Current Stem Cell Research & Therapy, 2017, *12*, 388-400

REVIEW ARTICLE

The Influence of Diabetes Mellitus on Proliferation and Osteoblastic Differentiation of MSCs

Jun Tan[1,2,3,#], Lihua Zhou[4,#], Yang Zhou[5,#], Peng Xue[1,2], Guangsheng Wu[1,2,6], Guangying Dong[1,2], Hang Guo[7] and Qintao Wang[1,2,*]

[1]*State Key Laboratory of Military Stomatology, Department of Periodontology, School of Stomatology, The Fourth Military Medical University, Xi'an, Shaanxi, P.R.China;* [2]*Shaanxi Key Laboratory of Stomatology, The Fourth Military Medical University, Xi'an, Shaanxi, P.R.China;* [3]*Department of Stomatology, Guangzhou General Hospital of Guangzhou Military Command, Guangzhou, Guangdong, P.R.China;* [4]*Department of Stomatology, General Hospital of Beijing Military Area, Beijing, P.R.China;* [5]*Department of Anesthesiology, Guangzhou General Hospital of Guangzhou Military Command, Guangzhou, Guangdong, P.R.China;* [6]*Department of Stomatology, Qingdao First Sanatorium of Jinan Military Area Command, Qingdao, Shandong, P.R.China;* [7]*Department of Anesthesiology, General Hospital of Beijing Military Area, Beijing, P.R.China*

ARTICLE HISTORY

Received: October 30, 2014
Revised: January 30, 2015
Accepted: February 3, 2015

DOI:
10.2174/1574888X10666151001114527

Abstract: ***Background:*** Diabetes mellitus (DM) is a widespread chronic metabolic disease which has high mortality due to its complications. In addition to traditional medication, stem cell transplantation therapeutics has become a brand-new and prospective remedy for DM. With strong self-renewal and multi-potential ability, mesenchymal stem cells (MSCs) are considered as ideal cell sources of cell therapy for DM and many other diseases. However, not only do endogenous MSCs fail to replace the impaired islet cells, but also transplanted MSCs fail to cure many patients complicated with DM. Besides, quite a few DM patients suffer from high risk of fracture and low efficiency of bone regeneration, which are often associated with the osteoblastic differentiation of MSCs. Recently, a number of researches have investigated that the changes in micro-environment by DM can affect biological characteristics of MSCs through many factors.

Summary: In this review, we summarize the developments in the influence of DM on proliferation and osteoblastic differentiation of MSCs, and moreover, osteoporosis, obesity and metabolism syndrome, as they are closely related to DM.

Keywords: Diabetes mellitus, mesenchymal stem cells, proliferation, osteoblastic differentiation, osteoporosis, obesity, metabolism syndrome.

1. INTRODUCTION

Diabetes mellitus (DM) is a group of chronic metabolic diseases characterized by hyperglycemia resulting from defects in insulin secretion, insulin action, or both [1]. It is divided into four categories: type 1 DM (T1DM, caused by an absolute deficiency of insulin secretion), type 2 DM (T2DM, caused by combination of resistance to insulin action and an inadequate compensatory insulin secretory response), gestational diabetes mellitus (GDM), and other special types of DM (caused by monogenetic defects in cell function, genetically determined abnormalities of insulin action, pancreas injury, excess amounts of antagonizing-insulin hormones, drugs, *etc.*) [1]. The overall prevalence of DM in Chinese adults is approximately 11.6% in 2010 [2], and the incident number of DM patients world-wide is 366 million in 2011 [3] which is expected to rise to 439 million in 2030 [4]. With a prevalent incidence, DM often causes long-term damage, dysfunction, failure of different organs [5] and late complications such as microvascular diseases including retinopathy, nephropathy, neuropathy, and macrovascular diseases including acute coronary syndrome, claudicatio intermittent, and stroke [1], which are related with high mortality. The treatment of DM and its complications are not only limited to pharmacotherapy but has also started to focus cell therapy.

Mesenchymal stem cells(MSCs), originated from cord blood, bone marrow, fat, odontogenic tissue, reproductive system, *ect*, are multi-potential with autocrine and paracrine secretion together with immuno-regulation function. Therefore, MSCs are attractive candidates for not only tissue engineering applications, but also for cell transplantation therapeutics for many diseases including DM, especially T1DM [6-8]. MSCs can secrete insulin by an adjusted 3-step proto-

*Address correspondence to this author at the Department of Periodontology, School of Stomatology, The Fourth Military Medical University, 145 West Changle Road, Xi'an, Shaanxi 710032, P.R. China; Tel: 86-29-84776096; Fax: 86-29-83223047; E-mails: wangqintao@hotmail.com and yznmbk@fmmu.edu.cn
[#]*These authors contributed equally to the work.*

2212-3946/17 $58.00+.00

col [9], differentiation protocol I and II [10], co-culture with islet cells [11], gene transfection [12] or gene expression control [13]. At the same time, MSCs are related with osteogenesis and regeneration in bone fracture through homing from the niche and osteogenic differentiation [14].

However, except for the failure to substitute endogenous MSCs for the damaged islet cells, there is a high percentage of inefficacies in many MSCs transplantation treatments on diabetes patients, and bone marrow MSCs (BMMSCs) from the non-obese diabetic mice even do not have therapeutic effect on T1DM [15]. Furthermore, patients both with type 1 and type 2 diabetes, whereas bone mineral density (BMD) is increased in T2DM and decreased in T1DM, have been reported to have higher relative risk of hip fracture [16] and problems in osteoblastogenesis and bone regeneration [17-19]. Hence, DM is believed to have direct detrimental effects leading to multiple micro-environmental defects on many types of cells, especially side effects on proliferation and osteoblastic differentiation of MSCs [20]. In this review, we summarize the influence of DM on proliferation and osteoblastic differentiation of MSCs and try to analyze its underlying mechanism. At the same time, analogous metabolic disorders which are often concomitant with DM are also mentioned.

2. INFLUENCE OF DM ON PROLIFERATION AND OSTEOBLASTIC DIFFERENTIATION OF MSCs

Previous researches on the relationships between DM and MSCs usually started by isolating MSCs from DM animal models or DM patients with a hyperglycemic micro-environment *in vivo*, or stimulating normal MSCs by high glucose or advanced glycation end-products to imitate the micro-environment of DM *in vitro*. Thus, we present the influence of DM on proliferation and osteoblastic differentiation of MSCs from different research approaches.

2.1. Influence of Hyperglycemia on MSCs

Animal models of DM have been established with systemic hyperglycemia *in vivo* [21] by intraperitoneal injection of streptozotocin (STZ) and/or high fatty and high sugar diet in Sprague-Dawley (SD) rats, Wistar rats, or other rats, which are widely used in studies on the relationship between DM and MSCs. Observations on gross specimen and MSCs isolated from the DM objects have been taken on this subject (Table **1**).

Animal studies *in vivo* have certificated the negative role that DM plays in proliferation and osteoblastic differentiation of MSCs. STZ-induced diabetic SD rats fed for 4 weeks, showing pronounced loss of mineral in the trabecular bone of tibia were scanned and many monocytes/macrophages and adipocytes were stained in the bone marrow [22]. When titanium alloy (Ti–6Al–4V) screw implants were implanted into lower mandibles of spontaneous type 2 diabetic Goto-Kakizaki rats, the onset of cell proliferation and osteoblastic differentiation of their mesenchymal progenitor cells in alveolar bone were delayed and then prolonged while compared with normal bone [23].

Abundant cell experiments display parallel results. When STZ-induced diabetic SD rats are fed for 10 weeks, BMMSCs are obtained from the tibia and femur. These cells display a more spread out and explanate appearance and a rounder shape prone to apoptosis, decreased vascular endothelial growth factor (VEGF) and insulin-like growth factor-1 (IGF-1) secretion which are related with inhibition of apoptosis and lower proliferative abilities [28]. The proliferation rate of BMMSCs isolated from diabetic SD rats, with a blood glucose level > 250 mg/dl, is also lower than non-DM-MSC and can be recovered by oxytocin treatment [24]. Besides, the cell proliferation ability alteration of the BMMSCs from the non-obese diabetic (NOD) mice is similar, and the cell-cycle-regulation protein p21 expression and nuclear factor kappa B (NF-κB) pathway correlated factor p65 level are significantly enhanced and further dysregulate the proliferation of the NOD-MSCs [15]. BMMSCs, isolated from STZ-induced type 1 diabetic Wistar rats after 12-week injection, unlike those from healthy rats, have slower proliferation speed, higher levels of p21 and apoptosis-associated maker p53, and poorer osteogenic differentiation *in vitro* [25, 30]. Nevertheless, both vitamin D and glucocorticoid receptors which control osteoblastic differentiation are upregulated [30]. BMMSCs obtained from C57BL/6 mice 90 days after diabetes induction are significantly less than those from age-matched normal mice, express higher level of CD 44 and less percentage of CD 90, and show no osteogenic potential [27].

