Genetic Alteration of Chinese Patient With Rectal Mucosal Melanoma

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Research Article

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Abstract

BACKGROUND

Rectal mucosal melanoma (RMM) is an aggressive disease with a poor prognosis. Due to the rarity of RMM, few studies about genetic mechanism of the malignant tumor have been conducted. This retrospective study aimed to analyze the mutation spectrum of RMM in China and lay a foundation for targeted therapy.

METHODS

Next-generation sequencing was performed in 36 patients with primary RMM in Peking University Cancer Hospital from May 2010 to March 2019. TMB estimates were determined applying the TruSight Oncology 500 targeted sequencing panel. Survival analysis was performed with Kaplan-Meier, log-rank test.

RESULTS

NRG1 deletions, BRAF mutations and mitotic index were significant prognostic factors in the univariate analysis. In multivariable analysis in overall survival of the prognostic factors in primary RMM patients, it revealed 2 significant variables: NRG1 deletions [HR = 8.830 (95%CI: 1.026–76.026), P = 0.047)]and BRAF mutations [HR = 7.877 (95%CI: 1.158–53.603), P = 0.035].

CONCLUSIONS

This is the first study to show genetic alterations exclusively to Chinese patients with RMM. Although the sample size of the current study is relatively small, we confirmed genetic mutations of RMM differs from cutaneous melanoma. Our study indicates that BRAF and NRG1 may be potential therapeutic targets for rectal mucosal melanoma treatment. AKT3/KCNH1 fusion was correlated with a poor prognostic of RMM.

Introduction

Mucosal melanoma (MM) is a malignant tumor arising from the aberrant growth of melanocytes in the mucosa lining internal tissues. As a subtype of melanoma, MM can occur in the mucous layer of different anatomical sites, such as the respiratory tract, gastrointestinal tract and urogenital tract. Compared with cutaneous melanoma (CM), MM appears to be particularly rare. In America, MM accounts for only 1.3% of all melanoma, while melanoma originated from skin accounts for 91.2% [1]. However, in a study enrolling 522 Chinese melanoma patients, the incidence of MM was 22.6% of all melanoma [2]. Anorectal mucosal melanoma (ARMM) is one of the most common types of MM and has less favorable prognosis [3]. Although melanoma occurring in rectum is more common than melanoma occurring in anus, most studies always include the two entities together [4, 5]. In addition, one study showed the incidence of rectal mucosal melanoma (RMM) consistently increased, and its increase rate has been higher than that of anal melanoma over the past decades [6]. However, whether melanoma was located in anus or rectum,

long-term prognosis was poor [7]. There is still a lack of knowledge about the etiology, pathogenesis and genetics for RMM owing to the rarity of the condition and a lack of reports in the literature, it makes difficult for proper diagnosis, treatment and predict of prognosis.

So far, for this rare tumor, a surgical procedure was recommended as the optimal treatment which can achieve a complete local excision [8, 9]. The addition of radiotherapy does not appear to improve outcomes for patients with RMM, even in those with more locally advanced disease [6]. Chemotherapy and biochemotherapy have shown limited efficacy without significant survival improvement in MM patients as in CM [10]. Despite surgical resection and emergence of various forms of adjuvant therapy, the overall prognosis of RMM remains dismal [11]. For CM, targeted therapy and immunotherapy have been shown to improve prognosis in advanced patients. However, at present, in terms of targeted therapy, the applicability of treatment based on BRAF inhibitors is limited because the incidence of BRAF mutations in MM is much lower than that in CM. Therefore, in future therapies, the discovery of potential therapeutic targets in RMM will be particularly important.

Some studies showed that mutational profiles of malignant melanomas in China are significantly different from Western countries [12, 13]. A whole genome landscape study also showed that MM arose different mutational characteristics in different sites [14]. Based on these differences, we need a more accurate genetic profiling of RMM in Chinese population. Therefore, we analyzed 36 formalin-fixed and paraffin-embedded (FFPE) tumors of RMM by next-generation sequencing (NGS). The purpose of this study was to analyze the mutant spectrum of RMM in China and lay the foundation for targeted treatment.

Method

Patients and pathology

We collected the clinical data and gene test results of 36 patients with RMM who were treated in Peking University Cancer Hospital from May 2010 to March 2019. Patients with melanoma that had an origin other than mucosa and patients with incomplete data were excluded from the present study. The demographic data of the patients were retrieved retrospectively, including their age at diagnosis, sex, treatment and follow-up results. All cases have FFPE samples available for analyses and they were confirmed diagnosed by 2 experienced pathologists in the pathology department of our hospital. The following primary tumor clinicopathological characteristics were assessed: depth of tumor invasion (lamina propria, submucosa, muscularis propria or serosa and beyond); pigment content; thickness; vascular invasion; perineural invasion; ulcer of primary focus; mitotic index and lymph node metastasis. This study was approved by the ethics committee of Peking University Cancer Hospital. All patients had informed consent and signed the informed consent.

