Tachykinins and hematopoietic stem cell functions: implications in clinical disorders and tissue regeneration

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1. ABSTRACT

Hematopoiesis is the process by which a limited number of hematopoietic stem cells (HSCs) maintain a functioning blood and immune system. In adults, hematopoiesis occurs in bone marrow and is supported by the microenvironment. The tachykinin family of peptides regulates hematopoiesis. Tachykinins can be released in bone marrow as neurotransmitters from innervating fibers, and from resident bone marrow cells. The hematopoietic effects by tachykinins involve four tachykinin genes, *Tac1-Tac4*. The latter is the most recently discovered member and encodes hemokinin-1, endokinin A, endokinin B, and two orphan peptides, endokinin C, and endokinin D. The alteration of normal hematopoietic functions by the tachykinins may result in the development of various pathologies. For example, *Tac1* is involved in myelofibrosis and in leukemia, both of which are dysfunction of hematopoietic stem cells. A comprehensive understanding of dysfunctions caused by the tachykinins requires further research since other cells, such as stromal cells and factors including cytokines, chemokines, and endopeptidases, are involved in a network in which the tachykinins have critical roles. Studies into the properties and functions of tachykinins, the biology of their receptors, and related molecules would provide insights into the development of aging disorders, hematopoiesis, other dysfunction, and may also lead to the discovery of novel and effective clinical therapies. Controversies on applications for hematopoietic stem cells in regenerative medicine are discussed. Despite these controversies, a detailed understanding on how the bone marrow microenvironment maintains pluripotency of hematopoietic stem cells would be useful to manipulate the system to acquire specialized cells for tissue repair.

2. HEMATOPOIESIS: AN OVERVIEW

In adults, the bone marrow is the major site of hematopoiesis. The process of hematopoiesis is explained by the development of immune and other blood cells from a relatively small number of self-renewing hematopoietic stem cells (1). The dynamic processes by which hematopoietic stem cells replenish the immune system designate the adult bone marrow as the major organ of the emerging immune system (2). A hematopoietic stem cell can be committed to common myeloid or lymphoid progenitors (1, 2). Common myeloid progenitors generate cell lineages that develop into dendritic cells, erythrocytes, megakaryocytes, and granulocytes (3). Common lymphoid progenitors mature towards B-, T-, natural killer and dendritic cells (4,5). T-cell development involves a complex process beginning from common lymphoid progenitors to mature pro-T-cells, followed by maturation and selection mainly in the thymus (6-8).

Hematopoiesis is regulated by soluble factors, extracellular matrix proteins, and cell-cell interactions that involve different families of adhesion molecules (Figure 1) (9, 10). Examples of soluble factors relevant to hematopoiesis are cytokines, neurotrophic factors, neuropeptides, and neurotransmitters (11-17). Bone marrow stromal cells are major sources of hematopoietic growth factors and extracellular matrix proteins (9). The stromal cells, through their expression of stromal derived growth factor (SDF-1α) and other molecules, could form cellular interactions with the hematopoietic stem cells and retain them within the bone marrow (9, 18). Bone marrow stromal cells, when established *in vitro*, comprise
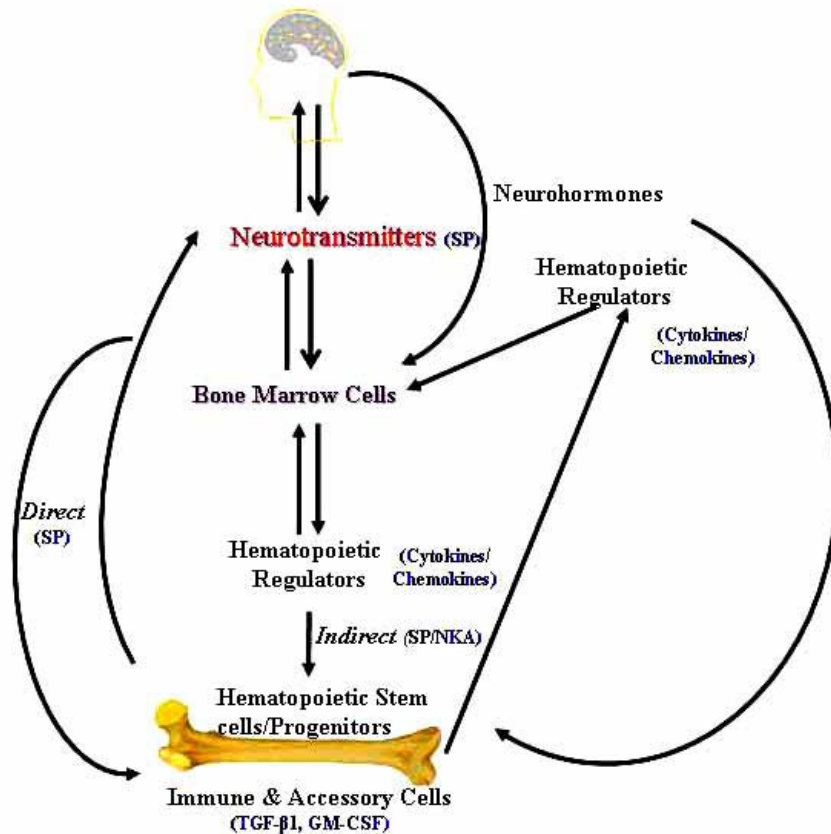
Figure 1. General pathways in the neural-hematopoietic axis. Shown is the brain releasing neurontransmitters, which induce changes in bone marrow (BM) cells such as cytokine/chemokine production. These changes affect brain function through retrograde feedback on the contacting neurons. Neurohormones are shown as another family of regulators that are derived from brain involvement, with hematopoietic effects. Both neurohormones and neurotransmitters are shown to affect hematopoiesis through direct and indirect mechanisms. Direct is described as the release of neurotransmitters that interact with hematopoietic stem cells and/or progenitors. Indirect indicates the induction of hematopoietic regulators (e.g., cytokines) by the neurotransmitter, SP. The molecules shown indicate the factors released/produced at each level of regulation as discussed in the text.

