Preclinical Development

Tyrosine Phosphatase Inhibitor-3 Sensitizes Melanoma and Colon Cancer to Biotherapeutics and Chemotherapeutics

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Abstract

Drug resistance is a major obstacle in cancer treatments and diminishes the clinical efficacy of biological, cytotoxic, or targeted therapeutics. Being an antiapoptotic mediator of chemoresistance in breast and lung cancer cells, MKP1 phosphatase might be targeted for overcoming chemoresistance and improving therapeutic efficacy. In this work, tyrosine phosphatase inhibitor-3 (TPI-3) was identified as a novel small molecule inhibitor of MKP1 and was capable of sensitizing tumors to bio- and chemotherapeutics in mice as a tolerated oral agent. Effective against recombinant MKP1, TPI-3 selectively increased MKP1 phosphosubstrates in Jurkat cells and induced cell death via apoptosis at nanomolar concentrations. TPI-3 also increased MKP1 phosphosubstrates in WM9 human melanoma cells and synergized with biotherapeutic IFNα2b in the growth inhibition of melanoma cells in vitro (combination index, <1). WM9 xenografts unresponsive to individual agents were significantly inhibited (62%, P = 0.001) in mice by a tolerated combination of oral TPI-3 (10 mg/kg, 5 d/wk) and IFNα2b. MKP1 expression was detected in human melanoma cell lines and tissue samples at levels up to six times higher than those in normal or nonmalignant melanocytes. TPI-3 also interacted positively with chemotherapeutics, 5-fluorouracil/leucovorin, against MC-26 colon cancer cells in vitro and in mice. Altogether, our data show the preclinical activities of TPI-3 in overcoming cancer resistance to bio- and chemotherapeutics, implicate MKP1 as a drug-resistant molecule in melanoma, and support the targeting of MKP1 for improving cancer therapeutic efficacy. Mol Cancer Ther; 9(8); 2287–96. ©2010 AACR.

Introduction

Drug resistance is common in many types of cancers. It could be intrinsic but is often acquired following initial treatments. It is a major obstacle for clinical efficacy of biological, cytotoxic, or targeted therapeutics (1–4). Identification of the mediators of drug resistance and understanding their mechanism(s) of action could provide insights for overcoming resistance and lead to novel approaches for improving cancer treatments.

MKP1 (mitogen-activated protein kinase phosphatase-1, dual-specificity phosphatase 1, and CL-100) was identified recently as a mediator of resistance to several chemotherapeutics in breast and lung cancer cells (5–7).

Transient or stable overexpression of MKP1 in breast cancer cells enhanced viability in the face of treatment with alkylating agents (mechlorethamine), anthracyclines (doxorubicin), and microtubule inhibitors (paclitaxel; ref. 5). Overexpression of MKP1 rendered lung cancer cells resistant to cisplatin, whereas knocking down MKP-1 expression with short interfering RNA sensitized lung cancer cells to cisplatin (6, 7). The clinical significance of these observations is indicated by the frequent overexpression of MKP1 in many cancer tissues including breast cancer, colon cancer, lung, prostate cancer, ovarian cancer, and pancreatic cancer (6, 8–12). The potential clinical effect is underscored by the association of MKP1 overexpression with early transformation and poor prognosis (9–11).

MKP1 mediates chemoresistance in a significant part via its antiapoptotic activity through dephosphorylating/inactivating c-Jun-NH2-kinase (JNK), a proapoptotic signaling molecule (13). Overexpression of MKP1 in breast cancer cells reduced caspase activation induced by chemotherapeutics (5). Knocking down MKP1 expression in cancer cells increased apoptosis (12, 14). Among the MKP1 substrates (JNK, p38, and ERK1/2; refs. 15, 16), JNK was reported as a key molecule in MKP1 antiapoptotic action. Inducible MKP1 expression blocked JNK activation and inhibited apoptosis (17), whereas suppression of MKP1 expression potentiates JNK activation coincident with apoptosis (14). Interestingly, MKP-1...
is also a negative regulator of innate and adaptive immune responses (18).

