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Aberrant cytoplasmic connexin43 expression as a helpful marker in vascular neoplasms

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Abstract

Background: Gap junctions consisting of connexins (Cx) are fundamental in controlling cell proliferation, differentiation, and cell death. Cx43 is the most broadly expressed Cx in humans and is attributed an important role in skin tumor development. Its role in cutaneous vascular neoplasms is yet unknown.

Methods: Fifteen cases each of cutaneous angiosarcoma (cAS), Kaposi sarcoma (KS), and cherry hemangioma (CH) were assessed by immunohistochemistry for expression of Cx43. Expression pattern, intensity, and percentage of positively stained cells were analyzed. Solid basal cell carcinomas served as positive and healthy skin as negative controls.

Results: Most cases of cAS presented with a strong Cx43 staining of almost all tumor cells, whereas endothelia of KS showed medium expression and CH showed mostly weak expression. In comparison with KS or cAS, the staining intensity of CH was significantly lower ($P \leq 0.001$). All tissue sections of both cAS and KS were characterized by a mostly diffuse, cytoplasmic staining pattern of the vascular endothelia. None of those showed nuclear staining.

Conclusion: The high-to-intermediate expression of Cx43 observed in all cases of cAS and KS suggests that this Cx may play a role in the development of malignant vascular neoplasms and serve as a helpful diagnostic marker.

KEYWORDS
cutaneous angiosarcoma, Cx43, hemangioma, immunohistochemistry, Kaposi sarcoma

1 | INTRODUCTION

Classification of cutaneous vascular anomalies remains a challenge because of their broad spectrum of clinical and histopathological appearances. According to the *20th International Society for the Study of Vascular Anomalies* (ISSVA) workshop, vascular anomalies should be classified as either vascular malformations (originating from a mesenchymal stem cell defect) or vascular tumors (true proliferative neoplasms).[1] Based on cellular behavior, the latter can be categorized as benign, locally aggressive/borderline, or malignant[1] and must be differentiated from vascular malformations especially from a therapeutic/prognostic point of view.[2] In general, the histopathologic growth pattern is decisive for distinction of benign and malignant lesions. While malignant tumors proliferate chaotically without a recognizable pattern, benign neoplasms follow a conservative organoid growth pattern. However, differential diagnosis of vascular tumors

can be challenging because both clinical features (ie, size and multifocality) and histomorphology with supplementary immunohistochemical staining (ie, expression of endothelial markers) are often quite similar.[3]

Gap junction (GJ)-mediated intercellular communication (GJIC) is a fundamental mechanism for the maintenance of cellular homeostatic balance. GJs are involved in the control of cell proliferation, differentiation, cell death, and gene expression.[4,5] Each GJ is composed of six connexin (Cx) proteins on each side of the channel that determine the permeability and regulatory properties of the GJ channel.[6] Cxs are transmembrane proteins expressed by various cell types including keratinocytes, fibroblasts, melanocytes, and endothelial cells that allow cell-to-cell communication via GJs and distant intercellular communication through hemichannel formation.[7,8] Therefore, they are crucial for normal tissue development, differentiation, and cell proliferation.[4,9-11] Cx43 is perceived to be the most broadly expressed Cx in humans, being also present in both the epidermis and dermis.[12,13] At present, there is evidence that Cx43 plays important roles both in wound repair and skin tumor development.[14-19] As the expression of Cx43 has not yet been studied in cutaneous vascular neoplasms, we here analyzed its expression in cAS and KS and compared it with CH and normal skin.

2 | MATERIALS AND METHODS

2.1 | Case selection and data assessment

Within a retrospective setting, we randomly retrieved 45 histopathological specimens from 45 individual patients with cutaneous angiosarcoma (cAS), Kaposi sarcoma (KS), or cherry hemangioma (CH) from our archives. In addition, clinical data were collected from the patients' files. The study was conducted according to the Declaration of Helsinki.

2.2 | Immunohistochemical studies

All paraffin-embedded sections were reviewed by one dermatopathologist (H. K.) and one dermatologist with special interest in dermatopathology (V. G. F.) under blinded conditions regarding prior diagnosis and the patients' clinical outcome at time of evaluation.

Immunohistochemistry for Cx43 was performed on 5-µm tissue sections, which were deparaffinized in xylol and rehydrated in graded alcohol. For antigen retrieval, slides were overlaid with antigen retrieval solution (Dako, Hamburg, Germany) and incubated in saturated steam for 30 minutes. For staining, slides were incubated with an anti-Cx43 antibody (#C6219, Sigma-Aldrich, Darmstadt, Germany; 1/4000 dilution) using the Dako Autostainer plus (Dako) and the Dako REAL Detection System Alkaline Phosphatase/RED (#K5005Dako, Dako).

