Immunohistochemical Study on Expression of CD34 in Tumors with Follicular Differentiation

Yong Gwan Baik, M.D., Ho Gyun Lee, M.D., Hye Rim Park, M.D.*,
Jin Hee Sohn, M.D.*, Jong Min Kim, M.D.

Departments of Dermatology and Pathology, Hallym University, College of Medicine, Seoul, Korea*

Background **: In normal hair follicles, CD34 is known to be expressed in the midfollicular areas around the bulge and in the outer root sheath.**

Object **: The purpose of this study is to know whether there is any difference in immunostaining patterns with anti-CD34 antibody among various tumors with follicular differentiation**

Method **: We performed immunoperoxidase staining(modified ABC technique) by using a monoclonal anti-CD34 antibody(QBEND10, IgG1) on the formalin-fixed, paraffin-embedded biopsy specimens of 11 basal cell carcinomas(BCCs), 10 trichoepitheliomas(TEs), 5 proliferating trichilemmal cysts(PTCs), 9 pilomatricomas, and 9 nevus sebaceuses.**

Results **: In normal hair follicles, strong staining around the follicles was observed just below the entrances of sebaceous ducts into the hair canals. The outermost layers of the outer root sheaths below the isthmic portions and above the papillae were also stained.**

Four out of 5 cases of PTC showed CD34-positive staining limited to the focal areas of 1-3 outer cell layers of tumor masses. Nine out of 10 cases of TE showed densely compact strong staining in the stroma just adjacent to the tumor. In BCCs, pilomatricomas, and nevus sebaceuses, there was no remarkable change in the tumor masses nor stromas just adjacent to the tumors.

Conclusion **: Anti-CD34 antibody staining patterns of TEs and PTCs are different from others, which will aid in the differentiation and clarifying the origin of tumors with follicular differentiation. (Ann Deramtol 8:(3)177~181, 1996).**

Key Words : CD34, Follicular differentiation

The human hematopoietic progenitor cell antigen(CD34) is a cell surface protein expressed by human hematopoietic progenitor cells, vascular endothelial cells and many mesenchymal tumors[1]. In normal skin, CD34 presence has been detected not only in endothelial cells and dermal dendritic cells, but also in the midfollicular areas around the bulge, which is the site of the putative hair follicle stem cells, and in cells of the outer root sheath just below the isthmus[2].

The benign tumors of hair follicular origin are various because they can differentiate toward each specific portion of hair structures. They may be related to the hair matrix, such as pilomatricoma; to the outer root sheath, such as proliferating trichilemmal cyst(PTC); or to a combination of both structures as components of the pilosebaceous complex, such as trichoepithelioma(TE). They also exhibit varying degrees of differentiation. Basal cell carcinoma(BCC) is the least differentiated[3]. Nevus sebaceus is thought to be derived from the primary epithelial germ and the epithelium of some nevus sebaceus shows

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Reprint request to : Yong Gwan Baik, M.D., Departments of Dermatology and Pathology*, Hallym University, College of Medicine, Seoul, Korea.
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trichilemmal differentiation[4].

In this study, we performed immunoperoxidase staining by using a monoclonal anti-CD34 antibody (QBEND10, IgG1) on the formalin-fixed, paraffin-embedded biopsy specimens to evaluate any difference in the expression patterns of CD34 among the tumors with follicular differentiation.

MATERIALS AND METHODS

Biopsy material

Eleven cases of BCC, 10 cases of TE, 5 cases of PTC, 9 cases of pilomatricoma, and 9 cases of nevus sebaceus were collected from the pathologic files of the Department of Pathology, Hallym University Hospital. Hematoxylin and eosin stained sections of all lesions were reviewed and the diagnoses were confirmed. All tissues had been fixed in formalin and then paraffin-embedded according to the conventional procedure.

Immunoperoxidase staining

An immunohistochemical study was performed on 5μm sections from formalin-fixed, paraffin-embedded biopsy specimens.

The immunohistochemical demonstration of CD34 was performed with the use of standard techniques and the avidin-biotin-peroxidase complex system. After deparaffinization with xylen and rehydration of the tissues with ethanol, endogenous peroxidase activity was blocked with 3% hydrogen peroxide in absolute methanol for 5 minutes. Each slide was treated sequentially with normal goat serum, monoclonal mouse antibody against CD34(QBEND/10, IgG1, Immunotech S.A., Marseille, France) at a dilution of 1:100, biotinylated antimouse IgG and antirabbit IgG (DAKO LSAB kit, USA) at a 1:200 dilution, and streptavidin-biotin- peroxidase complex(DA- KO LSAB kit, USA). The reaction was visualized by exposure to 3-amino-9-ethylcarbazole, and the slides were counterstained with hematoxylin and mounted.

