Life Sciences 232 (2019) 116598

Contents lists available at ScienceDirect

Life Sciences

journal homepage: www.elsevier.com/locate/lifescie

Review article

Strategies for elevating hematopoietic stem cells expansion and engraftment capacity

Mehdi Derakhshani[a,b], Hossein Abbaszadeh[a], Ali Akbar Movassaghpour[a], Amir Mehdizadeh[c], Majid Ebrahimi-Warkiani[d], Mehdi Yousefi[e,f,g,*]

[a] Hematology and Oncology Research Center, Tabriz University of Medical Sciences, Tabriz, Iran
[b] Student Research Committee, Tabriz University of Medical Sciences, Tabriz, Iran
[c] Endocrine Research Center, Tabriz University of Medical Sciences, Tabriz, Iran
[d] School of Biomedical Engineering, University Technology of Sydney, Sydney, New South Wales, 2007, Australia
[e] Stem Cell Research Center, Tabriz University of Medical Sciences, Tabriz, Iran
[f] Aging Research Institute, Tabriz University of Medical Sciences, Tabriz, Iran
[g] Department of Immunology, Faculty of Medicine, Tabriz University of Medical Sciences, Tabriz, Iran

ARTICLE INFO

Keywords:
Hematopoietic stem cells
Mesenchymal stem cells
Microfluidic system
Expansion

ABSTRACT

Hematopoietic stem cells (HSCs) are a rare cell population in adult bone marrow, mobilized peripheral blood, and umbilical cord blood possessing self-renewal and differentiation capability into a full spectrum of blood cells. Bone marrow HSC transplantation has been considered as an ideal option for certain disorders treatment including hematologic diseases, leukemia, immunodeficiency, bone marrow failure syndrome, genetic defects such as thalassemia, sickle cell anemia, autoimmune disease, and certain solid cancers. Ex vivo proliferation of these cells prior to transplantation has been proposed as a potential solution against limited number of stem cells. In such culture process, MSCs have also been shown to exhibit high capacity for secretion of soluble mediators contributing to the principle biological and therapeutic activities of HSCs. In addition, endothelial cells have been introduced to bridge the blood and sub tissues in the bone marrow, as well as, HSCs regeneration induction and survival. Cell culture in the laboratory environment requires cell growth strict control to protect against contamination, symmetrical cell division and optimal conditions for maximum yield. In this regard, microfluidic systems provide culture and analysis capabilities in micro volume scales. Moreover, two-dimensional cultures cannot fully demonstrate extracellular matrix found in different tissues and organs as an abstract representation of three dimensional cell structure. Microfluidic systems can also strongly describe the effects of physical factors such as temperature and pressure on cell behavior.

1. Introduction

Hematopoiesis is a continuous process which contributes to the production of all blood cells in the bone marrow, maintaining controlled differentiation and self-renewal features of HSCs, and the migration of mature cells into the bloodstream [1–4]. Hematopoietic stem cells (HSCs), as a rare cell population in adult Bone Marrow (BM), demonstrate a self-renewal capability and differentiation potentials into a full spectrum of blood cells. Common sources of HSCs are bone marrow, mobilized peripheral blood (MPB), and umbilical cord blood (UCB) [5]. Phenotypically, these cells show a $CD34^+$, $CD38^-$, $CD90^+$, Lin^-, and $CD45RA^-$ pattern as surface markers [6].

In patients lacking a human leukocyte antigen-matched marrow donor, human umbilical cord blood (HUCB) has been clinically considered as an alternative source of HSCs for allogeneic transplantation. The main advantages of UCB over stem cells are the relatively ease of procurement, the absence of donor risk, significantly reduced risk of infection transmission, and the rapid availability of the sample [7]. Ex vivo proliferation of the cells prior to transplantation has been introduced as a potential solution for low stem cell numbers. Extensive researches have been performed to determine the optimal conditions critical for ex vivo HSCs expansion, leading to various expansion techniques development [8,9]. Human bone marrow stem cell expansion in ex vivo cultures have been reported to have significant applications in transplantation, stem cell marking, and gene therapy [10]. In this review, specific HSC expansion techniques will be discussed which presents useful data for improvement of HSC engraftment and reducing related economic burden.

* Corresponding author at: Department of Immunology, School of Medicine, Tabriz University of Medical Sciences, Tabriz, Iran.
E-mail address: Yousefime@tbzmed.ac.ir (M. Yousefi).

https://doi.org/10.1016/j.lfs.2019.116598
Received 18 May 2019; Received in revised form 22 June 2019; Accepted 23 June 2019
Available online 25 June 2019

2. Mesenchymal stem cells (MSCs)

MSCs were first identified by Alexander Friedenstein [11]. These cells can be isolated from various sources including bone marrow, peripheral and umbilical cord blood, umbilical cord tissue, amniotic fluid, adipose tissue, synovium, skin, dental pulp, placental complex, and endometrium [12,13]. In 2006, the Mesenchymal and Tissue Stem Cell Committee of International society cell and gene therapy (ISCT) proposed a set of minimal criteria in order to standardize a current working definition for MSCs. First, MSCs should be plastic-adherent when maintained in standard culture conditions. Second, MSCs must express CD105, CD73, and CD90, and lack the expression of CD45, CD34, CD14 or CD11b, CD79 or CD19, and HLA-DR surface molecules. Third, MSCs must be differentiated into osteoblasts, adipocytes, and chondrocytes, in vitro [14]. MSC requires specific conditions to differentiate into osteoblasts and chondrocyte. TGF-β is a cytokine which inhibits MSCs differentiation into osteoblasts. In this regard, trombospondin-1(TSP-1) is essential for TGF-β activation. Kimberly Bailey Du Bose et al. [15]. have shown that following TSP-1 inhibition, TGF-β activation is reduced and MSCs differentiation to osteoblast is increased. TSP-1 also enhances MSCs proliferation and migration by PDGF protection against MSCs-derived protease [16]. Trombospondin-2(TSP-2) is also expressed on MSCs which exerts chondrogenic effects. TSP-2 leads to the differentiation of MSC into chondrocyte through P38MAPK, PKC, ERK and NOTCH signaling pathways [17]. The primary source of trombospondin production is mesenchymal stem cells. K.D. Hankenson et al. [18] Have shown that TSP-2 addition to MSCs derived from TSP-2 Null murine reduces proliferation of MSCs. MSCs possess high capacity for the secretion of soluble mediators, which contribute to the principle biological and therapeutic activities. MSCs secrete stromal derived factor-1 (SDF-1) [19], which plays a critical role in HSCs marrow niche homing [20]. MSCs constitutively secrete the IL-6, IL-7, IL-8, IL-11, IL-12, IL-14, IL-15, macrophage colony stimulating factor (M-CSF), Flt3 ligand, and stem cell factor (SCF), in vitro. MSCs can also be induced to further express IL-1α, leukemia inhibitory factor (LIF), granulocyte colony-stimulating factor (G-CSF), and granulocyte–macrophage colony-stimulating factor (GM-CSF) following IL-1α stimulation [21]. Moreover, several chemokine ligands can also be secreted by MSCs including CCL2, CCL4, CCL5, CCL20, CX3CL1, and CXCL8 [22]. Mesenchymal cells determine HSC fate through cell-to-cell interaction and cytokine secretion, increasing HSCs self-renewal and proliferation properties [23,24]. These cells also represent immunemodulatory properties reducing the incidence of graft versus host disease (GVHD) [25].

3. Niche

The niche concept was first coined by Schofield in the 1978 [21]. It refers to the in vivo microenvironment of bone marrow, which provides significant prerequisite place for self-renewal, differentiation, and survival of HSCs [26]. HSCs are located within the bone marrow microenvironment or niche, which regulates the quiescence, proliferation and differentiation of these cells [27]. Chemical signals such as binding and secretive molecules and physical signals like oxygen tension, shear stress, contractile forces and temperature originated from surrounding cells can modulate the HSC features like survival, differentiation and self-renewal [3,28,29].

