Electronic Supplementary Material (ESI) for Toxicology Research.

Supplemental results

Figure S1. The proportion of apoptotic cells after $NaAsO_2$ treatment. HEK293 cells were treated with various concentrations of $NaAsO_2$ for 24 h and stained with propidium iodide. Sub-G1 fractions of the cells were determined by flow cytometry (Lin,et al., J. Cell. Physiol. 205:428-436, 2005). Each value represents a mean ± standard deviation of three independent samples. Asterisks indicate significant differences as compared to that of untreated samples. *: $p < 0.05$.

Figure S2. Effect of various inhibitors on the lysosomal activity of arsenite-treated cells. HEK293 cells were treated with 20 μM $NaAsO_2$ for 24 h in the presence of (A) ERK (PD98059, 20 μM), JNK (SP600125, 15 μM) or p38 (SB202190, 10 μM) inhibitor and (B) apoptosis inhibitor (z-VAD-FMK, 20 μM). Lysosomal (acid phosphatase) activities were determined. Each value represents a mean ± standard deviation of three independent samples.

Figure S3. PTEN is not degraded through proteasome in arsenite-treated. Cells were treated with 20 μM $NaAsO_2$ for 24 h in the presence or absence of 20 μM MG132. PTEN levels in SF and InF were analyzed by immunoblotting. GAPDH and Lamin B1 were used as a loading control for SF and InF, respectively.

Figure S4. Effect of apoptosis inhibitor on factors altered by arsenite treatment. HEK293 cells were treated with 20 μM $NaAsO_2$ for 24 h in the presence of apoptosis inhibitor (z-VAD-fmk, 20 μM). Cells were harvested and mTOR, LC3 and p62 were visualized by immunoblotting. The phosphorylated mTOR level was indicated as pmTOR.

Figure S5. Effect of JNK inhibitor on the viability of arsenite treated cells. HEK293 cells were treated with 20 μM $NaAsO_2$ for 24 h in the presence of JNK inhibitor (SP600125, 15 μM). Cell viability was determined by the MTT assay. Each value represents a mean ± standard deviation of three independent samples. Asterisk indicates significant differences as compared to that of untreated samples. *: $p < 0.05$.

Figure S6. Effect of ERK inhibitor on the viability of arsenite-treated cells. HEK293 cells were treated with 20 μM $NaAsO_2$ for 24 h in the presence of ERK inhibitor (PD98059, 20 μM) with and without rapamycin (100 nM). Cell viability was determined by the MTT assay. Each value represents a mean ± standard deviation of three independent samples. Asterisks indicate significant differences as compared to that of untreated samples. *: $p < 0.05$.

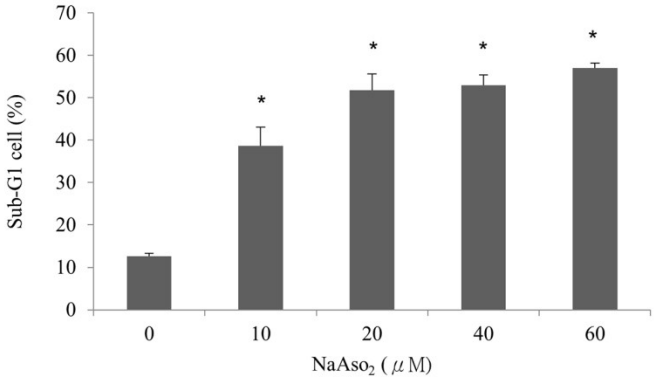

FIG. S1

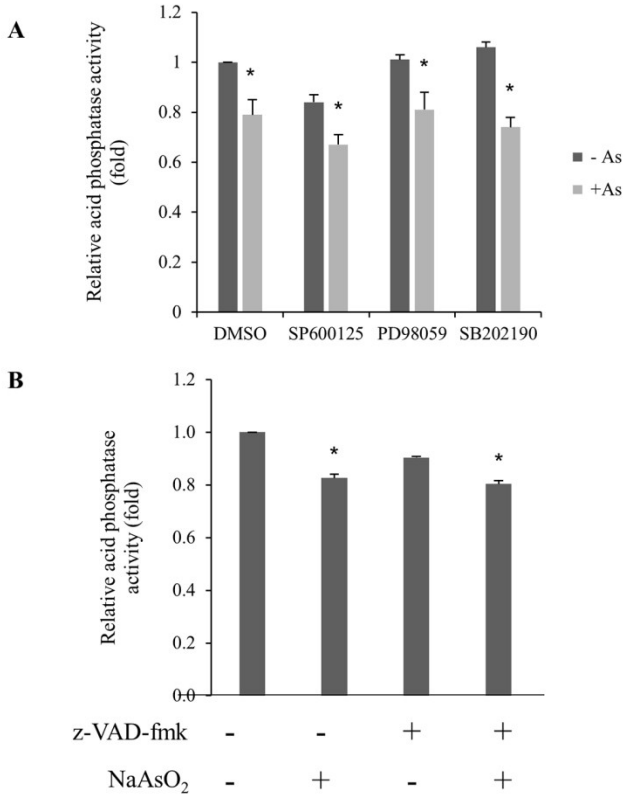
A
Relative acid phosphatase activity (fold)
1.2
1
0.8
0.6
0.4
0.2
0
DMSO
SP600125
PD98059
SB202190
- As
+As
B
Relative acid phosphatase activity (fold)
1.2
1.0
0.8
0.6
0.4
0.2
0.0
z-VAD-fmk - - + +
NaAsO2 - + - +

