Mitogen-activated Protein Kinase Phosphatase-1 (MKP-1) Expression Is Induced by Low Oxygen Conditions Found in Solid Tumor Microenvironments

A CANDIDATE MKP FOR THE INACTIVATION OF HYPOXIA-INDUCIBLE STRESS-ACTIVATED PROTEIN KINASE/c-Jun N-TERMINAL PROTEIN KINASE ACTIVITY

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Pathophysiological hypoxia is an important modulator of gene expression in solid tumors and other pathologic conditions. We observed that transcriptional activation of the c-jun proto-oncogene in hypoxic tumor cells correlates with phosphorylation of the ATF2 transcription factor. This finding suggested that hypoxic signals transmitted to c-jun involve protein kinases that target AP-1 complexes (c-Jun and ATF2) that bind to its promoter region. Stress-inducible protein kinases capable of activating c-jun expression include stress-activated protein kinase/c-Jun N-terminal protein kinase (SAPK/JNK) and p38 members of the mitogen-activated protein kinase (MAPK) superfamily of signaling molecules. To investigate the potential role of MAPKs in the regulation of c-jun by tumor hypoxia, we focused on the activation SAPK/JNKs in SiHa human squamous carcinoma cells. Here, we describe the transient activation of SAPK/JNKs by tumor-like hypoxia, and the concurrent transcriptional activation of MKP-1, a stress-inducible member of the MAPK phosphatase (MKP) family of dual specificity protein-tyrosine phosphatases. MKP-1 antagonizes SAPK/JNK activation in response to diverse environmental stresses. Together, these findings identify MKP-1 as a hypoxia-responsive gene and suggest a critical role in the regulation of SAPK/JNK activity in the tumor microenvironment.

We reported that the c-jun proto-oncogene is induced at the message and protein levels in hypoxic SiHa human squamous carcinoma cells (5). Further investigation of the mechanism of this induction demonstrated that activation of the c-jun promoter by hypoxia correlates with phosphorylation of the transactivation domain of the ATF2 transcription factor (13). Since c-Jun and ATF2 dimers are AP-1 complexes that bind to the c-jun promoter region (14), this finding suggested that hypoxic signals transmitted to the promoter are mediated in part by protein kinases that target both ATF2 and c-Jun. Stress-inducible protein kinases capable of activating the c-jun promoter include the SAPK/JNK and p38 MAPK families of the MAPK superfamily of serine/threonine kinases (15, 16). Since both SAPK/JNKs and p38 MAPK are sensitive to redox stresses, such as those associated with ischemia-reperfusion events (17–20), we investigated the effect of tumor-like hypoxia on their induction in transformed cells. In these studies, which are described in detail below, we observed that both SAPK/JNK and p38 MAPK activities are induced by hypoxia, but the inductions are transient. Because activated SAPK/JNKs and p38 MAPK can be deactivated by members of a family of dual-specificity phosphatases, called MAPK phosphatases (MKPs) (21–23), we hypothesized that the induction of these MAPKs in hypoxic cells is antagonized by redox-responsive members of the MKP family. In particular, we evaluated MKP-1 and -2 as possible contributors to this inhibitory activity, as they are widely expressed immediate-early gene products that are induced by a variety of stimuli (23–26).

Here, we report that hypoxia transiently induces SAPK/JNK as well as p38 MAPK activity in SiHa cells, and concurrently induces a SAPK/JNK phosphatase activity. This transient induction of SAPK/JNK activity correlates with both the transcriptional activation of the gene for the MKP family member MKP-1 and the enhanced expression of MKP-1 mRNA. The hypoxia-inducible expression of MKP-1 mRNA is reversible, returning to the aerobic level on reoxygenation. Together, these findings show that MKP-1 is a hypoxia-responsive phosphatase and imply that it contributes to the attenuation of SAPK/JNK activity stimulated in hypoxic cells. In the context of tumor
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biology, the poised and reversible responses of these MKP and MAPK pathways to hypoxic signals suggest that they are tightly regulated within the tumor microenvironment.