4. Perivascular niche

The bone marrow contains a vast network of sinusoidal vessels [30]. The vascular niche is in proximity to vascular endothelium. Cellular constituents of this niche are nestin$^+$ MSCs, *Lepr*-expressing perivascular stromal cells, sympathetic neurons, and CXCL12$^+$ adventitial reticular cells (CARs) in addition to endothelial cells [31–34] Fig. 1.

4.1. The role of the cells present in the Perivascular niche

4.1.1. Endothelial cells

Covering the luminal surface of the blood vessels, endothelial cells bridge the blood and sub tissues in the bone marrow, as well as, regeneration induction and HSCs survival [35,36]. Endothelial cells are not homogeneous in their morphology, gene expression, antigen composition distribution, and function [33]. These cells are surrounded with pericytes or CXCL12 adventitial reticular cells (CAR cell) including nestin+ CAR cells, as specialized pericytes [37]. Endothelial cells and hematopoietic cells originate from hemangioblast as a common multi potential precursor source [38,39]. Furthermore, cultivated endothelial cells isolated from non-hematopoietic organs can also maintain stem cells [40]. These cells have been proposed to support the hematopoiesis procedure by expression of surface markers including *E*-Selectin and upregulation of 'angiocrine' factors such as fibroblast growth factor 2 (FGF2), delta-like 1 (DLL1), insulin-like growth factor-binding protein 2 (IGFBP2), angiopoietin 1 (ANGPT1), desert hedgehog (DHH) and epidermal growth factor (EGF) [36,39,41,42].

4.1.2. Pericytes

Pericytes which are located in pericytic position adjacent to CAR cells, cover the wall of blood vessels [43] and can be identified by their location, as well as, surface markers such as platelet-derived growth factor receptor (PDGFR), CD146, neuron-glial 2 (NG2), SMA, desmin, and Nestin [44]. These cells are divided into sinusoidal and arterial subgroups, which can be detected in mice regarding the level of NES-GFP expression [45]. Significant reduction in HSCs number is the direct result of in vivo ablation of pericytes in bone marrow [32]. HSCs maintenance in the BM has been suggested to be regulated by leptin-receptor expressing pericytes [46].

4.1.3. CXCL12$^+$ adventitial reticular cells

CAR cells, closely related to a CD146$^+$ adventitial reticular cell, are associated with sinusoidal endothelium [32] and are responsible for significant roles in HSCs homing through CXCL12 production. FOXC1 transcription factor has been recently identified in CAR cells which plays an important role in HSCs maintenance, in vivo [47]. It has been reported that selective destruction of CAR cells can also reduce HSCs count [31].

4.1.4. Nerves

The fate of HSCs can also be modulated by sympathetic nervous system producing catecholamine, which is transmitted into the bone marrow microenvironment via nerve endings or the bloodstream to induce paracrine effects on HSCs [48]. The sympathetic nervous system is not involved in HSCs number maintenance, however, they influence HSCs mobilization [33]. Adrenergic signaling contributes in reduced CXCL12 synthesis, which facilitates the HSCs mobilization into peripheral blood [34,49].

5. Endosteal niche

The endosteal niche is characterized by trabecular or cortical bone close position. After transplantation, the adjacent to endosteum is one of the primary sites for HSC homing [50]. The endosteal niche includes certain essential cellular components such as osteoblast, osteoclast, and Nestin$^+$ MSCs [32,51–53].

5.1. The role of the cells present in the Endosteal niche

5.1.1. Osteoblasts

Osteoblasts along with osteoclasts regulate bone formation and resorption, respectively, within the bone marrow niche where pre-osteoclasts induce osteoblasts retraction [54]. Osteoblasts support the HSCs growth and regulate their numbers [51,52]. The interactions

