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ARTICLE

High frequency of *GNA14*, *GNAQ*, and *GNA11* mutations in cherry hemangioma: a histopathological and molecular study of 85 cases indicating *GNA14* as the most commonly mutated gene in vascular neoplasms

Jau-Yu Liau[1,2] · Jen-Chieh Lee[1,2] · Jia-Huei Tsai[1,2] · Chih-Chi Chen[2] · Yung-Chuan Chung[2] · Ying-Hao Wang[2]

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Abstract

Cherry hemangioma is the most common hemangioma in adult life. Neoplastic and non-neoplastic theories had both been proposed for its pathogenesis, but its nature is still poorly understood. We noted a significant subset of anastomosing hemangiomas and congenital hemangiomas harbored a population of small capillaries surrounded by a perivascular hyaline layer, reminiscent of the vessels seen in cherry hemangioma. Both anastomosing hemangioma and congenital hemangioma harbor recurrent mutations in exon 5 of *GNAQ* and its paralogues. In this study, we analyzed 68 cherry hemangiomas and 17 cherry hemangioma-like hemangiomas exhibiting additional non-classical features including markedly dilated, cavernous vessels, and/or a deep component extending to the deep dermis. By Sanger sequencing, *GNAQ*, *GNA11*, and *GNA14* exon 5 mutations were identified in 12, 4, and 32 cherry hemangiomas, respectively, and 5, 3, and 3 cherry hemangioma-like hemangiomas, respectively. MassARRAY analysis detected mutations (including exon 2 $GNAQ^{G48V}$ mutations) in additional 8 cherry hemangiomas and 3 cherry hemangioma-like hemangiomas. Overall, the cherry hemangiomas and cherry hemangioma-like hemangiomas had equal *GNA* mutation rates (82%), and *GNA14* and *GNAQ* mutations were present in approximately half of cherry hemangiomas and cherry hemangioma-like hemangiomas, respectively. All mutations were mutually exclusive. $KRAS^{G12V}$ mutation was also detected in one cherry hemangioma-like hemangioma without *GNA* mutations. In summary, our study demonstrated recurrent *GNA14*/*GNAQ*/*GNA11* mutations were present in the majority of this very common hemangioma and established its neoplastic nature. Our results also expanded the morphological spectrum of *GNA*-mutated hemangiomas to include tumors composed of cavernous-like vessels and indicated *GNA14* was the most commonly mutated gene in vascular tumors.

Introduction

Cherry hemangioma, also known as senile hemangioma or Campbell de Morgan spot, is the most common hemangioma in adult. Cherry hemangioma is rare before puberty but the number and incidence increase gradually with age, and it is extremely common in the elderly. Clinically, it typically, presents as a small circumscribed red to violaceous papule with a predilection for the trunk and upper extremities. Histologically, cherry hemangioma is characterized by a small dome-shaped lesion composed of thin-walled capillaries. The vessels are often surrounded by a hyalinized stroma.

The etiology of cherry hemangioma is poorly understood. A non-neoplastic nature has been suggested based on the absence of Ki-67 staining in the lesional cells [1].

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✉ Jau-Yu Liau
019188@ntuh.gov.tw

[1] Department of Pathology, National Taiwan University Hospital and National Taiwan University College of Medicine, Taipei, Taiwan

[2] Graduate Institute of Pathology, National Taiwan University College of Medicine, Taipei, Taiwan

However, a recent study reported 20% of cherry hemangioma harbored *RAS* mutations suggesting that at least some of them were benign vascular neoplasms [2].

In our recent study of anastomosing hemangioma (manuscript in submission), we noticed that in addition to the well-recognized anastomosing vascular channels, anastomosing hemangioma often also harbored a population of small to slightly dilated capillaries lined by flat or hobnailed endothelial cells and surrounded by a hyalinized perivascular sheath. These vessels often lacked the anastomosing character and were more frequently seen in the edematous or hyalinized areas. Although not emphasized in the literature, they appear to be a distinct histological feature. To our eyes, this type of vessels resembles the vessels in cherry hemangioma and has also been described in congenital hemangioma (in both the rapidly involuting and non-involuting variants) [3, 4]. Interestingly, anastomosing hemangioma and both variants of congenital hemangioma are now known to harbor mutations in *GNAQ* and its paralogues (*GNAQ* and *GNA14* in anastomosing hemangioma, and *GNAQ* and *GNA11* in congenital hemangioma) [5–7]. Based on the morphological similarities, we speculated that these genes might be involved in the pathogenesis of cherry hemangioma. In the present study, we showed that activating mutations in all these 3 genes, *GNA14* in particular, were present in the majority of cherry hemangioma and thus established its neoplastic nature.

