

Enhancing spontaneous stem cell healing (Review)

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Abstract. Adult stem cells are distributed throughout the human body and are responsible to a great extent for the body's ability to maintain and heal itself. Accumulating data since the 1990s regarding stem cells have demonstrated that the beneficial effects of stem cells are not restricted to their ability to differentiate and are more likely due to their ability to release a multitude of molecules. Recent studies indicated that $\leq 80\%$ of the therapeutic benefit of adult stem cells is manifested by the stem cell released molecules (SRM) rather than the differentiation of the stem cells into mature tissue. Stem cells may release potent combinations of factors that modulate the molecular composition of the cellular milieu to evoke a multitude of responses from neighboring cells. A multitude of pathways are involved in cellular and tissue function and, when the body is in a state of disease or trauma, a multitude of pathways are involved in the underlying mechanisms of that disease or trauma. 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 disease or tissue damage. Currently, efforts are aimed at defining, stimulating, enhancing and harnessing SRM mechanisms, in order to develop systems-based methods for tissue regeneration, develop drugs/biologics or other therapeutics and enhance the release of SRM into the body for natural healing through proper dietary, exercise and other lifestyle strategies.

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1. Introduction

In the post-genomic era, it is well-known that the phenotypic expression of normal and disease states is rarely predicted by the genome (1) and the genetic make-up of somatic cells may be altered during the lifespan of an individual (2). These phenotypic states reflect a complex set of interactions between the genome and numerous variables, including diet, lifestyle, epigenetics, toxins, non-genetic inheritable transmission and environment (3). Several of the disease phenotypes are currently considered to involve the underlying state of adult stem cells, which are located throughout the body and are involved in the maintenance, regeneration and repair of the majority, or all, human tissues. The pancreas may represent an exception, where the somatic cells themselves replicate (4). As regards aging, although the effects are tissue-dependent, certain tissues exhibit a decline in stem cell numbers during the aging process, while other tissues may exhibit an increase. However, it is well-known that aging is associated with a progressive decline in stem cell function in various tissues (5). This age-related decrease in stem cell function is partially a consequence of the number of times the stem cell replicates (6), although it is also considered to be associated with intrinsic and environmental changes in the stem cell niche (7), such as a decrease of the self-renewal molecule Upd within the stem cell niche, leading to a lower number of active stem cells (8). Thus, during aging, as the environment deviates from the ideal, the gradual decline in stem cell function may lead to an impaired ability of self-healing and may be directly associated with certain diseases (6,9).

2. Multiple stem cell types in healing

Multiple adult stem cell types are located in the majority of tissues in adult humans and reside in stem cell niches within various tissues, in either a dormant or an active state (10). The pool of active stem cells is readily available for tissue repair, regeneration and maintenance. To heal and maintain the various tissues of the body, including the skin (11), multiple stem cell types are often involved (12) and secrete a number of factors into the tissue to induce the healing response (13-15). Thus, stem cells in the human body may migrate to the area of diseased or damaged tissue and two or more stem cell types release their respective stem cell released molecules (SRM) into the damaged tissue (Fig. 1). Each stem cell type releases its own pool of SRM, each pool being a different collection of molecules, with a certain degree of overlap between pools.

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3. A multitude of molecules in healing

The release of factors from stem cells includes several molecules, such as cytokines, growth factors, interleukins, antioxidants, chaperone molecules, exosomes and microRNAs (15). The factors released from stem cells are collectively referred to as SRM, and when the SRM comes from two or more types of stem cells, the collection of molecules is called S²RM, reflecting the synergistic action of the two pools of SRM on the target tissue. The release of SRM is a key method for tissue maintenance, repair and regeneration, including immune modulation functions (15), whereas asymmetric regeneration of adult cells, with some of the replicated stem cells differentiating into mature cells, may be another method for tissue healing and maintenance (16).

4. Slowing the aging process of stem cells

If the human genome is not predictive of health and disease and human life is not predetermined through genetics, we must identify the factors that may be controlled in order to maintain a healthy state and enhance spontaneous stem cell healing. First, the data reported by Toledano *et al* (8) suggested that simply engrafting new or young stem cells into an old environment, such as the stem cell niche of an aged patient, may not lead to the optimal outcome regarding tissue regeneration and age-related conditions. Therefore, the stem cells themselves must be in an active state; however, the cells of the stem cell niche, other than the actual stem cells, must also be in a healthy and 'active' state and able to provide the proper signaling environment to maintain stem cell activity (17).

Telomeres in stem cells, similar to other cell types, are specialized chromatin structures at the ends of eukaryotic chromosomes that prevent the chromosome ends from being recognized as a DNA break. Although the pathways by which short telomeres promote aging have not been fully elucidated, telomere shortening may promote aging by inducing apoptosis and cell cycle arrest, thus leading to cell loss and tissue dysfunction. Furthermore, telomere shortening may impair the ability of stem cells to regenerate tissues, thus leading to tissue failure. It is well-known that shortened telomeres in stem cells impede their mobilization out of the niche, inhibit tissue repair and regeneration and suppress stem cell proliferation (18). Furthermore, stem cell telomeres are shortened with progressing age (18).

