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Neurogenic Potential of Human Dental Stem Cells

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ABSTRACT

Presently, both central and peripheral nervous system medicine is limited in the sources of stem/progenitor cells for regeneration/repair of damaged neurons. Classical animal studies have shown that dental soft tissue cells are derived from the ectoderm - neuroepithelium - neural crest - (ecto) mesenchymal craniofacial lineages, hence possessing a dormant neural identity. No such data was possible to obtain for humans until recent times. Here we review recent independent discoveries showing that adult dental pulp cells can express, under appropriate culture conditions, genes that (a) determine neural crest identity and (b) synthesize tau protein, conferring jointly on mesenchymal dental cell precursors a neuronal fate that has rarely been observed as their propensity to transformation into neurons; this potential could become a major source of new neurons for regenerative medicine in both the peripheral and central nervous systems.

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Neurons from Dental Cells

The Lack of Natural Neural Precursors Limits Regenerative Therapies for the Central and Peripheral Nervous System

The human nervous system is limited by neural progenitors necessary for the regeneration 42 and repair of damaged neurons and glia [1-3]. Despite the merits of natural neural stem cells (NSCs), the difficulty of isolating them from the brain and propagating them in vitro has hampered progress in their therapeutic use. Resident NSCs in the CNS are very few in number and lose their stemness with age, which reduces their potential for physiological neurodegeneration; they are located in hard-to-reach areas of the central nervous system of an adult, the biopsies for obtaining them are very small (1-2 ml), their postoperative enzymatic treatment affects the survival of NSCs, which, in addition, have a limited ability to proliferate in suspension and aggregate to form neurospheres the required homogeneity [4-6].

Dental-Derived Stem Cells Promise to Solve Problems in Nervous System Regenerative Medicine

Mesenchymal stem cells obtained from bone marrow, umbilical cord, placenta, and amniotic fluid are free of these disadvantages and, being multipotent (sometimes believed to reach the level of pluripotency [7]), promise to solve problems in human therapy [8]. However, despite encouraging preclinical results in various animal models of human diseases, the results of clinical trials have not yet confirmed their practical value for humans (eg [9]). This is largely due to the unfounded conventional wisdom in current biomedical science that any of these multipotent stem cells of different tissue origin can produce any cells for regenerative medicine. The shortcomings of this literature -expanded paradigm can only be seen in rigorous studies aimed at obtaining valuable information about the subtle epigenetic and stemness differences between stem cells of these structurally and physiologically distinct tissues. One important aspect of this problem is that although all of these stem cells can produce cells of mesodermal origin (skeletal bone, cartilage, muscle, fat), only stem cells from oral tissues are able to differentiate into both, mesodermal, predominantly bone cells, and also produce nerve cells, including cholinergic and dopaminergic neurons, applicable in the neuropathological field of regenerative medicine [10-20].

This is because the cells of the oral cavity (craniofacial cells in general) are endowed with the epigenetic identity of the ectodermal-neuroepithelial neural crest lineage, which is complemented by a secondarily occurring epithelial-mesenchymal transition at embryonic stages, ensuring the stemness of mesodermal -type cells. Therefore, the main problem to be solved is to make maximum use of this dual stemness, whose neural stemness lies dormant in vivo but can be activated in vitro under adequate conditions for research and medical use.

During Embryonic Development, the Neural Crest and its Derived Mesenchymal Regulators Endow Dental Stem Cell Progenitors with Dual (Neural Crest/Mesenchymal) Identity and Stemness of Neural/Bone Differentiation

