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The Gene Expression Profile Using cDNA microarray after treatment of Arsenic Compound (As_2O_3, As_4O_6) in SiHa Cell

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=ABSTRACT=

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Objective: To obtain information on the growth inhibition effect of arsenic compounds and gene expression profiles using cDNA microarray technique in SiHa cell lines.
Methods: We cultured 103 SiHa cell in 96 well plate and investigated growth inhibition effects using MTT assay and also we performed gene expression profile experiment using 384 cDNA chip in SiHa cell after exposure of arsenics (As_2O_3, As_4O_6 - 1 μM) for 48 hrs.
Results: Arsenics (As_2O_3, As_4O_6) inhibit the growth of SiHa cells (As2O3: 0.5, 1, 2, 3, 4, 5 μM - 9.2, 56, 89, 93, 96, 96%, As4O6: 0.5, 1, 2, 3, 4, 5 μM- 54, 84, 84, 85, 85, 87%) in 4 days culture. As_2O_3 and As4O6 induced apoptosis in SiHa cells. After exposure of As_2O_3, 47 genes were changed more than 2 times (eg, *thymidylate synthetase*, *cyclin B1*, *CDC 20*). In case of As_4O_6, 78 genes were changed more than 2 times (eg, *CDC 20*, *cyclin B1*, *primase*, *proliferating cell nuclear antigen*).
Conclusion: we observed arsenic compound (As_2O_3, As_4O_6) inhibit the growth of SiHa cell. In gene expression profiling experiment, 78 genes were changed the expression level 2 times more than that of reference RNA after treatment of As_4O_6 and 47 genes after treatment of As_2O_3. Through these results, we thought more study is needed in functional genomics after arsenic treatment at cervical cancer cells.

Key Words: Arsenic compound (As_2O_3, As_4O_6), Cervical cancer cell line (SiHa), cDNA chip

In order to reduce the mortality rate from cervical cancer, early diagnosis of cervical cancer has been developed for 20 years, and therapies such as surgical treatment, radiation therapy, immunotherapy and recently gene therapy have been developed and used. However, preventing relapse after treatment

remains a challenge in the treatment of cervical cancer.[1]

Human papillomavirus is known as a carcinogen-causing virus commonly found in cervical cancer tissues.[2] This virus induces the expression of E6 and E7, which are carcinogenic genes that it has after infection in human cells, respectively forms the complexity of p53 and pRB, which are cancer inhibition protein, to inactivate p53 and pRB, and promotes the degradation of p53 and pRB factors through Ubiquitin pathway.[3] Based on this, recently, gene therapy for inducing apoptosis of cervical cancer cells by inducing high expression of p53 protein by an artificial gene introduction in order to increase the concentration of p53 has been attempted.[4]

In general, arsenic is a deadly toxic substance of colorless, tasteless, odorless, and is dispersed as As_2S_2, As_2S_3, $KAsO_2$ in the natural world, in particular, arsenic trioxide (As_2O_3) and arsenic trisulfide (As_2S_3) have been used for a long time in the treatment of leukemia in China, and have been reported to have been used in oriental medicine in Korea and have been used in ancient Western medicine for a long time in the treatment of leukemia. Recently, since the announcement of November, 1996 that patients with leukemia can treat with arsenic trioxide (As_2O_3), studied by Chen Sai-silk and Chen Hui-shin of Shanghai Hematology Institute of Shanghai Second Medical University, research is being actively conducted.[2,5]

Recently, it has been reported that As_4O_6 selectively acts on cancer cells such as As_2O_3 to induce cell death of cancer cells, and it is known to induce cell death in NB4 cell even at a low concentration.[6] There is a report that As_2O_3 promotes cell division at low concentrations (0.1-0.5 μM) and induces cell death at high concentrations (0.5-2 μM) depending on the cell type.[2] In addition, the effects of inducing apoptosis and inhibiting the expression of E6 and E7, which are carcinogenic genes, has been reported for HPV16 metastatic cancer cells, but the mechanism is not clear yet.[7] Like this, the arsenic oxide compound has a simple structure and selectively acts on a carcinogenic gene or an expression product thereof, suggesting the possibility as an excellent chemotherapeutic agent having few side effects, but it is difficult to handle the toxicity of As_2O_3.[8]

The aim of this study was to investigate the effect of arsenic compounds (As_2O_3, As_4O_6) in the HPV-16 positive SiHa cell line to develop new therapies which are clinically more stable and effective in the treatment of cervical cancer through the mechanism of growth inhibition of arsenic compounds and the gene expression.

Research subjects and methods

1. Cell line and cell culture

The cervical cancer cell lines used in this study were HPV 16 positive SiHa cells with wild type p53 gene. Cell culture media were incubated with 5% fetal bovine serum (FBS, Gibco-BRL), 0.37% sodium bicarbonate (Amresco, Solon, Ohio) and 30 mM HEPES (Amresco) in Dulbecco's Modified Eagle Media (DMEM, Gibco- And streptomycin/penicillin (Gibco-BRL). These cell lines were cultured at 37 ° C in a CO_2 incubator.

2. Subjects and analsis methods

1) Cell growth inhibition assay-MTT assay

For MTT assay, SiHa cells were inoculated into 96-well plates at a density of 1×10^3 cells / well. After 24 hours, arsenic compounds were treated at 0, 0.5, 1, 2, 3, 4, 5μM and observed for 4 days.

MTT solution (Sigma, St. Louis, Mo.) was added to Wells in 20 μl and incubated in a CO_2 incubator for 4 hours. After removing all supernatant, add 100 μl / well of DMSO (Sigma) and then shake for 10 seconds on shaker and measure absorbance at 570 nm with ELISA-Reader (Spectromax 250, Molecular Devices). Absorbance measurements were repeated three times and the mean value was taken.

2) Measurement of DNA fragmentation Formation

After 24 hours after inoculating $5x10^5$ SiHa cells into a 100 mm dish, arsenic compounds (As_2O_3, As_4O_6) were treated at 0, 0.5, 1, and 2 μM. After 48 hours, the cells were collected and lysed with lysis buffer [0.8% SDS, 0.1 M NaCl, 0.1 M EDTA, 50 mM Tris-HCl (pH 8.0)] added 20 μg/ml of proteinase K (Sigma) and reacted at 56 ° C for 4 hours. After completion of the reaction, the protein was removed by treatment with Phenol / Chloroform, and genomic DNA was extracted. 5 μg of the extracted DNA was electrophoresed on 2% agarose gel at 50 V for 2 hours. After electrophoresis, the agarose gel was stained with EtBr solution (1 ug / ml, Sigma) and DNA ladder was confirmed on a UV illuminator.

