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Vanadate Induces G_2/M Phase Arrest in p53-Deficient Mouse Embryo Fibroblasts

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Vanadium compounds exert potent toxic and carcinogenic effects on a wide variety of biological systems. The mechanisms involved in their toxicity and carcinogenesis require investigation. Cell growth arrest and its regulation are important mechanisms in maintaining genomic stability and integrity in response to environmental stress. The p53 tumor suppressor plays a central role in the regulation of the normal cell cycle. To investigate the role of p53 in vanadate-induced cell growth arrest and its regulation, two cell lines—normal mouse embryo fibroblasts [p53(+/+)] and p53-deficient mouse embryo fibroblasts [p53(–/–)],— were used in this study. Flow cytometry was used to analyze cell growth arrest at G_0/G_1, S, or G_2/M phase. Western blotting analysis was performed to determine several cell growth regulatory proteins. The results showed that in p53(–/–) cells vanadate induced G_2/M phase arrest in a dose- and time-dependent manner without alteration of S phase. In p53(+/+) cells, vanadate treatment increased the S phase with no significant change in the G_2/M phase. Furthermore, Western blotting results showed that in p53(–/–) cells vanadate caused cdc25C degradation and activation of phospho-cdc2 without alteration of the p21 level. In p53(+/+) cells, vanadate increased the expression of p21 and degraded cdc25A instead of cdc25C without any effect on cdc2. These results demonstrate that vanadate induced G_2/M phase arrest in p53-deficient mouse embryo fibroblasts, and promoted S phase entry in p53 wild-type mouse embryo fibroblasts.

KEY WORDS: vanadium, cell cycle, G_0 phase, G_1 phase, G_2 phase, S phase, protein p53, oncogene protein p21, reactive oxygen species, protein kinases

Introduction

The ability of cells to maintain genomic integrity is vital for cell survival and proliferation. Lack of fidelity in DNA replication and maintenance results in deleterious mutations leading to cell death or cancer.[1] Surveillance systems exist to ensure proper completion of cell cycle progression. The transition stages of cell cycle are regulated by various protein kinases or their complexes composed of cyclin and cyclin-dependent kinases.[2] Checkpoints can induce a transient delay to provide the cells more time to repair the damage before progression to the next phase of the cell cycle.[1]

The p53 tumor suppressor gene is a transcription factor that plays an important role in signal transduction in response to DNA damage.[3] This gene mediates various cellular functions such as DNA repair, maintenance of genetic stability, and

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induction of cell cycle arrest or apoptosis. It has been reported that both p53-dependent and p53-independent pathways are involved in the cell cycle regulation.[4] Generally, p53 is considered an important factor in the regulation of the cell cycle. The p53-dependent cell growth arrest in G_1 phase is an important component of the cellular response to stress. A recent study indicated 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 the depletion of the substrates required for DNA synthesis.[5]

Vanadium is widely distributed in the environment and is a trace metal in biological systems.[6–8] Vanadate compounds are potent toxins and carcinogens.[9–12] Epidemiological studies have shown a correlation between vanadium exposure and the incidence of lung cancer in humans.[13,14] Vanadate compounds were reported to modify DNA synthesis and repair.[15–18] Vanadate was also shown to induce forward mutation and DNA-protein crosslinks in cultured mammalian cells.[19] It has also been reported that vanadate exhibits mitogenic effects by inhibiting the activity of tyrosine phosphatase, and triggering the autophosphorylation of tyrosine kinase on cell membrane receptors.[20] Vanadate regulates growth factor-mediated signal transduction pathways, promotes cell transformation, exerts inhibitory effects on certain enzymatic systems, and decreases cell adhesion.[21–23] Whereas the mechanisms of vanadate-induced toxicity and carcinogenicity remain to be investigated, reactive oxygen species (ROS) are considered to play an important role. Through ROS, vanadate compounds cause direct DNA damage, such as strand breaks and hydroxylation of dG residues,[24] activate certain nuclear transcription factors, such as AP-1,[25] NF-κB,[26] p53,[27] and nuclear factor of activated T cells (NFAT),[28] and induce apoptosis.[27,29] Our previous studies have found that vanadate-induced cell growth arrest is cell type specific. Vanadate caused G_2/M phase arrest in human epithelial A549 cell line, and this arrest is mediated by ROS.[30]

The purpose of the present study is to investigate the role of p53 in vanadate-induced cell growth arrest. We attempted to answer the following questions: Does vanadate induce cell cycle arrest in mouse embryo fibroblasts? Does p53 mediate the vanadate-induced cell cycle arrest? What checkpoints are involved in the cell cycle regulation?