The emergence of the neural crest during vertebrate evolution as a new autonomous source of stem cells (sometimes called the 4th germ layer) allowed the creation of additional structures, including craniofacial organs [21]. Classic embryological studies of the neural crest carried out in the last century by a French academic group, as well as more recent discoveries of its regulatory systems,

uncovered the main processes occurring at these stages [22-25]. Neural crest takes its origin in the dorsal aspect of neuroepithelium border as an array of lineages [26,27]. The basal gene regulatory "recipe" and the Sox transcription factor genes specify the neural crest segregation and lineage-specification of border derivative stem cells toward a huge number of structures [25, 28, 29]. Some of these cranial aspect cells undergo epithelial-mesenchymal transition, which makes them dual (epithelial/mesenchymal) stem ness cell lineages that migrate to sites of development of craniofacial tissues and the pharyngeal skeleton, including tooth buds [30-32]. Fine tracing techniques have made it possible to trace in animal models the migration of these human homologs to the first branchial arch, the initiation of tooth germs in contact with local mesodermal mesenchyme and the regulation of their subsequent morphogenesis [33,34]. In cultured mice and rat development the authors observed the contribution of early-emigrating posterior midbrain crest cells to mandibular molar tooth development [35, 36]. The presence of this process in human embryogenesis is evidenced by the detection of neural crest -specific markers, such as AP2, Sox10, p75, HNK1, in (i) early human embryos, in (ii) neural crest cells obtained in vitro from human pluripotent stem cells and (iii) in human dental stem cells [37-40].

The above results, along with the similarity in the structure of the dentition of mammals and humans, allow to extrapolate animal data to humans, although experimental evidence has not yet been obtained [41,42].

The following three questions of interest are still awaiting answers to prove the connection between neural crest and dental soft tissue: (1) dental stem cells do not exhibit neural crest markers and neuronal features in vivo, and exclusively exhibit a mesenchymal phenotype. and stemness; (2) dental stem cells do not produce neurons in vivo, and (3) the neuronal identity of cells with neuronal phenotype derived from dental stem cells to date has not been rigorously demonstrated. We recently decided to find out information about the state of neural crest-specific genes expressed in human embryos in mesenchymal cells of adult teeth [37].

Induction of Neural Crest-Specific Gene Expression in Human Adult Dental Cells

Addressing this issue, our group was able to identify neural crest markers such as Sox10 in SHED pulp [39] and in periodontal ligament [40] stem cells cultured in a medium meeting the following molecular requirements of neural crest: exclusion of fetal serum (or inhibition of Tgfbeta, a major inducer of EMT) and stimulation (eg with BIO) of the Wnt/beta-catenin pathway (a major inducer of MET and neural crest marker expression) [38-40, 43-45]. The above observations led us to the concept that the alternation of mesenchymal (bone-targeting) and epithelial (neural -targeting) epigenetic states requires the following two contrasting conditions: (1) Wnt/beta-catenin^{high} / Tgf-beta^{low} induces MET and neural stemness, while Wnt/beta-catenin^{low}/ / Tgf-beta^{high} induces EMT and mesenchymal epigenetic stemness.

In early embryogenesis, the initial neuroepithelial-neural crest lineages (including those that later will be the precursors of dental tissues), have neuronal identity/stemness maintained by the Wnt^{high}/Tgf/beta$^{low/no}$ state (see above) and are programmed to develop the autonomic nervous system [46]. Some lineages undergo partial EMT under the influence of elevated levels of Tgf/beta sufficient for the cells to individualize and become migratory. Tgf/beta levels increase, causing the cells to complete EMT and increase their mesenchymal stemness, ultimately acquiring the dual (neural/bone) stemness previously observed in corresponding animal neural crest cells (see [47]).

In migrating tooth precursors with such dual stemness, neural identity/stemness is suppressed by EMT factors, which leads to the establishment of mesenchymal cell development in dental tissues. The neural crest identity signature and neural stemness in these cells never become active in vivo but are akin for activation in vitro under the Wnt^{high}, Tgf/beta^{low} condition inducing MET to unveil their innate peripheral neural system destination. The concept presumes that this MET reverts the cells to their true, neural crest cell's epigenetic state.

The important assumption from these manifestations of dental cells is that dental cells have primarily neural crest-specific neural identity and neurogenic stemness, which is "sacrificed" for their being conferred a secondary, mesenchymal, stemness and developing craniofacial (including teeth) structures. Thus, craniofacial bone, muscle and fat cells stemness has embryologically ectodermal-neural crest identity unlike the rest of the skeletal cells that have mesodermal identity.

