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• GASTRIC CANCER •

Effect of *cis*-9,*trans*-11-conjugated linoleic acid on cell cycle of gastric adenocarcinoma cell line (SGC-7901)

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Abstract

AIM: **To determine the effect of *cis-9,*trans-11-conjugated linoleic acid (*c9,t*11-CLA) on the cell cycle of gastric cancer cells (SGC-7901) and its possible mechanism in inhibition cancer growth.**

METHODS: **Using cell culture and immunocytochemical techniques, we examined the cell growth, DNA synthesis, expression of PCNA, cyclin A,B_1,D_1,$p16^{ink4a}$ and $p21^{cip/waf1}$ of SGC-7901 cells which were treated with various *c9,t*11-CLA concentrations (25,50,100 and 200μmol·L^{-1})of *c9,t*11-CLA for 24 and 48h, with a negative control (0.1% ethane).**

RESULTS: **The cell growth and DNA synthesis of SGC-7901 cells were inhibited by *c9,t*11-CLA. SGC-7901 cells. Eight day after treatment with various concentrations of *c9, t*11-CLA mentioned above, the inhibition rates were 5. 92%,20.15%,75.61% and 82.44%,respectively and inhibitory effect of *c9,t*11-CLA on DNA synthesis (except for 25μmol/L,24h) showed significantly less ^{3}H-TdR incorporation than that in the negative controls ($P<0.05$ and $P<0.01$). Immunocytochemical staining demonstrated that SGC-7901 cells preincubated in media supplemented with different *c9,t*11-CLA concentrations at various times significantly decreased the expressions of PCNA (the expression rates were 7.2-3.0%,24h and 9.1-0.9% at 48h, respectively), Cyclin A (11.0-2.3%, 24h and 8.5-0.5%,48h), B_1 (4.8-1.8% at 24h and 5.5-0.6% at 48h)and D_1 (3.6-1.4% at 24h and 3.7%-0 at 48h) as compared with those in the negative controls(the expressions of PCNA, Cyclin A, B_1 and D_1 were 6.5% at 24h and 9.0% at 48h, 4.2% at 24h and 5.1% at 48h, 9.5% at 24h and 6.0% at 48h,respectively)($P<0.01$), whereas the expressions of $p16^{ink4a}$ and $p21^{cip/waf1}$, cyclin-dependent kinases inhibitors(CDKI), were increased.**

CONCLUSION: **The cell growth and proliferation of SGC-7901 cell is inhibited by *c9,t*11-CLA via blocking the cell cycle, with reduced expressions of cyclin A,B_1 and D_1 and enhanced expressions of CDKI($p16^{ink4a}$ and $p21^{cip/waf1}$).**

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INTRODUCTION

Gastric cancer is common in China[1-12], and it is currently thought to be caused by environmental factors, with diet being an important modifying agent[13-17]. Its mechanism of prevention and treatment still makes it become a hot spot in this area[18-39]. Its anticancerous potential Dietary fat has been implicated as an enhancing agent in carcinogenesis by both epidemiological and animal studies. Consumption of meat, specifically animal fat, has been implicated in a number of disease processes[40-42]. However, several epidemiological studies have suggested an association between increased consumption of meat and fat and decreased risk of stomach, mammary and esophageal cancers[43,44]. Among the fatty acids, only the essential fatty acid, linoleic acid (LA), has been clearly shown to enhance mammary tumorigenesis[43]. However, isomeric derivatives of *cis*-9,*cis*-12-octadecadienoic acid (linoleic acid, LA) containing a conjugated double-bond system (conjugated linoleic acid, CLA) showed inhibitory effect on carcinogenesis in animal studies[45,46]. CLA has a mixture of positional (9/11 or 10/12 double bonds) and geometric (various cis/trans combinations) isomers of LA formed by rumen and colon bacteria. The ability of CLA to prevent mammary and other tumors in rodents has been identified and has been the subject of several reviews[43]. There are eight potential isomers of CLA, but the *cis*-9, trans-11 and trans-9, *cis*-11 isomers are thought to be active as potential antioxidant and anticarcinogenic agents. Therefore, it is of interest to investigate more extensively the anticancer activities of CLA.

In the present study, we investigated the effect of *cis*-9, trans-11-CLA(*c9,t*11-CLA) on the cell cycle of human gastric adenocarcinoma cells (SGC-7901).

MATERIALS AND METHODS

Materials

*c9,t*11-CLA, a monoisomer of *c*-9,*t*11-octadecadienoic acid with 98% purity, was obtained from Dr. Rui-Hai Liu(Food Science and Toxicology, Department of Food Science, Cornell University, Ithaca, NY, USA). The *c9,t*11-CLA was dissolved in 96ml·L^{-1} ethanol, and was then diluted to the following concentrations: 0,25,50,100 and 200μmol·L^{-1}.

Methods

Cell culture Human gastric adenocarcinoma cells(SGC-7901), purchased from Cancer Research Institute of Beijing (China), were cultured in RPMI 1640 (Gibco) medium, supplemented with calf serum 100ml·L^{-1}, penicillin (100×103u·L^{-1}) and streptomycin (100mg·L^{-1}). The pH was maintained at 7.2-7.4, by equilibration with 5% CO_2.The temperature was maintained at 37℃. The cells were sub-cultured with a mixture Ethylenedinitrile tetraacetic acid (EDTA) and trypsin.

