Predictive Biomarkers and Personalized Medicine

Large-scale Analysis of PDGFRA Mutations in Melanomas and Evaluation of Their Sensitivity to Tyrosine Kinase Inhibitors Imatinib and Crenolanib

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Abstract

Purpose: Platelet-derived growth factor receptor \(\alpha\) (PDGFRA) is a target for tyrosine kinase inhibitor (TKI)-based targeted therapy. Dysregulation of PDGFRA has been reported in many cancers. However, PDGFRA mutations in melanomas have not been well studied. We analyzed the genetic mutations of PDGFRA in Chinese patients with melanoma and determined the inhibitory potency of TKIs, such as imatinib and crenolanib, on mutant PDGFRA.

Experimental Design: Of note, 351 melanoma tissue samples were examined for genetic mutations in exons 12, 14, and 18 of PDGFRA. Activities of mutations in response to imatinib and crenolanib were analyzed by Western blotting of tyrosine-phosphorylated PDGFRA and cell proliferation assays.

Results: PDGFRA mutations were observed in 4.6\% (16 of 351) of melanomas, and these mutations were mainly detected in acral and mucosal melanomas. PDGFRA mutations seem to be mutually exclusive with KIT mutations, but may coexist with BRAF and NRAS mutations. The genetic mutations of PDGFRA were unrelated to the age, thickness, and ulceration status of primary melanomas. Thirteen mutations were not reported before, and five (P577S, V658A, R841K, H845Y, and G853D) of them resulted in strong autophosphorylation of PDGFRA. Crenolanib showed higher potency than imatinib in inhibiting the kinase activity of PDGFRA. Except that V658A mutation was imatinib-resistant, all the other mutations were sensitive to both imatinib and crenolanib.

Conclusions: PDGFRA mutations are detected in a small population of melanoma patients. Our study suggests that patients with melanoma harboring certain PDGFRA mutations may benefit from imatinib and crenolanib treatment. Clin Cancer Res; 19(24); 6935–42. ©2013 AACR.

Introduction

Melanoma is the most aggressive skin cancer with the fastest increasing incidence rate. From 2005 to 2009, the age-adjusted incidence rate was 21.0 per 100,000 men and women per year in the United States (1). Metastatic melanoma is difficult to treat as standard chemotherapy [high-dose interleukin (IL)-2 and dacarbazine] cannot significantly improve the overall survival (OS; ref. 2). Targeted therapeutic agents have shown promising clinical efficacy, such as BRAF inhibitors (e.g., vemurafenib and dabrafenib; refs. 3, 4), KIT inhibitor (imatinib; refs. 5, 6), and MAP–ERK kinase (MEK) inhibitor (trametinib; ref. 7). Although about 50\% of melanomas harbor BRAF mutation in Caucasians (8), we have previously shown that KIT, BRAF, and NRAS mutation rates in Chinese patients with melanoma are 10.8\%, 25.5\%, and 7.2\%, respectively (9, 10). Therefore, uncovering other potential driver oncogenic mutations will help to identify patients with melanoma who may benefit from targeted therapeutic agents.

Platelet-derived growth factor receptor \(\alpha\) polypeptide (PDGFRA, also known as CD140A) belongs to the type III receptor tyrosine kinase (RTK) subfamily, and regulates cell proliferation, differentiation, survival, and tumour progression (11, 12). Dysregulation of PDGFRA has been reported in several cancers (12–16). PDGFRA is overexpressed in some melanomas and the increased copy number has also been reported previously (17, 18). The mutational analysis of PDGFRA is relatively limited in melanoma despite that...
Clinical Cancer Research