Materials and Methods

Reagents

Sodium metavanadate was obtained from Aldrich (Milwaukee, Wisconsin). RNase A and DMEM medium were purchased from Sigma (St. Louis, Missouri). Propidium iodide (PI) was from Molecular Probes (Eugene, Oregon). Fetal bovine serum (FBS) was from Gibco BRL (Life Technologies, Gaithersburg, Maryland). Antibodies against p21, cdc25A, cdc25C were from Santa Cruz Biotechnology (Santa Cruz, California). Phospho-cdc2^{tyr15} and secondary AP linked anti-rabbit IgG were from Cell Signaling (Beverly, Massachusetts).

Cell Culture

Normal mouse embryo fibroblasts [p53(+/+)] and p53-deficient mouse embryo fibroblasts [p53(–/–)] were incubated in DMEM medium with 10% FBS, 2 mM L-glutamine, and 1000 U/mL penicillin-streptomycin in an incubator at 5% CO_2 and 37°C.

Treatments

For the time-course study, cells were treated with 50 μM vanadate for 6, 12, 24, and 48 hours. For the dose-response study, cells were treated with 10, 25, 50, and 100 μM vanadate for 24 hours.

Measurement of Cell Cycle/DNA Content

DNA content in G_2/M phase was analyzed using flow cytometry according to the methods described previously.[31,32] Cells were fixed and permeabilized with 70% ethanol for more than 2 hours, 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 minutes at room temperature. Cell cycle analysis was performed by flow cytometry with at least 10,000 cells for each sample. The DNA content histogram was abstracted and the percentage of cells in G_2/M phase was calculated using ModFit LT software.

Western Blotting Analysis

Whole cell extracts were mixed with Tris-Glycine SDS sample buffer and the proteins were separated by Tris-Glycine gel electrophoresis. The resolved proteins were transferred to a PVDF membrane. Western blotting was performed using antibodies to p21, cdc25A, cdc25C, phospho-cdc2^{tyr15}, and secondary anti-rabbit IgG. After reaction with ECF substrate, the signal was detected using a Storm Scanner (Molecular Dynamics, Sunnyvale, California).

Statistical Analysis

All data were based on at least three independent experiments. Cell growth arrest data were presented as means +/– standard deviation and analyzed using one-way ANOVA with the Scheffe's test ($p < 0.05$ was considered statistically significant).

Results

Effects of Vanadate in Cell Cycle

To study vanadate-induced cell growth arrest, flow cytometry was used to measure the percentage of cells in each phase. Figure 1A shows a typical histogram of cell cycle. The first peak represents the G_0/G_1, the second peak the G_2/M. The S phase lies between the two peaks. Treatment of p53(–/–) cells with 50 μM vanadate for 24 hours increased the percentage of cells in the G_2/M phase (42% vs 18% in the control). Interestingly, treatment of p53(+/+) cells with vanadate did not change the percentage of cells in the G_2/M phase, but vanadate stimulation caused an increase in S phase.

Figure 1B shows the time dependence of vanadate-induced cell growth arrest in the G_2/M phase. In p53(–/–) cells, the percentage of cells in the G_2/M phase increased from 18% at the beginning of treatment to 42% when the cells were treated with vanadate for 24 hours. Conversely, in p53(+/+) cells the percentage of cells in the G_2/M phase did not change with vanadate exposure.

Figure 1C shows the dose-dependence of vanadate-induced cell cycle arrest in the G_2/M phase. As shown in this figure, at p53(–/–) cells there was a dose-dependent increase in the G_2/M phase. The highest percentage of cells in the G_2/M phase was 42% when the cells were treated with 50 μM vanadate for 24 hours. In p53(+/+) cells, the percentage of cells in the G_2/M phase was essentially unchanged regardless of the concentrations of vanadate.

Effects of Vanadate on p21

An important cell growth regulatory protein is p21. The protein level of this enzyme was measured by Western blotting. Figure 2A shows that treatment of p53(–/–) cells with 50 μM vanadate for 6 hours and 12 hours decreased the p21 level. No significant change was observed at 24 or 48 hours of vanadate treatment. The treatment of p53(+/+) cells with 50 μM of vanadate for 6 and 12 hours caused a weak induction of p21. A marginal increase in p21 was observed at 24 and 48 hours of 50 μM vanadate treatment.

Figure 2B shows the dose-dependence of vanadate treatment. In p53(–/–) cells at 24 hours incubation, vanadate did not cause p21 induction regardless of its concentrations. In p53(+/+) cells, 10 μM vanadate caused an observable p21 induction. An increase in vanadate concentrations did not enhance the p21 induction.