FIG. S2

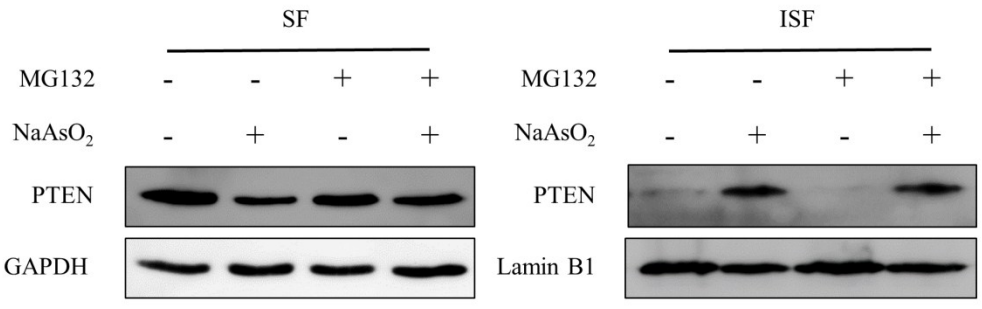
SF
ISF
MG132 - - + +
NaAsO2 - + - +
PTEN
GAPDH
MG132 - - + +
NaAsO2 - + - +
PTEN
Lamin B1

FIG. S3

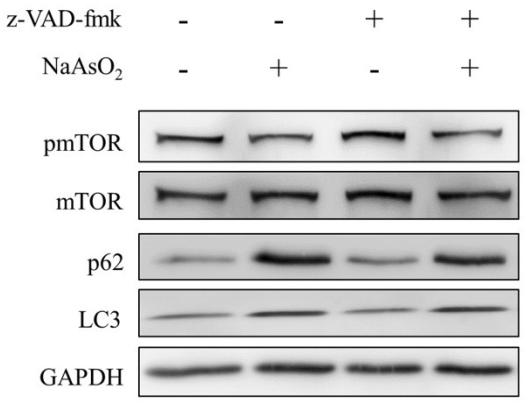
z-VAD-fmk - - + +
NaAsO2 - + - +
pmTOR
mTOR
p62
LC3
GAPDH

FIG. S4

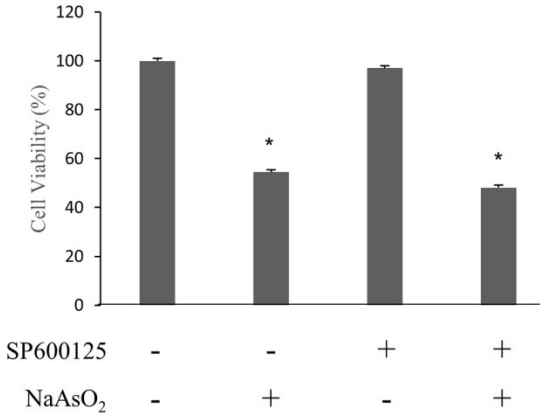
Cell Viability (%)
120
100
80
60
40
20
0
*
*
SP600125 - - + +
NaAsO2 - + - +

FIG. S5

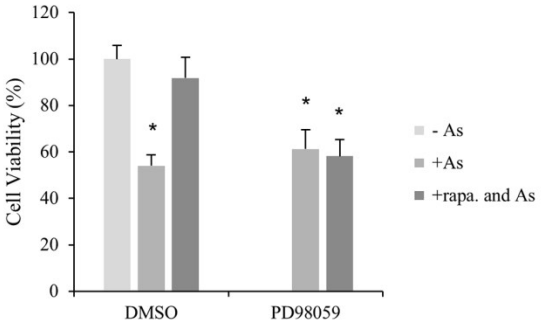
120
100
80
60
40
20
0
Cell Viability (%)
*
*
*
DMSO
PD98059
- As
+As
+rapa. and As

FIG. S6