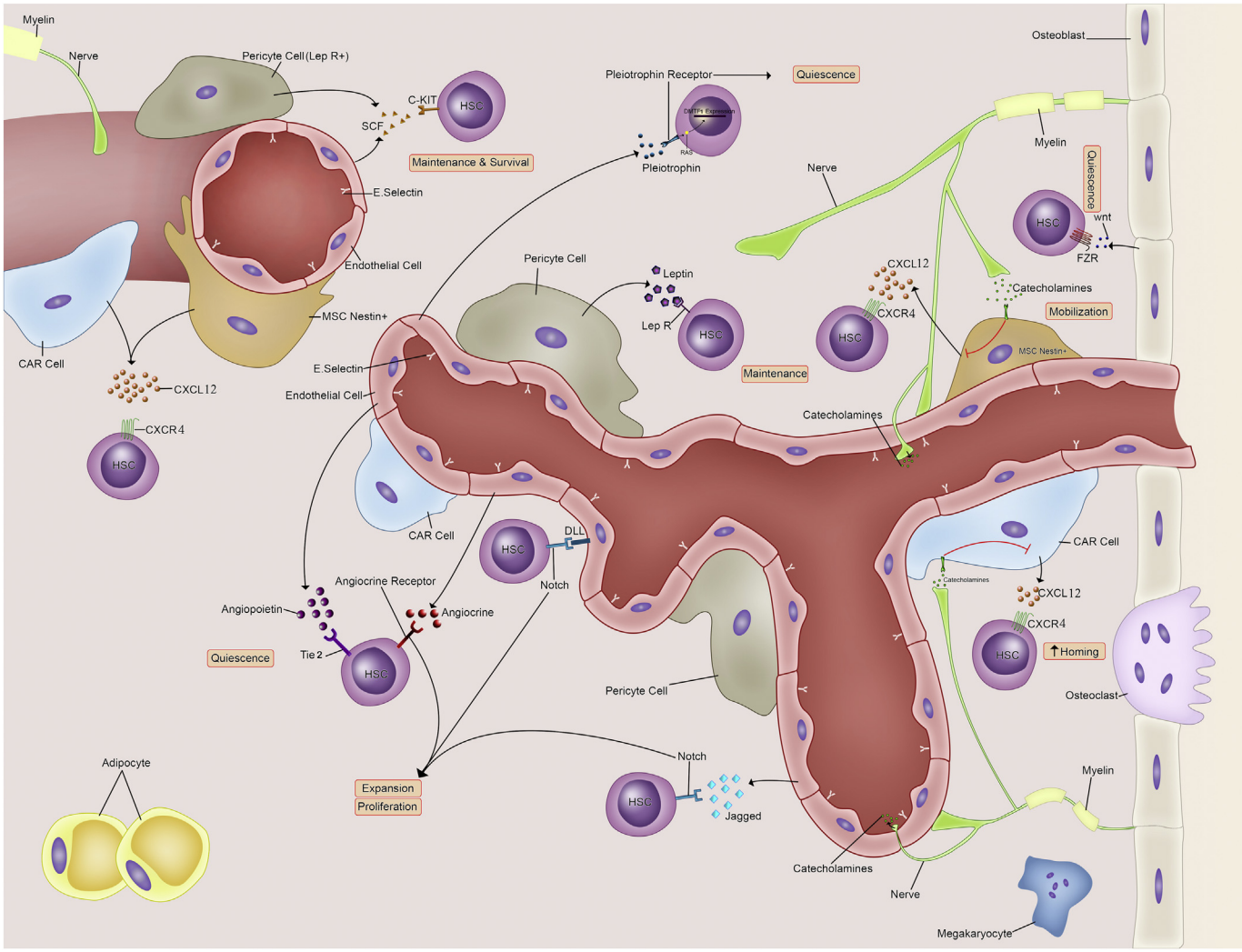

Fig. 1. Demonstration of the cells involved in HSC fate maintenance and regulation. Different cell types are involved in maintenance and regulation of HSC fate, including endothelial cells, CAR cells, nerves, pericyte cells, and mesenchymal stem cells, which control the HSC fate through production of cytokines and chemokines such as CXCL12, SCF, regulating factors (pleiotrophin, angiopoietin), and notch and WNT signaling pathways. Sympathetic nerves decrease CXCL12 production and consequently increase HSC proliferation through the released norepinephrine from nerve endings and their attachment to receptors on the HSC surface. CAR cells along with MSCs induce increased HSC homing via CXCL12 production. Pericyte cells play important role in HSC maintenance through leptin production and its attachment to leptin receptors on HSCs. By jagged and DLL production, which are the corresponding notch ligands on HSCs, endothelial cells contribute to increased HSCs proliferation. Moreover, endothelial cells induce HSC quiescence phase by angiopoietin 1 production and its attachment to Tie2. Endothelial cell and pericytes induce increased maintenance and survival of HSCs by secretion of SCF and its bonding to C-Kit on the surface of HSCs. Osteoblasts also induce quiescence phase in HSCs through Wnt production and attachment to HSC frizzele receptors. Pleiotrophin, produced by endothelial cells also induces quiescence phase in HSCs through RAS signaling pathway and activation of DMTF1 transcription factor. HSC: Hematopoietic stem cells, CAR cell: CXCL12+ adventitial reticular cells, MSC: Mesenchymal stem cell, DLL: Delta like Ligand, SCF: Stem cell factor or Kit Ligand, FZR: frizzele receptor, LepR :Leptin receptor, CXCR4: C-X-C chemokine receptor type 4, CXCL 12 : C-X-C chemokine Ligand 12.

between HSCs and N-cadherin+ osteoblasts are dependent to the stem cell quiescence regulation by Angiopoietin-1/Tie2 tyrosine kinase receptor and increase of HSC numbers through Jagged 1/Notch signaling [51,52,55]. Several studies have reported that osteoblasts are essential for stem cell number maintenance, so that, the loss of these cells results in the decreased numbers of HSCs. [51,52,56]

5.1.2. Osteoclasts

Osteoclasts are polykaryons derived from monocyte/macrophage lineage in the hematopoiesis process as the exclusive bone resorptive cell [57]. Bone-resorbing osteoclasts significantly contribute to stress-induced mobilization of HSCs via matrix metalloproteinase-9 (MMP-9) and CXCR4 dependent mechanisms. Defective HSC niche with decreased osteoblastic differentiation and relatively increased mesenchymal progenitor proportion has been reported in an impaired osteoblast activity mouse model [58].

6. Hematopoietic stem cell expansion

Bone marrow HSC transplantation has been considered as an ideal option for certain disorders treatment including hematologic diseases, leukemia, immunodeficiency, bone marrow failure syndrome, genetic defects such as thalassemia, sickle cell anemia, autoimmune disease and certain solid tumors [59]. Either the patient's own HSCs (autologous transplant) or HSCs derived from a donor with matched human leukocyte antigen (HLA) (allograft transplant) can be used as the cell sources for transplantation [60,61]. The cells used for HSCT can be isolated from different sources such as bone marrow, umbilical cord blood, and mobilized HSCs to peripheral blood through granulocyte-colony stimulating factor (G-CSF) induction [62].

The precise balance between different cell fates (quiescence, self-renewal, differentiation, apoptosis, and migration) acts as the determinant for HSC numbers in vitro and in vivo. The number of HSCs are strictly correlated with successful engraftments, as well as patient survival. The required number for a successful transplantation is $\geq 2.5 \times 10^7$ cell/kg human body weight [63]. Insufficient numbers of HSCs might cause impediment in successful transplantation. Extremely low frequency of HSCs in corresponding organs in some patients is a major problem along with their applications in transplantation. Therefore, HSCs must be expanded ex vivo in such cases. Moreover, the number of HSCs in umbilical cord blood is not enough for successful transplantation in adult population and they should also be expanded in vitro [64,65].

7. HSCs expansion strategies for transplantation

7.1. HSC co-culture

HSC fate can be modulated by bone marrow niche cells. In vitro HSCs culture conditions are mostly associated with differentiation or apoptosis. An increased expansion of hematopoietic cells has been observed in HSCs and different stromal cells, MSCs and endothelial cells co-culture [66–69]. Mesenchymal stem cells can improve HSC expansion by modulating cell-to-cell communications and secretory cytokine production [70–72]. It has been demonstrated in phase I [73] clinical trials that the co-culture of HSCs with mesenchymal stromal cells provides safe condition for transplantation, as well as, increased HSCs expansion rate along with faster Neutrophil and platelet recovery compared to the non-prior co-cultured HSCs [73]. Human fetal liver sinusoidal endothelial cells are the other cells used for HSC expansion in co-culture systems which are engineered to express adenoviral E4 or F1 genes (hFLSECs-E4orF1). Huilin et al. [74] demonstrated that co-culture of umbilical cord blood CD34+ HSCs with hFLSECs-E4orF1 culminates with 3.15-fold increase in expansion of CD34+ cells compared to control group. Yi Lou et al. [75] also reported the role of macrophages in fate determination of HSCs. M2 macrophages co-culture with umbilical cord blood CD34+ HSCs has resulted in 3.8-fold increase in the number of CD34+ cells compared to control group.

Osteoblasts-derived MSCs are also implemented in co-culture systems for expansion of hematopoietic stem cells. Mathew et al. [76] indicated that M-OST cells co-culture with CD34+ HSCs, will result in 3.7-fold increase in the number of CD34+ cells. It was also demonstrated that hCB CD34+ co-culture with M-OST leads to elevated levels of CXCR4 expression in CD34+ cells compared to control group. This process uses the wnt β-catenin signaling pathway.

Moreover, Kylie Mei Young et al. [77] reported a 6-fold increase in CD34+ cells number in the 7th day of culture after specific human liver-derived cell population with CD_{34}^{Lo} and CD_{133}^{Lo} surface markers co-culture with CD_{133}^{hi} and CD_{34}^{hi} HSCs, compared to the control group. Furthermore, they demonstrated that FL CD_{133}^{lo} and CD_{34}^{lo} cells increase the $CD34^+$ HSC number through production of soluble factors like SCF, IGF2, CXCL12, and AngPTLS.

Mkirre gene is the homologue of kirre gene in CP9 cells of mice that support HSCs in mice bone marrow. mkirre downregulation has led to abolished supporting capacity of these cells. Mutiur Rehman khan et al. [78] in a study also transferred mkirre gene, which encodes a type I transmembrane protein, into AFT24 cells and generated AFT24-hkirre cells. Direct co-culture of these cells with HSCs showed an almost 2.5 fold increase of $CD34^+CD38^-$ cells number in comparison to the control group Table 1.

7.2. Notch ligands

Notch signaling cascade is considered as an important pathway in lymphopoiesis and HSCs fate determination [79]. This pathway imposes positive effects on HSCs self-renewal under stressed hematopoiesis, however, no effects have been imposed in steady conditions [8]. Notch ligands include DLL family and Jagged which activate the notch signaling pathway, promoting HSCs ex vivo expansion [80]. Notch ligand related effects in HSCs are dose dependent. The expansion of HSCs in human cord blood is stimulated by Delta 1 under the low dosage condition, while, greater doses of a similar factor induces apoptosis [67].

