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Conjugated Linoleic Acid Isomers and Cancer[1,2]

Nirvair S. Kelley, Neil E. Hubbard, and Kent L. Erickson*

Department of Cell Biology and Human Anatomy, School of Medicine, University of California, Davis, CA 95616-8643

Abstract

We reviewed the literature regarding the effects of conjugated linoleic acid (CLA) preparations enriched in specific isomers, *cis*9, *trans*11-CLA (*c*9, *t*11-CLA) or *trans*10, *cis*12-CLA (*t*10, *c*12-CLA), on tumorigenesis in vivo and growth of tumor cell lines in vitro. We also examined the potential mechanisms by which CLA isomers may alter the incidence of cancer. We found no published reports that examined the effects of purified CLA isomers on human cancer in vivo. Incidence of rat mammary tumors induced by methylnitrosourea was decreased by *c*9, *t*11-CLA in all studies and by *t*10, *c*12-CLA in just a few that included it. Those 2 isomers decreased the incidence of forestomach tumors induced by benzo (a) pyrene in mice. Both isomers reduced breast and forestomach tumorigenesis. The *c*9, *t*11-CLA isomer did not affect the development of spontaneous tumors of the intestine or mammary gland, whereas *t*10, *c*12-CLA increased development of genetically induced mammary and intestinal tumors. In vitro, *t*10, *c*12-CLA inhibited the growth of mammary, colon, colorectal, gastric, prostate, and hepatoma cell lines. These 2 CLA isomers may regulate tumor growth through different mechanisms, because they have markedly different effects on lipid metabolism and regulation of oncogenes. In addition, *c*9, *t*11-CLA inhibited the cyclooxygenase-2 pathway and *t*10, *c*12-CLA inhibited the lipooxygenase pathway. The *t*10, *c*12-CLA isomer induced the expression of apoptotic genes, whereas *c*9, *t*11-CLA did not increase apoptosis in most of the studies that assessed it. Several minor isomers including *t*9, *t*11-CLA; *c*11, *t*13-CLA; *c*9, *c*11-CLA; and *t*7, *c*11-CLA were more effective than *c*9, *t*11-CLA or *t*10, *c*12-CLA in inhibiting cell growth in vitro. Additional studies with purified isomers are needed to establish the health benefit and risk ratios of each isomer in humans. J. Nutr. 137: 2599–2607, 2007.

Introduction

Conjugated linoleic acid (CLA)[3] was originally described as an anticarcinogen isolated from fried ground beef (1). At that time, specific isomers were not tested, but since then, CLA has been extensively studied both in vivo and in vitro as a possible anticarcinogen. That original study was published only a few years after linoleic acid (LA), a main fatty acid substrate for conversion to CLA, was shown to enhance tumorigenesis in a rat mammary tumor model (2). Although dietary fat had been implicated in altering carcinogenesis, researchers have now focused on specific fatty acids and their possible mechanism of action. Other specific fatty acids have been shown to alter tumorigenesis but will not be discussed here.

CLA is a collective term for isomers of LA that have conjugated double bonds. Depending on the position and geometry of the double bonds, several isomers of CLA have been identified (3). Most of the published studies have used a mixture of CLA isomers with 2 major forms, *cis*9, *trans*11-CLA (*c*9, *t*11-CLA) and *trans*10, *cis*12-CLA, (*t*10, *c*12-CLA), and a number of minor isomers (i.e. *t*7, *t*9-CLA; *c*9, *c*11-CLA; *t*9, *t*11-CLA; *c*10, *c*12-CLA; *t*10, *t*12-CLA; *t*11, *t*13-CLA; and *c*11, *c*13-CLA). Ruminant meat and dairy products are the major dietary sources of *c*9, *t*11-CLA and partially hydrogenated oils such as shortenings and margarines are the main sources of *t*10, *c*12-CLA as well as other isomers. Although in some early studies CLA intake was estimated to be 1 g/d, a recent report using food duplicate methodology suggests that average intake in the U.S. population is < 500 mg/d (4).