Translational Relevance

The incidence of melanoma is increasing worldwide. Similar to KIT, PDGFRA is a type III receptor tyrosine kinase (RTK) and a potential target for tyrosine kinase inhibitors (TKIs), such as imatinib and crenolanib, in cancer treatments. Genetic mutations of PDGFRA have been reported in many cancers, but its mutations in melanomas have not been well studied. Moreover, sensitivity of PDGFRA mutants to TKIs has not been fully evaluated. Defined sensitivity of mutant PDGFRA to TKIs might provide stratification criteria for patients with melanoma, who would benefit from TKI-based targeted therapy. Our study demonstrates that PDGFRA mutations are detected in a small population of melanoma patients, primarily in acral and mucosal melanomas. Our study suggests that a subgroup of patients with melanoma harboring PDGFRA mutations may benefit from TKIs, casting new light in the selection of melanoma patients sensitive to TKIs, such as imatinib and crenolanib.

DNA extraction and mutation detection

Genomic DNA was extracted from FFPE specimens using the QiAamp DNA FFPE Tissue Kit (Qiagen) according to the manufacturer’s instruction. Exons 12, 14, and 18 of PDGFRA were amplified by PCR in at least two separate preparations of genomic DNA. The primers and annealing temperatures were listed in Supplementary Table S1. The PCR products purification and sequencing were performed as described previously (9, 10).

Cells

293T (catalog no. ACC-635) and Ba/F3 (catalog no. ACC-300) cells were initially obtained from Leibniz Institute DSMZ (Braunschweig, Germany) and cultured as recommended. 293T cells are highly transfectable derivative of the human primary embryonal kidney cell line 293 (ACC-305), carrying a plasmid containing the temperature-sensitive mutant of SV-40 large T-antigen, which were authenticated by morphology assays (fibroblastoid cells growing adherently as monolayer), PCR assays of large T antigens, 293 cell-specific short tandem repeat markers, and cytogenetic karyotypes assays. Ba/F3 cells are IL-3-dependent murine pro-B cell line derived from C3H mouse strain, which were authenticated by morphology assays (single, round cells in suspension), PCR assays of mouse species-specific markers, immunologic assays (CD11b+ F4/80+), and cytogenetic karyotypes assays. Both cell lines were confirmed to be negative for the common microorganisms. Detailed information for authentication of these cells could be obtained online (http://www.dsmz.de).

Plasmid construction and lentiviral packaging

The wild-type (WT) PDGFRA cDNA was amplified from melanoma cDNA by PCR and subcloned into the expression vector pLenti PGK Neo DEST (w531-1; Addgene). All the PDGFRA-mutant cDNAs were generated by site-directed mutagenesis as previously described (25). All vectors were confirmed by bidirectional sequencing. The lentiviral expression vector, psPAX2 packaging vector, and pMD2.G envelop vector were added into 293T cells using Fugene 6 transfection reagent (Promega). The virus-containing supernatants were harvested, filtered through a 0.45-μm cellulose acetate filter (Millipore) at 48 and 72 hours, and concentrated using Lenti-X Concentrator (Clontech).

Cell cultures and transfections

293T cells were grown in Dulbecco’s modified Eagle medium (DMEM) medium (Invitrogen), supplemented with 10% FBS (Hyclone) and penicillin/streptomycin (Invitrogen). Ba/F3 cells (a kind gift from Dr. Wei Tong, Children’s Hospital of Philadelphia, University of Pennsylvania, Philadelphia, PA) were grown in RPMI-1640 medium with GlutaMAX (Invitrogen), supplemented with 10% FBS, 10 ng/mL IL-3 (Invitrogen), and penicillin/streptomycin. Virus was added onto 293T or Ba/F3 cells with polybrene. Ba/F3 clones were selected by 1 μg/mL G418 and followed by IL-3-independent growth. DNA from stably transfected cell

Materials and Methods

Tumor samples

A total of 351 archival formalin-fixed and paraffin-embedded (FFPE) melanomas (including 316 primary melanomas and 35 metastatic melanomas) were derived from Peking University Cancer Hospital & Institute (Beijing, China) between 2006 and 2011. Clinical data, including age, gender, stage, thickness, and ulceration, were collected. This study was reviewed and approved by the medical ethics committee of the Peking University Cancer Hospital & Institute.

one intronic mutation of PDGFRA has been reported in ocular melanoma (18–20). Some patients with gastrointestinal stromal tumors (GIST) bearing PDGFRA mutations have benefited from molecular therapeutic agents (21).

