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Original Contribution

ROLE OF REACTIVE OXYGEN SPECIES AND MAPKs IN VANADATE-INDUCED G_2/M PHASE ARREST

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Abstract—Cell growth arrest is an important mechanism in maintaining genomic stability and integrity in response to environmental stress. Using the human lung alveolar epithelial cancer cell line A549, we investigated the role of reactive oxygen species (ROS), extracellular signal-regulated protein kinase (ERK), and p38 protein kinase in vanadate-induced cell growth arrest. Exposure of cells to vanadate led to cell growth arrest at the G_2/M phase and caused upregulation of p21 and phospho-cdc2 and degradation of cdc25C in a time- and dose-dependent manner. Vanadate stimulated mitogen-activated protein kinases (MAPKs) family members, as determined by the phosphorylation of ERK and p38. PD98059, an inhibitor of ERK, and SB202190, an inhibitor of p38, inhibited vanadate-induced cell growth arrest, upregulation of p21 and cdc2, and degradation of cdc25C. In addition to hydroxyl radical ($^{\bullet}OH$) formation, cellular reduction of vanadate generated superoxide radical ($O_2^{\bullet -}$) and hydrogen peroxide (H_2O_2), as determined by confocal microscopy using specific dyes. Generation of $O_2^{\bullet -}$ and H_2O_2 was inhibited by specific antioxidant enzymes, superoxide dismutase (SOD) and catalase, respectively. ROS activate ERK and p38, which in turn upregulate p21 and cdc2 and cause degradation of cdc25C, leading to cell growth arrest at the G_2/M phase. Specific ROS affect different MAPK family members and cell growth regulatory proteins with different potencies. © 2003 Elsevier Inc.

Keywords—MAPKs, Cell cycle regulatory proteins, Reactive oxygen species, Growth arrest, Vanadate

INTRODUCTION

Vanadium is an essential transition trace element found in some plants and animals. It is widely distributed in rocks, soil, and to a lesser extent in water [1–3]. Vanadium compounds (V(V)) exert potent toxic effects on a wide variety of biological systems [1,4–8]. This metal regulates growth factor-mediated signal transduction pathways, promotes cell transformation, and decreases cell adhesion [9–11]. Occupational exposure to vanadium occurs in mining, petrochemical industries, and coal- and oil-fired plants. Epidemiological studies have shown a correlation between vanadium exposure and the incidence of lung cancer in humans [6,8,12,13]. Vanadium compounds were reported to modify DNA synthesis and repair [14–16]. Vanadate induced forward mutations and DNA-protein cross-links in cultured mammalian cells [17]. While the biochemical mechanism of vanadium carcinogenicity still is not fully understood, recent studies have indicated that vanadium-mediated generation of reactive oxygen species (ROS) may play an important role [18–26]. For example, through ROS, vanadium caused 2'-deoxyguanosine hydroxylation and DNA damage [26], apoptosis [27,28], and activation of nuclear transcription factors [21] AP-1 and NF-κB [29].

In mammalian cells, cell cycle transition is under the control of a tightly regulated network of cell division kinases (cdks) and numerous surveillance mechanisms, the so-called checkpoints [30]. In most normal cells, DNA damage arrests proliferation in G_1/S or G_2/M phase and then resumes proliferation after the damage is repaired [30]. The cell cycle controls the onset of DNA replication and mitosis to ensure the integrity of the

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genome [31–33]. Lack of fidelity in DNA replication and maintenance can result in deleterious mutations, leading to cell death or, in multicellular organisms, cancer [30].

Recent evidence indicates that ROS may function as intracellular messengers to modulate signaling pathways [34,35]. The changes of intracellular ROS have been detected in a variety of cells stimulated with cytokines, growth factors, and agonists of receptors [34,36,37]. Various experiments have shown that many protein kinases and transcription regulatory factors are activated under the conditions of oxidative stress [38–43]. Mitogen-activated protein kinases (MAPKs) cascades are protein kinase signal transduction pathways that have been remarkably conserved in evolution. They are differentially used to relay numerous extracellular signals within cells [44–46]. These MAPK cascades have been found to be involved in such diverse cellular functions as proliferation, differentiation, stress responses, and apoptosis.

Stress-activated protein kinases (SAPK)/Jun N-terminal kinase (JNK), p38, and extracellular signal-related kinase (ERK) are the most widely studied members of the MAPK family. Activation of MAPKs led to abnormal M phase transition in the cell cycle [47]. It has been reported that p38 functions as a component of the spindle assembly checkpoint in somatic cell cycles [48]. Although ROS are frequently mentioned in the literature to be inducers for MAPKs, many of the studies are indirect. For example, N-acetylcysteine (NAC) was used as an inhibitor [49] and the generation of ROS by the stimulant in cellular system was not well characterized. NAC can readily react with "–SH" group of the protein and affect its function in a mechanism other than the scavenging of ROS.