Effects of Vanadate on cdc25C

To investigate the involvement of cell cycle regulatory protein, the expression of cdc25C was examined in both p53(–/–) cells and p53(+/+) cells. Figures 3A and 3B show that in p53(–/–) cells, vanadate caused a time- and dose-dependent degradation of cdc25C. The cdc25C signal almost disappeared in cells treated with 50 μM vanadate for 24 and 48 hours (Fig. 3A, lanes 4 and 5) and 100 μM vanadate for 24 hours (Fig. 3B, lanes 4 and 5). However, in p53(+/+) cells the cdc25C did not exhibit any significant change regardless of incubation times (Fig. 3A) or treatment concentrations (Fig. 3B).

Effects of Vanadate on cdc25A

Tyrosine phosphatase cdc25A was detected using Western blotting (Fig. 4). In p53(–/–) cells, the

FIGURE 1. Effects of vanadate on cell growth arrest in both p53(–/–) and p53(+/+) cells. p53(+/+) and p53(–/–) cells are p53 wild type and p53 deficient mouse embryo fibroblasts, respectively. These two type of cells were incubated in DMEM medium with 5% FBS in a 100-mm dish. After 80–90% confluent, the cells were treated with vanadate for different times and different doses. The cells were harvested, and flow cytometry was used to measure DNA content. (A) The profile of a cell cycle, (a) control without vanadate treatment; and (b) treatment of the cells with 50 μM vanadate for 24 h. (B) The time-course of vanadate-induced cell growth arrest in G_2/M phase. (C) The dose-response of vanadate on cell cycle arrest at 24 h.
* $p < 0.05$ compared to control (one-way ANOVA with the Scheffe's test).

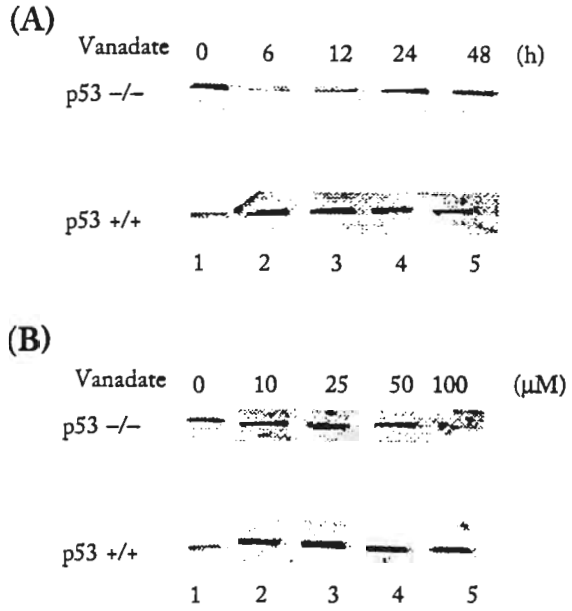

FIGURE 2. Effects of vanadate on cell growth inhibitory protein p21 in both p53(–/–) and p53(+/+) cells. The two types of cells were seeded in a 6-well plate then treated with 50 μM vanadate for 6, 12, 24, and 48 h (A), or treated with 10 μM, 25 μM, 50 μM, and 100 μM vanadate for 24 h (B). The whole-cell lysates were collected, and Western blotting was performed to detect the expression of p21. (A) Lane 1, control; lane 2, 6 h; lane 3, 12 h; lane 4, 24 h; and lane 5, 48 h. The concentration of vanadate was 50 μM. (B) Lane 1, control; lane 2, 10 μM; lane 3, 25 μM; lane 4, 50 μM; and lane 5, 100 μM vanadate. Incubation time was 24 h. Data are from a single preparation representative of 3 independent experiments.

expression of cdc25A did not change regardless of vanadate stimulation times (Fig. 4A) or doses (Fig. 4B). In p53(+/+) cells, 50 μM vanadate treatment caused maximal degradation of cdc25A at 48 hours (Fig. 4A, lane 5). The degradation of this protein was also observed when the cells were treated with 50 μM vanadate for 24 hours (Fig. 4B, lane 4).

Effects of Vanadate on cdc2

Another important kinase in the control of cell cycle progression from G_2 to M phase is cdc2. Western blotting was also used to study the effects of vanadate on this protein. In p53(–/–) cells, treatment with 50 μM vanadate activated phospho-cdc2 from 6 to 24 hours (Fig. 5A). Conversely, in p53(+/+) cells, vanadate caused a decrease in cdc2 at 12 hours (Fig. 5A, lane 3) and a complete inhibition at 24 hours (Fig. 5A, lane 4) when the cells were treated with 50 μM vanadate. In p53(–/–) cells, a dose-independent increase in cdc2 was observed after 10 to 50 μM vanadate. Treatment of p53(+/+) cells with 25 and 50 μM decreased cdc2 (Fig. 5B, lanes 3 and 4).