Feeding a mixture of CLA isomers to animals has been reported to alter chemically induced carcinogenesis, glucose and lipid metabolism, diabetes, body composition, and immune cell functions. Several reviews also indicate that feeding a mixture of CLA isomers hindered the growth of numerous types of tumors (5–9). Recently, purified isomers of CLA have become available for research studies. Results based on feeding CLA preparations enriched in individual CLA isomers indicate that the different isomers have distinct effects on tumorigenesis and lipid metabolism. A few of these studies with the isolated isomers may raise concerns regarding their safety. To the best of our knowledge, there is no published review that focused on the role of individual CLA isomers on tumorigenesis. The focus of this review is the effects of individual CLA isomers on the proliferation and apoptosis of tumor cells both in vivo and in vitro; potential mechanisms that may be involved were also addressed. We

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[3] Abbreviations used: AA, arachidonic acid; BP, benzo[a]pyrene; CLA, conjugated linoleic acid; COX-2, cyclooxygenase-2; ER, estrogen receptor; FLAP, 5-lipoxygtenase activating protein; 5-HETE, hydroxyeicosatetraenoic acid; LA, linoleic acid; 5-LOX, 5-lipoxygenase; MNU, methylnitrosourea; PG, prostaglandin; TPA, 12-*O*-tetradecanoylphorbol-13-acetate.
* To whom correspondence should be addressed. E-mail: klerickson@ucdavis.edu.

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included only those studies that examined the effects of purified individual CLA isomers on tumorigenesis, those that compared the effects of the purified isomers with each other, or with those of a mixture of CLA isomers. The literature reviewed here was published between 1999 and 2007. Initially, we summarize results from studies with CLA mixtures without referring to the original references.

Summary of studies with mixtures of CLA isomers

Although the main focus of this review is the effects of individual CLA isomers on tumorigenesis, we will initially summarize the effects of mixtures of CLA isomers on the development and progression of cancer in animal models. There are only a few epidemiological human studies in which the relationship between tissue CLA concentrations and tumor incidence has been examined. There are no published reports to our knowledge in which the effect of supplementing either a mixture of CLA isomers or those of individual CLA isomers on the incidence of cancer has been examined in humans.

Studies in animal models

Feeding a mixture of CLA isomers inhibited chemically induced tumors of the mammary gland, skin, colon, and forestomach in several animal models. The inhibition of tumorigenesis was dependent upon the dietary concentrations of the CLA mixtures, which varied from 0.05 to 1% of the diet (wt:wt). In humans, those dietary concentrations would be obtainable with either supplementation or a significant change in intake of CLA-rich foods. In those studies, the level and type of fat in the basal diet did not influence the inhibitory effects of CLA. The timing and duration of CLA feeding influenced the effectiveness of CLA in preventing tumorigenesis. When diets containing CLA were fed to prepubertal rats, inhibition of tumorigenesis continued after termination of CLA feeding. Both initiation and promotion of tumors induced by methylnitrosourea (MNU) administered to 56-d-old rats was inhibited when the animals were fed diets containing a mixture of CLA isomers during the early postweaning and prepubertal periods (10). In contrast, feeding diets containing CLA to postpubertal mice required a continuous intake of CLA to prevent carcinogen-induced tumorigenesis (11).

Dietary CLA, 1% (wt:wt), fed for 30 wk significantly decreased incidence of colon cancer induced by 1,2-dimethylhydrazine in 6-wk-old rats (12). Another study compared the effects of diets containing CLA or LA, 3.3% (wt:wt), on the development of pancreatic tumors induced by *N*-nitrosobis-2-oxopropylamine and their resultant hepatic metastasis in male Syrian hamsters. The number of pancreatic tumors did not differ when CLA- and LA-fed groups were compared; however, hepatic metastasis was significantly greater in the CLA group than in the LA group (13). Different types of tumors from various organs probably respond differently to CLA treatments. Additional details regarding the studies with a mixture of CLA isomers can be found in the reviews cited above.

