The American Journal of Pathology, Vol. 185, No. 10, October 2015

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The American Journal of
PATHOLOGY

ajp.amjpathol.org

Regenerative Medicine Theme Issue

GUEST EDITORIAL

Expanding Horizons of Cellular Plasticity in Regenerative Medicine

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Historical Perspective

Ancient alchemical obsession with the elixir of life preceded historical reports supporting regeneration in some animals and certain organs, as was immortalized in the classical Greek mythology of Prometheus and the regeneration of his liver. Validity to this mythology was provided by the French scientist René-Antoine Ferchault de Réaumur in 1712, who reported the occurrence of regeneration in crayfish.[1] In 1766, Peter Simon Pallas reported this phenomenon in flatworms of the genus *Planaria*, the experimental analysis of which was published later based on the work of John Graham Dalyell in 1814 and J.R. Johnson in 1822 (reviewed by Brøndsted[2]). Additional work by Abraham Trembley and Lazzaro Spallanzani extended the idea to include a wide range of phyla in the animal kingdom, including hydra, earthworms, snails, aquatic salamanders, tadpoles and frogs (reviewed by Dinsmore[3]). Our current aspiration for regenerative medicine therefore rests on the foundations laid by studies on all of the above mentioned organisms as well as on insects and zebrafish.[3]

The inspiration for mammalian regeneration is further refined by the proceedings of developmental biology (Figure 1).[1,2,4–10] During development, the inherent physiological plasticity of stem cells and primary progenitor cells prompts the onset of differentiation in response to the appropriate stimuli, resulting in the generation of cell types that are mature and specialized and functionally integrate into organs and tissues. The fetus is widely recognized for its perfect execution of tissue regeneration that is otherwise absent in adults.[11] Do drivers of the fetal repair process exist in the adult tissue either in part or in its entirety? And if the critical elements are present, how are they silenced? Or is it true that the entire fetal regenerative apparatus is obliterated leaving the adult tissue permanently deprived? In this issue of *The American Journal of Pathology*, we present the Regenerative Medicine Theme Issue, which explores our understanding of these processes as well as current advances in experimental models. The Review articles in this collection discuss macrophage plasticity and polarization, dysfunction of progenitor cells under conditions of diabetes, stem cell plasticity, and the emerging importance of miRNA in tissue regeneration. These Reviews provide critical insight into these complex unfolding frontiers of regenerative medicine.

Cellular Plasticity

Emerging evidence suggests a turning back of the dial of cellular plasticity in response to injury which could include the acquisition of multipotency or a reversion to stem-like state in an effort to support tissue repair.[12] Epithelial mesenchymal transitions (EMT), under conditions of wound healing, may be considered as a classical example of cellular plasticity. This was first demonstrated by the Hungarian pathologist Ödön (Edmund) Krompercher in 1908[10] in human skin and salivary gland tumors in which basal epithelial cells in contact with hyaline were found to transition to mesenchymal cells. However, the credit for the discovery of this phenomenon was given to Greenburg and Hay[13] for studies performed in adult and embryonic anterior

Accepted for publication June 22, 2015.

Supported by NIH grants GM069589, GM077185, GM108014, NS42617, NR015676, and NR 013898.

C.K.S. is Special Editor of the Regenerative Medicine Theme Issue.

Disclosures: None declared.

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http://dx.doi.org/10.1016/j.ajpath.2015.06.003

