ORIGINAL ARTICLE

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Expression of endoglin, CD105, in conjunctival melanocytic nevi: Is it suspicious like in thyroidology? *Oculi plus vident quam oculus*?

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SUMMARY

Objective: The aim of this study was to evaluate the expression of endoglin and its correlation with histopathological and clinical findings in conjunctival nevi.

Methods: The study included archival formalin-fixed, paraffin-embedded tissue sections of 44 patients with conjunctival nevi. Immunohistochemical staining for CD105 had been performed with monoclonal mouse antihuman CD105 antibodies. The intratumoral microvessel density for quantification of tumoral vascularization had been determined by this marker.

Results: The expression of CD105 was positive in 30 (68.2%) cases. There was a statistically significant difference in the level of CD105 expression regarding the histological type of nevus (p=0.03) and intralesional cysts status (p=0.02). Spearman's rho (ρ -0.316) revealed a significant negative correlation between the expression of endoglin and the histological type of nevus (p=0.03) and between the expression of endoglin and the presence of intralesional cysts (ρ -0.380, p=0.01).

Conclusion: This study suggests that endoglin could be a useful diagnostic and prognostic marker in differentiating between benign and malignant melanocytic ocular lesions.

Keywords: Endoglin. Conjunctiva. Nevus, Pigmented. Immunohistochemistry. Thyroid gland. Thyroidology.

INTRODUCTION

Conjunctival tumors frequently originate from pigment cells, usually possessing the structural configuration of melanocytic nevi[1,2]. Debate is still ongoing on the accurate differential diagnosis of the benign and malignant melanocytic proliferation of the conjunctiva. However, accuracy in the mentioned issue is crucial as it implies diverse ocular and systemic prognoses[3]. Ad initio, many studies recommended immunohistochemical markers to provide additional and supportive diagnostic and prognostic pieces of information, to date. Ad hoc, immunohistochemical expression of S100A1, S100A6, S100B, MelanA, CEA, HMB-45, MART-1, CD45, CD68, CD1a, Ki-67 nuclear proliferation protein, p16, p53, WT, and Bcl2 in conjunctival melanocytic lesions had been investigated, but still, this topic continues to receive serious needs for further investigations[4-12].

In the present study, it is purposed to investigate the endoglin (CD105) expression and its correlation to the histopathological and clinical features in the conjunctival nevi.

Endoglin is a homodimeric transmembrane glycoprotein, a component of the receptor complex of transforming growth factor beta1 (TGF-β1), a multifunctional cytokine with an important role in cell proliferation, differentiation, and migration[13]. Inhibition of CD105 expression enhances the ability of TGF-β1 to suppress growth and migration of cultured endothelial cells, and their capacity to form capillary tubes[14]. The evaluation of neovascularization by CD105 staining was found to be a useful prognostic indicator in different solid malignancies. In contrast to some other markers, expressed on endothelial cells of blood vessels in both normal tissue and malignant tumors, endoglin is mostly expressed on the peritumoral and intratumoral blood vessels, which makes it a potential molecular target for therapy[15].

The expression of endoglin has been investigated in uveal melanoma[6], cutaneous melanoma[7,8], and cutaneous melanocytic lesions[9]. There have been no published reports on CD105 expression in human conjunctival nevi so far.

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METHODS

This retrospective histopathological and immunohistochemical study of 44 surgically excised conjunctival nevi was conducted at the Institute for Pathology of the Faculty of Medicine, University of Kragujevac, Serbia. A total of 44 patients, 25 (56.8%) females and 19 (43.2%) males, who carried the diagnoses of conjunctival nevi, were examined and surgically treated in the tertiary referral center, Clinic for Eye Diseases, Clinical Center of Serbia, Belgrade, during the period from January 2000 to December 2002. The archival consultation files and 10% formalin-fixed, paraffin-wax-embedded tissue sections from the Eye Pathology Laboratory of the Clinic for Eye Diseases, Belgrade, were collected and retrospectively reviewed with approval from the institutional Ethics Committee of the University of Belgrade for the purposes of this study (approval nº:1009/3). The tumor data had also included intralesional cysts status, in terms of presence or absence. The patient charts had been reviewed for specific data as follows: gender, age at the time of excision, anatomic location of the lesion, duration of the process, clinical indications for excision, and follow-up period. These data had been collected and analyzed after the histopathological and immunohistochemical findings were determined.

