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Periodontal Ligament Stem Cells. A Literature Review on Its Properties

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ABSTRACT: Teeth represent a fascinating area of study in regenerative medicine, because of their unique and complex developmental origin. Several types of mesenchymal stem cells (MSC) have been characterized in the oral cavity, and those derived from the periodontal ligament (PDL) first isolated by our group in2005, can be expanded in a xeno- free medium preserving morphological features and markers associated with pluripotency. These postnatal MSC can be easily recovered by non invasive procedures and cultured. Thiscould facilitate the use of adult stem cells in human clinical regeneration therapy. In this review we summarize

the results of studies describing morphofunctional features, surface markers and multilineage differentiation capacity in vitro of PDL MSC obtained in our laboratories. In vivo characterization of PDLSC location and heterogeneity are still lacking. However we describe studies exploring the potential use of PDLSC to treat both periodontal diseases and regeneration of other tissues.

I. INTRODUCTION

"All cells come from cells" represent the paradigm trough which Rudolf Virchow (1821- 1902) postulates that all cells of the human body derive from a single cell and so starting regenerative medicine studies. Actually, the modern medicine, and in particular the regenerative medicine, focused its attention on adult stem cells, which are located in specific niches; they avoid the ethical and legal problems of earlier stem cells and they also can differentiate not only into their original source tissue, but also into cells of unrelated tissue. Stem cells derived from oral cavity are particularly interesting. It is well known that tooth development occurs through mutually inductive signalling between oral epithelium and ectomesenchyme. Cells originating from migrating neural crest cells represent a multipotent cell population derived from the lateral ridges of the neural plate during craniofacial development. PDL cells play an important role, not only in the maintenance of the periodontium but also in promoting periodontal regeneration. Recent studies have shown that proper manipulation of PDL cells is essential for tissue engineering. However, PDL cells are heterogeneous and include cells at different stages of differentiation and lineage commitment. PDL cells cultured at low densities exhibited replicative potential and formed colonies suggesting that PDL cells possess the ability both to proliferate and to produce colonies from a single cell.[1]

MORPHOLOGY AND STEM CELL- RELATED MARKER EXPRESSION OF PERIODONTAL LIGAMENT STEM CELLS.

Digirolamo et al. (1999)[2]showed that the replicative potential of the cells in culture was best predicted by a simple colony-forming assay when cells were plated at low densities, and the samples with the highest colony-forming efficiency also exhibited the greatest replicative potential. Seo et al. (2009)[3]reported that PDL stem cells (PDLSCs) are more proliferative than bone marrow derived MSCs . They showed that PDLSCs have a relatively low colony forming efficiency, whereas our study showed a much higher proliferation.

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This may be explained by the different method of cell culture, collagenase digestion or outgrowth methods.

Sakaguchi et al. (2004)[4] previously investigated the surface epitopes of bone marrow-derived MSCs. Although PDL cells are considered to be heterogeneous, it is still not clear how PDL cells express mesenchymal stem cell markers on their cell surface. FACS analysis of PDL cells demonstrated that the percentage positivity for CD34, CD45, CD117 (hematopoietic stem cell markers) and CD31 (endothelial cell marker) was less than 2%. This value was the same as in bone marrow-derived MSCs. These results indicate that the PDL cells were not contaminated by hematopoietic stem cells or endothelial cells. STRO-1 cells were originally identified as colony-forming osteogenic precursors isolated from bone marrow (Simmons PJ et al 1991)[5]

It has been reported that STRO-1-positive cells are present in human PDL tissues (Seo BM et al 2004)[3]In this study, they found that the STRO-1-positive/ALP-negative colony-forming efficiency decreased from 60% to 30% after 21 d of culture. On the other hand, the number of STRO-1-negative/ALP-positive colonies increased almost twofold during the same time-period. These observations suggest that the number of STRO-1-of an increase in ALP-positive colonies after 21 days of culture, and may be explained by the fact that the addition of ascorbic acid to the medium increases the ALP activity in PDL cells (Ishikawa S, et al 2004)[6], and STRO-1 expression disappears during maturation of the osteoblastic cell lineage . FACS analysis of PDL cells demonstrated that the expression of STRO-1 was very low and much less than bone marrow-derived MSCs.

However, the STRO-1-positive single-cell-derived colony-forming efficiency was 30%. This discrepancy may be explained by the different method of cell culture, colony-forming assay or cell-culture technique, and that the STRO-1-positive PDL cells possess high replicative potential and formed colonies readily in the colonyforming assay.