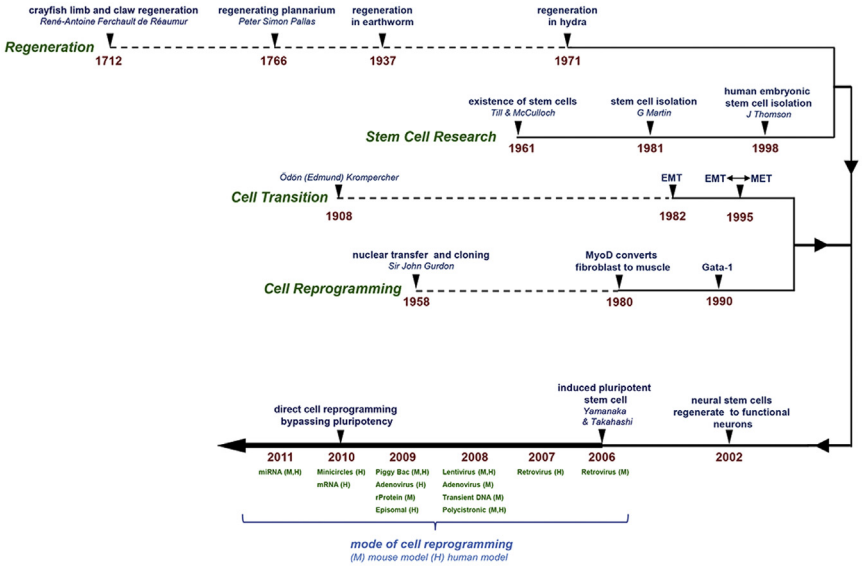

Figure 1 A schematic timeline of the history of regeneration, stem cell research, cellular transition, and cell reprogramming. Although these areas of research were initially considered to be different, they now form an integral part of regenerative medicine.[1,2,4–10]

lens tissue. Such cellular transition is commonly exhibited not only by stem cells but also by blood-borne monocyte-derived macrophages. Das et al[14] have addressed the significance of plasticity in macrophages and monocytes and their relevance to tissue regeneration and repair.

The search for transcription factors determining cell fates and reprogramming in mammals has been an ongoing quest boosted by the identification of Yamanaka factors, which were found to revert terminally differentiated cells to a pluripotent state. Furthermore, study of the hair follicle stem cell model has led to the recognition of superenhancers, dynamic support systems that serve as platforms for transcription factor binding in the process of cell plasticity regulation. Interestingly, these super-enhancers are exquisitely sensitive to fine tuning by master regulators such as SOX-9.[15] These observations lead to the following questions: if the above-said factors may revert cell phenotype, then why and how are these factors silenced in adult systems? And in adults, can these factors be unleashed in a regulated manner to achieve tissue regeneration?

Diabetic Complications and Chronic Inflammation

Diabetic complications compromise the function of progenitor cell populations. In this issue of the *AJP*, Rodrigues et al[16] address the effects of hyperglycemic memory on stem cells and provide guidance on how to use stem cell therapy under conditions of diabetes. In scenarios where one stem cell niche malfunctions, stem cells from other compartments are recruited to repair the damaged tissue. Grossly underrated compared to stem cells, the significance of macrophage plasticity in adult tissue repair is substantial. Das et al[15] critically address plasticity of monocytes and macrophages in the context of tissue repair and regeneration. Roughly 15 years ago, the pro-inflammatory M1 and pro-healing M2 phenotype of macrophages were considered to be two distinct populations of cells. Although this dichotomous paradigm explaining macrophage phenotype perpetuated for the better part of a decade, experimental observations challenging this overly simplified model continued to mount. It was soon recognized that M1 macrophages may transition to reparative M2 phenotype under supportive conditions.[17] These conditions that hold the key to the resolution of inflammation are of extraordinary interest.[18,19] Under pathological conditions such as diabetes, incompetence of the stem cell apparatus is further complicated by an arrest of M1 → M2 polarization resulting in accumulation of macrophages stalled in M1 and presenting a state of chronic inflammation.[20] Advancing M1 to M2 under conditions of diabetes represents a productive approach to break the deadlock of chronic inflammation and to resume the healing process.

