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Original research study

Correlation between Bcl-2 and Ki67 in radicular epitheliated granulomas

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Abstract

Aim

To assess the immunohistochemical expression of wild-type p53, Bcl-2 and Ki67 in the basal layer of proliferating radicular cysts.

Methods

A histopathological examination revealed the presence of proliferating radicular cysts in 22 specimens. Paraffin-embedded histological slices of these 22 radicular cysts were examined by immunohistochemistry for expression of wild-type p53, Bcl-2 and Ki67 in the basal layer of proliferating radicular cysts.

Results

A significant correlation was found between Bcl-2 and Ki67 ($p < 0.01$). Wild-type p53 was found to be positive in all patients, acting as a constant factor. The covariance effect of wild-type p53 on the correlation of Bcl-2 and Ki67 was found to be significant ($p < 0.05$).

Conclusion

The correlation of the oncoproteins Bcl-2 and Ki67 may be characterized by their synchronous presence. The basal layer mainly expressed this characteristic, indicating its importance in the regulation of epithelial apoptosis.

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Introduction

Intercellular signalling is important for tissue function (cell signal and transduction); consequently, each interruption of this operation may provoke an immune response[1]. The epithelium of tissues is composed of various layers depending on their embryonic origin[2]. Concretely, non-keratinized-type epithelium of radicular cysts resembles that of oral mucosa[3].

Previous researches indicated that communication between the cells of the epithelium and those of the underlying tissue contributes to the functional existence of the basement membrane and basal cells[4,5]. The basement membrane plays an important role in the creation, reformation and maintenance of epithelium by a complex biochemical activity that emanates from the loose connective tissue[5,6].

Moreover, the basement membrane of squamous epithelium includes a population of mesenchymal cells[7]. To date, there are no sound data about the possible effects of various oncoproteins on benign pathological epithelial linings such as epitheliated granulomas or radicular cysts. The explanation of the possible apoptotic failure in the stratified squamous epithelium of radicular cysts may suggest other growth mechanisms in these benign lesions. We aimed to investigate the expression of oncogenes wt p53, Bcl-2 and Ki67 that regulate different molecular actions such as tumour growth and suppression. Furthermore, we decided to investigate only the atrophic epithelium or granulomatous tissue containing epithelial lining, considering that the epithelium is under active biochemical activity.

Materials and methods

This study was approved by the local ethics committee of the Dental School. All patients who took part in this particular study ($n = 41$) gave their informed consent in accordance with the Declaration of Helsinki (1964) for human studies. Biopsy specimens were collected in excess of clinical needs from 41 patients with periapical lesions.

Exclusion criteria consisted of children and pregnant women or women who were trying to conceive. All patients accepted further histopathological processing of the biopsy specimen according to the information leaflet. Each biopsy specimen was immediately placed in 10% buffered formalin and was stored for future use. All diagnosed radicular epitheliated granulomas were further processed for immunohistochemistry.

All specimens contained only soft pathological tissue; hard tissues were left to avoid decalcification and embedded in paraffin for further processing.

From the formalin-fixed, paraffin-embedded tissues, 5-μm-thick sections were prepared and mounted on slides and stained with haematoxylin and eosin. Twenty-two specimens showed features of atrophic or proliferating radicular cyst epithelium. Atrophic epithelium was characterized when its thickness was only 10 cells. On the other hand, proliferating epithelium was diagnosed when multiple oval-shaped epithelial masses presented in the granulomatous field. Therefore, the diagnosis of atrophic or proliferating epithelium was made by the presence of atrophy of

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Materials and methods (Cont.)

the layers of the epithelium or by the presence of multiple epithelial linings in the form of arcades. All other specimens ($n = 19$) were excluded from the study according to the criteria specified prior to the study. The paraffin-embedded epitheliated granulomas were processed for immunohistochemical analysis of the expression of Ki67, Bcl-2 and wt p53 in the epithelial lining of proliferating cysts.

The reagents used were mouse monoclonal anti-Bcl-2 oncoprotein (ImmunoVision Technologies, California, USA), monoclonal mouse anti-human wt p53 protein (DacoCytomation, Glostrup, Denmark) and monoclonal mouse Ki67 antigen (Novocastra, Buffalo Grove, USA). The degree of staining was classified into mild (15/50 cells), moderate (16–35/50 cells) and severe (36–50/50 cells) in a randomly selected field of 50 epithelial cells.

