

Received: 2018.12.11
Accepted: 2019.02.19
Published: 2019.03.28

VEGFR-2 Is in a State of Activation in Hair Follicles, Sebaceous Glands, Eccrine Sweat Glands, and Epidermis from Human Scalp: An *In Situ* Immunohistochemistry Study of Phosphorylated VEGFR-2

Authors' Contribution:
Study Design A
Data Collection B
Statistical Analysis C
Data Interpretation D
Manuscript Preparation E
Literature Search F
Funds Collection G

ABCDEF G **Xian-Jie Wu**
ABCDEF G **Jing Jing**
ADG **Zhong-Fa Lu**
ABE **Min Zheng**

Department of Dermatology, The Second Affiliated Hospital, Zhejiang University School of Medicine, Hangzhou, Zhejiang, P.R. China

Corresponding Authors: Zhong-Fa Lu, e-mail: lzfskin@zju.edu.cn, Min Zheng, e-mail: minz@zju.edu.cn

Source of support: This research was supported by the Zhejiang Provincial Natural Science Foundation of China under Grant No. LY14H110002 and LZ17H110001; CMA – L'OREAL CHINA HAIR GRANT (No. H2014-005, H2010-06) and the National Natural Science Foundation of China (Grant no. 81472900, 81773350, and 81602776)

Background: Recent research reports that VEGFR-2 is expressed in the whole hair follicle, sebaceous glands, eccrine sweat glands, and epidermis. However, phosphorylated VEGFR-2 was not found, and it could not be ascertained whether the activated form of VEGFR-2 actually participates in the biological control of epidermal appendages. In this study we aimed to determine whether the VEGFR-2 pathway is directly involved in the daily regulation of epidermal appendages biology.

Material/Methods: In this study, we investigated the expression of phosphorylation of VEGFR-2 by immunohistochemical analysis in the epidermis and epidermal appendages in normal human scalp skin.

Results: Immunohistochemical analysis revealed phosphorylation of VEGFR-2 in a whole hair follicle, mainly in the infundibulum basal layer, hair cortex, and medulla in the isthmus, and matrix in the hair bulb. Phosphorylated VEGFR-2 also was found in the sebaceous glands, eccrine sweat glands, and epidermis.

Conclusions: Therefore, we suggest that VEGFR-2 activation is involved in routine regulation of human epidermal appendages.

MeSH Keywords: **Antibodies, Phospho-Specific • Hair Follicle • Scalp • Vascular Endothelial Growth Factor Receptor-2**

Full-text PDF: <https://www.basic.medscimonit.com/abstract/index/idArt/914570>

 1321  —  3  20



Background

An increasing number of studies show that VEGFR-2 not only participates in lymphangiogenesis [1], but also is involved in processes in other types of cells [2–5], including human epidermal cells and HaCat cells [6–8]. As this is a very interesting phenomenon, we have performed research on the hair follicle epithelium and found that VEGFR-2 can be detected in the outer root sheath (ORS), inner root sheath (IRS), hair follicle bulge cells, dermal sheath cells, and dermal papilla cells (DPCs) [9,10] and is involved in hair follicle ORS cell and DP cell biological activity *in vitro* [10–12]. We recently found that expression of VEGFR-2 in the mouse hair follicle epithelium changes with the hair cycle [13], indicating that it may have a direct role in hair cycle regulation in hair follicles.

Although VEGF and VEGFR-2 expression has been studied in human scalp hair follicles using immunofluorescence, no phosphorylated VEGFR-2 was detected. Therefore, it is not clear whether the VEGFR-2 pathway is directly involved in the daily regulation of hair follicle biology or merely plays a supplementary role under certain conditions. To clarify whether the form of VEGFR-2 that is expressed in hair follicle epithelia is actually involved in the daily biological control of hair follicles, we detected the expression of phosphorylated VEGFR-2 (p-VEGFR-2), as an activated form of VEGFR-2, in skin appendages.

Material and Methods

In this study, we obtained consent from all donors. All methods of our research were also approved by the Ethics Committee of the Second Affiliated Hospital, Zhejiang University School of Medicine, China.