The link between ROS and cell growth arrest has not yet been established. Many questions remain to be answered. For example, do ROS play a key role in the induction of cell growth arrest? Which species among ROS are involved? Are MAPKs involved in ROS-mediated cell growth arrest? Do MAPKs and ROS affect cell growth regulatory proteins? The present study attempts to answer these questions using vanadate as an inducer.

MATERIALS AND METHODS

Chemicals

Sodium metavanadate was purchased from Aldrich Chemical Co. (Milwaukee, WI, USA). RNase A and superoxide dismutase (SOD) were from Sigma Chemical Co. (St. Louis, MO, USA). Catalase was from Roche Molecular Biochemicals (Indianapolis, IN, USA). Propidium iodide (PI), 2', 7'-dichlorofluorescin diacetate (DCFH-DA), and dihydroethidium (HE) were from Molecular Probes (Eugene, OR, USA). Both F12K nutrient mixture medium and fetal bovine serum (FBS) were from Gibco BRL (Life Technologies, Gaithersburg, MD, USA). PD98058 and SB202190 were from Calbiochem (San Diego, CA, USA). Antibodies against p21 and cdc25C were from Santa Cruz Biotechnology (Santa Cruz, CA, USA). Antibodies against p38, ERK, and phospho-Cdc2 and second AP-linked antirabbit IgG were from Cell Signaling (Beverly, MA, USA).

Cell culture

The human alveolar epithelial cell line A549 was cultured in F12K nutrient mixture medium containing 10% FBS, 2 mM L-glutamine, and 1000 U/ml penicillin-streptomycin in an incubator at 5% CO_2 and 37°C.

Measurement of cell cycle/DNA content

DNA content was detected using flow cytometry [50,51]. A549 cells were fixed and permeabilized with 70% ice-cold ethanol for more than 2 h and incubated with the freshly prepared staining buffer (0.1% Triton X-100 in PBS, 200 μg/ml RNAase A, and 20 μg/ml PI) for 15 min at 37°C. Cell cycle analysis was performed by flow cytometry with at least 10,000 cells for each sample. The histogram was abstracted and the percentage of cells in the G_1/S and G_2/M phases were then calculated using ModFit LT software (Verity Software House, Topsham, ME, USA).

Western blot analysis

Whole cell extracts were mixed with Tris-Glycine SDS sample buffer and then subjected to Tris-Glycine gel electrophoresis. The resolved proteins were transferred to a PVDF membrane. Western blotting was performed using antibodies against p21, cdc25C, phospho-cdc2, p38, ERK, and second antirabbit IgG. After reaction with ECF substrate, the signal was visualized by blue laser scanning using a Molecular Dynamics STOM860 scanner (Molecular Dynamics, Sunnyvale, CA, USA).

Cellular hydrogen peroxide (H_2O_2) and superoxide anion ($O_2^{\bullet -}$) assay

Confocal microscopy was used to measure the generation of H_2O_2 and $O_2^{\bullet -}$. DCFH-DA is a specific molecular probe for H_2O_2 and HE is a specific dye for $O_2^{\bullet -}$ detection. The principle of this assay is that DCFH-DA diffuses through the cell membrane and is enzymatically hydrolyzed by intracellular esterases to nonfluorescent dichlorofluorescin (DCFH). In the presence of H_2O_2, this compound is rapidly oxidized to highly fluorescent dichlorofluorescein (DCF) [52–54]. The blue fluorescent dye HE is oxidized by $O_2^{\bullet -}$ to ethidium, which stains the

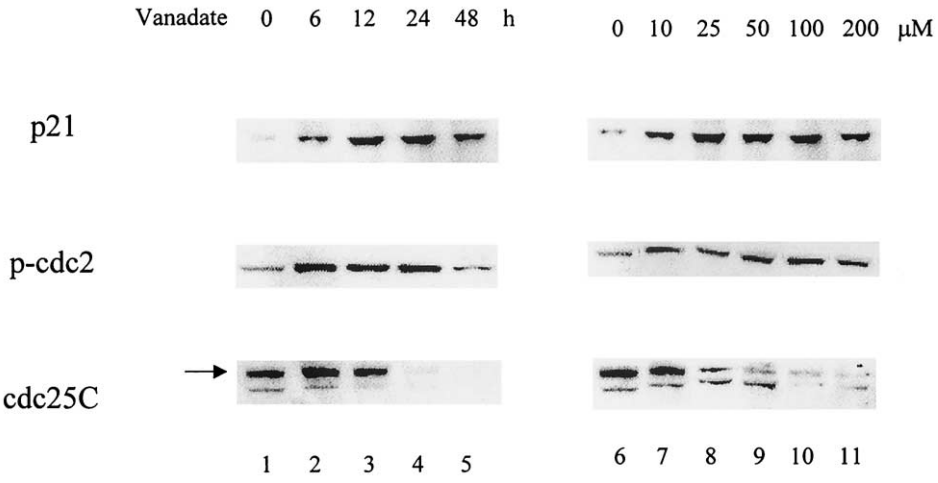

Fig. 1. Effects of vanadate on cell growth regulatory proteins. A549 cells were incubated in a 6-well plate until 80–90% confluent. After washing with PBS three times, cells were treated with 100 μM vanadate for different times or with different concentrations of vanadate for 24 h. The whole cell lysates were collected for Western blotting using specific antibodies to p21, cdc25C, and phospho-cdc2. Left panel: time dependence; the concentration of vanadate was 100 μM. Right panel: concentration dependence; the incubation time was 24 h.