Imatinib is a tyrosine kinase inhibitor (TKI) against BCR-ABL, KIT, and PDGFRA. It is the first-line therapy for chronic myelogenous leukemia and has been approved by U.S. Food and Drug Administration (FDA) for the treatment of patients with advanced GIST in 2002 (21, 22). The latest National Comprehensive Cancer Network (NCCN) guidelines have suggested imatinib as a treatment of KIT-mutated melanomas (23). Crenolanib is a PDGFR-specific TKI that significantly inhibits the kinase activity of PDGFRA (24).

Several phase II clinical trials are ongoing, including a trial of crenolanib. Several trials are detected in a small population of melanoma patients, primarily in acral and mucosal melanomas. One intronic mutation of PDGFRA has been reported in ocular melanoma (18–20). Some patients with gastrointestinal stromal tumors (GIST) bearing PDGFRA mutations have benefited from molecular therapeutic agents (21).

Considering that the mutational status of PDGFRA in melanomas has not been fully evaluated and that PDGFRA is a potential target for TKIs-based targeted therapy, we collected 351 melanoma tissue samples and examined the mutational status of PDGFRA. Kinase activities of these mutated PDGFRA as well as their sensitivity to imatinib and crenolanib were also evaluated.
lines was extracted and sequenced to confirm the presence of mutant $PDGFRA$ sequence.

**Immunoprecipitation and Western blotting**

Cells were lysed using PhosphoSafe Extraction Reagent (Millipore) and protein extracts were immunoprecipitated with rabbit anti-human $PDGFRA$ polyclonal antibody (C-20; Santa Cruz Biotechnology) and Protein A/G beads (Sigma-Aldrich). Western blotting was performed with mouse anti-phosphotyrosine antibody (4G10; Millipore) and $PDGFRA$ antibody. The procedures were carried out as described previously (26).

**Assessment of $PDGFRA$ kinase activity**

Imatinib and crenolanib were purchased from Selleckchem and resolved in dimethyl sulfoxide (DMSO) at the concentration of 10 mmol/L and stocked at $-80^\circ$C. Ba/F3 cells were treated with vehicle alone or various concentrations of kinase inhibitors for 90 minutes. Protein lysates were extracted and subjected to immunoprecipitation and Western blotting.

**Cell proliferation assays**

Ba/F3 cells transfected with various $PDGFRA$ mutants were seeded into 96-well plates at a density of $2 \times 10^4$ cells per well and incubated with different concentrations of imatinib or crenolanib for 72 hours, the proliferation and viability was measured by CCK-8 (Dojindo) according to the manufacturer’s instruction.

**Statistical analysis**

Statistical analyses were performed using SPSS 16.0 software. Continuous data such as age and thickness were described using mean $\pm$ SD for normally distributed data. The correlations between mutational status and clinical parameters were evaluated by $\chi^2$ test or Fisher exact test. All statistical analyses were two sided and $P < 0.05$ was considered as statistically significant.

**Results**

**Genetic mutations of $PDGFRA$ in melanoma subtypes**

Of a total of 351 melanomas analyzed, 16 (4.6%) of them were identified to contain $PDGFRA$ mutations. The mutation frequencies of $PDGFRA$ in acral, mucosal, melanomas on skin without chronic sun-induced damage (non-CSD) melanoma subtypes were 6.8% (9 of 132), 3.6% (4 of 110), and 1.8% (1 of 56), respectively. No $PDGFRA$ mutation was found in melanomas on skin with CSD (Table 3).

