The Genomic Profiling in Chinese Head and Neck Cancer and Incidence of NTRK Fusion

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Research

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Abstract

Background

Head and neck cancers are aggressive epithelial tumors and well recognized as a particularly challenging class of tumors to treat. Comprehensive molecular profiling is leading to the development of “personalized” or “precision” medicine. Here we report the genomic profiling of Chinese head and neck cancers and the incidence of *NTRK* aberrations.

Methods

We retrospectively analyzed the genetic aberrations in 127 patients of Chinese head and neck cancer. All the patients were detected by 1021-gene panel (including *NTRK1*, *NTRK2, NTRK3*) of hybridization capture-based next-generation sequencing with tumor tissue and matched peripheral blood control samples.

Results

This studied was inspired by the outcome benefit of a parotid cancer patient harboring *ETV6-NTRK3* fusion, who received crizotinib treatment and achieved a 2-year progression-free survival (PFS). Then, we reviewed 127 cases of head and neck cancer in our database. The most common histology type was HNSCC (79.5%). The genomic profiling indicated that both in our Chinese cohort and TCGA database, *TP53* is the most frequently mutated gene in head and neck cancer. The incidence of *NTRK* genetic aberrations was 7.9% (10/127) including *NTRK* fusion (n = 4, 3.1%) and *NTRK* mutation (n = 6, 4.7%). The most common fusion was *ETV6-NTRK3* (n = 3, 2.4%). Compared to NTRK-wt group, NTRK aberration group had more *APC* and *PTPRD* aberrations ($p < 0.05$). The association of genetic aberrations with tumor mutation burden (TMB) had been analyzed. NTRK fusion-group had a lower TMB compared to the NTRK-wt group ($p = 0.034$). *TP53* and *LRP1B* showed significant association with higher TMB (both $p < 0.01$), which may be potential markers of immunotherapy in head and neck cancer patients.

Conclusions

Our data is the first study to report the genomic profiling in Chinese head and neck cancers and the incidence of *NTRK* fusion. About 3% of Chinese head and neck patients may benefit from targeted therapy of *NTRK* inhibitors. An *ETV6-NTRK3* fusion patient reached a long-term response with crizotinib treatment,indicating crizotinib might be an alternative treatment option for patients with NTRK fusions.

Background

Head and neck cancer is the eighth most common cancers worldwide [1]. The latest statistics in China showed that the incidence of head and neck cancer is about 3.268% [2]. Squamous cell carcinoma (HNSCC) accounts for ~ 95% of head and neck cancer, includes cancers of oral cavity, oropharynx, hypo pharynx and larynx. HNSCC are aggressive epithelial tumors and well recognized as a particularly challenging class of tumors to treat. Sizable proportion of patients often develop recurrent, locally advanced and metastatic disease. Improvement in outcomes of these patients are urgent needed. Standard first-line therapy for metastatic disease is cetuximab plus chemotherapy with platinum and 5-fluorouracil, which provides median overall survival (OS) about 10 months and is associated with substantial toxicity [3]. Comprehensive molecular profiling is leading to the development of “personalized” or “precision” medicine. It is becoming standard practice for patients with advanced disease. Basket studies are very attractive because of targeting particularly genetic mutations regardless of the origin of tumor. They make precision medicine more attainable, especially for some rare or refractory cancers [4]. Through promoting molecular diagnosis and targeted therapies, treatment of certain head and neck cancers may soon be fundamentally transformed.

Neurotrophic-tropomyosin receptor tyrosine kinases (NTRKs) are composed of three transmembrane protein receptors TrkA, TrkB and TrkC (hereinafter referred to as TRK). They are encoded by the *NTRK1*, *NTRK2* and *NTRK3* genes, respectively [5]. Binding of neurotrophins to NTRKs activates the downstream signaling, such as phospholipase C-γ, MAPK and PI3K/ALK pathways [6], thus promoting the proliferation and survival of neuronal cells. A variety of mechanisms can cause the activation of TRK proteins, such as somatic *NTRK* mutations, splice variants and TRK overexpression [7]. Abnormal activation of NTRKs can induce neurogenic and non-neurogenic carcinogenesis [8, 9], of which *NTRK* fusions are the most common oncogenic mechanism [10]. The prevalence of *NTRK* fusions was 0.31% in adult tumors and 0.34% in pediatric tumors according to data from The Cancer Genome Atlas (TCGA) and the St Jude PeCan database, respectively [11]. Notably, *NTRK* fusions occurs more than 90% in some rare tumors, such as mammary-analog secretory carcinoma of the salivary gland (MSCC) and secretory breast carcinoma [11]. A small percentage of common cancers, including head and neck cancer, colorectal cancer and non-small cell lung cancer (NSCLC), also carry *NTRK* fusions [12]. Although the *NTRK* fusions are rare, the anti-tumor activity of such inhibitors is very significant in various cancer types harboring *NTRK* fusions [13–17].

Before the advent of specific NTRK inhibitors, several small molecular inhibitors have shown preclinical inhibitory activity against one or more NTRK receptors. They are originally approved by the US Food and Drug Administration (FDA) for other indications, such as cabozantinib (Cabometyx; Exelixis, South San Francisco, CA), crizotinib (Xalkori; Pfizer, New York, NY), and regorafenib (Stivarga; Bayer, Leverkusen, Germany) [11]. On Nov 26, 2018, the FDA accelerated the approval of larotrectinib (loxo-101, a selective inhibitor of TRK), for the treatment of locally advanced or metastatic solid tumor patients carrying NTRK gene fusion. Larotrectinib has been recommended by the NCCN guidelines as category 2a evidence for *NTRK*-positive head and neck cancers. NTRK diagnostic testing was also recommended by the NCCN guidelines (Head and Neck cancers. 2019, version 1). Personalized therapy is now possible for head and neck cancer patients. Nevertheless, the genetic alterations of *NTRK* in head and neck cancer are far from unclear.