Discussion

Damage to growing cells causes a temporary pause in the G_1/S or G_2/M phase until the damage is repaired. When damage is severe, cells may either undergo apoptosis or enter a dormant G_0 state.[33–35]

Similar to apoptosis or programmed cell death, cell growth arrest is a cell self defense system to prevent genetically damaged cells from passing to the next generation. The investigation of cell growth arrest upon exposure to a

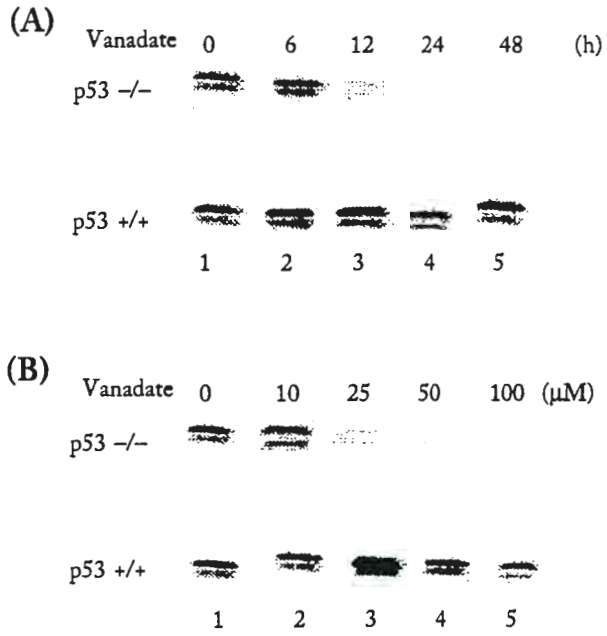

FIGURE 3. Effects of vanadate on cell growth regulatory protein cdc25C, according to the method described in Fig. 2. Western blotting was used to measure cdc25C level. (A) Lane 1, control; lane 2, 6 h; lane 3, 12 h; lane 4, 24 h; and lane 5, 48 h. The concentration of vanadate used was 50 μM. (B) Lane 1, control; lane 2, 10 μM; lane 3, 25 μM; lane 4, 50 μM; and lane 5, 100 μM vanadate. Incubation time was 24 h. Data are from a single preparation representative of 3 independent experiments.

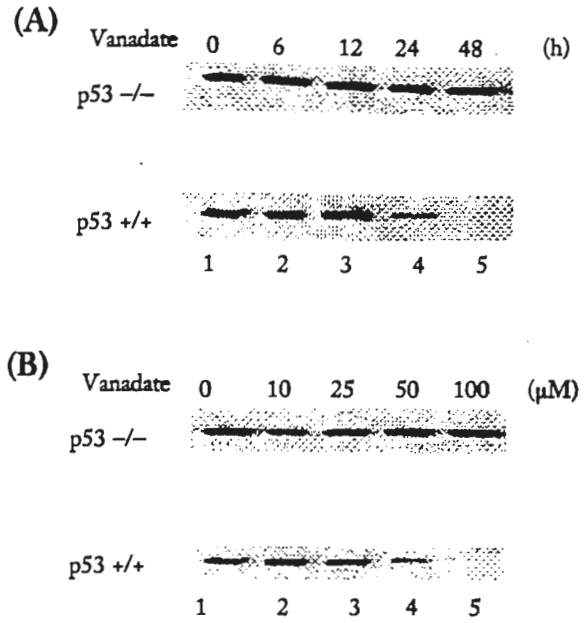

FIGURE 4 Effects of vanadate on cell growth regulatory protein cdc25A, according to method described in Fig. 2. Western blotting was conducted to determine the expression of cdc25A. (A) Lane 1, control; lane 2, 6 h; lane 3, 12 h; lane 4, 24 h; and lane 5, 48 h. The concentration of vanadate used was 50 μM. (B) Lane 1, control; lane 2, 10 μM; lane 3, 25 μM; lane 4, 50 μM; and lane 5, 100 μM vanadate. Incubation time = 24 h. Data are from a single preparation representative of 3 experiments.

carcinogen is an important part for the overall understanding of the mechanism of chemical-induced carcinogenesis.

Vanadate-containing compounds exert potent toxic and carcinogenic effects on a wide variety of biological systems, although the mechanisms involved are still unclear. Using human epithelial cell line A549 cells, our previous study has shown that vanadate is able to induce cell growth arrest in the G_2/M phase. This arrest is mediated by vanadate-induced ROS reactions. Mitogen-activated protein kinases (MAPKs) also play a critical role in the regulation of vanadate-induced cell growth arrest in the G_2/M phase.[30] The results from our laboratory demonstrated that vanadate enhanced S phase entry in C141 cells. The regulation of the S-phase enhancement is both p53- and p21-dependent (unpublished observations). The present study shows that vanadate induces G_2/M phase arrest in a dose- and time-dependent manner in p53(–/–) mouse embryo fibroblasts. Conversely, vanadate had no effect on G_2/M phase but increased the cell number in S-phase in p53(+/+) cells. In addition, hemopoietic cells that either lacked p53 gene expression or overexpressed a mutant form of the p53 gene exhibited a G_2 arrest after gamma irradiation, confirming that the G_2 arrest can be p53 independent.[36] A recent study from our group demonstrated that induction of GADD45α in arsenite-induced G_2/M phase arrest was p53-independent. Moreover, the activation of GADD45α by arsenite was higher in p53(–/–) cells than in p53(+/+) cells. All these data indicate that the G_2/M phase arrest can be p53 independent.[37]

