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Suppression of p53 and p21$^{CIP1/WAF1}$ reduces arsenite-induced aneuploidy

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Abstract

Aneuploidy and extensive chromosomal rearrangements are common in human tumors. The role of DNA damage response proteins p53 and p21$^{CIP1/WAF1}$ in aneugenesis and clastogenesis was investigated in telomerase immortalized diploid human fibroblasts using siRNA suppression of p53 and p21$^{CIP1/WAF1}$. Cells were exposed to the environmental carcinogen sodium arsenite (15 and 20 μM), and the induction of micronuclei (MN) was evaluated in binucleated cells using the cytokinesis-block assay. To determine whether MN resulted from missegregation of chromosomes or from chromosomal fragments, we used a fluorescent *in situ* hybridization with a centromeric DNA probe. Micronuclei were predominantly of clastogenic origin in control cells regardless of p53 or p21$^{CIP1/WAF1}$ expression. MN with centromere signals in cells transfected with NSC siRNA or Mock increased 30% after arsenite exposure, indicating that arsenite induced aneuploidy in the tGM24 cells. Although suppression of p53 increased the fraction of arsenite-treated cells with MN, it caused a decrease in the fraction of with centeromeric DNA. Suppression of p21$^{CIP1/WAF1}$ like p53 suppression decreased the fraction of with centromeric DNA. Our results suggest that cells lacking normal p53 function cannot become aneuploid because they die by mitotic arrest-associated apoptosis, whereas cells with normal p53 function that are able to exit from mitotic arrest can become aneuploid. Furthermore our current results support this role for p21$^{CIP1/WAF1}$. Since suppression of p21$^{CIP1/WAF1}$ caused a decrease in aneuploidy induced by arsenite suggesting that p21$^{CIP1/WAF1}$ plays a role in mitotic exit.

Keywords

aneuploidy; arsenite; p21$^{CIP1/WAF1}$; p53

1. Introduction

Aneuploidy and extensive chromosomal rearrangements are frequently found in human tumors (reviewed in [1]). Aneuploidy arises from chromosomal missegregation whereas chromosomal rearrangements arise from clastogenic induction of cycles of chromosome breakage and

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rejoining. Although aneuploidy may arise spontaneously, certain types of environmental agents induce aneuploidy by induction of chromosome loss or gain during cell division in somatic and germ cells [2]. Likewise, chromosome breakage arises from DNA strand breakage often caused directly or indirectly by environmental genotoxic agents. Arsenic in the trivalent form (arsenite) causes both aneuploidy [3,4] and clastogenesis [5]. Arsenic induces a mitotic delay and aneuploidy in diploid human fibroblasts and peripheral blood lymphocytes [3,4]. Both aneuploidy and clastogenesis have been proposed as mechanisms of arsenic-carcinogenesis [6].

Accurate chromosome segregation during mitosis is critical in the prevention of aneuploidy. In fact, loss of mitotic checkpoint, abnormal centrosome amplification and defects in the kinetochore-microtubule attachment are events that disrupt chromosome segregation and contribute to aneuploidogenesis [7]. The spindle assembly checkpoint, activated immediately upon entry into mitosis, is the main mitotic checkpoint controlling mitotic progression [8] and ensures proper alignment of the chromosomes at the metaphase plate before segregation. The major regulator of the spindle assembly checkpoint is the mitotic checkpoint complex composed of MAD2, BUB1 and MAD3/BUBR1 [9]. However, it is likely that regulation may occur by other pathways as ATM and $p21^{CIP1/WAF1}$ which suppress aneuploidy in a genome instability background [10]; p53 may also play a role in suppressing aneuploidy [11] by regulating mitotic progression either directly or indirectly through $p21^{CIP1/WAF1}$ (aka CDKN1A) [12].

Induction of p53 by mitotic checkpoint activation is essential to the conservation of normal ploidy caused by mitotic failure. In addition, p53 deficiencies induce insufficient mitotic arrest, compromise apoptosis, and can cause aneuploidy [13]. Apoptosis is an important defense mechanism to prevent the survival of aneuploid cells. Cells that proceed through a defective mitosis and slip through the arrest often undergo apoptosis in the ensuing pseudo-G1 phase. Paradoxically, p53 expression allows exit from arsenite-induced mitotic arrest in a $p21^{CIP1/WAF1}$-dependent manner. $p21^{CIP1/WAF1}$ is a cyclin dependent kinase inhibitor involved in both G_1 and G_2 checkpoints. Expression of $p21^{CIP1/WAF1}$ is suggested to play a role in release of cells from mitotic arrest induced by paclitaxel [14] as well as by arsenite [12].

Arsenite is an aneugenic agent and arsenite–induced apoptosis has been reported to be associated with activation of spindle checkpoint [15–17]. Chronic exposure to inorganic arsenic from drinking water has been associated with diabetes mellitus, cardiovascular disorders, and a variety of skin and internal organ cancers [6,18,19]. Paradoxically, arsenic trioxide has also been used as an effective chemotherapeutic agent in the treatment of patients with acute promyelocytic leukemia, particularly in patients showing resistance to therapy with all-*trans*-retinoic acid or other chemotherapeutic drugs [20]. Trivalent inorganic arsenic compounds, such as arsenic trioxide and arsenite, kill cancer cells by induction of apoptosis consequent to mitotic disruption [15,21–29].

Disruption of mitosis can be caused by interference with spindle function, destabilization of centrosomes, or disruption of mitotic spindles. Arsenite induces G_2 and mitotic arrest in cell lines that lack functional p53 [28,30,31]. We have previously demonstrated that arsenite induces accumulation of mitotic cells with non-functional p53, but not in p53-expressing cells. In addition, the p53-deficient cells underwent apoptosis, whereas most of p53-expressing cells exited from M phase [32]. Furthermore, p53-dependent $p21^{CIP1/WAF1}$ induction, releases cells from mitotic arrest and prevents the arrest-associated apoptosis [12]. Arsenic-induced mitotic arrest associated apoptosis is directly related to the functional activation of spindle checkpoint [15,17] and can be prevented by inhibition of the cyclin dependent kinase activity by roscovitine [15].

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In the present study, we investigated the effect of suppression of p53 and $p21^{CIP1/WAF1}$ on chemical-induced aneuploidy and clastogenesis in telomerase immortalized diploid human fibroblasts (tGM24 cells). Only cells that recover from mitotic disruption can become aneuploid, whereas the cells that do not recover will suffer mitotic arrest-associated apoptosis (*aka* mitotic catastrophe). Elucidation of this recovery process might provide information relevant to the mechanisms of both arsenic carcinogenesis and chemotherapy by mitotic disruption.

Here we tested the effect of siRNA suppression of p53 and $p21^{CIP1/WAF1}$ expression on genotoxicant-induced aneuploidy and clastogenesis in telomerase immortalized diploid human fibroblasts which are genetically stable cells. We compared chromosomal effects of arsenite with the microtubule disruptor colcemid and the DNA crosslinking agent mitomycin C.

