ONLINE FIRST

Re"evolutionary" Regenerative Medicine

Helen M. Blau, PhD

Jason H. Pomerantz, MD

THE POTENTIAL TO REGENERATE DAMAGED LIMBS AND hearts seems the subject of science fiction, but newts and zebrafish do it all the time. What can scientists learn from these simple creatures? Why have mammals not retained this remarkably useful property in the course of evolution? Can an evolutionary perspective on the mechanisms used by "lowly" organisms inform the approach to human tissue regeneration? Could this lead to the generation of abundant patient-specific differentiated cells for cell therapy, for elucidating disease mechanisms, for therapeutic drug screening? Recent studies suggest that this is possible.[1-3]

The newt's ability to regenerate its heart or an entire limb after amputation has attracted the attention of scientists for centuries, with the hope that it might be possible to mimic this remarkably useful capacity in humans. Pioneering work with this species has provided many seminal insights.[2,3] Limb regeneration entails regrowth of a diversity of tissues in their appropriate positions and proportions. This complex process is dependent on innervation and requires the orchestration of a dynamic interplay of cells.[2] Following amputation of a newt limb, a blastema, or cell aggregate, forms from which the limb develops. The blastema was long thought to comprise a cluster of multipotent cells that had to specialize anew. However, recent elegant lineage tracing studies have shown that the cells are not multipotent but instead remain dedicated cartilage, bone, neural, and muscle cells.[4] A critical step in this process of limb regeneration is the acquisition of proliferative potency. This is achieved by reentry into the cell cycle of postmitotic cells that retain their specified identity. A similar process is also observed in zebrafish heart regeneration.[3]

What if, as in newts, fully specialized, nondividing human cells could dedifferentiate and be pushed back just one step to a proliferative state? While retaining their identity or "sense of self," these cells could yield precise copies of themselves capable of regenerating the damaged tissues from which they arose. A crucial step would likely entail "lifting the brakes" on cell division, but only transiently, to avoid uncontrolled proliferation and tumor formation.

Author Interview available at www.jama.com.

How could dedifferentiation be achieved? Transient inhibition of tumor suppressors might play a role. It has been postulated that during evolution mammals lost regenerative potential as a trade-off for cancer protection. The tumor suppressor Rb, encoded by the retinoblastoma gene, is known as the eukaryotic "gatekeeper" of the cell cycle G1-S transition. Inactivation of Rb mediates newt regeneration.[5] In contrast, loss of Rb does not lead to mammalian cell dedifferentiation (eg, primary skeletal muscle cells).[1,6]

What might the additional mammalian brake on cell cycle reentry be? Cancer biologists had previously defined a critical function for the alternative reading frame protein (ARF; also known as p19ARF in mice and p14ARF in humans), a product of the mammalian ink4a tumor suppressor locus. ARF enforces cell cycle arrest and prevents tumorigenesis when Rb is inactivated (either by signaling or by mutation). In human cancers, ARF is frequently inactivated.[7] Even mature differentiated cells can become transformed when ARF is inactivated, if exposed to aberrant growth factor signals.[8] In evolution, ARF has no homologues in regenerative vertebrates (in contrast with the other product of the ink4a locus, p16). Indeed, ARF has not been identified in organisms lower on the evolutionary tree than chickens.[9] Thus, ARF was postulated to be the culprit. Remarkably, on transfection of inhibitory RNAs to both Rb and ARF, primary muscle cell nuclei initiated DNA synthesis. Thus, the double knockdown of these 2 tumor suppressors overcame the block to cell cycle reentry.[1]

A critical remaining question was whether cells that reentered the cell cycle could complete mitosis and proliferate following treatment with inhibitory RNAs targeting Rb and ARF. This was examined by testing differentiated mononuclear muscle cells, known as myocytes, that will not reenter the cell cycle regardless of the barrage of growth factors to which they are exposed.[1] To show definitively that a postmitotic cell could initiate division, a means of following single cells was essential. Using live cell laser capture catapulting, single myocytes were circumscribed and cut out

Author Affiliations: Baxter Laboratory for Stem Cell Biology, Stanford University School of Medicine, Stanford, California (Dr Blau); and Department of Surgery, University of California, San Francisco (Dr Pomerantz).

