SMGroup

Bone Marrow Stem Cells

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HISTORY

Bone marrow renewable cells was discovered in 1950 by noticing that radiated mice can reproduced blood cells after radiation when they transplanted with bone marrow cells [1]. These cells were studied and showed two definitive criteria of stemness, their ability to renew themselves and their ability to differentiate into various blood cell types [2,3]. The number of bone marrow cells required to protect radiated mice was determined by limiting dilution assays and spleen colony assay in 1960 [4]. Advancing of molecular techniques enable scientists to trace the origin of new blood cells using DNA identifying markers which is bacterial neomycin resistance (neo) gene in 1985 [5]. At 1988, other group purifies these cells from mouse bone marrow by utilizing relatively new technologies of multi-color fluorescence-activated cell sorting and monoclonal antibodies [6].

TYPES

Hematopoietic Stem Cells (HSCs)

Identification

HSCs represent about 0.05% of bone marrow cells. They are identified by lacking of mature markers and expressing of certain cell surface markers.

In mouse, HSCs identified by expressing of Ssca-1, c-kit, thy-1 and slamf-1 in Lin negative population [7]. While, in human express slightly different pattern of surface markers. They express CD34, c-kit and thy-1 in similar Lin negative population [8]. HSCs also contain ABCG2 transmembrane proteins [9]. By differentiation, the map of markers on the HSCs surface changes from one stage to other within Lin negative population toward mature blood cell phenotypes.

Isolation

Combination of cell surface markers that used to identify HSCs, also used to isolate them from mixed population utilizing Fluorescence Activated Cell Sorting **(FACS)** technology. Fluorescence-activated Cell Sorting **(FACS)** is a specialized type of flowcytometry that sorting particles depending on light scattering, granulation and fluorescent labels. The cell suspensions pass through a channel where they are scanned by light beams with different wavelength. After that, transmitted or emitted light detected and analyzed.

Cells could be sorted depending on size, shape, charges and fluorescent labeling.

Side population technique is a powerful assay to isolate HSCs. The principle of this assay is the ability of HSCs, which contain ABCG2 transmembrane protein, to efflux a stain called Hoechst 33342, while other cells that did not have ABCG2 stained with this stains. HSCs could be sub-populated by using different sets of markers.

Other sources of HSCs

HSCs were isolated from peripheral blood and umbilical cord blood [10]. Embryonic stem cells also were differentiated to produce hematopoietic stem cells population using specific culture condition [11]. Probably, the same result could be yield by using induced pluripotent stem cells.

Several factors are involved to produce HSCs from pluripotent stem cells including GFi1-b, cFos, Gata2, HoxA9, Erg, RORA, Sox4 and myb.

Differentiation of HSCs

HSCs develop all blood cell types. *In vivo*, specific conditions and certain factors implicated in HSCs differentiation and development including inflammation, tumor necrosis factors, vitamin D, prostaglandin E2 and chemokine CXCL12 [12].

Recent studies showed the “plasticity” of HSCs. HSCs have the ability to produce cell types other than blood lineages or even from different germ layer origin. For example, genetically

modified HSCs could produce myogenic progenitor when they transplanted into immunodeficient mice [13]. Moreover, transplantation of HSCs into animal model with Duchenne's muscular dystrophy reproduce muscle cells [14]. Injection of HSCs directly into the healthy myocardium adjacent to the injured area or mobilizing of HSCs to injured area using cytokines, produce new cardiac myocytes and cardiac blood vessels in adult mice [15]. This result was proved by using cell tracing techniques (LacZ transgenic mice) that showed the ability of HSCs to differentiated to cardiomyocytes and endothelial cells forming functional tissue [16]. Goolsby *et al*. demonstrated that CD34 positive cells from bone marrow express neural genes [17]. Other showed that HSCs produce neuronal phenotypes after transplantation into irradiated adult mice [18]. These finding may provide an alternative source of neurons in patients with neurodegenerative diseases or central nervous system injury.

