ISSN 1948-0210 (online)

World Journal of *Stem Cells*

World J Stem Cells 2018 June 26; 10(6): 57-77

Published by Baishideng Publishing Group Inc

WJSC World Journal of Stem Cells

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Monthly Volume 10 Number 6 June 26, 2018

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World Journal of Stem Cells
Volume 10 Number 6 June 26, 2018

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INDEXING/ABSTRACTING

World Journal of Stem Cells is now indexed in PubMed, PubMed Central, Science Citation Index Expanded (also known as SciSearch®), Journal Citation Reports/Science Edition, Biological Abstracts, and BIOSIS Previews.

NAME OF JOURNAL
World Journal of Stem Cells

ISSN
ISSN 1948-0210 (online)

LAUNCH DATE
December 31, 2009

FREQUENCY
Monthly

EDITORIAL OFFICE
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World Journal of Stem Cells
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7901 Stoneridge Drive, Suite 501, Pleasanton, CA 94588, USA
Telephone: +1-925-2238242
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E-mail: editorialoffice@wjgnet.com
Help Desk: http://www.f6publishing.com/helpdesk
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7901 Stoneridge Drive, Suite 501,
Pleasanton, CA 94588, USA
Telephone: +1-925-2238242
Fax: +1-925-2238243
E-mail: bpgoffice@wjgnet.com
Help Desk: http://www.f6publishing.com/helpdesk
http://www.wjgnet.com

PUBLICATION DATE
June 26, 2018

INSTRUCTIONS TO AUTHORS
http://www.wjgnet.com/bpg/gerinfo/204

ONLINE SUBMISSION
http://www.f6publishing.com

World Journal of Stem Cells

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World J Stem Cells 2018 June 26; 10(6): 57-65

DOI: 10.4252/wjsc.v10.i6.57

ISSN 1948-0210 (online)

REVIEW

Murine models based on acute myeloid leukemia-initiating stem cells xenografting

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Author contributions: All authors equally contributed to this paper with conception and design of the study, literature review and analysis, drafting and critical revision and editing, and final approval of the final version.

Supported by The project Competitiveness Operational Programme (COP) A1.1.4., No. P_37_798, Contract 149/26.10.2016 (MySMIS2014+: 106774).

Conflict-of-interest statement: No potential conflicts of interest.

Manuscript Source: Invited Manuscript

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Received: April 28, 2018
Peer-review started: April 28, 2018
First decision: May 11, 2018
Revised: May 24, 2018
Accepted: June 8, 2018
Article in press: June 8, 2018
Published online: June 26, 2018

Abstract

Acute myeloid leukemia (AML) is an aggressive malignant disease defined by abnormal expansion of myeloid blasts. Despite recent advances in understanding AML pathogenesis and identifying their molecular subtypes based on somatic mutations, AML is still characterized by poor outcomes, with a 5-year survival rate of only 30%-40%, the majority of the patients dying due to AML relapse. Leukemia stem cells (LSC) are considered to be at the root of chemotherapeutic resistance and AML relapse. Although numerous studies have tried to better characterize LSCs in terms of surface and molecular markers, a specific marker of LSC has not been found, and still the most universally accepted phenotypic signature remains the surface antigens CD34+CD38- that is shared with normal hematopoietic stem cells. Animal models provides the means to investigate the factors responsible for leukemic transformation, the intrinsic differences between secondary post-myeloproliferative neoplasm AML and *de novo* AML, especially the signaling pathways involved in inflammation and hematopoiesis. However, AML proved to be one of the hematological malignancies that is difficult to engraft even in the most immunodeficient mice strains, and numerous ongoing attempts are focused to develop "humanized mice" that can support the engraftment of LSC. This present review is aiming to in-

troduce the field of AML pathogenesis and the concept of LSC, to present the current knowledge on leukemic blasts surface markers and recent attempts to develop best AML animal models.

Key words: Acute myeloid leukemia; Leukemia-initiating stem cells; Antigen markers; Murine models; Xenografts

Core tip: The review is aiming to introduce the field of acute myeloid leukemia (AML) pathogenesis, the concept of leukemic stem cells, and also to present the current attempts to develop best AML animal models as means to investigate the factors responsible for leukemic transformation. Due to difficulties in engraftment of less aggressive AML samples, it is currently being attempted to develop humanized mice by introducing supporting human stromal cells as a source of proper cytokines, in a challenge to mimic an appropriate bone marrow niche able to support leukemic stem cells engraftment.

Mambet C, Chivu-Economescu M, Matei L, Necula LG, Dragu DL, Bleotu C, Diaconu CC. Murine models based on acute myeloid leukemia-initiating stem cells xenografting. *World J Stem Cells* 2018; 10(6): 57-65 Available from: URL: http://www.wjgnet.com/1948-0210/full/v10/i6/57.htm DOI: http://dx.doi.org/10.4252/wjsc.v10.i6.57

INTRODUCTION

Acute myeloid leukemia (AML) is an aggressive cancer characterized by unrestricted proliferation of functionally immature myeloid cells. High heterogeneity and variable expansion capacity of multiple clones within each patient[1,2], clinical and molecular differences between de novo and secondary AML, complicate even more treatment choices and make targeted therapy a goal yet to far to reach without using models that are able to simplify the multitude of mechanisms that might be involved in leukemogenesis.

In the last decades, murine models become very important tools in the field of preclinical research in oncology, hematology, and immunology, providing a platform for study of tumor biology and for *in vivo* evaluation of drugs in patient-derived xenograft tumors (PDX). Nowadays, a large variety of immunodeficient mice strains have emerged, able to support the xenografting and development of a complex human hemato-lymphoid system. The most difficult to reproduce is the immune system and the bone marrow (BM) microenvironment, mostly because of the differences between the signaling molecules responsible for the maturation of different hematopoietic cell populations[3]. Although the field of animal models has experienced a recent exponential growth through the development of IL2rg^{null} immunodeficiency mice, AML remains one of the hematologic malignancies difficult to engraft into the existing strains of mice due to the lack of a proper BM niche and absence of specific human growth factors and supporting stromal cells[4]. As a result several attempts were made to develop "humanized mice" that can better support myeloid leukemia-initiating stem cells xenografting.

This review is aiming to introduce AML pathogenesis and the concept of leukemic stem cells and the current most advanced strategies to overcome challenges in obtaining AML murine models.

AML PATHOGENESIS AND THE CONCEPT OF LEUKEMIC STEM CELLS

AML is a heterogeneous hematopoietic malignancy defined by clonal expansion of abnormally differentiated or undifferentiated myeloid progenitors (blasts) that accumulate in the BM and impair hematopoiesis, leading to multi-lineage cytopenias[5,6]. Blasts can also migrate from BM into peripheral blood and infiltrate other tissues[5].

AML can be divided in 3 categories taking into account their clinical ontogeny: Secondary AML (s-AML) occurred after leukemic transformation of a pre-existing myelodysplastic syndrome or myeloproliferative neoplasm, therapy-related AML (t-AML) developed in patients that received leukemogenic chemotherapy for antecedent non-myeloid malignancies and *de novo* AML generated in the absence of a previous stem cell disorder or a therapeutic exposure to cytotoxic drugs[7].

Despite recent progress in understanding AML pathogenesis and recognizing molecular subtypes of AML that have prognostic impact, AML is still characterized by poor outcomes, with a 5-year survival rate of only 30%-40%. The dismal prognosis is mainly related to high rate of relapse and refractory disease[2,8]. Patients with s-AML and t-AML display even a much worse prognosis, the median overall survival rate being 7 months. Notably, somatic mutations in *SRSF2, SF3B1*, *U2AF1*, *ZRSR2*, *ASXL1*, *EZH2*, *BCOR*, or *STAG2* proved to be highly specific for s-AML. They are acquired early in leukemogenesis and tend to persist during clonal remissions[7].