Cell growth curve The SGC-7901cells were seeded in six 24 well plates (Nuc,Co.); each well contained 2×10^4 cells. After 24h, the medium of different plates was replaced with media supplemented with *c*9, *t*11-CLA at different concentrations. On the next day, the numbers of cells of 3 wells from each plate were determined daily by using the trypan blue staining. The means were obtained on each of

eight days and were used to draw a cellular growth curve. The inhibitory rates(IR) on the 8th day was calculated, as follows:

$$IR(\%)=\frac{\text{Total Number of cells in negative control (8d)}-\text{Number of cells in test groups(8d)}}{\text{Total number of cells in negative control(8d)}}\times 100\%$$

[^{3}H]-Labeled precursor incorporation SGC-7901 cells (5×10^4/well in 24 well plate) were cultured in appropriate medium for 24h prior to beginning the experiment. The medium was, then, replaced with different concentrations *c9, t*11-CLA. After 18 and 42h, the cells were incubated with [^{3}H] thymidine (China Nucleus Institute, 0.5μci/mL, 1.0μci/well). After 6h the cells were harvested with trysin/EDTA. Cells were collected in an acetic fiber filter with cellular collector and washed three times with PBS. The filter was dried overnight at 37℃. The filter was transferred into liquid of scintillation(containing 1% po and 2% pop in xylene) and cpm value determined by liquid Scintillation Counter (LS6500, Beckmen Co.).

Cell samples

SGC-7901 cells were treated for 24 and 48h with various concentrations of *c9,t*11-CLA and collected by centrifugation. Specimens were fixed immediately in 40g·L^{-1} formaldehydum polymerisatum and embedded in paraffin. Gastric cancer tissue from a patient served as a reference.

Primary antibodyies

To examine the proliferating cell nuclear antigen(PCNA) in cell proliferation and to determine cyclins (A,B_1 and D_1) and cyclin-dependent kinases inhibitors ($p16^{ink4a}$ and $p21^{waf1}$) in cell cycle of SGC-7901, we used six primary antibodies: corresponding mouse monoclonal antibodies for cyclin B_1 and D_1, PCNA and $p21^{waf1}$ and corresponding rabbit polyclonal antibodies for cyclin A and $p16^{ink4a}$. PCNA, $p16^{ink4a}$, and cyclin D_1 were purchased from Calbiochem Co. USA; others from Zhongshan Co. China.

Immunocytochemistry

Immunocytochemical staining was performed on serial sections at room temperature, using the horseradish peroxidase method. The sections were deparaffinized in xylene and rehydrated through graded alcohol. The sections were incubated for 10min at 95℃ in 10mmol·L^{-1} sodium citrate(pH 6.0) buffer for PCNA staining. Endogenous peroxidases were inactivated by immersing the sections in hydrogen peroxide for 10min, and then were incubated for 10min with 100ml·L^{-1} normal goat serum in PBS to block non-specific binding. The sections were subsequently incubated overnight at 4℃ with relevant antibodies (1∶50 dilution) respectively. The next day, the sections were incubated with biotinylated anti-mouse or anti-rabbit IgG(Zhongshan Co. China) for 30min, followed by peroxidase-conjugated streptavidin(Zhongshan Co.China) for 30min. The chromogenic reaction was developed with DAB (diaminobenzidine) for 10min, and all sections were counterstained with hematoxylin. Controls consisted of omission of the primary antibody. The Positive Rate(PR) was calculated as follows:

$$PR(\%)=\frac{\text{Number of positive cells}}{\text{Total number}(2\times10^4)}\times 100$$

Statistical analysis

Analysis of data was performed using the student's *t* test or χ^2 test. A value of $P<0.05$ is considered to be statistically significant.

RESULTS

Effect of c9,t11-CLA on SGC-7901 cell growth

As shown in Figure 1, Growth of the cells in various concentrations of *c9, t*11-CLA did not differ from the negative control within 3d. After 3d, SGC-7901 cells incubated in 25 and 50μmol·L^{-1} of *c9,t*11-CLA grew at a lower rate than the negative control. While in 100 and 200μmol·L^{-1} concentrations of *c9, t*11-CLA, cell proliferation was significantly inhibited. The inhibitory rate of various *c9, t*11-CLA concentrations were 5.9%, 20.2%, 75.6% and 82.4%, respectively.

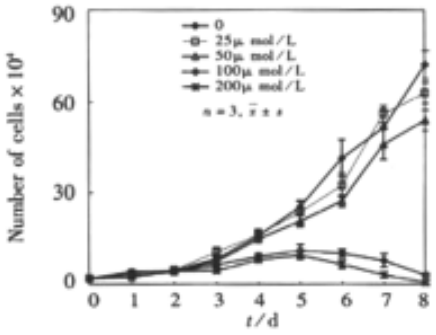

Figure 1 Growth curve of SGC-7901 cells cultured in various concentration of *c9, t*11-CLA

Effect on DNA synthesis

The effect of CLA on isotope incorporation into SGC-7901 cells are presented in Table 1. SGC-7901 cells preincubated in media supplemented with various *c9, t*11-CLA concentration (except for 25μmol/L, 24h) incorporated significantly less ^{3}H-TdR than did the negative control ($P<0.05$ and $P<0.01$, Table 1). The inhibitory rate (IR) displayed a dose-response relationship as the concentration of *c9, t*11-CLA increased.