When evaluating immunohistochemistry, we distinguished among membranous, cytoplasmic and nuclear patterns of staining. In addition, staining was marked as diffuse (when the entire tumor sample was stained) or focal (when some tumoral areas were not stained). Immunostaining was scored by analysis of five representative sections at ×20 magnification using a semi-quantitative approach (Table 1) that considers the percentage and intensity of positively stained tumor cells. Staining intensity was assessed in relation to the positive epidermal layer. Each tumor specimen was scored once; multiple tissue sections representing the same tumor were averaged. Any discrepancies were reassessed to yield an ultimate consensus. Solid basal cell carcinomas (n = 10) served as positive and healthy skin (fringe area of tissue sections after tumor surgery for epithelial neoplasms, n = 10) as negative control.

2.3 | Statistical analysis

Descriptive statistics are presented as mean ± SD. Unpaired *t* test was used to assess statistical significance (with *P* values at significance levels of ≤0.05 (*), ≤0.01 (**) or ≤0.001 (***) as indicated. Statistical analysis was performed using GraphPad Prism 5.0 (GraphPad software Inc., San Diego, California, USA).

3 | RESULTS

All tissue sections were unanimously diagnosed as cAS (n = 15), KS (n = 15), or CH (n = 15) by histomorphology and immunohistochemistry for ERG, CD31, CD34, and/or HHV8 where necessary. Table 2 provides selected clinical data including tumor sites for all patients included. All patients underwent surgery between 2006 and 2020. In the cAS cohort, most of the patients (67%) were males and the most common localization was the head area (forehead, cheek, nose, scalp; 73%). The median age in this cohort was 73 years (range 57-90). Most of the KS patients (67%) were males. The median age was 66 years (range 42-92) and tumors mostly localized at the lower extremities (foot, lower, or upper leg; 67%). In the CH cohort females prevailed (53%) and patients turned out

Positive cells, %	Points	Intensity	Points	Points subsumed	Total score
0	0	None	0	0–1	**0**
1-33	1	Weak	1	2–3	**1**
34-66	2	Intermediate	2	4–5	2
67-100	3	Strong	3	6	3

TABLE 1 Scoring of immunohistochemical connexin43 (Cx43) staining

TABLE 2 Patients' clinical characteristics and connexin43 (Cx43) staining

							Cx43	
Entity	Sex	Age	Localization	Management	Recurrence	Prognosis	Pattern	Score
cAS_1	M	57	Cheek	S, C/R	Y	Palliative	d, c	3
cAS_2	F	72	Trunk	S	Y	RFU	d, c	3
cAS_3	M	75	Temporal	S, C/R	Y	Palliative	d, c	2
cAS_4	M	73	Nose	S, R	Y	RFU	d, c	2
cAS_5	M	84	Nose	S, C/R	Y	Palliative	d, c	2
cAS_6	F	90	Scalp	S, R	Y	Palliative	d, c	3
cAS_7	M	61	Scalp	S, C/R	N	RFU	d, c	3
cAS_8	F	78	Trunk	S, C/R	Y	Palliative	d, c	3
cAS_9	F	69	Scalp	S, C/R	Y	RFU	d, c	3
cAS_10	M	84	Scalp	S, R	NA	LFU	d, c	2
cAS_11	M	79	Forehead	S, R	NA	LFU	d, c	3
cAS_12	M	64	Scalp	S, C/R	NA	RFU	d, c	3
cAS_13	M	66	Upper leg	S	Y	RFU	d, c	3
cAS_14	F	66	Trunk	S	NA	RFU	d, c	3
cAS_15	M	76	Forehead	S, C/R	NA	RFU	d, c	3
KS_1	F	88	Foot	R	N	Palliative	d, c	2
KS_2	M	66	Upper arm	IFNα	N	RFU	d, c	2
KS_3	M	52	Foot	R, IFNα	N	RFU	d, c	2
KS_4	M	69	Hand	R, IFNα	Y	LFU	d, c	2
KS_5	F	79	Lower leg	IFNα	Y	RFU	d, c	2
KS_6	M	64	Foot	IFNα	Y	RFU	d, c	2
KS_7	M	81	Foot	R, IFNα	Y	RFU	d, c	2
KS_8	M	64	Upper leg	R	Y	RFU	d, c	2
KS_9	M	53	Foot	HAART	N	RFU	d, c	2
KS_10	W	67	Upper leg	S	NA	LFU	d, c	2
KS_11	M	42	Upper leg	NA	NA	LFU	d, c	3
KS_12	M	92	Foot	R	Y	LFU	d, c	2
KS_13	W	49	Shoulder	R	Y	LFU	d, c	2
KS_14	W	88	Forearm	S	Y	LFU	d, c	2
KS_15	M	60	Forearm	S	Y	RFU	d, c	2
CH_1	M	52	Forearm	S	NA	NA	d, c/m	1
CH_2	M	68	Forearm	S	NA	NA	d, c/m	1
CH_3	F	59	Trunk	S	NA	NA	d, c/m	2
CH_4	M	52	Upper leg	S	NA	NA	d, c/m	2
CH_5	M	26	Nose	S	NA	NA	d, c/m	2
CH_6	F	51	Upper leg	S	NA	NA	d, c/m	2
CH_7	F	66	Trunk	S	NA	NA	f, c/m	1
CH_8	F	22	Trunk	S	NA	NA	f, c/m	1
CH_9	M	51	Trunk	S	NA	NA	f, c/m	1
CH_10	F	74	Upper leg	S	NA	NA	f, c/m	1
CH_11	F	45	Trunk	S	NA	NA	f, c/m	2
CH_12	F	40	NA	S	NA	NA	d, c/m	2
CH_13	F	54	Nose	S	NA	NA	d, c/m	1
CH_14	M	62	Trunk	S	NA	NA	d, c/m	1
CH_15	M	59	Trunk	S	NA	NA	d, c/m	1

