



Research paper

TP53 mutational landscape of metastatic head and neck cancer reveals patterns of mutation selection

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ABSTRACT

Background: Head and neck squamous cell carcinoma is a heterogeneous disease with respect to the anatomic site of the primary tumor. On the other hand, it is highly recurrent, and once metastatic, it is associated with poor prognosis. TP53 is the most commonly mutated gene in primary disease. TP53 mutations occur in different structural elements of the protein while the biological outcome can be diverse.

Methods: Here we aimed to find differences in the mutation profile of TP53 in primary and metastatic disease and the impact of TP53 mutations in metastasis, specific copy number alterations, tumor mutation burden and response to immune checkpoint inhibitors. Somatic mutation and clinical data for 512 primary and 134 metastatic biopsies were studied.

Findings: Overall TP53 mutation frequency is significantly lower in metastases compared to primary tumors. One the other hand, missense mutations in the DNA binding region are significantly enriched in metastases and are associated with a common fragile site in chromosome 11, leading to amplification and overexpression of genes with established role in metastasis. Finally, TP53 mutations are associated with higher TMB score in metastatic but not primary tumors, and poorer response to immune checkpoint inhibitors for the latter.

Interpretation: TP53 mutations affect clinical and molecular aspects of head and neck tumorigenesis including metastasis, genetic alterations and therapeutic response.

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1. Introduction

Head and neck squamous cell carcinoma (HNSCC) is a heterogeneous disease comprising neoplasias of the upper aerodigestive track, arising primarily in the oral cavity and the lips, the larynx and the pharynx. While early stage disease is efficiently treated with surgery or radiotherapy, a substantial fraction of patients develops distant metastases. Metastatic disease carries a dismal prognosis with a median overall survival of <1 year [1]. More recently, pembrolizumab and nivolumab, which are monoclonal antibodies against programmed death-1 (PD-1), were approved for the treatment of patients with recurrent/metastatic HNSCC with disease progression during or after a platinum-based therapy. Moreover, pembrolizumab was approved for use as first line treatment in combination with platinum and fluorouracil (FU) for all patients and as a single agent for

patients whose tumors express PD-L1 (Combined Positive Score [CPS] ≥ 1) as determined by a FDA-approved test [2].

During carcinogenesis, acquisition of mutations leads to the expression of potentially antigenic epitopes that can be recognized by the immune system. Tumor heterogeneity and clonal selection during metastasis influence in a highly dynamic manner the tumor neoantigen (TNA) evolution over space and time. High tumor mutation burden (TMB) or TNAs is associated with associated with better response to immune checkpoint blockade inhibitors [3]. Therefore, a better understanding of the mutational and TNA landscape in HNSCC metastasis is highly valuable to improve immune anticancer therapy in late-stage cancers.

In recent years, large-scale whole-genome sequencing (WGS) analysis efforts have focused on primary tumors. Despite the poor prognosis, metastatic cancers have been less comprehensively studied at the genome level, as biopsy tissue from metastasis is rarely available. As a result, the evolutionary dynamics of metastasis remain poorly understood. Mutations in the tumor suppressor protein TP53 is the most common genetic alteration in HNSCC and is found in approximately 70% of all cases, albeit with variable frequency among

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Research in Context

Evidence before the study

HNSCC is a highly recurrent metastasizing solid cancer with poor prognosis. The lack of tumor material has limited our knowledge about the molecular profile of metastases and the identification of biomarkers of prognostic and predictive value. TP53 is the most highly mutated gene in HNSCC, its mutation spectrum however in metastases and its predictive value in immunotherapy is not fully studied.

Added value of this study

In this study we uncover novel possible roles of TP53 mutations in metastasis and therapeutic response in HNSCC. We found that: i) the overall frequency of TP53 mutations is significantly lower in metastatic disease in comparison to primary tumors; ii) missense mutations in the DNA-binding region are significantly enriched in metastases; iii) missense mutations in the DNA binding region are associated with a common fragile site in chromosome 11, leading to amplification and overexpression of known metastasis drivers; iv) liver and lung metastases with TP53 mutations are associated with higher TMB score and poor response to therapy with immune checkpoint inhibitors.

Implications of all the available evidence

This study reveals a distinct mutation profile in TP53 in metastatic HNSCC. Missense mutations in the DNA binding region are enriched in metastatic lesions while the mutated TP53 has potentially predictive value in immunotherapy.

different head and neck regions [4]. TP53 is a DNA damage checkpoint protein preventing the accumulation of oncogenic mutations and genomic instability. Thus, the selection of TP53 inactivating mutations in head and neck epithelium in early stages of carcinogenesis enables pre-malignant or tumor cells to tolerate oncogene-induced DNA damage and replication stress, escaping apoptosis and senescence. TP53 is also known to promote epithelial-to-mesenchyme transition (EMT) and metastasis [5].

TP53 mutations occur mainly in the DNA-binding domain (DBD, amino acids 120–292), and are mostly missense mutations that cluster at several hotspot amino acid residues leading to loss of tumor suppressive activity. In some cases, specific missense mutations exhibit dominant-negative or gain-of-function (GOF) activity [6]. The DNA binding surface of the protein is formed by two loops (L2 and L3) which are stabilized by a third loop (L1) and a Zinc atom. Missense mutations in this region either affect directly DNA binding residues or lead to conformational defects abolishing DNA binding. In this study, we use published datasets from metastatic HNSCC to uncover selective forces that shape the mutational spectrum of TP53 in HNSCC metastasis, as well as to investigate whether TP53 mutational status in metastatic disease correlates with TMB and predicts therapeutic response to immune checkpoint inhibitors.