RNA & DNA extraction and Quality control

Both genomic DNA and total RNA were extracted from the same FFPE tissue sections using All Prep FFPE DNA/RNA Kit (Qiagen) Freshly cut FFPE tissue sections are treated by deparaffinization solutions and incubate in optimized lysis buffer that contains proteinase K. Under these conditions, RNA is released into solution, while genomic DNA and other insoluble material are precipitated. The sample is then centrifuged to give an RNA-containing supernatant and a DNA containing pellet, which then undergo separate purification procedures. The RNA-containing supernatant is incubated at 80°C to partially reverse formalin crosslinking. The supernatant is then mixed with Buffer RT and ethanol to provide appropriate binding conditions for RNA and applied to a RNeasy spin column. The bound RNA is treated with DNase to digest contaminating genomic DNA and then eluted in RNase-free water. For the DNA-containing pellet, lysed further in the presence of proteinase K and then incubated at 90°C reverses formalin crosslinking. Then sample is mixed with Buffer AL and ethanol to provide optimal DNA binding conditions and then applied to a DNeasy spin column. Genomic DNA binds specifically to the silica membrane and is washed with washing buffer and ethanol to remove contaminants. Pure DNA is then eluted in elute buffer.

Total extracted RNA was quantified using the Qubit Broad Range RNA Assay Kit (Thermo Fisher Scientific) and each RNA sample was assessed using RNA 6000 Nano Kit(Agilent)through Agilent Technologies 2100 Bioanalyzer (Agilent). DV200 value of $\geq$ 20% indicate High-quality RNA and would be used in following steps while samples with a DV200 value $<$ 20% may decrease the performance.

Library Preparation and Sequencing

The preparation of both RNA and DNA sample library were performed according to the Illumina'protocols about TruSight™ Oncology 500(TSO 500). Briefly, the purified RNA samples were diluted with nuclease-free water and then extended to synthesize the first chain,and completing the second chain synthesis by adding the RNase degradation template chain. Then, the cDNA was prepared by purifying and washing using the magnetic beads which is incubated at room temperature and resuspended with the buffer again. The cDNA and 90-250bp DNA fragment samples which were cut by Covaris M220 Focused-ultrasonicator TM (Covaris) were repaired at the end and added with a tail. After the connector was connected with it by ligase, the library was prepared after magnetic bead purification and label primer amplification. Then, after two-step capture and enrichment of specific capture probes for DNA and RNA samples, the DNA library and corresponding cDNA library of 8 samples was standardized by using the library homogenization method based on magnetic bead purification and sequenced with Illumina NextSeq 550Dx.

NGS Data Analysis and TMB Determination

TMB was calculated as the number of somatic, base substitution and indel alterations identified by NGS per Megabase (Mb). Analysis of raw sequencing data and TMB determination procession were conducted with the Illumina TSO 500 supporting software.

Statistical Analysis

The primary endpoint was overall survival (OS). OS was defined as the time interval between the date of tumor resection and date of death from any cause or the date of the last follow-up. SPSS software (SPSS, version 20.0) was used to perform statistical analysis. The overall survival rate was described using Kaplan-Meier survival curves with log-rank. The Cox proportional hazards model was used to perform univariate and multivariate analysis. And the hazard ratio and 95% confidence interval (95%CI) were recorded for parameters. The statistical significance level was set at 0.05.

Result

Demographic and clinical characteristics of the patients

A total of 36 patients with RMM were included in this study. Our clinicopathological analyze was predominantly based on the primary cases. The male/female ratio was 1:1.57, and the median age was 62 years. Other distribution of relevant parameters of 36 primary RMM patients is summarized in Table 1.

Table1 Distribution of relevant parameters associated with primary samples

Variables	n	%
Age, years		
Median	62	
Sex		
Male	14	38.9
Female	22	61.1
Depth of tumor invasion		
Lamina propria	0	0
Submucosa	10	27.8
Muscularis propria	20	55.6
Peri-rectal soft tissue	6	16.6
Serosa and beyond	0	0
Pigment content		
High	13	31.6
Low	14	38.9
No	6	16.7
Unknown	3	8.3
Thickness□mm		
Median	11	
Vascular invasion		
Absent	19	52.8
Present	17	47.2
Perineural invasion		
Absent	25	69.4
Present	11	30.6
Ulceration		
Absent	6	16.7
Present	30	83.3
Mitotic count,		

Median	16	
Lymph node metastasis		
Absent	12	33.3
Present	22	61.1
Unknown	2	5.6

Tumor clinicopathological characteristics

A majority of RMM patients had lymph node metastasis (61.1%), while 33.3% RMM did not occur and 5.6% remained undetermined. As RMM is not included in the American Joint Committee on Cancer (AJCC) melanoma staging system, we used the depth of tumor invasion similar to the T staging used in colonrectal cancer. The level of invasion for the non-perianal tumors was either submucosal (10/36, 27.8%), to muscularis propria (20/36, 55.6%) and beyond the muscularis propria into peri-rectal soft tissue (6/36, 16.6%). It is worth noting that most of patients presented with ulcers (83.3%). Vascular and perineural invasion were identified in 17 (47.2%) and 11 (30.6%) tumors, respectively. The median thickness of the tumor is 11 mm.

