HHS Public Access

Author manuscript

J Invest Dermatol. Author manuscript; available in PMC 2016 April 01.

Published in final edited form as:

J Invest Dermatol. 2015 October ; 135(10): 2536–2538. doi:10.1038/jid.2015.180.

Somatic V600E *BRAF* mutation in linear and sporadic syringocystadenoma papilliferum

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TO THE EDITOR

Syringocystadenoma papilliferum (SCAP) is a benign cutaneous hamartomatous tumor (Mammino and Vidmar, 1991) which rarely undergoes transformation to malignant syringocystadenocarcinoma papilliferum (SCACP) (Satter *et al.*, 2014). SCAP are thought to be sweat gland tumors, with evidence suggesting that they may arise from a multipotent progenitor (Yamamoto *et al.*, 2002).

Notably, SCAP rarely spontaneously arise within nevus sebaceus (NS) (OMIM 162900) lesions, caused by somatic mutations in *HRAS* and *KRAS*. Additional mutations necessary for SCAP development in NS have yet to be determined (Groesser *et al.*, 2012). Following a report that NS lesions result from *PTCH* deletion, *PTCH* deletion was also reported in some SCAP lesions (Boni *et al.*, 2001) though subsequent studies found that NS results nearly exclusively from somatic *RAS* mutation (Groesser *et al.*, 2012).

SCAP can develop spontaneously as a solitary lesion, or can appear in a linear pattern at birth following Blaschko's lines. Such linear patterns are rare and have not been reported to progress to malignancy. It is unclear if this is due to the small number of lesions observed, or to distinct molecular pathogenesis. Predicting that a somatic mutation would cause linear SCAP, we interrogated pathogenesis via paired whole exome sequencing (WES) of affected tissue and blood in one case.

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Work was done in New Haven, Connecticut

The authors state no conflict of interest.

This study was approved by the Yale Human Investigation Committee, and complies with the Declaration of Helsinki guidelines. Subjects provided written informed consent, except in the case of archival tissue samples, which were provided anonymized.

Our index case is a 12 year-old, otherwise healthy, girl with no other medical problems and no family history of any unusual nevi. At birth, an erythematous Blaschko-linear plaque composed of individual papules was noted. The lesion remained stable throughout childhood. (Figure 1, A). Histopathology revealed a cystic epithelial invagination containing papillae lined by columnar epithelium (Figure 1, B and C) and underlying dermis with abundant plasma cells.

WES was performed with mean coverage depth of 78x in blood and 104x in tissue (Supplementary Table 1). Comparison of single nucleotide variants (SNVs) and small insertions and deletions between tissue and blood revealed a single damaging somatic SNV: *BRAF* c.T1799A, p.V600E (Figure 2); 22% of reads were mutant in tissue (39/174) and blood had no mutant reads (0/192). Sanger sequencing confirmed this finding. Somatic loss-of-heterozygosity (LOH) and copy number variations were not identified (Supplementary Figure 2). The finding of a single somatic mutation without LOH provides evidence that *BRAF* V600E mutation is sufficient to cause SCAP. In analysis of ten solitary, sporadic SCAPs from unrelated subjects, we found *BRAF* V600E somatic mutations in four, using DNA from laser-capture microdissected normal epidermis as a control. To exclude other potential pathogenic *BRAF* mutations, we also screened exons 6, 8, 11, 12, 13 and 16 in which mutations have been previously found in cancer and cardiofaciocutaneous syndrome (Davies *et al.*, 2002; Niihori *et al.*, 2006), finding none.

SCAP develops in approximately 5% of NS lesions (Groesser *et al.*, 2012). To determine if such NS-associated SCAP lesions are also driven by *BRAF* mutation, we isolated DNA from four SCAPs that arose within *HRAS* G13R mutation-positive NS lesions. Sequencing revealed no *BRAF* mutations in these lesions which were histologically indistinguishable from *BRAF* V600E-positive lesions (Supplementary Table 2).

Recently, using a mutation-targeted assay to interrogate genes in the mitogen-activated protein kinase and phosphatidylinositol-3′-OH kinase signaling pathways, Shen *et al* found *BRAF* V600E in 12/23 screened sporadic SCAP and activating *HRAS* and *KRAS* mutations in 7/23 sporadic SCAP (Shen *et al.*, 2015). Consistent with our findings, *BRAF* mutations were not found in SCAP arising within NS. Notably, 5/6 RAS-positive lesions in the Shen study arose on the head or neck. We also identified a single archival sporadic SCAP sample, which arose on the scalp of a 16-year-old, in which we found a *HRAS* G13R mutation. There was insufficient tissue to determine if this lesion arose within an NS due to *RAS* mutation. There is precedent for focal neoplasia within RAS mutant tissue arising via copy number amplification of *HRAS* alleles as in spitz nevi arising within nevus spilus (Sarin *et al.*, 2013) and papillomas in HRAS G12V mice (Chen *et al.*, 2009). This is one possible explanation for distinct phenotypes of NS and SCAP despite bearing identical somatic RAS mutation.