2. Methods

2.1. HNSCC TCGA data

All meta-analyses performed in this manuscript used data generated by The Cancer Genome Atlas Research (TCGA) Network and retrieved from cBioPortal (<http://cbioportal.org>). Somatic mutation and clinical data for primary tumor samples were retrieved from the HNSCC Firehose legacy cohort that consists of 512 samples.

Mutational and clinical data from metastatic biopsies of 134 head and neck cancer patients were combined from three independent TCGA cohorts (Supplementary Table 1). More specifically, targeted sequencing and clinical data were retrieved for 31 patients from the recurrent and metastatic head and neck cancer cohort [7] (Study ID: HNC_MSKCC 2016), for 10 patients from the cohort of metastatic solid cancers [8] (Study ID: MST_MICH 2018) and for 93 patients from the TMB and immunotherapy cohort [9] (Study ID: TMB_MSKCC 2019). A flowchart of the analysis and the cohorts involved in each step are outlined in Supplementary Figure 1.

The recurrent and metastatic head and neck cancer cohort includes squamous cell carcinoma (HNSCC), adenoid cystic carcinoma (ACC), and other salivary and cutaneous cancers from 106 head and neck cancer patients with distant metastasis and from 66 patients with locoregional recurrence. Sequencing data of metastatic or recurrent tumors have been generated by exonic coverage of 410 cancer genes using the MSK-IMPACT (Memorial Sloan Kettering-Integrated Mutation Profiling of Actionable Cancer Targets) assay. Only HNSCC samples from distant metastases were selected for this study. Complete genomic and clinical data from this cohort are available in searchable form at http://www.cbioportal.org/study?id=hnc_mskcc_2016.

The cohort of metastatic solid cancers include whole exome sequencing data from 500 metastatic biopsies/primary normal pairs of 20 different cancer types. Metastatic HNSCC represent only the 2.0% of this cohort and the corresponding cases were selected for our study. Complete genomic and clinical data from this cohort are available in searchable form at https://www.cbioportal.org/study?id=metastatic_solid_tumors_mich_2017.

The TMB and immunotherapy cohort includes clinical and targeted next-generation sequencing data from 1662 cancer patients with advanced (stage IV) or metastatic disease that were treated with immune checkpoint inhibitors (ICIs). Advanced or metastatic head and neck cancer represent approximately 8.3% of this cohort while sequencing data from HNSCC metastatic biopsies were selected for this study. Complete genomic and clinical data from this cohort are available in searchable form at https://www.cbioportal.org/study?id=tmb_mskcc_2018.

Clinical and genomic data from HNSCC patients with primary advanced and metastatic disease that were treated with immune checkpoint inhibitors were retrieved exclusively from the TMB and immunotherapy TCGA cohort (Supplementary Table 2). Mutational and TMB data for 138 HNSCC primary and metastatic tumors in this cohort were generated by targeted next-generation sequencing using MSK-IMPACT 468, 410 and 310 panels. In this cohort, 130 HNSCC patients received anti-PD-1 or PD-L1 inhibitors and 8 received a combination of anti-CTLA-4 and anti-PD-1/PD-L1 therapy. TMB was calculated as the total number of somatic nonsynonymous mutations, normalized to the total number of megabases sequenced. Overall survival was measured from the date of first ICI treatment to time of death or most recent follow-up.

Our analyses relied exclusively upon patient data which are publicly available with no indication of bias in patient selection in the original studies. Although various clinicopathological data, patient features and life style information were available for both primary and metastatic cohorts, only age and gender could be directly compared. Unfortunately, information on parameters such as HPV status, smoking and drinking which could generate cohort imbalances was not available for all cohorts. Our analysis on gender and age patient data showed that there was absolutely no difference between primary and metastatic patients (Supplementary Figure 2).

2.2. TP53 structure and sequence analysis

Crystal structures of the human p53 core DNA-binding domain in complex with DNA (PDB IDs: 1TSR and 4IBV) [10,11] were retrieved from the RCSB Protein Data Bank and used to assign secondary

structure elements to TP53 DNA binding domain (DBD) sequence (UniProtKB: P04637). The structure alignment was performed using the DSSP [12] and Visual Molecular Dynamics (VMD) software [13].

2.3. Quantification and statistical analyses

Statistical differences in the frequency of missense mutations between metastatic and primary tumors for the beta-strands (S), α -helices (H) and the loops (L) of the TP53 core DNA-binding domain were determined by chi square test (χ^2). To identify co-occurring genomic alterations with specific TP53 mutation, we utilized the cBioPortal for Cancer Genomics “group comparison” suite of analysis (<https://www.cbioportal.org/>). More specifically, the group of primary HNSCC PanCancer Atlas tumors harboring missense mutations in the L1-S2 and S10-H2 TP53 regions ($N = 43$) was compared with the group of tumors from the same cohort that harbor missense TP53 mutations in the remaining part TP53 protein ($N = 140$). Statistical significance of group enrichments in copy number alterations was accompanied by p values derived from Pearson chi-squared test. Differences in TMB score between mutant and wild type TP53 tumors were examined using the non-parametric Mann-Whitney test. Kaplan-Meier survival test was used to analyze associations between TP53 mutation status and overall survival in patients treated with immunotherapy. Statistical analyses were performed using GraphPad Prism ver.7.0. A P value of less than 0.05 was considered statistically significant.

2.4. Ethics

Ethical approval was unnecessary because this work is a meta-analysis of previously published data.

2.5. Role of funders

This work was supported by a Horizon 2020 grant (801347) to AK, and a Greek General Secretariat for Research and Technology and the Hellenic Foundation for Research and Innovation (HFRI) grant (472–EpiNotch) to TR. Neither of the funding agencies had any role in study design, data collection and analysis, interpretation of the findings and writing of the manuscript.

