Various Expression of p63 in Ameloblastomas

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Several recent studies have detected genetic and cytogenetic alterations in epithelial odontogenic tumors. However, the detailed mechanisms of oncogenesis, cytodifferentiation, and tumor progression remain unknown. p63 as p53 homolog gene has been identified at loci 3q27-29. The p53 signaling cascade has an important role in oncogenesis or cyto-differentiation of odontogenic epithelium. Recently, several syndromes associated with p63 gene mutations have shown to include various tooth abnormalities of both the primary and permanent dentition. But little is known about p63 expression in odontogenic tumors, especially ameloblastomas. The purpose of this study was to examine various expression of p63 in ameloblastomas by immunohistochemistry and to clarify the possible biological role of p63 in ameloblastomas. 15 specimens including 6 follicular, 4 plexiform, 3 acanthomatous, and 2 granular cell types were fixed in 10% neutral formalin, 4um thick sections were used for routine H&E and immunohistochemical examinations. After immuno-histochemical satining, they were examined at a final magnification of 400X. For each case a minimum of 1000 nuclei located in the central and peripheral layers were counted in up to 10 consecutive microscopic fields per case. The immunoreactive cells were evaluated semiquantitatively. Immunoreactivity for p63 in all the types of ameloblastomas was higher in peripheral neoplastic cells than in central neoplastic cells, Keratinizing cells in acanthomatous ameloblastoma and granular cells in granular cell ameloblastoma showed markedly decreased reactivity for p63 in acanthomatous and granular cell ameloblastoma, Labelling index of acanthomatous, plexiform, and granular cell type was 86±11%, 81±17% and 83±15% in peripheral area while 88±14%, 82±11% and 76±10% in central area, respectively. Labelling index of follicular type was 17±4% in peripheral area while 21±3% in central area. There was no significant relationship between plexiform, acanthomatous, and granular cell type, while significant relationships between follicular and acanthomatous type, between plexiform and follicular type, and between granular cell and follicular type, respectively. It suggested that p63 expression could play an important role in the pathogenesis of ameloblastomas. Moreover plexiform, acanthomatous, and granular cell type would show more aggressive proliferative potentiality than follicular type.

Key words: Ameloblastomas, p63

I. Introduction

Odontogenic tumors arising from the odontogenic epithelium or from its remnants exhibit considerable histo-
cell, and desmoplastic types. Although several recent studies have detected genetic and cytogenetic alterations in these epithelial odontogenic tumors, the detailed mechanisms of oncogenesis, cytodifferentiation, and tumor progression remain unknown. p53 gene is a well-recognized tumor suppressor gene that is frequently altered in tumors. Its gene product is a transcriptional factor that regulates the expression of genes involved in cell cycle arrest or apoptosis in response to genomic damage or cell stress. p63 gene such as p53 homolog gene which has been identified at loci 3q27-29 and 1p36 in chromosome, respectively encode multiple proteins that have a significant degree of sequence homology, particularly in the transactivation, DNA-binding, and oligomerization domains. Although p63 gene might have a tumor suppressor function, genetic alterations are less frequent than those associated with p53.

The p53 signaling cascade has an important role in oncogenesis or cytodifferentiation of odontogenic epithelium in tooth germs and ameloblastomas. It is presented that EEC syndrome associated with p63 gene mutations has shown to include various tooth abnormalities of both the primary and permanent dentition. Recently, up-regulated expression and/or activity of p63 have been demonstrated in some malignancies, while the expression of p63 in ameloblastomas was rarely reported. The purpose of this study were to examine the expression and distribution of p63 in ameloblastomas by immunohistochemistry, and to clarify the possible role of p63 in the pathogenesis of ameloblastomas.

II. Materials and Methods

Surgically removed specimens from 15 patients with ameloblastomas at the Department of OMS, DKU, Dental Hospital were fixed in 10% neutral buffered formalin and were embedded in paraffin. The paraffin blocks were sliced into 4μm thick sections for routine histological and subsequent immunohistochemical examinations. Tissue sections were stained with H&E for histological diagnosis according to the WHO histological typing of odontogenic tumors. 15 ameloblastomas were histopathologically divided into 6 follicular, 4 plexiform, 3 acanthomatous, and 2 granular cell types.