EXPERIMENTAL PROCEDURES

Materials—The GST-ATF2(1–94) fusion protein was expressed from a pGEX-KG plasmid (obtained from Dr. John Kyriakis, Massachusetts General Hospital, Charlestown, MA), and the GST-JNK1(1–144) fusion protein was expressed from a pGEX-2T plasmid (obtained from Dr. James Woodgett, Ontario Cancer Institute, Toronto, Ontario). Mammalian expression vectors (pcDNAIII) for full-length mouse MKP-1 and rat MKP-2 cDNAs are described elsewhere (26). The following antibodies were purchased from Santa Cruz Biotechnology (Santa Cruz, CA): rabbit polyclonal anti-p38 MAPK antibody (p38 (C-20); cross-reactive with p38 MAPK), rabbit polyclonal SAPK/JNK1 antibody (JNK1 (F-3); immunizing antigen full-length recombinant human JNK1; cross-reactive with p54 SAPKα2/JNK2 and SAPKβ/JNK3), monoclonal anti-p46 SAPK/JNK1 antibody (JNK1 (P-3); immunizing antigen full-length recombinant human JNK1), rabbit polyclonal anti-SAPKα2/JNK2 (JNK2 (FL); immunizing antigen full-length recombinant human JNK2; cross-reactive with p46 SAPK/JNK1 and SAPKβ/JNK3), and monoclonal anti-phospho-SAPK/JNK antibody (p-JNK (G-7)); immunizing antigen amino acids 183–191 of human SAPK/JNK1 phosphorylated on Thr-183 and Tyr-185; identical with the correspond-
CA), streptavidin alkaline phosphatase (Vector), and the substrates nitro blue tetrazolium/5-bromo-4-chloro-3-indolyl phosphate (Life Technologies, Inc.). Alternatively, binding was detected by using an IgG antibody conjugated with horseradish peroxidase (IgG-HRP; Santa Cruz Biotechnology) diluted 1:1,000 in PBS/0.1% Tween 20, and the ECL Plus Western blotting detection system (Amersham Pharmacia Biotech).

**Assay for SAPK/JNK (Thr-183 + Tyr-185) Phosphorylation—**Aerobic and hypoxic SiHa cells were plated on ice in air or on Super Ice® cold packs in the anaerobic box, and the medium was warmed. Each dish was washed twice with 1 ml of ice-cold PBS containing 1 mM Na2VO4, 1 mM NaF, and 10 μM iodoacetamide, and then 200 μl of ice-cold lysis buffer (10 mM HEPES (pH 7.9), 1 mM EDTA, 60 mM KCl, 1 mM DTT, 0.5% Nonidet P-40, 1 mM Na2VO4, 50 mM NaF, 0.5 μM okadaic acid, 1 mM PMSF) were added. Degasged solutions were used with hypoxic conditions. The plates were scraped, and the resultant cell suspensions were transferred to gasket-cap microcentrifuge tubes for spinning at 15,000 × g for 10 min at 4 °C. Samples of the lysates were treated with 10 mM iodoacetamide washed with another 800 μl of ice-cold PBS-T, diluted with an equal volume of 200 μl of storage buffer (50 mM Tris·HCl (pH 8.3), 5 mM MgCl2, 0.1 mM EDTA, 40% glycerol) and frozen in liquid N2. To perform nuclear runoff transcription reactions, each sample of frozen nuclei was thawed at room temperature and added to 200 μl of 2× reaction buffer (10 mM Tris·HCl (pH 8.0), 5 mM MgCl2, 300 mM KCl, 2 mM DTT, 1 mM each of ATP, CTP, and GTP; 25 units/ml RNase inhibitor; Life Technologies, Inc.). Then 10 μl of 10 mM ATP ([γ-32P]ATP (Amersham Pharmacia Biotech) were added to each sample, and the samples were incubated at 30 °C for 30 min. The reactions were stopped by digestion with 10 μg of RNase-free DNase I (Life Technologies, Inc.) at 30 °C for 5 min. Nascent RNA samples were harvested and purified by the RNeasy® method (Midi Kit, Qiagen).

