汗腺腫における新規融合遺伝子CRTC3-MAML2とその病理組織学的意義

隈, 有希

<http://hdl.handle.net/2324/4060062>

出版情報 : Kyushu University, 2019, 博士 (医学) , 課程博士
バージョン :
権利関係 : © 2017 Elsevier Inc. All rights reserved.

Original contribution

A novel fusion gene *CRTC3-MAML2* in hidradenoma: histopathological significance^{☆,☆☆}

Yuki Kuma MD^{a,b}, Yuichi Yamada MD, PhD^a, Hidetaka Yamamoto MD, PhD^a, Kenichi Kohashi MD, PhD^a, Takamichi Ito MD^{a,b}, Masutaka Furue MD, PhD^b, Yoshinao Oda MD, PhD^{a,*}

^aDepartment of Anatomic Pathology, Graduate School of Medical Sciences, Kyushu University, Fukuoka, Fukuoka 812-8582, Japan

^bDepartment of Dermatology, Graduate School of Medical Sciences, Kyushu University, Fukuoka, Fukuoka 812-8582, Japan

Received 17 May 2017; revised 4 October 2017; accepted 11 October 2017

Keywords:

CRTC1;
CRTC3;
MAML2;
Hidradenoma;
Mucoepidermoid carcinoma;
Fusion gene

Summary Hidradenoma usually presents as a solitary, slow-growing, and solid or cystic nodular lesion, which arises in various anatomical sites. Its diagnosis is occasionally difficult because the tumor shares histological features with other cutaneous appendage tumors. Recently, *CRTC1-MAML2* fusion gene was reported in hidradenomas, with the fusion transcript being demonstrated in approximately 50% of cases. However, limited information is available regarding its clinical significance. Here, we investigated the relationship between the fusion gene and clinicohistopathological features. We reviewed 39 cases histologically diagnosed as hidradenoma. Reverse-transcription polymerase chain reaction (RT-PCR) was performed for all 39 cases, and fluorescence in situ hybridization was also performed for the RT-PCR-negative cases. The 39 tumors included 36 clear cell hidradenomas and 3 poroid hidradenomas. The details of the cellular components were as follows: clear cell-dominant type, 9 cases; polygonal cell-dominant type, 21 cases; and equally mixed type, 9 cases. There were no tumors with apparent mucinous cells. There were 8 tumors with prominent cystic change, 2 of which presented apocrine-like decapitated secretion. *CRTC1-MAML2* fusion was detected in 10 of the 39 tumors (26%) and *CRTC3-MAML2* fusion in 2 of the 39 (5%) by RT-PCR. *MAML2* gene rearrangement was detected in 11 of 27 fusion gene-negative cases by fluorescence in situ hybridization. Moreover, neither the fusion genes nor gene rearrangement was detected in prominent cystic tumors and poroid hidradenomas. We conclude that *CRTC1/3-MAML2* fusion gene analysis can be a useful method for diagnosing hidradenoma. Considering the histological and genetic similarity to mucoepidermoid carcinoma, hidradenoma may be a cutaneous counterpart of salivary gland mucoepidermoid carcinoma.

© 2017 Elsevier Inc. All rights reserved.

[☆] Competing interests: The authors have no conflicts of interest to declare.

^{☆☆} Funding/Support: This study was supported by a JSPS KAKEN grant (25293088, Tokyo, Japan).

* Corresponding author at: Department of Anatomic Pathology, Graduate School of Medical Sciences, Kyushu University, 3-1-1 Maidashi, Higashi-ku, Fukuoka, Fukuoka 812-8582, Japan.

E-mail address: oda@surgpath.med.kyushu-u.ac.jp (Y. Oda).

1. Introduction

Hidradenoma is a benign tumor of cutaneous adnexal origin, presenting as a slow-growing solitary mass, which is characterized by biphasic epithelial components and a solid or cystic structure [1,2]. In the current World Health Organization classification, it is defined as a benign adnexal neoplasm

with a limited degree of ductal differentiation, closely related to eccrine poroma [3]. Hidradenoma usually occurs on the head, neck, and limbs, and its local recurrence is uncommon [1,2]. Although several cases have been described as involving lymphatic vessels and lymph nodes, these cases were considered to involve benign metastasis or a tumor of uncertain malignant potential [4-7].

