CellBio, 2013, 2, 31-34
http://dx.doi.org/10.4236/cellbio.2013.22004 Published Online June 2013 (http://www.scirp.org/journal/cellbio)

Regeneration from a Cell Biological Perspective—Fascinating New Insights and Paradigms*

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Received February 16, 2013; revised March 17, 2013; accepted April 2, 2013

ABSTRACT

Regeneration research is more focused on translational values. However, lying at its very foundation is an understanding of how tissues and organs repair and renew themselves at the cellular level. The past decade has witnessed paradigm changing advances in regenerative biology, many of these stems from novel insights into stemness, pluripotency, cell death and their related intra- and inter-cellular biochemical and molecular processes. Some of these new insights are highlighted in the paragraphs that follow. We now have a much better understanding of how regeneration occurs in lower organisms. We have also discovered tools and means of nuclear reprogramming to generate induced pluripotency and changes in cell fate in mammalian models. With further research, there is reasonable hope that various obstacles of regeneration in humans can be better understood and tackled. As regeneration research enters a new era, *CellBio* welcomes timely review articles and original papers on the theme of "The Cell Biology of Regeneration".

Keywords: Inflammation; Induced Pluripotent Stem (iPS) Cells; Progenitor/Stem Cells; Regeneration; Reprogramming; Wnt

1. Introduction

The ability to regenerate injured tissues or organs, as well as rejuvenation of the senesced or aged, has been an elusive goal of ancient alchemy and modern biomedicine alike. Biologists have marveled at the ability of plants and lower animals to regenerate. Planarians and cnidarians could regenerate entire organism from small body fragments, or even dissociated single cells. However, for more complex animals, this regenerative capacity is apparently attenuated, or completely loss. While organ and limb regeneration are still readily observed in fishes, reptiles and amphibians, this almost never occurs to any significant extent in mammals. Even at the cellular level, one resigns to the vast amount of data demonstrating that whole tissues aside, most terminally differentiated cell types, such as brain neurons and skeletal muscle fibers, simply do not regenerate. While this latter notion remains accurate, the past few years have witnessed multiple advances that are paradigm changing in terms of our understanding of regeneration from a cell biological perspective. The following paragraphs highlight a few aspects of the novel insights associated with adult animal regeneration that have become clear after the turn of the century.

2. From cNeoblasts to Blastema Stem Cells-Endogenous Pluripotent and Multipotent Stem Cells Enable Regeneration

Whether complex tissues could be regenerated appears to depend primarily in the availability of stem cells, their relative lineage differentiation potency, and their state of quiescence (and how this latter state could be changed when the need for regeneration arises). At least in theory, stem/progenitor cells required for regeneration could exist as an ever present pool, or dedifferentiated from differentiated cells. To be able to account for their regenerative capacity at the organismal level, pluripotent, if not totipotent, stem cell types must exist in adult planarians and cnidarians, and for that matter widely distributed throughout the adult organism, Indeed, a population of undifferentiated adult dividing cells, the neoblasts, has been identified to be responsible for planarian regenerative capacity. Using a clonal analysis approach of lethal ionizing radiation followed by single-cell transplan-

*A preface to CellBio's thematic review series on "The Cell Biology of Regeneration".

tation in Schmidtea mediterranea, planarian clonogenic neoblasts (cNeoblasts) was shown to be able to differentiated to almost all known postmitotic cell types throughout the body. Intriguingly, single transplanted cNeoblasts could restore regeneration in a lethally irradiated worm [1]. On the other hand, tissue pluripotency in the cnidarian Hydra involves three independent cell lineages form the body of the polyp, namely epithelial stem cells from the ectodermal and endodermal layers respectively, as well as interstitial stem cells [2]. The epithelial stem cells are pluripotent [3] but the interstitial cells at best multipotent.

At a level of more modest regenerative capacity, reptiles and amphibians are able to generate severed limbs or other appendages. This is no mean feat as vertebrate appendages are composed of a mixture of tissue types from multiple germ layers. Regeneration in this regard is also dependent on resident stem cells [4]. The process begins with the formation of a blastema at the site if injury or amputation, which is a collection of progenitor cells that appear to be homogenous, but these are at best multipotent, with a good degree of lineage potential restriction [5]. The equivalent of a pluripotent planarian eNeoblast is most likely either completely absent in adult vertebrate tissues, or is not available in any significant numbers that would enable regeneration at a more massive scale. In mammals, limb regeneration is further reduced to the ability to regenerate digit tips, and this was recently shown to occur via ectodermal and mesodermal fate-restricted progenitors that regenerate their own lineages within the digit tip [6]. It is speculative at the moment as to why regenerative capacity reduces with complexity, or that a phenotype of having pluripotent stem cells at stock was selected against in higher vertebrates. One reason could be the difficulty in the maintenance of a large amount of pluripotent stem cells quiescent and the increase probability of malignant transformation. Understanding more about how lower organisms use their endogenous stem cells to regenerate may provide clues as to how endogenous stem cells in various niches of the adult human could be harness (or activated) to aid regeneration.

