Somatic Activating *RAS* Mutations Cause Vascular Tumors Including Pyogenic Granuloma

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TO THE EDITOR

Vascular anomalies comprise a spectrum of lesions subdivided into malformations and tumors by clinical behavior and histological features. Vascular malformations demonstrate slow progression, whereas vascular tumors proliferate rapidly with marked endothelial turnover (Enjolras *et al.*, 2007). Definitive diagnosis can be challenging, as lesion characteristics may not conform to existing classifications. Hence, many vascular anomalies are identified descriptively (Enjolras *et al.*, 2007). "Lobular capillary hemangioma" describes a vascular tumor with lobular architecture, including infantile hemangiomas (IHs) and other vascular lesions with lobules of proliferating endothelial cells, although the term is commonly used interchangeably for pyogenic granuloma (PG) (Enjolras *et al.*, 2007). PGs are benign, spontaneous vascular tumors with small vessels organized in lobules (Giblin *et al.*, 2007). Approximately 0.5% of skin nodules and 10% of head and neck hemangiomas are PGs, which often develop in children and adolescents and up to 5% of pregnant women, although rare congenital, disseminated cases occur (Giblin *et al.*, 2007; Browning *et al.*, 2009). Lesions appear as dome-shaped, bright red or blue protrusions that grow rapidly and are prone to hemorrhage (Enjolras *et al.*, 2007). PGs primarily occur on cutaneous or mucosal surfaces of the head, neck, and upper extremities, although cases within the gastrointestinal tract, urinary tract, eye, and the central nervous system have been reported (Ahuja *et al.*, 2013). Although infection, trauma, and angiogenic factor upregulation have been proposed, the etiology of PG remains unknown (Giblin *et al.*, 2007; Ahuja *et al.*, 2013).

Before the start of our investigation, the Yale Human Investigation Committee approved the study protocol, and all subjects provided written, informed consent. To identify somatic mutations driving the formation of lobular vascular tumors, we used pairwise whole-exome sequencing (WES) of blood and affected tissue in two patients. The first patient (VASC100) was a 5-year-old boy with a 1-cm compressible, pulsatile tumor on the left helix that had enlarged and reddened over 4 months (Figure 1a). Histologically, it contained lobules of benign-appearing endothelial cells (Figure 1b and c). The second patient (VASC101) was a healthy male infant who had a pedunculated vascular nodule on the medial left lower eyelid at birth undetected by prenatal ultrasound (Figure 1d). Despite prednisone and propranolol treatment, the lesion grew rapidly. Histopathology showed lobules of small caliber capillaries, extensive necrosis, and intervening foci with dilated vessels, some with valves (Figure 1e and f).

After pairwise WES, somatic mutations were identified (Supplementary Materials and Methods online). A heterozygous *KRAS* c.35G>A, p.G12D mutation and a heterozygous *NRAS* c.181C>A, p.Q61K mutation were found in VASC100 and VASC101 tumors, respectively (Table 1 and Supplementary Tables S1 and S2 online and Supplementary Figure S1 online). These mutations were confirmed by means of Sanger sequencing (Supplementary Figure S2 online). Neither data set had mutations in genes that were previously identified in vascular tumors or malformations, including *TEK*, *RASA1*, *CCM1/2/3*, *GLMN*, *VEGRF3*, *FOXC2*, *SOX18*, *ACVRLK1*, *MADH4*, and *ENG* (Ye *et al.*, 2011). No evidence of secondary somatic variants or regions of loss of heterozygosity was found, suggesting that both cases are true somatic RASopathies (Supplementary Figure S3 online).