Increasing evidence support the concept that a minor population of stem cells, named leukemia stem cells (LSCs), is responsible for leukemia initiation, disease progression and relapse, as well as drug resistance[9]. AML was among the first diseases in which the existence of cancer stem cells was documented using xenograft animal models[10].

LSCs are derived from transformed hematopoietic stem cells (HSCs) or downstream committed progenitors[11]. They are able to initiate the disease after transplantation into immunodeficient mice and are characterized by both unlimited self-renewal potential inducing disease in serial transplantation and capacity to

partially differentiate into non-LSC blasts that lack self-renewal properties and possibility of engraftment[12]. Although LSCs and non-LSC blasts harbor a common set of mutations there are epigenetic differences between them. A predominant hypo-methylation of *HOXA* gene cluster that has been involved in leukemogenesis represents a main feature of LSCs[13].

It is thought that, similarly to normal hematopoietic system, AML displays a hierarchical organization with LSCs on the top, being able to generate the whole population of AML blast cells[10]. Signaling pathways that control self-renewal of HSCs, such as Wnt/β-catenin, PI3K/Akt/mTOR, or Hedgehog, are also involved in LSC survival and expansion and can serve as therapeutic targets to facilitate eradication of LSCs[8,11]. Moreover, LSCs might escape apoptosis through up-regulation of NF-κB or downregulation of Fas/CD95. Additionally, CXCL12-CXCR4 axis promotes retaining of LSCs within the protective BM microenvironment[11].

The existence of a preleukemic stage in AML was proven by isolating from leukemia patients a population of HSCs that was found to bear some, but not all, of the mutations identified in the downstream leukemia. These preleukemic HSCs, that can be distinguished from LSCs by the surface antigen markers, TIM3 and CD99, are capable to generate bi-lineage engraftment in NOD.Cg-*Prkdc*^{scid}*Il2rg*^{tm1Wjl} (NSG) mice and the derived lymphoid and myeloid progeny display the same preleukemia mutations[14]. According to the currently proposed model of preleukemic clonal evolution, the first leukemia-related mutation has to occur in a cell that possesses self-renewal capacity or, alternatively, must confer self-renewal properties to a more differentiated progenitor[15]. By single-cell analysis it was shown that during the process of leukemogenesis, the preleukemic HSCs gradually acquire somatic mutations in a nonrandom pattern[16]. Thus, in the early phases of AML evolution there is enrichment for mutations in epigenetic modifiers such as *TET2*, *DNMT3A, IDH1/2*, and *ASXL1*. On the other hand, mutations in genes involved in signaling pathways and proliferation, such as *FLT3* and *KRAS* occur in later stages. Other leukemogenic mutations in genes like *NPM1*, *CEBPA*, and *WT1* can be found in preleukemic phase as well as in later stages[15,16].

The preleukemic HSCs that eventually give rise to AML persist in patient samples at diagnosis and are resistant to current chemotherapy, thus representing a source of disease recurrence[17].

LEUKEMIA-INITIATING STEM CELLS AND BLASTS

The identification of LSCs in AML plays an important role in disease diagnosis, prognosis and AML therapy monitoring, and also represents an important step in development of targeted therapy and drug discovery[9]. Although initial studies suggested that LSCs were CD34+CD38- and did not expressed other lineage markers[18-21], later studies proved that the LSC phenotype was more complex and heterogeneous[22,23]. At present, it is established that LSCs are characterized by increased or decreased expression of surface markers of normal myeloid precursors (CD34, CD38, CD33, CD13, CD117, and CD123), asynchronous expression of antigens determined by AML morphological subtype and by the LSC stage of differentiation (CD4, CD11b, CD14, CD15, CD36, CD61, CD64, CD71, *etc*.), as well as by aberrant expression of lymphoid antigens (cross-lineage expression) (CD2, CD5, CD7, CD19, CD22, CD56, Tim3, *etc*.)[24-30]. LSCs reside mainly in CD34+CD38- population, but may be present also in other cellular fractions, usually CD34+CD38+, and in some cases, in CD34- population[12,31]. Additional markers, more specific for the advanced characterization of cellular subpopulations in AML, include: CD90[32], CD96[33], CD123[34,35], CD47[36], CD44, C-type lectin-like molecule-1 (CLL1)[37], aldehyde dehydrogenase, *etc*[38]. Currently, standard diagnosis and sub-classification of AML integrate the study of cell morphology, genetics/cytogenetics and multi-parametric immuno-phenotyping. The antibody panels for surface markers used for sub-classification of each AML group are showed in Figure 1.

Methods commonly used to assess HSC properties are colony-forming cell (CFC) assay, long term culture (LTC), flow cytometry and competitive repopulation.

The CFC assay is an important tool used to evaluate the ability of the progenitor cells to proliferate and differentiate into multiple lineages. In order to produce colonies, cells are cultured in a semisolid medium, in the presence of appropriate cytokines for 7-14 d. Colonies are counted and characterized according to morphologic and phenotypic criteria. Although this short-term colony assay can determine the frequencies of hematopoietic progenitor cells in analyzed populations, still, it is not able to detect more immature progenitors or HSCs/LSCs. To overcome this limitation, the cells can be cultured for 5-8 wk on a stromal feeder layer that can provide a substrate and a source of cytokines and growth factors, in effort to mimic the *in vivo* niche conditions[39]. The long-term culture-initiating cells (LTC-ICs) can be evaluated by their capacity to generate CFCs in culture supernatant after 5 wk. This period allows CFCs present in the inoculum to terminally differentiate and the remaining CFCs may represent the progeny of LTC-ICs. Subsequent limiting dilution tests can be performed to determine the LTC-IC frequency[40]. Although this method facilitates the detection of more immature progenitor cells, it is time consuming and the presence of stromal cells can induce procedure variations and different outcomes[41,42].

Competitive repopulation represents the best method to assess the functional abilities of immature progenitor/stem cells by serial transplants in immunocompromised mice. This method is based on the ability

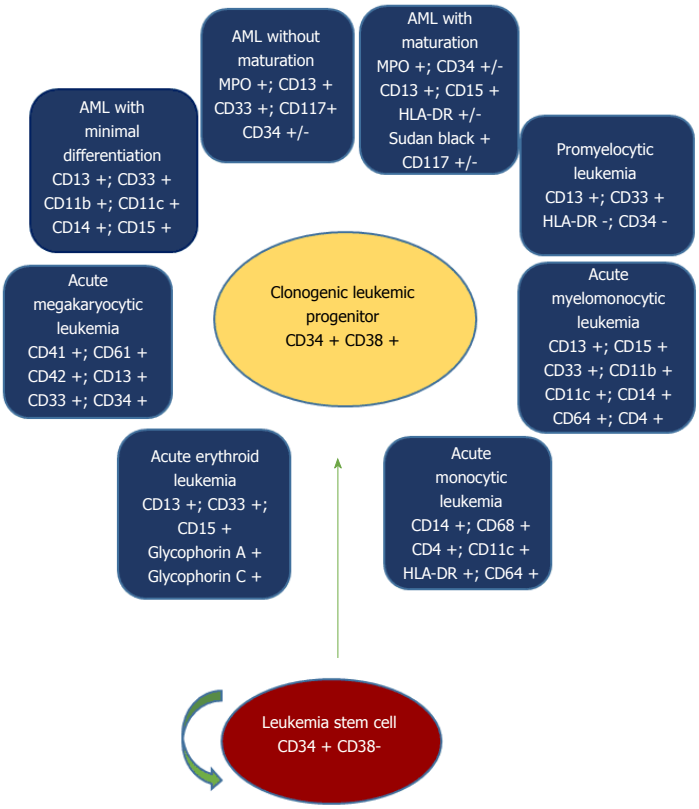

Figure 1 Advanced characterization of leukemic stem cell, clonogenic leukemic progenitors and various cellular subpopulations in acute myeloid leukemia. AML: Acute myeloid leukemia.

of cells that are investigated to compete with non-manipulated standard cells to repopulate the BM of an irradiated recipient[43].