**Nylon membrane (MSI) slot blots of 250 ng each of MKP-1, β-actin, and pBluescript II KS+ (pBSK, Stratagene, La Jolla, CA) cDNA were prepared by using a Hoefer PR 648 slot blot filtration manifold according to the manufacturer's instructions. The MKP-1 and β-actin cDNAs were inserted in pBSK, and the plasmids were linearized before blotting. Membranes were prehybridized at 42 °C for at least 4 h in 4× Denhardt's solution containing 1 μg/ml Saccharomyces cerevisiae tRNA, and hybridized at 42 °C for 36–48 h with 1–5 × 106 cpm of nascent RNA in 50% formamide hybridization solution (10 mM TES (pH 7.4), 500 mM NaCl, 2 mM EDTA, 0.4% SDS, 2 units/ml RNase inhibitor). After hybridization, the membranes were washed twice at 42 °C for 1 h with buffer A (10 mM Tris·HCl (pH 7.4), 300 mM NaCl, 2 mM EDTA), and then once at 42 °C for 30 min in buffer B (5 mM Tris·HCl (pH 7.4), 10 mM NaCl, 2 mM EDTA, 0.4% SDS). The blots were then washed twice in buffer A and incubated at 37 °C in buffer A containing 10 mg/ml RNase A. After washing the membranes in buffer A twice at 42 °C for 1 h, they were exposed to Kodak BioMax x-ray film for autoradiography.

**RESULTS**

**Hypoxia without Reoxygenation Transiently Induces Phosphorylation of the Transactivation Domains of the ATF2 and c-Jun Transcription Factors by SAPK/JNKs and p38 MAPK in SiHa Cells—**Previously, we reported that exposure of SiHa cells to a range of low oxygen conditions (pO2 < 0.1%) without reoxygenation caused transcriptional activation of c-jun (5) and phosphorylation of the ATF2 transcriptional domain (13). As mentioned above, the c-jun promoter is sensitive to activation by both SAPK/JNK and p38 MAPK members of the MAPK superfamily (15, 16). In the present study, we investigated the activation of these MAPKs by hypoxia (pO2 < 0.01%) by using aerobic and hypoxic SiHa cell lysates in the following assays: 1) kinase assays involving the GST-ATF2 (1–94) and GST-c-Jun (1–141) fusion proteins as substrates; and 2) immunocomplex kinase assays involving anti-phospho MAPK and anti-SAPK/JNK1 or –SAPK/JNK2 antibodies, and the GST-ATF2 (1–94) fusion protein as a substrate. To avoid possible effects of reoxygenation on SAPK/JNK and p38 MAPK activation, hypoxic cells were harvested for these assays exclusively under anaerobic conditions. It is important to note that, although time zero for hypoxia is defined as the start of the protocol described above, the c-jun activation of these MAPKs by hypoxia (#pO2 < 0.1%) without reoxygenation transiently induces phosphorylation of the ATF2 and c-Jun transcription domains of the MAPKs.

**Fig. 1. Hypoxia transiently induces both ATF2 and c-Jun kinase activities in SiHa cells.** Autoradiographs showing phosphorylation of the GST-ATF2 (1–94) and GST-c-Jun (1–141) substrates in kinase assays 200 μg SiHa cell lysates. Cells were incubated at 37 °C in 5% CO2/air (lanes 1 and 5) or under hypoxia (pO2 < 0.01%; lanes 2–4 and 6–8) for the indicated times. In this experiment and in all others, hypoxic cells were harvested for protein kinase assays under anaerobic conditions. For details, see “Results.”
stimulated in SiHa cells under low oxygen conditions, and that these activities peaked within 2–4 h of hypoxia. Both the degree of hypoxia (i.e. pO₂ and duration of exposure) and the cell type may be important determinants of the onset of SAPK/JNK and p38 MAPK activity. For example, in a previous study involving NIH 3T3 cells, c-Jun kinase activity was not detected following hypoxic exposures of less than 1 h at pO₂ ~ 0.1% (32). Fig. 2A shows that p38 MAPK activity was transiently stimulated in hypoxic SiHa cells under the same conditions as those that induced ATF2 kinase activity, and that this response persisted for at least 4 h of hypoxia. This induction of p38 MAPK activity was approximately 3-fold greater than that of the aerobic control (e.g. 3.2 ± 1.1 at 4 h, sample S.D., n = 4). For comparison, sorbitol (300 mM for 1 h) induced p38 MAPK activity by approximately 8-fold relative to the control (8.4 ± 3.1, n = 3, data not shown). Thus, as reported by others for heart (33, 34), p38 MAPK can be activated in a human carcinoma cell line by hypoxia. Fig. 2A also shows that these hypoxic conditions strongly and transiently induced SAPK/JNK1 activity relative to the aerobic control, giving a maximum value within the interval of 2–4 h of hypoxia (e.g. 31.8 ± 3.3 at 4 h, n = 3). This activation of SAPK/JNK1 is consistent with the finding shown in Fig. 1 of enhanced c-Jun kinase activity from SiHa cells exposed to identical hypoxic conditions. The Western blots shown in Fig. 2B demonstrate that total basal SAPK/JNK1 and p38 MAPK protein levels in SiHa cells did not change during hypoxic exposures of up to 6 h. These findings indicate that the transient induction of SAPK/JNK1 and p38 MAPK activities in hypoxic SiHa cells cannot be attributed to stress-induced MAPK protein synthesis and degradation. While ischemia-inducible p38 MAPK activity has been reported (33, 34), to our knowledge SAPK/JNK activation by hypoxia per se has not been established. Hypoxia was also found to induce both transient p38 MAPK and SAPK/JNK1 activities in identical experiments using immortalized mouse embryo fibroblasts (T-MEFs, obtained from Dr. Randall Johnson, University of California, San Diego; data not shown). This finding suggests that the activation of these stress-inducible MAPks by pathophysiological hypoxia can occur in a variety of mammalian cell types.