Also, intravenous injection of adult HSCs into animal model of tyrosinemia type I, help the mouse and restored the liver biochemical function [19]. Cross-sex was used to demonstrate that the origin of new hepatocyte in hepatic injured rat model is injected bone marrow stem cells [20].

In addition, haematopoietic stem cells are capable to develop insulin-expressing cells [21]. The ability of HSCs to produce cells of lung, GI tract, and skin was also studied [22].

Mesenchymal Stem Cells (MSCs)

Mesenchymal Stem Cells **(MSCs)** are fibroblast- like cells that origin from connective tissue (mesoderm). Sometime, MSCs are called stromal cells.

MSCs were described by Russian pathologist, Alexandar A, in 1924. However, their potential to generate other cell types was assessed by Friedenstein in the 1970 [23].

Identification

MSCs identified morphologically by fibroblast- like shape that present small cell body with small process. MSCs also characterized by plastic adherent growth pattern in normal medium condition.

More specifically, MSCs are identified by expression of CD105, CD73 and CD90, and lack expression of CD45, CD34, CD14 or CD11b, CD79alpha or CD19 and HLA-DR surface markers [24,25].

Isolation

MSCs isolated from mononuclear layer of bone marrow by using discontinuous gradient centrifugation. This method conducted by suspension of bone marrow cells in specific wash buffer carefully layered upon Ficoll separation medium. MSCs also isolated by immunomagnetic depletion techniques. Fluorescence-Activated Cell Sorting **(FACS)** used for characterization and further purification of MSCs utilizing specific cells surface markers [26].

Other sources of MSCs

MSCs were obtained from fetal tissue and most of adult tissue types including adipose tissue, amniotic fluid, amniotic membrane, dental tissues, endometrium, limb bud, menstrual blood, peripheral blood, placenta and fetal membrane, salivary gland, skin and foreskin, sub-amniotic umbilical cord lining membrane, synovial fluid and others [27].

Differentiation

In laboratory MSCs can differentiate into multilineage cell types including osteoblast, chondrocyte, adipocyte, cardiomyocyte, hepatocyte, endothelial and neural cells by using definitive media condition such as growth factor, genetic induction, microenvironments and biomaterial technology.

Within the same germ layer, mesoderm, MSCs induced to osteogenic differentiation by mixture of growth and transcription factor such as Dexamethasone **(Dex)**, β-glycerophosphate **(β-GP)**, and ascorbic acid Phosphate **(aP)**, Bone Morphogenetic Proteins **(BMPs)**, Vascular Endothelial Growth Factor **(VEGF)** and basic Fibroblast Growth Factor **(bFGF)** [28]. Recently, scaffold with biomaterial such as Silicate-substituted calcium phosphate **(Si-CaP)** are used to induce osteogenic differentiation of MSCs [29].

For adipogenetic deferentation, MSCs treated with dexamethasone, indomethacine, insulin and isobutyl methyl xanthine leading to express adipocytes-specific proteins and form lipid droplets [30].

Likewise, human MSCs derived from bone marrow have the ability to produce chondrocyte when they are cultured in standard condition containing DMEM media supplemented with Insulin-Transferrin-Selenium **(ITS)**, linoleic acid, selenious acid, pyruvate, ascorbate 2-phosphate, dexamethasone and transforming growth factor-β III (TGF-βIII) [31]. Mature chondrocyte that express chondrogenic transcription factors like Sox9, L-Sox5 and Sox6 could be obtained by further treatment with TGF-β family (TGF-β1, TGF-β2 and TGF-β3), Insulin Like Growth Factor-I **(IGF-I)** and BMP-2 [32,33].

MSCs also showed myogenic differentiation capability. Treatment of bone marrow MSCs with 5-azacytidine result in myotubes with multiple nuclei expressing myocyte-related genes, β-myocin heavy chain, α-cardiac actin and desmin [34,35].