Materials and methods

Tumor samples

Cherry hemangioma cases diagnosed in the Department of Pathology, National Taiwan University Hospital were retrieved for genetic analysis. In addition to classical cherry hemangiomas, we also included hemangiomas that gave an impression of cherry hemangioma at low power but exhibited non-classical histological findings including (1) tumors composed predominantly of markedly dilated and congested cavernous-like vessels, and/or (2) tumors with a deep component as vertically oriented tongue-like or rounded nodules that extended from the base of the main tumor to the deep reticular dermis or subcutis. We included these hemangiomas in this study because we speculated that they might be related to classical cherry hemangiomas. These hemangiomas were referred to as cherry hemangioma-like hemangiomas in this study. For comparison, a control group comprising 30 pyogenic granulomas (lobular capillary hemangiomas) was also studied. This study was approved by the Research Ethics Committee of National Taiwan University Hospital. The specimens were anonymous and analyzed in a blind manner.

Mutation analysis

Sanger sequencing

Briefly, 4–5 10-μm thick paraffin sections were cut from the block. The tumoral areas from the paraffin sections were dissected using sterilized razors under a microscope. Genomic DNA extraction was performed using a QIAamp DNA FFPE Tissue Kit (Qiagen, Santa Clarita, CA, USA) according to the manufacturer's protocol. Polymerase chain reaction was performed using primer pairs covering the mutation hot spots of *GNAQ* (codons 183 and 209), *GNA11* (codons 183 and 209), and *GNA14* (codons 179 and 205). The previously described M13-tagged primers were used for sequencing of exon 4 and exon 5 of *GNAQ* and *GNA11* [8]. The primer sequences for *GNA14* were: GGAGAGTCAGTTCCT CGTTC (exon 4, forward), AGCCTGGGCAGATTCTTCAT (exon 4, reverse); TACTGAGGACTCCAAGAGCA (exon 5, forward), and AGCATACCTCGTTGTCACAC (exon 5, reverse). The polymerase chain reaction was performed in an automatic DNA thermal cycler (PerkinElmer, Wellesley, MA, USA) with initial heating at 95 °C for 2 min followed by 30 cycles at 95 °C for 30 s, 68 °C for 30 s, 72 °C for 30 s, and finally, 72 °C for 10 min. After purification, direct sequencing was performed in an automated ABI 3730 sequencer (Applied BioSystems, Foster City, CA, USA).

MassARRAY analysis

MassARRAY analysis using the Sequenom MassARRAY platform with iPLEX gold chemistry (Sequenom, San Diego, CA, USA) was performed following the manufacturer's instructions. Customized primers were designed to detect the following non-synonymous mutations in *GNAQ*, *GNA11*, and *GNA14* based on the most common variants from the Catalog of Somatic Mutations In Cancer database and recent literatures: *GNAQ* (c.143G>T, p.G48V; c.142_143GG>TT, p.G48L; c.626A>C, p.Q209P; c.626A>T, p.Q209L; c.626A>G, p.Q209R; c.627A>C, p.Q209H; c.627A>T, p.Q209H), *GNA11* (c.626A>C, p.Q209P; c.626A>T, p.Q209L; c.626A>G, p.Q209R; c.627G>C, p.Q209H; c.627G>T, p.Q209H) and *GNA14* (c.614A>T, p.Q205L) [9, 10]. For *KRAS*, *NRAS*, *HRAS*, and *BRAF* mutations, another primer set was used to detect 65 non-synonymous mutations in hot spots of *RAS* and *BRAF* (Supplementary Table 1). Depending on the number of genes analyzed, 10 or 40 nanograms of genomic DNA were applied to the multiplex polymerase chain reaction and loaded onto a matrix pad of a SpectroCHIP (Sequenom). SpectroCHIPs were analyzed with MassARRAY Analyser 4. The mutation peaks were identified by a higher intensity than that of the non-mutation sample pool background. A cut-off value of 10% was used for mutation calling.

Table 1 Clinicopathological features and genetic changes of cherry hemangioma and cherry hemangioma-like hemangioma

	Cherry hemangioma ($N = 68$)	Cherry hemangioma-like hemangioma[a] ($N = 17$)
Sex (M:F)	49:19	11:6
Age (median)	21–88 (56)	35–77 (59)
Site		
Trunk	38	9
Head and neck	26	5
Extremities	4	3
Genetics		
GNAQ		
G48V	1	2
Q209H	10	3
Q209R	2	3
Q209P	1	0
GNA11 Q209H	5	3
GNA14		
Q205L (c.614A>T)[b]	34	3
Q205L (c.614_615AA>TT)	3	0
Total (%)	56 (82%)	14 (82%)

[a]One cherry hemangioma-like hemangioma harbored a $KRAS^{G12V}$ mutation

[b]In 5 cherry hemangiomas with $GNA14^{Q205L}$ mutation, the mutations were detected by MassARRAY only. Whether they were caused by c.614A>T or c.614_615AA>TT mutation could not be determined

Results

Clinicopathological features

Table 1 summarizes the clinical features. In total, 85 hemangiomas were studied, including 68 cherry hemangiomas and 17 cherry hemangioma-like hemangiomas from different individuals. For cherry hemangiomas, 49 patients were male and 19 were female with the patient age ranging from 21 to 88 years (median: 56); 38 were from the trunk whereas 26 and 4 were from the head and neck regions and extremities, respectively. For cherry hemangioma-like hemangiomas, 11 patients were male and 6 were female. Patient age ranged from 35 to 77 (median: 59). Nine, 5, and 3 tumors were from the trunk, head and neck regions and extremities, respectively.