Note: Age at time of biopsy [years].

Abbreviations: C, chemotherapy; c, cytoplasmic; cAS, cutaneous angiosarcoma; CH, cherry hemangioma; d, diffuse; F, female; f, focal; HAART, highly active anti-retroviral therapy, because of underlying HIV infection; IFNα, systemic therapy with interferon (IFN) α-2a (*Roferon*®); KS, Kaposi sarcoma; LFU, lost to follow-up; M, male; m, membranous; NA, not available/applicable; R, radiation; RFU, regular follow-ups; S, surgery.

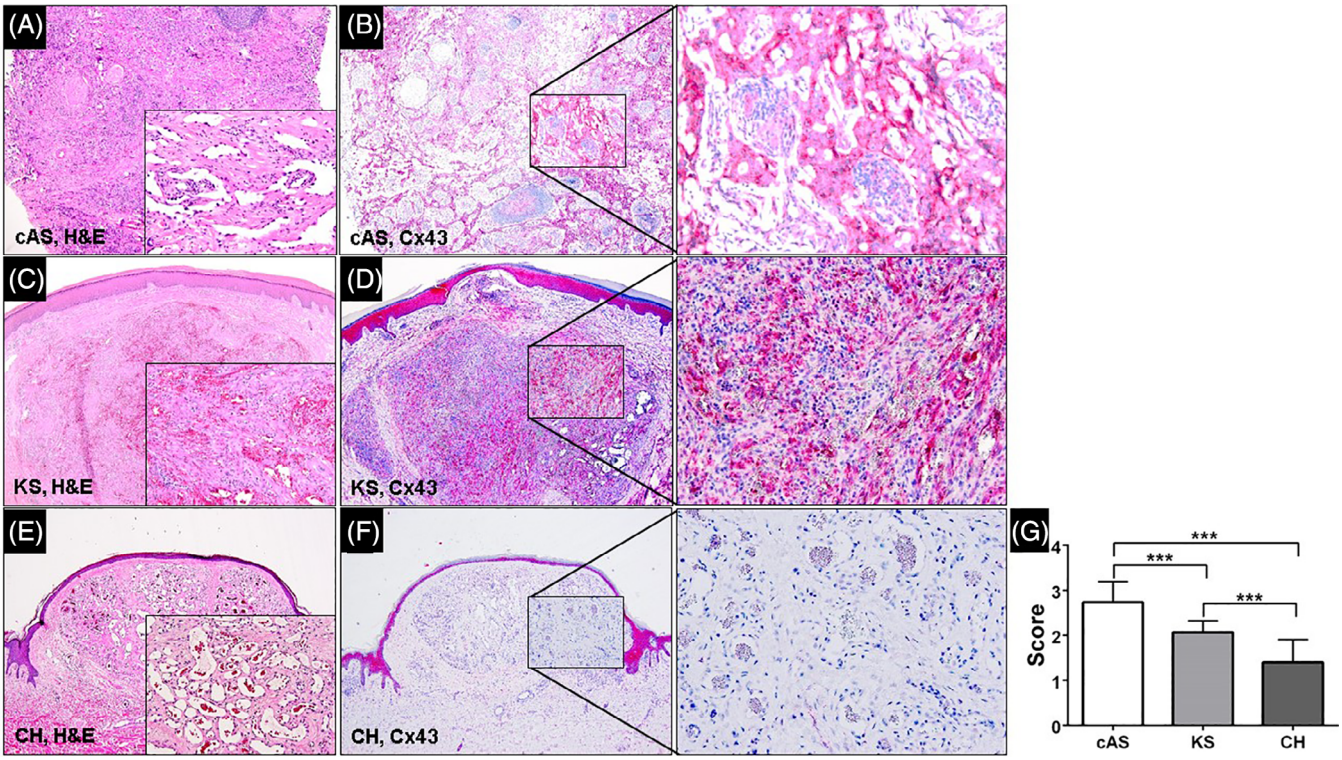

FIGURE 1 Immunohistochemical staining for connexin43 (Cx43) in vascular neoplasms. A,B, cAS; C,D, KS; and E,F, CH stained with H&E and Cx43, respectively. All micrographs were taken at ×40 magnification, inlays at ×200. G, Quantification of Cx43 staining according to a weak (1), medium (2) and strong (3) score, n = 15 each group. ***$P \leq 0.001$

