Stem Cell Expression of Odontogenic Tumors and Cysts

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Contributor: Eleni-Marina Kalogirou , Georgios Lekakis , Aristodimos Petroulias , Konstantinos Chavdoulas , Vasileios L. Zogopoulos , Ioannis Michalopoulos , Konstantinos I. Tosios

Stem cells have been associated with self-renewing and plasticity and have been investigated in various odontogenic lesions in association with their pathogenesis and biological behavior. Stem cells might be linked to the pathogenesis and clinical behavior of odontogenic pathologies and represent a potential target for future individualized therapies.

odontogenic tumor odontogenic cyst stem cell

1. Introduction

Odontogenic tumors and cysts are a diverse group of lesions that have in common their origin from cells participating in the normal process of tooth formation or odontogenesis. Benign and malignant odontogenic tumors are rare, representing less than 1% of oral tumors [1], and present a wide variety of clinical behavior and histopathological features. Some of them behave in a destructive manner, while others are slow-growing and may be even accidentally discovered during the microscopic examination of the follicular tissue surrounding an unerupted third molar (dental follicle, DF) [2].

The most common benign odontogenic tumor is ameloblastoma (AMBL), a locally infiltrative neoplasm with a high recurrence rate that may occasionally undergo malignant transformation [1]. AMBL often necessitates wide surgical excision, associated with high morbidity and necessitating extensive reconstructive surgery; thus, pharmaceutical-based management is a challenging treatment goal [3]. In contrast to AMBL, adenomatoid odontogenic tumor (AOT), another benign odontogenic tumor, has limited growth potential and significantly lower recurrence rate [1].

Odontogenic cysts are of developmental and inflammatory origin. The most common developmental odontogenic cyst is the dentigerous cyst (DC), which is associated with the crown of unerupted teeth, grows slowly, and rarely recurs [1][4]. Odontogenic keratocyst (OKC) is a developmental odontogenic cyst with great research and clinical interest, due to its high frequency and aggressive behavior, i.e., growth potential within jaw bones and high recurrence rate, as well as its occurrence as a manifestation of Gorlin–Goltz syndrome [1]. Radicular cyst (RC) is the most common among all odontogenic cysts and develops within the jawbones as a sequel of dental pulp necrosis [1][4].

The odontogenic tissues develop through constant epithelial–mesenchymal interactions, where stem cells play a pivotal role [5]. Odontogenic tumors and cysts purportedly arise from cells of the odontogenic tissues and their developmental remnants, such as dental lamina and epithelial rests of Malassez, where stem cells have been shown to exist [6][7]. Stem cells have been associated with self-renewing and plasticity, thus contributing to different organs' formation and regeneration [5], while, in cases of disturbed and uncontrolled proliferation, stem cells might promote tumorigenesis [8]. Moreover, based on their ability to differentiate various specialized subpopulations, stem cells have been linked to morphological heterogeneity and diverse biological behavior [9], features that characterize odontogenic pathologies.

Previous studies have focused on the expression of a limited number of stem cell markers in odontogenic tumors and cysts [10][11][12][13][14]; however, the complete stem cell gene expression portrait of odontogenic lesions remains elusive. Understanding the role of stem cells in odontogenic lesions can contribute to the development of personalized study models ("disease-in-a-dish" models), which are of great importance for the study of the pathogenesis of rare diseases [15]; identification of stem cell genes that might be targeted for personalized molecular treatments [16], in particular for the management of large osteolytic lesions; and the utilization of stem cells in regenerative techniques in the oral and maxillofacial region [17].

2. Stem Cell Expression Profile of Odontogenic Tumors and Cysts

Most relevant studies investigated AMBL, OKC, DC, and RC, the first two apparently due to their aggressive biological behavior [1], and the latter two probably due to the wide availability of tissue, as they are the most common odontogenic cysts [4]. In contrast, only a few studies included samples of malignant odontogenic tumors, profoundly due to the rarity of such lesions [1]. In most studies, the expression of stem cell markers was documented via immunohistochemistry and/or immunofluorescence. Although by modern molecular techniques, the expression of tens to hundreds of thousands of genes may be investigated in parallel [18], immunohistochemistry remains the gold-standard for the detection of the tissue-specific expression of proteins and their precise subcellular localization [19]. The latter may be important for unveiling their function, e.g., in case of transcription factors, where nucleo-cytoplasmic shuttling significantly influences their activity [20].

The SOX2 protein is encoded by the *SOX2* (SRY-box transcription factor 2) gene that is expressed in ESCs and adult tissue stem cells, and exerts an important role in the development of tissues of ectodermal origin, including the odontogenic epithelium [6][21]. SOX2 is also one of the main four "Yamanaka factors", i.e., transcription factors whose exogenous administration to differentiated somatic cells can induce their reprogramming into induced pluripotent stem cells (iPSCs), through the process of cellular reprogramming [22]. In addition to its role in the normal development and homeostasis of the covering mucosal epithelium, SOX2 participates in tumorigenesis, affecting the proliferation, apoptosis, and cell differentiation of malignant neoplasms originating from various tissues, such as oral and skin squamous cell carcinomas [23]. According to the meta-analysis, SOX2 has a remarkable ability in identifying cases of ACA over AMBL, its benign counterpart. In ACA, strong nuclear SOX2 expression was observed in areas with prominent cytological atypia and loss of the classical ameloblastic

morphology, whereas the few positive AMLB cases showed weak, focal SOX2 staining in peripheral or central cells of epithelial islands and strands [12][24][25]. Those findings suggest that SOX2 immunostaining could facilitate the diagnosis of ambiguous cases of ACA and reveal malignant transformation in AMBL [25].