2. Materials and methods

Chemicals

Fresh working aqueous solutions of sodium arsenite ($NaAsO_2$, Sigma, CAS 1327-53-3) were prepared on the day of treatment and filter prior to use. Colcemid (Gibco, CAS 477-30-5), an aneugenic agent and mitomycin C (Sigma, CAS 50-07-7), a DNA crosslinking agent, were used as positive controls of both clastogenic and aneugenic effects. Sterile water was used as the solvent for all chemicals (stock solutions). Cytochalasin B (Cyt B; Sigma, CAS 14930-96-2) was dissolved in DMSO (1 mg/ml) and kept at−20°C.

Cell culture

Telomerase-immortalized diploid human fibroblasts (HTERT-GM00024, “tGM24”) were obtained on Material Transfer Agreement with Geron Inc. as described previously [33]. tGM24 cells were grown in Eagle's minimal essential medium containing Earle's salts (Gibco-BRL) supplemented with non-essential amino acids (0.1 mM), L-glutamine (2 mM), penicillin (100 units/ml), streptomycin (100 μg/mL) and fetal bovine serum (10%, Hyclone). The cells were incubated at 37 °C, with 5% CO_2, and 90% relative humidity.

Arsenite toxicity

tGM24 cells were treated with $NaAsO_2$ at 5–50 μM for 24, 48, 72 and 144 h. Cultures were monitored with an inverted phase-contrast microscope and cell viability by AlamarBlue fluorescence assay as described [12].

Knock-down of $p53$ and $p21^{CIP1/WAF1}$ by siRNA transfection

RNA interference was carried out as described previously [12]. Briefly, cells were harvested from growing cultures and transfected with p53 and $p21^{CIP1/WAF1}$ Smartpool siRNAs (Dharmacon, Lafayette, CO). Mock transfections were performed without any siRNA. Nonspecific control transfections were performed with 2 μg of non-specific control (NSC) siRNA. Specific transfections were performed with 2 μg of TP53 siRNA or $p21^{CIP1/WAF1}$ siRNA; siRNA was transfected into the cells using Lipofectamine 2000 (Invitrogen). At 24 h post–transfection, the cells were treated for 24 h. Specific suppression of p53 and $p21^{CIP1/WAF1}$ was demonstrated in tGM24 cells by Western blot analysis of samples 72 h after induction by UV radiation.

Western blot analysis

Suppression of p53 and $p21^{CIP1/WAF1}$ was confirmed by western blot analysis performed described previously [12]. Membranes were stained with Ponceau stain to determine even gel loading and transfer to the membrane.

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Cytokinesis block micronucleus assay

The micronucleus assay was performed as described previously [34]. Briefly, 0.4×10^6 cells were seeded onto a cover slip in 60 mm dishes. After 24 h of culture, cells were exposed to $NaAsO_2$ (15 and 20 μM), colcemid (1 μM) or mitomycin C (1 μM) and also to cytochalasin B (3 μg/ml final concentration). After 24 h of exposure, the cells were harvested. Cells on the cover slips were fixed. Air-dried cover glasses were stained with Wright´s colorant and mounted on slides for microscopic evaluation.

The frequency of micronuclei (MN) was determined in 1000 binucleated cells per treatment group. Cell proliferation kinetics were analyzed by determining the frequency of mononucleated (Mono), binucleated (Bi) and polynucleated (Poly) cells, corresponding to 0, 1, and 2 or more *in vitro* cell divisions, respectively. Cytostatic activity was determined by calculating the percentage of binucleated cells. Three independent experiments were analyzed.

Fluorescence in situ hybridization (FISH) with a pancentromeric DNA probe

The chromosomal composition of MN was assessed by fluorescence *in situ* hybridization (FISH) technique to distinguish between aneugenic and clastogenic origin of the MN. For FISH analysis, samples were selected in which the quality was confirmed by observation after standard Wright´s staining. The FISH analysis using commercial human pancentromeric probes directly labelled with Cy3 (Cambio UK) was carried out according to the manufacturer ´s protocol with slight modifications. Briefly, the cells were treated with RNase (1 mg/ml) at 3700B0C for 15 min and proteinase K (50 ng/ml) at 37°C for 5 min. DNA was denatured with 70% formamide/2XSSC for 2 min at 70°C. The DNA probe was denatured for 10 min at 85° C and was added to the slide under reduced light. The slides were covered with a cover slip and sealed with rubber cement. Following an overnight hybridization at 37°C in a humidified box, slides were washed in 50% formamide/2XSSC for 5 min at 37°C. The cells were then counterstained with DAPI in antifade. One thousand binucleated cells with well-preserved cytoplasm were examined using a fluorescent microscope equipped with a double-band pass filter for visualization of Cy3 (red) and DAPI (blue) signals and single-band pass filter for visualization of DAPI signals alone. If one or several red spots (Cy3-labelled centromeres) were observed inside MN, the MN was classified as a MN with centromere signals or positive. While a MN without signals was classified as MN negative.

Statistical analysis

Normality of data was evaluated by the Kolmogorov–Smirnov test. The difference between groups for multiple variables was assessed with one-way analysis of variance (ANOVA) followed by Tukey´s post-test. The Fisher´s exact test was applied for comparisons between MN+ and MN− frequencies. Differences were considered significant at $p<0.05$.

3. Results

We sought to examine the role of p53 and $p21^{CIP1/WAF1}$ in suppressing aneuploidy and clastogenesis induced by arsenic in cells that were genetically stable. Thus, we chose to use a telomerase immortalized diploid human fibroblast cell line, tGM24. Like their non-immortalized forbears, the tGM24 cells are genetically stable and exhibit normal DNA repair functions [33]. The sensitivity to sodium arsenite ($NaAsO_2$) was evaluated in the tGM24 cells.

Cells treated with 0–50 μM were examined for signs of cytotoxicity at 24, 48, 72 and 144 h (Figure 1). Cell viability assay suggested that dose and time-dependent cytotoxicity was occurring because the apparent concentration - dependent decrease in AlamarBlue fluorescence increased with increasing time of incubation (Figure 1A). However, when cells were observed at $NaAsO_2$ concentrations up to 20 μM using phase contrast microscopy, it

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became apparent that the principle effect was a slowing of cell growth as indicated by the $NaAsO_2$ - concentration-dependent delay of increase in cell density (Figure 1B). Arsenite-treated cells had a similar morphology to non-treated cells at 5, 10 and 20 μM for 24 h. However, after 48 h when compared with non-treated cells, a decrease of growth was observed with concentrations up to 20 μM. It was noted that the cells grow more slowly but they did not die. Morphological impacts due to cytotoxicity were evident in cultures exposed at $NaAsO_2$ concentrations ≥ 30 μM. These results were essentially identical to those obtained in similar experiments conducted with non-immortalized GM24 cells [28].