However, some studies have shown the importance of p53 in G_2/M arrest. For example, p53 is required for the G_2 arrest in response to ionizing radiation in IMR-90 normal lung fibroblasts.[38] Other studies have indicated that inactivation of p53 by large T antigen is responsible for abrogating the G_2

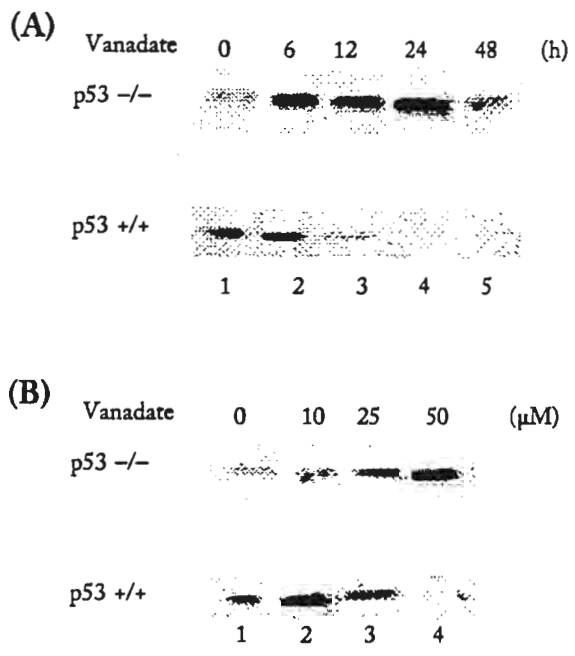

FIGURE 5. Effects of vanadate on cdc2, according to methods described in Fig. 2. The whole-cell lysates were used to perform Western blotting. (A) Lane 1, control; lane 2, 6 h; lane 3, 12 h; and lane 4, 24 h. The concentration of vanadate used was 50 μM. (B) Lane 1, control; lane 2, 10 μM; lane 3, 25 μM; and lane 4, 50 μM vanadate. Incubation time was 24 h. Data are from a single preparation representative of 3 experiments.

checkpoint.[39–41] Caffeine abrogated G_2 arrest in response to ionizing radiation in mouse embryo fibroblasts derived from p53-knockout mice and in rat embryo fibroblasts, expressing the dominant negative V143A mutant of human p53.[42]

Checkpoints are control mechanisms that ensure the proper timing of cell cycle events by enforcing the dependency of later events on the completion of early events. Movement of cells from G_2/M is regulated by both cyclin A and cdc2/cyclin B. The activity of cdc2/cyclin B kinases peaks in late G_2 and remains high until their degradation.[43] Earlier studies have shown that G_2/M arrest induced by a peroxovanadium compound was related to the reduced activity of $p34^{cdc2}$ and inhibition of cdc25C.[44] Our previous study also showed that sodium vanadate-induced G_2/M phase arrest was regulated by both phospho-$cdc2^{tyr15}$ and cdc25C.[30] γ-irradiation resulted in an accumulation of Thr-14/Tyr-15 phosphorylated cdc2, leading to the inhibition of cdc2/cyclin B activity.[2,45] It has been reported that sodium vanadate caused G_2/M arrest and Rb hypophosphorylation in T98 glioma cells.[46] The present study shows that vanadate increased phospho-cdc2 in p53(–/–) cells, but it decreased phospho-cdc2 in p53(+/+) cells.

