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CD10 AND CD34 AS MARKERS IN VASCULAR MALFORMATIONS WITH PIK3CA AND TEK MUTATIONS

Immunohistochemical Profile in Vascular Anomalies.

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ABSTRACT

Aims: Vascular malformations (vMs) encompass a wide range of diseases often associated with somatic or, more rarely, germinal genetic mutations. A mutation in the PIK3Ca/mTOR pathway is more often involved in various vMs. CD10 and CD34 are cellular markers that may play a role in mesenchymal differentiation and proliferation. The aim of our study was to find a possible link between the immunohistochemical expression of CD10 and CD34 in vMs and their relationship with mutations in the PIK3CA/mTOR signaling pathway.

Methods and Results: Our study on 58 samples of vMS showed that in endothelial cells CD10 was significantly expressed in PIK3CA mutated samples compared to samples without any mutation ($p<0.05$), especially and even more consistently when compared to samples with mutation in other pathways ($p <0.0001$). Conversely, in the same PIK3CA mutated samples, CD34 expression in endothelial cells was significantly reduced compared to samples either without any mutation or mutations in other pathways ($p<0.05$ and $p<0.0005$). Compared to samples with mutations in other pathways a significant overexpression of endothelial CD10 was also found in samples with TEK/TIE2 mutation, a gene linked to the PIK3CA/mTOR pathway ($p<0.01$). However, CD34 expression was not altered. In samples with PIK3CA mutation, the CD10 expression was significantly increased in the stroma as compared to samples with TEK/TIE2 gene or other genes mutations ($p<0.05$).

Conclusion: Therefore, CD10 and CD34 immunohistochemical profile could suggest/support the presence of mutations in PIK3CA/mTOR pathway in samples of vMs.

Keywords: vascular malformations; PIK3CA/mTOR pathway; CD10 and CD34 expression

1. INTRODUCTION

Vascular malformations (vMs) encompass a lot of different diseases that can cause overgrowth in different anatomic locations associated with somatic or, more rarely, germinal genetic mutations [1]. The genes that are more frequently involved are within the PIK3CA/mTOR cascade, the RAS-MAPK pathway and the GNAQ/GNA11 genes [2].

Immunohistochemistry (IHC) has become a daily tool that allows to distinguish vMs from vascular tumors and to identify endothelial, hematic and lymphatic cells. This is due to an increasing number of antibodies that characterize the different endothelial types, as well as their phase of differentiation [3-6].

In order to find a possible link between genetic and immunohistochemical patterns we investigated the expression of different genetic mutations and immunohistochemical markers according to the pathways that are more frequently involved in different types of vMs [7-8].

CD10 and CD34 expression were chosen as having a role in stem cells. In the fetus, CD10 plays a potential role in mesenchymal differentiation and proliferation [9]. At the same time, it is a marker of lipoblastoma and lipoblastomatosis. These diseases share a histopathology that is very similar to that of mesenchymal overgrowth related to vMs [10].

CD34 is a stem-cell marker expressed in endothelial cells in different phases of their development and differentiation. It is also a marker of stromal progenitor cells expressed in a mesenchymal proliferative component [11].

A lot of studies have shown expression of CD10 and CD34 as an epiphenomenon of different signaling pathways activation during cell development, differentiation and neoplastic transformation [12-17].

The aim of our study was to find a possible association between the immunohistochemical expression of CD10 and CD34 in vMs and their relationship with mutations in the PIK3CA/mTOR signaling pathway and related genes such as TIE2/TEK.

2. MATERIAL AND METHODS

2.1 Patient cohort

From 2015 to 2018 a total of 58 samples of vMs were examined from 53 outpatients collected from Buzzi Hospital and San Paolo Hospital, Milan, Italy. The study was performed after an informed consent was obtained by all patients or by their parents if minor, in accordance with the Helsinki Declaration.

2.2 Samples

Most samples were composed of skin and underlying tissues obtained during surgery performed for clinical reasons and a few from punch biopsy. Samples were fixed in formalin for histological and immunohistochemical evaluation. Further tissue was preserved in “RNA-Later preservation medium” (InVitrogen ThermoFischer Scientific) and a sample of peripheral blood in EDTA, 2.5 ml, was collected for molecular analysis. By comparing blood and tissue sample it was possible to differentiate somatic from germinal mutations.

2.3 Histological/immunohistochemical analysis

Sampling and staining were performed according to the "Procedures and operating instructions for diagnosis in vascular anomalies and pathology" [18]. Immunohistochemical study was performed using auto-stainer and antibodies Dako (Dakopatts, Denmark). CD31, D2-40, Ki67, SMA and WT-1 allowed to confirm the specific type of vM as: AVM (arterio-venous malformation); VM (venous malformation); CM (capillary malformation); LM (lymphatic malformation); CLM (capillary lymphatic malformation); CVM (capillary venous malformation); CLVM (capillary lymphatic venous malformation). Immunohistochemical evaluation of CD10 and CD34 expression was carried out by two independent investigators (L.M. and D.T.). The rare discrepancies in interpreting the results were checked by a third investigator (G.B.). CD10 and CD34 expression were evaluated separately in the endothelial cells of malformed vessels and in mesenchymal cells embedding the vMs, employing a score combining staining intensity and percentage according to Anam Ali [19] (Table 1 and Figure 1).

