Effects of inositol hexaphosphate on proliferation of HT-29 human colon carcinoma cell line

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Abstract

AIM: To investigate the effects of inositol hexaphosphate (IP$_6$) on proliferation of HT-29 human colon carcinoma cell line.

METHODS: Cells were exposed to various concentrations (0, 1.8, 3.3, 5.0, 8.0, 13.0 mmol/L) of IP$_6$ for a certain period of time. Its effect on growth of HT-29 cells was measured by MTT assay. The expressions of cell cycle regulators treated with IP$_6$ for 2 d were detected by immunocytochemistry.

RESULTS: IP$_6$ inhibited the HT-29 cell growth in a dose- and time-dependent manner. Analysis of cell cycle regulator expression revealed that IP$_6$ reduced the abnormal expression of P53 and PCNA and induced the expression of P21.

CONCLUSION: IP$_6$ has potent inhibitory effect on proliferation of HT-29 cells by modulating the expression of special cell cycle regulators.

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Key words: Phytic Acid; Colonic neoplasms; Cell proliferation

MATERIALS AND METHODS

INTRODUCTION

Colorectal cancer is the second most frequent cancer in Western countries[1], and the third leading cause of cancer deaths in the United States[2]. In China, the mortality rate of colorectal cancer is the fourth to sixth leading cause of cancer deaths[3]. Epidemiological studies have shown that high fiber foods, such as fruits, vegetables, whole grains and cereals, may protect against colorectal cancer[4-8]. Animal studies have shown that wheat bran has protective effect against colorectal cancer[9-14], which is attributed mostly to its high fiber content. Interestingly, many of the proposed protective mechanisms of wheat bran fiber, such as decreased transit time[15], increased bulk[16] and fermentation[17], are analogous to those of inositol hexaphosphate (IP$_6$ or phytic acid), which is a major fiber-associated component of wheat bran[18]. In some epidemiological studies, colorectal cancer-protective effect of fiber foods rich in IP$_6$, such as wheat bran has been observed[19], indicating that IP$_6$ may protect against colorectal cancer.

IP$_6$ is a naturally occurring polyphosphorylated carbohydrate, found in plants, particularly in cereals and legumes (0.4%-6.4%)[20]. It consists of a myo-inositol ring with six dihydrogen phosphate groups, assuming a chair conformation in dilute solution[21]. This unique structure empowers IP$_6$ with a chelating capacity of binding to polyvalent (both mono and divalent) cations. Some of these metal ions such as magnesium and zinc play an important role in stimulation of cellular proliferation[22]. This molecule is related to human health as an anti-nutrient. However, during the last decades it has been shown that IP$_6$ is also widely distributed in animal cells and tissues at substantial levels[23,24]. Especially, a strong anti-cancer activity of IP$_6$ has been demonstrated both in vivo and in vitro[25]. IP$_6$ exerts its anti-cancer activity by entering into cellular inositol hexaphosphate pool and affecting common cellular signal transduction pathways[26,27], but its mechanisms of action are still not completely understood.

This study was to examine the effect of IP$_6$ on growth of HT-29 human colon carcinoma cell line. The expressions of cell cycle regulators were assessed after IP$_6$ treatment.

Chemicals

IP$_6$ (a dodecasodium salt from rice) and 3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium (MTT) were purchased from Sigma (St Louis, MO, USA). DMEM/Ham F12 culture medium, fetal bovine serum and trypsin were from Gibco BRL (Grand Island, NY, USA). Rabbit polyclonal antibody to human P53, mouse monoclonal antibodies to human P21, PCNA and SP
histostain-plus kits were obtained from Santa Cruz Biotechnology (Santa Cruz, CA, USA).