Histologically, cherry hemangiomas were composed of vaguely lobulated small to mildly dilated thin-walled vessels lined by a single layer of endothelial cells. The endothelial cells were prominent, and some of them exhibited a hobnail-like appearance. The vessels were often surrounded by a layer of homogeneous eosinophilic matrix of variable thickness. The vessels were separated by an inconspicuous stroma but sometimes the perivascular hyaline zone extended to the intervascular areas. The perivascular eosinophilic matrix often imparted the lesion a distinct pinkish character at low power view. The overlying epidermis was attenuated, and the tumors often had a flat base roughly corresponding to the interface of the papillary and reticular dermis. Others exhibited a wedge-shaped configuration with the lower border extended to the mid reticular dermis. Histological features of a typical cherry hemangioma are demonstrated in Fig. 1, together with an anastomosing hemangioma for comparison.

The cherry hemangioma-like hemangiomas included in our study were also dome-shaped lesions covered by a flattened epidermis. However, 6 were composed of predominantly markedly dilated and congested vessels, often with a back-to-back arrangement. These vessels were much larger than those seen in classical cherry hemangiomas and the endothelial cells and the perivascular hyaline layer were more attenuated. Four tumors were composed of a superficial part that was identical to conventional cherry hemangioma, in addition to a deep component presenting as tongue-like or rounded nodules extending from the main tumor base to the deep dermis or even the subcutis. This deep component often demonstrated pronounced perivascular hyalinization. Seven tumors exhibited both non-classical features (i.e., dilated vessels and a deep component). When examined at higher magnification, 10 of the 13 tumors with cavernous-like vessels still harbored small vessels indistinguishable from classical cherry hemangioma, often at the superficial or peripheral aspect of the lesion. Examples of cherry hemangioma-like hemangioma are illustrated in Fig. 1.