Corresponding Authors: Helen M. Blau, PhD, Baxter Laboratory for Stem Cell Biology, Stanford University School of Medicine, 269 Campus Dr, CCSR 4215, Stanford, CA 94305-5175 (hblau@stanford.edu); and Jason H. Pomerantz, MD, Department of Surgery, University of California, San Francisco, 505 Parnassus Ave, Box 0932, San Francisco, CA 94143 (jason.pomerantz@ucsfmedctr.org).

©2010 American Medical Association. All rights reserved.

Downloaded from jama.ama-assn.org by Christopher Buttery on 29 December 2010

using a laser, which was then refocused to produce the energy to catapult the single cell on its tiny cut membrane into a capsule from which the captured cell was removed and plated in culture. Untreated individual myocytes survived and crawled off the membranes but never divided. In contrast, myocytes treated with inhibitory RNAs, to transiently reduce Rb and ARF, proliferated and gave rise to colonies. The clones obtained from the temporary "double knockdown" retained their identity and were functional. The cells reexpressed Rb and ARF, and differentiated in culture. Moreover, if introduced into a damaged muscle of a mouse limb, the cells repaired the damage. These findings suggest that "terminally differentiated" mammalian cells can be dedifferentiated to a proliferative state without becoming tumorigenic and can retain their identity.[1]

Why consider inducing regeneration of tissues by cell dedifferentiation? Currently, regeneration of most mammalian tissues is extremely limited. Some tissues harbor adult stem cells, and in some even this regenerative source seems absent. In the first case, regeneration is fueled by a very small population of adult stem cells, quiescent precursors that are stimulated to divide when needed for damage repair. In the second case, such as the heart, stem cells apparently do not exist. As a substitute, pluripotent embryonic stem cells or the remarkable induced pluripotent stem cells have been invoked as sources from which to generate cell types of interest.[10] Challenges include directing embryonic or induced pluripotent stem cells toward a single desired highly specialized cell fate and integrating those cells into the tissue following transplantation.[10] Dedifferentiation of cells from the damaged tissue could serve as a potent alternative. The cells would be plentiful, know their identity, have the desired tissue properties, and be located exactly where needed.

One major application of dedifferentiation is the regeneration of diverse human tissues. For instance, the induction of proliferation of healthy cardiomyocytes in the vicinity of infarcted myocardial tissue could potentially yield cells of the appropriate identity, resulting in cardiac regeneration rather than fibrosis. Could this be achieved by transiently suppressing Rb and ARF? A major attraction of this regenerative approach is that it mimics a process that nature already uses in regenerative species. Therefore, it is known to work. A potential caveat is that nature presumably had good reason to restrict proliferative promiscuity. Clearly, controlled dedifferentiation and only temporary loss of tumor suppression is critical.

Another major application of dedifferentiation is the potential to model human diseases and screen for drugs using cells derived by suppressing Rb and ARF. Possibly, in addition to muscle, cell types such as dopaminergic neurons, pancreatic islet cells, and cardiomyocytes could be proliferated. If these cells are obtained from human tissues by biopsy or at autopsy, they may closely replicate the disease phenotype in vitro. A major advantage would be that the cells have a specified identity, are only taken one step back to proliferate, and therefore should closely mimic their disease states of origin. Such cells should allow the regulatory networks underlying Parkinson disease, Alzheimer disease, diabetes, and heart disease, among others, to be discerned. The replication of a neuron of a defined nature may prove easier in some cases than deducing how to direct a pluripotent cell (derived from a patient's fibroblasts by induced pluripotent stem [iPS] cell technology) to adopt a particular desired specialized neuronal phenotype. It is possible that cells derived by dedifferentiation, as described herein, will complement pluripotent embryonic stem cells, iPS cells, and adult tissue–specific stem cells as sources for cell therapies, for creating in vitro disease models, and for discovering new therapeutic agents.