3. Results

3.1. TP53 mutations are less frequent in metastatic HNSCC

Our metastatic cohort included 134 HNSCC patients from three independent TCGA studies, with NGS data from metastatic biopsies [7–9]. As shown in Fig. 1A, the vast majority of metastases in this cohort involves the lung and liver in consistency with previous studies [14]. To identify differences in mutation profiles between HNSCC primary and metastatic tumors, exome or targeted MSK-IMPACT sequencing data from metastatic biopsies were compared against the exome sequencing data from primary tumors of the Firehose Legacy (TCGA) cohort that includes genomic and clinical data from 512 HNSCC patients [15]. This analysis revealed that metastatic tumors exhibit a substantially lower rate of TP53 mutations (38.8%) compared to primary ones (71.5%) (Fig. 1B).

To investigate whether the cohort composition with respect to the anatomic site of the primary tumor may affect the overall frequency of TP53 mutations, we analyzed the prevalence of TP53 mutations in tumors within each anatomic site. Our analysis showed that oral, laryngeal and hypopharyngeal carcinomas are characterized by a high frequency of TP53 mutations, while oropharyngeal carcinoma displays a low prevalence of TP53 mutations in the Firehose Legacy cohort (Fig. 1C). Notably, oropharyngeal carcinoma represents only 15% of primary tumors within this cohort. The identified association

of oropharyngeal carcinoma with wild type TP53 status in primary tumors is consistent with previous studies showing that oropharyngeal and nasopharyngeal squamous cell carcinomas are frequently driven by high-risk HPV and EBV infections, while harboring lower mutational load and wild type TP53 [16,17]. On the other hand, they represent distinct types of aggressive head and neck cancers with early age onset and higher tendency to metastasize to distant organs [14], a notion supported by our analysis which shows that a substantial percentage (36%) of metastatic cases originates from oropharyngeal and nasopharyngeal primary tumors (Fig. 1D). As expected, these metastases are primarily wild type for TP53 (Fig. 1D) affecting the overall frequency of TP53 mutations in our metastatic cohort (Fig. 1E).

3.2. Missense mutations in the DNA binding region are more common in metastatic cancers

The frequency of missense and truncating mutations in primary tumors (40.81 and 56.72%, respectively) and metastases (43.75 and 54.69%, respectively) was not affected. We thus decided to investigate whether the spectrum of missense mutations is altered in metastases. To this direction, we analyzed TP53 missense mutations in the same cohorts of primary and metastatic head and neck carcinomas with respect to TP53 secondary structure. Interestingly, in metastatic specimens we observed an enrichment in missense mutations within regions L1-S2 and S10-H2 (Fig. 2A and B). Crystallographic data indicate that the L1 loop and the H2 helix contact with DNA on a major groove while sheets S2 and S10 stabilize the DNA contact region [10] (Fig. 2C). Regions L1-S2 and S10-H2 (aa 113–127 and 272–287), comprised of amino acid residues located in opposite ends of the DBD sequence, come in proximity to interact with DNA (Fig. 2C). Approximately 43% of missense mutations in metastatic HNSCC but only 22% of respective mutations in primary carcinoma ($p < 0.05$), corresponding to 11.68% and 20.63% of the total TP53 mutations respectively ($p < 0.05$), are located therein (Supplementary Table 3). No statistical difference was observed in regions L2-H1-L2' and L3 (aa 164–194 and 237–250; Fig. 2D).

3.3. Amplification of the region 11q13.3 is associated with missense mutations in the DNA binding region

To gain further insight into the biological relevance of mutations within regions L1-S2 and S10-H2, we compared copy number variation (CNV), expression and mutation data between the group of patients carrying such mutations and those carrying nonL1-S2/S10-H2 TP53 missense mutations. For this analysis, we used the TCGA PanCancer Atlas cohort of primary HNSCC which contains a large cohort ($n = 515$) of HNSCC patients. A group of genes (*CCND1*, *ORAOV1*, *FGF19*, *FGF3*, *FGF4*, *ANO1*, *FADD*, *PPF1A1* and *CTTN*) located within chromosomal region 11q13.3 (Fig. 3A) is more frequently amplified and overexpressed in tumors with mutations in the L1-S2/S10-H2 regions ($p < 0.05$; Fig. 3B and C) in comparison to samples harboring missense mutations outside these regions. The observed amplification seems to be specific for this genomic region and not the result of general genomic instability as assessed by aneuploidy and global gene copy number alteration events (fraction genome altered; Fig. 3C). These findings imply that amplification of the 11q13.3 chromosomal region is associated with TP53 mutations located in the interface of the protein interacting with the DNA helix.

3.4. TP53 mutations are associated with increased TMB and worse survival in patients with metastatic disease

TP53 has been described as guardian of the genome because of its role in DNA damage response and cell cycle regulation [18]. Recent reports have uncovered a correlation between the TP53 mutation