There are two pathways associated with the activation of cdc2/cyclin B complex: (1) cdc25C phosphatase associates with cdc2/cyclin B complex and activates it by dephosphorylated Thr-14/Tyr-15.[47] This association between cdc25C and cdc2/cyclin B complex may be blocked through the actions of the Chk1 and Chk2 kinases, which phosphorylate cdc25C on serine 216.[1] This phosphorylation is necessary for cdc25C to bind to 14-3-3 proteins and its apparent sequestration from the cdc2/cyclin B complex.[48–50] (2) Transcriptional activation of $p21^{WAF1/CIP1}$ makes this protein bind to and inactivates the cdc2/cyclin B complex that is required for the cell cycle progression.[51-53] Our results show that (1) vanadate was able to cause degradation of cdc25C in p53(–/–) cells. The degradation of this protein leads to failure in dephosphorylation of cdc2 at Tyr15, resulting in inactivation of the cdc2/cyclin B complex. Instead of cdc25C, cdc25A, a checkpoint phosphatase for S phase, was degraded in vanadate-stimulated p53(+/+) cells. (2) Vanadate had no effect on p21 level in p53(–/–) cells regardless of treatment time and dose due to the deficiency of p53, although the activation of p21 might be p53-independent. Conversely, vanadate slightly increased the level of p21 protein in p53(+/+) cells. The explanation is that vanadate increased the percentage of S phase instead of change in the G_2/M phase in p53(+/+) cells. The S phase enhancement depended on p53 that was p21-dependent. It has been reported that cdc2 is likely to be inhibited by Thr-14/Tyr-15 phosphorylation, which is dephosphorylated by cdc25, whereas cdk2, which is an important checkpoint of S phase, is likely to be inhibited by association with p21 after UV radiation.[54] A similar mechanism probably exists in the vanadate-induced S-phase enhancement in mouse embryo fibroblasts, p53(–/–) cells, and p53(+/+) cells. The results from our previous study have also demonstrated that vanadate indeed increased p53 protein level, resulting in activation of p21. Both p53 and p21 levels decreased after pretreatment with pifithrin-α, a specific inhibitor of p53 in JB6 cells.[30]

In conclusion, vanadate is able to induce G_2/M phase arrest in p53 –/– cells. It causes degradation of cdc25C and inhibition of cdc2, while it does not alter the expression of p21 and cdc25A. In p53 +/+ cells, vanadate does not change the percentage of cells in G_2/M phase, but it increases the percentage of cells in S phase. Vanadate has no effect on cdc2 or cdc25C, but it increases the p21 level and degrades cdc25A.

References

1. Shackelford RE, Kaufmann WK, Paules RS. Cell cycle control, checkpoint mechanisms, and genotoxic stress. Environ Health Perspect 1999; 107 Suppl 1:5–24.
2. Paules RS, Levedakou EN, Wilson SJ, Innes CL, Rhodes N, Tlsty TD, Galloway TA, Donehower LA, Tainsky MA, Kaufmann WK. Effective G2 checkpoint function in cells from individuals with familial cancer syndromes. Cancer Res 1995; 55(8):1763–1773.
3. Almog N, Rotter V. Involvement of p53 in cell differentiation and development. Biochim Biophys Acta 1997; 1333(1):F1–F27.
4. Taylor WR, Stark GR. Regulation of the G2/M transition by p53. Oncogene 2001; 20(15):1803–1815.
5. Chan TA, Hwang PM, Hermeking H, Kinzler KW, Vogelstein B. Cooperative effects of genes

