Mitogen-Activated Protein Kinase Phosphatase-1 Is a Mediator of Breast Cancer Chemoresistance

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Abstract

The mitogen-activated protein kinase (MAPK) phosphatase (MKP)-1 is overexpressed in a large proportion of breast cancers, and in some systems interferes with chemotherapy-mediated proapoptotic signaling through c-Jun-NH²-terminal kinase (JNK). We therefore sought to examine whether MKP-1 is a mediator of breast cancer chemoresistance using A1N4-myc human mammary epithelial cells, and BT-474 and MDA-MB-231 breast carcinoma cells. Transient or stable overexpression of MKP-1 reduced caspase activation and DNA fragmentation while enhancing viability in the face of treatment with alkylating agents (mechlorethamine), anthracyclines (doxorubicin), and microtubule inhibitors (paclitaxel). This overexpression was associated with suppression of JNK activation, and JNK blockade alone induced similar effects. In contrast, reduction of MKP-1 levels using a small interfering RNA, or its targeted inactivation, enhanced sensitivity to these drugs, and this was associated with increased JNK activity. Pharmacologic reduction of MKP-1 by pretreatment with a novel p38 MAPK inhibitor, SD-282, suppressed MKP-1 activation by mechlorethamine, enhanced active JNK levels, and increased alkylating agent-mediated apoptosis. Combination treatment with doxorubicin and mechlorethamine had similar effects, and the enhanced efficacy of this regimen was abolished by forced overexpression of MKP-1. These results suggest that the clinical efficacy of combinations of alkylating agents and anthracyclines are due to the ability of the latter to target MKP-1. Moreover, they support the hypothesis that MKP-1 is a significant mediator of breast cancer chemoresistance, and provide a rationale for development and translation of other agents targeting MKP-1 into the clinical arena to overcome resistance and induce chemosensitization.

Introduction

Mitogen-activated protein kinase (MAPK) phosphatase (MKP)-1 is the prototypic member of a family of dual-specificity phosphatases that dephosphorylate tyrosine and threonine residues on target proteins. This phosphatase is best known for its specificity toward p44/42 MAPK (1), but recent studies showed that MKP-1 has a substrate preference for p38 MAPK and c-Jun-NH²-terminal kinase (JNK), and limits JNK activity (2, 3). Many chemotherapeutic agents induce apoptosis in part by activation of the JNK pathway (4), which causes mitochondrial cytochrome c release, leading to oligomerization of apoptotic protease activating factor-1 (Apaf-1). This cytochrome c/Apaf-1 complex recruits procaspase-9, inducing its autoactivation, with subsequent activation of the downstream effector caspase-3 (5–7). JNK family members are also involved in activation of programmed cell death by transcription-dependent processes, such as through death receptor induction (8). Given this role of JNK, the ability of MKP-1 to decrease JNK activation could be antia apoptotic. Indeed, in prostate cancer, MKP-1 expression was inversely related to apoptosis (9), and expression of MKP-1 conferred resistance to Fas-mediated apoptosis (10). In addition, conditional MKP-1 expression protected leukemia cells from UV-induced apoptosis (11), mediated the antiapoptotic effects of retinoids (12), and MKP-1 inhibition potentiated tumor necrosis factor-α–induced apoptosis in mesangial cells (13).

Studies have shown that MKP-1 is overexpressed by up to 5-fold or more (14) in primary samples from patients with breast malignancies. Such overexpression occurs in up to 80% or more of breast neoplasms ranging from carcinoma in situ to metastatic carcinoma (15, 16). In addition, MKP-1 can be further induced by genotoxic stress, such as radiation and alkylating agents (2), as well as by newer agents such as proteasome inhibitors (17). Induction of MKP-1 in response to stress is mediated in part through the p38 MAPK pathway (18), which itself is overexpressed and activated in breast cancer (14). Notably, p38 activation was found to be a poor prognostic sign in patients with lymph node–positive breast cancer (19). MKP-1 has also been identified as a putative mediator of glucocorticoid-induced survival signaling in breast cancer (20, 21). However, the significance of these findings to breast cancer therapy have not been established, nor has the potential to induce chemosensitization by targeting MKP-1 directly, or through p38 MAPK.

