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Genetic Variants in the Genes Encoding Rho GTPases and Related Regulators Predict Cutaneous Melanoma-specific Survival

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Abstract

Rho GTPases control cell division, motility, adhesion, vesicular trafficking and phagocytosis, which may affect progression and/or prognosis of cancers. Here, we investigated associations between genetic variants of Rho GTPases-related genes and cutaneous melanoma-specific survival (CMSS) by re-analyzing a published melanoma genome-wide association study (GWAS) and validating the results in another melanoma GWAS. In the single-locus analysis of 36,018 SNPs in

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CONFLICT OF INTEREST

The authors state no conflict of interest.

129 Rho-related genes, 427 SNPs were significantly associated with CMSS (P<0.050 and false-positive report probability <0.2) in the discovery dataset, and five SNPs were replicated in the validation dataset. Among these, four SNPs (i.e., *RHOU* rs10916352 G>C, *ARHGAP22* rs3851552 T>C, *ARHGAP44* rs72635537 C>T and *ARHGEF10* rs7826362 A>T) were independently predictive of CMSS (a meta-analysis derived $P=9.04\times10^{-4}$, 9.58×10^{-4}, 1.21×10^{-4} and 8.47×10^{-4}, respectively). Additionally, patients with an increasing number of unfavorable genotypes (NUGs) of these loci had markedly reduced CMSS in both discovery dataset and validation dataset ($P_{trend}=1.47\times10^{-7}$ and 3.12×10^{-5}). The model including the NUGs and clinical variables demonstrated a significant improvement in predicting the five-year CMSS. Moreover, rs10916352C and rs3851552C alleles were significantly associated with an increased mRNA expression levels of *RHOU* ($P=1.8\times10^{-6}$) and *ARHGAP22* ($P=5.0\times10^{-6}$), respectively. These results may provide promising prognostic biomarkers for CM personalized management and treatment.

Keywords

genome-wide association study; Rho GTPase; GTPase-activating protein; cutaneous melanoma-specific survival

Introduction

Cutaneous melanoma (CM), one of the most lethal skin cancers, is a leading cause of cancer mortality in the United States. In 2017, an estimated of 87,110 new cases will be diagnosed and 9,730 cases will die of CM [1]. Unlike several other major cancers, including lung, bronchus, colon and rectal cancers, that have manifested declining trends, CM has demonstrated a stably high mortality rate for the past two decades [2] and continues to represent a significant public health concern. Risk-stratified management, based on accurate staging systems and prognostic information, is a key in addressing CM-related mortality [3]. However, current staging systems have insufficient discriminative power to provide accurate clinical prognostication of the disease [4], thus hampering personalized clinical assessment. Therefore, there is an urgent need to identify new prognostic indicators improved discriminative power.

Germline genetic variants, such as single nucleotide polymorphisms (SNPs), may provide additional information beyond current clinical staging and pathologic prognostic assessment [5]. Recent years have witnessed much success of genome-wide association studies (GWAS) in identifying SNPs that are associated with increased CM risks [6]. Subsequent pathway analyses of GWAS datasets have further detected several functional SNPs that are associated with CM survival, after adjusting for clinical and pathologic prognostic features, including stage, and presence of primary tumor Breslow thickness and ulceration. Examples of proposed prognostic SNPs include those mapped to genes involved in angiogenesis and lymphangiogenesis pathways [7], Fanconi anemia pathway [8], Hippo pathway [9], Notch pathway [10] and vitamin D pathway [11]. In summary, analyzing genotyping data of genes functioning in pivotal biological pathways or processes can provide clues for molecular mechanisms underlying melanocyte carcinogenesis and CM progression.

Rho GTPases act as a molecular switch and have been implicated in controlling cell division, motility, cell adhesion, vesicular trafficking as well as phagocytosis and transcriptional regulation [12]. The activity of Rho proteins is determined by two different states: active GTP-bound states and inactive GDP-bound states that can be controlled by their regulatory proteins. Three classes of such regulatory proteins, including guanine nucleotide exchange factors (GEFs), upregulate Rho activity by catalyzing the exchange of GDP for GTP; GTPases-activating proteins (GAPs) inhibit Rho activity by stimulation of the GTP hydrolysis, while guanine nucleotide dissociation inhibitors (GDIs) act as molecular chaperones and prevent activation by sequestering GTPases away from GEFs [12].

Given their unique functions, Rho GTPases and their related regulators may be implicated in tumor progression. Evidence from the previous studies has indicated that deregulation of Rho GTPases and the related regulators is associated with cancer development, invasion and metastasis [13]. A series of melanoma studies have shown that the Rho GTPases and the related regulators play a vital role in melanoma cell motility and metastasis [14, 15]. Additionally, reports have also demonstrated that aberrant expression of *CDC42*, *RHOC*, *GEF-H1* and *DLC1* (one of the GAPs) is associated with CM survival [16–19]. Given these findings, we hypothesized that genetic variants in genes encoding Rho GTPases and the related regulators would be associated with CM-specific survival (CMSS).

