MAPKs Mediate S Phase Arrest Induced by Vanadate through a p53-Dependent Pathway in Mouse Epidermal C141 Cells

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Mitogen-activated protein (MAP) kinases play an important role in mediation of the signal transduction pathway in cellular response to genotoxic stress. Cell growth arrest is considered as an early stage in response to the genotoxic stress. p53 is well-known as a tumor suppression gene involved in both cell growth arrest and apoptosis. The present study investigated the involvement of MAP kinases in vanadate-induced cell growth arrest and the relationship of p53. DNA content analysis showed that vanadate-induced S phase arrest is time- and dose-dependent in p53 wild-type C141 cells but not in p53-deficient C141 cells. Western blotting results indicated that vanadate caused an inactivation of p-cdk2 at Thr160, which is an important kinase for the progression of S phase, and an increase in expression of p21, which is a key for S phase arrest. In p53-deficient cells, vanadate did not induce any observable change in p21 or p-cdk2 level. In addition, vanadate up-regulated phospho-p38 and ERK, two members of MAP kinases. At the same time, vanadate increased the p53 activity as measured by luciferase assay. Addition of PD98059 and SB202190, inhibitors of ERK and p38, respectively, decreased vanadate-induced S phase arrest, reduced p21 levels, restored activation of p-cdk2, and decreased p53 activity. The study demonstrated that vanadate-induced S phase arrest is mediated by both ERK and p38 in a p53-dependent pathway.

Introduction

Exposure of cells to genotoxic agents evokes a series of events leading to the activation of a wide spectrum of genes with diverse functions. A number of transcription factors as well as various gene products are involved in the response to mitogenic stimulation, illustrating that signal transduction pathways are involved in mediating the cellular response to stress and proliferative signals (*19*).

The mitogen-activated protein kinase (MAPK) pathway has a central role in cellular signaling (*20*). MAPKs include three major components which are extracellular signal-regulated kinases (ERK), stress-activated protein kinases (SAPK)/c-Jun N-terminal kinases (JNK), and p38/RK/CSBP kinases. These kinases play a key role in the activation of transcription factors and other regulatory proteins involved in activating gene expression (*8*). A common feature of MAPKs is their activation through phosphorylation of threonine and tyrosine residues in a homogeneous kinase subdomain VIII (*8*). Upon activation, MAPKs translocate into the nucleus where they phosphorylate transcription factors and thereby alter gene transcription patterns (*6, 26*). Over-activation of these protein kinases can lead to uncontrolled cell growth and tumorigenesis (*28*).

ERK is one of the most characterized signaling pathways that connect different types of membrane receptors to the nucleus after mitogenic stimulation (*12, 44*) or differentiation (*32*). The activation of ERK involves the activation of low-molecular-weight GTP-bonding proteins (Ras) at the plasma membrane and the sequential activation of a series of protein kinases: a MAPK kinase kinase (Raf-1) is activated and then by phosphorylation it activates a MAPK kinase (MEK1/2), which in turns phosphorylates ERK on threonine and tyrosine residues, leading to ERK activation (*53*). ERK is able to phosphorylate cytoplasmic and nuclear targets (*16, 29*). This pathway has been found to play a critical role in the control of cell proliferation via growth factor receptors and integrins (*22*). p38, another important member of MAPKs, is involved in inflammation, cell differentiation, cell cycle regulation, and cell death (*38*). The p38 pathway shares many similarities with other MAP kinase cascades. Similar to other MAPKs, p38 is activated by dual kinase, the MAP kinase kinase (MKK). Besides the same pathway as the ERK in Ras/Raf/MEK/MAPK, the Rho family, Rac, and cdc42 were also identified as potential regulators of the p38 pathway (*4, 40, 57*).

The p53 tumor suppressor protein appears to serve numerous functions during the cellular response to genotoxic stress, some of which act as a transcription factor to activate gene expression (*20*). p53 is considered an important mediator in the cell cycle. Various kinases

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including double-stranded DNA-activated protein kinase (DNA-PK), cyclin A- and cyclin B-associated kinase cdc2, ERK, and JNK have been implicated in p53 phosphorylation that in turn participates in regulation of p53 activity (*2*, *33*, *34*).