**Cell lines, culture conditions and IPs treatment**

The HT-29 human colon carcinoma cell line was obtained from Xiehe Medical University (Beijing, China). Cells were grown in DMEM/Ham F12 medium supplemented with 10% fetal bovine serum, penicillin (100 U/mL) and streptomycin (100 g/L) under standard culture conditions (37℃, 950 mL/L humidified air and 50 mL/L CO₂). A stock solution of 100 mmol/L IP₆ in distilled water was freshly prepared each time before use, the pH1 was neutralized with NaOH and sterilized by passing through a 0.22 μm membrane filter. Dilutions of 1.8, 3.3, 5.0, 8.0, 13.0 mmol/L in DMEM/Ham F12 medium were prepared from stock solution immediately before use. The DMEM/Ham F12 medium with equal volume of distilled water served as negative control.

**MTT assay**

Cell number was determined by colorimetric MTT assay[24]. MTT was dissolved in PBS at 5 g/L, filter-sterilized, diluted in the DMEM/Ham F12 medium, giving a final concentration of 1.0 g/L. For growth assay, cells were plated in 96-well microtiter plates (Costar, Cambridge, MA, USA) at a density of 2 × 10³ cells/well of 100 μL media. One of the IP₆ treatments was that cells were exposed to 1.8-13.0 mmol/L IP₆ for 6 h, 12 h, or 24 h, after which IP₆ was removed and cells were grown in culture media without IP₆. This treatment was given every other day and continued for 6 d. The other IP₆ treatment was that cells were treated with various concentrations of IP₆ continuously for 6 d, during which culture media were changed with fresh media every other day. At the indicated time the media were removed, 50 μL of MTT was added, and the incubation was continued for 4 h at 37℃. Individual cell viability was assessed by visualization of intracellular blue crystal formation by light microscopy. The precipitated formazan was dissolved with 150 μL of DMSO, and the absorbance was determined at 490 nm with a microplate autorader (EL311xs, Bio-Tek Instruments, Inc., Winooski, VT). Cell growth assay was repeated three times.

**Immunocytochemistry**

Immunocytochemical staining for P53, P21 and PCNA was carried out by the standard streptavidin-peroxidase-biotin technique (SP technique) using SP kit. Cells were treated with 1.8-13.0 mmol/L IP₆ for 2 d and collected by a brief trypsinization and plated on slides. The cells were fixed in acetone at -20℃ for 5 min. The endogenous peroxidase activity was quenched in a 3% solution of hydrogen peroxide for 15 min and blocked for 10 min. Cells were immunostained with monoclonal antibody (dilutions: P21 1:50, PCNA 1:80) and P53 polyclonal antibody (dilutions: 1:50) for 1 h at 37℃. After three further washes with PBS, a second biotinylated goat anti-rabbit or rabbit anti-mouse antibody was applied for 1 h at room temperature and then streptavidin conjugated to peroxidase was added. Following extensive washes with PBS, 3, 3-diaminobenzidine was used for color development, and hematoxylin was used for counterstaining. The negative controls were performed by substituting the primary antibody with PBS. Hematoxylin-stained cells were examined under light microscope and photographed. Cells not counterstained were measured by VIDAS2.1 image analysis system for absorbance because hematoxylin staining could affect the image-analysis results. Three highly magnified visual fields which were not overlapped were randomly selected to measure the absorbance of each field. The mean absorbance was calculated.

**Statistical analysis**

The experimental results were repeated three times and expressed as mean ± SD. Statistical analysis was carried out using one-way ANOVA. P < 0.05 was considered statistically significant. Statistical analyses were performed using SPSS 11.5 (SPSS Inc, Chicago, IL, USA).

**RESULTS**

**Effect of IP₆ on the growth of HT-29 cells**

Continuous treatment with IP₆ inhibited the proliferation of HT-29 cells (Figure 1A). The absorbance value for each IP₆ group was lower than that of control group. At the same time point, the absorbance value decreased with increasing IP₆ concentration. The absorbance values for the 8.0 mmol/L and 13.0 mmol/L IP₆ groups decreased on d 6 (P < 0.05). The effects of discontinuous treatment with IP₆ on the growth of HT-29 cells are shown in Figures 1B-1D. The treatment with IP₆ for 24 h inhibited the cell growth. But neither 6 h nor 12 h treatment showed dose- or time-dependent inhibition effects though the absorbance value for each IP₆ group was lower than that for control group.

