Malignant Proliferating Trichilemmal Tumors with CD34 Expression

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Malignant proliferating trichilemmal tumors (MPTT) are rare neoplasm arising from outer root sheath of hair follicle, the diagnosis of which is base essentially on histological features resulting in occasionally misdiagnosis of squamous cell carcinoma. In difficult cases, however, evaluation of additional parameters may be needed to differentiate benign proliferating trichilemmal tumor from MPTT or differentiate PTT and MPTT from squamous cell carcinoma. We report two cases of MPTT on which, in addition to histologic features, we have determined p53 immunohistochemical expression pattern, proliferative fraction, and CD34 expression. For comparison, concurrent proliferating trichilemmal tumors (PTT) and trichilemmal cysts (TC) as well as well-differentiated squamous cell carcinoma (SCC) were studied. The two MPTTs show expression of p53 with increased proliferative index as well as all three SCC. The PTTs and TCs stained negative and few basal cells for p53 and Ki-67, respectively. MPTTs exhibit CD34 immunoreactivity, indicating trichilemmal differentiation. The contrast p53 and Ki-67 expression pattern in MPTT and PTT may be helpful in the diagnosis of MPTT. Expression of CD34 may be an additional feature to distinguish MPTT from SCC.

Keywords: *Malignant proliferating trichilemmal tumor, CD34, p53*

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Proliferating trichilemmal tumor is a rare, usually benign, tumor of outer root sheath derivation. PTT occurs predominantly over the scalp of elderly women[1-4]. The histologic hallmark of PTT is the presence of trichilemmal keratinization-the abrupt transition of a nucleated epithelial cell to an anucleated, keratinized cell without the formation of granular layer[5]. More aggressive biological behavior, including capacity of invasion and metastasis[4-11,20,21], is seen in malignant proliferating trichilemmal tumor. The designation of MPTT has been made exclusively on a histologic basis of irregular infiltration into the surrounding dermis with a desmoplastic stromal response[6,19]. Ki-67 staining, used in combination with mitotic rate count to measure proliferation index, was increased in aneuploid MPTT[12-14]. Immunoreactivity for *p53* was detected in one instance of MPTT[15]. This positive *p53* immunostaining may be the reflection of a *p53* gene mutation in MPTT. In addition, loss of wild-type *p53* has been reported in association with malignant transformation of PTT[16]. PTT, both benign and malignant counterpart, is often confused histologically with squamous cell carcinoma. Area of trichilemmal keratinization is very helpful to distinguish PTT from SCC[4,6]. Immunohistochemical study was provided a useful tool in distinguishing MPTT from SCC. Regardless of atypia or invasion properties, most PTT showed positive staining for AE13 and AE14, monoclonal antibodies directed at pilar-type keratin polypeptides while SCC showed no staining[6]. Furthermore, some instances showed CD34, a marker of outer root sheath differentiation, immunoreactivity in PTT and carcinoma arising in PTT[17,18]. However, tumor with poorly differentiation was negative for CD34[14].

We have reported another two cases of MPTT. In addition to the clinicopathological features, we have analyzed proliferation index, *p53*, and CD34 immunoreactivity and compare these antigenic profiles

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with conventional TC, PTT, and SCC arising on scalp.

Material and Methods

Materials

Patient 1 was a 58-year-old Thai woman who had a non-ulcerated subcutaneous mass of left occiput measuring 3.2 x 2.2 x 1.5 cm, which had grown for 1 year. Her medical history was not contributory. No history suggestive of trauma and chronic irritation. An excision of the tumor was performed and a biopsy specimen was diagnosed as a metastatic squamous cell carcinoma. The patient was referred to the department of otolaryngology of our hospital for further investigation and treatment. Upon examination, there was a residual tumor close to the sutured wound, measuring 1.0x0.6x0.5 cm. No other regional lymphadenopathy was found. No evidence of metastasis was detected by chest roentgenogram, CT scan of the neck, MRI of the brain, bone scan, and ultrasonography of the upper abdomen. The residual mass was excised with 1 centimeter margin.