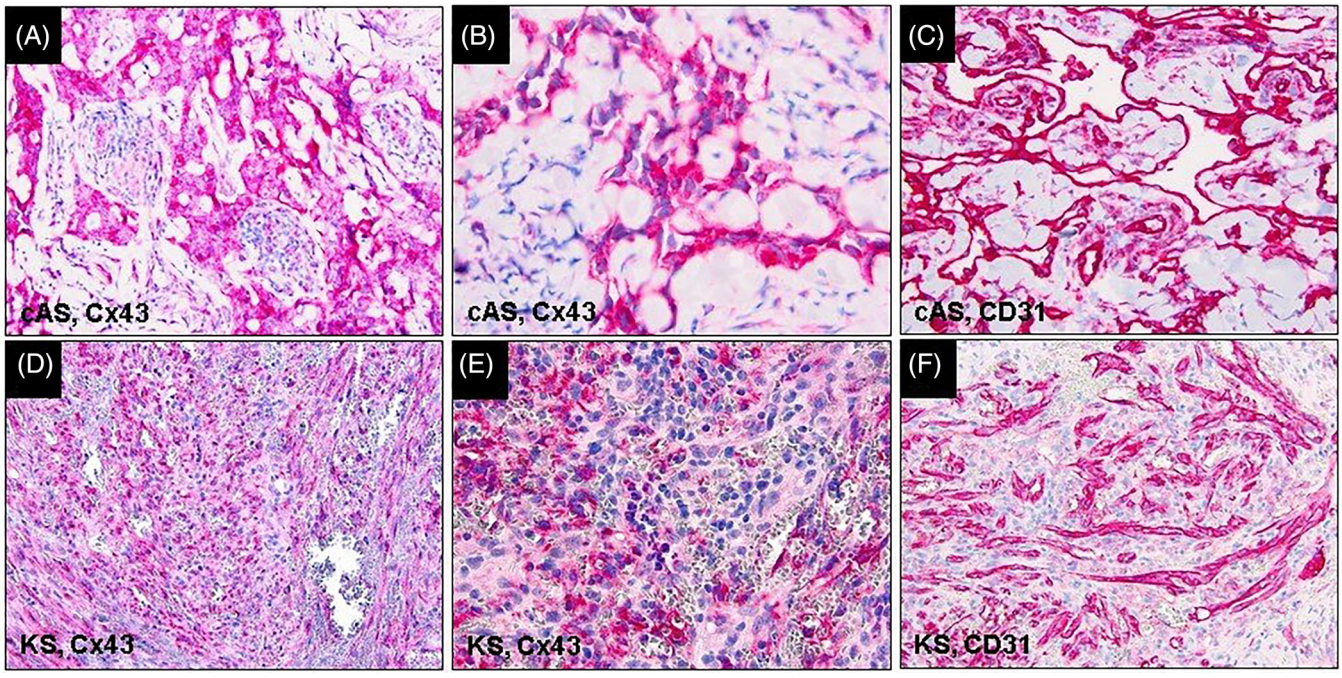

FIGURE 2 Immunohistochemical staining for connexin43 (Cx43) as compared to CD31 expression in vascular tumors. A-C, cAS and, D-F, KS stained for Cx43 and CD31. A,C,D,F, ×200 magnification; B,E, ×400 magnification

to be younger (median 52 years, range 22-74). CH lesions were predominantly present at the trunk (50%; one localization not available).

Table 2 also summarizes the anti-Cx43 staining patterns and scores. Most cases of cAS (73%) presented with a strong staining with almost all endothelial cells being positive (Figure 1A,B). In KS samples,