nucleus a bright fluorescent red. A549 cells were cultured in 6-well plates containing 5×10^5 cells in each well. The cells were treated with 100 μM vanadate for 1 h. DCFH-DA or HE (final concentration, 5 μM) was added to the cells and incubated for another 15–20 min prior to the measurement of fluorescence.

RESULTS

The effect of vanadate on the cell cycle

DNA content was measured by flow cytometry to investigate vanadate-induced cell growth arrest. Human alveolar epithelial cells (the A549 cell line) were first synchronized by serum starvation and then exposed to vanadate for 24 h with various concentrations of vanadate. The results show that exposure of the cells to 100 μM vanadate caused growth arrest at the G_2/M phase in agreement with a previous report [55]. At a vanadate concentration of 100 μM, vanadate-induced G_2/M phase arrest peaked (32%) at 24 h. The control cells without exposure to vanadate exhibited G_2/M at 9%. The effects of vanadate concentration in the ranges of 10–200 μM were examined after a 24 h exposure. An increase in vanadate concentration increased the percentage of G_2/M phase (Fig. 1B).

The effect of vanadate on cell growth regulatory proteins

Several cell growth regulatory proteins, p21, phospho-cdc2, and cdc25C, were examined by Western blotting. These proteins were chosen due to their involvement in the regulation of G_2/M phase arrest [56–58]. The results are shown in Fig. 1. Treatment of the A549 cells with 100 μM vanadate for different times increased the levels of both p21 and phosphorylated cdc2 (left panel). Treatment of the cells with 100 μM vanadate for 6 h slightly increased the cdc25C level. An increase in incubation time caused degradation of cdc25C; further exposure of the cells to 100 μM vanadate for 48 h completely degraded cdc25C.

The dose-dependent effects of vanadate on these three growth regulatory proteins also were examined. As shown in Fig. 1 (right panel), vanadate caused a dose-dependent increase in the levels of both p21 and phosphorylated cdc2. Vanadate at 25 μM caused degradation of cdc25C; at 100 μM, vanadate almost completely degraded this regulatory protein.

Activation of MAPK induced by vanadate

The influences of vanadate on the phosphorylation of ERK and p38 were examined by Western blotting using antibodies specific for the phosphorylated MAPKs in A549 cells. These cells were seeded in 6-well plates and cultured until 80–90% confluence. The cells were starved for 48 h in 0.1% FBS MEM and were treated with 100 μM vanadate for different times. Exposure of the cells to vanadate stimulated the phosphorylation of both ERK and p38 (Fig. 2). The phosphorylation was relatively rapid and remained at a high level from 1 to 4 h for both ERK and p38 (left panel). To study the dose dependence of the phosphorylation of both ERK and p38, A549 cells were treated with various concentrations of vanadate for 4 h. A dose-related increase in both ERK and p38 phosphorylation was observed in the cells

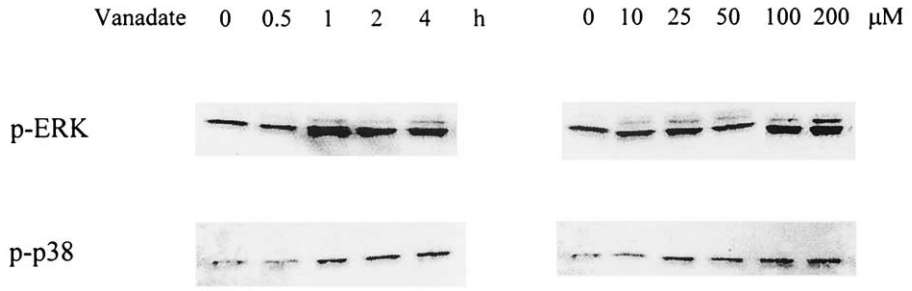

Fig. 2. Vanadate-induced phosphorylation of ERK and p38. A549 cells were seeded in 6-well plates. The cells were treated with 100 μM vanadate for 0.5 to 4 h or with different doses of vanadate for 4 h. Western blotting was performed as described in Fig. 2. Left panel: time dependence; the concentration of vanadate was 100 μM. Right panel: concentration dependence; the incubation time was 4 h. The designations "p" and "N-p" represent the phosphorylated and nonphosphorylated forms of the MAPKs.

treated with increasing concentrations of vanadate from 10 to 200 μM (right panel).