MURINE MODELS - WHICH ARE THE BEST CHOICE?

Animal models are used as replacement for human biological niches due to ethical restrictions in the use of human tissue samples from donors. Moreover, animal models accurately recapitulate human disease and have been an important tool in advancing the understanding of human pathology, and development of pre-clinical therapy. Small animals, such as mice and rats, are often used as a model for various diseases because of their ease in breeding, maintenance, and manipulation. In spite of these many advantages, there are limitations due to the disparities between the murine and human biological systems. Human immune system and the BM microenvironment are the most difficult to be reproduced in mouse models because of the differences in the signaling molecules responsible for the maturation of various hematopoietic cell populations[3]. As a result, many malignant hematopoietic and other hematologic disorders do not successfully engraft in conventional mice models.

AML is one of these hematologic malignancies that fail to properly graft into the existing strains of mice due to the lack of a proper BM niche, homing elements, absence of specific human growth factors and supporting stromal cells[4]. As a result, several attempts have been made to develop murine models that reproduce with fidelity human hematopoiesis, particularly the development of the myeloid line.

Early attempts to increase the support for myelopoiesis involved the use of mice injected with IL-3, GM-CSF, SCF[44], mice producing human TPO[45] or MISTRG mice strain which produces human tumor necrosis factor and IL-6[46]. These confirmed that the introduction of human genes into mice led to the production of functional proteins capable of supporting engraftment and proliferation of human grafts.

The following attempts were aimed to develop next-generation mouse models genetically engineered to support myeloid differentiation from human HSC. Thus, it was necessary to act at three major levels in order to induce tolerance in the murine host, provide a supportive niche, and support hematopoiesis/proliferation with appropriate growth factors and cytokines.

The first request was fulfilled by the development of mice that lacked the adaptive and innate immune compartment like NSG and NODShi.Cg-*Prkdc*^{scid}*Il2rg*^{tm1Sug} mice strains. These strains were developed on NOD *scid* immunodeficient mice by modifying them to bear mutations in the IL-2 receptor gamma chain gene th-

at induced either the absence or the presence of an nonfunctional truncated form of the receptor subunit. The gamma chain subunit is a major component of the IL2, IL4, IL7, IL9, IL15 and IL21 receptors, and is indispensable for binding and signaling of these cytokines[47,48].

The second condition, involved the ablation of mouse cells to create open niches for human transplanted cells. These were achieved through irradiation or depletion of mouse stroma *via* introducing mutations in *c-Kit* gene encoding for SCF receptor. SCF plays an important role in the maintenance and differentiation of HSCs[49]. The c-kit mutated mice strain, known as NODB6.SCID Il2rγ$^{-/-}$ Kit (W41/W41) (NBSGW) mice, supports engraftment studies with human HSCs without prior irradiation. McIntosh *et al*[49] showed that in peripheral blood, the median human CD45+ count in non-irradiated NBSGW mice was similar to the count in irradiated NSG. In BM a significant increase in CD45+ was observed in non-irradiated NBSGW (97%) compared to non-irradiated NSG (30%).

The third constraint regarding the need for supportive myeloid cytokines was overcome using animal models with transgenic expression of hSCF, hGM-CSF and hIL-3 on the NOD SCID background resulting the NSG-SGM3 mouse strain [also known as NOD.Cg-*Prkdc*^{scid}*Il2rg*^{tm1Wjl}Tg(CMV-IL3,CSF2,KITLG)/1Eav)]. Wunderlich *et al*[4] reported the development and use of NSG-SGM3 mouse for the engraftment of normal CD34+ and AML xenografts. The results showed an improvement in the expansion of normal human myeloid cells, as well as an enhanced engraftment of primary human AML samples. They injected five samples of primary AML in sub-lethally irradiated NGS and NSG-SGM3 mice. Only three samples from five engrafted in NSG mice, compared to five samples in NSG-SGM3 mice, showing variability in the engraftment potential across the AML samples, and that NSG-SGM3 strain was a better host for some subsets of AML, relative to NSG mice. Moreover, three of five samples with primary AML had higher BM engraftment level in NSG-SGM3[4]. Similar results regarding variable engraftment potential in immunodeficient mice strains were obtained by Klco *et al*[50] They injected blasts from six AML samples in tail vein of non-irradiated NSG and NSG-SGM3. The results showed that four samples had higher BM engraftment and CD34+ expression level in NSG-SGM3 than NSG mice.

Finally, we can conclude that next-generation humanized mouse models are able to support myeloid blast development and differentiation.

XENOGRAFT MOUSE MODELS USED FOR ACUTE MYELOID LEUKEMIA

Cell line derived xenografts

AML represents a heterogeneous disease including several subtypes which are characterized by specific fusion oncogenes as a result of chromosome abnormalities. The fusion oncogenes in AML are associated with different clinical and laboratory characteristics, highlighting the different ways of malignant transformation in this disease. A study focused on the evaluation of four important AML fusion oncogenes reported that MLL-AF9 and NUP98-HOXA9 had very similar effects *in vitro* on primary human CD34+ cells, resulting in erythroid hyperplasia and an obvious blockage in erythroid and myeloid maturation while AML1-ETO and PML-RARA produced only modest effects on myeloid and erythroid differentiation. Moreover, MLL-AF9, NUP98-HOXA9 and AML1-ETO fusion oncogenes generated a significant increase in long-term proliferation and self-renewal of CD34+ cells. The characterization of gene profiles determined by AML fusion oncogenes can be considered an important tool for the discovery of new potential drug targets. In this study, two different time patterns of gene deregulation as result of fusion of these oncogenes were observed: MLL-AF9 and NUP98-HOXA9, caused gene deregulation 3 d after transduction, while gene deregulation by AML1-ETO and PML-RARA appeared within 6 h. Interestingly, p53 inhibitor MDM2 was upregulated by AML1-ETO at 6 h suggesting that MDM2 upregulation was involved in cell transformation, being related to AML1-ETO[51].

Wei *et al*[52] evaluated the *in vitro* and *in vivo* effects of *MLL-AF9* gene fusion in human CD34+ cord blood cells using retroviral vectors. Thus, MA9 transduced cells became immortal and doubled in number every 2-3 d. The expression of CD33, CD11b, CD13, CD14 and CD15 suggested a myelo-monocytic lineage. Moreover, long-term cultured MA9 cells failed to differentiate towards the erythroid or B lymphoid lineages, remaining cytokine and FLT3L dependent for growth. In non-obese diabetic/severe combined immunodeficient [NOD/SCID (NS)], NS-β2M-/- (NS-B2M) and NS mice, MA9 cells induced acute myeloid, lymphoid, or mixed-lineage leukemia with blast cells present in the peripheral blood, BM, spleen and liver. Gene expression profile of MLL-AF9 transduced cells was similar to human AML with 11q23 translocations, Rac signaling pathway being the most affected pathway and a promising therapeutic target in MLL-rearranged AML[52].

