Human Umbilical Cord Blood-Derived Mesenchymal Stem Cells (hUCB-MSCs): Isolation, Wnt Signaling, Differentiation Capacity and Therapeutic Applications

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ABSTRACT

Human umbilical cord blood derived mesenchymal stem cells are regarded as an alternative source of bone marrow derived mesenchymal stem cells because collection of cord blood is less invasive and that the differentiating potential of bone marrow MSCs decreases with age. hUCB-MSCs are regulated by an endogenous level of Wnt signaling involved in mesenchymal differentiation. Moreover, the migratory potential of MSCs works as a major tool to correct inherited disorders of tissue repair. Hence, this review focuses on the current knowledge about the isolation, the understanding of the molecular mechanisms and therapeutic implications of hUCB-MSCs that regulate self-renewal and lineage-specific differentiation.

Keywords: Human Umbilical Cord Blood Derived Mesenchymal Stem Cells, Umbilical Cord, Mesenchymal Stem Cells, Bone Marrow, Differentiaiton, Wnt Signaling

1. MESENCHYMAL STEM CELLS: DISCOVERY

German pathologist Cohnheim first suggested the presence of nonhematopoietic stem cells in bone marrow. Friedenstein and colleagues commenced the work which stated that the bone marrow contains cells which can differentiate into other mesenchymal stem cells and fibroblasts as well. These cells are now collectively called either as mesenchymal stem cells (MSCs), the reason being that they possess the ability to differentiate into mesenchymal-type cells, or as marrow stem cells, since they appear to originate from marrow which comprises of complex assemblage of supporting structures [1].

2. MESENCHYMAL STEM CELLS: SOURCES

In 1976 Friedenstein indentified the sources of MSCs as a fibroblast-like cellular population in the bone marrow (BM) and that MSCs are a rare, heterogeneous, stromal population of multipotent

nonhematopoietic progenitor cells which possessed the potential to differentiate into multiple mesenchymal lineages including bone, fat and cartilage [2].

Currently, groups have found new potential alternative sources of MSCs and have relied on new sources such as adipose tissue, the synovium, the fetal liver, deciduous teeth, amniotic fluid, the UCB, and UC [2-4]. The UCB and UC, in particular Wharton's Jelly are being considered relatively more as a promising alternative source of MSCs. They also hold importance because of their extraction procedures as they lack ethical controversies, accessibility as they are produced in abundance and involve painless procedures for donors, and a lower risk of contamination thus makes them a promising tool as a source of MSCs.

3. HUMAN UMBILICAL CORD BLOOD- DERIVED MESENCHYMAL STEM CELLS (HUCB-MSCS): ISOLATION

For more than 10 years, the transplantation of cord blood has been a pivotal part of clinical practice. Moreover, the umbilical cord blood (UCB) has also been proved to be a vital source for hematopoietic stem cells. However, the prospect of using UCB for MSC is still a matter of consideration. Some groups have established that UCB possess traces of MSCs but on the contrary some groups have substantiated that UCB cannot be considered a rich source of MSCs[2]. The total number of MSCs per UCB unit is directly proportional to their proliferation rate which appears to be slightly low and a small amount of UCB samples proved relatively to be a good source of MSCs[2,4]. The reason behind the increasing attention of UC is because they possess high amounts of MSCs which has been proved by several groups to be isolatable from the tested samples[3]. Various types of hUC-MSCs have been identified depending upon the protocol used and as well as the part of UC used were: UC matrix stem cells, UC perivascular cells, UC stromal Cells, Wharton's jelly stem cells, and cord-lining membrane MSCs [2-5]. Many approaches have been incorporated to isolate hUC-MSCs which are primarily based on enzymatic treatment or explantation techniques. The enzymatic digestion involved for the isolation of hUC-MSCs has been based either on the use of collagenase alone in combination with other enzymes namely trypsin and hyaluronidase[2]. The processes have involved either use or removal of the cord blood vessels or after the dissection of the UC into small pieces. Moreover, the hUC-MSCs are then cryopreserved and expanded only when the initial thawing has occurred[4]. After thawing, these cells are then used as a tool for the survival and maintenance of their differentiation potential by incorporating cryopreservation which has either involved the differentiation potential to occur in the presence of dimethyl sulphoxide by cooling them in a computer-controlled programmable cooler or using dimethyl sulphoxide-free cryoprotectant solutions[2,3]. It was also reported that the hUC-MSCs

which were thawed, grown easily and all the other related parameters like phenotypes, population doubling times and adipogenic abilities brought them into limelight.

