Hindawi Publishing Corporation
ISRN Stem Cells
Volume 2013, Article ID 784541, 12 pages
http://dx.doi.org/10.1155/2013/784541

Review Article

The Systems Biology of Stem Cell Released Molecules—Based Therapeutics

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Received 20 February 2013; Accepted 2 April 2013

Academic Editors: A. Chapel and B. Rogister

Most therapeutics are based on the traditional method of reductionism where a clinically defined condition is broken down into a defined biochemical pathway underlying the condition, then a target in the pathway is identified, followed by developing a drug to interact with the target, modifying the target such that the disease is ameliorated. Biology acts as a system, therefore reductionist approaches to developing therapeutics are limited in therapeutic value because disease or traumatized tissue involves multiple underlying pathways, only a part of the pathways underlying the disease is manipulated by the traditional therapeutic. Much data regarding stem cells shows that their beneficial effects are not restricted to their ability to differentiate, but is more likely due in large part to their ability to release a multitude of molecules. Stem cells release potent combinations of factors that modulate the composition of the cellular milieu to evoke a multitude of responses from neighboring cells. Therefore, stem cells represent a natural systems-based biological factory for the production and release of a multitude of molecules that interact with the system of biomolecular circuits underlying an indication. Current research includes efforts to define, stimulate, enhance, and harness stem cell released molecules (SRM) to develop systems-therapeutics.

1. Introduction

In the postgenomic era, where even individual somatic cells display genetic heterogeneity [1], knowing the sequence of the genome has limited predictive value in disease diagnosis and treatment [2, 3]. Thus new diagnostic and therapeutic regimens are needed beyond those that rely on simple genomics [4]. While research and development costs in the pharmaceutical industry continue to increase, the number of new approved drugs is on a steady decline and new paradigms for drug development are being proffered [5–7]. Following the rapid emergence of *in vitro* and *in silico* screening tools, including molecular and genetic tools, there have now been advances in systems-based tools necessary to describe the effects of drug candidates within the complex biochemical pathways of intact, fully assembled living networks [8]. The pharmaceutical industry has thus realized the need to develop innovative strategies and new technologies to identify and develop new drug candidates, moving away from the over reliance of nonpredictive genetic tools [4]. One of the newly identified strategies and technologies is systems biology. As an example of a systems biology technique, a new analytical tool has emerged called metabolomics, which is a systems biology approach to measuring metabolites throughout the cell or defined tissue to collectively measure the activity of multiple biochemical pathways in one experiment [9, 10]. Many more systems biology-based analytical tools are being used and further developed such as proteomics, genomics, or connectomics to name a few, and indeed metabolomics has seen further development into such subdisciplines as tracer-based metabolomics [10]. The rationale behind those terms, using omics, is to convey that the proteins, or genes, or connections in the brain need to be thought of as operating within a system.

However, unlike the use of systems biology in describing a circuit within biology or describing analytical methods of drug development, the development of therapeutics that act in a systems manner rather than a in reductionist manner has received little attention.

2. Systems Analysis

The systems biology analytical approaches have now been used to better understand the mechanisms underlying diseases, for example, alcohol abuse and dependence [11], and to understand the mechanisms of drug actions and failures. For example, Gleevec is one of the first drugs designed through a rational, targeted approach to treat chronic myeloid leukemia (CML). Studies employing tracer-based metabolomics have been used to understand the mechanism of CML, and the mechanism of failure when some patients develop resistance to Gleevac and the CML again expresses itself [9].

In this case a reaction network analysis employed tracer-based metabolomics using a 13C-labeled glucose tracer to study the distribution of the tracer among other metabolites. In brief, this methodology allows one to define those multiple biochemical pathways that are active for any given phenotype, whether this happens to be the normal phenotypic state or a state in which a disease and/or a therapeutic is present. These studies of Gleevac employing metabolomics were able to show additional metabolic pathways that became active in the tumor in response to Gleevac and therefore allowed the cancer to remain active despite the Gleevac treatment.

3. The Emergent Properties of Systems Therapeutics

Let us consider a traditional reductionist-based therapeutic versus a newer system-based therapeutic. As opposed to traditional reductionist approaches, where one molecule is developed to target and perturb one pathway in a system, stem cells actively contribute to their environment by releasing multiple cytokines, growth factors, extracellular matrix (ECM) molecules, micro-RNA, antioxidants, and other molecules that act either on themselves (autocrine actions) or on neighboring cells (paracrine actions) to exert their therapeutic actions (Figure 1). Indeed, the pathway shown in Figure 1 for SRM instead of the pathway for differentiation as the key mechanism for tissue repair and other actions of adult stem cells is described in this paper. Adult stem cells are known to be involved in the daily maintenance of tissue, as well as in tissue regeneration and repair, and are even likely to be involved in immune system function [12] and to fight infection [13]. Therefore, a clearer understanding of stem and progenitor cell biomolecule production may yield new insights into the regulation of cell phenotypes, better define the functional role of stem cells in tissue maintenance, replication, and repair processes, better determine appropriate cell sources for specific tissue repair and regeneration applications, and lead to the development of drugs/biologics and other therapeutics. As such, current research directions include efforts to define, stimulate, enhance, and harness the stem cell released molecules and their autocrine and paracrine mechanisms for regenerative medicine and therapeutic development.