The previous-excised specimen was reviewed together with the residual tumor. Both of them showed typical features of MPTT. Grossly, the residual tumor was nodular and located in the subcutis. The cut surface was yellow to white. Microscopic examination revealed a lobulated expansive mass of squamous epithelium without direct connection to the overlying epidermis. The lobules separated by loose edematous stroma and filled centrally with homogeneous acellular eosinophilic material derived from trichilemmal keratinization (Fig.1). In addition, the squamoid tumor cells manifested large, hyperchromatic nuclei with irregular nuclear membranes surrounded by abundant eosinophilic cytoplasm. Foci of single cell necrosis and abrupt keratinization were identified. Some cells showed clear or vacuolated cytoplasm but not predominate. Within the bands of squamoid cells, smaller basaloid cells were seen palisading at the periphery. The periphery of the bands and lobules also exhibited a thick, hyaline, eosinophilic basement membrane which was focally disrupted by cords of atypical squamous epithelium (Fig. 2).

Cords of atypical squamous epithelium extended into the surrounding dermis with a desmoplastic stromal response (Fig. 3). Numerous mitoses, many of which were atypical, were detected, averaging 10-15 mitoses per 10 high-power fields (Fig. 4). The margins of the previous-excised specimen contained the neoplastic cells. The second excision showed tumor-free margins.

Patient 2 was a 41-year-old Thai woman

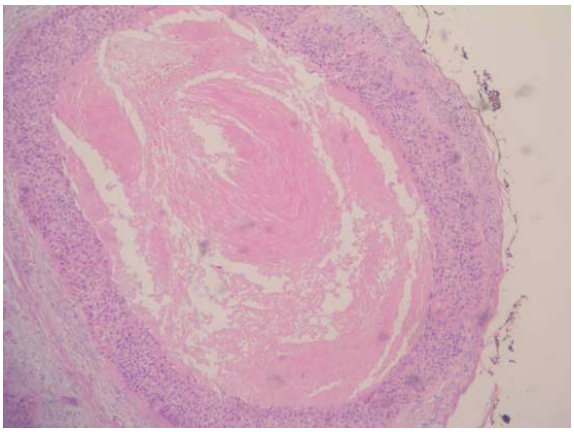

Fig.1B. Central cystic formation with internal pilar-type keratin

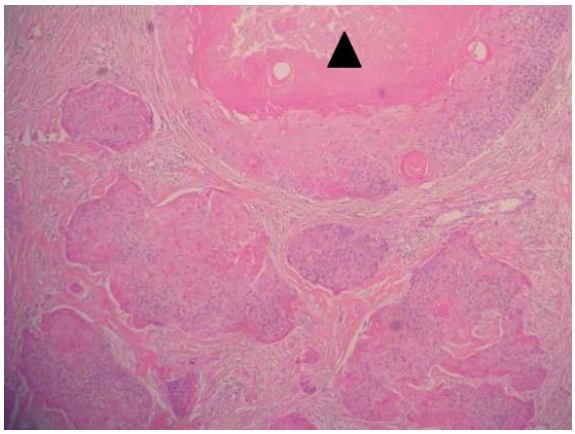

Fig.1 A. Irregular lobules and islands of squamous epithelium with abrupt central keratinization (“)

