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Osteogenic differentiation of mesenchymal stem cells from dental bud: Role of integrins and cadherins

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ABSTRACT

Several studies have reported the beneficial effects of mesenchymal stem cells (MSCs) in tissue repair and regeneration. New sources of stem cells in adult organisms are continuously emerging; dental tissues have been identified as a source of postnatal MSCs. Dental bud is the immature precursor of the tooth, is easy to access and we show in this study that it can yield a high number of cells with ≥95% expression of mesenchymal stemness makers and osteogenic capacity. Thus, these cells can be defined as Dental Bud Stem Cells (DBSCs) representing a promising source for bone regeneration of stomatognathic as well as other systems. Cell interactions with the extracellular matrix (ECM) and neighboring cells are critical for tissue morphogenesis and architecture; such interactions are mediated by integrins and cadherins respectively. We characterized DBSCs for the expression of these adhesion receptors and examined their pattern during osteogenic differentiation. Our data indicate that N-cadherin and cadherin-11 were expressed in undifferentiated DBSCs and their expression underwent changes during the osteogenic process (decreasing and increasing respectively), while expression of E-cadherin and P-cadherin was very low in DBSCs and did not change during the differentiation steps. Such expression pattern reflected the mesenchymal origin of DBSCs and confirmed their osteoblast-like features. On the other hand, osteogenic stimulation induced the upregulation of single subunits, αV, β3, α5, and the formation of integrin receptors α5β1 and αVβ3. DBSCs differentiation toward osteoblastic lineage was enhanced when cells were grown on fibronectin (FN), vitronectin (VTN), and osteopontin (OPN), ECM glycoproteins which contain an integrin-binding sequence, the RGD motif. In addition we established that integrin αVβ3 plays a crucial role during the commitment of MSCs to osteoblast lineage, whereas integrin α5β1 seems to be dispensable. These data suggest that functionalization of biomaterials with such ECM proteins would improve bone reconstruction therapies starting from dental stem cells.

1. Introduction

Numerous studies have reported beneficial effects of multipotent MSCs in tissue repair and regeneration (Marx and Harrell, 2014; Otte et al., 2013; Yamaguchi, 2014). These cells can be isolated from many different adult tissues, are self-renewable and can differentiate into all cell lineages that form mesenchymal and connective tissues (Yamaguchi, 2014; Barthes et al., 2014; Paschos and Brown, 2014).

The most well characterized source for MSCs is still the bone marrow, however in the past decade, populations of stem cells have been isolated from different dental tissues (Gronthos et al., 2000; Miura et al., 2003). A population of adult stem cells isolated from dental pulp (DP) tissues and designed as dental pulp stem cells (DPSCs), has been identified as a promising source of MSCs for tissue engineering (Gronthos et al., 2000; Daltoe et al., 2014; Mori et al., 2011; Mori et al., 2010; Spath et al., 2010; d'Aquino et al., 2009; Mangano et al., 2010; Galli et al., 2011; Mangano et al., 2011).

Abbreviations: MSCs, mesenchymal stem cells; DBSCs, dental bud stem cells; ECM, extracellular matrix; FN, fibronectin; VTN, vitronectin; OPN, osteopontin; BMMSCs, bone marrow MSCs; DP, dental pulp; DPSCs, dental pulp stem cells; DFSCs, dental follicle stem cells; ALP, alkaline phosphatase; ARS, Alizarin red staining.

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MSCs have also been identified in human Dental Follicle (DF) and Dental Bud (DB). DF is the loose connective tissue sac that surrounds the dental bud, which is the unerupted deciduous tooth. These tissues are more undifferentiated than mature Dental Pulp, thus dental bud is a more productive tissue of MSCs than mature DP and would have alternative applications in bone and periodontal tissue engineering (Huang et al., 2009; Shi et al., 2001).