In our treatment center, a IV-stage parotid cancer patient harboring *ETV6-NTRK3* fusion underwent crizotinib treatment and achieved a long-term PFS without severe adverse effect. We then retrospectively analyzed the genetic aberrations in 127 patients of Chinese head and neck cancer by hybridization capture-based next-generation sequencing (NGS) of 1021-gene panel. The clinical and molecular characteristics of patients with *NTRK* genetic aberrations were further analyzed.

Materials And Methods

Ethical statement

This study was approved by the institutional review board of Nanjing Medical University. The data released from TCGA database did not require informed patient consent because cancer is a reportable disease in the US.

Patients and clinical tissues

The present study retrospectively enrolled 404 head and neck cancer patients who underwent a next-generation sequencing assay in the Geneplus-Beijing Ltd. (Beijing, China) between March 2016 and November 2019. All participants have signed the written informed consent. Either fresh tissues, or formalin-fixed paraffin-embedded (FFPE) tissues, or malignant effusion, and matched peripheral blood were obtained from each patient. To ensure the effectiveness of the analysis, patients were screened according to the detected panel and samples. The exclusion criteria were: 1. patients undergo NGS but not the 1021-gene panel; 2. patients with only liquid tumor sample;3. patients with multiple primary tumors. As a result, a total of 127 head and neck cancer patients of Chinese population were finally included in our study.

DNA extraction and qualification

DNeasy Blood & Tissue Kit (Qiagen, Hilden, Germany) was used for the extraction of tissue samples. The DNA concentration and the size distribution of the DNA were measured using a Qubit fluorometer (Invitrogen, Carlsbad, CA, USA) and the Qubit dsDNA HS (High Sensitivity) Assay Kit (Invitrogen), and Agilent 2100 BioAnalyzer and the DNA HS kit (Agilent Technologies, Santa Clara, CA, USA), respectively. All steps of DNA extractions were performed according to the manufacturer's instructions [18].

Next-generation sequencing

The DNA got above were used to construct sequencing libraries with the KAPA DNA Library Preparation Kit (Kapa Biosystems, Wilmington, MA, USA) according to the manufacturer's protocol. The constructed libraries were hybridized to custom-designed biotinylated oligonucleotide probes (Roche NimbleGen, Madison, WI, USA) for target enrichment. The probes cover 1021 cancer-related genes (Supplementary Table 1). The captured DNA fragments were amplified and pooled to generate multiplex libraries. DNA sequencing was performed using the HiSeq 3000 Sequencing System (Illumina, San Diego, CA, USA) with 2 × 101-bp paired-end reads. Single nucleotide variants (SNVs) were called using MuTect (version 1.1.4) and NChot; small insertions and deletions (Indels) were called by GATK. Copy number variations (CNVs) were detected using Contra (2.0.8), structure variations (SVs) were detected with BreakDancer. All final candidate variants were verified with the integrative genomics viewer browser. Tumor mutational burden was defined as the number of somatic non-synonymous mutations per megabase including SNVs, insertions, and deletions of the panel region [19].

Statistical analysis

Somatic mutation data of 522 HNSCC patients in the TCGA database was downloaded from cBioPortal [20]. Chi-square test or Fisher exact test were used to assess categorical variables. Differences between two groups were examined with two-taild unpaired Mann-Whitney test. Statistical analyses were performed using Prism analysis and graphic software (GraphPad) version 8.0.1. Maftool package, an R Bioconductor package, was used to analyze genetic aberrations in different pathway [21]. A two-sided *P* value of less than 0.05 was considered statistically significant.

Table 1
Patient characteristics for the 127 head and neck patients

Characteristics	Total, N(%)	NTRK		P value*
		GA, N(%)	WT, N(%)	
Patient number	127	10	117	
Median age, years(range)	53(14–83)	46(14–76)	53(24–83)	0.442
Gender				0.914
Male	97(76.4%)	8(80.0%)	89(76.1%)	
Female	30(23.6%)	2(20.0%)	28(22.3%)	
Histology type				0.0001
HNSCC	101(79.5%)	6(60.0%)	95(81.2%)	
Adenocarcinoma	3(2.4%)	3(30.0%)	0	
Malignantpleomorphicadenoma	2(1.6%)	1(10.0%)	1(0.9%)	
Mucoepidermoid carcinoma	4(3.1%)	0	4(3.4%)	
HNMUCM	9(7.1%)	0	9(7.7%)	
Clear cell carcinoma	1(0.8%)	0	1(0.9%)	
Basal cell carcinoma	1(0.8%)	0	1(0.9%)	
Adenoid cystic carcinoma	6(4.7%)	0	6(5.1%)	
Clinical stage				0.388
I/II	4(3.2%)	0	4(3.4%)	
III	11(8.7%)	1(10.0%)	10(8.5%)	
IV	53(41.7%)	6(60.0%)	47(40.2%)	
NA	59(46.4%)	3(30.0%)	56(47.9%)	
Previous treatment				0.559
No	40(31.5%)	2(20.0%)	38(32.5%)	
Yes	80(63.0%)	8(80.0%)	72(61.5%)	
NA	7(5.5%)	0	7(6.0%)	

GA: genetic aberration; WT: wild type; HNSCC: Head and neck squamous cell carcinoma; HNMUCM: Head and neck mucosal melanoma; NA: not available

* P values were calculated by T-test, Chi-square test or Fisher's exact test.

Table 2
Genetic aberrations of *NTRK* in 10 patients with head and neck cancer

Sample index	Age	Gender	Histology type	Stage	Gene	Type of NTRK genetic aberration	Nucleotide	Amino acid
190023521	NA	Male	HNSCC	□	NTRK1	MISSENSE	c.2008G > T	p.G670C
190021869	55	Male	HNSCC	□	NTRK1	MISSENSE	c.1030G > A	p.G344R
180021091	23	Male	HNSCC	□	NTRK1	MISSENSE	c.1888G > A	p.V630M
180010277	78	Female	Adenocarcinoma	III	NTRK1	MISSENSE	c.2239G > C	p.E747Q
180008022	54	Male	HNSCC	IV	NTRK3	MISSENSE	c.842C > T	p.T281I
190000065	65	Male	HNSCC	□	NTRK1	CNV	/	/
190017115	77	Male	Adenocarcinoma	IV	NTRK3	AGBL1-NTRK3 fusion	EX22:EX3	AGBL1(PMT..IVS22)_NTRK3(IVS3..PMT
190000716	62	Female	HNSCC	IV	NTRK3	ETV6-NTRK3 fusion	EX5:EX15	ETV6(PMT..IVS5)_NTRK3(IVS14..END)
180004283	27	Male	Malignantpleomorphicadenoma	IV	NTRK3	ETV6-NTRK3 fusion	EX5:EX15	ETV6(PMT..IVS5)_NTRK3(IVS14..END)
180004284	31	Male	Adenocarcinoma	IV	NTRK3	ETV6-NTRK3 fusion	EX5:EX15	ETV6(PMT..IVS5)_NTRK3(IVS14..END)