RESULTS

In normal dermis, vascular endothelial cells and scattered dendritic cells were CD34-positive. In normal hair follicles, strong staining around the follicles was observed just below the entrances of the sebaceous ducts into the hair canals. The pattern of staining was diffuse and no discernible structure was identified (Fig. 1). The outermost layers of the outer root sheaths below the isthmic portions and above the papillae were also stained mainly in a pericellular pattern(Fig. 2).

Four out of 5 cases of PTCs showed CD34-positive staining limited to the focal areas of the tumor masses(Fig. 3). In PTCs with CD34-positive staining, only the outer 1-3 cell layers were stained with CD34 in a pericellular pattern and the intensity of the staining was the strongest in the outermost cell layers. Nine out of 10 cases of TEs showed band-like densely compact, strong staining in the stroma just adjacent to the tumor lobules so that almost no structures were discernible in the stained area(Fig. 4). In BCCs, pilomatricomas, and nevus sebaceuses, there was no remarkable change in the tumor masses nor stromas.

Table The summary of the results of CD34 expression

Diagnosis	No. of cases	CD34 expression	
		Stroma adjacent to tumor	Tumor
BCC	11	◆ loose	-
TE	10	■ dense(9)/loose(1)	-
PTC	5	loose	+ (4/5)*
Pilomatricoma	9	loose	-
Nevus sebaceus	9	loose	-

*Limited to the focal areas of tumor masses

◆ loose : CD34-positivity in loosely scattered dendritic cells

■ dense : CD34-positivity as band-like, dense staining in the stroma just adjacent to the tumor lobules

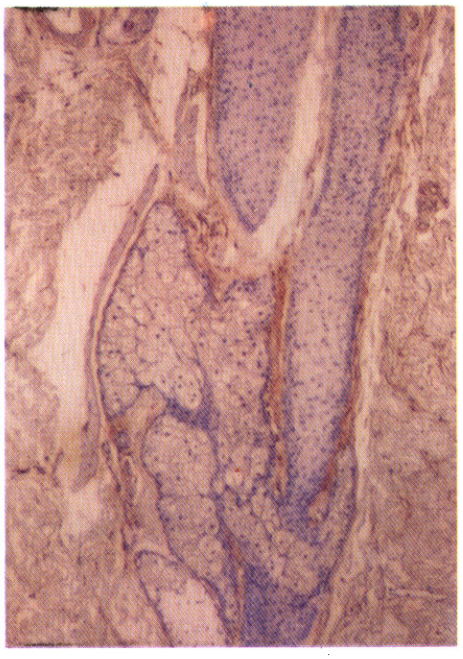

Fig. 1. Strong staining around the follicle was observed just below the entrance of the sebaceous duct into the hair canal (Streptavidin-biotin complex method, ×200).

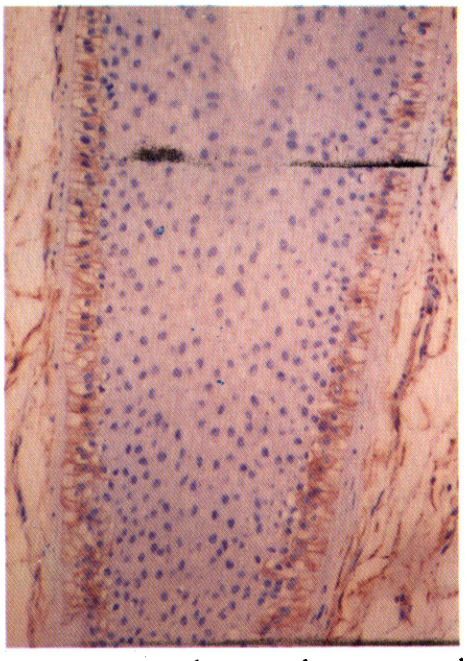

Fig. 2. CD34 positively stains the outermost layer of the outer root sheath below the isthmic portion and above the papilla (Streptavidin-biotin complex method, ×400).

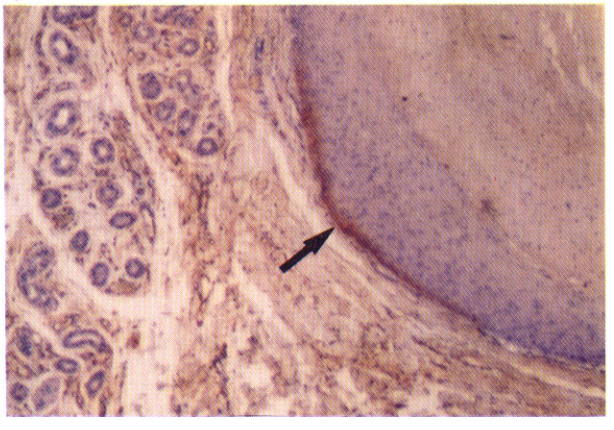

Fig. 3. Proliferating trichilemmal cyst showed CD34-positive staining limited to focal area (arrow) (Streptavidin-biotin complex method, ×100).

