Original Article
Local transplantation of osteogenic pre-differentiated autologous adipose-derived mesenchymal stem cells may accelerate non-union fracture healing with limited pro-metastatic potency

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Abstract: Fracture non-union is a serious complication in orthopedic clinical practice. Mesenchymal stem cells are believed to play a vital role in fracture healing process. Among various origins of mesenchymal stem cell, adipose derived stem cells hold great promise especially in clinical milieu. However, the wide spread application of mesenchymal stem cell based therapy is impeded by the pro-metastasis nature of the mesenchymal stem cell itself. Based on the findings from previous studies, we hypothesize that local transplanted osteogenic pre-differentiatiated adipose stem cell may promote the non-union fracture healing. Moreover, the pre-differnetiation stem cells by down-regulating the expression of CCL5 and CCL2. This novel osteogenic pre-differnetiation technique may help clinical orthopedists to resolve the refractory non-union cases and shed new light on other stem cell based therapies to counteract to avoid the pro-metastasis nature of the mesenchymal stem cells.

Keywords: ADSC, pre-differentiation, fracture non-union, pro-metastatic

Introduction

Nonunion of bone fracture is a rare but refractory complication in orthopedic clinical practice, which can severely compromise prognostic function. Based on research datum, in the United states, 5% to 10% of all diaphyseal fractures will encounter delayed union or nonunion problem [1-5]. Major symptoms of fracture nonunion include persistent pain, stiffness of surrounding joints and limb disability which ultimately causing unemployment. Therefore fracture nonunion imposes both economical and psychological burden on patients as well as their families [6, 7].

At least three cellular events are required to initiate fracture healing including chemo-attractive recruitment, inductive proliferation, and osteogenic differentiation [8-12]. Recently more and more evidence show that mesenchymal stem cell (MSC) population is the fundamental ancestor of these cellular assemblages and plays a vital role in fracture repair and nonunion

pathology. Following bone fracture, disrupted bone matrix and degranulated platelets release various cytokines to the fracture site forming a chemo-attractive environment which recruit MSC to the damaged area [13]. Moreover, communication with the local cell population occurs to stimulate MSC osteoblastic capabilities. Carter DR et al reported favorable biologic and mechanical environments result in proliferation and differentiation of MSC to osteoblasts and chondrocytes [14]. Furthermore, by studing 35 nonunion patient's bone marrow, Hernigou and Beaujean reported that reduction in bone-producing stem cell population in the fracture hematoma may contribute to bone consolidation malfunction [15]. Also, they found that there were smaller stem cell morphology in the marrow of synovial pseudarthrosis patient than that of control group patient which give a hint on the relationship between MSC disorder and nonunion development [16].

As a promising cell source, MSC are demonstrated capable to self-renew and possess

multi-potent differentiation properties [18]. Also, evidence show that MSC exhibit non-immunogenic or hypo-immunogenic properties [17]. Barrilleaux and colleagues isolated fibroblast-like MSC from the stromal cell population from various tissues [18]. Bone marrow has been used as the major source of MSC and under appropriate conditions bone marrow derived MSC can be selectively induced into osteogenic lineage [19]. However, the harvest of bone marrow MSC is a highly invasive and painful procedure which prompts the quest for alternative sources from which to isolate MSC. Moreover, Bone marrow cellularity declines with age, and there is also a decrease in the prevalence of connective-tissue progenitors with increasing age which hinder the bone marrow stem cells in a wide range of clinical applications [20].

Like bone marrow, adipose tissue is meso-derm-derived organ containing stromal population such as microvascular endothelial cells, smooth muscle cells and stem cells [21]. These cells can be enzymatically isolated from adipose tissue (commonly from lipoaspirate) and separated from the buoyant adipocytes by centrifugation. A more homogeneous population emerges in culture under conditions supportive of MSC growth. From the last decade, this population which is termed with generic nomenclature, adipose-derived stem cell (ADSC) has been identified as possessing many of the properties of its counterpart from bone marrow including extensive self-renewal potential and the capacity to undergo multilineage differentiation [22-25]. Furthermore, the phenotypic and gene expression profiles of ADSC are similar to MSC obtained from bone marrow [23, 25].

Zuk and colleagues reported that ADSC can be expanded in vitro for extended periods [22]. Since humans have abundant subcutaneous fat deposits, ADSC can easily be isolated by conventional liposuction procedures, thus over-coming the tissue morbidity associated with bone marrow aspiration. Furthermore, the MSC frequency in bone marrow is somewhere between 1 in 25,000 to 1 in 100,000 cells whereas ADSC constitute approximately 2% of lipoaspirate cells [25-28]. Due to its abundance, relatively easy harvest, and high MSCs frequency, adipose derived stem cell might be a solid starting basis for further development of stem cell therapies.