The power of system therapeutics versus reductionist therapeutics can be shown specifically for SRM. For example, Eppler et al. [14] have shown that one component of SRM alone, namely, VEGF, when administered intravenously has a half-life of only 30 minutes. Rapid degradation of the growth factor results from denaturation, oxidation, and proteolysis [15]. However, when SRM is administered as the whole collection of molecules, instead of just one isolated molecules, including extracellular chaperones and antioxidants, the half-life is greatly extended [16–18]. Likewise, the power of SRM in wound healing compared to one isolated factor, such as PDGF, is dramatic [16]. Thus, harnessing the power of stem cells promises to bring a systems approach to tissue repair where a multitude of molecules are used to effectuate a system, or circuit, of mechanisms important for mimicking the healing properties inherent in the native tissue.

Genomic and proteomic approaches have recently been used to characterize biomolecule production by stem cells to analyze the SRM in cell-conditioned media [19–23], or in the extracellular space [24]. Stem cells are capable of producing a broad spectrum of cytokines, chemokines, growth factors, antioxidants, microRNA, ECM molecules, and chaperone proteins [24–27]. While the majority of published reports to date focus on adult multipotent stem cells (i.e., mesenchymal stem cells (MSCs), adipose derived stem cells (ASCs), and hematopoietic stem cells (HSCs)), several studies have also examined pluripotent stem cell (i.e., embryonic stem cell (ESC) and induced pluripotent stem cell (iPSC)) and lineage-restricted progenitor cell (i.e., skeletal myoblast (skMb)) SRM production.

Growth factors secreted by a number of stem/progenitor cell populations are capable of promoting cell proliferation, cytoprotection, chaperone activity, immunomodulation, and migration. Stem and progenitor cells can also protect other cells from damaging oxygen-free radicals through the production of antioxidants and antiapoptotic molecules, while these cells also release angiogenic factors, antifibrotic factors, factors responsible for ECM homeostasis such as collagens, matrix metalloproteinases (MMPs), and their tissue-derived inhibitors (TIMPs), and anti-inflammatory or immunosuppressive factors. Additionally, stem/progenitor cells not only release the aforementioned factors but also consume proapoptotic and inflammatory molecules. Because many exogenous cell therapies for tissue repair and regeneration typically involve transplantation of cells into ischemic tissue with varying degrees of inflammation, stem/progenitor cells may also produce a variety of molecules that serve to mediate tissue repair and regeneration via anti-apoptotic, immunosuppressive, antioxidative, proliferative, and angiogenic mechanisms. Therefore, novel research directions aspire to use stem and progenitor cells as biologically complex drug production and delivery vehicles to orchestrate molecular signaling that facilitates tissue regeneration and repair and pain reduction. Further, the need for the use of "systems therapeutics," where more than one molecule, rather a combination of molecules, is used to develop therapeutics, has been demonstrated [16]. As an example, in considering wound healing, mechanical strain applied to human fibroblasts differentially regulates skeletal myoblast differentiation through a combination of molecules and not just IL-6 as previously hypothesized [28]. The potential to use a stem cell-based model for the development of systems therapeutics for

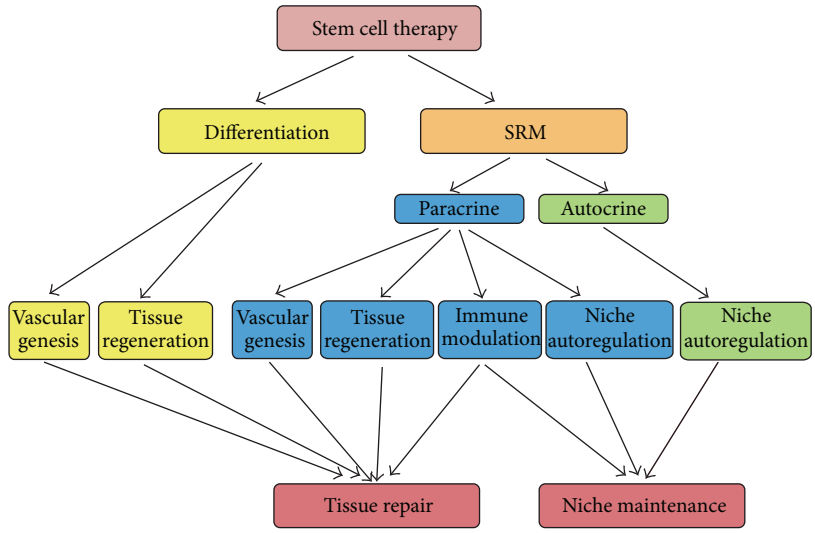

Figure 1: The actions of adult stem cells to achieve therapeutic value are carried out through two main pathways: (1) differentiation into new tissue and (2) stem cell released molecules (SRM). Up to 80% of the healing actions of stem cells have been seen to occur through the paracrine and autocrine actions of the SRM. The paracrine and autocrine actions of the SRM help to generate tissue, modulate the immune system, and regulate the stem cell niche.