Previous studies indicated that exercise, lifestyle and diet may lengthen telomeres and improve stem cell function. Exercise was shown to lengthen or preserve human telomere length (19,20) and combat the effects of stress on telomere length (21). Furthermore, Kadi and Ponsot (22) demonstrated that telomeres in skeletal muscle are dynamic structures affected by their environment. When satellite cells were heavily recruited for regenerative events, as in the skeletal muscle of athletes, telomere length was found to be either significantly shortened or maintained, and was even found to be longer compared to that in non-trained individuals. Evidence suggested that physical activity may preserve or lengthen telomeres, although exhaustive exercise, such as that performed by athletes, may shorten the telomeres in skeletal muscle (23). Moreover, exercise induces autophagy (24), thus clearing the

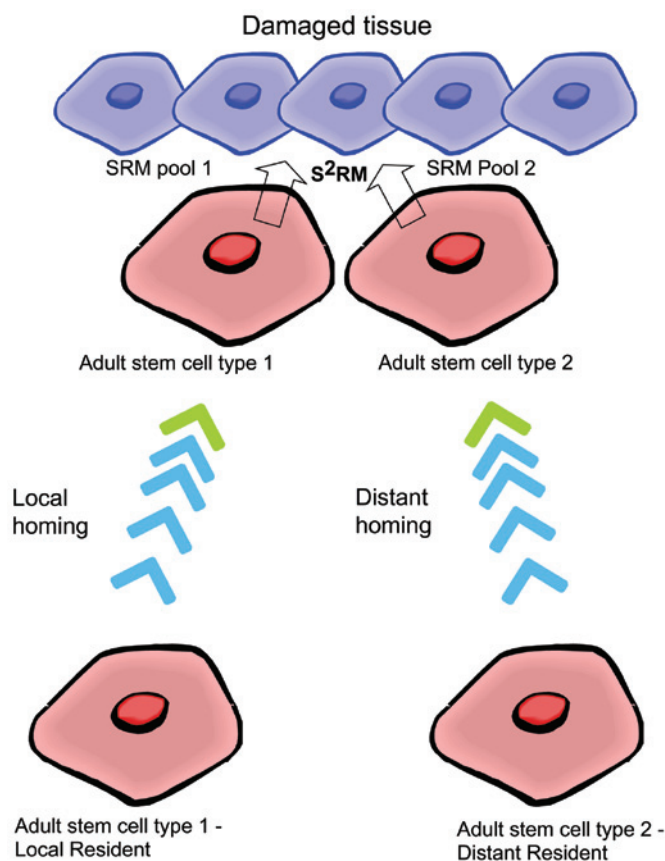


Figure 1. Two stem cell types home into the damaged tissue and release stem cell released molecules (SRM) to induce healing. The SRM from stem cell type 1 is different from that of type 2. The two SRM pools act synergistically and are referred to as S²RM.

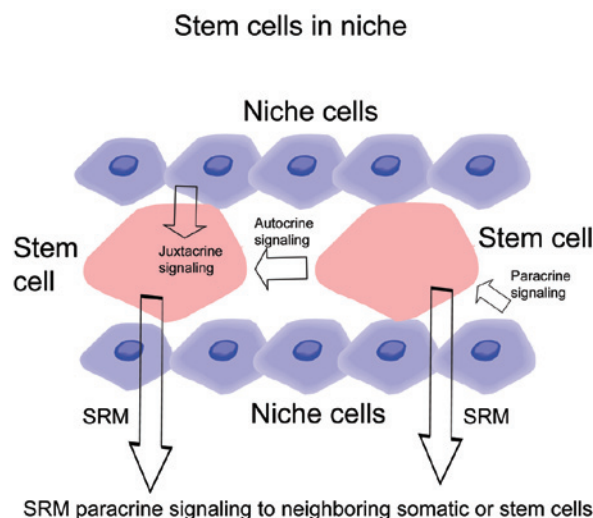


Figure 2. Adult stem cells exist within niches, where a rich interaction occurs between the stem cells and other niche cells, such as endothelial cells and adipocytes. SRM, stem cell released molecules.

debris from the stem cell niche and allowing the stem cells and the niche to properly interact and, therefore, enhance stem cell function. Valero *et al* (25) demonstrated that stem cells release SRM during exercise. Maguire and Friedman (15,26)

also demonstrated that SRM may exert multiple effects on the body, including pain reduction, tissue regeneration, immune system modulation and possible telomere lengthening. Those data suggested that exercise may induce several useful mechanisms that may optimize stem cell function and enable the highly beneficial release of SRM and a multitude of beneficial downstream physiological effects.