**Hypoxia-inducible SAPK/JNK Activation Involves Both SAPKα/JNK2 and SAPKβ/JNK1**—The anti-SAPKα/JNK1 antibody used for the immunoprecipitations shown in Fig. 2A cross-reacts with both human SAPKα/JNK2 and SAPKβ/JNK3 (see “Experimental Procedures”). Thus, it is possible that other members of the SAPK/JNK family (16, 35) can contribute to the activity immunoprecipitated by the anti-SAPKα/JNK1 antibody. To confirm that SAPKβ/JNK1 is activated by hypoxia, an identical immunoprecipitation study was performed involving a monoclonal antibody specific for p46 SAPKβ/JNK1. Fig. 3A shows that hypoxia transiently stimulated p46 SAPKβ/JNK1 activity relative to the aerobic control, giving a maximum induction within 2–4 h of stress (e.g. 2.8 ± 1.0 at 2 h, n = 3). The difference in the fold-induction of SAPKβ/JNK1 activity detected by the monoclonal compared with the polyclonal SAPKβ/JNK1 antibody can be attributed in part to the lower aerobic background signal consistently found with the polyclonal antibody. In addition, the larger -fold induction in Fig. 2A may reflect the contribution of more than one SAPK/JNK to the signal. The Western blot shown in Fig. 3B confirms that the monoclonal antibody detected p46 SAPKβ/JNK1 in SiHa cells and that the total basal level of this SAPKβ/JNK1 isoform remained constant for up to 6 h of hypoxia. Fig. 3C shows that SAPKα/JNK2 was also transiently activated in hypoxic SiHa cells within 2–4 h of stress (e.g. 3.3 ± 1.2 at 4 h, n = 3), and Fig. 3D indicates that total basal SAPKα/JNK2 protein levels remained constant for at least 6 h of hypoxia. Finally, using a specific cDNA probe for SAPKβ/JNK3 (36), no signal was detected on a Northern blot of SiHa cell total RNA (data not shown) indicating that SAPKβ/JNK3 is not significantly expressed in these cells. It has been reported that SAPKβ/JNK3 is primarily expressed in neuronal tissue (16, 36) whereas SiHa cells are of cervical origin. Together, these studies demonstrate

![Image](image-url)
that the transient SAPK/JNK activity induced in hypoxic SiHa cells consists of contributions from both SAPK\(_\gamma/JNK1\) and SAPK\(_\alpha/JNK2\).