2.4 Molecular analysis

DNA was extracted with conventional spin column technique. Next Generation Sequencing (NGS) was performed using a MiSeq Illumina instrument for a custom panel of genes, choosen from literature [20-22] and from ISSVA Classification of mutation involved in vMs [2]. Those cases that resulted negative after the first probing underwent a second NGS aimed at finding those cases with a low frequency of mutations. All positive cases were then confirmed with a different approach with a Nextera XT Illumina protocol using custom primers in order to obtain an ultradeep coverage as previously described [23]. Cases were considered mutated when at least two different examinations had the same results. The cases were divided in the following groups according to the genetic analysis: 1) mutations in the PIK3CA (PIK3CA-group); 2) mutations in the specific gene TEK/TIE2 (TEK-group); 3) mutations not related to the PIK3CA pathway (no-PIK3CA-group); 4) without any mutation (neg-group).

2.5 Statistical analysis

Data were deemed significant ($p<0.05$) after a one-way ANOVA followed by the Tukey's multiple comparisons. The results are expressed as the mean value ± SEM. Statistical analysis was performed by GraphPad Instat software (La Jolla, CA, USA) and graph illustrations by GraphPad Prism software (La Jolla, CA, USA).

3. RESULTS

3.1 Clinical data

We evaluated 58 specimens of 53 patients with vMs (we had obtained 3 biopsies in one patient and 2 biopsies in 3 patients). The median age was of 15,3 years, with a male to female ratio 21:32. In 23 patients a syndrome was diagnosed on the basis of clinical features while in 19 patients vMs were associated with only soft tissue overgrowth; 2 cases were characterized by macroglossia and 3 cases by macrodactily alone. The remaining 6 patients had no alterations except vMs.

3.2 Histological data

Histological examination showed a prevalence of LM with or without capillary and/or venous component (26 combined and 10 pure). Pure VM, CM and AVM were present respectively in 6, 10 and 4 specimens. The remaining cases were CVM (2 cases). Histological data were confirmed by IHC used for the evaluation of vascular anomalies [3,5]. Clinical data and histotype are summarized in Table 2.

3.3 Molecular analysis

The molecular analysis showed several types of mutated genes, mainly in the PIK3/mTOR pathway. We found 28 cases with a gene mutation in PIK3CA while a mutation in the TEK gene was found in 6 cases.

In 7 and 2 cases respectively, we found mutations in GNAQ e GNA11 genes; in 2 cases the KRAS gene was mutated; in one case SOS1 and MAP2K1 genes were mutated while in another case the NF1 gene was affected. In 11 cases we were unable to find mutations in genes that are usually linked to vMs (Table 3).

3.4 Immunohistochemistry

In the malformed vessels, CD10 endothelial expression was present in 41 out of 58 specimens with varying score: 13 (1+); 11 (2+) and 16 (3+). It is worth to be noted that in every case where CD10 was positive in malformed vessels, the normal vascular component was negative. Endothelial cells expressed CD34 in 47 specimens with 11 (1+), 10 (2+) and 26 (3+) score in malformed vascular component. In these cases, the antibody expression was maintained also in not malformed component, as it usually happens. CD10 and CD34 were expressed in 28 specimens simultaneously. However, analyzing further slices we found one of the following: a) co-expression of the two antibodies in the same malformed vessels; b) vessels in part positive for CD10 and in part for CD34, in a mutually exclusive way. The stroma expressed CD10 antigen in 30 specimens; here, too, they were present with different intensity and distribution: 9 (1+), 11 (2+) and 10 (3+). These were often consistent with the endothelial expression but sometimes weaker and in rare

cases stronger. Finally, stromal cells CD34 expression was maintained in 51 specimens with different intensity. Table 3 and Figure 3 show endothelial and stromal CD10 and CD34 expression in groups with different mutations (PK3CA, TEK and other mutations).

3.5 Statistical analysis

Statistical analysis showed a significantly higher expression of CD10 in endothelium of both PIK3CA and TEK-group compared to cases in which the mutation in genes of other pathways was found ($p<0.0001$ and $p<0.01$). The CD10 stromal expression of the PIK3CA-group, although with less statistical significance, remained higher than that of both the TEK and no-PIK3CA-groups ($p<0.05$). Conversely, the PIK3CA-group showed a statistically significant reduction in CD34 endothelial expression compared to the no-PIK3CA-group ($p<0.0005$). However, they did not present substantial differences of expression as compared to the TEK-group. Finally, all the groups maintained the stromal expression of CD34 (Figure 3). The neg-group showed a similar CD10 and CD34 expression, both in endothelial and stromal cells, compared to TEK and no-PIK3CA groups.

4. DISCUSSION

Under the term vMs it has been recognized a lot of different diseases, simple or associated with complex syndromes, sporadic or more rarely familiar [1,24], histologically recognizable also with the help of IHC [3,25].

The introduction of NGS allowed to investigate the genetic basis of these anomalies [26] and the recent literature has identified genes and pathways that are more often associated with these diseases. Typical examples are: PIK3CA-mTOR pathway and its related genes as TEK/TIE2, RAS-MAPK pathway and GNAQ and GNA11 genes [8,24].

Mutations in these genes are usually associated with discernible clinical and histological features. PIK3CA mutations are more frequent in LM, pure or combined with other vMs. These are also related to syndromes with overgrowth, named PROS, acronym for PIK3CA related overgrowth spectrum [27].

TEK/TIE2 mutations are often related to VMs [28], while GNAQ and GNA11 gene mutations are related frequently with Sturge Weber Syndrome and CMs [29-30] and RASA1 mutations are associated to AVMs and CM-AVM [7].

However, the correlation between genotype and phenotype is not always present. So, despite of a clinical and/or histological picture we can surmise a specific gene mutation without certainty.