7.3. Wnt signaling and CHIR99021

Wnts are secretory lipoproteins which bind to frizzele receptors [81]. Wnt signaling plays essential roles in modulating HSCs and keeps them in quiescence phase [82]. Similar to notch ligands, wnt signaling effects are also dose-dependent, so that, HSC expansion is induced in lower doses, while, increased doses result in HSCs exhaustion [83]. Soluble wnts such as wnt3a and wnt5a also elevate HSC activity. It has been reported that LT-HSCs treatment with Wnt5a in ex vivo cultures results in HSC implantation capacity loss [84,85]. In contrast, glycogen synthase kinase 3B (GSK3B) inhibitor treatment, which activates wnt signaling pathway, also induces human HSC engraftment and expansion in xeno-grafted mice [86]. Umbilical cord blood HSCs and osteoblasts-derived MSC co-culture can also culminate the HSCs expansion through wnt and β-catenin signaling pathways, in vitro [87]. CHIR99021 is a GSK3B inhibitor which induces the B glycosidase overexpression and *wnt* pathway activation along with embryonic stem cell (ESC) self-renewal ability support [88,89]. Hung et al. [89], have also reported that the administration of M-TOR pathway (CHIR99021) and GSK3B (Rapamycin) inhibitors in ex vivo HSCs culture using cytokine free medium, results in LT-HSC functions maintenance. It has also been reported that CHIR99021 and Rapamysin combination provokes cell cycle without apoptosis induction.

7.4. Sonic hedgehog (Shh)

Shh protein can impose positive effects on human HSCs proliferation and regeneration in SCID/NOD mice [67]. It has also been indicated that inhibition of GSK3B can modulate certain pathways including shh, which induces elevated regeneration [90]. Shh induced expansion is dependent on BMP4, which is the downstream pathway for shh, and its elimination inhibits such expansion [8]. BMPs are members of transforming growth factor β (TGFβ) superfamily that negatively regulate HSCs in mice endosteal niche [91]. Human HSCs via BMP receptors expression improve the HSC maintenance and proliferation [92,93]. A lower dose of TGFβ2 has been reported to induce the Lin-, sca-1^+ and Kit^+ cells proliferation in C57BL/6 mice [94,95]. According to Nakauchi et al. [85] findings, unmyelinated Schwann cells-derived TGFβ is necessary for HSCs hibernation maintenance.

7.5. Fibroblast growth factor (FGF)

Mice bone marrow analysis has revealed the expression of FGF receptor on every long term mouse bone marrow HSCs [96]. FGF1 and FGF2 supplementation in serum free culture medium of inseparable mice osteoblasts also support the HSCs expansion and regeneration [96–98]. Additionally, FGF receptor derivatives have been used for expansion and survival support of both short and long term HSCs in culture medium [99].

7.6. Insuline-like growth factor (IGF) and Parathyroid hormone (PTH)

Cassellia et al. [100] suggested IGF1 as a completely essential factor for osteoblasts-medoated endosteal niche formation. IGF1 gene knock-downing or IGF1 inhibition will prevent endosteal niche formation and HSC engraftment. IGF is also a downstream molecule of PTH which plays important roles in the HSC expansion regulation.

PTH through osteoblasts survival and activation plays an important

Table 1
Various methods used for Human/Murine HSC expansion.

Methods	Effects	Mechanism	Source of HSC	Reference
Co-culture with mesenchymal stromal cell	• Homing induction • Self-renewal induction and expansion of HSC	• SDF1 expression • Cell-to-cell interaction, cytokine secretion, wnt and β-catenin signaling pathways	• Hu CB HSC	• 70,72,73
Co-culture with hFLSECs-E4 or F1	• Expansion of the HSC	• Unknown	• Hu CB HSC	• 74
Co-culture with M2 macrophages	• Expansion of the HSC	• Unknown	• Hu CB HSC	• 75
Co-culture with M-OST cell	• Expansion of the HSC • Induction of homing	• Wnt and β-catenin signaling pathways • expression of CXCR4	• Hu CB HSC	• 76
Co-culture with FL CD34^{lo},CD133^{lo}	• Expansion of the HSC • Induction of self- renewal	• Production of SCF, IGF2, CXCL12, and AngPTLS.	• Hu CB HSC	• 77
Co-culture with AFT24-hkirre cells	• Expansion of the HSC • Induction of self- renewal	• Unknown	• Hu CB HSC	• 78
Treatment with Notch ligands	• Positive effects on self-renewal under stress hematopoiesis • Promoting ex vivo expansion	• Notch signaling pathway	• Human bone marrow CD34+ Lin-,CD38-	.,80
Activation of *Wnt* signaling	• Induction of engraftment and expansion	• GSK3B inhibitor treatment	• Hu CB HSC	0.82,83
Administration of CHIR99021	• Induction of self- renewal and maintenance of LT-HSC functions	• Inhibition of GSK3B and M-TOR pathway • Inhibition of β glucosidase	• LSK Cell(Murine)	. 89
Treatment with *SHH*	• Induction of elevated regeneration • Expansion of the HSC	• Modulating of SHH pathway using GSK3B inhibitor through BMP4 pathway	• LSK Cell(Murine) • Human bone marrow CD34+ Lin-,CD38-	• 90 0.92,93
FGF1 and FGF2 supplementation	• Supported expansion and regeneration of HSC	. Unknown	• LSK Cell(Murine)	• 96–99
PTH treatment	• Enhancement of mobilization from bone marrow into peripheral blood • Improved implantation capacity • Induction of homing	• SDF1 expression on osteoblasts increased levels of cytokines such as IL6, IL11, GM-CSF, and C-kit	• LSK Cell(Murine)	• 97.98,103
Administration of serum free culture systems with certain cytokines and Anti LILRB2	• Expansion of the HSC	• Unknown	• Hu CB HSC	• 107
Treatment with Pleiotrophin	• Induction of HSC quiescence phase	• Up-regulation of RAS/MEK signaling	• LSK Cell(Murine) • Hu CB HSC	• 110,111
Treatment with fucosylation enzymes	• Induction of homing and engraftment	• Induction of fucosylation levels on selectin ligands	• Hu CB HSC	• 116
Complement component (C3a)	• Induction of homing and engraftment	• Induction of CXCR4 expression on HSC membrane	• Human bone marrow CD34+ Lin-,CD38-	• 117,118
Valproic acid	• Induction of HSC expansion	• Inhibition of Histon de acetylation	• Hu CB HSC	• 121–123
Garcinol	• Expansion of the HSC	• Inhibition of p53 acetylation	• Hu CB HSC	• 120
HOXB4 overexpression or direct injection	• Expansion of the HSC	• Unknown	• LSK Cell(Murine)	• 121
Administration of Valporic acid and neurotrophic factor	• Induction of proliferation	• Epigenetic modification	• Hu CB HSC	• 124
Administration of 5aza and TSA	• Expansion of HSC and induction of self-renewal	• Inhibition of DNA methyl transferase • Expression of HOXB4, BMi1, and GATA2	• Human CD34+ HSC	• 125,126
Treatment with PI-C	• Induction of proliferation	• Down regulation of mtln4	• LSK Cell(Murine)	• 131
C7 administration	• Expansion of the HSC	• Unknown	• Hu CB HSC	• 132
Administration of Sb203580	• Expansion of HSC and induction of self-renewal	• Inhibition of P_{38}MAPK pathway	• LSK Cell(Murine)	• 133,134
Administration of Caspase inhibitors like ZVADFMK and ZLLYFMK	• Expansion of HSC with myeloid cell lineage differentiation	• Inhibition of Caspase and Calpain related apoptosis • Induction of BCL-2 expression • Reduction of caspase 3 expression	• Hu CB HSC	• 150,151
TEPA administration	• Ex vivo increasing expansion of HSC	• Cupper elimination • Reduction in the activity of cytochrome oxidase	• Hu CB HSC	• 153,154

(*continued on next page*)

Table 1 (*continued*)

