Elevated expression of c-kit in small venous malformations of blue rubber bleb nevus syndrome

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Abstract

The blue rubber bleb nevus syndrome (BRBNS, syn. bean syndrome) is a rare disease characterized by multiple cutaneous and gastrointestinal venous malformations associated with severe bleeding. However, the underlying molecular mechanisms are unknown and no targeted therapeutic approach exists to date. Here we report the case of a 19-year-old male patient with severe BRBNS in whom we analyzed the expression of tyrosine kinases frequently involved in tumor development by immunohistochemistry (vascular endothelial growth factor receptor-2, stem cell growth factor receptor (c-kit), platelet-derived growth factor receptor-β, and stem cell tyrosine kinase-1). A prominent expression of c-kit was detectable in smaller blood vessels, which also showed a moderate expression of the proliferation marker MIB1. Surprisingly, other growth factor receptors stained negatively. We therefore conclude that pharmacological inhibition of the c-kit signaling pathway in cavernous hemangiomas by selective kinase inhibitors may offer options in the treatment of BRBNS patients.

Introduction

The blue rubber bleb nevus syndrome (BRBNS) is a rare vascular syndrome characterized by venous malformations (cavernous hemangiomas) in the skin, gastrointestinal tract, and less frequently in the liver, spleen, lung, and nervous system.[1,2] In cases where the skin is affected, extensive plum-purple papules and nodules are located predominantly on the upper extremities and the trunk. About 200 BRBNS cases have been reported in the literature to date. Main clinical complications especially in gastrointestinal lesions are chronic and acute hemorrhage with consequent iron deficiency, anemia, and death.[3-5] However, the underlying molecular mechanisms responsible for the uncontrolled growth of venous blood vessels are not known. In one case, a gain-of-function mutation in TIE2 (syn. TEK tyrosine kinase, endothelial) was detected in a patient with pancreatic lymphangioma associated with BRBNS;[6] however, it is unclear whether aberrant expression or activation of tyrosine kinases is a general relevant mechanism in the pathogenesis of BRBNS. In this context, administration of highly selective tyrosine kinase inhibitors would offer novel pharmacological options in the treatment of BRBNS patients.

Materials and Methods

Specimens (2 cm in diameter) of a venous malformation from a 19-year-old patient were obtained. After paraffin embedding, 5 µm-sections were stained immunohistochemically using antibodies recognizing vascular endothelial growth factor receptor 2 (VEGFR2, 1:200, R&D-Systems, Wiesbaden, Germany), stem cell growth factor receptor (c-kit, 1:20, Dako, Hamburg, Germany), platelet-derived growth factor receptor-β (PDGFR-β, 1:100, Calbiochem, Schwalbach/Ts, Germany), and stem cell tyrosine kinase 1 (FLT3, 1:100, Abcam, Cambridge, UK), as well as CD31 (1:25, Dako) and MIB1 (1:200, Dako), using the EnVision™ system (Dako) according to the manufacturers' instructions. With the exception of VEGFR2 staining (EDTA buffer, pH 9.0), all slides were pretreated with citrate buffer (pH 6.1). Respective positive controls were used for all staining (VEGFR2: ductal invasive breast carcinoma; c-kit: skin; PDGFR-β: skin; FLT3: thymus). Samples were stained with hematoxylin-and-eosin (H/E) and carefully reviewed by the study pathologist (CM). The specific immunohistochemical staining and the number of MIB1 positive cells were evaluated semi-quantitatively. Mutational analyses of c-kit exons 9, 11, 13, 14, and 17 were performed on genomic DNA extracted from paraffin-embedded BRBNS tissue as described previously.

Results

We report on a 19-year-old male with multiple, slowly growing, cutaneous malformations, predominantly in mechanically stressed regions of the skin (feet, waist). Initially, the boy presented early after birth with a large hemangioma of the right shoulder resulting in a diagnosis of Kasabach-Merritt syndrome. Because glucocorticoid therapy did not result in a clinical reduction of hemangioma size, several surgical interventions were attempted.