NA: not available; CNV: copy number variation; HNSCC: Head and neck squamous cell carcinoma

Results

Analysis of genetic aberrations in Chinese Head and Neck cancers

The clinical characteristics of all the patients were shown in Table 1. The median age of diagnosis was 53, ranged from 14 to 83 years. 97 (76.4%) patients were male and the most common histology type was HNSCC (79.5%). NGS of all 127 patients with sufficient tumor tissue and peripheral blood control samples was done. As shown in Fig. 1a, the most frequently altered genes were *TP53* (43.3%), *CDKN2A* (18.9%), *MLL2* (13.4%), *LRP1B* (11.0%) and *TERT* (11.0%). Pathway analysis was done by maftool package with all the mutant genes. Most of the mutated genes clustered in RTK-RAF pathway, followed the by cell cycle and Notch pathways (Fig. 2a). Genetic aberrations (GAs) in RTK-RAF pathway were shown in Fig. 2b. Together with *NTRK1* and *NTRK3*, the *NTRK* genes were the most aberrated ones, followed by *KRAS, MET* and *EGFR*. Co-existence of mutations detected in 127 patients was also analyzed. Except for *CDKN2A* and *CDKN2B*, there was no significant coexistence between the other mutations (Supplementary Table 2). We also consulted the TCGA database and retrieved genetic aberration information of 522 HNSCC patients for analysis [22–27]. The top 5 altered genes were *TP53* (68.4%), *CDKN2A* (50.4%), *PIK3CA* (27.8%), *FAT1 (27.6%)* and *LRP1B* (27.6%) (Supplementary Fig. 1). The top altered genes were compared (Supplementary Fig. 2). *TP53* and *CDKN2A* were the common altered gene in both TCGA database and our cohort. The mutations in TCGA database for *NTRK1/2/3* genes were 2.87%, 2.11% and 1.15%, respectively. However, only 2 gene fusions were detected (0.38%).

Incidence rate of NTRK genetic aberrations in Chinese Head and Neck cancers and Molecular characteristics

A total of 10 genetic aberrations of *NTRK* genes were identified. The *NTRK* alterations included four (3.1%) *NTRK3* fusions, four *NTRK1* missense mutation, one *NTRK1* copy number variation (CNV) and one *NTRK3* missense mutation (Table 2). The most common fusions were *ETV6-NTRK3* (n = 3, 2.4%). No fusion of *NTRK1* or *NTRK2* was detected. For the *NTRK* genetic aberration (NTRK-GA) group, besides *NTRK*, *TP53* (50%) was still the most common altered gene, along with *APC* (30%), *CCND1* (20%), *LRP1B* (10%) (Fig. 1b). No co-existence of mutations was detected in the NTRK-GA group (Supplementary Table 3). Clinical parameters of our cohort between NTRK-GA group and wild type (NTRK-wt) group were similar (Table 1), except for pathological subtype. Then, top 10 frequently altered genes of NTRK-GA group and NTRK-wt group were compared and shown in Fig. 3a. *TP53* and *NOTCH1* gene were highly altered in both groups. Except for *NTRK1* and *NTRK3*, the alteration frequencies of *APC* and *PTPRD* were significantly higher in the NTRK-GA group compared to the NTRK-wt group (Fig. 3).

Recently, immune checkpoint inhibitors such as pembrolizumab and nivolumab are approved for the NHNSCC patients [28]. TMB is considered as an important biomarker for immunotherapy. The association between *NTRK* mutation and TMB was carried out. Median TMB was 0.5 mutations per megabase (mut/MB) in the NTRK-fusion group, significantly lower compared to 3.0 mut/MB in the NTRK-wt group ($p = 0.034$) (Fig. 4a). On the contrary, the median TMB was much higher in NTRK-mutation group (11.1 mut/MB) compared to that in the NTRK-wt group (3.0 mut/MB, $p = 0.032$) (Fig. 4b).

Other potential biomarker in Head and Neck cancers

The association between top 10 frequently altered genes of NTRK-GA group and TMB was also carried out. *TP53* mutation was significantly associated with higher TMB ($p < 0.0001$, Fig. 4c). Recent studies have indicated an association between *LRP1B* mutation and TMB in both melanoma and NSCLC patients [29, 30]. In the present study, we also found that *LRP1B* mutation was associated with higher TMB. The median TMB of LRP1B-mut group was 10.0 mut/MB, significantly higher than that in the LRP1B-wt group (3.0 mut/MB, $p = 0.0009$, Fig. 4d). Then, double mutations of *TP53* and *LRP1B* was observed in some patients. The significantly higher TMB was found in double-mut group than that in the double-wt group (11.00 vs 2.88 mut/MB, $p < 0.0001$, Fig. 4e).

Case with ETV6-NTRK3 fusion

During the present study, a 27-year-old male patient was diagnosed as lung metastasis of parotid carcinoma by wedge resection of right lower lung in our hospital on Mar, 2016. NGS detected *ETV6-NTRK3* fusion. Five months after 6 cycles of chemotherapy, the disease progressed because of increased lung metastasis. Larotrectinib has not been approved by FDA and not available at that time. Fortunately, crizotinib was reported to have inhibitory effect on *NTRK* fusions [31]. Crizotinib was administered on Aug, 2017. Then the patient underwent regular computed tomography (CT) examination in outpatient. The latest CT on Sep, 2019 showed that some lesions in the lung were shrunk, but one of them enlarged with major diameter from 1.4 cm to 1.8 cm, without metastasis to other sites (Fig. 5). Then he received third line chemotherapy. In brief, the patient harboring *ETV6-NTRK3* fusion reached a PFS of nearly two years.