Methods	Effects	Mechanism	Source of HSC	Reference
HSC culture in the present of cytokines and NAM EX-527	• Inhibition of differentiation and improvment of transplantation	• Inhibition of SIRT1 and NAD-dependent ribosyl transferases • Reduction of p21 expression • Inhibition of SIRT1	• Hu CB HSC • Hu CB HSC	• 155,156
Treatment with *PGE2*	• Induction of proliferation and self-renewal	• Induction of homing, proliferation, and survival genes expression	• Hu CB HSC • LSK Cell(Murine)	• 162, 164
Treatment with TPO	• Expansion of HSC and induction of self-renewal	• Induction of HOXB4 gene transcription through expression of USFI	• LSK Cell(Murine)	• 169,170
Administration of AHR antagonist (SR1)	• Expansion of HSC and induction of homing	• Induction of CXCR4	• Human Mobilized peripheral blood CD 34+ Cells	• 176,177
Co-administration of UM171 with SR1	• Expansion of HSC • Enhancement of differentiation of pluripotent stem cells to HSC	. Unknown	• Human CD34+,CD43+ HSC	• 179,180
EDAG inhibition	• Reduction in the numbers of G1 phase HSCs, induction of survival capacity, reduction of apoptosis	• Unknown	• Human CD34+ HSC	• 184
Administration of NR101	• Expansion of HSC, improving maintenance of colony forming capacity	• Activation of C-MPL downstream signaling pathway	• Human bone marrow CD34+ Lin-,CD38-	• 171
Inhibition of P18 (INK4C) by XIE18–6, Compound 40, P18in003,P18in011	• Expansion of HSC and induction of self-renewal maintenance	• Inhibition of p18	• LSK Cell(Murine)	• 185,186
N-Acetyl cysteine	• Expansion of the HSC • Induction of engraftment	• Inhibition of P38 MAPK pathway	• Hu CB HSC	• 183,184
Anti miRNA15b and anti miRNA219b	• Expansion of the HSC	• Inhibition of mirRNA15b & mirRNA219B	• Hu CB HSC	• 182
∝−Tocopherol	• Expansion of HSC	• Unknown	• LSK Cell(Murine)	• 144
CAEP	• Expansion of the HSC	• Induction of kit –ligand, HIFα,VEGF&SDF-1	• Hu CB HSC	• 148,149
Leptin	• Expansion of HSC	• Activation of Jak2	• LSK Cell(Murine)	• 143
10,074-G5	• Induction of proliferation	• Inhibition of C-Myc	• LSK Cell(Murine)	• 140–142
L-NIL	• Expansion of HSC	• Inhibition of iNOS	• Human CD34+ HSC	• 139
Dastinib	• Induction of proliferation	• Inhibition of c-kit signaling	• LSK Cell(Murine)	• 137

SDF-1: Stromal cell-derived factor 1, Hu CB HSC: Human umbilical cord blood hematopoietic stem cell, hFLSECs-E4 or F1: Human fetal liver sinusoidal endothelial cells which are engineered to express adenoviral E4 or F1 genes, M-OST cell: Osteoblasts-derived MSCs, CXCR4: C-X-C chemokine receptor 4, SCF: Stem cell factor, IGF: Insulin-like growth factor, ANGPTLs: Angiopoietin–like proteins, CXCL12: C-X-C chemokine ligand −12, GSK3B inhibitor: glycogen synthase kinase 3B inhibitor, LSK-Cell: Lin-,Sca-1+,c-Kit + HSC, BMP4: Bone morphogenic protein 4, Shh; Sonic hodge hog, FGF; Fibroblast growth factor, PTH; Parathyroid hormone HOX−B4: Homeobox protein Hox−B4, 5AZA&TSA: 5aza-2deoxycitidine and trichostatin, BMI-1: B cell-specific Moloney murine leukemia virus integration site 1, PI-C: Polycitidylic acid, Mtln 4: Matrilin 4, Sb203580: small molecule inhibitor of P38 MAPK, P38 MAPK: P38 mitogen-activated protein kinase, BCL-2: B-cell lymphoma 2, TEAP: Tetra ethylene pentamin NAM; Nicotine amid, SIRT1: Sirtuin 1, PGE2: Prostaglandin E2, TPO: Thrombopoietin, AHR: AryL Hydrocarbon receptor, *SR1*: Stem regenin 1, EDAG: Erythroid differentiation associated gen, C-MPL: myeloproliferative leukemia protein, CAPE: Cafeic Acid phenethly ester, C7: Imidazole analogue, 10,074-G5: C-myc inhibitor, iNOS: Inducible nitric oxide synthase.

role in calcium hemostasis and bone remodeling regulation [101]. PTH exerts its signaling effects through PTHrPR receptors on osteoblasts [102]. It has been shown that mice treatment with PTH results in HSC mobilization enhancement from bone marrow into peripheral blood. Adams et al. [92] have reported that PTH treatment along with cyclophosphamide chemotherapy protects mice HSCs. Additionally, PTH administration for six weeks after implantation can improve HSCs implantation capacity. Furthermore, Jung et al. [93] have also shown that SDF1 is increasingly expressed on osteoblasts following PTH injection and improves HSC homing [103]. Administration of exogenous PTH also contributes to the elevation of certain cytokines levels such as IL6, IL11, GM-CSF, and C-kit, which are essential in HSC proliferation and differentiation [101].

7.7. Angiopoietin–like proteins (ANGPTLs)

ANGPTLs include intensively glycosylated secretory proteins which play significant roles in inflammation, cancer, metabolism, and hematopoiesis [104]. Different ANGPTLs are involved in HSCs expansion stimulation in mice bone marrow [66,67,105]. Akhter et al. [95] have indicated that ANGPTLs 1, 2, 3, 4, 6, and 7 are essential in expansion and survival of mice HSCs. Recent data also indicated that LILRB2 acts as receptor for multiple ANGPTLs. ANGPTL2 by binding to LILRB2, induces notch signaling pathway activation of which ultimately activates MYC gene expression [106]. Considering these data, serum free culture systems including certain cytokines and Anti LILRB2 have been designed to improve the expansion of human umbilical cord HSCs by 5 fold after 10 subcultures [107].

7.8. Pleiotrophin

Pleiotrophin is encoded by PTN gene on 7q33 chromosome and is known as Heparin-Binding Brain Mitogen (HBBM) or neurite growth-promoting factor 1 [108]. It is expressed in central and peripheral nerve system, lungs, kidney, gut, and bones [109]. It is also expressed by sinusoidal endothelial cells in bone marrow and improves the survival rate of radiated mice and expansion of LT-HSCs in mice bone marrow and human umbilical cord blood [110,111]. The pleiotrophin-related HSCs quiescence is mainly depended on RAS oncogenes expression. Following pleiotrophin-mediated RAS/MEK signaling activation, DMTF1 transcription factor is activated and increases the HSC quiescence phase [111].

7.9. Fucosylation enzymes

One of the important strategies for the improvement of human HSC homing and engraftment is to increase fucosylation levels in selectin ligands expressed on HSC membrane [112]. These ligands are fucosylated by $\alpha 1 \rightarrow 3$ fucosyltransferase, so that insufficient $\alpha 1 \rightarrow 3$ fucosylation contributes to lower attachment capacity to P-selectin and *E*-selectin and ultimately decreased human HSC homing following transplantation [113]. Administration of guanosine diphosphate fucose and fucosyltransferase VI and VII can improve fucosylation levels on selectin ligands and enhance $CD_{34}{}^{+}$ engraftment and homing [113,114]. According to jay myers et al. [115], HSCs lack homing and self-renewal abilities in fucosyl transferase defected mice. Atashi et al. [116] also reported that treatment of $CD_{133}{}^{+}$ umbilical cord blood HSCs with fucosyltransferase VI in protein coated nanoscaffold medium, contributes to elevated expression of CXCR4, VLA4, VLA5, LFA1, and E-cadherin on HSC surface.