Table 1 Inhibitory effect of c9, t11-CLA on DNA synthesis in SGC-7901 cells (n=6)

*c*9,*t*11-CLA (μmol/L)	^{3}H-TdR incorporation (cpm, $x\pm s$)		Inhibitory Rate (%)	
	24h	48h	24h	48h
0	2165±172	3598±603	-	-
25	1810±505	3093±323	16.4	14.0
50	2208±291	2640±607[a]	-2.0	26.6
100	2065±261	2063±495[b]	4.6	42.7
200	472±260[b]	88±15[b]	78.2	97.5

[a]$P<0.05$, [b]$P<0.01$ *vs* negative control

Cell proliferation

As shown in Figure 2, expression raues of PCNA (Figure 3.1) on SGC-7901 cells gradually decreased after SGC-7901 cells were incubated with different concentrations of *c9,t*11-CLA at various times. Moreover, SGC-7901s cell expressed significantly less PCNA than did the negative control ($P<0.01$). The expression rate displayed a dose-response relationship as the concentrations of CLA increased.

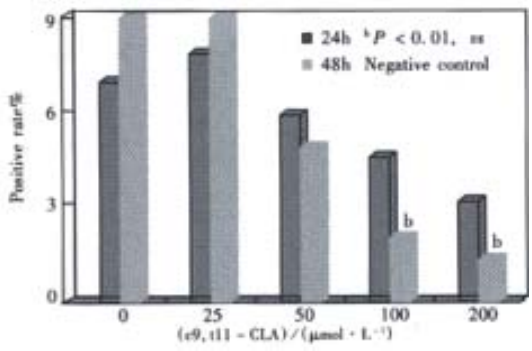

Figure 2 Expression of PCNA on SGC-7901 cells treated with *c9, t*11-CLA

Expressions of cyclin A,B_1 and D_1 and p16^{ink4a}, p21^{waf1}

The the expression of rates cyclin A, B_1, and D_1 (Figure 3.2-4)on SGC-7901 cells was decreased (Table 2) after SGC-7901 cell were incubated with different concentrations of *c9, t*11-CLA for 24h and 48h while cyclin-dependent kinases inhibitors (*P*16^{ink4a}, and *P*21^{waf1}) increased (Table 3; Figure 3.5-6).