fibroblasts (major subset) and minor subsets: endothelial cells, macrophages, reticular cells, and adipocytes (10). However, the stromal cells, which are fibroblastoid, could be selected as purified cultures for experimental analyses. The stromal cells act as the major support cells for hematopoiesis by instructing lineage commitment and differentiation at various regions within the hierarchy of cell lineages (10). Stromal cells are involved in the protection of hematopoietic stem cells from potential insults by chemical exposures, viral infection, and anoxia (19-21). The stromal cells can respond to various stimuli and produce factors that act through autocrine and/or paracrine mechanisms to regulate hematopoiesis (10).

3. NEUROENDOCRINE-IMMUNE-HEMATOPOIETIC AXIS

Cytokines are considered as prototypic hematopoietic regulators (16). Neuropeptides, neurotransmitters, neurotrophic factors, neurohormones and other hormones regulate hematopoiesis (Figure 1) (22-24).

Although hematopoietic regulators are considered to be functionally redundant, they nonetheless exert specific functions in a particular microenvironment. There is no direct association between a particular family of hematopoietic regulators and function since the hematopoietic function of a growth factor is not `mutually exclusive' of other mediators within the bone marrow microenvironment (14). Rather, different types of hematopoietic growth factors form a complex/interactive network that lead to the ordered biological functions in the bone marrow. Despite extensive research on the roles of cytokines on hematopoiesis, the roles of other families have yet to be unraveled. Detailed understanding on the roles of neurotransmitters, neurotrophic factors, and neurohormones on hematopoietic modulation could have two major contributions to the field: I. Better understanding of the complex network of soluble factors in bone marrow and II. Add to the understanding of the biology within the neuroendocrine-immune-hematopoietic axis (25-38).

The anatomical substrates underlying the neural-hematopoietic axis include the sympathetic and peptidergic

innervation of the bone marrow (27-32). A similar relationship exists for thymus and secondary lymphoid organs (33). Functional studies also support a neuroendocrine-hematopoietic link. Stimulation of sympathetic trunks in rats led to changes in BM cellularity and organ distribution (34). In other studies, altered hematopoiesis has been demonstrated in experimentally induced epileptic seizures; and induction of thymoma (35, 36). These are exciting observations since they show direct links between the brain, tumor development, and hematopoiesis.

In human, hematopoiesis is suppressed in areas of the bone below spinal cord injury (37). We showed evidence for hematopoietic activity in the periphery of patients with spinal cord injury (38). Due to ethical reasons, it is not possible to perform studies using human models in order to understand whether spinal cord injury causes extramedullary hematopoiesis or whether the hematopoietic progenitors are unable to be retained in the bone marrow when the nerve is damaged. The latter is the favored hypothesis since extramedullary hematopoiesis would lead to an enlarged spleen, which has not been reported for spinal cord injured patients. Bone marrow innervation allows for anterograde and retrograde communication within the neural-hematopoietic axis (37-45). The retrograde arm could transport soluble factors and ligand-receptor complexes from the bone marrow to neural bodies (39). The anterograde arm could release neurotransmitters from the autonomic postganglionic endings to the primary and secondary lymphoid organs.