Specimens

Normal scalp specimens were obtained from 11 donors (mean \pm SD age 28.90 \pm 5.24 years, range 20–35 years old) by cosmetic surgery at the Department of Plastic Surgery and Dermatology. Mouse anti-human polyclonal p-VEGFR-2 (Tyr1175) antibodies were purchased from Cell Signaling Technology (Perom, MI, Italy). Horseradish peroxidase-labeled rabbit anti-mouse secondary antibody was obtained from Dako Cytomation (Denmark).

Immunohistochemistry analysis *in situ*

Immunohistochemistry analysis was performed *in situ* using our previously reported methods [10]. Normal scalp samples were embedded in paraffin and cut into 5–10 mm sections. The sections were placed on slides, which were covered with poly-L-lysine. To eliminate endogenous peroxidase activity, after dewaxing in water, all sections were incubated in 3%

hydrogen peroxide at room temperature for 5–10 min. After washing with distilled water, all slides were immersed in 0.1% PBST for 5 min. For antigen retrieval, all sections were boiled in an oven. The sections were incubated at room temperature with 5–10% normal goat serum (diluted in PBS) for 10 min to prevent nonspecific binding, and then overnight at 4°C with mouse anti-human polyclonal antibodies (diluted in 1: 100 with 5% BSA in PBS) against p-VEGFR-2 (Tyr1175). The sections were rinsed 3 times in PBST and incubated in the secondary antibody labeled with biotin (diluted in 1: 200 with 1% BSA-PBS) at 37°C for 30 min. After washing in PBST 3 times, the sections were incubated at 37°C for 30 min with horseradish streptavidin-labeled with peroxidase (diluted with PBS). The sections were washed 3 times in PBST and then stained with 3,3'-diaminobenzidine (DAB). The slides were thoroughly washed in tap water, and nuclear labeling was achieved with hematoxylin. The positive control was sections from a human umbilical vein, and the negative control was mouse IgG.

Results

Detection of the expression of p-VEGFR-2 in anagen hair follicles from human scalp by immunohistochemistry

p-VEGFR-2-staining was detected in almost the whole hair follicle, including the ORS, IRS, matrix, medulla, cortex, hair bulb, DP, and dermal sheath. The staining for p-VEGFR-2 was detected in the infundibulum and was much more intensive in its basal layer, while it was negative in the upper hair shaft. In the isthmus, the staining for p-VEGFR-2 was detected in both the IRS and ORS, and it was also visible in the hair cortex and medulla. Enhanced staining was detected in the cortex and medulla; however, it was decreased in the ORS. In the hair bulb, moderate staining for p-VEGFR-2 was detected in the outermost basal cells and DP (Figure 1).

Detection of the expression of p-VEGFR-2 in sebaceous glands from human scalp by immunohistochemistry

Both the lobules and ducts of the sebaceous glands (SGs) showed intense staining for p-VEGFR-2, especially from the ostium to the hair follicle. Undifferentiated peripheral cells showed moderately intense staining for p-VEGFR-2, although staining was seldom detected in the differentiated cells near the center, such as in lobulated mature SGs and degenerated cells (Figure 2).

Detection of the expression of p-VEGFR-2 in eccrine sweat glands from human scalp by immunohistochemistry

There was strong positive staining for p-VEGFR-2 in eccrine sweat gland (ESG) secretory parts and ESG ducts in the derma.