all tissues throughout the body is evidenced by the general rule that stem cells are located throughout the body for the purposes of tissue repair and regeneration, although there may be exceptions to the rule as evidence suggests for pancreatic beta cells, which may replicate themselves [29].

4. Stem Cell Paracrine Actions and Immune Modulation

The injection of stem cells into damaged tissue has been observed to repair tissue through the relative contribution of two mechanisms: (1) differentiation of the stem cells into mature, healthy tissue and (2) paracrine and autocrine actions, that is, the release of molecules (SRM) into the extracellular space. The relative contributions of the two mechanisms have been estimated to be 20–50% of the effects resulting from differentiation, whereas the actions of the SRM account for 50–80% of the effects [30]. When administered systemically, only a small proportion of infused stem cells home to the damaged tissue, whereas the majority of cells were found entrapped in other organs including lungs [31–33]. Homing of the stem cells to the target tissue is controlled by many factors [34, 35], and because of many other factors, including local hypoxia, oxidative stress, and inflammation in the targeted damaged tissue, the retention of transplanted stem cells is poor and the low cell survival reduces the therapeutic effects [36].

Human MSCs and embryonic stem cell-derived MSCs (ESC-MSCs) are immunotolerant and may modulate the immune response alone or when cotransplanted with other cell types. MSCs express MHC class I molecules, including HLA-A, -B, and -C, but not MHC class II molecules, such as HLA-DR or costimulatory molecules (e.g., CD40, CD80 and CD86) [37–40]. Human adipose-derived mesenchymal stem cells (ASCs), ESC-MSCs, and umbilical cord blood-derived mesenchymal stem cells (UCBs) have also been shown to share similar surface immunophenotypes [21, 41–43]. The immunosuppressive characteristics of MSCs were first reported in an *in vivo* model using MSCs to delay rejection of skin grafts in a baboon through suppression of lymphocytes [44]. Subsequent research has focused on defining the role of these cells in modulating host immune response and on the utility of these cells as "protectors" or chaperones for other cell types during cell transplantation.

Although cells communicate with each other via released molecules such as short peptides, proteins, nucleotides, and lipids that bind to surface receptors on neighboring cells, an additional mechanism exists whereby eukaryotic cells communicate with each other through the release of microparticles and exosomes in their extracellular environment. Microparticles are a heterogeneous population of spherical structures with a diameter of 100–1000 nm, which are released by budding of the plasma membrane (ectocytosis) as phospholipid vesicles that express antigens specific of their parental cells [45]. Distinct from microparticles, exosomes are membrane vesicles with a diameter of 40–100 nm, formed by endocytosis, a process involving the sequestration of plasma membrane proteins within the exosomes. Exosomes are stored intracellularly in endosomal compartments and are secreted when these multivesicular structures fuse with the cell plasma membrane [46]. Exosomes display a broad spectrum of bioactive substances on their surface, carry a concentrated set of proteins, lipids, and even nucleic acids that are taken up by other cells, and regulate their function [47]. Barile et al. [48] have shown that stem cells release exosomes and may be involved in numerous regulatory pathways, including angiogenesis. Sahoo et al. [49, 50] have shown that exosomes mediate many of the paracrine angiogenic actions of human stem cells and that the exosomes

provide a means for preserving the integrity of the molecules contained within the exosome.