Archival in 10% formalin-fixed, paraffin-embedded tissue sections was cut at 3 µm. Representative paraffin sections, mounted on high adhesive slides (Star Frost, Waldemar Knittel GmbH, Braunschweig, Germany), were heated at 55ºC to melt the paraffin, deparaffinized in xylene (three times, 5 min each), and rehydrated with a graded series of alcohol. Standard hematoxylin-eosin staining for the type of nevus differentiation and Alcian Blue-Periodic Acid Schiff pH 2.5 (AB-PAS) staining for testing and differentiation of neutral (red) from the acid (blue) mucins were performed in separate sections.

Antigen retrieval was enhanced by autoclaving of slides in sodium citrate buffer (pH 6.0) for 30 min. After cooling for about 30 min, the slides were washed again in phosphate-buffered saline (PBS). Endogenous peroxidase activity was blocked by treating the sections with 0.3% (v/v) H_2O_2 in methanol for 25 min and then the slides were washed again. The sections were then incubated with the primary antibodies overnight at 4ºC. Staining was performed with antibodies for the identification of CD105 (monoclonal mouse anti-human CD105, endoglin, Clone SN6h, 1:50, DAKO, Glostrup, Denmark). Incubation with secondary antibody was performed with Vectastain Elite ABC Kit (Vector Laboratories, Burlingame, CA, USA) at room temperature for 1 h. Visualization of immune complexes was performed with 3,3'-diaminobenzidine (DAB) chromogen solution. Slides were counterstained with Mayer's hematoxylin and mounted on Canada balsam. For negative controls, the primary antibody was replaced by PBS.

The microvessel density (MVD) was calculated by immunostaining for CD105, by searching the most vascularized area ("hotspots") at low magnification (200×) using light microscope (Olympus BH-2). Then, CD105-positive vessels in five of these fields at high magnification (400×) were counted according to the Weidner's procedure[10]. A countable single microvessel was considered any immunopositive (clearly stained) endothelial cell or endothelial cell cluster that is separated from the adjacent microvessels, tumor cells, and other connective tissue elements[10]. The mean of the vessels in five fields was used as MVD. The immunohistochemical staining intensity was graded as follows: negative: –, weak/mild: +, moderate: ++, or strong: +++, depending on the expression of endoglin (–: absent; +: 1–5 positive cells; ++: 5–10 positive cells; +++: more than 10 positive cells). The immunohistological specimens were evaluated by two independent experienced pathologists; in all cases, agreement was reached.

SPSS version 20.0 (SPSS Inc., Chicago, IL, USA) was used for statistical data processing. The results were analyzed by using the descriptive statistics and Mann-Whitney U test or the Kruskal-Wallis test depending on the distribution and number of compared groups. Nonparametric variance analysis was performed by Spearman's rank correlation coefficient calculation. A value of $p<0.05$ was considered statistically significant.

RESULTS

In this study, 44 nevi in 44 patients, 25 (56.8%) females and 19 (43.2%) males, with a mean age 31.36±18.715 years (range 7–73 years), were included. Excision of conjunctival melanocytic lesion was done in all patients. One or more clinical indications for excision were as follows: suspicious clinical appearance, accelerated growth, or color change of the lesions. Bulbar conjunctiva was the most common location of the tumor. In all, 28 (63.6%) of 44 nevi was located on nasal quadrant and 16 (36.4%) on temporal region. The tumor anterior margin was located on the edge of the cornea in 9 (20.5%) lesions and on plica semilunaris in 7 (15.9%) cases.

