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Rx for Tissue Restoration: Regenerative Biology and Medicine

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Vertebrates regenerate tissues in three ways: proliferation of cells that maintain some or all of their differentiated structure and function, dedifferentiation of mature cells followed by proliferation and redifferentiation into the same cell type or transdetermination to another cell type, and activation of restricted lineage stem cells, which have the ability to transdetermine to different lineages under the appropriate conditions. The behavior of the cells during regeneration is regulated by growth factors and extracellular matrix molecules. Some non-regenerating tissues are now known to harbor stem cells which, though they form scar tissue *in vivo*, are capable of producing new tissue-specific cells *in vitro*, suggesting that the injury environment inhibits latent regenerative capacity. Regenerative medicine seeks to restore tissues via transplantation of stem cell derivatives, implantation of bioartificial tissues, or stimulation of regeneration *in vivo*. These approaches have been partly successful, but several research issues must be addressed before regenerative medicine becomes a clinical reality.

An emerging approach to the restoration of damaged tissues and organs is regenerative biology and medicine. Born of the disciplines of cell, molecular, and developmental biology, and nourished by advances in genomics, proteomics, and bioinformatics, this multidisciplinary field has the potential to revolutionize our ability to replace damaged tissues. Regenerative biology seeks to understand the mechanisms of regeneration in a variety of multicellular organisms where it occurs naturally. Regenerative medicine is the application of this understanding to disinhibit, stimulate, or recreate these mechanisms in damaged human tissues that fail to regenerate. The purpose of this article is to review briefly what we know about the mechanisms of regeneration and the current status of regenerative medicine.

The Biology of Regeneration

Natural mechanisms of regeneration fall into three categories: compensatory hyperplasia, dedifferentiation/ transdetermination, and activation of stem cells.

Compensatory hyperplasia

Compensatory hyperplasia is defined here as the proliferation of cells to restore tissue mass while maintaining most or all of their differentiated functions. Liver regeneration is the classic example of compensatory hyperplasia, but blood vessels and newt cardiac muscle also appear to regenerate in this way.

In the rat partial hepatectomy model of liver regeneration, hepatocytes divide first, followed by nonparenchymal cells (biliary epithelial cells; fenestrated endothelial cells; Kupffer cells (the macrophages of hepatic sinusoids); and Ito cells) which surround hepatocytes and store vitamin A, synthesize connective tissue proteins and secrete several growth factors. The molecular biology of liver regeneration has been studied extensively (Fausto, 1995; Michaelopoulos and DeFrances, 1997; Trembly and Steer, 1998). Hepatocyte growth factor (HGF) appears to be the initial mitogenic stimulus, but hepatocytes cannot respond to HGF without first being primed (made competent). The main priming event appears to be the upregulation of the urokinase plasminogen activator (uPA) receptor within one minute of the operation, although the signals that trigger this upregulation are unknown. uPA is activated by binding to the receptor, initiating a proteolytic cascade that converts plasminogen to plasmin, which in turn activates matrix-degrading matrix metalloproteinases (MMPs), releasing matrix-bound HGF and perhaps other growth factors. uPA activates the free HGF, which then binds to the c-Met receptor on hepatocytes. This triggers a receptor tyrosine kinase signal transduction pathway, leading to entry into G1. Epidermal growth factor (EGF), tumor necrosis factor alpha (TNF-alpha), interleukin 6 (Il-6) and transforming growth factor alpha (TGF-alpha) are also mitogenic for hepatocytes. TNF-alpha and IL-6 are produced by

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Kupffer cells, EGF is supplied by Brunner's glands in the duodenum, and TGF-alpha is produced by hepatocytes. TNF-alpha, EGF, and IL-6 interact to upregulate the transcription factors NF-kappa B and Stat 3, leading to the activation of a variety of immediate early genes encoding a cascade of transcription factors essential for progression to the G1/S transition point. How liver regeneration is halted following proliferation is not well understood, but TGF-beta may play a role in the process because it is a mitosis inhibitor whose production increases in the liver once restorative mass is approached.

Angiogenesis, the formation of new blood vessels from existing vessels, is a crucial regenerative response in all injured tissues, which also occurs by compensatory hyperplasia. Endothelial cells are activated by injury and release plasminogen activator, uPA, and collagenase which break down the surrounding basement membrane. The cells undergo partial dedifferentiation and lose their tight junctional complexes, allowing migration of the cells through the fragmented membrane into the perivascular space (Madri et al., 1996). Continued expression of MMPs allows the cells to cut a path through the ECM. Endothelial cells in the parent vessel proliferate to provide a continuous cell supply and many of the endothelial cells in the new vessels proliferate as well. As the endothelial sprout proliferates distally, the more proximal cells form new capillaries by flattening, arranging themselves into a tube, re-establishing tight junctions, and reconstituting the basement membrane. At the same time, mesencymal cells replicate and differentiate into vascular smooth muscle. Pericytes, mesenchymal cells embedded in the basement membrane of the capillary, may contribute to mechanical stability of the new vessel (Lindhal et al., 1997).

Many *in vitro* and some *in vivo* studies indicate that angiogenesis is activated and regulated by growth factors produced by macrophages and other cells in the wound space (Clark, 1996; Madri et al., 1996; Tomanek and Schatteman, 2000). Endothelial cell activation can be triggered *in vitro* by fibroblast growth factor-2 (FGF-2), TGF-beta1, IL-1, and TNF-alpha. Migration of endothelial cells is modulated by a variety of growth factors, including FGF-2, TGF-beta, and platelet derived growth factor (PDGF). In addition, migrating endothelial cells express platelet endothelial cell adhesion molecule-1 (PECAM-1) in a diffuse pattern on their surfaces, suggesting that this adhesion molecule plays a role in endothelial cell migration. Proliferation and differentiation of endothelial cells and mesenchymal cells is regulated by platelet derived-endothelial growth factor (PD-ECGF), PDGF, TGF-alpha and beta, and TNF-alpha. Vascular endothelial growth factor (VEGF), a heparin-binding dimeric protein related to PDGF, is a key molecule in angiogenesis, acting specifically on endothelial cells. VEGF and its receptor, Flk-1/KDR, has been shown to be critical for vasculogenesis in the embryo (Ferrara, 2000) and is synthesized in large quantities by the epidermis of healing skin wounds, indicating that it plays a role in blood vessel regeneration (Clark, 1996). Two members of the angiopoietin family, angiopoietins1 and 2 and their receptor, tie-2, modulate VEGF-induced angiogenesis in the adult (Asahara et al, 1999). Beta 1,3, and 5 integrins, fibronectin, laminin, and thrombospondin are essential for cell migration and normal endothelial tube formation (Tomanek and Schatteman, 2000).