In order to establish the changes in the cell proliferation in relation to functional status of p53 and $p21^{CIP/WAF1}$, we analyzed the cell proliferation kinetics in tGM24 cells after silencing with the corresponding siRNAs. Suppression of p53 and $p21^{CIP/WAF1}$ was confirmed by Western blot (Figure 2). Effects on cell proliferation were evaluated by the scoring of mono, bi and polynucleated cells (Figure 3A, B). Suppression of p53 produced a small non-significant increase in the percentage of binucleated cells (Figure 3C). In contrast suppression of $p21^{CIP/WAF1}$ induced a significant decrease in the percentage of binucleated cells (Figure 3C), indicating a slowing of cell proliferation and suggesting that $p21^{CIP/WAF1}$ may play a role in mitotic exit. $NaAsO_2$ treatment decreased the percent binucleated Mock and NSC transfected cells (Fgiure 3C) indicating a slowing of cell proliferation. Only cells treated with 15 μM arsenite had a significant increase in cell proliferation when p53 was suppressed (Figure 3C).

Suppression of $p21^{CIP/WAF1}$ did not alter cell proliferation in tGM24 cells treated with 15 or 20 μM of arsenite (Figure 3C). It should be noted that mitomycin C and colcemid significantly impair cell proliferation kinetics as indicated by the large increases in percent mononucleated cells (Figures 3Biv, 3Bv,) and deceases in binucleated cells (Figure 3C) compared to controls (Figure 3Bi). Although siRNA knockdown of p53 and $p21^{CIP/WAF1}$ did not impact proliferation of tGM24 cells treated with colcemid, knockdown of p53, but not $p21^{CIP1/WAF1}$, increased proliferation of cells treated with mitomycin C suggesting that cell cycle arrest caused by mitomycin C was p53 dependent but $p21^{CIP1/WAF1}$ independent.

The effects of p53 and $p21^{CIP1/WAF1}$ suppression on MN induction were tested using the cytokinesis block assay (Figure 4) in tGM24 cells. Addition of cytochalasin B leads to binucleated cells due to inhibition of cytokinesis but not of karyokinesis (Figure 4A). Suppression of $p21^{WAF1/CIP1}$, but not p53, caused a significant increase in MN in control cells (Figure 4B). MN were induced by $NaAsO_2$ and colcemid independent of the suppression of p53 or $p21^{CIP1/WAF1}$ and the induction showed $NaAsO_2$ dose response in Mock, NSC and p53 siRNA transfected cells. MN induction was further increased by p53 suppression with all given treatments. Suppression of $p21^{CIP1/WAF1}$ significantly further increased MN in cells treated with colcemid but not mitomycin C or $NaAsO_2$.

To determine whether MN resulted from missegregation of chromosomes or from chromosomal fragments, we used fluorescent in situ hybridization (FISH) with a centromeric DNA probe (Figure 5). MN that are positive for centromeric DNA (MN+, Figure 5B) are derived from whole chromosomes whereas MN without centromeric DNA (MN−, Figure 5C) are derived from chromosome fragments. Although suppression of p21 in control cells increased the percentage of cells with MN (Figure 4B), it did not change the fraction of MN+ in control cells (Figure 6). Likewise, suppressing p53 did not change the ratio of MN+/MN− in control cells.

MN+ cells transfected with NSC siRNA or Mock increased 30% after arsenite exposure (both 15 and 20 μM), indicating that arsenite induced aneuploidy in the tGM24 cells. Similar results were observed in colcemid treated cells. Although suppression of p53 increased the fraction of arsenite-treated cells with MN (Figure 4B), it caused a decrease in the fraction of MN+ cells

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treated with 15 μM $NaAsO_2$ (Figure 6). This change in MN+/MN− ratio also was significant but not as great in cells treated with 20 μM $NaAsO_2$ (Figure 6). Thus, when the MN+/MN− ratio was evaluated in 15 μM $NaAsO_2$ treated cells, the suppression of p21 had no effect on the MN+/MN− ratio, but 20 μM $NaAsO_2$ treated cells did show a significant effect on the MN +/MN− ratio and decreased the fraction of MN+ similar to p53 suppression. Suppression of $p21^{CIP1/WAF1}$ dramatically suppressed the percentage of MN+ in colcemid treated cells (Figure 6) while increasing the total fraction of cells that had MN (Figure 4). Surprisingly, p53 suppression resulted in an increase in the percentage of aneuploid MN which showed centromere signals in mitomycin C-treated tGM24 cells.

4. Discussion

The induction of cytogenetic alterations by arsenite is probably not due to its direct damage effect on DNA. Arsenite is a genotoxic agent that affects cell division and the mitotic spindle apparatus, resulting in the loss or gain of whole chromosomes, thereby inducing aneuploidy. In the current study, we show that the suppression of p53 is associated with a reduction of aneuploidy induced by arsenite in telomerase immortalized diploid human fibroblasts. Arsenic induces mitotic arrest and subsequent apoptosis in cancer cell lines and p53 expression has previously been shown to affect the ability of cells to escape from mitotic arrest induced by arsenic [12,31,32]. Our results suggest that cells lacking normal p53 function cannot become aneuploid because they die by mitotic arrest-associated apoptosis, whereas cells with normal p53 function that are able to exit from mitotic arrest can become aneuploid. Furthermore our current results support this role for $p21^{CIP1/WAF1}$. Since suppression of $p21^{CIP1/WAF1}$ caused a decrease in aneuploidy induced by $NaAsO_2$ (20 μM) suggesting that $p21^{CIP1/WAF1}$ plays a role in exit from $NaAsO_2$ – induced mitotic delay (Figure 7). These observations are consistent with the role of p53 induced $p21^{CIP/WAF1}$ in allowing cells arrested in mitosis by arsenite to exit the block [12]. Thus, cells that lack $p21^{CIP1/WAF1}$ and don't divide cannot become aneuploid. On the other hand, cells not exposed to genotoxic agents and lacking $p21^{CIP1/WAF1}$ exhibited an increase in MN−, indicating an increase in clastogenesis.

Aberrations in the centrosome duplication cycle result in the formation of monopolar or multipolar spindles, leading to aneuploidy (reviewed in [1]). There is evidence linking p53 and centrosome amplification [7,11]. Centrosome amplification in tumors and cell lines has been linked to numerous genetic aberrations, including the loss of the tumor suppressor protein p53 and the deregulated expression of its regulators (Mdm2) and downstream targets ($p21^{CIP/WAF1}$, Gadd45) [35]. Our data indicate that arsenite-induced aneuploidy is clearly reduced in tGM24 cells when p53 was suppressed. These results are further supported by the findings that arsenite induces centrosome amplification prior to the induction of mitotic arrest and apoptosis [21,36]. In addition to arsenite-induced abnormal centrosome amplification, subsequent mitotic arrest has been associated with HSP70, a protein involved in organization of the spindle apparatus [21].

It should be noted that it is known that p53 plays a key role in the mitotic checkpoint. We found that the suppression of p53 inhibits the arsenite-induced aneuploidy. Our findings are supported by the role of p53 in mitotic checkpoint activation. Induction and/or activation of p53 seems to be essential for protecting cells against the development of abnormal levels of chromosomal ploidy that would be induced should the cells escape the checkpoint [11].