In vitro studies have indicated regulatory effects of neurotransmitters on hematopoiesis (46-50). The relevance for these studies is underscored by the identification of bone marrow nerve fibers that are immuno-positive for different neurotransmitters; and the expressions of their respective receptors on bone marrow cells (38). The neuroendocrine-hematopoietic link could also occur indirectly by neurohormones produced through the hypothalamus-pituitary-adrenal axis. An example of this is shown by the ability of prolactin and adrenocorticotrophin to affect hematopoiesis (51).

4. TACHYKININS AND NEUROKININ (NK) RECEPTORS

The Tac1 gene (also referred as Preprotachykinin-I, or Preprotachykinin-A) is a single copy gene with 7 exons, encoding several evolutionary conserved peptides that belong to the tachykinin family (51-54). The tachykinins share a common sequence in their -COOH termini (52). The *Tac1* transcript is alternately spliced into one of four transcripts: α-, ß-, γ- or δ-PPT-I (55). The major peptides of *Tac1* are substance P and neurokinin A. Substance P is encoded by Exon 3, which is present in each of the four types of transcripts (53). Neurokinin-A, on the other hand, is encoded by Exon 6 and is present on only two transcripts, ß- and γ-PPT-I (56).

Substance P is the most studied peptide among the tachykinins (57).

Substance P is an undecapeptide that is widely distributed in the central and peripheral nervous systems (52). Substance P is released in the periphery as a neurotransmitter, and is also produced from non-neural cells (57). The latter includes immune and bone marrow stromal cells (38, 57). The expression of *Tac1* appears to be tissue-specific. For example, *Tac1* is constitutively expressed in neurons and its peptides are stored in synaptic vesicles (27-29), while its induction in bone marrow stromal cells requires cell stimulation (38). *Tac1* could be induced by different stimuli including cytokines, neurotrophic factors, lippopolysaccharide, glucocorticoids, neuropeptides, and neuronal depolarization (57). Substance P has been reported to regulate its production (58). Whether this self-induction of substance P is directly or indirectly mediated through other soluble factors remains to be determined. Non-neural functions of the *Tac1* peptides include immune and hematopoietic modulation (38). Substance P has a role in T-cell development after the T-cell progenitors have migrated from the bone marrow to the thymus (59). The Tac1 gene has been linked to several clinical disorders (described below). Recently, a third tachykinin gene, *PPT-C* (also referred to as *Tac4*) has been identified. *Tac4* peptides have been grouped under the category of tachykinins (60).

In humans, the *PPT-C* mRNA primary transcript which codes for hemokinin 1 (HK-1) can be alternately spliced to produce the additional tachykinins, endokinin A (EKA) and endokinin B (EKB) and two orphan tachykinin-gene related peptides (EKC and EKD) (61, 62). *Tac4* has 5 exons with exon 2 being the HK-1 coding sequence (62). *Tac4* is primarily expressed in non-neural cells such as hematopoietic cells where its peptides act in an autocrine and paracrine manner to regulate hematopoiesis (62). As compared to the expression of *Tac1* in neural tissues, *Tac4* is mostly expressed in peripheral tissues (61). It has been proposed that *Tac4* produces peptides that are functional substance P agonists (61).