Being an antiapoptotic mediator of chemoresistance in several types of cancers (6–12, 19), MKP1 is a potential cancer therapeutic target. Indeed, MKP1-inhibitory compounds active at low micromolar ranges induced cancer cell death in culture and sensitized cancer cells to chemotherapeutics in vitro (20). However, the effectiveness and safety of targeting MKP1 as an anticancer strategy in vivo remain to be established. MKP1 inhibitors with preclinical antitumor activity in vivo have not been reported. It has not been clear whether MKP1 is also significant in cancer resistance to biotherapeutics.

In this work, a novel MKP1 inhibitor was identified and called tyrosine phosphatase inhibitor-3 (TPI-3). Being a small organic compound (274 Da) with defined structure (Fig. 1) and of little prior interest, TPI-3 has not been reported for biological activities. The compound was included in chemical collections for high-throughput screening and found inactive for the measles virus RNA polymerase or 14-3-3 molecule (PubChem). Herein, we provide preclinical evidence demonstrating that TPI-3 improved the efficacy of bio- and chemotherapeutics for melanoma and colon cancer in mice as a tolerated oral agent. Our data suggest that targeting MKP1 could be an effective and tolerated strategy for overcoming resistance to multiple types of anticancer agents and implicate TPI-3 as a promising lead compound for further development.

Materials and Methods

Cells, cell culture, and reagents

Cancer cell lines including Jurkat (21), WM9 (22), A375 (23), MCF-7 (24), DU145 (25), SW620 (26), and MC-26 (27) were obtained from colleagues and cultured in RPMI 1640 or DMEM supplemented with 10% FCS. The effects of chemical compounds on cancer cell growth in culture were quantified by MTT assays following established procedures (28). Normal human melanocytes and human melanoma cell lines were from the institutional melanoma core.

Recombinant human IFNα2b (specific activity, 2 × 10^8 units/mg protein, Intron A, Schering-Plough), recombinant MKP1 and recombinant SHP-1 have been described previously (29). TPI-2 (L6, Chembridge), its analogues (a1-a6, Chembridge), 5-fluorouracil (5FU; Sigma), and leucovorin (LV; Sigma) were purchased from the indicated commercial sources. Antibodies to pERK1/2, ERK1/2, p-p38, pJNK, pLck-pY394, pZAP70, and actin were obtained from a commercial source (Cell Signaling).

Chemical library, databases, and phosphatase assay

A library of drug-like chemical compound was from a commercial source (Chembridge). Upon identification of TPI-2 as a MKP1-inhibitory lead, we sought to derive improved compounds. However, the lack of crystal or solution structure of MKP1 has prevented the rational design for analogues. Moreover, the limited sequence homology (∼50%) of the MKP1 protein to the other related phosphatases with resolved structures also hindered the development of a computer-assisted three-dimensional model. Accordingly, we initially focused on the identification and evaluation of analogues with chemically structural similarities to TPI-2. Analogues of TPI-2 were identified from chemical databases by computer-assisted structure analyses. Briefly, chemical structure of TPI-2 was compared with individual structures in commercial chemical databases (Chembridge and Asinex) of approximately

Figure 1. Identification of MKP1 inhibitors TPI-2 and TPI-3. A, chemical structure of TPI-2 (L6). B, Jurkat cells treated with TPI-2 for 30 min were analyzed by Western blotting to quantify pERK1/2 and ERK1/2 levels. C, chemical structures of TPI-2 analogues. D, activities of recombinant MKP1 in the presence of TPI-2 or its analogues (1 μg/mL) in phosphatase assays (mean ± SD of duplicates).
1 million small organic compounds, using the software at the commercial sites for calculating structural similarities with TPI-2. Six compounds with similarities ≥70% with TPI-2 were selected and purchased from a commercial source (Chembridge). Their effects on the phosphatase activity of recombinant MKP1 in vitro were evaluated following established procedures (29).

**Induction and detection of cellular phospho-proteins**

Cells in culture medium were treated with agents for designated times and concentrations at 37°C and lysed on ice for 30 minutes in cold lysis buffer [1% NP40, 50 mmol/L Tris (pH 7.4), 150 mmol/L NaCl, 20 mmol/L NaF, and 0.2 mmol/L Na3VO4] containing a cocktail of proteinase inhibitors (10 mL/tablet; Sigma). The lysates were cleared by centrifuging (14,000 rpm, 10 minutes) in a microfuge at 4°C to remove insoluble parts, mixed with equal volumes of 2× SDS-PAGE sample buffer, boiled for 5 minutes, and analyzed by SDS-PAGE/Western blotting with commercial antibodies as described previously (28, 30, 31). Relative intensities of phospho-protein bands were quantified through densitometry analysis.