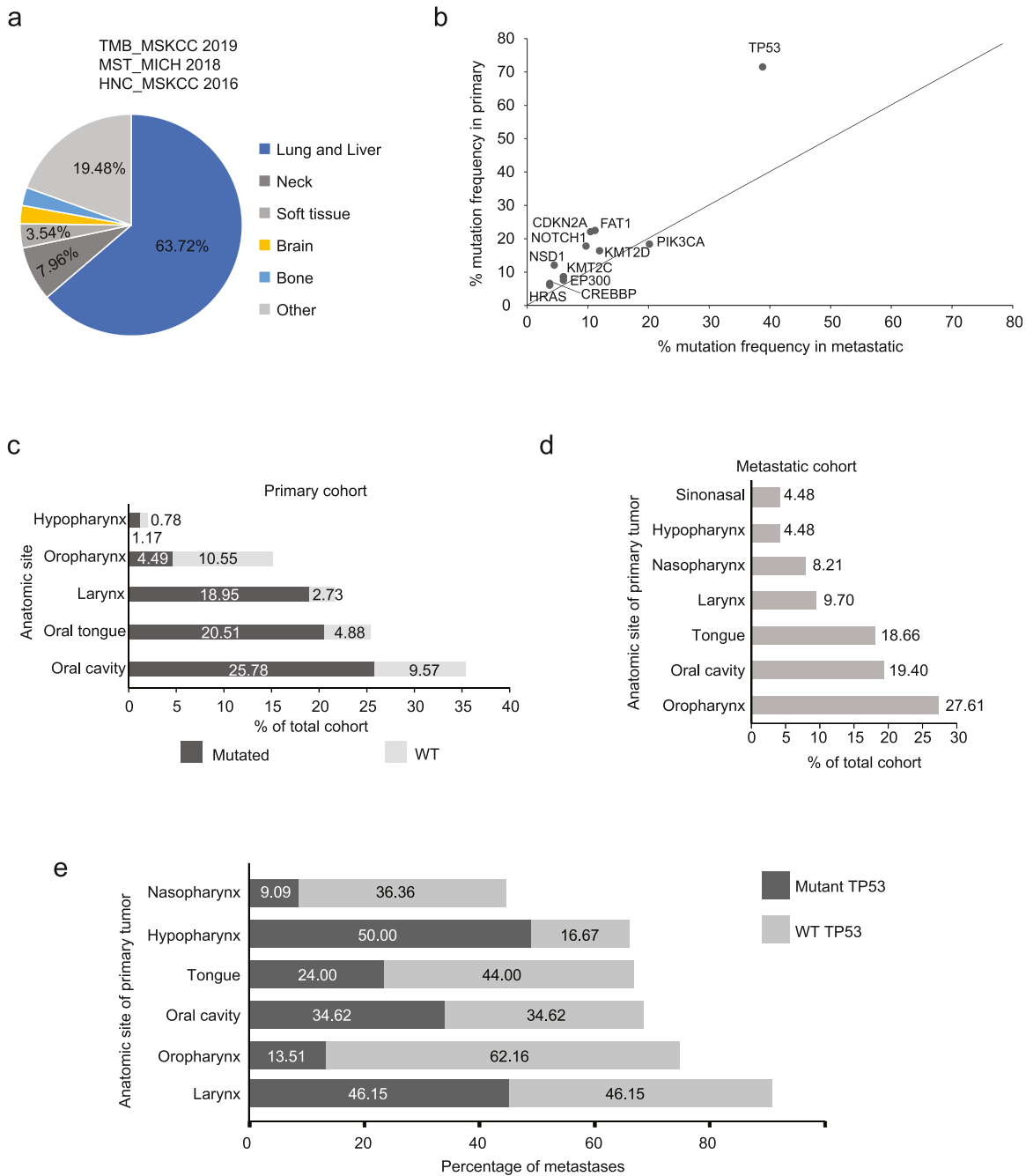


Fig. 1. HNSCC metastasizing pattern and TP53 mutation status. (a) Metastasizing pattern of HNSCC. (b) Mutation frequency of the most commonly mutated genes in primary HNSCC in comparison to metastatic disease. (c) Frequency of TP53 mutations in primary tumors ($n = 512$) from different anatomical sites. (d) Bargraph indicating the contribution of different primary tumor sites to metastatic disease. (e) TP53 mutation status in metastatic disease with respect to anatomic site of primary tumor.

status and genomic instability in cancer [19]. Tumor mutation burden (TMB) has emerged as a predictive biomarker in immunotherapy response [9]. Given the role of TP53 in DNA damage response and repair, we sought to investigate the impact of TP53 loss on TMB and therapeutic benefit from immune checkpoint inhibitors in patients with metastatic HNSCC. Because the metastasis landscape of HNSCC is quite heterogeneous while metastases to the lung and liver account for >63% (Fig. 1C), we focused our analysis on samples from lung and liver metastases from the TMB_MSKCC 2019 study [9] which is the only one assessing TMB scores and provides clinical data from immunotherapy. As Fig. 4A indicates, TP53 mutations are twice as common (64% vs. 32%) in primary sites in comparison to metastatic cancer tissue equally affecting missense and truncating mutations. Surprisingly, both the overall mutation count and the TMB score are affected

by the TP53 mutation status in metastatic but not in primary tissue. Specifically, mutation count and TMB are significantly ($p = 0.0006$ and 0.0079 , respectively) higher in TP53 mutant tumors (Fig. 4A). High TMB is associated with better response to immune checkpoint inhibitors. In this study, however, patients with metastatic disease carrying TP53 mutations respond poorly to therapy ($p = 0.0258$), despite high TMB score (Fig. 4B). TP53 status in patients with primary disease receiving the same therapy does not correlate with clinical outcome ($p = 0.462$). These data indicate that the TP53 mutation status could have predictive value in immunotherapy in patients with metastatic but to primary disease.

To investigate whether our observations are relevant for other solid malignancies, we analyzed publically available sequencing data from metastatic cohorts from six more tumor types. As