**Effects of IP₆ on the expression of P53, P21 and PCNA**

Compared to the control, the expression of P53 protein in HT-29 cells was decreased after 2 d of IP₆ treatment at different concentrations (P < 0.05) (Table 1 and Figure 2A, Figure 2B).

Compared to the control, treatment of HT-29 cells with IP₆ at various concentrations for 2 d increased the expression of P21 (P < 0.05) (Table 2 and Figure 2C, Figure 2D).

Compared to the control, the expression of PCNA decreased after treated with IP₆ at different concentrations for 2 d (P < 0.05) (Table 3 and Figure 2E, Figure 2F).

The absorbance values assayed by MTT after IP₆ treatment for different periods of time are listed in Tables 4, 5 , 6, and 7.

| Table 1 P53 expression in IP₆-treated HT-29 cells (mean ± SD) |
|---------------------------------------------------------------|
| Concentration of IP₆ (mmol/L) | Absorbance value    |
| Control             | 0.6772 ± 0.0095     |
| 1.8                 | 0.6161 ± 0.0203     |
| 3.3                 | 0.5996 ± 0.0205     |
| 5.0                 | 0.6067 ± 0.0130     |
| 8.0                 | 0.5871 ± 0.0159     |
| 13.0                | 0.5817 ± 0.0158     |
DISCUSSION

Uncontrolled proliferation is one of the most important characteristics of malignant cells due to the aberrations of cell cycle regulators such as mutation, activation or inactivation of genes. Identification of cell cycle regulator specificity of anti-tumor drugs is essential to understand the mechanisms of their action.

MTT assay in this study showed that the growth of HT-29 cells was inhibited after continuous IP6 treatment for 2-6 d (P < 0.05). The effect enhanced with increasing IP6 concentration and prolonged treatment time, suggesting that the inhibition effects of IP6 are dose- and time-dependent.

To confirm our data we used another proliferating marker, proliferating cell nuclear antigen (PCNA) which is essential for both DNA replication and repair. During DNA replication, PCNA forms a ring structure clamping the synthesized DNA to the DNA polymerases δ and ε to ensure continuation of the replication process. In case of DNA damage, PCNA binds to the over-expressed P21waf1/cip1 leading to inhibition of PCNA-dependent replication, but it does not affect the DNA repair function attained by PCNA. Thus, PCNA is expressed in both cycling and non-cycling cells. Immunocytochemistry in this study showed that IP6-treated cells reduced PCNA expression compared with control cells (P < 0.05), although the dose-dependent inhibition was not obvious, which was in agreement to the low proliferation rate observed in MTT assay, indicating that IP6 inhibits proliferation of HT-29 cells.

**Table 2** P21 expression in IP6-treated HT-29 cells (mean ± SD)

| Concentration of IP6 (mmol/L) | Absorbance value |
|-------------------------------|------------------|
| Control                       | 0.486 ± 0.0486   |
| 1.8                           | 0.601 ± 0.0152   |
| 3.3                           | 0.513 ± 0.0336   |
| 5.0                           | 0.603 ± 0.0105   |
| 8.0                           | 0.608 ± 0.0086   |
| 13.0                          | 0.598 ± 0.0163   |

**Table 3** PCNA expression in IP6-treated HT-29 cells (mean ± SD)

| Concentration of IP6 (mmol/L) | Absorbance value |
|-------------------------------|------------------|
| Control                       | 0.6407 ± 0.0096  |
| 1.8                           | 0.6361 ± 0.0087  |
| 3.3                           | 0.5904 ± 0.0302  |
| 5.0                           | 0.4520 ± 0.0495  |
| 8.0                           | 0.4788 ± 0.0357  |
| 13.0                          | 0.5006 ± 0.0403  |
Since IP6 inhibits cell growth, we studied the regulators of cell cycle. P53, a tumor suppressor protein, is a nuclear transcription factor that controls cell cycle progression and plays a role in G1/S checkpoint of cell cycle allowing the repair of damaged DNA. Mutations and deletions of the tumor suppressor gene p53 have been identified in about 50% of colorectal carcinomas and are associated with poor prognosis due to its weaker ability to inhibit cell proliferation. The half-life of wild-type P53 is very short and difficult to detect, while the mutant P53 protein has a much longer half-life and can be examined by conventional immunohistochemical technology. Rodrigues NR et al showed that over-expression of p53 is synonymous with mutation and HT-29 cells have mutations in codon 273 of the p53 gene, so HT-29 cells overproduce mutant p53 antigen. In our study, the immunocytochemical results showed that in IP6-treated cells the abnormal expression of P53 protein decreased compared to control ($P < 0.05$), indicating that IP6 reduces the expression of mutant P53 protein. It was reported that treatment of HT-29 cells with IP6 increases the level of wild-type P53.

