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GNAQ Mutations in Diffuse and Solitary Choroidal Hemangiomas

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Abstract

Purpose: *GNAQ* mutations have been identified in port wine stains (both syndromic and non-syndromic) and melanocytic ocular neoplasms. This study investigates the presence of *GNAQ* mutations in diffuse- (those associated with Sturge-Weber syndrome (SWS)) and solitary choroidal hemangiomas.

Participants: Tissue from 11 patients with the following diagnoses: port wine stain (n = 3), diffuse choroidal hemangioma (n = 1), solitary choroidal hemangioma (n = 6), choroidal nevus (n =1)

Methods: Ten specimens were interrogated with Memorial Sloan Kettering-Integrated Mutation Profiling of Actionable Cancer Targets (MSK-IMPACT), a hybridization capture-based next-generation sequencing assay for targeted deep sequencing of all exons and selected introns of 468 key cancer genes in formalin-fixed, paraffin-embedded tumors. Digital polymerase chain reaction was used to detect *GNAQ* Q209 mutation in one specimen.

Main outcomes: Detection of *GNAQ* codon-specific mutation

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Results: Activating somatic *GNAQ* mutations (c.547C>T; p.Arg183Cys) were found in 100% (3 of 3) of the port wine stain and in the diffuse choroidal hemangioma. Somatic *GNAQ* mutations (c.626A>T;p.Gln209Leu) were found in 100% (6 of 6) of the solitary choroidal hemangiomas and (c.626A>C;p.Gln209Pro) in the choroidal nevus.

Conclusion: *GNAQ* mutations occur in both diffuse and solitary hemangiomas, although, at distinct codons. An R183 codon is mutant in diffuse choroidal hemangiomas, consistent with other Sturge-Weber vascular malformations. By contrast, solitary choroidal hemangiomas have mutations in the Q209 codon, similar to other intraocular melanocytic neoplasms.

Precis:

Both diffuse and solitary choroidal hemangiomas have activating mutations in GNAQ, although they are present in distinct codons.

Introduction

Mutations in *GNAQ* or *GNA11* result in dysregulation of the mitogen-activated protein kinase (MAPK), which influences gene transcription and results in cellular proliferation.[1] A group of vascular and pigmented neoplasms is recognized to have *GNAQ/11* mutations, including both benign and malignant variants (Table 1).[2–202122] Many of these lesions have been identified in the eye or the periocular tissues. The most frequently mutated lesions include 82–95% of choroidal nevi and melanomas, which have mutually exclusive mutations in *GNAQ* or *GNA11*, most commonly at the Q209 codon.[11] With similar frequency, port wine stains (either non-syndromic or related to Sturge-Weber syndrome (SWS)) have *GNAQ* mutations, but at codon R183.[2]

The eyes of patients with SWS characteristically develop an intraocular vascular malformation, termed diffuse choroidal hemangioma. To date, there is a single published case that identifies a GNAQ R183Q mutation in this intraocular lesion[23]. However, there is no published genetic information regarding the non-syndromic counterpart, solitary choroidal hemangiomas. These solitary lesions are typically discovered later in life, can be asymptomatic and unlike diffuse hemangiomas in SWS are not associated with ipsilateral glaucoma. In this study, we investigated whether diffuse and solitary choroidal hemangiomas harbor *GNAQ/11* mutations with particular attention to the codon involved.

Methods

This retrospective study was approved by the Institutional Review Board of Memorial Sloan Kettering Cancer Center (MSKCC), the New York Eye and Ear Infirmary of Mount Sinai and the University of Washington School of Medicine and Public Health and adhered to the Declaration of Helsinki. At the participating institutions, choroidal hemangioma specimens were retrieved from the archives of the Department of Pathology. The specimens were obtained through a search of the respective database, patient and tissue records. The cohort included 11 specimens: 1 diffuse choroidal hemangioma, three port wine stains (2 related to SWS and one non-SWS), six solitary choroidal hemangiomas and one choroidal nevus. An ophthalmic pathologist at each institution made the initial pathological diagnosis. All intraocular tumor specimens were obtained from enucleated eyes. The correct diagnosis was

confirmed and the amount of lesional tissue assessed for suitability for inclusion in the study by MSKCC pathology (KB). Ten of 11 samples were investigated with the Memorial Sloan Kettering Integrated Mutation Profiling of Actionable Cancer Targets (MSK-IMPACT) Assay. Due to a small quantity of tissue, one specimen (number 8) was studied using the digital droplet polymerase chain reaction (ddPCR) assay for the *GNAQ* residue Q209 mutation.