**Induction and quantification of apoptotic cells**

Jurkat cells were cultured for 16 hours in the presence of TPI-2, a1, a2, or TPI-3 (0, 0.1, and 1 μg/mL, respectively), washed thrice in PBS, and stained with the DNA dye 7-AAD (Invitrogen) and phycoerythrin-conjugated Annexin V antibody (BD Biosciences) in darkness at room temperature for 30 minutes. Following staining, the samples were washed thrice, resuspended in 200 μL of 1% paraformaldehyde solution, and analyzed (20,000 cells/sample) using a BD FACSCalibur cytometer as described previously (32, 33).

**Statistical analysis**

Median effect analysis (34), which provides the most general form of studying the interactions between drugs, was used to analyze the interaction between TPI-3 and IFNαβ. Median effect plots were generated for IFNα2b alone, TPI-3 alone, and the combination in inhibiting the growth of WM9 melanoma cells in culture. The combination index (CI) was determined and plotted versus the fraction affected (fa). Data were analyzed in both modes, mutually exclusive and mutually nonexclusive. The interaction between two mutually nonexclusive drugs is described by the equation CI = D1/Dx1 + D2/Dx2 + D1D2/Dx1Dx2, where Dx1 and Dx2 are the doses of drug 1 and drug 2 that are required to inhibit growth x%. D1 and D2 in combination also inhibit growth x% (i.e., drug 1 and drug 2 are isoeffective). When CI < 1, drugs are synergistic, when CI = 1, drugs are additive, and when CI > 1, drugs are antagonistic. Student's t test was used to assess the significance of the effects of different treatments against tumors in mice.

**Evaluation of MKP1 expression in melanoma cell lines and melanoma tissues**

The expression levels of MKP1 transcripts in normal human melanocytes and human melanoma cell lines were quantified by gene expression array analysis following established procedures (35). Briefly, RNA samples were harvested from cells (~80% confluence) lysed in TRIzol (Invitrogen) and evaluated with the Illumina Sentrix Human-6_v2 Expression BeadChip technology. Data were analyzed with BeadStudio V3.2 software. Total cell lysates were also prepared (28) from cancer cell lines and endothelial cell line EA.hy926 (36). The protein levels of MKP1 in the lysates were quantified by SDS-PAGE/Western blotting (28) using a commercial antibody for the phosphatase (Santa Cruz Biotechnology).

MKP1 expression in human melanoma tissue samples was investigated via immunohistochemistry analysis of an institutional melanoma tissue microarray. The array consisted of formalin-fixed, paraffin-embedded tissues from human melanoma samples of radial growth phase (15) or vertical growth phase (11), and tissue samples of nonmalignant human nevus (5). Sections of the array were prepared and stained for immunohistochemistry (32) with a commercial MKP1 antibody (Santa Cruz Biotechnology) that was reported for immunohistochemical detection of the human molecule (37). The relative MKP1 staining levels in melanoma cells and adjacent benign melanocytes in the tissue samples were estimated via microscopy.

**Animal and animal studies**

To evaluate the interactions of TPI-3 with IFNαβ against melanoma in vivo, athymic nude mice (nu/nu, NCR, female, 6 weeks old; Taconic Farms) were inoculated (s.c.) in the flanks with WM9 human melanoma cells (2 × 106 cells/site). On day 4 postinoculation, the mice were subjected to treatment with vehicle control, TPI-3 (10 mg/kg, orally 5 d/wk) via oral gavage, IFNα2b (500,000 units/mouse, s.c., 5 d/wk), or the combination. The TPI-3 dose was chosen based on its tolerance in mice in a pilot experiment. The dose of IFNα2b used for treatment was comparable with those in previous studies (28, 38) but was given only 5 days per week instead of the previous daily treatment. For assessing interactions between TPI-3 and 5FU/LV, BALB/c mice (female, 6 weeks old; Taconic Farms) were inoculated with MC-26 colon cancer cells (s.c., 106 cells/site). Starting on day 4 postinoculation, the mice were treated with vehicle control, TPI-3 (10 mg/kg, orally 5 d/wk), 5FU/LV (50 mg/kg–100 mg/kg, i.p., weekly), or the combination. The 5FU/LV treatment was similar to those reported previously (39). Tumor volume was measured and calculated using the formula for a prolate spheroid (V = 4/3 π a2b; ref. 40). Mouse viability and body weights were also recorded. Internal organs of the mice were inspected visually upon their termination at the end of the experiment. All studies involving mice were approved by the Institutional Animal Care and Use Committee of the Cleveland Clinic.
Results