controlling the G(2)/M checkpoint. Genes Dev 2000; 14(13):1584–1588.
6. Chasteen ND. The biochemistry of vanadium. Struct Bond 1983; 53:107–137.
7. Kustin K, McLeod G, Gilbert TR, Briggs LBR. Vanadium and other metal ions in the physiological ecology of marine organisms. Struct Bond 1983; 53:139–185.
8. Nriagu JO, Pacyna JM. Quantitative assessment of worldwide contamination of air, water and soils by trace metals. Nature 1988; 333(6169):134–139.
9. Boyd DW, Kustin K. Vanadium: a versatile biochemical effector with an elusive biological function. Adv Inorg Biochem 1984; 6:311–365.
10. Erdmann E, Werdan K, Krawietz W, Schmitz W, Scholz H. Vanadate and its significance in biochemistry and pharmacology. Biochem Pharmacol 1984; 33(7):945–950.
11. Leonard A, Gerber GB. Mutagenicity, carcinogenicity and teratogenicity of vanadium compounds. Mutat Res 1994; 317(1):81–88.
12. Younes M, Strubelt O. Vanadate-induced toxicity towards isolated perfused rat livers: the role of lipid peroxidation.pa: Toxicology 1991; 66(1):63–74.
13. Hickey RJ, Schoff EP, Clelland RC. Relationship between air pollution and certain chronic disease death rates. Multivariate statistical studies. Arch Environ Health 1967; 15(6):728–738.
14. Stock P. On the relations between atmospheric pollution in urban and rural location and mortality from cancer, bronchitis, pneumonia, with particular reference to 3,4-benzopyrene, beryllium, molybdenum, vanadium and arsenic. Br J Cancer 1965; 14:397–418.
15. Carpenter G. Vanadate, epidermal growth factor and the stimulation of DNA synthesis. Biochem Biophys Res Commun 1981; 102(2):1115–1121.
16. Hori C, Oka T. Vanadate enhances the stimulatory action of insulin on DNA synthesis in cultured mouse mammary gland. Biochim Biophys Acta 1980; 610(2):235–240.
17. Sabbioni E, Pozzi G, Pintar A, Casella L, Garattini S. Cellular retention, cytotoxicity and morphological transformation by vanadium(IV) and vanadium(V) in BALB/3T3 cell lines. Carcinogenesis 1991; 12(1):47–52.
18. Smith JB. Vanadium ions stimulate DNA synthesis in Swiss mouse 3T3 and 3T6 cells. Proc Natl Acad Sci USA 1983; 80(20):6162–6166.
19. Nechay BR, Nanninga LB, Nechay PS. Vanadyl (IV) and vanadate (V) binding to selected endogenous phosphate, carboxyl, and amino ligands; calculations of cellular vanadium species distribution. Arch Biochem Biophys 1986; 251(1):128–138.
20. Yin X, Davison AJ, Tsang SS. Vanadate-induced gene expression in mouse C127 cells: roles of oxygen derived active species. Mol Cell Biochem 1992; 115(1):85–96.
21. Cruz T F, Morgan A, Min W. In vitro and in vivo antineoplastic effects of orthovanadate. Mol Cell Biochem 1995; 153(1–2):161–166.
22. Stern A, Yin X, Tsang SS, Davison A, Moon J. Vanadium as a modulator of cellular regulatory cascades and oncogene expression. Biochem Cell Biol 1993; 71(3–4):103–112.
23. Swarup G, Cohen S, Garbers DL. Inhibition of membrane phosphotyrosyl-protein phosphatase activity by vanadate. Biochem Biophys Res Commun 1982; 107(3):1104–1109.
24. Shi X, Jiang H, Mao Y, Ye J, Saffiotti U. Vanadium(IV)-mediated free radical generation and related 2'- deoxyguanosine hydroxylation and DNA damage. Toxicology 1996; 106(1–3):27–38.
25. Ding M, Li JJ, Leonard SS, Ye JP, Shi X, Colburn NH, Castranova V, Vallyathan V. Vanadate-induced activation of activator protein-1: role of reactive oxygen species. Carcinogenesis 1999; 20:663–668.
26. Ye J, Ding M, Zhang X, Rojanasakul Y, Nedospasov S, Vallyathan V, Castranova V, Shi X. Induction of TNF alpha in macrophages by vanadate is dependent on activation of transcription factor NF-kappaB and free radical reactions. Mol Cell Biochem 1999; 198(1–2):193–200.
27. Huang C, Zhang Z, Ding M, Li J, Ye J, Leonard SS, Shen HM, Butterworth L, Lu Y, Costa M, Rojanasakul Y, Castranova V, Vallyathan V, Shi X. Vanadate induces p53 transactivation through hydrogen peroxide and causes apoptosis. J Biol Chem 2000; 275(42):32516–32522.
28. Huang C, Ding M, Li J, Leonard SS, Rojanasakul Y, Castranova V, Vallyathan V, Ju G, Shi X. Vanadium-induced nuclear factor of activated T cells activation through hydrogen peroxide. J Biol Chem 2001; 276(25):22397–22403.
29. Ye J, Ding M, Leonard SS, Robinson VA, Millecchia L, Zhang X, Castranova V, Vallyathan V, Shi X. Vanadate induces apoptosis in epidermal JB6 P+ cells via hydrogen peroxide-mediated reactions. Mol Cell Biochem 1999; 202(1):9–17.
30. Zhang Z, Huang C, Li J, Leonard SS, Lanciotti R, Butterworth L, Shi X. Vanadate-induced cell growth regulation and the role of reactive oxygen species. Arch Biochem Biophys 2001; 392(2):311–320.
31. Nicoletti I, Migliorati G, Pagliacci MC, Grignani F. Riccardi C. A rapid and simple method for measuring thymocyte apoptosis by propidium iodide stain-