Materials and Methods

Study populations and genotyping data

We used a GWAS dataset from The University of Texas MD Anderson Cancer Center (MDACC) study as the discovery dataset and another GWAS dataset from the Nurses' Health Study and the Health Professionals Follow-up Study conducted by Harvard Brigham and Women's Hospital as the validation dataset. The study protocols were approved by Institutional Review Boards at both MDACC and Harvard Brigham and Women's Hospital with a written informed consent from each of the subjects.

The MDACC discovery dataset included 858 non-Hispanic white CM patients who had complete information for clinical variables [8]. The genotypes were called by using the BeadStudio algorithm at John Hopkins University Center for Inherited Disease Research. Genome-wide imputation was conducted with the MACH software based on the 1000 Genomes Project, phase I v2 CEU data. SNPs with a minor allele frequency ≥ 0.05, a genotyping rate ≥ 95%, and Hardy-Weinberg equilibrium *P*-value ≥ 1×10^{-5} were included in the present study. The MDACC dataset can be accessed at the Database of Genotypes and Phenotypes (dbGaP: http://www.ncbi.nlm.nih.gov/gap) with an accession number phs000187.v1.p1.

The replication dataset from Harvard GWAS have been described previously [11, 20]. Genotyping was performed using the Illumina HumanHap610 array. Genome-wide imputation was also performed using the MACH program based on the 1000 Genomes Project (Utah Residents with Northern and Western European Ancestry data, phase I v3). SNPs with imputation quality $r^2 \geq 0.8$ and minor allele frequency ≥ 0.05 in each study were

used in the present study. This led to 409 non-Hispanic white patients to be included in the validation and final analysis [11].

Gene and SNP selection

Based on the search of gene bases of the HUGO Gene Nomenclature Committee at the European Bioinformatics Institute (HGNC: http://www.genenames.org/), 20 genes encoding Rho GTPases, 66 genes encoding Rho GEFs and 50 genes encoding Rho GAPs were identified. Through the literature we used [21], we identified only three genes (*ARHGDIA*, *ARHGDIB* and *ARHGDIG*) that encode Rho GDIs in humans. In total, 129 autosome genes encoding the Rho GTPases and related regulators were selected after excluding nine genes in the X chromosome and one pseudogene (Table S1). SNPs within these 129 genes and their 2-kb flanking regions were extracted from the MDACC dataset.

Statistical methods

CMSS was defined as the time from the diagnosis of disease to the date of CM-related death or the date of the last follow-up, whichever came first. Deaths with non-CM causes were considered censored. Cox proportional hazards regression analysis was conducted to assess associations between SNPs (with an additive model) and CMSS using the GenABEL package of R software. Although the Bonferroni method for multiple test correction can control the family-wise error rate efficiently, assuming that all SNPs under investigation are independent, it will lead to an over-correction due to a high level of correlations among SNPs in GWAS studies, particularly as a result of imputation that provided the majority of SNPs used in the present pathway-based hypothesis-driven study. Therefore, we used the less strident false-positive report probability (FPRP) method for multiple test correction to generate a better discriminatory set of SNPs from the MDACC study for further validation in the Harvard study [22]. We assigned a prior probability of 0.1 to detect a HR of 1.5 for the genotypes and alleles of SNPs with an elevated risk. Only those SNPs with a FPRP value < 0.2 were considered worthy of subsequent validation in the Harvard dataset. Linkage disequilibrium (LD) analysis was performed by HaploView 4.2 according to European populations from the 1000 Genomes Project with pairwise r^2=0.6 as a cut-off value. Potential functions of SNPs were predicted by RegulomeDB (http://www.regulomedb.org/), SNPinfo Web Server (http://snpinfo.niehs.nih.gov/) and HaploReg [23]. The stepwise Cox regression model including validated SNPs and clinical variables was performed to choose the independent SNPs. Pooled hazards ratio (HR) and 95% confidence interval (CI) were calculated by the meta-analysis using PLINK 1.07. Cochran's Q statistics and I^2 were carried out to access an inter-study heterogeneity. Fixed-effects models were used when no heterogeneity was found between two studies (Q-test *P*-value > 0.10 and I^2 < 25.0%); otherwise, random-effects models were used. To evaluate the joint effects of the SNPs, we combined risk genotypes and risk alleles of each identified SNP into two different variables as the number of unfavorable genotypes (NUGs) and the number of risk alleles, respectively, and both were used as a genetic risk score for further analysis. Kaplan-Meier estimation of survival functions and Log-rank tests were used to evaluate the combined effects of risk genotypes on CMSS. The receiver operating characteristic (ROC) curve was performed to estimate area under the curve (AUC) from the logistic regression model. Delong's test was perform to compare the AUCs across different models. A time-dependent ROC analysis was

performed with the survival ROC package of R software [24]. The expression quantitative trait loci expression quantitative trait loci (eQTL) analysis was performed using data from the 1000 Genomes Project and the GTEx Portal [25, 26]. All analyses were performed using SAS (version 9.1.3; SAS Institute, Cary, NC), unless otherwise specified. Figure S1 provides the study flow chart, illustrating procedures of analyses performed in the present study.