4.1. Stem Cells and the Immune System. Recent *in vitro* and *in vivo* studies show that human MSCs can regulate immune responses through mechanisms that employ cells of both the innate and adaptive immune systems. MSCs influence T cell, B cell, natural killer (NK) cell, dendritic cell (DC), macrophage, and neutrophil immune activity. Other data suggest that MSCs not only inhibit T-cell proliferation, cytokine activity, and cytotoxicity attributed to MSC secretion of several factors including TGF-β1 [51, 52], HGF [52], nitric oxide [53], indoleamine 2,3-dioxygenase (IDO) [51, 54, 55], and prostaglandin E2 (PGE_2) [52, 56], but also stimulate the aforementioned cells through the secretion of cytokines IL-1 and -6 and the chemokine CCL5, also known as RANTES [57]. IDO has also been shown to play a role in T-cell apoptosis [55, 58]. MSCs inhibit B-cell proliferation, maturation, migration, and immunoglobulin and antibody production [59]. Secretion of IL-6 by MSCs may mediate their inhibitory effects on B-cells. However, the exact molecules and mechanisms responsible have yet to be fully elucidated [60]. MSCs can have an inhibitory effect on immature and mature DC phenotype, maturation, activation, and antigen presentation, and these effects are thought in part to be due to MSC IL-6, M-CSF, and PGE_2 release [61–63]. MSCs may also modulate immune response through inhibition of DC maturation and subsequent DC inhibition of T-cell proliferation [62]. In addition, MSCs inhibit NK cell proliferation, cytokine production, and cytotoxicity through IDO, TGF-β, HLA-G, and PGE_2 [64, 65]. ESC-MSCs have also been implicated in impeding cell lysis by NK cells through the downregulation of cell surface receptors necessary for NK cell activation [43]. Data also suggest that MSCs play a role in macrophage and neutrophil function. MSC IL-1 receptor agonist secretion inhibits TNF-α production by activated macrophages [66] and that IL-6 secretion protects neutrophils from apoptosis [67]. These studies demonstrate that MSCs act to modulate the host immune response and protect other cells from innate and adaptive immune responses.

5. Stem Cell Paracrine Actions and Tissue Regeneration

Studies continue to demonstrate that most organ systems of the body have a resident pool of dormant somatic, tissue-specific stem cells ready to be activated in the case of injury or disease. However, in many cases of traumatic injury or disease and aged and damaged tissue, the quantity and potency of endogenous stem cell populations are insufficient to regenerate the injured tissue. Therefore, research has focused on exogenous or nontissue-specific stem and progenitor cell sources for tissue repair and regeneration, such as banked stem cells and/or autologous adipose-derived stem cell therapy. Early efforts to use stem cells for therapeutic purposes centered on the directed differentiation of these cells to the intended cell phenotypes and functional improvements in a number of tissues were observed with cell transplantation and attributed to stem or progenitor cell differentiation. By the 1990s however many studies demonstrated that the functional improvements attributed to stem cells may be due to the SRM actions in the host tissue rather than cell differentiation and repopulation [68].

5.1. Protective and Regenerative Properties of Stem Cells in CNS. Stem and progenitor cell SRM has been implicated in repair and regeneration of the CNS following traumatic injury or disease. Recent directions in research on neurodegenerative diseases such as amyotrophic lateral sclerosis, Parkinson's disease (PD), and Huntington's disease aim to elucidate the SRM and their subsequent neuroprotective properties. There is evidence suggesting that human neural stem cells (NSCs), human UCBs, and murine MSCs secrete glial cell- and brain-derived neurotrophic factors (GDNF and BDNF), IGF-1, and VEGF that may protect motor neurons, thereby prolonging the lifespan of the animal into which they were transplanted [69–73]. In addition, NSCs have been shown to secrete NGF and neurotrophin-3 (NT-3) [71, 72], and studies in humans by Crigler et al. [73] and collaborators have shown that NGF injection into the brain improves cognitive function in Alzheimer's Disease [74]. However, the work of Crigler et al. [73], although being seminal in the field of Alzheimer's, represents a reductionist approach where only one of the many SRMs from stem cells is given to the patient.

The secretion of GDNF, BDNF, and NGF by MSCs have been implicated in increased dopaminergic neuron survival in *in vitro* and *in vivo* models of PD, and anti-inflammatory SRMs from MSCs has been shown to attenuate microglia activation, thereby protecting dopaminergic neurons from death [74, 75, 74]. MSCs and UCBs are also capable of releasing NT-3 that supports the survival and differentiation of existing neurons and encourages the growth and differentiation of new neurons and synapses [76, 77]. In addition, human UCBs secrete antioxidants, NGF, VEGF, and basic FGF (bFGF), which may lend to the neuroprotective properties of these cells [76, 78].

Exogenous SRMs from MSCs determine the lineage specification of NSCs and neuronal progenitor cells (NPCs). More specifically, MSCs can differentially release, given the culture conditions, soluble factors that drive NSCs and NPCs to either the neurogenic or astrocytic phenotype [79]. Further, MSCs secrete trophic factors that provide support to NSC- or NPC-derived neuronal cells in ischemic tissues and promote neurite and axonal outgrowth *in vitro* and *in vivo* [79]. Transplantation of MSCs into ischemic brain resulted in the recovery of motor function in rats, and the authors suggest that such an effect is due to SRMs from the MSCs resulting in inhibited scar formation and apoptosis, increased angiogenesis, and neuronal commitment of NSCs and NPCs [80]. The aforementioned neuroprotective and cell fate effects of MSCs can be attributed to the SRM, containing many factors including NGF, VEGF, antioxidants, and bone morphogenic protein-(BMP-)4 [81, 82]. In a mouse model of schizophrenia, transplanted MSCs were shown to increase the levels of brain neurotrophic factors and attenuate the schizophrenic behavior [83].