In conclusion, the re"evolutionary" approach to regenerative medicine, the transient dual knockdown of tumor suppressors, ARF and Rb, addresses one critical obstacle to translating the potent regenerative capabilities of newts to mammals. The adaptation of a mechanism that nature has previously designed and successfully exploited is highly appealing. Dedifferentiation may provide insights into human disease, lead to drug discovery, and a means for regenerating tissues. Perhaps newts can give humans, as well as themselves, a hand.

Published Online: December 21, 2010. doi:10.1001/jama.2010.1938
Conflict of Interest Disclosures: All authors have completed and submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest and none were reported.
Funding/Support: This work was supported by grant F32 AR051678 and a University of California, San Francisco Program for Breakthrough Biomedical Research Opportunity Award (Dr Pomerantz); and grants AG009521, AG020961, HL096113, HL100397, and AR059565 from the National Institute of Health, grant TR6025-09 from the Leukemia and Lymphoma Society, grant 34-2008-623 from the Juvenile Diabetes Research Foundation, grants RT1-01001 and RB1-01292 from the California Institute for Regenerative Medicine, and support from the Baxter Foundation (Dr Blau).
Role of the Sponsors: The funding sponsors had no role in the preparation, review, or approval of the manuscript.
Online-Only Material: The author interview is available at http://www.jama.com.
Additional Contributions: We thank Kostandin Pajcini, PhD, for his major experimental contributions without whose efforts this work could not have been achieved. Dr Pajcini did not receive any compensation for his contribution.

REFERENCES

1. Pajcini KV, Corbel SY, Sage J, Pomerantz JH, Blau HM. Transient inactivation of Rb and ARF yields regenerative cells from postmitotic mammalian muscle. *Cell Stem Cell*. 2010;7(2):198-213.
2. Brockes JP, Kumar A. Comparative aspects of animal regeneration. *Annu Rev Cell Dev Biol*. 2008;24:525-549.
3. Poss KD. Advances in understanding tissue regenerative capacity and mechanisms in animals. *Nat Rev Genet*. 2010;11(10):710-722.
4. Kragl M, Knapp D, Nacu E, et al. Cells keep a memory of their tissue origin during axolotl limb regeneration. *Nature*. 2009;460(7251):60-65.
5. Tanaka EM, Gann AA, Gates PB, Brockes JP. Newt myotubes reenter the cell cycle by phosphorylation of the retinoblastoma protein. *J Cell Biol*. 1997;136(1):155-165.
6. Huh MS, Parker MH, Scimè A, Parks R, Rudnicki MA. Rb is required for progression through myogenic differentiation but not maintenance of terminal differentiation. *J Cell Biol*. 2004;166(6):865-876.
7. Sherr CJ, McCormick F. The RB and p53 pathways in cancer. *Cancer Cell*. 2002;2(2):103-112.
8. Bachoo RM, Maher EA, Ligon KL, et al. Epidermal growth factor receptor and Ink4a/Arf: convergent mechanisms governing terminal differentiation and transformation along the neural stem cell to astrocyte axis. *Cancer Cell*. 2002;1(3):269-277.
9. del Arroyo AG, Peters G. The Ink4a/Arf network: cell cycle checkpoint or emergency brake? *Adv Exp Med Biol*. 2005;570:227-247.
10. Yamanaka S, Blau HM. Nuclear reprogramming to a pluripotent state by three approaches. *Nature*. 2010;465(7299):704-712.

©2010 American Medical Association. All rights reserved.

Downloaded from jama.ama-assn.org by Christopher Buttery on 29 December 2010