Oncogenic GNAQ and GNA11 Mutations in Uveal Melanoma in Chinese

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Abstract

**Purpose:** To examine whether GNAQ and GNA11 somatic mutations previously identified in uveal melanomas of Caucasians are associated with uveal melanomas in Chinese patients.

**Methods:** Uveal melanomas treated by primary enucleation in Chinese patients underwent a mutation analysis of GNAQ and GNA11 with sequencing of exon 5 and exon 4.

**Results:** The study included 50 patients with uveal melanoma and with a mean age of 47.6±13.0 years. During the follow-up of at least 3 years, 20 (40%) patients developed extraocular metastases. The frequencies of GNAQ and GNA11 somatic mutations in uveal melanoma were 18% (9/50) and 20% (10/50), respectively. The mutations occurred exclusively in codon 209 of exon 5. No mutations were detected in exon 4. Mutations affecting codon 209 in GNAQ were c.626A>G (C209F) (78%) and c.626A>T (Q209L) (22%). Mutations affecting codon 209 in GNA11 were exclusively c.626A>T (Q209L) (100%). In none of the tumors, mutations of BRAF and NRAS were detected. GNAQ/11 mutations were marginally (P=0.045) associated with optic disc involvement. In Kaplan-Meier analysis, metastasis-free survival was not significantly (P=0.94) associated with GNAQ/11 mutations.

**Conclusions:** Mutations of GNAQ and GNA11 can be found in Chinese patients as in Caucasian patients with uveal melanoma, with a higher frequency reported for Caucasian patients.

Introduction

Uveal melanoma is the most common intraocular malignant tumor in adults, which arises from melanocytes of the choroid, ciliary body, and iris. It accounts for approximately 5% of all melanomas and represents the second most common form of melanoma [1,2]. Uveal melanoma has a strong tendency to metastasize to the liver and there have been no effective treatments for the metastases. The survival time after the detection of metastasized uveal melanoma, with a mean age of 47.6±13.0 years, during the follow-up of at least 3 years, 20 (40%) patients developed extraocular metastases. The frequencies of GNAQ and GNA11 somatic mutations in uveal melanoma were 18% (9/50) and 20% (10/50), respectively. The mutations occurred exclusively in codon 209 of exon 5. No mutations were detected in exon 4. Mutations affecting codon 209 in GNAQ were c.626A>G (C209F) (78%) and c.626A>T (Q209L) (22%). Mutations affecting codon 209 in GNA11 were exclusively c.626A>T (Q209L) (100%). In none of the tumors, mutations of BRAF and NRAS were detected. GNAQ/11 mutations were marginally (P=0.045) associated with optic disc involvement. In Kaplan-Meier analysis, metastasis-free survival was not significantly (P=0.94) associated with GNAQ/11 mutations.

GNAQ and GNA11 encode Gqα, a member of the q class of G-protein alpha-subunits which are involved in mediating signals between G-protein-coupled receptors (GPCRs) and downstream effectors [15,16]. Recently, oncogenic mutations in the G-protein α-subunit q class were found in about 83% of uveal melanomas with constitutive an activation of downstream MAPK signaling [13]. Glu209 and Arg183 are conserved in the guanosine triphosphate (GTP) binding site of Gq subunits and essential in GTP hydrolysis. The substitutions of glutamine or arginine in GNAQ or GNA11 mutations cause constitutive G-protein activation due to a reduced GTP hydrolysis [17,18]. Matthew et al. reported that R183 mutated oncoproteins were less potent than that of Q209 [19]. GNAQ or GNA11 mutations lead to an upregulating activation of the MAPKinase pathway and appear to be major contributors to the development of uveal melanomas [12,13].
The landmark studies on the association of uveal melanoma with GNAQ and GNA11 mutations were conducted in Caucasian populations while the status of GNAQ and GNA11 mutations in uveal melanomas of Chinese has not been investigated yet. Considering the genetic variations and their role in tumor genesis in different ethnic groups, we investigated the status of GNAQ and GNA11 mutations in uveal melanoma of Chinese patients to decrypt potential oncogenic differences between Caucasian and Chinese populations. It may promote the understanding of molecular mechanisms and of the pathogenesis of uveal melanomas in patients of different ethnic background.

Methods

The study included globes with uveal melanomas arising either from the ciliary body or choroid. The study was approved by the medical ethics committee of Beijing Tongren Hospital and was conducted according to the Declaration of Helsinki Principles. All participants gave their written informed consent. The diagnosis of uveal melanoma was performed in several steps. For each tumor, five metastasis-free patients had to have a follow-up of at least 3 years. A detected metastasis was used as the primary end point. All participants were informed about the possibility of a metastatic disease, whereby death due to other causes was treated as censored. P-values were based on two-sided tests and, if less than 0.05, were regarded as statistically significant.