Although hidradenoma had earlier been considered to be a tumor of eccrine origin [1,8], a previous investigation suggested that it could harbor both apocrine and eccrine features [9]. Histologically, the tumor is composed of several types of cell, namely, clear or pale cells, squamoid polygonal cells, and mucinous cells [1,10,11]. Transition among these cell types is frequently seen. The tumor presents lobular foci of bilayered tumor epithelial cells located in the dermis together with variously sized tubular lumina. In the solid part of the tumor, it consists of various types of epithelial tumor cells such as clear cells, squamoid cells, and mucinous cells in various proportions.

Owing to its histopathological overlap with other skin appendage tumors, it is occasionally challenging to make a correct diagnosis; no reliable diagnostic marker for hidradenoma has yet been established. Some investigations reported the presence of the *CRTC1-MAML2* fusion gene in hidradenoma cases, and the utility of this fusion gene as a diagnostic marker of hidradenoma has drawn attention [12-14]. On the other hand, the *CRTC1/CRTC3-MAML2* fusion gene and rearrangement of the *MAML2* gene have been detected in mucoepidermoid carcinoma (MEC) at a high frequency. About 30%-80% of MEC cases possess the *CRTC1-MAML2* fusion gene, whereas 6% have the *CRTC3-MAML2* fusion gene [15,16].

In this study, we explored *CRTC1*- and *CRTC3-MAML2* fusion genes in hidradenomas, examined the relationship between fusion gene patterns and histopathological features, and investigated their diagnostic significance.

2. Materials and methods

2.1. Patients and tissue samples

This study was conducted in accordance with the principles embodied in the Declaration of Helsinki and was approved by the Ethics Committee of Kyushu University (nos. 25-111 and 25-143). Informed consent was obtained from the subjects or guardians. We examined 39 paraffin-embedded samples diagnosed as primary cutaneous hidradenoma and 1 paraffin-embedded sample of primary cutaneous hidradenocarcinoma registered between 1984 and 2016 at the Department of Anatomic Pathology, Graduate School of Medical Sciences, Kyushu University, Japan. All cases were treated with surgical excision, and the surgical margins were negative in all cases except 1. Clinicopathological data were retrieved from the patient files at the Department of Dermatology, Kyushu University Hospital.

The diagnosis of hidradenoma was made in accordance with the latest edition of the World Health Organization

classification [3]. Based on this classification, we included poroid hidradenoma, which is composed of poroid cells and cuticular cells and which was defined as a variant of poroma by Abenoza and Ackerman [8]. In this study, we classified tumors as “C > P” when the proportion of clear cells was higher than that of polygonal cells and “P > C” when polygonal cells exceeded clear cells. The cases with equal levels of these 2 types of cell were classified as “C = P.”

Clinical details and follow-up information were obtained by reviewing medical records of Kyushu University Hospital. Follow-up information was available for 37 of 39 patients.

2.2. Reverse-transcription polymerase chain reaction for *CRTC1*-, *CRTC2*-, or *CRTC3-MAML2* fusion

Total RNA was isolated from paraffin-embedded tissue using the miRNeasy FFPE Kit (Qiagen, Valencia, CA), and first-strand cDNA was synthesized using Superscript III Transcriptase (Invitrogen, Carlsbad, CA), in accordance with the manufacturer's instructions. To detect *CRTC1*-, 2-, and 3-*MAML2* fusion, we performed reverse-transcription polymerase chain reaction (RT-PCR) using primers and cycle conditions as reported previously [1]. The primer sets are listed in Table 1.

The PCR products were electrophoresed in 2% agarose gel, and the sequence was confirmed by direct sequencing methods using an ABI3500xL genetic analyzer (Applied Biosystems, Foster City, CA). Amplification of 189-base pair (bp) products of the *PGK* gene and 100-bp products of *PGKp* was used to test the quality of the extracted RNA.

2.3. Detection of *MAML2* break by fluorescence in situ hybridization analysis

First, the areas for cell counting were determined by hematoxylin-eosin slide review. Then, a 3- μ m-thick formalin-fixed, paraffin-embedded section was placed onto a positively charged slide. Fluorescence in situ hybridization (FISH) was performed with ZytoLight SPEC *MAML2* Dual Color Break Apart Probe (11q21) (ZytoVision GmbH,

