SQUAMOUS ODONTOCgenic Tumor of the MANDIBLE: A CASE REPORT DEMONSTRATING IMMUNOEXPRESSION OF NOTCH1, 3, 4, JAGGED1 AND DELTA1

C. H. Siar1, K. Nakano2, K. H. Ng3, M. Tomida4, H. Nagatsu5, T. Kawakami2

1Department of Oral Pathology, Oral Medicine and Periodontology, Faculty of Dentistry, University of Malaya, Kuala Lumpur, Malaysia, 2Department of Oral Pathology and Medicine, Graduate School of Medicine, Dentistry and Pharmaceutical Sciences, Okayama University, Okayama, Japan

Abstract

Background: Squamous odontogenic tumor (SOT) is a rare benign odontogenic epithelial neoplasm. A slow-growing painless expansive swelling is the common presenting symptom. Histopathologically, SOT can be easily misdiagnosed as an acanthomatous ameloblastoma. Although Notch receptors and ligands have been shown to play a role in cell fate decisions in ameloblastomas, the role of these cells signaling molecules in SOT is unknown.

Case report: This paper describes a case of SOT affecting the anterior mandible of a 10-year-old Indian female. The patient was treated by local surgical excision and there has been no follow-up clinical record of recurrence 5 years after primary treatment. Histopathological examination revealed a solid, locally-infiltrative neoplasm composed of bland-looking squamoid islands surrounded by a mature fibrous connective tissue stroma and the diagnosis was SOT. Immunohistochemical evaluation showed positive reactivity of varying intensity in the neoplastic epithelial cells for notch1, notch3, notch4, and their ligands Jagged1 and Delta1. Expression patterns showed considerable overlap. No immunoreactivity was detected for notch2 and Jagged2.

Conclusions: Present findings suggest that notch receptors and their ligands play differential roles in the cytodifferentiation of SOT.

Key words: solitary odontogenic tumor, Notch signaling, immunohistochemistry, cytodifferentiation

INTRODUCTION

Squamous odontogenic tumor (SOT) is a rare tumor with less than 50 cases reported [1]. It was first described as a distinct entity by Pullon et al. in 1975 [2]. The aetiopathogenesis of this benign locally-invasive odontogenic epithelial neoplasm is unclear. Clinico-pathologically, three main types are identified: intraosseous [1], mural (mural SOT-like proliferations in cyst) [3] and extraosseous forms [4]. SOT affects a wide age range, shows a slight male preponderance and occurs more frequently in the mandible [1]. Aggressive [5] and multifocal [6] variants have been reported. Histopathologically it is composed of islands of well-differentiated non-keratinizing squamous epithelium surrounded by a mature fibrous connective tissue [1]. There is no cellular atypia. In the epithelial islands, cystic degeneration as well as calcification may occur. Invasion into cancellous bone may be present [7].

Mammalian Notch is a four-member family of receptors (Notch1-4) that mediates short-range events [8, 9]. The Notch receptor is a single transmembrane protein containing distinct structural extracellular and intracellular domains. The structure of the four Notch receptors is highly homologous with only some differences in these domains. Notch signaling pathway is activated when cell surface-anchored ligands (Jagged1, Jagged2, Delta1, Delta3 and Delta4) from neighboring cells bind the receptors and trigger the proteolytic cleavage of Notch receptors. The activation of Notch signaling pathway leads to different outcomes ranging from control of proliferation to apoptosis, differentiation, maintenance of stemness and cell fate decision [9]. Deregulation of Notch signaling has been implicated in some genetic diseases and tumorigenesis [10]. Notch signaling in a variety of tumors can be either oncogenic or tumor suppressive, depending on the specific cellular context, also in odontogenic neoplasms [11-13].

The potential role for Notch signaling pathway in the development and cytodifferentiation of odontogenic neoplasms has gained attention only recently. In others [14] and our studies [15-17], notch expression was observed in plexiform and follicular ameloblastoma [4, 15], ameloblastic carcinoma [16] and ameloblastic fibroma [17] but not in the odontogenic myxoma [17]. A search of the English language literature disclosed that Notch signaling activity in SOT is not known. In this report, the expression patterns of notch1-4 and their ligands, Jagged1, Jagged2 and Delta1 in a case of SOT are presented and the significance of these findings speculated.
unknown duration in her anterior mandible. No further clinical or radiographic information was available as to the presentation of this lesion in the jaw. A pre-operative diagnosis of ossifying fibroma was made. The lesion was surgically excised under general anesthesia, and submitted for histopathological examination. No follow-up information was available as to the outcome of the patient five years after primary treatment.