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In subsequent years, additional hemangiomas developed within dermal tissue. Owing to the patient's young age and good compliance, offline targeted therapeutic approaches were considered in order to improve his quality of life. H/E-staining revealed multiple sub-epidermal and differentially sized cavernous hemangiomas (Figure 1A). All analyzed hemangiomas were lined with CD31-positive cells (Figure 1B). Less than 5% of these endothelial cells stained positively for the nuclear proliferation marker MIB1 (Figure 1C). In order to identify growth factor signaling pathways possibly involved in angiogenesis and vasculogenesis of cutaneous lesions in BRBNS patients, we analyzed the expression of four different known angiogenic markers including tyrosine kinases (VEGFR2, c-kit, PDGFR-β, FLT3). No detectable membranous or cytoplasmic signals were detected for FLT3, VEGFR2, or PDGFR-β in the endothelial positive cells of venous malformations, while all controls showed a regular staining pattern (data not shown). In contrast, c-kit showed positivity in the cytoplasm of endothelial cells with preference for smaller sized vessels in the venous malformation (Figure 1D-F). In order to define whether common activating mutations participate further in the activation of the SCF/c-kit pathway, mutation analysis of the c-kit exons 9, 11, 13, 14, and 17 were performed. No mutations were detected in these genomic regions.

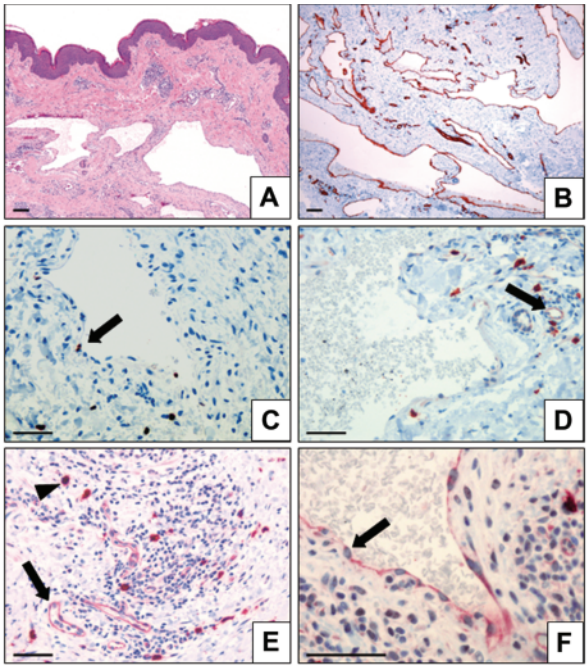

Figure 1. Histopathology and immunohistochemical analyses of resected hemangioma specimen. (A) Conventional H/E staining of cavernous hemangiomas. Immunohistochemical staining with (B) the endothelial cell marker CD31, and (C) the nuclear proliferation marker MIB1 (arrow: positive cell). (D-F) Staining of c-kit revealed an exclusive staining in endothelial cells of intermediate and small venous malformations (arrows) but not of larger sized vessels. Note inflammatory cells as internal positive control (arrowhead). Scale bars: 100 µm.

Discussion

The BRBNS (synonym: bean syndrome) is characterized by slow and permanent growth of cavernous hemangiomas in the skin and gastrointestinal mucosa. BRBNS patients suffer from severe complications such as rupture of larger malformations with potentially life-threatening intestinal bleeding.[4,7-8] Our results together with published data suggest that this moderate but steady increase of vessel size is based on the low number of dividing endothelial cells.[9]

Because the underlying molecular mechanisms of this disease are not understood, no specific therapeutic approaches have been developed to date. Nobuhara and colleagues correlated the presence of an activating mutation in the tyrosine kinase receptor TIE2 (synonym: CD202b, TIE2R849W) with the development of hemangiomatosis.[6] Although it has been demonstrated that this gain-of-function mutation is essential for proper venous morphogenesis,[10] additional data analyzing the potential relevance of other cell growth regulating tyrosine kinases is missing. Therefore, we systematically analyzed the expression of known angiogenic markers and found that most of these factors are not expressed in CD31 positive cells of hemangiomas. However, c-kit was detectable predominantly in smaller sized vessels within the specimens of our patient, suggesting that the SCF (stem cell factor)/c-kit signaling axis is involved in the constant growth of the venous malformations. Normal endothelial cells of adult vessels did not show c-kit expression, whereas at least partial c-kit positivity has been reported in angiosarcomas.[11] In addition, c-kit is indeed known as a stem cell marker that has also been described in the context of angiogenesis.[12-14] C-kit inhibition by selective small molecules such as imatinib mesylate currently represents the gold standard in the treatment of gastrointestinal stromal tumors (GISTs) and hematologous malignant diseases.[15-17] In addition, a second generation of c-kit inhibitors for the treatment of imatinib mesylate-refractory patients (e.g. AMG-706 and AMN-107) currently is being tested in clinical trials.[17] Although no mutations were found in described exons of c-kit, a remarkable expression of c-kit is notable. Thus, targeting slowly cycling endothelial cells using c-kit-specific inhibitors might represent a novel therapeutic option for BRNBS or patients with smaller and slowly progressing lesions.

