HHS Public Access

Author manuscript

J Invest Dermatol. Author manuscript; available in PMC 2015 December 01.

Published in final edited form as:

J Invest Dermatol. 2015 June ; 135(6): 1698–1700. doi:10.1038/jid.2015.55.

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

Young H. Lim[1,2,5], **Stephanie R. Douglas**[3], **Christine J. Ko**[1,2], **Richard J. Antaya**[1,4], **Jennifer M. McNiff**[1,2], **Jing Zhou**[1,2,5], **Yale Center for Genome Analysis**, **Keith A. Choate**[1,2,5,*], and **Deepak Narayan**[3,*]

[1]Department of Dermatology, Yale University School of Medicine, New Haven, Connecticut, USA

[2]Department of Pathology, Yale University School of Medicine, New Haven, Connecticut, USA

[3]Section of Plastic Surgery, Department of Surgery, Yale University School of Medicine, New Haven, Connecticut, USA

[4]Department of Pediatrics, Yale University School of Medicine, New Haven, Connecticut, USA

[5]Department of Genetics, Yale University School of Medicine, New Haven, Connecticut, USA

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, while 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 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, though rare congenital, disseminated cases occur (Browning *et al.*, 2009; Giblin *et al.*, 2007). 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

Correspondence: Keith A. Choate, M.D., Ph.D. Departments of Dermatology, Pathology, and Genetics, Yale University School of Medicine, 333 Cedar Street, New Haven, CT 06519 USA, keith.choate@yale.edu.

*These authors contributed equally to this work.

Work was done in New Haven, Connecticut, USA.

Conflict of interest

The authors state no conflict of interest.

(Ahuja *et al.*, 2013). Although infection, trauma, and angiogenic factor upregulation have been proposed, the etiology of PG remains unknown (Ahuja *et al.*, 2013; Giblin *et al.*, 2007).

Prior to 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 utilized pairwise whole-exome sequencing (WES) of blood and affected tissue in two patients. The first (VASC100) was a 5 year-old boy with a 1cm compressible, pulsatile tumor on the left helix which had enlarged and reddened over four months (Figure 1a). Histologically, it contained lobules of benign-appearing endothelial cells (Figure 1b, c). The second (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, f).

Following 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 and Supplementary Figure S1 online). These mutations were confirmed via Sanger sequencing (Supplementary Figure S2 online). Neither dataset had mutations in genes 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 (LOH) was found, suggesting both cases are true somatic RASopathies (Supplementary Figure S3 online). Total number of somatic mutations were comparable between blood and tissue, demonstrating no increased mutation burden in tumor.

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*, while 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 infantile hemangiomas (Leon-Villapalos *et al.*, 2005) but immunoreactivity was negative in 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 *VEGFR* receptor (Ye *et al.*, 2011). While 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 expressing activated *KRAS* show increased VEGF synthesis, via 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 a *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 COX-2, which increases 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-MAPK activity in vascular tumorigenesis. PG occurs in Costello syndrome due 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). Additionally, 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 (Palomba *et al.*, 2012; Reifenberger *et al.*, 2004).

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, like VASC101, are unresponsive to such interventions (Wine Lee *et al.*, 2014). These cases may harbor *RAS* mutations, and might respond to farnesyl transferase inhibitors (FTIs) 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.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgements

We would like to thank Lynn Boyden for critical review of the manuscript, Rong-Hua Hu and Vincent Klump for technical assistance, and members of the Yale Center for Genome Analysis, including Richard P. Lifton, Shrikant M. Mane, and Kaya Bilguvar. This study was supported by a Doris Duke Charitable Foundation Clinical Scientist Development Award to K.A.C. and by the Yale Center for Mendelian Genomics (NIH U54 HG006504). Y.H.L. was supported by the Medical Scientist Training Program at Yale University.