Newts can survive excision of 30-50% of the heart ventricle (Becker et al., 1974; Bader and Oberpriller 1978). Amputation of the ventricular apex results in the partial dedifferentiation of cardiomyocytes adjacent to the wound, resulting in an embryonic type organization of patches of myofibrils scattered throughout the cytoplasm (Nag et al., 1979). Intercalated discs are lost, resulting in the formation of mononucleate cells that re-enter the cell cycle and achieve high levels of tritiated thymidine labeling and mitotic indices, over 24% and 2.6%, respectively (Oberpriller and Oberpriller, 1991; Rumyantsev, 1991).

In vitro studies indicate that partially dedifferentiated adult newt cardiomyocytes maintain their contractility after each mitosis, just like embryonic and fetal cardiomyocytes (Nag et al., 1979). Once the mass of the ventricle is restored, the cells withdraw from the cell cycle and redifferentiate. Cultured adult rat cardiomyocytes come to resemble fetal cardiomyocytes in morphology and synthesis of the myosin heavy chain (Eppenberger et al., 1988). These cells can synthesize DNA at relatively low levels compared to the newt, but do not undergo any appreciable mitosis (Claycomb, 1992; Borisov, 1998).

Dedifferentiation/transdetermination

The urodele amphibians (salamanders and newts) are the only vertebrates able to provide embryonic-like progenitor cells for regeneration via the dedifferentiation of mature cells. The dedifferentiated cells do not maintain their differentiated functions, but proliferate and then either differentiate back into the cells of origin, or become transdetermined to differentiate into another cell type. Labeling studies have shown that during redifferentiation, blastema cells derived from skeletal cells redifferentiate into skeletal cells but fibroblasts and myogenic cells can transdetermine to become skeletal cells as well (Steen, 1968; Lo et al., 1993; Casimir et al., 1988). In contrast, there is no evidence that skeletal cells can transdetermine to muscle or connective tissue cells during regeneration. Urodeles use these mechanisms to regenerate a wide variety of tissues and complex structures, including tails, limbs, jaws, lens, neural retina, spinal cord, and intestine (Stocum, 1995).

Regeneration by dedifferentiation has been extensively studied in the limb. Amputation of the urodele limb

results in the breakdown of ECM at the wound site, liberating chondrocytes, osteocytes, muscle cells, fibroblasts, and Schwann cells from their tissue organization (Stocum, 1995). The liberated cells lose their phenotypic specializations and revert to a morphology that resembles mesenchymal cells of the limb bud. These progenitor cells proliferate to form a conical regeneration blastema which then continues to grow and to form the missing limb structures in a pattern identical to the original.

The matrix is degraded by proteases, particularly by the acid hydrolases such as cathepsin D, acid phosphatase (Ju and Kim, 1998), beta-glucuronidase, and carboxylic ester hydrolases (Schmidt, 1966), and by MMPs, particularly MMP 2 and 9 (gelatinases) and MMP3/10a and b (stromelysins) (Yang et al., 1999; Miyazaki et al., 1996; Park and Kim, 1999; Carinato et al., 2000). Acid hydrolase and MMP activity is sharply increased in animals treated with retinoic acid, which causes more extensive dedifferentiation than normal (Ju and Kim, 1994; Park and Kim, 1999). Acid hydrolases are undoubtedly released after amputation from injured and dying cells, and are particularly active in chondroclasts and osteoclasts, which degrade cartilage and bone matrix in regenerating limbs (Stocum, 1979). The cell types that produce MMPs and other proteases for histolysis have not been clearly defined, but candidates are macrophages, the wound epithelium that covers the blastema, and the dedifferentiated mesenchymal cells themselves. The cessation of matrix degradation coincides with the beginning of redifferentiation and is likely to involve the upregulation of tissue inhibitors of metalloproteinases (TIMPS) as well as down regulation of MMP genes. The matrix of the mature limb is replaced by a more limb-bud type matrix rich in collagen I, hyaluronate, and fibronectin (Stocum, 1995).

We do not have a clear understanding of the mechanisms that regulate dedifferentiation. Degradation of the ECM by proteases would break contacts between adhesion proteins, such as integrins, and ECM molecules such as collagen and fibronectin, leading to changes in cell shape and reorganization of the actin cytoskeleton. This reorganization could, in turn, activate signal transduction pathways that induce patterns of gene activity involving the upregulation of enzymes that dismantle the phenotype-specific internal structure of the cells. Consistent with this idea, the ECM molecules tenascin, osteonectin, and thrombospondin promote reorganization of the actin cytoskeleton in cultured bovine arterial endothelial cells by modulating adhesive contacts (Sage and Bornstein, 1991) and tenascin is known to be upregulated in regenerating limbs (Onda et al., 1991). Gelatinase A (GL-A), a MMP involved in connective tissue remodeling and tumor invasion, is activated in cultured fibroblasts with stress fibers when they are treated with cytochalasin D. The activation is mediated by a membrane-type MMP that cleaves the GL-A propeptide (Tomasek et al., 1997).

Transdetermination following dedifferentiation is the mechanism by which the neural retina and lens are regenerated in the amphibian eye. Cutting the optic nerve and blood vessels to the eye results in degeneration of the neural retina (NR). The NR and optic nerve are then regenerated from the retinal pigmented epithelium (RPE) (Klein et al., 1990; Mitashov, 1996; Raymond and Hitchcock, 1997). The RPE cells enlarge, change from a cuboidal to a columnar shape, depigment, and proliferate to form a layer of pseudostratified cells typical of a germinative neuroepithelium. Newt RPE cells express an antigen that binds the mouse monoclonal antibody RPE-1. The depigmented RPE cells continue to express this antigen as they proliferate. Expression of RPE-1 is extinguished as the cells express NR cell markers (Negeshi et al., 1992; Mitashov, 1996). This pattern suggests that dedifferentiating RPE cells may continue to express genes specific for the RPE as they undergo transdetermination to patterns of gene activity characteristic of NR cells. The dedifferentiated cells give rise to the several cell layers characteristic of the NR, with photoreceptors next to the RPE, ganglion cells toward the vitreous chamber, and the outer and inner nuclear layers in between. The optic nerve is re-formed by bundles of axons growing from the regenerated ganglion cells. The axons of the optic nerve re-establish the correct connections to the optic tectum, and vision is recovered (Sperry, 1944; Gaze 1959).