7.10. Complement components

C3a, as a member of complement component, can improve the CXCR4 expression on HSC membrane by bonding to Hematopoietic stem and progenitor cell and induces HSCs enhanced homing and engraftment in bone marrow niche [117]. Nie et al. [105] demonstrated that CXCR4 signaling is vital for HSC quiescence phase maintenance and the use of CXCL12 inhibitor in culture medium can promote HSCs entrance to cell cycle and proliferation. C3a-C3aR signaling pathway influences the SDF1-related homing process by improved response to SDF1, binding to VCAM1, expression and secretion of matrix metalloproteinase 9 and migration via sub-endothelial membrane [117,118].

7.11. Epigenetic modifiers

Various studies have demonstrated epigenetic effects on self-renewal and differentiation of HSCs. Elizalde et al. [119] have suggested histone deacetylase 3 as an important factor in HSC expansion, so that its inhibition leads to increased expansion in vitro. Nishino et al. [120] have also reported that Garcinol, as a nonspecific inhibitor of histone acetyl transferase, restrains P53 acetylation on lysine 382 (K382), causing the elevated expansion levels of human umbilical cord blood HSCs. Additionally, histone deacetylase inhibition by Valproic acid leads to enhanced expression of CD90, CD117, CD49F, CXCR4 and HOXB4 on HSCs, as well as aldehyde dehydrogenase increased activity, resulting in improved HSCs ex vivo homing and expansion [121–123]. Akin et al. [124] have demonstrated that implementation of epigenetic modifiers such as valproiec acid and neurotrophic factor will result in increased umbilical cord blood HSC proliferation.

In adult population, HSCs are usually in quiescent phase and their activation is very crucial for cell division. Araki et al. [125] have used 5aza-2deoxycitidine and trichostatin A (5aza/TSA) as chromatin remodeling agents in culture mediums. Data revealed the 12-fold increase in $CD_{34}{}^{+}$ and $CD_{38}{}^{-}$ HSCs expansion following 5aza/TSA treatment. Additionally, the genes involved in self-renewal process including HOXB4, BMi1, and GATA2 were overexpressed and the expression of cell cycle genes including C-MYC were down-regulated after this treatment [126].

Gene expression analysis in mice and human bone marrow HSCs has also revealed the HOXB expression in mice and human primary HSCs [127]. Furthermore, HOXB4 overexpression or direct injection of HOXB4 contributes to HSCs improved expansion both in vivo and ex vivo [128].

G9a and GLP are strictly conserved lysine methyltransferases which play important roles in gene expression and chromosome structures and can inhibit gene transcription via lysine 9 methylation on histone 3. Unc0638 is a small molecule inhibitor of G9a/GLP. Xioa chen et al. [129] showed that treatment with unc0638 will increase the bone marrow derived $CD_{34}tCD_{38}{}^{Lo}$ HSCs expansion rate up to 3-folds.

7.12. Matrilins and polycytidylic acid (PI:C)

Matrilins, including matrilin 1, 2, 3, and 4 are oligomeric adaptor proteins in extracellular matrix. Matrilin 4 (mttn4) is expressed in HSCs that are in quiescence phase and prevents further HSC proliferation [130]. It has been shown that the lack of mttn4 due to decreased expression of CXCR4 in HSCs, leads to the increased engraftment capacity, mobilization, and faster and better recovery of blood cells. Hannah Uckelmann et al. [131] have also indicated that mice treatment with PI·C induces an intensive IFNα reaction and down-regulates the mttn4 expression on HSC surface by IFNα, leading to increased HSC proliferation.

7.13. C7 and SB203580

C7 is a small imidazole analogue molecule with the 383.12 g/mol molecular weight [131]. Sudipto BARI et al. [132] reported that C7 can improve the expansion of umbilical cord blood HSCs [1] by $CD_{28}{}^{-}$, $CD_{24}{}^{+}$, $CD_{49}{}^{F^{-}}$, $CD_{90}{}^{+}$, and $CD_{45}R_{A}$-CD markers and [2] by $CD_{90}{}^{+}$, $CD_{45}RA^{-}$, $CD_{38}{}^{-}$, and $CD_{49}F^{+}D_{34}{}^{+}$ CD markers by 4 to 5 fold in comparison to the control group.

Furthermore, Sb203580 is another small molecule inhibitor of P_{38}MAPK pathway with the molecular weight of 377.43 g/mol [110]. P38MAPK pathway in response to ROS induces oxidative stress and causes decreased lifespan and self-renewal capability of HSPCs, however, its effects can be limited by P_{38}MAPK inhibitors [133,134]. Young Wang et al. [135] have illustrated that c-kit+, Lin-, and Scal+ (LKS^+ cell) derived from murine bone marrow, can increase the expansion and self-renewal properties of these cells in the presence of Sb203580, in vitro.

7.14. Dastinib

Dastinib is a second generation tyrosine kinase inhibitor which is used in the treatment of CML and ALL with positive Philadelphia chromosome. Dastinib can inhibit certain tyrosine kinases such as C-Kit, PDGFR, PDGFRB [136]. C-Kit is essential for HSCs maintenance in quiescence phase. Johanna M.Duyvestyn et al. [137] have also shown that Dastinib provokes LT-HSCs entrance to cell cycle and raises proliferation through C-Kit signaling inhibition, indicating the role of c-kit signaling pathway in maintaining the hematopoietic stem cell self-renewal capacity.

7.15. Nitric oxide

Nitric oxide is a free radical with limited lifetime, which has three isoforms. Isoform I is Ca^{2+} dependent, isoform III is produced by endothelial cells and isoform II or I NOS is not Ca^{2+} dependent. Isoform II causes $CD_{34}{}^+$ HSCs increased differentiation in HL60 monocyte cells [138].

Sigrun Reykdal et al. [139] also demonstrated that L-NIL treatment as an inhibitor of iNOS, increases the expansion of $CD_{34}{}^+$ HSCs by 13.4 fold compared to the control group in day 7 of culture. Moreover, it has been shown that L-NIL treatment decreases the apoptosis compared to the control group.

7.16. 10074-G5

C-Myc is the homologue of viral oncogene (V-Myc) in Myelocytomatosis bird retrovirus. C-Myc regulates specific procedures such as proliferation, cell growth, differentiation, angiogenesis, and apoptosis. Esther Baena et al. [140] showed that prenatal C-Myc inactivation results in the accumulation of LSK-CELL in mice bone marrow. They also showed that the inactivation of C-Myc gene leads to increased expression of the P21. Wilson et al. [141] also suggested that C-Myc elimination leads to increased number of Lin^- hematopoietic stem cells. Additionally, Merve Aksoz et al. [142] indicated that treatment of $LSKCD_{34}{}^{lo}$ cells with 10,074-G5 as a C-Myc inhibitor, increases cell population by 2-fold compared to the control group. Inhibition of CDKIs like P18, P21, P27, and P57 also induces the $LSKCD_{34}{}^{lo}$ cells entrance into cell cycle and proliferation. Moreover, 10,074-G5 contributes to Varberg effect through the genes involved in glycolysis which plays a crucial role in proliferation.

7.17. Leptin

Leptin is a protein encoded by obesity gene, which controls food and energy usage through different signaling pathways. It is also involved in hematopoiesis. Leptin receptor expression is observed in different hematopoietic organs such as liver, spleen, and bone marrow. Leptin receptor is also presented on mice and human HSCs. Leptin also induces the proliferation of HSCs and myeloid progenitors and has synergistic effects along with stem cell factor (SCF) in HSC proliferation.

Carolc Dias et al. [143] have also suggested that treatment of mice HSCs with LEP5, which is a synthetic fragment of Leptin, leads to 2-fold increase in mice HSC population through JAK2 activation.

7.18. ∝− Tocopherol

∝−Tocopherol is a member of vitamin E family with an important role in cell signaling, inflammation, apoptosis, and cell proliferation. Amanda Nogueira pedro et al. [144] demonstrated that ∝−Tocopherol use increases the mice LSK cells up to 2-fold.