MN frequency was induced in cells treated with sodium arsenite, mitomycin C and colcemid. These results are consistent with the induction of DNA damage in cells treated with genotoxic agents [34]. Since the formation of MN were evaluated in cells with one cell division, it could indicate that in presence of DNA damage, the cells can ultimately undergo an aberrant mitosis and enter the ensuing G_1. More importantly, a higher induction of MN was observed in cells

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treated with all the chemicals when p53 was suppressed. As compromised function of p53 is linked to centrosome amplification, it is possible that the micronuclei formation is due to centrosome amplification during prolonged mitotic arrest induced by arsenic.

Current data show that the $p21^{CIP/WAF1}$ suppression causes a decrease in nuclear proliferation index in control cells suggesting that $p21^{CIP/WAF1}$ may play a role in mitotic exit by inhibiting the cyclin B dependent kinase CDK1 (CDC2). It has been shown that suppression of $p21^{CIP/WAF1}$ increased the occurrence of mitotic catastrophe (apoptotic mitotic death) in arsenite treated TR9-7 cells [12]. Also when A375 melanoma cells arrested in mitosis by arsenite were treated with roscovitine (a chemical inhibitor of CDK1), they exited from arsenite induced mitotic arrest [15]. Considered together, the release from mitotic arrest by both $p21^{CIP1/WAF1}$ and roscovitine suggest that inhibition of CDK1 activity is essential to release from mitotic arrest induced by arsenite. Data obtained with $p21^{CIP/WAF1}$ suppression in control cells support the hypothesis that inhibition of CDK1 by $p21^{CIP/WAF1}$ is involved in the mitotic exit network (Figure 7). Furthermore, we know that in p53 deficient cells, $p21^{CIP/WAF1}$ is still expressed although not as high as in cells with functional p53. It may be that the mitotic arrest is not as complete in p53 deficient cells as it is in $p21^{CIP/WAF1}$ suppressed cells. Hence, the micronuclei formation may be dependent on partial mitotic progression that is less likely in p21 $^{CIP/WAF1}$ suppressed cells.

In a recent study, Yu et al [37] examined the gene expression changes in response to arsenite using a microarray-based analysis. Although arsenite induces apoptosis and cell cycle arrest in a dose-dependent manner in both wildtype and p53 deficient mouse embryonic fibroblasts, arsenite induces differential gene pathways leading to cell cycle arrest and apoptosis in p53+/+ cells versus p53−/− cells [37]. Yu et al. revealed also that arsenite induced p53-dependent gene expression alterations in DNA damage and cell cycle regulation genes in p53+/+ cells, while in the p53−/− cells, arsenite induced a significant up-regulation of apoptosis-related genes (Noxa) and down-regulation of genes involved in the regulation of immune modulation.

The present study demonstrates a direct correlation between the arsenite-induced aneuploidy and the suppression of p53 and $p21^{CIP/WAF1}$. Increase in MN frequency could be consequence of increases in abnormal centrosome number which give rise to aneuploidy by altering the number of spindle poles. Considering our findings we propose that p53 expression in the presence of arsenite might accelerate the chromosomal instability and aneuploidy in p53+/+ cells. However, given that p53 is only a part of the regulatory mechanism that maintains p53 functions, future studies are needed to better understand arsenic-induced aneuploidy.

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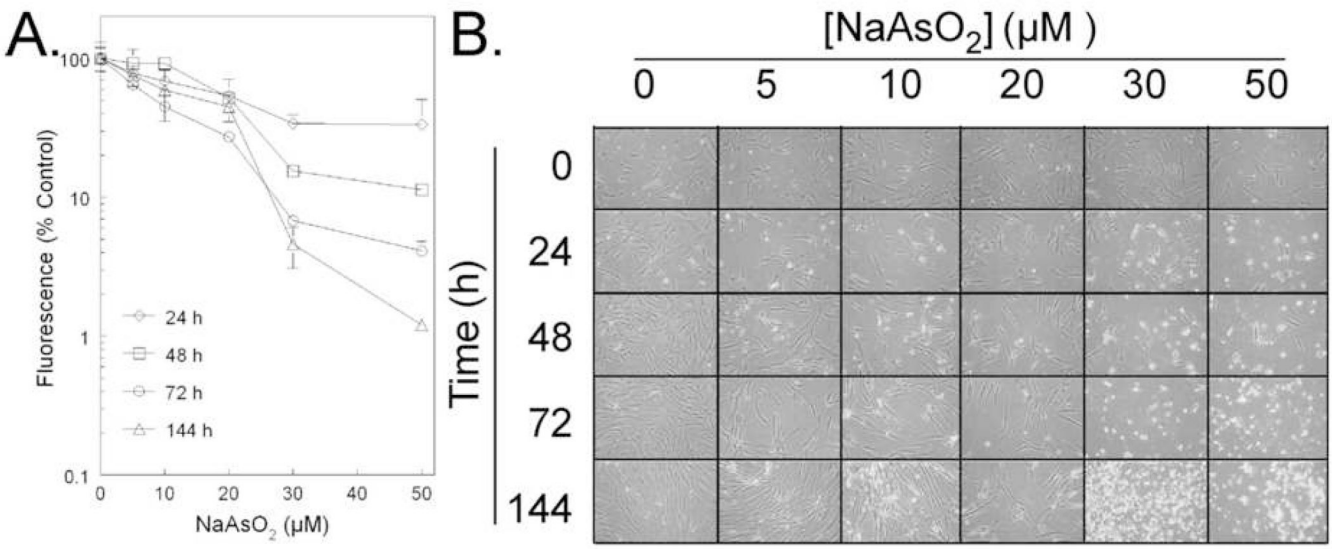

Fig. 1.

Analysis of arsenite-induced sensitivity in tGM24 fibroblasts. Cells were treated with the indicated concentrations of arsenite for 24 to 144 h. A) Cell viability assay using AlamarBlue. Data are reported arbitrary fluorescence units above the blank versus time. B) Following the exposure, morphological changes were monitored with a phase-contrast microscope.

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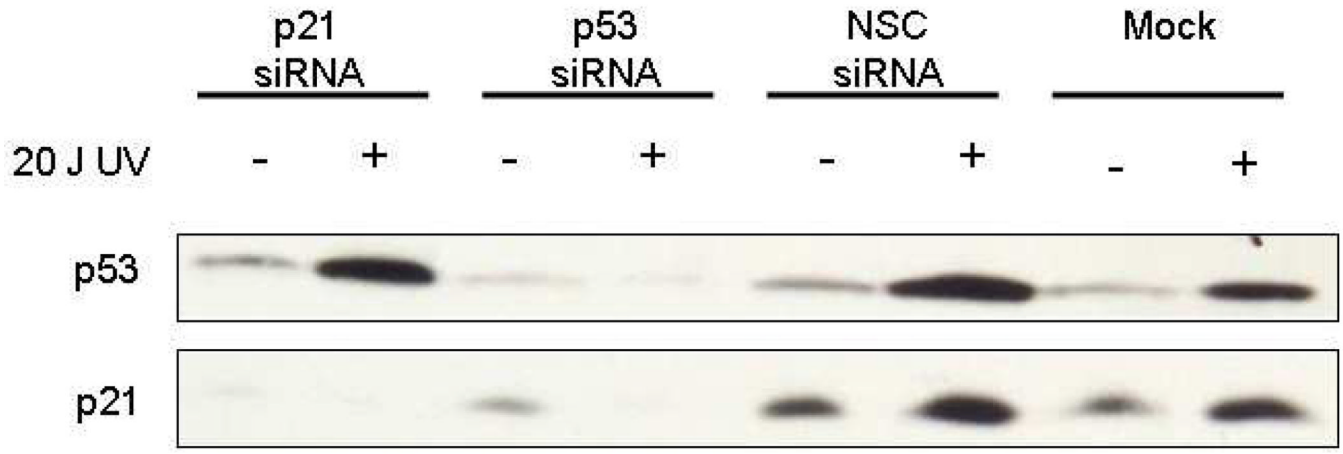

Fig. 2.