**Hypoxia Induces a Phosphatase Activity in SiHa Cells That Dephosphorylates the TPY Signature Motif of SAPK/JNKs—**

The finding of a transient activation of both SAPK/JNKs and p38 MAPK in hypoxic cells suggested a hypoxia-inducible negative regulatory mechanism for these MAPKs. To investigate this possibility, we focused on the attenuation of hypoxia-inducible SAPK/JNK activity because it has a strong response in SiHa cells. Fig. 4 shows that endogenous SAPK/JNKs in hypoxic SiHa cells are transiently phosphorylated during 2–4 h of stress on Thr-183 and Tyr-185 in the activating TPY signature motif ([15, 16]). In addition, unlike anisomycin, hypoxia seems to preferentially phosphorylate/activate p46 isoforms of SAPK/JNKs in SiHa cells (the antibody recognizes the phosphorylated TPY motif in both SAPK\(_\alpha/JNK2\) and SAPK\(_\gamma/JNK1\); see “Experimental Procedures”). This finding parallels that of the immunocomplex kinase assay shown in Fig. 3 for the monoclonal antibody specific for p46 SAPK\(_\gamma/JNK1\), in which transient SAPK/JNK activation occurred within 2–4 h of hypoxia. Although an adducted GST-ATF2-(1–94) fusion protein rather than an immunoprecipitating antibody was used to isolate
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Fig. 6. The induction of MKP-1/1CL100 expression by hypoxia involves transcriptional activation and not mRNA stabilization. A, autoradiographs of a Northern blot of total RNA from hypoxic SiHa cells (pO_2 < 0.01%; lanes 1–6) and UV-treated aerobic SiHa cells (5% CO_2/air; lanes 7–12) harvested at the indicated times after exposure to the transcriptional inhibitor actinomycin D (5 μg/ml). Time zero was defined as 10 min after the addition of actinomycin D at 37 °C. Hypoxic cells were incubated with actinomycin D and harvested under anaerobic conditions at 37 °C. B, autoradiograph of nylon slot blots of linear MKP-1 cDNA, β-actin cDNA, and plasmid pBSK (pBSK) hybridized with nascent RNA from SiHa cells nuclei labeled with [32P]UTP, as described under “Experimental Procedures.” RNA was obtained from aerobic SiHa cells, TPA-treated aerobic SiHa cells (100 ng/ml for 1 h), and hypoxic SiHa cells (pO_2 ≤ 0.01% by 2 h). Each strip contained triplicate slot blots of each plasmid DNA (250 ng/slot blot).

 activated SAPK/JNKs for the anti-phospho-SAPK/JNK Western blot, both p46 and p54 SAPK/JNK isoforms bind to this adducted protein (Fig. 4, anisomycin lane). Interestingly, using a similar assay, others have reported preferential activation of a p46 SAPK/JNK isoform in cells stimulated by TNFα (37). Together, the findings shown in Figs. 1–4 provide strong evidence to support the hypothesis that pathophysiological hypoxia induces both SAPKα/JNK2 and SAPKβ/JNK1 activity by phosphorylation on the TPY signature motif, with a possible preference for activation of the p46 isoforms. In addition, the decline in (Thr-183+Tyr-185)-phosphorylated SAPK/JNK protein in SiHa cells by 4 h of hypoxia (Fig. 4) is consistent with the stimulation of a specific phosphatase activity capable of antagonizing concurrent SAPK/JNK activation.

Hypoxia Stimulates Expression of the MAPK Phosphatase MKP-1 in SiHa Cells—Activation of SAPK/JNKs can be antagonized by members of the MKP family of dual-specificity phosphatases (22, 23, 31, 38). Prompted by evidence that the MKP gene family members MKP-1/CL100 and MKP-2 are stress-inducible (23, 26, 31), we investigated whether the SAPK/JNK phosphatase activity induced in hypoxic SiHa cells could be associated with the accumulation of the mRNAs for these MKPs. Although originally identified as specific for ERK1/2 dephosphorylation (24, 25), recent reports provide evidence that MKP-1 and MKP-2 can also recognize SAPK/JNKs (22, 23, 38, 39). Fig. 5A shows that MKP-1 mRNA accumulated in hypoxic SiHa cells as early as 2 h and remained elevated for up to 24 h of stress. This mRNA accumulation ranged from 2- to 4-fold relative to that in aerobic cells (e.g. 1.8 ± 0.2 at 2 h of hypoxia, n = 3), and returned to the aerobic level by 2 h of reoxygenation (Fig. 5B). In contrast, MKP-2 mRNA accumulation did not change appreciably in response to hypoxia (Fig. 5A). For comparison, UV radiation, a strong inducer of MKP-1 expression in some cells (31), caused a 3-fold accumulation relative to the control (3.5 ± 0.8, n = 3) of MKP-1 mRNA in SiHa cells (data not shown). These findings indicate that MKP-1 is a candidate for a hypoxia-inducible SAPK/JNK phosphatase activity in SiHa cells.
The MAPK superfamily of proline-directed Ser/Thr kinases includes the mitogen-responsive members ERK1/2, the SAPK/JNKs, p38 MAPKs, Fos-related kinase or FRK, and ERK3/BMK (15, 16, 43–45). Signaling pathways for the MAPKs involve the general sequence MAPK kinase kinase \( \Rightarrow \) MAPK kinase \( \Rightarrow \) MAPK. The MAPK kinases are dual-specificity protein kinases that phosphorylate their substrates on threonine and tyrosine within the conserved signature motif TXY (X is E for ERK1/2, P for SAPK/JNKs, and G for p38 MAPKs) (15, 16). Deactivation of these signaling cascades at the MAPK level is critically dependent on dephosphorylation of the TXY motif by members of the MKP family of dual-specificity phosphatases (21, 23). Currently, the known MKP family includes nine members (22, 23, 26, 46, 47). Although various MKPs can dephosphorylate different members of the MAPKs, individual members of the family possess some substrate specificity (23, 38, 46–48). For example, mouse MKP-1 (3CH134/ERP; related to human CL100) preferentially recognizes ERK1/2, SAPK/JNK, and p38 MAPK, whereas rat MKP-2 (related to human VH-2) preferentially recognizes ERK2 and SAPK/JNK2, but not p38 MAPK (23–25, 38). While some MKPs have tissue-specific patterns of expression (47), MKP-1 and MKP-2 are widely distributed. Moreover, like other immediate-early gene products (e.g., c-Jun), their expression is responsive to various environmental stimuli, including mitogens, oxidative stress, UV radiation, heat shock, and alkylating agents (22, 23, 31, 49).