ECM molecules, particularly laminin, appear to be important regulators of NR regeneration. Transdetermination of *Rana* tadpole RPE cells is promoted by culturing them on a laminin substrate (Reh et al., 1987). During NR regeneration in these tadpoles, the first new NR cells arise in association with the vitreal vascular membrane, which contains a high concentration of laminin (Reh and Nagy, 1987). These observations suggest that laminin might regulate gene activity leading to dedifferentiation and transdetermination by intracellular signaling mechanisms resulting from cytoskeletal changes mediated by cell adhesion proteins.

Several observations from experiments on chick embryos have demonstrated the importance of FGF-2 as a signaling molecule in regenerating the NR (Park and Hollenberg, 1993). The neural retina of stage 22-24 chick embryos does not normally regenerate after retinectomy, but if contact between the outer layer of the optic cup (which forms the RPE) and inner layer (which forms the NR layers) is prevented, the RPE differentiates as NR (Orts-Llorca and Genis-Galvez, 1960). Both the RPE and NR of adult rat eyes express FGFs and their receptors (Noji et al., 1990). Regeneration of the NR from the RPE can be induced by implanting a piece of NR into the vitreous chamber (Coulombre and Coulombre, 1970). The effect of NR implants on regeneration is mimicked by implanting

blocks of ethyelene/vinyl coacetate polymer impregnated with FGF-2 or, at higher concentrations, FGF-1 (Park and Hollenberg, 1991). The polarity of the regenerated neural retina is reversed in both cases; i.e. photoreceptors facing the implants, implying that cells experiencing the highest concentration of FGF signal become photoreceptors. FGF-2 induces RPE cells to form NR cells *in vitro* (Pittack et al., 1991). Furthermore, inclusion of FGF-2 in the medium of cultured chick stage 9-10 optic vesicles caused the RPE to differentiate as retina, producing an eye with a double retina with normal polarity (Pittack et al., 1997). Collectively, these results suggest that signals from the NR, as yet unknown, inhibit the RPE from developing as NR during embryogenesis and that during normal regeneration of the NR from the RPE in amphibians, the RPE may be the source of FGF-2 which acts to stimulate regeneration in a polarized fashion. FGFs and their receptors have also been shown to be a key regulatory factor in urodele lens regeneration (Del Rio-Tsonis et al., 1997; 1998).

The mechanism by which RPE cells dedifferentiate to regenerate the NR in adult newts again is not well understood. In the chicken, however, a gene called *Mitf*, which encodes a basic helix-loop-helix-leucine-zipper protein, appears to play an important role in the differentiation of pigmented epithelial cells. Overexpression of *Mitf* inhibits FGF-2-induced dedifferentiation and transdetermination of cultured RPE cells. Conversely, induction of dedifferentiation and transdetermination of RPE cells by FGF-2 inhibits *Mitf* activity, suggesting that downregulation of this gene is essential for NR (and lens) regeneration (Mochii et al., 1998). It will be important to assess the expression patterns, in the newt embryonic and regenerating retina, of other genes known to be essential to retinal development. For example, *pax-6* is expressed in the embryonic retina and regenerating lens of the newt (Del Rio-Tsonis et al., 1995). The *Sonic hedgehog* and *patched* genes are expressed in adjacent domains in the developing mouse retina. Treatment of cultured mouse NR cells with the amino- terminal fragment of the Sonic hedgehog protein promotes their proliferation (Jensen and Wallace, 1997).

Stem cells

All the tissue types of vertebrates arise from pluripotent embryonic stem cells (ESCs). These cells become progressively more restricted in their developmental potential as they divide. They express different sets of cell surface proteins, transcription factors, and other molecular markers at each restriction point on their way to forming individual cell phenotypes. ESC cultures have been established from embryos of a wide variety of vertebrates, including humans (Shamblott et al., 1998; Thomson et al., 1998). ESCs differentiate randomly *in vitro* or when implanted into adult tissues, but integrate perfectly into virtually all tissues when injected into embryos.

Late in embryonic or fetal life, subpopulations of lineage-restricted cells are set aside as stem cells to provide for growth after hatching or birth, and in some tissues, for regeneration throughout life. The most salient features of these adult stem cells are the capacity for self-renewal and differentiation as they divide (Morrison et al., 1997). They may be multipotent, bipotent, or unipotent, depending on the tissue in which they reside. Regeneration by stem cells is the most common avenue of tissue restoration in multicellular adult organisms. Mammalian tissues known to regenerate from stem cells are blood, bone, muscle, epithelia, endothelium, olfactory bulb of the brain, and severely damaged liver. Stem cell activation, proliferation and differentiation in each case is regulated by multiple signaling molecules, such as growth factors and cytokines (Fuchs and Segre, 2000; Weissmann, 2000). Here, the focus will be on what tissues harbor stem cells and the developmental potential of these cells.

Bone marrow harbors two stem cell populations, hematopoietic stem cells (HSCs), and mesenchymal stem cells (MSCs), which can give rise to bone, cartilage, skeletal muscle, fibroblasts, adipocytes, and endothelial cells of blood vessels (Prockop, 1997). Endothelial cell precursors have been isolated from human, rabbit, and mouse peripheral blood and have been shown by marking experiments to be incorporated into the endothelium of regenerating capillaries in mice and rabbits (Asahara et al., 1997). The fact that blood vessel regeneration is accomplished by dual mechanisms may reflect the importance of angiogenesis to regeneration in general. Stem cells for cartilage and bone also reside in the periosteum of the bone, and stem cells for muscle (satellite cells) reside between the sarcolemma and basal lamina of myofibers. In fish, amphibians, and lizards, ependymal cells act as stem cells to regenerate the spinal cord (Chernoff, 1996; Clarke and Ferretti, 1998). Neural stem cells have been identified in the dentate gyrus of the hippocampus, as well as in the ependyma and subventricular zone of the spinal cord. Stem cells in the dentate gyrus of the rat proliferate and form new neurons in response to learning-enriched environments, leading to the speculation that birth of new neurons plays a role in life-long learning and memory (Gage, 2000). Evidence for this idea has been obtained from experiments in which rats were given the drug methylazoxymethanol (MAM), which kills proliferating cells, and then tested for their ability to remember a trace conditioning involving the timing and temporal order of events known to be specifically dependent on the hippocampus. BrdU labeling studies showed that memory was impaired when the number of proliferating NSCs was reduced by 80% (Shors et al., 2001).