Abbreviations used

PG	pyogenic granuloma
SNV	single nucleotide variation
LOH	loss of heterozygosity
IH	infantile hemangioma
IGV	integrated genome viewer

References

Ahuja T, Jaggi N, Kalra A, et al. Hemangioma: review of literature. The journal of contemporary dental practice. 2013; 14:1000–1007. [PubMed: 24685812]

Browning JC, Eldin KW, Kozakewich HP, et al. Congenital disseminated pyogenic granuloma. Pediatric dermatology. 2009; 26:323–327. [PubMed: 19706097]

Eerola I, Boon LM, Mulliken JB, et al. Capillary malformation-arteriovenous malformation, a new clinical and genetic disorder caused by RASA1 mutations. American journal of human genetics. 2003; 73:1240–1249. [PubMed: 14639529]

Enjolras, O.; Wassef, M.; Chapot, R. Introduction: ISSVA classification. Cambridge University Press; Cambridge, UK: 2007.

Fisher GH, Wellen SL, Klimstra D, et al. Induction and apoptotic regression of lung adenocarcinomas by regulation of a K-Ras transgene in the presence and absence of tumor suppressor genes. Genes & development. 2001; 15:3249–3262. [PubMed: 11751631]

Giblin AV, Clover AJ, Athanassopoulos A, et al. Pyogenic granuloma - the quest for optimum treatment: audit of treatment of 408 cases. Journal of plastic, reconstructive & aesthetic surgery : JPRAS. 2007; 60:1030–1035. [PubMed: 17478135]

Kranenburg O, Gebbink MF, Voest EE. Stimulation of angiogenesis by Ras proteins. Biochimica et biophysica acta. 2004; 1654:23–37. [PubMed: 14984765]

Leon-Villapalos J, Wolfe K, Kangesu L. GLUT-1: an extra diagnostic tool to differentiate between haemangiomas and vascular malformations. British journal of plastic surgery. 2005; 58:348–352. [PubMed: 15780229]

Meadows KN, Bryant P, Vincent PA, et al. Activated Ras induces a proangiogenic phenotype in primary endothelial cells. Oncogene. 2004; 23:192–200. [PubMed: 14712224]

Morice-Picard F, Ezzedine K, Delrue MA, et al. Cutaneous manifestations in Costello and cardiofaciocutaneous syndrome: report of 18 cases and literature review. Pediatric dermatology. 2013; 30:665–673. [PubMed: 24283439]

Palomba G, Colombino M, Contu A, et al. Prevalence of KRAS, BRAF, and PIK3CA somatic mutations in patients with colorectal carcinoma may vary in the same population: clues from Sardinia. Journal of translational medicine. 2012; 10:178. [PubMed: 22931052]

Reifenberger J, Knobbe CB, Sterzinger AA, et al. Frequent alterations of Ras signaling pathway genes in sporadic malignant melanomas. International journal of cancer Journal international du cancer. 2004; 109:377–384. [PubMed: 14961576]

Tang B, Reardon W, Black GC, et al. Congenital ulcerating hemangioma in a baby with KRAS mutation and cardio-facio-cutaneous syndrome. Clinical dysmorphology. 2007; 16:203–206. [PubMed: 17551339]

Wine Lee L, Goff KL, Lam JM, et al. Treatment of pediatric pyogenic granulomas using beta-adrenergic receptor antagonists. Pediatric dermatology. 2014; 31:203–207. [PubMed: 24138457]

Ye C, Pan L, Huang Y, et al. Somatic mutations in exon 17 of the TEK gene in vascular tumors and vascular malformations. Journal of vascular surgery. 2011; 54:1760–1768. [PubMed: 21962923]