We previously reported that MKP-1 was induced by proteasome inhibitors (17) and played an antia apoptotic role through effects on JNK (22). This prompted us to extend our studies to chemotherapeutic agents that were clinically relevant to breast malignancies, including alkylating agents, anthracyclines, and taxanes. Stable and transient MKP-1 overexpression inhibited the ability of drugs in these classes to induce apoptosis in association with decreased JNK activation. In contrast, MKP-1 suppression with a small interfering RNA (siRNA), or targeted deletion, enhanced chemosensitivity and JNK activity. Pharmacologic MKP-1 inhibition through the use of a p38 MAPK inhibitor or doxorubicin enhanced alkylating agent–mediated apoptosis, and this was abolished by forced MKP-1 overexpression. Taken together, these studies support the hypothesis that MKP-1 is a significant mediator of both de novo and inducible breast cancer chemoresistance. Furthermore, they suggest that strategies
targeting MKP-1, such as through p38 inhibition, may prove fruitful in overcoming chemoresistance and inducing chemosensitization.

**Materials and Methods**

**Materials.** Phosphatase inhibitors deltamethrin and nodularin were from Calbiochem-Novabiochem Corp., whereas sodium orthovanadate was from Sigma Chemical Co. Phenylmethylsulfonyl fluoride (PMSF) was from Fisher Scientific. The p38 MAPK inhibitor SD-282 (23) was from Scios, Inc. Stock solutions were prepared in DMSO (SD-282 and phosphatase inhibitors; Fisher Scientific), 100% ethanol (PMSF; Mallinckrodt Baker, Inc.), or PBS (sodium orthovanadate). These reagents were used at concentrations indicated in the text, with a final vehicle concentration no >0.5%. All other chemicals were obtained from Fisher Scientific.

**Cell lines and cell culture.** A1N4-myc human mammary epithelial cells transformed by c-myc (24), and BT-474 (25) and MDA-MB-231 (26) carcinoma cells served as human breast cancer models. Preparation of MDA-MB-231 cells overexpressing MKP-1, or vector-bearing controls, was described previously (22), as was the cloning of BT-474 cells expressing an siRNA targeting MKP-1 (siMKP), or a scrambled sequence control (ssMKP; ref. 27). Mouse embryo fibroblasts (MEF) from homozygous MKP-1 knockout mice (28), and wild-type controls, were from the Bristol Myers Squibb Research Institute. All cells were propagated in incubators providing a humidified atmosphere with 5% CO2 (17, 22, 27, 29).

**Adenovirus-mediated expression.** Recombinant adenoviral plasmids expressing MKP-1 and green fluorescent protein (GFP), or GFP alone as a control, were constructed using the pAdEasy vector system (Stratagene; ref. 22). For adenoviral infections, cell lines were plated at 0.5 × 10⁶ cells per well in 96-well plates for apoptosis assays, or at 1 × 10⁶ cells per well in 24-well plates for Western blotting. Cells were allowed to recover overnight and exposed to viral particles using a multiplicity to yield 80% to 100% infection, based on GFP expression evaluated by immunofluorescence microscopy using a Zeiss Axioplan microscope (Carl Zeiss Optical, Inc.). Treatments of interest were applied 24 h later under conditions detailed in the text. For expression of dominant negative c-Jun (dn-c-Jun), adenovirus expressing GFP as a control, or dn-c-Jun, was obtained from Vector Biolabs. Cells were exposed to a multiplicity of infection of 75 for either viral preparation, and the further manipulations described were done 24 h later.

**Western blotting.** Total cellular extracts were prepared in lysis buffer containing PBS, SDS, deoxycholate, and NP40 with protease and phosphatase inhibitors, and subjected to Western blotting (22). JNK activation status was determined using rabbit polyclonal antibodies recognizing active, dually phosphorylated (Thr183/Tyr185) p54/64 JNK (Cell Signaling Technology, Inc.). Total JNK levels were determined using rabbit polyclonal JNK antibody recognizing p46 JNK (Santa Cruz Biotechnology). Rabbit polyclonal C-19 antibody to MKP-1 was used to assess MKP-1 levels (Santa Cruz Biotechnology). As a loading control in addition to JNK, a rat monoclonal antibody recognizing heat shock cognate protein (HSC)-70 was used (StressGen Biotechnologies Corp.). To quantify protein bands, autoradiographs were scanned with an Agfa Duoscan T2500 scanner (Agfa Corp.) into Adobe Photoshop 5.0 (Adobe Systems, Inc.), and densitometry was done using NIH Image version 1.61.