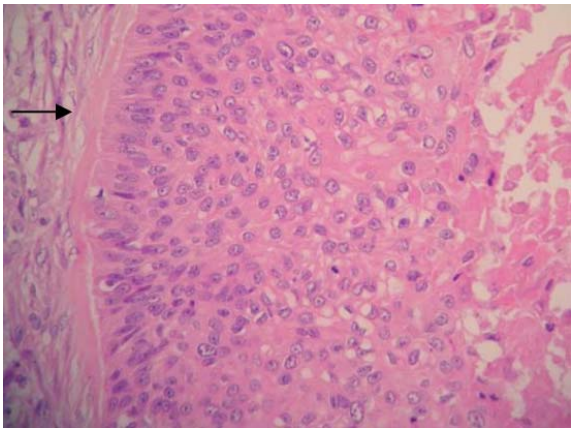

Fig.2. Eosinophilic thickened basement membrane and peripheral palisading

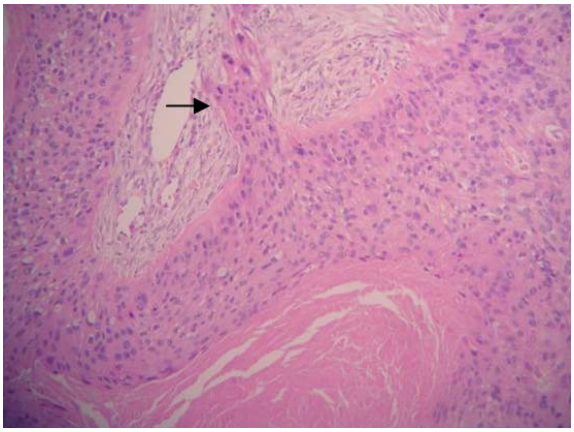

Fig. 3. Invasion of squamous epithelium into surrounding tissue

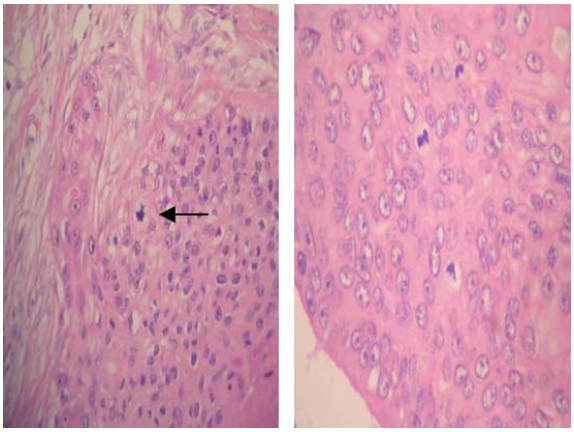

Fig. 4. Nuclear pleomorphism, numerous mitoses, and atypical mitotic figure (arrow)

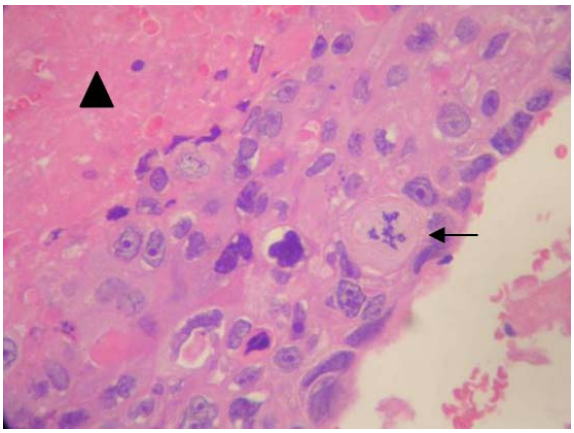

Fig. 5. A band of squamous epithelium showing trichilemmal keratinization (“). Cells show maked pleomorphism, hyperchromasia, and atypical mitosis (arrow)

present with a scalp mass at postauricular area which had been slow growing for 1 year. One month prior to her evaluation, she noticed a rapid increase in size. The patient had a history of a breast carcinoma 6 years ago, which was treated by modified radical mastectomy, radiotherapy, and chemotherapy. Currently, the patient has no evidence of disease. She had no prior trauma to this area, and had no prior dermatologic complaints. The lesion was locally excised and clinically thought to be a metastatic lesion of the breast carcinoma. The previous-excised specimen was diagnosed as a MPTT. The patient was referred to the department of plastic surgery of our hospital for proper management. Physical examination revealed a residual tumor in the previous-biopsy site, measuring 1.0 x 0.8 x 0.6 cm. No palpable regional lymph node was found. No evidence of metastatic was detected by chest roentgenogram and bone scan. The residual mass was excised with 1-cm margin and reached the skull.