Could the IHC help us to improve in detecting the genes involved in the genesis of vMs?

In this study we recognized that the combined evaluation of CD10 and CD34 expression in endothelial and stromal cells is a possible tool to suggest or support the presence of a mutation in specific pathways.

CD10 and CD 34 expression gained our attention as stem cells markers.

CD10 is an important regulator shared by different tissues and it has been used as a cell surface marker of stem cells in many normal tissues [31].

We found CD10 expression both in endothelial and stromal cells in the cases with mutations in PIK3CA/mTOR pathway. More precisely, in PIK3CA-group, CD10 was often expressed more intensely in endothelial than stromal cells (figure n.3). CD10 positive endothelium belongs to venous, capillary or lymphatic vessels. CD10 positive stromal cells were present from superficial dermis to adipose or fibrous-adipose tissue and showed the same (or slightly less than so) intensity of staining of endothelial cells. Its expression in malformed vessels of PIK3CA-group was an unexpected yet easy detectable finding although it is not used as endothelial marker, while CD10 expression in lipomatosis and fibro-lipomatosis was already described [10]. The relationship between CD10 expression and the mutation of PIK3CA is not clear. The CD10 signaling pathway interferes with PIK3CA pathway in different points, directly and indirectly through PTEN, finally influencing endothelial cell growth and angiogenesis [32-33]. However, we do not know the cause-effect relationship between PIK3CA gene mutation and CD10 expression, because the former could interfere on the latter. For this reason we speculate that a primary alteration of PIK3CA pathway interferes with the CD10 expression through a feed-back positive mechanism. One case with PIK3CA mutation did not show CD10 endothelial expression. This data could be explained by the inadequacy of the sample or simply due to tissue over-fixation.

We found an association between CD10 endothelial expression in cases with other mutations in the PIK3CA pathway, as in TEK. However, in TEK-group a high endothelial CD10 expression was not accompanied by the same strong expression in the soft tissue surrounding the vMs (figure n.4). These data support our idea that there is a relationship between a PIK3CA pathway gene mutations and the expression of CD10. The reason why this marker is reduced in the stroma of the mutated TEK-group remains unexplainable at present. It might be related to the fact that the TEK gene is involved in the PIK3CA/mTOR pathway in an indirect way only. Anyway the lower CD10 expression found in the stromal cells of the TEK-group is a feature that allows to distinguish that from PIK3CA-group.

The expression of CD34 is less significant than that of CD10 to differentiate the PIK3CA-group from the others, even if there is a statistical difference between PIK3CA and no-PIK3CA-group. Conversely, TEK-group maintained a high

expression of CD34 in endothelial cells and in stromal tissue too (figure n.5). The intensity was lower as compared to the no-PIK3CA-group and neg-group even if with lower statistical relevance. The association between decreased expression of CD34 in endothelium and mutation in PIK3CA pathway can be related to a CD34 low expression in lymphatic vessels, that are more frequently associated with PIK3CA mutation if malformed [10,34]. On the other side CD34 positive, multipotent mesenchymal stem cells have been shown to have a greater propensity for endothelial trans-differentiation [11] and the complexity of expression of CD34 in endothelium during the different phases of angiogenesis and differentiation could be influenced by the effects of PIK3CA mutations in lowering its expression. Finally, the alternating expression patterns of CD10 and CD34 that we found in some endothelial cells of PIK3CA-group and especially in TEK-group was already documented in different tissues of fetuses where their alternating expression was suggested to have a role in differentiation and proliferation [9].

In conclusion, in our experience, a vM can show a peculiar immunohistochemical profile that can suggest the presence of a specific mutation. In particular, the expression of CD10 and CD34 could suggest or support the alteration of PIK3CA/mTOR pathway. Indeed, samples with PIK3CA gene mutation usually express high level of CD10 with a mutual decrease in CD34 expression both in endothelial and stromal cells. Also, cases with a mutation in TEK gene showed high CD10 expression limited to the endothelium. On the other side, cases with low CD10 and high CD34 expression in endothelial and stromal cells could correspond to vMs without genetic mutation of the PIK3CA pathway.

In any case, the expression of CD10 and CD34 could be useful when gene analysis has not yet been conducted or gives erratic results. This last possibility could be due to the low mutation frequency of the PIK3CA pathway genes found in some cases. Furthermore, the evaluation of the expression of these markers could be particularly useful when the vMs network is partially hidden by soft tissue overgrowth.

At last it could be of interest to take in mind that CD10 is an enzyme against which inhibitors drugs are used in cardiovascular diseases [35]. The confirmation of CD10 overexpression in PIK3CA mutated vMs could be the first step to think it as a possible target for a pharmacologic treatment also in this kind of diseases.

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REFERENCES

[1] Wassef M, Blei F, Adams D, et al. Vascular Anomalies Classification: Recommendations From the International Society for the Study of Vascular Anomalies. Pediatrics. 2015; 136(1):203-214. http://www.pediatrics.org/cgi/doi/10.1542/peds.2015-0688

[2] ISSVA Classification of Vascular Anomalies ©2018 International Society for the Study of Vascular Anomalies Available at "issva.org/classification" Accessed July 14, 2019

[3] Al Dhaybi R, Powell J, McCuaig C, et al. Differentiation of vascular tumors from vascular malformations by expression of Wilms tumor 1 gene: evaluation of 126 cases. J Am Acad Dermatol. 2010;63(6):1052-1057.