**Apoptosis assays.** Programmed cell death was evaluated as described (22) using the apoptosis-specific Cell Death Detection ELISAPLUS™ kit (Roche Applied Science). As a confirmatory assay in some cases, caspase activation was evaluated using the Apo-ONE Homogeneous Caspase-3/7 Assay kit (Promega Corporation). Additionally, cell proliferation and

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**Figure 1.** MKP-1 suppresses chemotherapy-mediated apoptosis. A, MKP-1 was stably overexpressed in MDA-MB-231 human breast carcinoma cells from pcDNA3/MKP-1 (pcMKP) and compared with the pcDNA3 vector control (pcDNA; left). These cells were treated with 1 μmol/L doxorubicin (dox), 10 μmol/L mechlorethamine (mech), or 100 nmol/L paclitaxel (pac) for 18 h, and programmed cell death was quantified with an assay of caspase-3/7 activity. Caspase activity was expressed as a fold increase over vehicle-treated controls, which were arbitrarily set at 1.0. Columns, mean from 12 independent experiments; bars, SE. MKP-1 was also transiently overexpressed in MDA-MB-231 cells from an adenoviral vector along with GFP (Ad-MKP/GFP), and compared with the vector control (Ad-GFP; right) as above. Statistical comparisons were done as described: **, P < 0.05; ***, P < 0.01. B, A1N4-myc human mammary epithelial cells were infected with either Ad-GFP/MKP-1 or Ad-GFP as a control; 24 h later, these cells were exposed to doxorubicin, mechlorethamine, or paclitaxel as described above. Apoptosis was evaluated with an assay detecting oligonucleosomal DNA fragmentation and expressed in relation to vehicle-treated controls, which were arbitrarily set at 1.0 (top). Columns, mean from 10 independent experiments; bars, SE. These cells were also analyzed for their viability after the described manipulations using the WST-1 reagent (bottom). Viability was expressed in relation to vehicle-treated controls, which were arbitrarily set at 100%. Columns, mean from 10 independent experiments; bars, SE. C, BT-474 human breast carcinoma cells were infected with adenoviral constructs, treated as described above, and then assayed for apoptosis (top) and viability (bottom).
Results

Effect of MKP-1 overexpression on chemotherapy-mediated apoptosis. To examine the influence of MKP-1 on chemotherapy-induced cell death in breast carcinoma models, MDA-MB-231 cells were stably transfected with pcDNA3-MKP-1, resulting in a 2-fold increase in MKP-1 (Supplementary Fig. S1A) compared with pcDNA3 controls. These cells were treated with the anthracycline doxorubicin, the alkylating agent mechlorethamine, or the microtubule inhibitor paclitaxel, and assayed for apoptosis. Mechlorethamine was used rather than cyclophosphamide because, although the latter is more clinically relevant, it must be transformed in vivo to the active metabolite 4-hydroxycyclophosphamide (30), and cannot be used in vitro. All three chemotherapeutics induced apoptosis, as measured by enhanced caspase-3/7 activity, in both cell lines (Fig. 1A, left). This enhancement was consistently greater, however, in the control MDA-MB-231/pcDNA3 cells, which did not overexpress MKP-1. In the case of mechlorethamine, for example, this alkylator induced 5.9-fold more caspase activity in MDA-MB-231/pcDNA3 cells, but only 2.6-fold more in MDA-MB-231/pcDNA3-MKP-1 cells (P < 0.01).