Isolation and purification of DNA

Microdissection was performed on the ten formalin-fixed and paraffin-embedded (FFPE) samples on 10μm-thick unstained sections, using hematoxylin and eosin-stained (H&E) sections as a guide. The DNeasy Tissue Kit (Qiagen) was used for DNA extraction according to manufacturer's recommendations. The Nano-Drop 8000 (Thermo Scientific) and Qubit (Life Technologies) were employed to quantify the extracted DNA. The minimum concentration of formalin fixed paraffin embedded DNA was 250ng.

Exon-capture sequencing

Genetic alterations in *GNAQ/11* were profiled using the IMPACT assay (Integrated Mutation Profiling of Actionable Cancer Targets). As previously described in detail,[24,25] this assay employs solution phase hybridization-based exon capture and massively parallel DNA sequencing to interrogate all protein-coding exons and select introns of 410 oncogenes, tumor suppressor genes, and members of pathways considered actionable by targeted therapies. The concentration of genomic DNA and DNA sequence library are both tested at the beginning of the assay before and after exon capture. If the yield is too low at any of these steps, the sample is designated as "failed": this designation did not apply to any specimens studied.

Digital drop polymerase chain reaction assay (ddPCR)

Assays specific for the detection of GNAQ-p.Q209R-626A>G were designed and ordered through BioRad (Hercules, CA): (Unique Assay ID: dHsaMDS971099482). Cycling conditions were tested to allow for optimal annealing/extension temperature in addition to optimal separation of positive from empty droplets. All reactions were executed on a QX200 ddPCR system (BioRad, Hercules CA). Plates were read and analyzed with the QuantaSoft software (BioRad, Hercules, CA) to evaluate the number of droplets positive for mutant DNA, wild-type DNA, both, or neither.

Results

All eleven specimens contained activating somatic *GNAQ* mutations in either c.547C>T; p.Arg183Cys (p.R183Q), c.626A>C;p.Gln209Pro (p.Q209P) or c.626A>T;p.Gln209Leu (p.Q209R). Table 2 provides a summary of the results.

Discussion

SWS (also known as encephalofacial angiomatosis) is a neurocutaneous disorder and manifests clinically as a vascular malformation involving the skin, neural tissue, and eye.

It occurs sporadically from an activating mutation in *GNAQ* at codon R183.[2] Previously published reports have confirmed the presence of this mutation in many characteristic features of SWS including cutaneous capillary malformations (port wine stain)[2,3,6] in addition to gingival lesions,[5,6] leptomeningeal angiomatosis[2,7,8] and even the adjacent cortex/white matter.[7] A single published case identifies the presence of the hallmark genetic aberration in the intraocular tumor associated with this syndrome[23]. In this study, we confirm that the intraocular manifestation of SWS, a diffuse choroidal hemangioma, has a mutation in *GNAQ* at the R183Q codon.

Solitary choroidal hemangioma is a circumscribed vascular tumor histopathologically similar to the diffuse counterpart, although its clinical presentation and features are distinct. One of the central questions in this present study was whether solitary and diffuse choroidal hemangiomas share the same mutational profile. We discovered that they both exhibit mutations in *GNAQ*, but at different codons: diffuse hemangiomas at R183Q, which is consistent with SWS, and solitary hemangiomas at Q209. The latter is similar to other solitary uveal neoplasms: choroidal nevi and uveal melanoma.

The significance of these specific codons has been debated in the literature in relation to other *GNAQ* mutated lesions. Mutations in either R183 or Q209 both have a gain-of-function effect with hyperactivation of downstream MAPKinase pathways.[1] However, they have a distinct impact on G-alpha protein structure and different modulation of signal intensity.[2,5,9,26] For example, mutations in codon 183 have “weaker and less promiscuous” effects on signaling[2]. In contrast, mutations in codon 209 result in *complete* inactivation, thereby resulting in a more profound activation of downstream MAPKinase signaling.[2,3] In Table 1, note that Q209 inhibition appears to occur more often in lesions that are solitary and deeply or viscerally located.

The question becomes how we can relate this knowledge to diffuse and solitary hemangiomas. It has been proposed that the more severe Q209 inhibition (compared to R183) may explain the presence of this mutation in “tumors” (which we interpret as mass-producing neoplasms with a potential for significant growth) and *not* stationary malformations.[3,11,13]Akin to Happle’s theory, which states that less severe mutations are more sustainable in the germline,[27] could it be possible that less severe mutations are supportable more often in malformations than tumors? Both SWS and Phakomatosis pigmentovascularis have the less severe R183 mutations identified in their characteristic malformations.[9] Corresponding to this, we identified the R183 mutation in diffuse capillary hemangioma, which is consistent with this lesion being a malformation related to SWS.

