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Research Paper

TP53 Mutation as Potential Negative Predictor for Response of Anti-CTLA-4 Therapy in Metastatic Melanoma

Wenjing Xiao [a], Nan Du [b], Taoyuan Huang [c], Jinan Guo [d], Xingkui Mo [e], Tao Yuan [e], Yong Chen [f], Ting Ye [g], Chunwei Xu [h], Wenxian Wang [i], Guoqiang Wang [j], Shangli Cai [j], Jing Chen [g,*]

[a] Department of Tumor Radiotherapy, The Affiliated Hospital of Qingdao University, No. 16, Jiangsu Road, Shinan District, Qingdao, PR China
[b] Department of Oncology, The First Affiliated Hospital, Chinese PLA General Hospital, No. 51, Fucheng Road, Beijing, PR China
[c] Department of Dermatologic Surgery, Dermatology Hospital of Southern Medical University, Guangdong Province Dermatology Hospital, No. 2, Lujing Road, Yuexiu District, Guangdong Province, PR China
[d] The Department of Urology, The Second Clinical Medical College of Jinan University (Shenzhen people's Hospital), Shenzhen Urology Minimally Invasive Engineering Center, Shenzhen, PR China
[e] Department of Orthopaedics, The Third Affiliated Hospital of Kunming Medical University, Tumor Hospital of Yunnan Province, No 519, Kunzhou Road, Xishan District, Kunming, Yunnan Province, PR China
[f] Department of musculoskeletal surgery, Fudan University Shanghai Cancer Center, Department of Oncology, Shanghai Medical College, Fudan University, Shanghai, PR China.
[g] Cancer Center, Union Hospital, Tongji Medical College, Huazhong University of Science and Technology, Wuhan, PR China
[h] Department of Pathology, Fujian Cancer Hospital, Fujian Medical University Cancer Hospital, Fuzhou, Fujian, PR China
[i] Department of Chemotherapy, Zhejiang Cancer Hospital, Hangzhou, Zhejiang, PR China
[j] The Medical Department, 3D Medicines Inc., Shanghai, PR China

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ABSTRACT

TP53 has been proved to be associated with cytotoxic T-cell induced apoptosis, however, the association between TP53 and the benefit of immunotherapy in melanoma has not been studied. In the present study, we examined the relationship between TP53 mutation and response to CTLA-4 blockade in metastatic melanoma by analyzing the data from one public cohort consisting of 110 patients with metastatic melanoma. The sequencing, mRNA and survival data of 368 patients with skin melanoma from The Cancer Genome Atlas (TCGA) was used to explore the underlying mechanism. TP53 mutation was associated with significant poorer progression-free survival (HR, 2.25; 95% CI, 1.15–4.37; P = 0.014), poorer overall survival (HR, 2.05; 95% CI, 1.02–4.13; P = 0.040) and trend of poorer response (OR, 0.20; 95% CI, 0.02–1.62; P = 0.131). The correlations were significant in multivariate analysis including lactate dehydrogenase, tumor mutational burden and tumor stage (P < 0.05). In TCGA, no association was observed between TP53 mutation and survival (P = 0.55). The mRNA expression of FAS was lower in patients with TP53 mutation than TP53 wild-type. Our findings suggest that TP53 mutation is a potential negative predictor of metastatic melanoma treated with CTLA-4 blockade.

1. Introduction

In the last decade, immune checkpoint blockades (ICBs) of targeting cytotoxic T-lymphocyte antigen-4 (CTLA-4), programmed death receptor-1 (PD-1) and its associated ligand (PD-L1) as monotherapy or combination therapy, have revolutionized the treatment of metastatic melanoma [1–4]. Unfortunately, only a subset of treated patients responds to the current immunotherapy. Therefore, it has become a challenge to identify clinically useful biomarkers that can distinguish patients who may respond or resist to ICBs.

Biomarkers including PD-L1 expression, tumor mutational burden (TMB), tumor-infiltrating lymphocytes, micro-satellite instability and immune gene signatures have been shown to be associated with the clinical benefit of ICBs [5]. Among these biomarkers, TMB is associated with better clinical outcome in metastatic melanoma treated with anti-CTLA-4 or anti-PD-1 therapy [6–8]. However, high TMB cannot guarantee response to ICBs, suggesting that other independent variables may exist to predict the clinical outcome beyond the existing biomarkers.

TP53, a well-known tumor suppressor and transcriptional activator or repressor, is the most frequently mutated genes in cancer [9], mutation of which allows tumor evasion and induces rapid tumor progression [10]. Preclinical studies have illustrated TP53-induced enhancement of cytotoxic T-cell (CTL) response by up-regulating major histocompatibility complex I (MHC I) and FAS [11,12]. In MHC antigen presentation pathway, oligopeptides generated via proteasome degradation require transporter associated with antigen processing 1 (TAP1)-mediated transport to rough endoplasmic reticulum and endoplasmic reticulum aminopeptidase 1 (ERAP1)-mediated trimming to fit

* Corresponding author.
E-mail address: Chenjingunion@163.com (J. Chen).

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the length criterion for MHC I presentation. The transcription of both TAP1 and ERAP1 are directly modulated by TP53, and the inactivity of mutant TP53 decreased their de novo protein synthesis and thereby the surface level of MHC-peptide complex, resulting in down-regulated immune surveillance [13,14]. Moreover, the gene of FAS is also targeted by the transcriptional activation of TP53. Thus, mutant TP53 diminishes its surface level in tumor cells and therefore inhibits CTL-induced apoptosis [12]. However, in a recent clinical study of lung adenocarcinoma, TP53 has been shown to be associated with higher TMB and better outcome of anti-PD-1 therapy [15]. The predictive value of TP53 mutation in patients treated with ICBs seems to be controversial and needs to be further illustrated.

