Doctoral Dissertation
Doctoral Program in Bioengineering and Medical-Surgical Sciences (36th Cycle)

The impact of biological aging on periodontal ligament-derived stem cells and its implications for clinical periodontal regeneration: foundation for a translational approach.

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Summary

Periodontal regeneration is the most desirable clinical outcome in the management of intraosseous and furcation periodontal defects. The aim is the complete restoration of the lost periodontal anatomy, i.e. the formation of new cementum, periodontal ligament (PDL) and alveolar proper bone. However, surgical techniques and biomaterials currently used limited the efficacy of periodontal regeneration. Cell therapy using autologous mesenchymal stem cells from the PDL (PDLSCs) may emerge as a solution to restore the architecture of the original periodontium due to their plasticity and ability to differentiate into osteo/cementoblast and periodontal ligament cell lines. However, a thorough characterization of these cell populations in patients affected by periodontitis is lacking, as well as understanding the impact of inflammatory-driven senescence and epigenetic aging on their regeneration potential.

This study aimed to conduct a comparative analysis between PDLSCs from healthy individuals (hPDLSCs) and those with periodontitis (pPDLSCs) to identify potential functional disparities attributed to their respective origins. The assessment encompassed colony-forming unit efficiency, multilineage differentiation capacity, immunophenotype, stemness, and senescence status, which were examined through flow cytometry, immunofluorescence, and β-galactosidase staining. Gene expression profiles were determined using RT-PCR. Moreover, a clinical transability of the findings was attempted, by assessing whether the presence of active inflammation and the expression of the senescence-associated secretory phenotype (SASP) in the gingival crevicular fluid (GCF) influenced the early wound healing (EHI) and the one-year outcomes of periodontal regeneration.

Both hPDLSCs and pPDLSCs exhibited similarities in their immunophenotype and their ability to differentiate into multiple lineages. However, pPDLSCs demonstrated a higher frequency of a senescent phenotype, expressing significantly

more p16 and p21 genes and, contemporary, more stemness genes such as OCT4. Moreover, pPDLSCs showed a higher expression pattern of P2X7R, a novel pro-inflammatory molecule involved in the purinergic signalling pathways. To investigate whether this senescent phenotype could be reverted, we tested the effect of an investigational small molecule inhibitor targeting DNA methyltransferase, known as RG108. RG108 application did not impact the proliferation and apoptosis of PDLSCs and had a non-significant effect on hPDLSCs. In contrast, a notable reduction in p16 and p21 expression was observed in pPDLSCs following treatment with 100μM RG108, together with an elevation in SOX2 and OCT4. Furthermore, the subset of PDLSCs co-expressing OCT4 and p21 diminished, and the adipogenic potential increased in pPDLSCs after RG108 treatment. Regarding the clinical findings, the presence of active inflammation (bleeding on probing) was a negative predictor for EHI at 2 weeks after surgery, as well as for the achievement of clinical success at one year. At the same time, a higher expression of SASP factors in the GCF (IL-1β, IL-6, MMP-8 and MMP-9) positively correlated with EHI, as well as with final probing pocket depth and clinical attachment level, suggesting a potential effect of inflammation and senescence on the attainment of periodontal regeneration.

In summary, pPDLSCs displayed a differential phenotypic and functional behavior compared to hPDLSCs, presenting a higher degree of cellular senescence. This phenotype was partially reversed through RG108. Clinically, among the other important parameters affecting the clinical outcomes of periodontal regeneration, SASP expression level represented a prognostic factor which deserves further investigation. The importance of addressing cellular senescence and biological aging to enhance the success of periodontal regeneration may broaden new horizons for future research and therapeutic applications.