Discussion

Previously studies have reported that the *NTRK* fusion were most prevalent in some rare cancers, and they occurred in a very small proportion of common cancer types [32]. However, the incidence of *NTRK* fusion in head and neck cancers varies greatly with pathological types. Up to 90% of patients with MASC, a subset of salivary cancer, harbor NTRK rearrangement. Whereas the frequency of thyroid cancer was about 2.34%~6% [11, 32]. Therefore, we analyzed a relatively large sample of Chinese head and neck cancer patients with NGS sequencing. We demonstrate that this group of head and neck cancer was characterized by heterogeneous genotype, which offered potential targeted therapy for patients with different genotypes.

To the best of our knowledge, the present study was the first to provide an overview of genetic aberrations in a relatively large cohort of Chinese head and neck cancer patients. Firstly, the genomic profiling of Chinese head and neck cancer was analyzed. The common genetic aberrations of 1021 genes were identified, which were also compared with TCGA database. Then, we did the pathway analysis of genetic aberrations. The RTK-RAS pathway was mostly affected by genetic aberrations including *EGFR, MET, NTRK*, which may provide potential therapeutic targets for patients with such driver genes.

In view of the long-term response of the patient with *ETV6-NTRK3* fusion to crizotinib, *NTRK* genomic alterations were analyzed in our cohort. *NTRK* fusions were observed in 3.1% of Chinese head and neck cancers. The common fusion gene was *ETV6-NTRK3*. Additional genomic alterations of *NTRK* occur in 4.7% of samples. Whereas according to the TCGA database, the frequency of *NTRK* fusion in HNSCC was only 0.38%. This suggests that the frequency of *NTRK* fusion may vary from races, just like the *EGFR* mutation.

NTRK1/2/3 fusions are the most common mechanisms of oncogenic TRK activation [10]. Upon neurotrophic binding, the TRK fusion products can active downstream pathways the same as the full-length TRK proteins [33–35]. *ETV6-NTRK3* was first discovered in congenital fibrosarcoma tumors by Sorensen et al. in 1998 [36]. Although this *NTRK3* rearrangement results in a chimeric protein lacks the SHC binding site of TrkC, it leads to the same major signaling cascades activation as the full-length TrkC: the PI3K and MAPK pathways [37]. Genetically engineered mouse models of *ETV6-NTRK3* and *BCAN-NTRK1* fusions have found that the presence of these fusion genes triggers carcinogenesis that could be sensitive to TRK inhibitors [38, 39].

Targeting fusions has shown marked anti-tumor activity. Examples include imatinib for *BCR-ABL* fusion chronic myeloid leukemia, crizotinib and alectinib for *EML4-ALK* fusion NSCLC. Of importance, 32 molecules have demonstrated inhibitory activity against *NTRK* fusions [11]. Five of these small inhibitors are originally approved by the FDA for other indications, including crizotinib. The case in our study was administered crizotinib and had achieved a PFS of nearly two years. Larotrectinib is a TRK-selective inhibitor and has been explored in three clinical trials for cancer patients harboring *NTRK* fusions. Response rate achieved 76%, regardless of tumor origin, *NTRK* fusion type or upstream partner [13]. It has been approved by the FDA for *NTRK*-positive cancer recently. Entrectinib is another first-generation *NTRK* inhibitor and has been approved by the FDA on Aug, 2019. The *ETV6-NTRK3* rearrangement has been proved to be both sensitivity to larotrectinib and entrectinib [17]. Other fusion forms such as *CTRC-NTRK1*, *SQSTM1-NTRK1* and *LMNA-NTRK1* are also sensitive to larotrectinib, entrectinib or both of them [11, 16, 40]. However, new fusion genes have been identifying and their sensitivity to *NTRK* inhibitors is not currently known.

Several point mutations, especially NTRK kinase domain mutations, have been reported to be associated with larotrectinib or entrectinib resistance [7, 11]. Second-generation *NTRK* inhibitor LOXO-195 was designed to overcome secondary resistance and has shown promising preliminary clinical activity [41]. The present study detected 5 missense mutations which has never been reported, and 3 of them located in the kinase domain (Fig. 6). Furthermore, compared with NTRK-wt group, *APC* and *PTPRD* mutations were more common in NTRK-GA group. *APC* and *PTPRD* have been reported to associated with inferior outcome of HNSCC [42, 43]. The potential interactions between these two genes and *NTRK* aberrations need further study.

With the success of immunotherapy in many common cancers, it may also be a treatment strategy in head and neck patients. We also found that *NTRK*-fusion group had a significantly lower TMB compared with NTRK-wt group. This observation was consist with a previous report [11]. It seems that tumors harboring driver-gene mutations or fusions tend to have a lower number of mutation burden [25]. As mentioned above, point mutations are considered associated with resistance to *NTRK* inhibitors. Interestingly, TMB was significantly higher in the NTRK-mutation group. Therefore, immunotherapy can be considered for patients after failure to *NTRK* inhibitors. Moreover, the association between TMB and top 10 frequently altered genes of NTRK-GA group were analyzed. *TP53* and *LRP1B* mutations were associated with higher TMB, which was consistent with that in melanoma and NSCLC. So, for head and neck patients without driver genes, *TP53/LRP1B* may be another biomarker for immunotherapy, especially those harboring double mutations.

Our observations have meaningful implications for future clinical trial settings. Genomic profiling helped better understanding and treating head and neck tumors. First, the analysis of *NTRK* fusion identified that about 3% of Chinese head and neck patients may benefit from targeted therapy of *NTRK* inhibitors. Second, our study also provides potential markers for immunotherapy. Several limitations were in the present study. First, our sample was mainly HNSCC. Thyroid cancer and some rare pathological types of head and neck cancers had not been recruited. Second, the possibility of sample size bias cannot be excluded. Thirdly, our study was limited to retrospective analysis and some of the results could not be verified. Despite these limitations, the current study provides a genomic landscape of *NTRK* alterations among head and neck cancer in a Chinese population.