There are few reports but parallel results about MSCs isolated from DM patients. Adipose-tissue-derived mesenchymal stem cells (ASCs) obtained from T2 diabetic patients (dASCs) are demonstrated to have reduced proliferative capacity and osteogenic differentiation potential. Cell death related tumor necrosis factor-α (TNF-α) exhibits higher expression in dASCs, which elevates the expression of *caspase 3*, *caspase 8*, and *p53*. Besides, genes involved in insulin resistance, such as *adiponectin* and *resistin*, express abnormally [29].

As the ability of reacting to ectogenic signals and adapting to the changed micro-environment is impaired in MSCs, although preconditioned by growth factors such as IGF-1 and fibroblast growth factor-2 (FGF-2) in combination for 1h can augment the function of BMMSCs from STZ-induced diabetic C57BL/6 wild-type mice at 60 days [31], diabetic BMMSCs from Wistar rats failed to have higher colony forming units-fibroblast (CFU-f) efficiency and osteogenic differentiation potential after pre-culture on the fibrin scaffolds [26], and the reduced proliferation and osteogenic potential of dASCs are irreversible when treated with insulin [29].

Nevertheless, some different outcomes about the changes in the ability of proliferation are recognized. For example, BMMSCs isolated from diabetic mice after 90 days of induction display similar proliferation potential as the healthy cells [27], and the proliferation ability of BMMSCs is even enhanced in diabetic rats after 4-week injection [30]. These inconsistent results may be caused by different diabetic conditions such as the course of disease, the duration of high glucose in serum of animals, the culture environment *in vitro*, and the species, body tolerance and physiological status of animals.

On the whole, hyperglycemia has negative effects on the proliferation and osteoblastic differentiation of MSCs, and the possible mechanism has been explored by a series of researches described hereinafter.

2.2. Influence of High Glucose on MSCs

Needless to say, high glucose (HG) *in vitro* can mimic the changed micro-environment of DM and is associated with the characteristics and function of MSCs. There have been a number of researches on the influence of HG on the proliferation and osteoblastic differentiation of MSCs, while conflicting results have been observed, and yet the mechanism is not very clear (Table **2**).

High concentration of D-glucose, not L-glucose is identified to have effects on MSCs [36]. On an average, high glucose induces cellular senescence, impairs proliferation ability, and decreases the number of colony forming units in

Table 1. Hyperglycemia's influence on the proliferation and osteoblastic differentiation of MSCs.

Authors, Year	Animal Model/Patient	Blood Glucose	Cells	Proliferation	Osteoblastic Differentiation	Molecular Changes
Gu Z *et al.*, 2013 [15]	NOD mice	-	BMMSCs	The cell proliferation ability of the BMMSCs was significantly decreased.	-	The expressions of p21 and p65 were increased.
Kim YS *et al.*, 2013 [24]	STZ-induced diabetic 12-week SD rats	Higher than 250 mg/dl	BMMSCs	Proliferation rate was significantly reduced in 7 days and was restored by oxytocin.	-	-
Zhao YF *et al.*, 2013 [25]	STZ-induced type 1 diabetic 4-week-old male Wistar rats 12-week after injection	Higher than 16.7 mmol/l	BMMSCs	Significantly lower at days 3, 5, and 7 than those of control ones.	Osteogenic differentiation was poorer *in vitro* and osteogenic potential was attenuated *in vivo*.	*IR*,*IGF-1*, *IGF-1R* and *IRS-1* gene expressions and the protein levels of IGF-1R, IRS-1and p-ERK were decreased.
Stolzing A *et al.*, 2011 [26]	STZ-induced diabetic 2 to 3-month-old male Wistar rats 12-week after injection	-	BMMSCs	-	The CFU-f assays of BMMSCs from diabetic rats were lower than those of normal ones in osteogenic growth medium either after culturing on fibrin or not.	-
Ezquer F *et al.*, 2011 [27]	STZ-induced diabetic C57BL/6 mice 90-day after injection	Higher than 400 mg/dl for more than 2 months	BMMSCs	Viable BMMSCs were less abundant in the diabetic bone marrow but they displayed the same proliferation potential.	The diabetic BMMSCs had no osteogenic potential.	-
Jin, P *et al.*, 2010 [28]	STZ-induced diabetic SD rats weighting 200–220 g 10-week after injection	Higher than 16.7 mmol/l	BMMSCs	Proliferative abilities at day 3, 5 and 7 were lower and apoptosis rate was higher in the early phase.	-	VEGF and IGF-1 production were decreased significantly.
Cramer C *et al.*, 2010 [29]	T2DM patients	The average HbA1c of DM donors was 10.75±2.65.	ASCs	Diabetic ASCs had lower population doublings than those of normal ASCs.	The *ALP* expression decreased significantly.	Apoptosis genes *caspase 3*, *caspase 8*, and *p53* were upregulated by a high expression of TNF-α. Genes such as *adiponectin* and *resistin* expressed abnormal levels.
Stolzing A *et al.*, 2010 [30]	STZ-induced diabetic 3-month-old male Wistar rats 4-week/12-week after injection	Higher than control ones	BMMSCs	While the sizes of the colonies were smaller, CFU numbers increased in 4-week diabetic rats but declined in 12-week ones.	-	ROS production, p21, p53, AGE and RAGE levels were increased. Both vitamin D and glucocorticoid receptors expressed high levels.

Table 2. HG's influence on the proliferation and osteoblastic differentiation of MSCs.

Authors, Year	Glucose Concentration, Time	Cells	Proliferation	Osteoblastic Differentiation	Molecular Changes
Wang A *et al.*, 2014 [22]	25.6 mmol/L in osteogenic medium for 4 weeks	SD rat BMMSCs	-	There were much less mineralization and more lipids.	The cytosolic UDP-GlcNAc and UDP-GlcUA were elevated.
Rinker TE *et al.*, 2014 [32]	high in osteogenic medium in a hydrogel-based 3D culture platform for a week	BMMSCs	Clonogenicities of BMMSCs were degraded when mono- and co-cultured with osteoblasts but unchanged when cultured with adipocytes.	-	-
Kim SY *et al.*, 2013 [33]	30 mmol/L in osteogenic medium for 4 or 7 days	hPDLSCs	-	The ALP activity in cells, *ALP*, *Runx2*, *OSX* and *FRA1* gene expressions, Runx2, OSX, OPN, and COLIA protein levels were descended.	The ROS generation was increased, the protein levels of PI3K and β-catenin, and Akt phosphorylation were decreased.
Zhu G *et al.*, 2013 [34]	25 mmol/L for a week	MSCs	Most MSCs were obstructed in the G0/G1 phase in first 2 days.	-	The miR-32-5p expressed significantly lower and PTEN expressed higher. Akt phosphorylation and cyclin D1 expression were inhibited, and GSK-3β phosphorylation was promoted.
Dhanasekaran M *et al*, 2013 [35]	25mmol/L for 15d and 21d	hASCs from subcutaneous fat, omentum fat and hBMMSCs	The proliferation ability was not influenced by HG.	The mineralization of calcium deposits did not change significantly.	-
Kim YJ *et al.*, 2012 [36]	25 and 50 mmol/L for 2 weeks	hASCs	MSCs' senescence was promoted and cell proliferation was diminished.	The osteogenic differentiation was inhibited.	The miR-486-5p expression was increased and SIRT1 expression was decreased.
Keats E *et al.*, 2012 [37]	25mmol/L for 1, 4, and 12 days	hBMMPCs	The cell number was significant decreased in day 1 but turn to the same as control cells by day 4.	*Runx2* and *osterix/SP7* were reduced.	-
Stolzing A *et al.*, 2012 [38]	25mmol/L for 2 weeks	rat nonadherent BMMSCs	The colony formation of BMMSCs was declined.	The osteoblastic differentiation ability of BMMSCs was suppressed.	-
Ryu JM *et al.*, 2010 [39]	25mmol/L for 72h and in addition to osteogenic induction medium for 2 weeks	hMSCs	The CFU of hMSC was increased.	The osteogenic differentiation capability was not impaired.	Cyclin D1/E and TGF-β1 expressions were elevated, $[Ca^{2+}]$ was increased, pan PKC, p44/42, p38 MAPKs, Akt and mTOR phosphorylation levels were enhanced. Relevant inhibitors could reverse such variations.

Table (2) contd….