A surprising discovery has been that stem cells are capable of switching from one lineage to another,

depending on the microenvironment in which they find themselves. This developmental plasticity has been revealed by experiments on mice and birds in which marked stem cells were transplanted into embryos, irradiated adult hosts, *scid* mice, or injury environments, or were manipulated *in vitro*. By these means, it was shown that marrow stem cells can give rise to hepatic stem cells and cardiomyocytes (Petersen et al., 1999; Makino et al., 1999), satellite cells of skeletal muscle can transdetermine into cardiomyocytes when transplanted into injured heart muscle (Atkins et al., 1999), and muscle stem cells can be transformed into blood cells (Jackson et al., 1999). Remarkably, neural stem cells can form a wide range of other cell types, including blood cells, hepatocytes, intestine, skeletal muscle, and cardiac muscle (Bjornson et al., 1999; Clarke et al., 2000).

Commonalities of regeneration-competent cells

The common feature of all regeneration-competent cells, regardless of their state of differentiation, is that they are not terminally differentiated; i.e., they can be activated by initial signals in the injury environment in a way that permits them to re-enter the cell cycle. What these initial signals are, is not clear, but *in vivo,* activation is invariably followed by the dissolution of whatever ECM surrounds the cells, suggesting that the organization of the ECM is an important regulator of their state of differentiation. Loss of this organization, with the concomitant release of growth factors bound to ECM components, activates signal transduction systems which respond to alterations in the actin cytoskeleton resulting from the uncoupling of adhesion molecules and ECM, and to the binding of signaling molecules to transmembrane receptors. In the case of tissues regenerating by dedifferentiation/transdetermination (limb and pigmented retina), major loss of phenotypic characteristics takes place and the original differentiated cell function is lost, permanently or temporarily. In the case of compensatory hyperplasia (liver, endothelial cells, urodele cardiomyocytes), loss of differentiated phenotype is less drastic and some or all differentiated cell functions are maintained. Stem cells are by definition relatively undifferentiated and activation sets them on the road to completing their developmental program. Regardless of their differences in extent of phenotypic alterations, all activated regeneration-competent cells become competent to enter G1, re-express cell-cycle genes, and undergo mitosis. A critical point is the G1/S transition, which requires the phosphorylation of the retinoblastoma protein, releasing E2F transcription factors that activate genes essential for DNA synthesis (Weinberg, 1995).

Non-regenerating mammalian tissues have repressed regenerative capacity

There is substantial evidence that several non-regenerating mammalian tissues contain regeneration-competent cells, but that this competence is negated by the injury environment. Mammalian cardiomyocytes respond to injury by partial dedifferentiation, but the number of proliferating cardiomyocytes is very small and the lesion is patched by the rapid proliferation of scar-forming fibroblasts (Borisov, 1998). Transfection of cultured adult rat ventricular cardiomyocytes with retroviral constructs containing E2F-1 cDNA induces re-entry into S phase (Kirschenbaum et al., 1996). Axons of spinal cord neurons begin to re-grow after injury, but re-growth is quickly inhibited by toxic calcium influx, and synthesis of myelin proteins and chondroitin sulfate proteoglycans by oligodendrocytes (Chernoff, 1996; Filbin, 2000). Substantial numbers of axons will re-grow completely across the lesion if antibodies are generated to myelin proteins or if a bridge of peripheral nerve sheath (Schwann cells) through which the axons can grow (Aguayo, 1985). Ependymal cells, which form glial scar tissue after spinal cord injury, can form neurons when cultured under the right conditions (Gage, 2000). Mammalian retina does not regenerate, but stem cells capable of differentiating *in vitro* into retina-specific cell types have been identified in the pigmented ciliary margin of the mouse eye (Tropepe et al., 2000) and dissociated human pigmented epithelial cell lines from a eighty-year old donor eye were able to transdetermine *in vitro* into neuronal and lens cells (Eguchi, 1998). These observations suggest that stem cells capable of participating in regeneration may be widespread in the body. In support of this idea, cells with the properties of MSCs have been isolated from the connective tissues of virtually all mammalian tissues and organs, even those which do not regenerate (Young et al., 1999).

Regenerative Medicine

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

Cell transplants and bioartificial tissues

Lineage-restricted mouse glial precursor cells, derived by the controlled differentiation of ESCs *in vitro*, have been injected into the spinal cord and brain of 7-day old mutant rats to cure a myelin deficiency that mimics human Pelizaeus-Merzbacher disease (Brustle et al., 1999). Probes for proteolipid protein, mouse satellite DNA, and glial fibrillary acidic protein showed clearly that the injected cells differentiated into astrocytes and myelinating oligodendrocytes which remyelinated spinal cord and brain axons. Marked human fetal brain cells (53-57 days post-conception) transplanted into the brains of fetal or neonatal rats were integrated into

every region of the host brain and differentiated site-specifically in the appropriate organizations (Flax et al., 1998; Brustle et al., 1998). Clinical trials of human fetal neural cell transplants for Parkinson's and Huntington's diseases have shown some symptomatic relief, but how effective such transplants will be in the long run is unknown, because cell survival has proved problematic in these transplants (Bjorklund and Lindvall, 2000). ESC-derived neural and glial precursors injected into paralyzing lesions of rat spinal cords nine days after the injury spread throughout the lesion and differentiated into neurons, astrocytes, and oligodendrocytes (McDonald et al., 1999). The rats appeared to exhibit some functional recovery consisting of awkward stepping movements of the hindlimbs, but nothing approaching full functional recovery.

Fetal cardiomyocytes or cardiomyocytes randomly differentiated from mouse ESCs become stably integrated into the ventricular muscle, differentiate into mature cardiac muscle, and appear to function in concert with the host muscle, thus indicating their potential in restoring injured heart muscle (Soonpa et al., 1994; Klug et al., 1996). 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 their potential for treatment of problems such as urinary incontinence and perhaps diseases such as muscular dystrophy (Chancellor et al., 2000).