In contrast, the codon Q209 mutation in solitary choroidal hemangiomas is more compatible with these lesions being tumors, rather than with a developmental aberration that yields a stationary malformation. As such, it would point toward solitary choroidal hemangiomas as acquired lesions (and therefore absent at birth –a contentious subject), much along the same lines as other *GNAQ* Q209 mutant lesions such as choroidal nevi, uveal melanomas. To add complexity to this topic, it may not simply be distinct genomics that differentiate the final phenotype, but we have to also consider the contribution of epigenetics and even environmental factors.

Cuoto et al. have hypothesized that an early versus late origin of the mutation defines whether the pathology is syndromic or non-syndromic[4]. If this proves to be accurate, then it would follow that: *if* solitary choroidal hemangiomas were merely the non-syndromic counterpart to diffuse choroidal hemangiomas, they would share *GNAQ* mutations at the same codon and be detected at birth. However, solitary hemangiomas are typically detected in adulthood with a characteristic progressive clinical course and have distinct codon mutations at Q209, making them unlikely to represent the non-syndromic version of diffuse choroidal hemangiomas.

The 183-codon-specific mutation in capillary hemangiomas is recognized to be rich in endothelial cells[4]. Presumably, even though solitary choroidal hemangiomas and choroidal nevi/melanomas share mutations in the *same* gene (*GNAQ*), and at the *same* codon (Q209), the cell harboring the mutation differs in these lesions: occurring at the endothelial cell in solitary hemangiomas and the melanocyte in nevi/melanomas. We hope future studies will prove this hypothesis to be correct and to confirm our present findings with a larger scale study. With the recent discovery of a natural compound (FR900359) which targets and deactivates the aberrant G alpha q signaling,[28] the identification of *GNAQ* mutations in both diffuse and solitary choroidal hemangiomas may have promising clinical applications.

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Table 1:

Published literature on GNAQ mutations

Diagnosis	Mutated Codon			
	GNAQp.R183	GNA11p.R183	GNAQp.Q209	GNA11p.Q209
SWS port wine stain	12/13 (88%)[2,3], 18/20 (90%)[6]			
non-SWS port wine stain	23/26 (92%)[2,3]			
SWS gingval lesion	1 case[5], 19/20 (95%)[6]			
SWS CNS (leptomeningeal angiomatosis)	15/18 (83%)[2], 9/9 (100%)[7], 12/15 (80%)[8]			
SWS CNS (cortex/white matter)	7/9 (78%)[7]			
Extensive dermal melanocytosis	1/3 (33%)[9]		1/3 (33%)[9]	
Phakomatosis pigmentovascularis	2/8 (25%)[9]	4/8 (50%)[9]		
Lobular capillary hemangioma		2/3 (66%)[12]		
Port wine stain extemity		3/7 (43%)[4]		
Congenital hemangioma			8/16 (50%)[13]	4/16 (25%)[13]
Anastamosing hemangioma			9/13 (69%)[14]	
Hepatic small vessel neoplasm			2/3 (66%), 2/8 (25%)[15]	
Common blue nevus		1/41 (2.4%)[10,11]	4/10 (40%)[21], 39/60 (65%)[10,11]	4/60 (6.7%)[10,11]
Blitz nevi			1/7 (14%)[16]	
Uveal melanoma	4/145 (2.8%)[10,11]	3/145 (2.1%)[10,11]	73/163 (44.8%)[10,11]	52/163 (31.9%)[10,11]
Melanosis oculi	1/11 (9.1%)[10,11]		2/20 (10%)[10,11]	1/20 (5%)[10,11]
Choroidal nevi			7/16 (44%)[17]	8/16 (50%)[17]
Melanocytoma (iris, optic n)			2/6 (33%)[18]	
Melanocytoma (ciliochoroid)			2/2 (100%)[19]	
Melanocytoma (CNS)			8/16 (50%)[20]	
Conjunctival blue nevus			2/2 (100%)[22]	

SWS = Sturge-Weber syndrome, CNS = central nervous system

Table 2:

Pathological diagnosis and GNAQ mutations

Specimen	Pathological Diagnosis	GNAQ mutation
1	SWS Diffuse Choroidal Hemangioma	p.R183Q
2	SWS Port Wine Stain	p.R183Q
3	Non SWS Port Wine Stain	p.R183Q
4	SWS Port Wine Stain	p.R183Q
5	Solitary Choroidal Hemangioma	p.Q209R
6	Solitary Choroidal Hemangioma	p.Q209R
7	Solitary Choroidal Hemangioma	p.Q209R
8	Solitary Choroidal Hemangioma*	p.Q209R
9	Solitary Choroidal Hemangioma	p.Q209R
10	Solitary Choroidal Hemangioma	p.Q209R
11	Choroidal Nevus	p.Q209P

* = by ddPCR assay, SW = Sturge-Weber syndrome