In the ontogenesis of teeth, there are not one, but two parallel stem cell lineages, the initial, leading to the formation of SHED, provisional rootless milk teeth of children, and the following it lineage of permanent teeth. Each of these lineages dominates one of the dual cells' stemness: deciduous tooth stem cells have higher mesenchymal stemness, while permanent tooth stem cells show higher neural stemness [48-51]. These differences must be considered when planning their regenerative/therapeutic applications [51].

In this regard, supernumerary teeth, known in odontological practice as hyperdontia, which currently serve to study the mechanisms of embryopathogenic phenomena in the dental system, can become an additional source of SHED stem cells. A case of supernumerary tooth grown in one of the female newborn twins is a subject of our ongoing study which has shown that the proliferation-differentiation properties of the pulp derived cells were indistinguishable from those in normal SHED except for an enhanced osteogenesis. Remarkably, Tatullo's group described a case of non-symptomatic multiple bilateral hyperdontia with normal karyotype [52].

The Neural Regeneration/Restoration Potential of Human Dental Stem Cells Occurring Through Paracrine Mechanisms

Recent progress in understanding the dual stemness of dental stem cells extends their applicability not only to diseases of mesoderm-derived tissues such as skeletal bone/muscle/fat but also to ectodermal tissues such as cranial, oral and neural tissues cells [8]. These advantages have become the basis for the significant and intensive contribution of several collaborative groups, raising at a strategic level the research of the potential of these stem cells in regenerative medicine in general and directly in the treatment of tissues of the maxillofacial region and in dentistry in particular [53]. effectively revolutionizing old classic dental clinical approaches with innovative, powerful methodologies. This is facilitated by new discoveries in the field of paracrine mechanisms of the therapeutic action of stem cells, which are gradually replacing previous ideas that the therapeutic effect of stem cells lies in their differentiation into cells that replace local diseased cells. Research is currently focusing on molecules that stem cells produce as secretomes with soluble and exocellular bioactive molecules integrated into vesicles, such as nano-sized

exosomes (30–100 nm), phospholipid macromolecules containing cytokines and microRNAs released in target tissues and having specific effects [54]. Compelling data from these studies indicate that future regenerative therapies may be based on these cell-free principles. To confirm the possibility of such treatment, the presence in the conditioned medium of human bone marrow MSCs of a package of physiologically active effectors- insulin-like growth factor-1, VEGF, Tgf -beta, interleukins 3 and 6, and hepatocyte growth factor. and monocyte chemoattractant proteins -1 was shown (e.g. [55]). These and other bioactive factors secreted by stem cells contribute to specific immunomodulatory and various therapeutic effects. Their discovery and characterization are an important step of modern science towards a better understanding of the important role of the intracellular (at the level of tissues and organs) regulatory network, in which paracrine and cell-replacement modes of intercellular interaction can lead to physiological regeneration and alleviation of pathological conditions.

Additionally, scaffold materials combined with MSC-EVs improved bone regeneration in vivo [56]. In this aspect of regenerative medicine promoters, the dual stemness of dental stem cells is an outstanding advantage, and although their primary neural crest-endowed neurogenesis is suppressed in vivo in dental stem cells, this potential can be unlocked in vitro and develop ed into various types of nerve cells [12, 13, 22, 46]. Notably, dental stem cells were among the first to have a known paracrine mechanism for delivering neurotrophic, neuroprotective and neuroregenerative factors to maintain trigeminal nerve integrity [57, 58]. Currently, knowledge of this potential of stem cells is becoming a direction for studying the secretomes they produce in the processes of tissue regeneration/repair. Owing to their dual stemness, dental cells secrete two classes of molecules with physiological regenerative activity: (1) molecules for differentiation/functioning/restoration of dentin and other oral structures of ectomesenchymal origin and (2) molecules for trophism/protection/regeneration of neurons [59, 60]. Molecules of both types can be found in the conditioned medium of MSCs obtained from teeth, capable of influencing and improving regenerative processes. The secretome obtained from teeth showed stronger neuroregenerative and neuroprotective effects compared to the secretome obtained from other MSC sources. According to a meta-analysis of 15 studies, seven reported the therapeutic benefit of the conditioned medium on neurological diseases and three reported on joint/bone-related defects [61]. For these reasons, secretomes from dental MSCs may represent the most promising approach for the treatment of neurodegenerative diseases, nerve damage, and bone repair (review [62]). Thus, this dual stemness with respect to the mesodermal (bone) and ectodermal (neural) lineages makes dental stem cells an outstanding tool in regenerative medicine.