Results

Basic characteristics of study populations

The present study included 858 patients from the MDACC GWAS and 409 patients from the Harvard GWAS (Table S2). All the subjects were non-Hispanic white. The details of clinical information including age, sex, tumor stage, Breslow thickness, ulceration of tumor, tumor cell mitotic rate and survival outcomes were available in the MDACC study, while only age, sex and survival outcomes were available in the Harvard study. In the MDACC study, slightly more patients were men (496, 57.8%) and older than 50 years old (487, 56.8%), having a median follow-up time of 81.1 months and 95 (11.07%) died of CM at the last follow-up. Univariate Cox regression analysis indicated that age, sex, stage, Breslow thickness, ulceration and mitotic rate were significantly associated with CMSS. In the Harvard study, however, much more patients were women (271, 66.3%) and older than 50 years old (337, 82.3%), having a relatively longer median follow-up time (179 months) and 57 (11.5%) died of CM at the last follow-up. Univariate Cox regression analysis indicated that only age was significantly associated with CMSS.

Survival analysis of SNPs and CMSS

As shown in Figure S1, a total of 5,289 genotyped and 30,732 imputed SNPs were extracted in the MDACC discovery dataset. We found that 2,453 SNPs were significantly associated with CMSS at $P < 0.05$ in the single-locus analysis with an additive genetic model by Cox regression analysis, in which 427 SNPs had FPRPs < 0.20. Then, those loci were further subjected for validation. As summarized in Table 1, five SNPs in four genes remained statistically significant with $P < 0.05$ in the Harvard study and in the same direction of effects as detected by the MDACC study. *RHOU* rs10916352, *ARHGAP22* rs3851552, *ARHGAP44* rs72635537 and *ARHGEF10* rs7826362 were significantly associated with poorer survival, while SNP *RHOU* rs7555155 was associated with better survival in both studies. Meta-analysis confirmed that the same associations remained, and the five SNPs were not significantly heterogeneous in effects across the two studies.

Four independent SNPs as CM survival predictors

We further performed LD analysis of the two SNPs in *RHOU*, and found that they were in moderate LD ($r^2 = 0.66$). Functional prediction by SNPinfo and RegulomeDB indicated that *RHOU* rs3851552, *ARHGAP22* rs3851552 and *ARHGEF10* rs7826362 had a RegulomeDB scores of 5, 6 and 5, respectively, which suggests that these SNPs may be located in the transcription factor binding or DNase I regulating sites (Table S3). We also searched for their moderate linked SNPs ($r^2 \geq 0.60$) and made further functional annotation by HaploReg (Table S4). For example, SNP rs7555155 may disrupt the motif of Zfp105, whereas rs10916352 is located in the DNase I hypersensitive sites and may disrupt the motifs of

Foxq1, GR and HNF1, and has a linear correlation with mRNA expression of its corresponding gene *RHOU*. Two SNPs in ARHGAP44, in a moderate linkage with our identified SNP rs72635537, were predicted to disrupt protein motifs. Considering all the functional prediction results of the five SNPs, we included *RHOU* rs10916352, *ARHGAP22* rs3851552, *ARHGAP44* rs72635537 and *ARHGEF10* rs7826362 as functional SNPs to build the model for CMSS prediction. They remained significantly associated with CMSS when included together with clinical characteristics in a stepwise Cox model in MDACC study (Table S5). Taken all together, we selected *RHOU* rs3851552, *ARHGAP22* rs3851552, *ARHGAP44* rs72635537 and *ARHGEF10* rs7826362 as the final independent SNPs for further analyses (Regional association plots were shown in Figure 1). In the MDACC study, risk of death was significantly increased with the number of rs10916352 C, rs3851552 C, rs72635537 T and rs7826362 T alleles (trend test: $P = 0.012$, 0.016, 0.004 and 0.012, respectively, Table 2) and similar results were observed in the Harvard study (trend test: $P = 0.047$, 0.024, 0.018 and 0.022, respectively, Table 2). Consistently, individuals with genotypes of rs3851552 CC+TC, rs7263553 TT+CT and rs7826362 TT+AT had a poorer CMSS, compared with those harboring the wild-type genotypes of each SNP in the MDACC study ($P = 0.004$, 0.003 and 0.029, respectively) and the Harvard study ($P = 0.005$, 0.019 and 0.045, respectively). However, the significant dominant effect of rs10916352 CC+GC genotypes was observed in the MDACC study ($P = 0.003$), but not in the Harvard study ($P = 0.167$).