7.19. Cafeic Acid phenethly ester (CAPE)

CAPE is one of the derivatives of honey wax that has anti-oxidant and anti-inflammatory properties [145]. It has been shown that CAPE has a non-competitive inhibitory effect on 5-lipoxygenase enzyme [146]. The findings also suggest that CAPE has anti-metastatic and anti-tumor properties by reducing the production of matrix metalloproteinase x-2 and -9 (MMP) and vascular endothelial growth factor (VEGF) in CT26 cells [147]. Wang et al. [148] proved that CAPE contributes to elevated expression of Kit-Ligand and heme oxygenase-1, both of which have significant roles in the regulation of hematopoietic stem progenitor cells (HSPC) function.

Lui Yimming et al. [148] also reported a 2.5-fold increase in umbilical cord blood HSPCs number after CAPE treatment in comparison to control group through increased expression of Kit LiGand (SCF) and HIF-l∝. Additionally, Xiao fang Chen et al. [149] have shown that CAPE increases the homing of LSK cell by upregulation of HIF-lα, VEGF-A and stromal-derived cellular factor 1α (SDF-1α).

7.20. ZVADFMK and ZLLYFMK

One of the problems of HSC culture is the Caspase and Calpain related apoptosis which causes transplantation failure. Sangeethav et al. [150] reported that the administration of general Capase and calpain inhibitors including ZVADFMK and ZLLYFMK in $CD_{34}{}^+$ HSC culture media can result in elevated expansion and differentiation into myeloid cell lineage. These two substances lead to increased expression of anti-apoptotic proteins such as bcl-2, and on the other hand, they induce downregulation of proteins involved in apoptosis, including Caspase 1, Caspase 3, and Fas [151].

7.21. Tetraethylene pentamin (TEPA)

Cupper acts as a cofactor of the cytochrome oxidase enzyme, which is the key enzyme in the mitochondrial respiratory chain. Cupper deficiency disrupts the ATP production via the citric acid cycle and changes the energy production to the Embden–Meyerhof–Parnas [152]. It has been reported that high amounts of Cu in culture medium leads to enhanced HSCs differentiation. Therefore, administration of TEPA as a copper schellator, eliminates Cu from the HSC expansion supporting medium, ex vivo [153,154]. Peled et al. [153] reported that $CD_{133}{}^+$ HSCs culture in IL6, TPO, SCF, FL, and copper schellator (TEPA) enriched medium Leads to increased number of HSC by 89 fold compared to the control group.

7.22. Sirtuin1 inhibitor

Sirtuin1 is a member of NAD+ dependent histone deacetylase class III family [155]. Sirtuin1 removes acetyl group from histones and non-histone proteins such as transcription factors [152]. Several studies have shown that Sirtuin1 has a significant role in cellular processes such as inflammation, differentiation, and cell survival [156–159]. Nicotine amide (NAM) is a form of vitamin B3 that prevents HSC differentiation and improves transplantation success [160]. It has been shown that NAM causes a significant increase in homing without changing CXCR4 expression level, indicating the effect of NAM on CXCR4 downstream signaling pathway [155]. NAM also inhibits SIRT1 and NAD-dependent ribosyl transferases [161]. Peled et al. [155] have demonstrated that HSC culture in the present of cytokines and NAM leads to the elevated

numbers of CD_{34}^{+} and CD_{38}^{-} HSCs and reduced number of differentiated $CD_{11C^{+}}$, $CD_{14^{+}}$, $CD_{11}b^{+}$ cells. They also indicated that EX-527 as a SIRT4 specific inhibitor prevents differentiation of umbilical cord blood HSCs [155].

7.23. Prostaglandin E2

Prostaglandin E2 is the most active prostaglandin in mammalian cells which is involved in various processes such as proliferation and apoptosis [162]. Prostaglandin E2 was first discovered as a chemical substance for stem cells support and HSC proliferation and self-renewal induction in Zebra fish [163]. Data have revealed that ex-vivo mice HSC treatment with PGE2 for two hours leads to significant raise in regenerated HSC numbers compared to the control group [164]. This agent also enhances the expression of homing genes (CXCR4), proliferation genes (cyclinD1) and survival genes in umbilical cord blood HSC. The exact action mechanism of PGE2 is still unclear. However, its interaction with wnt-β-catenin pathway has been suggested [165]. Data from a phase I clinical trial study on 16 cord blood samples treated with 16-dimethyl prostaglandin E2, have indicated safe and accelerated neutrophil recovery in patients receiving this treatment compared to the control group [166].

7.24. Thrombopoietin (TPO) and NR101

Thrombopoietin (TPO), as the primary regulator of platelet production, shares a high degree of importance in HSC biology [167,168]. Either alone or in combination with cytokines such as IL3, FLT3L, and SCF, TPO induces HSC proliferation, in vitro. Keita et al. [169] demonstrated that TPO induces the USF1 expression which is a positive regulator of HOXB4 gene transcription. SCF1 expression contributes to the elevated HOXB4, which in turn leads to improved HSC self-renewal and repopulation. Yagi et al. [170] have also indicated that TPO induces self-renewal and expansion in both LT-HSC and ST-HSC. NR101 is also a C-MPL or thrombopoietin receptor agonist which increases the CD_{34}^{+} cells proliferation rate [171]. NR101 to bind to the C-MPL transmembrane domain also requires a histidine residue. Additionally, it causes the increased expansion of $CD_{34}^{+}CD_{38}^{-}$ cells and the maintenance of colony forming capacity of the cells relative to recombinant human TPO. After binding to C-MPL, NR101 induces the activation of C-MPL downstream signaling pathway including JAK2, STAT5, STAT3 [171].

7.25. AryL Hydrocarbon receptor (AHR) antagonist

It has been reported that nearly 70–80% of HSCs are in quiescence status, in normal hematopoiesis (G0 phase) [172]. AHR is a member of BHLB-PAS transcription factor superfamily which includes AHR1, AHR2, AHR receptors and play an important role in destination determining of HSCs [173]. AHR interacts with Rb protein which is a G1 cell cycle regulator. Other genes such as HES1 and C-Myc are also under direct modulation of AHR [174,175]. AHR antagonists increase the HSC expression of superficial CXCR4 which is involved in homing process [176]. Scientists have introduced SR1 as AHR antagonist for optimum HSC expansion. Additionally, Boitano et al. [177] have proposed Stem Regenin 1 as AHR antagonist to induce nearly 50-fold increase of human HSC expansion compared to control group.

7.26. UM171

During screening of chemical substances which can increase the HSC expansion, UM171, a Pyrimido-Indol derivative analogue, was discovered. Despite SR1, this component activates AHR signaling pathway [178]. Li et al. [179] reported that UM171 induces HSPCs differentiation from pluripotent stem cells. Furthermore, UM171 co-administration with SR1, has resulted in significant improvement of cells expansion. UM171 treatment also has yielded about 13-fold increase in LT-HSC compared to the control group [180]. CD201, CD36, PF4, and GYPB are several genes and surface markers that cause increased expression in UM171-treated HSCs [180,181].

7.27. Anti-miRNAs

One of the important factors in self renewal maintenance of HSCs is the Sall4 transcription factor. This factor contains zinc finger domain belonging to the spalt1 like protein family, located on chromosome 20 and is expressed in CD_{34}^{+} HSCs. miRNA15b and miRNA219b are the main inhibitors of this transcription factor. Anti-miRNA15b and anti-miRNA219b administration in HSC cultures has been associated to increased HSC expansion, in vitro [182].