Results

Our study included 50 globes with uveal melanoma treated by primary enucleation. The mean age was 47.6 ± 13.0 years (Table 2). During the follow-up, 10 patients developed extraocular metastases and 10 patients had died from metastases. There were 4 patients as censors with follow-up data before the reference time horizon. All the metastasis-free patients had a follow-up of longer than 3 years. In detail, the metastasis free survival was 94% for 1-year, 90% for 2-years and 87% for 3-years follow-up in the wild type group, 89% for 1-year, 78% for 2-years and 78% for 3-years follow-up in the GNAQ mutation group, and 90% for 1-year, 80% for 2-years and 80% for 3-years follow-up in the GNA11 mutation group.

To investigate mutations within GNAQ and GNA11, we amplified mutation hotspot regions (exon 4 and 5) of GNAQ and GNA11 and sequenced the purified PCR products directly. Sequencing of GNAQ and GNA11 was successful in all 50 tumors. Among the 50 samples screened for GNAQ and GNA11 mutations, the overall mutation frequency was 38% (19/50), with 18% (9/50) for GNAQ and 20% (10/50) for GNA11, respectively. These figures were lower than mutation frequencies reported in Caucasian patients [12–14]. In all samples, the GNAQ or GNA11 mutations were mutually exclusive and occurred exclusively at codon 209. Mutations affecting codon 209 in GNAQ were c.626A>C(Q209P) (78%) and c.626A>T(Q209L) (22%). These mutations predicted substitution by proline (Q209P) in 78% of samples that were analyzed and by leucine (Q209L) in 22% of the samples. Mutations affecting codon 209 in GNA11 were exclusively c.626A>T(Q209L) (100%) (Tables 3). These mutations predicted substitution by leucine (Q209L) in all samples analyzed. In addition, all of these tumors had been sequenced for BRAF and NRAS hotspots, but no mutations were found, which were consistent with previous reports.

Examining correlations between GNAQ mutations or GNA11 mutations and the clinical-pathologic features in univariate analysis revealed a statistically association between the presence of GNAQ/11 mutations and optic disc involvement (P = 0.045) and a statistically weak association between male gender and presence of GNAQ/11 mutations (P = 0.04) (Table 4). In a logistic multivariate analysis with GNAQ/11 mutations as the dependent variable, Fisher’s exact test, analysis of variance and log-rank test were applied. The Kaplan-Meier method and the log-rank test were used for survival analyses. The primary end point for metastasis-free survival was defined as the time to the development of metastatic disease, whereby death due to other causes was treated as censored. P-values were based on two-sided tests and, if less than 0.05, were regarded as statistically significant.

Table 1. Used Primer Sequences.

| Primer Name | Sequence |
|-------------|----------|
| GNAQexon 5F | 5'-GACTGGATGATCATCGTATT-3' |
| GNAQexon 5R | 5'-AGGGCCTTCCCTCTGTTG-3' |
| GNAQexon 4F | 5'-TGGCTTTGCGGCTGGTG-3' |
| GNAQexon 4R | 5'-GGCAATGGACGCCCTCAGTG-3' |
| GNA11exon 5F | 5'-AAGCGTCTGCCCCTGTT-3' |
| GNA11exon 5R | 5'-AGGGTCCACCTGCGTGG-3' |
| GNA11exon 4F | 5'-GGTGTTTGGTGCTGTTG-3' |
| GNA11exon 4R | 5'-GGCAATGGACGCCCTCAGTG-3' |
| GNAQ/11 | 5'-GGCAATGGACGCCCTCAGTG-3' |
variable and gender and optic disc involvement as independent variables showed that GNAQ/11 mutations remained to be significantly (P = 0.034; Odds ratio (OR): 4.76; 95% Confidence Interval (CI): 1.12, 20.2) associated with an optic disc involvement while gender was no longer significantly associated (P = 0.07; OR: 3.26; 95%CI: 0.92, 11.5).

In the Kaplan-Meier analysis, the difference in metastasis-free survival among patients with GNAQ or GNA11 mutations and patients without the mutations was not statistically significant (P = 0.94), indicating that GNAQ and GNA11 mutations were not significantly associated with metastasis (Fig. 1). Due to the relatively small number of patients, a multivariable survival analysis was not performed.