Conclusions

In summary, the present study evaluated a large retrospective cohort to investigate the genomic profiling of Chinese head and neck cancer patients using NGS for the first time. *NTRK* genetic aberrations provided potential therapeutic strategies for some patients. It would be of interest to explore the function of all the *NTRK* genetic aberrations, not only gene fusion. Next generation of *NTRK* inhibitors should be rational designed to any of common *NTRK* alterations. Furthermore, immunotherapy is a possible therapeutic strategy for patients with mutations in *TP53/LRP1B.*

Supplementary Files List

Additional file 1: Supplementary Fig. 1. Landscape of genetic alternations in 522 HNSCC from TCGA database. Genetic aberration frequencies of top 60 genes were showed. Top, the mutation numbers of each sample. Right, the mutation percentage of each gene in the total group.

Additional file 2: Supplementary Fig. 2. Comparation of frequently altered genes between TCGA group and our cohort. Statistical analysis is performed using the Fisher's exact test. *p < 0.05; ** p < 0.01; ***p < 0.001.

Additional file 3: Supplementary Table 1. List of target regions of the pan-cancer 1021-gene panel.

Additional file 4: Supplementary Table 2. The co-occurrence of mutations in 127 patients.

Additional file 5: Supplementary Table 3. The co-occurrence of mutations in 10 patients harboring *NTRK* genetic aberrations.

Abbreviations

NGS: next-generation sequencing; PFS: progression-free survival; OS: overall survival; HNSCC: head and neck squamous cell carcinoma; TMB: tumor mutation burden; NTRK: neurotrophic-tropomyosin receptor tyrosine kinase; MSCC: mammary-analog secretory carcinoma of the salivary gland; NSCLC: non-small cell lung cancer; FDA: the US Food and Drug Administration; FFPE: formalin-fixed, paraffin-embedded; SNV: single nucleotide variant; CNV: copy number variation; SV: structure variation; GA: genetic aberration; WT: wild type; mut/MB: mutations per megabase; CT: computed tomography

Declarations

Acknowledgements

Not applicable.

Authors' contributions

Jiali Xu and Lianke Liu made substantial contributions to the design of the study. Jiali Xu and Rong Wang contributed to the literature review, manuscript preparation and editing. Tongshan Wang and Tingting Wang made contributions to the data collection and manuscript editing. Tingting Wang, Dejian Gu and Yuange He performed the bioinformatics analysis. Yongqian Shu and Rongrong Chen made contributions to the manuscript review. Rongrong Chen and Lianke Liu are responsible for the quality of the overall manuscript. All authors read and approved the final manuscript.

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Availability of data and materials

All data presented or analyzed in this study are included either in this article or in the additional files

Ethics approval and consent to participate

This study was approved by the institutional review board of Nanjing Medical University. The data released from TCGA database did not require informed patient consent because cancer is a reportable disease in the US.

Consent for publication

All authors have consented to publication of the results presented in this manuscript.

Competing interests

Dejian Gu, Yuange He and Rongrong Chen are employees of Geneplus-Beijing Ltd. All other authors declared no conflict of interest.