PDL cells have been shown to form mineralized matrix in the presence of ascorbic acid, dexamethasone, and β-glycerophosphate, which suggests that PDL cells possess osteogenic potential Mukai M, et al. (1993)[7] and Seo et al. (2004)[3]have shown that PDL cells have the potential to differentiate into other cell lineages, such as adipocytes and osteoblastic cells . The Alizarin-Red positive colony-forming efficiency was 27% in the presence of osteogenic differentiation medium. Furthermore, the Oil Red-O-positive colony-forming efficiency was 20% in the presence of adipogenic differentiation medium. The differentiation occurred primarily at the center of the colonies, and the few adipocytes that formed near the periphery migrated towards the center (Sekiya I et al 2004)[8]. It may be, for this reason,that the cells in the center of the colony shifted to a differentiation phase earlier than those in the periphery of the colony. Approximately 30% of 400 PDL cells possessed replicative potential and 30% of total colonies displayed multipotency.

Although it has been reported that MSCs in human bone marrow exist at a rate of approximately 1 per 100,000 cells, Sakaguchi et al. (2004) [4]showed that cultured MSCs possessed 27% colony forming efficiency, and 40–50% multipotential on young donors. This may be the result of a high adherence potential to the culture dish and a high proliferative potential of MSCs.

It is still not clear how many stem cells are present in PDL tissues; however, identification of cells that possess stem cell properties in the cultured PDL cell population, is definitely important for clinical application in the near future. As human bone marrow-derived MSCs transplantation is recognized as a possible option for treatment of periodontal defects (Kawaguchi H et al 2004)[9], these results indicate that human PDL cells will be a useful source for periodontal regenerative therapies. Further investigation will be necessary in order to clarify the relevance of the different stem cell properties in donors.

PDL, A NICHE OF NEURAL CREST STEM CELLS:

During embryogenesis, the periodontal ligament consists of cells derived from the dental follicle which are considered to be neural crest-derived ectomesenchymal cells (Ten Cate AR ;1997)[10]. The neural crest comprises a highly pluripotent cell population that migrates towards the first arch to participate in the formation of the teeth. The neural crest can differentiate into ectodermal and mesodermal cell types (Le Douarin NM et al 2004).[11]Using human periodontal ligament cell cultures, were able to identify, by immunocytochemistry, a small proportion of cells positive for HNK-1 and p75, suggesting the presence of undifferentiated cells with characteristics similar to those of neural crest cells. These markers have been used to identify neural crest stem cells derived from animal embryonic

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tissues and also from human adult tissue. Moreover, nestin-positive markers in periodontal ligament cultures by immunocytochemistry and RT-PCR techniques. Nestin corresponds to an intermediate filament protein expressed in neuroectodermal progenitors , including neural crest cells . The existence of a small population of neural crest-like cells (< 10%) that express nestin, HNK-1 or p75, possibly corresponding to a population of stem cells or multipotent progenitors residing in the human periodontal ligament.

Seo et al. 2004 [3] and Nagatomo et al. 2006[12] demonstrated that periodontal ligament stem cells produce mineralized nodules or Oil-red positive lipidic vacuoles when cultured in osteogenic-inductive or adipogenic inductive environments, respectively. Under appropriate culture conditions in the present study we observed the formation of calcium deposition after staining with the Von Kossa stain, and noted the presence of adipocite-like cells containing Oil-red-O stained vesicles, suggesting the potential of differentiation to osteoblast and adipocite, respectively. In addition, the presence of a smooth muscle actin positive cells, suggesting the capacity for myofibroblastic differentiation. Taken together, these results demonstrate that human periodontal ligament cells exhibit the capacity for differentiation into several mesenchymal lineages, in accordance with previous studies.

MULTIPOTENCY OF PDLSCs.

Recently, Techwattanawisal et al. 2007[13] and Widera et al. 2007[14]identified in periodontal ligament cultures of rats and human, respectively, multipotent adult cells capable of differentiating into neural and mesodermal progenitors, therefore suggesting that the periodontal ligament contains cells with neural crest characteristics. Moreover, cells with the potential for neural differentiation were identified in deciduous and permanent dental pulps (Gronthos S et al 2002)[15] cells derived from human periodontal ligament produced mesodermal and ectodermal phenotypes. They observed, at the mRNA level, positive gene expression for nestin, b-tubulin III, neurofilament M, microtubule-associated protein 2 and peripherin, suggesting neural differentiation. The expression of nestin and b-tubulin III was also confirmed at the protein level. In addition, the expression of both b-tubulin III and a-smooth muscle actin suggested the presence of bipotent or multipotent progenitors. Alternatively, this effect could be caused by the phenomenon of reprogramming or transdifferentiation_ of differentiated cells, such as myofibroblasts (a-smooth muscle actin-positive cells), because the neural crest derivatives display great plasticity in vitro (Real C, et al 2005)[16.]Moreover, the gene expression of protein zero, an earlier marker of glial cells (Hagedorn L 1999)[17].