Identification of MKP1 inhibitors TPI-2 and TPI-3

Given the role of MKP1 in cancer chemoresistance, we sought to identify small molecule inhibitors for the phosphatase. Lead six (L6; Fig. 1A), a compound from a chemical library, increased MKP1 phosphosubstrate pERK1/2 (41) in Jurkat leukemia cells (Fig. 1B) in which MKP1 and the substrate were expressed (42). This prompted us to investigate the MKP1 inhibitory activities of L6 and its analogues, six of which (L6a1-6; Fig. 1C) were identified based on structural similarities to the parental compound. L6 and analogue L6a6 reduced the activity of recombinant MKP1 in vitro, whereas the other analogues were less effective or not effective (Fig. 1D). These data indicated L6 and L6a6 as MKP1 inhibitors. They were named as TPI-2 and TPI-3 for further investigation.

The potency and selectivity of the compounds for cellular MKP1 were evaluated via quantification of cellular MKP1 phosphosubstrates levels. TPI-2 and TPI-3 increased all three of the MKP1 phosphosubstrates in Jurkat cells with effective concentrations at 0.1 to 1 μg/mL (Fig. 2A, left), suggesting a capacity to inhibit the cellular MKP1 (cellular IC50, 0.1–1 μg/mL). Consistent with their lack of activity in vitro (Fig. 1D), analogue L6a1 only increased pERK1/2 but not the other MKP1 phosphosubstrates whereas L6a2 did not increase any of the three phosphosubstrates (Fig. 2A, left). Suggesting a selective action, TPI-2 at 0.001 to 1 μg/mL had no apparent effect on SHP-1 phosphosubstrates (pLck-pY394 and pZAP-70) in Jurkat cells (Fig. 2B and D), in which the expression of these molecules were reported (43), or on the general cellular phospho-proteins (Fig. 2C). Similarly, TPI-3 treatment also had little effect on the cellular SHP-1 substrates (Fig. 2D). In further support, TPI-2 and TPI-3 at 1 μg/mL or lower concentrations had little effect on the activity of recombinant SHP-1 (data not shown). Moreover, the durability of cellular MKP1 phosphosubstrates induced by TPI-3 was substantial at more than 4 hours (Fig. 2A, right), indicating a significant effect of TPI-3 on these substrates. These data together provided evidence that intracellular MKP1 was selectively inhibited by TPI-2 or TPI-3 at ~1 μg/mL for a duration of more than 4 hours.

TPI-2 and TPI-3 induce cell death via apoptosis

Being an antiapoptotic molecule in cancer cells, MKP1 might be targeted with inhibitors to induce cell death via apoptosis. TPI-2, TPI-3, and the negative control analogues a1 and a2 were therefore evaluated for their capacities to induce Jurkat cell apoptosis and affect cancer cell viability in vitro.

Apoptotic cells, as indicated by surface Annexin V expression, were induced markedly by TPI-2 (52%) and TPI-3 (58%) in Jurkat cells cocultured with compounds at 1 μg/mL for 16 hours (Fig. 3A). The two leads at a lower dose (0.1 μg/mL) also induced apoptotic cells with less potency (~23–28%; Fig. 3A). Analogue a1 failed to induce cell apoptosis under comparable conditions, whereas a2 was modestly effective only at 1 μg/mL (Fig. 3A). Jurkat cells were completely dead when cultured in the presence of TPI-2 or TPI-3 at 0.3 μg/mL or higher levels for 6 days (Fig. 3B), but were only partially inhibited (<50%) by the other analogues (Fig. 3B). TPI-2 and TPI-3 also induced cell death in a panel of cancer cell lines in vitro whereas the control a2 was not effective (Fig. 3C–H). The responsive cell lines include those of melanoma (WM9 and A339), breast cancer...
(MCF-7), prostate cancer (DU-145), and colon cancer (SW620 and MC-26).