6. Protective and Regenerative Properties of Stem Cells in the Cardiovascular System

Improvements in myocardial function following stem cell transplantation were originally attributed to stem cell differentiation into cardiomyocytes within host myocardium. During the last decade studies have shown that few, if any, exogenous stem cells actually engraft and differentiate within the heart tissue [84–87]. Explanations for observed functional improvements have been suggested by *in vitro* and *in vivo* studies that demonstrated that SRMs from MSCs are cytoprotective and reduce apoptosis and necrosis in cardiomyocytes and other myocardial cell populations [88–92]. ESC transplantation into infarcted myocardium also attenuates cell apoptosis, hypertrophy, and fibrosis because of their SRMs [93]. Cardioprotective SRMs include bFGF, VEGF, PDGF, IL-1β, IL-10, stem cell-derived factor-(SDF-) 1, HGF, IGF-1, thymosin-β4, and Wnt5a [94], and the pool of SRMs from stem cells grown in hypoxic conditions have a more favorable effect than those grown under normal conditions [95].

The functional benefits of stem cell transplantation have been attributed to several other mechanisms in addition to cardioprotection. Secretion of SRMs such as bFGF, HGF, angiopoietin-1 and -2 (Ang-1 and -2), VEGF, and cysteine-rich protein 61 by MSCs and ASCs leads to increased vascular density and blood flow in ischemic myocardium, resulting in increased perfusion and function [96]. In addition, cardiac levels of IL-1β and TNF-α and SRMs implicated in angiogenesis were shown to be elevated following MSC transplantation. The expressions of SDF-1, IGF-1, HGF, and PEDF are known to promote repair and regeneration by facilitating circulating progenitor cell recruitment to damaged tissues. Further, the SRMs, HGF and IGF-1, are essential for activation of cardiac stem cells that may contribute to nourishment of the stem cell niche and to endogenous repair mechanisms. Stem cell transplantation may also attenuate left ventricular chamber dilation postmyocardial infarction (MI) via SRMs including collagens, TGF-β, MMPs, TIMPs, serine proteases, and serine protease inhibitors that act to reestablish ECM homeostasis and inhibit cardiac fibrosis [97]. The SRMs of MSCs *in vitro* include a number of molecules implicated in ECM synthesis and remodeling, such as collagen I and III, MMPs, and TIMPs, and inhibit cardiac fibroblast proliferation and collagen synthesis [98, 99]. Mesenchymal stem cells have been shown to improve cardiac conduction in an *in vitro* model by upregulation of connexin 43 through paracrine signaling of SRM [100].

Skeletal myoblasts (SkMbs) are myogenic progenitor cells possessing the ability to expand and form new fibers following muscle injury. SkMbs *in vitro* express a number of antiapoptotic genes (BCL-2 and BAG-1) and genes associated with ECM remodeling (MMP-2, -7, and -9) and a number of SRMs implicated in angiogenesis, proteases involved in matrix remodeling, and cytokines involved in apoptosis when cultured *in vitro* under normal and hypoxic conditions [84]. Several clinical trials have demonstrated improved left ventricular functional outcomes in patients treated with SkMbs [101].

7. Wound Healing

Wound healing is a good example of a well-studied systems biology cascade of events including cell migration, proliferation, ECM remodeling and angiogenesis [102]. Stem and progenitor cell SRMs implicated in immunoregulation, cell proliferation, migration, neovascularization, and ECM synthesis and remodeling, may accelerate wound healing when administered to the site of injury. Studies of MSC, ASC, and amnion-derived progenitor cell SRM administration to dermal wounds have demonstrated positive results. In addition to their previously discussed immunosuppressive properties, MSCs and ASCs administered to chronic wounds enhanced capillary density. Studies determined that MSCs induced neovascularization was carried out through SRMs, including proangiogenic factors Ang-1 and VEGF within the wound beds [103]. ASCs implanted into chronic dermal wounds enhanced granulation tissue thickness, epithelialization, and capillary formation by SRMs including bFGF, PDGF, VEGF, and HGF. Human amniotic progenitor cells have been shown to inhibit neutrophil and macrophage migration to the site of injury through SRMs including migration inhibitory factor and the suppression of IL-1α and -1β. Amniotic progenitor cells also release anti-inflammatory SRMs that prevent apoptosis and enhance wound healing. Though SRM actions of stem and progenitor cells are now known to play many roles in wound healing, the entirety of constituents in the SRM by various progenitor cell populations and their role in wound healing has yet to be thoroughly characterized.