Both residual mass and previous-excised specimen were examined microscopically. They showed typical features of MPTT. Grossly, the residual tumor was yellow-to-white rubbery ill-defined nodule and located in subcutis. The overlying epidermis showed a linear surgical scar. Microscopic examination of both specimens revealed variable-sized lobules, nests, and bands composed of squamous epithelium that was well demarcated from surrounding tissue with abrupt central keratin formation. Individual cell keratinization and squamous eddy formation were also detected. Peripheral palisading was occasionally present and bands were often surrounded by thickened refractile basement membrane. The tumor cells showed

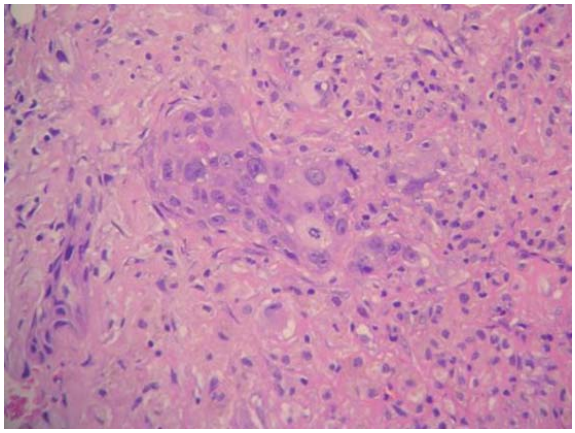

Fig. 6. Cord of atypical squamous epithelium showing stromal invasion with desmoplastic reaction. Abnormal mitosis also seen

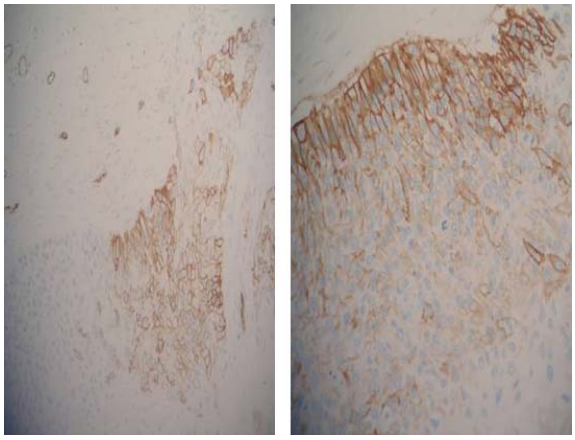

Fig.7. Case-1 MPTT showing strong cell membrane-staining with monoclonal antibody CD34 in patchy pattern

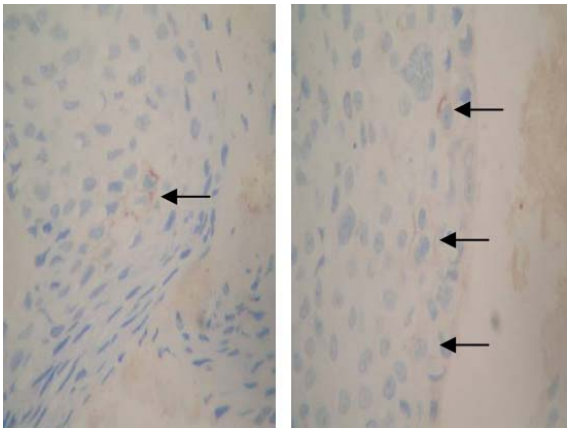

Fig. 8. Case-2 MPTT exhibits rare tumor cells immunoreactivity for CD34 (arrow)