Studies in humans

Few epidemiological human studies have investigated the relationship between CLA intake or tissue CLA concentrations and tumor incidence. Unfortunately, those studies have yielded far less conclusive results than studies conducted in animals. In 1 study (14), 55- to 69-y-old female subjects were followed for 6 y; they answered self-administered questionnaires regarding the amount of their dietary CLA intake, family incidence of cancer, and other risk factors (11). Throughout the course of the study, 941 of the 62,573 women reported incidences of breast cancer. Contrary to the expected negative association, results of this study showed a weak positive relationship between breast cancer incidence and the intake of CLA.

CLA levels in the serum and breast adipose tissue were used to analyze the relationship between breast cancer and CLA (15,16). In a study of postmenopausal women, the levels of serum and dietary CLA were significantly lower in breast cancer patients than in control subjects (15). In contrast, CLA concentrations in breast adipose tissue were not directly correlated in women with and without breast cancer (16). Thus, a limited number of studies do not allow us to establish whether CLA can provide any protection in humans against cancer of any site.

Studies with purified isomers

In vivo animal studies with purified isomers of CLA. The studies that reported the effects of individual isomers of CLA on tumorigenesis were summarized (**Table 1**). Rats fed diets containing butter enriched in *c*9, *t* 11-CLA, purified *c*9, *t* 11-CLA, or a mixture of CLA isomers (0.8%) for 1 mo (between d 23 and d 55) had reduced mammary epithelial mass, size of the terminal end buds, and mammary tumor development (17). This demonstrated that purified *c*9, *t*11-CLA fed either as butter fat or as an isomer to the diet was as effective as a mixture of CLA isomers in reducing the number of MNU-induced mammary tumors. Those authors also examined whether vaccenic acid, which can be metabolized to *c*9, *t*11-CLA, would inhibit MNU-induced tumorigenesis (18). Feeding 1% *c*9, *t*11-CLA- or 2% vaccenic acid-containing diets started after the injection of MNU and continued for 6 wk. Diets containing both fatty acids reduced the MNU-induced premalignant lesions by 50% compared with a control group fed regular butter. Also, CLA concentrations in the tissues and the concentration of *c*9, *t*11-CLA in the mammary gland was 4-fold greater in the vaccenic acid group than the control group. Those results showed that rats could metabolize vaccenic acid to *c*9, *t*11-CLA, which was effective in decreasing tumorigenesis. Both *c*9, *t*11-CLA and *t*10, *c*12-CLA CLA also decreased the number of premalignant lesions by ~35% at 6 wk and decreased the number of mammary tumors by ~40% at 24 wk after MNU treatment (19). The authors concluded that *c*9, *t*11- and *t*10, *c*12- CLA isomers were equally effective in reducing MNU-induced tumorigenesis. Rats fed diets with sunflower oil containing *c*9, *t*11-CLA or a mixture decreased tumor incidence by 45% (20). Neither of the CLA-containing diets altered the time required to detect palpable tumors (latency). Results from these rat studies showed that *c*9, *t*11- and *t*10, *c*12-CLA were as effective as the CLA mixture in reducing mammary tumors induced by MNU.

In a study with salad oil supplemented with 75 or 98% pure *c*9, *t*11-CLA or 98% pure *t*10, *c*12-CLA, incidences and multiplicity of benzo[a]pyrene (BP)-induced forestomach tumors in mice were differentially altered dependent on the isomer used and its concentration (21). Incidences were 100, 75, 69, and 54% in control, 75% pure *c*9, *t*11-CLA, 98% pure *c*9, *t*11-CLA, and 98% pure *t*10, *c*12-CLA, respectively. Thus, *t*10, *c*12-CLA resulted in lower incidence of tumors than *c*9, *t*11-CLA, but the tumor size did not differ between the 2 isomers. In a transplantable mouse