Another AML subtype with a particularly poor outcome is characterized by the t(6;9)(p22;q34) chromosome rearrangement which generates DEK-NUP214 chimeric gene. Qin *et al*[53] developed an AML model harboring DEK-NUP214, using CD34+ human hematopoietic progenitor cells and MO7e cell lines xenografted into immunocompromised mice that expressed human myeloid cell growth factors. The M07e human megakaryoblastic leukemia cell line was strictly dependent on either IL-3 or GM-CSF for survival; retroviral expression of this fusion gene in IL-3 dependent M07e cell line induced a cytokine independence and increased colony formation ability in soft-agar. DEK-NUP214 expression also modified the differentiation of human cord blood CD34+ progenitor

cells, which expressed myeloid lineage markers (CD13+), with small subsets showing T- (CD3+) and B- (CD19+) cell linage markers. The obtained results suggested that DEK-NUP214 was involved in leukemic transformation and differentiation of myeloid cells. In this study, CD34+ progenitor cells obtained from three different umbilical cord blood samples and transduced with chimeric DEK-NUP214 were engrafted in NSG-SGM3 mice strain. Interestingly, two months after transplantation, almost 20% of peripheral blood cells from the transplanted mice displayed a human-specific CD45 immuno-phenotype with CD45+CD13+CD34+CD38+ cells. The analysis of peripheral blood smears also showed the typical human AML cell morphology with a larger nucleus and reduced cytoplasmic ratio. Therefore, the study demonstrated that DEK-NUP214 could transform human CD34+ progenitor cells and induced human AML *in vivo*. Gene profiling of this model revealed that several genes of HOX family (*HOXA9, 10, B3, B4* and *PBX3*) were highly upregulated. In this AML model pathways involving KRAS, BRCA1 and ALK were significantly dysregulated[53].

Similar results were obtained in case of t(8;13)-(p11;q12) chromosome translocation which led to ZMYM2-FGFR1 chimeric kinase, characteristic for another AML subtype. Human CD34+ cells harboring ZMYM2-FGFR1 transplanted into immune-compromised mice developed myeloproliferative disease that progressed to AML. Mice displayed hepatosplenomegaly, hypercellular BM and a CD45 + CD34 + CD13+ immunophenotype[54].

Preclinical cancer research remains essential for the discovery and the development of new therapies in case of the most advanced cancers. Various cancer cell lines have been developed and used for the study of cancer but with a great disadvantage that they do not really reflect the behavior of the original cancer cells, due to the artificial nature of their culture conditions.

Patient derived xenografts - patient stem-cells derived xenografts

PDX models established by transplanting patient cancer cells into immunocompromised mice represent an important tool in cancer research. They have a great potential to offer important information on cancer biology and to guide the therapeutic approach. Unlike cell lines derived from primary tumors that might have lost their original characteristics due to a prolonged *in vitro* growth, PDX mouse models seemed to be able to overcome this issue[55,56]. Many studies demonstrated that PDX models kept the most important features of the original tumor including histology, genomic pattern, cellular heterogeneity, and more important, drug responsiveness or personalized drug selection[57].

The development of PDX models of AML allows us to monitor *in vivo* the progression of the disease and to evaluate the efficacy of an experimental treatment on tumor growth using imaging techniques[58].

A first full study on the engraftment ability of a large cohort of AML samples in immunodeficient animal models was published by Kennedy *et al*[59] who transplanted BM or peripheral blood cells from 307 AML patients intra-femorally into sublethally irradiated NOD. SCID mice pre-treated with an anti-CD122 antibody. AML xenografts were obtained in 44% of cases, leukemic engraftment being associated with a higher white cell count in peripheral blood (mean of 92 x 10^9/L in engrafters *vs* 67 x 10^9/L in non-engrafters, $P = 0.01$). Moreover, results showed that complete remission was achieved in only 51% of patients whose diagnostic samples established AML xenografts, compared to 80% of non-engrafting samples ($P < 0.0001$). As a conclusion, AML xenografting was successful when using samples from AML patients with aggressive disease and with a poor response to standard induction therapy.

OVERCOMING CHALLENGES IN PATIENT-DERIVED XENOGRAFTS OF AML

Recent experiments are trying to improve mouse experimental models of AML, aiming to engraft with a higher success rate even less aggressive leukemia samples. Most of them are based on immunodeficient mice with humanized microenvironment created by injection of human mesenchymal stem cell (MSC) that provide a better niche for leukemic blast engraftment.

An interesting approach was that of Reinisch *et al*[60], who used human MSC grafts injected subcutaneously in NSG mice to form a humanized microenvironment named "ossicles", in which they subsequently injected (8 wk later) human HSCs and AML blasts. The final aim was to analyze the engraftment capacity the resulting niche. After 6-10 wk, the "ossicles" showed human BM–like functions and morphology and allowed enhanced engraftment of primary patient-derived AML.

A further attempt employed humanized niches based on genetically modified MSCs to express huIL-3 and TPO. Carretta *et al*[61] implanted subcutaneously these human MSCs in ceramic scaffolds or Matrigel in NGS mice, and 6 to 8 wk later transplanted CD34+ enriched AML blasts in the ectopically engineered BM niches. The engraftment capacity was then compared with the one from non-engineered MSC niches. The results showed that leukemic blasts efficiently engrafted in both models with no significant differences. An unexpected result was that CD33+-sorted myeloid clones from the animal model failed to self-renew in secondary recipients, probably due to overexpressed IL-3 and TPO cytokines from modified microenvironment that might have affected a proper self-renewal of myeloid blasts.

An important challenge for obtaining mouse models valuable as preclinical models is the capability of PDX cells to authentically mimic the heterogeneity of the initial disease. The xenograft mouse model of AML has been used mainly to study primary transplantation and further serial experiments were performed to

verify self-renewal competence or stability of gene expression profiles of engrafted cells. However, this model was rarely employed to investigate deeply AML biology or therapy[62]. Most of the published results revealed that PDX cells resembled the primary samples in terms of gene expression profiles but sub-clonal profiles were often not reflecting the primary sample. Another important drawback was the inability of the most proposed models to sensitively and repetitively monitor disease progression or drug effects. These were determined at single time points by invasive procedures or post mortem. However, researchers tried to overcome these challenges by proposing a better control of PDX cells. This control aimed to check the pattern of alterations in mutational or antigen expression possibly occurred during engraftment. For better monitoring disease progression or drug effects, recombinant luciferase enabled bioluminescence *in vivo* imaging has been proposed to facilitate *in vivo* monitoring of PDX AML cells as a quantitative, sensitive, reliable method for quantifying leukemia initiating cells[62].

CONCLUSION

Mouse models were of tremendous importance for understanding the molecular etiology of leukemia, proven to be valuable tools to facilitate preclinical *in vivo* studies.

Most of the studies verified that PDX models kept the most important features of the original tumor. However, mouse models should be controlled more carefully before and after xenotransplantation, especially in serial transplantation experiments, in order to ensure that the heterogeneity of the original sample is conserved and genetic drift is not modifying genetic, phenotypic or functional characteristics of the original disease.

Prospectively, advancements allowing repetitive, reliable, sensitive and fast studies, able to evaluate the efficacy of an experimental treatment in well genetically defined and heterogeneous subgroups of AML, will represent valuable tools to improve the individualized xenograft mouse model of AML and drastically reduce the number of mice to be used in these kind of experiments.

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P- Reviewer: Kiselev SL, Liu L, Scarfì S, Song YH
S- Editor: Wang JL **L- Editor**: A **E- Editor**: Tan WW

World Journal of Stem Cells

Submit a Manuscript: http://www.f6publishing.com

World J Stem Cells 2018 June 26; 10(6): 66-77

DOI: 10.4252/wjsc.v10.i6.66

ISSN 1948-0210 (online)

REVIEW

Applications of stem cells in orthodontics and dentofacial orthopedics: Current trends and future perspectives

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Author contributions: All authors contributed to the manuscript.

Conflict-of-interest statement: No potential conflicts of interest relevant to this article were reported.