Despite all the advantages MSC therapies possess, the potential risks of inducing uncon-trolled cell growth pose a serious threat to the recipient patient. In 2007, Weinberg and colleagues demonstrate that when mixed with otherwise weakly metastatic human breast carcinoma cells, human bone-marrow-derived mesenchymal stem cells greatly increase the metastatic potency of the cancer cells. Furthermore, they showed that mesenchymal stem cells when stimulated by the breast cancer cells produce chemokine CCL5 (also called RANTES) which in turn acts in a paracrine fashion on the cancer cells to enhance their motility, invasion and metastasis [29].

Hypotheses

Local transplantation of autologous osteogenic pre-differentiated ADSCs to the fracture gap of non-union fracture might be able to promote non-union fracture healing by stimulating local angiogenesis, producing abundant calcium deposit in early phase of post-surgical facture healing and matching the transplanted ADSCs osteogenic function to the fracture healing time frame. Moreover, by limiting cell self-renewal capacity, osteogenic pre-differentiation treatment might greatly prevent the transplanted mesenchymal stem cell from neoplasia and cancer metastasis promoting behavior which will potentially facilitate the wide spread use of mesenchymal stem cell therapy. Based on all the facts above, we hypothesize that local transplantation of autologous osteogenic pre-differentiated ADSCs holds the promise to enhance non-fracture healing and reduce risk of uncontrolled cell growth.

Evaluation of the hypothesis

Match ADSCs's osteogenic function to fracture repair time frame

Previous studies have shown that it normally take more than two to three weeks for ADSCs to exhibit calcified extracellular matrix in osteo-genic induction culture [30]. Moreover, in vivo study showed human ADSCs possesses the capacity to form osteoid on appropriate biomaterials [30]. Consistently, Jaiswal and colleagues demonstrated JNK activation occurred on day 13 to day 17 in the osteogenic differentiation process, which was associated with extracellular matrix synthesis and increased calcium deposition, the two hallmarks of bone formation [31]. Based on these evidence, in our hypothesis, we first isolate autologous ADSCs using the patient's own adipose tissue. Next,

the ADSCs are pre-differentiated with osteogenic induction medium in culture for 14 days. Then, the pre-differentiated ADSCs are locally transplanted into the the fracture gap of non-union patient in ten non-union repair surgery. This procedure may help to match the osteogenic function of ADSCs to the subsequent fracture healing time frame and aid to facilitate early production of osteoid and wound calcification, which may accelerate the stabilization of facture site and enhance the prognosis of non-union repair surgery.

Avoid the risk of mesenchymal stem cell promoted cancer metastasis

There is a long history of clinical and experimental observations showing that metastases frequently occur at sites of injury. As stem cells preferentially migrate to tumors and sites of tissue injury [32], they may prepare the injured sites for subsequent colonization. Once there, hematopoietic stem cells are recruited to a so-called 'premetastatic niche', where they reorganize the matrix and establish sites at which tumor cells proliferate more frequently than at other locales to develop tumor metastases [33]. Recent studies elucidated that the human bone-marrow-derived mesenchymal stem cell (MSC) is recruited in large numbers and integrate into the stroma of developing tumors [34]. Moreover, Weinberg's team revealed that that human mesenchymal stem cells, by secreting the CCL5, act in a paracrine fashion on the cancer cells to enhance their motility, invasion and metastasis [29]. Also, CCL2 was shown to partly mediate the interaction between breast cancer cells and MSCs [35]. These findings suggest a potential adverse effect of MSC mediated therapy, which may promote the otherwise weakly metastatic cancer cells in patients to increase metastatic potency and eventually cause the spread of cancer. On the other hand, Djouad's data demonstrated that, as the MSC differentiating into chondrocytes, CCL5 expression is downregulated on day 7 under chondrogenic induction [36]. In addition, Molloy's study showed that MSC's secretion of CCL2 is dramatically decreased on day 10 and 14 during differentiation into osteoblasts [35]. Accordingly, in our current hypothesis, a 14 days of osteogenic pre-differentiation would potentially abrogate the crosstalk effect between ADSCs and cancer cells via downregulating the CCL5 and CCL2 expression both of which play pivotal role in mediating metastasis of cancer cells. Furthermore, pre-differentia-

tion of ADSCs also serve to reduce the possibility of uncontrolled cell proliferation and increase the functional cell fraction in the total transplanted cells.

Conclusion

Fracture non-union is a clinically refractory complication which seriously affecting the life quality of patients. Recently, MSC mediated therapy emerged as a promising solution to enhance the wound healing capacity. Among all source of MSC, ADSCs possess many advantages over other origins such as abundant sources, easy to harvest, and high MSCs frequency. However, despite MSC has been a hot spot of researches for over a decade, few have been interpreted into clinical therapy owing to its pro-metastatic nature. Based on reported studies, we present a hypothesis that local transplantation of autologous osteogenic pre-differentiated ADSCs into the fracture gap holds great promise to enhance non-union fracture healing while remitting the risk of systemic and local tumor metastasis. Our current hypothesis, if proven to be valid, will not only promote the outcome of facture non-union cases with enhanced bone formation capacity, but also will shed new light on the other applications of stem cell based therapies.

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Disclosure of conflict of interest

None.

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