References

1. Crepeau J, Poliquin J. The blue rubber bleb nevus syndrome. J Otolaryngol 1981;10:387-90.
2. Haiping P, Guoqing L, Zhongshu Y. Blue rubber bleb naevus syndrome. Eur J Surg 2001;167:628-30.
3. Atten MJ, Ahmed S, Attar BM, et al. Massive pelvic hemangioma in a patient with blue rubber bleb nevus syndrome. South Med J 2000;93:1122-5.
4. Kanai M, Noike M, Masaki C, et al. Severe gastrointestinal bleeding during pregnancy in a case of blue rubber bleb nevus syndrome. Semin Thromb Hemost 2005;31: 284-9.
5. Wong YC, Li YW, Chang MH. Gastrointestinal bleeding and paraparesis in blue rubber bleb nevus syndrome. Pediatr Radiol 1994;24:600-1.
6. Nobuhara Y, Onoda N, Fukai K, et al. TIE2 gain-of-function mutation in a patient with pancreatic lymphangioma associated with blue rubber-bleb nevus syndrome: report of a case. Surg Today 2006;36:283-6.
7. Domini M, Aquino A, Fakhro A, et al. Blue rubber bleb nevus syndrome and gastrointestinal haemorrhage: which treatment? Eur J Pediatr Surg 2002;12:129-33.
8. Hansen LF, Wewer V, Pedersen SA, et al. Severe blue rubber bleb nevus syndrome in a neonate. Eur J Pediatr Surg 2009;19: 47-9.
9. Villalain L, Junquera LM, Diaz M, et al. Blue rubber bleb nevus syndrome: im munohistochemical study. Ann Otol Rhinol Laryngol 2003;112:647-50.
10. Vikkula M, Boon LM, Carraway KL 3rd, et al. Vascular dysmorphogenesis caused by an activating mutation in the receptor tyrosine kinase TIE2. Cell 1996;87:1181-90.
11. Miettinen M, Sarlomo-Rikala M, Lasota J. KIT expression in angiosarcomas and fetal endothelial cells: lack of mutations of exon 11 and exon 17 of C-kit. Mod Pathol 2000; 13:536-41.
12. Li TS, Hamano K, Nishida M, et al. CD117+ stem cells play a key role in therapeutic angiogenesis induced by bone marrow cell implantation. Am J Physiol Heart Circ Physiol 2003;285:H931-7.
13. Li TS, Hayashi M, Liu ZL, et al. Low angiogenic potency induced by the implantation of ex vivo expanded CD117(+) stem cells. Am J Physiol Heart Circ Physiol 2004;286: H1236-41.
14. Fiedler W, Staib P, Kuse R, et al. Role of angiogenesis inhibitors in acute myeloid leukemia. Cancer J 2001;7:S129-33.
15. Cervantes F, Hernandez-Boluda JC, Steegmann JL, et al. Imatinib mesylate therapy of chronic phase chronic myeloid leukemia resistant or intolerant to interferon: results and prognostic factors for response and progression-free survival in 150 patients. Haematologica 2003;88:1117-22.
16. Tallman MS. Advancing the treatment of hematologic malignancies through the development of targeted interventions. Semin Hematol 2002;39:1-5.
17. Muler JH, Baker L, Zalupski MM. Gastrointestinal stromal tumors: chemotherapy and imatinib. Curr Oncol Rep 2002;4:499-503.