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Figures

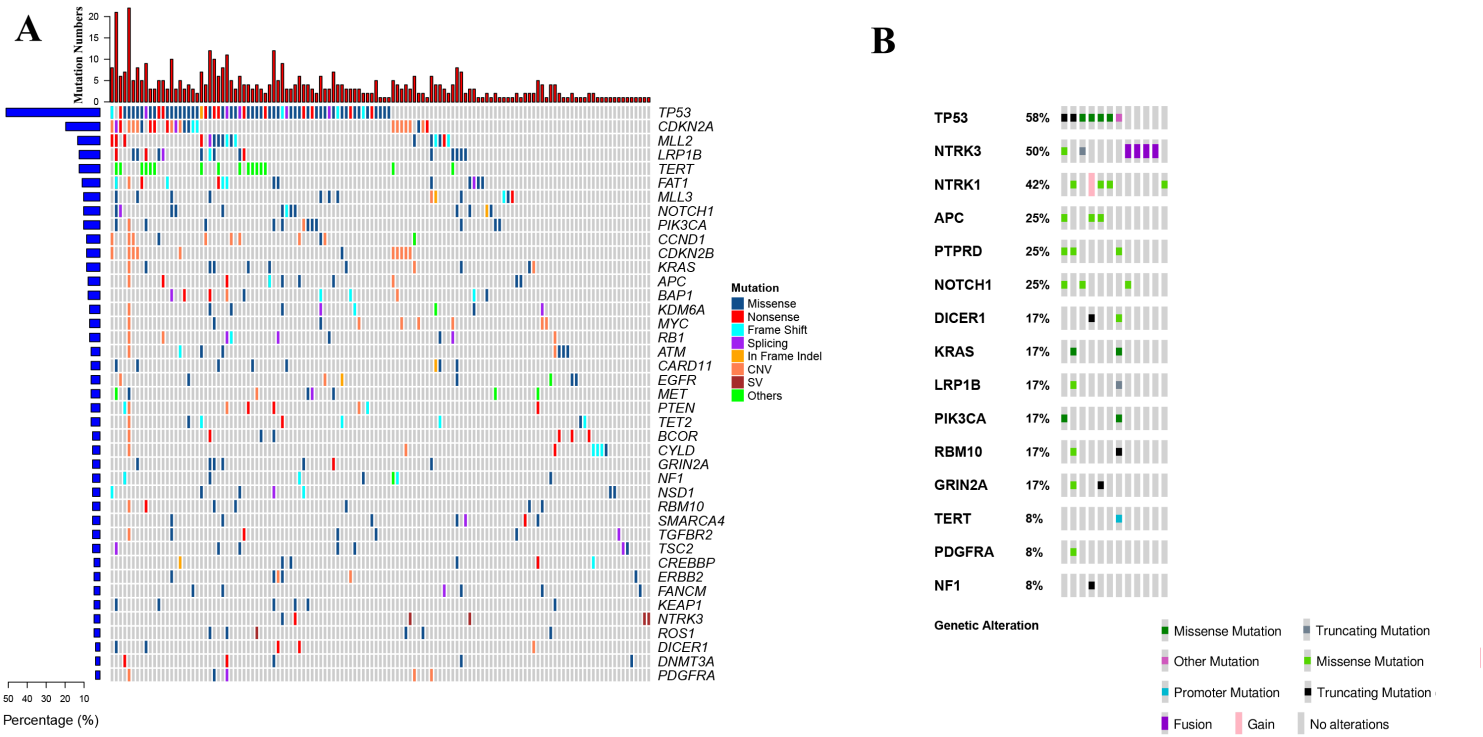

Figure 1

Genetic aberrations of head and neck cancers in a Chinese cohort. (a) Landscape of genetic alternations in 127 Chinese head and neck patients. Genes that have undergone genetic aberration in at least 5 samples. Top, the mutation numbers of each sample. Left, the mutation percentage of each gene in the total group. (b) Top ten genes in the NTRK-GA group.

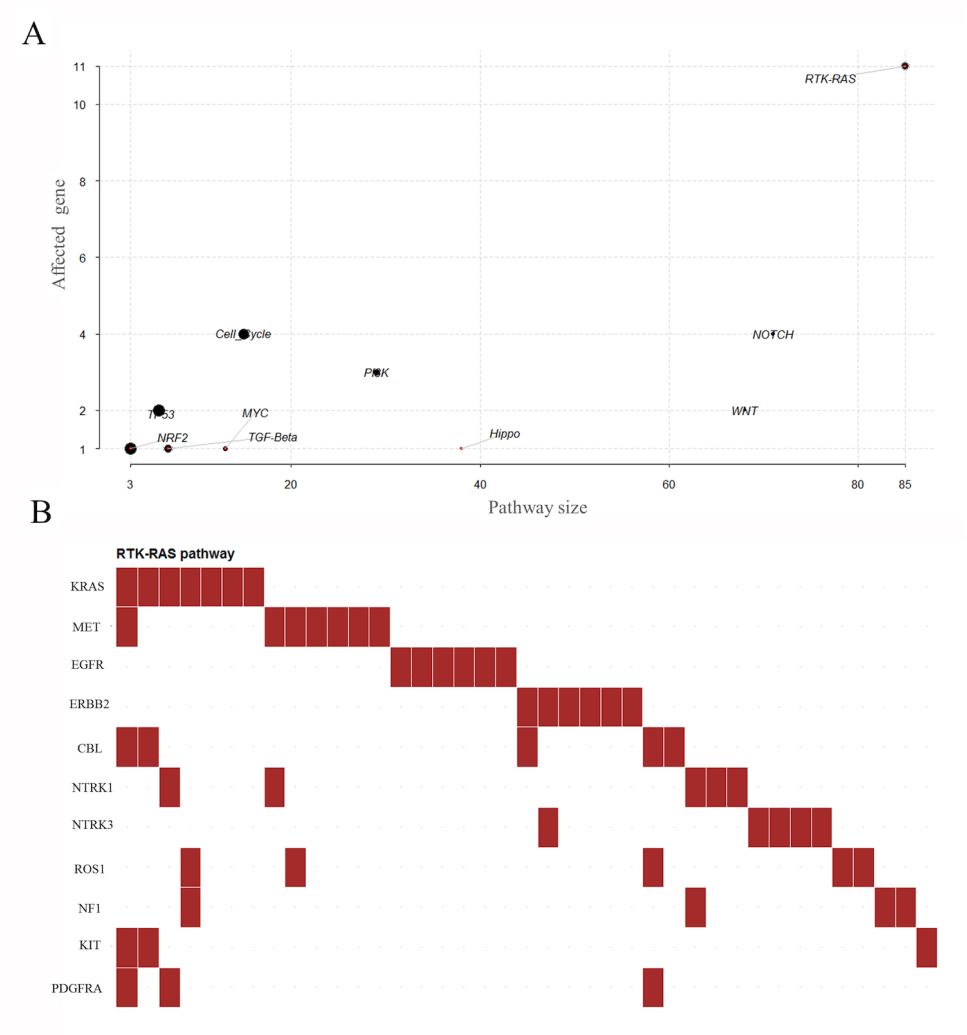

Figure 2

Genetic aberrations in the pathway. (a) Numbers of affected gene in difference pathway. (b) Genetic aberrations in RTK-RAF pathway.