Vanadate is an essential trace element. It is widely found in occupational and environmental systems. Epidemiological studies have shown a correlation between vanadium exposure and the incidence of lung cancer in humans (*21*, *37*, *42*). Vanadium compounds were reported to modify DNA synthesis and repair (*9*, *21*, *42*) and induce mutations and DNA−protein cross-links (*9*, *18*, *30*, *49*, *59*). Vanadate is able to induce G_2/M phase arrest through a MAPK pathway mediated by reactive oxygen species (ROS) in A549 cells. A study from our group also indicated that vanadate caused p53-dependent S phase arrest in mouse epidermal JB6 cells.

The purposes of the present study are as follows: (1) to examine of effects of vanadate on MAPKs; (2) to understand the relationship between MAPKs and cell growth arrest induced by vanadate; and (3) to investigate the role of p53 in vanadate-induced cell growth arrest.

Materials and Methods

Reagents. Sodium metavanadate was purchased from Aldrich (Milwaukee, WI). Dulbecco's modified Eagle's medium (DMEM) and Eagle's minimal essential medium (MEM) were from Sigma (St. Louis, MO). Fetal bovine serum (FBS) was from Gibco BRL (Life Technologies, Gaithersburg, MD). Antibody against p21 was from Santa Cruz Biotechnology (Santa Cruz, CA). Antibodies against phospho-cdk2, p38 and ERK, and secondary AP-linked anti-rabbit IgG were from Cell Signaling (Beverly, MA).

Cell Culture. The JB6 P^+ mouse epidermal cell line, C141, cells were stably transfected with p53 luciferase reporter, and cultured in MEM medium containing 5% FBS, 2 mM L-glutamine, and 1000 units/mL penicillin−streptomycin in an incubator at 5% CO_2 and 37 °C.

Determination of Lactate Dehydrogenase Leakage. Activity of lactate dehydrogenase (LDH) leakage was measured using an FARA Chemical Analyzer with the test kit (Roche, Gaithersburg, MD). The total LDH activity was measured after cells were disrupted using ultrasonication. LDH leakage (%) was calculated as (LDH activity in medium/total LDH activity) × 100.

Measurement of Cell Cycle/DNA Content. DNA content in the G_1, S, and G_2/M phase was analyzed using flow cytometry (*39*, *45*). Cells were fixed and permeabilized with 70% ethanol for more than 2 h, and then incubated with the freshly prepared staining buffer (0.1% Triton X-100, 200 μg/mL RNase A, and 20 μg/mL PI) for 30 min at room temperature. For flow cytometric analysis, at least 10 000 cells were used in each sample. The DNA content histogram was abstracted, and the percentage of cells in S phase was calculated using ModFit LT software.

Western Blotting Analysis. Whole cell extracts were mixed with Tris−glycine SDS sample buffer, and then Tris−glycine gel electrophoresis was performed. The resolved proteins were transferred to a PVDF membrane. Western blotting was performed using antibodies against p21, phospho-cdk2 at Thr160, ERK, and p38, and secondary anti-rabbit IgG. After reaction with ECF substrate, the signal was detected using a Storm Scanner (Molecular Dynamics, Sunnyvale, CA).

Luciferase Assay for p53 Activity. After C141 cells were 80−90% confluent, 1×10^4 cells were placed into a 96-well plate with 100 μL of MEM medium in each well. The cells were treated by vanadate with different times and doses. At various time points, the cells were extracted with lysis buffer. The p53 luciferase activity was measured using a luminometer (Moonlight 3010). The results were expressed as p53 activity relative to the control.