3. Rising from the Ashes of the Dead

Injury often causes massive cell death. Attraction of immune cells to the site of injury underlies the associated inflammatory responses, which together create a non-conducive post-injury environment that is conventionally viewed to be hostile, impairing the survival of spared cells as well as anti-proliferative against regenerating cells. This view may be overtly oversimplified, as recent work suggest that both apoptosis (or more accurately, programmed cell death) and inflammation play important roles in triggering regeneration from cnidarian to vertebrates. Midgastric bisection of Hydra precipitates a rapid wave of apoptosis and transient release of Wnt3 among interstitial cells at the head regenerating end, and the latter activates the canonical Wnt/β-catenin pathway in neighbouring cycling cells to enhance cell cycle progression [7]. This sort of apoptotic cell-induced compensatory cell proliferation has also been documented in regeneration models of higher organisms, including Drosophila wing disc regeneration [8] and tail regeneration in the tadpoles of Xenopus laevis [9].

The adult mammalian brain is a well-known organ where regeneration is particularly restrictive. In fact, it was believed for a long time prior to the identification and characterization of adult neurogenic regions that adult neurogenesis (*i.e.* the formation of new neurons from progenitors) [10] does not occur in the mammalian brain. The much simpler fish brain, on the other hand, could regenerate to a significant degree. Neuroinflammation characterizing cases of acute ischemic or traumatic injuries, as well as more chronic neurodegenerative diseases in human brain pathology, is widely recognized as a major barrier to regeneration of any kind. Interestingly, recent findings points to inflammation as being required and sufficient for enhancing the proliferation of neural progenitors and their subsequent neurogenesis in the adult zebra fish brain [11]. In connection with apoptosis-driven regeneration discussed above, Wnt signalling appears to be a key pathway in balancing brain damage and repair. Exogenous Wnt3a injected into mouse striatum was recently shown to enhance neurogenesis and significantly functional recovery after ischemic injury [12]. Wnt signalling components are only present in immune cells as well as brain glia cells in adult mammals, and the crosstalk between these cells in a post-injury inflammatory setting, particularly in influencing neurogenesis [13,14], could be exploited for therapeutic intervention purposes. Regenerative capacities are not only conserved between lower vertebrates and mammals in terms of signalling. It is worth noting that both neurogenic adult neural progenitors in fish and mammals have a similar morphological phenotype and niche—they all appear to be derived from ventricular radial glia [15,16].

4. Starting Over-Nuclear Reprogramming to Pluripotency, Multipotency or Alternative Fates

Erasure of epigenetic markings of differentiation and aging, as well as induction of pluripotency, occur naturally during reproduction, be it in the case of a budded Saccharomyces. cerevisiae daughter cell or after the fusion of a spermatozoa and an ovum in humans. The ability of an enucleated ovum to reprogram somatic cell nuclei to a state of pluripotency underlies the promise of somatic cell nuclear transfer (SCNT) for the generation

of embryonic stem cells, and thus materials for autologous transplantation (or for cloning). Just as the community begins to feel that perhaps efficient SCNT-based reprogramming is for some reason unachievable for primates and humans, the discovery of induced pluripotency [17] literally changed overnight the way many approach the subject. The technology is based on a deceptively simple concept that nuclear reprogramming could be achieved by the introduction and expression of the four Yamanaka factors (Oct3/4, Sox2, Klf4 and c-Myc), or a subset of these in combination with others genes/compounds, into easily sampled somatic cells such as fibroblasts or keratinocytes. These genes initiate a cascade of changes in genetic and epigenetic profiles, converting differentiated somatic cells these over a period of time into pluripotent stem cells [18]. Work on or related to induced pluripotent stem (iPS) cells has now amassed more than 4500 PUBMED entries, and related new findings are being made at an unprecedentedly fast pace.

Of particular therapeutic interest is the potential of iPS methods to generate individual-specific autologous cells or tissues that are safe for grafting. In accordance to the generalized notion that grafting differentiated cells runs a lower risk of tumorigenesis, researchers quickly develop methods of direct reprogramming of fibroblast into differentiated cell types of other lineages, such as neurons [19], cardiomyocytes [20] or endothelial cells [21] without passage through the undifferentiated pluripotent iPS stage. Modifications of factors and culture methods allowed the generation of multipotent neural progenitors [22-24] and hematopoietic progenitors [25]. Beyond providing therapeutic materials, the seemingly limitless lineage conversion to either fully differentiated cell types or more immediate progenitors from clinically accessible cells like fibroblasts will also greatly advance studies on disease etiology and development. Granted that nuclear reprogramming may be incomplete in the case of iPS cells and residual epigenetic memories of the cell of origin may limit their usefulness, the paradigm shift in terms of research approach using iPS-based methods has clearly revolutionize regenerative biology.

5. Acknowledgements

The author is supported by NUS Graduate School for Integrative Sciences and Engineering (NGS), and declares no conflict of interest.

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