Macrophage Fate at the Site of Tissue Repair

Current understanding of macrophage transdifferentiation is mostly supported by observations on cellular transition with cells co-expressing macrophage and endothelial markers. Although in some cases evidence from lineage tracing studies are present, the mechanistic underpinnings defining macrophage plasticity remain obscure. Examples of such plasticity include transdifferentiation of macrophages to endothelial progenitor cells to support tissue vascularization.[21] Transdifferentiation of monocytes and macrophages to functional endothelial cells has been demonstrated by overexpression of proteins like vascular endothelial growth factor (VEGF)[21] and pleiotrophin.[22] This *in vivo* reprogramming of cellular identity using direct transdifferentiation strategies has been also demonstrated in the mouse brain,

spinal cord, heart, pancreas, and liver. Recently, a remarkable advancement in regenerative medicine was achieved by introducing a defined cocktail of transcription factors directly into the adult somatic cells resulting in reprogramming of adult cells from one type to the other.[23] This is exemplified in studies that converted exocrine cells from the pancreas into beta cells.[24] Similar goals may be achieved by the use of purified recombinant proteins or whole-cell extracts isolated from either embryonic stem cells or genetically engineered HEK293 cells.[25] Although this protein-based, non-viral approach is attractive at first sight, its efficiency is poor and therefore not best suited for therapeutic interventions.[26]

Challenges in Gene Delivery and miRNA Solutions

In regenerative medicine, the therapeutic utility of *in vivo* reprogrammed cells in tissue repair and regeneration largely depends on the safe, efficient, and robust delivery of reprogramming factors. In this context, Smith and Zhang[27] deconstruct the complexities related to reprogramming adult cell identity *in vivo*. Although viral transduction is commonly practiced for gene delivery in laboratory animals, barriers to adopt such an approach for human applications are substantial. Genomic integration following viral transduction increases the risk of modifying recipient chromosomal locus making the recipient genomic loci unstable.[28] Although the integration frequency of adenovirus into chromosomal DNA *in vitro* was estimated to be in the order of 10^{-3} to 10^{-5} events per cell,[29] caution should be exercised while assessing *in vivo* risks based on such *in vitro* data. Do RNA viruses integrate into the host genome? Although theoretical arguments rule out that possibility presenting Sendai viruses as a better choice, Arenavirus reverse-transcribed genome has been detected in infected mice.[30] Furthermore, *Bornaviridae*, *Filoviridae*, and *Totiviridae* sequences have successfully integrated into several mammalian genomes indicating that possibility of integration of RNA virus into the host genome may not be summarily rejected.[31–33] Taken together, viral gene delivery is not a safe proposition from a translational perspective.

In pursuit of virus-free transduction strategies, recent studies have demonstrated pluripotent stem cell generation *in vitro* using minicircle DNA constructs in human adipose stromal cells and *in vivo* using hydrodynamic tail–vein injection of DNA constructs in the adult mouse liver.[34,35] Low efficiency of these approaches represent a serious concern.[36] mRNA-based reprogramming represents another option for virus-free transduction.[37] However, limitations of such approach include inherent complexities related to cellular procedures and purification of reprogrammed cells.[37] In addition, the expression of reprogramming factors is robust for approximately 24 hours after mRNA transfection. Unfortunately, there is a long two- to three-week lag between expression of reprogramming factor proteins and induction of pluripotency in human cells. Finally, repeated transfections that are needed to generate induced pluripotent stem cells is time intensive.[37] In this theme issue, we have reviewed the emergent significance of miRNA in tissue repair and regeneration.[38] The observation that miRNA does not integrate into the genome makes miRNA-based therapeutic strategies translationally valuable.[39]

Concluding Remarks

Success in regenerative medicine will be measured by its impact on functional biological outcomes. It relies on using the body's own regenerative capabilities to restore the function of damaged and degenerating cells, tissues, and organs. Although the discovery of novel principles governing cellular plasticity represents major advancements in cell biology, unless such principles are leveraged to restore functional outcomes *in vivo*, milestones in regenerative medicine will remain unmet. For example, the study of macrophage plasticity in chronic wounds would require that functional wound macrophages be isolated from the wound site as opposed to the study of differentiated monocytes isolated from peripheral blood.[40] To successfully rescue and restore a diseased or degenerating tissue, discovery in regenerative medicine must involve appropriate preclinical and clinical experimental models that approach the complexities of the actual pathology. Toward this end, we hope this Review Series will stimulate discussion and further interest this important area of research.

Acknowledgment

I thank Drs. Subhadip Ghatak and Shomita Mathew-Steiner for assistance with developing this Guest Editorial.

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