Table 1 Primer list

Gene	Forward/reverse	Primer
<i>PGK</i>	Forward	cagtttgagctcctggaag
<i>PGK</i>	Forward	cagccctaagtcaacttag
<i>PGK</i>	Reverse	ggccaagagatgcagtgccag
<i>CRTC1</i>	Forward	tcgcgctgcacaatcagaag
<i>CRTC1</i>	Forward	gaggtgatgaaggacctgag
<i>CRTC2</i>	Forward	ttgcgctgcagaagcagctg
<i>CRTC2</i>	Forward	ggaggtgatgatggacatcg
<i>CRTC3</i>	Forward	tcgcgctgcacacgcagaga
<i>CRTC3</i>	Forward	cagagacaggccgaggagac
<i>MAML2</i>	Reverse	ggctgcttgcgttggcagg
<i>MAML2</i>	Reverse	ttgctgttggcaggagatag

Bremerhaven, Germany) and the Histology FISH Accessory Kit (ZytoVision GmbH, Bremerhaven, Germany) in accordance with the manufacturer's protocols. After counterstaining with DAPI/DuraTect-Solution (ZytoVision GmbH, Bremerhaven, Germany), we examined the sample under an Olympus BX51 (Olympus, Tokyo, Japan) fluorescence microscope using a $\times 100$ objective. Fifty randomly selected nonoverlapping tumor cell nuclei were examined for the presence of yellow (normal) or green and red (chromosomal breakpoint) fluorescent signals. The sample was considered positive when $>10\%$ of nuclei showed a breakpoint signal.

3. Results

3.1. Clinical and pathological features

Comprehensive clinicopathological data of all 39 hidradenoma patients are shown in Table 2. Twenty patients were male and 19 were female, with a mean age of 51.9 years. Locations included scalp ($n = 8$), face ($n = 8$), extremities ($n = 7$), and trunk ($n = 14$). In 1 case, the location was unknown. All tumors were located in the dermis to subcutis. All cases were treated surgically with curative intent, and the surgical margins were negative in 38 cases. Follow-up information was available for 37 of 39 patients. There were no cases with recurrence or metastasis.

All tumors were well-demarcated masses in the dermis, composed of multiple cells (clear cells and eosinophilic polygonal cells) and accompanied by luminal formation (Fig. 1A–C). There were 8 patients with prominent cystic change (Fig. 1D and E), 2 of whom presented apocrine-like decapitated secretion (Fig. 1F and G). The tumors were initially diagnosed as clear

cell hidradenoma (36 cases) and poroid hidradenoma (3 cases) (Fig. 1H and I, Table 2). Component cells consisted of clear cells or polygonal cells, and there were no cases with mucinous cells. The numbers of cases in the different categories were as follows: clear cell–dominant type (Fig. 1J and K), 9 cases; polygonal cell–dominant type (Fig. 1L and M), 21 cases; and equally mixed type, 9 cases (Table 2).

3.2. CRTC1/3-MAML2 fusion gene

CRTC1-MAML2 fusion transcript was detected in 10 (26%) of the 39 cases, and *CRTC3-MAML2* fusion transcript was detected in 2 (5%) of the 39 cases (Table 2, Fig. 2). Sequence analysis of the PCR products confirmed that, in all cases, exon 1 of *CRTC1* or exon 1 of *CRTC3* was fused to exons 2–5 of *MAML2* (Fig. 2). No *CRTC1/3-MAML2* fusion gene was detected in the cases diagnosed as poroid hidradenoma.

3.3. FISH for the MAML2 gene

FISH for the breaking apart of *MAML2* was performed on 27 specimens of RT-PCR–negative cases and 1 specimen of an RT-PCR–positive case, and a split signal was detected in 12 cases. *MAML2* gene split signals were found in $>60\%$ of tumor cells (average 33.6 of 50 cells, median 35) (Fig. 3); however, in only 1 case were the split signals present in 15% of the tumor cells. Gene rearrangement was observed in all of the clear cells and polygonal cells, and there was no relationship between the existence of a split signal and the kind of component cells. In contrast, no *MAML2* gene rearrangement was observed in 6 tumors with prominent cyst formation and stromal cells in all examined cases.

3.4. Statistical analysis

We analyzed the relationship between clinical matters and presence or absence of expression of *MAML2* fusion gene. Regarding recurrence and metastasis, there were no cases and analysis was impossible. Statistical analysis of the size of the 33 available cases was as follows; in the *MAML2* gene rearrangement–positive cases, the average of major diameter was 21.5 mm, and in the case of *MAML2* gene rearrangement–negative cases, it was 24.7 mm. There was no statistical difference of tumor size between the fusion gene–positive and –negative cases by Student *t* test.