### Table 4
Absorbance values assayed by MTT after continuous treatment with IP6 (mean ± SD)

| IP6 (mmol/L) | Time (d) | 0         | 2         | 4         | 6         |
|--------------|----------|-----------|-----------|-----------|-----------|
| Control      |          | 0.0847 ± 0.0021 | 0.1487 ± 0.0031 | 0.3083 ± 0.0240 | 0.3450 ± 0.0056 |
| 1.8          |          | 0.0857 ± 0.0045 | 0.1293 ± 0.0049 | 0.2320 ± 0.0155 | 0.3097 ± 0.0180 |
| 3.3          |          | 0.0837 ± 0.0042 | 0.1203 ± 0.0075 | 0.1770 ± 0.0227 | 0.2537 ± 0.0300 |
| 5.0          |          | 0.0873 ± 0.0057 | 0.1233 ± 0.0029 | 0.1547 ± 0.0042 | 0.2077 ± 0.0153 |
| 8.0          |          | 0.0857 ± 0.0021 | 0.0940 ± 0.0035 | 0.0873 ± 0.0042 | 0.0577 ± 0.0025 |
| 13.0         |          | 0.0847 ± 0.0032 | 0.0660 ± 0.0010 | 0.0530 ± 0.0026 | 0.0510 ± 0.0036 |

### Table 5
Absorbance values assayed by MTT after IP6 treatment for 6 h (mean ± SD)

| IP6 (mmol/L) | Time (d) | 0         | 2         | 4         | 6         |
|--------------|----------|-----------|-----------|-----------|-----------|
| Control      |          | 0.0847 ± 0.0021 | 0.1487 ± 0.0031 | 0.3083 ± 0.0240 | 0.3450 ± 0.0056 |
| 1.8          |          | 0.0857 ± 0.0045 | 0.1397 ± 0.0023 | 0.2823 ± 0.0344 | 0.3303 ± 0.0080 |
| 3.3          |          | 0.0837 ± 0.0042 | 0.1333 ± 0.0015 | 0.2317 ± 0.0192 | 0.3383 ± 0.0025 |
| 5.0          |          | 0.0873 ± 0.0057 | 0.1277 ± 0.0025 | 0.2593 ± 0.0131 | 0.3240 ± 0.0082 |
| 8.0          |          | 0.0857 ± 0.0021 | 0.0940 ± 0.0035 | 0.0873 ± 0.0042 | 0.0757 ± 0.0025 |
| 13.0         |          | 0.0847 ± 0.0032 | 0.1157 ± 0.0035 | 0.2237 ± 0.0215 | 0.3020 ± 0.0080 |