For immunohistochemical staining, the paraffin sections on poly-L-lysine-coated glass slides were deparaffinized, immersed in methanol with 0.3% hydrogen peroxide to quench endogenous peroxidase activity. And then they were rinsed for 20min with PBS. After treatment with normal serum for 30 min, the sections were incubated with primary antibodies at 4°C overnight in a humidified chamber. The applied antibodies were mouse anti-p63 monoclonal antibody (Santa Cruz Biotechnology, USA; subclass IgG2a; diluted at 1:100, clone 4A4(Dako, Denmark)). The sections were washed three times with PBS at RT. The standard streptavidin-biotin-peroxidase complex method was performed to bind the primary antibodies, and reaction products were visualized by immersing the sections in 0.03% diaminobenzidine solution containing 2 mM hydrogen peroxide for 1-3 min with the use of Envision system(Dako, Denmark). Nuclei were lightly counterstained with Mayer's hematoxylin, mounted with permanent mounting medium, and examined by light microscopy.

Control slides confirmed to be unstained. Immunohistochemical reactivity and distribution for p63 was evaluated in peripheral epithelial cells and central epithelial cells. To evaluate the p63 expression, a mean percentage of positive nuclei cells/all the counted cells (Labelling Index) derived from the analysis of 100 cells in 10 random areas at 40 magnification, A semi-quant-
titative assessment of p63 expression was performed. Significant differences \( p < 0.05 \) between groups were determined.

III. Results

Immunohistochemical reactivity for p63 was detected in the nuclei of neoplastic odontogenic epithelial cells, while stromal cells in ameloblastomas were faintly reactive with anti-p63 antibody (Fig. 1, a-d). Ameloblastomas showed p63 reactivity in most peripheral columnar or cuboidal cells and in fewer central polyhedral cells in most ameloblastoma (Fig. 1, a-d), especially in acanthomatous and granular cell type.

Central polyhedral cells in plexiform ameloblastoma were far more positive for p63, while rarely positive in acanthomatous and granular cell type. Keratinizing cells in acanthomatous ameloblastoma and granular cells in granular cell ameloblastoma showed markedly decreased reactivity for p63 in acanthomatous and granular cell ameloblastoma (Fig. 1, c, d).

Labelling index of plexiform, acanthomatous, and granular cell type was significantly higher than that in follicular type. Labelling index of acanthomatous, plexiform, and granular cell type was 86±11%, 81±17% and 83±15% in peripheral area while 88±14%, 82±11% and 76±10% in central area, respectively. Labelling index of follicular type was 17±4% in peripheral area while 21±3% in central area. There was no significant relationship between

Fig. 1. A. Mainly distribution of p63 expression in peripheral cell layer of plexiform type. B. Scattered distribution of p63 expression were decreased in follicular type. C. Mainly distribution of p63 expression in peripheral cell layer of granular cell type while rare in granular cell area. D. Mainly distribution of p63 expression in peripheral cell layer of acanthomatous type while rare in keratinizing cell area.
plexiform, acanthomaous, and granular cell type, while significant relationships between follicular and acanthomaous type, between plexiform and follicular type, and between granular cell and follicular type, respectively.

IV. Discussion

p63 expression is only restricted to certain tissues, including the skin, bladder, prostate, uterus, mammary gland, and skeletal muscle compared to p53 expression\(^\text{11}\). It is reported that mice lacking p63 gene have defects in their limb, craniofacial, and epidermal development, suggesting that p63 is essential for various aspects of ectodermal differentiation\(^\text{16,17}\). And also mutations of p63 gene have recently been shown to cause several inherited human syndromes with abnormal limb development and/or ectodermal dysplasia, often accompanying abnormal tooth development ranging from enamel hypoplasia to a tooth loss\(^\text{16,17}\).