Bioartificial tissues and organs can be made by combining cells with biomaterial scaffolds that can be molded into the shape of the tissue or organ (Langer, 1992). Examples of some of these scaffolds are collagen I, polyesters, polyanhydrides, hydroxyapatite, and pig small intestine submucosa (Stocum, 1998). In the case of small three-dimensional or large thin-section tissues that can survive by diffusion, the tissue could be constructed completely *in vitro* prior to implantation; i.e., the cells combined with the scaffolds could be allowed to grow and differentiate to completely fill the scaffold. In the case of larger three-dimensional tissues, the scaffold would have to be seeded *in vitro* with multiple small foci of cells and the construct immediately implanted so that it can become vascularized to support tissue growth and differentiation. To date, the most successful bioartificial tissues have been skin equivalents using collagen or polyglycolic acid mesh scaffolds, and, to a lesser extent, bone substitutes made by seeding ceramic scaffolds with bone marrow cells (Stocum, 1998).

A major issue in the use of cell transplants and bioartificial tissues are cell sources and the immunological and bioethical questions they raise. Use of allogeneic cells requires immunosuppression to avoid immunorejection. In addition, the use of human fetal cells or ESC derivatives raises moral and religious issues concerning sanctity of life. Thus there is great interest in using autogeneic cells as sources of transplants. A procedure to repair defects in articular cartilage by expanding chondrocytes obtained by biopsy from a healthy area of cartilage (Brittberg et al, 1994), is now in clinical use. The restricted lineage stem cells of adults, particularly the MSCs of bone marrow, are of special interest, given the ease of obtaining them, the ability to expand them *in vitro,* and their plasticity.

Stimulation of regeneration in vivo

The stimulation of regeneration *in situ* from regeneration-competent cells is clearly the most desirable way to restore damaged tissues, because it eliminates the need for cell culture and immunosuppression, and negates bioethical issues. The current strategy employs natural or synthetic materials to bridge lesions in order to promote the migration, proliferation, and differentiation of local regeneration-competent cells within the bridge. Numerous tissues have been induced to regenerate using such scaffolds, including skin, bone, blood vessels, dura mater, tendon, peripheral nerve, esophagus, urinary bladder, and spinal cord axons (Stocum, 1999). For example, dermis does not normally regenerate. There is some evidence, based on the *in vitro* behavior of fibroblasts derived from the dermis or hypodermis, that in an excisional skin wound, regeneration-competent dermal fibroblasts are inhibited by scar-forming hypodermal fibroblasts (Gross, 1996). The presence of a biomaterial scaffold in a skin wound might selectively promote the proliferation of dermal fibroblasts, leading to regeneration. Similarly, re-growth of spinal cord axons has been stimulated by protecting them from inhibitory factors, either by treatment with antibodies to myelin proteins (Filbin, 2000) or by bridging lesions with biomaterials that promote axon re-growth (Holmes et al., 2000).

Research Issues

There are a number of research issues to be addressed before regenerative medicine becomes a clinical reality (Stocum, 1999; Picciolo and Stocum, 2001). First, are all cells potentially regeneration-competent? This would seem to be quite possible, given the range of differentiated states exhibited by known regeneration-competent cells. Furthermore, every somatic cell except the B-cells of the immune system carries the same genome, which can be re-programmed to create clonal progeny (Lanza et al., 2000). What we need to know is how to deactivate blockades to cell cycling and stimulate controlled proliferation. Depending on the cell type, this process might also require various degrees of dedifferentiation, redifferentiation, and transdetermination.

Second, what are the gene activities that distinguish regeneration from scar tissue formation? Comparative approaches, using a variety of animal models, parti-

cularly strong regenerators (not just rodents), will be useful in answering this question. For example, species differences in regenerative ability can be compared. Newt myotubes, which break up into mononucleate cells, dedifferentiate, and undergo mitosis during limb regeneration *in vivo* (Lo, et al., 1993). These myotubes can respond *in vitro* to serum factors by phosporylating the Rb protein, whereas mouse myotubes cannot (Tanaka et a.l, 1997). The nuclei of the cultured newt myotubes enter S-phase, but do not undergo mitosis. The active factor in serum to which the newt myotubes are responding is a protein activated by thrombin, which is elevated during the dedifferentiation phase of newt limb regeneration (Tanaka et al., 1999). These observations suggest that the difference in response is due to lack of a receptor for the protein in the mouse cells, or the presence of a factor (s) inhibitory to the protein's activity.

Third, what are the effects of tissue mass and age on regeneration? For example, minced muscle regenerates well in rats, less well in larger animals such as guinea pig, rabbit, cat, and dog, and not at all in humans (Borisov, 1999). Larger muscle masses may fail to regenerate because of difficulties with revascularization and innervation, and slow resorption of degenerating myofibers. Muscle regeneration is poorer in older rats than younger ones. If minced muscle is reciprocally transplanted between old and young rats, the quality of regeneration changes t match that of the host, implying that it is the local and/or systemic environment that determines regenerative quality, not changes in the ability of satellite cells to respond (Borisov, 1999).

Fourth, what are the specific culture conditions that will allow us to direct the differentiation of stem cells to the desired cell phenotypes? This is an important question with regard to both ESCs and the restricted lineage stem cells of adults. Some progress has been made in directing the differentiation of ESCs to precursors of glial and neural cells, and of MSCs to various cell types (Brustle et al., 1999; Pittenger et al., 1999), but the sets of factors required to direct differentiation to most cell types remain largely unknown.

Other important issues are how to insure a high degree of cell survival after transplantation, the development of biomaterials to induce regeneration *in vivo* or construct bioartificial tissues which incorporate physical and chemical cues and signals essential for cell migration, proliferation and differentiation, and the standardization of procedures for producing and testing the components used in the various strategies of regenerative medicine.

Research directed at these issues is proceeding on a wide front and at a rapid pace. It is not unreasonable to believe that, within two to three decades, we will know enough about the developmental signals required for regeneration, how many of these signals remain in the injury environments of specific tissues, the capacities of these tissues to respond to the signals, how to provide signals and response mechanisms where they do not exist, and how to neutralize inhibitors, to restore the original structure and function of tissues that do not regenerate naturally.

References

Aguayo AJ (1985) Axonal regeneration from injured neurons in the adult mammalian central nervous system. In: Cotman CW (ed), Synaptic Plasticity, Guilford Press, New York, pp 457-483.