Vi. Tau Appears in The Embryonic Stages of Specification by Neural Crest of Mesenchymal Progenitors of Dental Stem Cells to Complement their Neuronal Fate

Considering the above, we investigated the presence of tau in both stem cells of primary teeth (SHED) and stem cells of permanent teeth (in preparation). In both lineages, tau was present in significant quantities [63]. As a specific goal of the present study, we reprogrammed cells from one of the third molars and established a neural crest and mesenchymal embryonic developmental stages, of which only the latter stage cells contained tau protein. We expect that we have established a single tooth-specific epigenetic lineage in which the same tau was present in the adult and in its past embryonic stage.

Tau has not yet been detected in human dental stem cells (e.g. [64-68]) or in neurons reported to develop from dental cells [12,13]. Authors of publications in this area have not appreciated the importance of tau in neurogenesis and axonal growth. In some of them, MAP2 was found, which, along with tau, is required for the stability and dynamics of the microtubule cytoskeleton [69-71]. However, MAP2 alone is not enough, since it is tau that is involved in structural polarization and in the organization of the axonal microtubule delivery system for the flow of cellular organelles and molecules [72]. MAP2 function is limited to cell body microtubules and dendrites [73]. The demonstration of the presence of tau in dental stem cells is, in our opinion, the first convincing evidence of their endowment with a neuronal fate, thereby confirming the possibility of obtaining neurons from dental stem cells Figure 1 schematically presents the available information, including our data, on tau during the in vivo development of human embryonic stem cells (hESCs) and the in vitro development of hESCs/iPSCs into dental cells; the established and putative stages of tau gene expression along with neural crest and mesenchymal stem cell identity genes, are indicated. In humans, neural crest stem cells were discovered in the early embryonic stages C12 – C20, but the cells expressing them could not then be traced until adulthood, when the cells produced from these precursors express mesenchymal markers in developed teeth; notably, as indicated above, neural crest markers can be detected in these neural crest derived tooth mesenchymal cells under appropriate MET-inducing culture conditions [37, 39, 40].