MDA-MB-231–based cell lines overexpressed MKP-1 but this level tended to decrease with propagation, and similar findings were noted in other cells. Therefore, an adenoviral-based expression system was used, allowing MKP-1 overexpression by up to 5-fold or more (Supplementary Fig. S1B). Doxorubicin, mechlorethamine, and paclitaxel induced caspase-3/7 activity in MDA-MB-231 cells infected with Ad-GFP or Ad-MKP/GFP (Fig. 1A, right), but this caspase activation was blunted by MKP-1. Paclitaxel, for example, enhanced caspase-3/7 activity by 4.3-fold in MDA-MB-231/Ad-GFP cells, but only 2.3-fold in MDA-MB-231/Ad-MKP/GFP cells (P < 0.01).

It was of interest to confirm this antiapoptotic role of MKP-1 in other models and to use other means to quantify programmed cell death. To this end, A1N4-myc and BT-474 cells were infected with Ad-MKP/GFP or Ad-GFP and treated as described above. Exposure of A1N4-myc/Ad-MKP/GFP cells to chemotherapeutics enhanced apoptosis, as measured by the generation of oligonucleosomal DNA fragmentation, by an average of 2.2-fold above vehicle-treated controls (Fig. 1B, top). Overexpression of GFP alone, however, allowed these drugs to induce programmed cell death to a greater extent, with an average 5.4-fold increase. This was accompanied by a preservation of viability in the Ad-MKP/GFP–infected cells compared with their Ad-GFP counterparts (Fig. 1B, bottom). Chemotherapeutic treatments resulted in an average 22% decline of viability in A1N4-myc/Ad-GFP cells, but only 2% in A1N4-myc/Ad-MKP/GFP cells. Similar results were obtained with BT-474 cells, where apoptotic induction was blunted (Fig. 1C, top), and viability was preserved to a greater extent (Fig. 1C, bottom) by Ad-MKP/GFP. These findings supported the hypothesis that MKP-1 overexpression protected breast carcinoma cells from chemotherapy-mediated apoptosis.

Overexpression of MKP-1 and chemotherapy-mediated JNK activation. Several classes of chemotherapeutics, including anthracyclines (31, 32), alkylating agents (33), and taxanes (34–36), induce apoptosis in part through JNK activation. To determine the effect of these chemotherapeutics, and of MKP-1, on JNK, Western blotting was used to detect activated, dually phosphorylated JNK. Doxorubicin, mechlorethamine, and paclitaxel induced JNK activation in A1N4-myc, BT-474, and MDA-MB-231 cells infected with Ad-GFP (Fig. 2A). Overexpression of both GFP and MKP-1, however, dramatically blunted the ability of these drugs to enhance JNK activity. For example, in A1N4-myc cells, the anthracycline, alkylator, and microtubule inhibitor increased phosphorylated (phospho-)JNK levels by 3.5-, 4.7-, and 1.6-fold more, respectively, in Ad-GFP–infected cells compared with Ad-MKP/GFP cells. To verify that this JNK repression played a role in suppressing
chemotherapy-mediated apoptosis, A1N4-myc cells were infected with Ad-GFP or with Ad-dn-c-Jun (37). When these cells were treated with doxorubicin, mechlorethamine, or paclitaxel (Fig. 2B), apoptosis-associated DNA fragmentation was induced in A1N4-myc/Ad-GFP cells. However, suppression of just one of the downstream effectors of JNK, c-Jun, decreased programmed cell death induced by these chemotherapeutics. These studies documented that MKP-1 overexpression and suppression of apoptosis was associated with blunted JNK activity and that this change played a role in modulating the levels of programmed cell death.

**Genetic suppression of MKP-1 and chemotherapy-mediated apoptosis.** If MKP-1 plays an antiapoptotic role, then inhibiting its expression should enhance chemosensitivity. To evaluate this possibility, genetic approaches were studied, including the use of an siRNA and targeted gene disruption. BT-474 cells stably expressing ssMKP, a control siRNA that does not target any known genes (22, 27), were treated with doxorubicin, mechlorethamine, or paclitaxel, all of which induced apoptosis (Fig. 3A). In BT-474 cells stably expressing siMKP, however, which suppressed MKP-1 expression (refs. 22, 27; Supplementary Fig. S1C), the extent of apoptosis was significantly enhanced, by an average of 2.2-fold. Although siMKP decreased MKP-1 levels, it did not abolish them; thus, we sought to study the effect of complete MKP-1 abrogation, such as might be possible in the future with a direct pharmacologic MKP-1 antagonist. MEFs from MKP-1 knockout (MKP−/−) mice, in which both MKP-1 alleles were disrupted (28), were therefore compared with wild-type (MKP+/+) controls. Doxorubicin, mechlorethamine, or paclitaxel induced apoptosis in MKP+/+ controls (Fig. 3B); however, MKP−/− cells were dramatically more sensitive. Treatment with doxorubicin, for example, resulted in a 12-fold increase of apoptosis in MKP−/− MEFs relative to wild-type controls, whereas for mechlorethamine there was a 10-fold difference. These studies supported the possibility that targeting MKP-1 represents a rational means of overcoming chemoresistance in breast malignancies.