3) RNA extraction

After 24 hours of SiHa cell inoculation in a 100 mm dish with 5×10^5 concentrations, 2 μM of arsenic compounds (As_2O_3, As_4O_6) were treated and cultured for 48 hours. After the incubation, the supernatant was removed and the cells were washed with PBS buffer and then total RNA was extracted using TRI reagent (MRC. Chincineti Ohio). The extracted RNA was diluted to 1/500 and the purity and amount of RNA were determined by measuring OD at 260 nm and 280 nm of a spectrophotometer (Amersharm Pharmacia Biotech., Buckinghamshire, UK).

4) DNA chip analysis

4-1) DNA chip

The 384 DNA chip used in this experiment was purchased from Macrogen Inc. (Seoul, Korea).

4-2) 384 cDNA list

1. Hs.240534 "1-acylglycerol-3-phosphate O-acyltransferase1 (lysophosphatidic acid acyltransferase, alpha)"
2. Hs.87417 cathepsin L2
3. Hs.144058 DKFZP564C103 protein
4. Hs.7301 G protein pathway suppressor 2
5. Hs.186711 hypothetical protein FLJ20070
6. Hs.15087 KIAA0250 gene product
7. Hs.52081 KIAA0867 protein
8. Hs.285754 met proto-oncogene (hepatocyte growth factor receptor)
9. Hs.51 "phosphatidylinositol glycan, class A (paroxysmal nocturnal hemoglobinuria)"
10. Hs.77028 protein translocation complex beta
11. Hs.153022 "TATA box binding protein (TBP)-associated factor, RNA polymerase I, C, 110kD"
12. actin
13. Hs.264981 2'-5'oligoadenylate synthetase 2
14. Hs.85963 "CD36 antigen (collagen type I receptor, thrombospondin receptor)-like 2
15. Hs.134200 DKFZP564C186 protein
16. Hs.79222 "galactosidase, beta 1"

17. Hs.225767 IDN3 protein
18. Hs.118978 KIAA0256 gene product
19. Hs.104305 KIAA0926 protein
20. Hs.101448 metastasis associated 1
21. Hs.283006 "phospholipase C, beta 4"
22. Hs.108969 PTD008 protein
23. Hs.155188 "TATA box binding protein (TBP)-associated factor, RNA polymerase II, F, 55kD"
24. actin
25. Hs.67896 7-60 protein
26. Hs.79197 "CD83 antigen (activated B lymphocytes, immunoglobulin superfamily)"
27. Hs.3804 DKFZP564C1940 protein
28. Hs.76392 "gamma-glutamyl hydrolase (conjugase, folylpolygammaglutamyl hydrolase)"
29. Hs.7089 insulin induced protein 2
30. Hs.74579 KIAA0263 gene product
31. Hs.173656 KIAA0941 protein
32. Hs.154672 "methylene tetrahydrofolate dehydrogenase (NAD+ dependent)
33. Hs.54941 "phosphorylase kinase, alpha 2 (liver)"
34. Hs.75082 "ras homolog gene family, member G (rho G)"
35. Hs.60679 "TATA box binding protein (TBP)-associated factor, RNA polymerase II, G, 32kD"
36. tubulin
37. Hs.1211 "acid phosphatase 5, tartrate resistant"
38. Hs.82906 "CDC20 (cell division cycle 20, S. cerevisiae, homolog)"
39. Hs.24766 DKFZP564E1962 protein
40. Hs.26655 "glucose-6-phosphatase, transport (glucose-6-phosphate) protein 1"
41. Hs.111577 integral membrane protein 2C
42. Hs.31463 KIAA0281 gene product
43. Hs.172825 KIAA1037 protein
44. Hs.155462 "minichromosome maintenance deficient (mis5, S. pombe) 6"
45. Hs.77274 "plasminogen activator, urokinase"
46. Hs.274494 RBP1-like protein
47. Hs.32935 "TATA box binding protein (TBP)-associated factor, RNA polymerase III, C, 90kD"
48. tubulin
49. Hs.180952 "actin, beta"
50. Hs.172405 cell division cycle 27
51. Hs.16492 DKFZP564G2022 protein
52. Hs.172153 glutathione peroxidase 3 (plasma)
53. Hs.271986 "integrin, alpha 2 (CD49B, alpha 2 subunit of VLA-2 receptor)"
54. Hs.96485 KIAA0290 protein
55. Hs.227835 KIAA1049 protein
56. Hs.47007 mitogen-activated protein kinase kinase kinase 14
57. Hs.1050 "pleckstrin homology, Sec7 and coiled/coil domains 1 (cytohesin 1)"
58. Hs.79372 "retinoid X receptor, beta"
59. Hs.115256 telomerase reverse transcriptase
60. lambda 564
61. Hs.10247 activated leucocyte cell adhesion molecule
62. Hs.77204 "centromere protein F (350/400kD, mitosin)"
63. Hs.72157 DKFZP564I1922 protein

64. Hs.5233 glutathione S-transferase M4
65. Hs.83968 "integrin, beta 2 (antigen CD18 (p95), lymphocyte function-associated antigen 1"
66. Hs.155979 KIAA0295 protein
67. Hs.126084 KIAA1055 protein
68. Hs.7510 mitogen-activated protein kinase kinase kinase 7
69. Hs.44 "pleiotrophin (heparin binding growth factor 8, neurite growth-promoting factor 1)"
70. Hs.75879 ribosomal protein L19
71. Hs.7733 tetratricopeptide repeat domain 1
72. lambda 564
73. Hs.28914 adenine phosphoribosyltransferase
74. Hs.1708 "chaperonin containing TCP1, subunit 3 (gamma)"
75. Hs.165998 DKFZP564M2423 protein
76. Hs.226795 glutathione S-transferase pi
77. Hs.99995 intercellular adhesion molecule 3
78. Hs.5716 KIAA0310 gene product
79. Hs.285211 KIAA1067 protein
80. Hs.86575 mitogen-activated protein kinase kinase kinase kinase 1
81. Hs.99890 "polymerase (DNA directed), delta 1, catalytic subunit (125kD)"
82. Hs.275865 ribosomal protein S18
83. Hs.125359 Thy-1 cell surface antigen
84. lambda 1.0k
85. Hs.75081 adenomatosis polyposis coli
86. Hs.20295 "CHK1 (checkpoint, S.pombe) homolog"
87. Hs.107747 DKFZP566C243 protein
88. Hs.180577 granulin
89. Hs.86958 "interferon (alpha, beta and omega) receptor 2"
90. Hs.7911 KIAA0323 protein
91. Hs.106711 KIAA1085 protein
92. Hs.227400 mitogen-activated protein kinase kinase kinase kinase 3
93. Hs.74598 "polymerase (DNA directed), delta 2, regulatory subunit (50kD)"
94. Hs.169407 "SAC2 (suppressor of actin mutations 2, yeast, homolog)-like"
95. Hs.82962 thymidylate synthetase
96. lambda 1.0k
97. Hs.1217 adenosine deaminase
98. Hs.79018 "chromatin assembly factor 1, subunit A (p150)"
99. Hs.224137 DKFZP566D143 protein
100. Hs.79000 growth associated protein 43
101. Hs.5337 "isocitrate dehydrogenase 2 (NADP+), mitochondrial"
102. Hs.103915 KIAA0346 protein
103. Hs.278442 KIAA1090 protein
104. Hs.82979 mitogen-activating protein kinase kinase kinase kinase 2
105. Hs.46964 "polymerase (DNA-directed), mu"
106. Hs.278431 "SCO (cytochrome oxidase deficient, yeast) homolog 2"
107. Hs.5831 "tissue inhibitor of metalloproteinase 1 (erythroid potentiating activity, collagenase inhibitor)"
108. actin
109. Hs.8402 adenylate cyclase 3