The effect of inhibitors of ERKs and p38 on vanadate-induced cell growth arrest

ERK and p38 may affect vanadate-induced cell growth arrest through a signal transduction network. To test the role of ERK and p38 in vanadate-induced growth arrest, the A549 cells were exposed to 100 μM vanadate for 24 h and cell cycle arrest was monitored by flow cytometry. As shown in Fig. 3, exposure of the cells to vanadate caused cell growth arrest with 32% of the cells being retained in the G_2/M phase. For examination of the role of ERK in vanadate-induced cell growth arrest, the cells were pretreated with PD98059—a specific MEK1-ERK pathway inhibitor—before the exposure of cells to vanadate. The results show that pretreatment of the cells

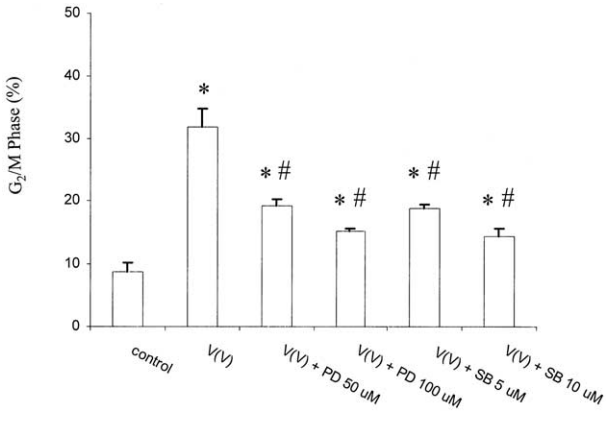

Fig. 3. The effects of ERK and p38 inhibitors on vanadate-induced cell growth arrest at G_2/M phase. A549 cells were incubated in a 100 mm dish and pretreated with either PD98059 (an ERK inhibitor) or SB202190 (a p38 kinase inhibitor) for 0.5 h prior to vanadate treatment (100 μM) for 24 h. Cell growth arrest was analyzed by DNA content using flow cytometry, as described in Fig. 1. *A significant increase from control without vanadate treatment; #a significant decrease from vanadate-treated positive control.

with PD98059 resulted in an inhibition of vanadate-induced cell growth arrest, decreasing the percentage of cells in the G_2/M phase from 33% to: 20% for 50 μM PD98059 and 16% for 100 μM PD98059. Pretreatment of the cells with SB202190—a specific inhibitor of p38—also inhibited vanadate-induced cell growth arrest, decreasing the percentage of cells in the G_2/M phase from 33% to: 19% for 5 μM SB202190 and 16% for 10 μM SB202190. Both of these two inhibitors alone had no effect on cell cycle and the G_2/M phases stayed the same as control (data not shown). These results indicate that both ERK and p38 are involved in vanadate-induced cell growth arrest.

Inhibition of ERK and p38 inhibitors on cell growth regulatory proteins

To further show that vanadate-induced cell growth arrest is mediated by ERK and p38, we examined the effects of PD98059 and SB202190 on vanadate-induced p21 and phospho-cdc2 levels and cdc25C degradation. As shown in Fig. 4, pretreatment of the cells with PD98059 or SB202190 decreased the levels of p21 and phospho-cdc2. Both of these two inhibitors partially blocked the degradation of cdc25C observed after vanadate exposure.

Generation of H_2O_2 and $O_2^{\bullet -}$ by vanadate-stimulated cells

Confocal microscopy, together with ROS dyes, was used to measure H_2O_2 and $O_2^{\bullet -}$ generated by vanadate-stimulated cells. Although selective dyes (DCFH-DA for H_2O_2 and HE for $O_2^{\bullet -}$) were employed, it is still possible that these dyes may not be totally specific. To overcome this potential problem, catalase and SOD were used in combination with the dye staining. Cells were cultured in a 6-well plate. After reaching 80–90% confluence, cells were treated with 100 μM vanadate for 1 h. The cells were pretreated with 500 U/ml SOD or 5000 U/ml cata-