[4] Trindade F, Tellechea O, Torrelo A, et al. Wilms Tumor 1 Expression in Vascular Neoplasms and Vascular Malformations. Am J Dermatopathol. 2011; 33:569–572

[5] Breiteneder-Geleff S, Soleiman A, Kowalski H, et al. Angiosarcomas express mixed endothelial phenotypes of blood and lymphatic capillaries: podoplanin as a specific marker for lymphatic endothelium. Am J Pathol. 1999;154(2):385-394

[6] Kahn HJ, Bailey D, Marks A. Monoclonal antibody D2-40, a new marker of lymphatic endothelium, reacts with Kaposi's sarcoma and a subset of angiosarcoma. Mod Pathol. 2002;15(4):434-440.

[7] Greene AK, Goss JA. Vascular Anomalies: From a Clinicohistologic to a Genetic Framework. Plast Reconstr Surg. 2018;141(5):709e-717e

[8] Ten Broek RW, Eijkelenboom A, van der Vleuten CJM, et al. Comprehensive molecular and clinicopathological analysis of vascular malformations: A study of 319 cases. Genes Chromosomes Cancer. 2019;58(8):541-550

[9] Kim JH, Hwang SE, Yu HC, et al. Distribution of CD10-positive epithelial and mesenchymal cells in human mid-term fetuses: a comparison with CD34 expression. Anat Cell Biol. 2014;47(1):28-39.

[10] Requena L, Kutzner H. Lipoblastoma and lipoblastomatosis. In: Requena L, Kutzner H, eds. Cutaneous Soft Tissue Tumors. Philadelphia, PA: Wolters Kluwer; 2015: 855-858

[11] Sidney LE, Branch MJ, Dunphy SE, et al. Concise review: evidence for CD34 as a common marker for diverse progenitors. Stem Cells. 2014;32(6):1380-1389

[12] Suzuki T, Ino K, Kikkawa F, et al. Neutral endopeptidase/CD10 expression during phorbol ester-induced differentiation of choriocarcinoma cells through the protein kinase C- and extracellular signal-regulated kinase-dependent signalling pathway. Placenta. 2002;23(6): 475-482.

[13] Takata M, Yao T, Nishiyama KI, et al. Phenotypic alteration in malignant transformation of colonic villous tumours: with special reference to a comparison with tubular tumours. Histopathology. 2003;43(4):332-339.

[14] Affara NI, Trempus CS, Schanbacher L, et al. Activation of Akt and mTOR in CD34+/K15+ keratinocyte stem cells and skin tumors during multi-stage mouse skin carcinogenesis. Anticancer Res. 2006;26(4B):2805-2820.

[15] Bilalovic N, Sandstad B, Golouh R, et al. CD10 protein expression in tumor and stromal cells of malignant melanoma is associated with tumor progression. Mod Pathol. 2004;17(10):1251-1258.

[16] Barth PJ, Westhoff CC. CD34+ fibrocytes: morphology, histogenesis and function. Curr Stem Cell Res Ther. 2007;2(3):221-227

[17] Nimphius W, Moll R, Olbert P, et al. CD34+ fibrocytes in chronic cystitis and noninvasive and invasive urothelial carcinomas of the urinary bladder. Virchows Arch. 2007;450(2):179185.

[18] Moneghini L, Mihm MC, Fulcheri E.Procedures and operating instructions for diagnosis in vascular anomalies and pathology. Pathologica. 2019;111(1):48-50

[19] Ali A, Asif M, Ahmad B, et al. Stromal Expression of CD10 by Immunohistochemistry in Odontogenic Keratocyst (OKC), Dentigerous and Radicular Cysts and Its Correlation with Local Recurrence and Aggressive Behaviour. Asian Pac J Cancer Prev. 2019;20 (1):249253

[20] Nopajaroonsri C., Aubrey A. Lurie. Venous aneurysm, arterial dysplasia, and near-fatal hemorrhages in neurofibromatosis type I - Human Pathology. 1996 ;27(9):982-985

[21] Nikolaev S.I., Vetiska S., Bonilla X., et al. Somatic Activating KRAS Mutations in Arteriovenous Malformations of the Brain. N Engl J Med. 2018; 378:250-261

[22] Tao Hong, Yupeng Yan, Jingwei Li, et al. High prevalence of KRAS/BRAF somatic mutations in brain and spinal cord arteriovenous malformations. Brain 2019;142; 23–34

[23] Kuentz P, St-Onge J, Duffourd Y, et al. Molecular diagnosis of PIK3CA-related overgrowth spectrum (PROS) in 162 patients and recommendations for genetic testing. Genet Med. 2017;19(9):989-997

[24] Blei F. Overgrowth Syndromes With Vascular Anomalies. Curr Probl Pediatr Adolesc Health Care. 2015; 45:118-131

[25] Mulliken JB, Glowacki J. Hemangiomas and vascular malformations in infants and children: a classification based on endothelial characteristics. Plast Reconstr Surg. 1982;69(3):412-22

[26] Lindhurst MJ, Sapp JC, Teer JK, et al. A mosaic activating mutation in AKT1 associated with the Proteus syndrome. New Engl J Med. 2011; 365:611–619.

[27] Keppler-Noreuil KM, Rios JJ, Parker VE, et al. PIK3CA-related overgrowth spectrum (PROS): diagnostic and testing eligibility criteria, differential diagnosis, and evaluation. Am J Med Genet A. 2015;167A (2):287-295.

