Expression of the antiapoptotic survivin in the adenomatoid odontogenic tumors

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Abstract

Background: Adenomatoid odontogenic tumor (AOT) is an uncommon benign epithelial odontogenic tumor. Expert morphological diagnosis is required to establish differential diagnosis, in particular from ameloblastoma, thus preventing extensive surgery.

Objective: To clarify the histopathological features of AOT, as well as to assess the immunohistochemical expression of survivin in AOT.

Material and methods: Ten specimens of AOT, and one specimen of tooth germ as a control were collected from the archival files of Oral Pathology Department, Faculty of Dentistry, Alexandria University, Egypt. The expression of survivin was assessed by immunohistochemical methods.

Results: Our results revealed that all the cases of AOT, as well as the tooth germ were immunoreactive to survivin.

Conclusion: The expression of survivin in AOT, suggested that the family protein survivin contributes to the biological properties of AOT, such as cell survival, proliferation, differentiation and tissue structuring, as well as cellular regulation during tooth development.

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Keywords: Adenomatoid odontogenic tumors; Survivin; Apoptosis

1. Introduction

Adenomatoid odontogenic tumor (AOT) is an uncommon benign epithelial odontogenic tumor, representing almost 3% of all odontogenic tumors [1].

The origin of AOT remains uncertain. This has posed problems in its classification. AOT was included in the spectrum of mixed odontogenic tumors in 1992 WHO classification [2]. Lately, it was considered as an epithelial odontogenic tumor in 2005 WHO classification. Philipsen et al. [3], had strongly reported that AOT was derived from dental lamina or its remnants, although there is evidence that the tumor cells are derived from enamel organ epithelium. Most reports [4,5] have shown that AOT commonly presents as a slowly growing, painless mass often involving the anterior maxilla, and with 3 clinical subtypes, all with identical histology: follicular type (73%), the extra-follicular variant (24%), and finally the peripheral form.
that is the most infrequent (3%). AOT is regarded as benign neoplasm by most authors, although some have classified them as hamartomas [6,7].

In spite of previous confusing denominations, such as adeno-ameloblastoma, or Adenomatoid ameloblastic tumors [8], AOT is a benign tumor with a very low rate of recurrence, and appears most often in the second decade of life [1].

Survivin belongs to apoptosis inhibitor gene family, in which the proteins characterized by a domain of about 70 amino acids, termed baculovirus inhibitor of apoptosis proteins (IAPs). Survivin is a multifunctional protein that suppresses apoptosis by inhibiting one or more caspases and regulates mitosis by interacting with other chromosomal passenger protein [9–11].

Some studies have shown that some oncogenic mutations can disrupt apoptosis, leading to tumor initiation, progression or metastasis [12,13]. In mammalian cells, apoptosis is mainly modulated by two protein families, the bcl-2 and (IAPs) to which survivin belongs [14].

Survivin protein is expressed highly in most human tumors and fetal tissues [15], but is completely absent in differentiated cells [16]. This fact therefore makes survivin an ideal target for cancer therapy as cancer cells are targeted while normal cells are left alone [17]. Furthermore, survivin is likely to be involved in tumor cells resistance to radiation and chemotherapeutic drugs, and high expression levels of survivin also correlates with increased rate of tumor recurrence [14,18]. In this study, the reason for choosing survivin marker was its previously documented potential therapeutic target for epithelial odontogenic tumor.

This work aimed to clarify the histopathological features of AOT as well as to assess the immunohistochemical expression of survivin in AOT.

2. Material and methods

Ten surgical specimens of AOT were selected from the files of Oral Pathology Department, Faculty of Dentistry, Alexandria University, through the period from 2005 to 2013. The studied cases include nine follicular AOT and one extrafollicular AOT. One biopsy specimen of normal tooth germ was taken as control.

2.1. Immunohistochemistry

All samples were fixed in buffered formalin and embedded in paraffin, four-micrometer sections were obtained from each tumor and dispensed on coated slides. Sections were deparaffinized and immersed in methanol with 3% hydrogen peroxide. They were submitted to antigen retrieval solution before applying the anti-survivin (dilution 1:1000 polyclonal, Dako Corp, Carpentaria, CA, USA) antibody and incubated for 30 min at room temperature. The reaction was amplified using a streptavidin-biotin peroxidase immunostaining method. Diaminobenzidine was used as chromagen: followed by counter-staining with Mayer's hematoxylin, and finally cover slipped.

2.2. Immunohistochemical assessment

The immunostaining reaction of survivin was evaluated according to the intensity and pattern of distribution. The positive immunohistochemical staining for survivin appeared as nuclear brownish reaction and sometimes cytoplasmic reaction was noted.

Nuclear or cytoplasmic staining for survivin were scored as positive when staining reaction was noted in more than 10% of the tumor cells, and negative when staining reaction was noted in less than 10% of the tumor cells [19].