These results showed that TPI-2 and TPI-3 had pro-apoptotic activity in Jurkat cells (Fig. 3) at concentrations effective for MKP1 inhibition (Fig. 2), and suggested a similar action for the leads in various cancer cell lines in vitro.

**TPI-3 interacts positively with IFNα2b in growth inhibition of WM9 melanoma in vitro and in mice**

Being an antiapoptotic mediator of chemoresistance, MKP1 might be targeted with inhibitors for increased responses to cancer therapeutics. Accordingly, TPI-3 was evaluated for its ability to overcome drug resistance in cancer cells. Because WM9 human melanoma cells were resistant to growth inhibition by melanoma therapeutic IFNα2b (28), the capacity of TPI-3 to sensitize melanoma cells to IFNα2b in vitro and in vivo was determined.

TPI-3 enhanced the growth inhibition of WM9 cells by IFNα2b in culture (Fig. 4A) in a synergistic manner (CI < 1.0) as defined by median effect analysis (40). The two agents also interacted positively against WM9 xenografts in nude mice (Fig. 4B). Their combination significantly inhibited WM9 tumor growth (62%, \( P < 0.001 \)), in contrast to the limited effects from single agents (Fig. 4B). It remained effective beyond the point when the control mice had to be terminated due to large tumor burden and tumor ulceration (Fig. 4B). TPI-3 alone and its combination were tolerated: all the mice survived to the end of study with comparable body weights (Fig. 4C) and no obvious abnormalities in behaviors or gross anatomy (data not shown).

For mechanistic insights, the effect of TPI-3 on phosphosubstrates of MKP1 in WM9 cells in culture was determined. TPI-3 increased cellular pJNK levels and was effective starting at \( \sim 0.1 \, \mu\text{g/mL} \) (Fig. 4D). pJNK in WM9 cells was also increased by TPI-2 but not by control analogues a1 or a2 (Fig. 4D). Cellular pERK1/2 and pp38 were present at high basal levels in WM9 cells and was 10 times higher than those in Jurkat cells (data not shown). They were not increased further by TPI-3 treatment (Fig. 4D). The samples were further evaluated for pStat1 and pStat3 molecules. Cellular levels of pStat1 and pStat3 in WM9 cells were not significantly affected by treatments with TPI-3 (0.001–1 \( \mu\text{g/mL} \)) under these experimental conditions (data not shown).
These results showed that TPI-3 sensitized IFNα2b-refractory WM9 melanoma to the cytokine in vitro and in vivo. Consistent with targeting MKP1, TPI-3 increased pJNK levels in melanoma cells that expressed the phosphatase. Its lack of effect on cellular pERKs and pp38 was likely due to high basal levels of the phosphoproteins, which were downstream events of the activating B-RafV600E mutation (44, 45) that was common in melanoma (46) and detected in WM9 cells (data not shown). Given the synergy between TPI-3 and IFNα2b (Fig. 4), additional studies of TPI-3 on IFNα2b signaling in WM9 cells might provide mechanistic insights.

MKP1 is expressed at significant levels in human melanoma cell lines and melanoma tissue samples

The capacity of TPI-3 to sensitize WM9 melanoma to IFNα2b supported targeting MKP1 for improving melanoma treatments. However, it was unclear whether and at what levels MKP1 was expressed in human melanoma. We therefore assessed the expression of MKP1 in human melanoma cell lines and melanoma tissue samples.

MKP1 transcripts in human melanoma cell lines and normal human melanocytes cultured under comparable conditions were quantified by gene expression array analysis as described previously (35). The MKP1 transcripts were significantly higher (~1- to 6-fold) in the melanoma cell lines in comparison with normal human melanocytes (Fig. 5A). Expression of the MKP1 protein in human melanoma cell lines and several other cancer cell lines was verified (Fig. 5B and C).