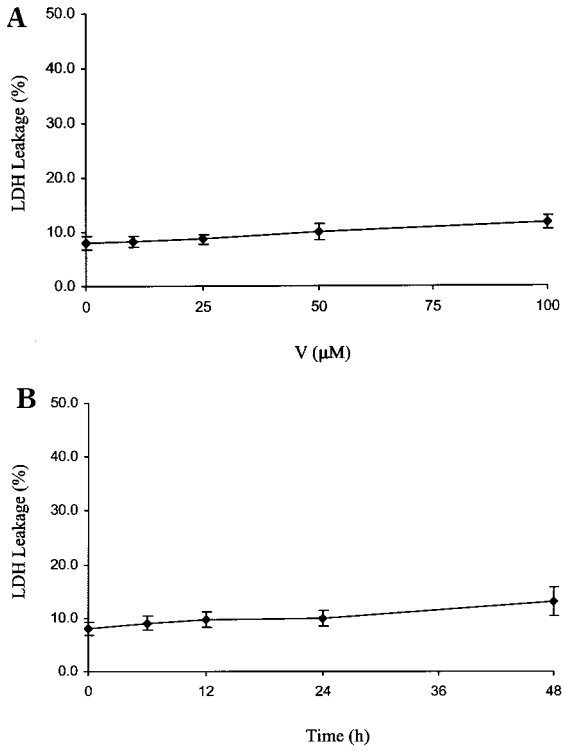

Figure 1. Leakage of cytosolic lactate dehydrogenase (LDH) from C141 cells exposed to various concentrations of vanadate (A) for 24 h or to 50 μM vanadate for various times (B).

Statistical Analysis. All data were based on at least three independent experiments. Relative p53 activity was presented as means ± SD and analyzed using one-way ANOVA with the Scheffe's test. A p value less than 0.05 was considered statistically significant.

Results

Toxicity of Vanadate. The toxicity of vanadate in C141 cells was examined by measuring LDH leakage. Figure 1A shows that at concentrations up to 100 μM for an incubation time of 24 h vanadate did not exhibit any significant toxicity. No significant toxicity was observed for 50 μM vanadate incubation times up to 48 h (Figure 1B). These results indicate that at the concentrations used in the present study, vanadate did not cause any significant toxic effect.

Effects of Vanadate on Cell Cycle. To study the effects of vanadate on cell growth arrest in C141 cells, flow cytometry was performed to analyze DNA content. Figure 2 shows that vanadate caused a time-dependent increase at S phase in C141 cells. The percentage of cells at S phase was 14.40% in 50 μM vanadate-treated C141 cells for 6 h (b) instead of 8.02% in control without stimulation (a). The percentage of cells at S phase was the highest (30.03%) at 24 h treatment (d). The results from the dose-dependent study are shown in Figure 3. In this study, C141 cells were treated with 10, 25, 50, and 100 μM vanadate for 24 h. The percentage of the cells at S phase was 13.35%, 18.45%, 30.03, and 25.82% when the cells were treated with 10, 25, 50, and 100 μM vanadate, respectively. In contrast, in p53 −/− cells, the percentage of the cells at S phase remained at the control levels regardless of vanadate treatment times or doses (data not shown).

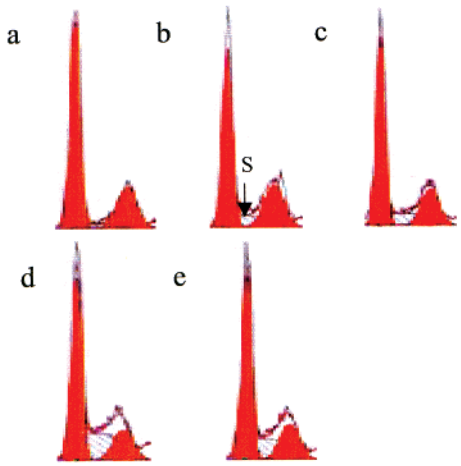

Figure 2. Time dependence of vanadate-induced cell growth arrest in C141 cells. C141 cells were suspended in 5% fetal bovine serum (FBS) MEM in a 100 mm dish. After 80% confluence, cells were washed with PBS 3 times, and treated with 50 μM vanadate for various times. a, control; b, 6 h; c, 12 h; d, 24 h; and e, 48 h. Cells were harvested, and DNA content was measured by flow cytometry. Data are from a single preparation representative of three independent experiments.