Results

All the specimens were extracted from the anterior maxilla. Ten of them were related to lateral incisors, eight were associated with central incisors and four were extracted from the canine teeth. A total of ten epitheliated granulomas were found in men with a mean age of 41 years, whereas the remaining was found in women with a mean age of 48 years.

The histopathological examination described earlier proved the presence of radicular cyst epithelium in 22 cases. The remaining cases were otherwise specified and finally excluded from the study. The statistical evaluation of the data revealed that wt p53 was positive in all the patients diagnosed with epitheliated granulomas, (Figure 1). Therefore, wt p53 may be considered as a constant feature. The oncoprotein Bcl-2 was detected immunohistochemically in 11 cases (Figure 2). According to the data, Ki67 was found to be positive in 12 cases (Table 1 and Figure 3). The Pearson's correlation analysis revealed significant correlation between Bcl-2 and Ki67 ($p < 0.01$). Wt p53 was not evaluated because it was considered to be constant for our sample. The analysis of variance revealed that the influence of Bcl-2 (independent variable) on Ki67 (dependent variable) was significant ($p < 0.05$). To exclude the possibility of bias, the factor F showed values of numerical independence. The degree of immunostaining is presented in Table 2.

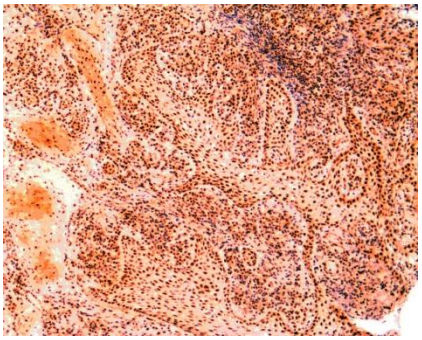

Figure 1: The proliferating epithelium is widely distributed into the connective tissue. Wt p53 is strongly immunostained in the atrophic epithelium.

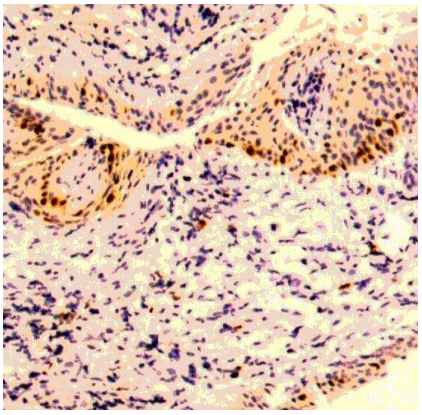

Figure 2: The atrophic epithelium initiates the process of microcystic development. Bcl-2 is moderately immunostained mainly on basal cells of the atrophic epithelium.

Discussion

Epithelium is a very important tissue for studying the effect of various macromolecules such as oncoproteins.

Immunohistochemistry is one of the most reliable techniques for identifying oncoproteins such as wt p53, Bcl-2 and Ki67. The role of these macromolecules in the prognosis and treatment outcome of various malignancies has been extensively evaluated[8,9]. There is a lack of evidence of their effect in benign pathological epithelial tissues, such as radicular epitheliated granulomas, to consider these oncoproteins as important assessment tools. Oncoproteins affect the growth and suppression of tumour tissues[10]. Homeostasis between these two actions is necessary for all living tissues. When one of these two complicated actions is altered, the cellular growth and apoptosis equilibrium is affected. Apoptosis or programmed cell death may be characterized as time-related or induced one[11].

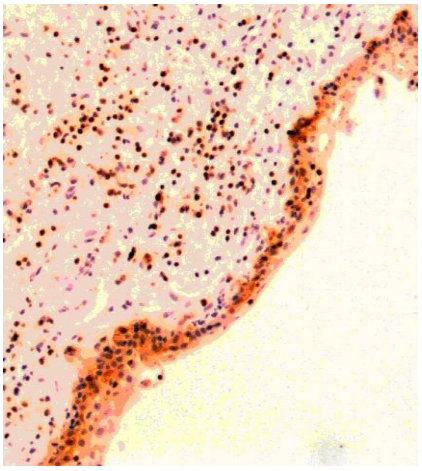

Figure 3: The atrophic epithelium showed a continuity, explaining the unicentral cystic environment. Ki67 is strongly immunostained on basal cells.

