Annals of Diagnostic Pathology 17 (2013) 518–521

Contents lists available at ScienceDirect

Annals of Diagnostic Pathology

Expression of cell cycle and apoptosis-related proteins in ameloblastoma and keratocystic odontogenic tumor

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ARTICLE INFO

Keywords:
Ki-67
KCOT
Ameloblastoma
Apoptosis
Proliferation

ABSTRACT

Tumors arising from epithelium of the odontogenic apparatus or from its derivatives or remnants exhibit considerable histologic variation and are classified into several benign and malignant entities. A high proliferative activity of the odontogenic epithelium in ameloblastoma (AM) and keratocystic odontogenic tumor (KCOT) has been demonstrated in some studies individually. However, very few previous studies have simultaneously evaluated cell proliferation and apoptotic indexes in AM and KCOT, comparing both lesions. The aim of this study was to assess and compare cell proliferation and apoptotic rates between these two tumors. Specimens of 15 solid AM and 15 KCOT were evaluated. The proliferation index (PI) was assessed by immunohistochemical detection of Ki-67 and the apoptotic index (AI) by methyl green-pyronin stain. KCOT presented a higher PI than AM ($P < .05$). No statistically significant difference was found in the AI between AM and KCOT. PI and AI were higher in the peripheral cells of AM and respectively in the suprabasal and superficial layers of KCOT. In conclusion, KCOT showed a higher cell proliferation than AM and the AI was similar between these tumors. These findings reinforce the classification of KCOT as an odontogenic tumor and should contribute to its aggressive clinical behavior.

1. Introduction

Odontogenic tumours and tumour-like lesions constitute a group of heterogeneous diseases that range from hamartomatous or non-neoplastic tissue proliferations to benign neoplasms to malignant tumours with metastatic potential. They are derived from epithelial, ectomesenchymal and/or mesenchymal elements of the tooth-forming apparatus. Odontogenic tumours are rare, some even extremely rare, but can pose a significant diagnostic and therapeutic challenge [1]. Ameloblastoma is the most common locally aggressive odontogenic tumor of jaws, accounting for approximately 1% of all oral tumors [2].

Based mainly on clinical behavior and prognosis, there are two clinicopathological variants of intraosseous ameloblastoma: conventional ameloblastoma (CA), also known as solid or multicystic ameloblastoma, and unicystic ameloblastoma (UA) [3]. The most common subtypes are follicular and plexiform. Acanthomatous, granular cell, desmoplastic and basal cell are the less common histopathologic patterns [4].

Keratocystic odontogenic tumor (KCOT) defined by the World Health Organization (WHO), is a benign, intraosseous neoplasm of dental origin, with a characteristic lining of parakeratinized stratified squamous epithelium, prominent palisaded basal cell nuclei and potential for aggressive, infiltrative behaviour" [1]. Increased activity of the epithelium, confirmed by previous studies that have compared KCOTs with other odontogenic cysts, may explain the high recurrence rates of KCOTs [5–7].

Since cell population and turnover are controlled by a balance between cell proliferation and programmed cell death, cell proliferation and apoptosis are fundamental events for the development and tissue homeostasis [8]. However, few studies have evaluated apoptosis related proteins and apoptotic index in the epithelial lining of the odontogenic cysts and tumors.

Apoptosis, also known as programmed cell death or physiologic cell death, plays an important role in the development and maintenance of homeostasis within all multicellular organisms , controlling cell turnover in normal and neoplastic tissues in cooperation with cell proliferation [8–10].

A higher proliferation activity in AM epithelium in relation to KCOT epithelial lining has been demonstrated [5,11,12], even though opposite results have also been reported [13,14]. Several studies have evaluated apoptosis and related apoptotic factors in the odontogenic epithelium of AM and epithelial lining of KCOT [6,7,13,15–20]. Nevertheless, despite the fact that the cell population is controlled by the balance between cell proliferation and apoptosis, very few studies have evaluated simultaneously apoptotic and proliferation indexes in AMs and KCOTs. Therefore, the aim of this study was to evaluate and compare the cell proliferation index (PI), and the apoptotic index (AI) in AM and KCOT.

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http://dx.doi.org/10.1016/j.anndiagpath.2013.06.006

2. Material and Methods

2.1. Tissues and samples

A total of 15 solid AM and 15 KCOT from archival formalin - fixed, paraffin embedded specimens were included in the study.

2.2. Assessment of the proliferation index (PI)

The PI was assessed by immunohistochemical detection of the cell proliferation marker Ki-67. Four μm sections from the paraffin-embedded samples were utilized. Tissue sections were dewaxed with xylene, hydrated using graded alcohols, and treated with 0.6% H2O2 in methanol to eliminate endogenous peroxidase activity. Antigen retrieval was conducted by heating in a 0.01 M citrate buffer (pH 6.0) for 30 minutes. Subsequently, the anti-Ki-67 monoclonal antibody was used (clone MM1, diluted 1:100; Novocastra Laboratories, Newcastle, UK). The LSAB + kit (Dako Corporation, Carpinteria, USA) was used for application. of the biotinylated link antibody and peroxidase-labeled streptavidin, according to the manufacturer's instructions. The reactive products were visualized by immersing the sections for 3 min in 0.03% diaminobenzidine solution, containing 2 mM H2O2. The sections were then counterstained with Mayer's hematoxylin, dehydrated, and mounted. Sections of oral squamous cell carcinoma with known Ki-67 immunoreactivity were used as a positive control. Negative control was determined by omission of the primary antibody. Cell counts were made at ×400 magnification, using an eyepiece grid in light microscopy for at least 10 fields. Epithelial cells with distinct brown nuclear staining were regarded as Ki-67 positive (Fig. 1). The percentage of positive cells was then calculated to obtain the PI. PI was evaluated in the whole epithelium of AM and KCOT as well as independently in peripheral and central cells of the AM epithelium and in basal, suprabasal and superficial layers of KCOT epithelium.