[28] Limaye N, Wouters V, Uebelhoer M, et al. Somatic mutations in angiopoietin receptor gene TEK cause solitary and multiple sporadic venous malformations. Nat Genet. 2009;41(1):118124

[29] Shirley MD, Tang H, Gallione CJ, et al. Sturge-Weber syndrome and port-wine stains caused by somatic mutation in GNAQ. N Engl J Med. 2013;368(21):1971-1979

[30] Couto JA, Ayturk UM, Konczyk DJ, et al. A somatic GNA11 mutation is associated with extremity capillary malformation and overgrowth. Angiogenesis. 2017;20(3):303-306

[31] Bühring HJ, Battula VL, Treml S, et al. Novel markers for the prospective isolation of human MSC. Ann N Y Acad Sci. 2007; 1106:262-271.

[32] Maguer-Satta V., Besançon R., Bachelard-Cascales E. Concise review: neutral endopeptidase (CD10): a multifaceted environment actor in stem cells, physiological mechanisms, and cancer. Stem Cells. 2011;29(3):389-396.

[33] Jana Sayantan H, Jha Bharti M, Patel Chandni, et al. D10-A new prognostic stromal marker in breast carcinoma, its utility, limitations and role in breast cancer pathogenesis. Indian J Pathol Microbiol. 2014;57(4):530-536.

[34] Fina L, Molgaard HV, Robertson D et al. Expression of the CD34 gene in vascular endothelial cells. Blood. 1990; 15;75(12):2417-2426

[35] McMurray JJ, Packer M, Desai AS et al. Angiotensin-neprilysin inhibition versus enalapril in heart failure. N Engl J Med. 2014; 371 (11): 993–1004.

Figure legends

Figure 1 Combined Score of CD10 and CD34 in endothelial and stromal cells

Figure 2 CD10 and CD34 expression in endothelium and stromal compartment. The bar graphs represent the expression level of: CD10 in the endothelium (panel A), CD10 in the stromal compartment (panel B), CD34 in the endothelium (panel C) and CD34 in the stromal compartment (panel D). Data are reported as immunohistochemical score (IHC score). The PIK3CA-group refers to the samples (n = 28) bearing PIK3CA mutation; the neg-group (n = 11) refers to the samples bearing no mutations; the TEK-group (n = 6) refers to the samples bearing TEK mutation; the no-PIK3CA-group refers to the samples (n = 13) bearing mutations other than PIK3CA. Significance was evaluated by one-way ANOVA, followed by Tukey's multiple comparisons post-test. Panel A: ANOVA, P<0.0001; Tukey's post-test: *, P < 0.05; **, P < 0.01; ****, P < 0.0001. Panel B: ANOVA, P = 0.0024; Tukey's post-test: *, P < 0.05. Panel C: ANOVA, P = 0.0006; Tukey's post-test: *, P < 0.05; ***, P < 0.0005. Panel D: ANOVA, P = ns.

Figure 3 Expression of CD10 and CD34 in endothelium and stroma in samples with mutation of different genes (PK3CA group, TEK group and no-PIK3CA group)

Table 1: Combined Score achieved summing Proportion and Intensity Score

Proportion Score (P) (%)	**Intensity score (I)**
0- 0 ≤10	0-Negative
1- 11≤25	1-Weak staining
2- 26≤50	2-Moderate staining
3- >50	3-Intense staining
Combined Score (T)	
(T) = Proportion score (P) + Intensity score (I)	
Score 0-1	Negative
Score 2	1+
Score 3 - 4	2+
Score 5 - 6	3+

Table 2: Clinical data and histotype. (y): years; F: female; M: male; PWS: Parker-Weber Syndrome; SWS: Sturge-Weber Syndrome; KTS: Klippel-Trenaunay Syndrome; GSD: Gorham-Stoud Disease. Multiple biopsies: Patient 27 (3 samples); Patients 22, 29 and 37 (2 samples)