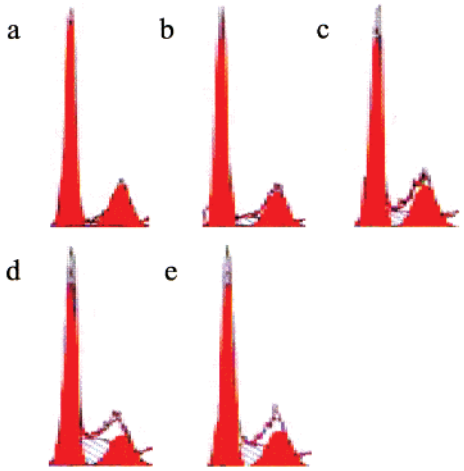

Figure 3. Concentration dependence of vanadate-induced cell growth arrest in C141 cells. These cells were suspended in 5% fetal bovine serum (FBS) MEM in a 100 mm dish. After 80% confluence, cells were washed with PBS 3 times, and treated with vanadate at various concentration for 24 h. a, control; b, 10 μM; c, 25 μM; d, 50 μM; and e, 100 μM. Cells were harvested, and DNA content was measured by flow cytometry. Data are from a single preparation representative of three independent experiments.

Effects of Vanadate on Cell Growth Regulatory Proteins. Western blotting was used to examine the effects of vanadate on proteins p21 and p-cdk2. Protein p21 is an important kinase for S phase arrest while p-cdk2 is for S phase progression. In p53-deficient cells, vanadate did not induce a significant alteration in the p21 or p-cdk2 level (data not shown). In C141 cells, vanadate caused an increase in the p21 level with a peak at 25 μM (Figure 4). At the same time, vanadate inactivated phosphorylation of p-cdk2 at Thr160 in a time- and dose-dependent manner.

Effects of Vanadate on ERK and p38. Western blotting was performed to study the effects of vanadate on ERK and p38. As shown in Figure 5, lanes 1 and 2, vanadate at 50 μM caused phosphorylation of both ERK and p38. An increase in incubation time enhanced the phosphorylation (lanes 3−6). Vanadate-induced ERK and

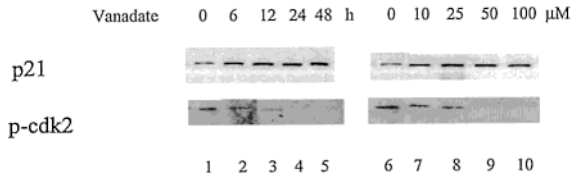

Figure 4. Effects of vanadate on growth regulatory proteins in C141 cells. The cells were treated with 50 μM vanadate for 6, 12, 24, and 48 h and with 10, 25, 50, and 100 μM vanadate for 24 h. The whole cell lysates were collected for western blotting using specific antibodies against p-cdk2 and p21. Lanes 1 and 6, control; lane 2, 50 μM, 6 h; lane 3, 50 μM, 12 h; lanes 4 and 9, 50 μM, 24 h; lane 5, 50 μM, 48 h; lane 7, 10 μM, 24 h; lane 8, 25 μM, 24 h; lane 10, 100 μM, 24 h. The results are representative of three independent experiments.

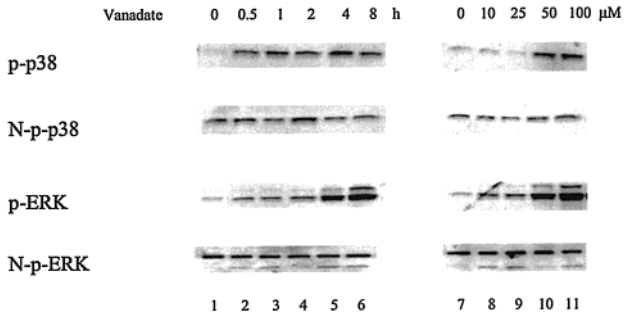

Figure 5. Activation of p38 and ERK by vanadate in C141 cells. C141 cells were seeded in a 6-well plate. Western blotting was performed as described in detail under Materials and Methods. Lanes 1 and 7, control; lane 2, 50 μM, 0.5 h; lane 3, 50 μM, 1 h; lane 4, 50 μM, 2 h; lanes 5 and 10, 50 μM, 4 h; lane 6, 50 μM, 8 h; lane 8, 10 μM, 4 h; lane 9, 25 μM, 4 h; lane 11, 100 μM, 4 h. The results are representative of three independent experiments.