Follow-up examinations were made at 6- to 12-month intervals, and the patients were followed up for a mean of 8.9 years. During the follow-up period, there were no recorded data on recurrence or malignancy.

Histopathologically, the 44 excised lesions had revealed the compound nevus in 33 (75.0%) patients, subepithelial nevus in 7 (15.9%) patients, combined nevus in 2 (4.5%) patients,

junctional nevus in 1 (2.3%) patient, and ceruleus nevus in 1 (2.3%) patient. The cysts were noted in 9 (20.5%) nevi, of which 7 (15.9%) were compound nevi and 2 (4.5%) were subepithelial nevi.

The expression of CD105 was positive in 30 (68.2%) cases. Out of these, 24 (80%) were compound nevi, 2 (6.7%) combined nevi, 2 (6.7%) subepithelial nevi, 1 (3.3%) junctional nevus, and 1 (3.3%) ceruleus nevus. The majority of CD105-positive conjunctival nevi showed weak CD105 expression, with 27 (61.4%) cases showing the weak (+) CD105 staining intensity and 3 (6.8%) cases, including 1 compound nevus, 1 junctional nevus, and 1 combined nevus, showing moderate (++). The CD105-positive vessels were absent in 14 (31.8%) conjunctival nevi (Figure 1).

We have investigated the association of the presence of CD105-positive blood vessels with histology. There was statistically significant difference in the level of CD105 expression regarding the histological type of nevus (p=0.04). The CD105 expression was significantly higher in compound nevi. Of all subepithelial nevi, only one showed a low (+) CD105 expression, and in other subepithelial nevi, the CD105-positive vessels were absent (Figure 2).

The CD105 expression was moderate (++) in 3 (6.8%) of 44 conjunctival nevi, 1 junctional nevus, 1 compound (Figure 3) nevus with the presence of marginal mitotic activity, and 1 combined nevus in which the greater part of the lesion was in a deeper subepithelium, composed of spindle-shaped cells and strongly pigmented.

We have also investigated the association between the presence of CD105-positive blood vessels and the intralesional cysts status. There was statistically significant correlation between the CD105 expression and intralesional cysts status in conjunctival nevi (p=0.03). The CD105 expression was significantly lower in nevi with intralesional cysts. The CD105-positive vessels were present in three of nine nevi with intralesional cysts. The statistical analysis showed that there was no significant correlation between CD105 expression and nevi location (p=0.43).

Nonparametric variance analysis by Spearman's rho (ρ -0.316) revealed significant negative correlation between expression of endoglin and histological type of nevus (p=0.03), which means that as nevus is in deeper layers of the conjunctiva, the expression of endoglin is weaker. A significant negative correlation between expression of endoglin and the presence of intralesional cysts was also confirmed with nonparametric variance analysis by Spearman's rho (ρ -0.380, p=0.01), which means that the more the intralesional cysts, the weaker the expression of endoglin.

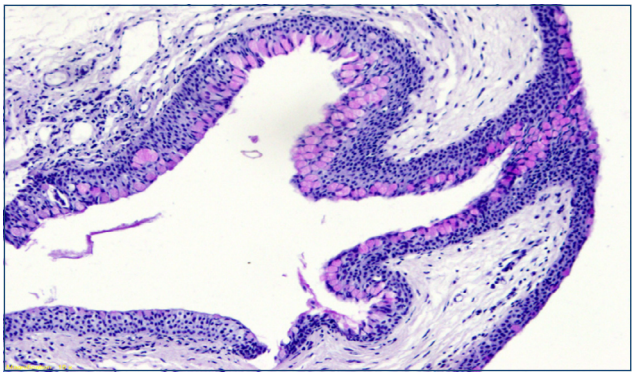

Figure 2. The naevus subepithelial plica semilunaris with cystic formation that is in continuity with cover epithelium. In superficial third of the epithelium (periluminal), mucinous epithelium transformation with PAS+ neutral mucins is present and the CD105 expression is negative (Immunohistochemistry, AB-PAS; Original magnification, 200×).