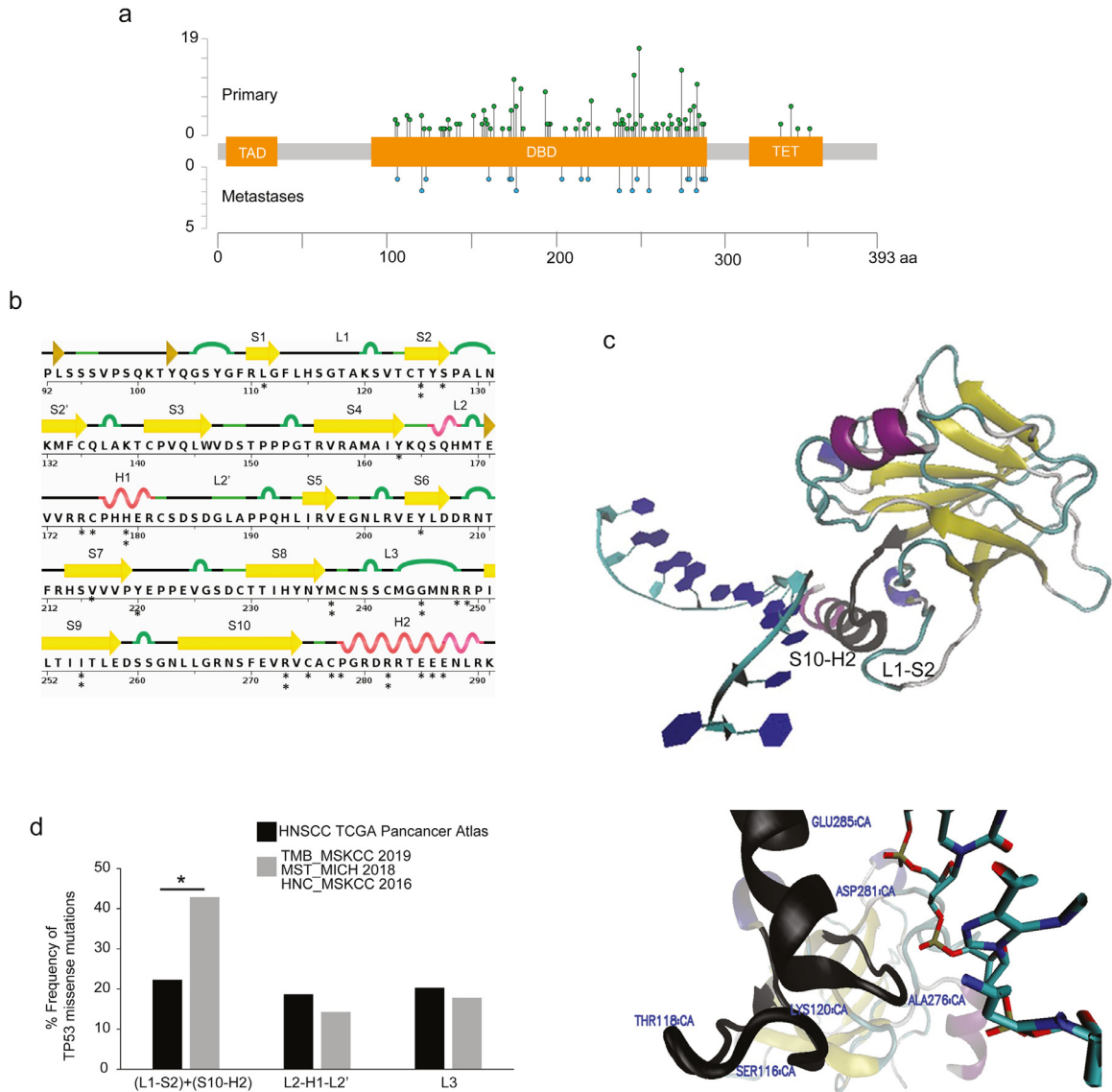


Fig. 2. Spectrum of missense TP53 mutations. (a) Distribution of mutations in the TP3 protein from primary (above) and metastatic (below) HNSCC. (b) Sequence and positioning of secondary structure elements within the TP53 DBD. Missense mutations are indicated with stars below the respective amino acid residue. (c) Position of the S10-H2 and L1-S2 domains (top) and critical amino acid residues mediating interaction with the DNA (bottom) in the tertiary structure of the monomeric TP53 DBD:DNA complex. (d) Frequency of missense TP53 mutations within different DBD structural elements. Statistical analysis was performed with chi-square test. * indicates $p < 0.05$.

Supplementary Figure 4a indicates, HNSCC is the only tumor type in which TP53 mutations are less common in metastases compared to primary tumors. On the contrary, TP53 mutations are invariably more frequent in metastases. Although prostate adenocarcinoma and breast carcinoma show a trend towards enrichment of L1-S2/S10-H2 mutations in metastases, only HNSCC shows a statistically significant enrichment which is accompanied by a reduction in non L1-S2/S10-H2 TP53 mutations (Supplementary Figure 4b and Supplementary Table 3). Analysis of CNA data for the same tumor types showed that the high frequency of chromosome 11q13.3 region amplification in tumors bearing L1-S2/S10-H2 TP53 mutations is a feature unique to HNSCC (Supplementary Table 4). Moreover, we assessed the association between TP53 mutation status and immunotherapy response, as indicated by overall survival, in patients with lung and liver metastases for which data was available (NSCLC, skin cutaneous melanoma-SKCM, colorectal adenocarcinoma-COAD and bladder carcinoma-BLCA). As Supplementary Figure 4c indicates, the TP53 mutation status has no predictive value in immunotherapy treatment, although cohort sizes are admittedly small. It is worth

mentioning however, that melanoma patients with TP53 mutations seem to respond better ($p = 0.0888$). These findings underscore the complexity of TP53 biology, which despite the several decades of studies remains largely elusive.