110. Hs.19554 chromosome 1 open reading frame 2
111. Hs.98693 DKFZP586J0917 protein
112. Hs.73172 growth factor independent 1
113. itag6
114. Hs.23263 KIAA0350 protein
115. Hs.72172 KIAA1115 protein
116. Hs.170027 "mouse double minute 2, human homolog of; p53-binding protein"
117. Hs.127007 "potassium channel, subfamily K, member 5 (TASK-2)"
118. Hs.313 "secreted phosphoprotein 1 (osteopontin, bone sialoprotein I, early T-lymphocyte activation 1)"
119. tp53
120. actin
121. Hs.1239 "alanyl (membrane) aminopeptidase"
122. cldn7
123. Hs.22981 DKFZP586M1523 protein
124. Hs.115352 growth hormone 1
125. Hs.23119 ITBA1 gene
126. Hs.168052 KIAA0421 protein
127. Hs.131728 KIAA1140 protein
128. Hs.172210 MUF1 protein
129. Hs.30743 preferentially expressed antigen in melanoma
130. semp1
131. Hs.80598 "transcription elongation factor A (SII), 2"
132. tubulin
133. Hs.105445 "aldolase A, fructose-bisphosphate"
134. Hs.157113 "coenzyme Q, 7 (rat, yeast) homolog"
135. Hs.4766 DKFZP586O0120 protein
136. Hs.65149 growth hormone 2
137. Hs.2340 junction plakoglobin
138. Hs.214646 KIAA0447 gene product
139. Hs.26229 KIAA1366 protein
140. Hs.24297 multiple endocrine neoplasia I
141. Hs.173609 pregnancy specific beta-1-glycoprotein 1
142. Hs.153003 serine/threonine kinase 16
143. Hs.155202 "transcription elongation factor B (SIII), polypeptide 3 (110kD, elongin A)"
144. tubulin
145. Hs.234726 alpha-1-antichymotrypsin
146. Hs.179573 "collagen, type I, alpha 2"
147. Hs.6285 DKFZP586P0123 protein
148. Hs.273457 "guanine nucleotide binding protein (G protein), beta polypeptide 2"
149. Hs.65114 keratin 18
150. Hs.29956 KIAA0460 protein
151. Hs.128867 KIAA1372 protein
152. Hs.170120 muscle specific gene
153. Hs.225932 pregnancy specific beta-1-glycoprotein 7
154. Hs.48915 serine/threonine kinase 6
155. Hs.125156 "transcriptional adaptor 2 (ADA2, yeast, homolog)-like"

156. lambda 564
157. Hs.74561 alpha-2-macroglobulin
158. Hs.75617 "collagen, type IV, alpha 2"
159. Hs.151696 DKFZP727G051 protein
160. her2
161. Hs.182265 keratin 19
162. Hs.239686 KIAA0462 protein
163. Hs.86392 KIAA1402 protein
164. Hs.1345 mutated in colorectal cancers
165. Hs.82741 "primase, polypeptide 1 (49kD)"
166. Hs.112049 SET binding factor 1
167. Hs.27299 transcriptional regulator protein
168. lambda 564
169. Hs.3763 "amyloid beta (A4) precursor protein-binding, family B, member 1 (Fe65)"
170. Hs.108885 "collagen, type VI, alpha 1"
171. Hs.171695 dual specificity phosphatase 1
172. Hs.51043 hexosaminidase B (beta polypeptide)
173. Hs.195850 "keratin 5 (epidermolysis bullosa simplex, Dowling-Meara/Kobner/Weber-Cockayne types)"
174. Hs.158095 KIAA0484 protein
175. Hs.109315 KIAA1415 protein
176. Hs.78934 "mutS (E. coli) homolog 2 (colon cancer, nonpolyposis type 1)"
177. Hs.279558 PRO0478 protein
178. Hs.74564 "signal sequence receptor, beta (translocon-associated protein beta)"
179. Hs#S1972073 "transducer of ERBB2, 2"
180. lambda 1.0k
181. Hs.120 "anti-oxidant protein 2 (non-selenium glutathione peroxidase, acidic calcium-independent)"
182. Hs.151242 "complement component 1 inhibitor (angioedema, hereditary)"
183. Hs.183 Duffy blood group
184. Hs.88556 histone deacetylase 1
185. Hs.278441 KIAA0015 gene product
186. Hs.200595 KIAA0562 gene product
187. Hs.274396 KIAA1423 protein
188. Hs.11000 MY047 protein
189. Hs.6451 PRO0659 protein
190. Hs.50002 "small inducible cytokine subfamily A (Cys-Cys), member 19"
191. Hs.74137 transmembrane trafficking protein
192. lambda 1.0k

Results

1. Inhibition of growth of SiHa cell line by As_2O_3 and As_4O_6

To identify the anticancer effects of As_2O_3 and As_4O_6 on cervical cancer cell lines, we used an HPV 16

positive SiHa cell line with wild type p53 gene. Arsenic compounds, As_2O_3 and As_4O_6, were treated at 0.5, 1, 2, 3, 4, and 5 μM in SiHa cells, respectively. As a result, cell growth was decreased by 9.2, 56, 89, 93, 96 and 96% in As_2O_3 and 54, 84, 84, 85, 75 and 87% in As_4O_6, respectively. (Fig. 1)

