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

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Jiali Xu, Rong Wang, Tongshan Wang, Tingting Wang, Yongqian Shu, Dejian Gu, Yuange He, Rongrong Chen, Lianke Liu

Jiali Xu
The First Affiliated Hospital of Nanjing Medical University

Rong Wang
The First Affiliated Hospital of Nanjing Medical University

Tongshan Wang
The First Affiliated Hospital of Nanjing Medical University

Tingting Wang
The First Affiliated Hospital of Nanjing Medical University

Yongqian Shu
The First Affiliated Hospital of Nanjing Medical University

Dejian Gu
Geneplus-Beijing Ltd.

Yuange He
Geneplus-Beijing Ltd.

Rongrong Chen
Geneplus-Beijing Ltd.

Lianke Liu
The First Affiliated Hospital of Nanjing Medical University

Corresponding Author
liulk_oncology@sina.com
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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 occur 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-tailed 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.

| 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          |     |
| Malignantpleomorphica   | 2(1.6%)     | 1(10.0%) | 1(0.9%)   |     |
| Genotype            | Cases | Controls | P Value |
|---------------------|-------|----------|---------|
| Mucoepidermoid carcinoma | 4(3.1%) | 0 | 0.388 |
| HNMC | 9(7.1%) | 0 | 0.388 |
| Clear cell carcinoma | 1(0.8%) | 0 | 0.388 |
| Basal cell carcinoma | 1(0.8%) | 0 | 0.388 |
| Adenoid cystic carcinoma | 6(4.7%) | 0 | 0.388 |
| Clinical stage       |       |          |         |
| I/II                | 4(3.2%) | 0 | 0.388 |
| III                 | 11(8.7%) | 1(10.0%) | 0.388 |
| IV                  | 53(41.7%) | 6(60.0%) | 0.388 |
| NA                  | 59(46.4%) | 3(30.0%) | 0.388 |
| Previous treatment   |       |          |         |
| No                  | 40(31.5%) | 2(20.0%) | 0.559 |
| Yes                 | 80(63.0%) | 8(80.0%) | 0.559 |
| NA                  | 7(5.5%) | 0 | 0.559 |

GA: genetic aberration; WT: wild type; HNSCC: Head and neck squamous cell carcinoma; HNMC: 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          | IV    | NTRK1| MISSENSE                        | c.2008G > T| p.G670C    |
| 190021869    | 55  | Male   | HNSCC          | IV    | NTRK1| MISSENSE                        | c.1030G > A| p.G344R    |
| 180021091    | 23  | Male   | HNSCC          | IV    | 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          | IV    | 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   | Malignant pleomorphic adenoma | 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%), LRPIB (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 alterations 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.** Comparison 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; MCC: 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.

Author details

1 Department of Oncology, The First Affiliated Hospital of Nanjing medical university, 300 Guangzhou Road, Nanjing 210029, China

2 First clinical medical college, Nanjing Medical University, 818 Tianyuan East Road, Nanjing 210029, China

3 Geneplus-Beijing Ltd., Medical Park Road, Zhongguancun Life Science Park, Beijing 102206, China

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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.
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Figure 2

Genetic aberrations in the pathway. (a) Numbers of affected gene in difference pathway. (b) Genetic aberrations in RTK-RAF pathway.
Figure 2

Genetic aberrations in the pathway. (a) Numbers of affected gene in difference pathway. (b) Genetic aberrations in RTK-RAF pathway.
Figure 3

Comparison 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.
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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 4

Comparison 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).
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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.
Figure 6

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

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