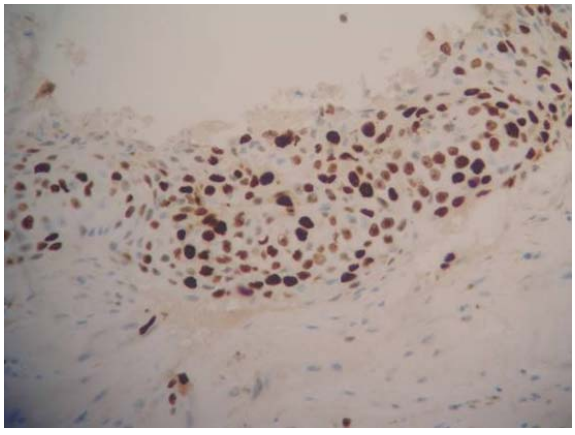

Fig. 9. Immunoreactivity for Ki-67 of MPTT

moderated to marked pleomorphism, hyperchromasia, and high mitotic activity (18-20 mitoses per 10 high-power fields) with atypical mitotic figures (Fig. 5). Foci of dermal invasion by cords of atypical tumor cells were observed (Fig. 6). The first-excised specimen margins of resection were involved by tumor cells. The re-excised specimen showed negative margins. A diagnosis of MPTT was then made.

Methods

Case studied

Skin biopsy paraffin blocks were retrieved from the files of the department of Anatomical pathology, Army Institute of Pathology, Bangkok, Thailand. For further comparison, we evaluated the 2-reported cases of MPTT, 3 cases of PTT, 3 cases of TC, and 3 cases of well-differentiated SCC from scalp. All tissue specimens had been fixed in neutral-buffered formalin and routinely processed.

Immunohistochemistry

Immunohistochemical studies were performed using anti-p53 protein mouse monoclonal antibody, clone BP53-12 (BioGenex, California, USA, diluted 1:1000 with PBS), monoclonal mouse antibody against CD34 (QBEND/10, DakoCytomation, Denmark, diluted 1:500), and Ki-67 (BioGenex, California, USA)

Results

Both cases of MPTT show variable expression pattern of CD34 from less than 1% (case 2, Fig. 8) to 20% (case 1, Fig. 7), which are suggested as degree of differentiation toward outer root sheath of hair follicle.

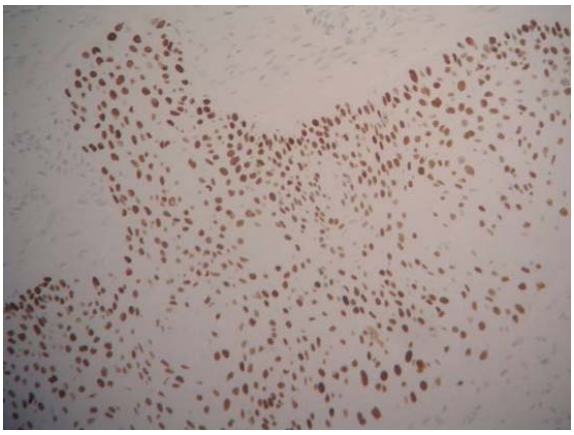

Fig. 10. Diffuse and strongly nuclear staining of p53 in both MPTTs

However, all positive cells in both cases showed definitely membrane staining and normal vascular endothelial cells as well as normal outer root sheath cells, which were served as internal control, were strongly positive for CD34.

In contrast, in all 3 cases of SCC as well as all 3 cases of TC and PTT were negative for CD34 staining.

Ki-67 positivity showed a significantly increasing in both MPTTs (Fig. 9), where as TCs and PTTs showed only focal nuclear staining of basal cells. The same Ki-67 staining pattern as the both MPTTs was seen in 3 cases of SCC.

Immunoreactivity for *p53* was detected in all cases of MPTT and SCC in strongly diffuse nuclear staining pattern (Fig. 10); where as only one of 3 cases of PTT contained isolated *p53* positive cells which were restricted to the basal layer. All TCs and the rest of PTTs were negative for *p53* immunostaining.