7.1. Osteogenic SRM Mechanisms. Tissue healing has been demonstrated to be a scar-free process in the fetus. Recent studies by Zimber et al. [104] have shown that culture of neonatal stem cells in conditions that mimic the fetal environment induces SRM that is similar to that of the SRM produced by fetal cells. The difference in the bone-forming ability of children and adults has been attributed to differential expression of SRMs such as TGF-β1, TGF-β3, and bFGF. Microarray analysis of calvarial regenerates from juvenile and adult mice demonstrated a marked increase of proosteogenic cytokines (e.g., BMP-2, -4, and -7 bFGF, and IGF-2) in juvenile samples. In addition, increased levels of bone-related ECM proteins, such as procollagens Col6a1, Col3a1, and Col4a1, as well as MMP-2 and -14, pleiotrophin, and cathepsin K, were found in juvenile regenerates compared to those of adults [105]. Given the SRM profiles of certain stem and progenitor cell populations *in vitro* and *in vivo*, these cells may provide a cell source capable of modulating bone regeneration through paracrine actions and regulating scar formation in adults.

Many studies using stem and progenitor cells to regenerate tissues following disease or traumatic injury have been promising, and further characterization of the various stem and progenitor cell populations and subpopulations and their secretion profiles, differentiation potential, and ability to release SRMs in adequate amounts to produce functional benefits in patients is needed. Further methods to modulate the paracrine actions of stem and progenitor cells and capture the SRM produced by these cells are

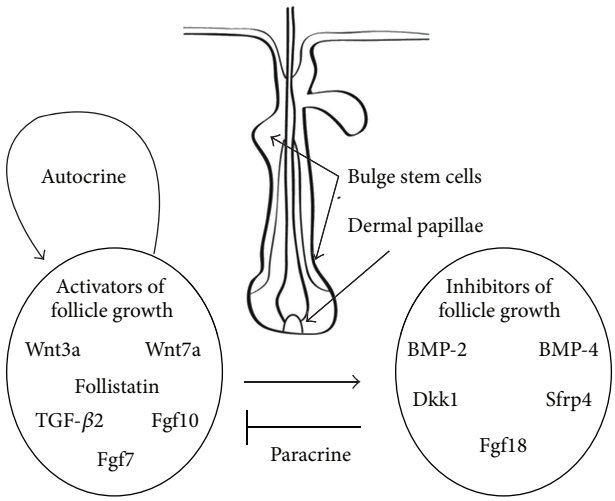

Figure 2: The hair follicle is an important model system for understanding stem cell and SRM function. Although not fully understood, a number of paracrine and autocrine functions have already been defined.

currently under investigation and are successfully used in cosmetic and therapeutic products, such as the Dermagraft (Shire Regenerative Medicine, San Diego, CA, USA) dermal substitute that contains fibroblast-derived SRM.

8. Mucosal SRM Mechanism

The oral mucosa plays critical roles in protection, sensation, and secretion and can be classified into masticatory, lining, and specialized mucosa that are known to be functionally, histologically, and clinically distinct. Each type of oral mucosa is believed to develop through discrete molecular mechanisms, which remain unclear. MicroRNAs (miRNAs) are 19 to 25 nt noncoding small single-stranded RNAs that negatively regulate gene expression by binding target mRNAs. miRNAs are crucial for fine-tuning of molecular mechanisms. Mesenchyme miRNAs were shown to have an indirect effect on lining mucosal epithelial cell growth/differentiation [106]. SRM has been observed to regenerate oral and vaginal mucosa when topically applied to the tissue (Maguire and Friedman, personal observation).

9. Hair Follicle and Hair Growth

The mammalian hair follicle serves as a model system (Figure 2) for stem cell dynamics underling cellular renewal processes [107] and involves SRM such as Wnt ligands secreted from hair follicle epithelium for the growth of hair [108]. The hair follicle is divided into a permanent upper region, which consists of the infundibulum and isthmus, and a variable lower region, that is, the actual hair-shaft factory that contains the hair matrix, differentiated epithelial cells and dermal papilla (DP) cells. DP cells are responsible for the production of dermal-cell populations such as dermal sheath (DS) cells, and generate dermal fibroblasts and adipocytes. After morphogenesis, various stem cell types are maintained in distinct regions of the follicle. For example, follicle epithelial cells are found in the follicle stem cell niche of the bulge region; multipotent mesenchymal precursors are found in DP cells; neural crest-derived melanocyte progenitors are located in the subbulge region and follicle epithelial stem cells in the bulge region. The follicle variable region mediates the hair cycle, which depends on the activation of follicle epithelial stem cells in the bulge stem cell niche during the telogen-to-anagen transition. This transition includes phases of growth (anagen), apoptosis-driven regression (catagen), and relative quiescence (telogen), whereas the organogenesis of most organs is induced only once during embryogenesis. The dermal papilla is a source of Wnt ligands, but it is also maintained in anagen by Wnt3a and Wnt7a ligands. Further, when the ligand catenin is artificially elevated in resting stem cells, hair follicles are induced to begin a new round of hair growth [109]. In contrast, cyclic BMP expression has been observed in adipocytes that reside in extrafollicular space, and data suggest that high levels of BMP signaling can maintain bulge stem cells in a quiescent state during telogen. Although the influence of particular activators and inhibitors on hair growth is a developing model system, precisely how the activators and inhibitors of follicle growth interact with one another has not yet been well characterized.