The 2 types of fusion transcripts were mutually exclusive in individual cases. Among the component cells, there was no histopathological difference between *MAML2* fusion–positive and –negative cases, between the cases with *CRTC1-MAML2* and *CRTC3-MAML2* fusion genes, and between the exon variants of *CRTC1/3-MAML2* fusion genes (Table 3). Tumors with prominent cystic change lacked the fusion genes and gene rearrangement, with statistical significance ($P < .0001$, Fisher exact test).

Table 2 Clinicopathological data

Age, y (range, mean $\pm$ SD)	6–85 (51.9 $\pm$ 20.3)
Sex, n (%)	
Male	20 (51.3)
Female	19 (48.7)
Tumor site, n (%)	
Scalp	8 (20.5)
Face	7 (17.9)
Trunk	10 (25.6)
Extremities	13 (33.3)
Unknown	1 (2.6)
Size, mm (range, mean $\pm$ SD)	5–60 (17 $\pm$ 14.4)
Component cell, n (%)	
C > P	9 (23.0)
C = P	9 (23.0)
P > C	21 (53.8)
Pathological diagnosis, n (%)	
Clear cell hidradenoma	36 (92.3)
Poroid hidradenoma	3 (7.7)

Abbreviation: C, clear cell, P, polygonal cell.

Fig. 1 The variety of histological findings was as follows: A, Clear cell > polygonal eosinophilic cell type (case 34); *CRTC3-MAML2* fusion was detected in this lesion. B, Clear cell component (case 34). C, Polygonal cell component (case 34). D and E, Lesions mainly involving cyst formation (case 19); no *CRTC-MAML2* fusion was detected in this lesion. F and G, Presenting apocrine-like decapitated secretion (case 28); no *CRTC-MAML2* fusion was detected in this lesion. H, Poroid hidradenoma (Case 32). I, It consisted of poroid cells and cuticular cells (case 32); no *CRTC-MAML2* fusion was detected in this lesion. J and K, Clear cell > polygonal eosinophilic cell type (case 35); *CRTC1-MAML2* fusion was detected in this lesion. L and M, Polygonal eosinophilic cell > clear cell type (case 2).

4. Discussion

Historically, hidradenoma was considered to be a tumor of eccrine origin [1,8]; however, a recent investigation suggested that it can harbor both apocrine and eccrine features. Moreover, the tumor presents a distinct histologic appearance characterized by lobules composed of epithelial cells of at least 2

types: myoepithelial cells and glycogen-containing clear cells. Therefore, it was thought to be a variation of myoepithelioma [17]. At present, the origin of hidradenoma is estimated to be outer cells of the sweat gland from the sweat duct, which are thought to be stem cells that are closer anatomically to the deep secretory part than poroma. The results in the present study confirmed that the tumor cells presented a variety of types of

Fig. 2 The results of RT-PCR and direct sequencing. The conspicuous bands of *CRTC1/3-MAML2* transcriptional product were highlighted by UV illumination, and direct sequencing confirmed them (case 33 and case 34).

epithelial differentiation and that all of the hidradenomas occurred in the dermis. However, the etiology and mechanism of tumorigenesis in this condition could not be characterized.

Fig. 3 The results of FISH. FISH revealed split signals of the *MAML2* gene (see arrows) in about 67.2% of the tumor cells (case 10).

Although it is occasionally challenging to diagnose hidradenoma because of its histological overlap with other skin appendage tumors, no useful diagnostic marker for hidradenoma has yet been established. According to an investigation reported in 2005, the existence of the *CRTC1-MAML2* fusion gene was demonstrated in hidradenomas of the skin [12], and the rate of cases with this fusion gene was subsequently reported to be about 50% [13,14]. There were cases with *MAML2* gene rearrangement carrying unknown patterns of the *CRTC1-MAML2* fusion gene, and there might be more unknown patterns of *MAML2*-associated fusion genes. In this study, we reviewed 39 cases of hidradenoma by gene analysis of *CRTC1*-, *CRTC2*-, and *CRTC3-MAML2* fusions using RT-PCR. The *CRTC1-MAML2* and *CRTC3-MAML2* fusion transcripts were detected in 26% and 5% of hidradenoma cases, respectively. These 2 fusions were mutually exclusive. To the best of our knowledge, this is the first report describing the detection of *CRTC3-MAML2* fusion in hidradenoma. Together with the results of FISH, 23 of the 39 cases (59%)