DBSCs give rise to all the tissues present in the mature tooth: enamel, dentin, pulp, cement, and periodontal ligament. We show in the present paper that the central part of the bud, corresponding to the dental papilla, contains the MSCs which can differentiate into osteoblast-like cells. To obtain these postnatal stem cells, the extraction of a tooth before its eruption is required; however, the early removal (age 7–12) of the dental bud of third molars yields excellent stem cells. Furthermore, patients selected for the extraction are often subjects that will undergo dental crowding which is worsened by wisdom teeth eruption, thus they will not lose a functional tooth. Since the stemness and osteogenic commitment of DFSCs has been previously established (Mori et al., 2012), these cells represent an excellent source for tissue engineering therapy and we can speculate on their possible use for bone regeneration of stomatognathic and other systems. In order to obtain an efficacious reconstruction of bone tissue, stem cells must grow and differentiate in osteogenic conditions on biomaterial scaffolds to be subsequently implanted *in vivo*. However the new bone formation or the osteointegration with the resident bone tissue sometimes result to be ineffective due to a failed recruitment and adhesion of stem cells on the scaffold. To achieve the correct tissue architecture during morphogenesis, cells must interact each other and with ECM. These interactions are mediated by two classes of adhesion receptors, respectively cadherins and integrins (Weber et al., 2011; Chen and Gumbiner, 2006; Berrier and Yamada, 2007). Cadherins are single chain transmembrane glycoproteins that mediate cell–cell adhesion and interfere with intracellular signaling, in particular the Wnt/b-catenin pathway (Nelson and Nusse, 2004; MacDonald et al., 2009). They control proliferation, differentiation and survival of MSCs, which express low levels of multiple cadherins and their pattern changes as stem cells are committed to a differentiated cell lineage. Several *in vivo* and *in vitro* studies have established a main role of cadherins, in particular N-cadherin and cadherin-11, in osteoblastogenesis and bone formation either by controlling cell-cell adhesion or interacting with Wnt intracellular signaling (Schambony et al., 2004; Bienz, 2005; Brembeck et al., 2006). Expanding the knowledge on cadherin expression in MSCs of dental origin would be fundamental to promote the possible use of these cells in bone reconstruction therapies. Thus, as already demonstrated for DFSCs (Mori et al., 2012), the stemness and osteogenic commitment of DBSCs was confirmed. Further, these cells were characterized for the expression of cadherins and their pattern examined during osteogenic differentiation. To achieve the goal of bone regeneration and osteointegration, it would be also important to examine cell–matrix interaction mediated by integrins. These are a family of cell surface receptors that primarily mediate adhesion of cells to the ECM proteins. Integrins are heterodimeric transmembrane proteins, consisting of associated α and β subunits, with a large extracellular domain and a short cytoplasmic tail. Differently combined α and β subunits result in a variety of integrin heterodimers that can bind a specific repertoire of ECM proteins. Among others are fibronectin, laminin, collagens, tenascin, vitronectin, osteopontin, bone sialoprotein, and dentin matrix protein-1 (Docheva et al., 2007). Besides, integrins transmit crucial signals inside the cells. Adhesion of MSCs to ECM is still poorly characterized, as well as protein matrix role in regulating osteogenic differentiation. Thus we characterized our cell model of DBSCs for the expression of four integrin subunits and receptor heterodimers and then analyzed the effect of DBSC adhesion on three ECM glycoproteins containing the integrin-binding sequence RGD, namely FN, VTN, and OPN.

2. Results

2.1. Immunophenotype of DBSCs

DBSCs from 20 donors were tested for the criteria to be called MSCs (Dominici et al., 2006), as was done for DFSCs (Mori et al., 2012), in order to ensure that these cells had actually stem and mesenchymal feature. Flow cytometric analysis of research accepted MSC surface markers was performed and all populations exhibited ≥95% expression of CD73, CD90, and CD105 while were negative for CD45, a common leukocyte antigen. DBSC samples from three donors were selected to perform the experiments, since they showed higher and similar percentage values of stemness marker expression. Fig. 1 upper panel, shows flow cytometry histograms from one representative DBSC culture.

2.2. Cadherin expression profile in DBSCs reflected their mesenchymal origin

Expression of “classical” cadherins was investigated in undifferentiated DBSCs and during their osteogenic differentiation. The cells were kept in favoring conditions for MSC maintenance and were characterized for cadherin expression. Subcellular localization showed a high expression of N-cadherin, as well as for cadherin-11 (Fig. 1 A–B). E-cadherin was expressed to a lesser extent and P-cadherin was poorly expressed (Fig. 1 C–D). Notably, only N-cadherin was markedly localized at the cell–cell contact, perhaps contributing to maintain the stem cells niche in the dental bud.

Thus cadherin expression profile was evaluated during osteogenic differentiation. As already demonstrated for DFSCs, (Mori et al., 2012) DBSCs cultured under osteogenic conditions differentiated into osteoblast-like cells producing mineralized matrix nodules and expressed the typical osteoblastic markers Runx-2, alkaline phosphatase (ALP), and Collagen I (Coll I) (data not shown). Cadherin expression profile was analyzed by Western blotting during the different steps of osteogenic differentiation. As shown in Fig. 1 panel E, N-cadherin was expressed in undifferentiated DBSCs, thus confirming the subcellular observation; its expression remained constant after 7 days of osteogenic differentiation, but a slight decrease initiated after 14 days keeping a low expression of differentiation at 21 and 28 days. Similarly, cadherin-11 was expressed in undifferentiated DBSCs and its expression did not change after 7 days of differentiation, however after 2 weeks cadherin-11 strongly increased and remained highly expressed also at the later stages. Thus, while N-cadherin and cadherin-11 were present in undifferentiated DBSCs and their expression underwent changes during the osteogenic process, expression of E-cadherin and P-cadherin was low in DBSCs and did not change during the differentiation steps. Such expression profile of cadherins in DBSCs, and more precisely the changes undergoing cadherin-11 and N-cadherin (increasing and decreasing respectively), confirmed that these stem cells can acquire an osteoblastic phenotype under appropriate osteogenic conditions. The subcellular localization of cadherins was observed only during the early phases of osteogenic differentiation (3–7 days), since these cells usually reach the confluence in few days and grow in multilayers, thus preventing their microscopic observation. E-cadherin and P-cadherin were poorly expressed and homogenously distributed in DBSCs and their localization did not change after 3–7 days of osteogenic trigger (data not shown) as their expression until 28 days of differentiation (Fig. 1 panel E). Conversely N-cadherin and cadherin-11 changed their localization in response to osteogenic stimulation. In Fig. 1 (bis) F on time zero (T0) N-cadherin was localized at the cell–cell adhesion sites, being probably involved in the formation of adherens junction complexes as revealed by the “red bridges” merging with actin cytoskeleton marked by Phalloidin and resulting in an intense yellow staining. After 3 days N-cadherin remained localized in the adherens junction complex, while after 7 days N-cadherin lost the characteristic bridge-like