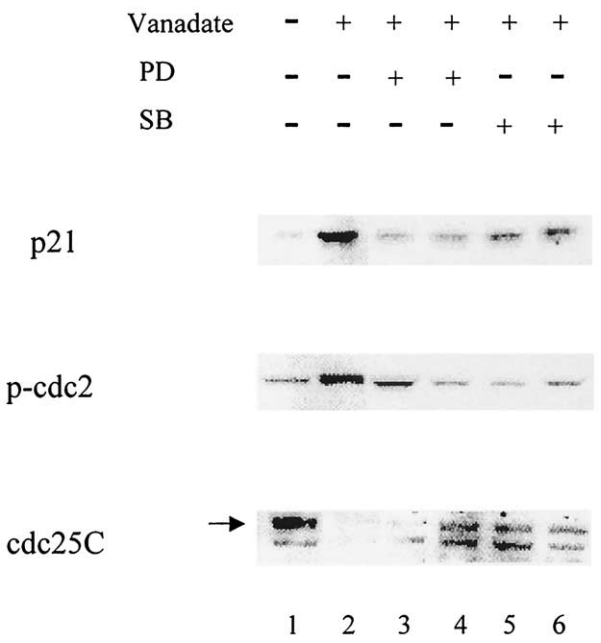

Fig. 4. The effects of ERK and p38 inhibitors on vanadate-regulated cell growth regulatory proteins. The cells were pretreated with PD98059 or SB202190 for 0.5 h prior to vanadate treatment (100 μM, 24 h). Proteins p21, phospho-cdc2, and cdc25C were analyzed using Western blotting. Lane 1: control without vanadate; lane 2: vanadate; lane 3: vanadate + 50 μM PD; lane 4: vanadate + 100 μM PD; lane 5: vanadate + 5 μM SB; and, lane 6: vanadate + 10 μM SB.

lase for 0.5 h before treatment with the 100 μM vanadate. DCFH-DA or HE was added to the cells and incubated for another 15–20 min at 37°C. Then, the cells were washed with PBS twice and collected for analysis using confocal microscopy.

As shown in Fig. 5A, in the presence of DCFH-DA, treatment of the cells with 100 μM vanadate for 1 h resulted in a green fluorescence, which was evidence of vanadate-induced generation of H_2O_2. Pretreatment with catalase, a specific scavenger of H_2O_2, sharply decreased the intensity of the green color, confirming the H_2O_2 generation. Figure 5B shows the generation of $O_2^{\bullet -}$. The control cells were blue due to the addition of HE (left panel). In the presence of $O_2^{\bullet -}$, blue HE was oxidized to ethidium that fluoresces red. The red color observed in vanadate-treated cells (right panel) indicated that vanadate indeed caused the generation of $O_2^{\bullet -}$. The addition of SOD, a scavenger of $O_2^{\bullet -}$, blocked the conversion of HE to ethidium. Therefore, the cells remained blue (left panel) and red color did not appear (right panel), confirming that $O_2^{\bullet -}$ was indeed produced.

The effects of antioxidants on ERK and p38

In the previous sections, we have shown that vanadate-stimulated cells are able to generate H_2O_2 and $O_2^{\bullet -}$. Since both ERK and p38 are oxidative stress-sensitive kinases [18], we examined the effects of antioxidants on vanadate-induced ERK and p38 activation. As shown in Fig. 6, SOD, formate, and catalase inhibited activation of ERK and p38 kinase. The inhibitory effect of catalase was more potent than that of formate ($^{\bullet}$OH radical scavenger) and SOD. These results indicate that all ROS members were involved in the activation of MAPKs.

The effects of antioxidants on growth regulatory proteins

The effects of antioxidants on several growth regulatory enzymes were examined. The results are shown in Fig. 7. SOD, formate, and catalase all exhibited an inhibitory effect on vanadate-induced p21 level, with catalase showing the strongest effect of the three. Neither SOD nor formate had any observable effect on vanadate-induced phosphorylation of cdc2. However, catalase abolished it. Both SOD and formate partially blocked the degradation of cdc25C. In contrast, catalase enhanced the extent of vanadate-induced cdc25C degradation. These results show ROS are involved in the regulation of these growth regulatory proteins. Specific ROS affect different regulatory proteins and exhibit different potencies.

DISCUSSION

Although vanadate-containing compounds exert potent toxic and carcinogenic effects on a wide variety of biological systems, the mechanisms of their actions remain to be investigated. We hypothesize that vanadate-mediated free radical reactions may cause oxidative stress and play a key role in the mechanism of vanadate-induced carcinogenesis [24,25]. ROS generated by vanadate-mediated reactions are able to damage DNA [26]. Normally, if the cell is injured by external agents, it will respond to such injury by the activation of signal transduction pathways that control the modulation of transcription factors and regulation of gene expression while transiently delaying cell cycle progression to allow the repair of damaged DNA [42,59]. Alternatively, if the damage is too severe to be adequately repaired, the cell may undergo apoptosis or enter an irreversible senescence-like state [31]. Our recent studies have shown that vanadate causes cell growth arrest and apoptosis through H_2O_2-mediated reactions [27,28,55]. The present study shows that vanadate is able to induce cell growth arrest and regulate cell growth regulatory enzymes through MAPKs and ROS.