Manuscript source: Invited manuscript

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Received: March 27, 2018
Peer-review started: March 28, 2018
First decision: April 16, 2018
Revised: April 19, 2018
Accepted: May 9, 2018
Article in press: May 10, 2018
Published online: June 26, 2018

Abstract

A simple overview of daily orthodontic practice involves use of brackets, wires and elastomeric modules. However, investigating the underlying effect of orthodontic forces shows various molecular and cellular changes. Also, orthodontics is in close relation with dentofacial orthopedics which involves bone regeneration. In this review current and future applications of stem cells (SCs) in orthodontics and dentofacial orthopedics have been discussed. For craniofacial anomalies, SCs have been applied to regenerate hard tissue (such as treatment of alveolar cleft) and soft tissue (such as treatment of hemifacial macrosomia). Several attempts have been done to reconstruct impaired temporomandibular joint. Also, SCs with or without bone scaffolds and growth factors have been used to regenerate bone following distraction osteogenesis of mandibular bone or maxillary expansion. Current evidence shows that SCs also have potential to be used to regenerate infrabony alveolar defects and move the teeth into regenerated areas. Future application of SCs in orthodontics could involve accelerating tooth movement, regenerating resorbed roots and expanding tooth movement limitations. However, evidence supporting these roles is weak and further studies are required to evaluate the possibility of these ideas.

Key words: Alveolar bone grafting; Dentofacial deformities; Distraction osteogenesis; Guided tissue regeneration; Orthodontics; Orthodontic tooth movement; Orthognathic surgery; Periodontitis; Root resorption; Stem cells

Core tip: Stem cell therapy has multiple applications in the field of orthodontics and dentofacial orthopedics. Recent researches have demonstrated advantageous use of stem cells (SCs) for correction of craniofacial anomalies, rapid consolidation phase of distraction osteogenesis, reconstruction of temporomandibular joint and stability of palatal expansion. SCs also could be used to regenerate infrabony alveolar defects and move

the teeth into regenerated areas. Future application of SCs in orthodontics could involve accelerating tooth movement, regenerating resorbed roots and expanding tooth movement limitations.

Safari S, Mahdian A, Motamedian SR. Applications of stem cells in orthodontics and dentofacial orthopedics: Current trends and future perspectives. *World J Stem Cells* 2018; 10(6): 66-77 Available from: URL: http://www.wjgnet.com/1948-0210/full/v10/i6/66.htm DOI: http://dx.doi.org/10.4252/wjsc.v10.i6.66

INTRODUCTION

Orthodontics involves treatment of dental malocclusions and correction of dentofacial deformities. The aim of orthodontic treatment is to achieve facial aesthetics and improve oral health related quality of life[1,2]. The prevalence of dental malocclusion varies in different communities and have been reported to be 22.5% to 93%[3-6]. Orthodontic treatment of malocclusions has several shortcomings such as prolonged treatment time, apical root resorption, tooth movement limited to alveolar bone and difficulties to overcome periodontal defects.

Although facial anomalies and jaw base deformities are less frequent compared to simple dental malocclusions, they are more burdensome[7]. About 5% of orthodontic patients could be considered as handicapped and need multidisciplinary treatments[8]. Current treatment modalities of craniofacial deformities can reduce the severity of these deformities but their final aesthetic outcomes are still not pleasing.

Stem cells (SCs) are self-renewal cells that could differentiate toward various cells under suitable conditions[9]. Various sources for harvesting SCs have been introduced such as muscle, dermis, bone marrow, adipose tissue, periosteum, blood, umbilical cord, synovial membrane and teeth[10,11]. Among these sources, some are easily accessible in orthodontics. As extraction of primary teeth or permanent premolar or wisdom teeth is common interventions in orthodontic treatment of malocclusions, SCs sources from the teeth could be gained without extra morbidity. Several studies have revealed differentiation and proliferation potential of mesenchymal stem cells (MSCs) obtained from dental pulp, periodontal ligament or human exfoliated deciduous teeth[12-15].

Nowadays, MSCs could be considered as "research trends" in the field of biology and medicine and their application in regenerative medicine is growing. Some modalities involve direct plantation of MSCs into the defect site while others use proper scaffolds to support the cells. In bone tissue engineering, MSCs are carried by an osteoconductive scaffold and differentiated toward osteogenic cells using osteoinductive growth factors[16]. Several types of scaffolds and growth factors have been used for regeneration of craniofacial bone defects including orthodontic related bone defects[17-19]. The aim of the current study was to review applications of SCs in treatment of dentofacial defects and deformities and to propose possible advantages of SC therapy in enhancing orthodontic treatments.

APPLICATIONS IN DENTOFACIAL ORTHOPEDICS

To evaluate the uses of SCs in dentofacial orthopedics, application of SCs in treatment of dentofacial anomalies and temporomandibular joint (TMJ) disorders as well as their possible role in distraction osteogenesis (DO) and maxillary expansion have been discussed (Figure 1).

Dentofacial anomalies

Craniofacial deformities such as congenital and developmental malformation and those resulting from trauma, tumor resection and nonunion of fractures, are common clinical problems in craniofacial surgery, which are difficult to remedy. Current surgical techniques in various combinations, autogenous, allogeneic, and prosthetic materials have been used to achieve bone and soft tissue reconstruction[20]. These approaches have several complications such as insufficient autogenous resources, donor site morbidity, contour irregularities, postoperative pain, additional cost, long surgical time and postsurgical reabsorption, disease transmission, major histoincompatibility, graft versus-host disease (GVHD), immunosuppression, unpredictable outcome for tissue formation and infection of foreign material[21-24]. In order to overcome these complications, stem cell-based tissue regeneration offers a promising approach to provide an advanced and reliable therapeutic strategy for craniofacial tissue reconstruction[25]. In the current review, regenerative approaches for two types of craniofacial anomalies are presented; cleft lip and palate (CLP) (for hard tissue regeneration) and hemifacial microsomia (HFM) (for soft tissue regeneration).

CLP is one of the most prevalent congenital anomalies which results from fusion failure of nasal process and oropalatal shelves. The prevalence of this malformation is 0.36-0.83 in 1000 live-born infants[26]. Alveolar bone defect, problem in swallowing and pronunciation, facial deformity, missing teeth, and maxillary deformity can be seen in CLP patients[27]. Repair of the malformed alveolar bone is critical for oronasal fistula closure, maxilla unification, tooth eruption, and support of the alar base[28,29]. The gold standard treatment for alveolar reconstruction in CLP patients is autogenous cancellous bone grafts[30] since they are immunologically inert and potential suppliers of cells with osteoconductive and osteoinductive properties[31,32]. The commonest site for acquiring autogenous bone for grafting is the anterior iliac crest[33]. An overall success rate for iliac crest bone grafting to the alveolar cleft with respect to bone resorption is 88%[34]. With the advent of tissue en-

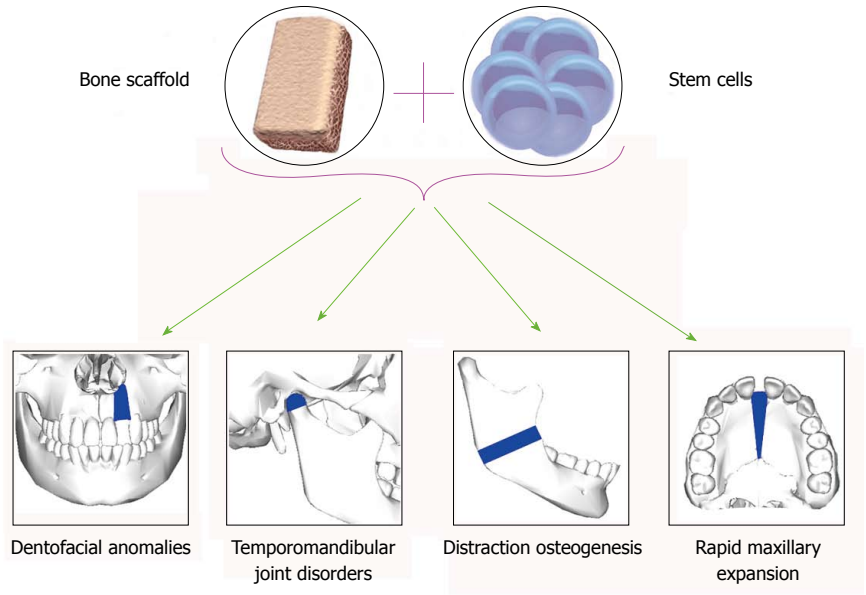

Figure 1 Applications of stem cells (alone or in conjugation with bone scaffolds) in dentofacial orthopedics.

gineering techniques, alternatives to the traditional iliac crest bone grafting techniques are available. MSCs have been shown to have the ability to form new bone when transplanted[35].