4. IN-VITRO NEURAL CELLS DIFFERENTIATION AND WNT SIGNALING

Many groups have reported the In-Vitro differentiation of hUC-MSCs into neural cells. Apart from identifying MSCs through morphologic or phenotypic characteristics, reports have suggested that MSC populations can also be identified by differentiating into bone, fat and cartilage In-Vitro[2]. To induce the differentiation of MSCs to osteoblasts In-Vitro involves a classic method which involves incubating a confluent monolayer of MSCs with ascorbic acid, β-glycerophosphate, and dexamethasone for 2-3 weeks [1,2]. It was further reported that the MSCs were able to form the aggregates or nodules and thus reflecting their osteogenecity[2]. In neuron-conditioned medium, the Wharton's jelly culture has also been shown to differentiate into neuron-like cells[1]. These cells expressed neuron-specific proteins such as NeuN and Neurofilament[1,2]. The kainate receptor and glutamate decarboxylase were also expressed as subunits for mRNA[6]. Due to the generation of the inward current in response to glutamate, even after 10 d in culture, it was observed that 87% of cells were found to be differentiated and functional[2,7]. Keeping in mind the same neural induction strategies two groups drew conclusions that the neural differentiation which was obtained from 9 of 10 UCs raised information that cells with neural morphologies were shown to be positive for neuron-specific enolase, an NSC marker[2,6]. The other group also involved an ideal three-step neural induction method involving basic fibroblast growth factor, then followed by β-mercaptoethanol with neurotrophin-3(NT-3) and lastly an amalgamation of NT-3, nerve growth factor, and brain derived neurotrophic factor (BDNF) which gained them information that even observing after 14 d, 60% of the hUC derived cells expresses MAP-2along with 32% glial fibrillary acidic protein, which justifiably proved their role of differentiating into neurones and astrocytes[2,6,7].

It should be taken into consideration that undifferentiated MSCs also express many neural cell-related mRNAs and proteins. In addition to this, many unexpected and misleading effects such as variations in cell morphology due to actin cytoskeleton disruption, cell shrinkage has occurred when involving In-Vitro neuronal differentiation protocols[2]. Such reports thus urge to find out the basic fundamentals lying at the molecular level which could help in justifying the potential of MSCs to differentiate into neural cells.

Wnt proteins comprises of the most pivotal families of signaling molecules in development. They are also involved in the regulation of cell proliferation and motility, production of cell polarity, and description of cell fate[8]. It has been reported that BMP-2 has been involved to initiate alkaline

phosphatase and mineralization via Wnt signaling in mesenchymal cell lines [8,9]. Some groups have reported that RT-PCR could be used as a tool to determine that which Wnt-related genes were expressed by primary MSCs [8]. Canonical Wnt signalling was mimicked in MSCs using a conditioned medium which inhibited GSK-3β and induced β-catenin stabilization[9]. The Wnt signaling pathway was incorporated in MSCs through the protocol [9] and the work provided evidence that canonical Wnt signaling is functional in MSCs as β-catenin is present in the nucleus of MSCs resulting in the inhibition of GSK-3β because of the Wnt 3a treatment and Li+ application [8]. It should also be taken into account that more new techniques should be able to determine how mesenchymal differentiation is mediated through Wnt signaling in MSCs. Some groups have also suggested that if purified bioactive Wnt proteins, or inhibitors such as Dkk-1, if undergone molecular manipulation may act as a promising tool for controlling MSC self-renewal and differentiation [8]. An important aspect, laid down by a group that Wnt3a and 5b have been used in the induction of myogenesis[8,9]. Wnt 1,3a,4,7a, and 7b have been found to promote proliferation and chondrogenic differentiation, and in contrast Wnt5a and 11 retard these processes[8,9]. The functional mechanism which activate and binds the Wnt proteins are the binding affinity to Fz receptors which is likely to create stimulation within the MSCs [8]. Hence, it is required to initiate the processes by which an understanding of signaling mechanisms involving Wnt Signaling in MSCs can be elucidated out.