Combined effects of the four independent SNPs

For ease of interpretation of the joint effect of the four significant SNPs, we combined risk genotypes of rs10916352 CC+GC, rs3851552 CC+TC, rs7263553 TT+CT and rs7826362 TT+CT into a single variable as number of unfavorable genotypes (NUGs) (Table 3). The trend test indicated that an increased number of NUGs was associated with an increased risk of death in both the MDACC ($P = 1.47\times10^{-7}$) and Harvard studies ($P = 3.12\times10^{-5}$). We further divided the combined NUGs into two groups: a low-risk group (0–2 NUGs) and a high-risk group (3–4 NUGs), and found that the hazards ratio (HRs) of death for the high-risk group was 2.62 times [95% confidence interval (CI) = 1.73–3.96, $P = 5.39\times10^{-6}$] and 2.52 times (95% CI = 1.43–4.45, $P = 1.43\times10^{-3}$) in the MDACC and Harvard studies, respectively (Table 3), when compared with the low-risk group. For the visual effect, we used Kaplan-Meier curves to depict associations between NUGs and CMSS (Figure 2). We also performed the genetic risk score analysis by using the method of simple additive summing up the number of risk alleles in both the MDACC and Harvard studies. As with a small number of events in lower and higher risk categories (Table S6), a new combined model was employed for survival analysis (Table S7). Individuals with either 3–4 or 5–7 risk alleles had an increased HR, compared with those with 0–2 risk alleles in the MDACC study. The trend test showed that an HR significantly increased as the number of risk alleles increased, which was also consistently observed in the Harvard study. Additionally, it is apparent that results of the combined analysis of risk alleles are very consistent with that of risk genotypes in the both datasets.

Stratified analyses for associations between NUGs and CMSS

As shown in Table S8, compared with those with 0–2 NUGs, those with 2–4 NUGs had significantly poorer CMSS in the presence or absence of clinical variables in most of the stratified subgroups, except for the subgroups of metastasis and mitotic rate ≤ 1 /mm^2. Heterogeneity was observed only in the subgroup of stage (P = 0.008).

Time dependent AUC and ROC curves for CMSS prediction

Using time-dependent AUC of the ROC curves as criteria, we further evaluated predictive value of the unfavorable genotypes. As shown in Figure 2, the time-dependent AUC plot indicated an improved prediction performance with the addition of NUGs to the model with clinicalpathologic factors from the beginning of the follow-up and remaining over time, compared with clinicalpathologic factors only. As classification of five-year CMSS, the AUC was significantly increased from 86.0% to 88.5% (P = 0.019), when adding NUGs to the clinical variables as classifiers in the ROC curve (Figure 2).

eQTLs analyses

We further conducted eQTLs analysis using data from the GTEx Portal, which only included *RHOU* rs10916352 and *ARHGAP22* rs3851552 in transformed fibroblasts derived from donors' tissues. Rs10916352C and rs3851552C alleles were associated with a significant increase in mRNA expression levels of *RHOU* ($P = 1.8\times10^{-6}$) and *ARHGAP22* ($P = 5.0\times10^{-6}$) in an additive genetic model (Figure 3), respectively. However, no significant associations were observed in 373 Europeans from the 1000 Genomes Project (data not shown).

Discussion

In the present study, we evaluated associations of germline genetic variants in genes encoding Rho GTPases and the related regulators with CMSS, using available genotyping data from two published CM GWAS datasets. We found that genetic variants of *RHOU* rs10916352, *ARHGAP22* rs3851552, *ARHGAP44* rs72635537 and *ARHGEF10* rs7826362 may individually or jointly modulate CMSS. We also observed that incorporating the number of NUGs of these risk SNPs significantly improved prediction accuracy of the model including the variables known to predict CMSS. Our results suggested the potential biological roles of Rho GTPases in CM progression.

The most crucial function of the Rho GTPases, which is correlated with progression of cancer, is the regulation of actin and cytoskeleton organization involved in cancer invasion and migration. The available information on the functions of Rho proteins is mostly derived from the study of three members: Rac1, RhoA and Cdc42. The underlying mechanisms include regulating the formation of lamellipodia and membrane ruffles, focal adhesion complexes and contractile actomyosin filaments, and formation of filopodia [27]. Abnormal expression of RHO genes has been observed to be associated with invasion of several tumor types, including breast cancer, gastric carcinoma, testicular germ cell tumors, and colon cancer [13] as well as melanoma [19]. However, no studies have reported a role of genetic variants encoding Rho and the related regulators in predicting clinical outcomes of cancer.

Our analysis identified four significant SNPs mapped to four genes encoding a member of Rho GTPases (*RHOU*), two members of GAPs (*ARHGAP22* and *ARHGAP44*), and a member of GEFs (*ARHGEF10*). *RHOU* is upregulated by the Wnt1 signaling in Wnt1-transformed mouse mammary cells to promote filopodium formation and stress fiber dissolution [28], and has been reported to regulate tumor cell invasion in prostate cancer by functioning similarly as the Cdc42 small GTPase [29]. While ARHGAP22 and ARHGAP44 trigger local Rac-GTP hydrolysis, thus reducing actin polymerization required for filopodia formation [14, 30]. For example, in melanoma cell movement, ARHGAP22 can be activated to suppress mesenchymal movement by inactivating Rac [14]. *ARHGEF10* is located near a cancer related region, chromosome arm 8p. Loss of chromosome arm 8p has been found in urothelial carcinoma and other epithelial cancers and associated with more advanced tumor stage [31]. Genetic variant in *ARHGEF10* may affect the binding affinity of the Sp1 transcriptional factor, which in turn may increase transcription of the *ARHGEF10* gene, leading to high expression of RhoA [32]. Considering these crucial biological implications, we inferred that the four genes may play a part in tumor progression. As the four identified genes hosting the four significant SNPs, respectively, we conjectured that there might be a strong combined effect of these four SNPs on survival of CM patients. Indeed, our analysis confirmed that the combined effect of the four risk genotypes outweighed that of individual genotypes, hinting the existence of a possible interaction network among the four genes. Their molecular mechanism in melanoma invasion and migration is worthy for further study.