Endokinins have been implicated in the development of T- and B-cell lymphopoiesis, mucosal inflammation, granulomatous responses, and platelet aggregation (61). HK-1 has also been implicated in immune regulation such as plasma extravastion and mast cell degranulation (60). Despite the claim that HK-1 and the endokinins share physiological properties with substance P, there are reports of distinct properties (60). For example, while HK-1 mediates the proliferation of interleukin 7-expanded B cells, and promotes the survival of bone marrow B-lineage cells, substance P has no observed effect on these functions (60). Furthermore, while substance P indirectly affects myeloid proliferation through cytokine production in bone marrow stroma, HK-1 influences pre-B cell survival and proliferation directly through binding to B-cell progenitors (60). Interestingly, the bone marrow cells (70Z/3) used showed no evidence of the three cloned NK receptor subtypes (60). Since, substance P had no effect on the survival and proliferation
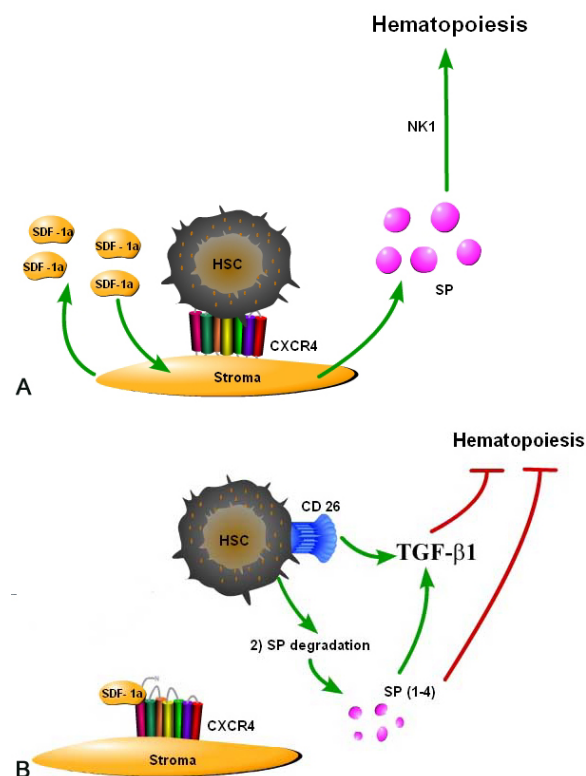
Figure 2. A. Hematopoiesis demonstrated during stimulation. The chemokine, SDF-1α, through CXCR4 receptor, induces the production of SP in stromal cells. SP then stimulates hematopoiesis by inducing the proliferation and differentiation of HSCs and their progenies. B. Negative effects on SP-mediated hematopoietic stimulation. The endopeptidase CD26 degrades SP to the amino terminal fragment, which produces TGF-β1 from stromal cells. TGF-β1 acts as a negative regulator on hematopoiesis (87, 88).

of these B lineage cells and HK-1 did, this may suggest HK-1 to act via another receptor rather than with the current known NK receptors (60).

The tachykinins bind with different affinities to three mammalian G protein-coupled, 7-transmembrane receptors: NK1, NK2 and NK3 (55). Substance P and Neurokinin-A exhibit binding preferences for NK1 and NK2 respectively (55). Tac4 peptides have been shown to bind the NK receptor subtypes, although they show preference for NK1 (61, 62). In addition to binding the NK subtypes, the tachykinins can also interact with non-neurokinin membrane molecules (63). NK1 and NK2 are mostly expressed in peripheral tissues, and NK3 is found mostly in the brain (52). NK1 and NK2 are widely expressed, e.g. mammary epithelial, BM cells (hematopoietic and mesenchymal type) (64). In unstimulated bone marrow stroma, NK1 expression requires cell stimulation, whereas NK2 is constitutively expressed (65). When NK1 is induced by cell stimulation, NK2 is concomitantly down regulated (65). This type of

yin-yang expression between NK1 and NK2 appears to be due to receptor crosstalk (65). We refer to crosstalk as a method by which the expression of one NK subtype affects the expression of the other. NK1 can undergo reversible desensitization with high concentrations and prolonged exposure of the ligand (66). Optimal stimulation of NK1 results in internalization, and consequently, cell activation (66). NK receptors and their ligands are central to the cellular and humoral parts of the complex networks involved in bone marrow biology (57). NK receptors are expressed on cells within hematopoietic lineages (46, 59, 60, 65), indicating that the tachykinins could directly regulate hematopoiesis, in addition to indirect effects through stromal cells (38).

This discovery of new tachykinins combined with experimental data argues for a fourth NK receptor (62). For example, EKC and EKD have weak binding affinity for each NK receptor (62). Similarly, NKB has been shown to induce oedema formation in the mouse lung and liver, independent of the NK subtypes (62). There are alternate explanations that might explain the confounding data. For example, tachykinins might bind different isoforms of a particular subtype (62). In addition, the tachykinins might interact with various conformations of the current NK receptor subtypes and/or molecules mimicking the NK receptors (62, 65, 67) Despite the aforementioned argument, the existence of a fourth NK receptor is still a valid argument (60).

