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Perspective Article

Regenerative biology and medicine

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Abstract

The replacement of damaged tissues and organs with tissue and organ transplants or bionic implants has serious drawbacks. There is now emerging a new approach to tissue and organ replacement, regenerative biology and medicine. Regenerative biology seeks to understand the cellular and molecular differences between regenerating and non-regenerating tissues. Regenerative medicine seeks to apply this understanding to restore tissue structure and function in damaged, non-regenerating tissues. Regeneration is accomplished by three mechanisms, each of which uses or produces a different kind of regeneration-competent cell. Compensatory hyperplasia is regeneration by the proliferation of cells which maintain all or most of their differentiated functions (e.g., liver). The urodele amphibians regenerate a variety of tissues by the dedifferentiation of mature cells to produce progenitor cells capable of division. Many tissues contain reserve stem or progenitor cells that are activated by injury to restore the tissue while simultaneously renewing themselves. All regeneration-competent cells have two features in common. First, they are not terminally differentiated and can re-enter the cell cycle in response to signals in the injury environment. Second, their activation is invariably accompanied by the dissolution of the extracellular matrix (ECM) surrounding the cells, suggesting that the ECM is an important regulator of their state of differentiation. Regenerative medicine uses three approaches. First is the transplantation of cells into the damaged area. Second is the construction of bioartificial tissues by seeding cells into a biodegradable scaffold where they produce a normal matrix. Third is the use of a biomaterial scaffold or drug delivery system to stimulate regeneration *in vivo* from regeneration-competent cells. There is substantial evidence that non-regenerating mammalian tissues harbor regeneration-competent cells that are forced into a pathway of scar tissue formation. Regeneration can be induced if the factors leading to scar formation are inhibited and the appropriate signaling environment is supplied. An overview of regenerative mechanisms, approaches of regenerative medicine, research directions, and research issues will be given.

Keywords: Compensatory Hyperplasia, Dedifferentiation, Stem Cells, Regenerative Biology, Regenerative Medicine

The cost of tissue damage and loss due to degenerative disease and injury is enormous in terms of health care dollars, lost economic productivity, diminished quality of life, and premature death. We are able to regenerate some tissues, such as blood, blood vessels, epithelia, liver, bone, muscle, and fingertips, but many others, such as spinal cord, heart muscle, and pancreas respond to injury by the formation of scar tissue. Tissues like bone and muscle, even though they regenerate after fracture or tearing, cannot regenerate across a large gap. Two clinical approaches now used to replace damaged or failing organs and tissues are organ transplantation and implantation of bionic devices. Donor shortages and the side effects of required immunosuppression are drawbacks of the former, while use of the latter is limited by our inability to engineer and manufacture artificial tissues and organs that duplicate the durability, strength, form, function, and biocompatibility of natural tissues. In the 21st century, however, there will be a new approach to tissue replacement, regenerative biology and medicine[1], born of the disciplines of cell and developmental biology and biomaterials science, and nourished by advances in genomics, proteomics, biotechnology, and information science and technology. Regenerative biology seeks to understand the mechanisms of regeneration and how they differ from repair by scar tissue formation. Regenerative medicine seeks to apply this understanding to restore the structure and function of tissues that do not regenerate naturally.

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Mechanisms of regeneration

Multicellular organisms use three mechanisms of regeneration: compensatory hyperplasia, dedifferentiation/transdetermination of mature cells, and activation of stem cells[2-5]. The regeneration-competent cells involved in each case exhibit different states of differentiation, but have two common features. First, they are not ***terminally*** differentiated and thus can respond to signals in the injury environment that promote re-entry into the cell cycle. Second, activation of the cells is invariably accompanied by the dissolution of surrounding ECM, uncoupling cell adhesion molecules (e.g., integrins) from ECM molecules. This uncoupling alters the actin cytoskeleton, activating signal transduction systems coupled to the cytoskeleton. At the same time, growth factors and other signaling molecules bound to ECM components are released and bind to transmembrane receptors that activate signal transduction pathways.