By searching public data from the GTEx Portal, we found that variant alleles of *RHOU* rs10916352C and *ARHGAP22* rs3851552C were significantly associated with mRNA expression levels of the corresponding genes in skin fibroblasts. This biologic evidence demonstrated that *RHOU* and *ARHGAP22* expression may be mediated by these putatively functional SNPs, possibly explaining the associations with CMSS. It has been reported that other micro-environmental factors, such as endothelial cells, immune cells, soluble molecules, and the extracellular matrix, can interact with host fibroblasts to drive tumor progression and even drug resistance [33]. A member of Rho GAPs, ARHGAP35, has been also reported to regulate expression of Cav1 in fibroblasts and facilitates remodeling of peri- and intra-tumoral microenvironments to promote tumor invasion [34]. As the CMSS-associated SNPs in *RHOU* and *ARHGAP22* can modulate the expression of the corresponding mRNA in the skin fibroblasts, their roles in regulation of melanoma microenvironment are warrant to be investigated.

There are several limitations of the present study. The first limitation is the lack of complete clinical data in the Harvard dataset used for validation. In addition, neither of the two datasets had information on any systemic therapies received by the patients with an advanced or aggressive disease. However, no heterogeneity was observed when the two datasets were combined, not in the results of their meta-analysis. Second, we used a less stringent FPRP method to control for multiple comparisons in the discovery dataset [35]. Although this may lead to more false positive findings, it is noteworthy that consistent effects of the identified SNPs on CMSS in both the discovery and validation datasets were observed and that two SNPs, *RHOU* rs10916352 and *ARHGAP22* rs3851552, have potential functions in regulating mRNA expression. Third, although we demonstrated independent and combined effects of the four genetic variants on CMSS, no direct biological experiments

were conducted in vitro or in vivo for additional validations. Further functional investigations are warranted to investigate the exact function of these SNPs or genes on melanoma progression.

In conclusion, our present study identified the role of *RHOU* rs10916352, *ARHGAP22* rs3851552, *ARHGAP44* rs72635537 and *ARHGEF10* rs7826362 in CMSS as assessed in two independent GWAS datasets. Given the importance of Rho GTPases and the related regulators in the invasion and migration of cancer cell, these genetic variants may represent promising prognostic biomarkers for CM personalized management and treatment.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Abbreviations

CM	cutaneous melanoma
SNP	single nucleotide polymorphism
GWAS	genome-wide association study
GEF	guanine nucleotide exchange factor
GAP	GTPases-activating protein
GDI	guanine nucleotide dissociation inhibitor
CMSS	CM-specific survival
MDACC	The University of Texas MD Anderson Cancer Center
FPRP	false-positive report probability
LD	linkage disequilibrium
HR	hazards ratio
CI	confidence interval

ROC	receiver operating characteristic
AUC	area under the curve
eQTL	expression quantitative trait loci
NUG	number of unfavorable genotype
RHOU	ras homolog family member U
ARHGAP22	Rho GTPase activating protein 22
ARHGAP44	Rho GTPase activating protein 44
ARHGEF10	Rho guanine nucleotide exchange factor 10

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Novelty and Impact

Rho GTPases control cell division, motility, adhesion, vesicular trafficking and phagocytosis, which may affect progression and/or prognosis of cancers. In the current study, we investigated associations between genetic variants of Rho GTPases-related genes and cutaneous melanoma survival by using datasets from two genome-wide association studies. Four SNPs in four genes, *RHOU, ARHGAP22*, *ARHGAP44*, and *ARHGEF10*, showed individually or jointly predicted effects on survival, suggesting a potential role of those genes in melanoma progression.