BRAF is a serine kinase, which plays a crucial role in the RAS-RAF-MEK-ERK signaling pathway, and mutations including V600E have been found in about 50% of melanomas, and

in colon, lung and ovarian cancers (Davies *et al.*, 2002). Oncogenic *BRAF* mutations discovered to date are restricted to exons 11 and 15 (Davies *et al.*, 2002), which encode the P-loop and the activation domains (Wan *et al.*, 2004) that typically interact to inactivate the enzyme. The constitutively active *BRAF* V600E mutation lies within the activation loop, disrupting this interaction (Wan *et al.*, 2004). Constitutional expression of *BRAF* V600E causes early embryonic lethality in mice (Dhomen *et al.*, 2010), and keratin-14 driven expression leads to perinatal lethality with craniofacial defects (Krishnaswami *et al.*, 2014), epidermal thickening and loss of keratinocyte differentiation markers.

RAS-RAF-MEK-ERK dysfunction has also been observed in RASopathies with skin and other organ abnormalities. Weakly-activating *BRAF* mutations cause cardiofaciocutaneous syndrome (CFC) (OMIM 115150), featuring craniofacial abnormalities, intellectual disability and cardiomyopathy (Niihori *et al.*, 2006).

CFC can demonstrate hyperkeratotic skin lesions on extensor surfaces of the limbs and on the scalp and nipples, or generalized ichthyotic scale (Niihori *et al.*, 2006). The marked epithelial hyperplasia of SCAP may be due to stronger *BRAF* activation by the V600E mutation than by more weakly-activating mutations in CFC, though further experimental investigation is warranted.

Despite SCAP's limited malignant potential, it may be clinically important to consider the possibility of transformation to carcinoma and risk of internal malignancy in patients presenting with large mosaic SCAP lesions at birth. Mosaic disorders have been shown to extend beyond the epidermis to affect other tissues including melanocytes, bone, and neural tissue (Lim *et al.*, 2013).

Since *BRAF* mutations are found in cancer, therapeutics targeting mutant *BRAF* have been developed. Vemurafenib, originally developed to treat melanoma, targets cells with a V600E mutation (Bollag *et al.*, 2010). This or similar therapeutics may provide benefit for patients with *BRAF* V600E-positive SCAP lesions that are intractable to resection as well as for patients with SCACP.

Materials and Methods

Human Subjects

This study was approved by the Yale Human Investigation Committee, and complies with the Declaration of Helsinki guidelines. Subjects provided written informed consent, except in the case of archival tissue samples, which were provided anonymized.

DNA Extraction

For linear SCAP, DNA was directly extracted from a punch biopsy of and excised lesion. Fat and underlying dermis were trimmed to leave clinically homogeneous lesional tissue.

For archival SCAP specimens, 2–3 1mm cores were taken from the center of lesional tissue based upon a hematoxylin-eosin stained slide from an adjacent section. DNA from formalin-fixed paraffin-embedded (FFPE) archival tissue samples was extracted using an FFPE

extraction kit (QIAGEN, Valencia, CA). DNA was extracted from fresh tissue and blood via standard methods.

Whole Exome Sequencing

DNA was sheared, and captured using EZexome V2 capture probes (Roche). Paired-end sequencing was performed on an Illumina HiSeq2000. Raw reads were aligned to the hg19 reference genome using BWA-mem [1]. PCR duplicates were excluded and reads were trimmed to fit the targeted regions. Variants (SNVs and indels) were called using SAMtools [2], and common variants (dbSNP 137) were excluded. A Perl script was used to identify mutations with increased non-reference reads in tissue versus blood, and manually filtered for novel, coding mutations with ≥4 non-reference reads in tissue and ≤3 non-reference reads in blood. Mutations were manually inspected using the Integrative Genomics Viewer to ensure that reads were not mismapped [3].

Copy Number Variation and Loss-of-Heterozygosity

Copy number variation and loss-of-heterozygosity events were evaluated using CoNIFER [4] (267 control exomes, SVD 20).