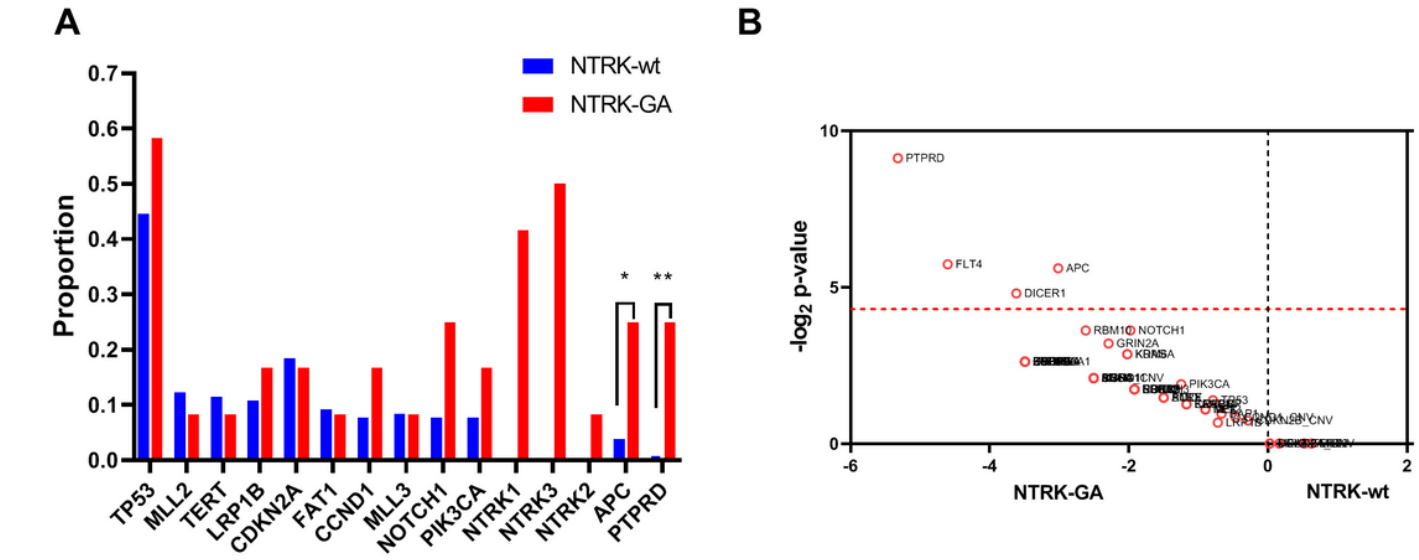

Figure 3

Comparation of frequently altered genes of NTRK-GA group and NTRK-wt group. (a) Top 10 frequently altered genes of NTRK-GA group and NTRK-wt group. P values for APC and PTPRD are 0.010 and 0.016, respectively. (b) The alteration frequencies of APC and PTPRD gene mutation are significantly higher in the NTRK-GA group. Statistical analysis is performed using the Fisher's exact test. *p < 0.05; ** p <0.01.

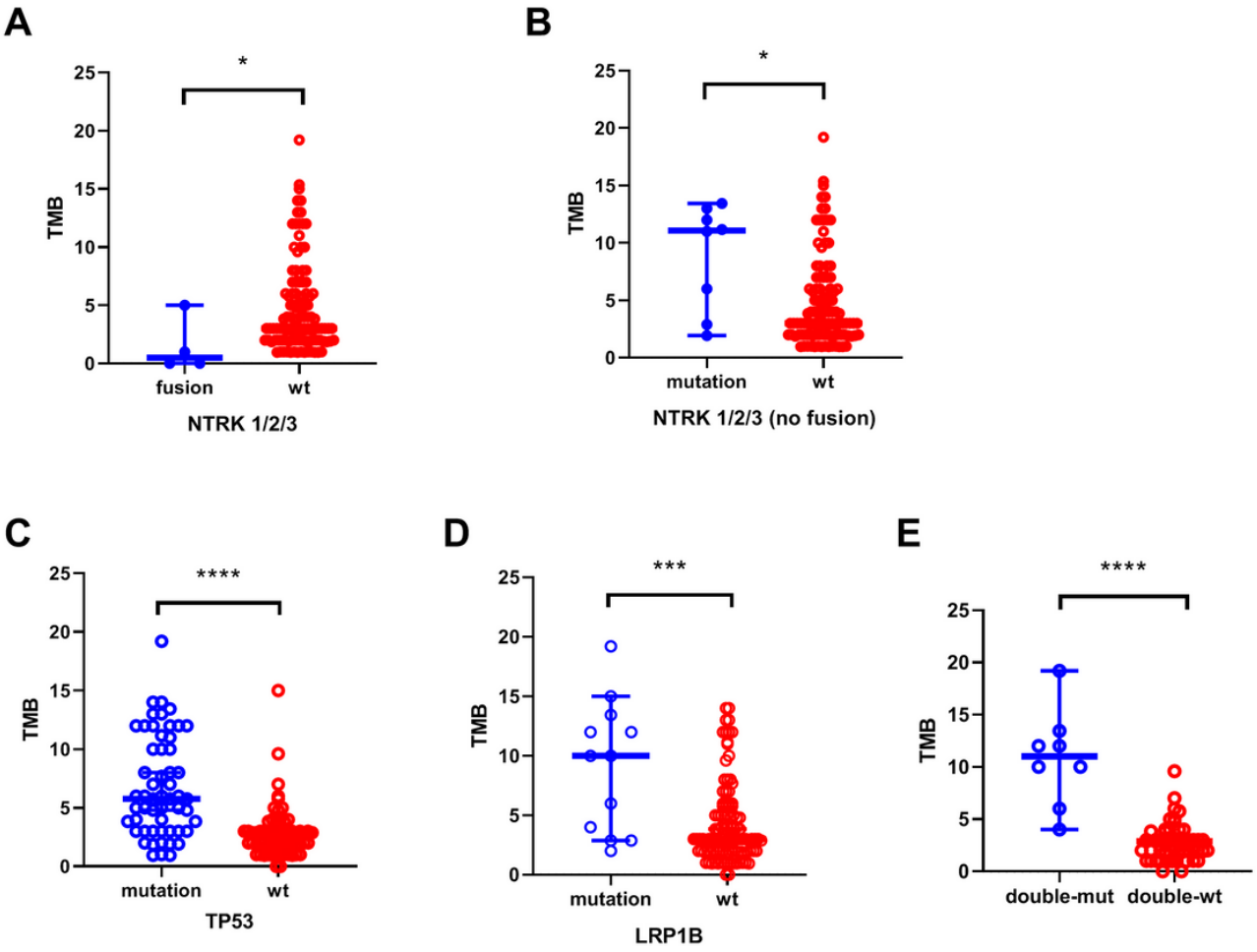

Figure 4

Comparation of TMB in different groups. Differences of TMB between NTRK-wt group and NTRK-fusion (a) or NTRK-mutation group (b). Differences of TMB according to TP53 (c), LRP1B (d) mutation status, and double mutation (both TP53 and LRP1B mutated) (e). P values for a, b, c, d, e are 0.034, 0.032, <0.001, <0.001 and <0.001, respectively. The median and standard deviation are indicated by the thick horizontal line. Statistical analysis is performed using the Mann-Whitney test. *p < 0.05; ** p <0.01; ***p <0.001; **** p<0.0001.