Compensatory hyperplasia is the proliferation of cells to restore tissue mass and integrity while maintaining most or all of their differentiated functions. The classic example of regeneration by this means is the liver[3]. After partial hepatectomy, all the cell types of the liver (hepatocytes, Kupffer, Ito, bile duct epithelial, and fenestrated epithelial cells) divide while maintaining all of their functions. Blood vessels and injured newt heart muscle are also regenerated by compensatory hyperplasia[2]. Angiogenesis occurs by the proliferation of endothelial cells in the walls of venules. The endothelial cells rearrange themselves into tubes after each mitosis. Newt cardiomyocytes undergo partial disorganization of their myofibrils and divide, maintaining their contractility after each mitosis, just like embryonic and fetal cardiomyocytes.

Dedifferentiation is the loss of phenotypic specialization by differentiated cells to produce embryonic-like progenitor cells that retain none of their mature functional specializations. These "blastema" cells proliferate and then either differentiate back into the cell types of origin or transdetermine to become a different cell type. Urodeles (salamanders and newts) are the divas of dedifferentiation. They regenerate a wide variety of tissues and complex structures by this means, including tails, limbs, jaws, lens, neural retina, spinal cord, and intestine[4].

The activation of lineage-restricted stem cells set aside late in embryonic or fetal life is the most common mechanism by which adult mammalian tissues regenerate. These cells, like all others, are derived from pluripotent stem cells (embryonic stem cells, ESCs) that comprise the early embryo[6], except that they do not complete their differentiation program. Mammalian tissues known to regenerate via stem cell proliferation and differentiation are blood, bone, skeletal muscle, epithelia, and olfactory bulb of the brain. In addition, blood vessels can regenerate via endothelial stem cells located in the stroma of the bone marrow, and liver can regenerate from a population of oval epithelial cells located in the bile ductules. A discovery of great interest is that stem cells can be switched from one lineage to another, depending on what kind of microenvironment they are placed in[7-9]. Marrow stem cells can give rise to hepatic stem cells or cardiomyocytes, stem cells of skeletal muscle can differentiate into cardiomyocytes when transplanted into injured heart muscle and into blood cells, and neural stem cells can differentiate into a wide range of other cell types, including blood cells, hepatocytes, intestine, skeletal muscle, and cardiac muscle.

Regeneration by dedifferentiated cells and stem cells recapitulates embryonic development

Regeneration by dedifferentiated cells or stem cells largely recapitulates the embryonic program that produced the original tissue. A good example is the repair of a long bone after fracture[10-12]. After an initial phase of hemostasis and inflammation, periosteal osteoprogenitor cells differentiate directly into bone on either side of the fracture to form a hard callus. Simultaneously, periosteal and marrow stromal cells proliferate to form a soft callus bridging the fracture. The cells of the soft callus repeat the steps of endochondral bone embryogenesis. They condense and differentiate into chondrocytes that secrete cartilage-specific matrix. This cartilage follows the same pattern of hypertrophy, calcification, invasion by blood vessels, and replacement by bone as in embryonic bone development or in postnatal growth plates. Synthesis of matrix components such as type I, IX, and X collagens, aggrecan, fibronectin, osteonectin, osteopontin, and osteocalcin appear to follow temporal and spatial patterns identical to those in developing and growing bones. Cartilage formation and replacement are controlled by the same factors as in embryonic development. BMPs, TGF-beta, and FGF-1 and their receptors are expressed during chondrogenesis. As the cartilage matures, *ihh* and *gli 1* transcripts are detected. Transcripts for the transcription factor, Cbfa1, are detected in newly forming bone, coincident with the expression of the *osteocalcin* gene. Though not yet verified, it is likely that other features of embryonic endochondral bone formation, such as the upregulation during condensation of N-cadherin, NCAM, fibronectin, and the heparin-binding protein Cyr61, and regulation of the rate of transition of proliferating chondrocytes to hypertrophying chondrocytes by the Ihh-PTHrP and Delta/Notch signaling pathways, are similar in the differentiating soft callus.

Regenerative medicine

Regenerative biology has led to three approaches of regenerative medicine: cell transplants, implantation of bioartificial tissues, and the induction of regeneration from residual tissues *in vivo*.