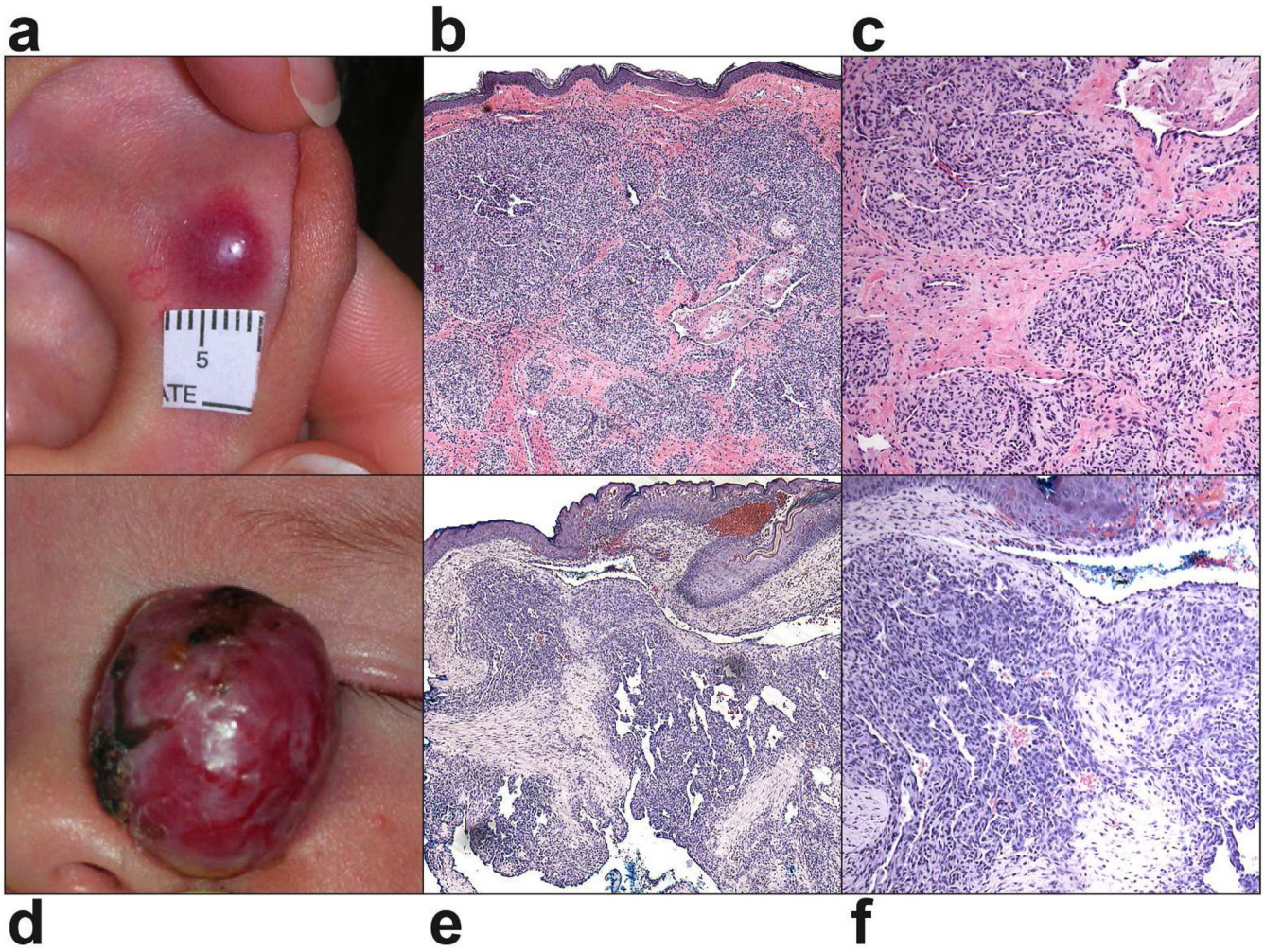

Figure 1. Clinical and histological features of vascular tumors

(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, 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 vessles (not shown). (b,e) 4X, scale bar = 100μm; (c,f) 10X, scale bar = 100μm.

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

Patient ID, histopathological diagnosis, age at time of presentation, sex, site of lesion, mutation information (gene, base change, and amino acid change) and method of detection (WES = whole exome sequencing; SS = Sanger sequencing) are presented.