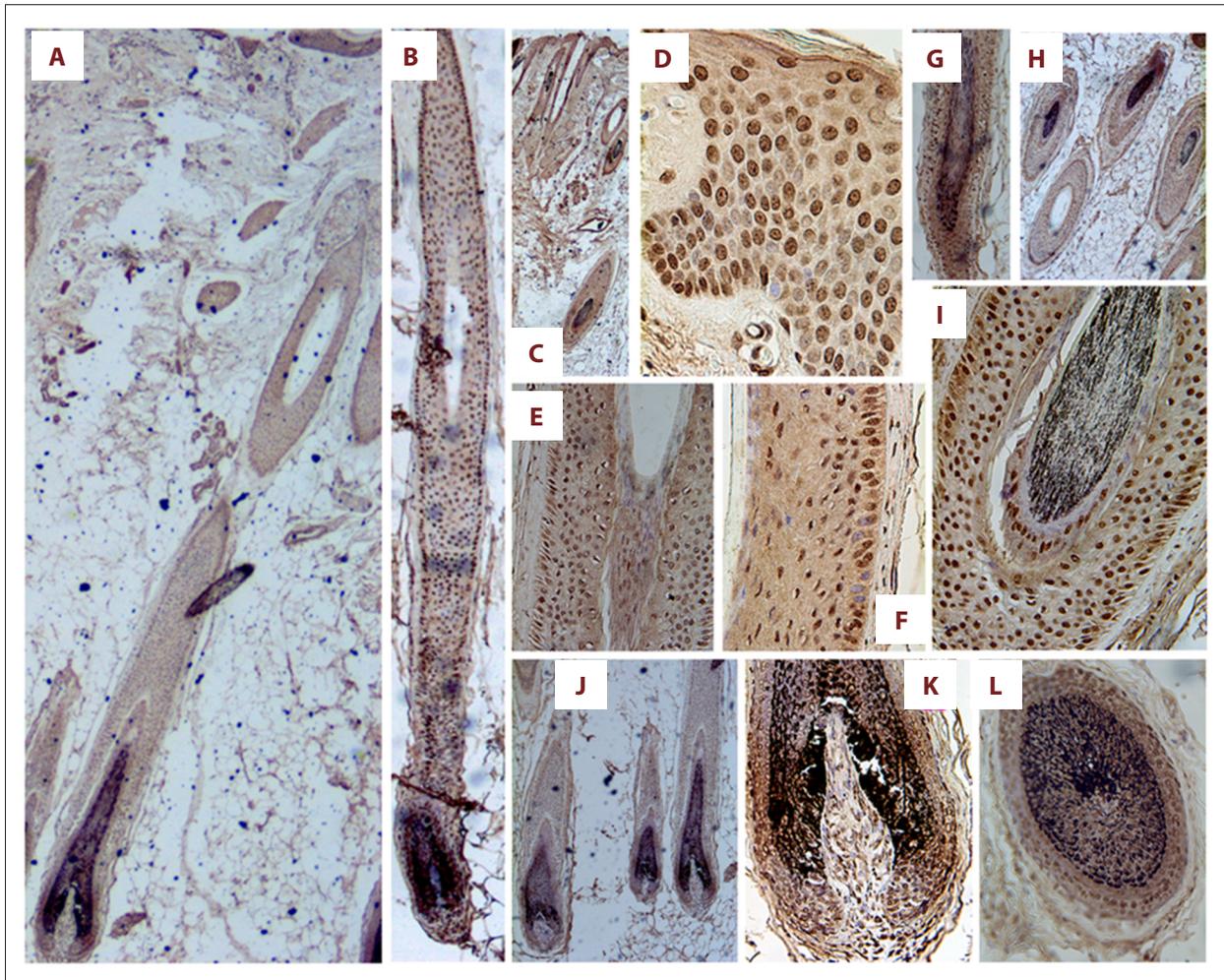


Figure 1. The detection of the expression of p-VEGFR-2 in various parts of the hair follicle by immunohistochemistry. p-VEGFR-2-staining was detected in almost the whole hair follicle and epidermis (A–C). In the epidermis, staining was intense in the basal and spinous layers, but was decreased in the granular layer, although it was also visible in the cuticle (C, D). In the infundibulum, the staining for p-VEGFR-2 was detected more strongly in its basal layer, but it was decreased in the central part (C, E, F). In the isthmus, staining for p-VEGFR-2 was detected in both the IRS and ORS, and it was also visible in the hair cortex and medulla, while it was negative in the cortex and medulla close to the upper hair shaft (G–I). In the hair bulb, staining for p-VEGFR-2 was detected in each layer, with much more intense staining detected in the matrix, and then it decreased in the outermost layer and DP (J–L). The whole dermal sheath of the hair follicle was positive for staining for p-VEGFR-2. Nuclei are shown in blue and p-VEGFR-2 is shown in brown; p-VEGFR-2 is stained in the epithelial cell membrane, cytoplasm, and nucleus, but perinuclear staining is especially strong; **A** – whole skin; **B** – whole hair follicle; **C** – epidermis and infundibulum; **D** – epidermis; **E** – infundibulum; **F** – infundibulum; **G** – isthmus; **H** – isthmus; **I** – isthmus; **J** – hair bulb; **K** – hair bulb; **L** – hair bulb.