Some case reports and case series studies reported results of MSCs usage to regenerate alveolar cleft[36]. Composite scaffold of demineralized bone mineral and calcium phosphate loaded with MSCs showed 34.5% regenerated bone in the cleft area in one case and in the other there was 25.6% presentation of bone integrity[37]. About 50% fill of the bone defect was measured after placement of the scaffold, growth factor and MSCs in cleft area[38], whereas 79.1% bone regeneration has been reported in the another study[22]. Autogenous osteoblasts cultured on demineralized bone matrix showed more reduction in defect size in comparison to control group[39]. About 90% defect correction of soft palate defect has been reported 14 d after injection of autologous MSCs[40]. Biomaterial seeded with autogenous osteogenic cells into the alveolar cleft resulted in spontaneously eruption of canine in its proper place after eighteen months[41]. Poly-L-lactic acid with osteogenically differentiated fat-derived stem cells showed substantial bone regeneration in palatal defect[42]. The mean pain score, including both intensity and pain frequency and donor site morbidity was greatest at all-time points in traditional iliac crest bone graft and least at all-time points in tissue engineering[31].

Thus, it can be concluded that SCs seem to possess favorable potential for bone regeneration in oral and maxillofacial region and use of them in alveolar defect repair, reduce defect size by bone formation[37,39,42], have less postoperative morbidity compared to autogenous bone grafting[31] and help the teeth in the defect area to erupt in their proper position[41].

HFM is a rare, multi-systemic congenital disease. It is considered to be the product of unilateral abnormal morphogenesis of the first and second pharyngeal arches. HFM is a frequently encountered form of congenital facial malformation, ranking second only to cleft lip and palate[43]. The fundamental features of HFM include unilateral hypoplasia of the craniofacial skeleton and its overlying soft tissue[44]. Autologous fat grafting is considered to reconstruct soft tissue defect in the treatment of congenital malformations as well as post-traumatic malformations[45]. To overcome problems associated with fat grafting, such as unpredictable clinical results and a low rate of graft survival, many innovative efforts and refinements of surgical techniques have been reported[46]. Use of adipose derived stromal cells (ASCs) for tissue regeneration has attracted attention recently.

Patients with HFM which have been grafted with supplementation of ASCs Showed 88% of fat volume surviving after 6 mo in comparison to control group which was 54%[46]. Also, residual graft volumes of ASCs enriched grafts was significantly higher in comparison to control group[45].

Studies are ongoing, and as results are reported, it will be crucial to evaluate the long term outcome of such procedures. The current evidence suggests that use of ASCs for soft tissue reconstruction may enhance angiogenesis[47], improve the survival of grafts[45,46] and thus reduce atrophy[47].

Temporomandibular joint disorders

The temporomandibular joint (TMJ) is comprised of both osseous and cartilaginous structures. It is enclosed in a capsule that is lubricated with synovial fluid and serves as an important growth site during postnatal development with two articular surfaces that can adapt to changing environment conditions[48,49]. The mandibular condyle grows by proliferation of the progenitor/SCs that differentiate into chondrocytes[49,50] leading to formation

and increase of cartilage matrix, which will be replaced with lamellar trabecular bone[51]. As SCs possess the ability to differentiate into chondrogenic and osteogenic cells, they could be used for both maintenance of mandible in new position and repair of TMJ lesions.

Forward positioning of mandible, for example in functional therapy, leads to increase in the number of mesenchymal cells (stem/progenitor cells) in the temporal fossa, which resulted in new cortical bone formation[52]. Thus, the question arises as to whether the injection of SCs into articular space accelerates bone formation in the temporal fossa? This issue requires Further targeted researches.

TMJ is prone to injuries, tumors, osteoarthritis, rheumatoid arthritis and congenital anomalies. Approximately 10 million individuals in the United States have been affected by temporomandibular disorders (TMD)[53]. TMD manifest as pain, myalgia, headaches, and structural destruction, collectively known as degenerative joint disease[54]. The primary methods used to reconstruct the TMJ includes autogenous bone grafting such as harvesting from the rib, or the use of alloplastic materials, with neither being ideally suited for the task and sometimes leading to unwanted adverse effects. The major and final option for those patients with advanced degenerative diseases is surgical replacement of the mandibular condyle[55]. These approaches have complications such as immunorejection, infection, implant wear, dislocation, suboptimal biocompatibility, donor site limitation and morbidity, and potential pathogen transmission[56,57]. To overcome these disadvantages, strategies have been found to engineer osteochondral tissue, such as that found in the TMJ, will produce tissue that is both biologically and mechanically functional used. Recently, these cells have attracted much interest to joint reconstruction.

Engineering a TMJ-like osteochondral graft has been studied in several studies. The culture of human umbilical cord matrix (HUCM) SCs in growth medium containing chondrogenic factors, showed the HUCM SCs can outperform the TMJ condylar cartilage cells[58]. Rat bone marrow MSCs which encapsulated in poly (ethylene glycol)-based hydrogel molded into the shape of a cadaver human mandibular condyle, demonstrated two stratified layers of histogenesis of cartilaginous and osseous phenotypes[59,60]. Porcine MSCs which had been cultured in osteogenic induction medium and were seeded onto a poly DL-lactic–co-glycolic acid scaffold, formed the construct had a shape that closely resembled to the model condyle and it's radiodensity was between that of the normal condyle and that of control scaffolds[61].

Because of fibrocartilaginous structure of disk, there has been little success in the manufacture of synthetic TMJ discs rather than bone and cartilage and attention has turned to tissue engineering to reconstruct the disc[62]. In one study, Combination of polylactide acid discs with adipose tissue stem cell demonstrated the potential to development a tissue-engineered TMJ disc[63].

While animal studies are in progress to replicate bone the osteochondral interface to engineer TMJ, yet no clinical trials on humans have been done. These data revealed possibility of application of SCs in combination with different scaffolds as a promising approach to regenerate osteochondral tissues of TMJ and ultimately the joint disk.

Distraction osteogenesis

DO which is regarded as "endogenous bone tissue engineering" has been widely applied in orthopedic surgery for correction of limb length and also in the treatment of many craniofacial deformities[64]. DO is done by creating a corticotomy, placing a rigid distractor across the cut bone and gradually activating the device[65]. The mechanism of osteogenesis and gap repair initiated by an immediate inflammatory response that leads to the recruitment of MSCs and subsequent differentiation into chondrocytes that produce cartilage and osteoblasts which form bone[66]. Despite its great advantages, long treatment periods and fibrous union or even non-union of bone are possible major draw backs impeding its widespread clinical application[67,68].

Efforts have been made to accelerate osteogenesis in the distraction Gap, shorten the consolidation period and reduce complications such as the development of nonunion, infection, or fracture.