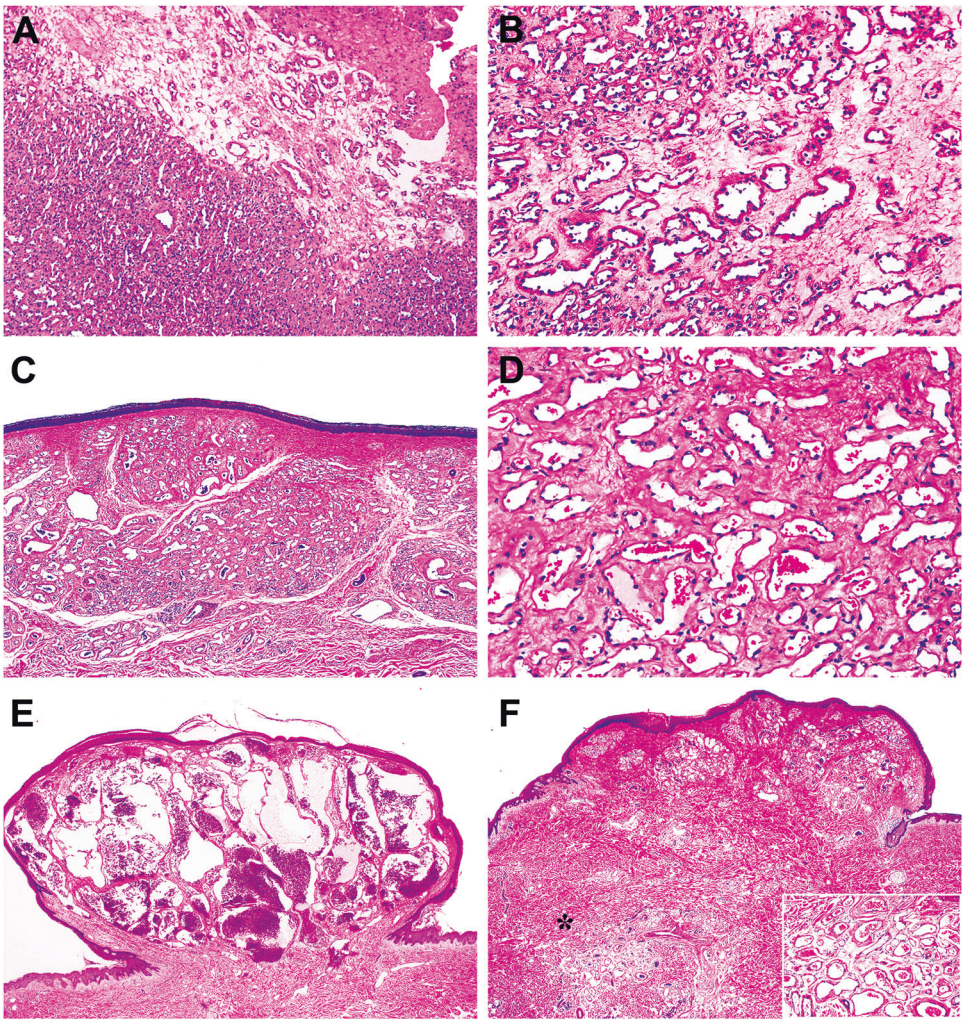

Fig. 1 **a** An anastomosing hemangioma showing the characteristic anastomosing growth of the endothelial cells. In addition, small capillary vessels lined by prominent, hobnail-like endothelial cells and surrounded by a perivascular hyalin layer were present in the edematous stroma. These vessels were highlighted in **b**. **c** A cherry hemangioma composed of vaguely lobulated vessels with perivascular hyalinization was shown. The vessels, highlighted in **d**, resembled the non-anastomosing vessels of an anastomosing hemangioma. **e** A dome-shaped hemangioma exhibiting a low power silhouette of cherry hemangioma but was composed of markedly dilated and congested, cavernous-like vessels. **f** A hemangioma showing a superficial part identical to a cherry hemangioma but additionally had a deep component in the deep dermis (asterisk). The vessels of the deep component (inset) often showed pronounced perivascular hyalinization

Mutation detection by Sanger sequencing and MassARRAY

Sanger sequencing for exon 5 of *GNAQ*, *GNA11*, and *GNA14* were performed in all cases. By Sanger sequencing, mutually exclusive *GNAQ*, *GNA11*, and *GNA14* exon 5 mutations were detected in 12, 4, and 32 tumors, respectively, of the 68 cherry hemangiomas (71% collectively), and in 5, 3, and 3 tumors, respectively, of the 17 cherry hemangioma-like hemangiomas (65%). Representative histologies and sequencing chromatographies are shown in Fig. 2. All *GNA14* mutations were Q205L. Most $GNA14^{Q205L}$ mutations were caused by c.614A>T transversions; however, 3 tumors harbored c.614_615AA>TT mutations with the same amino acid change. MassARRAY analysis verified these mutations (c.614_615AA>TT mutation could not be distinguished from c.614A>T mutation) and additionally detected $GNAQ^{G48V}$, $GNAQ^{Q209R}$, $GNA11^{Q209H}$, and $GNA14^{Q205L}$ mutations in 1, 1, 1, and 5 cherry hemangiomas, respectively, as well as $GNAQ^{G48V}$ and $GNAQ^{Q209H}$ mutations in 2 and 1 cherry hemangioma-like hemangiomas, respectively. Overall, *GNAQ*, *GNA11*, and *GNA14* mutations were present in 14, 5, and 37 of the 68 cherry hemangiomas, respectively (combined: 82%), and 8, 3, and 3 of the 17 cherry hemangioma-like hemangiomas, respectively (combined: 82%). Except for $GNAQ^{G48V}$ mutations, all other *GNA* mutations occurred at hotspots of exon 5, specifically at codon 209 of *GNAQ* and *GNA11* and codon 205 of *GNA14*. All mutations were mutually exclusive. In 20 tumors, including 15 with and 5 without *GNA* mutations, MassARRAY was also used to detect *RAS* and *BRAF* mutations. A $KRAS^{G12V}$ mutation was detected in 1 cherry hemangioma-like hemangioma that was wild-type for *GNAQ*/*GNA11*/*GNA14*. The $KRAS^{G12V}$ and $GNAQ^{G48V}$ mutations were verified by Sanger sequencing (Fig. 2). The overall mutation rates were highly similar for these 2 groups. Figure 3 shows representative histologies and MassARRAY spectra of the hemangiomas, and Table 1 summarizes the genetic alterations of these 2 groups.

In 20 cherry hemangiomas, sequencing for exon 4 was also performed and showed no mutation. No *GNAQ*, *GNA11*, or *GNA14* exon 5 mutations were identified in the 30 pyogenic granulomas.

