Supplementary Online Content

Sarin KY, Sun BK, Bangs CD, et al. Activating *HRAS* mutation in agminated Spitz nevi arising in a nevus spilus. *JAMA Dermatol*. Published online July 24, 2013. doi:10.1001/jamadermatol.2013.4745.

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This supplementary material has been provided by the authors to give readers additional information about their work.

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eMethods

Exome sequencing

DNA was isolated from tissue samples using the Qiagen Blood/Tissue kit (Qiagen, Valencia, CA). Illumina libraries were made using NEBNext reagents (New England Biolabs, Ipswich, MA) and the resulting libraries were subjected to exome enrichment using the Agilent SureSelect All-Exon v2 kit (Agilent, Santa Clara, CA). 100 bp paired-end library reads were generated on Illumina HiSeq 2000 (Illumina, San Diego, CA) to an 80x average depth of coverage.

The reads were assembled against the human reference genome GRCh37(hg19) using DNANexus (www.dnanexus.com) and the DNANexus cancer nucleotide algorithm was used to call single nucleotide variants in the lesional samples as compared with normal skin using default parameters. Non-synonymous somatic coding variants from the lesional tissue were intersected to identify common variants present in both samples. This resulted in 27 recurrent mutations (eTable). The resulting mutation list was further filtered using the following criteria: (1) adequate coverage of greater than 3 reads in all samples, (2) threshold of greater than 5 percent mutant reads in the lesional samples, and (3) the absence of the mutation in the normal skin control. The *HRAS* point mutation (c.37G→C) was the only common variant identified that remained after our quality filters.

Resequencing Validation

DNA was amplified for Sanger resequencing using HRAS primers 5′-CCTATCCTGGCTGTGTCCTG-3′ and 5′-CAGGAGACCCTGTAGGAGGA-3′. Initial Sanger sequencing using these primers on total DNA extracted from the nevus spilus sample did not detect the *HRAS* point mutation (c.37G→C, p.Gly13Arg) (eFigure 2). We hypothesized that this

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was due to the extremely low proportion of melanocytes in the nevus spilus tissue. Restriction enzyme digestion was performed to enrich the proportion of the mutant sequence and improve the sensitivity to detect the HRAS point mutant. The HRAS point mutation (c.37G→C) disrupts the Aci1 restriction enzyme site. Thus digestion of the HRAS exon2 amplicon with Aci1 specifically cleaves the WT sequence but leaves the mutant sequence intact (eFigure 1). DNA from the Spitz nevi, nevus spilus, and normal skin was treated with Aci1 for 8 hours followed by PCR amplification using two independent sets of internal HRAS exon 2 primers 5′-CTCACCTCTATAGTGGGGTCGT-3′, 5′-TGAGGAGCGATGACGGAATA-3′ and primers 5′-ATGACGGAATATAAGCTG-3′, 5′-CTCTATAGTGGGGTCGTA-3′. These primers span the digestion site and only amplify intact DNA thereby enriching for the mutant sequence. Subsequent Sanger sequencing reproducibly demonstrated the HRAS mutation (c.37G→C) in the nevus spilus and Spitz nevi but not in the adjacent normal skin (eFigure 2).

Copy Number Analysis

The SeqGene-CNV algorithm was employed to determine copy number changes using circular binary segmentation.[1] Such algorithms on massive parallel sequencing offers an alternative to array-comparative genome hybridization, and in some cases, offers high precision that traditional array based methods for the detection of copy number changes.[2] Validation with dual-color fluorescent in situ hybridization was performed on tissue sections of archived paraffin-embedded nevi using a Texas Red-labeled *HRAS* probe along with a reference FITC-labeled centromeric probe against chromosome 11 (Abnova, Taipei city, Taiwan).

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References

1. Deng X. SeqGene: a comprehensive software solution for mining exome- and transcriptome-sequencing data. *BMC Bioinformatics.* 2011;12:267.
2. Chiang DY, Getz G, Jaffe DB, et al. High-resolution mapping of copy-number alterations with massively parallel sequencing. *Nat Methods.* Jan 2009;6(1):99-103.