Authors, Year	Glucose Concentration, Time	Cells	Proliferation	Osteoblastic Differentiation	Molecular Changes
Weil BR *et al*., 2009 [40]	20 and 30 mmol/L for 24h or 48h	hMSCs	The proliferation rate was not affected in either concentration.	-	Productions of VEGF, HGF, or FGF2 didn't change, and neither JAK/STAT nor p38 MAPK phosphorylation was activated.
Liu Z *et al*., 2009 [41]	15, 30, and 50 mmol/L for 48h	Rat MAPCs	The proliferation rate was decreased in a high concentration of 50 mmol/L.	-	VEGF expression was suppressed and inhibited JAK2/STAT3 phosphorylation.
Li YM *et al*., 2007 [42]	11 and 25 mmol/L for 4 day and 4 weeks	hMSC-TERT	The proliferation was stimulated dose dependently in both short- and long-term culture.	-	Txnip mRNA expression was enhanced upon HG concentrations from 5.6 mmol/L to 25 mmol/L both after 4 days and 4 weeks.
	11, 25, and 40 mmol/L for 4 day and 4 weeks	primary hMSC	The proliferation was invariable for 4 days while decreased slightly but significantly after long-term exposure.	The degree of mineralization was enhanced in 25 mmol/L glucose media after 4 weeks.	Txnip mRNA level increased in 40 mmol/L glucose media after 24h.
Gopalakrishnan V *et al*., 2006 [43]	16.5 and 49.4 mmol/L for 3, 7, and 28 day	rat BMMSCs	Proliferation of BMMSCs was decreased.	ALP activity was descended, areas stained for collagen and mineralized nodules were smaller.	-

BMMSCs obtained from rats [44]. Rat MSCs cultured in 25mmol/L D-glucose media for 48h mostly stay in unipartite G0/G1 phases and express significantly lower level of miRNA-32-5p, high level of phosphatase and tensin homologs deleted on chromosome (PTEN) which is a primary control channel of inflammation and diabetes. Then, Akt phosphorylation and cyclin D1 expression are inhibited, and glycogen synthase kinase-3β (GSK-3β) phosphorylation is promoted [34]. After 3-day- and 7-day-culture in higher glucose concentration in the presence of an osteogenic medium, proliferation of rat BMMSCs and alkaline phosphatase (ALP) activity are attenuated, and the areas stained for collagen and mineralized nodules are smaller than that in normal medium after 4 weeks [22, 43]. In addition, insulin and estradiol are able to attenuate such negative effect of high glucose on osteogenic differentiation of rat BMMSCs [43]. MSCs expanded in suspension are also affected by high glucose. In spite of a more homogenous stage of differentiation comparing with adherent MSCs, rat nonadherent BMMSCs have decreased colony formation and osteogenic differentiation ability when cultured in HG-containing medium than that treated with classical medium [38]. In a hydrogel-based 3D culture platform, BMMSCs viability and clonogenicity are equally cut down when mono- and co-cultured with osteoblasts under high glucose conditions, but they remain the same when cultured with adipocytes at high glucose levels [32]. Similarly, human bone marrow mesenchymal progenitor cells (hBMMPCs) treated with high glucose (25mmol/L) showed an impaired osteogenic differentiation potential [37]. Human ASCs exposed to high glucose show promoted senescence, and diminished proliferation and osteogenic differentiation as well. MiR-486-5p, which is associated with aging and senescence, is found to increase and down regulate the expression of a regulator of metabolic disorders and metabolic---silent information regulator (SIRT1) [36].

Periodontitis is regarded as the sixth complication of DM; therefore, the periodontal ligament stem cells (PDLSCs) are affected by the micro-environment of DM, too. The osteogenic activity of hPDLSCs cultured with high glucose (30 mmol/L) is significantly lower than that of the control group at an earlier stage. During this process, reactive oxygen species (ROS) generation is increased, the protein expression of phosphatidylinositol 3-kinase (PI3K) and β-catenin, and protein kinase B (PKB/ Akt) phosphorylation are decreased. However, this suppression can be revised by hesperetin, an antioxidant and free radical scavenger, through altering the abovementioned molecules [33].

Even though many evidences of the attenuated effects of HG on bionomics of MSCs have been enumerated, a coincidence of the no detrimental effect on proliferation and differentiation of MSCs appears between HG and DM. hBMMPCs cultured with high glucose concentration (25mmol/L) showed a significant decrease in cell number only on the first

day but turn to parallel to control cells by day 4 [37]. At both 24 and 48 h, glucose concentration from 5.5mmol/L to 30mmol/L does not affect the proliferation of hMSCs together with production of VEGF, hepatocyte growth factor (HGF), or FGF2, and neither janus-activated kinase singal transducers and activators of transcriprion (JAK/STAT) nor p38 mitogen-activated protein kinase (MAPK) phosphorylation was activated [40]. Rat multipotent adult progenitor cells (MAPCs), which are included in BMMSCs, do not have a lower proliferation rate when cultured with high glucose (15 and 30 mmol/L) unless cultured in a concentration of 50 mmol/L than that of control (D-glucose concentration of 5.5 mmol/L). The released VEGF expression decreased until a glucose concentration of 30 mmol/L for 48h [41]. Another study discovered that HG (25mmol/L) does not impact either proliferation or osteogenic differentiation ability of ASCs from subcutaneous fat, omentum fat and BMMSCs [35]. The facts show that short-term exposure to relatively high glucose concentration neither affects the proliferative capacity of MSCs nor their ability to produce paracrine growth factors [40].

Moreover, HG can have an even enhanced influence role on MSCs sometimes. Although HG makes the proliferation rate of primary hMSCs invariable after 4 days and decreases it slightly after long-time culture (4 weeks), proliferation of telomerase-immortalized MSCs (hMSC-TERT) is dose-dependently enhanced by high glucose concentrations in both short-term (4 day) and long-term exposure (4 weeks), together with the apoptosis-related protein Txnip. HG even significantly enhances primary osteogenic differentiation of hMSCs *in vitro* [42]. When cultured with 25 mmol/L D-glucose for 72h, hMSCs from umbilical cord blood have more colony forming units than that with 5 mmol/L glucose, and maintain the undifferentiated status and multipotency. Cyclin D1/E and transforming growth factor-β1 (TGF-β1) expression were elevated, [Ca^{2+}] was increased, pan protein kinase C (PKC), p44/42, p38 MAPKs, Akt and mammalian target of rapamycin (mTOR) phosphorylation levels were enhanced. However, their relevant inhibitors could reverse such variations [39].

The constant or increased proliferation of MSCs in early stage of the stimulus by HG may be due to several factors which are still not clear. One factor is probably a stress reaction on the alteration of micro-environment to coping with apoptosis. Another one may be a temporary response to a rise in serum glucose [30]. MSCs may be resistant to high glucose toxicity in early period of diabetes pathogenesis but a proapoptotic situation is motivated simultaneously. Furthermore, different cell types can also have various responses to HG. On account of the confused relationship between HG and proliferation and osteoblastic differentiation of MSCs, some studies have been completed to reveal the function of hyperglycemia/HG's toxic product as follows.

2.3. Advanced Glycation End-products on MSCs

In advanced diabetes mellitus, long-term hyperglycemia makes glucose react with proteins, lipids and nucleic acids forming unstable Schiff bases, which are modified to form Amadori products and then advanced glycation end products (AGEs) [45, 46]. AGEs, especially biologically potent AGE2 and AGE3, exist for weeks, and interaction between AGEs and the receptor for AGEs (RAGE) is related with DM and almost all the complications of DM [45, 47-49] and are documented to affect MSCs [47] (Table **3**).

AGEs have been first reported to inhibit the proliferation and multipotential differentiation of MSCs in 2005. The cells treated with AGE-2 and AGE-3 show a dose-dependent (1–100μg/ml) decrease in proliferation, and a declined osteogenic differentiation ability, in which AGE-RAGE interaction is involved [54]. AGE- bovine serum albumin (BSA) inhibits the proliferation of MSCs from SD rats time-and dose-dependently (6-24h, 25-200 μg/ml), via activating ROS generation and p38 phosphorylation, and inducing pro-inflammatory chemokines/cytokines which can inhibit MSCs growth in turn [53]. Furthermore, the apoptosis of BMMSCs is found to be increased significantly by AGEs no lower than 200μl/ml, with caspases activity, TNF production/secretion, p38 phosphorylation, and oxidative stress (OxS) augmented [51]. The osteoblastic differentiation of clonal stromal cells isolated from the bone marrow of BC8 mice (ST2 cells) is inhibited by AGE2 or AGE3 alone dose-dependently from 10-200μg/ml, perhaps through decreasing osterix (OSX) expression and partly through increasing RAGE expression, while high glucose alone or in combination with AGEs has no effect on these mouse stromal ST2 cells [52]. Moreover, AGE3 significantly inhibits the mineralization in both ST2 cells and human MSCs, and inhibits the differentiation and mineralization of both cells with partially binding to RAGE and increasing TGF-β signaling, according to small interfering RNA (siRNA) transfection technique [50].

AGEs of 50-100μg/ml are also reported to reduce the proliferation of BMMSCs from Wistar rats at day 5, and semblable trend is presented in the mRNA expression levels of osteocalcin (OCN) after a 14-day induction. However, AGEs of lower concentration (≤30 μg/ml) are found to increase proliferation due to oxidative effect. Furthermore, various concentrations fail to affect the expression of sialoprotein and runt-related transcription factor 2 (Runx2) [30]. These consequences may be due to the alarm reaction of MSCs in prophase stimulus and a short time in differentiation induction.

