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 large 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-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. In contrast, no targetable receptor was detectable in these genomic regions.

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. 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; 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, Schwabach/Ts., Germany), and stem cell tyrosine kinase 1 (FLT3, 1:100, Abcam, Cambridge, UK), as well as CD31 (1:125, 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). Respectively 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 semiquantitatively. 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.

In subsequent years, additional hemangiomas developed within dermal tissue. Owing to the patient’s young age and good compliance, off-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, mutational analysis of the c-kit exons 9, 11, 13, 14, and 17 were performed. No mutations were detected in these genomic regions.
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.\(^4,5\) 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.\(^6\)

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.\(^7\) Although it has been demonstrated that this gain-of-function mutation is essential for proper venous morphogenesis,\(^8\) 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.\(^9\) In addition, c-kit is indeed known as a stem cell marker that has also been described in the context of angiogenesis.\(^10,11\) 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 hematological malignant diseases.\(^12-14\) In addition, a second-generation of c-kit inhibitors for the treatment of imatinib mesylate-refractory patients (e.g. AMG-706 and AMN-107) is currently being tested in clinical trials.\(^15\) 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 BRBNS or patients with smaller and slowly progressing lesions.

References