MAPKs can be activated by many stress signals [29,60,61]. These kinases are serine/threonine protein kinases that participate in signal transduction of many

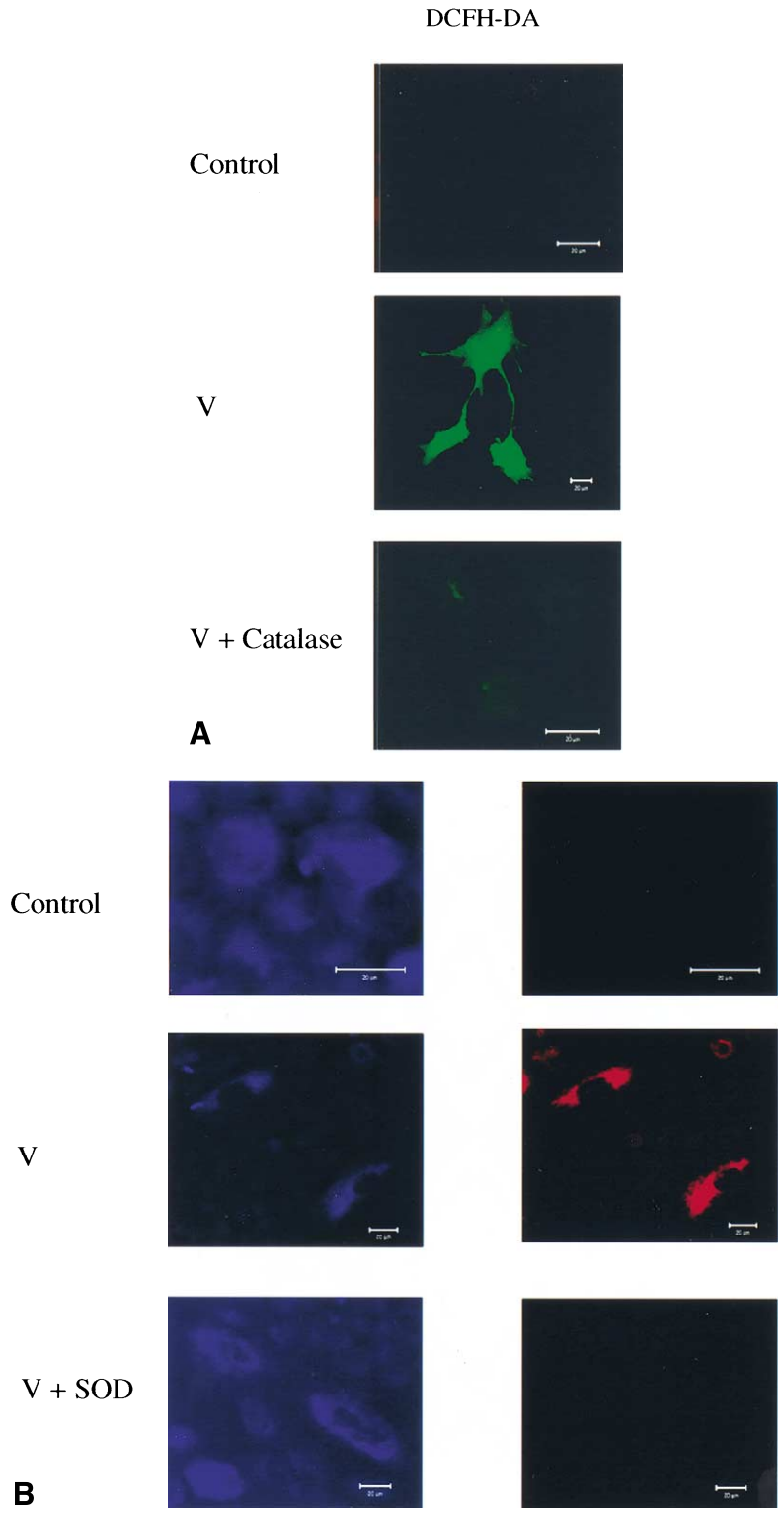

Fig. 5. Formation of H_2O_2 and $O_2^{\bullet -}$ induced by vanadate and the effects of catalase and SOD detected by confocal microscopy. Cells were cultured in a 6-well plate. After reaching 80–90% confluence, cells were treated with 100 μM vanadate for 1 h. For the evaluation of antioxidant effects, the cells were pretreated with 500 U/ml SOD or 5000 U/ml catalase for 0.5 h before treatment with 100 μM vanadate. DCFH-DA or HE was added to the cells and incubated for another 15–20 min at 37°C. Then, the cells were washed with PBS twice and collected for analysis using confocal microscopy. (A) The generation of H_2O_2; (B) the generation of $O_2^{\bullet -}$ (left: blue channel; right: red channel).