Intense staining for p-VEGFR-2 was found in the cuboidal cells of the secreting gland and the inner cuboidal cells in the basal part of the ductus (Figure 3).

Discussion

It has been believed that VEGF can accelerate hair regeneration by inducing the formation of new blood vessels around the hair follicle [14]. In fact, we previously identified VEGFR-2

and p-VEGFR-2 expression on human hair ORS cells *in vitro* by RT-PCR and Western blot analysis, and it has since been confirmed that VEGF can directly regulate the hair follicle through VEGFR-2 [9–13]. However, the consequences of VEGFR-2 expression in hair follicles is not clear.

Previous studies have shown that VEGFR-2 expressed in the whole hair follicle [9–13] is not an activated form, and this finding did not show that VEGFR-2 actually routinely participates daily in the biological control of hair follicles, although we

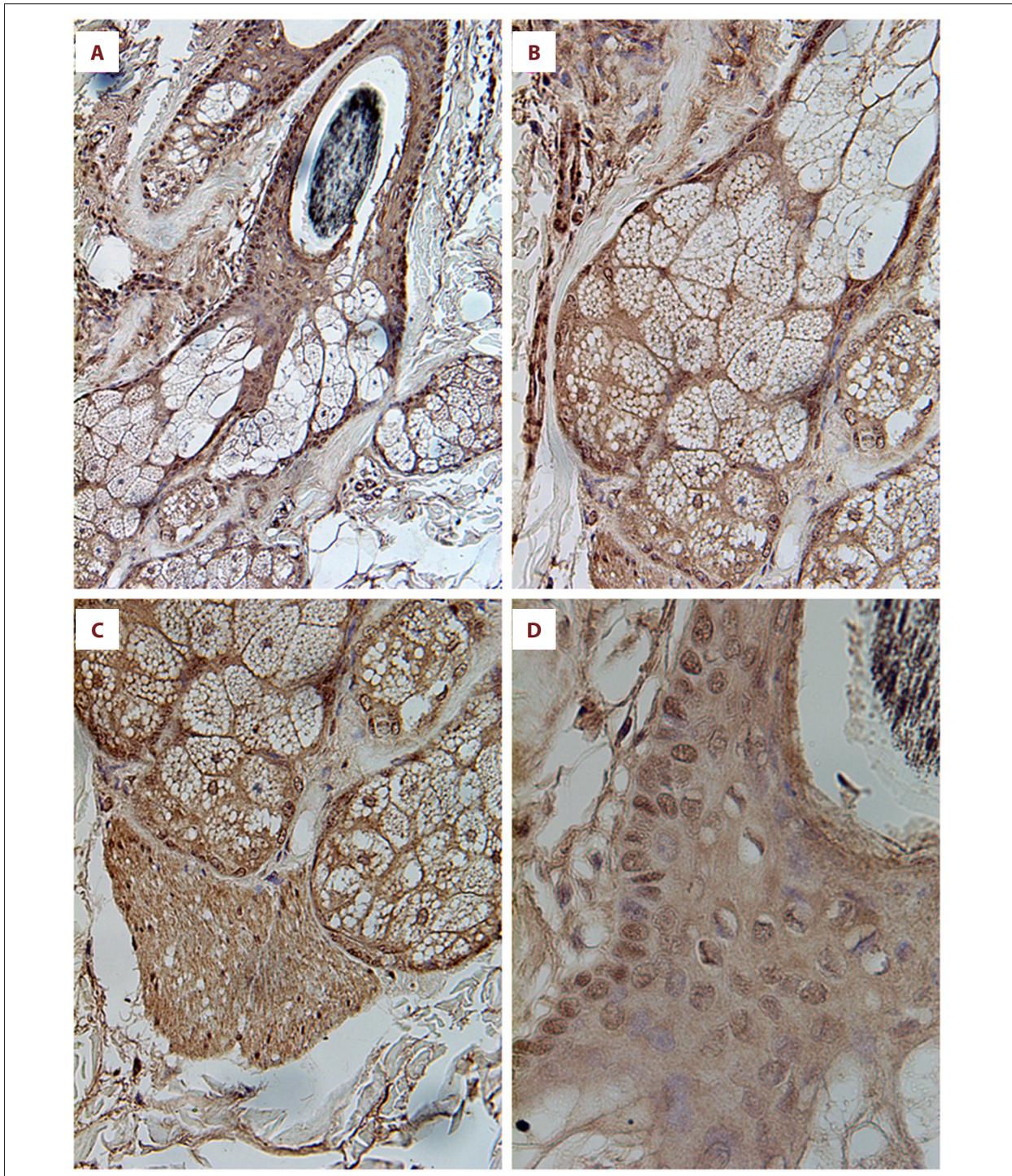


Figure 2. The detection of the expression of p-VEGFR-2 in SGs by immunohistochemistry *in situ*. Both the ducts and lobules of the SGs show rich staining for p-VEGFR-2 (A). In SGs, undifferentiated peripheral cells showed moderately intense p-VEGFR-2 staining, but staining was seldom observed in the differentiated cells near the center in lobulated mature SGs and degenerated cells, and there was negative staining for p-VEGFR-2 in sebum (B, C). Much more intense staining for p-VEGFR-2 was observed in the SG ducts, especially from the ostium to the hair follicle (A, D). Nuclei are shown in blue, p-VEGFR-2 is shown in brown; p-VEGFR-2 is stained in the epithelial cell membrane, cytoplasm, and nucleus, and perinuclear staining is especially strong; A – SG ducts ostium to hair follicle; B – lobulated mature SGs; C – lobulated mature SGs; D – SG ducts ostium to hair follicle.