In order to demonstrate the association between TP53 mutation and clinical outcome of ICBs, we analyzed the data from the largest available cohort of metastatic melanoma treated with anti-CTLA-4 with both genomic and clinical data [16]. The sequencing, mRNA and survival data from The Cancer Genome Atlas was also analyzed to explore the possible underlying mechanism.

2. Materials and Methods

2.1. Clinical Cohorts

The whole-exome sequencing and clinical data of 110 patients with metastatic melanoma treated with ipilimumab were obtained from the public cohort [16]. The Allen cohort is the largest cohort available in melanoma treated with ICBs with both genomic and clinical data. The genomic, survival and mRNA data of 368 patients with skin cutaneous melanoma (SKCM) was obtained from The Cancer Genome Atlas (TCGA) (www.cbioportal.org). The estimated $CD8^+$ T cell infiltration data of TCGA samples was obtained from a previous study [17]. Most of the patients enrolled in TCGA were early stages.

2.2. Study Design

Any mutation in coding region of TP53 was determined as TP53 mutation. TMB was defined as the total number of nonsynonymous mutation in coding region. TMB-high group was defined as TMB ≥ median, while TMB-low group was defined as TMB < median. Indel burden was defined as the total number of insert and deletion mutation in coding region. The primary outcome was PFS, which was calculated from the date of first immunotherapy administration to disease progression or death due to any cause. The secondary outcome was OS, which was calculated from the date of first immunotherapy administration until death due to any cause and response rate. We first determined the association between TP53 and PFS or OS using univariate and multivariate models. Then we examined the association between TP53 and response in univariate and multivariate models. As previously reported, patients were stratified into response groups based on RECIST criteria [18], duration of OS and duration of PFS [16].

2.3. Statistical Analyses

Data analyses were performed using SPSS 20.0 (SPSS Inc., Chicago, IL). Survival description was illustrated by the Kaplan-Meier curves, with P value determined by a log-rank test. Hazard's ratio (HR) was determined through the univariate and multivariate Cox regression. The associations between response and variables were examined by a univariate logistic regression. Variables with significant P values or interest were included into multivariate logistic regression. Continuous variables were compared by Mann-Whitney *U* test. False discovery rate (FDR) was used to estimate the significance of differences between the mRNA expression levels. All reported P values were two-tailed, and $P < 0.05$ and $FDR < 0.05$ is considered statistically significant.

3. Results

3.1. Patient Cohort

110 patients with metastatic melanoma treated with anti-CTLA-4 were included in this analysis. The baseline characteristics were summarized in Table 1. 78 of 110 patients (70.9%) were male and the median age of the cohort was 61.5 years (range, 18 to 86 years). Most patients (90.9%) were stage 4. The median TMB count was 203.5 (range, 12–5976). Patients with TMB ≥ median were defined as TMB-high. 34 patients (30.9%) harbored $BRAF^{V600E}$ mutation and 10 patients (9.1%) carried TP53 mutation including 6 missense mutation, 3 nonsense mutation and 1 splice site mutation.

3.2. Association Between TP53 and Survival Outcomes of Anti-CTLA-4 Therapy

The detailed baseline characteristics and clinical outcomes of patients with TP53 mutation were summarized in Supplemental Table S1. We first analyzed whether TP53 mutation status was associated with the survival outcomes of anti-CTLA-4 in metastatic melanoma. Patients with TP53 mutation obtained poorer PFS compared to wild-type TP53 (median PFS: 2.5 months vs. 2.8 months, $P = 0.014$, Fig. 1a). Previous studies have demonstrated that TMB and LDH are associated with the clinical benefit of ICBs in melanoma ([7,16,19]. In the present study, univariable analysis revealed significant association between poorer PFS and LDH-abnormal or TP53 mutation, while no relation between PFS and TMB as continuous variable or binary variable with median as cut-off (Table 2). In a multivariate model including tumor stage, LDH, TMB and TP53, TP53 mutation and LDH-abnormal remained an independent prognostic indicator for poorer PFS (Table 2). TMB ≥ median showed borderline improvement in PFS (HR, 0.69; 95% CI, 0.45 to 1.04; $P = 0.077$; Table 2).

In consistence with PFS, poorer OS was discovered in patients with TP53 mutation compared to patients with wild-type TP53 (median OS: 5.5 months vs. 9.6 months, $P = 0.04$, Fig. 1b). Univariate and multivariate analysis exposed significant association between OS and TP53 status, LDH or stage (Table 3). No association was discovered between OS and TMB as continuous variable, while stratifying patients as per median TMB revealed signification relation between TMB ≥ median and better OS (Table 3), suggesting that TMB alone may not be sufficient enough to predict the clinical outcomes of anti-CTLA-4 treatment. In consideration of a previous study demonstrating that TP53 mutation is involved in the carcinogenesis in cutaneous melanoma [20], we further

Table 1
Baseline characteristics.

Variable	Total (n = 110)
Sex, n (%)	
Male	78 (70.9%)
Female	32 (29.1%)
Age, median (range), years	61.5 (18–86)
Stage, n (%)	
Stage 3	10 (9.1%)
Stage 4	100 (90.9%)
Primary, n (%)	
Skin	92 (83.6%)
Occult	14 (12.7%)
Mucosal	4 (3.6%)
Baseline LDH, n (%)	
Normal	58 (52.7%)
Abnormal	48 (43.6%)
Unknown	4 (3.6%)
Tumor mutational burden, median (range)	203.5 (12-5976)
$BRAF^{V600E}$ mutation	34 (30.9%)
TP53 mutation	10 (9.1%)

LDH, lactate dehydrogenase.

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