The MKP1 protein was detected in tissue samples of advanced human melanoma by immunohistochemistry at levels approximately three times higher than those in benign melanocytes or melanocytic nevus cells (Table 1; Fig. 5D). MKP1 protein was present predominantly in the nuclei, as reported for other types of cancer cells (5, 7). Significant MKP1 staining (+++) was detected in ~90% of the tissue samples of radial or vertical growth melanoma in contrast with the weaker MKP1 staining in nonmalignant human melanocytes (0/+ or nevus cells (+) (Table 1; Fig. 5D).

MKP1 was therefore expressed in human melanoma cells and melanoma tissues and often at levels several folds higher than those in normal or nonmalignant melanocytes.

TPI-3 interacted positively with 5FU/LV against MC-26 colon cancer tumors in mice

To extend the observed activity of TPI-3 to sensitize melanoma response to IFNα2b, the capacity of TPI-3 to sensitize other types of cancers to chemotherapeutics was investigated. For this, we determined the capacity of TPI-3 to sensitize MC-26 colon cancer tumors in vivo to 5FU/LV, a backbone of chemotherapy (47). Consistent with the reported expression of MKP1 in colon cancer tissues (8), MKP1 protein was detected in MC-26 cells (data not shown).
MC-26 tumor growth was inhibited 78% by the combination, significantly more than by 5FU/LV (54%, \( P = 0.011 \)) or TPI-3 (18%, \( P = 0.004 \)) as single agents (Fig. 6A). The increased inhibition of tumor growth by the combination suggested a positive interaction between TPI-3 and 5FU/LV. TPI-3 and 5FU also interacted positively in growth inhibition of MC-26 cells in culture (Fig. 6B). Supporting a mechanism of action targeting MKP1 for sensitization, TPI-3 increased MKP1 phosphosubstrates in MC-26 cells (Fig. 6C). The compound also had the capacity to interact with 5FU against human colon cancer cells (HT-29) \( \text{in vitro} \) (Fig. 6D).

**Discussion**

In the current study, we provide evidence for the first time that MKP1 inhibitor TPI-3 sensitized cancer cells to IFN\(\alpha_2\)b and 5FU \(\text{in vitro} \) and in mice. TPI-3 sensitized WM9 human melanoma cells \(\text{in vitro} \) and WM9 xenografts to the biotherapeutic IFN\(\alpha_2\)b (Fig. 4). It also sensitized MC-26 colon cancer cells to colon cancer chemotherapeutic 5FU \(\text{in vitro} \) and in mice (Fig. 6). These actions of the compound were likely mediated via targeting MKP1 given that TPI-3 was an inhibitor of recombinant MKP1 (Fig. 1) and was capable of increasing MKP1 phosphosubstrates in both WM9 and MC-26 cells (Figs. 4 and 6). Our data thus indicate that targeting MKP1 could be an effective strategy for sensitizing differential cancers to both biotherapeutics and chemotherapeutics \(\text{in vitro} \) and \(\text{in vivo} \). Moreover, the tolerance of TPI-3 and its combinations with cancer therapeutics suggests that the strategy is safe and supports further translational evaluation. Additional studies are clearly needed to define the role of MKP1 as the target of TPI-3. In particular, assessment of the responses of cancer cells with differential MKP1 expression levels to TPI-3 could be of significance and might provide mechanistic insights.

The effectiveness and tolerance of TPI-3 in sensitizing melanoma and colon cancer to therapeutics in mice also suggested the potential of the compound as a lead for developing MKP1-targeted agents to overcome drug resistance.
resistance. The oral availability (Figs. 4 and 6) of TPI-3 further increases its attractiveness for translational development. In addition, its chemical characteristics (data not shown) show no violation of Lipinski’s rule of five and the extensions (48), indicating good druggability. Moreover, the higher potency of TPI-3 in comparison with its structurally related TPI-2, L6a1, or L6a2, suggests potential and insights for improving potency via structural modifications. In addition, TPI-3 analogues may be exploited for developing more selective inhibitors. TPI-3 displayed selective actions for MKP1 as it was inactive for SHP-1 under these experimental conditions (Fig. 1 and 2). It is also encouraging that L6a1, although inactive for MKP1 (Figs. 1 and 2), selectively increased pERK1/2 (Fig. 2A), suggesting that it targets a pERK1/2-specific phosphatase (e.g., MKP3; ref. 18). The limited structural differences between TPI-3 and L6a1 (Fig. 1) implicate the feasibility of modulating target specificity via minor chemical variations. Whereas the full spectrum of TPI-3–targeted phosphatases remains to be established, it is worth noting that several inhibitors targeting multi-kinases have been approved as effective and tolerated cancer therapeutics (e.g., imatinib and sunitinib), suggesting translational potential for inhibitors with multi-phosphatase targets. Altogether, our data provide a basis for developing refined inhibitors against MKPs (or other phosphatases) and for biological evaluations of new candidate inhibitors.