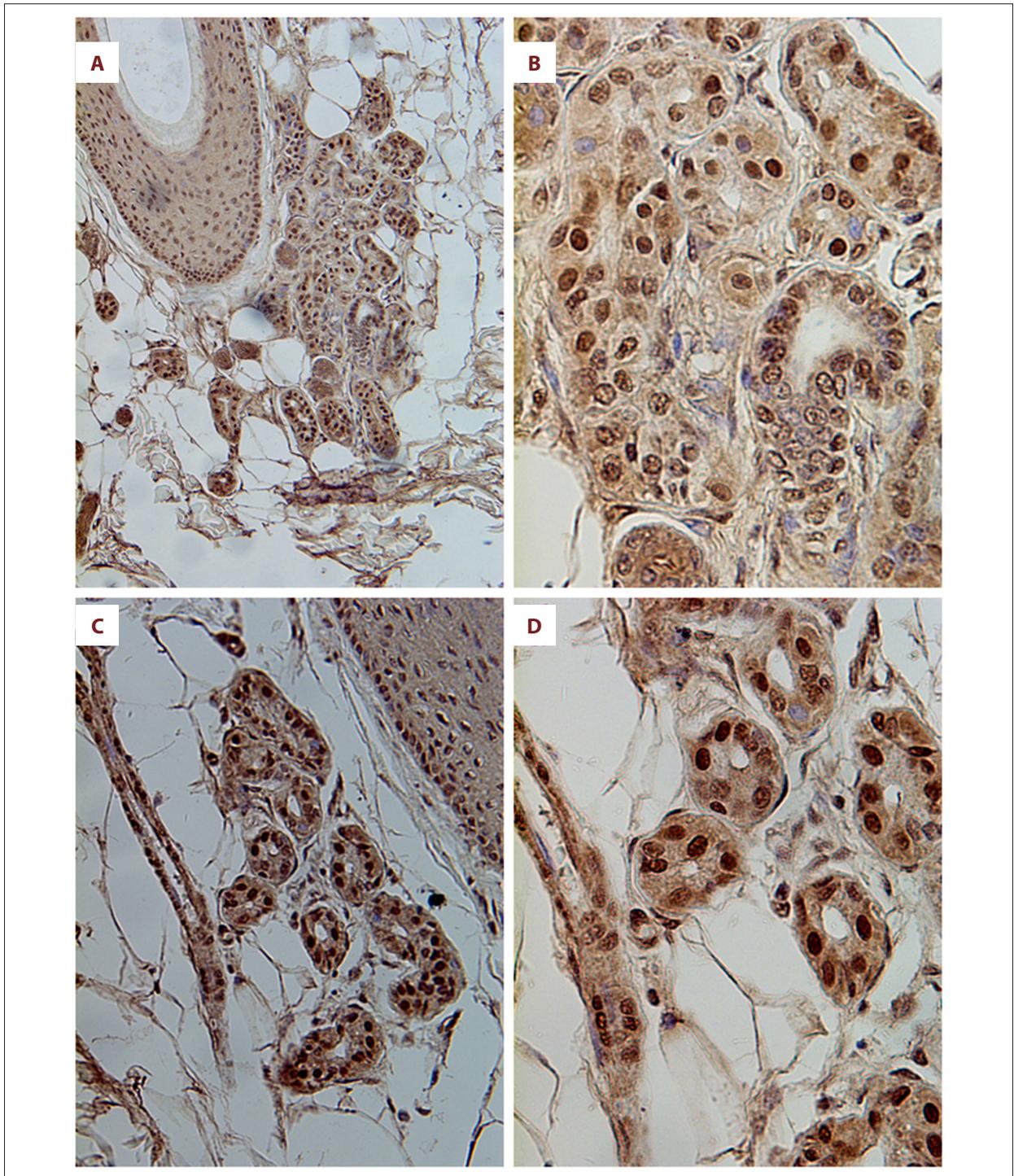


Figure 3. Detection of p-VEGFR-2 expression in ESGs by *in situ* immunohistochemistry. In an ESG, the intense staining for p-VEGFR-2 was detected in the secretory site and the dermal part of the duct (A). Both the inner cuboidal cells in the basal part of the ductus and the cuboidal cells of the secreting gland showed intense staining for p-VEGFR-2 (B, D); however, no staining for p-VEGFR-2 was detected in the secretory cavity or the ESG duct cavity (C, D). Nuclei are shown in brown; p-VEGFR-2 is stained in the epithelial cell membrane, cytoplasm and nucleus, and perinuclear staining is especially strong. A – secreting gland; B – secreting gland; C – ductus; D – ductus.

have confirmed recently that VEGF₁₆₅ can activate VEGFR-2 in cultured human ORS cells *in vitro* and function in ORS cells proliferation, adhesion, migration, and differentiation [10,11]. Our work described herein provides further evidence that VEGFR-2 is activated in a variable manner in hair follicles, epidermis, ESGs, and SGs. Our study suggests that VEGF directly plays a routine and important role in hair follicle growth.

Interestingly, we found that intense staining for p-VEGFR-2 is mainly confined to the matrix, cortex, and medulla. These sites are considered to be important to hair growth [15]. Considering the expression of VEGF₁₆₅ in the DP [14], we speculate that VEGFR-2 is activated by exogenous VEGF₁₆₅ from the DP, which may promote the proliferation of matrix cells for hair shaft formation. The inner hair sheath is thought to be strongly involved in shaping of the hair shaft [15, 16], indicating that VEGFR-2 activation in the IRS is involved in hair shaft differentiation in the anagen phase. When the hair shaft has grown to maturity though the infundibulum, VEGFR-2 gradually transitions to its inactive state.