**Mutation types of $PDGFRA$ in melanoma**

Sixteen melanomas were found to harbor $PDGFRA$ mutations and 18 different mutations were detected, all of which were heterozygous point mutations (Table 2 and Supplementary Figs. S1–S3). In addition to previously reported five mutations (V561A, K646E, H816Y, L839P, and Y849C; refs. 27–30), the other 13 mutations were novel (Table 2). No common mutation was found. Among the 18 $PDGFRA$ mutations, five mutations were located in juxta-

**Table 1. Genetic mutations of $PDGFRA$ in melanoma subtypes**

<table>
<thead>
<tr>
<th>Subtype</th>
<th>No.</th>
<th>Positive</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acral</td>
<td>132</td>
<td>9</td>
<td>6.8</td>
</tr>
<tr>
<td>Mucosal</td>
<td>110</td>
<td>4</td>
<td>3.6</td>
</tr>
<tr>
<td>Non-CSD</td>
<td>56</td>
<td>1</td>
<td>1.8</td>
</tr>
<tr>
<td>CSD</td>
<td>18</td>
<td>0</td>
<td>0.0</td>
</tr>
<tr>
<td>Unknown</td>
<td>35</td>
<td>2</td>
<td>5.7</td>
</tr>
<tr>
<td>Total</td>
<td>351</td>
<td>16</td>
<td>4.6</td>
</tr>
</tbody>
</table>

Abbreviation: Unknown, melanomas of unknown primary lesion.

membrane domain (exon 12, 27.8%), five and eight mutations were identified in the first and second tyrosine kinase domain separately (exon 14, 27.8%; exon 18, 44.4%). A premature stop mutation (Q639stop) was observed in one patient. Two patients harbored two different missense point mutations simultaneously, one harbored two mutations in exon 14 (V685A) and 18 (R841K) separately, and the other harbored two mutations both in exon 12 (V561A and Q579R). No mutation was detected in codon 842, which was the most prevalence mutation reported in GISTs (13). All the mutations, including those obtained from The Cancer Genome Atlas (TCGA) database and those reported here, are illustrated in Fig. 1 (31). To exclude the possibility that the detected mutations were due to polymorphisms, we extracted DNA from the peripheral blood mononuclear cells from all the 16 patients harboring $PDGFRA$ mutations and examined the mutation status of $PDGFRA$. No mutations in exons 12, 14, and 18 of $PDGFRA$ were detected, indicating that the mutations detected by us are indeed somatic mutations.

To analyze the relationship of $PDGFRA$ mutations to other important mutations in melanoma, we further screened the $KIT$, $BRAF$, and $NRAS$ sequences as previously described (9, 10). We found two $BRAF^{V600E}$ mutations, one $NRAS^{Q61E}$ mutation and zero $KIT$ mutation in samples harboring $PDGFRA$ mutations. Four $BRAF$ mutations, five $NRAS$ mutations, and zero $PDGFRA$ mutation were found in 36 samples containing $KIT$ mutations (data not shown).

**Correlation of $PDGFRA$ mutations to the clinical characteristics of melanoma**

The summary of correlations between $PDGFRA$ mutations and clinical characteristics is shown in Table 3. Both the age and the proportion of gender were not significantly different between patients with $PDGFRA$ mutations and those without a $PDGFRA$ mutation.

As the Breslow thickness of melanoma is an important prognostic indicator (32), we analyzed the thickness of melanoma samples. Among the 310 samples with thickness data available, the average thickness of samples with $PDGFRA$ mutations was 5.85 mm $\pm$ 2.66 mm, whereas
that of samples without PDGFRA mutations was 4.87 mm ± 2.64 mm (P = 0.194).

Ulceration is another adverse prognostic factor for melanoma (32). The overall ulceration rate of 311 available samples was 58.8% (183 of 311). The ulceration rate in acral, mucosal, non-CSD, and CSD were 63.1%, 54.2%, 56.4%, and 61.1%, respectively. The ulceration rate was not significantly different in patients with PDGFRA mutations and those without PDGFRA mutations (P = 0.437).