Other manipulations are required to induce MSCs to skeletal and smooth muscles by NOTCH gene transfection and treatment with TGF-β2, respectively [36].

Bone marrow MSCs have the ability to form neural cells. Naghdi *et al*. reported that treatment of MSCs with Beta-Mercaptoethanol **(BME)** and followed by Nerve Growth Factor **(NGF)** induce neural markers such as NeuroD, Microtubule-associated Protein-2 **(MAP-2)** and anti-synapsin-I forming cholinergic phenotype [37]. Other factors such as Hepatocyte Growth Factor **(HGF)**, FGF,

Epidermal Growth Factor **(EGF)**, insulin, retinoic acid, valproic acid, and hydrocortisone were reported to have neurological differentiation effects on MSCs [38-40].

MSCs produce hepatocyte in stepwise. Firstly, MSCs treated with EGF, bFGF and nicotinamide added into Dulbecco's Medium **(IMDM)**, followed by culturing in IMDM supplemented with oncostatin M, dexamethasone and ITS (insulin, transferrin, selenium) to form cells that express hepatic specific markers such as albumin, α-fetoprotein, Nuclear Factor 4 α **(HNF-4α)** [41]. Newly formed hepatocyte like cells restore have the ability for albumin synthesis, glycogen storage, urea secretion. Moreover, transplantation of these cells into hepatic injured mice restores liver function and reduces transaminases activity [42].

Many studies demonstrated that mesenchymal stem cells derived from bone marrow could produce insulin-producing cells by several protocols such as genetic manipulation, culture condition supplementation. Details of protocols were mentioned in the chapter of "Adult stem cells and diabetes ".

Immuno regulatory properties of MSCs

MSCs are the most immune-tolerable type of stem cells due to specific features of MSCs including low expression of major histocompatible and stimulatory proteins [43]. Moreover, bone marrow derived MSCs secrete immunosuppressers such as Leukemia inhibitory factor, Vascular Endothelial Growth Factor **(VEGF)**, Insulin-Like Growth Factor 1 **(IGF-1)**, basic Fibroblast Growth Factors **(bFGF)**, Hepatocyte Growth Factor **(HGF)**, IL-6 and CCL-2 and interferon gamma [44-46].

CLINICAL APPLICATION OF BMSCS

HSCs

Hematopoietic stem cells were uses mainly for management of hematological disordered. Chronic Myeloid Leukemia **(CML)** is a myeloproliferative disorder of granulocyte white blood cells, neutrophils, eosinophils and basophils, characterized by Philadelphia chromosomal. According to American Cancer Society **(ACS)**, CML represent 10% of newly diagnosed leukemia. ACS also estimate that 1,070 people will die of CML in the United States for 2016 [47]. Recently, CML was treated by autologous stem cell transplantation which showed that 65% of patients survive more than 5years [48]. Moreover, allografting treatment of CML with HSCs offers a high rate of cure in the pediatric population [49].

Kumar *et al.* reported that High dose chemotherapy followed by autologous HSCs transplant improve the survival rate of patients with advanced multiple myeloma [50].

Patients with relapsed Hodgkin's disease that treated with HSCs beside chemotherapy showed better remission than patients treated with chemotherapy alone [51].

HSCs were also used to manage autoimmune diseases. Patients with severe Systemic Lupus Erythematous **(SLE)** improved without need of immunosuppressive drugs when they were

treated with high dose of chemotherapy with HSCs transplantations [52]. Thus, Hematopoietic Stem Cell Transplantation **(HSCT)** considered a potential therapy for severe rheumatoid arthritis [53].

Infusion of bone marrow derived cells into patient with acute myocardial infraction improved Left Ventricular Ejection Fraction **(LVEF)** and reduced Left Ventricular End-Diastolic Volume **(LVEDV)**, Left Ventricular End-Systolic Volume **(LVESV)** and infract size. In addition to reduce the risk of death significantly. The effects of these cells last for more than one year [54].