3. THE INFLUENCE OF CERTAIN DM-CORRELATED DISEASES ON MSCS

Partly resulted from inadequate endogenic secretion, deficient action of insulin on target tissues is the basis of the abnormalities in carbohydrate, fat, and protein metabolism in diabetes [1]. Thus endocrinic and immune diseases such as thyroid dysfunction [55], hyperparathyroidism [56], and rheumatoid arthritis [57], which are demonstrated to have positive correlations with DM, can also bring about abnormalities of bone metabolism [58-60]. While on the contrary, few reports [61] have been presented about the influence of these illnesses on the biological properties of MSCs. More research work might be required in these areas. Furthermore, some DM-correlated metabolic diseases, obesity, metabolism syndrome and osteoporosis, which are common comorbidities of DM and always interact with DM, have been recognized to affect MSCs as well. A outline on those metabolic diseases is given below.

Table 3. AGEs' influence on the proliferation and osteoblastic differentiation of MSCs.

Authors	AGE Concentrations	Cells	Proliferation	Osteoblastic Differentiation	Molecular Changes
Notsu M *et al.*, 2014 [50]	200 μg/ml AGE-3	Mice BMMSCs (ST2 cells) hMSCs	-	The mineralization was suppressed.	RAGE was binded partially with AGEs and TGF-β expression was increased.
Weinberg E *et al.*, 2014 [51]	10–400 mg/ml AGE	Rat BMMSCs	Apoptosis of BMMSCs was increased significantly by AGEs no lower than 200μl/ml.	-	Caspases activity, TNF production/secretion, p38 MAPK activation, and ROS generation were augmented.
Okazaki K *et al.*, 2012 [52]	10-200μg/ml for mineralization analysis 200μg/ml for growth and protein detection AGE-2, and AGE-3	ST2 cells	The time-dependent growth curve was shifted downward during 5 days.	ALP staining and activities were inhibited for 4 days, and mineralization stainings were attenuated at day 21.	Protein expressions of OSX and Runx2 were decreased at day 14 and RAGE expression was increased at first 3 days.
Yang K *et al.*, 2010 [53]	25-200 μg/ml AGE	Rat BMMSCs	Proliferation was inhibited.	-	Pro-inflammatory chemokines/cytokines such as *Ccl2*, *Ccl3*, *Ccl4* and *Il1b* were induced, and ROS generation and p38 phosphorylation were activated.
Stolzing A *et al.*, 2010 [30]	5-100μl/ml for proliferation 5-500μl/ml for differentiation AGE	Rat BMMSCs	Proliferation of MSCs was decreased by higher concentrations but increased by AGEs no higher than 30μl/ml.	The mRNA expression levels of *OCN* were lower but those of sialoprotein and *Runx2* were not.	-
Kume S *et al.*, 2005 [54]	1–100μg/ml AGE-2 and AGE-3	hMSCs	The growth curve was shifted downwards.	The mineralization was less.	AGE-RAGE interaction was involved. ROS generation was stimulated.

3.1. Definition of Obesity, Metabolism Syndrome and Osteoporosis and Relationship among Them and DM

According to the standardization of World Health Organization (WHO) in 1997, obesity is defined as BMI [body mass indices, the ratio of body weight (kg) to height (m^2)] ≥ 30, or waist circumference 94 cm (man)/ 80 cm (woman).

Obesity has been identified as a risk factor for many metabolic disorders, especially diabetes mellitus, and many patients with T2DM are over-weight. As obesity often accompanies insulin resistance, the incidence rate of T2DM raises rapidly along with that of obesity.

Metabolism syndrome(MS), including constellation of metabolic abnormalities such as insulin resistance, glucose intolerance, dyslipidemia (particularly hypertriglyceridemia and low levels of HDL), central obesity, hypertension, and a high proportion of atherosclerotic disease [62], is one of the pathophysiological bases and risk factors of DM and comes with obesity [63, 64].

Besides, both obesity and MS have been associated with increased bone fracture risk and osteoporosis [65-67].

Osteoporosis has been characterized as a bone metabolism disorder of decreased bone strength which leads to an increased risk of fracture [68]. Epidemiological study and Meta-analysis showed that patients with DM are at increased risk of osteoporosis, osteopenia, fractures, as well as impaired bone healing and regeneration [30, 69, 70]. As the skeletal integrity and the bone turnover are demonstrated to be affected by diabetes through insulin resistance, AGEs and IGF-I accumulation [71, 72], and T1DM enhance bone destruction in periodontitis through the receptor activator for nuclear factor-κ B ligand (RANKL) signaling pathway [73], diabetic bone disease, such as osteoporotic fracture, which is considered to be an overlooked complication of diabetes [74].

Therefore, DM, osteoporosis, obesity and metabolism syndrome are correlated, and their influences on MSCs are more or less similar.

3.2. Influence of Obesity and Metabolism Syndrome on MSCs

Redundant fat is the main harmful factor in obesity and metabolism syndrome. The co-existence of MSCs with

mouse fat *in vitro* [75] or marrow adipocytes *in vivo* [76] can attenuate their osteogenic differentiation potential.

Fructose-rich diet (FRD)-induced MS SD rats display delayed bone healing, besides, BMMSCs from them show decreased osteogenic potential and express lower Runx2/ peroxisome proliferator-activated receptor (PPARγ) ratio than control rats BMMSCs [62]. In human, BMI plays an important role in biologic characteristics of MSCs. Mesenchymal stem-like cells from endometrium (eMSCs) isolated from overweight BMI women have significantly lower cloning efficiency than those from normal BMI women [77]. There is an inverse correlation with BMMSCs not only between BMI and ALP activity [78], but also between BMI and osteogenic response of mechanical strain *in vitro* [79], suggesting that higher BMI might compromise in the osteogenic differentiation potential of BMMSCs. Similarly, ASCs isolated from patients with overweight BMI have reduced proliferation, lower colony-forming unit potential, and compromised osteogenic capacity than lean BMI-ASCs *in vitro* [80].

However, there are some protective factors in fat tissue. One is adiponectin as previously described; another is free fatty acids (FFAs). In mainly FFAs contained high-fat diet (HFD) obese mouse model, higher BMD is observed and BMMSCs isolated from the proximal femurs show increased CFU-osteo assay and down regulated gene expression and protein levels of PPARγ. FFAs stimulated BMMSCs also present promoted mineralization [66]. In addition, the negative influence of obesity on MSCs can be reversed by low magnitude mechanical signals [81].

3.3. Influence of Osteoporosis on MSCs

In ovariectomized (OVX) osteoporosis rat model, separated MSCs show lower colony-forming and growth rate, down-regulated osteogenic genes and less calcium nodules than those from healthy rats [82-84].

BMMSCs from osteoporotic postmenopausal women have a lower proliferation rate and impaired osteogenic differentiation ability as proved by the ALP activity, osteogenic genes such as *OCN* and *Runx2*, and calcium phosphate deposition compared with control cells [85-88]. However, proliferation rate and differentiation potential of ASCs are less effected by osteoporosisas compared to BMMSCs [89].

Synthesizing less type I collagen [90], the osteoporotic BMMSCs have lower leptin (a bone-protective hormone) binding capacity during osteogenic induction [91], impaired leptin cell response [92], and partly defective bone morphogenetic protein (BMP-2) signal transduction [87]. The cells are also unresponsive to the osteoblastic-differentiation-related extracellular regulated protein kinases (ERK)1,2 MAPK signal pathway [86], express decreased osteogenic differentiation promoter miR-21 [88], and over express osteoblastic differentiation inhibitors miR-705 and miR-3077-5p which are mediated by TNF-α and ROS through NF-κB pathway [93], in contrast with the healthy MSCs. Otherwise, gene expression analysis demonstrates that the transcriptomes of BMMSCs from primary osteoporosis show distinct signatures and little overlap with those of non-osteoporotic cells [94].

4. THE POSSIBLE MECHANISM OF DM'S INFLUENCE ON MSCS

As mentioned earlier, various even conflicting results have been reported on the relationship between DM and proliferation and osteoblastic differentiation of MSCs. Although the exact mechanism is not quite clear, we can estimate some possible modulating molecules and pathways according to the researches aforementioned, and hypothesize some other probable factors which have been demonstrated to be involved in DM and the function of MSCs, respectively.

4.1. Probable Modulating Molecules and Pathways

Impairment of biological characteristics of MSCs can be owing to mitochondrial dysfunction, glucose-induced replicative senescence, metabolic disorders, and signaling pathways blocked by a diabetic environment [28, 42]. Sometimes, the decrease of cell number is responsible for the reduced osteoblastogenesis [42]. More importantly, as cell expansion depends on the balance of proliferation and apoptosis, and bone marrow metabolism emphasizes the biologic balance between osteoblastogenesis and adipogenesis, a deal of molecules and pathways can alter the balances [95]. For example, adipogenic gene expression notes that a cross-talk between BMMSCs and adipocytes may emerge [32], and one of the fundamental mediators of diabetic pathology, cytosolic UDP-GlcNAc, is suggested to have critical roles in regulating the diversion which divides MSCs to an adipogenic pathway by high glucose [22]. On the basis of limited investigations mentioned above, we sketch out possible modulating molecules and pathways involved in the mechanism of DM's influence on proliferation and osteoblastic differentiation of MSCs as follows (Fig. **1**).