Table 1. Time-Dependence of Vanadate-Induced p53 Activity[a]

incubation time (h)	relative p53 activity
6	1.44 ± 0.23
12	2.15 ± 0.19
24	3.71 ± 0.38[b]
48	5.33 ± 0.53[b]

[a] A total of 1×10^4 cells were seeded into a 96-well plate with 100 μL of medium in each well. The cells were treated with 50 μM vanadate for various times. The cells were extracted with lysis buffer. The p53 luciferase activity was measured using a luminomater (Moonlight 3010). The results were expressed as p53 activity relative to control. Each point represents ±SD of three independent experiments. [b] $p < 0.05$ compared to control (one-way ANOVA with Scheffe's test).

p38 phosphorylation were also dose-depedent (lanes 7−11).

Effects of Vanadate on p53 Activity. Luciferase assay was used to determine the p53 activity in C141 cells. The vanadate-induced increase in p53 activity was time-dependent as shown in Table 1. When the C141 cells were treated with 50 μM vanadate for 6 h, the p53 activity increased 1.5-fold compared to the control. The relative p53 activity increased from 1.5-fold to 6-fold when vanadate treatment time was increased from 6 to 48 h. Vanadate treatment also caused a dose-dependent increase in p53 activity (Table 2). It was about 5-fold when cells were treated with 100 μM vanadate for 24 h.

Effects of MAPK Inhibitors on MAPKs. PD98059, an inhibitor of ERK, and SB202190, an inhibitor of p38, were used to determine their effects on vanadate-induced up-regulaton of MAPKs. Figure 6 showed that treatment of the cells with 50 or 100 μM PD98059 almost abolished

Table 2. Dose-Dependence of Vanadate-Induced p53 Activity[a]

vanadate concentration (μM)	relative p53 activity
10	1.61 ± 0.28
25	2.64 ± 0.32[b]
50	3.71 ± 0.38[b]
100	5.44 ± 0.66[b]

[a] A total of 1×10^4 cells were seeded into a 96-well plate with 100 μL of medium in each well. The cells were treated with vanadate at various concentrations for 24 h. The cells were extracted with lysis buffer. The p53 luciferase activity was measured using a luminomater (Moonlight 3010). The results were expressed as p53 activity relative to control. Each point represents ±SD of three independent experiments. [b] $p < 0.05$ compared to control (one-way ANOVA with Scheffe's test).

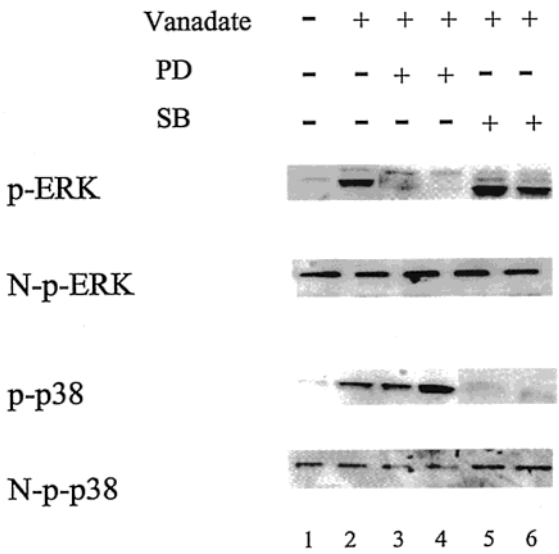

Figure 6. Inhibitory effects of PD98059 and SB202190 on vanadate-activated ERK and p38 in C141 cells. C141 cells were incubated in a 6-well plate in MEM medium containing 5% FBS. The cells were pretreated with PD or SB for 0.5 h prior to vanadate treatment (4 h). The whole cell lysates were used to analyze phospho-p38 and ERK by Western blotting. Lane 1, control without vanadate; lane 2, 50 μM vanadate; lane 3, 50 μM vanadate + 50 μM PD; lane 4, 50 μM vanadate + 100 μM PD; lane 5, 50 μM vanadate + 10 μM SB; lane 6, 50 μM vanadate + 20 μM SB. The results are representative of three independent experiments.

vanadate-induced elevation of p-ERK (lanes 3 and 4). In contrast, SB202190 had no effect on p-ERK (lanes 5 and 6). Pretreatment of the cells with 5 or 10 μM SB202190 dramatically decreased p-p38 as expected. PD98059 did not inhibit it.

