their potential. They say something profound about the plasticity of cells in response to stress, and have great implications for how we understand cancer."

It is proven mature cells can be manually persuaded to dedifferentiate into pliable and potent Induced Pluripotent Stem Cells (iPSC [4]s) via complex *in vitro* manipulation. But science has not firmly established mature cells *naturally* dedifferentiate to more pliable states in adult mammals. The above three papers hint this can occur, if under defined circumstances, their authors say.

There are key differences between the “other three” and the “acid” papers. First, the main stressors differed: none of the others used acid. Second, the “acid” work involved damaging mature cells engineered to light up via a GFP (green fluorescent protein) reporter when dedifferentiating to a point where they expressed the pluripotency gene *Oct4—all in vitro*. By contrast, in the “other three,” researchers damaged cells *in vivo* (animals), then tracked their responding *endogenous* dedifferentiation via advanced lineage tracing techniques. (In two of the papers, they tracked dedifferentiation of mature neighboring cells. In the third, they tracked dedifferentiation of damaged mature cells *themselves*—and also, perhaps, neighboring mature cells.)

Third, dedifferentiation in the “other three” was limited to epithelial cells. The “acid” work claimed cells dedifferentiated in many tissues.

Finally, in the “other three,” the cells didn’t go embryonic, as claimed in the “acid” work. They reverted to an intermediate, pliable adult stem cell and stem-cell-like state—in what seems a natural response to stem cell injury in some epithelial tissues; a natural response to *mature* cell injury in others.

There were other differences. The above appear key.

Two Papers: Dedifferentiation in Response to Stem Cell Injury

Of “the other three,” the paper garnering most attention was published in Nature *[5]* in November. The Harvard crew of Jayaraj Rajagopal reported mature cells respond to stem cell ablation in trachea epithelia by dedifferentiating into cells that proliferate—then redifferentiate into mature cells, presumably to repair: “Fully committed (mature) secretory cells respond to stem cell ablation by proliferating and converting into functional epithelial stem cells.”

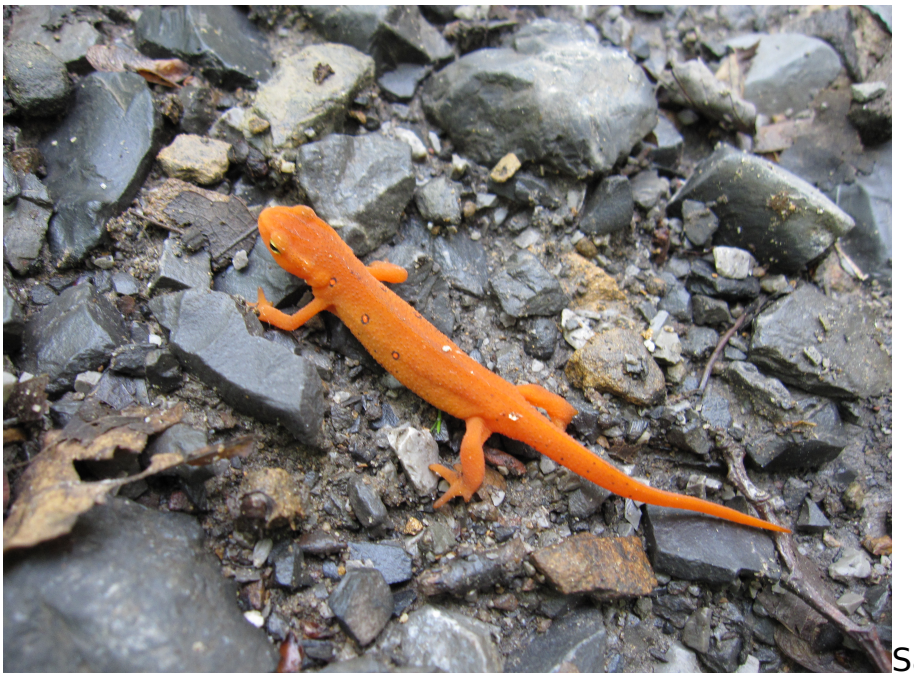
“We showed the cells have a surprising ability to go `backwards’ in their natural lineage,” Rajagopal said via email. Some cells may not “lock or hold on to their identity” as tightly as thought, he said.

The group reported cells would *not* dedifferentiate if touching a native stem cell: “The ability of basal stem cells to prevent the dedifferentiation of (mature) secretory cells has many implications for tissue biology in general, as stem cells and their progeny can now be seen to reciprocally modulate one another.”

That feedback resembled that occurring in newt dopamine neurons, wrote University of Florida geneticist Malcolm Maden in a December 2013 *Cell Stem Cell* commentary: “This is a striking example of universality of mechanisms and suggests a deep evolutionary origin for the cell behavior.”

Rajapopal emailed that his dedifferentiation was inversely proportionate to maturity, as in classic amphibian [6] work. His team reported: “Our findings have broad implications for cancer biology, as they point to an underlying physiologic cell plasticity that could be co-opted in tumorigenesis. Indeed, some lung cancers seem able to resist chemotherapy by using a lineage conversion into a different tumour

subtype."



Said Bhandoola via email: "I find myself wondering if chemotherapy itself might be a source of stimuli that can enhance or encourage this conversion. Relapses might not only be due to resistant cells, but created by the chemo."

Emailed first author Purushothama Rao Tata: "We believe that under conditions such as injury, stress, infections, or inflammation, differentiated cells may acquire either a stem cell property, or an embryonic state. Our study, and other recent studies in kidney, adrenal gland, and stomach indicate that cells are much more plastic than previously thought."