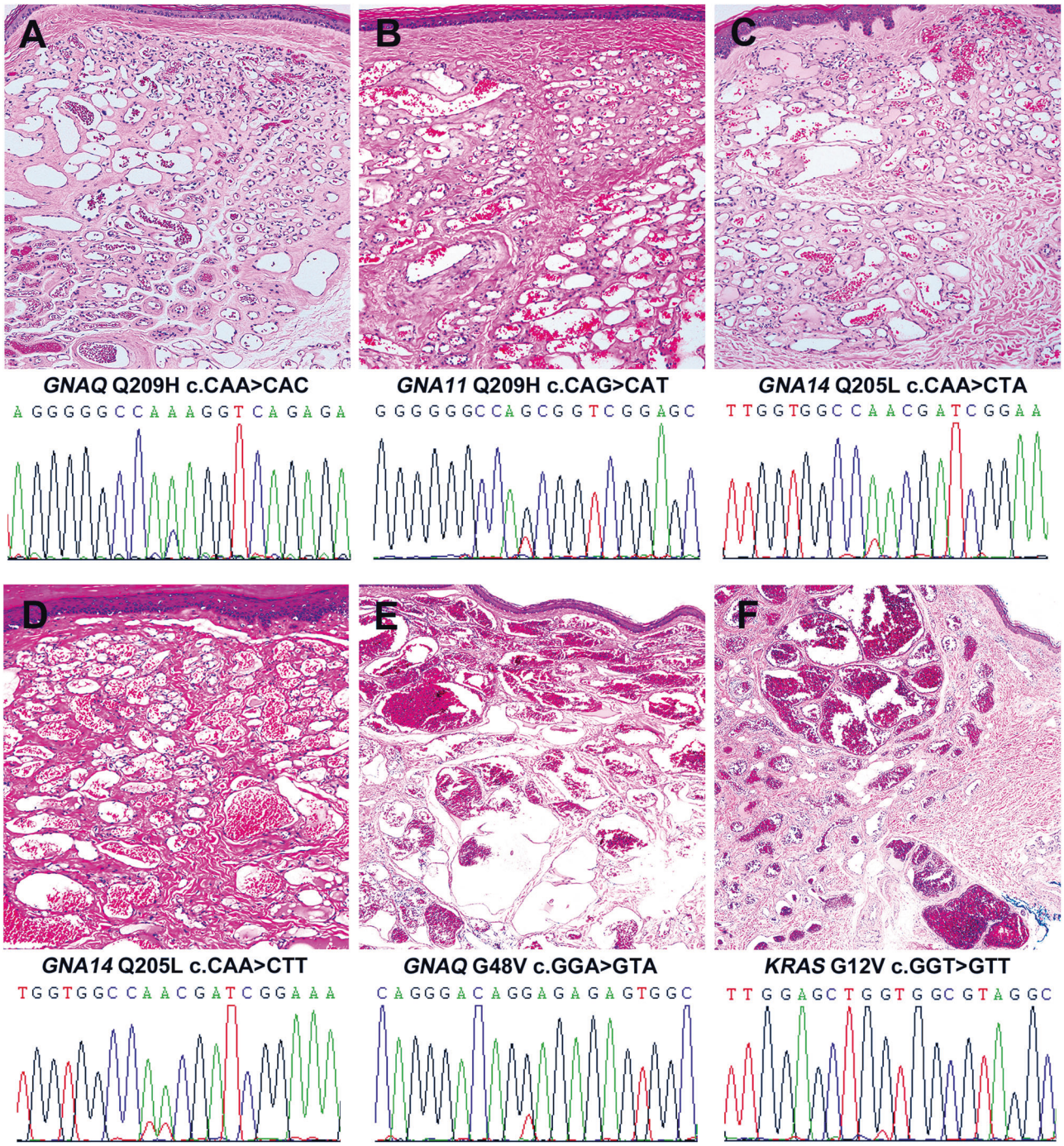

Fig. 2 Representative histologies and sequencing chromatographies. **a–d** Cherry hemangiomas with $GNAQ^{Q209H}$, $GNA11^{Q209H}$, $GNA14^{Q205L}$ (c.614>A>T), and $GNA14^{Q205L}$ (c.614_615AA>TT) mutations, respectively. **e** A cherry hemangioma-like hemangioma composed of markedly dilated and congested vessels with $GNAQ^{G48V}$ mutation. **f** A cherry hemangioma-like hemangioma with markedly dilated vessels and a deep component with $KRAS^{G12V}$ mutation.

Histopathological and molecular correlations

No apparent associations were noted between *GNA* mutations and the clinicopathological features in each group. However, *GNA14* was the most commonly mutated gene in cherry hemangiomas (37/68, 54%). By contrast, *GNAQ* mutations prevailed in cherry hemangioma-like hemangiomas (8/17, 47%). In the cherry hemangioma group, all but 3 *GNAQ* Q209 mutations were Q209H whereas Q209R tied Q209H in the cherry hemangioma-like hemangioma group. Two of the $GNAQ^{Q209R}$-mutated cherry hemangioma-like hemangiomas were composed of cavernous-like vessels. In