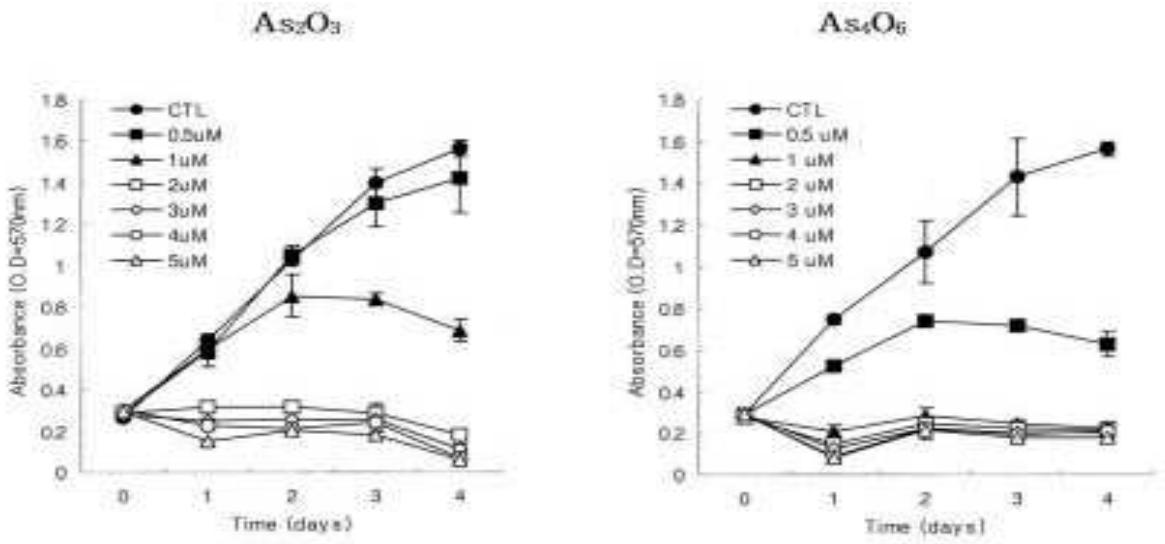


Fig. 1. The growth of human cervical cancer cell line was suppressed by As_2O_3, As_4O_6

2. Induction of apoptosis by As_2O_3 and As_4O_6 in cervical cancer cell line

DNA fragmentation assay was performed to determine whether the inhibitory effect of As_2O_3 and As_4O_6 on cell growth of cervical cancer cells is due to cytotoxicity or apoptosis. DNA ladder was observed in all cases where 0, 0.5, 1, 2 μM of As_2O_3 or As_4O_6 was treated with SiHa cell line. (Fig. 2)

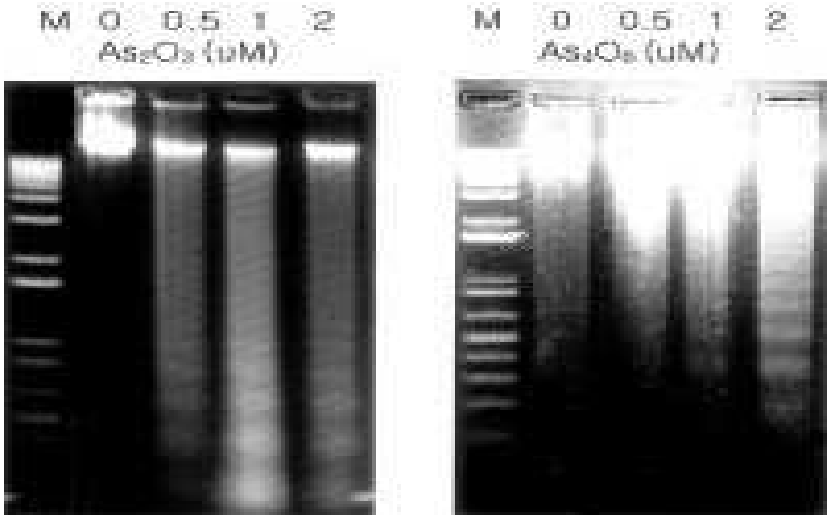


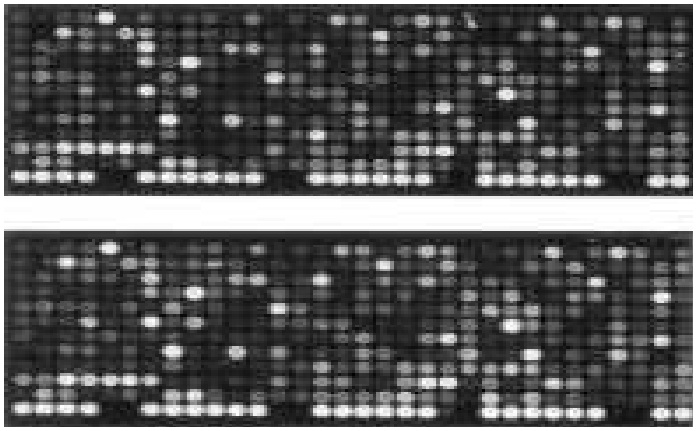
Fig. 2. Induction of apoptosis by arsenic compound (As2O3, As4O6) treatment was checked by DNA fragmentation assay

3. Comparison of gene expression by treatment with As_2O_3 and As_4O_6 using DNA chip

384 DNA chip was used for comparative analysis of gene expression by As_2O_3 and As_4O_6. SiHa cell lines were treated with 1 μM of As_2O_3 and As_4O_6 for 48 hours. Total RNA was extracted and

hybridized to 384 DNA chips, followed by scanning for analyzing gene expression changes. As a result, in the case of As_2O_3, about 47 mutations were found in the genes such as thymidylate synthetase, cyclin B1 and CDC 20 (cell division cycle 20) (Table 1). In the case of As_4O_6, more than 78 times of genes such as CDC20 (cell division cycle 20), cyclin B1, primase and proliferating cell nuclear antigen were changed. The two arsenic compounds showed that 32 genes such as UDP-glucose dehydrogenase were changed in common. (Table 3)

As_2O_3



As_4O_6

Fig. 3. Overlaid image of cDNA filter hybridized with nontreated (red) and 1 μg arsenic compounds (As_2O_3, As_4O_6) treated (green) in SiHa cells after 48 hrs. Down-regulated genes in red, up-regulated genes in green.