5. TACHYKININS AND STROMAL DERIVED FACTOR-1 α (SDF-1α)

The chemokine SDF-1α acts as a mediator within the neural-hematopoietic axis partly via Tac1 expression (68). SDF-1α interacts with the seven transmembrane G-protein coupled receptor, CXCR4 (68). Under homeostatic conditions, SDF-1α functions as a chemoattractant and induces the binding of hematopoietic stem cells to stromal cells (Figure 2). Recent studies have reported small increases in SDF-1α to induce Tac1 in stromal cells to produce substance P (68). The production of substance P then stimulates hematopoiesis via NK1, underscoring the the important roles of SDF-1α in the neural-immune-hematopoietic axis (68).

Reports indicate that the interactions between stromal cells and hematopoietic stem cells can be affected by the expression of the endopeptidase CD26, also known as dipeptidylpeptidase IV (38). CD26 expression results in downregulation of SDF-1α, which causes hematopoietic stem cells to be released from stromal cells and migrate towards the periphery (Figure 2). Perhaps CD26 may have a negative feedback role by inactivating excess SDF-1α with concomitant degradation of substance P (38). These are interesting findings since the interplay among these cells and molecules might explain the self-renewal properties of hematopoietic stem cells and provide insight into the mechanisms by which the bone marrow maintains homeostasis.

6. CLINICAL RELEVANCE FOR TACHYKININS/NEUROKININ RECEPTORS

Structural similarity between NK1 and fibronectin causes the high affinity ligand for NK-1 to interact with fibronectin (69). This is significant because fibronectin is a component of extracellular matrix proteins in the BM (9). Furthermore, both NK1 and fibronectin are relevant to hematopoiesis (9, 38). Fibronectin-Substance P interaction appears to protect substance P from endogenous endopeptidases (67). Despite this advantage, the protective functions of fibronectin for substance P could lead to pathophysiology as substance P accumulates, as reported for patients with myelofibrosis (64). The link between tachykinins/NK receptors and clinical disorders is underscored in past clinical trials (70). Substance P is linked to inflammation in the periphery, and inflammatory-mediated changes in the brain (71, 72). *Tac1* peptides regulate normal functions in primary and secondary lymphoid organs (57). When the functions of *Tac1* peptides are dysregulated, immune disorders could occur (57). Substance P appears to facilitate viral infections including HIV-1 (73). Substance P/NK receptors are also implicated in malignancies and hematological and solid tumors (62, 74-76). Dysregulated production of substance P and its receptors are linked to Parkinson's, Alzheimer's, other nervous system inflammation, depression, traumatic brain injury, predisposition to anxiety, ischemia of the brain, and epileptic seizures (77-83). We have reported links between surgical trauma-induced head injuries and hematopoietic dysregulation (84). In other studies we have reported hematopoietic and immune dysfunctions in a rat model of kindled epilepsy (35).

Due to the fact that bone marrow continually replenishes the immune system via hematopoiesis, the roles of tachykinins-NK-receptors in hematopoiesis will add a neglected area of hematopoiesis: neuroendocrine-immune-hematopoietic axis. Ultimately, the proposal will be relevant to novel and/or improved treatments for disorders that disrupt the bone marrow, e.g. anemia, myeloma, myelo- and lymho-proliferative disorders. Of note is the report that viral proteins have also been reported to mimic the tachykinins. Virokinin, released from viral-infected cells, shows similarity to tachykinins by immunoreactive methods, and also interacts with the NK1 receptor (85). Hemokinin-1 has been show to regulate lymphopoiesis in both B- and T- cell compartments (60).

A wide body of literature shows compelling evidence that psychological stress leads to immune suppression. Since the hematopoietic system is the site where the immune cells are generated, these studies could open avenues for targeted research with *in vivo* models. Santarelli *et al* reported on a role for the NK1 receptor in anxiety and stress-related responses (81). The authors used robust analyses with NK1 knockout mice and pharmacological agents. The pre-clincial trials suggest therapeutic benefits with NK1 antagonists in brain-associated disorders, which correlate with the animal models that support NK1 as a therapeutic target (86). Ongoing and future clinical trials will determine whether NK1 could be used in central disorders such as anxiety, or as a modulator of hematopoiesis, or bone marrow-resident tumor cells (74). Regardless, experimental evidence indicates that NK1-mediated central effects, whether they are mediated through the hypothalamus-pituitary-adrenal axis, could influence the hematopoietic system.