**Histopathology**

Microscopic examination of the lesional area disclosed a solid, locally-infiltrative, benign odontogenic epithelial neoplasm. It was composed of bland-looking islands of well-differentiated squamous epithelium set against a mature fibrous connective tissue stroma (Fig. 1). These tumor islands did not show any evidence of peripheral columnar cells, reversal nuclear polarity or central stellate reticulum-like cells. Cellular atypia was absent. There was central keratinization and cystic degeneration. Foci of dystrophic calcifications and occasional clear cell clusters were noted. These histopathological findings led us to diagnose the lesion to be SOT.

**Immunohistochemistry**

The immunohistochemical detection of Notch receptors and ligands was performed using the Envision technique as previously described [14]. Primary antibodies used are detailed in Table 1. Stromal endothelium and fibroblasts served as internal positive controls while negative controls were performed by substituting the primary antibody with phosphate-buffered saline.

SOT showed positive expression for Notch1, Notch3 and Notch4 in the well-differentiated squamous epithelial islands, central keratinization and cystic degeneration (Fig. 2). Notch2 was not detected. Expression for Jagged1 was moderate while that for Delta1 was weak within the neoplastic epithelium. Jagged2 was consistently absent. Clear cell nests and dystrophic calcific foci showed similar expression patterns (Fig. 3). Stromal endothelium and fibroblasts

**Table 1.** Details of primary antibodies used in this examination.

| Antibody | Manufacturer | Product       | Dilution |
|----------|--------------|---------------|----------|
| Notch1   | Abcam        | Rabbit polyclonal | 1:500    |
| Notch2   | R&D System   | Goat polyclonal | 1:500    |
| Notch3   | Abcam        | Rabbit polyclonal | 1:500    |
| Notch4   | Abcam        | Rabbit polyclonal | 1:500    |
| Jagged1  | Abcam        | Goat polyclonal | 1:500    |
| Jagged2  | Abcam        | Rabbit polyclonal | 1:500    |
| Delta1   | R&D System   | Mouse monoclonal | 1:200    |

**Table 2.** Expression of Notch signaling molecules in odontogenic neoplasms as examined by ISH and IHC.

| Studies                        | Notch1 | Notch2 | Notch3 | Notch4 | Jagged1 | Jagged2 | Delta1 |
|--------------------------------|--------|--------|--------|--------|---------|---------|--------|
| **Ameloblastoma (n = 22) [14]** |         |        |        |        |         |         |        |
| Peripheral cells               | -      | +      | -      | NA     | -/+     | NA      | -/+    |
| Central cells                  | +/+++  | +++    | +/++   | NA     | +       | NA      | +++    |
| Keratinizing cells             | -      | -      | -      | NA     | -       | NA      | -      |
| Granular cells                 | -      | -      | -      | NA     | -       | NA      | -      |
| Stromal cells                  | +/+++  | +++    | +/++   | NA     | +++     | NA      | +++    |
| **Ameloblastic carcinoma (n = 1) [16]** |         |        |        |        |         |         |        |
| Peripheral cells               | +      | NA     | NA     | NA     | NA      | NA      | NA     |
| Central cells                  | +++    | NA     | NA     | NA     | NA      | NA      | NA     |
| **Ameloblastic fibroma (n = 1) [17]** |         |        |        |        |         |         |        |
| Peripheral cells               | +      | NA     | NA     | NA     | NA      | NA      | NA     |
| Central cells                  | +++    | NA     | NA     | NA     | NA      | NA      | NA     |
| Dental papilla-like cells      | +++    | NA     | NA     | NA     | NA      | NA      | NA     |
| **Odontogenic myxoma (n = 1) [17]** |         |        |        |        |         |         |        |
| Dental papilla-like cells      | -      | NA     | NA     | NA     | NA      | NA      | NA     |
| **Squamous odontogenic tumor (n = 1)*** |         |        |        |        |         |         |        |
| Peripheral cells               | ++     | -      | +      | +++    | +/+     | -       | -/+    |
| Central cells                  | ++     | -      | +      | +++    | +/+     | -       | -/+    |
| Keratinizing cells             | ++     | -      | +      | +++    | +/+     | -       | -/+    |
| Cystic degeneration            | +      | -      | +      | +++    | +/+     | -       | -/+    |
| Clear cells                    | ++     | -      | +      | +++    | +/+     | -       | -/+    |
| Dystrophic calcifications      | ++     | -      | +      | +++    | +/+     | -       | -/+    |