Recognizing that PGs share the lobular architecture found in VASC100 and 101 tumors, we screened paired lesional and normal tissue of 40 archival PG samples from adolescent patients (age <18) for *RAS* mutation via Sanger sequencing. This age range was selected as our exome cases were young children, and PGs commonly affect this age group. Four (4/40, 10%, VASC102, VASC103, VASC104, and VASC105) had somatic *RAS* mutations (Table 1) and all showed lobules of small caliber vessels (Supplementary Figures S4a–d and S5a–d online). Both VASC102 and VASC104 PGs had a heterozygous c.182A>G, p.Q61R mutation in *HRAS*, whereas VASC103 and VASC105 PGs had a heterozygous c.145G>A, p.E49K and a heterozygous c.37G>A, p.G13S mutation in *HRAS*, respectively (Table 1 and Supplementary Figures S4e, f and S5e, f online). VASC105 had additional heterozygous *HRAS* mutations c.44G>A, p.G15D and c.100C>T, p.P34S of unknown significance. The erythrocyte-type glucose transporter GLUT1 is a specific marker of IHs (Leon-Villapalos *et al.*, 2005), but immunoreactivity was negative in

Abbreviations: IH, infantile hemangioma; MAPK, mitogen-activated protein kinase; PG, pyogenic granuloma; VEGF, vascular endothelial growth factor; WES, whole-exome sequencing

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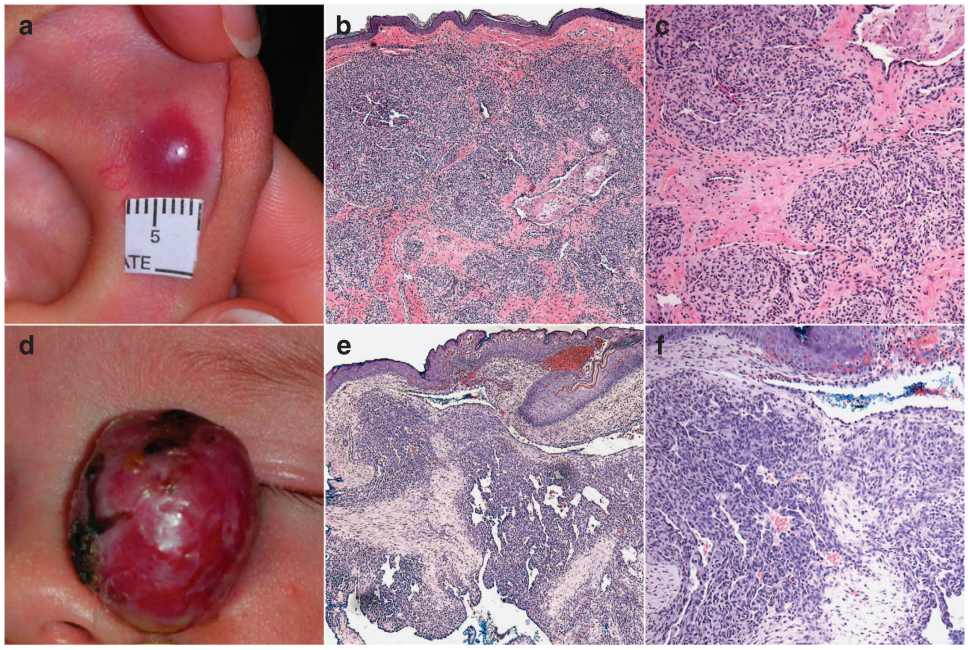

Figure 1. Clinical and histological features of vascular tumors. (**a**–**c**) VASC100, a 5-year-old boy, who presented with a 1-cm reddish lesion on the left ear. Histopathology demonstrates a lobular organization of small caliber vessels. (**d**–**f**) VASC101, a male infant with a large pedunculated vascular nodule involving the left lower eyelid, which was present since birth. The tumor was difficult to classify, but there were foci of lobules of small capillaries, necrosis (not shown), and intervening areas composed of more dilated valvular vessels (not shown). (**b**, **e**) Magnification ×4, bar = 100 μm; (**c**, **f**) ×10, bar = 100 μm.