4. Discussion

In our analysis, the mutational spectrum of TP53 was compared between primary and metastatic HNSCC using publicly available TCGA mutational data. This analysis showed that metastatic tumors exhibit a substantially lower rate of TP53 mutations than primary ones. Analysis of the anatomic location of primary tumors and the metastasizing patterns in our metastatic cohort revealed that a significant portion of metastases arise from the oropharynx and nasopharynx. Human papillomavirus-positive oropharyngeal squamous cell carcinoma (HPV⁺ OPC) represents about the 60% of oropharyngeal cancer [20] while nasopharyngeal cancer (NPC) is associated with EBV in more than 50% of cases worldwide [21,22]. HPV and EBV positivity in OPC and NPC cancer, respectively, carries a good prognosis in

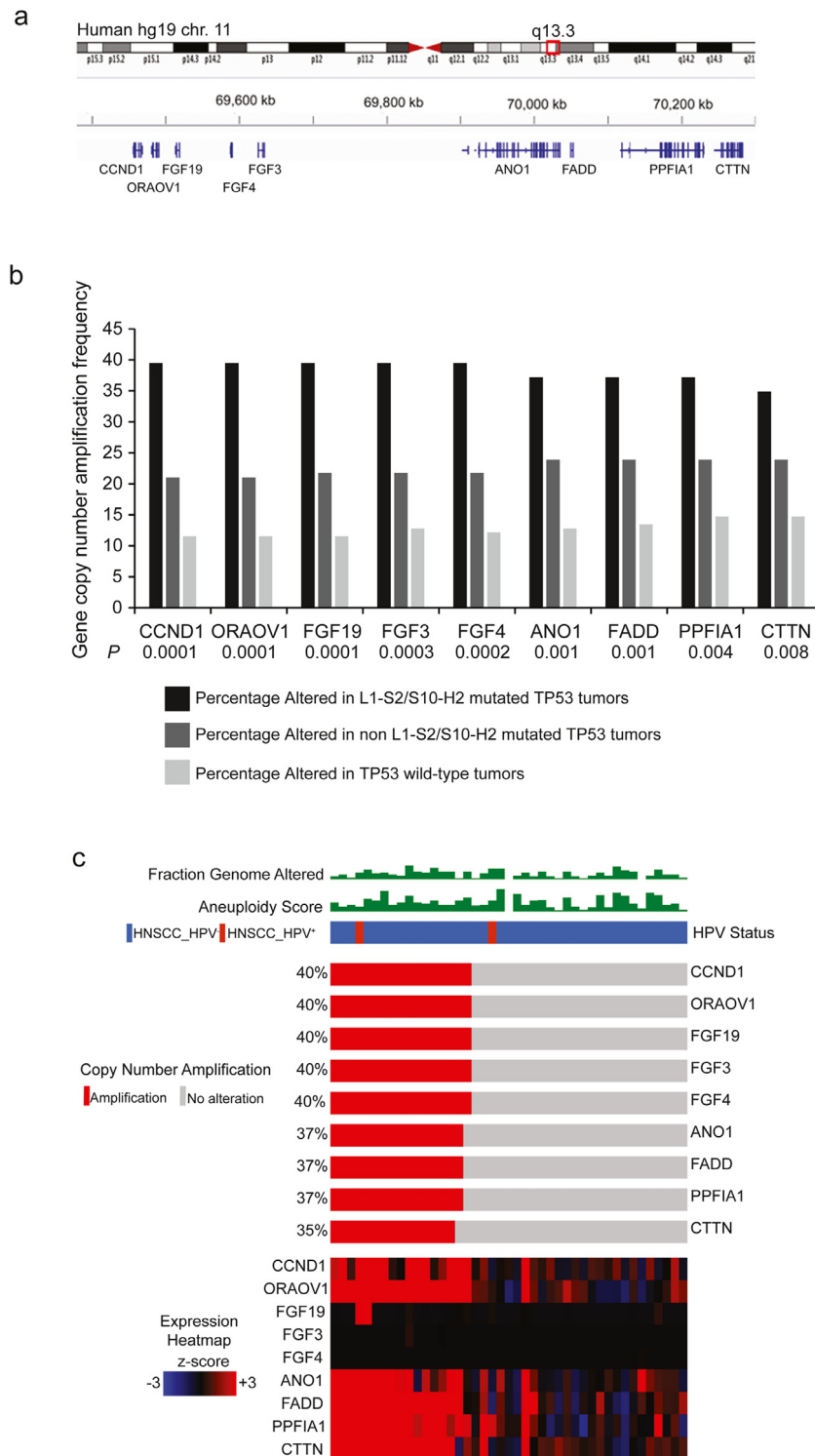


Fig. 3. Genomic alterations associated with TP53 missense mutations within the DNA contact interface. (a) Schematic representation of chromosome 11 and the q13.3 region and genes therein. (b) Comparison of frequency of copy number amplifications in the indicated genes between tumors with TP53 missense mutations within and outside the L1-S2/S10-H2 region as well as tumors with wild type TP53. P values underneath the gene names were calculated with Pearson χ^2 test. (c) Oncoprint and expression heatmap indicating association of copy number gain with transcriptional upregulation of 11q13.3 genes.

patients with primary disease [23,24]. On the other hand, metastasis is the leading cause of death in HPV⁺ OPC and EBV⁺ NPC due to their high potential for distant recurrence [25,26]. TP53 inactivating mutations are very rare in HPV and EBV-driven OPC and NPC carcinogenesis since the oncoviral proteins E6 and BZLF1 respectively, bind TP53 and interact with ubiquitin–protein ligase complexes causing its

proteolytic degradation [27,28] explaining the lower rate of TP53 mutations in the metastatic cohort.