Previous study revealed that p53 expression in tooth germs was much lower than expression of its upstream regulators, MDM2 and p14ARF\(^\text{15}\). In immunohistochemistry, p63 expression in mesenchymal cells in tooth germs was observed chiefly in epithelial components, suggesting that p63 might be involved in epithelial differentiation during tooth development. In tooth germs, p63 expression was found in most cells of inner and outer enamel epithelium and dental lamina and in fewer cells of stratum intermediate and stellate reticulum, p63 positive cells were more numerous than p73 positive cells in stratum intermediate and stellate reticulum, p63 mRNA was predominantly expressed in its DN isoform of two isoforms. These results in tooth germs were similar to those in human keratinocytes\(^\text{19}\), suggesting that p63 might play differential roles in developing odontogenic epithelium.

p63 mRNA expression in a limited number of neoplastic tissues suggested that p63 might plays a role in differentiation and/or proliferation of ameloblastoma cells. Ameloblastomas had slightly higher expression of p63 mRNA than did tooth germs\(^\text{22}\). These features suggested that p63 might play an oncogenic role in odontogenic epithelium, While p53 gene is mutated in more than 50% of human cancers, mutations of its homolog p63 gene are infrequent\(^\text{12}\). Recently, it was presented that p63 amplification and/or overexpression had been identified in nasopharyngeal, bladder, prostate, skin, and esophageal carcinomas\(^\text{18-21}\). In the present study, immunoreactivity for p63 in all the types of ameloblastomas was higher in peripheral neoplastic cells than in central neoplastic cells. It was reported that the expression of p63 isoforms in ameloblastomas were similar to that in squamous cell carcinomas of the head and neck\(^\text{23-24}\). Proliferative activity in ameloblastomas was known to be higher in peripheral neoplastic cells than in central neoplastic cells\(^\text{13-15}\). In the present study, immunoreactivity for p63 in ameloblastomas was found evidently in neoplastic cells neighboring the basement membrane, and higher labelling index in peripheral area. Follicular ameloblastoma showing the lowest labelling index suggested that it might had less proliferative activity. These features suggested that p63 expression might be associated with proliferation of neoplastic odontogenic epithelial cells, Immunohistochemically increased expression of p63 in epithelial odontogenic tumors, especially in ameloblastic carcinomas, as compared with tooth germs, suggested that p63 might be involved in oncogenesis and moreover malignant transformation of odontogenic epithelium. Basal cell and desmoplastic ameloblastoma showed higher expression of some apoptosis inhibitory factors, including bcl-2, bcl-x, and survivin, than other subtypes, and these ameloblastoma variants were
considered to possess a high potential for cell survival.\(^{26,27}\) p63 immunoreactivity was slightly higher in basal cell and desmoplastic ameloblastoma than in other subtypes\(^{22}\). Present study showed that labelling index of follicular type was the lowest, while there was no significant difference between labelling index of plexiform, acanthomatous, and granular cell type. These features suggested that DNp63 expression in ameloblastomas might act with the bcl-2 and IAP family members to inhibit apoptosis induction\(^{22}\). Moreover plexiform, acanthomatous, and granular cell type might show more aggressive proliferative potentiality than follicular type. Although basal cell and desmoplastic ameloblastoma exhibited p63 reactivity in most neoplastic cells, distribution in another types was lower\(^{22}\). And also staining intensity in follicular ameloblastoma was the lowest in this study. Desmoplastic ameloblastoma demonstrated significantly higher p63 expression than acanthomatous and granular cell ameloblastoma\(^{22}\), while in this study, immunoreactivity for p63 was significantly higher in plexiform ameloblastoma than in follicular ameloblastoma. Another previous study has revealed that expression of p53, MDM2, and p14ARF is higher in plexiform ameloblastoma than in follicular ameloblastoma\(^ {15}\). These features suggested that genomic damage or cell stress might be greater in plexiform ameloblastoma than in follicular ameloblastoma, being involved in tissue structuring of ameloblastomas. These genomic change would be involved in granular and acanthomatous type according to these results. In the present study, p63 reactivity in acanthomatous and granular cell ameloblastoma was markedly decreased in keratinizing cells and granular cells. Previous studies have detected increased apoptotic cell death in keratinizing cells and granular cells of these ameloblastoma subtypes\(^ {13,20}\). These features suggested that induction of apoptosis by TAp63 might be minimal during terminal differentiation of neoplastic cells in acanthomatous and granular cell ameloblastoma\(^ {22}\).

In conclusion, expression of p63 in ameloblastomas suggested that p63 might play a role in differentiation and proliferation of ameloblastomas. In addition, different distribution of expressed p63 suggested that this p53 homolog might differentially function in neoplastic odontogenic epithelial cells of ameloblastoma.

### V. References


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