

Expression of Notch1 in Ameloblastoma and Correlation with CBCT Imaging Subtypes

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Objective: Ameloblastoma (AM) is a prevalent benign odontogenic tumor affecting the jaw bone. Its potential for recurrence and malignant transformation is closely associated with the regulation of signaling pathways. The objective of this study was to examine the expression and clinical significance of Notch1 in AM, along with exploring the correlation between Notch1 expression and AM imaging patterns.

Methods: The streptavidin-peroxidase (S-P) immunohistochemical technique was employed to detect the expression of Notch1 protein in the oral mucosa of 70 AM patients and 25 normal controls. Additionally, the preoperative cone-beam computed tomography (CBCT) images of the patients were classified, and the results were analyzed in relation to the expression of Notch1 protein in AM.

Results: The positive expression rate of Notch1 in the 70 AM patients was 74.29% (52/70), while the Notch1 expression rate in 26 oral mucosa patients was 19.23% (5/26), indicating a statistically significant difference ($P < 0.05$). The expression rate of Notch1 was 58.06% in single-room AM and lower in multi-room AM (85.29%) and honeycomb AM (100%) ($P < 0.01$). However, there was no significant difference in the expression rate of Notch1 between multi-room AM and honeycomb AM ($P > 0.05$).

Conclusion: Notch1 may serve as an important diagnostic indicator in AM, and the apoptosis inhibition ability and cell proliferation ability of single-room AM are lower compared to those of the room and honeycomb types.

Key words: Receptor, Notch1, Immunohistochemistry, Ameloblastoma, Cone-Beam Computed Tomography

Introduction

Ameloblastoma (AM) stands as the predominant benign neoplasm within the oral and maxillofacial domain, garnering

significant interest owing to its pronounced recurrence propensity and the potential for malignant progression [1-4].

Through intricate scrutiny of AM's etiology, researchers have delineated a multifaceted interplay of signaling pathways [5-7]. The Notch signaling pathway, a pivotal regulatory axis, has emerged as a focal point of scholarly inquiry and investigation. Operating as a linchpin in governing fundamental biological processes like cellular proliferation, differentiation, and apoptosis [8-10], deregulated Notch pathway activation or inhibition may intricately intertwine with AM's pathogenesis. Consequently, delving deeply into the precise involvement of the Notch signaling pathway in AM genesis and its modulator mechanisms holds promise in furnishing critical theoretical underpinnings and clinical directives for forthcoming therapeutic modalities. The Notch signaling pathway, a vital cell-cell interaction mechanism, serves as pivotal regulator of cell proliferation, differentiation, adhesion, apoptosis, and epithelial mesenchymal transition, among other fundamental biological processes [8]. This intricate signaling cascade primarily comprises receptors, ligands, and signal transduction molecules, exerting a significant influence on cellular behavior during intercellular interactions [8,9]. In the realm of normal growth and development, the Notch signaling pathway's functionality is indispensable, intricately involved in fundamental physiological phenomena like embryonic development, organogenesis, and cell fate determination [8-12]. By modulating the activation and inhibition of the Notch signaling pathway, cells establish communication channels and synchronize their activities in biological processes, thus facilitating typical tissue and organ development and functional maintenance. However, deregulation of the Notch signaling pathway can result in various pathologies, including tumorigenesis. Within AM, Notch1 plays a pivotal role in this signaling cascade, and its aberrant expression is intricately linked to the onset and progression of AM. Research indicates an up regulation of Notch receptors (Notch 3 and 4) and their corresponding ligands (Jagged1 and Delta1) in AM patients [13,14]. These observations underscore the significance of Notch1 in the pathophysiology of AM. Consequently, a thorough investigation into Notch1 expression in AM and its clinical implications holds substantial value in elucidating the disease's pathogenesis. To delve deeper into the expression of Notch1 in AM and its clinical significance, this research employed immunohistochemical staining to assess Notch1 expression levels in AM tissues relative to normal tissues. Additionally, an investigation was conducted into the correlation between Notch1 expression levels and AM

patterns observed in cone-beam computed tomography (CBCT) images. The findings of this study not only elucidate the role of Notch1 in AM pathogenesis but also establish a robust scientific foundation for early AM diagnosis, identification of therapeutic targets, and surgical interventions. Through an examination of Notch1 expression profiles in AM and its association with imaging characteristics, the aim is to contribute positively to enhancing the diagnostic and treatment standards for AM, ultimately enhancing patient survival rates.

Materials and Methods

Research Object

The study included a total of 70 patients who underwent AM resection at the Department of Oral and Maxillofacial Surgery, Affiliated Hospital of Chifeng University, Inner Mongolia, China. The excised AM tissues were carefully fixed with formalin and subsequently embedded in paraffin wax, while the oral mucosa samples obtained during dental implantation from 25 patients were also embedded in paraffin wax, serving as control specimens. These tissue samples were appropriately stored in the Department of Pathology, Affiliated Hospital of Chifeng University, Inner Mongolia, China. Simultaneously, the corresponding preoperative CBCT scans of the AM patients were collected for further analysis.