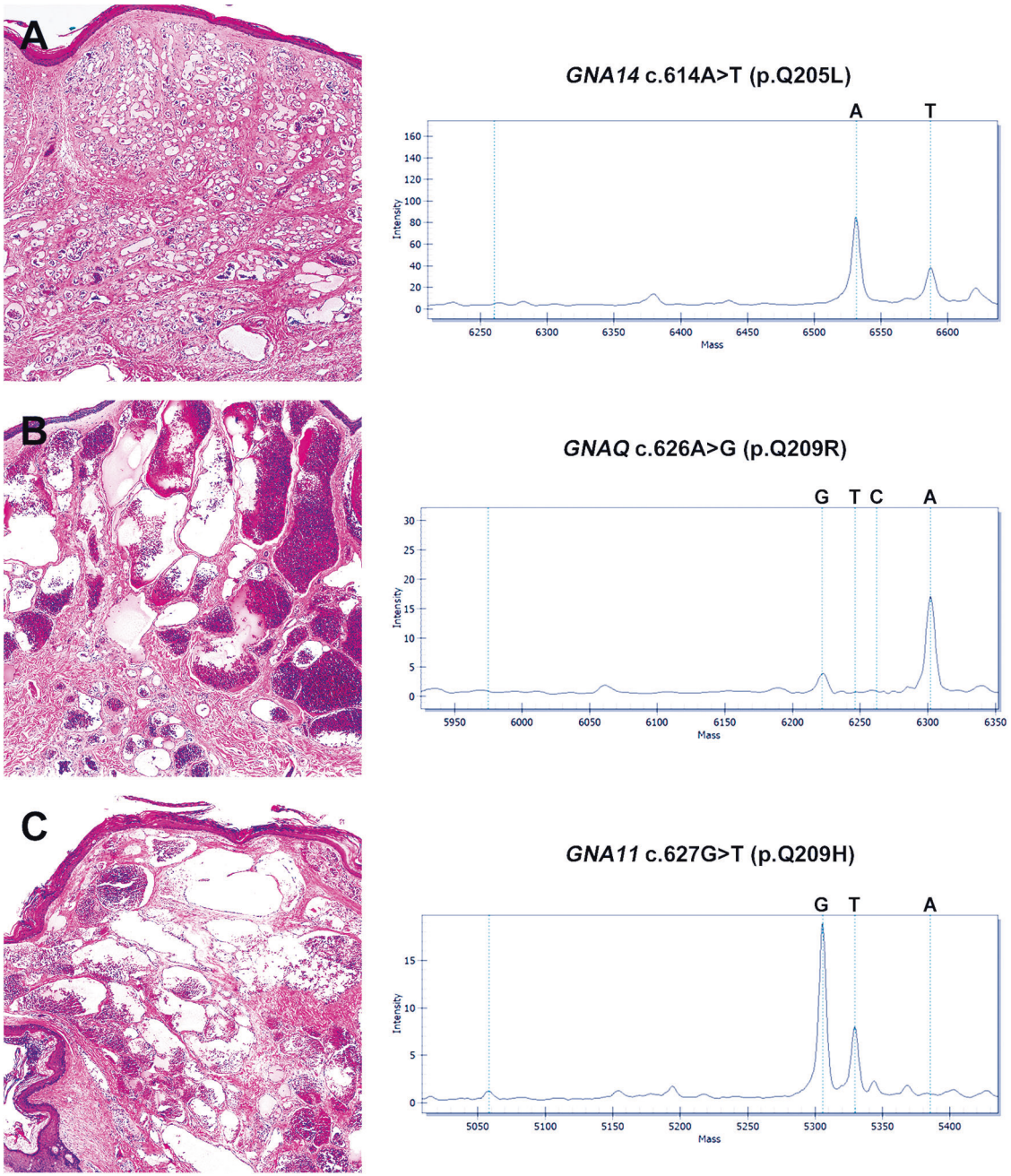

Fig. 3 Representative histologies and MassARRAY spectra. **a** A cherry hemangioma (**a**) with *GNA14*^{Q205L} mutation and 2 cherry hemangioma-like hemangiomas (**b**, **c**) with *GNAQ*^{Q209R} and *GNA11*^{Q209H} mutation, respectively

addition, the genetic profile of cherry hemangioma-like hemangiomas appeared to be more diverse; 2 tumors had *GNAQ*^{G48V} mutations, and the only *RAS*-mutated tumor in our study was also in this group. However, except for *KRAS*^{G12V} and *GNAQ*^{Q209P} mutations, all other mutations were shared by these 2 groups and most of the cherry hemangioma-like hemangiomas exhibited focal areas reminiscent of the classical cherry hemangiomas suggesting that they were closely related. Finally, none of the tumors exhibited eosinophilic hyaline globules (thanatosomes) or extramedullary hematopoiesis, unlike other hemangiomas with *GNA* mutations such as congenital hemangioma and anastomosing hemangioma. A few examples in our series, particularly those with cavernous-like vessels, demonstrated focal thrombosis and changes of organization; however, none of them exhibited anastomosing growth of the endothelial cells to the extent that was encountered in anastomosing hemangioma.