Confirmation of p53 and $p21^{CIP/WAF1}$ knockdown. Western blot analysis of p53 and $p21^{CIP/WAF1}$ in tGM24 fibroblasts unexposed and exposed to UV radiation. Uniformity of loading and transfer were confirmed by staining the membrane with Ponceau Red.

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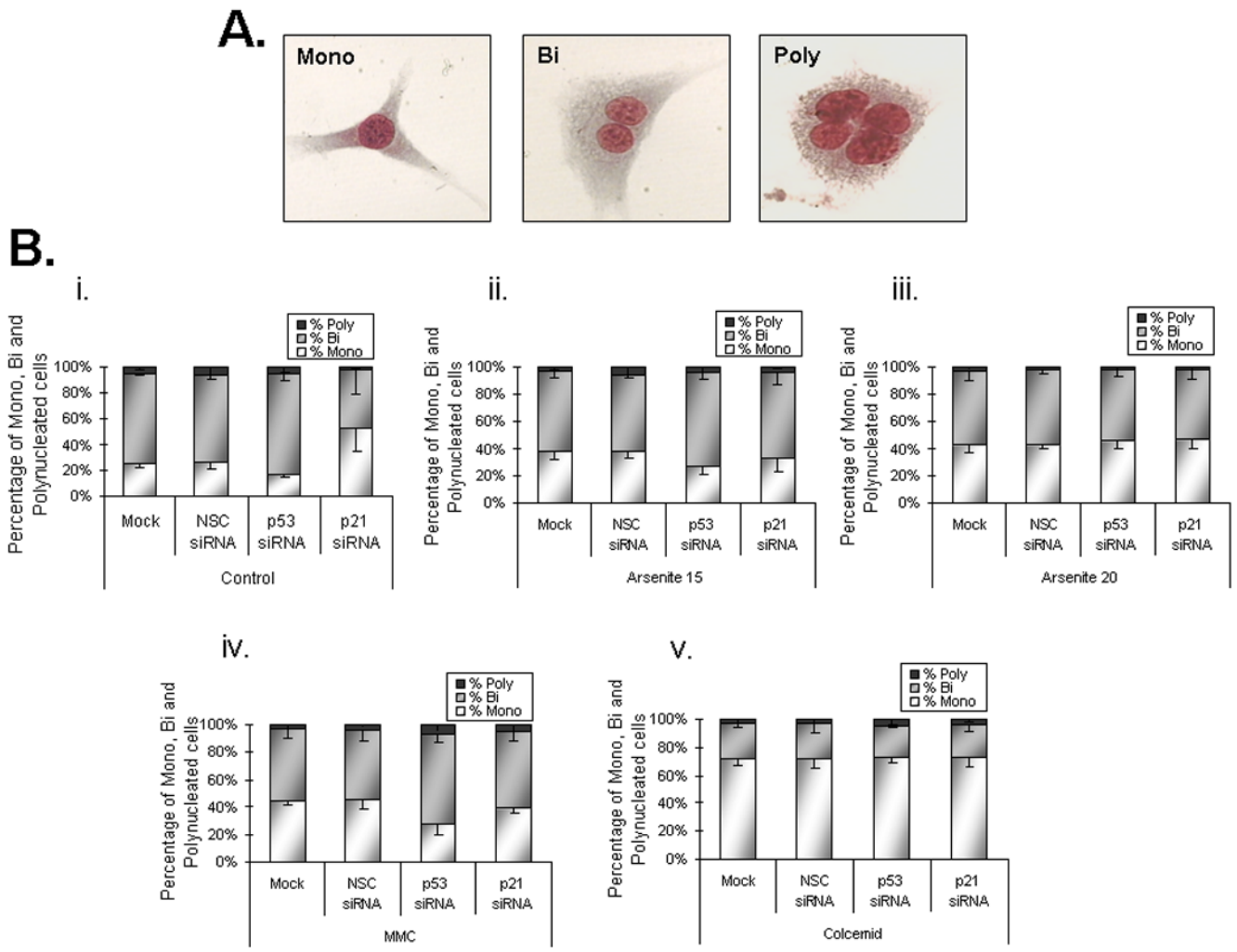

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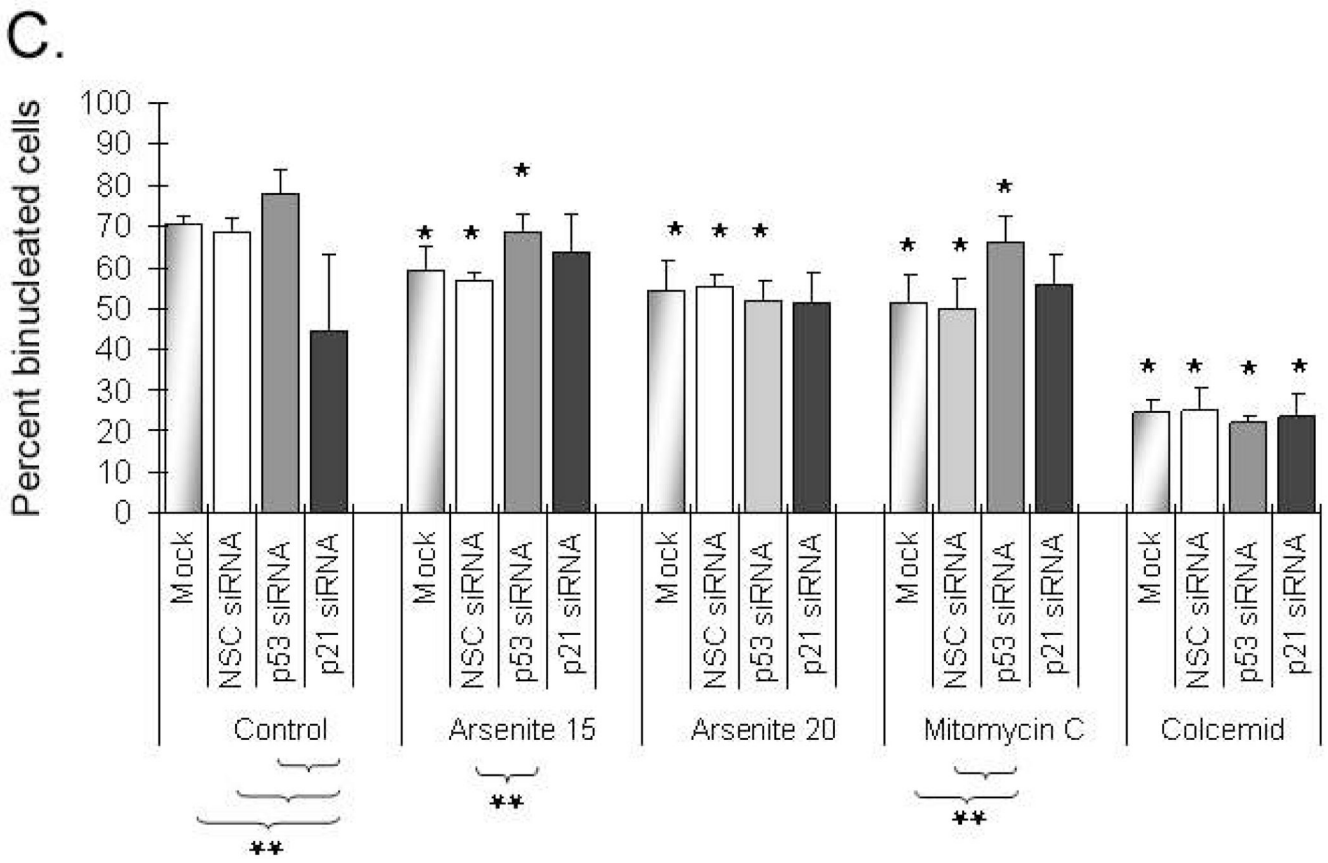