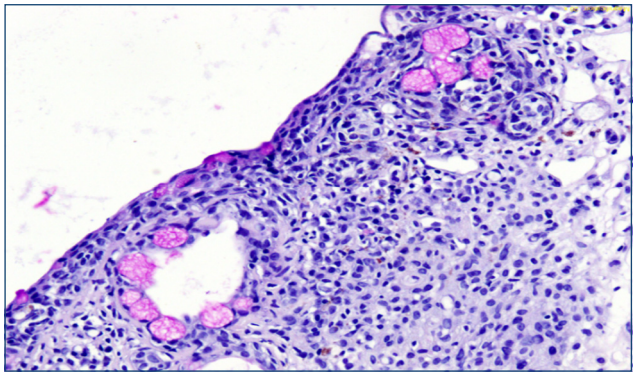

Figure 1. The naevus compositus conjunctivae associated with chronic conjunctivitis and mucinous cysts and filled with PAS+ neutral purple-red mucins. A focal mucinous metaplasia is present on the surface of epithelium and the CD105 expression is negative (Immunohistochemistry, AB-PAS; Original magnification, 400×).

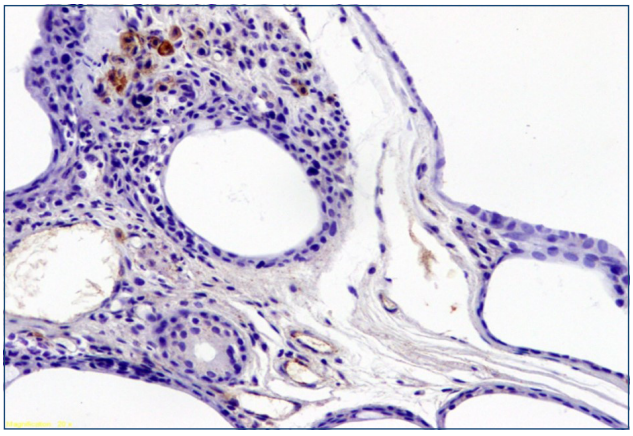

Figure 3. A compound nevi with a moderate expression of CD105 in the individual endothelial cells.

DISCUSSION

In a study, CD105 immunolabeling has been investigated with 35 uveal melanomas[6] and concluded that, since the staining for some nonendothelial cells has been described in benign cutaneous melanocytic lesions[9], further specific investigations are needed to confirm the CD105 specificity for proliferating endothelial cells in ocular melanocytic tumors. To the best of our knowledge, since the expression of endoglin in human conjunctival nevi has not been studied to date, we were interested to do that investigation. Our clinical characteristics of the investigated nevi and histopathological distribution of the types of nevi were quite similar to other studies. Compound nevus was the most common diagnosis, and bulbar lesions were the most common site[2,11,12]. In 30 out of 44 lesions, endoglin was weakly or moderately positive while it was not identified in 14 (31.8%) lesions. The association between the number of CD105-positive nevi and the histological type of conjunctival nevi was investigated in our study. The evolution of conjunctival nevi starts from an initial junctional phase to a compound and finally subepithelial nevus. Junctional nevi, characterized of nevus cells located at the epithelium and substantia propria interface, are found only early in life. In compound nevi, nevus cells are located in the substantia propria and junctional area. Subepithelial nevus, over time, becomes located in substantia propria, entirely beneath the epithelium[2]. Our study showed a statistical significant correlation between CD105 expression and the histological type of conjunctival nevi. The CD105 expression was significantly higher in compound nevi compared to subepithelial and becomes weaker as nevus cells pass into the deeper layers of the conjunctiva. These results correspond to the fact that subepithelial nevus is characterized by calm epithelium in which there is no nevus cells, or any cell activity, and never shows malignant transformation[2].