Next-generation sequencing

We performed NGS of tumors from 36 patients with RMM, and assessed the single nucleotide polymorphisms (SNP) and the copy number variations (CNV). Overall, 183 genes were found to be enriched in the RMM patient samples. Of the 36 samples included in our study, the average of TMB was 9.15/Mb. Microsatellite-instability status was determined for 36 cases, all of which were classified as microsatellite stable (MSS).

The genes significantly enriched for mutations in RMM were summarized in Supplementary table 1 and Fig. 1. NF1 represented the most frequently mutated gene (22.2%) in RMM although the oncogenicity of a majority of NF1 mutations remained inconclusive. KIT was the second most common mutated gene among all patients which was observed in 16.7% of all cases. Importantly, there were 4 cases of KIT mutation at site p.L576P, the other three cases were mutated at different sites. SF3B1 were identified in 5 patients (13.9%), 4 of whom presented with SF3B1 hotspot R625H mutation in these cases. In addition, the BRAF mutations were diverse with BRAF V600E mutations accounting for 25.0% (1/4) of all BRAF mutations in RMM. Some variants have also been previously reported in RMM, including SPTA1, BCR, TP53, GNAS and BARD1.

We then identified genes with significant CNV. Table 2 and Fig. 2 summarizes the genes significantly mutated genes affected by CNV in RMM. In our study, MYC (9/36, 25.0%), CCND3 (7/36,19.4%) and PDGFRA (7/36, 17.1%) were the most common genes with CNV amplifications. KIT amplifications were

detected in 16.7% of cases and they were frequently amplified on chromosome 4. Of note, copy number deletions were respectively found in PTEN (13.9%) and NRG1(8.3%) of our cohort.

Table 2
Multivariable analysis in overall survival of the prognostic factors in primary rectal mucosal melanoma patients

Variable	HR(95%CI)	*P*-value
Depth of tumor invasion		0.991
Submucosa	1	
Muscularis propria	0.957 (0.235–3.897)	
Perirectal	1.049 (0.203–5.411)	
Mitotic index		0.362
≤ 16	1	
> 16	1.687 (0.548–5.192)	
Lymph node metastasis		0.120
Absent	1	
Present	3.045 (0.747–12.419)	
Unknown	-	
Ulceration		0.062
Absent	1	
Present	4.228 (0.932–19.181)	
NRG1 Deletion		0.047
No	1	
Yes	8.830 (1.026–76.026)	
BRAF Mutation		0.035
No	1	
Yes	7.877 (1.158–53.603)	
BARD1 Mutation		0.765
No	1	
Yes	1.373 (0.172–10.935)	
HR, hazard ratio; CI, confidence interval		

Overall survival analysis

We next analyzed the association of patients' OS with clinicopathological characteristics and gene alterations. Of all 36 patients, 12 were alive at the end of follow-up. Prognosis for all patients was poor with median OS of only 15.7 months (25% – 75% quartiles: 11.3 months–40.8 months). Mitotic index (the median of mitotic index served as the threshold value for grouping) and lymph node metastasis were found to be significant in univariate analysis of OS (P = 0.044 and P = 0.045, respectively, Fig. 3A and Fig. 3B), no differences in OS were observed for other clinicopathological characteristic, as shown in Supplementary table 2. In addition, we found that patients with BRAF mutation had significantly shorter OS compared to non-mutated (P = 0.008, Supplementary table 1, Fig. 3C). The difference between OS and mutational status of KIT, SF3B1, NF1, and TP53 was not significant. NRG1 copy number deletion was present in a total of 8.3% of the cohort and its correlation with OS remained significant (P < 0.001, Table 2, Fig. 3D). However, we did not observe a significant difference of OS between MYC amplification and MYC non-amplification in RMM patients, although this gene seemed to be more amplified. In addition, we found AKT3/KCNH1 fusions and BARD1 mutation in these samples were significantly associated with OS (P = 0.021 and P = 0.040, respectively, Fig. 3E and Fig. 3F). Other genes' alterations were all not statistically significant associated with OS, as shown in Supplementary table 1 and Table 2.

Finally, we built a multivariate model, besides OS, we put the variables which were significant prognostic parameters (P < 0.100) in univariate analysis into our multivariate model. It revealed 2 significant variables: NRG1 deletions [HR = 8.830 (95%CI: 1.026–76.026), P = 0.047)]and BRAF mutations [HR = 7.877 (95%CI: 1.158–53.603), P = 0.035]. The results were shown in Table 3.