**Suppression of MKP-1 and JNK activation status.** To further validate the hypothesis that MKP-1 was influencing chemotherapy-induced apoptosis through JNK, BT-474/siMKP, BT-474/ssMKP, and MKP+/+ and MKP−/− MEFs treated as above were analyzed by Western blotting. Doxorubicin, mechlorethamine, and paclitaxel enhanced the abundance of activated, dually phospho-JNK-1/2 (Fig. 4A), by 2.0-, 30.7-, and 3.5-fold, respectively, in BT-474/ssMKP cells. In BT-474/siMKP cells, however, these agents induced enhanced levels of JNK activity, as reflected by a 4.4-, 144.0-, and 4.9-fold increase, respectively, in phosphorylated JNK-1/2. In the MEF model system, the three chemotherapeutics induced a modest increase in phospho-JNK in wild-type MKP+/+ cells (Fig. 4B). Targeted MKP-1 disruption enhanced the sensitivity of JNK to

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**Figure 3.** Suppression or inactivation of MKP-1 enhances chemotherapy-mediated apoptosis. A, BT-474 cells stably expressing an siRNA to MKP-1 (siMKP) or a scrambled sequence control (ssMKP) were treated with 1 μmol/L doxorubicin, 10 μmol/L mechlorethamine, or 100 nmol/L paclitaxel for 18 h and analyzed with a DNA fragmentation assay as described previously. Columns, mean fold increase in apoptosis from 12 independent experiments; bars, SE. Statistical comparisons were done as described. *, P < 0.05; **, P < 0.01. B, wild-type MEFs (MKP+/+) or homozygous MKP-1 knockout MEFs (MKP−/−) were treated and analyzed as above. Columns, mean fold increase in apoptosis from 10 independent experiments; bars, SE.

**Figure 4.** Reduction of MKP-1 augments JNK activation. A, BT-474/ssMKP-1 (ss) and BT-474/ssMKP-1 (si) cells treated as above were analyzed for their content of activated JNK by Western blotting for phospho-JNK-1/2 levels, and the fold increase is expressed in relation to vehicle-treated controls, which were arbitrarily set at 1.0, after adjusting for equivalent loading of the JNK-1 control. Each panel is a representative result from one of two independent experiments. B, MKP-1 (+/+) and knockout (−/−) MEFs were treated with doxorubicin, mechlorethamine, or paclitaxel, and analyzed for their JNK activation status as described above.
activation by anthracyclines, alkylating agents, and microtubule inhibitors, in association with the increased apoptotic susceptibility of these cells (Fig. 3).

**Impact of p38 MAPK inhibition on MKP-1 and apoptosis.** In addition to the basal overexpression of MKP-1 seen in breast tumors, further MKP-1 induction occurs in response to genotoxic stressors, such as alkylating agents and proteasome inhibitors, in part through activation of p38 MAPK (18, 38, 39). Because clinically relevant direct pharmacologic MKP-1 inhibitors are not yet available, we considered the possibility that targeting p38 could serve to enhance chemosensitivity. BT-474 cells treated with mechlorethamine underwent programmed cell death in association with JNK activation, but this alkylation also substantially induced MKP-1 (Fig. 5A). Pretreatment with the p38 inhibitor SD-282 decreased mechlorethamine-mediated MKP-1 induction from 15.0- to 2.4-fold above controls. This was associated with a superinduction of JNK, with phospho-JNK levels increasing from 14.7- to 59.5-fold above vehicle-treated controls. To evaluate if this pharmacologic manipulation resulted in enhanced alkylating agent-mediated apoptosis, BT-474 cells were treated with mechlorethamine in the presence or absence of the p38 MAPK inhibitor (Fig. 5B). When MKP-1 was overexpressed to high levels due to p38 activation (Fig. 5A), mechlorethamine activated programmed cell death. However, when mechlorethamine-mediated MKP-1 induction was blunted with SD-282, higher levels of apoptosis were induced ($P < 0.05$).