The major finding of this study is that exposure of human carcinoma cells to tumor-like low oxygen conditions (3, 28, 50) stimulates transient SAPK/JNK activity while simultaneously activating transcription of the \( \text{MKP-1} \) gene. Thus, \( \text{MKP-1} \) is a hypoxia-responsive gene. In terms of functional expression, we demonstrated that \( \text{MKP-1} \) inhibits hypoxia-inducible SAPK/JNK1 activity in co-transfected SiHa cells.2 Taken with our observation that hypoxia SiHa cells contain a (Thr-183 \( \text{cis} \) Ser)-phosphorylated phosphatase (183 Tyr) (Fig. 4), these findings suggest that MKP-1 contributes to the attenuation of SAPK/JNK activation in transformed cells exposed to pathophysiological hypoxia. In support of this idea, others have reported that an MKP such as \( \text{MKP-1} \) antagonizes transient SAPK/JNK activation in mitogen-stimulated Jurkat human T-cells (51) and in rat mesangial cells treated with TNF-\( \alpha \) (52, 53). To establish the contribution of endogenous \( \text{MKP-1} \) to hypoxia-inducible SAPK/JNK dephosphorylation, it will be necessary to obtain effective anti-MKP-1 antibodies for immunodepletion and immunoprecipitation studies, as commercially available antibodies are either nonspecific or cross-react with multiple MKPs (23, 26).

\( \text{MKP-1} \) is regarded as an immediate-early gene (31, 39, 42, 54), but little is known concerning the transcriptional and post-transcriptional controls on its expression and/or activity. At the protein level, \( \text{MKP-1/CL100} \) has a short half-life (54), suggesting that it is targeted for rapid proteolysis like other immediate-early genes. At the transcriptional level, a model for stress-inducible \( \text{MKP-1} \) expression has been proposed in which SAPK/JNKs transcriptionally activate the \( \text{MKP-1} \) gene in a negative feedback loop (22, 39). Consistent with this model, the promoter region for the human \( \text{MKP-1} \) gene (i.e., \( \text{MKP-1/CL100} \)) contains cis-acting elements for AP-1 and ATF/CREB transcription factors (42) both of which are physiological targets of SAPK/JNKs and/or p38 MAPK (16, 55). However, we observed that activation of the \( \text{MKP-1/CL100} \) promoter in SiHa cells occurs by 2 h of the initiation of hypoxia (Fig. 6), overlapping with the onset of transient SAPK/JNK activity (i.e., 2–4 h of hypoxia, Figs. 2–4). We also determined that hypoxia-inducible expression of the mouse \( \text{MKP-1} \) gene (i.e. 3CH134/ERP) does not require c-Jun, using c-Jun null T-MEFs (56).2 Interestingly, it has been reported that the activation of MAPKs including SAPK/JNKs is not sufficient for the induction of \( \text{MKP-1} \) expression in rodent fibroblasts (57–59). Although these findings do not necessarily exclude a model of hypoxia-inducible \( \text{MKP-1/CL100} \) expression involving a SAPK/JNK feedback loop, they suggest that other models are also possible. For example, the hypoxia response of the \( \text{MKP-1/CL100} \) promoter may be mediated by its Sp1 and/or CRE sites, shown to be hypoxia-responsive elements in some systems (60, 61). Alternatively, hypoxia-responsive elements may be present at distant sites in the regulatory regions of the \( \text{MKP-1/CL100} \) gene, as has been demonstrated for the human erythropoietin and mouse heme oxygenase-1 genes (62, 63). Given this potential complexity, it is likely that identifying the hypoxia-responsive elements in the \( \text{MKP-1/CL100} \) gene will require detailed knowledge of both the 5' and 3'-regulatory regions.