Effects of MAPK Cell Growth Arrest. As shown in Figure 7, for untreated C141 cells, the percentage of cells at S phase was 8.02% (a). Treatment of the cells with 50 μM vanadate for 24 h increased the S phase percentage to 30.02 (b). Pretreatment with the ERK inhibitor PD98059 decreased the percentage of the C141 cells at S phase to 11.86% at 50 μM and to 8.92% at 100 μM (c and d). Similarly, after addition of 5 or 10 μM SB202190, the percentage of the cells at S phase was 16.95% or 14.97% (e and f). The results show that inhibition of ERK or p38 decreases S phase arrest in vanadate-treated C141 cells.

Effects of MAP Kinase Cell Growth Regulatory Proteins. As expected, pretreatment of C141 cells with PD98059 or SB202190 decreased the vanadate-induced p21 level compared to the vanadate treatment alone (Figure 8). The amount of proteins was almost at the same level as the control without stimulation. Pretreatment of the cells with these two inhibitors restored vanadate-inactivated p-cdk2.

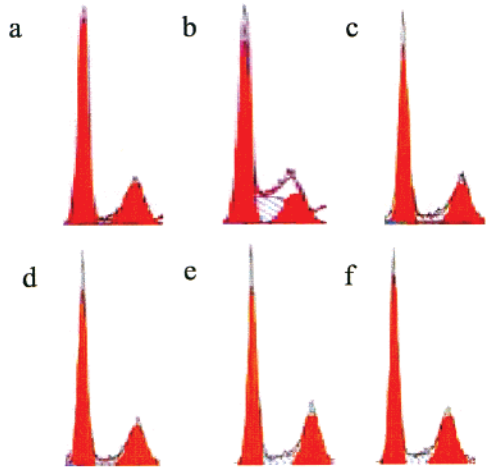

Figure 7. Effects of MAPK inhibitors on vanadate-induced cell growth arrest in C141 cells. The cells were incubated in a 100 mm dish and pretreated with PD98059 or SB202190 for 0.5 h before vanadate treatment (50 μM, 24 h). After 24 h, cells were harvested, and DNA content was measured by flow cytometry. a, control without vanadate treatment; b, 50 μM vanadate; c, 50 μM vanadate + 50 μM PD; d, 50 μM vanadate + 100 μM PD; e, 50 μM vanadate + 10 μM SB; f, 50 μM vanadate + 20 μM SB.

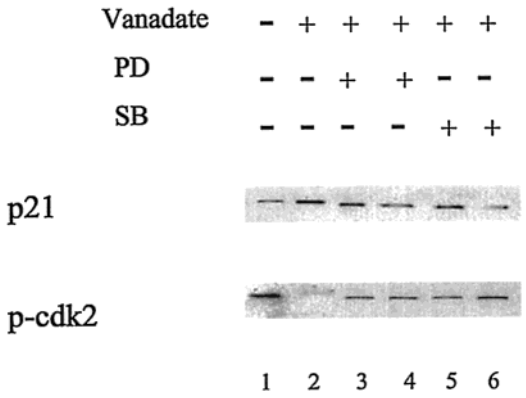

Figure 8. Inhibitory effects of MAPK inhibitors on vanadate-regulated growth proteins in C141 cells. The cells were incubated in a 6-well plate. These cells were pretreated with PD or SB for 0.5 h before vanadate stimulation (50 μM, 4 h). The whole cell lysates were collected for western blotting using specific antibodies against p-cdk2 and p21. Lane 1, control; lane 2, 50 μM vanadate; lane 3, 50 μM vanadate + 50 μM PD; lane 4, 50 μM vanadate + 100 μM PD; lane 5, 50 μM vanadate + 10 μM SB; lane 6, 50 μM vanadate + 20 μM SB. The results are representative of three independent experiments.

Effects of MAPKs on p53 Activity. As shown in Table 3, 50 μM vanadate caused a 3.7-fold increase in p53 activity. The relative p53 activity was sharply decreased when C141 cells were incubated with 50 and 100 μM PD98059, respectively. Similarly, pretreatment with SB also decreased the p53 activity.