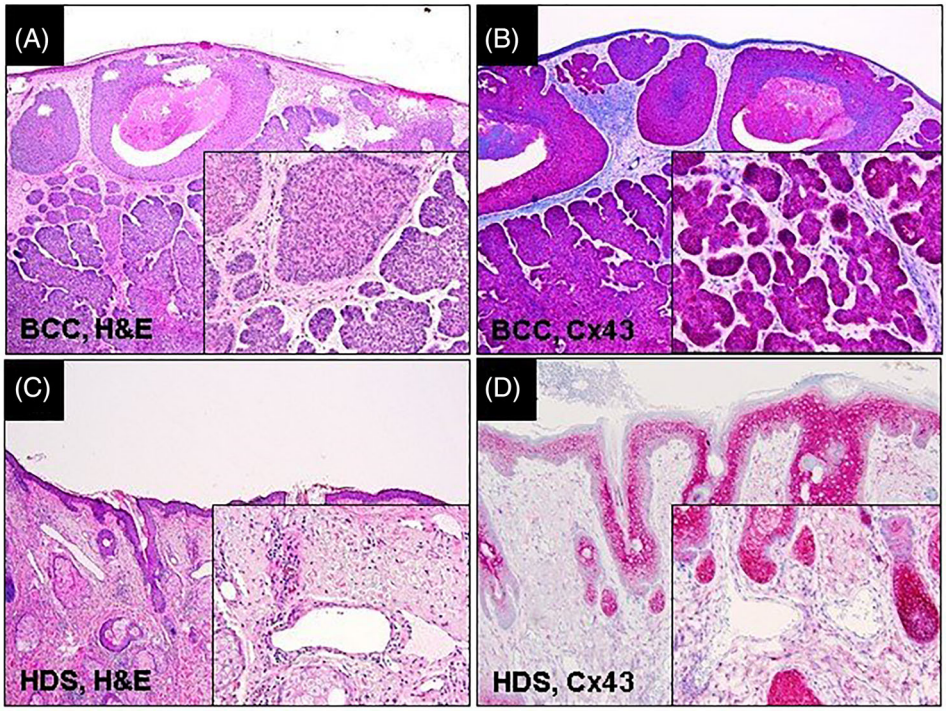

FIGURE 3 Positive and negative controls. Basal cell carcinoma stained with, A, H&E and, B, connexin43 (Cx43) serving as positive control. Healthy donor skin (HDS) stained with, C, H&E and, D, Cx43 serving as negative control. All ×40 magnification, inlays ×200

the score for both the percentage of Cx43-positive cells and the staining intensity was lower (Figure 1C,D) while endothelia of CH stained considerably weaker (Figure 1E,F). Overall, the staining intensity of CH was significantly lower than that in KS or cAS (Figure 1G; $P \leq 0.001$). All tissue sections of both cAS (Figure 1B, inlay) and KS (Figure 1D, inlay) showed an essentially identical immunohistochemical pattern with a mostly diffuse and cytoplasmic staining of the vascular endothelia while CH slides were difficult to assess because of their physiological single layer of endothelial cells and an overall weak staining. We observed a diffuse staining pattern with cytoplasmic and/or membranous localization in most cases of CH (67%). None of the investigated vascular neoplasms presented with a nuclear staining. All cases (except a third of CH samples that showed focal staining) were characterized by a homogenous staining pattern over the whole tissue slide.

As compared to immunohistochemical staining with CD31, malignant vascular tumors (cAS, KS) expressed a similar staining pattern for Cx43 with prominent lining of endothelial cells and some diffusely positive tumor cells and fibroblasts in the periphery (Figure 2). All reviewed basal cell carcinomas were strongly positive for Cx43 (Figure 3A,B), whereas healthy skin showed no Cx43 staining of endothelia of the superficial vascular plexus but positivity of the suprabasal epidermis and hair follicles (Figure 3C,D).

4 | DISCUSSION

Cx43 is attributed an important role in skin tumor development[14-20] and tumor angiogenesis.[21] We therefore aimed at gaining novel insight into the expression of Cx43 in cutaneous vascular neoplasms. In order to obtain the most differentiated picture possible between benign and malignant entities, we chose to investigate benign CH,[22] borderline malignant KS,[23] and highly aggressive cAS[24] as reference cases from our archives. Using tissue samples that could be clearly attributed to either entity by histomorphology and if necessary immunohistochemistry, we attempted to assess the potential relevance of anti-Cx43 immunolabeling for their diagnosis.

CHs, also known as Campbell de Morgan spots, adult hemangiomas or senile angiomas, are circumscribed vascular proliferations of newly formed venule-like blood vessels in a lobular pattern in the papillary dermis without signs of malignant morphology.[22] KS, on the other hand, is considered a borderline malignant tumor derived from lymphatic endothelial cells.[23] cAS is a rare aggressive soft tissue neoplasm arising from endothelial cells. All forms of cAS have a very poor prognosis and recur frequently.[24] The usually ill-defined lesions are characterized by irregular, anastomosing, and dilated vascular structures with pleomorphic endothelium that may be misinterpreted as hemangioma or lymphangioma, particularly in cases where only a superficial biopsy specimen is available. Poorly differentiated cAS can be difficult to distinguish from other cutaneous carcinomas such as KS or melanoma.[25] Therefore, it is useful to assess a panel of endothelial markers such as von Willebrand factor, CD31, CD34, FLI1, and ERG since individual antibodies tend to be variably positive in different tumors. As for KS, CD31, and CD34 are the most frequently used immunohistochemical markers in supporting the diagnosis of cAS.[26-28] Even if labeling for CD31 is widely applied in the diagnosis of malignant vascular tumors,[24] there may be diagnostic pitfalls since CD31 is also expressed by other cell lineages.[29]