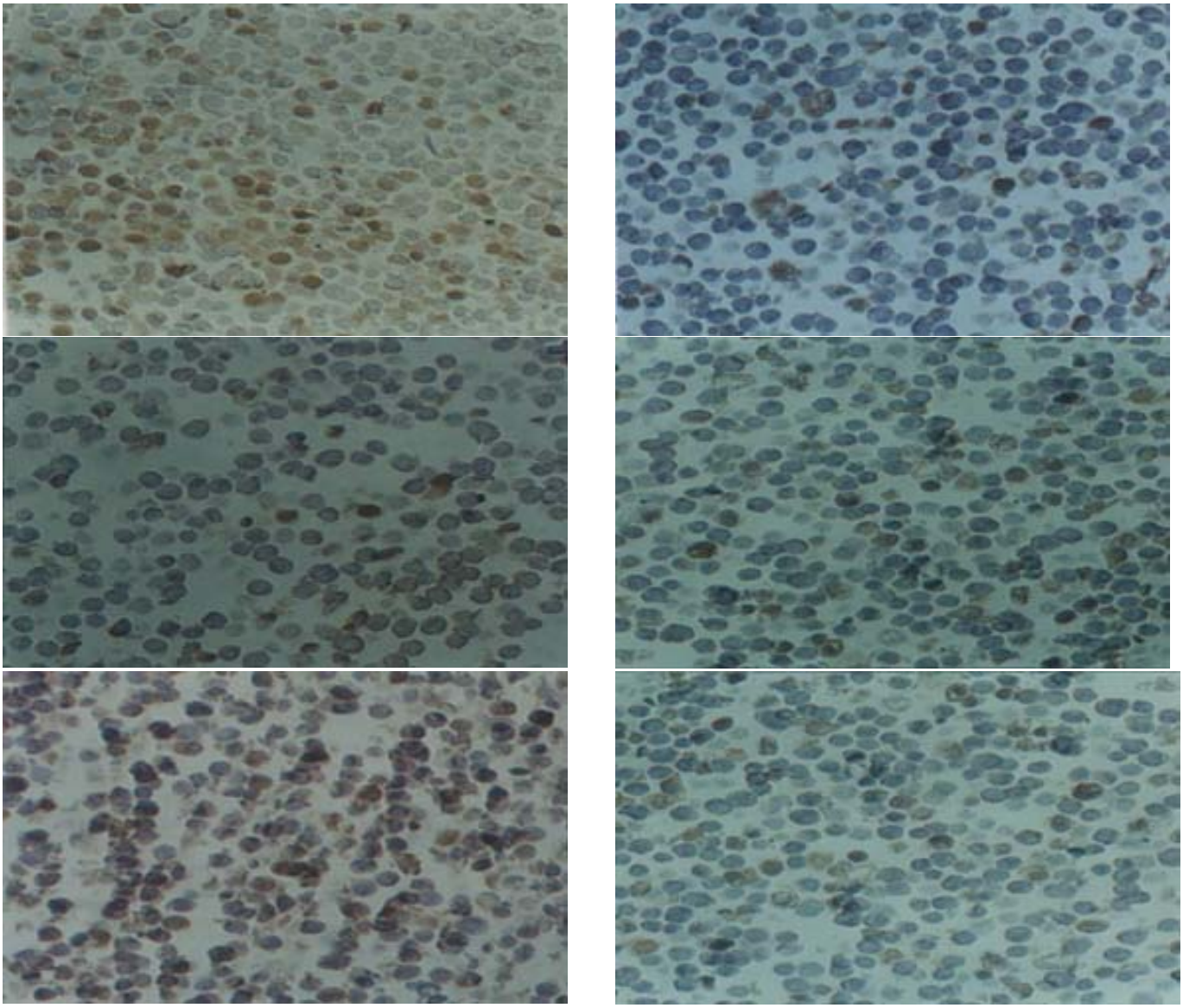

Figure 3 A: The expression of PCNA on SGC-7901 cells of the negative controls (immunocytochemistry staining SP method, original magnification ×400); B: The expression of cyclin A on SGC-7901 cells of the negative controls (immunocytochemistry staining SP method, original magnification ×400); C: The expression of cyclin B_1 on SGC-7901 cells of the negative controls (immunocytochemistry staining SP method, original magnification ×400); D: The expression of cyclin D_1 on SGC-7901 cells of the negative controls (immunocytochemistry staining SP method, original magnification ×400); E:The expression of *p*16^{inf4a} on SGC-7901 cells of *c9,t*11-CLA group (100μmol·L^{-1}) (immunocytochemistry staining SP method, original magnification ×400); F:The expression of *p*21$^{cip/waf1}$ on SGC-7901 cells of *c9,t*11-CLA group (100μmol·L^{-1}) (immunocytochemistry staining SP method, original magnification ×400)

Table 2 Positive rates of cyclin A, B_1, and D_1 on SGC-7901 cells treated with *c9,t11*-CLA(%)

c9,t11-CLA (μmol/L)	24h			48h		
	Cylin A	Cylin B_1	Cyclin D_1	Cylin A	Cylin B_1	Cyclin D_1
0	10.7	4.2	9.5	5.9	5.1	6.0
25	11.0	4.8	3.6[b]	8.5	5.5	3.7[b]
50	7.9	2.5	3.5[b]	5.0	3.1[b]	3.7[b]
100	4.4[b]	2.6[b]	2.1[b]	1.3[b]	0.7[b]	0.6[b]
200	2.3[b]	1.8[b]	0.4[b]	0.5[b]	0.6[b]	0

[b]*P*<0.01 *vs* negative control

Table 3 Positive rates of *p16^{ink4a}* and *p21^{waf1}* on SGC-7901 cells treated with *c9,t*11-CLA(%)

*c9,t*11-CLA (μmol/L)	24h		48h	
	p16^{ink4a}	p21^{waf1}	p16^{ink4a}	p21^{waf1}
0	1.0	0.2	0.8	0.6
25	0.7	1.4[b]	0.2	0.8
50	1.4	1.0[b]	3.0[b]	2.5[b]
100	2.8[b]	4.1[b]	4.6[b]	3.8[b]
200	3.6[b]	5.2[b]	5.0[b]	6.3[b]

[b]*P*<0.01 *vs* negative control

DISCUSSION

CLA is a naturally occurring fatty acid in animal's food. Dietary sources of CLA include grilled beef, cheese, and related foods[47]. Another source of CLA is its endogenous generation via the carbon centered free radical oxidation of linoleic acid[45]. Over the past ten years, a number of research works of animal experiments have supported the observation that CLA is an effective chemopreventive agent of cancer, and that it can inhibit carcinogenesis of different tissues at different stages of induction by chemical agents[44,45]. Several investigators in our group have reported that *c9, t*11-CLA is an effective agent to prevent carcinogenesis[48,49] and cancer[50-52]. Zhu's study[48] demonstrated that *c9,t*11-CLA could significantly inhibit the mice forestomach neoplasia induced by B(a)P(50mg·kg^{-1}) in post-initiation in short term(23weeks).The incidences of tumors of mice in the B(a)P group, B(a)P with high dose CLA(5μL·g^{-1}) group and B(a)P with low dose CLA(2.5μL·g^{-1}) group were 100%, 60% and 69% respectively (P <0.05). Xue's research[49] also indicated that the incidence of neoplasm in mouse forestomach in the B(a)P group,75% pure *c9,t*11-CLA group, 98% pure *c9,t*11-CLA group and 98% pure t10,c12-CLA group were 100.0%, 75.0%, 69.2%, and 53.8%, respectively. This maybe due to an inhibition mitogen of activated protein kinase(MAPK)-a way to reduce carcinogenesis. The data from our research group suggested that *c9,t*11-CLA could inhibit proliferation of cancer cells, i.e. SGC-7901 cells[50] and MCF-7 cells[51,52], and induced cancer cell (SGC-7901) apoptosis [53]. Moreover, the inhibiting effect of *c9,t*11-CLA on SGC-7901 cell proliferation may be related to cell cycle.