Our results also provide the first evidence implicating MKP1 overexpression as a drug-resistant mechanism in melanomas. MKP1 overexpression was detected in human melanoma cell lines and advanced human melanoma tissues (Fig. 5). Its functional significance in therapeutic resistance was suggested by the sensitization of WM9 melanoma to IFNα2b in vitro and in mice (Fig. 4). Advanced melanoma responds poorly (~10%) to each of the three approved melanoma treatments (IFNα2b, interleukin-2, and dacarbazine; ref. 49). As indicated by TPI-3 activity in sensitizing WM9 melanoma to IFNα2b (Fig. 4), MKP1-targeted agents might be combined with melanoma therapeutics as more efficacious treatment options. Indeed, TPI-3 also near significantly increased WM9 tumor growth inhibition induced by the dacarbazine analogue temozolomide or its combination with methoxyamine (ref. 50; data not shown). Moreover, the capacity of TPI-3 to sensitize WM9 melanoma (Fig. 4), which harbors the B-RafV600E mutant with heightened downstream pERK1/2 levels (Fig. 4) also suggests that TPI-3 and other MKP1 inhibitors might be complementary with B-Raf inhibitors in antitumor actions and could be exploited for combination treatments. Mutations of B-Raf, N-Ras, and c-kit have been reported in melanoma, leading to active oncogenic molecules. Because MKP1

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While our data are promising, further research is needed to fully understand the potential of TPI-3 and other MKP1 inhibitors in the treatment of melanoma.

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*Our unpublished data.*
substrates are signaling molecules downstream of the oncoproteins, targeting MKP1 with TPI-3 is expected to be effective in melanoma with each of the mutant genotypes. Comparative evaluation of TPI-3 responses by melanoma cells with differential genotypes will be informative in this regard and could have implications for MKP1-targeted agents. Interestingly, low MKP1 expression levels were detected in ~10% samples of the advanced melanoma tissues (Fig. 5) and may correlate with better clinical responses that might be exploited for preselecting potential responding cases.

Among the three MKP1 phosphosubstrates, pJNK was the only one that was consistently induced by TPI-3 in Jurkat, WM9, and MC-26 cells (Figs. 2, 4, and 6). Thus, pJNK was implicated as a key mediator for TPI-3 in proapoptotic action and antitumor action, and could be further evaluated to define the mechanism of action of the compound and its significance as a biomarker. Because MKP1 negatively regulates innate and adaptive immune responses (18), targeting the phosphatase might lead to immune cell activation. Indeed, TPI-2 and TPI-3 were capable of increasing mouse splenocyte IFNγ+ cells in vitro,7 although immune cell activation was apparently not required for TPI-3 sensitization of WM9 melanoma to IFNα2b because the sensitization occurred in vitro in the absence of immune cells and in T cell–deficient nude mice (Fig. 4). Despite its effectiveness in inducing cancer cell death in vitro (Fig. 3), TPI-3 did not exhibit significant activity as a single agent against WM9 xenografts and had only a modest effect on MC-26 tumors (Figs. 4 and 6). This might be related to the lower doses (≤10 ng/mL) of TPI-3 required for sensitization in comparison with its death-induction doses (~100 ng/mL; Figs. 3 and 4). Taken together, our results provide a strong basis for elucidating the mechanism of action of MKP1 inhibition and for potential translation in future studies.

Disclosure of Potential Conflicts of Interest

T. Yi has a patent pending for TPI-3 and analogs.

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