Discussion

The proliferating trichilemmal tumor occurs most commonly as a solitary tumor on the scalp of elderly women[22]. They are thought to initially develop as a focus of epithelial proliferation in trichilemmal cyst[23]. Perhaps as a consequence of trauma or chronic inflammation, but it may arise de novo or develop from an organoid nevi[24]. True malignant transformation is rare. The histologic characteristics of MPTT are a high mitotic rate, atypical mitotic figures, nuclear pleomorphism, tumor invasion in adjacent structures, and the presence of metastatic lesions.

Controversy exists over the histologic criteria of the diagnosis of malignant versus benign lesions. The notion that marked nuclear pleomorphism and mitotic activity, indistinguishable from squamous cell carcinoma, are allowable in benign PTT was advanced by Brownstein and Arluk[3]. There is no question that significant cytologic atypia may be present in PTT that ultimately have a benign outcome. However, it should be noted that the case of Mori et al, which resulted in patient death from metastatic disease did not show infiltrative growth.

Immunohistochemical studies may provide a useful tool in detecting malignancy. Staining against proliferating cell nuclear antigen in TCs and benign PTTs is positive in only the basal layer, while PTTs with either focal areas of malignant change or overt carcinoma both exhibit increased staining in the malignant areas[12], as illustrated by the 2 reported-cases and the six–comparative-cases of TC and PTT.

Furthermore, in a previous study of a series of cutaneous tumors, positive immunostaining for *p53* was detected in one instance of MPTT[15]. The 2 reported-cases of MPTT showed strongly diffuse nuclear staining for *p53*, which was contrast to the six-comparative-cases of TC and PTT. Although one case of PTT contained *p53*-positive cells, the staining pattern differs from those shown in the MPTT as well as SCC.

Our 2 cases of MPTT showed CD34 immunoreactivity supporting the differentiation toward the outer root sheath. These results accordance with Haas et al[18], who elucidated a case of anaplastic carcinoma arising in proliferating trichilemmal cyst. The lesion showed expression of CD34, indicating trichilemmal differentiation. With regard to CD34 expression, although all two cases were morphologically definite MPTT these results are similar to those found previously in anaplastic carcinoma in proliferating trichilemmal cyst.

The immunohistochemical studies result are summarized in the table.
Summary of results

	p53	Ki-67	CD34
Trichilemmal cyst (3)	Negative (3)	Basal cell positive <5% (3)	Negative (3)
Proliferating trichilemmal tumor (3)	Negative (2) Isolated basal cell weakly positive (1)	Basal cell positive <5% (3)	Negative (3)
Malignant proliferating trichilemmal tumor (2)	Diffuse and strongly positive (2)	30% (1) 40% (1)	Patchy positive 20% (1) Rarely positive <1% (1)
Well differentiated squamous cell carcinoma (3)	Diffuse and strongly positive	60% (1) 20% (1) 30% (1)	Negative (3)

*( ) = number of case

CD34-negative MPTT has been reported[14]. Our two cases, however, showed numerical difference of CD34 immunoreactivity. We interpret this variation of CD34 expression as a consequence of degree of tumor differentiation which ranges from CD34-negative undifferentiated phenotype to strongly-CD34-positive well-differentiated phenotype. Presence of CD34 immunoreactivity may well be of value in distinction between MPTT and other lesions that can have a similar histopathological picture such as squamous cell carcinoma. However, a negative result of CD34 immunostaining should be interpreted with caution.

In conclusion, combination of Ki-67 and *p53* immunohistochemical studies may provide a useful data which help to distinguish MPTT from benign PTT but should be evaluated in cases with histological features of hesitant malignancy. The variation of CD34 expression suggests that there is the degree of tumor differentiation. Presence of CD34 immunoreactivity may help to distinguish MPTT from squamous cell carcinoma.