**Interaction between anthracyclines and alkylators at the level of MKP-1.** We previously reported that anthracyclines, including doxorubicin and epirubicin, specifically suppressed MKP-1 transcription by a direct effect on its promoter (27). The above results with p38 inhibitors suggested, therefore, that the known clinical efficacy of the anthracycline/alkylating agent regimen against breast cancer might be due to its effect on MKP-1. To test this, A1N4-myc cells were infected with Ad-GFP or Ad-MKP/GFP, and then treated with vehicle, doxorubicin, mechlorethamine, or both. Lower MKP-1 levels were induced in these experiments because a lower multiplicity of infection with adenovirus vectors was used. This was necessary to allow for the detection of the higher levels of apoptosis anticipated due to the application of two antineoplastic agents as opposed to one, as well as the effect of adenoviral infection itself, within the linear range of the assay in use. A1N4-myc/Ad-GFP cells treated with the combination showed a reduction in MKP-1 compared with cells treated with the alkylator alone (Fig. 6A, left), and this was associated with enhanced phospho-JNK induction (Fig. 6A, left). Programmed cell death (Fig. 6B, left) was induced by the combination to a greater extent than a simple sum of the two agents, indicating the likely presence of synergy. In A1N4-myc/Ad-MKP/GFP cells, however, MKP-1 overexpression blunted JNK activation by the alkylating agent/anthracycline regimen (Fig. 6A, left). Notably, in the presence of higher levels of irrepressible MKP-1, this combination regimen was less active in inducing enhanced levels of programmed cell death in comparison with control, GFP-expressing counterparts (Fig. 6B, left). Under these conditions, mechlorethamine and doxorubicin had only an additive effect.

To confirm these findings, we studied BT-474 cells, and found that BT-474/Ad-GFP cells treated with the combination showed a reduction in MKP-1 compared with cells treated with the alkylating agent alone (Fig. 6A, right). This was associated with enhanced phospho-JNK induction (Fig. 6A, right), and levels of programmed cell death that were greater than the sum of the effect of each agent alone (Fig. 6B, right), supporting the presence of synergy. In BT-474/Ad-MKP/GFP cells, however, MKP-1 overexpression blunted JNK induction by the alkylating agent/anthracycline regimen (Fig. 6A, right). Importantly, in the presence of higher levels of irrepressible MKP-1, this combination regimen no longer induced apoptosis at levels higher than that achieved by either agent alone (Fig. 6B, right), supporting the presence of antagonism. These findings strongly support the hypothesis that MKP-1 suppression by anthracyclines or p38 MAPK inhibitors is a rational approach to chemosensitization to agents such as alkylators that further induce MKP-1 levels.
Discussion

Chemotherapeutic agents can be limited in their ability to induce beneficial antitumor effects by chemoresistance, which can occur in de novo or inducible forms. One example of a pathway that contributes to both is the nuclear factor-kB (NF-kB), which is activated at baseline in many cancers, including some breast malignancies, and can be further induced by genotoxic stressors, such as chemotherapeutics (40). Downstream consequences of NF-kB activation include the induction of survival-promoting Bcl-2 and inhibitor of apoptosis protein families that mediate chemoresistance. Strategies that block NF-kB activity, including overexpression of the inhibitory protein IκB, or its stabilization through proteasome inhibition that blocks IκB degradation (41), have been proven to overcome chemoresistance and to induce chemosensitization.