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eTable. List of Common Non-synonymous Single-Nucleotide Variants in Spitz1 and Spitz2 Called by DNAnexus Prior to Our Filters[a]

Gene	Chr	Position	Mutation	Spitz1	Spitz2	Nl Skin
PLEKHG5	1	6533393	G→C	3(6)	3(5)	0(0)
ATP13A2	1	17312743	C→T	6(14)	2(5)	7(8)
NBPF10	1	145299805	A→C	19(58)	5(32)	17(35)
NBPF10	1	145299809	G→A	154(1374)	5(37)	18(40)
OTOF	2	26741961	G→A	10(19)	3(6)	6(7)
ARL6IP6	2	153575305	G→T	10(13)	3(5)	0(3)
MUC4	3	195509987	C→T	26(182)	7(52)	10(76)
HLA-DRB5	6	32487165	G→C	3(95)	5(47)	0(100)
BCLAF1	6	136599885	C→A	16(99)	7(35)	6(59)
TCP10L2	6	167592601	G→A	7(57)	7(17)	5(30)
MUC17	7	100678932	A→G	77(698)	11(98)	38(89)
MUC17	7	100678935	C→T	77(693)	11(98)	38(88)
SLC39A4	8	145641564	T→G	17(17)	8(8)	10(10)
HRAS	**11**	**534286**	**C→G**	**8(44)**	**8(33)**	**0(39)**
OSBPL5	11	3114190	C→T	7(12)	2(4)	9(10)
ALX4	11	44331509	C→G	8(8)	5(5)	3(3)
TAS2R43	12	11243913	T→G	4(4)	4(4)	1(1)
CSPG4	15	75969335	C→T	4(8)	4(9)	0(0)
NUBP1	16	10837913	C→G	5(6)	3(5)	1(3)
PTRF	17	40574760	A→T	4(12)	3(7)	1(2)
LOXHD1	18	44126909	T→C	17(37)	7(16)	19(23)
PALM	19	731144	A→G	15(15)	4(4)	4(4)
ZFR2	19	3831525	C→A	8(8)	4(4)	2(2)
SIRPB1	20	1585397	T→C	6(6)	5(5)	1(1)
EWSR1	22	29695754	G→A	4(30)	3(7)	2(22)
PLXNB2	22	50722134	T→C	18(39)	8(11)	17(18)
MAGEC1	X	140994142	C→A	40(67)	9(9)	21(39)

[a]The number of mutant reads and total coverage in each sample is displayed as: mutant reads (total number of reads). Chr indicates chromosome.

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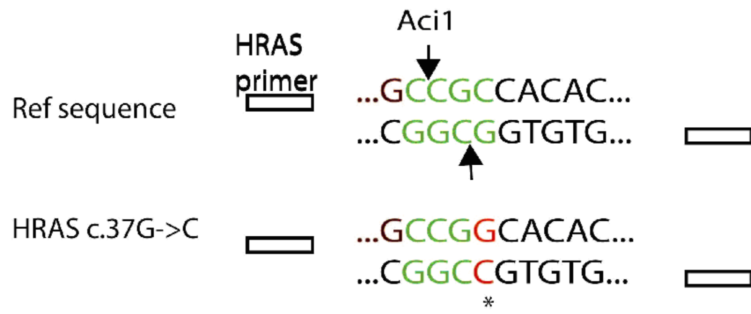

eFigure 1. *HRAS* Point Mutation (c.37G→C) Disrupts the Aci1 Digestion Site

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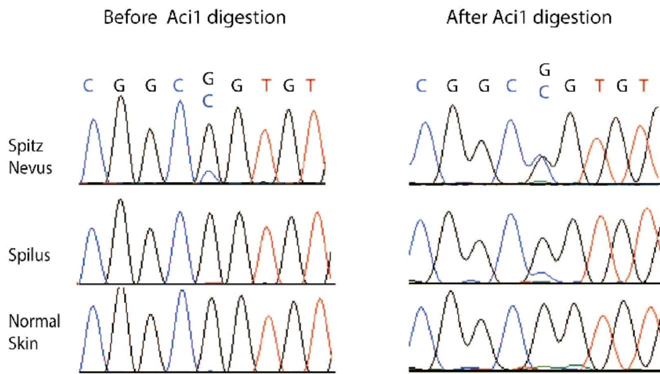

eFigure 2. Enrichment of *HRAS* Point Mutation (c.37G→C) After Aci1 Digestion

Sanger sequencing of Aci1 digested HRAS exon 2 DNA enriched for the mutant DNA strand and reproducibly detected the HRAS point mutation (c. 37G→C) in the Spitz nevus and nevus spilus but not in the normal skin sample.

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