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Discussion (Cont.)

The objective of this study was to investigate the possible correlation between oncoproteins with different functions in proliferating radicular cysts. The macromolecule wt p53 was found in all cases (Figure 1). This molecular structure is essential for the suppression of various tumours[12]. This concept supported the role of wt p53 in apoptosis as a regulatory factor. One of the major functions of wt p53 was found to be the initiation of apoptosis when the DNA damage is irreparable[13]. Therefore, wt p53 may be characterized as an inductive molecule of nucleotide repair[14]. The other functions of wt p53 in DNA repair and in cell cycle were not assessed in our study. Our findings showed that wt p53 was positive in the basal layer of all proliferating radicular cysts. The possible apoptotic role of wt p53 on the basement membrane of proliferating radicular cysts may be based on cellular interaction between superficial epithelium and lamina propria. Because of the absence of vascular system in the benign epithelium, the cellular interaction of basal layer with lamina propria via the basement membrane is essential for regulatory reasons. The findings of this research revealed a constant value of wt p53, which is estimated to have a covariance effect on Ki67 and Bcl-2.

The macromolecule Ki67 has been found to be oncogenic in various neoplasms[15]. The positivity for Ki67 on the basal layer may be explained by the presence of active proliferating cysts (Figure 2). In our sample, it was present in 12 cases, which theoretically suggests rapid cell growth or turnover. Due to these very features of rapid growth, we suggest that these lesions may be more difficult to treat with conventional methods such as endodontic treatment. Generally, the positive expression of Ki67 in the cells of basal layer may indicate the grade of epithelial proliferation.

The last investigated apoptotic Bcl-2 was expressed immunohistochemically in 11 cases (Figure 3). This macromolecule was characterized by the degree of controlling the rate of apoptosis by the tissue[16]. The biomarker Bcl-2 showed a significant correlation with Ki67 positivity. According to our data, it is highly likely ($p < 0.01$) that Bcl-2 and Ki67 were expressed proportionally. This coexistence indicates that the basal layer of proliferating radicular cysts might have an essential role in apoptotic stages.

The attachment of the basement membrane on epithelium is regulated through various junctions such as hemidesmosomes[17]. A study on mice showed that the absence of the basement membrane hemidesmosomes may cause detachment of epithelial tissue[18]. Therefore, the cellular interaction between the basal layer of epithelium and the basement membrane is considered to be essential for the growth of radicular cyst lining. The non-keratinized stratified squamous epithelium contained living cells in the superior epithelial layers, which could easily be assessed by the presence of cellular nuclei. Hypothetically, this may have another significant role in the proliferation of the cyst lining since all the superficial cells are active. Therefore, the transmission of various cellular nutrients from lamina propria to the superficial cells of the proliferating epithelium may occur. This nutrient transmission may take place in the proliferation of the stratified squamous epithelium.

In the presence of altered expression of oncoproteins on the basal layer using immunochemical methods, we suggest that proliferating cysts may grow rapidly because of the existence of pathological cellular interactions between the lamina propria and epithelial layers (at a microenvironmental level).

Table 1 Cross-tabulation of Bcl-2 and Ki67 showing strong proportional correlation.

		Bcl-2		
		Positive	Negative	Total
Ki67	Positive	9	3	12
	Negative	2	8	10
Total		11	11	22

Table 2 Staining degree of the oncoproteins.

Stain	p53	Ki67	Bcl-2
Mild	3	7	8
Moderate	9	6	3
Severe	10	0	1

Conclusion

In conclusion, immunohistochemical expression of the oncoproteins wt p53, Bcl-2 and Ki67 in the basal layer may indicate the growth aspect of the proliferating stratified squamous epithelium[10]. The prognostic value of this expression should be considered only in vitro since there is no reliable conservative technique for evaluating the growth status of the cyst lining at present. Developments in optical technology (elastic scattering spectroscopy and Raman spectroscopy) in the future may enable an in vivo assessment. The correlation of Bcl-2 and Ki67 clearly indicates the interactive effect between these two oncoproteins to control growth and apoptotic indexes.