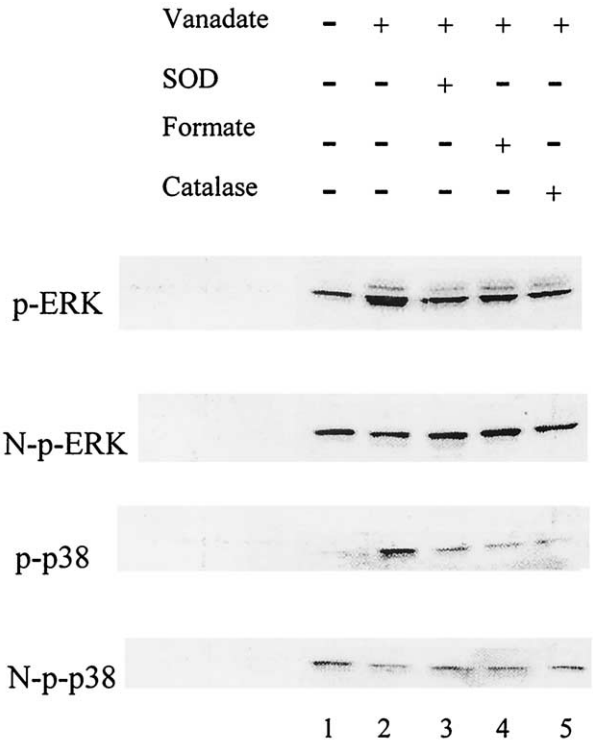

Fig. 6. The effects of antioxidants on vanadate-activated ERK and p38. A549 cells were seeded in a 6-well plate and pretreated with 500 U/ml SOD, 300 μM sodium formate, or 5000 U/ml catalase for 0.5 h before treatment with vanadate (100 μM). After 4 h, cells were harvested and Western blotting was used to analyze phosphorylation of ERK and p38. The designations "p" and "N-p" represent the phosphorylated and nonphosphorylated forms of the MAKPs.

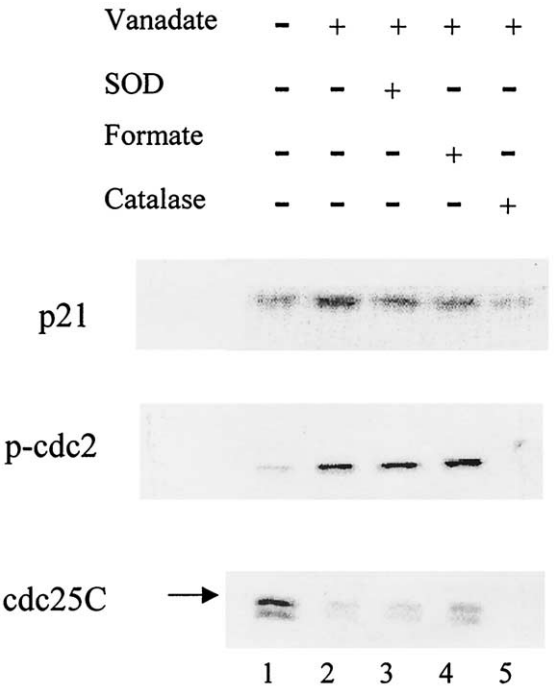

Fig. 7. The effects of antioxidants on vanadate-regulated cell growth regulatory proteins. A549 cells were incubated in a 6-well plate and pretreated with SOD, sodium formate, or catalase for 0.5 h before treatment with vanadate (100 μM). After 24 h, cells were collected for Western blotting using specific antibodies to p21, cdc25C, and phospho-cdc2.

extracellular stimuli, including UV light, bacterial derivatives, and growth factors. These stress-activated kinases have been implicated in the control of several diverse biological processes including cell proliferation, development, apoptosis, the response to stress, and the production of inflammatory cytokines [62]. Activation of these kinases is marked by phosphorylation of serine/threonine amino residues in their protein molecules. They act as signal transducers at the end of kinase cascades, which transmit signals to the nucleus. Activation of these kinases leads to the induction of transcription factors that in turn regulate gene expression in the nucleus and cell proliferation.

Recent studies have shown that MAPKs signaling pathways regulate the eukaryotic cell cycle [62]. It has been reported that growth factors can trigger entry into the G_1 phase of the cell cycle via activation of the Ras signaling pathway [63,64]. MAPKs also have been implicated in this process. In *Xenopus* oocytes, activation of MAPKs is necessary for the progression from meiotic prophase 1 to metaphase 2 [65]. After fertilization, inactivation of MAPKs is essential for the first G_2 to M progression [66]. The present study shows that ERK and p38 are involved in vanadate-induced cell growth arrest because both PD98059 and SB202190 exhibited inhibitory effects, with SB202190 being the more potent of the two. Inhibition of ERK or p38 also inhibited vanadate-induced expression of p21 and phospho-cdc2 and degradation of cdc25C. It appears that ERK and p38 regulate cell growth arrest at the G_2/M phase through these cell growth regulatory enzymes.