TABLE 1 Effect of CLA isomers on in vivo tumorigenesis

Animals	Tumors	CLA	Results	Comments	Reference
Female Sprague Dawley rats, 23 d old	Mammary; Induced by single dose of 50 mg/kg MNU[1] given at age of 55 d	Regular and CLA-enriched butters with 0.8% *c*9, *t*11-CLA, *t*10, *c*12-CLA or mixture; CLA diets fed for 30 d prior to MNU	All CLA preparations equally effective in reducing mammary epithelial mass, TEB size, and tumor yield	Both *c*9, *t*11- and *t*10, *c*12- CLA inhibited tumorigenesis equally	14
Female Sprague Dawley rats, 45 d old	Mammary; Induced by single dose of 50 mg/kg MNU, given at age of 50 d	Regular butter, 2% vaccenic acid, 1% *c*9, *t*11-CLA; CLA diets fed for 6 wk after MNU injections	Both vaccenic acid and *c*9, *t*11-CLA diets reduced number of IDP lesions by 50%	Vaccenic acid as effective as *c*9, *t*11-CLA	14
Female Sprague Dawley rats, 50 d old	Mammary; Induced by single dose of 50 mg/kg MNU, given at age of 55 d	0.5% *c*9, *t*11-CLA or *t*10, *c*12-CLA; CLA diets fed for 6 or 24 wk after MNU	Both isomers decreased premalignant lesions by 33–36% at 6 wk and no. of mammary tumors by 35–40% at 24 wk	Both *c*9, *t*11- and *t*10, *c*12- CLA equally inhibited tumorigenesis	16
Female Sprague Dawley rats, 47 d old	Mammary; Induced by single dose of 25 mg/kg MNU, given at age of 47 d	1% *c*9, *t*11-CLA or CLA mix; CLA diets fed for 6 or 20 wk after MNU	Both CLA preparations decreased tumor incidence by 45%; neither reduced latency	CLA effects vary on tumor incidence and latency	17
Kun Ming female mice, 9 wk old	Forestomach; induced by 50 mg/kg BP, 4 times for 2 wk	0.8% *c*9, *t*11-CLA 75% pure, 0.5% *c*9, *t*11-CLA 98% pure, 0.5% *t*10, *c*12-CLA 98% pure; CLA diets fed for 7 wk after BP	Tumor incidence was 100, 75, 69, and 54% in control, *c*9, *t*11-CLA 75 and 98% pure, and *t*10, *c*12-CLA 98% pure. All CLA preparations reduced tumor diameter.	Pure preparations of both isomers were more effective than less pure *c*9, *t*11-CLA	18
BALB/C female mice, 5 wk old	Mammary line 4526 injected into mammary fat pad or tail vein	0.1 or 0.25% *c*9, *t*11-CLA; 0.1 or 0.25% *t*10, *c*12-CLA; 0.25%; CLA mixture fed for 4 wk	Neither CLA preparation affected tumor latency or growth. All CLA preparations decreased metastatic lung tumor load.	Effects of 2 isomers may not be additive, may share similar mechanisms for metastasis	19
C57BL/6 Min male mice, 6 wk old	Small and large intestine; genetically induced	1% *c*9, *t*11-CLA or *t*10, *c*12-CLA; CLA diets fed for 8 wk	No. of adenomas increased by *t*10, *c*12- CLA not by *c*9, *t*11-CLA.	*t*10, *c*12-CLA increased size of colon tumors	20
FVB mice, wild or transgenic, weanling/ post puberty	Mammary; genetically induced	0.5% *c*9, *t*11-CLA or *t*10, *c*12-CLA; CLA diets started at weanling or post puberty and continued until tumor diameters reached 18–22 mm	*t*10, *c*12-CLA increased lobular hyperplasia and decreased tumor latency; *c*9, *t*11-CLA had no effect.	*t*10, *c*12-CLA enhanced mammary tumor development	21

[1] IDP, intraductal proliferation; TEB, terminal end bud.

mammary tumor model that spontaneously metastasizes to the lung, CLA isomers lacked a differential effect but did reduce metastasis depending on the concentration of the individual isomers (22). Mice were fed diets containing either no CLA; 0.1 or 0.25% *c*9, *t*11-CLA; the same 2 concentrations of *t*10, *c*12-CLA; or 0.25% of a 50:50 mixture of the 2 isomers. Neither of the individual isomers nor the mixture had an effect on latency nor primary tumor growth when compared with those in the group without CLA. However, all diets containing CLA significantly reduced pulmonary tumor burden after spontaneous metastasis, as well as the implantation and survival of the metastatic cells. The higher concentration of either isomer had a significantly greater effect on decreasing metastatic nodule size and the total lung tumor burden.