* Present study; NA: Not available; (-) : negative; (+): mild; (++) moderate; (+++): strong immunopositivity
were variably positive for notch receptors and ligands. Furthermore, there were some notch1,3,4 positive cells scattered in the stromal connective tissue.

Staining intensity of SOT was analyzed according to the criteria of published studies [14, 16, 17] (Table 2) where expression results of immunohistochemistry were taken into account.
notch1 activity was detected in the peripheral and central stromal cells, and these findings suggested that notch1 plays some roles in the cytological differentiation of odontogenic epithelium. In the developing tooth, notch receptors participate in cell fate decisions by the process of lateral inhibition or inductive signaling. In the developing tooth, notch receptors and ligands have been found to be expressed in dental epithelium and/or ectomesenchyme, suggesting that notch signaling might be involved in the cellular differentiation of the odontogenic apparatus [1].

In summary, a case of SOT is reported here and its notch immunoperoxidase profile defined. As a wide range of neoplasms including odontogenic neoplasms occurs in the oral and craniofacial regions, research on cell differentiation phenomenon and cell signaling factors is currently ongoing [11-17, 20-25]. Studies on larger series of SOT are also recommended to help refine the role of these signaling molecules in the development of this neoplasm.

ACKNOWLEDGMENTS: This research was supported jointly by the University of Malaya Research Grant FS170/2008C and Grant-in Aid for Scientific Research (C) (20592349) from the Japan Society for the Promotion of Science.