Table 3
Univariate analysis of copy number variations in overall survival of the prognostic factors in RMM

Variables	n	HR(95%CI)	*P*-value
MYC Amplification			0.764
No	27	1	
Yes	9	1.168 (0.432–3.227)	
CCND3 Amplification			0.505
No	29	1	
Yes	7	0.693 (0.236–2.036)	
PDGFRA Amplification			0.421
No	29	1	
Yes	7	0.608 (0.181–2.043)	
FGF6 Amplification			0.778
No	30	1	
Yes	6	0.856 (0.290–2.523)	
KIT Amplification			0.823
No	35	1	
Yes	6	0.870 (0.259–2.931)	
PTEN Deletion			0.114
No	31	1	
Yes	5	2.269 (0.822–6.259)	
CCNE1 Amplification			0.942
No	32	1	
Yes	4	1.046 (0.310–3.526)	
FGF23 Amplification			0.994
No	32	1	
Yes	4	1.005 (0.296–3.413)	
NRG1 Deletion			0.001

RMM, rectal mucosal melanoma; HR, hazard ratio; CI, confidence interval

Variables	n	HR(95%CI)	*P*-value
No	33	1	
Yes	3	16.478 (3.269–83.066)	
RMM, rectal mucosal melanoma; HR, hazard ratio; CI, confidence interval			

Discussion

MM is rare, in a retrospective cohort involving 446 Chinese patients with melanoma, they were known to behave more aggressive and were associated with shorter survival than cutaneous lesions [15]. Although similar research has been reported in the literature before, no specific mucosal site has been under investigation [13, 16, 17]. In this study, we retrospectively performed genomic profiling of 36 cases with RMM using NGS in order to provide a reference for the clinical prognosis of the tumor and further targeted intervention therapy.

A review of the histopathology revealed multiple adverse prognostic pathological factors including deep tumor thickness, ulceration, high tumor mitotic rate and lymphovascular invasion. Likewise, in our study, mitotic index and lymph node metastasis may be risk factors of prognostic significance. Previous study also found the presence of lymph nodes at the initial presentation profoundly affected survival [18]. Interestingly, such unfavorable prognostic value had no statistical significance in multivariate analysis. This may be related to limited cases in our study.

In the study of genetic change, we found mutations in common genes involved in MAPK signaling pathway, including mutations in NF1, KIT, BRAF, and NRAS. A significant higher proportion of NF1 single-nucleotide variant (SNV), insertion and stop-gain mutations were observed. Notably, similar to the previous findings [19], nine harbored clearly NF1 inactivating mutations in our study for RMM. Point mutations in KIT genes were also common, consistent with the report of genetic alterations of KIT in ARMM, and more mutations in L576P were found in our study [20]. A number of KIT inhibitors, such as imatinib, sunitinib, dasatinib, and nilotinib, which have shown variable clinical activity in the treatment of KIT mutated MM [21]. This provides a reliable theory for targeted therapy of RMM.

Furthermore, BRAF was mutated gene with a frequency of 17%, which was primarily missense mutation, including BRAF V600 substitutions. A comparison has showed that whereas CM presented a vast majority of V600 mutation (more than 90%), MM were characterized by a high prevalence of non-V600 mutations (37%)(for example, G469A and D594G are found frequently in MM) [22].This was consistent with our research: out of all the BRAF single-nucleotide alterations that has detected in this study, the BRAF (V600E) mutation accounts for 25%, while non-V600 mutation accounts for 75%(R146Q, G469A and D594G) of all cases. Our survival analysis for the entire cohort showed point mutation of BRAF was related to poor prognosis. Existing research shows that small molecule inhibitors (vemurafenib and dabrafenib) of BRAF V600E/K–mutant induce tumor regression and improve survival in melanoma patients compared to chemotherapy [23, 24].However, BRAFV600E/K mutations are less common in MM,

rendering them cannot be treated with BRAF/MEK inhibitors. Currently, only few studies have investigated the molecular mechanisms of non-BRAF V600 mutations, they show these mutants are able to promote MEK phosphorylation in a CRAF-dependent manner by directly binding to and activating CRAF to drive the MAPK pathway [25]. Therefore, much more experiments in vivo are still need to be researched intensively in future. Two BRAF gene amplifications were also observed in this study. It is worth noting that BRAF amplification results in BRAF over-expression has been reported as one of the mechanisms responsible for acquired resistance to BRAF and/or MEK inhibitor [26, 27].Our results show that the BRAF-KCTD7 fusion gene also occur in RMM, although they are rare. KCTD7 can regulate neuronal autophagy and its bi-allelic mutations could cause severe neurodevelopmental diseases [28], however, its biological role with BRAF remains unclear. Only one study identified a novel ZNF767-BRAF gene fusion that displayed resistance to the BRAF inhibitor vemurafenib in respiratory MM patients [29].