Recent data from the Kipnis Lab (27) also demonstrated that the immune system regulates the neurogenic stem cell niche, suggesting that those factors boosting immune function may also boost stem cell-based self renewal. Fig. 2 illustrates some of the key signaling pathways in the stem cell niche. It is crucial to identify the factors that boost immune system function, allowing the proper function of the neurogenic niche, or even of all stem cell niches throughout the body. The answer again lies with exercise, diet and lifestyle. These lifestyle changes may induce autophagy, so that the niche is cleared of debris, thus optimizing stem cell activation and migration from the niche to the damaged tissue. The lifestyle changes may also enhance SRM-mediated paracrine and autocrine signaling and, at least in some stem cell types, is likely to increase juxtacrine signaling (28).

Calorie restriction (CR) may extend the life span and ameliorate age-related pathologies in the majority of investigated species; however, the mechanisms underlying these effects have not been fully elucidated. A number of studies demonstrated that CR acts in part by enhancing the function of tissue-specific stem cells (29). Even a short-term CR may significantly enhance stem cell availability and activity, as observed in muscle tissue (30).

Paneth cells, a key constituent of the intestinal stem cell (ISC) niche, which reside adjacent to stem cells, may augment stem cell function in response to CR. CR acts by reducing mechanistic target of rapamycin complex 1 signaling in Paneth cells and the ISC-enhancing effects of CR may be mimicked by rapamycin (29). In another study, CR was shown to enhance adult stem cell availability and function in muscle, improving the efficacy of recovery from injury and the engraftment of transplanted cells (30). Those studies indicated that metabolic factors are crucial in the regulation of stem cell function and that this regulation may improve the organism's natural spontaneous stem cell healing. The restriction of calorie ingestion, without malnutrition, prolongs lifespan and promotes healthy aging in numerous animal species and may be at least partly attributed to the enhanced stem cell function. Campbell and Campbell (31) reported sound evidence supporting the reduction of protein intake to ~10% of the caloric intake in order to increase lifespan and reduce the risk of disease. Furthermore, Fontana *et al* (32) demonstrated that reduced protein intake reduces the risk of cancer. However, the effect of specific dietary restrictions has not been clearly determined regarding stem cells.

Stress. Stress exerts a number of debilitating effects on health. As demonstrated by a recent animal model of cancer and cancer survival, Rottweilers are prone to cancer and, despite a body riddled with cancerous growths, a certain percentage of Rottweilers live to an old age and die due to causes other than cancer. Furthermore, cancer in these animals tends to develop in distinct regions of the body (33). A recent study by Waters (33) demonstrated that the senior animals expressed

little stress, as revealed by their behavior. Furthermore, the animals exhibiting little behavioral stress also exhibited low levels of cortisol. Low blood levels of cortisol would not have been predicted in these aged dogs based on the literature. Although exhibiting low levels of cortisol, those animals were able to increase their cortisol levels when challenged, which reflects normal adrenal function.

Tumors are characterized by the disordered structure of extracellular matrix (ECM) and unregulated cell growth. Cells present within the tumor interact, not only in an autocrine or paracrine manner, but also with the ECM components. The ECM is a dynamic niche involved in cancer progression (34) and adult stem cells secrete a number of matrix molecules that appear to be important for tissue growth regulation. It is well-known that the stress-induced secretion of cortisol disrupts the ECM (35) and that the SRM may mitigate the effects of stress (36). Furthermore, in the stress pathway, stem cells are inhibited from being activated and released into the damaged tissue (37,38). An important component of the response to stress, the autonomic nervous system, appears to directly or indirectly mediate stem cell function in the stromal tissues in several parts of the body and regulate cancer growth and dissemination (39). Thus, stress likely affects a number of pathways throughout the body, deregulating the ECM. Therefore, stress may be an important contributor to cancer development and progression, as well as other conditions, including disruption of epidermal function (40).

5. Conclusions

Reverse-engineering the endogenous stem cell mechanisms and their control mechanisms underlying tissue maintenance and healing may be instructive for an understanding of lifestyle and therapeutic development that may enhance the maintenance and healing functions of the human body. Traditional drug development is based on the investigation of chromatin regulators and transcription factors that may affect stem cell maintenance, particularly in tumor development, and the evidence indicates that such manipulations of adult stem cell function in aged individuals must be performed with caution, considering the increased risk of cellular transformation. However, previous studies on the effect of exercise, environmental enrichment, SRM and parabiosis on aged stem cells revealed that rejuvenation of stem cells may be achieved without the induction of neoplastic properties.

Elucidating the synergistic or antagonistic roles of different chromatin and stem cell niche regulators, such as SRM and S²RM, and their primary targets, as well as the external signaling pathways responsible for that regulation, is crucial for restoring the regenerative potential of aging adult stem cells and their niches in a controlled manner. The enhancement of the regenerative potential of dormant endogenous stem cells and their SRM may be a promising avenue of prevention and treatment of several age-dependent and immune diseases and other types of trauma that are characterized by tissue degeneration, leading to enhanced spontaneous stem cell healing and resulting tissue healing and regeneration, as envisioned by such early pioneers as the famous aviator and biomedical engineer Charles Lindbergh and his partner, Nobel Laureate Alexis Carrel (41).

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