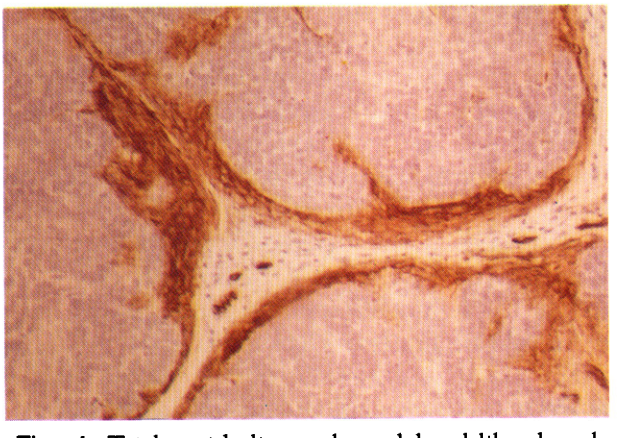

Fig. 4. Trichoepithelioma showed band-like densely compact, strong staining for CD34 in the stroma just adjacent to the tumor lobules (Streptavidin-biotin complex method, ×100).

The results are summarized in Table.

DISCUSSION

The CD34 antigen is a 115-kd transmembrane glycoprotein initially detected on human hematopoietic progenitor cells, and subsequently it has been demonstrated to be found also on vascular endothelial cells, on a distinctive population of dermal dendritic/spindle-shaped cells, on the perifollicular area of the middle portion of the follicles, and on spindle-shaped cells in the basement membrane zone of the eccrine glands[5].

Increasing evidence has been accumulated that anti-CD34 is a rather nonspecific marker for mesenchymal tumors, and that it not only stains vascular tumors and acute leukemia cells, but also dermatofibrosarcoma protuberans, epithelioid sar-

coma, leiomyoma, leiomyosarcoma, perpheral nerve sheath tumors, neurofibroma, clear cell sarcoma, malignant fibrous histiocytoma, and neuroma[6-8].

In normal hair follicles, CD34 staining is strongly positive in the perifollicular areas around the bulge, which is the site of the putative hair follicle stem cells[1,6]. This location led Nickoloff[1] to speculate that these CD34-positive dermal cells might represent translocated hematopoietic progenitor cells that could be interacting with the epithelial stem cells of the bulge. Furthermore, it was observed that in fetal skin, CD34 positive cells form a nest around the base of developing follicles[9].

The outer root sheath cells expressed CD34 at the outermost layer, mostly in the inferior portion above the papilla. Miyauchi et al[10] used Ki-67 to investigate the mitotic activities of hair and hair follicles. They observed that there were two different patterns of cytoplasmic staining in the outer root sheath, i.e. the strong staining of the innermost cells and weaker staining of the other outer root sheath cells in the isthmus. Ki-67 reactivity of the innermost cell layer was observed at the anagen stage and was regularly seen from the upper bulb to the isthmus. It means that there is heterogeneity between the cells of the outer root sheath, and it may explain the expression patterns of PTCs, which differentiate toward the outer root sheath. In the present study, CD34 was expressed focally in the outermost 1-3 cell layers of PTCs, similar to the immunostaining pattern of the outer root sheath of normal hair follicles.

TEs contain a distinctive fibrotic stroma similar to the stroma surrounding hair follicles in normal skin, and papillary mesenchymal bodies which resemble hair papillae. Therefore, it is believed that they are related to the combined components of the pilosebaceous complex[11]. Staining with anti-CD34 antibody showed densely compact, strong staining in the stroma just adjacent to the tumors. In relation to normal hair follicles, perifollicular staining for CD34 was most intense in the angled area between the sebaceous duct and follicular canal. The fact that the stromas of BCCs showed no remarkable change appears somewhat confusing considering Nikoloff's[1] speculation. There are three possible explanations. First is that none of our cases of BCC showed differentiation toward the hair follicle. Second is that protease production in the region of BCC tumors had removed the antigen from the cell surface. BCCs or their stroma are known to produce a number of protease, including type I collagenase, type IV collagenase, stromelysin 3, endoprotease, and glycoprotease that can cleave the CD34 antigen from cell surface[9,12]. The third explanation is that the stroma of BCC does not differentiate toward the expression of CD34 antigen.

Nevus sebaceuses are thought to be derived from the primary hair germ and develop epidermal hyperplasia as they age. Morioka[4] asserted that more than half the cases with epidermal hyperplasia were essentially due to trichilemmoma and it is possible that trichilemmomas may express CD34 in tumor masses like PTCs. In the present study, the hyperplastic epidermis in all cases of nevus sebaceus showed negative staining for CD34, so it is suggested that the hyperplastic epidermis might be unrelated to trichilemmoma.

Pilomatricoma is a tumor with differentiation toward hair cortex cells. They were not expected to express CD34, like the hair matrix or hair cortex, and they did not.

We conclude that anti-CD34 antibody staining patterns of TEs and PTCs are different from others, which will aid in the differentiation and clarifying the origin of tumors with follicular differentiation. However, further studies are required to understand and explain their diversity more precisely.

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