Neuregulins (NRGs) are a large subclass of polypeptide growth factors of the epidermal growth factor (EGF) family [30]. The NRG1 (neuregulin-1) gene has been proposed both as a candidate oncogene and a candidate tumor suppressor gene [31]. On one hand, it can specifically bind to the extracellular domain the receptor tyrosine kinase ERBB3 and ERBB4 to alter receptor conformation and promote dimerization with ERBB2 [30].Receptor hetero dimerization promotes autophosphorylation of the cytoplasmic tyrosine residues, resulting in the activation of downstream PI3Kinase and MAPKinase signaling pathways [32]. NRG1/ERBB3 signaling was able to negatively regulate melanocyte (MC) differentiation and pigmentation while promoting proliferation [32]. On the other hand, the NRG1 gene is frequently silenced by methylation in breast cancers and NRG1 may be the principal tumor suppressor gene that leads to loss of 8p in many breast and other epithelial cancers [31].Loss of the NRG1 gene was detected in 7.3% (3 out of 36) of RMM patients and is associated with poor prognosis in our cohort. Previous studies have shown that over expression of NRG1 leads to the activation of ERBB3/ERBB2 signaling and a poor prognosis. One of the reasons might be the paracrine effect of NRG1 enhances the resistance to RAF and MEK inhibitors [33, 34].However, no loss mutation of NRG1 has been reported in RMM previously. Future studies will be needed to understand the biological significance of mutated NRG1 in RMM patients and to understand how this influence melanoma progression. The NRG1 gene has been shown to display seemingly contradictory functions: the induction of tumorigenesis and the induction of apoptosis [35]. Therefore, as a critical tumor-associated gene, NRG1 may be a potential therapeutic target for melanoma treatment.

As a binding partner of BRCA1, BRCA1-associated RING Domain 1 (BARD1) has been extensively investigated in multiple cancers. Alterations of the BARD1/BRCA1 pathway have also been shown to play a significant role in breast and ovarian cancer. However, BARD1 mutations are associated with few cases of non-BRCA1/BRCA2-related sporadic breast and ovarian tumors and account for only a small fraction of cases of familial breast cancer overall [36]. In our cohort, BARD1 showed relatively high frequent mutations and are negatively correlated with the prognosis of RMM. This is in line with observations in ovarian and breast carcinomas, where BARD1 mutations might be a poor prognostic factor [37]. Interesting to note, emerging data suggest that BARD1 can have both tumor-suppressor gene and oncogene functions in tumor initiation [38]. Studies suggesting that other cancer predisposition genes

can increase the risk of melanoma is also available in the literature [39]. Our study provides insights into the functions of new gene mutation of RMM, and offers new opportunities for therapeutic intervention in cancer. Further complete gene sequencing or whole genome sequencing projects are warranted to investigate the contribution of rare variants of BARD1 in conferring cancer risk.

Our data suggest that some patients with RMM also harbor gene fusions, rendering them potential candidates for targeted therapy. In our study, AKT3/KCNH1 fusion was found to correlate with poor prognosis. In the previous literature, a recurrent MAGI3-AKT3 fusion was found to be enriched in triple-negative breast cancer [40]. However, no literature related to AKT3 gene fusion was found in melanoma. Akt3 has been only shown to promote early melanoma development with the mutant BRAFV600E in a cooperatively acting manner and is an important mediator of cell survival and drug resistance in melanomas [41, 42]. KCNH1 is a CNS-localized Eag voltage-dependent potassium channel, it shows a function in proliferation of melanoma cells [43]. Furthermore, it aberrantly expressed in 3.4% of all human colorectal cancer and was an independent marker of adverse prognosis [44, 45]. In conclusion, AKT2 and KCNH1 might be used as a potential prognostic marker as well as a potential therapeutic target for RMM. Fusions involving neurotrophic-tropomyosin receptor kinase (NTRK) genes are known drivers of oncogenesis. Consistent with findings of Cecilia et al, we also found an NTRK2 fusion in a mucosal primary tumor [46]. Entrectinib, a potent oral inhibitor of the tyrosine kinases, has been shown remarkably effective targeting NTRK fusions [47]. Larotrectinib had marked and durable antitumor activity in patients with TRK fusion–positive cancer and had also shown great efficacy in recent clinical trials [48]. All of these may throw light on the target therapy for the MM with NTRK fusions.

Conclusions

To the best of our knowledge, this is the first study to show genetic alterations exclusively to Chinese patients with RMM. We performed NGS of 36 primary RMM specimens to describe the mutational characteristics and tackle the mechanism of this rare tumor. Although the sample size of the current study is relatively small, we confirmed genetic mutations of RMM differs from cutaneous melanoma. BRAF and NRG1 may be potential therapeutic targets for melanoma treatment. This established a rationale for that several genes could serve as therapeutic targets. We also observed that AKT3/KCNH1 fusion was correlated with the poor prognostic of RMM. However, further validation with a larger cohort and in vitro experiments are needed to confirm these findings.