Interestingly, Cx43 expression assessed by immunohistochemical scoring correlated with the aggressiveness of the vascular neoplasm

in our study. However, scoring has not been evaluated to allow prognostic conclusions for cAS or KS. The expression of Cxs had earlier been described to correlate with a bad prognosis in some tumoral lineages,[12] but Cxs levels may paradoxically predict a better prognosis in other tumors depending on the entity.[30-32]

Cxs are normally expressed on cell surfaces as membranous proteins that build blocks of GJs, which play a role in regulating cell proliferation and apoptosis.[33] Previous studies suggested that aberrant cytoplasmic localization of Cxs and, therefore, disturbance of GJIC could be of relevance for carcinogenesis, invasion, and metastasis in carcinomas, melanoma, and leukemia.[33-37] Cx components are stored in the cytoplasm and dysfunctional trafficking decreases the uptake of Cxs by the cell membrane from the cytoplasm.[33,38,39] Cx43 forms channels in the cell membrane which result in a membranous staining upon immunolabeling with an anti-Cx43 antibody. However, in KS and cAS staining is mainly found in the cytoplasm of tumor cells. Therefore, we assume that the aberrant cytoplasmic expression of Cx43 in malignant vascular tumors might be related to a defect in GJ assembly associated with increased Cx retention as well. This lack of GJIC by retention of Cx43 has also been observed in gastric,[40,41] pancreatic,[42] and breast cancer.[43,44] In addition, an abnormal or aberrant cytoplasmic expression seen upon immunohistochemical staining is not uncommon in other oncoproteins. For example, CD117 is expected to show a membranous staining pattern, whereas a cytoplasmic and globular/dot-like staining is also frequently encountered.[45] In accordance with these findings and a similar report on atypical fibroxanthoma,[18] our data suggest that Cx43 may play a role in tumorigenesis of KS and cAS. The localization of Cx43 in CH could not be reliably evaluated due to the morphological properties of benign vascular tumors such as CHs. Therefore this study can only hypothesize that in benign vascular tumors Cx43 is localized in membranes and/or cytoplasm. However, it has to be kept in mind that because of a lack of Ki-67 labeling and failure of growth of the endothelial cells in culture,[46] CH has been suggested of being rather a soft tissue overgrowth than a true neoplasm. In contrast, Groesser et al[47] reported *RAS* mutations in 20% of cases, indicating that at least some cases were neoplastic in nature. This is in accordance with recent studies reporting that the majority of investigated CHs showed MIB1-positive cells[22] and diffusely expressed *WT-1*, *GNA14*, *GNAQ*, and *GNA11* mutations, thus providing solid evidence to establish it as a benign vascular neoplasm.[48] For this reason, it is speculative if Cx43 in CHs should show a membranous or cytoplasmic staining.

5 | CONCLUSION

The prominent expression of Cx43 in all cases of cAS and KS studied suggests that this Cx may play a role in the development of malignant vascular neoplasms. Our observations may help to contribute to our understanding of angiomatous lesions and perhaps facilitate classification of vascular tumors in the further course. Larger patient cohorts are necessary to validate our findings and to study their potential diagnostic and prognostic/predictive value.

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CONFLICT OF INTEREST

The authors declare no potential conflict of interest.

AUTHOR CONTRIBUTIONS

Verena Gerlinde Frings and Hermann Kneitz have made substantial contributions to conception and design and have acquired the data. Verena Gerlinde Frings has analyzed as well as interpreted the data and drafted the manuscript. Bastian Schilling, Matthias Goebeler, and Hermann Kneitz have critically revised the manuscript. All authors have given final approval of the version to be published.

DATA AVAILABILITY STATEMENT

Further research data are not shared. All necessary data are available in the manuscript.