Neurological disorders and spinal cord injury affect millions of peoples worldwide that mostly result from loss of functional cells. Replacement of lost cells is a goal of regenerative medicine. Important sources of new neural cells are embryonic and adult stem cells. Injection of purified CD34(+) and CD133(+) stem cells in spinal cord of patients with spinal cord injury result in segmental sensory and motor improvement in 37% and 10% of patients, respectively [55]. Burt *et al.* demonstrated that Autologous transplantation non-myeloablative hematopoietic stem cells restore neurological function of patients with relapsing-remitting Multiple Sclerosis **(MS)** who had not responded to treatment with interferon beta [56].

Combined infusion of insulin-secreting adipose-derived mesenchymal stromal cells with bone marrow-derived hematopoietic stem cells into patients with type 1 diabetes mellitus decrease HbA1c level with increase of c-peptide [57].

MSCs

MSCs showed high plasticity that develops to several types of cells. Many of Bone diseases end up with lose of degeneration of cellular composition which put them as a target of regenerative medicine. Osteogenesis Imperfect **(OI)** is a congenital disorder characterized by week bone composition lead to bone fracture due to a deficiency of Type-I collagen [58]. Engraftment of bone marrow stem cells from sibling or allogeneic source in children affected with OI reveal increase in growth and growth velocity with significant reduction in bone fracture frequencies [59,60]. Total body bone mineral content of patients received MSCs increase by 45-77% compared to control group [61].

Osteoporosis is a bone diseases characterized by loss of bone mass because of increased bone resorption compared to formation rate. This condition leads to increased risk of falls and recurrent fractions. Some studies referred the disturbance of formation and reabsorption rate to the decrease of osteoblast development from progenitor cells [62]. Improvement of Bone Mineral Density **(BMD)** at the Lumbar Spine **(LS)** and Femoral Neck **(FN)** of the patients with osteoporosis after allogeneic stem cell transplantation treated with zoledronic acid. Treatment with zoledronic acid increase osteogenic progenitors in the stromal cell compartment [63].

Long bone fracture and defect were treated by both techniques, scaffold and mesenchymal stem cells transplantation. Scaffold of hydroxyl apatite matrix or ceramics mixed with mesenchymal

stem cell improve limb function and Harris Hip Scores [64,65]. Autologous fibrin clot was used instead of synthetic scaffold to embed MSCs demonstrates effective and safe in the long-term healing of bone non-unions [66].

Similar techniques, using different scaffold enriched with growth factor, was used to improve Cranio-facial defects result in fill around 51.3% of alveolar pre-maxillary clefts [67]. Other study demonstrated that MSCs therapy generated 80% of the original jawbone deficiency after traumatic injury [68]. Intervertebral Disc Degeneration **(DD)** is a common disease effecting 71% in men and 77% in women aged under 50 years, and >90% in both men and women aged over 50 years [69]. DD causes acute or chronic low back or neck pain sporadic tingling or weakness through the knees, hands, and fingers. When expanded MSCs *ex vivo* were injected into degenerative inter-vertebral discs or pulposus area of patient suffering from back pain and lumbar spinal canal stenosis, improvement of patients was observed and confirmed by radiography and computer tomography by producing chondrocyte-like cells and increasing matrix synthesis [70,71].

Repairing of cartilage, tendon and ligaments, disorders by using MSCs treatments was approved in animal models, mainly horses [72]. Clinical uses of MSCs in tendon and ligament repair not yet published. Cardiovascular disease is a major global health problem. Around 17.5 million people died from CVDs in 2012, representing 31% of all global deaths mainly (80%) from heart attacks and strokes [73].

Clinical trials from different countries demonstrated that bone marrow mesenchymal cell injection intracoronary or intravenous into acute myocardial infraction patients from either allogeneic [74] or autologous [75] origin is effective and safe. Cardiac function of patients was improve by reducing Left Ventricular End-Systolic Volume **(LVESV)** also, ventricular volumes, wall thickness, and systolic wall thickening showed improvement when measured by Magnetic Resonance Imaging **(MRI)** or Computed Tomography **(CT)** [75]. There is no significant difference between allogeneic and autologous efficiency and safety when they introduced transendocardial injection in patients with ischemic cardiomyopathy [76].