Interestingly, we observed that p-VEGFR-2 is mainly present in undifferentiated peripheral cells of SGs. Sebaceous gland cells are involved in the synthesis and metabolism of lipids and secrete sebum via holocrine secretion for purposeful self-destruction of its primary cellular unit [17,18]. Thus, VEGFR-2 is actually activated in the daily routine of undifferentiated

sebocytes, and it should participate in sebocytes in the proliferative state, such that the synthesis and metabolism of lipids promotes SG secretion. p-VEGFR-2 was also detected in cuboidal cells in ESGs. Cuboidal cells are found both in the basal region of the ductus and in the secretory portion; 2 types of cuboidal cells are involved in sweat secretion, and secretion is controlled by the central nervous system [19,20]. However, VEGFR-2 activation in the daily routine of cuboidal cells remains completely unknown. It is possible that VEGFR-2 activation combined with different levels of neuronal central has a role in sweat secretion regulation in ESGs. Further functional research is warranted on the roles of VEGFR signaling activation in SGs and ESGs.

Conclusions

In conclusion, using immunohistochemistry analysis, we revealed that VEGFR-2 is always activated in the epidermis, anagen hair follicle, SGs, and ESGs, suggesting that the VEGFR-2 signal is involved in the daily regulation of physiological functions of skin appendages. However, the specific regulatory mechanism requires further research.