Discussion

Vanadate compounds exhibit potent toxic and carcinogenic effects on various biological systems. They are able to modify DNA synthesis and repair (*9*, *21*, *42*, *48*), induce mutation and DNA−protein cross-links (*37*), inhibit tyrosine phosphatase, and autophosphorylate tyrosine kinase on the cell membrane receptor (*56*). It

Table 3. Effects of MAPKs on p53 Activity[a]

MAP kinase inhibitors	relative p53 activity
0	3.71 ± 0.38
50 μM PD	0.95 ± 0.17[b]
100 μM PD	0.42 ± 0.15[b]
5 μM SB	0.87 ± 0.23[b]
10 μM SB	0.24 ± 0.06[b]

[a] A total of 1×10^4 cells were incubated in a 96-well plate. The cells were pretreated with PD98059 or SB202190 for 0.5 h prior to vanadate (50 μM) stimulation. After 24 h, the cells were extracted with lysis buffer. The p53 luciferase activity was measured using a luminomater (Moonlight 3010). The results were expressed as p53 activity relative to control. Each point represents ±SD of three independent experiments. [b] $p < 0.05$ compared to vanadate treatment (one-way ANOVA with Scheffe's test).

has been reported that vanadate causes DNA damage (*46*), and activates certain transcription factors, such as AP-1 (*13*), NF-κB (*55*), p53 (*25*), and nuclear factor of activated T cells (NFAT) (*23*). It has also been reported that vanadate is able to induce apoptosis and cell growth arrest (*25, 54, 58*). Our laboratory has shown that vanadate caused G_2/M phase arrest through ROS-mediated reactions (*58*). The detailed mechanisms of the signal transduction pathway involved in vanadate-induced cell growth arrest are still unclear. The results from the present study show that: (1) vanadate caused S phase arrest in C141 cells in a time- and dose-dependent manner but not in p53-deficient cells; (2) vanadate up-regulated p21 and decreased p-cdk2; (3) it activated two important members of the MAPK family, ERK and p38; (4) vanadate increased p53 activity; (5) vanadate-induced S phase arrest was decreased by inhibition of either ERK or p38; (6) inhibition of MAPKs decreased vanadate-induced p21 and restored activation of p-cdk2; and (7) inhibition of MAPKs reduced vanadate-induced p53 activity.

Cellular stress, particularly that involving damage to the cell's replicative machinery, causes delay in cell cycle progression in virtually all living organisms. The cell cycle delay or arrest is considered important after DNA damage, since this delay or arrest allows the cells time to recover and/or repair damaged DNA prior to replication and mitosis. Earlier studies have shown that vanadate caused G_2/M arrest in T98 glioma cells, and both $p34^{cdc2}$ and cdc25C participated in the regulation of growth arrest at this phase (*11, 14*). The results from our laboratory also indicated that vanadate caused G_2/M phase arrest in A549 cells through ROS-mediated reactions (*58*). The present study shows that vanadate induced S phase arrest in C141 cells.

Cell cycle checkpoints monitor movement through the cell cycle, survey for cell damage, and induce a pause in cell cycle progression when necessary. Cyclins and cyclin-dependent kinases (CDKs) are key regulators of the eukaryotic cell cycle (*41*). Cdk2 is associated with cyclin A and cyclin E, and the respective complexes are believed to control S phase and the G_1/S transition (*17*). The activity of cdk2 is regulated by an intricate system of protein−protein interactions and phosphorylation. The activity of this kinase is increased by phosphorylation of Thr160 and inhibited by phosphorylation of Thr-14 and Tyr-15. The activity of cdk2 is also negatively regulated by its binding to protein inhibitors. One of the important inhibitors is p21, which is a downstream protein of p53. In addition to inhibiting the kinase activity by binding to the complex, p21 also blocks the phosphorylation of the activating threonine residue in CDKs (*3*). It has been reported that p21 is probably sufficient for the inhibition of cdk2 (*41*). The results from the present study showed that in C141 cells vanadate indeed decreased phosphorylation of cdk2 at Thr160. In the p53 −/− cells, vanadate treatment did not alter the percentage of the cells at S phase, and both p21 and p-cdk2 remained at the same levels as the control without vanadate stimulation (data not shown).