Abbreviations

Abbreviation	Full name
95%CI	95% confidence interval
AJCC	American Joint Committee on Cancer
ARMM	Anorectal mucosal melanoma
BARD1	BRCA1-associated RING Domain 1
CM	Cutaneous melanoma
CNV	Copy number variations
EGF	Epidermal growth factor
FFPE	Formalin-fixed and paraffin-embedded
HR	Hazard ratios
Mb	Megabase
MC	Melanocyte
MM	Mucosal melanoma
MSS	Microsatellite stable
NGS	Next-generation sequencing
NRGs	Neuregulins
NTRK	Neurotrophic-tropomyosin receptor kinase
OS	Overall survival
RMM	Rectal mucosal melanoma
SNP	Single nucleotide polymorphisms
SNV	Single-nucleotide variant

Declarations

Ethics approval and consent to participate

All analyses of human data conducted in this study were approved by the Ethics Committee of Peking University Cancer Hospital and all methods were carried out in accordance with relevant guidelines and regulations. Written informed consent was obtained from all participants. This study was performed in accordance with the Declaration of Helsinki.

Consent for publication

Not applicable.

Availability of data and materials

All analyzed data are included in this published article and its supplementary information file. The original data are available from the corresponding author on reasonable request.

Conflict of interest

The authors declare no competing interests.

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Authors' contributions

ZW L and L S conceived and designed the experiments. H L, LJ Y, YM L, DL W, XJ L, NN H and Y K performed the experiments. H L and LJ Y analyzed the data. DL W, XJ L and NN H contributed the materials/analysis tools for the study. H L and YM L updated the follow-up data for all cases. H L wrote the paper. All authors have read and approved the final manuscript. Co-published data notes will be linked to the research article the data support.