Asahara T, Murohata T, Sullivan A, Silver M, van der Zee R, Li T, Witzenbichler B, Schatterman G, and Isner JM (1997) Isolation of putative progenitor endothelial cells for angiogenesis. *Science* 275: 964-967.

Asahara T, Takahashi T, Masuda H, Kalka C, Chem D, Iwaguro H, Inai Y, Silver M, and Isner M (1999) VEGF contributes to postnatal neovascularization by mobilizing bone marrow- derived endothelial cells. *EMBO J* 18: 3964-3972.

Atkins B, Lewis C, Kraus, Hutcheson A, Glower DD, and Taylor DA (1999) Intercardiac transplantation of skeletal myoblasts yields two populations of striated cells in situ. *Ann Thorac Surg* 67: 124-129.

Bader D and Oberpriller JO (1978) Repair and reorganization of of minced cardiac muscle in the adult newt (*Notopthalmus viridescens*). *J Morphol* 155: 349-358.

Becker RO, Chapin S, and Sherry R (1974) Regeneration of the ventricular myocardium in amphibians. *Nature* 248: 145-147.

Bjorklund A and Lindvall O (2000) Cell replacement therapies for central nervous system disorders. *Nature Neurosci* : 537-544.

Bjornson R, Rietze R, Reynolds BA, Magli MC, and Vescovi AL (1999) Turning brain into blood: a hematopoietic fate adopted by adult neural stem cells *in vivo*. *Science* 283 534-537.

Borisov AB (1998) Cellular mechanisms of myocardial regeneration. In: Ferretti P and Geraudie, J (eds), Cellular and Molecular Basis of Regeneration, John Wiley & Sons, New York, pp 335-354.

Borisov AB (1999) Regeneration of skeletal and cardiac muscle in mammals: do non-primate models resemble human pathology? *Wound Rep Reg* 7: 26-35.

Brittberg M, Lindahl A, Nilsson AA, Ohlsson C, Isaksson O, and Peterson L (1994) Treatment of deep cartilage defects in the knee with autologous chondrocyte transplantation. *New Eng J Med* 331: 899-895.

Brustle O, Choudhary K, Karram K, Huttner A, Murray K, Dubois-Dalcq M, and McKay RDG (1998) Chimeric brains generated by intraventricular transplantation of fetal human brain cells into embryonic rats. *Nature Biotechnol* 16: 1040-1044.

Brustle O, Jones KN, Learish R, Karram K, Choudhary K, Wiestler OD, Duncan ID, and McKay RDG (1999) Embryonic stem cell-derived gliall precursors: a source of myelinating transplants. *Science* 285: 754-756.

Carinato M, Walter BE, and Henry JJ (2000) *Xenopus laevis* gelatinase B (Xmmp-9): development, regeneration, and wound healing. *Dev Dyn* 217: 377-387.

Casimir CM, Gates PB, Patient, and Brockes JP (1988) Evidence for dedifferentiation and metaplasia in amphibian limb regeneration from inheritance of DNA methylation. *Development* 104: 657-668.

Chancellor MB, Yokoyama T, Tirney S, Mattes CE, Ozawa H, Yoshimura N, de Groat WC, and Huard J (2000) Preliminary results of myoblast injection into the urethra and bladder wall: a possible method for the treatment of stress urinary incontinence and impaired detrusor contractility. *Neurourol Urodyn* 19: 279-287.