Conflicts of interest

None.

References:

1. Yehya AHS, Asif M, Petersen SH et al: Angiogenesis: Managing the culprits behind tumorigenesis and metastasis. *Medicina (Kaunas)*. 2018; 54: pii: E8
2. Melincovici CS, Bosca AB, Susman S et al: Vascular endothelial growth factor (VEGF) – key factor in normal and pathological angiogenesis. *Rom J Morphol Embryol*, 2018; 59: 455–67
3. Corti F, Simons M: Modulation of VEGF receptor 2 signaling by protein phosphatases. *Pharmacol Res*, 2017; 115: 107–23
4. Mohamed TK, Batran RZ, Elseginy SA et al: Synthesis, anticancer effect and molecular modeling of new thiazolopyrazolyl coumarin derivatives targeting VEGFR-2 kinase and inducing cell cycle arrest and apoptosis. *Bioorg Chem*, 2019; 85: 253–73
5. Marini M, Manetti M, Sgambati E: Immunolocalization of VEGF/VEGFR system in human fetal vomeronasal organ during early development. *Acta Histochem*, 2019; 121: 94–100
6. Man XY, Yang XH, Cai SQ et al: Immunolocalization and expression of vascular endothelial growth factor receptors (VEGFRs) and neuropilins (NRPs) on keratinocytes in human epidermis. *Mol Med*, 2006; 12: 127–36
7. Man XY, Yang XH, Cai SQ et al: Overexpression of vascular endothelial growth factor (VEGF) receptors on keratinocytes in psoriasis: regulated by calcium independent of VEGF. *J Cell Mol Med*, 2008; 12: 649–60
8. Yang XH, Man XY, Cai SQ et al: Expression of VEGFR-2 on HaCaT cells is regulated by VEGF and plays an active role in mediating VEGF induced effects. *Biochem Biophys Res Commun*, 2006; 349: 31–38
9. Man XY, Yang XH, Cai SQ et al: Expression and localization of vascular endothelial growth factor and vascular endothelial growth factor receptor-2 in human epidermal appendages: A comparison study by immunofluorescence. *Clin Exp Dermatol*, 2009; 34: 396–401
10. Wu XJ, Zhu JW, Jing J et al: VEGF165 modulates proliferation, adhesion, migration and differentiation of cultured human outer root sheath cells from central hair follicle epithelium through VEGFR-2 activation *in vitro*. *J Dermatol Sci*, 2014; 73: 152–60
11. Li W, Lu ZF, Man XY et al: VEGF upregulates VEGF receptor-2 on human outer root sheath cells and stimulates proliferation through ERK pathway. *Mol Biol Rep*, 2012; 39: 8687–94
12. Li W, Man XY, Li CM et al: VEGF induces proliferation of human hair follicle dermal papilla cells through VEGFR-2-mediated activation of ERK. *Exp Cell Res*, 2012; 318: 1633–40
13. Wu XJ, Jing J, Lu ZF, Zheng M: Expression and localization of VEGFR-2 in hair follicles during induced hair growth in mice. *Arch Dermatol Res*, 2018; 310: 591–98
14. Bassino E, Gasparri F, Giannini V, Munaron L: Paracrine crosstalk between human hair follicle dermal papilla cells and microvascular endothelial cells. *Exp Dermatol*, 2015; 24: 388–90
15. Martel JL, Badri T: *Anatomy, head, hair, follicle*. 2018
16. Harland DP, Plowman JE: Development of hair fibres. *Adv Exp Med Biol*, 2018; 1054: 109–54
17. Zouboulis CC, Picardo M, Ju Q et al: Beyond acne: Current aspects of sebaceous gland biology and function. *Rev Endocr Metab Disord*, 2016; 17: 319–34
18. Hoover E, Krishnamurthy K: *Physiology, sebaceous glands*. 2018
19. Bovell DL: The evolution of eccrine sweat gland research towards developing a model for human sweat gland function. *Exp Dermatol*, 2018; 27: 544–50
20. Cui CY, Schlessinger D: Eccrine sweat gland development and sweat secretion. *Exp Dermatol*, 2015; 24: 644–50