Cells for transplantation can be obtained from biopsies of differentiated tissue, adult stem cell populations, the controlled differentiation of ESCs, or from fetal tissues. The feasibility of using cell transplants to replace damaged neural tissue or correct genetic lesions has been demonstrated by numerous investigations[13]. For example, lineage-restricted mouse glial

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precursor cells, derived by the controlled differentiation of ESCs *in vitro*, differentiated into oligodendrocytes when transplanted into the spinal cord and brain of 7-day old mutant rats, curing a myelin deficiency that mimics human Pelizaeus-Merzbacher disease[14]. A subpopulation of rat muscle satellite cells with high survival capabilities differentiates into smooth muscle when injected into the wall of the injured bladder, thus showing the potential of these cells for treatment of problems such as urinary incontinence and perhaps diseases such as muscular dystrophy[15].

Bioartificial tissues and organs are made by combining cells with biomaterial scaffolds that can be molded into the shape of the tissue or organ[16]. Examples of some scaffold materials are collagen I, polyesters, polyanhydrides, hydroxyapatite ceramics, and pig small intestine submucosa. The most successful bioartificial tissues to date are skin equivalents using collagen or polyglycolic acid mesh scaffolds and bone substitutes made by seeding ceramic scaffolds with bone marrow stroma cells.

A current strategy to regenerate tissues *in vivo* is to bridge lesions with biomaterial scaffolds that stimulate the migration, proliferation, and differentiation within the bridge of local regeneration-competent cells, and/or neutralizing molecules in the injury environment that are inhibitory to regeneration. Tissues that have been induced to regenerate include skin, bone, blood vessels, dura mater, tendon, peripheral nerve, esophagus, urinary bladder, and spinal cord[1,2,17]. In no case, however, has the original structure and function of the tissue been completely restored.

There is substantial evidence that several non-regenerating mammalian tissues contain regeneration-competent cells, but that participation of these cells in regeneration is thwarted by the injury environment[1,2]. A good example is ependymal cells of the spinal cord, which form glial scar tissue after spinal cord injury, but which can form new neurons when cultured under the right conditions. Mammalian retina does not regenerate, but stem cells capable of differentiating *in vitro* into retina-specific cell types have been isolated from the pigmented ciliary margin of the mouse eye[18] and dissociated human pigmented epithelial cell lines from an 80-year old donor eye were able to differentiate as neuronal and lens cells[19]. Thus the induction of regeneration *in vivo* may be "simply" a matter of providing an environment favorable to repair by regeneration rather than by scar tissue formation.

Research issues for regenerative biology and medicine

A number of research issues in regenerative biology must be resolved before the potential of regenerative medicine is realized. First, how ubiquitous are regeneration-competent cells in the human body? How many tissue types harbor stem cells that are normally inhibited from participating in regeneration? How many types of differentiated mammalian cells can be induced to divide in the differentiated state or to dedifferentiate? Even cells considered to be terminally differentiated are theoretically regeneration-competent, given that every somatic cell except the B-cell carries a complete genome which can be re-programmed, given the appropriate conditions.

Second, how can we stimulate or re-program potentially regeneration-competent cells to re-enter the cell cycle, proliferate, and differentiate in the correct tissue organization? Understanding how to do this requires first that we have a complete inventory of the molecular differences between regenerating and non-regenerating tissues. Molecular comparisons between regeneration-competent and incompetent tissues, using a variety of animal models, will be useful in obtaining this inventory. A related question concerns the specific culture conditions that will allow us to direct the differentiation of cultured stem cells, whether autogeneic or allogeneic, for use in transplantation or bioartificial tissue construction. The question of directed differentiation includes the development of biomaterials which incorporate physical and chemical cues and signals essential for cell migration, proliferation, and differentiation.

Third, what are the effects of tissue mass and age on regenerative ability? We know, for example, that minced muscle regenerates well in young rats, but less well in larger animals such as guinea pig, rabbit, cat, and dog, and not at all in humans. Furthermore, muscle regeneration is poorer in old rats than young ones. Do these mass and age-related effects apply equally well to all tissues? If so, how might we reverse them?

And finally, clinical regenerative medicine will require the standardization of procedures for producing and testing the components used in the various strategies of regenerative medicine, as well as the education of physicians in the basic biology underlying regenerative therapies[20].

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