Discussion

Our hospital-based study showed that mutation frequency of GNAQ and GNA11 in Chinese patients with uveal melanomas was 18% (9/50) for GNAQ and 20% (10/50) for GNA11 with an overall frequency of 38% (19/50). These results on a relatively low percentage of uveal melanomas showing mutations in GNAQ and GNA11 are different from the findings obtained in previous studies on Caucasian patients. Van Raamsdonk and her colleagues detected GNAQ mutations in 46% of uveal melanomas [12]. In our study, the GNAQ mutation frequency was 18%, which was lower than the frequency in studies on Caucasian patients. It has remained unclear however, whether the difference in the reported frequencies of GNAQ mutations between the study populations allowed conclusions on inter-ethnic differences.

Interestingly with respect to cutaneous melanomas, the frequency of BRAF mutations was 66% in Caucasian patients with cutaneous melanomas while the frequency was only 25.5% in Chinese patients with cutaneous melanomas [9,20]. Curtin et al. reported that mutations of KIT gene were detected in 29% of Caucasian patients with cutaneous melanomas while the frequency was 11% in a Chinese cohort with cutaneous melanomas [21,22]. These data may suggest that Caucasians and Chinese may differ in the genetic background of cutaneous melanomas. It has remained unclear whether any analogies between cutaneous melanomas and uveal melanomas can be drawn. It has also remained elusive whether the difference in iris and skin pigmentation between Caucasians and Chinese may play a role in the development of uveal melanoma, with fair complexion and light irides being generally considered risk factors for uveal melanoma [23].

Table 3. Mutations Found in GNAQ and GNA11 in Chinese Patients with Uveal Melanoma.

| Gene       | Mutation     | n    | Total (%) |
|------------|--------------|------|-----------|
| GNAQ exon 5| c.626A>C (Q209P) | 7    | 77.8%     |
|            | c.626A>T (Q209L) | 2    | 22.2%     |
| GNAQ exon 4| –             | 0    | 0         |
| GNA11 exon 5| c.626A>T (Q209L) | 10   | 100%      |
| GN11Q exon 4| –             | 0    | 0         |

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## Table 4. Associations of GNAQ and GNA11 Mutations with Clinical-Pathologic Features of Uveal Melanoma in Chinese Patients.

| Factors                               | Total n | GNAQmut, n (%) | GNA11mut, N (%) | WT, N (%) | P-Value | GNAQ/11mut, N (%) | WT, N (%) | P-Value |
|----------------------------------------|---------|----------------|-----------------|-----------|---------|------------------|-----------|---------|
|                                        | 50 (100%) | 9 (18%)        | 10 (20%)        | 31 (62%)  | 0.11    | 19 (38%)         | 31 (62%)  | 0.043   |
| Gender                                 | 20 (40%) | 6 (12%)        | 5 (10%)         | 9 (18%)   | 0.11    | 11 (22%)         | 9 (18%)   | 0.043   |
|                                        | 30 (60%) | 3 (6%)         | 5 (10%)         | 22 (44%)  | 0.043   | 8 (16%)          | 22 (44%)  | 0.043   |
| Age (Years)                            | 47.6 (23–78) | 43.8 (32–58) | 47.6 (25–68)    | 48.7 (23–78) | 0.62 | 45.8 (25–68) | 48.7 (23–78) | 0.45 |
|                                        | 27 (54%) | 7 (14%)        | 5 (10%)         | 15 (30%)  | 0.34    | 12 (24%)         | 15 (30%)  | 0.31    |
|                                        | 23 (46%) | 2 (4%)         | 5 (10%)         | 16 (32%)  | 0.34    | 7 (14%)          | 16 (32%)  | 0.31    |
| Largest Basal Tumor Diameter (mm)      | 14.7 (6—20) | 13.8 (9–20) | 15.5 (7–20)     | 14.8 (6–20) | 0.53 | 14.7 (7–20) | 14.8 (6–20) | 0.93 |
|                                        | 32 (64%) | 7 (14%)        | 4 (8%)          | 21 (42%)  | 0.22    | 11 (22%)         | 21 (42%)  | 0.48    |
|                                        | 18 (36%) | 2 (4%)         | 6 (12%)         | 10 (20%)  | 0.22    | 8 (16%)          | 10 (20%)  | 0.48    |
| Tumor Thickness (mm)                   | 9.5 (4–20) | 9.6 (5–14) | 10.7 (5–20)     | 9.1 (4–16) | 0.39 | 10.2 (5–20) | 9.1 (4–16) | 0.26 |
|                                        | 26 (52%) | 4 (8%)         | 4 (8%)          | 18 (36%)  | 0.61    | 8 (16%)          | 18 (36%)  | 0.27    |
|                                        | 24 (48%) | 5 (10%)        | 6 (12%)         | 13 (36%)  | 0.61    | 11 (22%)         | 13 (26%)  | 0.61    |
| Epithelioid Cell Type                  | 8 (16%) | 1 (2%)         | 3 (6%)          | 4 (8%)    | 0.54    | 4 (8%)          | 4 (8%)    | 0.72    |
|                                        | 42 (84%) | 8 (16%)        | 7 (14%)         | 27 (54%)  | 0.54    | 15 (30%)         | 27 (54%)  | 0.54    |
| Scleral Invasion                       | 32 (64%) | 4 (8%)         | 7 (14%)         | 21 (42%)  | 0.41    | 11 (22%)         | 21 (42%)  | 0.41    |
|                                        | 18 (36%) | 5 (10%)        | 3 (6%)          | 10 (20%)  | 0.41    | 8 (16%)          | 10 (20%)  | 0.41    |
| Ciliary Body Involvement               | 9 (18%) | 0 (0%)         | 3 (6%)          | 6 (12%)   | 0.25    | 3 (6%)          | 6 (12%)   | 1.00    |
|                                        | 41 (82%) | 9 (18%)        | 7 (14%)         | 25 (50%)  | 0.25    | 16 (32%)         | 25 (50%)  | 0.25    |
| Optic Disc Involvement                 | 12 (24%) | 4 (8%)         | 4 (8%)          | 4 (8%)    | 0.045   | 8 (16%)          | 4 (8%)    | 0.045   |
|                                        | 38 (76%) | 5 (10%)        | 6 (12%)         | 27 (54%)  | 0.045   | 11 (22%)         | 27 (54%)  | 0.045   |