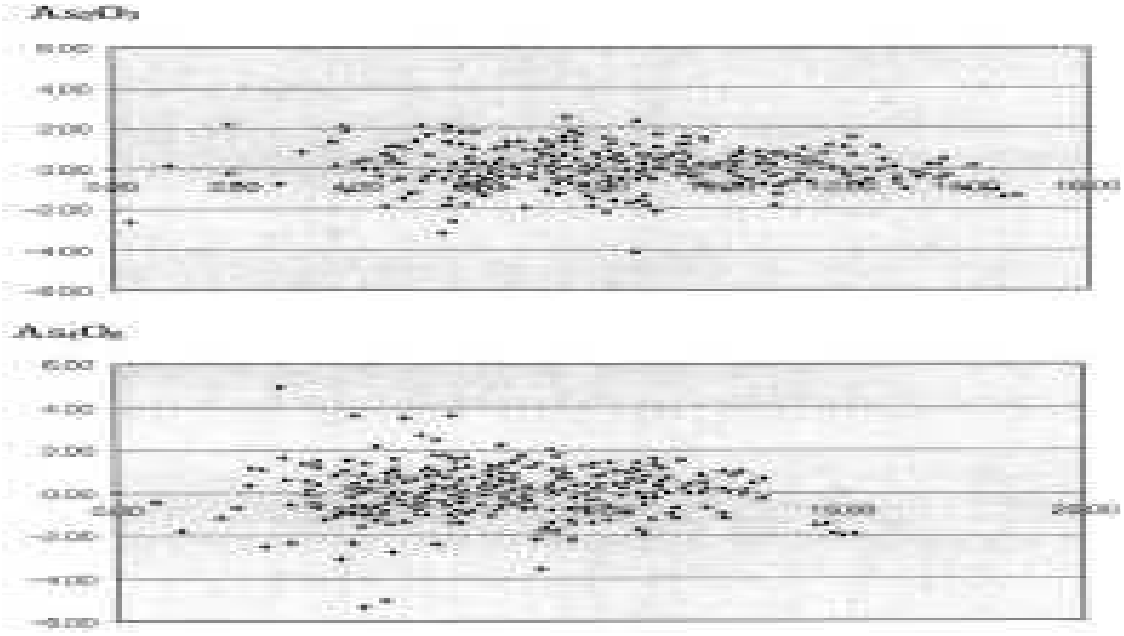


Fig. 4. Comparision of gene expression profile between untreated -(cy3) and arsenic compounds (As_2O_3, As_4O_6) treated SiHa cell (cy5). Y axis value is normalized with 2log (3D/5D). Above 2 means 22, 4 time higher expression in control, and below -2 means 22, 4 times lower in arsenic compounds treated SiHa cell.

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Table 1. Forty seven genes and ESTs (out of total 384) whose expression was changed at least two-fold of exposure of As_2O_3 (1 uM) for 48 hrs is SiHa cell

Gene/Protein	cDNA array results			
	2log (3F)	2log (5F)	2log (3F/5F)	$2^{2log(3F/5F)}$
Up-regulated gene				
snuportin-1	4.43	8.56	-4.13	17.5
Hs. 134200 DKFZP562C186 protein	6.32	10.30	-3.98	15.8
tumor differentially expressed 1	9.04	10.86	-1.82	3.5
Hs.85963 "CD36 antigen"	5.40	7.21	-1.81	3.5
her2	7.07	8.67	-1.58	3.0
integral membrane protein 2C	6.65	8.23	-1.60	3.0
membrane protein, palmitoylated 1 (55 kD)	6.44	7.91	-1.47	2.8
tp53	7.39	8.73	-1.34	2.5
UDP-glucose dehydrogenase	9.70	10.94	-1.24	2.4
Hs.171695 dual specificity phosphatase 1	11.23	12.41	-1.17	2.3
TATA box binding protein (TBP)-associated factor, RNA polymerase III, C, 90 kD	6.04	7.12	-1.08	2.1
cathepsin D (lysosomal aspartyl protease)	9.04	10.09	-1.05	2.1
Down-regulated gene				
Hs.106794 KIAA0584 protein	8.85	5.96	2.88	7.4
thymidylate synthetase	9.96	7.47	2.49	5.6
Hs.268530 G protein pathway suppressor 1	12.83	10.45	2.38	5.2
Hs.74561 alpha-2-macroglobulin	9.66	8.65	1.01	2.0
cyclin B1	10.93	8.61	2.33	5.0
DKFZP586J0619 protein	4.09	1.92	2.17	4.5
CHK1 (checkpoint, S.pombe) homolog	7.65	5.48	2.17	4.5
alanyl (membrane) aminopeptidase	7.21	5.09	2.12	4.3
collagen, type IV, alpha 2	5.92	3.80	2.12	4.3
ATPase, H+ transporting, lysosomal (vacuolar proton pump)	7.42	5.52	1.90	3.7
mutated 1 colorectal cancers	9.38	7.54	1.83	3.6
bladder cancer overexpressed protein	7.53	5.76	1.77	3.4
centromere protein F (350/400 kD, mitosin)	7.73	5.96	1.77	3.4
CDC20 (cell division cycle 20, S. cerevisiae, homolog)	10.64	8.96	1.68	3.2
DKFZp434A0131 protein	6.87	5.18	1.68	3.2
1-acylglycerol-3-phosphate O-acyltransferase 1	9.34	7.67	1.66	3.2
Hs.79241 B-cell CLL/lymphome 2	13.07	11.38	1.69	3.2
proliferating cell nuclear antigen	10.98	9.44	1.54	2.9
splicing factor, arginine/serine-rich 1 (splicing factor 2, alternate splicing factor)	13.71	12.18	1.53	2.9
primase, polypeptide 1 (49 kD)	9.60	8.13	1.48	2.8
collagen, type 1, alpha 2	8.84	7.37	1.47	2.8
CD83 antigen (activated B lymphocytes, immunoglobulin superfamily)	11.10	9.68	1.42	2.7
mutS (E. coli) homolog 2 (colon cancer, nonpolyposis type 1)	8.75	7.42	1.33	2.5
mitogen-activated protein kinase kinase kinase kinase 1	8.91	7.59	1.33	2.5
adenosine deaminase	8.03	6.71	1.32	2.5
cytochrome P450, subfamily I (dioxin-inducible), polypeptiede 1	7.81	6.50	1.31	2.5
methylenetetrahydrofolate dehydrogenase (NADP+ dependent)	10.69	9.47	1.22	2.3
minichromososme maintenance deficient (mis5, S. pombe) 6	10.55	9.33	1.22	2.3
cell division cycle 27	9.40	8.21	1.18	2.3
protein phosphatase 1G (formerly 2 G), magnesium-dependent, gamma isoform	12.95	11.78	1.17	2.2
cell division cycle 27	8.39	7.26	1.13	2.2
serine/threonine kinase 2	8.32	7.22	1.11	2.2
ATP synthase, H+ transporting, mitochondrial F0 complex, subunit c (subunit 9)	13.12	12.03	1.09	2.1
IDN3 protein	10.03	8.97	1.06	2.1
Hs.74561 alpha-2-macroglobulin	9.66	8.65	1.01	2.0

Table 2. Seventy eight genes and ESTs (out of total 384) whose expression was changed at least two-fold of exposure of As_4O_6 (1 μM) for 48 hrs in SiHa cell