Furthermore, the quantitative analysis performed in the present study indicated that SOX2 expression between OKC and AMBL is different. This finding is significant as it could be associated with differences in the pathogenesis of those lesions, while diagnostically it could be utilized in the identification of ameloblastic transformation in OKC. Previous studies have shown nuclear expression of SOX2 [6] in the dental lamina, focally in the dental lamina rests included in DFs [26][27], and cytoplasmic expression of SOX2 in ameloblasts, odontoblasts, and inner enamel epithelium cells of human fetuses at the bell stage of odontogenesis [28]. In OKC, SOX2 positivity was stronger and more diffuse in the intermediate epithelial layers [26][29][30], composed of cells with squamous differentiation, compared to the basal layer composed of cells with a preameloblast-like cellular phenotype [31]. Taken together, these findings agree with a theory based on the comparative transcriptomics analysis between OKC and AMBL [30], suggesting that OKC may develop from cells arrested at the dental lamina or bud stage, while AMBL progenitor cells may be more differentiated and may have reached the bell stage of odontogenesis [32].

The expression of OCT4 (Octamer-binding transcription factor 4), a member of the Pit-Oct-Unc (POU) family of transcription factors, encoded by the *POU5F1* gene, is evaluated in multiple studies; however, divergent results were found, even between studies using anti-OCT4 antibodies from the same vendor [10][33]. However, further evaluation shows that four studies reporting no or limited OCT4 expression in benign tumors and developmental cysts used monoclonal antibodies [10][12][32][34], while one study showing OCT4 positivity applied a polyclonal antibody [33] and the relevant information was not available in another study [14]. In studies including both AMBL and OKC samples, the number of OCT4 positive cases was the same [33] or slightly higher in the OKC group [32], and the meta-analysis showed that OCT4 cannot be applied for distinguishing between OKC and AMBL. Interestingly, a study employing a monoclonal anti-OCT4 antibody found strong nuclear staining in the epithelial islands in 85% of ACA cases, while all AMBL samples were negative [12]. OCT4 has a vital role in the maintenance of self-renewal and pluripotency of ESCs [35], and regulates cell fate decisions by conducting an autonomous, but also synergistic, action with SOX2 [36]. OCT4 is another “Yamanaka factor”, crucial for the establishment of iPSCs [22], and has been implicated in the initiation and progression of several malignant tumors [37].

Of note, except for SOX2 and OCT4, the other two “Yamanaka factors” [22], i.e., KLF4 (Krüppel-like factor 4) and c-Myc, were also found to be expressed in some odontogenic lesions [26][38]. KLF4 is implicated in various cellular processes, including cell proliferation, apoptosis, and differentiation, and promotes terminal epidermal differentiation by inducing the expression of epithelial molecules and suppressing the expression of mesenchymal molecules [39][40]. RNA-seq analysis showed that the *KLF4* gene was upregulated in OKC compared to DF, and immunohistochemistry showed strong nuclear expression of the KLF4 protein, mainly in intermediate layers, and focally weak expression in the basal layer of OKC epithelia [26]. c-Myc is a proto-oncoprotein that acts as a transcription factor involved in cellular proliferation, apoptosis, and inhibition of differentiation [41]. Immunohistochemical expression of c-Myc was observed in the epithelial cells of most AMBL, AOT, and OKC cases, and in half or less than half of cases of RC and DC, respectively [38]. It is worth mentioning that among all

odontogenic pathologies, OKC is the only one documented to express all four “Yamanaka factors”. As the endogenous expression of these factors has been linked to a higher intrinsic potential of somatic cells for cellular reprogramming [42][43], further research is warranted to elucidate whether cells isolated from OKC samples are amenable to reprogramming.

The expression of CD44, a surface glycoprotein encoded by the *CD44* gene, was also investigated in many studies. CD44 acts as a core receptor for hyaluronic acid, regulating cell adhesion to extracellular matrix elements [44], and participates in the organization of a microenvironment conducive to the proliferation and stemness of tumor cells [45]. Strong membranous CD44 expression was observed in several odontogenic lesions, including AMBL, OKC, DC, and RC [11][12][33][46][47][48][49].

It is suggested that stem cells may be linked to the development and clinical behavior of odontogenic pathologies and represent a potential target for future individualized therapeutic approaches. The outstanding discriminative ability of SOX2 for OKC vs. AMBL, may be associated with their origin from cell populations at distinct stages of odontogenesis.

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