Rajapopal used a "secretory cell lineage tracing mouse model (Scgb1a1-creER- with fluorescent reporter) which specifically labels only secretory cells and not basal (stem) cells," Tata emailed. Injury came via diptheria-toxin expressing basal (stem) cells, which were ablated after inhalation of doxycycline. There was no native stem cell contamination. "We did not find any labeled basal cells (0 out of 2159) in the Scgb1a1 lineage-labeled airways...An independent study by Brigid Hogan (Duke University), who originally generated this mouse model, also did not find any labeled basal cells (0 out of 5604 cells from 19 mice), supporting its fidelity and specificity. This data in aggregate (0 out of 7763) makes the possibility of `inadvertently marked' original basal cells virtually non-existent."

In the second paper, a team led by the Utrecht Institute's Hans Clevers reported in a recent *Cell* [7] that mature Troy+ "chief" cells can act as stem cells, generating all lineages of the *stomach* epithelium. Using lineage tracing for a Troy-eGFP-ires-CreERT2 allele, the group found single-marked chief cells can generate entirely labeled gastric units in months—"a phenomenon that accelerates upon tissue damage," the report read. When local stem cells were ablated via the chemo 5-Fu, mature cells acted "as quiescent reserve stem cells. These observations challenge the notion that stem cell hierarchies represent a one-way street."

Said the report: “The gastric corpus thus appears to contain two stem cell populations: an actively dividing population located in the isthmus…and a small population of reserve stem-like chief cells…The unique property of the Troy cell as a fully differentiated cell with the capability to act as a multipotent stem cell represents a surprising example of plasticity.”

Emailed first author Daniel Stange: “Our lineage tracing showed that, starting from the chief cells, we could over time mark all other cell types in the stomach epithelium. So (resulting cells) are multipotent. Nevertheless, it might be that the chief cells dedifferentiated towards only one or more other cells (omnipotent), and some of those cells can then form the remaining cells. This possibility cannot be distinguished by lineage tracing.”

Stange emailed that endogenous dedifferentiation papers may soon be increasing along with lineage tracing mouse models. “More CreERT2 mice for all kinds of cells are generated. So we will see more studies in this direction.”

A *Nature* commentary [8] by Stanford University’s Tushar Desai and Mark Krasnow said the stomach and trachea work “challenge the primacy of undifferentiated resident stem cells, given that mature cells can substitute for their function and even make new ones.”

Third Paper: Dedifferentiation in Response to Mature Cell Injury

Then there is Harvard’s Benjamin Humphreys, who reported in a January 2014 *Proceedings* [9] *of the National Academy of Sciences* that, in kidney, when mature epithelial cells near the nephron are injured (by “ischemia,” or blocked blood/oxygen) they act like stem cells. Lineage tracing revealed mature proximal tubule cells upregulate stem-cell markers and proliferate. “Our work demonstrates that fully differentiated epithelia repair proximal tubule without any contribution from a pre-existing intra-tubular stem cell population.” (The mouse model possessed a CreERt2 cassette knocked into the sodium-dependent inorganic phosphate transporter SLC34a1 locus of its genome. This is expressed only in differentiated cells.)

In a statement, Humphreys said: “I’m not saying kidney stem cells don’t exist, but in tissues where cell division is very slow during homeostasis, there may not have

been an evolutionary pressure for stem cell mechanisms of repair."

Noted Humphreys by email: "Our work shows definitively that fully differentiated proximal tubule epithelial cells undergo dedifferentiation and proliferation after acute injury, and that this is the predominant mechanism of repair in kidney. It is now increasingly recognized that dedifferentiation is an important repair mechanism across organs... What is less clear, and an important future area of study, is whether dedifferentiated kidney epithelial cells gain multi-potent and stem-cell-like capacities, or whether they remain lineage-restricted."

Humphreys' paper shows injury to *mature* cells *also* spurs dedifferentiation—in the injured cells themselves, he says. But, as with the above papers, *uninjured* mature cells may also have dedifferentiated in response to *neighbors'* injury.

Humphreys emailed he believes most of his dedifferentiation occurs in injured mature cells, but it could also be occurring in uninjured mature cells reacting to others' injury. "We cannot rigorously distinguish between the two. We know it is *mature* cells undergoing the dedifferentiation, proliferation and re-differentiation into fully differentiated proximal tubule cells after an injury stimulus. Whether an 'uninjured' differentiated cell dedifferentiates in response to injury of a neighbor —we can't assess. It is biologically plausible, since injury to a neighboring cell might induce loss of adherens junctions, proteins in the injured cell, leading to disruption of this cell-cell contact—which could induce a dedifferentiation response...Our injury is a fairly global one (ischemia) that should not *necessarily* be expected to severely injure one cell, yet leave a neighbor unaffected. But I can't prove that. So I would say that both are possible interpretations."

The upshot: some mammal cells appear to naturally respond to certain forms of stress by dedifferentiating into more potent cells. Repeated, researchers say, this may well impact regenerative medicine.

Links:
[1] http://www.nature.com/nature/journal/v505/n7485/full/nature12968.html
[2] http://www.biosciencetechnology.com/articles/2014/04/scientist-said-he-may-have-made-stap-cells%E2%80%94just-riken-called-fraud
[3] http://www.biosciencetechnology.com/blogs/2014/02/new-stem-cell-sagas-0
[4] http://www.cell.com/abstract/S0092-8674(07)01471-7
[5] http://www.nature.com/nature/journal/v503/n7475/full/nature12777.html
[6] http://www.pnas.org/content/100/14/8048.full
[7] http://www.cell.com/abstract/S0092-8674(13)01145-8
[8] http://www.nature.com/nature/journal/v503/n7475/abs/nature12706.html#affil-auth
[9] http://www.pnas.org/content/early/2013/10/08/1310653110.short?rss=1