Fig. 3.

Effects on cell proliferation kinetics by suppression of p53 and $p21^{CIP1/WAF1}$ in tGM24 fibroblasts. A) Representative images of mononuclear, binuclear and polynuclear cells are shown. B) Analysis of mononucleated (Mono), binucleated (Bi) and polynucleated (Poly) cells. C) Analysis of the cytostatic effect. *Y*-axis indicates the percentage of binucleated cells (BN) graphed as bars. Cells were incubated with sodium arsenite (15 and 20 μM), mitomycin C and colcemid for 24 h. Knock-down of p53 and $p21^{CIP1/WAF1}$ by siRNA transfection was performed as indicated (Materials and methods). Data represent the mean ± S.D. calculated from three independent experiments. *$p \leq 0.05$ with respect to corresponding control cells; **$p \leq 0.05$ with respect to each treatment as indicated by brackets (ANOVA test).

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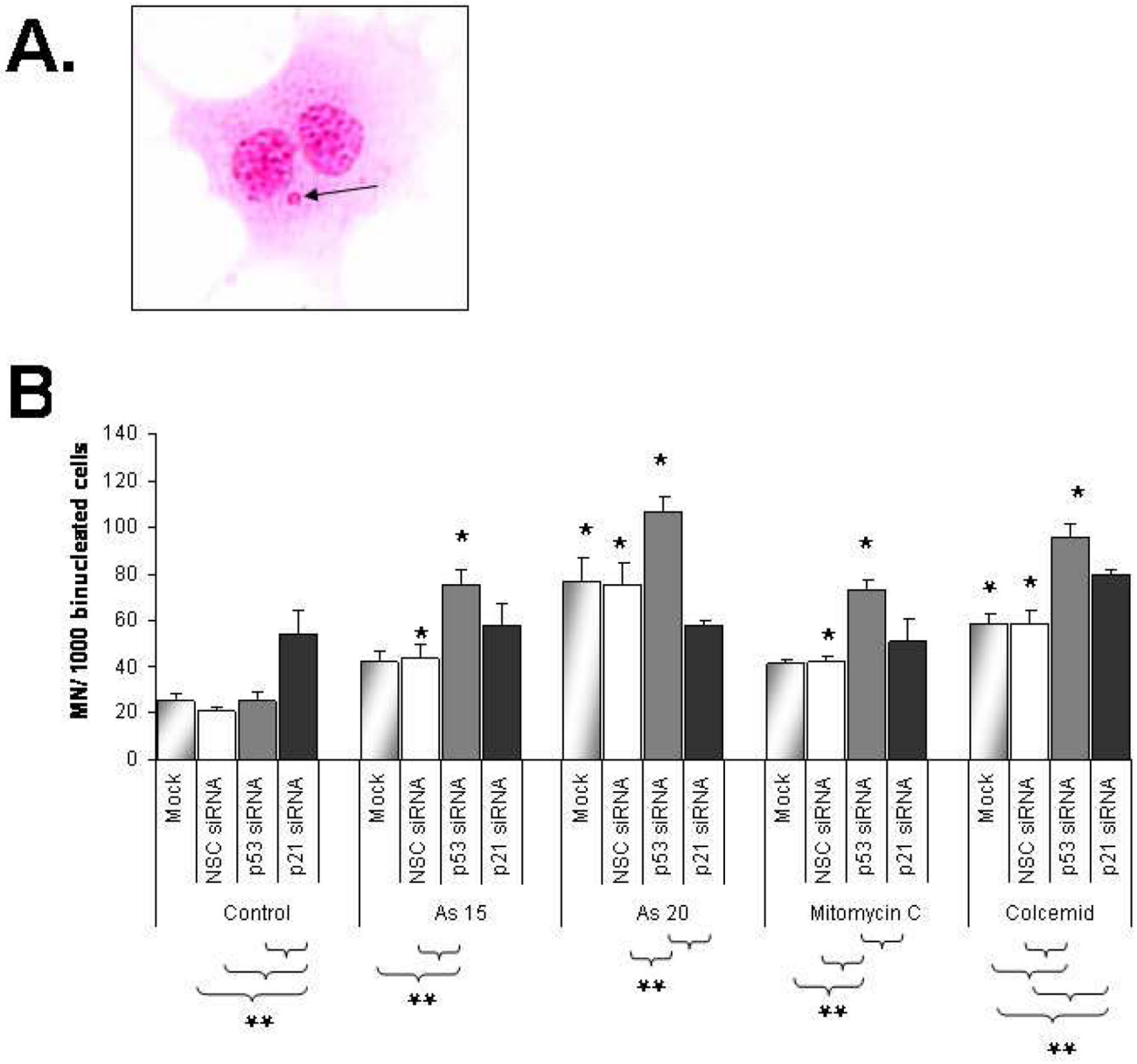

Fig. 4.

Effects on cell proliferation kinetics by suppression of p53 and $p21^{CIP1/WAF1}$ in tGM24 fibroblasts. A) Representative images of mononuclear, binuclear and polynuclear cells are shown. B) Analysis of mononucleated (Mono), binucleated (Bi) and polynucleated (Poly) cells. C) Analysis of the cytostatic effect. *Y*-axis indicates the percentage of binucleated cells (BN) graphed as bars. Cells were incubated with sodium arsenite (15 and 20 μM), mitomycin C and colcemid for 24 h. Knock-down of p53 and $p21^{CIP1/WAF1}$ by siRNA transfection was performed as indicated (Materials and methods). Data represent the mean ± S.D. calculated from three independent experiments. *$p \leq 0.05$ with respect to corresponding control cells; **$p \leq 0.05$ with respect to each treatment as indicated by brackets (ANOVA test).