Sanger Sequencing

Kapa 2G polymerase was used for PCR. The following primers were used for amplification and sequencing:

BRAF_exon11_F: TTCTGTTTGGCTTGACTTGAC

BRAF_exon11_R: GACTTGTCACAATGTCACCAC

BRAF_exon15_F: TCATAATGCTTGCTCTGATAGGA

BRAF_exon15_R: GGCCAAAAATTTAATCAGTGGA

HRAS_exon2_F: CTCCTTGGCAGGTGGGGCAG

HRAS_exon2_R: AGCCCTATCCTGGCTGTGTCCTG

KRAS_exon2_F: TGAGTTTGTATTAAAAGGTACTGGTGGAG

KRAS_exon2_R: AACTTGAAACCCAAGGTACATTTCAG

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

We would like to thank Young Lim, Evelyn Lily and Lynn Boyden for review of the manuscript and Nouf Hijazi for technical assistance. This work was supported by the Yale Center for Mendelian Genomics (NIH U54 HG006504). JLL is supported by the Medical Scientist Training Program (NIH NIGMS GM007205) and is a recipient of a clinical research mentorship award from Doris Duke Charitable Foundation.

Abbreviations

SCAP	syringocystadenoma paplliliferum
SCACP	syryingocystadenocarcinoma Papilliferum
KEN	keratinocytic epidermal nevus
NS	nevus sebaceus
CFC	cardiofascialcutaneous syndrome
SNV	single nucleotide variation
LOH	loss of heterozygosity

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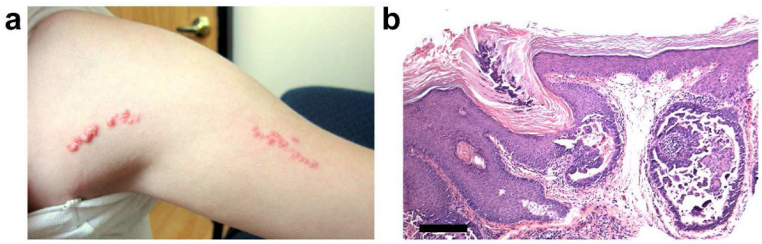
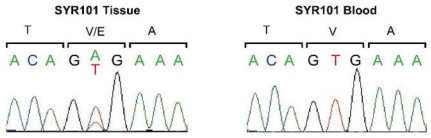


Figure 1.

Clinical and histological features of linear SCAP. (A) Linear pink hyperkeratotic papules in a 12-year-old girl have been present since birth. (B) Histopathology demonstrates a cystic epithelial lesion containing papillary projections lined by columnar epithelium and stromal plasma cell infiltration. Scale bar = 500 um.

a

Sample	Position (hg19)	Gene	Base Change	Protein Effect	# of reads tissue ref	# of reads tissue non-ref	# of reads blood ref	# of reads blood non-ref	*p*-value
SYR101	chr7: 140453136	***BRAF***	T>A	p.V600E	174	39	192	0	3.43×10^{-12}



b

Sample ID	Age at Excision	Mutation	Location and Associated Findings
SYR102	29 years	*BRAF* p.V600E	Scalp
SYR103	59 years	None found	Scalp
SYR104	48 years	None found	Scalp
SYR105	25 years	*BRAF* p.V600E	Temple
SYR106	53 years	None found	Scalp
SYR107	64 years	None found	Scalp
SYR108	54 years	None found	Shoulder
SYR109	16 years	*HRAS* p.G13R	Scalp
SYR110	46 years	*BRAF* p.V600E	Scalp
SYR111	24 years	*BRAF* p.V600E	Thigh
SYR112	12 years	*HRAS* p.G13R	Scalp, Arose within an NS
SYR113	39 years	*HRAS* p.G13R	Scalp, Arose within an NS
SYR114	41 years	*HRAS* p.G13R	Scalp, Arose within an NS
SYR115	13 years	*HRAS* p.G13R	Scalp, Arose within an NS

Figure 2.

WES demonstrates *BRAF* V600E somatic mutation in SCAP. (A) WES was performed paired samples and SNVs and insertions or deletions (indels) were filtered to identify protein damaging variants not found in control exomes. Remaining SNVs were then ranked by fisher score for tissue specificity. Only *BRAF* V600E surpassed genome wide significance for tissue specificity (2.4×10^{-6}), and was confirmed by Sanger sequencing. No other mutations demonstrated a p-value less than 1×10^{-4}. There were 39 non-reference reads and 147 reference reads in tissue at this site, demonstrating the presence of wild-type admixture. No non-reference reads were found in blood. Sanger sequencing confirmed that SYR101 has a tissue-specific *BRAF* V600E mutation. (B) 4 out of 10 sporadic SCAP demonstrated V600E mutations identified via Sanger sequencing. No other damaging mutations were found in exons 6, 8, 11, 12, 13, 15 or 16 in any of the samples. None of the 4 SCAP arising from within an NS that were screened demonstrated a V600E mutation.