Table 3 Factors associated with *CRTC1/3-MAML2* expression

Parameters	<i>CRTC1-MAML2</i>	<i>CRTC3-MAML2</i>	<i>MAML2</i> rearrangement	Positive	Negative	Total
Component cell						
C > P	2	1	4	7	2	9
C = P	2	0	3	5	4	9
P > C	6	1	4	11	10	21
Total	10	2	11	23	16	39
Average of major diameter (mm)				21.5	24.7	
Pathological diagnosis						
Clear cell hidradenoma	10	2	11	23	13	36
Poroid hidradenoma	0	0	0	0	3	3
Total	10	2	11	23	16	39

possessed *MAML2* gene translocation. The rate at which the *CRTC1-MAML2* fusion gene was present is almost the same as described in previous reports [13,14]. The results of this study suggest that *CRTC1/3-MAML2* fusion gene analysis and *MAML2* gene FISH would be useful methods to diagnose hidradenoma. Meanwhile, hidradenoma should be diagnosed based on histopathological findings because of the absence of *MAML2* gene translocation in about half of cases.

A previous report showed the relationship between histology and genetics in hidradenoma and revealed the tendency for the clear cell variant to be exhibited in *CRTC1-MAML2* fusion gene-positive cases [13]. In this study, we examined the relationship between histopathological findings and genetics and demonstrated negativity for a fusion gene in predominant cystic tumors and poroid hidradenomas. There was no statistically significant relationship between the existence of gene rearrangement and other histological features. Poroid hidradenoma was considered to be a variant of poroma by Abenoza and Ackerman [8], and the results obtained in this study may confirm the preexisting concept that poroid hidradenoma is different from hidradenoma from a genetic perspective. Prominent cystic change of hidradenoma is a distinctive characteristic from ordinary hidradenoma, and the finding of fusion gene negativity may suggest that hidradenoma with prominent cystic change has a genetically different background from ordinary hidradenoma. It was suggested that the *CRTC1/3-MAML2* fusion may be an important driver event in the development of tumors with a glandular morphology but not sufficient for malignant transformation.

Salivary gland tumors and skin appendage tumors are similar to each other; in recent years, it has become well known that polymorphic adenoma and skin mixed tumors are essentially the same tumor [18]. In addition, some tumors have the common features of arising at salivary glands and skin appendages, such as mixed tumor, adenoid cystic carcinoma, primary cutaneous adenoid cystic carcinoma, and cylindroma [19,20]. Mucoepidermoid carcinoma is a relatively common tumor in salivary glands, whereas there are currently far fewer reports of MEC of the skin. It was recognized that hidradenoma histologically resembles MEC and that they share a genetic feature, *CRTC1-MAML2*, although they have different

malignant potentials. In this investigation, the existence of the *CRTC3-MAML2* fusion gene was demonstrated as a new pattern of *MAML2*-associated gene fusion in hidradenoma. The relationship between hidradenoma and MEC was thought to be more intimate because they share the same pattern of a chimeric gene. Hidradenoma may thus be a genuine cutaneous counterpart of MEC of the salivary glands. On the other hand, their distinct classifications should be retained because of their differences in biological behavior.

From now on, emphasis should be placed on genetic diagnosis in the diagnostic pathology of the skin, as well as in that of lymphoma and soft-tissue sarcoma. In common with MEC, the fusion genes of *CRTC1* and *CRTC3* are expressed in a mutually exclusive manner, and the proportions of the tumors harboring the above translocations are similar to each other. Previous observations indicated that *CRTC1-MAML2* fusion was etiologically linked to benign and low-grade malignant tumors originating from diverse exocrine glands [13,14,21]. Cases expressing the *CRTC3-MAML2* fusion gene in MEC are associated with good clinicopathological features with a younger age at onset than *CRTC1-MAML2* cases [16]. The same phenomena may occur in hidradenoma; however, the accumulation of further cases is necessary because we detected only 2 cases with the *CRTC3-MAML2* fusion gene in this investigation.

In conclusion, we detected an unknown pattern of gene fusion, namely, the *CRTC3-MAML2* fusion gene, in hidradenoma. Hidradenoma may be a cutaneous counterpart of salivary gland MEC in view of their histological and genetic similarity.

References

- [1] Winkelmann RK, Wolff K. Histochemistry of hidradenoma and eccrine spiradenoma. *J Invest Dermatol* 1967;49:173-80.
- [2] Johnson Jr BL, Helwig EB. Eccrine acrospiroma: a clinicopathologic study. *Cancer* 1969;23:641-57.
- [3] Leboit PE, Burg G, Weedon D, et al, editors. World Health Organization classification of tumours, pathology & genetics, skin tumours. Lyon: IARC Press; 2006. p. 143.