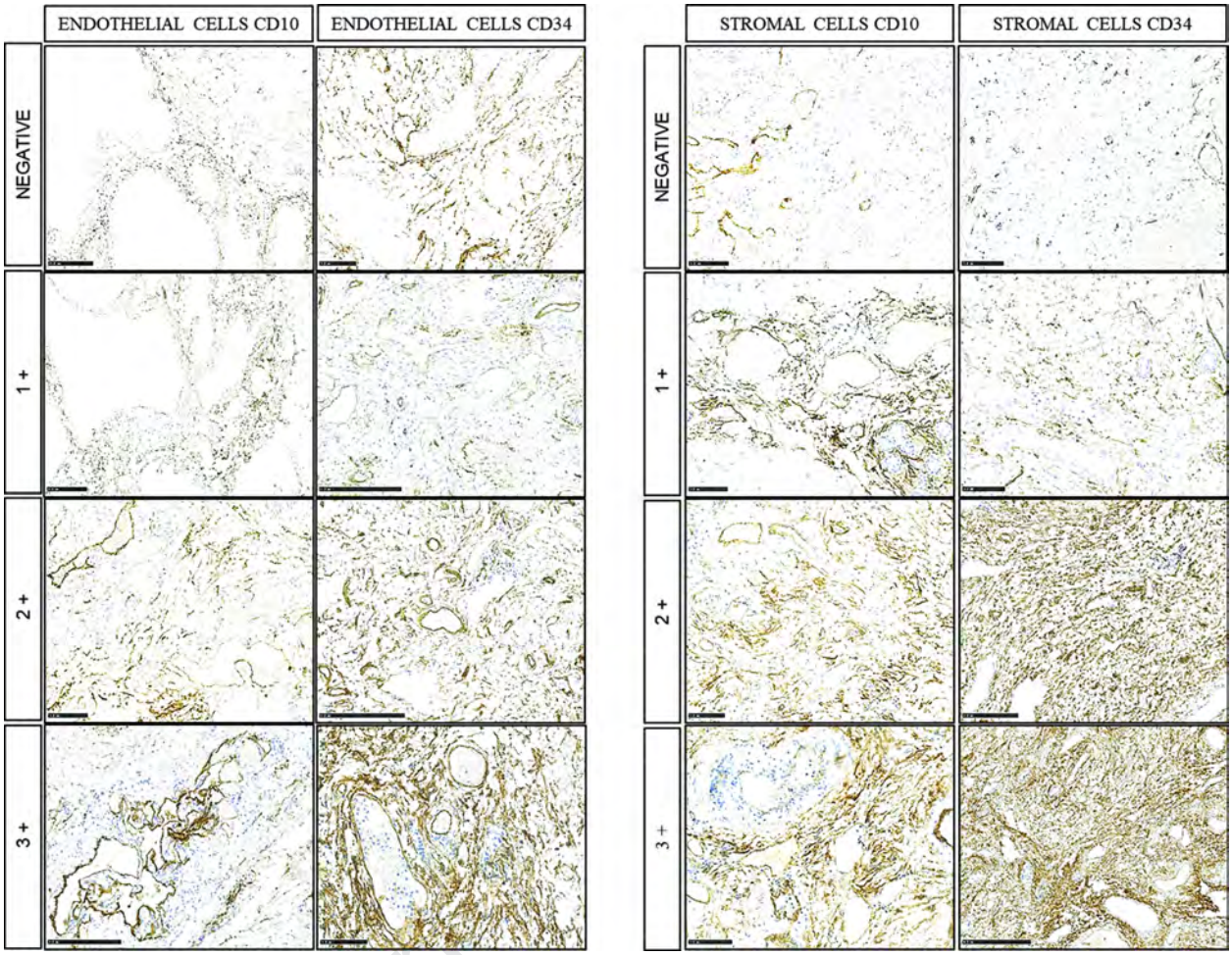
Patients	Age (y)	Gender	Phenotype	Histotype	Site
1	7	M	Overgrowth	LVM	right lower limb
2	18	F	KTS	CLM	left lower limb
3	32	F	KTS	VM	left lower limb
4	43	M	VM	VM	cheek
5	9	M	Overgrowth	CLVM	right lower limb
6	15	M	Overgrowth	CM	right hemisome and bilateral toes
7	20	M	Overgrowth	VM	left hemisome
8	5	F	Overgrowth	LM	right trunk
9	1	M	Macrodactily	LVM	left hand second finger
10	11	F	KTS	CLM	left leg
11	20	F	PWS	CLVM	face, trunk, right footh second finger
12	1	F	Overgrowth	LM	left foot
13	1	F	Overgrowth	LM	right hemiface
14	7	M	Overgrowth	CM	right hip
15	4	M	Overgrowth	LM	left lower limb
16	14	M	Overgrowth	CLM	face, trunk and lower limbs
17	6	F	LM	LM	left thigh
18	21	F	KTS	CLVM	left lower limb
19	5	F	Overgrowth	LVM	chest and axillary bilaterally
20	9	F	Macrodactily	CM	left foot second finger
21	13	F	KTS	LVM	left lower limb
22	13	F	KTS	LVM	left lower limb
22	5	F	KTS	CLM	left upper limb
23	24	M	KTS	VM	left lower limb and abdomen
24	8	F	Overgrowth	CLVM	left trunk
25	57	F	Macroglossia	LVM	tongue
26	3	M	Macroglossia	LM	tongue
27	5	M	KTS	LM	left foot
27	5	M	KTS	CLVM	toe right foot
27	5	M	KTS	LVM	left lower limb
28	15	F	Overgrowth	CLVM	right hemisome
29	26	M	KTS	CLM	bilateral lower limbs
29	27	M	KTS	CLM	bilateral lower limbs
30	9	F	KTS	CVM	left lower limb
31	23	F	Macrodactily	CLM	left toes and right foot
32	12	F	Overgrowth	CLM	left lower limb
33	20	F	AVM	AVM	right hemiface