Gene/Protein	cDNA array results			
	2log (3F)	2log (5F)	2log (3F/5F)	$2^{2log(3F/5F)}$
Up-regulated gene				
Hs.171695dual specificity phosphatase1	11.75	14.79	-3.04	8.2
Hs.77274 "plasminogen activator, urokinase"	5.21	7.37	-2.17	4.5
DKFZP434J154 protein	7.29	9.43	-2.14	4.4
UDP-gluoose dehyorogenase	8.97	10.90	-1.93	3.8
Hs.69855 NRAS-related gene	11.15	13.06	-1.91	3.8
Hs.85963 CD36 antigen (collagen type I receptor, thrombospondin receptor)-like 2 (lysosoma)	6.82	8.61	-1.79	3.5
tumor differentiallty expressed 1	9.06	10.80	-1.74	3.3
Hs.52081 KIAA0867 protein	7.01	8.64	1.63	3.1
growth hormone 2	3.47	5.05	-1.58	3.0
cathepsin D (lysosomal aspartyl protease)	8.43	9.82	-1.40	2.6
transcriptional regulator protein	7.57	8.96	-1.39	2.6
her2	8.07	9.38	-1.31	2.5
vav 1 oncogene	8.88	10.18	-1.30	2.5
SEC24 (S. oerevisiae) related gene family, member B	9.84	11.09	-1.25	2.4
spinocerebellar ataxia 2 (divopontocerebellar ataxia 2, autosomal dominant, ataxin 2)	11.36	12.56	-1.20	2.3
sudD (suppressor of bimD6, Aspergillus nidulans) homdog	10.38	11.57	-1.18	2.3
caoherin 5, VE-cadherin (vascular epithelium)	5.79	6.94	-1.15	2.2
Machaob-Joseph disease (spinocerebellar ataxia 3, divopontocerebellar ataxia 3, autosoma)	6.63	7.73	-1.10	2.1
proteaseome (prosome, macropain) 26S subunit, ATPase, 4	10.43	11.44	-1.01	2.0
Down-regulated gene				
CDC20 (cell division cycle 20, S. cerevisiae, homolog)	8.33	3.37	4.96	31.1
cyclin B1	10.51	6.91	3.59	12.1
primase, polypeptide 1 (49 kD)	8.51	4.93	3.58	12.0
thymidylate synthetase	9.48	5.96	3.52	11.5
mutated in colorectal cancers	9.89	6.29	2.69	6.4
ADP-ribosyltransferase (NAD+: poly (ADP-ribose) polymerase)	9.06	6.61	2.45	5.5
methylenetetrahydrofolate dehydrogenase (NADP+ dependent), methenyltetrahydrofolate cyclo	10.17	7.94	2.23	4.7
mitogen-activated protein kinase kinase kinase kinsae kinase 1	7.50	5.36	2.14	4.4
proliferating cell nuclear antigen	10.96	8.97	1.99	4.0
ATP synthase, H+ transporting, mitochondrial F0 complex, subunit c (subunit 9), isoform 1	10.89	9.12	1.77	3.4
collagen, type I, alpha 2	8.37	6.62	1.75	3.4
DKFZP586K0919 protein	10.16	8.41	1.74	3.3
periodontal ligament fibroblast protein	8.56	6.84	1.71	3.3
minichromosome maintenance deficient (mis5, S. pombe) 6	10.76	9.07	1.69	3.2
melanoma adhesio molecule	10.69	9.07	1.62	3.1
proteasome (prosome, macropain) activator subunit 3 (PA28 gamma; Ki)	8.80	7.18	1.62	3.1
NADH dehydrogenase (ubiquinone) 1 alpha subcomplex, 5 (13 kD, B13)	8.54	6.94	1.61	3.0

CHK1 (checkpoint, S.pombe) homolog	7.36	5.77	1.59	3.0
ATP synthase, H+ transporting, mitochondrial F0 complex, subunit c (subunit 9), isoform 2	12.68	11.13	1.55	2.9
CD83 antigen (activated B lymphocytes, immunoglobulin superfamily)	9.82	8.28	1.55	2.9
splicing factor, arginine/serine-rich 1 (splicing factor 2, alternate splicing factor)	13.14	11.64	1.50	2.8
mutS (E. coli) homolog 2 (colon cancer, nonpolyposis type 1)	8.43	6.97	1.46	2.8
protein phosphatase 1 G (formerly 2 C), magnesium-dependent, gamma isoform	12.18	10.75	1.44	2.7
HSPC133 protein	9.57	8.17	1.30	2.6
SAC2 (suppressor of actin mutation 2, yeast, homolog)-like	11.60	10.21	1.39	2.6
protein phosphatase 2, regulatory subunit B (B56), gamma isoform	11.27	9.89	1.38	2.6
dynactin 1 (p150, Glued (Drosophila) homolog	6.55	5.18	1.37	2.6
IDN3 protein	10.07	8.72	1.35	2.6
Hs. 79732 FIBULIN 1	10.15	8.83	1.32	2.5
DKFZP564M2423 protein	12.43	11.12	1.31	2.5
NPD002 protein	9.25	7.95	1.30	2.5
creatine kinase, brain	10.97	9.70	1.27	2.4

Down-regulated gene

SET binding factor 1	8.99	7.74	1.25	2.4
capping protein (actin filament) muscle Z-line, alpha 1	11.03	9.82	1.21	2.3
collagen, type VI, alpha 2	8.48	7.27	1.21	2.3
chorionic gonadotropin, beta polypeptide	9.70	8.49	1.21	2.3
palmitoyl-protein thioesterase 1 (ceroid-lipofuscinosis, neuronal 1, infatile)	9.96	5.77	1.19	2.3
trinucleotide repeat containing 5	9.80	8.62	1.18	2.3
serine/threonine kinase 2	7.62	6.45	1.17	2.3
NRAS-related gene	11.86	10.69	1.17	2.2
proteaseome (prosome, macropain) activator subunit 1 (PA28 alpha)	9.12	7.96	1.16	2.2
Hs.74561 alpha-2-macroglobulin	8.60	7.45	1.15	2.2
electron-transfet-flavoprotein, alpha polypeptide (glutaric aciduria II)	10.39	9.24	1.15	2.2
secreted protein. acidic, cysteine-rich (osteonectin)	12.41	11.27	1.14	2.2
Hs.209100 DKFZP434C171 protein	11.69	10.55	1.14	2.2
RAB6 interacting, kinesin-like (rabkinesin6)	8.14	7.02	1.12	2.2
plasminogen activator, urokinase	6.82	5.71	1.11	2.2
farnesyl diphosphate synthase (farnesyl pyrophosphate synthetase, dimethylallyltranstransfer)	10.13	9.03	1.10	2.1
huntingtin (Huntingtom disease)	5.77	4.71	1.06	2.1
prolactin regulatory element binding	7.97	6.93	1.04	2.1
v-Ki-ras2 Kirsten rat sarcoma 2 viral oncogene homolog	6.78	5.74	1.03	2.0
pleiotrophin (heparin binding growth factor 8, neurite gorwth-promoting factor 1)	7.36	6.34	1.02	2.0
RBP1-like protein	11.83	10.81	1.02	2.0
site-1 protease (subtilisin-like, sterol-regulated, cleaves sterol regulatory element binding prc)	10.27	9.26	1.01	2.0
nucleophosmin (nucleolar phosphoprotein B23, numatin)	13.54	12.54	1.01	2.0
c-src tyrosine kinase	8.90	7.89	1.00	2.0
vimentin	13.92	12.92	1.00	2.0
Hs.274275 hypothetical protein FLJ10254	13.65	12.65	1.00	2.0
cyclin-dependent kinase 4	11.12	10.13	1.00	2.0

Table 3. Thirty two genes and ESTs (out of total 384) whose expression was commonly changed after exposure of arsenic compounds (As_2O_3, As_4O_6, 1 μM) for 48 hrs in SiHa cell