Recently, because of the role of MSCs in osteogenesis, many researchers have successfully documented the ability of SCs on promoting bone formation and shortening the consolidation period during DO. For this purpose various sources of SCs such as human exfoliated deciduous teeth (SHED)[69], bone marrow[70-77] and adipose tissue[78-80] have been used in studies. In some studies, alone MSCs[71,79,81,82], in the others, gene transferred MSCs[72,76-78,83] and factors[75,84,85] have been used to enhance bone regeneration following distraction osteogenesis. The modifications such as use of scaffolds[75], demineralized bone matrix[74] and Platelet-rich Plasma[73] have been done in some studies.

The injection of MSCs 1 d before onset of distraction resulted in increase in new bone volume in the distracted callus and the bone mineral density (BMD)[81], MSCs injection after distraction was complete showed higher radiodensity of the distraction zone and grater histologically callus, new bone volume and thickness of the new trabeculae[71] and doing this intervention on the first day of consolidation resulted in greater biomechanical strength and increase in total and compact bone ratio in regenerate bone[82]. The injection of SHED during osteotomy period showed higher percentage of newly formed bone after 2, 4, and 6 wk[69]. One study revealed that callus density, the ossification rate, quality of newly formed bone and the number of active cells in bone formation were higher in group which osteoblast-differentiated stem cell were injected to distraction site compared to control group and stem cell group[79]. Addition of MSCs sheet fragments yielded significant increases in bony union, more intensive

bone formation on histomorphometric analysis and higher peak load on biomechanical testing[70]. MSCs transfected with bFGF showed excellent bone formation and higher BMD and bone mineral content (BMC) in the distracted callus[76]. The use of MSCs osteogenic differentiation using FGF-2 and confirm cell integration with a gelatin-based Gelfoam scaffold, demonstrated less interfragmentary mobility, more advanced gap obliteration, higher mineral content and faster mineral apposition[75]. One study suggested that gene therapy using rhRunx2-modified ASCs promoted new bone formation during osteoporotic mandibular DO[78]. Application of ASCs transfected with pEGFP-OSX showed the highest BMD, thickness of new trabecula (TNT), and the volumes of the newly generated cortical bone (NBV1) and the cancellous bone (NBV2) in the distraction zones[78]. Excellent bone formation and highest BMD, TNT and NBV in the distraction zones was observed in groups that MSCs transfected with OSX[72]. The injection of MSCs transfected with Bone Morphogenic Protein (BMP) showed greater bone formation and earlier mineralization in the distracted callus[77], more mature medullary cavity[83], better bone quality and higher trabecular parameters (trabecular thickness, trabecular number, volumetric bone mineral density at tissue, and bone volume fraction) at the second and fourth weeks of the consolidation period[86] and acceleration of osteogenesis[87]. The use of stromal cell-derived factor-1 (SDF-1) facilitated migration of MSCs into osteogenesis site[84]. The addition of MSCs transfected with recombinant plasmids pIRES-hBMP2-hVEGF165 at the beginning of distraction is more ideal than the start of latency period[85].

These data shows that SCs from Various sources, alone or in combination of genes and factors, in different phases of treatment can lead to an increase in new bone volume and quality[69,71,72,77,78,81,86], bone mineral density[71,72,76,78,81], trabecular thickness[71,78,86], biomechanical strength[70,82].

Rapid maxillary expansion

Maxillary constriction can be associated with several problems that include occlusal disharmony and esthetics as well as such functional difficulties as narrowing of the pharyngeal airway, increased nasal resistance, and alterations in tongue posture, resulting in retroglossal airway narrowing and mouth breathing[88-90]. Maxillary constriction can be corrected with slow orthodontic expansion, rapid maxillary expansion (RME), surgically assisted rapid palatal expansion or a two-segmented Le Fort I-type osteotomy with expansion[91]. RME is indicated in patients younger than 12 years, who have lateral discrepancies involving several teeth, whether the constriction is skeletal, dental or a combination of both[92]. It is an effective orthopedic procedure to open the midpalatal suture, providing appropriate and stable maxillary width increase and re-stablish balance between the width of the jaws[93,94].

RME is similar to DO histologically. During RME, a gap in the midpalatal suture is created which is filled with blood and granulated tissue and followed by active bone formation. The expanded arch width relapses unless followed by an appropriate retention period. Therefore, providing a strategy to accelerate bone formation in the midpalatal suture might shorten treatment and retention period, achieve stability and prevent relapse. Because of the ability of SCs to differentiate into osteogenic cells, injection of SCs seems to have the ability to accelerate the process of bone formation. This was studies in one study by Ekizer *et al*[95]. In their animal study, local injection of MSCs into intermaxillary suture after force application resulted in increased new bone formation in the suture by increasing the number of osteoblasts and new vessel formation[95]. Thus, locally applied MCSs to the expanded maxilla might be a useful and practical treatment strategy to accelerate new bone formation in midpalatal suture and to shorten the treatment and retention period for patients undergoing orthopedic maxillary expansion.

APPLICATIONS IN ORTHODONTICS

To evaluate the uses of SCs in orthodontics, current evidence regarding application of SCs in expanding the limitations of orthodontic tooth movement (OTM), tooth movement into periodontal defects, accelerating OTM and treatment of external root resorption (ERR) have been reviewed (Figure 2).

Expanded envelope of discrepancy

The extent of OTM is limited by several factors including the anatomy of the alveolar bone, pressures exerted by soft tissues, periodontal tissue attachment levels, neuromuscular forces and lip–tooth relationships[96,97]. The anteroposterior, vertical, and transverse millimetric range of treatment possibilities in orthodontics can be expressed as an "envelope of discrepancy"[98] Gingival recession occurs secondarily to an alveolar bone dehiscence, if overlying tissues are stressed during OTM beyond this envelope. Sites in which the buccal or lingual bone cortex and covering gingival tissue are thin, such as lower incisors in patients with a prominent chin and compensation in the form of lingual tipping of these teeth are at particular risk of bone defects like fenestrations and dehiscence[99,100].

SCs have the potential to generate different tissues, including bone, thereby stem cell therapy is a promising approach to alveolar bone regeneration[101]. Some researches have applied stem cell therapy in case of bone ridge augmentation in humans and mainly used bone marrow cells[102-104]. The outcome of alveolar bone regeneration showed a tendency to enhance bone formation[105]. Hence, bone regeneration methods using SCs might provide an approach for expanding limitations of envelope of discrepancy.

As a hypothesis, relying on the results of alveolar

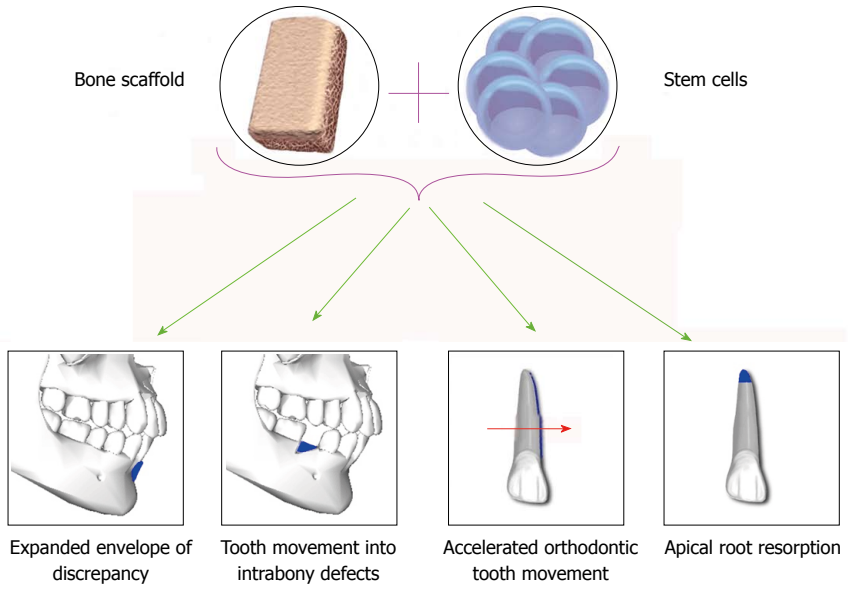

Figure 2 Possible applications of stem cells (alone or in conjugation with bone scaffolds) in orthodontics.

bone augmentation studies, it might be possible with the aid of stem cell based osteogenesis to horizontally augment the ridge in order to extend the tooth movement extent and to overcome some anatomical boundaries.