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kinase. Van Raamsdonk and her colleagues reported that most of GNAQ and GNA11 mutations occurred at codon 209, and few others occurred at codon 183 [12,13]. In a similar manner, Onken and his colleagues found that 49% of primary uveal melanomas harbored activating mutations in GNAQ at codon 209 [14]. Consistent with previous studies, our data confirmed that GNAQ and GNA11 mutations in exon 5 occurred exclusively in codon 209. Although we did not detect mutations in exon 4, the relatively small number of patients included into our study may not allow concluding that mutations did generally not occur in exon 4 in a study population as ours. Interestingly, mutations in exon 4 appear also in Caucasians in a very low frequency [13].

In previous studies, associations between GNAQ and GNA11 mutations and clinical-pathologic features and prognosis were examined intensively. Van Raamsdonk and her colleagues reported that GNA11 mutations were more common in locally advanced primary tumors and in melanomas originating from the peripheral choroid or ciliary body [13]. But these associations were not statistically significant. In the study of Onken and colleagues, GNAQ mutations were not significantly associated with any clinical or histopathological parameter nor correlated with tumor progression [14]. Onken and coworkers concluded that the GNAQ mutation may be an early or initiating event in the tumorigensis. Koopmans and his colleagues reported that patient survival in uveal melanoma was not correlated with oncogenic mutations in GNAQ and GNA11 [24]. It is in agreement with our study in which GNAQ/11 mutations were not significantly associated with age, largest tumor basis diameter, tumor thickness, tumor cell type, scleral invasion or ciliary body involvement, with these parameters being associated with a poor prognosis of uveal melanomas. Consequently, we also did not find statistically significant associations of GNAQ/11 mutations with metastasis.

Interestingly, GNAQ/11 mutations were associated with uveal melanomas involving optic disc in our study. Future studies may address whether this finding could be due to site-specificity. Specific windows for GNAQ signaling in terms of location, cell type and developmental time were previously reported, such as that GNAQ mutations induced melanocytic proliferations spared epithelial structures [16]. Our study did not allow addressing whether the association between GNAQ/11 mutations and uveal melanomas involving optic disc would be a parallel to the previous observation of two choroidal melanocytomas harboring mutations in GNAQ with one of them transforming into uveal melanoma [25].

Potential limitations of our study should be mentioned. First, it was a hospital-based study with the inherent risk of a referral bias. Second, the study sample was relatively small, so that the power of the statistical analysis was limited. Strength of the study was that it was the first study to investigate the frequency of GNAQ/11 mutations in uveal melanomas of Chinese patients.

In conclusion, mutations of GNAQ and GNA11 can be found in Chinese patients as in Caucasian patients with uveal melanoma, with the reported frequency being higher in Caucasian patients. Future studies may address whether as suggested by our study, the frequency of these mutations is indeed lower in Chinese than in Caucasians.

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Author Contributions
Conceived and designed the experiments: XX WBW BL FG ZZ JBJ. Performed the experiments: XX WBW BL FG ZZ. Analyzed the data: XX WBW BL. Contributed reagents/materials/analysis tools: XX WBW BL. Contributed to the writing of the manuscript: XX WBW BL FG ZZ JBJ.
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