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Figures

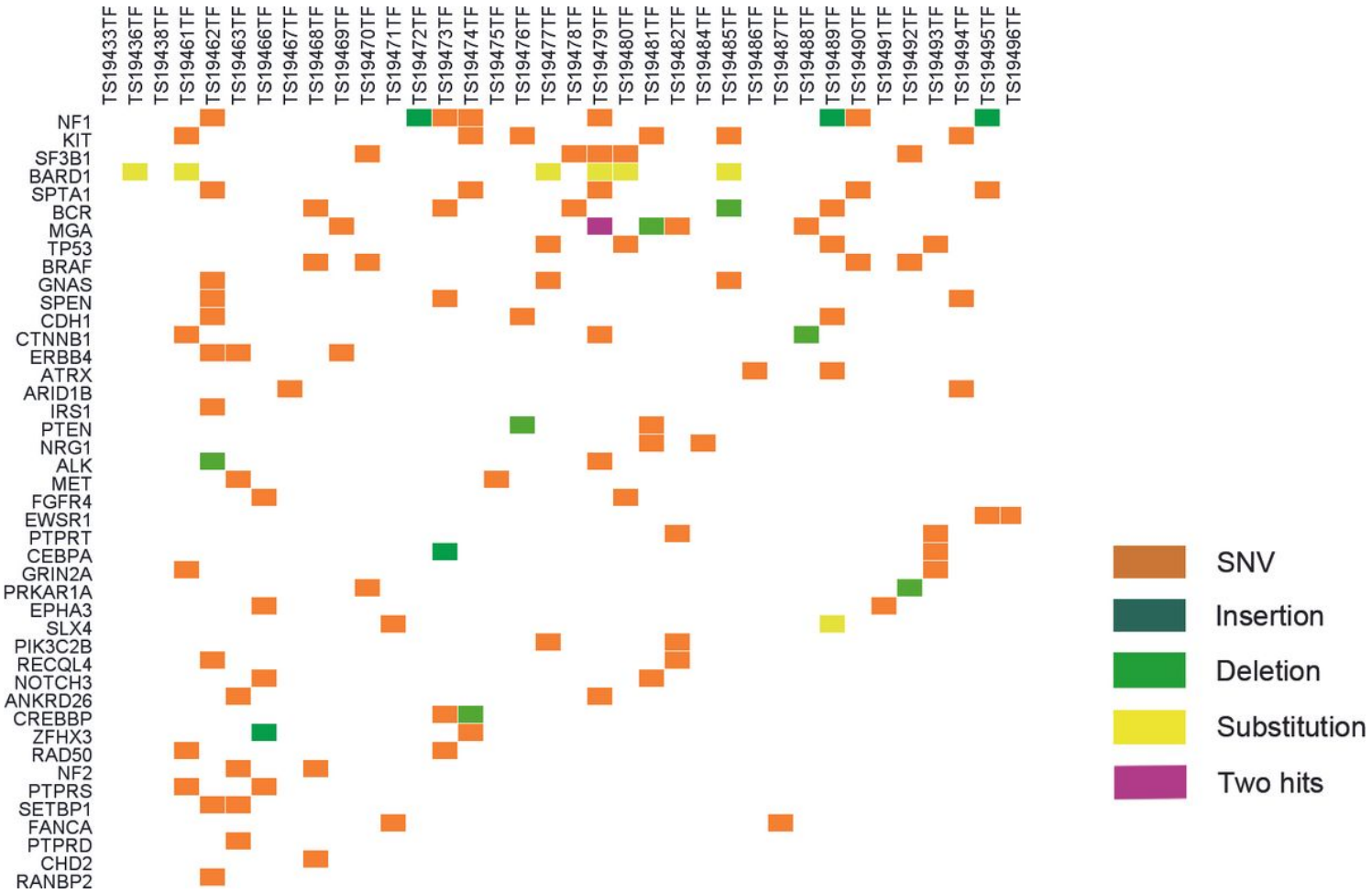

Figure 1

Spectrum of genetic variations. At least two mutation occurred in 36 samples. The variants included single-nucleotide variations (SNV), insertion, deletion and substitution. Arranged by frequency.

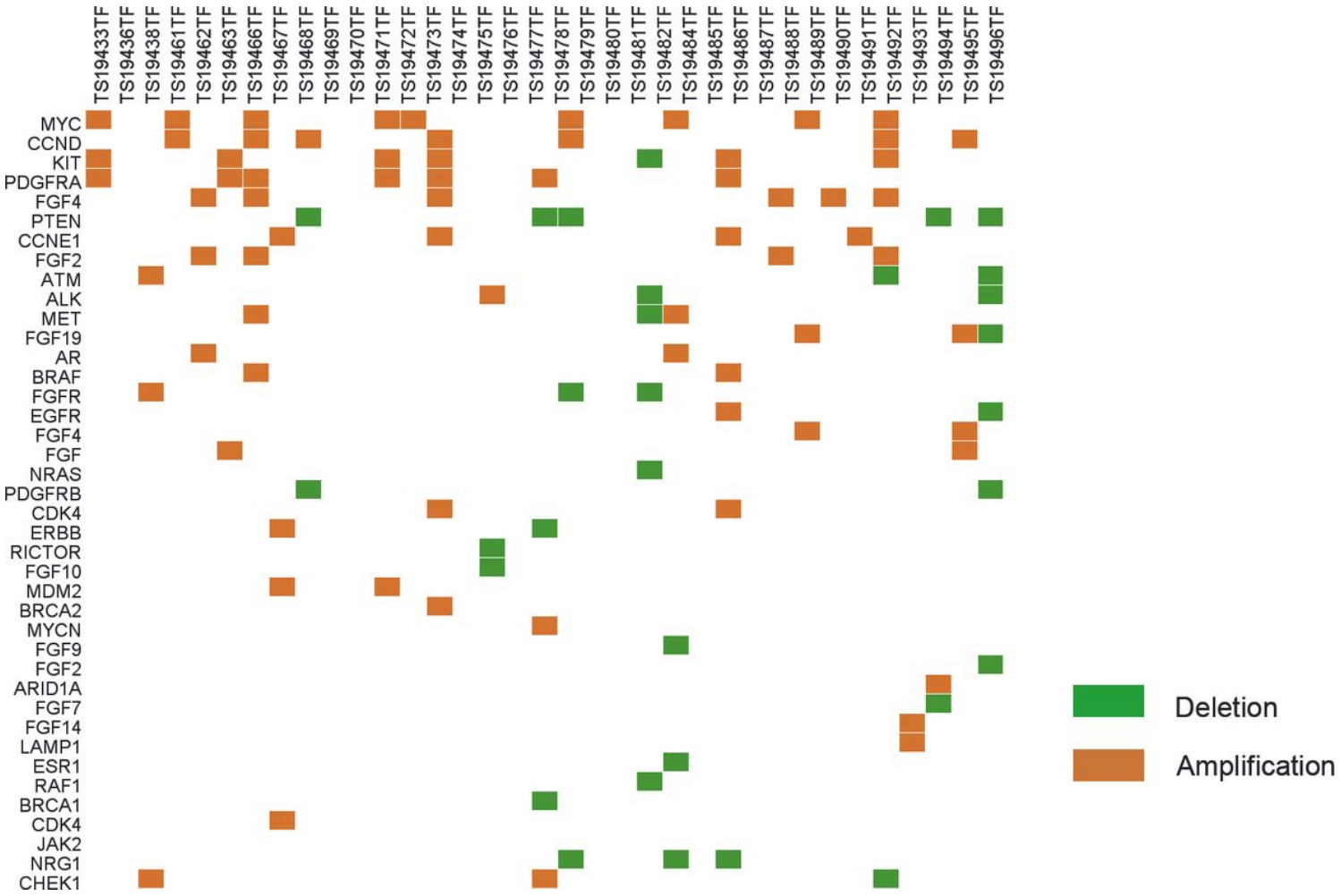

Figure 2

Spectrum of copy number variations (CNV). At least one mutation occurred in 36 samples. The variants included deletion and amplification. Arranged by frequency.