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REFERENCES

1. Wassef M, Borsik M, Cerceau P, et al. Classification of vascular tumours and vascular malformations. Contribution of the ISSVA 2014/2018 classification. *Ann Pathol*. 2021;4(1):58-70.
2. Brahmbhatt AN, Skalski KA, Bhatt AA. Vascular lesions of the head and neck: an update on classification and imaging review. *Insights Imaging*. 2020;11(1):19.
3. Antonescu C. Malignant vascular tumors—an update. *Mod Pathol*. 2014;27(suppl 1):S30-S38.
4. Herve JC, Derangeon M. Gap-junction-mediated cell-to-cell communication. *Cell Tissue Res*. 2013;352(1):21-31.
5. Carette D, Gilleron J, Chevallier D, Segretain D, Pointis G. Connexin a check-point component of cell apoptosis in normal and physiopathological conditions. *Biochimie*. 2014;101:1-9.
6. Harris AL. Connexin channel permeability to cytoplasmic molecules. *Prog Biophys Mol Biol*. 2007;94(1–2):120-143.
7. Willecke K, Dermietzel R, Druge PM, et al. Biochemical and genetic investigations on gap junctions from mammalian cells. *Biophys Struct Mech*. 1982;9(2):103-107.
8. Bai D, Wang AH. Extracellular domains play different roles in gap junction formation and docking compatibility. *Biochem J*. 2014;458(1):1-10.
9. Mese G, Richard G, White TW. Gap junctions: basic structure and function. *J Invest Dermatol*. 2007;127(11):2516-2524.
10. Villanelo F, Escalona Y, Pareja-Barrueto C, Garate JA, Skerrett IM, Perez-Acle T. Accessing gap-junction channel structure-function relationships through molecular modeling and simulations. *BMC Cell Biol*. 2017;18(suppl 1):5.
11. Wong P, Laxton V, Srivastava S, Chan YW, Tse G. The role of gap junctions in inflammatory and neoplastic disorders (review). *Int J Mol Med*. 2017;39(3):498-506.
12. Aasen T, Leithe E, Graham SV, et al. Connexins in cancer: bridging the gap to the clinic. *Oncogene*. 2019;38(23):4429-4451.
13. Zhang XF, Cui X. Connexin 43: key roles in the skin. *Biomed Rep*. 2017;6(6):605-611.