7.28. N-Acetyl cysteine

N-Acetyl cysteine molecule is one of the inhibitors of P38 MAPK pathway. Jing Zou et al. [183] indicated that umbilical cord blood CD_{133}^{+} HSC treatment with N-Acetyl cysteine leads to decreased ROS formation and increased HSC expansion about 14.5-fold, compared to control group. Linping Hu et al. [184] have shown that the amount of reactive oxygen species in NOD/SCID mice bone marrow is high, which results in decreased engraftment of human umbilical cord blood hematopoietic stem cell in the recipient mice. Additionally, *N*-acetyl-L-cysteine treatment in mice has resulted in decreased ROS and increased hematopoietic stem cell engraftment by 2.3-fold compared to the control group.

7.29. P18 inhibitors

Among INK4 protein family, INK4C or P18 has an important role in cell cycle regulation at phase G1. P18 modulates the cell cycle through inhibition of CDK4/6. The essential role of P18 in HSC self-renewal maintenance has also been indicated. Lack of P18 helps HSCs to overcome the exhaustion process while P18 inhibitors can be useful in HSCs expansion increase.

P18 In003 and P18 Ino011 are synthetic inhibitors of P18. It has been proved that LSK cell treatment with these inhibitors increases the engraftment capacity after 16 weeks compared to the control group [185]. Moreover, the population of LSK cells treated with P18 In003 and P18 Ino011 has been increased by nearly 4-fold in comparison to control group [185].

Compound 40 is another synthetic inhibitor of P18, so that, treatment of mice LT-HSC with this compound has resulted in increased expansion of the cells by 2.61 fold. Furthermore, the umbilical cord blood HSCs population has been increased by 30% after treatment with compound 40 [186].

In Xiang. Qunxie et al. [186] study, treatment of $CD_{34}^{+}CD_{49}^{f+}$ HSCs with XIE18-6 as a P18 inhibitor for 7 days, showed a 30% increase in HSCs expansion. Moreover, 10% increase in the LT-HSC population with initial phenotype was also observed in comparison to the control group. XiE 18-6 also caused 1.5-fold increase in the number of LT-HSCs and HSPCs after treatment.

7.30. Erythroid differentiation-associated gen (EDAG)

EDAG is a multifunctional transcription factor which is expressed in CD_{34}^{+} HSCs and is involved in cell apoptosis, differentiation, and proliferation [187]. Zhao et al. [188] have reported that EDAG increases the number of umbilical cord blood HSCs in G1 phase. In order to validate this hypothesis, remarkable decrease in G1 phase HSC numbers and notable increase in survival capacity, as well as decreased apoptosis have been observed following EDAG gene knock down.

7.31. IGFBP2

As a member of IGFBPs family, IGFBP2 controls the function, distribution, and activity of IGF1 and IGF2 [189]. This protein is expressed in fetus, different adult tissues, and biologic fluids [190]. IGFBP2 has two mechanisms of action: 1) internal IGFBP2 promotes cell survival by binding to integrin, 2) secretive IGFBP2 bind to surface receptors and regulate proliferation and cell mobilization [190–192]. Huynh Et al [193] illustrated that IGFBP2 expressed by mice bone marrow stromal cell, support HSC functions. Moreover, in IGFBP2-null mice, the number and functions of HSCs are limited due to increased and decreased apoptosis and cell cycle, respectively.

8. Microfluidic devices

Microfluidic devices are known as micro total analysis system (MTAS) or LaB onchip [194]. Recent developments in microfluidic systems have resulted in on chip culture advancements, because of the limitations of conventional culture methods [195,196]. Certain problems in precise understanding of dynamic cell response in physiologic conditions have resulted in the development of microfluidic systems in cell culture field. These systems are used to facilitate cell feeding and waste discarding.

8.1. Type of cell culture

In vitro cell culture depends on the growth control capacity and cell preservation in lab environment [195]. Microfluidic systems provides culture and analysis facilities in micro scales. Compared to conventional cell culture methods, the microfluidic cell culture (MCC), provides benefits such as flexibility of designed equipments, direct link to downstream analytical systems, perfusion culture availability, automation and very small sample amount, and cell numbers [195].

8.2. Two-dimentional (2D) culture

In 2D culture methods, cells are cultivated as suspension or adherent. Two types of cells which require fluidic suspensions include mammals blood cells and yeast cells [197]. Therefore, most of the cells should be cultured on a cell layer in order to preserve their important phenotypic features [197].

Of important factors in microfluidic systems that affects cell adhesion to the surface is treatment and coating of the surface with hydrophilic substances; since, primary matters used to produce microfluidic systems like PDMS are hydrophilic and prevent cell adhesion to the surface [198,199]. In order to reduce the hydrophobicity, certain techniques such as ultra violet radiation, laser or oxidation by plasma oxygen are used [198,200]. Implementation of extracellular matrix proteins like collagen, fibronectin, and laminin are other strategies for hydrophobicity reduction [201,202]. These extracellular matrix proteins bind to the PDMS surface by hydrogen or polar bonds.

8.3. Three-dimensional (3D) culture

2D cultures cannot fully demonstrate extracellular matrix found in different tissues and organs as an abstract representation of three dimensional cell structure. 3D cultures are much more efficient in providing environment similar to in vivo condition. Microfluidic systems provide spatial control, perfusion merge, and over-control of gradients. Several required substrates for 3D cultures are capability of interaction with microfluidic chips, hydrogel-based and gel free patterns production [203,204]. Hydrogel based pattern is mostly used in 3D cultures. Hydrogels are natural hydrophilic substances which are used in 3D cultures construction in laboratory models [205,206]. Among 3D cultures, vascular models have certain values such as spatial and temporary gradient control, spatial co-culture and perfusion. Thus, they are suitable for better understanding the in vitro tumor angiogenesis and the effects of environmental factors on this process [207].

Microfluidic systems can strongly describe the effects of physical factors like temperature and pressure on cell behavior [208]. Cellular interactions are achieved based on cell-to-cell interplay or paracrine signaling mechanisms which determines cell phenotype in response to stimuli. Microfluidic systems permits detection of specific signals in single cell scale [196,209].

One of the methods for cell interactions investigation is the co-culture process. In the first step of this process, the cells are separately cultured and then dynamic interactions can be observed. This procedure can be performed in microfluidic systems via several processes such as mixing culture, micro valve, micro gaps, and droplet technology. Functional features and the combination of extracellular matrix can directly affect cell functions including growth, differentiation, death, and cell morphology. Therefore, extracellular matrix is crucial in cell fate determination. Various materials are used in extracellular matrix construction of including natural (like fibrin, hyaluronan, and nectin) or synthetic (polietilenglicol) polymers. Huei wen wu et al. [210]. have successfully implemented microfluidic systems for separation and counting of umbilical cord blood HSCs. Stefan siber et al. [211] have also demonstrated that after four weeks of HSC culture in microfluidic 3D medium, CD_{28}^{-} and CD_{34}^{+} cells construct almost 31% of the whole cell population. On the other hand, it has been revealed that CFU-G, CFU-M and less prevalent CFU-GEMM population is increased in 3D culture medium after 4 weeks, compared to their initial numbers.

9. Conclusion

The critical aims in HSC expansion include 1) increase of umbilical cord blood HSCs number for transplantation, 2) decrease of neutrophil and platelet recovery period after transplantation, 3) transplantation improvement without the risk of GVHD, and 4) cost effectiveness of the technique. Since, HSCs can be drawn into various fates such as differentiation, self-renewal, apoptosis, and migration, we have to understand HSC interplay with the other cells and in vivo extracellular matrix. HSCs usually have tendency toward differentiation and apoptosis in culture medium and in order to overcome this problem certain components are currently being used to increase the self-renewal capacity and decrease apoptosis and differentiation rates.

Acknowledgements

This work has been done as part of the M.Sc. thesis for Mehdi Derakhshani. This study was supported by Stem Cell Research Center at Tabriz University of Medical Sciences,Iran [Grant No 61897]

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