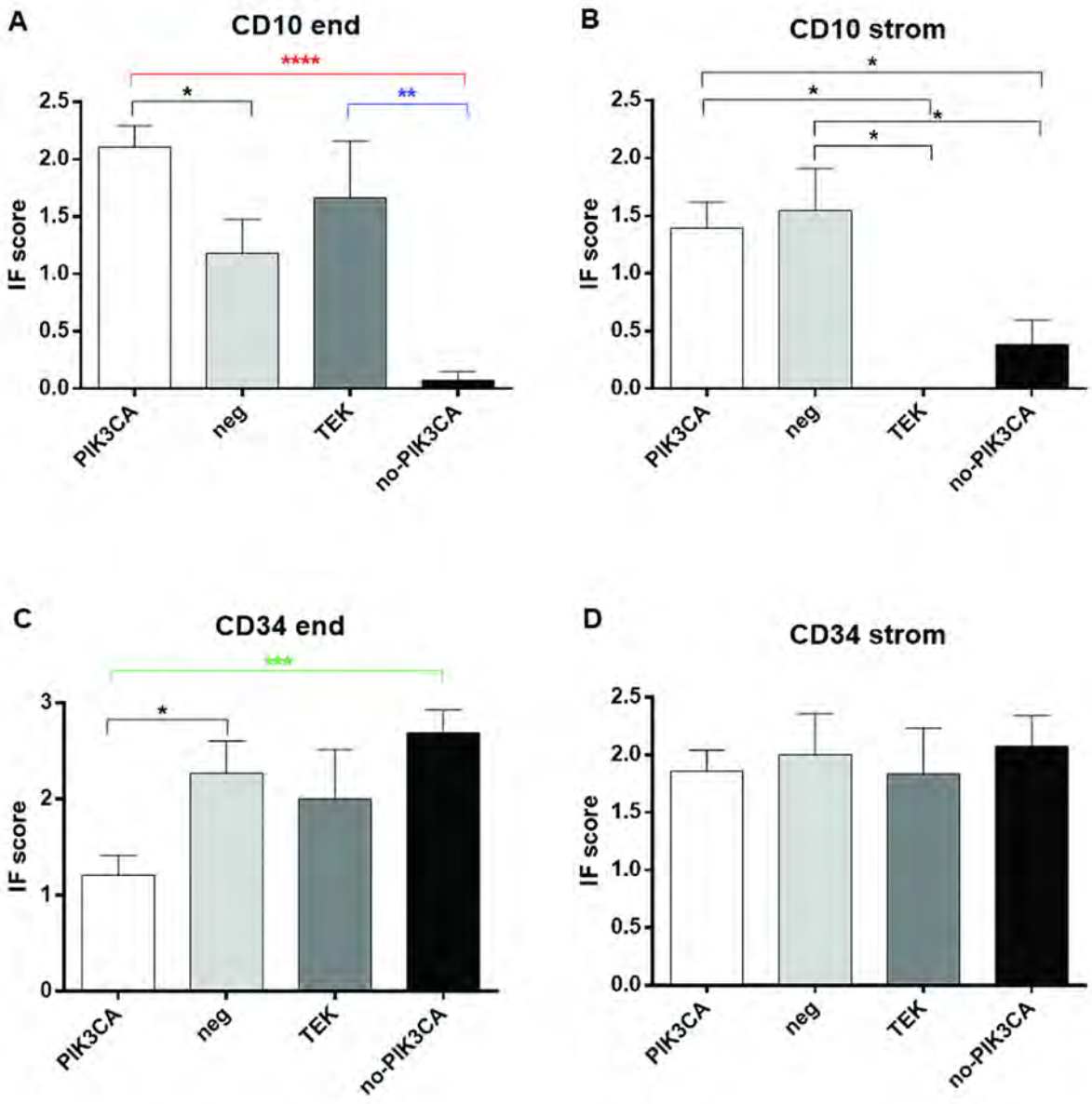
34	17	F	LM	LM	left hemiface
35	15	M	GSD	LVM	hepatic hilus
36	19	M	VM	AVM	lower limb
37	17	F	Overgrowth	CLM	left lower limb
37	17	F	Overgrowth	CLM	left lower limb
38	14	F	Overgrowth	CM	left lower limb
39	17	F	Overgrowth	CLM	right lower limb and trunk
40	8	M	Overgrowth	VM	feet, front and toungue
41	16	M	PWS	CVM	limbs and face
42	4	F	PWS	CM	left hemisome
43	13	F	SWS	CLM	right cervical region
44	22	F	SWS	CM	right upper lip
45	1	F	SWS	LM	left thigh
46	2	F	PWS	CM	left arm
47	0.5	M	SWS	CM	right lower limb
48	6	F	SWS+KTS	CM	left hemisome
49	10	F	SWS	CM	back
50	15	F	KTS	LM	left lower limb and genitalia
51	37	M	Overgrowth	AVM	right hemiface
52	17	M	KTS	VM	right lower limb
53	52	M	AVM	AVM	tongue