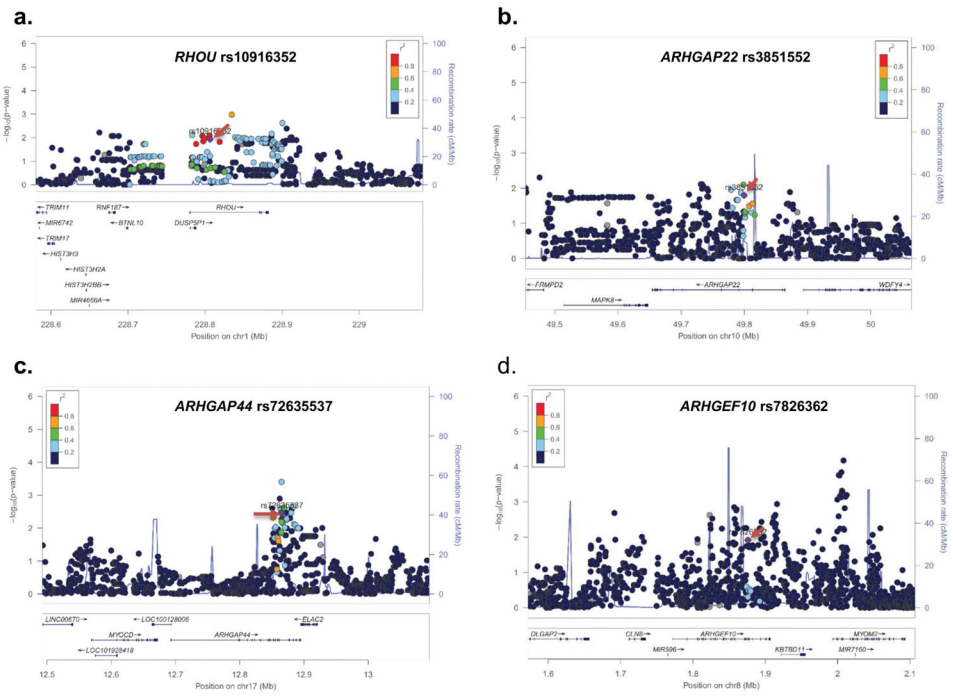

Figure 1.

Regional association plot for the independent SNPs in The University of Texas MD Anderson cancer Center (MDACC) dataset. Single nucleotide polymorphisms (SNPs) in the region of 200 kb up- or down-stream of *RHOU* rs10916352 (a), *ARHGAP22* rs3851552 (b), *ARHGAP44* rs72635537 (c), and *ARHGEF10* rs7826362 (d). Data points are colored according to the level of linkage disequilibrium (LD) of each pair of SNPs based on the hg19/1000 Genomes European population. The left-hand y-axis shows *P* values for associations with individual SNPs, which is plotted as −log10 (*P*) against chromosome base-pair position; the right-hand y-axis shows the recombination rate estimated from HapMap Data Rel 22/phase II European population; the selected SNPs were pointed with the red arrows.

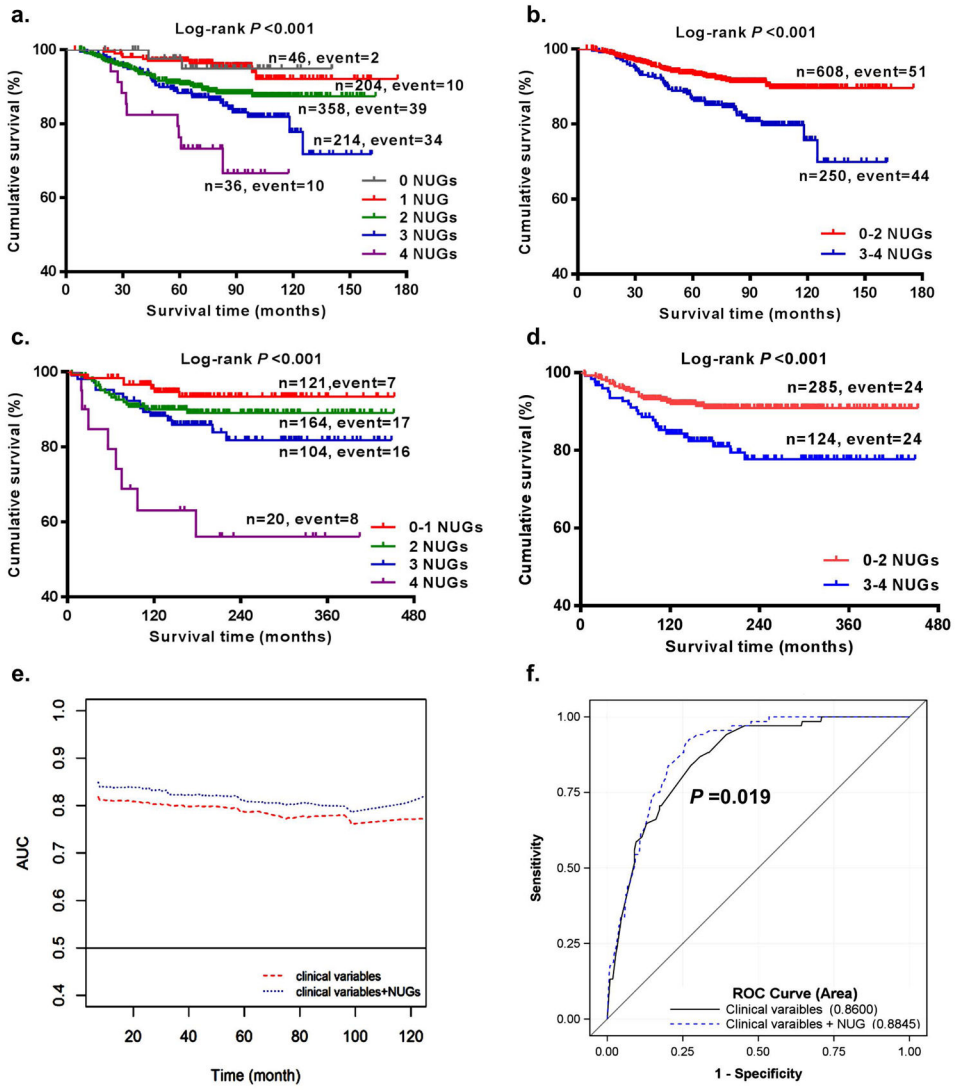

Figure 2.