References

1. Jones EW. Proliferating epidermoid cysts. Arch Dermatol 1966; 94: 11-9.
2. Dabska M. Giant hair matrix tumor. Cancer 1971; 28: 701-6.
3. Brownstein MH, Arluk DJ. Proliferating trichilemmal cyst: a simulant of squamous cell carcinoma. Cancer 1981; 48: 1207-14.
4. Sau P, Graham JH, Helwig EB. Proliferating epithelial cysts. Clinicopathological analysis of 96 cases. J Cutan Pathol 1995; 22: 394-406.
5. Satyaprakash AK, Sheehan DJ, Sangueza OP. Proliferating trichilemmal tumors: a review of the literature. Dermatol Surg 2007; 33: 1102-8.
6. Ye J, Nappi O, Swanson PE, Patterson JW, Wick MR. Proliferating pilar tumors: a clinicopathologic study of 76 cases with a proposal for definition of benign and malignant variants. Am J Clin Pathol 2004; 122: 566-74.
7. Folpe AL, Reisenauer AK, Mentzel T, Rutten A, Solomon AR. Proliferating trichilemmal tumors: clinicopathologic evaluation is a guide to biologic behavior. J Cutan Pathol 2003; 30: 492-8.
8. Amaral AL, Nascimento AG, Goellner JR. Proliferating pilar (trichilemmal) cyst. Report of two cases, one with carcinomatous transformation and one with distant metastases. Arch Pathol Lab Med 1984; 108: 808-10.
9. Batman PA, Evans HJ. Metastasising pilar tumour of scalp. J Clin Pathol 1986; 39: 757-60.
10. Weiss J, Heine M, Grimmel M, Jung EG. Malignant proliferating trichilemmal cyst. J Am Acad Dermatol 1995; 32: 870-3.
11. Park BS, Yang SG, Cho KH. Malignant proliferating trichilemmal tumor showing distant metastases. Am J Dermatopathol 1997; 19: 536-9.
12. Rutty GN, Richman PI, Laing JH. Malignant change in trichilemmal cysts: a study of cell proliferation and DNA content. Histopathology 1992; 21: 465-8.
13. Sleater J, Beers B, Stefan M, Kilpatrick T, Hendricks J. Proliferating trichilemmal cyst. Report of four cases, two with nondiploid DNA content and increased proliferation index. Am J Dermatopathol 1993; 15: 423-8.
14. Herrero J, Monteagudo C, Ruiz A, Llombart-Bosch A. Malignant proliferating trichilemmal tumours: an histopathological and immunohistochemical study of three cases with DNA ploidy and morphometric evaluation. Histopathology 1998; 33: 542-6.
15. Urano Y, Oura H, Sakaki A, Nagae H, Matsumoto K, Fukuhara K, et al. Immunohistological analysis of P53 expression in human skin tumors. J Dermatol Sci 1992; 4: 69-75.
16. Takata M, Rehman I, Rees JL. A trichilemmal carcinoma arising from a proliferating trichilemmal cyst: the loss of the wild-type p53 is a critical event in malignant transformation. Hum Pathol 1998; 29: 193-5.
17. Poblet E, Jimenez-Acosta F, Rocamora A. QBEND/10 (anti-CD34 antibody) in external root sheath cells and follicular tumors. J Cutan Pathol 1994; 21: 224-8.
18. Haas N, Audring H, Sterry W. Carcinoma arising in a proliferating trichilemmal cyst expresses fetal and trichilemmal hair phenotype. Am J Dermatopathol 2002; 24: 340-4.
19. Mehregan AH, Lee KC. Malignant proliferating trichilemmal tumors—report of three cases. J Dermatol Surg Oncol 1987; 13: 1339-42.
20. Saida T, Oohara K, Hori Y, Tsuchiya S. Development of a malignant proliferating trichilemmal cyst in a patient with multiple trichilemmal cysts. Dermatologica 1983; 166: 203-8.
21. Mathis ED, Honningford JB, Rodriguez HE, Wind KP, Connolly MM, Podbielski FJ. Malignant proliferating trichilemmal tumor. Am J Clin Oncol 2001; 24: 351-3.
22. Lever WF, Schaumburg-Lever G. Tumours of the