The presence of intralesional epithelial cysts is very characteristic and diagnostically useful feature of conjunctival nevi[11]. Intralesional epithelial cysts are less frequent in early lesions. In long-standing nevi, cysts may occupy most of the volume of the lesion and the melanocytic component may not be apparent and their presence may help differentiate such conjunctival nevi from other amelanotic conjunctival lesions. In contrast, conjunctival cysts are extremely rare in primary acquired melanosis and melanoma[2,11]. When we analyzed the correlation of the presence of CD105-positive blood vessels with the intralesional cysts status, results showed that expression of CD105 was significantly lower in nevi with intralesional cysts and that the more intralesional cysts are present when the expression of endoglin becomes weaker.

Many ophthalmology studies evaluated MVD using biomarkers such as CD34, CD31, vWF, and CD105 and demonstrated that CD105 is specifically overexpressed on endothelial cells of all angiogenic tissues, including tumors, but only weakly or not at all on those of normal tissues[3,6,16,17]. In recent years, the antiangiogenic therapy represents a promising approach for cancer treatment[18,19]. How to improve the benefit from these therapies and how to check patient response are leading goals for investigators. The value of using tumor MVD as a prognostic and antiangiogenic treatment efficacy indicator for a wide range of cancers is still the aim of many studies[20]. To date, bevacizumab is an antiangiogenic antibody approved for clinical indications. However, essentiality is ongoing in order to develop more antibodies that have targets highly expressed on tumor endothelium. CD105 represents a promising marker of angiogenesis that requires to be further investigated, in terms of its therapeutic relevance in cancer. In this context, Karmani et al.[21] suggest, in vivo, that the potential use CD105, in terms of indirectly iodinated anti-CD105 mAbs (D-KRYRR peptide as a linker [I125-KRYRR-anti-CD105-mAbs]) regarding its directly usage (125-anti-CD105 mAbs) for the thyroid tumor imaging and for therapeutic purposes in thyroidology. Understanding and recognizing the benefits and limitations of microvessel density, further investigations of characteristics of molecules expressed by endothelial cells will certainly improve the efficacy of antiangiogenic agents and proper guidelines for effective therapy in human malignancies. The importance of evaluating the CD105 expression in some ocular tumors has been already indicated, but there is a need for further detailed investigations of endoglin expression in the eye in order to determine its importance as a prognostic and antiangiogenic treatment efficacy indicator.

CONCLUSION

Herewith, in the present study, the first demonstrating the CD105 expression in conjunctival nevi, weak expression of endoglin was exhibited in the majority of investigated nevi. As such, this study demonstrated a statistically significant correlation of endoglin and histological type of nevus, also a statistically significant correlation of endoglin and the presence of intralesional cysts. Of note, this association suggests that endoglin could be a useful diagnostic and prognostic marker for ocular melanocytic lesions, but the further confirmatory study is required in order of affirming its specificity in differentiating between benign and malignant melanocytic ocular lesions and its clinical, predictive, and therapeutic potential.

AUTHORS' CONTRIBUTIONS

DD: Conceptualization, Data curation, Formal Analysis, Investigation, Methodology, Project administration, Resources, Software, Validation, Visualization, and Writing – original draft. **SJ**: Conceptualization, Data curation, Formal Analysis, Investigation, Methodology, Project administration, Resources, Software, Validation, Visualization, and Writing – original draft. **DS**: Investigation, Methodology, Project administration, Software, Supervision, Validation, Visualization, Writing – original draft, and Writing – review & editing. **IS**: Investigation, Methodology, Software, Supervision, Visualization, Writing – original draft, and Writing – review & editing. **DD**: Investigation, Methodology, Software, Supervision, Visualization, Writing – original draft, and Writing – review & editing.

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