Although the marker of differentiated glial cells (glial fibrillary acidic protein) was not detected and the periodontal ligament- derived cells also showed differentiation potential for the glial phenotype. It is important to emphasize that peripherin is an intermediate filament protein associated with peripheral neurons that are neural crest derived (Parysek LM et al 1988)[18]. These results corroborate the findings of Techawattanawisal et al. and Widera et al. obtained with rat and human periodontal ligament, respectively, suggesting that human periodontal ligament may be a source of stem cells or of multipotent progenitors with neural crest characteristics. Presence of early markers of neural crest cells (HNK-1, p75 and nestin) as well as markers of differentiated ectodermal cells (neurons and glial cells) and mesodermal cells (myofibroblasts, osteoblasts and adipocytes). In addition,also observed cells with markers for both neuronal-specific (b-tubulin III) and myofibroblast-specific (a-smooth muscle actin) proteins. These results suggest that periodontal ligament cultures contain a heterogeneous population of cells with different level of maturity, including a small population of multipotent progenitors.

PDLSCS IN REGENERATIVE MEDICINE

Production of culture medium of PDLS Cs from MS patients under hypoxic conditions induced a potent modulation of oxidative stress, autophagic and apoptotic markers when injected in mice where EAE was induced expression, and i ncreased expression of γ[19], with reduction of IL - 17 and Interferon- beclin- 1 and LC3, princip al markers of autophagy. This was correlated w ith a strong increase in the content of the anti - inflammatory cytokine IL- 37, a member of th e IL- 1 family.[19] PDLSC conditioned medium inc reased the functionality of the PI3K/Akt/mTOR ax is, and also reduced inflammation and oxidative stress in injured NSC- 34 neurons, restoring B DNF production.[20] Moreover, CM was revealed to contain NT3, and IL- 10, suggesting a neuro protective effect as the result of the presence o f growth factors and immunomodulatory cytokin es. Since MS is one of the prominent disorders wh

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ere clinical trials with MSCs are being conducted[21]t hese novel findings may indicate the possible ben eficial effects of treatment with PDLSCs or the ir secretome, which may represent a great adva ncement for safety and availability of such therapeu tics. The secretome of MSCs acts as master reg ulator of the so- called neurogenic niche[22]a reserv oir of regenerating neuronal cells with a great t herapeutic potential. Experimental autoimmune e ncephalomyelitis (EAE), the animal model of m ultiple sclerosis (MS), has focused the attention of our group, since injection of PDLSCs at th e beginning of the disease (i.e. 14 days after i mmunization with myelin peptides) decreased si gns of inflammation and demyelination in the s pinal cord of the animals, both through the inc reased production of neurotrophic factors and t he suppression of inflammatory mediators.[23] Th e conditioned medium of the same cells were able to reduce inflammatory damage in the sa me model and purified extracellular vescicles from PDLSCs obt ained a similar effect. The vescicles were found to contain anti- inflammatory stained positive for surface mesenchymal antigens CD90 and ,β cytokines IL- 10 and TGF- CD29, and were a mi xed population of exosomes and shedding vescicles . The same result was produced by injecting con ditioned medium or vescicles obtained from pat ients suffering from relapsing- remitting MS.[24]

This was proof of a pathology- independent fu nctional niche of PDLSCs, which may be of i mportance for several other neurodegenerative a nd inflammatory diseases.

II. CONCLUSION

Restoration of tissues destroyed by periodontitis to their original form and function has been a longstanding goal of periodontal therapy. However, our current available regenerative therapies are crude and of poor clinical predictability. There is need for novel regenerative technologies to be developed based on contemporary understanding. In order for this to become a reality it will be necessary for us to obtain a complete understanding of periodontal development and the progenitor cells involved in this process. Subsequent tissue-engineering approaches may then be developed using these progenitor cells within a matrix scaffold, together with the introduction of various signaling molecules in an orderly temporal and spatial sequence. Studies on both embryonic cells and adult stem cells should continue as part of a collective effort to expand our knowledge on how cells function and what fails in the disease process. It is this combined and solid knowledge base that will underpin future treatment modalities and ultimately make stem cell-based tissue engineering and gene therapy a realistic alternative in periodontal regeneration.

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