Although not established in vivo, an epitope of \( \text{MKP-1} \) can be phosphorylated by SAPK/JNK2 in vitro (64), raising the possibility that stress-inducible \( \text{MKP-1} \) activity could be regulated at the post-translational level by phosphorylation as well as by proteolysis. Hypoxia can modulate the activities of protein phosphatases (65, 66) and activate protein kinases (8–10, 32, 33, 60, 66, 67). If \( \text{MKP-1} \) and SAPK/JNK activation in hypoxic SiHa cells are interrelated, it is conceivable that early signals for their induction share upstream activators. We observed that genistein (50 \( \mu \text{M} \)), a broadly active PTK inhibitor (68), inhibited hypoxia-inducible SAPK/JNK and p38 MAPK activity in SiHa cells, while suramin (0.3 \( \mu \text{M} \)), which disrupts receptor PTK oligomerization (69), had no effect.2 These findings are consistent with a role for non-receptor PTK activity in the activation of SAPK/JNK and p38 MAPK pathways by hypoxia. Members of the Src family of PTKs have been implicated in hypoxia-responsive signaling pathways (10, 66, 67). Finally, a report demonstrating that both an MKP protein and the upstream SAPK/JNK activator MEKK-1 are components of the \( \text{IxB} \) kinase complex (64) suggests an integrating mechanism for the upstream regulation of redox-responsive MKP and MAPK pathways. The potential role of multi-protein complexes in the transmission of signals generated by hypoxia and reoxygenation is an important area for further research (70).

Up-regulation of normal \( \text{MKP-1} \) mRNA and protein has been detected in clinical specimens of a group of early stage carcinomas and in various stages of breast and prostate carcinoma (71–74). The biological function of \( \text{MKP-1} \) activity in tumors is not clear, but it is reasonable to hypothesize that the induction of \( \text{MKP-1} \) expression in hypoxic or reoxygenated tumor microenvironments is associated with stress-inducible MAPK activation. Evidence has been presented showing that both SAPK/JNKs and p38 MAPK can promote apoptosis in cells exposed to toxic stimuli (reviewed in Refs. 75 and 76). If \( \text{MKP-1} \) inhibits SAPK/JNK or p38 MAPK-dependent apoptosis by preventing prolonged MAPK activation (51–53), then stress-inducible \( \text{MKP-1} \) expression may contribute to the net growth of a solid tumor. In support of an anti-apoptotic function for \( \text{MKP-1} \) in tumors, patterns of \( \text{MKP-1} \) mRNA expression in early stage prostate carcinoma specimens were found to be inversely correlated with apoptosis as determined by a TUNEL assay and with SAPK/JNK1 protein expression (73, 74). As opposed to stress-inducible apoptosis mediated by prolonged SAPK/JNK and/or p38 MAPK activity, in some cell types \( \text{MKP-1} \) may actually promote apoptosis in response to a transient receptor-dependent signal (77). Because overexpressed \( \text{MKP-1} \) can down-regulate ras-dependent mitogenic signals (21, 78), the
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suggestion has also been made that it could act as a tumor suppressor (72). Regardless of the potential role of MKP-1 in oncogenesis, observations of heterogeneous, up-regulated MKP-1 expression in human tumor specimens provide evidence of an important contribution of this MKP to tumor pathophysiology, and suggest that it may be protective for hypoxic cells.

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