Gene/Protein	cDNA array results $<2^{2\log(SF)S}$	
	As_2O_3	As_4O_6
Up-regulated gene		
UDP-glucose dehydrogenase	2.40	3.80
tumor differentially expressed 1	3.50	3.30
Hs.85963 "CD36 antigen (collagen type Ⅰ receptor, thrombospondin receptor)-like 2"	3.50	3.50
Hs.171695 dual specificity phosphatase 1	2.30	8.20
her2	3.00	2.50
cathepsin D (lysosomal aspartyl protease)	2.10	2.60
Down-regulated gene		
ADP-ribosyltransferase (NAD+; ploy (ADP-ribose) polymerase)	2.6	5.5
ATP synthase, H+ tansporting, mitochondrial F0 complex, subunit c (subunit 9), isoform 2	2.1	2.9
CD83 antigen (activated B lymphocytes, immunoglobulin superfamily)	2.7	2.9
CDC20 (cell division cycle 20, S. cerevisiae, homolog)	3.2	31.1
CHK1 (checkpoint, S.pombe) homolog	4.5	3.0
chorionic gonadotropin, beta polypeptide	2.3	2.3
collagen, type Ⅰ, alpha 2	2.8	3.4
collagen, type Ⅳ, alpha 2	4.3	2.3
creatine kiinase, brain	2.5	2.4
cyclin B1	5.0	12.1
Hs.74561 alpha-2-macroglobulin	2.0	2.2
IDN3 protein	2.1	2.6
melanoma adhesion molecule	2.8	3.1
methylenetetrahydrofolate dehydrogenase (NADP+ dependent)	2.3	4.7
minichromosome maintenance deficient (mis5, S. pombe) 6	2.3	3.2
mitogen-activated protein kinase kinase kinase kinase 1	2.5	4.4
mutated in colorctal cancers	3.6	6.4
mutS (E. coli) homolog 2 (colon cancer, nonpolyposis type 1)	2.5	2.8
nucleophosmin (nucleolar phosphoprotein B23, numatrin)	2.1	2.0
primase, polypeptide 1 (49 kD)	2.8	12.0
proliferating cell nuclear antigen	2.9	4.0
protein phosphatase 1 G (formerly 2 C), magnesium-dependent, gamma isoform	2.2	2.7
RAB6 interacting, kinesin-like (rebkinesin 6)	2.6	2.2
serine/threonine kinase 2	2.2	2.3
splicing factor, arginine/serine-rich 1 (splicing factor 2, alternate splicing factor)	2.9	2.8
thymidylate synthetase	5.6	11.5

Review

Arsenic is a colorless, tasteless, odorless poison that is distributed in the natural world as As_2S_3, As_2S_3 and $KAsO_2$. Especially arsenic trioxide (As_2O_3) and arsenic trisulfide (As_2S_3) have long been used for treatment of leukemia in China.[9] Since arsenic compounds are known to cause skin cancer in the 1820s, arsenic compounds have begun to be accepted as environmental carcinogens and have been considered as co-mutagen and co-carcinogen in human skin and lungs.[10-12] Recently it has been known that arsenic trioxide (As_2O_3) can treat patients with leukemia. Therefore, interest in arsenic

compounds is increasing and researches are actively being conducted.[13] Arsenic disulfide-containing arsenic compounds such as As_2O_3 have been reported to be effective in the treatment of patients with acute promyelocytic leukemia (APL).[14] There was no evidence of bone marrow suppression when As_2O_3 was used as a therapeutic agent. In a study of 15 patients with acute promyelocytic leukemia, none of the patients treated with Arsenic showed significant platelet reduction or anemia. The clinical usefulness of As_2O_3 in acute promyelocytic leukemia has also been confirmed in patients resistant to conventional chemotherapy. In vitro experiments on the molecular biology of acute promyelocytic leukemia cells, it shows that the anticancer effect of As_2O_3 is due to down-regulation of bcl-2 expression and direct induction of cell death through regulation of PML/RARα/PML protein.[10,15] Furthermore, caspase activity by As_2O_3 is involved in As_2O_3-induced apoptosis in acute promyelocytic leukemia cells. It has been reported that As_2O_3 and melarsoprol, an organic arsenic, down-regulate Bcl-2 protein expression in myeloid leukemia cell lines such as HL-60, U937, and KG-1 and cell death occurs through caspase activity in B-cell leukemia cell line.[3,15] Based on these reports, cell death by arsenic compounds has been reported in other tumor cells such as esophageal cancer, neuronal subpopulations, head and neck tumors, and cervical cancer.[6] It is known to cause cell death in cervical cancer cells as well as bladder cancer, leukemia, gastrointestinal tumors (human gastric cancer cell line, MGC 803), esophageal cancer, neuronal subpopulations, head and neck tumors.[14,16] It shows in this experiment that the molecular structure of As_4O_6 is known and its toxicity is less than that of As_2O_3 and its anticancer effect is excellent. However, research on this substance is not yet active. Therefore, we examined the effect of As_4O_6 on the growth of cervical cancer cell line, SiHa cells. As a result, the cell growth was inhibited when treated with a low concentration of arsenic compounds and the cell growth was remarkably inhibited based on 2 μM as in other studies. However, As_4O_6 showed strong inhibitory effect on cell proliferation as compared with As_2O_3 at 0.5 μg concentration.

With the recent development of cDNA microarray, cDNA chip technology and high-throughput method to monitor gene expression, it has become possible to simultaneously analyze thousands of gene expressions. As a result, based on the change of various gene expressions in cancer tissues, a new indicator of cancer classification can be established. In addition, there is evidence that the gene expression profile reflects the drug sensitivity of cancer cells.[17] The use of DNA chips in this study has become a very important clue in solving one of the most serious dilemmas that cannot predict the response of cancer in individual cancer patients when clinically confronted while treating the patients.[18]

Based on the DNA chip technology, molecular genetic studies related to radiation sensitivity became possible, and in the course of treatment, genes and genes regulatory pathways associated with cell resistance were identified. One of the goals of using this technology is to identify cancers that are sensitive to radiation and treat cancer with radiation alone. Another goal is to identify those cancers that are not controlled by combined therapies (cancer, radiation) leading to the development of treatment.