3. Results

Ten cases of adenomatoid odontogenic tumor (AOT) and one tooth germ were analyzed for the expression of antiapoptotic protein survivin. The tooth germ was considered as control. A written informed consent was taken from all the patients. This was approved by the research ethics committee.

3.1. Clinical findings

The median age of the patients was 14 years, ranging from 4 to 25 years. All the studied cases were females. The most common site of occurrence was the anterior portion of maxilla in the canine area (Fig. 1).

3.2. Histopathological results

The Adenomatoid odontogenic tumor cases exhibited spindle shaped epithelial cells that form whorled masses in scant fibrous stroma (Fig. 2a), as well as characteristic preameloblast like cells forming duct like structure (Fig. 2b, c). Some foci of basophilic cementum-like structures were noted scattered throughout the stroma (Fig. 2d).
3.3. Immunohistochemical results

Immunohistochemical reactivity for survivin was detected as a brownish reaction observed in cytoplasm as well as in the nucleus. The examined tooth germ specimen was positive to survivin. Immunosignals were more evident in the inner enamel epithelium than in outer enamel epithelium and dental lamina (Fig. 3a). Expression of antiapoptotic protein survivin was detected in 100% of the studied cases of AOT.

Intense survivin immunoexpression was detected in the odontogenic spindle epithelial cells projecting from the cyst lining (Fig. 3b, c, d, e), as well as in the pre-ameloblast like cells forming the duct like structures (Fig. 3f).

4. Discussion

The development of a tumor, whether benign or malignant involves a series of genetic events with abnormal activation of cellular oncogenes or inactivation of tumor suppressor genes [20,21]. Some of these events aim to stimulate the release of certain substances inside the tumor cells which maintain their viability and enhance their survival.

Survivin, a member of IAP-family, is essential for cell division, as well as inhibitor of apoptosis with dual roles in promoting cell proliferation, and preventing apoptosis [22,23], it is considered as protein that interfaces life and death. Unlike bcl-2 family members that induce their antiapoptotic effects through regulating the intrinsic pathway, IAPs act predominantly by directly binding and inhibiting initiators as well as caspases, thus providing a separate and non-redundant pathway of cell viability in cancer [24].
Many researchers have worked on survivin, investigating its presence in different kinds of neoplastic and preneoplastic lesions all over the body. Among the researched tumors are malignancies of lung, prostate, breast carcinomas, malignant lymphoma, neuroblastoma [25–27], as well as preneoplastic lesions as polyps of colon [28] and breast adenomas [26]. However, very few works have been done on survivin in association with odontogenic tumors. Moreover, there are no reported data concerning its expression in AOT.

Apoptosis, also known as programmed or physiological cell death, plays diverse roles in embryogenesis and normal homeostasis, as well as in oncogenesis [29,30]. Apoptotic processes are modulated by various factors, which have inhibitory or stimulatory effects. Survivin, is a unique bifunctional protein which suppresses apoptosis by inhibiting caspase 3 and 7 as well as regulate the G2/M phase of the cell cycle by associating with mitotic spindle microtubules [14].

In the present research, the specimen of tooth germ examined for the presence of survivin, showed positive immunoreactions to antiapoptotic survivin in the inner and outer enamel epithelium. This is in accordance with Kumanoto et al. [31]. They attributed that survivin plays a role in cell survival and differentiation throughout the different phases of tooth development. Moreover, Vohtokari et al. [32], reported that, expression of survivin during embryonic and fetal development may contribute to tissue homeostasis and differentiation. Furthermore, they stated that apoptotic cell death has a significant role in tooth development.

AOT is an uncommon benign and usually cystic tumor of odontogenic epithelial origin with duct-like structures, and exerts various degrees of inductive
effects on adjacent mesenchyma [7]. The increasing number of reports on AOT points to the fact that the tumor develops more frequently than formerly expected [33].

In the present work, the 10 examined cases of AOT, showed positive immunoreactions to survivin. The reaction was detected mainly in the duct-like structure as well as the spindle cells forming the whorled pattern. This is in agreement with Kumanoto et al. [31], in their work on ameloblastoma. They interestingly stated that all the peripheral layers of ameloblastomatous follicles were stained intensely by survivin. Moreover, some investigators [34] detected two pattern for ameloblastoma: an antiapoptotic proliferating area in the outer layer (periphery), that morphologically resemble the preameloblasts or ameloblast-like-cell, and a pro-apoptotic differentiating region in the inner layer (center). This explains the positive reaction to survivin in the examined cases of AOT. These findings suggest that survivin expression is associated with cell proliferation and differentiation of AOT cell.

It should be noted that the dynamics of molecules and genetic interplay cannot be reflected in their entirely by immunohistochemistry at any particular point in time [35,36]. Therefore, ultrastructural studies or more sophisticated methods, such as cytogenetics and molecular analysis may be needed to clarify the histogenesis and behavior of AOT. In conclusion, the expression of survivin in AOT, suggests that it contributes to the biological properties of AOT, such as cell survival, proliferation, differentiation and tissue structuring, as well as to cellular regulation during tooth development.

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