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References

1. Pieri L, Domenici L, Romagnoli P. Langerhans cells differentiation: a three-act play. Ital J Anat Embryol. 2001 Jan-Mar;106(1):47–69.
2. Barbieri CE, Tang LJ, Brown KA, Pietenpol JA. Loss of p53 leads to increased cell migration and up-regulation of genes involved in invasion and metastasis. Cancer Res. 2006 Aug 1;66(15):7589–97.
3. Ricucci D, Pascon EA, Ford TR, Langeland K. Epithelium and bacteria in periapical lesions. Oral Surg Oral Med Oral Pathol Oral Radiol Endod. 2006 Feb;101(2):239–49.
4. Cheung KH, Leung GP, Leung MC, Shum WW, Zhou WL, Wong PY. Cell–cell interaction underlies formation of fluid in the male reproductive tract of the rat. J Gen Physiol. 2005 May;125(5):443–54.
5. Andriani F, Margullis A, Lin N, Griffey S, Garlick JA. Analysis of microenvironmental factors contributing to basement membrane assembly and normalized epidermal phenotype. J Invest Dermatol. 2003 Jun;120(6):923–31.
6. Andriani F, Garfield J, Fusenig NE, Garlick JA. Basement membrane proteins promote progression of intraepithelial neoplasia in 3-dimensional models of human stratified squamous epithelium. Int J Cancer. 2004 Jan 20;108(3):348–57.
7. Franz M, Richter P, Geyer C, Hansen T, Acuna LD, Hyckel P, et al. Mesenchymal cells contribute to the synthesis and deposition of the laminin-5 gamma2 chain in the invasive front of oral squamous cell carcinoma. J Mol Histol. 2007 Jun;38(3):183–90.
8. Rothenberg SM, Ellisen LW. The molecular pathogenesis of head and neck squamous cell carcinoma. J Clin Invest. 2012 Jun 1;122(6):1951–7.
9. Florescu A, Simionescu C, Ciurea R, Pitru A. p53, Bcl-2 and Ki67 immunoexpression in follicular solid ameloblastomas. Rom J Morphol Embryol. 2012;53(1):105–9.
10. Suzuki T, Kumamoto H, Kunimori K, Ooya K. Immunohistochemical analysis of apoptosis-related factors in lining epithelium of radicular cysts. J Oral Pathol Med. 2005 Jan;34(1):46–52.
11. Yoshida M, Yasuda T, Hiramitsu T, Ito H, Nakamura T. Induction of apoptosis by anti-CD44 antibody in human chondrosarcoma cell line SW1353. Biomed Res. 2008 Feb;29(1):47–52.
12. Nemes JA, Deli L, Nemes Z, Márton IJ. Expression of p16(INK4A), p53, and Rb proteins are independent from the presence of human papillomavirus genes in oral squamous cell carcinoma. Oral Surg Oral Med Oral Pathol Oral Radiol Endod. 2006 Sep;102(3):344–52.
13. Xiong L, Kou F, Yang Y, Wu J. A novel role for IGF-1R in p53-mediated apoptosis through translational modulation of the p53-Mdm2 feedback loop. J Cell Biol. 2007 Sep;178(6):995–1007.
14. Chang YC, Liao CB, Hsieh PY, Liou ML, Liu YC. Expression of tumor suppressor p53 facilitates DNA repair but not UV-induced G2/M arrest or apoptosis in Chinese hamster ovary CHO-K1 cells. J Cell Biochem. 2008 Feb;103(2):528–37.
15. Simpson PT, Da Silva LM, Lakhani SR. In situ carcinoma – can we predict which patient will come back with a recurrence? Cancer Cell. 2007 Nov;12(5):409–11.
16. Todaro M, Lombardo Y, Francipane MG, Alea MP, Cammareri P, Iovino F, et al. Apoptosis resistance in epithelial tumors is mediated by tumor-cell-derived interleukin-4. Cell Death Differ. 2008 Apr;15(4):762–72.
17. Mirancea N, Hausser I, Metze D, Stark HJ, Boukamp P, Breitkreutz D. Junctional basement membrane anomalies of skin and mucosa in lipoid proteinosis (hyalinosis cutis et mucosae). J Dermatol Sci. 2007 Mar;45(3):175–85.
18. van der Neut R, Krimpenfort P, Calafat J, Niessen CM, Sonnenberg A. Epithelial detachment due to absence of hemidesmosomes in integrin beta 4 null mice. Nat Genet. 1996 Jul;13(3):366–9.

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