ing and flow cytometry. J Immunol Methods 1991; 139(2):271–279.
32. Sgonic RWG. Methods for the detection of apoptosis. Int Arch Allergy Immunol 1994; 105:327–332.
33. Irani K, Xia Y, Zweier JL, Sollott SJ, Der CJ, Fearon ER, Sundaresan M, Finkel T, Goldschmidt-Clermont PJ. Mitogenic signaling mediated by oxidants in Ras-transformed fibroblasts. Science 1997; 275(5306):1649–1652.
34. Morgan DO. Principles of CDK regulation. Nature 1995; 374(6518):131–134.
35. Ye J, Wang S, Leonard SS, Sun Y, Butterworth L, Antonini J, Ding M, Rojanasakul Y, Vallyathan V, Castranova V, Shi X. Role of reactive oxygen species and p53 in chromium(VI)-induced apoptosis. J Biol Chem 1999; 274(49):34974–34980.
36. Kastan M B, Onyekwere O, Sidransky D, Vogelstein B, Craig RW. Participation of p53 protein in the cellular response to DNA damage. Cancer Res 1991; 51:6304–6311.
37. Chen F, Zhang Z, Leonard SS, Shi X. Contrasting roles of NF-kappaB and JNK in arsenite-induced p53-independent expression of GADD45alpha. Oncogene 2001; 20(27):3585–3589.
38. Thompson DA, Belinsky G, Chang TH, Jones DL, Schlegel R, Munger K. The human papillomavirus-16 E6 oncoprotein decreases the vigilance of mitotic checkpoints. Oncogene 1997; 15(25):3025–3035.
39. Avantaggiati M L, Carbone M, Graessmann A, Nakatani Y, Howard B, Levine AS. The SV40 large T antigen and adenovirus E1a oncoproteins interact with distinct isoforms of the transcriptional co-activator, p300. EMBO J 1996; 15(9):2236–2248.
40. Chang T H, Ray FA, Thompson DA, Schlegel R. Disregulation of mitotic checkpoints and regulatory proteins following acute expression of SV40 large T antigen in diploid human cells. Oncogene 1997; 14(20):2383–2393.
41. Eckner R, Ludlow JW, Lill NL, Oldread E, Arany Z, Modjtahedi N, DeCaprio JA, Livingston DM, Morgan JA. Association of p300 and CBP with simian virus 40 large T antigen. Mol Cell Biol 1996; 16(7):3454–3464.
42. Powell S N, DeFrank JS, Connell P, Eogan M, Preffer F, Dombkowski D, Tang W, Friend S. Differential sensitivity of p53(–) and p53(+) cells to caffeine-induced radiosensitization and override of G2 delay. Cancer Res 1995; 55(8):1643–1648.
43. Pines J, Hunter T. Isolation of a human cyclin cDNA: evidence for cyclin mRNA and protein regulation in the cell cycle and for interaction with p34cdc2. Cell 1989; 58(5):833–846.
44. Faure R, Vincent M, Dufour M, Shaver A, Posner BI. Arrest at the G2/M transition of the cell cycle by protein-tyrosine phosphatase inhibition: studies on a neuronal and a glial cell line. J Cell Biochem 1995; 59(3):389–401.
45. Kaufmann W K, Levedakou EN, Grady HL, Paules RS, Stein GH. Attenuation of G2 checkpoint function precedes human cell immortalization. Cancer Res 1995; 55(1):7–11.
46. Chin, L S, Murray SF, Harter DH, Doherty PF, Singh SK. Sodium vanadate inhibits apoptosis in malignant glioma cells: a role for Akt/PKB. J Biomed Sci 1999; 6(3):213–218.
47. Strausfeld U, Labbe JC, Fesquet D, Cavadore JC, Picard A, Sadhu K, Russell P, Doree M. Dephosphorylation and activation of a p34cdc2/cyclin B complex in vitro by human CDC25 protein. Nature 1991; 351(6323):242–245.
48. Brown A L, Lee CH, Schwarz JK, Mitiku N, Piwnica-Worms H, Chung JH. A human Cds1-related kinase that functions downstream of ATM protein in the cellular response to DNA damage. Proc Natl Acad Sci USA 1999; 96(7): 3745–3750.
49. Peng CY, Graves PR, Thoma RS, Wu Z, Shaw AS, Piwnica-Worms H. Mitotic and G2 checkpoint control: regulation of 14-3-3 protein binding by phosphorylation of Cdc25C on serine-216. Science 1997; 277(5331):1501–1505.
50. Sanchez Y, Wong C, Thoma RS, Richman R, Wu Z, Piwnica-Worms H, Elledge SJ. Conservation of the Chk1 checkpoint pathway in mammals: linkage of DNA damage to Cdk regulation through Cdc25. Science 1997; 277(5331):1497–1501.
51. el-Deiry W S, Tokino T, Velculescu VE, Levy DB, Parsons R, Trent JM, Lin D, Mercer WE, Kinzler KW, Vogelstein B. WAF1, a potential mediator of p53 tumor suppression. Cell 1993; 75(4):817–825.
52. Harper J W, Adami GR, Wei N, Keyomarsi K, Elledge SJ. The p21 Cdk-interacting protein Cip1 is a potent inhibitor of G1 cyclin- dependent kinases. Cell 1993; 75(4):805–816.
53. Xiong Y, Hannon GJ, Zhang H, Casso D, Kobayashi R, Beach D. p21 is a universal inhibitor of cyclin kinases. Nature 1993; 366(6456):701–704.
54. Poon RY, Jiang W, Toyoshima H, Hunter T. Cyclin-dependent kinases are inactivated by a combination of p21 and Thr-14/Tyr-15 phosphorylation after UV-induced DNA damage. J Biol Chem 1996; 271:13283–13291.