Periodontal regeneration

Periodontal complications are one of the most actual side effects linked to the orthodontics. It can be found in various forms, from gingivitis to periodontitis, dehiscence, fenestrations, interdental fold, gingival recession or overgrowth, black triangles[100]. Periodontal regeneration has been defined as the formation of new cementum, alveolar bone, and a functional periodontal ligament on a previously diseased root surface[106]. The current treatment approaches include the use of surgery, guided tissue regeneration (GTR), bone fillers and growth factors and application of bioactive molecules to induce regeneration[107,108]. Based on the differential potential capability of SCs and their ability of renewal *via* mitosis[109], they have the quality to regenerate damaged tissues, hence they can be used for regeneration of periodontium.

Periodontal defects could be a challenging situation both pre and post orthodontic treatment. On one hand, because of the increasing number of adult patients seeking orthodontic treatment, encountering the periodontally involved patients may be a potential problem for every practitioner. It has been suggested that, by moving the teeth into infrabony defects, we can achieve the regeneration of the attachment apparatus[110]. Accordingly with the combination of periodontal regeneration treatments such as GTR and OTM, it might be possible to reduce infrabony defect and upgrade periodontal health[111]. On the other hand, periodontal defects such as fenestration, dehiscence and attachment loss are among common complications of orthodontic treatments[112].

Several reports on application of SCs for regeneration of periodontal tissues have been published. In a study, induced pluripotent SCs have been implanted into a mouse periodontal fenestration defect model with a silk fibroin scaffold in combination with enamel matrix derivative gel. As a result, higher rate of cementum and alveolar bone formation was observed[113]. Also, it has been shown that the bone marrow derived mesenchymal stem cells (BM-MSC)-treated wounds exhibited significantly accelerated wound closure, with increased re-epithelialization, cellularity, and angiogenesis[114]. In another study conditioned medium (CM) obtained from PDLSCs were transplanted into a rat periodontal defect model and consequently PDLSC-CM enhanced periodontal regeneration by suppressing the inflammatory response *via* TNF-α production[115]. Incubation of induced PDLSCs with dentin non collagenous proteins *in vivo* revealed that cementum-like tissues formed along the chemical-conditioned root dentin surface, enhanced alkaline phosphatase (ALP) activity, increased matrix mineralization, and upregulated expression of mineralization-associated genes[116]. One study has revealed that autologous PDLSCs obtained from extracted teeth of the miniature pigs which were transplanted into the surgically created periodontal defect areas were capable of regenerating periodontal tissues, leading to a favorable treatment for periodontitis[117]. PDLSCs were delivered onto suitable collagen sponges and implanted into periodontal defects of immunodeficient nude rats in an *in vivo* study, as a result reformation of periodontal ligament-like tissue, collagen fibers, and elements of bone was observed[118]. In another *in vivo* study, PDLSCs sheet were transferred to a miniature pig periodontitis model. Significant peri-

odontal tissue regeneration was achieved in both the autologous and the allogeneic PDLSCs transplantation[119]. Using amniotic membrane for transferring PDLSCs for periodontal regeneration in a rat periodontal model as a new method of transplantation is also being suggested in a study[120].

According to aforesaid studies, human adult PDLSCs are capable of regenerating elements of bone and collagen, since the periodontitis is a chronic disease, it may benefit from such stem cell based therapies[114,117-119]. Thus the use of PDLSC transplantation in periodontal therapies can reduce treatment time and better outcomes followed by patient comfort, however, due to complex structure of periodontium, regeneration is a feasible and yet complicated procedure and may need pluripotent SCs and more investigations.

Accelerated OTM

OTM is achieved by the remodeling of periodontal ligament (PDL) and alveolar bone in response to mechanical loading[121,122]. The initiating inflammatory event at compression sites is caused by constriction of the PDL microvasculature, resulting in a focal necrosis, followed by recruiting of osteoclasts from the adjacent marrow spaces[123]. These osteoclasts are mostly derived from hematopoietic SCs[124]. Hence, SCs could be used to accelerate OTM by providing progenitor cells.

The development of new methods to accelerate OTM has been sought by clinicians as a way to shorten treatment times, reduce adverse effects such as pain, discomfort, dental caries, and periodontal diseases, and minimize iatrogenic damages such as root resorption and the subsequent development of non-vital teeth[125]. There are surgical methods like surgically-facilitated orthodontic therapy or corticotomy[126], periodontally accelerated osteogenic orthodontics[127] and some nonsurgical procedures such as systemic/local administration of chemical substances like epidermal growth factor, parathyroid hormone, 1,25-dihydroxyvitamin d 3, osteocalcin and prostaglandins, resonance vibration, static or pulsed magnetic field, low-intensity laser irradiation therapy[128].

In a study, increased PDL progenitor cells with suppressed expression of type I collagen (Col-I) were observed during orthodontic force application, whilst after force withdrawal they increase in Col-I expression, which suggests that PDLSCs are able to respond to orthodontic mechanical forces with suppressed collagen expression[129]. This ability of SCs could be used to accelerate OTM in response to orthodontic forces. When orthodontic force is applied, tooth movement is hindered until the necrosis is removed, leading to the clinical manifestation of a delay period. Hypothetically, transplantation of SCs in pressure sites may speed up the process, resulting in accelerated OTM.

ERR

ERR is a common and unfavorable side effect of orthodontic treatment[130,131], which any specialist may encounter. Many factors seems to be involved in ERR such as genetics, individual biological variability, age, sex, and orthodontic forces and treatment duration[132,133]. Orthodontic forces yet seem to be the main etiologic factors. ERR may lead to loss of tooth structure such as cementum and in more advanced stages, dentin, however no specific treatment has been introduced so far. One possible treatment modality could be regeneration of resorbed roots by application SCs and tissue engineering.

In severe cases ERR may cause poor prognosis of tooth, resulting in tooth loss. Regeneration of these lesions increases the longevity of tooth and may play an important role in facilitating the treatment. In a study designed to induce de novo cementum formation by SC therapy, MSCs driven from periodontal ligament in *in vivo* transplantation were able to form cellular cementum-like hard tissue containing embedded osteocalcin-positive cells[134]. According to studies in which the whole tooth structure has been bioengineered and transplanted into Rodent[135,136] and beagle dogs[137] models, it might be possible to regenerate the damaged tooth structure such as dentin and cementum and in the future to achieve a bioengineered functional human tooth structure.

Although it seems that there is a long way until regeneration of the teeth materials, cementogenesis and regeneration of dental structures through stem cell based therapies could be anticipated.

CONCLUSION

The current review showed application of SCs alone or in conjugation with bone scaffold or growth factors in surgical correction of dentofacial deformities, TMJ defects, and alveolar bone lesions. Recent studies show that SCs could improve treatment results and reduce treatment duration. Use of SCs is associated with accelerated healing and less morbidity compared to current surgical approached. Also, SCs could be used in DO surgeries and RME to increase consolidation rate and reduce relapse.

The contemporary evidence reveals feasibility of use of SCs for accelerating OTM, regenerating resorbed roots, expanding limitations of OTM while preserving periodontal health. In addition, SCs could be used for regeneration of periodontal tissues both pre and post OTM. *In vivo* studies are required to assess the possibility of such interventions.

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P- Reviewer: Chivu-Economescu M **S- Editor**: Ji FF **L- Editor**: A
E- Editor: Tan WW

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