### Table 6
Absorbance values assayed by MTT after IP6 treatment for 12 h (mean ± SD)

| IP6 (mmol/L) | Time (d) | 0 | 2 | 4 | 6 |
|--------------|----------|---|---|---|---|
| Control      |          | 0.0847 ± 0.0021 | 0.1487 ± 0.0031 | 0.3083 ± 0.0240 | 0.3450 ± 0.0056 |
| 1.8          |          | 0.0857 ± 0.0045 | 0.1383 ± 0.0065 | 0.2660 ± 0.0056 | 0.3267 ± 0.0050 |
| 3.3          |          | 0.0837 ± 0.0042 | 0.1230 ± 0.0043 | 0.1990 ± 0.0554 | 0.3220 ± 0.0105 |
| 5.0          |          | 0.0873 ± 0.0057 | 0.1250 ± 0.0017 | 0.2290 ± 0.0350 | 0.3217 ± 0.0076 |
| 8.0          |          | 0.0857 ± 0.0021 | 0.1103 ± 0.0038 | 0.2693 ± 0.0711 | 0.2723 ± 0.0025 |
| 13.0         |          | 0.0847 ± 0.0032 | 0.0957 ± 0.0035 | 0.2363 ± 0.0299 | 0.2107 ± 0.0135 |

### Table 7
Absorbance values assayed by MTT after IP6 treatment for 24 h (mean ± SD)

| IP6 (mmol/L) | Time (d) | 0 | 2 | 4 | 6 |
|--------------|----------|---|---|---|---|
| Control      |          | 0.0847 ± 0.0021 | 0.1487 ± 0.0031 | 0.3083 ± 0.0240 | 0.3450 ± 0.0056 |
| 1.8          |          | 0.0857 ± 0.0045 | 0.1323 ± 0.0040 | 0.2547 ± 0.0153 | 0.3330 ± 0.0122 |
| 3.3          |          | 0.0837 ± 0.0042 | 0.1277 ± 0.0099 | 0.2300 ± 0.0161 | 0.3123 ± 0.0115 |
| 5.0          |          | 0.0873 ± 0.0057 | 0.1317 ± 0.0032 | 0.2137 ± 0.0168 | 0.2990 ± 0.0190 |
| 8.0          |          | 0.0857 ± 0.0021 | 0.1067 ± 0.0021 | 0.1757 ± 0.0116 | 0.2093 ± 0.0105 |
| 13.0         |          | 0.0847 ± 0.0032 | 0.0807 ± 0.0021 | 0.0650 ± 0.0036 | 0.0857 ± 0.0051 |
the expression of wild-type P53 and down-regulates the expression of mutant P53 to control cell cycle check-point and prevent progression of cells to the DNA synthesis phase (S phase) of the cell cycle. But the exact mechanism by which IP6 affects p53 is not clear and needs further study.

P21\(^{waf1/cipl}\) is an inhibitor of cyclin dependent kinases (CDKS) that are required for the cells to enter the S-phase of the cell cycle.\(^{[30]}\) The gene encoding P21\(^{waf1/cipl}\) is transcriptionally regulated by the protein product of the gene p53. Over-expression of P21\(^{waf1/cipl}\) is growth inhibitory, possibly by inhibiting the activity of cyclin/CDK complex\(^{[30]}\) which binds to the C-terminal domain of PCNA. The resulting P21-PCNA complex blocks the ability of PCNA to process DNA polymerase in DNA replication. Thus P21\(^{waf1/cipl}\) may act as a tumor suppressor because of its role in growth control.\(^{[39,40]}\) In the present study, the expression of P21 was increased after IP6 treatment for 2 d (\(P < 0.05\)). After counterstaining with hematoxylin, untreated cells were stained purple while IP6-treated cells were stained yellow, indicating that expression of P21 is higher in IP6-treated cells. High-expression of P21\(^{waf1/cipl}\) leads to decreased nuclear expression of PCNA, which is in agreement with our results.

In summary, IP6 remarkably inhibits proliferation of HT-29 human colon carcinoma cell line. IP6 exerts its inhibitory effect in part by affecting special cell cycle regulators and reduces over-expression of mutant P53 and stimulates expression of wild-type P53 and P21\(^{waf1/cipl}\). P21\(^{waf1/cipl}\) binds to PCNA, thus preventing PCNA-dependent cellular proliferation. In our immunocytochemical experiments, cells grew very slowly and were not adhered in media with high IP6 dose, fell off and died very soon. The effect of 13.0 mmol/L IP6 on expression of genes was less than that of 8.0 mmol/L IP6, partly due to the rapid death of cells in 13.0 mmol/L IP6, indicating that that IP6 has no significant effect on the expression of genes. Furthermore, neither significant dose-dependent effect of IP6 was observed on the expressions of cell cycle regulators nor obvious correlation among these indexes was found, possibly owing to the short period of IP6 treatment (only 2 d), suggesting that the effects of IP6 on gene expressions are relatively weak.