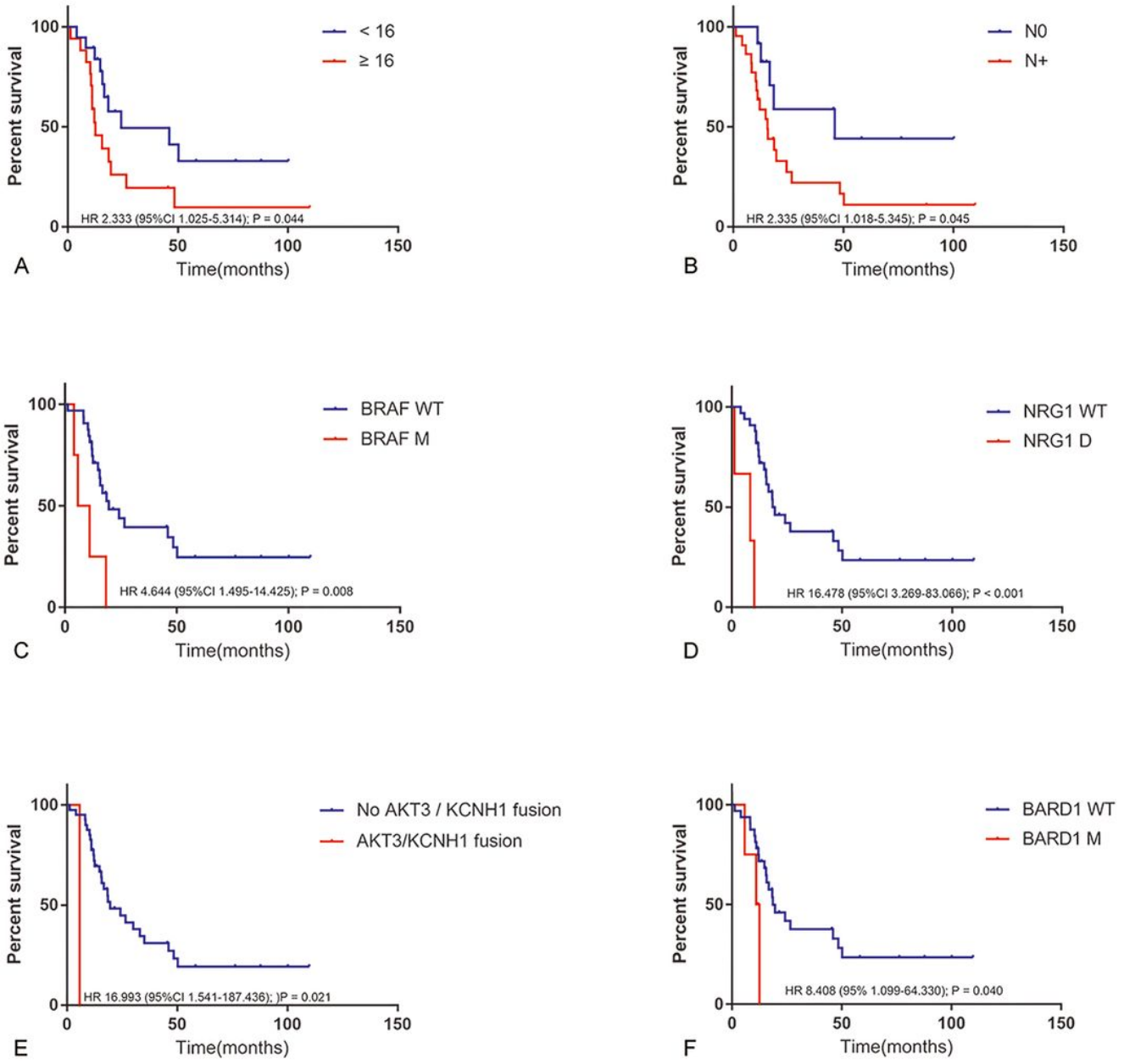

Figure 3

Kaplan-Meier survival curves of overall survival (OS). Kaplan-Meier survival curves of OS for pathological features and molecular changes of rectal mucosal melanoma were performed. (A) The increased mitotic index has a trend with decreased OS (p = 0.044). (B) The lymph node metastasis has a trend with decreased OS (p = 0.045). (C) The BRAF mutation has a trend with decreased OS (p = 0.006). (D) The NRG1 copy number deletion has a trend with decreased OS (p = 0.001). (E) The AKT3/KCNH1 fusions has a trend with decreased OS (p = 0.021). (F) The BARD1 mutation has a trend with decreased OS (p = 0.040).

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