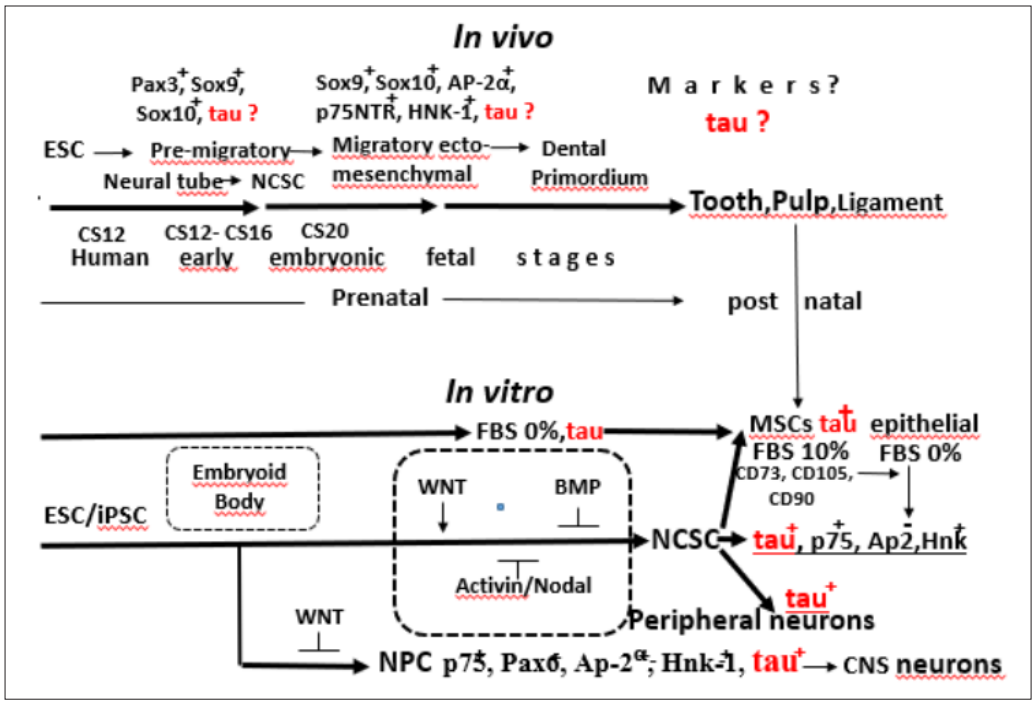

Figure 1: Scheme of cumulative data on the development of human pluripotent stem cells (ESC, iPSC) in vivo and in vitro towards adult tooth stem cells

In vivo differentiation of ESCs into adult dental stem cells demonstrates the expression of neural crest identity genes on early embryonic C12-C20 stages, which, as shown on animal models, but not in humans, specifies these cells as progenitors of mesenchymal stem cells of adult teeth.

It is not known whether tau gene expression occurs in vivo [37].

In vitro, ESC/iPSCs' differentiation to cells of CNS and neural crest lineages, the latter ones converting into mesenchymal cells of adult teeth; the possibility of unveiling of neural crest genes' expression is shown to complement the here shown expression of tau in embryonic and in adult tooth mesenchymal cells. NPC-neural progenitor cells [33, 39, 40].

Tau in Dental Stem Cells Exhibited Some of the Features Known for Alzheimer's Tauopathy

In an ongoing study we explore a model of reprogramming of tau-containing dental cells to iPSCs and their differentiation to dental cell progenitors with reactivated tau expression to reconstruct the whole ontology of tau in human dental pulp stem cells as potential sources of neurons for research and clinical application. At the same time, we raised the possibility that tau in dental cells may be useful for better understanding the role of tau in neurodegenerative tauopathies. To our knowledge, crucial information about the involvement of tau in neurodegeneration comes mainly from biopsy material from fixed postmortem brains in advanced stages of both the disease and the transformation of tau into neurofibrillary tangles, NFTs [74]. The lack of sufficient information about the initial stages of transformation of normal tau into NFT to show the dependence of pathogenesis on this event makes the current view on tauopathies elusive. The use of 12 anti-tau antibodies highlighted tau epitopes and thus the presence of tau in stem cells of primary teeth (SHED) and compared these results with the tau of postmortem AD brain samples [63]. Eleven antibodies bound to epitopes both normal tau in dental cells and to modified tau (NFT) in Alzheimer's disease brain samples, demonstrating that the regions of tau where the epitopes are located are conserved in normal (dental) and in diseased (brain) cells. The epitope of the 12th antibody, AT100, bound NFT of Alzheimer's disease brains but did not bind tau of normal dental cells, and since this antibody is known as a major diagnostic tool for the disease, its failure to bind to normal tau is taken as evidence that its epitope is not formed in normal cells, but is formed in aggregated NFT tau cells, in accordance with previously described process [75]. As an additional property linked to neurodegeneration, dental cells were shown to contain the amyloid precursor (APP) and GSK3β (68) kinase (see [63]) associated with the tau pathological modifications.

Conclusion

The properties of human dental cells described here, combined with additional data from other studies, including the easy availability from discarded teeth, potential for long-term in vitro proliferation, expression of the neural crest identity and of tau protein genes, make them the most effective source of neurons. for regenerative medicine of the nervous system.

Conflict of Interest

The authors declare no conflict of interest.

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Authors Contributions

Conceptualization: **KG**; Bibliography Administration: **KG**; Funding Acquisition: **KG**, Writing – Original Draft and Writing, Review & Editing: **KG, SLMO**

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