10. Synergistic Stem Cell SRM Interactions

Multiple adult stem cell types are located in most tissues of the adult human and reside in stem cell niches within the various tissues in either a dormant state or an active state [110], and the pool of active stem cells are readily available for tissue repair, regeneration, and maintenance functions. To heal and maintain the various tissues of the body, including the skin [111], multiple stem cell types are often involved [112] and secrete a number of factors into the tissue to induce the healing response [113]. Thus, stem cells in the human body will mobilize into the area of diseased or damaged tissue, utilizing two or more types of stem cells, and the two or more stem cell types will release their respective SRM (stem cell released molecules) into the damaged tissue (Figure 3). As shown in Figure 4, each stem cell type releases its own pool of SRM, with each pool being a different collection of molecules but showing some overlap between pools.

The latest data demonstrate that the regenerative properties of the SRM are enhanced by the secretion of SRM from more than one stem cell type. For example, in muscle repair, bidirectional signaling between fibroblasts and myocytes is required for efficacy, and one pool of SRM from the fibroblasts is insufficient to induce repair [114]. Stem cells and progenitor cells communicate with one another through paracrine actions of SRM to develop emerging phenotypes [115]. In wound healing, multiple stem cell types are likely involved in the wound healing cascade [116], acting through paracrine mechanisms of SRM [117]. Therefore, the development of therapeutics should not only employ a system biology-based approach where a full complement of molecules derived from stem cells is used to target multiple

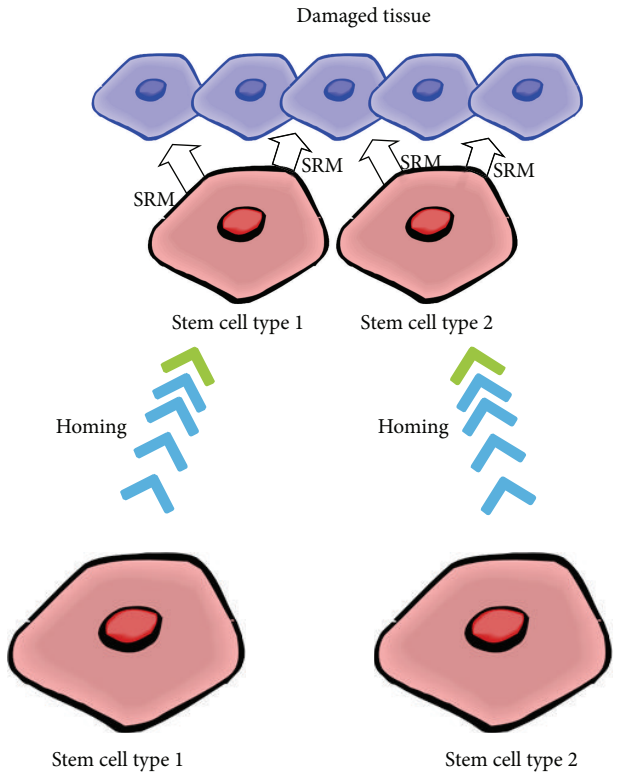

Figure 3: Two types of stem cell home into the damaged tissue and release SRM (stem cell released molecules) into the damaged tissue to induce healing. SRM from stem cell type 1 is different from that of type 2, and when the two SRM pools act together, they are synergistic and called S^2RM.

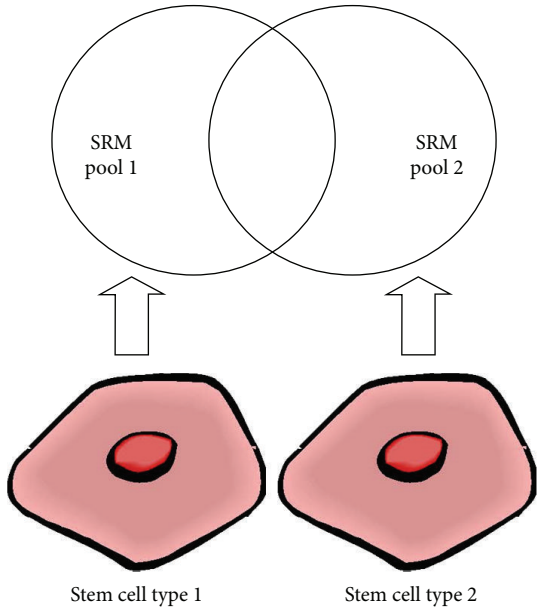

Figure 4: Two types of stem cell release SRM (stem cell released molecules). Although there is overlap in the molecule released by stem cells 1 and 2, SRM from stem cell type 1 is different from that of type 2, and when the two SRM pools act together, they are synergistic and called S^2RM.

pathways underlying the diseased or damaged tissue but should also employ an approach that uses multiple molecules (SRM) released from two or more types of stem cells to effectuate a delivery of molecules to multiple pathways underlying the diseased or damaged tissue. Nature often uses more than one type of stem cell to induce a natural healing process, and to mimic the natural healing process, one should use, just as nature would do, SRM from one or more stem cell types. Thus a therapeutic delivery system should therefore employ a synergistic group of SRM from two or more stem cell types for the induction of healing. Current therapeutic development in the commercial sector using such a synergistic, systems biology stem cell approach is under way at such companies as BioRegenerative Sciences, Inc. (San Diego, CA, USA).