2.3. Assessment of the Apoptotic Index (AI)

The AI was assessed quantitatively by morphological evaluation of sections stained by methyl green-pyronin. The methyl green component appears to be specific under certain conditions for DNA and the pyronin specific for RNA .Thus it was thought that the methyl green might emphasise apoptotic cells due to the staining of pyknotic nuclei , and pyronin may highlight apoptotic cells due to increase in mRNA levels. The methyl green pyronin technique is more simple to perform and has advantage of only highlighting cells undergoing cell death or division [21]. Four μm sections from the paraffin-embedded samples were used. Tissue sections were dewaxed with xylene and hydrated using graded alcohols. The sections were stained for 5 minutes in a solution prepared with 70 mL of 2% methyl green and 30 mL of 1% pyronine. Subsequently, the sections were enveloped in a paper filter for 5 minutes and washed individually in distilled water. The sections were evaluated under light microscopy and, if the specimens appeared as exceedingly red, they were washed again with 80% ethanol at 5 °C. The sections were then dehydrated and mounted. Cell counts were made at ×1000 magnification, using an eyepiece grid under light microscopy for at least 10 fields. Epithelial cells with morphologic characteristics of apoptosis were regarded as apoptotic cells .Apoptotic cells were defined as those which showed more intense nuclear and cytoplasmic staining by methyl green pyronin [21] (Fig. 2). The percentage of apoptotic cells was then calculated to obtain the AI in whole epithelium of AM and KCOT as well as independently in peripheral and central cells of the AM epithelium and in basal, suprabasal and superficial layers of KCOT epithelial lining.

2.4. Statistical analysis

Differences in PI and AI between AM and KOT were analyzed using the Mann–Whitney test. Differences in PI and AI between the epithelium lining layers of KCOT as well as between peripheral and central cells of AM were analyzed using the Wilcoxon test. Data were analyzed using Biostat 4.0 software (Optical Digital Technology, Belém, PA, Brazil). Tests were considered significant when their *P*-values were < .05.

3. Results

PI was higher in KCOT than in AM ($P < .05$) (Table 1).

No significant difference in AI was observed between KCOT and AM ($P > .05$) (Table 1).

In AM, PI in the peripheral cells was higher than in the central cells ($P < .05$). No statistically significant difference was observed in AI of these two areas ($P > .05$) (Table 2).

In KCOT, PI was higher in the suprabasal layer than in the basal layer ($P < .05$). PI in these two layers was higher than in the superficial layer ($P < .05$) (Table 3). In KCOT, no statistically significant difference was observed in AI between the basal and suprabasal layers ($P > .05$). Nevertheless, AI in these two layers was lower than in the superficial layer ($P < .05$) (Table 3).

4. Discussion

Odontogenic cysts and tumors are unique to the jaws and originate from the tissue associated with tooth development. They comprise an unusually diverse group because odontogenesis is a complicated

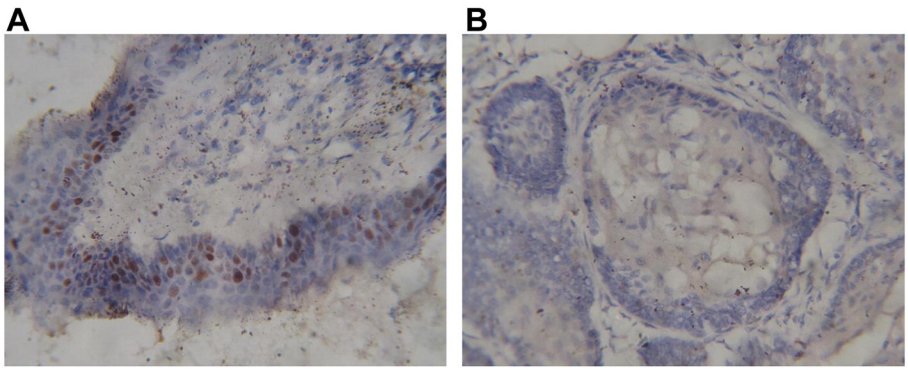

Fig. 1. Keratocyst odontogenic tumor: (A). Immunohistochemical reactivity for Ki-67. Odontogenic epithelium cells with distinct brown nuclear staining were regarded as Ki-67 positive (original magnification ×400). Ameloblastoma: (B). Immunohistochemical reactivity for Ki-67. Odontogenic epithelium cells with distinct brown nuclear staining were regarded as Ki-67 positive (original magnification: ×400).