To investigate whether the metastasis in HNSCC is associated with a selection process related to remaining TP53 transactivation activity, we evaluated the pattern of missense mutations in metastatic HNSCC biopsies. We observed a significant enrichment in

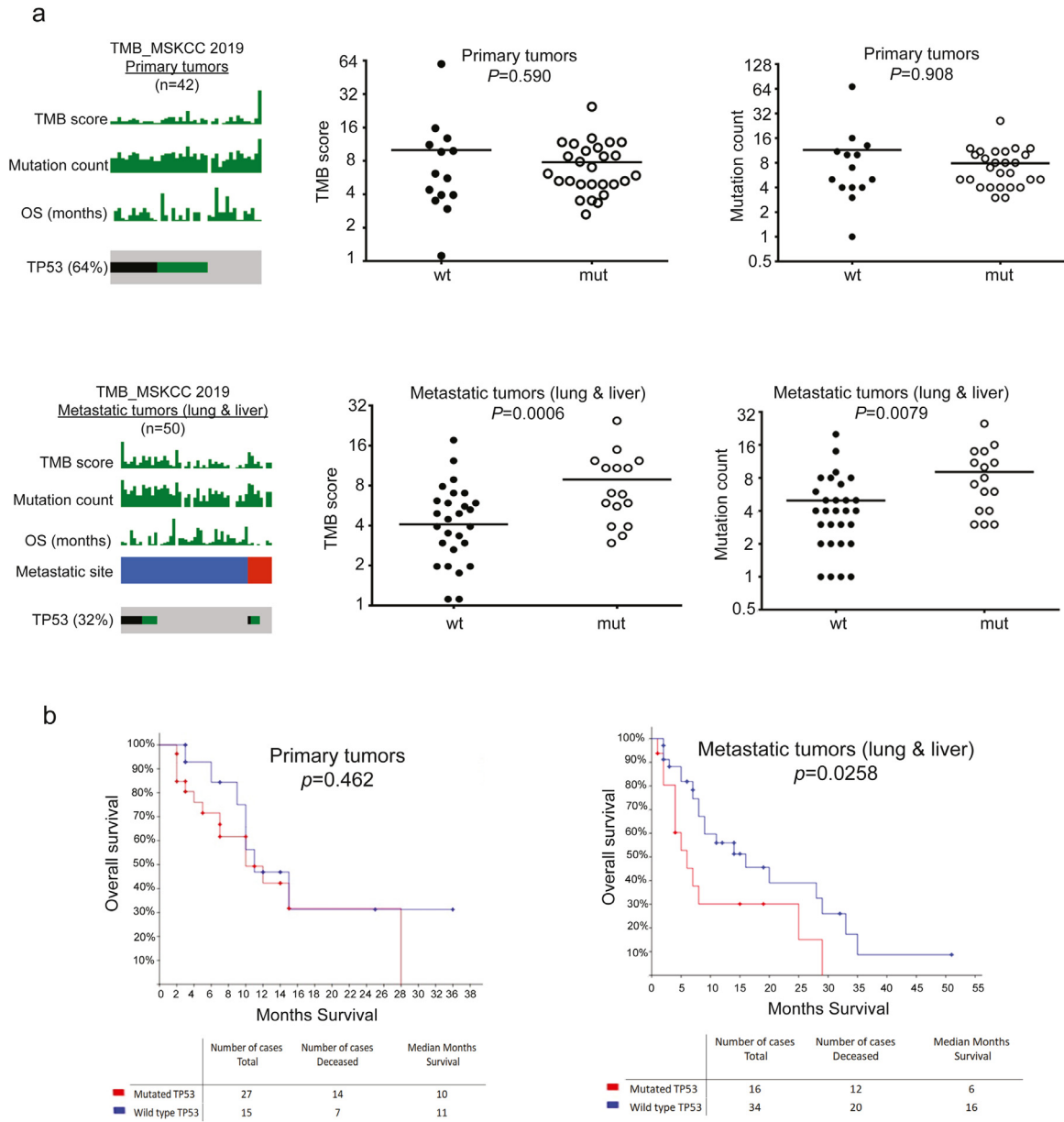


Fig. 4. TP53 mutations are associated with higher TMB score and worse overall survival in patients with metastatic disease undergoing immunotherapy. (a) Oncoprints (left) indicating mutation count, TMB score, overall survival and TP53 mutation status in primary tumors (top) and lung/liver metastases (bottom). In the TP53 mutation status bar, black color corresponds to truncating mutations while green corresponds to missense mutations. For the metastatic cohort, the site of metastasis is indicated in blue for lung and in red for liver metastasis. Also, boxplots (right) indicating mutation count and TMB score with respect to TP53 mutation status are shown. Mann-Whitney U test was performed. (b) Kaplan-Meier survival curves indicating overall survival of patients with primary or metastatic disease undergoing therapy with immune checkpoint inhibitors with respect to TP53 mutational status.

missense mutations located in the L1-S2 and S10-H2 regions that are in close proximity with DNA. A similar enrichment, albeit not statistically significant was observed in metastatic breast and prostate cancer but no other cancer type. With respect to the p53 three-dimensional structure, short helix H2 (residues P278-E287) and loop L1 (residues F113-T123) form the DNA major groove-binding surface, and are essential for DNA recognition. Helix H2 is spatially positioned near L1 and hence these elements are likely to dynamically interact through short-range communications [29]. L1 is the most dynamic loop among L1, L2 and L3. Beta-sheet S2/S2' that flank L1 is essential for the stabilization and proper positioning of L1 to DNA, mainly through the hydrogen bond between S2 residues S116 and C124 [30], whereas beta sheet S10 adjacent to helix H2 has a stabilizing role on both L1 and H2 [31].

Functional data from site-directed mutagenesis studies or studies that include synthetic libraries of mutant TP53 variants, have revealed that missense mutations in the L1-S2 and S10-H2 domains are associated with loss of transactivation potential towards p53 target genes [32]. The enrichment of the metastatic cohort with tumors harboring missense mutations in these domains may indicate that TP53 variants lacking DNA binding capacity and transactivation activity have higher metastatic potential or selective advantage when invading and colonizing distant organs, compared to tumors harboring TP53 missense mutations that retain a level of WT TP53 activity.