- [4] Hernández-Pérez E, Cestoni-Parducci R. Nodular hidradenoma and hidradenocarcinoma. A 10-year review. *J Am Acad Dermatol* 1985;12:15-20.
- [5] Stefanato CM, Ferrara G, Chaudhry IH, et al. Clear cell nodular hidradenoma involving the lymphatic system: a tumor of uncertain malignant potential or a novel example of “metastasizing” benign tumor? *Am J Surg Pathol* 2012;36:1835-40.
- [6] Au B, Sidiropoulos KG, Ghazarian D, Sidiropoulos M. Lymph node involvement by a clear cell nodular hidradenoma-like tumor of uncertain malignant potential. *Am J Dermatopathol* 2016. <https://doi.org/10.1097/DAD.0000000000000808> [Epub ahead of print].
- [7] Tingaud C, Costes V, Frouin E, et al. Lymph node location of a clear cell hidradenoma: report of a patient and review of literature. *J Cutan Pathol* 2016;43:702-6.
- [8] Abenzo P, Ackerman AB. Hidradenomas. In: Abenzo P, Ackerman AB, editors. *Neoplasms with eccrine differentiation*. Philadelphia: Lea and Febiger; 1990. p. 311-50.
- [9] Lim SC, Lee MJ, Lee MS, Kee KH, Suh CH. Giant hidradenocarcinoma: a report of malignant transformation from nodular hidradenoma. *Pathol Int* 1998;48:818-23.
- [10] Czamecki DB, Aarons I, Dowling JP, Lauritz B, Wallis P, Taft EH. Malignant clear cell hidradenoma: a case report. *Acta Derm Venereol* 1982; 62:173-6.
- [11] Park HJ, Kim YC, Cinn YW. Nodular hidradenocarcinoma with prominent squamous differentiation: case report and immunohistochemical study. *J Cutan Pathol* 2000;27:423-7.
- [12] Behboudi A, Winnes M, Gorunova L, et al. Clear cell hidradenoma of the skin-a third tumor type with a t(11;19)-associated TORC1-MAML2 gene fusion. *Genes Chromosomes Cancer* 2005;43:202-5.
- [13] Winnes M, Mölne L, Suurküla M, et al. Frequent fusion of the CRTC1 and MAML2 genes in clear cell variants of cutaneous hidradenomas. *Genes Chromosomes Cancer* 2007;46:559-63.
- [14] Kyrpychova L, Kacerovska D, Vanecek T, et al. Cutaneous hidradenoma: a study of 21 neoplasms revealing neither correlation between the cellular composition and CRTC1-MAML2 fusions nor presence of CRTC3-MAML2 fusions. *Ann Diagn Pathol* 2016; 23:8-13.
- [15] Nakano T, Yamamoto H, Hashimoto K, et al. HER2 and EGFR gene copy number alterations are predominant in high-grade salivary mucoepidermoid carcinoma irrespective of MAML2 fusion status. *Histopathology* 2013;63:378-92.
- [16] Nakayama T, Miyabe S, Okabe M, et al. Clinicopathological significance of the CRTC3-MAML2 fusion transcript in mucoepidermoid carcinoma. *Mod Pathol* 2009;22:1575-81.
- [17] Lever W, Castleman B. Clear cell myoepithelioma of the skin. *Am J Pathol* 1952;28:691-9.
- [18] Hirsch P, Helwig EB. Chondroid syringoma. Mixed tumor of skin, salivary gland type. *Arch Dermatol* 1961;84:835-47.
- [19] North JP, McCalmont TH, Fehr A, van Zante A, Stenman G, LeBoit PE. Detection of MYB alterations and other immunohistochemical markers in primary cutaneous adenoid cystic carcinoma. *Am J Surg Pathol* 2015;39:1347-56.
- [20] Fehr A, Kovács A, Löning T, Frierson Jr H, van den Oord J, Stenman G. The MYB-NFIB gene fusion-a novel genetic link between adenoid cystic carcinoma and dermal cylindroma. *J Pathol* 2011;224:322-7.
- [21] Larsen WJ. *Human embryology*. 3rd ed. Philadelphia: Churchill Livingstone; 2001. p. 351-78 and p. 465-80.