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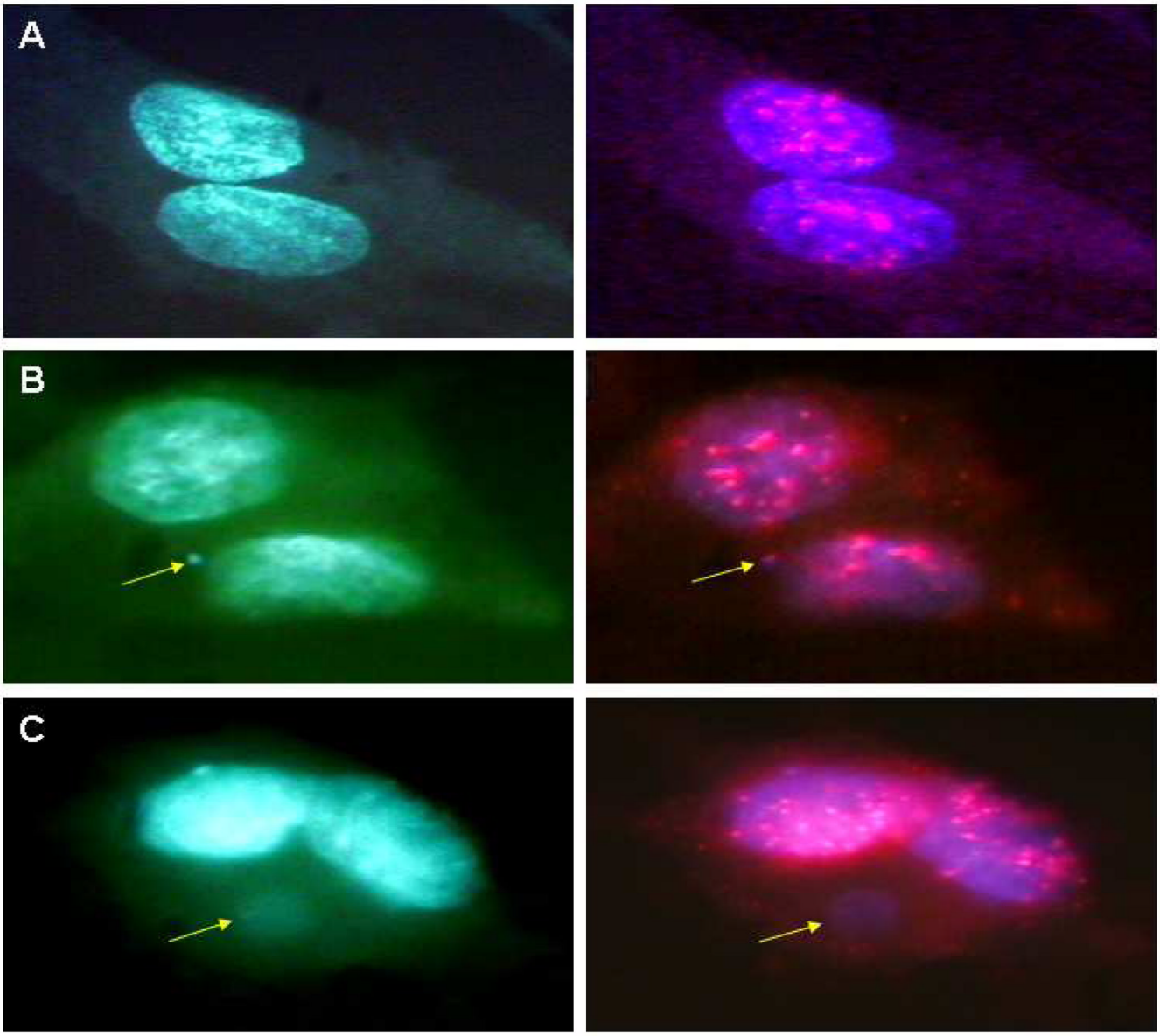

Fig. 5.

Fluorescence in situ hybridization (FISH) images of centromeric DNA probe hybridization in tGM24 cells. Only cells with well-preserved cytoplasm were examined under DAPI filter (left panels) and centromeric regions in the same cells with the filter for visualization of Cy3 (merged image in right panels). A) Typical binucleated cell. B) Binucleated cell with a signal in MN (MN+; arrows). C) Binucleated cell without a centromeric signal (MN−; arrows).

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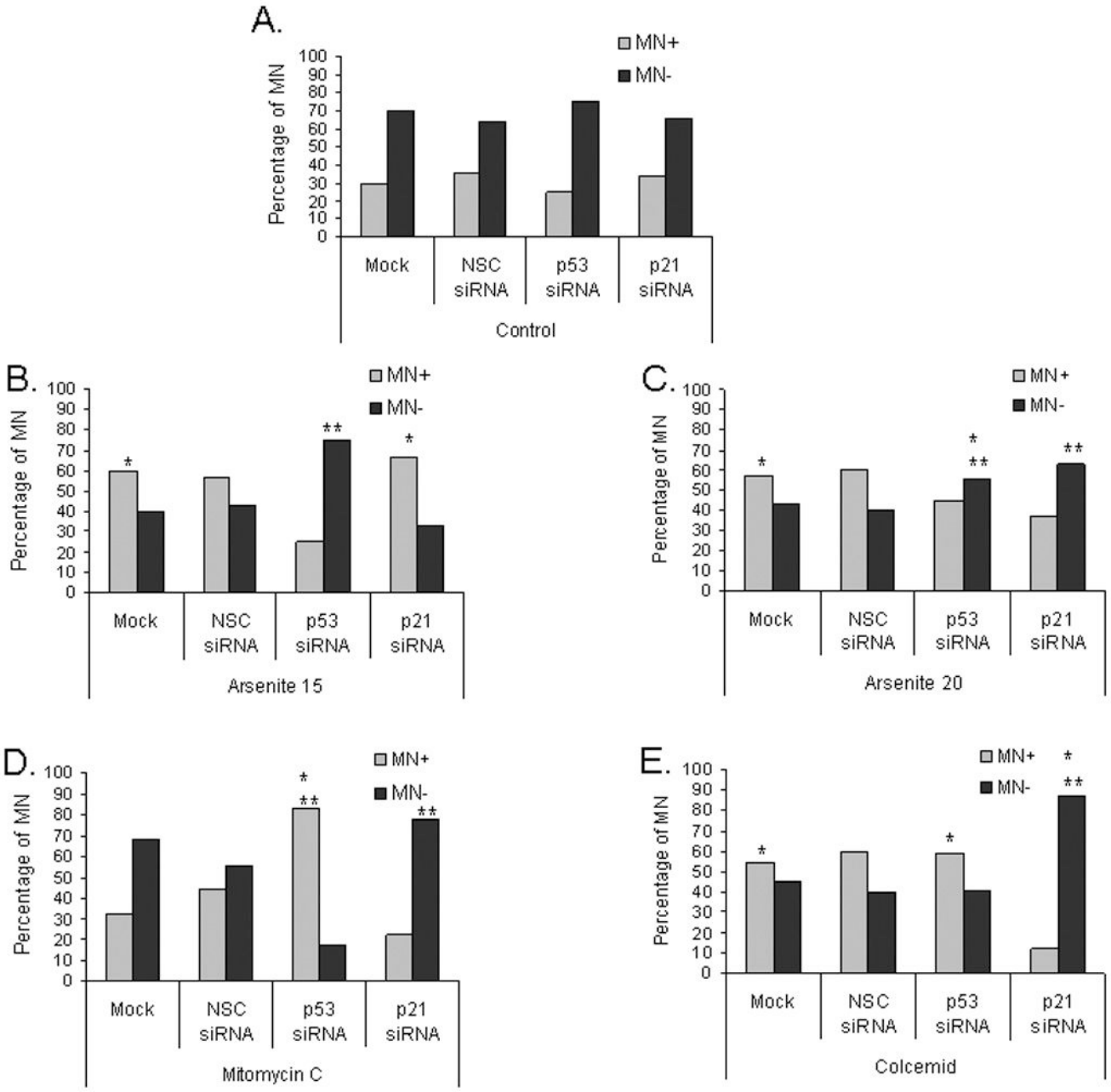