14. Tittarelli A, Guerrero I, Tempio F, et al. Overexpression of connexin 43 reduces melanoma proliferative and metastatic capacity. *Br J Cancer*. 2015;113(2):259-267.
15. Luu S, Bell C, Schneider S, Nguyen TA. Connexin 26 and connexin 43 in canine mammary carcinoma. *Vet Sci*. 2019;6(4):101.
16. Hofmann F, Navarrete M, Alvarez J, et al. Cx43-gap junctions accumulate at the cytotoxic immunological synapse enabling cytotoxic T lymphocyte melanoma cell killing. *Int J Mol Sci*. 2019;20(18):4509.
17. Aasen T, Mesnil M, Naus CC, Lampe PD, Laird DW. Gap junctions and cancer: communicating for 50 years. *Nat Rev Cancer*. 2016;16(12):775-788.
18. Fernandez-Flores A, Varela-Vazquez A, Mayan MD, Fonseca E. Expression of connexin 43 by atypical fibroxanthoma. *J Cutan Pathol*. 2021;48(2):247-254.
19. Fernandez-Flores A, Varela-Vazquez A, Suarez Penaranda JM, Mayan MD, Fonseca E. Expression of connexin 43 in 32 cases of Merkel cell carcinoma. *Am J Dermatopathol*. 2020;42(3):178-185.
20. Cogliati B, Vinken M, Silva TC, et al. Connexin 43 deficiency accelerates skin wound healing and extracellular matrix remodeling in mice. *J Dermatol Sci*. 2015;79(1):50-56.
21. Wang WK, Chen MC, Leong HF, Kuo YL, Kuo CY, Lee CH. Connexin 43 suppresses tumor angiogenesis by down-regulation of vascular endothelial growth factor via hypoxic-induced factor-1 alpha. *Int J Mol Sci*. 2014;16(1):439-451.
22. Fernandez-Flores A, Colmenero I. Campbell de Morgan spots (cherry angiomas) show endothelial proliferation. *Am J Dermatopathol*. 2018;40(12):894-898.
23. Kaposi M. Idiopathisches multiples Pigmentsarkom der Haut. *Arch f Dermatol*. 1872;4:265-273.
24. Young RJ, Brown NJ, Reed MW, Hughes D, Woll PJ. Angiosarcoma. *Lancet Oncol*. 2010;11(10):983-991.
25. Patel RM, Billings SD. Cutaneous soft tissue tumors that make you say, "oh $*&%!". *Adv Anat Pathol*. 2012;19(5):320-330.
26. Rao P, Lahat G, Arnold C, et al. Angiosarcoma: a tissue microarray study with diagnostic implications. *Am J Dermatopathol*. 2013;35(4):432-437.
27. Miettinen M, Lindenmayer AE, Chaubal A. Endothelial cell markers CD31, CD34, and BNH9 antibody to H- and Y-antigens—evaluation of their specificity and sensitivity in the diagnosis of vascular tumors and comparison with von Willebrand factor. *Mod Pathol*. 1994;7(1):82-90.
28. Al-Daraji W, Husain E, Zelger BG, Zelger B. A practical and comprehensive immunohistochemical approach to the diagnosis of superficial soft tissue tumors. *Int J Clin Exp Pathol*. 2009;2(2):119-131.
29. McKenney JK, Weiss SW, Folpe AL. CD31 expression in intratumoral macrophages: a potential diagnostic pitfall. *Am J Surg Pathol*. 2001;25(9):1167-1173.
30. Huang RP, Fan Y, Hossain MZ, Peng A, Zeng ZL, Boynton AL. Reversion of the neoplastic phenotype of human glioblastoma cells by connexin 43 (cx43). *Cancer Res*. 1998;58(22):5089-5096.
31. Fukushima M, Hattori Y, Yoshizawa T, Maitani Y. Combination of non-viral connexin 43 gene therapy and docetaxel inhibits the growth of human prostate cancer in mice. *Int J Oncol*. 2007;30(1):225-231.
32. Ableser MJ, Penuela S, Lee J, Shao Q, Laird DW. Connexin43 reduces melanoma growth within a keratinocyte microenvironment and during tumorigenesis in vivo. *J Biol Chem*. 2014;289(3):1592-1603.
33. Kanczuga-Koda L, Sulkowski S, Lenczewski A, et al. Increased expression of connexins 26 and 43 in lymph node metastases of breast cancer. *J Clin Pathol*. 2006;59(4):429-433.
34. Nomura S, Maeda K, Noda E, et al. Clinical significance of the expression of connexin26 in colorectal cancer. *J Exp Clin Cancer Res*. 2010;29(1):79.
35. Liu Y, Zhang X, Li ZJ, Chen XH. Up-regulation of Cx43 expression and GJIC function in acute leukemia bone marrow stromal cells post-chemotherapy. *Leuk Res*. 2010;34(5):631-640.
36. Zhang D, Kaneda M, Nakahama K, Arii S, Morita I. Connexin 43 expression promotes malignancy of HuH7 hepatocellular carcinoma cells via the inhibition of cell-cell communication. *Cancer Lett*. 2007;252(2):208-215.
37. Chen JT, Cheng YW, Chou MC, et al. The correlation between aberrant connexin 43 mRNA expression induced by promoter methylation and nodal micrometastasis in non-small cell lung cancer. *Clin Cancer Res*. 2003;9(11):4200-4204.
38. Krutovskikh V, Mazzoleni G, Mironov N, et al. Altered homologous and heterologous gap-junctional intercellular communication in primary human liver tumors associated with aberrant protein localization but not gene mutation of connexin 32. *Int J Cancer*. 1994;56(1):87-94.
39. Krutovskikh VA, Troyanovsky SM, Piccoli C, Tsuda H, Asamoto M, Yamasaki H. Differential effect of subcellular localization of communication impairing gap junction protein connexin43 on tumor cell growth in vivo. *Oncogene*. 2000;19(4):505-513.
40. Tang B, Peng ZH, Yu PW, Yu G, Qian F. Expression and significance of Cx43 and E-cadherin in gastric cancer and metastatic lymph nodes. *Med Oncol*. 2011;28(2):502-508.
41. Li CH, Hao ML, Sun Y, Wang ZJ, Li JL. Ultrastructure of gap junction and Cx43 expression in gastric cancer tissues of the patients. *Arch Med Sci*. 2020;16(2):352-358.
42. Liang QL, Wang BR, Chen GQ, Li GH, Xu YY. Clinical significance of vascular endothelial growth factor and connexin43 for predicting pancreatic cancer clinicopathologic parameters. *Med Oncol*. 2010;27(4):1164-1170.
43. Kanczuga-Koda L, Sulkowska M, Koda M, et al. Expression of connexin 43 in breast cancer in comparison with mammary dysplasia and the normal mammary gland. *Folia Morphol*. 2003;62(4):439-442.
44. Kazan JM, El-Saghir J, Saliba J, et al. Cx43 expression correlates with breast cancer metastasis in MDA-MB-231 cells in vitro, in a mouse xenograft model and in human breast cancer tissues. *Cancers (Basel)*. 2019;11(4):460.
45. Fletcher CD, Berman JJ, Corless C, et al. Diagnosis of gastrointestinal stromal tumors: a consensus approach. *Hum Pathol*. 2002;33(5):459-465.
46. Tuder RM, Young R, Karasek M, Bensch K. Adult cutaneous hemangiomas are composed of nonreplicating endothelial cells. *J Invest Dermatol*. 1987;89(6):594-597.
47. Groesser L, Peterhof E, Evert M, Landthaler M, Berneburg M, Hafner C. BRAF and RAS mutations in sporadic and secondary pyogenic granuloma. *J Invest Dermatol*. 2016;136(2):481-486.
48. Liau JY, Lee JC, Tsai JH, Chen CC, Chung YC, Wang YH. High frequency of GNA14, GNAQ, and GNA11 mutations in cherry hemangioma: a histopathological and molecular study of 85 cases indicating GNA14 as the most commonly mutated gene in vascular neoplasms. *Mod Pathol*. 2019;32(11):1657-1665.

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