Among the 16 patients with PDGFRA mutations, stage data were available in 15 patients. The percentages of PDGFRA mutations at stage I, II, III, and IV were not significantly different from those with WT PDGFRA (P = 0.217). Among the 6 patients in stage III and IV of disease with PDGFRA mutations, tumor samples from primary sites and metastatic sites were available for 3 of them. Mutational analysis showed that the PDGFRA mutations were the same for the primary and metastatic melanomas.

In addition, the relationship of PDGFRA mutations with melanoma subtypes was analyzed. In the 316 patients with subtype information available, the PDGFRA mutation frequency was not significantly different in melanomas

**Table 2.** Mutation types of PDGFRA in melanoma

<table>
<thead>
<tr>
<th>Exon</th>
<th>Nucleotide</th>
<th>Amino acid</th>
<th>No.</th>
<th>KIT</th>
<th>BRAF</th>
<th>NRAS</th>
<th>Subtype</th>
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<tr>
<td>12</td>
<td>T1682C</td>
<td>V561A</td>
<td>1</td>
<td>WT</td>
<td>WT</td>
<td>WT</td>
<td>Acral</td>
</tr>
<tr>
<td></td>
<td>G1702A</td>
<td>D568N</td>
<td>1</td>
<td>WT</td>
<td>WT</td>
<td>WT</td>
<td>Acral</td>
</tr>
<tr>
<td></td>
<td>C1729T</td>
<td>P577S</td>
<td>1</td>
<td>WT</td>
<td>WT</td>
<td>WT</td>
<td>Acral</td>
</tr>
<tr>
<td></td>
<td>A1736G</td>
<td>Q579R</td>
<td>1</td>
<td>WT</td>
<td>WT</td>
<td>WT</td>
<td>Acral</td>
</tr>
<tr>
<td></td>
<td>C1751T</td>
<td>S584L</td>
<td>1</td>
<td>WT</td>
<td>WT</td>
<td>WT</td>
<td>Mucosal</td>
</tr>
<tr>
<td>14</td>
<td>G1897A</td>
<td>A633T</td>
<td>1</td>
<td>WT</td>
<td>V600E</td>
<td>WT</td>
<td>Unknown</td>
</tr>
<tr>
<td></td>
<td>C1915T</td>
<td>Q639stop</td>
<td>1</td>
<td>WT</td>
<td>WT</td>
<td>WT</td>
<td>Acral</td>
</tr>
<tr>
<td></td>
<td>A1936G</td>
<td>K646E</td>
<td>1</td>
<td>WT</td>
<td>WT</td>
<td>WT</td>
<td>Unknown</td>
</tr>
<tr>
<td></td>
<td>T1973C</td>
<td>V658A</td>
<td>1</td>
<td>WT</td>
<td>WT</td>
<td>WT</td>
<td>Mucosal</td>
</tr>
<tr>
<td></td>
<td>C1988T</td>
<td>A663V</td>
<td>1</td>
<td>WT</td>
<td>WT</td>
<td>Q61L</td>
<td>Acral</td>
</tr>
<tr>
<td>18</td>
<td>C2446T</td>
<td>H816Y</td>
<td>1</td>
<td>WT</td>
<td>WT</td>
<td>WT</td>
<td>Mucosal</td>
</tr>
<tr>
<td></td>
<td>A2489G</td>
<td>K830R</td>
<td>1</td>
<td>WT</td>
<td>WT</td>
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<td>Acral</td>
</tr>
<tr>
<td></td>
<td>A2500G</td>
<td>I834V</td>
<td>1</td>
<td>WT</td>
<td>WT</td>
<td>WT</td>
<td>Acral</td>
</tr>
<tr>
<td></td>
<td>T2516C</td>
<td>L839P</td>
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<td>WT</td>
<td>WT</td>
<td>WT</td>
<td>Mucosal</td>
</tr>
<tr>
<td></td>
<td>G2522A</td>
<td>R841K</td>
<td>1</td>
<td>WT</td>
<td>WT</td>
<td>WT</td>
<td>Mucosal</td>
</tr>
<tr>
<td></td>
<td>C2533T</td>
<td>H845Y</td>
<td>1</td>
<td>WT</td>
<td>V600E</td>
<td>WT</td>
<td>Non-CSD</td>
</tr>
<tr>
<td></td>
<td>A2546G</td>
<td>Y849C</td>
<td>1</td>
<td>WT</td>
<td>WT</td>
<td>NA</td>
<td>Acral</td>
</tr>
<tr>
<td></td>
<td>G2588A</td>
<td>G853D</td>
<td>1</td>
<td>WT</td>
<td>WT</td>
<td>WT</td>
<td>Acral</td>
</tr>
</tbody>
</table>