Loss of TP53 activity has been associated with tumor invasion due to the transcriptional downregulation of E-cadherin [33]. Several lines of evidence further indicate that TP53 GOF mutations drive an epithelial-mesenchymal transition (EMT) transcriptional program and tumors harboring such mutations have higher metastatic

potential [5]. Interestingly, several TP53 GOF mutations that are associated with EMT and metastasis, cluster within the S10-H2 region. For instance, R273H, a TP53 GOF mutation located in this region, has been found to promote cell migration, invasion *in vitro* and tumor metastasis *in vivo* [34]. Notably, R280K, another TP53 GOF mutation in the S10-H2 region, was found to promote TGF- β -mediated cancer cell spread [35]. Similarly, the GOF variant R282W, which is also located in the H2 helix, has been found to inhibit the expression levels of Kruppel-like-factor 17 (KLF17), a well-known transcriptional suppressor of EMT-related genes such as ID1, E-cadherin, ZO-1, vimentin and fibronectin [36]. On the other hand, in the L1-S2 region, the GOF variant K120R has been found to diminish the ability of TP53 to liberate the pro-apoptotic protein BAK from anti-apoptotic oncoprotein MCL-1 and subsequently to promote apoptosis [37].

Somatic copy-number alterations (SCNAs) are a hallmark of cancer. Different mechanisms that are associated with replication stress and loss of replication fidelity such as non-homologous end joining, alternative or micro-homology-mediated end joining in replication fork stalling or collapse [38] and aberrant replication or re-replication [39] have been described to induce SCNAs. Replication stress has also been linked to increase of common fragile site (CFS) breaks in metaphase chromosomes [40]. TP53 has a broad role in responding to replication stress and gene copy number variation, sustaining S/G2 arrest after loss of replication fidelity [41]. More recently, studies have also demonstrated the localization of p53 at replication forks, providing evidence that p53 is directly interacting with the replisome and altering its composition in response to replication stress [42]. It is largely agreed that TP53 loss increases replication stress which is a dynamic inducer of CFSs and CNVs. In this context, recent pan-cancer studies have shown that TP53 mutations are associated with SCNAs in all serous ovarian and breast carcinoma samples, as well as in a large fraction of lung, head and neck squamous cell carcinomas and endometrioid tumors of the serous subtype [43].

Missense mutations within different regions of TP53 can vary in their impact on the remaining protein activity and therefore on the levels of tumor replication stress and genomic instability. In agreement with this notion, we observed a significant association of L1-S2/S10-H2 missense mutations with chromosomal gain of 11q13.3 locus and copy number amplification of genes therein, associated with increased transcription levels. Several studies in the past have identified 11q13.3 gain to promote metastatic disease progression in head and neck and other cancer types [44,45]. However, our PanCancer analysis showed significantly lower levels of chromosome 11q13.3 CNAs in NSCLC, PRAD, BRCA, BLCA and SKCM which did not correlate with the TP53 mutation pattern.

The association of 11q13.3 gain with metastasis also implies that this cytogenetic alteration might augment the invasiveness of tumor cells and therefore is selected during the metastatic process. In this context, several studies have shown that gene amplification and overexpression of CCND1, ANO1, PPFIA1, CTTN has been associated with tumor invasion and metastasis due to the ability of these genes to regulate cell adhesion and migration [46].

Since the 2016 FDA approval of nivolumab and pembrolizumab for the treatment of patients with recurrent or metastatic HNSCC (R/M-HNSCC) that had progressed after platinum-based chemotherapy, several trials are investigating whether anti-PD-1 and anti-PD-L1 therapy could replace or integrate with standard of care chemotherapy as first line treatment. According to recent peer-reviewed results of the phase III Keynote-048 trial, pembrolizumab monotherapy or pembrolizumab with chemotherapy improved overall survival compared with cetuximab plus chemotherapy [2]. Moreover, in June 2019, FDA approved pembrolizumab for first-line treatment of R/M-HNSCC.

TMB has emerged as a predictive biomarker in immunotherapy response across many tumor types including HNSCC [9]. NGS studies have shown a clear association between TP53 mutated tumors with high TMB levels and robust clinical benefit from ICIs in NSCLC

[47,48]. In this direction, we evaluated the association between TP53 mutation status, TMB score and overall survival in primary and metastatic (lung and liver) HNSCC tumors of patients treated with ICIs. Interestingly, in our study, TP53-mutant tumors that have metastasized to the lung and liver showed a statistically significant worse response to immunotherapy compared to wild-type TP53 tumors, despite their significantly higher TMB score. On the other hand, we did not identify a predictive role for TP53 mutational status or a positive association with TMB score in primary tumors. No correlation between TP53 mutation status and response to ICIs was observed in metastatic NSCLC, BLCA and COAD, while SKCM TP53 mutated tumors show a better response, not reaching statistical significance, though. Our findings suggest that mutated TP53 is a potential negative predictor of response to immunotherapy in metastatic HNSCC but not in other solid malignancies currently treated with immune checkpoint inhibitors.

In summary, metastatic HNSCC is a highly heterogeneous disease at the genetic level since the primary tumor can arise from different anatomic locations. Our findings demonstrate that metastatic HNSCC is characterized by a lower frequency of TP53 mutations compared to primary HNSCC tumors possibly due to the high metastatic potential of HPV⁺ and EBV⁺ oropharyngeal and nasopharyngeal tumors that do not harbor TP53 mutations. We also demonstrate that TP53 missense mutations in metastatic HNSCC are enriched in the L1-S2 and S10-H2 domains that are critical for the TP53 DNA binding activity. Finally, mutations in TP53 could serve as a potential biomarker for guiding immune checkpoint inhibitor therapy in metastatic HNSCC.

Contributors

AK and TR designed and performed the analysis, and wrote the manuscript.

Declaration of Competing Interest

The authors declare that they have no conflict of interest.

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Supplementary materials

Supplementary material associated with this article can be found in the online version at doi:10.1016/j.ebiom.2020.102905.

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