The four independent SNPs and melanoma survival. **a–d.** Kaplan–Meier survival curves of the combined risk genotypes: the exact numbers of unfavorable genotypes (NUGs) (a) in MDACC study and (c) in the Harvard study; dichotomized groups of the NUGs (b) in the MDACC study and (d) in the Harvard study. **e–f.** Time-dependent area under the curve (AUC) and receiver operating characteristic (ROC) curve estimation for prediction of melanoma-specific survival. (e) Time-dependent AUC estimation, based on age, sex, Breslow thickness, stage, ulceration, mitotic rate and the NUGs in the MDACC study and (f) five-year melanoma-specific survival prediction by ROC curve in the MDACC study.

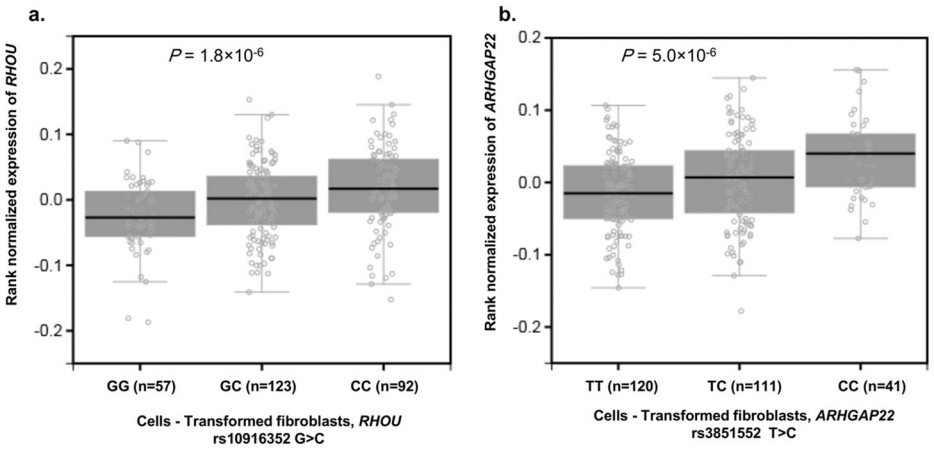

Figure 3.

The expression quantitative trait loci analysis (eQTLs) from the Genotype-Tissue Expression (GTEx) project for (a) *RHOU* rs10916352 and (b) *ARHGAP22* rs3851552 in an additive genetic model.

Table 1

Meta-analysis of five validated SNPs using two published melanoma GWAS datasets

SNP	Gene	Position	Allele[a]	MDACC (n=858)[b]				Harvard (n=494)[c]			Meta-analysis			
				EAF	HR (95%CI)	*P*	FPRP	EAF	HR (95%CI)	*P*	$P_{\text{-het}}$	I^2	HR (95%CI)	*P*
rs7555155[d]	*RHOU*	1q42.13	C/T	0.49	0.59 (0.44–0.80)	0.001	0.024	0.50	0.65 (0.44–0.98)	0.039	0.700	0	0.61 (0.48–0.78)	6.63E-05
rs10916352[e]	*RHOU*	1q42.13	G/C	0.50	1.48 (1.11–1.98)	0.008	0.118	0.50	1.51 (1.01–2.25)	0.046	0.949	0	1.49 (1.18–1.88)	9.04E-04
rs3851552[d]	*ARHGAP22*	10q11.22	T/C	0.35	1.45 (1.08–1.96)	0.015	0.184	0.36	1.61 (1.07–2.43)	0.024	0.691	0	1.50 (1.18–1.91)	9.58E-04
rs72635537[e]	*ARHGAP44*	17p12	C/T	0.08	1.99 (1.28–3.11)	0.002	0.170	0.11	1.90 (1.12–3.24)	0.018	0.897	0	1.96 (1.39–2.75)	1.21E-04
rs7826362[e]	*ARHGEF10*	8p23.3	C/T	0.31	1.48 (1.08–2.03)	0.015	0.196	0.29	1.58 (1.07–2.35)	0.022	0.790	0	1.52 (1.19–1.94)	8.47E-04

Abbreviations: SNP, single nucleotide polymorphism; GWAS, genome-wide association study; MDACC, The University of Texas M.D. Anderson Cancer Center; EAF, effect allele frequency; HR, hazards ratio; FPRP, false positive report probability; CI, confidence interval; P_{het}, *P* value for heterogeneity by Cochrane's Q test.

[a] Reference allele/effect allele.

[b] Adjusted for age, sex, Breslow thickness, stage, ulceration, and mitotic rate in the additive model.