NF-κB-p53/p21 pathway is involved in the proliferation and apoptosis of MSCs. Cell cycle regulatory protein p21 can induce replication senescence in nucleus but has anti-apoptotic effect in cytoplasm [15]. Nucleus p21 and its upstream regulator p53 are enhanced in DM originated MSCs and to suppress the proliferation of MSCs via activating NF-κB pathway by AGEs [15, 30]. The decreased MSCs' proliferation ability is also caused by high expression of TNF-α owing to hyperglycemia and AGEs to start apoptosis via caspase pathway and p38 MAPK pathway [29, 51].

IGF-1 deficiency is the crucial character of T1DM. The decreased gene and protein expression of IGF-1 related factors [25, 28] in DM MSCs can inhibit the IGF-1 pathway and then block ERK pathway, as to downregulate the cell proliferation, osteoblastic differentiation and skeletal development [25].

PI3K/Akt pathway is critical for bone development and skeletal remodeling, bone formation and osteoblastic survival [33], and activation of mTOR is a central regulator of translation and cell proliferation [39]. PI3K/Akt-mTOR pathway can be blocked by HG [33] through increasing the expression of PTEN by low level of miR-32-5p [34], to diminish the duplication and osteoblastic differentiation of MSCs. ROS is considered to bi-directionally regulate proliferation and apoptosis through oxidative stress. The ROS generation has been found enhanced by HG and high level of AGEs [30, 33, 53, 54] to downregulate PI3K/Akt and then

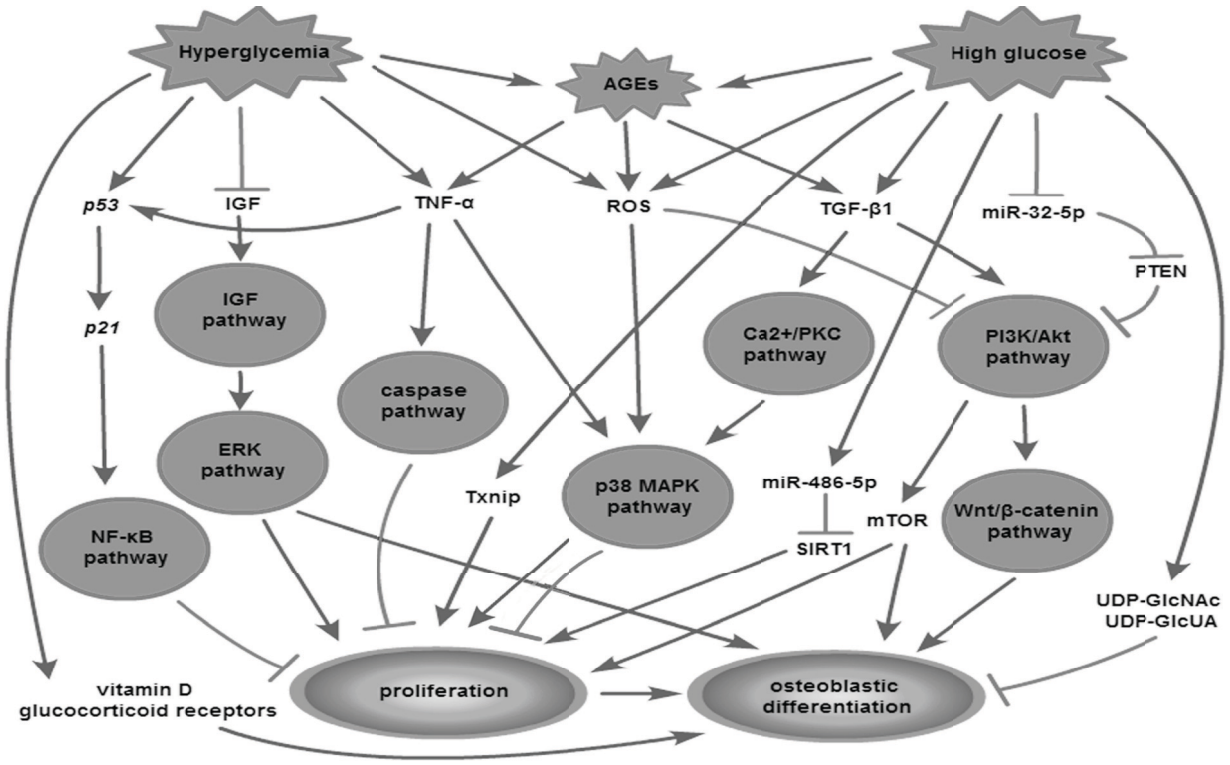

Fig. (1). Possible modulating molecules and pathways involved in the mechanism of DM's according to the researches in this review.

Wnt/β-catenin signaling pathway, which is critical for bone development and skeletal remodeling [33]. High expression of cell proliferation and osteogenesis regulator TGF-β stimulated by AGEs is believed to lead higher apoptosis and lower mineralization [50, 51], but TGF-β1 is found to activate PI3K/Akt/mTOR pathway and cell-cycle regulator Ca^{2+}/PKC/p38 MAPKs pathway [39]. However, p38 MAPK pathway is responsible for improving expression of pro-inflammatory cytokines and apoptosis, and ROS-p38 MAPK pathway activated by AGEs is also found responsible for the inhibition of MSC proliferation [53]. On the other hand, the upregulation of vitamin D and glucocorticoid receptors which control osteoblastic differentiation by HG, perhaps because of lower cortisol ligand or non-function of themselves, can be regarded as a compensatory mechanism of the MSCs [30]. High expression of Txnip induced by HG to avoid apoptosis has also displayed remarkable resistance of MSCs to HG [42]. These contrary results may be due to the mediation with different activating levels of different pathways associated with the same bioactive molecule, and the previous studies are far from enough.

4.2. Other Potential Factors

Apart from those relatively confirmative factors mentioned above, there are several probable factors such as fat-related factors and pro-inflammatory cytokines, which relate to DM and alter proliferation and osteoblastic differentiation ability of MSCs, respectively. Though no research has clarified the roles played by these endogenous cytokines directly in the influence of DM on MSCs, we can infer some probable functions of them from a series of interrelated experiments, which could be new research subjects.

The osteoblast-adipocyte ratio in the bone marrow and their differentiation are always modified [95], and then fat-related factors involved in DM may also impact cell increment and osteogenesis. PPARγ is a master regulator in adipogenesis, inflammation, lipid biosynthesis, and glucose metabolism [96], which suppresses osteogenesis through inhibiting the transcriptional activity of *Runx2* [97] and decreasing activities of the TGF-β/BMP signaling pathways [98]. Its expression is found to be increased in T1 diabetic mice [99], and L-SACC1 mice, a murine model of impaired insulin clearance to imitate hyperinsulinemia [100]. One co-repressor of *PPARγ2*, small leucine zipper protein (sLZIP), can enhance *Runx2* transcriptional activity to promote osteoblastic differentiation of hMSCs [101]. Adiponectin (APN), which is one of the adipokines provided by white adipose tissue (WAT) and reduced in DM, is presumed to be potent insulin-sensitized by binding its receptors and activating pathways such as AMPK and PPARγ, and has antidiabetic and antiatherogenic effects [102, 103]. APN is found to regulate the mobilization and recruitment of BMMSCs in mice to participate in bone repair and regeneration by regulating stromal cell-derived factor (SDF)-1. It can be assumed that PPARγ and APN might be target points on osteoblastic differentiation of BMMSCs in DM.

Inflammation has been considered to lead to and to appear in DM through insulin resistance [104], and many pro-inflammatory cytokines may be produced when DM occurs.The risk of developing T2DM rises with increasing concentrations of pro-inflammatory mediators such as soluble intracellular adhesion modecule-1 (sICAM-1), and soluble vascular cell adhesion molecule-1 (sVCAM-1) [105]. ICAM suppresses MSCs osteogenic differentiation and matrix min-

eralization *in vivo*, enhances MSCs proliferation through inhibition of the ERK/MAPK and NF-κB pathways or activation of the PI3K/AKT pathway *in vitro* [106]. High-mobility group box 1 (HMGB1), an evolutionarily conserved chromosomal protein, which is recognized to be a potent innate inflammatory mediator, can be released extracellularly by dead and dying cells including damaged pancreatic β-cells in T1DM and is regarded as a potent innate immune mediator to enhance diabetes onset [107-109] and a bone resorption signal [110]. HMGB1 is found to inhibit the proliferation of hBMMSCs but induce their osteoblastic differentiation in a 7-day-culture [111]. Furthermore, although pro-inflammatory cytokine IL-17 is observed in the gingival crevicular fluid (GCF) of patients with type 2 diabetes with chronic periodontitis, compared to patients without diabetes [112, 113], it is found to accelerate proliferation and osteoblastic differentiation of hMSCs [114] but restrains the osteogenic ability of osteoblast precursor cells [115]. The complex capabilities of these pro-inflammatory mediators may explain the contrary effects of DM and HG on proliferation and osteoblastic differentiation of MSCs.