As shown in Figure 1, *c9, t*11-CLA at various concentrations in 8 days reduced the proliferative activity of SGC-7901 cells and its inhibitory rates were from 5.92% to 82.44%, but the mechanism of such inhibition of *c9,t*11-CLA has not been clarified. However, we discovered that SGC-7901 cells supplemented with *c9,t*11-CLA incorporated significantly less [^{3}H] thymidine than negative controls (shown in Table I). The inhibitory rates of, from 16.4% to 78.2% after incubating with *c9,t*11-CLA for 24h and from 14.0% to 97.5% after 48h displayed a dose-response relationship.In the meantime, we investigated further the expressions of PCNA and protein from cell cycle such as cyclins and cyclin-dependent kinase inhibitors (CDKI) on SGC-7901 cells treated with various concentrations of *c9,t*11-CLA. PCNA (proliferating cell nuclear antigen) plays an essential role in both the replication and repair of DNA, and is an essential component of the DNA replication machinery, acting as the processing factor for polymerases äandå.In addition to its role in replication, PCNA is not only required for base excision-repair of nucleotides, but also binds to cell cycle regulatory proteins such as *p*21 and Gadd45 [54]. In this study, we discovered that the expression of PCNA on SGC-7901 cells gradually decreased with increasing concentrations of *c9,t*11-CLA in comparison with negative controls(showed in Figure 2). In other words, DNA replication lessens, thereby resulting in slower on SGC-7901 cell proliferation.

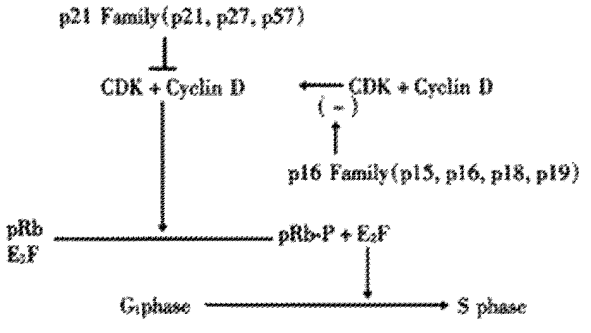

Figure 4 The relationship between CDKI(*p*16 and *p*21) and cyclins in G_1/S transition

The fundamental task of the cell cycle is to ensure that DNA is faithfully replicated once during S phase and that identical chromosomal copies are distributed equally to two daughter cells during M phase. The cell cycle is a complex process, regulated by many factors, which can be divided into three groups: cyclins(A,B, D,E....H); cyclin-dependent kinases (CDK, including CDK_1-CDK^a-7); CDK inhibitors (CDKI, including *p*16 family and *p*21 family). They are balanced through mutual interactions. Uncontrolled cell proliferation is the hallmark of cancers which are the result of damage to genes that directly regulate their cell cycles. Using immunocytochemical technique to detect expressions of cyclins and CDKI, we demonstrated that the expressions of cyclin A,B_1 and D_1 on SGC-7901 cells treated with various concentrations *c9,t*11-CLA were reduced, whereas expressions of CDKI($p16^{in4a}$ and $p21^{waf1}$) increased, as compared with those of negative controls. Successive actions of CDKs promote cell-cycle progression in mammalian cells. Various cyclins bind and activate CDKs at specific times during the cell cycle.

Mammalian cyclin A activates CDK_2[55] in S-phase and CDK_1 (Cdc_2) in G_2- and M-phases. One important mechanism that enables sequential activation of cyclin-CDK complexes is the periodic synthesis and destruction of cyclins. Cyclin A expression starts late in G_1-phase and is increasing through S- and G_2-phase before the protein is degraded in M-phase. The cell cycle-dependent expression of cyclin B_1 is critical for the proper timing of a cell's entry into mitosis which is dependent both upon the binding of CDK_1 to cyclin B_1, as well as a series of phosphorylation and dephosphorylation events. The cyclin B_1 protein accumulates during interphase and peaks at the G_2-M phase transition [56]. One of the crucial substrates of G_1 phase CDK, including CDK_4 in the complex with D-type cyclins(cyclin D_1,D_2 and D_3), is Rb protein (pRb), which is the product of the retinoblastoma susceptibility gene. Rb protein plays an important role in the regulation of the G_1 to S phase progression in normal cells and the function of pRb is regulated by phosphorylation. Thus, during the G_0 and G_1 phase, Rb protein is in an un- or underphosphorylated stage and binds to E_2F family transcription factors. Cylin Ds/CDK_4 becomes activated around the mid G_1 phase, resulting in the accumulation of increasingly phosphorylated, inactive forms pRb. This causes the release of E_2F family transcription factors which induce the expression of S-phase genes by positive regulation through E_2F-binding sites(see Figure 4) [57]. It is also known that abrogation of the functions of Cylin A prevents entry into the S phase. From the beginning of the S phase Rb protein remains in the hyperphosphorylated inactive state until the end of M phase; such condition is thought to be due to both cylin A/CDK_2 and Cylin A,B_1/Cdc_2 in catalyzing the phosphorylation reaction [58]. $p16^{ink4a}$ is the founder member of a family of proteins with the ability to inhibit CDK_4 and the CDK_4-related kinase CDK_6.