Our current results support the possibility that MKP-1 is an important mediator of de novo breast cancer chemoresistance because its overexpression to levels comparable with those in clinical samples inhibited the proapoptotic activity of an anthracycline, an alkylating agent, and a microtubule inhibitor (Fig. 1). This is in agreement with recent reports that MKP-1 may play a role in the glucocorticoid-mediated resistance seen in MCF-7 breast carcinoma cells in response to paclitaxel and doxorubicin (20, 21). Moreover, siRNA-mediated MKP-1 inhibition, or its complete abolition by targeted disruption, significantly enhanced the proapoptotic activity of these drugs (Fig. 3) that have clinical relevance to breast cancer in a number of settings, including adjuvant therapy (42). Mechanistic studies suggested that this effect of MKP-1 was mediated, at least in part, through its ability to modulate the activation of JNK. Overexpression of MKP-1, which inhibited apoptosis, was associated with decreased JNK activity (Fig. 2), whereas MKP-1 inhibition, which augmented apoptosis, resulted in enhanced phospho-JNK levels (Fig. 4), reflective of increased JNK activity. Also, inhibition of c-Jun, one of the downstream JNK mediators, inhibited chemotherapy-mediated apoptosis (Fig. 2), further validating the link between MKP-1, JNK, and programmed cell death.

Clinically relevant, direct MKP-1 inhibitors have not yet been developed; however, the current study suggests that they could be applicable to breast cancer, where up to 80% or more of primary samples overexpress this phosphatase (15, 16). It should be noted that MKP-1 is overexpressed in other malignancies as well, such as prostate cancer (9, 10, 16, 43). Given the early evidence of an inverse relationship between MKP-1 and apoptosis in prostate cancer (9), it is tempting to speculate that MKP-1 may mediate de novo chemoresistance in that malignancy. Our studies therefore provide an impetus for further preclinical development and clinical translation of direct MKP-1 inhibitors to overcome de novo chemoresistance. In that regard, recent results from Vogt et al. (44) and Lazo et al. (45), describing the identification of novel
agents that specifically inhibit MKP-1 in vitro at micromolar concentrations, are encouraging, and suggest that it may be possible to develop even more potent, clinically applicable inhibitors. MKP-1 is part of the stress response pathway and can be induced further by chemotherapeutics such as alkylating agents in a p38 MAPK-dependent process (18). Given the prominent role of alkylators in breast cancer therapy, we studied the effect of combinations with a p38 inhibitor and an anthracycline, which both suppress MKP-1. Regimens incorporating either a p38 inhibitor and an alkylator, or MKP-1 and doxorubicin (Fig. 5), resulted in enhanced proapoptotic activity, suppression of MKP-1, and enhanced JNK activation. Moreover, forced overexpression of MKP-1 suppressed the ability of the anthracyline/alkylating agent regimen to induce greater levels of programmed cell death, supporting the importance of inhibition of MKP-1 expression in this process. These results suggest that the clinical relevance of alkylating agent/MKP-1, which for many years were standards of care for adjuvant breast cancer therapy (42), may be due in part to doxorubicin-mediated suppression of cyclophosphamide-induced MKP-1. They also suggest that incorporation of p38 MAPK inhibitors, some of which are currently in clinical trials (46), may be a rational approach to chemosensitization to alkylating agents either in addition to, or in place of, doxorubicin. The latter approach has the potential of reducing the anthracyline-mediated cardiac toxicity associated with breast cancer chemotherapy (47). Moreover, inhibition of p38 may have other beneficial effects, such as suppression of P-glycoprotein (48), of activation of heat shock protein-27 (49), and of the AKT8 virus oncogene cellular homologue/protein kinase B (50), all of which may play roles in chemoresistance. It is also tempting to speculate that p38 MAPK activation, which is seen in a large proportion of breast neoplasms (14), and in some studies has portended a poor clinical prognosis (19), may have its effect through MKP-1-mediated chemoresistance.

Taken together, these studies strongly implicate MKP-1 as an important mediator of de novo and inducible chemoresistance. Approaches to inhibit MKP-1, such as through the development of pharmacologic agents that directly block its phosphatase activity, or through the use of p38 MAPK inhibitors that act indirectly by blocking its transcription, therefore merit further investigation as potential mechanisms to induce chemosensitization and overcome breast cancer chemoresistance.

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