Chernoff EAG (1996) Spinal cord regeneration: a phenomenon unique to urodeles? *Int J Dev Biol* 40: 823-832.
Clark RAF (1996) Wound repair: overview and general considerations. In: Clark RAF (ed), The Molecular and Cellular Biology of Wound Repair, Plenum Press, New York, pp 3-50.
Clarke JD and Ferrertti P (1998) CNS regeneration in lower vertebrates. In: Ferretti P and Geraudie J (eds), Cellular and Molecular Basis of Regeneration, John Wiley & Sons, New York, pp 255-272.
Clarke DL, Johansson CB, Wilbertz J, Veress B, Nilsson E, Karlstrom H, Lendahl U, and Frisen J (2000) Generalized potential of adult neural stem cells. *Science* 288: 1660-1663.
Claycomb WC (1992) Control of cardiac muscle cell division. *Trends Cardiovasc Med* 2: 231-236.
Coulombre JL and Coulombre AJ (1970) Influence of mouse neural retina on regeneration of chick neural retina from chick embryonic pigmented epithelium. *Nature* 228: 559-560.
Del Rio-Tsonis K, Washabaugh CH, and Tsonis PA (1995) Expression of *pax-6* during urodele eye development and lens regeneration. *Proc Natl cad Sci USA* 92: 5092-5096.
Del Rio-Tsonis K, Jung JC, Chiu I-M, and Tsonis PA (1997) Conservation of fibroblast growth factor function in lens regeneration. *Proc Natl Acad Sci USA* 94: 13701-13706.
Del Rio-Tsonis K, Trombley M, McMahon G, and Tsonis PA (1998) Regulation of lens regeneration by fibroblast growth factor receptor 1. *Dev Dyn* 213 140-146.
Eguchi G (1998) Transdifferntiation as a basis of eye lens regeneration. In: Ferretti P and Geraudie J (eds), Cellular and Molecular Basis of Regeneration, John Wiley & Sons, New York, pp 207-229.
Eppenberger ME, Hauser J, Baechi T, Schaub M, Brunner UT, Dechesne CA, and Eppenberger HM (1988) Immunocytochemical analysis of the regeneration of myofibrils in long-term cultures of adult cardiomyocytes of the rat. *Dev Biol* 130: 1-15.
Fausto N (1995) Liver stem cells. In: Arias IM, Boyer JL, Fausto N, Jacoby WB, Schacter DA, and Shafritz DA (eds), The Liver: Biology and Pathology, 3rd Ed, Raven Press, New York, pp 1501-1518.
Ferrara N (2000) The role of vascular endothelial growth factor in angiogenesis. In: Angiogenesis in Health and Disease. Basic Mechanisms and Clinical Applications, Marcel Dekker Inc, New York, pp 47-73.
Filbin MT (2000) Axon regeneration: vaccinating against spinal cord injury. *Curr Biol* 10: 100-103.
Flax JD, Aurora, Yang C, Simonin , Wills AM, Billinghurst LL, Jendoubi M, Sidman R, Wolfe J, Kim SU, and Snyder EV (1998) Engraftable human neural stem cells respond to developmental cues, replace neurons, and express foreign genes. *Nature Biotechnol* 16: 1033-1039.
Fuchs E and Segre JA (2000) Stem cells: a new lease on life. *Cell* 100: 143-156.
Gage FH (2000) Mammalian neural stem cells. *Science* 287: 1433-1438.
Gaze RM (1959) Regeneration of the optic nerve in *Xenopus laevis*. *Quart J Exp Physiol* 44: 209-308.
Gross J (1996) Getting to mammalian wound repair and amphibian limb regeneration: a mechanistic link in the early events. *Wound Rep Reg* 4: 190-202.
Holmes T, de Lacalle S, Su X, Liu G, Ricj A, and Zhang S (2000) Extensive neurite outgrowth and active synapse formation on self-assembling peptide scaffolds. *Proc Natl Acad Sci USA* 97: 6728-6733.
Jackson KA, Mi T, and Goodell MA (1999) Hematopoietic potential of stem cells isolated from murine skeletal muscle. *Proc Natl Acad Sci USA* 96: 14482-14486.
Jensen AM and Wallace VA (1997) Expression of *Sonic hedgehog* and its putative role as a precursor cell mitogen in the developing mouse retina. *Development* 124: 363-371.
Ju B-G and Kim W-S (1994) Pattern duplication by retinoic acid treatment in the regenerating limbs of Korean salamander larvae, *Hynobius leechii* correates well with the extent of dedifferentiation *Dev Dyn* 199: 253-267.
Ju B-G and Kim W-S (1998) Upregulation of cathepsin D expression in the dedifferentiating salamander limb regenerate and enhancement of its expression by retinoic acid. *Wound Rep Reg* 6: S349-S358.
Kirshenbaum, AM, Chakraborty S, and Schneider MD (1996) Human E2F-1 reactivates cell cycle progression in ventricular myocytes and represses cardiac gene transcription. *Dev Biol* 179: 402-411.
Klein LR, MacLeish PR, and Wiesel TN (1990) Immunolabeling by a newt retinal pigment epithelium antibody during retinal development and regeneration. *J Comp Neurol* 293: 331-339.
Klug MG, Soonpaa MH, Koh GY, and Field LJ (1996) Genetically selected cardiomyocytes from differentiating embryonic stem cells form stable intracardiac grafts. *J Clin Invest* 98: 1-9.
Langer R and Vacanti JP (1992) Tissue engineering. *Science* 260: 920-926.
Lanza RP, Cibelli JB, Blackwell C, Cristofalo VJ, Francis MK, Baerlocher GM, Mak J, Schertzer M, Chavez EA, Sawyer N, Lansdorp PM, and West MD (2000) Extension of cell life span and telomere length in animals cloned from senescent somatic cells. *Science* 288: 665-668.
Lindhal P, Johansson BR, Leveen P, and Betsholtz C (1997) Pericyte loss and microaneurysm formation in PDGF-B-deficient mice. *Science* 277: 242-245.
Lo DC, Allen F, and Brockes JP (1993) Reversal of muscle differentiation during urodele limb regeneration. *Proc Natl Acad Sci USA* 90: 7230-7234.
Madri JA, Sankar S, and Romanic AM (1996) Angiogenesis. In: Clark RAF (ed), The Molecular and Cellular Biology of Wound Repair, 2nd Ed, Plenum Press, New York, pp. 355-372.
Makino S, Fukuda K, Miyoshi S, Konishi F, Kodoma H, Pan J, Sano M, Takahashi T, Hori S, Abe H, Hata J, Umezawa A, and Ogawa S (1999) Cardiomyocytes can be generated from marrow stromal cells *in vitro*. *J Clin Invest* 103: 697-705.
McDonald JW, Liu XZ, Qu Y, Liu S, Mickey SK, Turetsky D, Gottleib DL, and Choi DW (1999) Transplanted embryonic stem cells survive, differentiate, and promote recovery in injured rat spinal cord. *Nat Med* 12: 1410-1412.
Michaelopoulos GK and DeFrances MC (1997) Liver regeneration. *Science* 276: 60-66.
Mitashov VI (1996) Mechanisms of retina regeneration in urodeles. *Int J Dev Biol* 40: 833-844.
Miyazaki K, Uchiyawa K, Imokawa Y, and Yoshizato K (1996) Cloning and characterization of cDNAs for matrix metalloproteinases of regenerating newt limbs. *Proc Natl Acad Sci USA* 93: 6819-6824.
Mochii M, Mazaki Y, Mizuno N, Hayashi H, and Eguchi G (1998) Role of Mitf in differentiation and transdifferentiation of chicken pigmented epithelial cell. *Dev Biol* 193: 47-62.
Morrison SJ, Shah NM, and Anderson DJ (1997) Regulatory mechanisms in stem cell biology. *Cell* 88: 287-298.
Nag AC, Healy CJ, and Cheng M (1979) DNA synthesis and mitosis in adult amphibian cardiac muscle cells *in vitro*. *Science* 205: 1281-1282.
Negeshi K, Shinagawa S, Ushijima M, Kaneko Y, and Saito T (1992) An immunohistochemical study of regenerating newt retinas. *Dev Brain Res* 68: 255-264.
Noji S, Matsuo T, Koyama E, Yamaai T, Nohno T, Matsuo N, and Taniguchi S (1990) Expression pattern of acidic and basic fibroblast growth factor genes in adult rat eyes. *Biochem Biophys Res Commun* 168: 343-349.
Oberpriller JO and Oberpriller JC (1991) Cell division in adult newt cardiac myocytes. In: Oberpriller JO, Oberpriller JC, Mauro A (eds), The Developmental and Regenerative Potential of Cardiac Muscle, Harwood Press, New York, pp 293-311.
Onda H, Poulin ML, Tassava RA, and Chiu I-M (1991) Characterization of a newt tenascin cDNA and localization of tenascin mRNA during newt limb regeneration by in situ hybridization. *Dev Biol* 148: 219-232.
Orts-Llorca F and Genis-Galvez JM (1960) Experimental pro-