The immunoregulatory function of MSCs rationalized the use of MSCs to treat autoimmune diseases. MSCs ameliorate the multiorgans dysfunctions effects of systemic lupus erythrematosus. The beneficial effects of MSCs were evaluated by serologic markers and renal function [77]. MSCs suppress T- cell activity by increasing Indoleamine 2,3-Dioxygenase **(IDO)** in lupus patients [78]. Despite the ability of MSCs to produce neural cells phenotypes [79], clinical trials could not reported a significant improvement of patients with chronic multiple sclerosis treated with autologous bone marrow derived MSCs [80].

Other study concerning with transplantation of BM-MSCs into patients with multiple sclerosis showed clinical improvements without radiological evidences. However, no side effects were observed [81].

Preclinical study demonstrated significant decreased of arthritis score by introducing bone marrow derived MSCs plus bortezomib. However, MSC-only, bortezomib-only groups are not improved [82].

Spinal cord injuries lead to serious impairments of muscles, sensation and autonomic functions including Loss of movement, Loss of sensation, Loss of bowel or bladder control, Exaggerated reflex activities or spasms, Changes in sexual function, sexual sensitivity and fertility, Pain or an intense stinging and difficulty in breathing, coughing or clearing secretions from your lungs.

From 3.6 to 195.4 patients per million around the world exposed to Traumatic Spinal Cord Injuries **(TSCI)**. These data extracted from 41 countries [83].

Fourteen patients suffered from chronic spinal cord injury were transplanted with MSCs showed variable improvements in tactile sensitivity and eight subjects developed lower limbs motor functional gains, principally in the hip flexors. Moreover nine patients of them demonstrated urological function improvement [84]. Other clinical study reported neurological function improvement of patients with cervical spinal cord injury who are received bone marrow derived MSCs [85]. However, the sample size of these studies is small which need further large clinical trials.

Despite the ability of MSCs to produce neural cells phenotypes [79], clinical trials could not reported a significant improvement of patients with chronic multiple sclerosis treated with autologous bone marrow derived MSCs [80]. Cirrhosis is a replacement of liver cells with scare tissue which impaired liver function including jaundice, ascites, palmar erythema, liver tenderness and enlargement , Spider angiomata, hypogonadism, Gynecomastiableeding, and, Portal hypertension effects such as splenomegaly, esophageal varices and caput medusa. Liver Cirrhosis result in serious complication including liver damage and dysfunction, and may lead to liver cancer.

Several factors are implicated in liver cirrhosis. For example, Alcohol, toxins, viral, bacterial and parasite infection, congenital causes such as hereditary hemochromatosis, alpha 1-antitrypsin deficiency, cystic fibrosis, Wilson's disease and others.

Replacement of degenerative hepatic cells, either by whole liver transplantation or by cellular therapy, is the standard management of liver failure. Autologous bone marrow derived MSCs infusion into four patients with decompensated liver cirrhosis result in improvement the quality of life of all four patients with increase of both physical and mental scale [86]. Larger study where the MSCs injected through intrahepatic or intraspleen routes, showed similar improvement of liver function [87]. MSCs treatment of patients with hepatic cirrhosis caused by alcohol or viral infection demonstrated improvement of histological scale and liver biochemical function [88,89]. Similar results were obtained by infusion of hematopoietic stem cells. In clinical trial repeated by the same research group with patient suffered from hepatic cirrhosis caused by viral and non-viral infection [90,91].

Transplantation of bone marrow derived MSCs into kidney transplanted patients protected them from graft dysfunction through immunomedulatory effects by reducing circulating CD8 T-cells [92].

Co-transplantation of MSCs and HSCs for treatment of patients with malignant lymphomas induces faster production of lymphocyte [93].

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