Methods

The specimens were sectioned using streptavidin-peroxidase (S-P) immunohistochemistry. A concentrated monoclonal antibody against Notch1 mice, purchased from ZYMED Company in the United States, was used as the primary antibody at a working concentration of 1:50. The S-P immunohistochemical kit Universal SP-9000 Kit, obtained from DA KO in Denmark, was used according to the kit instructions. PBS was used as a negative control instead of the primary antibody.

The color intensity was scored as follows: colorless (0 points), light yellow (1 point), brown (2 points), and dark brown (3 points). Five high-power fields ($\times 40$) were randomly observed in each section under a light microscope, and 50 cells were counted to calculate the percentage of positive cells. The scoring criteria were as follows: 0 points (no positive cells), 1 point (1%-10%), 2 points (11%-50%), 3 points (51%-80%), and 4 points

(81%-100%). The final total score was calculated by multiplying the color intensity score and the percentage score. The scoring categories were as follows: 0 points (-), 1-4 points (+), 5-8 points (++) and 9-12 points (+++).

Statistical Analysis

SPSS software (version 22.0, SPSS Inc., Chicago, IL, USA) was used to perform all statistical analyses. The statistical data were subjected to analysis using the χ^2 test or Fisher exact probability method, and a significance level of $P < 0.05$ was considered statistically significant.

Ethical Statement

This retrospective study received approval from the Ethics Committee of the Mongolian National University of Medical Sciences (2023/3-04), ensuring compliance with ethical standards.

Results

Patient general data

Among the cohort of 70 patients diagnosed with AM, 39 were of male gender while 31 were female, with an average

age of 32.15 ± 17.40 years. Of the 25 patients who provided samples of oral mucosa, 13 were males and 12 were females, with an average age of 33.25 ± 21.22 years. Following the diagnostic criteria for image classification of AML as outlined in the publication "Oral and Maxillofacial Medical Imaging Diagnostics" edited by Zhang Zuyan [13], the patients with AM who underwent CBCT were categorized into 31 cases of single-room types, 34 cases of multi-room types, and 5 cases of honeycomb types.

The expression of the Notch1 protein in oral mucosa and AM was observed

The Notch1 protein was predominantly localized in the cytoplasm and/or nucleus, exhibiting a yellow or brown-yellow appearance in the form of particles. A minimal level of expression was detected in the epithelial cells of oral mucosal tissue (Fig 1-A,B), while AM showed predominantly mild to moderate positive staining (Fig 1-C,D), as illustrated in Table 1.

Table 1. Difference of Notch1 protein expression in oral mucosa and AM

Groups	n	Notch1								χ^2	P-value
		%	+	%	++	%	+++	%			
Oral mucosa	26	21	76.12	5	19.23	0	0	0	0	23.82	0.00
AM	70	18	25.71	22	31.43	25	35.72	5	7.14		

Note: - Negative, + Positive, % Percentage

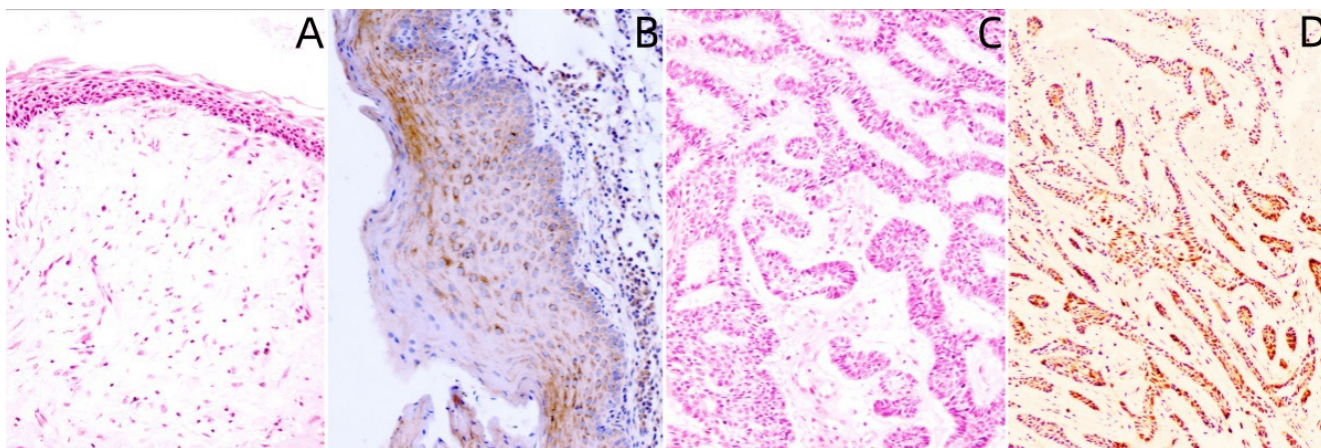


Figure 1. Expression of Notch1 protein in oral mucosa and AM

Fig.1-A Histopathology of oral mucosa (×100), Fig.1-B Immunohistochemical characteristics of Notch1 in oral mucosa (×100), Fig.1-C Histopathology of AM (×100) and immunohistochemical characteristics of Notch1 in AM (×100)