epidermal appendages. In: Lever WF, Schaumburg-Lever G, editors. Lever's histopathology of the skin. 7th ed. Philadelphia: JB Lippincott; 1990: 589-91.
23. Poiares Baptista A, Garcia E Silva L, Born MC. Proliferating trichilemmal cyst. J Cutan Pathol 1983; 10: 178-87.
24. Rahbari H, Mehregan AH. Development of proliferating trichilemmal cyst in organoid nevus. Presentation of two cases. J Am Acad Dermatol 1986; 14: 123-6.
25. Mori O, Hachisuka H, Sasai Y. Proliferating trichilemmal cyst with spindle cell carcinoma. Am J Dermatopathol 1990; 12: 479-84.

เนื้องอก Proliferating Trichilemmal ชนิดร้ายแรงมีการแสดงออกของ CD34

โกสินทร์ ชัยชำนาญ, กัลตังต์ สัตยสุนทร, สุรศักดิ์ พุทธานุภาพ, อัครเดช อัตถะอินทรีย์,

เนื้องอก proliferating trichilemmal ชนิดร้ายแรงเป็นเนื้องอกที่พบได้ยาก กำเนิดจากเยื่อบุรากขนชั้นนอกของรูขุมขน การวินิจฉัยโรคนี้อาศัยลักษณะทางจุลกายพยาธิวิยาเป็นสำคัญ ทำให้ในบางครั้งมีการวินิจฉัยผิดพลาดเป็น squamous cell carcinoma. อย่างไรก็ตามในกรณีที่ยากต่อการวินิจฉัยการประเมินลักษณะเพิ่มเติมอื่นอาจจำเป็นต่อทั้งการแยกเนื้องอก proliferating trichilemmal ชนิดไม่ร้ายแรงออกจากชนิดร้ายแรง และการแยกเนื้องอก proliferating trichilemmal ออกจาก squamous cell carcinoma ในที่นี้เรารายงานเนื้องอก proliferating trichilemmal ชนิดร้ายแรง 2 กรณีและมีการศึกษารูปแบบการแสดงออกทาง immunohistochemistry ของ CD34, p53, และดัชนีการเพิ่มจำนวนเพิ่มเติมจากลักษณะทางพยาธิวิทยา นอกจากนี้เรายังศึกษา เนื้องอก proliferating trichilemmal ชนิดไม่ร้ายแรงและถุงน้ำ trichilemmal รวมทั้ง squamous cell carcinoma เพื่อเปรียบเทียบกันอีกด้วย ผลการศึกษาพบว่าเนื้องอก proliferating trichilemmal ชนิดร้ายแรงทั้งคู่มีดัชนีการเพิ่มจำนวนสูงขึ้นและมีการแสดงออกของ p53 เป็นไปในทางเดียวกันกับ squamous cell carcinoma ส่วนเนื้องอก proliferating trichilemmal ชนิดไม่ร้ายแรงและถุงน้ำ trichilemmal ไม่พบการแสดงออกของ p53 และดัชนีกานเพิ่มจำนวนไม่ได้สูงขึ้น รวมทั้งยังพบการแสดงออกของ CD34 ในเนื้องอก proliferating trichilemmal ชนิดร้ายแรง ซึ่งบ่งชี้ถึงพัฒนาการจากรูขุมขน สรุปได้ว่าเนื้องอก proliferating trichilemmal ชนิดไม่ร้ายแรงและชนิดร้ายแรงมีรูปแบบการติดสีของ Ki-67 และ p53 ที่ตรงข้ามกัน ลักษณะเช่นนี้อาจช่วยในการวินิจฉัยเนื้องอก proliferating trichilemmal ชนิดร้ายแรง นอกจากนี้การแสดงออกของ CD34 ในเนื้องอก proliferating trichilemmal ชนิดร้ายแรงอาจเป็นลักษณะเพิ่มเติมที่ช่วยแยก เนื้องอก proliferating trichilemmal ชนิดร้ายแรงกับ squamous cell carcinoma