The present study is merely a preliminary investigation of IP6 on colon cancer. The results are also limited although the effects of IP6 can be seen. Additional research is needed to explore the mechanisms of IP6 in cell proliferation and differentiation, apoptosis, and potential therapeutic value of IP6.

REFERENCES
1. Steiner H, Buschmann T, van der Linden M, Fels LM, Lippert H, Reymond MA. The role of proteomics in the diagnosis and outcome prediction in colorectal cancer. Technol Cancer Res Treat 2002; 1: 297-304
2. Price AS. Primary and secondary prevention of colorectal cancer. Gastroenterol Nurs 2003; 26: 73-81
3. Zhang YZ, Li SY. New technologies of early diagnosis on colorectal cancer. Shijie Huaren Xiaohua Zazhi 2004; 12: 1200-1205
4. Steinmetz KA, Kushi LH, Bostick RM, Folsom AR, Potter JD. Vegetables, fruit, and colon cancer in the Iowa Women's Health Study. Am J Epidemiol 1994; 139: 1-15
5. Greenwald P, Lanza E, Eddy GA. Dietary fiber in the reduction of colon cancer risk. J Am Diet Assoc 1987; 87: 1178-1188
6. Hellszuer K, Block G, Blumberg J, Diplock AT, Levine M, Marnett LJ, Schulpin RF, Spence JT, Simic MG. Summary of the round table discussion on strategies for cancer prevention: diet, food, additives, supplements, and drugs. Cancer Res 1994; 54: 2044s-2051s
7. Howe GR, Benito E, Castellote R, Cernée J, Estèe J, Gallagher RP, Iscovitch JM, Deng-ao J, Kaaks R, Kune GA. Dietary intake of fiber and decreased risk of cancers of the colon and rectum: evidence from the combined analysis of 13 case-control studies. J Natl Cancer Inst 1992; 84: 1887-1896
8. Slavin J, Jacobs D, Marquart L. Whole-grain consumption and chronic disease: protective mechanisms. Nutr Cancer 1997; 27: 1-21
9. Barbolt TA, Abraham R. The effect of bran on dimethylhydrazine-induced colon carcinogenesis in the rat. Proc Soc Exp Biol Med 1978; 157: 656-659
10. Barbolt TA, Abraham R. Dose-response, sex difference, and the effect of bran in dimethylhydrazine-induced intestinal tumorigenesis in rats. Toxicol Appl Pharmacol 1980; 55: 417-422
11. Reddy BS, Mori H. Effect of dietary wheat bran and dehydrated citrus fiber on 3,2'-dimethyl-4-aminobiphenyl-induced intestinal carcinogenesis in F344 rats. Carcinogenesis 1981; 2: 21-25
12. Alabaster O, Tang Z, Frost A, Shivapurkar N. Effect of betacarotene and wheat bran fiber on colonic aberrant crypt and tumor formation in rats exposed to azoxymethane and high dietary fat. Carcinogenesis 1995; 16: 127-132
13. McIntyre A, Gibson PR, Young GP. Butyrate production from dietary fibre and protection against large bowel cancer in a rat model. Gut 1993; 34: 386-391
14. Alabaster O, Tang ZC, Frost A, Shivapurkar N. Potential synergism between wheat bran and psyllium: enhanced inhibition of colon cancer. Cancer Lett 1995; 75: 53-58
15. Eastwood MA. The physiological effect of dietary fiber: an update. Am J Nutr 1992; 12: 19-35
16. Weisburger JH, Reddy BS, Rose DP, Cohen LA, Kendall ME, Wynder EL. Protective mechanisms of dietary fibers in nutritional carcinogenesis. Basic Life Sci 1993; 61: 45-63
17. Velázquez OC, Lederer HM, Rombeau JL. Butyrate and the colonocyte. Implications for neoplasia. Dig Dis Sci 1996; 41: 727-739