REFERENCES
1. Reichart PA. Squamous odontogenic tumour. In: Barnes L, Eveson JW, Reichart P, Sidransky D (eds) World Health Organization Classification of Tumours: Pathology and genetics of tumours of the head and neck. 301, 2005, IARC Press, Lyon, France.
2. Pullon PA, Shafer WG, Elzay RP, Kerr DA, Corio RL. Squamous odontogenic tumor: Report of six cases of a previously undescribed lesion. Oral Surg Oral Med Oral Pathol 1975; 40: 616-630.
3. Oliveira JA, Costa IM. Squamous odontogenic tumor-like proliferations (SOT-LP) versus intraosseous squamous cell carcinoma in residual cyst. J Oral Maxillofac Surg 2006; 64: 1325.
4. Baden E, Doyle J, Mesa M, Fabie M, Lederman D, Eichen M. Squamous odontogenic tumor: Report of three cases including the first extracolonic case. Oral Surg Oral Med Oral Pathol 1993; 75: 733-738.
5. Ruhin B, Raoul G, Kolb F, Casiraghi O, Lecomte-Houcke M, Cassagne S, Aszuna O, Ferri J. Aggressive maxillary squamous odontogenic tumour in a child: histological dilemma and adaptive surgical behaviour. Int J Oral Maxillofac Surg 2007; 36: 864-866.
6. Leider AS, Jonker LA, Cook HE. Multicentric familial squamous odontogenic tumor. Oral Surg Oral Med Oral Pathol 1989; 68: 175-181.
7. Kim K, Mintz SM, Stevens J. Squamous odontogenic tumor causing erosion of the lingual cortical plate of the mandible: a report of 2 cases. J Oral Maxillofac Surg. 2007; 65: 1227-1231.
8. Miele L. Notch signaling. Clin Cancer Res 2006; 12: 1074-1079.
9. Artavanis-Tsakonas S, Rand MD, Lake RJ. Notch signaling: cell fate control and signal integration in development. Science 1999; 284: 770-776.
10. Leong KG, Karsan A. Recent insights into the role of Notch signaling in tumorigenesis. Blood 2006; 107: 2223-2233.
11. Nakano K, Nagatsuka H, Tsujigawa H, Gunduz M, Katase N, Siar CH and Kawakami T. Immunohistochemical characteristics of odontogenic neoplasms and their physiological counterparts. J Hard Tissue Biol 2008; 17: 79-90.
12. Kawakami T and Nagatsuka H. Cell differentiation of neoplastic cells originating in the oral and craniofacial regions. pp 1-56, Nova Science Publishers, Inc. New York, 2009.
13. Kawakami T, Nagatsuka H, Nakano K, Shimizu T, Tsujigawa H, Hasegawa H and Nagai N. Chapter I: Cell differentiation of neoplastic cells originating in the oral and craniofacial regions. In Ivanova LB ed., Cell Differentiation Research Developments, p1-30, Nova Science Publishers, Inc. New York, 2008.
14. Kumamoto H, Ohki K, Ooya K. Expression of notch signaling molecules in ameloblastomas. J Oral Pathol Med 2008; 37: 228-234.
15. Siai CH, Ng KH, Ariff Z, Muraki E, Shimizu T, Tsujigawa H, Nagatsuka H, Nagai N and Kawakami T. A case report of ameloblastoma of the mandible with examination of Notch signaling. Oral Med Pathol 2006; 11: 35-39.
16. Nakano K, Siai CH, Tsujigawa H, Nagatsuka H, Nagai N, Kawakami T. Notch signaling in benign and malignant ameloblastic neoplasms. Eur J Med Res 2008; 13: 476-480.
17. Nakano K, Chelvanayagam P, Born K, Siai CH, Ng KH, Nagatsuka H and Kawakami T. A study of recurrent giant odontogenic myxoma of the mandible with immunohistochemical examination of Notch. Oral Med Pathol 2008; 12: 53-56.
18. Tatemoto Y, Okada Y, Mori M. Squamous odontogenic tumor: immunohistochemical identification of cytokeratins. Oral Surg Oral Med Oral Pathol 1980; 67: 63-67.
19. Misiadis TA, Regaudiat I, Gridley T. Role of Notch signaling pathway in tooth morphogenesis. Arch Oral Biol 2005; 50: 137-140.
20. Chuah KS, Siai CH, Nakano K, Nagatsuka H, KhooSP, Ng KH and Kawakami T. Wingless-type protein-1 (Wnt-1) expression in primary conventional and unicystic ameloblastomas and their recurrent tumors. J Hard Tissue Biol 2009; 18: 63-70.
21. Nagatsuka H, Katsue N, Pwint HP, Tsujigawa H, Siai CH, Nakajima M, Naomoto Y, Tamamura R, Kawakami T and Gunduz M. Role of Heparanase in the release of heparin sulphate binding growth factors in odontogenic tumors. Oral Med Pathol 2009; 13: 81-89.
22. Han PP, Nagatsuka H, Tamamura R, Katsae N, Bernard M, Hu H, Takagi S, Ishida N, Nakano K, Kawakami T and Gunduz M. Heparanase and its related molecules in odontogenic tumors. Oral Med Pathol 2009; 13: 81-89.
23. Han PP, Tamamura R, Katsae N, Fujii E, Okauchi M, Jin T, Siai CH and Nagatsuka H. Differential distribution of type IV collagen α1 to α6 chains suggests distinct molecular interaction between the epithelial and mesenchymal components of benign odontogenic tumors. J Hard Tissue Biol 2008; 17: 23-30.
24. Han PP, Tamamura R, Katsae N, Fujii E, Okauchi M, Jin T, Siai CH and Nagatsuka H. Differential distribution of type IV collagen α1 to α6 chains suggests distinct molecular interaction between the epithelial and mesenchymal components of benign odontogenic tumors. J Hard Tissue Biol 2008; 17: 23-30.
25. Heikinheimo K, Mori K, Nagatsuka H and Happonen RP. Transforming growth factor beta (TGF-β) gene family members in developing and neoplastic odontogenic tissues. J Hard Tissue Biol 2006; 15: 1-5.

Received: September 30, 2009 / Accepted: October 14, 2009

Address for correspondence:
Keisuke Nakano, DDS, PhD,
Hard Tissue Pathology Unit
Matsumoto Dental University Graduate School of Oral Medicine
1780 Hidaka-Gobara
Shiojiri, 399-0781 Japan
Phone and Fax: +81-(0)263-51-2035
E-mail: keisuke1@po.mdu.ac.jp