[c] Adjusted for age and sex in the additive model.

[d] Genotyped SNP in the MDACC study.

[e] Imputed SNP in the MDACC study.

Table 2

Associations between the four independent SNPs and the CMSS in the MDACC and Harvard studies

Genotype	MDACC				Harvard			
	All	Death (%)	HR (95% CI)[a]	*P*[a]	All	Death (%)	HR (95% CI)[b]	*P*[b]
RHOU rs10916352								
GG	231	12 (5.2)	1.00		109	9 (8.3)	1.00	
GC	404	54 (13.4)	2.50 (1.33–4.69)	0.005	195	22 (11.3)	1.40 (0.64–3.06)	0.395
CC	223	29 (13.0)	2.49 (1.27–4.91)	0.008	105	17 (16.2)	2.22 (0.99–5.01)	0.054
Trend test				0.012				0.047
CC+GC	627	83 (13.2)	2.50 (1.36–4.58)	0.003	300	39 (13.0)	1.67 (0.81–3.46)	0.167
ARHGAP22 rs3851552								
TT	361	31 (8.6)	1.00		168	10 (6.0)	1.00	
TC	386	52 (13.5)	1.99 (1.26–3.16)	0.003	190	32 (16.8)	2.85 (1.40–5.81)	0.004
CC	111	12 (10.8)	1.71 (0.85–3.46)	0.135	51	6 (11.8)	2.18 (0.79–6.02)	0.134
Trend test				0.016				0.024
CC+TC	497	64 (12.9)	1.94 (1.24–3.03)	0.004	241	38 (15.8)	2.72 (1.36–5.47)	0.005
ARHGAP44 rs72635537								
CC	725	73 (10.1)	1.00		327	33 (10.1)	1.00	
CT	124	21 (16.9)	2.12 (1.29–3.48)	0.003	78	14 (17.9)	2.06 (1.10–3.86)	0.024
TT	9	1 (11.1)	2.09 (0.28–15.8)	0.477	4	1 (25.0)	2.54 (0.35–18.59)	0.359
Trend test				0.004				0.018
TT+CT	133	22 (16.5)	2.12 (1.29–3.46)	0.003	82	15 (18.3)	2.09 (1.13–3.85)	0.019
ARHGEF10 rs7826362								
AA	409	34 (8.3)	1.00		210	19 (9.0)	1.00	
AT	373	49 (13.1)	1.52 (0.97–2.39)	0.071	158	21 (13.3)	1.65 (0.88–3.07)	0.116
TT	76	12 (15.8)	2.20 (1.13–4.31)	0.021	41	8 (19.5)	2.46 (1.07–5.65)	0.033
Trend test				0.012				0.022
TT+AT	449	61 (13.6)	1.62 (1.05–2.51)	0.029	199	29 (14.6)	1.81 (1.01–3.24)	0.045

Abbreviations: SNP, single nucleotide polymorphism; CMSS, cutaneous melanoma-specific survival; MDACC, The University of Texas M.D. Anderson Cancer Center; HR, hazards ratio; CI, confidence interval.

[a]Adjusted for age, gender, Breslow thickness, stage, ulceration, and mitotic rate.

[b] Adjusted for age and gender.

Table 3

Associations between NUGs and CMSS in patients of the MDACC study and Harvard study

Genotype	MDACC				Harvard			
	All	Death (%)	HR (95% CI)[b]	P[b]	All	Death (%)	HR (95% CI)[c]	P[c]
NUG[a]								
0	46	2 (4.3)	1.00		19	0 (0)	1.00[d]	
1	204	10 (4.9)	1.34 (0.29–6.26)	7.07E-01	102	7 (6.9)		
2	358	39 (10.9)	2.84 (0.68–11.85)	1.51E-01	164	17 (10.4)	1.96 (0.81–4.74)	1.34E-01
3	214	34 (15.9)	5.09 (1.22–21.28)	2.59E-02	104	16 (15.4)	2.96 (1.21–7.20)	1.71E-02
4	36	10 (27.8)	9.96 (2.17–45.75)	3.12E-03	20	8 (40.0)	10.49 (3.76–29.28)	7.25E-06
Trend test				1.47E-07				3.12E-05
0–2	608	51 (8.4)	1.00		285	24 (8.4)	1.00	
3–4	250	44 (17.6)	2.62 (1.73–3.96)	5.39E-06	124	24 (19.4)	2.52 (1.43–4.45)	1.43E-03

Abbreviations: NUGs, number of unfavorable genotypes; CMSS, cutaneous melanoma-specific survival; MDACC, The University of Texas M.D. Anderson Cancer Center; HR, hazards ratio; CI, confidence interval.

[a] Unfavorable genotypes were rs10916352 CC+CG, rs3851552 CC+TC, rs72635537 TT+CT, and rs7826362 TT+AT.

[b] Adjusted for age, gender, Breslow thickness, stage, ulceration, and mitotic rate.

[c] Adjusted for age and gender.

[d] Combined the individuals with NUGs of 0 and 1 as reference.