18. Graf E, Eaton JW. Dietary suppression of colonic cancer. Fiber or phytate? Cancer 1985; 56: 717-718
19. Harland BF, Oberleas D. Phytate in foods. World Rev Nutr Diet 1987; 52: 235-259
20. Johnson LF, Tate ME. Structure of “phytic acids”. Can J Chem 1969; 47: 63-73
21. Mills BJ, Broghamer WL, Higgins PJ, Lindeman RD. Inhibition of tumor growth by zinc depletion of rats. J Nutr 1984; 114: 746-752
22. Bunce CM, French PJ, Allen P, Mountford JC, Moor B, Greaves MF, Michell RH, Brown G. Comparison of the levels of inositol metabolites in transformed haemopoietic cells and their normal counterparts. Biochem J 1993; 289 (Pt 3): 667-673
23. Grases F, Simonet BM, Prieto RM, March JG. Phytate levels in diverse rat tissues: influence of dietary phytate. Br J Nutr 2001; 86: 225-231
24. Shamsuddin AM, Vuicenic I, Cole KE. IP6: a novel anti-cancer agent. Life Sci 1997; 61: 343-354
25. Shamsuddin AM. Inositol phosphates have novel anticancer function. J Nutr 1995; 125: 725S-732S
26. Warbrick E. PCNA binding through a conserved motif. Bioessays 1998; 20: 195-199
27. Lehmann AR. Dual functions of DNA repair genes: molecular, cellular, and clinical implications. Bioessays 1998; 20: 146-155
28. Kelman Z. PCNA: structure, functions and interactions. Oncogene 1997; 14: 629-646
29. Cayrol C, Knibiehler M, Duchmann B. p21 binding to PCNA causes G1 and G2 cell cycle arrest in p53-deficient cells.
30 Aranda FI, Laforga JB. Cellular proliferation in breast ductal infiltrating carcinoma. Correlation with clinical and histopathological variables. Pathol Res Pract 1997; 193: 683-688
31 Allred DC, Harvey JM, Berardo M, Clark GM. Prognostic and predictive factors in breast cancer by immunohistochemical analysis. Mod Pathol 1998; 11: 155-168
32 Cox LS, Lane DP. Tumour suppressors, kinases and clamps: how p53 regulates the cell cycle in response to DNA damage. Bioessays 1995; 17: 501-508
33 Lowe SW, Ruley HE, Jacks T, Housman DE. p53-dependent apoptosis modulates the cytotoxicity of anticancer agents. Cell 1993; 74: 957-967
34 Lowe SW, Bodis S, McClatchey A, Remington L, Ruley HE, Fisher DE, Housman DE, Jacks T. p53 status and the efficacy of cancer therapy in vivo. Science 1994; 266: 807-810
35 Culotta E, Koshland DE Jr. p53 sweeps through cancer research. Science 1993; 262: 1958-1961
36 Rodrigues NR, Rowan A, Smith ME, Kerr IB, Bodmer WF, Gannon JV, Lane DP. p53 mutations in colorectal cancer. Proc Natl Acad Sci USA 1990; 87: 7555-7559
37 Saied IT, Shamsuddin AM. Up-regulation of the tumor suppressor gene p53 and WAF1 gene expression by IP6 in HT-29 human colon carcinoma cell line. Anticancer Res 1998; 18: 1479-1484
38 Levine AJ. p53, the cellular gatekeeper for growth and division. Cell 1997; 88: 323-331
39 Malkowicz SB, Tomaszewski JE, Linnenbach AJ, Cangiano TA, Maruta Y, McGarvey TW. Novel p21WAF1/CIP1 mutations in superficial and invasive transitional cell carcinomas. Oncogene 1996; 13: 1831-1837
40 Xiong Y, Hanocom GJ, Zhang H, Casso D, Kobayashi R, Beach D. p21 is a universal inhibitor of cyclin kinases. Nature 1993; 366: 701-704

S-Editor Wang J L-Editor Wang XL E-Editor Bai SH