Studies involving cervical squamous cell carcinoma cells through DNA chips have reported that the expression of the following specific genes is up to 4.1 times higher in radioresistant cells than in radiosensitive cells. It is reported that the genes that are overexpressed in radiation resistant cell lines include metal-regulatory transcription factor-1, cytochrome P450 CYP1B1, adenomatosis polyposis coli, translation elongation factor-1, and cytochrome-c oxidase and the genes that are overexpressed in radiation sensitive cell lines include transcription factor NF-kappa-B, metalloprotein inhibitor-1 precursor, superoxide dismutase-2, insulin-like growth factor binding protein-3, guanine

nucleotide-binding protein and transforming growth factor beta induced protein.[19]

In this experiment, As_2O_3 and As_4O_6 were administered to SiHa cell line. When compared, genes increased the expression at the same time were UDP-glucose dehydrogenase, CD36 antigen (collagen type Ⅰ receptor), thrombospondin receptor, dual specificity phosphatase 1 and cathepsin D (lysosomal aspartyl protease), etc. In case of dual specificity phosphatase 1 gene involved in signal transduction was increased 2.3 times when treated with As_2O_3 and 8.2 times when treated with As4O6. At the same time, the genes whose expression are decreased are 26 genes including CDC20 (cell division cycle 20, S. cerevisiae, homolog), cyclin B1, primase, polypeptide 1 (49 Kd), thymidylate synthetase and proliferating cell nuclear antigen. In case of the gene of CDC20, cyclin B1, thymidylate synthetase, proliferating cell nuclear antigen which is involved in the cell cycle, it decreased 3.2, 5.0, 5.6 and 2.9 times when treated with As_2O_3 and 31.3, 12.1, 11.5 and 4.0 times when treated with As_4O_6.

As such, even if the same gene was treated with different arsenic compounds, the degree of expression was different and a large number of other genes were differentially expressed in each arsenic compound. The significance of the difference in the degree of expression is thought to be necessary for further study.

These studies confirmed the inhibition of cell growth and its mechanism after administration of arsenic compounds in cervical cancer cell lines and this cell death is associated with caspase activity and examined gene expression that is increased or decreased using DNA chip. In fact, It is thought that designating about 500-1,000 specific genes related to cervical cancer and observing the changes of these genes will be helpful for the selection of treatment modalities such as the prognosis of patients before chemotherapy or radiation therapy and anticancer drugs.

In this way, it can be concluded that a number of gene changes occur simultaneously due to a chain reaction caused by drugs from the outside or genes introduced from, and it is thought that further studies are needed.

- References -

1. Chikashi Kihara, Tatsuhiko Tsunoda, Toshihiro Tanaka, Hideaki Yamana, Yoichi Furukawa, Kenji Ono, et al. Prediction of sensitivity esophageal tumors to adjuvant chemotherapy by cDNA microarray analysis of gene-expression profiles. Cancer research 2001; 61: 6474-9.
2. Zheng J, Deng YP, Lin C, Fu M, Xiao PG, Wu M. Arsenic trioxide induces apoptosis of HPV16 DNA-immortalized human cervical epithelial cells and selectively inhibits viral gene expression. Int J Cancer 1999; 82: 286-92.
3. Wang J, Sampath A, Raychaudhuri P, Bagchi S. Both Rb and E7 are regulated by the ubiquitin proteasome pathway in HPV-containing cervical tumor cells. Oncogene 2001; 20: 4740-9.
4. Xiao-Hua Jiang, Benjamin Chun-Yu Wong, Siu-Tsan Yuen, Sh-Hu Jiang, Chi-Hin Cho, Kam-Chuen Lai, et al. Arsenic trioxide induces apoptosis in Human gastric cancer cells through up-regulation of P53 and activation of capsase-3. Int Cancer 2001; 91: 173-9.
5. Zhu XH, Shen YL, Jing YK, Cai X, Jia PM, Huang Y. et al. Apoptosis and growth inhibition in malignant lymphocytes after treatment with arsenic trioxide at clinically achievable concentrations. J Natl Cancer Inst 1999; 91: 772-8.
6. Gurr JR, Bau DT, Liu F, Lynn S, Jan KT Dithiothreitol enhances arsenic trioxide-induced apoptosis in NB4 cells. Mol Pharmacol 1999; 56: 102-9.
7. Mattias Nees, Joel M. Geograpegan, Tehila Hyman, Stephan Frank, Lance Miller, Craig D. Wood

Worth. Papillomavirus Type 16 Oncogenes downregulate expression of Interferon-responsive gene and upregulate proliferation associated and NF-kB-responsive genes in cervical keratinocytes. Journal of virology 2001; 4283-96.

8. Buzard GS, Kasprzak KS. Possible roles of nitric oxide and redox cell signaling in metal-induced toxicity and carcinogenesis. J Environ Pathol Toxicol Oncol 2000; 19: 179-99
9. Murgo AJ, McBee WL, Cheson BD. Clinical trials referral resource. Clinical trials with arsenic trioxide. Oncology(Huntingt) 2000; 14: 206, 211, 215-6.
10. Boonchai W, Walsh M, Cummings M, Chenevix-Trench G. Expression of p53 in arsenic-related and sporadic basal cell carcinoma. Arch Dermatol 2000; 136: 195-8.
11. Buzard GS, Kasprzak KS. Possible roles of nitric oxide and redox cell signaling in metal-induced toxicity and carcinogenesis. J Environ Pathol Toxicol Oncol 2000; 19: 179-99.
12. Ding M, Shi X, Castranova V, Vallyathan V. Predisposing factors in occupational lung cancer: inorganic minerals and chromium. J Environ Pathol Toxicol Oncol 2000; 19: 129-38.
13. Simeonova PP, Wang S, Toriuna W, Kommineni V, Matheson J, Unimye N, et al. Arsenic mediates cell proliferation and gene expression in the bladder epithelium: association with activating protein-1 transactivation. Cancer Res 2000; 60: 3445-531.
14. Lu T, Liu J, LeCluyse EL, Zhou YS, Cheng ML, Waalkes MP. Application of cDNA microarray to the study of arsenic-induced liver diseases in the population of Guizhou, China. Toxicol Sci 2001; 59: 185-92.
15. Perkins C, Kim CN, Fang G, Bhalla KN. Arsenic induces apoptosis of multi drug-resistant human myeloid leukemia cells that express Bcr-Abl or overexpress MDR, MRP, Bcl-2, or Bcl-x(L). Blood 2000; 95: 1014-22.
16. Jiang XH, Chun-Yu Wong B, Yuen ST, Jiang SH, Cho CH, Lai KC, et al. Arsenic trioxide induces apoptosis in human gastric cancer cells through up-regulation of p53 and activation of caspase-3. Int J Cancer 2001; 91: 173-9.
17. Chen GQ, Zhu J, Shi XG, Ni JH, Zhong HJ, Si GY. et al. In vitro studies on cellular and molecular mechanisms of arsenic trioxide (As_2O_3) in the treatment of acute promyelocytic leukemia: As_2O_3 induces NB4 cell apoptosis with downregulation of Bcl-2 expression and modulation of PML-RAR alpha/PML proteins. Blood 1996; 88: 1052-61.
18. Lu M, Levin J, Sulpice E, Sequeira-La Grand A, Alemany M, Caen JP, et al. Effect of arsenic trioxide on viability, proliferation, and apoptosis in human megakaryocytic leukemia cell lines. Exp Hematol 1999; 27: 845-52.
19. Shen L, Chen TX, Wang YP, Lin Z, Zhao HJ, Zu YZ, et al. As_2O_3 induces apoptosis of the human B lymphoma cell line MBC-1. J Biol Regul Homeost Agents 2000; 14: 116-9.