MAPKs play a pivotal role in the regulation of cell growth arrest, survival, differentiation, and apoptosis (*15, 36*). Once they are activated, MAPKs regulate gene expression through phosphorylation of downstream transcription factors. Generally, activation of the ERK cascade is involved in mitogenesis and differentiation, whereas both p38 and JNK are believed to mediate cell growth arrest and apoptosis (*15, 36*). In *Xenopus* egg, ERK is activated during the cells arrested in the M phase. Addition of PD98059 decreased the M phase arrest (*5*). Another study showed that PD98059 reverted the G_1 phase arrest as well as pRB hyperphosphorylation induced by the growth inhibition agent TPA (*1*). Furthermore, inhibition of ERK kinase activity substantially abrogates the UV induction of the GADD45 promoter that is an important checkpoint responsible for G_2/M phase arrest (*52*). The results from our laboratory also showed that ERK was involved in vanadate-induced G_2/M phase arrest (unpublished observation). In contrast, a previous study also found that persistently activated ERK may cause unregulated growth of fibroblasts and tumor formation in mice (*31*). The inhibition of ERK activity induced a dose-dependent growth arrest in the G_0/G_1 phase with up-regulation of cdk2 and hypophosphorylation of RB (*27*). The present study showed that vanadate activated phosphorylation of ERK as early as 0.5 h. Addition of PD98059 not only decreased vanadate-induced S phase arrest, but also reduced the p21 level and restored p-cdk2 inactivated by vanadate, suggesting that ERK is involved in vanadate-induced S phase arrest in C141 cells. In addition, the importance of p38 in cell growth became apparent due to the observation that overexpression of p38 in yeast led to a significant decrease in proliferation (*60*). The p38 was activated in cultured mammalian cells when these cells were arrested in M phase by disruption of the spindle with nocodazole (*50*). Activation of p38 is also believed to be involved in the spindle assembly checkpoint of somatic cell cycles (*35*). G_1 arrest in NIH3T3 cells caused by microinjection of cdc42 is p38-dependent (*35*). Disruption of fission yeast p38 cascades, Wis1-Spc/Sty1, leads to cell size enlargement and cell division arrest, suggesting that the Wis1-Spac1 MAPK cascade is linked to the G_2/M cell cycle control mechanism (*47*). The regulation of cdc25B phosphorylation by p38 is a critical event for initiating the G_2/M phase checkpoint after UV exposure (*7*). The present study indicates that p38 was activated by the vanadate treatment in a time- and dose-dependent manner. Inhibition of p38 phosphorylation by SB202190 decreased vanadate-induced S phase arrest. Similarly, p38 is also involved in vanadate-regulated protein p21 and p-cdk2.

p53 mediates several cellular functions such as induction of cell cycle arrest or apoptosis in response to DNA damage and helps to preserve genetic stability (*51*). It has been reported that p53-dependent cell growth arrest in G_1 phase is an important component of the cellular

response to stress (*51*). A recent study indicates that p53 controls the entry into mitosis when cells enter G_2 phase with damaged DNA or when they are arrested in S phase due to depletion of the substrates required for DNA synthesis (*10*). It has been reported that activation of p53 following DNA damage induces expression of p21, which in turn may be responsible for the cell cycle arrest (*41*). In addition, inhibition of either ERK or p38 decreased the p53 activity induced by vanadate. The p53 activation correlated with prominent activation of ERK1/2 MAP kinases that resulted from colcemid-stimulated development of focal adhesions that caused G_1 phase arrest (*43*). It has also been reported that phosphorylation of p53 by p38 plays a role in p53-dependent transcription (*24*).

In summary, vanadate induced S phase arrest in p53 wild-type C141 cells, up-regulated p21, and inactivated cdk2. Vanadate caused activation of both ERK and p38. Vanadate also increased p53 activity. The inhibition of either ERK or p38 decreased vanadate-induced S phase arrest, reduced p21 level and p53 activity, and increased phophorylation of cdk2. The present study indicates that vanadate-induced S phase arrest is mediated by MAPKs through p53-dependent pathway.

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