7. TACHYKININS AND MEDICINE

Like most G-protein coupled receptors, the hematopoietic effects mediated by the tachykinins are partly controlled by a yin-yang relationship in the expressions of two subtypes, NK1 and NK2 on the hematopoietic supporting layer, bone marrow stroma. Based on the hematopoietic functions mediated by the NK receptors, and the cytokine production that resulted from their activation (38), we deduce that the mechanisms by which NK1 and NK2 regulate hematopoiesis might occur at two levels. Firstly, activation of NK1 leads to the induction of GM-CSF. The second messenger signals induced by GM-CSF have the capability of phosphorylating the unactivated NK2. This could lead to minimal activation of NK2, leading to the production of low levels of TGF-β1. This premise is based on previous studies, which showed that NK2 activation induced high levels of TGF-β1 in bone marrow cells (65). The stimulatory hematopoietic effects by high production of GM-CSF, with low levels of the hematopoietic suppressor, TGF-β1 could still cause an increase in hematopoietic activity (65). However, the activity would be balanced by TGF-β1 to prevent abnormally high hematopoietic activity. Given these arguments, we premise that NK1 and NK2 act in concert to cause hematopoietic homeostasis. Secondly, activation of NK2 leads to increased induction of TGF-β1, and reduced production of GM-CSF, consequently dampening hematopoietic activity, but not severe enough to cause bone marrow ablation.

Our research group has been involved in seminal work on the role of tachykinins and hematopoiesis, and coined the term, neural-hematopoiesis axis. However, in the past, skeptics among hematologists have exhibited low confidence in this area. This has led to the detriment of the field, with a lapse in original research. Various laboratories have begun to apply the information gained on the basic biology of tachykinins and hematopoiesis to bone marrow dysfunction. The Tac1 gene has been found to be required for the entry of breast cancer cells into the bone marrow, and its expression is critical to allowing the breast cancer cells to enter the bone marrow at an early period of cancer, long before cancer development (74). In other studies, substance P, the major *Tac1* peptide, is highly produced in leukemia cells and its production could lead to bone marrow fibrosis (87). In cases where substance P levels might be high, increased proliferation of the hematopoietic progenitors is a risk for mutation, which could cause leukemia and myeloproliferative disorders. It is established that psychological stress causes cancer relapse and immune suppression. Thus, the proposed studies are significant to bringing the neural-hematopoietic axis towards an understanding of the development of hematopoietic stem cell disorders. The cartoon, shown in Figure 1, summarizes

the salient points discussed in this brief review. Neurotransmitters are shown to be released from neural and non-neural sources. Although the cartoon highlights bone marrow cells (BM), neurotransmitters can be produced by immune and other cells (38). In addition to neurotransmitters, the brain could also initiate the production of neurohormones, through the hypothalamus-pituitary-adrenal axis. Both of these brain-derived families of molecules can affect hematopoietic functions through direct and indirect mechanisms. Direct effects could occur by interactions between the neurotransmitters and their cognate receptors; indirect binding could occur through the release of hematopoietic growth factors, such as cytokines and chemokines, which could regulate hematopoietic functions.

8. CONCLUSION

As discussed above, hematopoietic stem cells have the ability to home from the bone marrow to the periphery. Current research and translational studies are involved in taking advantage of the ability of stem cells to home to areas of injuries with the intent to repair tissues. If these studies are successful, then the phenomenon of homing by hematopoietic stem cells may have a prominent role in stem cell therapy. For example, in a case where a patient with myocardial infarct is brought to an emergency room, time is a crucial factor that determines outcome. The desire is to stop further cardiac tissue death and possibly halt cardiac arrest. If therapies allow for attraction of hematopoietic stem cells to the damaged heart to halt and/or repair damage, this would be a rapid and non-invasive method to treat this type of pathology as compared to current therapies. Even though hematopoietic stem cells have been implicated in the formation of cell other than immune and blood cells, their plasticity is not fully understood and is currently a controversial issue. Further research is needed to understand the communication between hematopoietic stem cells and their microenvironment to bring this type of therapy to translation. In the case of their use in cardiac repair, detrimental effects may result if undesired tissue growth occurs in the damaged region.

The potential use of hematopoietic stem cells in medicine and aging is not exclusive of tissue repair. Expansion of hematopoietic stem cells, which continues to elude current research, would provide cheaper and more efficient approaches than the current method of harvesting and transplantation of hematopoietic stem cells. This would eliminate several problems and hazards faced by potential recipients and donors by current methods. With further research on hematopoietic stem cells and an understanding of regulation by cytokines and the microenvironment, scientists may be able to take advantage of the intrinsic and extrinsic properties of hematopoietic stem cells for effective use in clinical applications, and also to understand how hematopoietic dysfunctions could occur.

9. ACKNOWLEDGEMENT

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