Discussion

Cherry hemangioma is the most common hemangioma in adult. It is particularly prevalent in elderly people, and hence is also named senile hemangioma. The etiology is poorly understood. Beside age, which appears to be the most common etiologic factor, exposure to chemicals such as sulfur mustard gas and bromide and hormone factors have been implicated in some cases [11–13]. The latter is exemplified by occurrence of cherry hemangiomas in pregnant women and spontaneous involution after delivery [14]. Cases of eruptive cherry hemangiomas have been reported to be associated with multicentric Castleman disease, multiple myeloma, and ECHO virus infection [15–17]. Because of the lack of Ki-67 labeling and failure of growth of the endothelial cells in culture, it has been suggested not to be a true neoplasm but a soft tissue overgrowth comprising mature vessels resembling the dermal venules [1]. However, Groesser et al. reported *RAS* mutations in 20% of cases, indicating that at least some cases were neoplastic in nature [2]. In addition, a recent study using more sensitive immunohistochemistry reported all studied cherry hemangiomas had MIB1-labeled cells, albeit with a low labeling index on average (~2%) [18]. The authors also found that all cherry hemangiomas diffusely expressed WT-1, a marker that in vascular lesions usually suggests neoplastic processes [19]. In this study, we showed presence of mutually exclusive *GNA14*, *GNAQ*, and *GNA11* mutations in the majority of the tumors, thus providing solid evidence to establish it as a benign vascular neoplasm. In retrospect, there have been hints in the literature that suggest the involvement of these genes in the pathogenesis of cherry hemangioma. Wu et al. reported a patient with segmental dyschromatosis, blue nevi and cherry hemangiomas, and a family with familial nevus flammeus and early onset cherry hemangiomas was reported by Gao et al. [20, 21]. Blue nevus and nevus flammeus were both known to carry *GNA* mutations, although the mutation repertoire was different from cherry hemangioma (see below). During the revision of the manuscript, we noted that a recent study identified *GNAQ* and *GNA11* mutations in 5 of 10 cherry hemangiomas by targeted next generation sequencing (Q209 mutations in 4; $GNAQ^{R183G}$ mutation in one) [22]. However, *GNA14* mutations were not detected as *GNA14* was not included in the panel. By contrast, *GNA14* mutations were identified in approximately half of cherry hemangiomas in our study. Our and the recent studies strongly indicated that *GNA* mutations underlie the great majority of cherry hemangiomas. We also provided histopathological and molecular correlations and expanded the morphological spectrum of *GNA*-mutated vascular tumors.

GNAQ encodes the alpha q subfamily of the heterotrimeric guanine nucleotide-binding protein (Gαq), which couples the 7-transmembrane domain receptors on the cell membrane to intracellular signaling pathways, including the PI3K/AKT and MAPK pathways, and promotes cellular proliferation, survival, and protein synthesis [23]. Sharing 90% in their amino acid sequence homology, *GNAQ* and *GNA11* play important and overlapping roles in human neoplasia. Activating mutations in codon 183 or 209 of either gene are present in the majority of uveal and meningeal melanocytic tumors and blue nevi [23–25]. In vascular lesions, *GNAQ* R183 mutations can be detected in up to 90% of port-wine stain and Sturge-Weber syndrome [26]. Phakomatosis pigmentovascularis, a mosaic genetic disorder characterized by capillary malformation, dermal melanocytosis and Ota nevus, is associated with postzygotic *GNA11* or *GNAQ* R183 mutations [27]. Recently, recurrent $GNA11^{R183C}$ mutation was identified in patients with extremity capillary malformation and overgrowth [28]. In addition to capillary malformations, *GNAQ* and *GNA11* mutations have been identified in several types of hemangiomas as well, including congenital hemangioma (*GNAQ* and *GNA11*), anastomosing hemangioma (*GNAQ*), and hepatic small vessel neoplasm (*GNAQ*) [6, 7, 9]. We also identified recurrent *GNA11* mutations in anastomosing hemangioma (manuscript in submission). Interestingly, the mutation sites seem to be correlated with the histotypes of the vascular lesions. Codon 183 and 209 mutations were characteristically seen in capillary malformations and hemangiomas, respectively. Remarkably, it has been demonstrated that R183 mutations only partially abrogate the GTPase activity, whereas Q209 mutations completely inactivate the enzyme, indicating a stronger oncogenic potential of Q209 mutations [24]. This biochemical difference seems to be reflected by the clinical phenotypes (i.e., R183 mutations in capillary malformations and Q209 mutations in vascular tumors).

In Gαq subfamily, *GNA14* also shows a high degree of homology to *GNAQ*. *GNA14* mutations have not been found in melanocytic tumors or capillary malformations, but recently recurrent $GNA14^{Q205L}$ mutation (which corresponded to Q209L of *GNAQ* and *GNA11*) was identified in several types of vascular tumors including anastomosing hemangioma, hepatic small vessel neoplasm and in one case each of kaposiform hemangioendothelioma, tufted angioma, and pyogenic granuloma [5, 9, 29]. In human umbilical vein endothelial cells the $GNA14^{Q205L}$ mutant protein was capable of activating the MAPK pathway, inducing cellular morphology change and rendering them growth factor-independent [29]. To our knowledge, all Q205L mutations were caused by c.614A>T transversions in previous studies. However, 3 cherry hemangiomas in our study carried novel c.614_615AA>TT mutations, resulting in the same Q205L mutation. In Catalog of Somatic Mutations In Cancer database double nucleotide mutations resulting in *GNAQ* or

GNA11 Q209L mutation have also been identified in uveal melanomas and blue nevi.