Relationship between Notch1 protein expression and CBCT image typing of AM

In 70 samples, the Notch1 protein expression was observed to be 58.06% (18/31) in single-room AM, 85.29% (29/34) in multi-room AM, and 100% (5/5) in honeycomb AM. Notably, the

expression of Notch1 protein in single-room AM was significantly lower compared to both multi-room AM and honeycomb AM (P < 0.01), while no significant difference was observed between the expression of Notch1 protein in multi-room AM and honeycomb AM (P > 0.05). The detailed results are presented in Table 2.

Table 2. Relationship between expression of Notch1 protein and CBCT image typing of AM

Groups	Types	n	Notch1				%
			-	+	++	+++	
I	single-room	31	13	9	7	1	58.06
II	multi-room	34	5	10	17	2	85.29
III	honeycomb	5	0	3	1	2	100

Note: - Negative, + Positive, % Percentage
Group I was compared with group II and group III, P < 0.05

Discussion

The precise orchestration of the cell cycle is essential in cellular biology, with its intricate regulatory mechanisms serving as vital components in upholding cellular homeostasis and proliferation [8,9]. Central to this regulatory framework is the cell cycle-dependent protein kinase (CDK), which assumes a pivotal role. Human cells harbor various principal CDKs, encompassing CDK1 (CDC2), CDK2, CDK4, CDK5, CDK6, and CDK7 (CAK), each uniquely contributing to the regulation of distinct phases within the cell cycle. Notch1, a cell cycle-dependent gene, assumes a

pivotal role in cell cycle regulation. Notch1 engages with CDK4 to constitute the

Notch1-CDK4 complex, which subsequently triggers the activation of the CDK2/CyclinE complex. This cascade of events culminates in the phosphorylation of Retinoblastoma (Rb) protein, precipitating cellular progression into the mitotic phase and facilitating the perpetuation of the cell cycle. The intricate interplay and signaling pathways how cased herein not only underscore the intricacies of cell cycle control but also furnish a

crucial framework and avenue for delving deeper into biological phenomena like cell proliferation and differentiation.

In this study, the expression of Notch1 in AM was significantly higher than that in normal oral mucosa. The reason for this result may be that under the stimulation of some adverse factors, the apoptosis of some odontogenic epithelium [2] (such as enamel forming organs, oral mucosal epithelium, and reduced enamel epithelium) was inhibited and abnormal proliferation occurred, leading to the occurrence of AM. Therefore, the up regulated expression of Notch1 protein in AM may suggest that this protein is one of the diagnostic markers of AB.

CBCT imaging holds significant importance in the assessment and detection of jaw bone density as well as the identification of tumors and is considered one of the primary diagnostic methods for AM [16, 17]. CBCT images typically exhibit single-room, multi-room, or honeycomb patterns [18]. The expression rate of Notch1 protein varies among different types of AM as observed in CBCT images. Specifically, the expression rate of Notch1 protein is significantly lower in single-room AM compared to multi-room and honeycomb AM. However, there is no significant difference in the expression rate between multi-room and honeycomb AM. Nevertheless, considering that Notch1 can promote cell proliferation, it suggests that the cell proliferation ability differs based on CBCT image type. Cellular AM demonstrates higher cell proliferation ability while single-chamber AM exhibits lower cell proliferation ability, indicating potential weakness in invasion and implying a better prognosis for patients. This finding aligns with some scholars' belief regarding the low postoperative recurrence rate associated with single-room AM [19,20].

To summarize, different surgical approaches can be considered for adolescents with various types of CBCT images in AM treatment. For instance, single-room AM can be utilized, along with the use of first-stage decompression and second-stage radical surgery methods [19-21]. These approaches not only minimize surgical trauma but also have a lesser impact on patients' maxillofacial function and appearance. However, it is challenging to preserve the multi-room and cellular AM treatment.

However, the initiation and progression of tumors are multifactorial in nature. This research exclusively employed immunohistochemical techniques to scrutinize the Notch1 protein expression in AM at the protein level. The investigation revealed an up-regulation of Notch1 protein expression in AM,

mirroring observations in various other malignancies [24-26]. Nevertheless, elucidating the precise mechanistic involvement of the Notch signaling pathway in AM necessitates substantiation through further comprehensive studies at the gene and cellular strata. Currently, therapeutic investigations focused on Notch are in the research phase, with selective Notch inhibitors poised to assume a pivotal role in tumor treatment[27-30]. As specific Notch inhibitors progress, the practice of suppressing Notch protein expression for tumor prevention and treatment is anticipated to gain prevalence. This study will serve as a valuable resource for chemoprevention of AM, the utilization of selective Notch inhibitors, and the targeted therapy of Notch, thereby advancing the field of oncology.

Conflict of interests

The authors declare no competing interests.

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