Abbreviations: NA, not available; Unknown, melanomas of unknown primary lesion.

**Figure 1.** Distribution of mutations in PDGFRA. PDGFRA somatic mutations found in our study and TCGA database were shown. The mutations found in our study were underlined. Boxes represent functional domains: I–V, five immunoglobulin-like domain; TM, transmembrane domain; JM, juxtamembrane domain; TK1, tyrosine kinase domain 1; KI, kinase insert domain; TK2, tyrosine kinase domain 2.
subtypes ($P = 0.419$). Nevertheless, $PDGFRA$ mutations were more commonly seen in acral and mucosal melanomas, whereas non-CSD and CSD melanomas were less likely to harbor $PDGFRA$ mutations. When comparing $PDGFRA$ mutations in three subtypes of melanomas (acral, mucosal, and nonacral cutaneous melanomas), we found that the $PDGFRA$ mutation frequency was still not significantly different ($P = 0.176$).

Functional analysis of $PDGFRA$ mutations

To investigate whether the somatic mutations could induce $PDGFRA$ protein auto-activation, 12 novel missense mutants were overexpressed in 293T cells. The tyrosine phosphorylation levels of these PDGFRAs were analyzed by Western blotting. Because D842V was demonstrated to be the most common activating mutation in GISTs, it was used as the positive control. Strong phosphorylation was observed in P577S, V658A, R841K, H845Y, and G853D mutations without the stimulation of PDGF ligand, whereas the other mutations showed weak to medium phosphorylation (Fig. 2A). The five mutations were then transfected into Ba/F3 cells, and the results showed that transfected Ba/F3 cells could grow after G418 selection and withdrawal of IL-3 (Fig. 2B). Therefore, $PDGFRA$ gain-of-function mutations may be gain-of-function mutations. The substitution mutations P577S and V658A may be homologs for the P573S and V654A mutations reported in KIT respectively (33, 34).

In vitro sensitivity of $PDGFRA$ gain-of-function mutations to imatinib and crenolanib

To analyze the effects of imatinib and crenolanib on gain-of-function mutations of $PDGFRA$, the representative $PDGFRA$ mutants were expressed in Ba/F3 cells. The tyrosine phosphorylation status of PDGFRAs was detected under different drug concentrations. As others have demonstrated that D842Y was sensitive to imatinib while D842V was resistant to imatinib, they were used as positive and negative control separately (13). Phosphorylation induced by D842Y was completely inhibited at the concentration of 1 $\mu$mol/L while D842V was still strongly phosphorylated (Supplementary Fig. S4). D842V was fully inhibited at the crenolanib concentration of 0.1 $\mu$mol/L, being consistent with Heinrich and colleagues’ report (24). The six testing mutations were all sensitive to crenolanib and the phosphorylation was completely inhibited at the concentration of 1 $\mu$mol/L. V561A, P577S, R841K, H845Y, and G853D were sensitive to imatinib, only V658A was imatinib-resistant. Therefore, imatinib and crenolanib had same inhibitory efficiency on V561A and G853D, whereas crenolanib showed stronger inhibitory effect than imatinib on P577S, V658A, R841K, and H845Y (Fig. 3A).