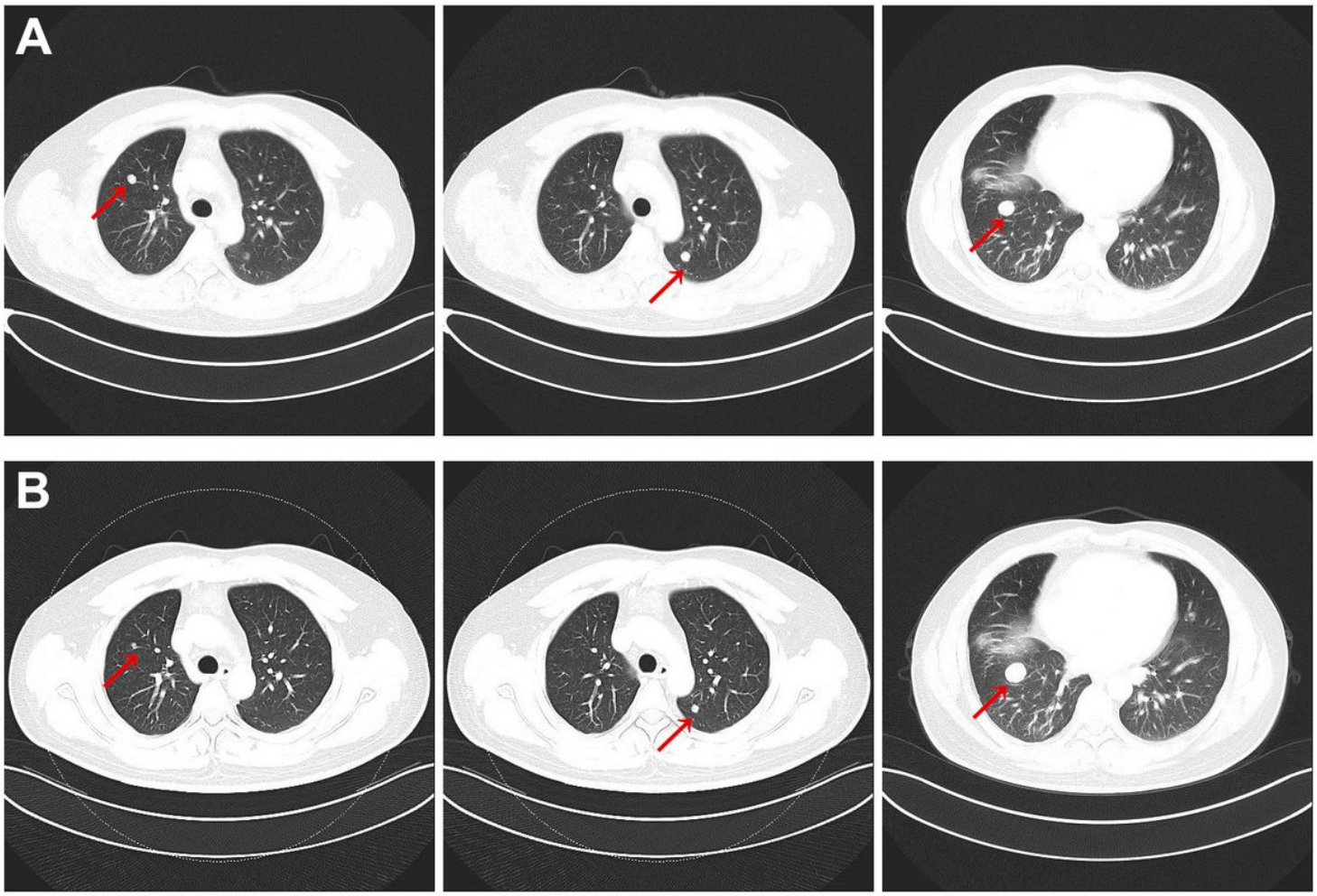

Figure 5

Imaging changes of lung metastatic lesions of the patient. (a) Before crizotinib treatment (Jul, 2017). (b) Two years after crizotinib treatment (Sep, 2019).

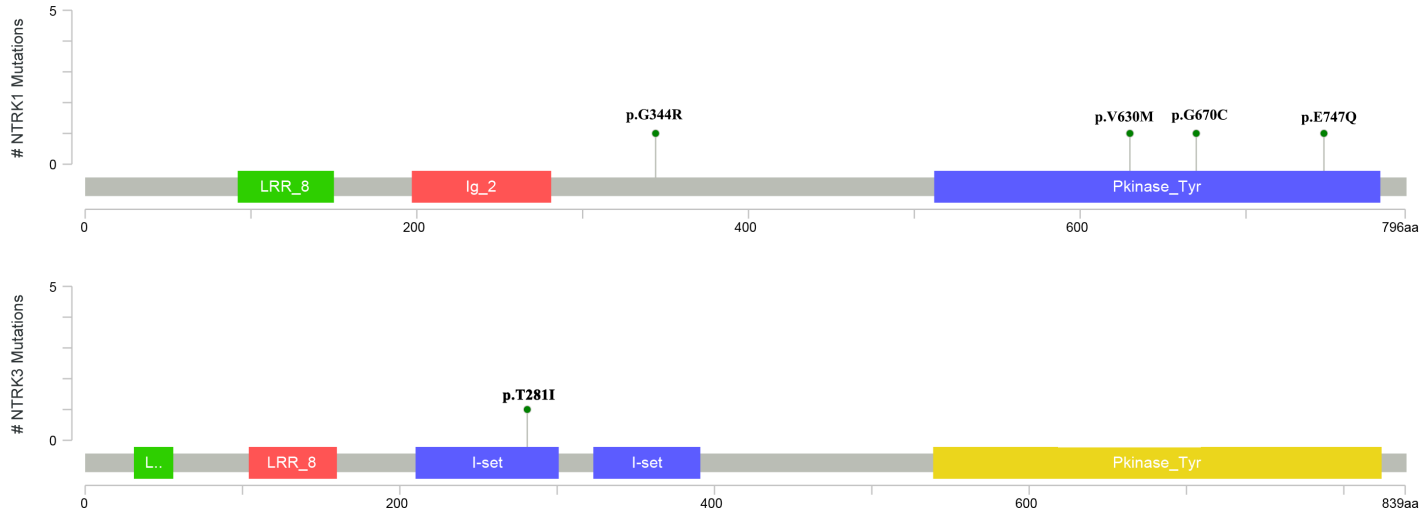

Figure 6

Schematic representation of 5 missense mutations detected in NTRK1 gene and NTRK3 gene.

Supplementary Files

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