CONCLUSION AND PROSPECTIVES

In conclusion, according to previous studies on MSCs isolated from DM animals/patients, and MSCs stimulated by HG and AGEs, DM and its intimate-correlated diseases have attenuating long-term effects on proliferation and osteoblastic differentiation of MSCs, but differences still exist depending on the research subjects and culture conditions, *etc*. The mechanism is so complex that the cross-talk and even network-talk of different signals and pathways need to be further studied. Besides, the mechanism from organelle level such as mitochondria and endoplasmic reticulum which are sensitive under stress might be a fresh research orientation. Clarifying the mechanism of proliferation and osteoblastic differentiation of MSCs altered by DM micro-circumstance will help to elevate the quality of MSCs in DM patients, to find new therapy target spots on the osteogenesis complications of DM, and to enhance practical cell transplantation therapy for DM patients.

CONFLICT OF INTEREST

The authors are sure that no conflict of interest exists in this review.

ACKNOWLEDGEMENTS

This work is supported by the National Nature Science Foundation of China (Grant No. 81271137 and 81470742).

REFERENCES

[1] American Diabetes Association. Diagnosis and classification of diabetes mellitus. Diabetes Care 2014; 37 Suppl 1 S: 81-90.

[2] Xu Y, Wang L, He J, *et al.* Prevalence and control of diabetes in Chinese adults. JAMA 2013; 310(9): 948-59.

[3] Rewers M. Challenges in diagnosing type 1 diabetes in different populations. Diabetes Metab J 2012; 36(2): 90-7.

[4] Shaw JE, Sicree RA, Zimmet PZ. Global estimates of the prevalence of diabetes for 2010 and 2030. Diabetes Res Clin Pract 2010; 87(1): 4-14.

[5] Jackuliak P, Payer J. Osteoporosis, fractures, and diabetes. Int J Endocrinol 2014; 2014; 820615.

[6] Mabed M, Shahin M. Mesenchymal stem cell-based therapy for the treatment of type 1 diabetes mellitus. Curr Stem Cell Res Ther 2012; 7(3): 179-90.

[7] Carlsson PO, Schwarcz E, Korsgren O, Le Blanc K. Preserved beta-cell function in type 1 diabetes by mesenchymal stromal cells. Diabetes 2015; 64(2): 587-92.

[8] Wu H, Mahato RI. Mesenchymal stem cell-based therapy for type 1 diabetes. Discov Med 2014; 17(93): 139-43.

[9] Gao F, Wu DQ, Hu YH, Jin GX. Extracellular matrix gel is necessary for *in vitro* cultivation of insulin producing cells from human umbilical cord blood derived mesenchymal stem cells. Chin Med J (Engl) 2008; 121(9): 811-8.

[10] Prabakar KR, Dominguez-Bendala J, Molano RD, *et al.* Generation of glucose-responsive, insulin-producing cells from human umbilical cord blood-derived mesenchymal stem cells. Cell Transplant 2012; 21(6): 1321-39.

[11] Karaoz E, Ayhan S, Okcu A, *et al.* Bone marrow-derived mesenchymal stem cells co-cultured with pancreatic islets display beta cell plasticity. J Tissue Eng Regen Med 2011; 5(6): 491-500.

[12] Guo QS, Zhu MY, Wang L, *et al.* Combined transfection of the three transcriptional factors, PDX-1, NeuroD1, and MafA, causes differentiation of bone marrow mesenchymal stem cells into insulin-producing cells. Exp Diabetes Res 2012; 2012 672013.

[13] Li HT, Jiang FX, Shi P, *et al. In vitro* reprogramming of rat bone marrow-derived mesenchymal stem cells into insulin-producing cells by genetically manipulating negative and positive regulators. Biochem Biophys Res Commun 2012; 420(4): 793-8.

[14] Weaver AS, Su YP, Begun DL, *et al.* The effects of axial displacement on fracture callus morphology and MSC homing depend on the timing of application. Bone 2010; 47(1): 41-8.

[15] Gu Z, Jiang J, Xia Y, *et al.* p21 is associated with the proliferation and apoptosis of bone marrow-derived mesenchymal stem cells from non-obese diabetic mice. Exp Clin Endocrinol Diabetes 2013; 121(10): 607-13.

[16] Vestergaard P. Discrepancies in bone mineral density and fracture risk in patients with type 1 and type 2 diabetes--a meta-analysis. Osteoporos Int 2007; 18(4): 427-44.

[17] Hie M, Iitsuka N, Otsuka T, Tsukamoto I. Insulin-dependent diabetes mellitus decreases osteoblastogenesis associated with the inhibition of Wnt signaling through increased expression of Sost and Dkk1 and inhibition of Akt activation. Int J Mol Med 2011; 28(3): 455-62.

[18] Xu F, Dong Y, Huang X, *et al.* Decreased osteoclastogenesis, osteoblastogenesis and low bone mass in a mouse model of type 2 diabetes. Mol Med Rep 2014; 10(4): 1935-41.

[19] Hamann C, Goettsch C, Mettelsiefen J, *et al.* Delayed bone regeneration and low bone mass in a rat model of insulin-resistant type 2 diabetes mellitus is due to impaired osteoblast function. Am J Physiol Endocrinol Metab 2011; 301(6): E1220-8.

[20] Fadini GP, Ferraro F, Quaini F, Asahara T, Madeddu P. Concise review: diabetes, the bone marrow niche, and impaired vascular regeneration. Stem Cells Transl Med 2014; 3(8): 949-57.

[21] Velander P, Theopold C, Hirsch T, *et al.* Impaired wound healing in an acute diabetic pig model and the effects of local hyperglycemia. Wound Repair Regen 2008; 16(2): 288-93.

[22] Wang A, Midura RJ, Vasanji A, Wang AJ, Hascall VC. Hyperglycemia diverts dividing osteoblastic precursor cells to an adipogenic pathway and induces synthesis of a hyaluronan matrix that is adhesive for monocytes. J Biol Chem 2014; 289(16): 11410-20.

[23] Colombo J S, Balani D, Sloan A J, *et al.* Delayed osteoblast differentiation and altered inflammatory response around implants placed in incisor sockets of type 2 diabetic rats. Clin Oral Implants Res 2011; 22(6): 578-86.

[24] Kim Y S, Kwon J S, Hong M H, *et al.* Restoration of angiogenic capacity of diabetes-insulted mesenchymal stem cells by oxytocin. BMC Cell Biol 2013; 14: 38.

[25] Zhao YF, Zeng D L, Xia LG, *et al.* Osteogenic potential of bone marrow stromal cells derived from streptozotocin-induced diabetic rats. Int J Mol Med 2013; 31(3): 614-20.

[26] Stolzing A, Colley H, Scutt A. Effect of age and diabetes on the response of mesenchymal progenitor cells to fibrin matrices. Int J Biomater 2011; 2011: 378034.

[27] Ezquer F, Ezquer M, Simon V, Conget P. The antidiabetic effect of MSCs is not impaired by insulin prophylaxis and is not improved by a second dose of cells. PLoS One 2011; 6(1): e16566.

[28] Jin P, Zhang X, Wu Y, *et al.* Streptozotocin-induced diabetic rat-derived bone marrow mesenchymal stem cells have impaired abilities in proliferation, paracrine, antiapoptosis, and myogenic differentiation. Transplant Proc 2010; 42(7): 2745-52.

[29] Cramer C, Freisinger E, Jones RK, *et al.* Persistent high glucose concentrations alter the regenerative potential of mesenchymal stem cells. Stem Cells Dev 2010; 19(12): 1875-84.

[30] Stolzing A, Sellers D, Llewelyn O, Scutt A. Diabetes induced changes in rat mesenchymal stem cells. Cells Tissues Organs 2010; 191(6): 453-65.

[31] Khan M, Akhtar S, Mohsin S, SNK, Riazuddin S. Growth factor preconditioning increases the function of diabetes-impaired mesenchymal stem cells. Stem Cells Dev 2011; 20(1): 67-75.

[32] Rinker TE, Hammoudi TM, Kemp ML, Lu H, Temenoff JS. Interactions between mesenchymal stem cells, adipocytes, and osteoblasts in a 3D tri-culture model of hyperglycemic conditions in the bone marrow microenvironment. Integr Biol (Camb) 2014; 6(3): 324-37.

[33] Kim SY, Lee JY, Park YD, *et al.* Hesperetin alleviates the inhibitory effects of high glucose on the osteoblastic differentiation of periodontal ligament stem cells. PLoS One 2013; 8(6): e67504.

[34] Zhu G, Chai J, Ma L, Duan H, Zhang H. Downregulated microRNA-32 expression induced by high glucose inhibits cell cycle progression via PTEN upregulation and Akt inactivation in bone marrow-derived mesenchymal stem cells. Biochem Biophys Res Commun 2013; 433(4): 526-31.

[35] Dhanasekaran M, Indumathi S, Rajkumar JS, Sudarsanam D. Effect of high glucose on extensive culturing of mesenchymal stem cells derived from subcutaneous fat, omentum fat and bone marrow. Cell Biochem Funct 2013; 31(1): 20-9.

[36] Kim YJ, Hwang SH, Lee SY, *et al.* miR-486-5p induces replicative senescence of human adipose tissue-derived mesenchymal stem cells and its expression is controlled by high glucose. Stem Cells Dev 2012; 21(10): 1749-60.