11. Chaperone Molecules

Of the 200 proteins secreted by neural cells identified in one study, a large number of proteins with molecular chaperone and antioxidant properties were secreted, and different pools of proteins were secreted by nerve, astrocytes, and neural stem cells [24]. Chaperones are essential for cell-to-cell trafficking of mobile transcription factors and important in chaperone-dependent protein trafficking for stem cell function [27]. Chaperone molecules are able to recognize folded or unfolded states of the client, exist in the intra- and extracellular space, and enable movement of the client through different cellular or tissue compartments [118]. Chaperones can be released into the extracellular space by passive and active mechanisms and are involved in immune responses [119]. Epigenetics and chromatin assembly at telomeres in stem cells has been shown to be regulated by chaperones [120]. Clusterin, a ubiquitous and highly conserved secreted protein, is an efficient EC clusterin that potently inhibits stress-induced protein aggregation by ATP-independent binding to nonnative proteins to form soluble, high-molecular-weight complexes. More recently, it was established that haptoglobin is also an abundant EC with a chaperone action similar to clusterin. Both ECs are highly conserved, widely distributed glycoproteins that are present in most physiological fluids, including plasma and cerebrospinal fluid [121].

12. Signaling in the Stem Cell Niche

Age-related decrease in stem cell function is partially a consequence of the number of times that stem cell replicates [122] but is also believed to be related to intrinsic and environmental changes in the stem cell niche [123], involving, for example, a decrease of the self-renewal molecule Upd within the stem cell niche and leading to fewer active stem cells [124]. During aging, numerous intrinsic and extrinsic changes occur that result in altered stem-cell behavior and reduced tissue maintenance and regeneration. As an example, in the *Drosophila* testis, aging results in a marked decrease in the self-renewal factor Unpaired (Upd), leading to a concomitant loss of germline stem cells [124]. In a murine model, Guo et al. [125] have shown that stem cells secrete cytokines and

other molecules that enhance cell survival, are antiapoptotic, and stimulate colony formation of hematopoietic progenitor cells. Further, Davey and Zandstra [126] have shown that stem cell autocrine factor gp130 ligand buffers stem cells against differentiation.

Telomere length of cells, including stem cells, is predictive of the cells ability to function properly, including the ability of the stem cell to leave the stem cell niche and home to damaged tissue [127]. Evidence suggests that physical activity preserves or lengthens telomeres, but exhaustive exercise such as that in athletes may shorten the telomeres in skeletal muscle [128]. Moreover, exercise induces autophagy [129], thus clearing the debris from the stem cell niche and allowing the stem cells and the niche to properly interact and therefore enhancing stem cell function. Valero et al. [130] have shown that stem cells release SRM during exercise. Maguire and Friedman [16] have shown that SRM has multiple effects in the body, including pain reduction, tissue regeneration, immune system modulation, and possible telomere lengthening properties. These data suggest that exercise will induce many useful mechanisms that will induce better stem cell function, highly beneficial release of SRM, and a multitude of beneficial downstream physiological effects.

In conclosion, traditional drug development will explore chromatin regulators and transcription factors that affect stem cell maintenance, especially in tumor development, and evidence indicates that such manipulations of adult stem cell function in the aged must be carried out cautiously, given the increased risks of cellular transformation. However, studies on the effect of SRM, exercise, environmental enrichment, and parabiosis on aged stem cells and other cells have revealed that rejuvenation of stem cells and other cells can be achieved without the induction of neoplastic properties.

Elucidating the synergistic or antagonistic roles of different chromatin, cellular, and stem cell niche regulators, such as SRM and S^2RM, and their primary targets and the external signaling pathways responsible for that regulation will be important for restoring regenerative potential to aging cells, adult stem cells, and their niches in a controlled manner. Engineering techniques that enhance and/or mimic the regenerative potential of dormant endogenous stem cells and their SRM will be a promising avenue to prevent and treat a number of age-dependent and immune diseases and other traumas characterized by tissue degeneration, leading to enhanced spontaneous stem cell healing, and lead to what biomedical engineer Charles Lindbergh and his partner, Nobel Laureate Alexis Carrel [131], envisioned long ago in their attempts as early pioneers to regenerate organs.

Conflict of Interests

Greg Maguire and Peter Friedman have an interest in ownership of BioRegenerative Sciences, Inc.

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