The INK4 family is composed of four members in mammalian organisms: $p16^{ink4a}$, $p15^{ink4b}$, $p18^{ink4c}$, and $p19^{ink4d}$. The four mammalian INK4 proteins have similar biochemical properties: all of them bind to CDK_4 and CDK_6 and inhibit the kinase activity of the $CDK_{4\text{-}6}$/Cylin D complexes(Figure 4)[59]. The INK4 inhibitor causes G_1 arrest indicating that the phosphorylation of pRb on residues specific for CDK_4(and possibly CDK_6) is critical for G_1/S progression. While $p21^{CIP1/waf1}$ family, comprising $p21^{Cip1/waf1}$, $p27^{CIP1}$ and $p57^{CIP2}$, bind to a variety of CDKs and cyclins, preferentially to cyclin/CDK complexes rather than monomeric forms and also inhibit performed active cyclin/CDK complexes(see Figure 4)[59]. In addition to its role as a CDKI, $p21^{Cip/waf1}$ has been shown to block DNA replication by direct interaction with PCNA mentioned above. However, $p21^{CIP1/waf1}$ does not inhibit the PCNA-dependent nucleotide excision-repair of DNA. In deed, DNA damage leads to an increase in the level of *p*53, and result in *p*21-mediated cell cycle arrest in the G_1 phase, which persists

until DNA repair is completed[60].Thus, it is proposed that $p21^{Cip/waf1}$ plays an important role under such conditions as terminal differentiation and cell senescence.

In conclusion, *c9,t*11-CLA may inhibit cell growth and proliferation by a decrease in the expressions of cyclin A,B_1 and D_1 and an increase in that of CDKI($p16^{ink4a}$ and $p21^{Cip1/waf1}$) on SGC-7901 cells in comparison with the negative controls. This result suggested that the inhibition effect of *c9,t*11-CLA on SGC-7901 cell proliferation is related to the cell cycle. The whole mechanism of the action of *c9,t*11-CLA on SGC-7901 cell cycle further research.

REFERENCES

1 Gao HJ,Yu LZ,Bai JF,Peng YS,Sun G,Zhao HL,Miu K,Lu XZ,Zhang XY,Zhao ZQ. Multiple genetic alterations and behavior of cellular biology in gastric cancer and other gastric mucosal lesions:*H.pylori* infection, histological types and staging. *World J Gastroenterol* 2000;6:848-854

2 Deng DJ, E Z. Overview on recent studies of gastric carcinogenesis: human exposure of N nitrosamides. *Shijie Huaren Xiaohua Zazhi* 2000; 8:250-252

3 Deng DJ. progress of gastric cancer etiology: N-nitrosamides 1999s. *World J Gastroenterol* 2000;6:613-618

4 Li DG, Wang ZR, Lu HM. Pharmacology of tetrandrine and its therapeutic use in digestive diseases. *World J Gastroenterol* 2001;7:627-629

5 Niu WX, Qin XY, Liu H, Wang CP. Clinicopathological analysis of patients with gastric cancer in 1200 cases. *World J Gastroenterol* 2001;7: 281-284

6 Kimura K. Gastritis and gastric cancer. Asia. *Gastroenterol Clin North Am* 2000;29:609-621

7 Hua JS. Effect of *Hp*: cell proliferation and apoptosis on stomach cancer. *Shijie Huaren Xiaohua Zazhi* 1999;7:647-648

8 Xia HX, Zhang GS. Apoptosis and proliferation in gastric cancer caused by Hp infection. *Shijie Huaren Xiaohua Zazhi* 1999;7:740-742

9 Cai L, Yu SZ, Ye WM, Yi YN. Fish sauce and gastric cancer: an ecolog ical study in Fujian Province, China. *World J Gastroenterol* 2000;6: 671-675

10 Cai L, Yu SZ, Zhang ZF. *Helicobacter pylori* infection and risk of gastric cancer in Changle County, Fujian Province, China. *World J Gastroenterol* 2000;6:374-376

11 Cai L, Yu SZ. A molecular epidemiologic study on gastric cancer in Changle, Fujian Province. *Shijie Huaren Xiaohua Zazhi* 1999;7:652-655

12 Cai L,Yu SZ,Zhang ZF.Glutathione S-transferases M1,T1 gentypes and the risk of gastric cancer: A case-control study. *World J Gastroenterol* 2001;7:506-509

13 Sugie S, Okamoto K, Watanabe T, Tanaka T, Mori H. Suppressive effect of irsogladine maleate on N-methyl-N-nitro-N-nitrosoguanidine (MNNG)-initiated and glyoxal-promoted gastric carcinogenesis in rats. *Toxicology* 2001;166:53-61

14 Palli D, Russo A, Saieva C, Salvini S, Amorosi A, Decarli A. Dietary and familial determinants of 10-year survival among patients with gastric carcinoma. *Cancer* 2000;89:1205-1213

15 Mathew A,Gangadharan P,Varghese C,Nair MK.Diet and stomach cancer: a case-control study in South India. *Eur J Cancer Prev* 2000;9:89-97