To confirm the observations, cell proliferation assays were performed in selected $PDGFRA$ mutants. IL-3–independent growth of V658A and D842V was inhibited by crenolanib, while still sustained in the presence of imatinib. R841K and D842Y induced IL-3–independent growth was almost fully inhibited by either imatinib or crenolanib at

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Mutation</th>
<th>WT</th>
<th>$P$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (yr)</td>
<td></td>
<td>55.1 ± 11.9</td>
<td>54.3 ± 14.7</td>
</tr>
<tr>
<td>Gender (F, %)</td>
<td></td>
<td>9 (56.2)</td>
<td>177 (52.8)</td>
</tr>
<tr>
<td>Thickness (mm)</td>
<td></td>
<td>5.85 ± 2.66</td>
<td>4.87 ± 2.64</td>
</tr>
<tr>
<td>Ulceration N (%)</td>
<td></td>
<td>9 (69.2)</td>
<td>174 (58.4)</td>
</tr>
<tr>
<td>Stage N (%)</td>
<td></td>
<td>0 (0.0)</td>
<td>12 (100.0)</td>
</tr>
</tbody>
</table>

Subtypes N (%) |          | 0.419 |
| Acral          |          | 9 (6.8) | 123 (93.2) |
| Mucosal        |          | 4 (3.6) | 106 (96.4) |
| Non-CSD        |          | 1 (1.8) | 55 (98.2) |
| CSD            |          | 0 (0.0) | 18 (100.0) |

Abbreviations: Unknown, melanomas of known primary.
Moreover, P577S and G853D induced IL-3–independent growth could also be inhibited by either imatinib or crenolanib to different extent. However, both imatinib and crenolanib could not significantly inhibit IL-3–dependent growth of Ba/F3 cells even at the concentration of 1 μmol/L (data not shown).

Discussion

The treatment of advanced melanoma is still challenging. Ipilimumab (Yervoy) and vemurafenib (Zelboraf) have been approved by the FDA for the treatment of advanced melanoma. Ipilimumab is the only monotherapy reported to prolong OS with the longest duration time, but the overall response rate is only limited, and side effects can be severe due to hepatotoxicity and immune-related adverse events (35, 36). Unlike immunotherapy, targeted therapeutic agents showed fewer side effects. Vemurafenib is the first targeted inhibitor approved by FDA and European Commission for the treatment of patients with advanced melanoma. About 50% of metastatic melanomas harboring BRAV600E and majority of these patients responded to vemurafenib, with a median OS of approximately 16 months. But the response only lasts 5 to 8 months, and most patients eventually progressed because of acquired resistance (3, 8, 37, 38). The prevalence of BRAF mutation is only 25.5% in Chinese patients with melanoma, which was due to acral and mucosal melanomas have much lower BRAF mutation rates (8, 10). KIT mutation is another common genetic aberration in acral and mucosal melanomas, as acral and mucosal melanomas are the common subtypes in China; the total mutation rate of KIT in Chinese patients with melanoma is much higher than that in Caucasians, such as in Australia and Portland (39, 40). The rate of total disease control was about 50% when patients with melanoma harboring KIT mutations were treated with imatinib, but acquired resistance developed within 3 to 4 months (5, 6). These data indicate that other druggable therapeutic targets are needed for melanoma.