[37] Keats E, Khan ZA. Unique responses of stem cell-derived vascular endothelial and mesenchymal cells to high levels of glucose. PLoS One 2012; 7(6): e38752.

[38] Stolzing A, Bauer E, Scutt A. Suspension cultures of bone-marrow-derived mesenchymal stem cells: effects of donor age and glucose level. Stem Cells Dev 2012; 21(14): 2718-23.

[39] Ryu JM, Lee MY, Yun SP, Han HJ. High glucose regulates cyclin D1/E of human mesenchymal stem cells through TGF-beta1 expression via Ca2+/PKC/MAPKs and PI3K/Akt/mTOR signal pathways. J Cell Physiol 2010; 224(1): 59-70.

[40] Weil BR, Abarbanell AM, Herrmann JL, Wang Y, Meldrum DR. High glucose concentration in cell culture medium does not acutely affect human mesenchymal stem cell growth factor production or proliferation. Am J Physiol Regul Integr Comp Physiol 2009; 296(6): R1735-43.

[41] Liu Z, Lei M, Jiang Y, *et al.* High glucose attenuates VEGF expression in rat multipotent adult progenitor cells in association with inhibition of JAK2/STAT3 signalling. J Cell Mol Med 2009; 13(9B): 3427-36.

[42] Li YM, Schilling T, Benisch P, *et al.* Effects of high glucose on mesenchymal stem cell proliferation and differentiation. Biochem Biophys Res Commun 2007; 363(1): 209-15.

[43] Gopalakrishnan V, Vignesh RC, Arunakaran J, Aruldhas MM, Srinivasan N. Effects of glucose and its modulation by insulin and estradiol on BMSC differentiation into osteoblastic lineages. Biochem Cell Biol 2006; 84(1): 93-101.

[44] Stolzing A, Coleman N, Scutt A. Glucose-induced replicative senescence in mesenchymal stem cells. Rejuvenation Res 2006; 9(1): 31-5.

[45] Goh SY, Cooper ME. Clinical review: The role of advanced glycation end products in progression and complications of diabetes. J Clin Endocrinol Metab 2008; 93(4): 1143-52.

[46] Singh R, Barden A, Mori T, Beilin L. Advanced glycation end-products: a review. Diabetologia 2001; 44(2): 129-46.

[47] Brownlee M. Biochemistry and molecular cell biology of diabetic complications. Nature 2001; 414(6865): 813-20.

[48] Chang PC, Chien LY, Yeo JF, *et al.* Progression of periodontal destruction and the roles of advanced glycation end products in experimental diabetes. J Periodontol 2013; 84(3): 379-88.

[49] Nogueira-Machado JA, Chaves MM. From hyperglycemia to AGE-RAGE interaction on the cell surface: a dangerous metabolic route for diabetic patients. Expert Opin Ther Targets 2008; 12(7): 871-82.

[50] Notsu M, Yamaguchi T, Okazaki K, *et al.* Advanced glycation end product 3 (AGE3) suppresses the mineralization of mouse stromal ST2 cells and human mesenchymal stem cells by increasing TGF-beta expression and secretion. Endocrinology 2014; 155(7): 2402-10.

[51] Weinberg E, Maymon T, Weinreb M. AGEs induce caspase-mediated apoptosis of rat BMSCs *via* TNFalpha production and oxidative stress. J Mol Endocrinol 2014; 52(1): 67-76.

[52] Okazaki K, Yamaguchi T, Tanaka K, *et al.* Advanced glycation end products (AGEs), but not high glucose, inhibit the osteoblastic differentiation of mouse stromal ST2 cells through the suppression of osterix expression, and inhibit cell growth and increasing cell apoptosis. Calcif Tissue Int 2012; 91(4): 286-96.

[53] Yang K, Wang XQ, He YS, *et al.* Advanced glycation end products induce chemokine/cytokine production via activation of p38 pathway and inhibit proliferation and migration of bone marrow mesenchymal stem cells. Cardiovasc Diabetol 2010; 9: 66.

[54] Kume S, Kato S, Yamagishi S, *et al.* Advanced glycation end-products attenuate human mesenchymal stem cells and prevent cognate differentiation into adipose tissue, cartilage, and bone. J Bone Miner Res 2005; 20(9): 1647-58.

[55] Palma CC, Pavesi M, Nogueira VG, *et al.* Prevalence of thyroid dysfunction in patients with diabetes mellitus. Diabetol Metab Syndr 2013; 5(1): 58.

[56] Taylor WH, Khaleeli AA. Coincident diabetes mellitus and primary hyperparathyroidism. Diabetes Metab Res Rev 2001; 17(3): 175-80.

[57] Jiang P, Li H, Li X. Diabetes mellitus risk factors in rheumatoid arthritis: a systematic review and meta-analysis. Clin Exp Rheumatol 2014.

[58] Garin MC, Arnold AM, Lee JS, Robbins J, Cappola AR. Subclinical thyroid dysfunction and hip fracture and bone mineral density in older adults: the cardiovascular health study. J Clin Endocrinol Metab 2014; 99(8): 2657-64.

[59] Gianotti L, Tassone F, Baffoni C, *et al.* Relationship between insulin sensitivity and bone mineral density in primary hyperparathyroidism. Clin Endocrinol (Oxf) 2014; 81(3): 350-5.

[60] Mosaad YM, Hammad EM, Fawzy Z, *et al.* Vitamin D receptor gene polymorphism as possible risk factor in rheumatoid arthritis and rheumatoid related osteoporosis. Hum Immunol 2014; 75(5): 452-61.

[61] Boeloni JN, de MON, Silva JF, *et al.* Osteogenic differentiation of bone marrow mesenchymal stem cells of ovariectomized and non-ovariectomized female rats with thyroid dysfunction. Pathol Res Pract 2013; 209(1): 44-51.

[62] Felice JI, Gangoiti MV, Molinuevo MS, McCarthy AD, Cortizo AM. Effects of a metabolic syndrome induced by a fructose-rich diet on bone metabolism in rats. Metabolism 2014; 63(2): 296-305.

[63] Asghar O, Alam U, Hayat SA, *et al.* Obesity, diabetes and atrial fibrillation; epidemiology, mechanisms and interventions. Curr Cardiol Rev 2012; 8(4): 253-64.

[64] James WP. The epidemiology of obesity: the size of the problem. J Intern Med 2008; 263(4): 336-52.

[65] Strotmeyer ES, Cauley JA, Schwartz AV, *et al.* Nontraumatic fracture risk with diabetes mellitus and impaired fasting glucose in older white and black adults: the health, aging, and body composition study. Arch Intern Med 2005; 165(14): 1612-7.

[66] Lv S, Wu L, Cheng P, *et al.* Correlation of obesity and osteoporosis: effect of free fatty acids on bone marrow-derived mesenchymal stem cell differentiation. Exp Ther Med 2010; 1(4): 603-10.

[67] Shin JH, Kim SH, Yu SH. Metabolic syndrome and chronic kidney disease as risk factors of osteoporosis. Clin Nephrol 2014; 81(1): 1-8.

[68] Siris ES, Adler R, Bilezikian J, *et al.* The clinical diagnosis of osteoporosis: a position statement from the National Bone Health Alliance Working Group. Osteoporos Int 2014; 25(5): 1439-43.

[69] Dhaon P, Shah VN. Type 1 diabetes and osteoporosis: A review of literature. Indian J Endocrinol Metab 2014; 18(2): 159-65.

[70] Janghorbani M, Van Dam RM, Willett W C, Hu FB. Systematic review of type 1 and type 2 diabetes mellitus and risk of fracture. Am J Epidemiol 2007; 166(5): 495-505.

[71] Carnevale V, Romagnoli E, D'Erasmo L, D'Erasmo E. Bone damage in type 2 diabetes mellitus. Nutr Metab Cardiovasc Dis 2014.

[72] Yamaguchi T, Sugimoto T. Bone metabolism and fracture risk in type 2 diabetes mellitus [Review]. Endocr J 2011; 58(8): 613-24.

[73] Silva JA, Lopes Ferrucci D, Peroni LA, *et al.* Periodontal disease-associated compensatory expression of osteoprotegerin is lost in type 1 diabetes mellitus and correlates with alveolar bone destruction by regulating osteoclastogenesis. Cells Tissues Organs 2012; 196(2): 137-50.

[74] Hamann C, Kirschner S, Gunther KP, Hofbauer LC. Bone, sweet bone--osteoporotic fractures in diabetes mellitus. Nat Rev Endocrinol 2012; 8(5): 297-305.

[75] Rosen CJ, Ackert-Bicknell C, Rodriguez JP, Pino AM. Marrow fat and the bone microenvironment: developmental, functional, and pathological implications. Crit Rev Eukaryot Gene Expr 2009; 19(2): 109-24.

[76] Duque G. As a matter of fat: New perspectives on the understanding of age-related bone loss BoneKEy-Osteovision 2007; 4(4): 12.