Our previous studies have shown that vanadate-stimulated cells consumed molecular oxygen that generated ROS in the process of cellular vanadate reduction [59]. Using ESR spin trapping, we showed generation of the $^{\bullet}OH$ radical in vanadate-stimulated cells [55]. H_2O_2 serves as a precursor for the generation of this radical. The present study shows that H_2O_2 and $O_2^{\bullet -}$ were generated in the reduction of vanadate by the cells, as detected by confocal microscopy in combination with specific scavengers for these species—catalase and SOD. From the results obtained in this study and those reported previously [55], the following can be concluded: (i) molecular oxygen was consumed to generate $O_2^{\bullet -}$ radical during the cellular reduction of vanadate, with flavoenzyme/NADPH as a major reductant; (ii) the $O_2^{\bullet -}$ radical produced H_2O_2 upon dismutation; and, (iii) H_2O_2

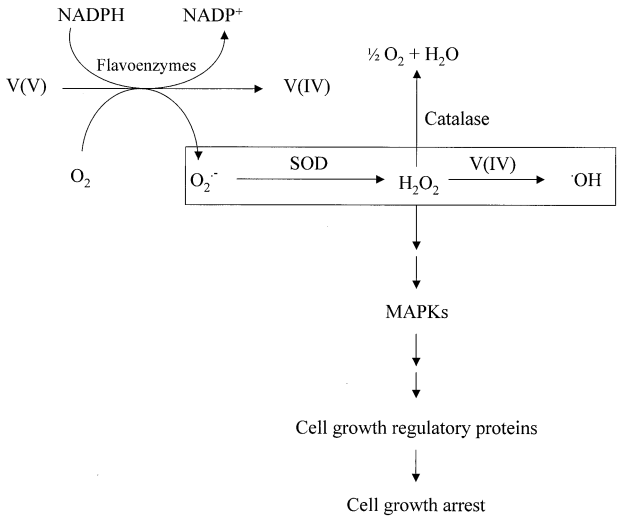

Fig. 8. The possible mechanism of vanadate-induced ROS generation and cell growth arrest.

produced $^{\bullet}OH$ radical via a Fenton-like reaction ($V(IV) + H_2O_2 \rightarrow {}^{\bullet}OH + OH^- + V(V)$).

Thus, in vanadate-stimulated cells, a whole spectrum of ROS was generated. ROS can also be generated as a result of normal oxygen metabolism in mitochondria. When generation of ROS exceeds the capability of the cellular defense system, consisting of small molecules and cooperative redox enzymes, oxidative stress occurs, resulting in DNA damage, protein modification, and other cellular responses. At these oxidative stress conditions, signaling protein kinases and transcription regulatory factors are activated. The present study shows that ROS scavengers inhibited the activities of ERK and p38, with different scavengers exhibiting different potencies, indicating that ERK and p38 are two important, early response kinases in response to vanadate-induced oxidative stress.

The results obtained from the present study show that vanadate also affected several cell growth regulatory proteins, namely p21, phospho-cdc2, and cdc25C, although different reactive oxygen species exhibited different potencies. All of these proteins are involved in the regulation of the G_2/M arrest [56–58]. The effects of vanadate on these regulatory proteins became observable at 6 h. It appears that ERK and p38 acted on the upstream of these growth regulatory proteins, as shown by the following observations: (i) both ERK and p38 inhibitors reduced the effects; and, (ii) while ERK and p38 were activated in a relatively short time, the effects of vanadate on the growth regulatory proteins were observed after a relatively long time. Therefore, it can be concluded that ROS generated by the stimulation of the cells with vanadate acted upstream of ERK and p38 and caused activation of these MAP kinases. ERK and p38 affected the cell growth regulatory proteins p21, phospho-cdc2, and cdc25C, leading to the cell growth arrest. The possible mechanism of vanadate-induced ROS generation and cell growth arrest is shown in Fig. 8.

Increasing evidence indicates a vital role for ROS in mediating cellular responses by various extracellular stimulators [67]. ROS can be generated from many exogenous and endogenous sources in biological systems. Exogenous sources of ROS include tobacco smoke, toxic gases, vapors, chemicals, pollutants present in ambient air, asbestos, metals, and mineral particles [68]. It is possible that these agents may activate MAPKs and alter the status of growth regulatory proteins, leading to cell growth arrest.

The results obtained from the present study support the following conclusions: (i) vanadate induces cell growth arrest at the G_2/M phase; (ii) vanadate causes phosphorylation of ERK and p38 by acting on their upsteam regulatory proteins; (iii) vanadate upregulates p21 and cdc2 and causes the degradation of cdc25C through both ERK and p38 pathways; (iv) during the cellular reduction of vanadate, molecular oxygen is reduced to generate ROS; (v) ROS are involved in vanadate-induced activation of ERK and p38, expression of p21 and cdc2, and degradation of cdc25C; and, (vi) it may be speculated that other metal carcinogens and mineral particles, such as chromium [69–72], cobalt [73], nickel [74], and silica [75], that are ROS-promoting agents may also cause cell growth arrest by a mechanism similar to that of vanadate.

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