In this study, we have examined the frequency and function of PDGFRα mutations in a cohort of 351 Chinese patients with melanoma. The overall mutation rate in our study was 4.6%. Mutations of PDGFRα are more likely to be happened in acral and mucosal melanomas, as indicated by higher mutation incidence than other subtypes. On the basis of the literature to date, only few studies have examined PDGFRα mutation analysis in melanoma, and no PDGFRα exon mutation was reported before (18–20). There were only 9 cases of acral and 13 mucosal melanomas in these reports. The discrepancy may be due to small sizes of their cohorts, especially the acral and mucosal samples were limited. Although KIT and PDGFRα belong to the same RTK subfamily, the PDGFRα mutation frequency in Chinese patients with melanoma is much lower than KIT (10.8%; ref. 9). The PDGFRα mutations were all detected in KIT WT melanomas and in none of the KIT-mutant melanomas. Recently, TCGA database published a dataset of exon sequencing results of 225 cutaneous melanomas. In this cohort, 23 different PDGFRα mutations were found in 21 melanomas (9.3%; 21 of 225), whereas only 3.1% (7 of 225) harbored KIT mutations (31). Their PDGFRα mutation frequency was higher than that in our cohort, which may be due to the fact...
that whole exons were sequenced, whereas we only sequenced the mutation hotspot of PDGFRA; in their cohort, the frequency of PDGFRA mutations in exon 12, 14, and 18 was 1.3% (3 of 225). In addition, the cases in the TCGA database were most likely from Caucasian patients and race difference may also play a role. In the PDGFRA mutation samples reported by TCGA, six were found with BRAF mutations and eight were found with NRAS mutations simultaneously; in the seven melanoma samples with KIT mutations, two NRAS mutations were detected. On the basis of our data and the data from TCGA, PDGFRA and KIT mutations seem to be mutually exclusive. Although we detected three PDGFRA mutations (A633T, H845Y, and A663V) in melanomas that simultaneously contained BRAFV600E or NRASQ61K mutations, two of them (A633T and A663V) did not cause auto-phosphorylation of PDGFRA, suggesting that these PDGFRA mutations may not affect the kinase activity and additional studies are needed to explore the function of these mutations.

Unlike BRAF mutations, there is no hotspot in PDGFRA mutations, and no overlapping mutation was found in different patients in our study or in the TCGA database. Exons 12, 14, and 18 of PDGFRA, which are more commonly mutated in other malignancies, encode for juxtamembrane domain, ATP-binding domain, and phosphotransferase domain, respectively. Our results showed no significant difference in the incidence rates of the three exons, quite different from the mutation distribution in GISTs with the highest mutation frequency detected within exon 18 (13, 41). Together with our functional analysis and the previous studies, almost half of the mutations found in our study can induce strong and constitutive activation of PDGFRA, although we cannot exclude the possibility that weak activation may also exert same effects on the downstream pathways.

Imatinib has been used for the treatment of patients harboring PDGFRA mutations for years. The mutational status of PDGFRA may be a significant predictive factor for response to imatinib. Similar to the facts in GISTs that PDGFRA mutation status affect the response to imatinib, our results showed that the sensitivity of PDGFRA to imatinib was correlated with the mutational types, with five of them (V561A, P577S, R841K, H845Y, and G853D; in except of V654A substitution) in inhibiting PDGFRA mutations, evidenced by the fact that all the mutations causing auto-phosphorylation could be fully inhibited by crenolanib. It has been reported that imatinib and crenolanib had different activity spectrum according to the PDGFRA mutation status, and crenolanib was more potent in imatinib-resistant mutations (e.g., D842V, D842I, etc.), whereas imatinib was more potent in inhibiting V561D mutation (24). On the basis of our study, it seems that crenolanib has a broader efficacy spectrum than imatinib in inhibiting PDGFRA activity.

To sum up, our study has confirmed that a small population of Chinese patients with melanoma harbor PDGFRA mutations. Gain-of-function mutations of PDGFRA can lead to ligand-independent activation of PDGFRA and this can be inhibited by TKIs. Our study suggested that patients with melanoma harboring PDGFRA mutations may respond to TKIs-based targeted therapy, thus warranting phase II clinical trials of imatinib or crenolanib in these patients.

Disclosure of Potential Conflicts of Interest
No potential conflicts of interest were disclosed.

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Large-scale Analysis of *PDGFRA* Mutations in Melanomas and Evaluation of Their Sensitivity to Tyrosine Kinase Inhibitors Imatinib and Crenolanib

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