16 Palli D. Epidemiology of gastric cancer: an evaluation of available evidence. *J Gastroenterol* 2000;35:84-89

17 Palli D, Russo A, Ottini L, Masala G, Saieva C, Amorosi A, Cama A, D'Amico C, Falchetti M, Palmirotta R, Decarli A, Costantini RM, Fraumeni JF Jr. Red meat, family history, and increased risk of gastric cancer with microsatellite instability. *Cancer Res* 2001;61:5415-5419

18 Tu SP, Jiang SH, Tan JH, Jiang XH, Qiao MM, Zhang YP, Wu YL, Wu YX. Proliferation inhibition and apoptosis induction by arsenic trioxide on gastric cancer cell SGC-7901. *Shijie Huaren Xiaohua Zazhi* 1999;7:18-21

19 Chen YQ, Shen HX, Zhou SJ, Chen L, Yu X, Lu LP. Experimental study of the inhibitory effect of fufang shenqitang on gastric adenocarcinoma of rats. *Shijie Huaren Xiaohua Zazhi* 1999;7:117-119

20 Gao GL, Yang Y, Ren CW, Yang SF. Effect of vitamin D3 on forestomach carcinogenesis induced by N methyl N'nitro N nitroso-guanidine and tween 20 in rats. *Shijie Huaren Xiaohua Zazhi* 1999;7:763-765

21 Xiao B, Xiao LC, Lai ZS, Zhang YL, Zhang ZS, Zhang WD. Experimental study of the inhibition affect of Zhen'ailing on the growth of gastric cancer in vitro. *Shijie Huaren Xiaohua Zazhi* 1999;7:951-954

22 Xin Y, Zhao FK, Zhang SM, Wu DY, Wang YP, Xu L. Relationship between CD44v6 expression and prognosis in gastric carcinoma patients. *Shijie Huaren Xiaohua Zazhi* 1999; 7:210-214

23 Li XS, Zhang XJ, Sun YR, Wang XF, Liu HW, Li W, Sa HJ, Liu HY, Zhang XM, Yang JC. Studies on cytokine activity in sera and ascites of patients with digestive tumor. *Shijie Huaren Xiaohua Zazhi* 1999;7:13-14

24 Wang SW, Xie YH, Li YR, Lin HS, Zhu LZ. Antineoplastic effect and short-term effect on advanced malignant tumor of digestive tract of Chinese herbs antike. *Shijie Huaren Xiaohua Zazhi* 1999;7:236-239

25 Liu HF, Liu WW, Fang DC, Men RF, Wang ZH. Apoptosis and its relationship with Fas ligand expression in gastric carcinoma and its precancerous lesion. *Shijie Huaren Xiaohua Zazhi* 1999;7: 561-563

26 Zhou HP, Wang X, Zhang NZ. Early apoptosis in intestinal and diffuse gastric carcinomas. *World J Gastroenterol* 2000;6:898-901

27 Liu XB, Li L, Zhuang BZ, Jiang YD, Wang JH. Cyclin E expression in gastric carcinoma and its clinicopathological significance. *Shijie Huaren Xiaohua Zazhi* 1999;7:656-658

28 Wang XS, Wang RB, Zhang ZL, Cao TJ, Jin WD. Effect of short time heating with MMC and 5-FU on cancer cells. *Shijie Huaren Xiaohua Zazhi* 1999;7:576-578

29 Zhang FX, Zhang XY, Fan DM, Deng ZY, Yan Y, Wu HP, Fan JJ. Antisense telomerase RNA induced human gastric cancer cell apoptosis. *World J Gastroenterol* 2000;6:430-432

30 Zhao AG, Yang JK, Zhao HL. Chinese Jianpi herbs induce apoptosis of human gastric cancer grafted onto nude mice. *Shijie Huaren Xiaohua Zazhi* 2000;8:737-740

31 Zhu ZH, Xia ZS, He SG. The effects of ATRA and 5Fu on telomerase activity and cell growth of gastric cancer cells *in vitro*. *Shijie Huaren Xiaohua Zazhi* 2000;8:669-673

32 Xia ZS, Zhu ZH, He SG. Effects of ATRA and 5-Fu on growth and telomerase activity of xenografts of gastric cancer in nude mice. *Shijie Huaren Xiaohua Zazhi* 2000;8:674-677

33 Guo YQ, Zhu ZH, Li JF. Flow cytometric analysis of apoptosis and proliferation i-n gastric cancer and precancerous lesion. *Shijie Huaren Xiaohua Zazhi* 2000;8:983-987

34 Gu QL, Li NL, Zhu ZG, Yin HR, Lin YZ. A study on arsenic trioxide inducing *in vitro* apoptosis of gastric cancer cell lines. *World J Gastroenterol* 2000;6:435-437

35 Wu K, Shan YJ, Zhao Y, Yu JW, Liu BH. Inhibitory effects of RRRa2tocopheryl succinate on benzo(a)pyrene (B(a)P)a 2induced forestomach carcinogenesis in female mice. *World J Gastroenterol* 2001;7: 60-65

36 Wu K, Liu BH, Zhao DY, Zhao Y. Effect of vitamin E succinate on expression of TGF-β-1, c-jun and JNK1 in human gastric cancer SGC-7901 cells. *World J Gastroenterol* 2001;7:83-87