[77] Murakami K, Bhandari H, Lucas ES, *et al.* Deficiency in clonogenic endometrial mesenchymal stem cells in obese women with reproductive failure--a pilot study. PLoS One 2013; 8(12): e82582.

[78] Du HM, Zheng XH, Wang LY, *et al.* The osteogenic response of undifferentiated human adipose-derived stem cells under mechanical stimulation. Cells Tissues Organs 2012; 196(4): 313-24.

[79] Friedl G, Windhager R, Schmidt H, Aigner R. The osteogenic response of undifferentiated human mesenchymal stem cells (hMSCs) to mechanical strain is inversely related to body mass index of the donor. Acta Orthop 2009; 80(4): 491-8.

[80] Frazier TP, Gimble JM, Devay JW, *et al.* Body mass index affects proliferation and osteogenic differentiation of human subcutaneous adipose tissue-derived stem cells. BMC Cell Biol 2013; 14: 34.

[81] Luu YK, Capilla E, Rosen CJ, *et al.* Mechanical stimulation of mesenchymal stem cell proliferation and differentiation promotes osteogenesis while preventing dietary-induced obesity. J Bone Miner Res 2009; 24(1): 50-61.

[82] Wang Q, Zhao B, Li C, *et al.* Decreased proliferation ability and differentiation potential of mesenchymal stem cells of osteoporosis rat. Asian Pac J Trop Med 2014; 7(5): 358-63.

[83] Bian Q, Liu SF, Huang JH, *et al.* Oleanolic acid exerts an osteoprotective effect in ovariectomy-induced osteoporotic rats and stimulates the osteoblastic differentiation of bone mesenchymal stem cells *in vitro*. Menopause 2012; 19(2): 225-33.

[84] Yang Z, Huang JH, Liu SF, *et al.* The osteoprotective effect of psoralen in ovariectomy-induced osteoporotic rats via stimulating the osteoblastic differentiation from bone mesenchymal stem cells. Menopause 2012; 19(10): 1156-64.

[85] Rodriguez JP, Garat S, Gajardo H, Pino AM, Seitz G. Abnormal osteogenesis in osteoporotic patients is reflected by altered mesenchymal stem cells dynamics. J Cell Biochem 1999; 75(3): 414-23.

[86] Rodriguez JP, Rios S, Fernandez M, Santibanez JF. Differential activation of ERK1, 2 MAP kinase signaling pathway in mesenchymal stem cell from control and osteoporotic postmenopausal women. J Cell Biochem 2004; 92(4): 745-54.

[87] Prall WC, Haasters F, Heggebo J, *et al.* Mesenchymal stem cells from osteoporotic patients feature impaired signal transduction but sustained osteoinduction in response to BMP-2 stimulation. Biochem Biophys Res Commun 2013; 440(4): 617-22.

[88] Yang N, Wang G, Hu C, *et al.* Tumor necrosis factor alpha suppresses the mesenchymal stem cell osteogenesis promoter miR-21 in estrogen deficiency-induced osteoporosis. J Bone Miner Res 2013; 28(3): 559-73.

[89] Chen HT, Lee MJ, Chen CH, *et al.* Proliferation and differentiation potential of human adipose-derived mesenchymal stem cells isolated from elderly patients with osteoporotic fractures. J Cell Mol Med 2012; 16(3): 582-93.

[90] Rodriguez JP, Montecinos L, Rios S, Reyes P, Martinez J. Mesenchymal stem cells from osteoporotic patients produce a type I collagen-deficient extracellular matrix favoring adipogenic differentiation. J Cell Biochem 2000; 79(4): 557-65.

[91] Hess R, Pino AM, Rios S, Fernandez M, Rodriguez JP. High affinity leptin receptors are present in human mesenchymal stem cells (MSCs) derived from control and osteoporotic donors. J Cell Biochem 2005; 94(1): 50-7.

[92] Astudillo P, Rios S, Pastenes L, Pino AM, Rodriguez JP. Increased adipogenesis of osteoporotic human-mesenchymal stem cells (MSCs) characterizes by impaired leptin action. J Cell Biochem 2008; 103(4): 1054-65.

[93] Liao L, Yang X, Su X, *et al.* Redundant miR-3077-5p and miR-705 mediate the shift of mesenchymal stem cell lineage commitment to adipocyte in osteoporosis bone marrow. Cell Death Dis 2013; 4: e600.

[94] Benisch P, Schilling T, Klein-Hitpass L, *et al.* The transcriptional profile of mesenchymal stem cell populations in primary osteoporosis is distinct and shows overexpression of osteogenic inhibitors. PLoS One 2012; 7(9): e45142.

[95] Nuttall ME, Gimble JM. Controlling the balance between osteoblastogenesis and adipogenesis and the consequent therapeutic implications. Curr Opin Pharmacol 2004; 4(3): 290-4.

[96] Kawai M, Rosen CJ. PPARgamma: A circadian transcription factor in adipogenesis and osteogenesis. Nat Rev Endocrinol 2010; 6(11): 629-36.

[97] Jeon MJ, Kim JA, Kwon SH, *et al.* Activation of peroxisome proliferator-activated receptor-gamma inhibits the Runx2-mediated transcription of osteocalcin in osteoblasts. J Biol Chem 2003; 278(26): 23270-7.

[98] Moerman EJ, Teng K, Lipschitz DA, Lecka-Czernik B. Aging activates adipogenic and suppresses osteogenic programs in mesenchymal marrow stroma/stem cells: the role of PPAR-gamma2 transcription factor and TGF-beta/BMP signaling pathways. Aging Cell 2004; 3(6): 379-89.

[99] Botolin S, Faugere MC, Malluche H, *et al.* 0Increased bone adiposity and peroxisomal proliferator-activated receptor-gamma2 expression in type I diabetic mice. Endocrinology 2005; 146(8): 3622-31.

[100] Huang S, Kaw M, Harris MT, *et al.* Decreased osteoclastogenesis and high bone mass in mice with impaired insulin clearance due to liver-specific inactivation to CEACAM1. Bone 2010; 46(4): 1138-45.

[101] Kim J, Ko J. A novel PPARgamma2 modulator sLZIP controls the balance between adipogenesis and osteogenesis during mesenchymal stem cell differentiation. Cell Death Differ 2014; 21(10): 1642-55.

[102] Kadowaki T, Yamauchi T. Adiponectin and adiponectin receptors. Endocr Rev 2005; 26(3): 439-51.

[103] Kadowaki T, Yamauchi T, Kubota N, *et al.* Adiponectin and adiponectin receptors in insulin resistance, diabetes, and the metabolic syndrome. J Clin Invest 2006; 116(7): 1784-92.

[104] Agrawal NK, Kant S. Targeting inflammation in diabetes: Newer therapeutic options. World J Diabetes 2014; 5(5): 697-710.

[105] Julia C, Czernichow S, Charnaux N, *et al.* Relationships between adipokines, biomarkers of endothelial function and inflammation and risk of type 2 diabetes. Diabetes Res Clin Pract 2014; 105(2): 231-8.

[106] Xu FF, Zhu H, Li XM, *et al.* Intercellular adhesion molecule-1 inhibits osteogenic differentiation of mesenchymal stem cells and impairs bio-scaffold-mediated bone regeneration *in vivo*. Tissue Eng Part A 2014; 20(19-20): 2768-82.

[107] Han J, Zhong J, Wei W, *et al.* Extracellular high-mobility group box 1 acts as an innate immune mediator to enhance autoimmune progression and diabetes onset in NOD mice. Diabetes 2008; 57(8): 2118-27.

[108] Bianchi ME. DAMPs, PAMPs and alarmins: all we need to know about danger. J Leukoc Biol 2007; 81(1): 1-5.

[109] Luft FC. A high mobility group box-containing transcription factor leads to diabetes risk. J Mol Med (Berl) 2006; 84(12): 985-7.

[110] Yang J, Shah R, Robling A G, *et al.* HMGB1 is a bone-active cytokine. J Cell Physiol 2008; 214(3): 730-9.

[111] Meng E, Guo Z, Wang H, *et al.* High mobility group box 1 protein inhibits the proliferation of human mesenchymal stem cells and promotes their migration and differentiation along osteoblastic pathway. Stem Cells Dev 2008; 17(4): 805-13.

[112] Atieh MA, Faggion CM, Jr., Seymour GJ. Cytokines in patients with type 2 diabetes and chronic periodontitis: A systematic review and meta-analysis. Diabetes Res Clin Pract 2014; 104(2): e38-45.

[113] Ribeiro FV, de Mendonca AC, Santos VR, *et al.* Cytokines and bone-related factors in systemically healthy patients with chronic periodontitis and patients with type 2 diabetes and chronic periodontitis. J Periodontol 2011; 82(8): 1187-96.

[114] Huang H, Kim HJ, Chang EJ, *et al.* IL-17 stimulates the proliferation and differentiation of human mesenchymal stem cells: implications for bone remodeling. Cell Death Differ 2009; 16(10): 1332-43.

[115] Kim YG, Park JW, Lee JM, *et al.* IL-17 inhibits osteoblast differentiation and bone regeneration in rat. Arch Oral Biol 2014; 59(9): 897-905.