Fig. 6.

Suppression of p53 and p21$^{CIP1/WAF1}$ reduces arsenite-induced aneuploidy. MN frequency was evaluated in tGM24 fibroblasts treated with sodium arsenite (15 and 20 μM), mitomycin C and colcemid for 24 h. Knock-down of p53 and p21$^{CIP1/WAF1}$ by siRNA transfection was performed as indicated (Materials and methods). Data show the percentage of cells containing MN centromere positive (MN+) or MN centromere negative (MN−). *$p \leq 0.05$ with respect to corresponding control cells; **$p \leq 0.05$ with respect to each treatment as indicated by brackets (Fisher´s exact test).

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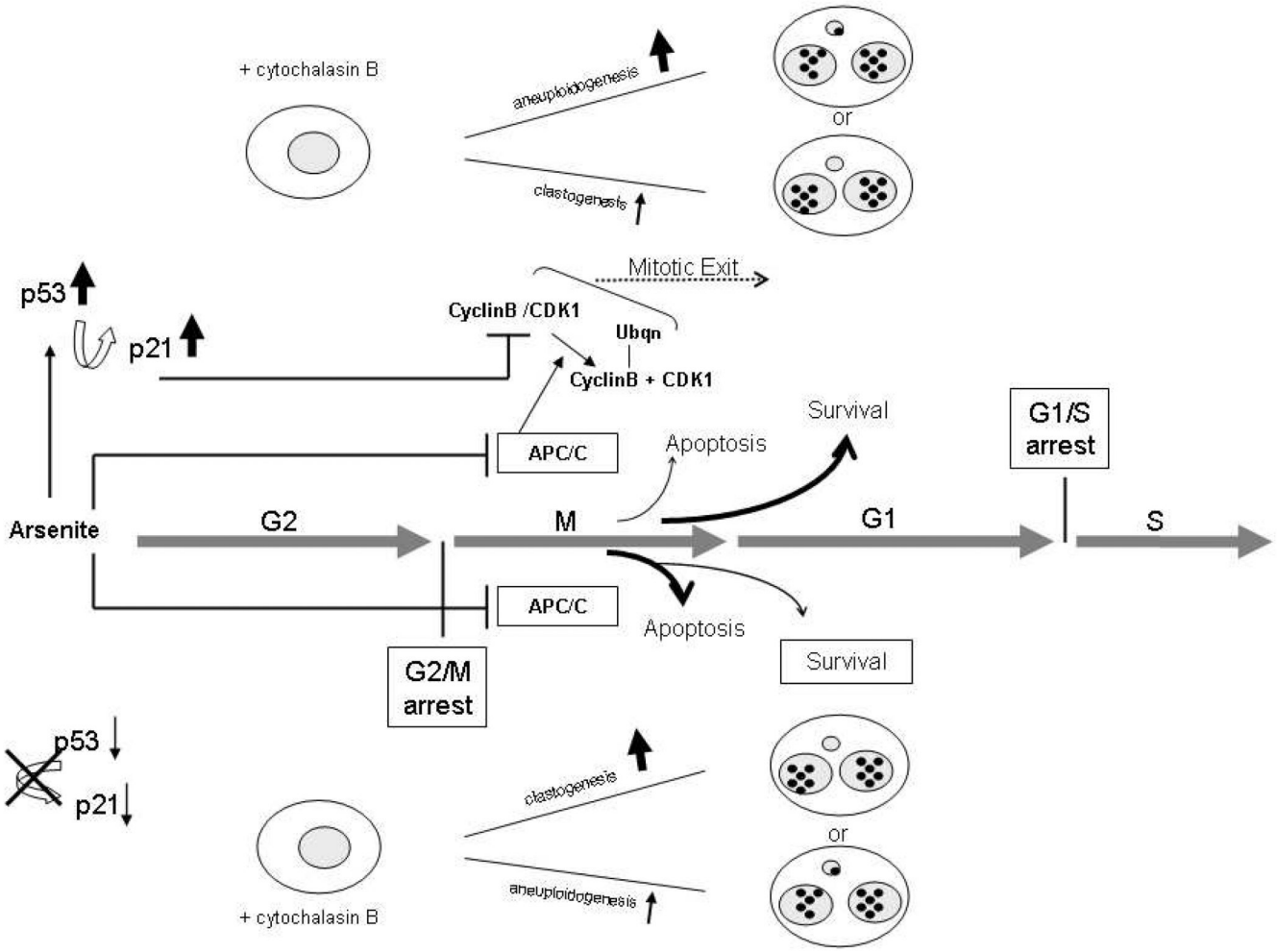

Fig. 7.

Schematic of proposed role of $p21^{CIP1/WAF}$ induction in exit from $NaAsO_2$-induced mitotic delay and the effect on aneuploidy. Cyclin B is normally targeted for proteasomal degradation by polyubiquitinylation by the anaphase promoting complex/cyclosome (APC/C) and thus CDK1 activity is decreased allowing mitotic exit. $NaAsO_2$ induces mitotic delay or mitotic arrest by stabilization of cyclin B and maintenance of CDK1 activity [12,16] most likely a result of inhibition of APC/C. Thus, inhibition of CDK1 activity is essential to exit from $NaAsO_2$ – induced mitotic arrest [15]. Cells unable to exit mitosis suffer mitotic catastrophe and die by apoptosis [12], whereas the cells that recover from mitotic disruption can become aneuploid, $NaAsO_2$ induces p53 in mitotically arrested cells, which in turn induces the cyclin dependent kinase inhibitor $p21^{CIP1/WAF1}$ which inhibits CDK1 activity allowing mitotic exit and avoidance of apoptosis.