Table 1. *RAS* mutation identified in six vascular tumors

Patient	Diagnosis	Age/sex	Site of lesion	Gene	Base change	Protein change	Method
VASC100	Vascular tumor	5/M	Ear	*KRAS*	G>A	G12D	WES
VASC101	Vascular tumor	0/M	Lower eyelid	*NRAS*	C>A	Q61K	WES
VASC102	PG	12/M	Back	*HRAS*	A>G	Q61R	SS
VASC103	PG	14/M	Abdomen	*HRAS*	G>A	E49K	SS
VASC104	PG	2/F	Cheek	*HRAS*	A>G	Q61R	SS
VASC105	PG	15/M	Back	*HRAS*	G>A	G13S	SS

Abbreviations: A, adenine; C, cytosine; F, female; G, guanine; M, male; PG, pyogenic granuloma; SS, Sanger sequencing; WES, whole-exome sequencing. Patient ID, histopathological diagnosis, age at the time of presentation, sex, site of lesion, mutation information (gene, base change, and amino-acid change), and method of detection are presented.

all six of our cases (Supplementary Figure S6a–f online).

Previously identified somatic mutations in vascular tumors or malformations have involved key angiogenic players such as *TEK*, a tyrosine kinase involved in vascular remodeling, and the vascular endothelial growth factor receptor (*VEGFR*) (Ye *et al.*, 2011). Although somatic mutation in *RAS* has not been reported in human PG or vascular tumors, *RAS* signaling is associated with angiogenesis and vascular proliferation (Kranenburg *et al.*, 2004). Tumor cells that express activated *KRAS* show increased VEGF synthesis, by stabilization of *VEGF* mRNA or increased phosphorylation of HIF-1a, a transcription factor for VEGF (Kranenburg *et al.*, 2004). Transgenic *KRAS* G12D mice spontaneously develop multiple vascular tumors, with endothelial cells demonstrating an *RAS*-dependent increase in *VEGF* and *Flk-1* mRNA (Fisher *et al.*, 2001). *RAS* mutant endothelial cells acquire an angiogenic phenotype, including membrane ruffling, branching morphogenesis, increased DNA synthesis, and cell migration (Meadows *et al.*, 2004). Constitutively active *HRAS* G12V fibroblasts and *KRAS* mutant intestinal epithelial cells demonstrate enhanced expression of cyclooxygenase-2, which increases the synthesis of proangiogenic cytokines and prostaglandins, further stimulating these factors via positive feedback (Kranenburg *et al.*, 2004). For archival PG lesions without *RAS* mutations, it is possible that they harbor mutations in distinct regulators of angiogenesis, or other genes in the *RAS* pathway.

Germline RASopathies provide further evidence for Ras-mitogen-activated protein kinase (MAPK) activity in vascular tumorigenesis. PG occurs in Costello syndrome owing to *HRAS* mutations (Morice-Picard *et al.*, 2013), and a congenital ulcerating hemangioma was reported in a case of cardio-facio-cutaneous syndrome with a germline *KRAS* mutation (Tang *et al.*, 2007). In addition, germline mutations in *RASA1*, a *RAS* p21 protein activator, cause capillary malformation–arteriovenous malformation (CM–AVM), which features an increased number of dermal capillaries (Eerola *et al.*, 2003).

Our reported *RAS* mutations have been found in cancer, including codon 12, 13, and 61 mutations, which are well-established hotspots for constitutive activation of Ras-MAPK signaling. The *HRAS* E49K variant in VASC103 is at a less commonly implicated site; to date, mutations at this position have only been reported in somatic *NRAS*- and *KRAS*-driven neoplasms (Reifenberger *et al.*, 2004; Palomba *et al.*, 2012).

Our identification of somatic *RAS* mutations in vascular tumors has clinical relevance. Current therapies against these lesions are limited to steroids and β-blockers, which achieve mixed results, often limited to tumor size reduction without resolution (Wine Lee *et al.*, 2014). Some infantile vascular tumors, such as VASC101, are unresponsive to such interventions (Wine Lee *et al.*, 2014). These cases may harbor *RAS* mutations, and might respond to farnesyl transferase inhibitors or Raf/Mek/Erk inhibitors, which block signaling upstream or downstream of *RAS*. The finding that *RAS* mutation drives vascular tumors provides potential opportunities to develop targeted therapies for current drug-resistant lesions.

CONFLICT OF INTEREST

The authors state no conflict of interest.

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SUPPLEMENTARY MATERIAL

Supplementary material is linked to the online version of the paper at http://www.nature.com/jid

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