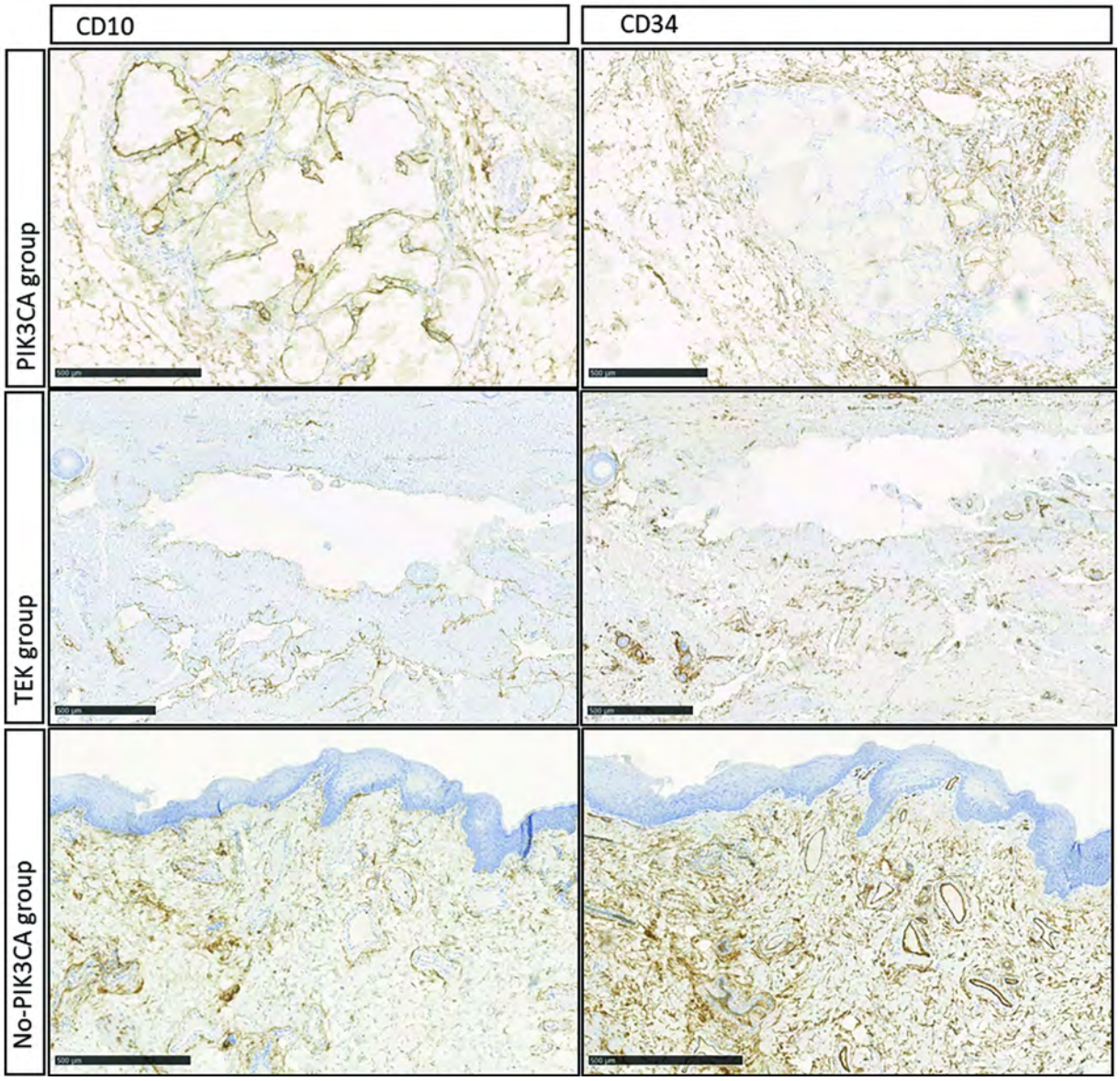
Table 3: CD10 and CD34 endothelial (end.) and stromal (str.) score related to genes mutations (mut.)

Patients	CD10 end.	CD10 str.	CD34 end.	CD34 str.	mut.
1	3	1	2	3	PIK3CA
2	3	0	1	1	PIK3CA
3	1	0	1	3	PIK3CA
4	3	1	1	1	PIK3CA
5	3	3	0	0	PIK3CA
6	1	0	3	2	PIK3CA
7	2	1	3	2	PIK3CA
8	2	2	1	3	PIK3CA
9	3	3	1	2	PIK3CA
10	2	0	2	3	PIK3CA
11	1	0	1	1	PIK3CA
12	2	2	0	3	PIK3CA
13	3	3	0	2	PIK3CA
14	1	1	3	1	PIK3CA
15	1	2	2	0	PIK3CA
16	1	0	2	3	PIK3CA
17	0	3	3	0	PIK3CA
18	3	2	0	2	PIK3CA
19	2	1	2	1	PIK3CA
20	0	0	2	2	PIK3CA
21	3	3	0	3	PIK3CA
22	3	3	1	2	PIK3CA
22	3	2	0	2	PIK3CA
23	3	2	2	2	PIK3CA
24	3	0	0	3	PIK3CA
25	2	1	1	2	PIK3CA
26	3	3	0	2	PIK3CA
27	2	0	0	1	PIK3CA
27	0	0	3	3	NEG.
27	0	0	3	3	NEG.
28	3	3	1	1	NEG.
29	1	3	2	0	NEG.
29	1	3	3	0	NEG.
30	1	2	3	2	NEG.
31	1	0	3	3	NEG.
32	2	2	3	2	NEG.
33	0	1	3	3	NEG.
34	2	1	0	2	NEG.
35	2	2	1	3	NEG.
36	1	0	3	3	TEK
37	1	0	3	2	TEK
37	0	0	3	0	TEK

38	2	0	2	2	TEK
39	3	0	3	2	TEK
40	3	0	1	2	TEK
41	0	0	3	3	GNA11
42	0	0	3	2	GNA11
43	0	0	3	3	GNAQ
44	0	1	3	3	GNAQ
45	0	0	3	3	GNAQ
46	0	0	3	2	GNAQ
47	0	2	3	0	GNAQ
48	0	0	0	1	GNAQ
49	0	0	3	2	GNAQ
50	1	0	3	2	KRAS
51	0	2	3	3	KRAS
52	0	0	2	1	NF1
53	0	0	3	2	SOS1MAP2


ENDOTHELIAL CELLS CD10
ENDOTHELIAL CELLS CD34
STROMAL CELLS CD10
STROMAL CELLS CD34
NEGATIVE
1 +
2 +
3 +
NEGATIVE
1 +
2 +
3 +


A
CD10 end
IF score
PIK3CA
neg
TEK
no-PIK3CA
B
CD10 strom
IF score
PIK3CA
neg
TEK
no-PIK3CA
C
CD34 end
IF score
PIK3CA
neg
TEK
no-PIK3CA
D
CD34 strom
IF score
PIK3CA
neg
TEK
no-PIK3CA


CD10
CD34
PIK3CA group
TEK group
No-PIK3CA group

HIGHLIGHTS

- Some vascular malformations are related to a lot of gene mutations as PIK3CA, TEK, GNAQ and GNA11
- CD10 and CD34 are two stem progenitor cell/mesenchymal markers
- Their expression is unknown in vascular malformations
- A correlation between CD and CD34 expression in endothelium and stroma was found in vascular malformation with defined gene mutations
- This discover could be useful in diagnosis and therapy of these diseases