duction of retinal septa in the chick embryo: differentiation of pigment epithelium into neural retina. *Acta Anat* 42: 31-70.
Park C and Hollenberg MJ (1991) Induction of retinal regeneration *in vivo* by growth factors. *Dev Biol* 148: 322-333.
Park C and Hollenberg MJ (1993) Growth factor-induced retinal regeneration *in vivo*. *Int Rev Cytol* 146: 49-71.
Park I-S and Kim W-S (1999) Modification of gelatinase activity correlates with the dedifferentiation profile of regenerating axolotl limbs. *Mol Cells* 9: 119-126.
Petersen BE, Bowen WC, Patrene KD, Mars M, Sullivan AK, Murase N, Boggs SS, Greenberger JS, and Goff JP (1999) Bone marrow as a potential source of hepatic oval cells. *Science* 284: 1168-1170.
Picciolo GL and Stocum DL (2001) ASTM lights the way for tissue engineered medical products standards. *ASTM Standardization News* 29: 30-35.
Pittack C, Jones M, and Reh TA (1991) Basic fibroblast growth factor induces retinal pigment epithelium to generate neural retina *in vitro*. *Development* 113: 577-588.
Pittack C, Grunwald GB, and Reh TA (1997) Fibroblast growth factors are necessary for neural retina but not pigmented epithelium differentiation in chick embryos. *Development* 124: 805-816.
Pittenger MF, Mackay AM, Beck S, Jaiswal RK, Douglas R, Mosca JD, Moorman MA, Simonetti DW, Craig S, and Marshak DR (1999) Multilineage potential of adult human mesenchymal stem cells. *Science* 284: 143-147.
Prockop DJ (1997) Marrow stromal cells as stem cells for non-hematopoietic tissues. *Science* 276: 71-74.
Raymond PA and Hitchcock PF (1997) Retinal regeneration: common principles but a diversity of mechanisms. *Adv Neurol* 72: 171-184.
Reh TA and Nagy Y (1987) A possible role for the vascular membrane in retinal regeneration in *Rana catesbiena* tadpoles. *Dev Biol* 122: 460-482.
Reh TA, Nagy T, and Gretton H (1987) Retinal pigment epithelial cells induced to transdifferentiate to neurons by laminin. *Nature* 330: 68-71.
Rumyantsev PP (1991) Reproduction of cardiac myocytes developing *in vivo* and its relationship to processes of differentiation. In: Rumyantsev PP (ed), Growth and Hyperplasia of Cardiac Muscle, Harwood Press, New York, pp 70-159.
Sage H and Bornstein P (1991) Extracellular matrix proteins that modulate cell-matrix interactions. *J Biol Chem* 266: 831-834.
Schmidt AJ (1966) The Molecular Basis of Regeneration: Enzymes. Illinois Monogr Med Sci Vol 6 (4), University of Illinois Press, Urbana.
Shamblott MJ, Axelman J, Wang S, Bugg EM, Littlefield JW, Donovan J, Blumenthal PD, Huggins GR, and Gearhart JD (1998) Derivation of pluripotent stem cells from cultured human primordial germ cells. *Proc Natl Acad Sci USA* 95: 13726-13731.
Shors TJ, Mieswgaes G, Beylin A, Zhao M, Rydel T, and Gould E (2001) Neurogenesis in the adult is involved in the formation of trace memories. *Nature* 410: 372-375.
Soonpa MH, Koh GY, Klug MG, and Field LJ (1994) Formation of nascent intercalated discs between grafted fetal cardiomyocytes and host myocardium. *Science* 264: 696-698.
Sperry RW (1944) Optic nerve regeneration with return of vision in anurans. *J Neurophysiol* 7: 57-69.
Steen TP (1968) Stability of chondrocyte differentiation and contribution of muscle to cartilage during limb regeneration in the axolotl (*Siredon mexicanum*). *J Exp Zool* 167: 49-78.
Stocum DL (1979) Stages of forelimb regeneration in *Ambystoma maculatum*. *J Exp Zool* 209: 395-416.
Stocum DL (1995) Wound Repair, Regeneration, and Artificial Tissues. RG Landes, Austin.
Stocum DL (1998) Regenerative biology and engineering: strategies for tissue restoration. *Wound Rep Reg* 6: 276-290.
Stocum DL (1999) Regenerative biology: a millennial revolution. *Sem Cell Dev Biol* 10: 433-440.
Tanaka EM, Gann AF, Gates PB, and Brockes JP (1997) Newt myotubes re-enter the cell cycle by phosphorylation of the retinoblastoma protein. *J Cell Biol* 136: 155-165.
Tanaka EM, Dreschel DN, and Brockes JP (1999) Thrombin regulates S phase re-entry by cultured newt myoblasts. *Curr Biol* 9: 792-799.
Thomson JA, Itskovitz-Eldor J, Shapiro SS, Waknitz MA, Swiergiel JJ, Marshall V, and Jones JM (1998) Embryonic stem cell lines derived from human blastocysts. *Science* 282: 1145-1147.
Tomanek RJ and Schatteman GC (2000) Angiogenesis: new insights and therapeutic potential. *Anat Rec* 261: 126-135.
Tomasek JJ, Halliday NL, Updike DL, Ahern-Moore JS, Vu T-KH, Liu RW, and Howard EW (1997) Gelatinase A activation is regulated by the organization of the polymerized actin cytoskeleton. *J Biol Chem* 272: 7482-7487.
Trembly JH and Steer CJ (1998) Perspectives on liver regeneration. *J Minn Acad Sci* 63: 37-46.
Tropepe V, Coles BLK, Chiasson BJ, Horsford DJ, Elia AJ, McInnes RR, and van der Kooy D (2000) Retinal stem cells in the adult mammalian eye. *Science* 287: 2032-2036.
Weinberg RA (1995) The retinoblastoma protein and cell cycle control. *Cell* 81: 323-330.
Weissman IL (2000) Stem cells: units of development, units of regeneration, and units in evolution. *Cell* 100: 157-168.
Yang EV, Gardiner DM, and Bryant SV (1999) Expression of *Mmp-9* and related matrix metalloproteinase genes during axolotl limb regeneration. *Dev Dyn* 216: 2-9.
Young HE, Steele TA, Bray RA, Detmer K, Blake LW, Lucas PW, and Black AC (1999) Human pluripotent and progenitor cells display cell surface cluster differentiation markers CD10, CD13, CD56, and MHC class-I. *Soc Exp Biol Med* 221: 63-71.

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