5. APPLICATIONS AND UNIQUENESS OF HUCB-MSCS AS COMPARED WITH OTHER SOURCES OF STEM CELLS

The hUC contains varied stem cell types, involving MSCs [2]. From the last 20 years, CB has emerged successfully to be a feasible clinical alternative to BM transplantation. Moreover, CB banking systems have been developed worldwide holding importance for incorporating technical requirements for clinical class cellular products [2-4]

The immunosuppressive properties and varied capacity to differentiate into various lineages [4], have made the MSCs to take the flagship by acting as a responsible tool in the field of cell based therapies, primarily focusing in regenerative medicine. The reports have also suggested that hUCB-MSCs have a vital role in the maintenance of peripheral tolerance and transplantation tolerance. Due to this property, they have been regarded as a favourable choice in cellular therapy for graft-*versus*-host disease (GVHD) and also acts as a protective measure in solid-organ grafts from being rejected [2,11].They possess various advantages like they are produced in very large quantities, taking into account the total number of births occurring worldwide each year, they are considerably the cheapest source as they are still regarded as medical waste in the delivery rooms, they are even free from any legal issues and can be easily gathered, manipulated without causing any threat to the

baby or mother, hence avoiding invasive and uncomfortable BM aspiration techniques [2,10]. They are also associated with low immunogenicity in clinical applications [2] followed up by a lower risk of viral contamination [2]. Further applications in disease models make them unique as they are involved in cartilage regeneration where hUCB-MSCs possess higher chondrogenic differentiation potential among other mesodermal differentiation potentials which aids in regeneration of damaged cartilage [11]. It has been reported that thrombospondin-1, 2(TSP-1,2) which works as an anti-inflammatory agent in rheumatoid arthritis functions by inhibiting the production of proinflammatory mediators such as interferon-γ and tumour necrosis factor-α which in turn works by causing depletion of synovium residing T cells and finally reduces the infiltration of monocytes in articular tissues [11]. Hence, such paracrine actions might help in the stimulation of the regeneration process [11]. hUCB-MSCs are not limited to this but also acts as a defensive tool for glioma [10,11]. It is an interesting fact that MSCs have been shown to migrate towards glioma [11]. This characteristic can be used in tumour therapy. IL-12, IFN-β, and cytosine deaminase have been used as therapeutic agents in MSCs-mediated delivery [11]. They also hold extreme importance in the application of ischemic brain damage in the way that when intravenous administration of hUCB-MSCs was done it brought a reduction in the occurrence of the behavioural deficits [11]. It was reported by a group that it differentiated into neurones and astrocytes in and around endothelial cells [2]. Their results suggested that hUCB-MSCs could be involved in the clinical trials for ischemia. The applications of hUCB-MSCs for lung diseases can have a striking mechanism [11]. They work by immune modulation and differentiation potential as well. Once the hUCB-MSCs were administered it was seen that they were able to decrease the dead cells, myeloperoxidase activity and also the level of IL-6 mRNA. Strikingly, the increased level of TNF-α,TGF-β mRNA were decreased by the administration of hUCB-MSCs thus acting as a definitive and promising tool in the regeneration process[10,11].

6. CONCLUSION

MSCs are multipotent and nonhematopoietic progenitor cells which holds pivotal importance in the area of tissue regeneration. The fact that hUCB-MSCs holds much greater value than BM-MSCs emerges them out successfully. The differentiation of hUCB-MSCs into various lineages via Wnt signaling holds promising information which if investigated further can be beneficial in dealing with the respective immune modulatory functions.

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