37 Cui RT, Cai G, Yin ZB, Cheng Y, Yang QH, Tian T. Transretinoic acid inhibits rats gastric epithelial dysplasia induced by N-methyl-N-nitro-N-nitrosoguanidine: influences on cell apoptosis and expression of its regulatory genes. *World J Gastroenterol* 2001;7:394-398

38 He XS, Su Q, Chen ZC, He XT, Long ZF, Ling H, Zhang LR. Expression, deleton and mutation of p16 gene in human gastric cancer. *World J Gastroenterol* 2001;7:515-521

39 Liu S, Wu Q, Chen ZM, Su WJ. The effect pathway of retinoic acid through regulation of retinoic acid receptor in gastric cancer cells. *World J Gastroenterol* 2001;7:662-666

40 Zhang B, Zhang LH, Li N, Wang YJ, Li JS. Effects of n-3 fatty acids on glucose metabolism of septic rats and its mechanism. *Shijie Huaren Xiaohua Zazhi* 1999;7:335-337

41 Lewls CJ, Yetley EA. Health claim and observational human data: relation between dietary fat and cancer. *Am J Clin Nutr* 1999;69: 1357-1364

42 Mantzioris E, Cleland LG, Gibson RA, Neumann MA, Demasi M, James MJ. Biochemical effects of a diet containing foods enriched with n-3 fatty acid. *Am J Clin Nutr* 2000; 72:42-48

43 MacDonald HB. Conjugated linoleic acid and disease prevention: a review of current knowledge. *J Am Coll Nutr* 2000;19:111-118

44 Kimoto N, Hirose M, Futakuchi M, Iwata T, Kasai M, Shirai T. Site-dependent modulating effects of conjugated fatty acids from safflower oil in a rat two-stage carcinogenesis model in female Sprague-Dawley rats. *Cancer Lett* 2001; 168: 15-21

45 Ip C, Banni S, Angioni E, Carta G, McGinley J, Thompson HJ, Barbano D, Bauman D. Conjugated linoleic acid-enriched butter fat alters mammary gland morphogenesis and reduces cancer risk in rats. *J Nutr* 1999;129:2135-2142

46 Brodie AE, Manning VA, Ferguson KR, Jewell DE, Hu CY. Conjugated linoleic acid inhibits differentiation of pre- and post-confluent 3T3-L1 preadipocytes but Inhibits cell proliferation only in preconfluent cells. *J Nutr* 1999; 129: 602-606

47 Parodi PW. Conjugated linoleic acid and other anticarcinogenic agents of bovine milk fat. *J Dairy Sci* 1999;82:1339-1349

48 Zhu Y, Qiu J, Chen BQ, Liu RH. The inhibitory effect of conjugated linoleic acid on mice forestomach neoplasia induced by benzo(a) pyrene. *Zhonghua Yufang Yixue* 2001;35:19-22

49 Xue YB, Chen BQ, Liu JR, Zheng YM, Liu RH. The inhibition of mouse forestomach neoplasm induced by B(a)p through MAPKs pathway by conjugated Linoleic acid. *Zhonghua Yufang Yixue* 2001;35:1-4

50 Liu JR, Chen,BQ, Liu RH, Lu GF, Zhu Y, Han XH. The Inhibited effect on gastric carcinoma cell induced by conjugated linoleic acid (CLA). *Weisheng Yanjiu* 1999;28:353-357
51 Liu JR, Chen BQ, Xue YB, Han XH, Yang YM, Liu RH. Inhibitory effect of conjugated linoleic acid on the *in vitro* growth of human mammary cancer cells (MCF-7). *Zhonghua Yufang Yixue* 2001;35:244-247
52 O'Shea M, Devery R, Lawless F, Murphy J, Stanton C. Milk fat conjugated linoleic acid (CLA) inhibits growth of human mammary MCF-7 cancer cells. *Anticancer Res* 2000;20:3591-3601
53 Liu JR, Chen BQ, Deng H, Han XH, Liu RH. Cellular apoptosis induced by conjugated linoleic acid in human gastric cancer (SGC-7901) cells. *Gongye Weisheng Yu Zhiyebing* 2001;27:129-133
54 Tsurimoto T. PCNA binding proteins. *Front Biosci* 1999; 4: D849-858
55 Mani S, Wang C, Wu K, Francis R, Pestell R. Cyclin-dependent kinase inhibitors: novel anticancer agents. *Expert Opin Investig Drugs* 2000;9: 1849-1870
56 Ito M. Factors controlling cyclin B expression. *Plant Mol Biol* 2000;43: 677-690
57 Pyronnet S, Sonenberg N. Cell-cycle-dependent translational control. *Curr Opin Genet Dev* 2001;11:13-18
58 Viallard JF, Lacombe F, Belloc F, Pellegrin JL, Reiffers J. Molecular mechanisms controlling the cell cycle: fundamental aspects and implications for oncology. *Cancer Radiother* 2001;5: 109-129
59 Israels ED,Israels LG. The cell cycle. *Stem cells* 2001;19:88-91
60 Sowa Y, Sakai T. Butyrate as a model for "gene-regulating chemoprevention and chemothera py". *Biofactors* 2000;12:283-287

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