Ultrastructurally cherry hemangiomas have been shown to be composed of capillaries and post-capillary venules with multiple layers of basement membrane [30]. These findings were in line with the observed histological findings (i.e., the vessels are often surrounded by an eosinophilic hyaline layer). However, the vessels of cherry hemangioma showed a spectrum of sizes, ranging from small capillaries to dilated and congested vessels with an attenuated wall. In this study, besides classical cherry hemangiomas, we also included hemangiomas that superficially resembled cherry hemangiomas but exhibited non-classical features. Both groups had equal *GNA* mutation rates. The presence of similar mutation profile in our study suggested that these hemangiomas are related to cherry hemangiomas. However, *GNA14* was the most commonly mutated gene in cherry hemangiomas whereas in cherry hemangioma-like hemangiomas *GNAQ* mutations were more common. In addition, *GNA* exon 5 mutations are now known to be present in several different kinds of hemangiomas and the presence of even identical mutations is not a testimony that they should be classified together. Two cherry hemangioma-like hemangiomas composed of markedly dilated cavernous-like vessels in our study harbored $GNAQ^{Q209R}$ mutations suggesting that this variant, which appears to be rare in the Catalog of Somatic Mutations In Cancer database, might be associated with this particular morphological phenotype. For cherry hemangioma-like hemangioma with a deep component, the presence of the deep component may argue that the lesion is secondary to a vascular malformation. However, these hemangiomas were clinically solitary lesions acquired in adult life and were histologically composed of similarly appearing vessels with a circumscribed border suggesting a clonal lesion. There were no remarkable pathological changes or evident dysplastic vessels in adjacent skin to suggest a hamartomatous or malformative process. Furthermore, in vascular lesions *GNA* exon 5 mutations are more characteristically seen in hemangiomas. Therefore, we favored that they were vascular neoplasms. We speculated that in classical cherry hemangioma, the vessels are derived from the capillaries in the dermal papillae and the venules in the superficial vascular plexus, whereas in cherry hemangioma-like hemangioma with a deep component, the drainage venules in the reticular dermis are also involved.

In our study, $GNAQ^{G48V}$ mutation was identified in 3 tumors. This mutation was not recorded in the Catalog Of Somatic Mutations In Cancer database but has been reported as a rare variant in uveal melanoma [10]. Recurrent G48L mutation was also described recently in hepatic small vessel neoplasm [9]. These findings strongly suggest that codon 48 is a mutation hotspot of *GNAQ* and is implicated in the pathogenesis of melanocytic and vascular tumors.

Activating mutations in exon 5 of *GNAQ*/*GNA11*/*GNA14* are now known to be present in congenital hemangioma, anastomosing hemangioma and hepatic small vessel neoplasm. All of them are characterized by proliferation of predominantly small capillaries; other commonalities include eosinophilic hyaline globules, extramedullary hematopoiesis and hobnailed endothelial cells. Because of similar genetic changes and overlapping histological features, they may be unified within the spectrum of *GNA*-mutated capillary hemangiomas. However, we have expanded the histopathological features of *GNA*-mutated hemangiomas to include tumors composed of markedly dilated, cavernous-like vessels. Further clinicopathological studies are necessary to characterize the full morphological spectrum of *GNA*-mutated vascular tumors and determine the nosological relationships of these hemangiomas.

Twenty hemangiomas were also analyzed for *RAS*/*BRAF* mutations by MassARRAY. A $KRAS^{G12V}$ mutation was identified in one cherry hemangioma-like hemangioma (5%) that lacked *GNA* mutations. *RAS* mutations, particularly *HRAS* mutations, were recently reported in 20% (5/25) of cherry hemangiomas [2]. This discrepancy might be partly attributed to the relatively small case numbers. *GNA* mutations are also capable of activating the MAPK pathway. Therefore, these findings indicate that MAPK pathway activation is present in the great majority of cherry hemangioma and plays crucial roles in its pathogenesis.

Our study had several limitations. First, we chiefly used Sanger sequencing to detect the mutations. Although we also used MassARRAY to increase the sensitivity, only certain specific mutation variants could be detected. Therefore, we might have missed rare mutation variants, and the actual mutation rate might be even higher than the figure we presented here. Unfortunately, our attempt to apply next generation sequencing failed because of insufficient DNA concentrations in most of our samples. Second, the cherry hemangiomas we studied were all from different individuals. Cherry hemangiomas sometimes present with numerous eruptive lesions. Whether eruptive lesions also share identical genetic alterations with the sporadic counterpart is yet to be determined.

In summary, we have identified highly frequent *GNA14*, *GNAQ,* and *GNA11* mutations, particularly $GNA14^{Q205L}$ mutation, in the majority of cherry hemangiomas, thus establishing their neoplastic nature. We also characterize a group of cherry hemangioma-like cutaneous hemangiomas that exhibit non-classical histological features and yet share identical mutations, and thus broaden the histopathological spectrum of *GNA*-mutated hemangiomas. Since cherry hemangioma is extremely common in general population, our findings also indicate

that *GNA14* is the most commonly mutated gene in vascular tumors.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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