The effects of 2 purified CLA isomers on the development of intestinal tumors were compared in Min mice (23). These mice have a mutation of the APC gene that leads to neoplasia at multiple sites of both the small and large intestine. In contrast to the tumor development in this mouse model, most human intestinal tumors occur in the large intestine. In this study, Min mice were fed either a diet with sunflower and rapeseed oils or a diet also containing 1% *c*9, *t*11-CLA or *t*10, *c*12-CLA (23). The total number of adenomas did not differ among the 3 dietary groups, but the sizes of the adenomas were significantly greater in the distal part of small intestine in mice fed the diet containing *t*10, *c*12-CLA compared with the control group. Tumor size and numbers in the *c*9, *t*11-CLA group did not differ from the control group. These results suggested that *t*10, *c*12-CLA may act as a growth promoter in small intestine carcinogenesis. Those results are in contrast to reports citing a reduction in the number of tumors of several digestive organs after feeding a mixture of CLA isomers (6,8). In another study (24), FVB/J female mice with altered erbB2 gene expression in mammary epithelium were fed diets with or without 0.5% *c*9, *t*11-CLA or

*t*10, *c*12-CLA until the tumor diameter reached ~20 mm. When CLA feeding of the genetically modified mice started at weaning, *t*10, *c*12-CLA accelerated mammary tumor development and decreased the median time required for tumor development compared with the *c*9, *t*11-CLA and control groups. Results were similar when feeding the experimental diets started after puberty. In the control wild-type mice, the number of terminal end buds increased by 30-fold after feeding the *t*10, *c*12-CLA-containing diet. Collectively, the results from this 1 study (24) demonstrated that *t*10, *c*12-CLA accelerated mammary tumor development, whereas the *c*9, *t*11-CLA isomer had no effect. In the aggregate of all murine tumor types studied in vivo with purified CLA isomers, *t*10, *c*12-CLA reduced tumorigenesis in 6 types and increased tumorigenesis in 2. The *c*9, *t*11-CLA isomer reduced tumorigenesis in 6 studies but had no effect in 2 others. However, summarizing the effects of dietary CLA over several tumor types from multiple sites may not be appropriate, because different mechanisms can be associated with tumorigenesis of different tumor types and different stages of tumor progression, especially when the methods for tumor induction are quite different. Sufficient studies with appropriate animal models that parallel human pathogenesis need to be completed before definitive conclusions can be extrapolated to human malignancy.

In vitro studies with purified isomers of CLA. Reports that investigated the effects of individual CLA isomers on the growth, viability, or apoptosis of tumor cell lines were summarized (**Table 2**). Most of these studies focused on tumor cell lines derived from mammary gland, prostate, and digestive tract or their metastasis and used *c*9, *t*11-CLA and *t*10, *c*12-CLA isomers; the CLA concentrations used ranged from 1 to 200 μmol/L with treatments lasting 2–11 d.

Growth of human breast cancer MCF-7 cells treated with 10 μmol/L *c*9, *t*11-CLA, *t*10, *c*12-CLA, or a mixture of different CLA isomers for 4 d was reduced by 60, 40, and 25%, respectively (25). In that study, *t*10, *c*12-CLA inhibited growth induced by insulin and estrogen but not that induced by epidermal growth factor; *c*9, *t*11-CLA failed to inhibit growth induced by all 3 of those growth factors. Also, *t*10, *c*12-CLA but not *c*9, *t*11-CLA increased the apoptosis of cells cultured with insulin. These results suggest that the 2 CLA isomers may reduce tumor cell numbers through different mechanisms. Growth of estrogen receptor (ER)-positive MCF-7, but not ER-negative MDA-MB-231 cells, was inhibited by a CLA mixture (26). Thus, ER may play a role in mediating the CLA effects on mammary tumors. Additional studies examined the effects of 5 individual CLA isomers at 100 and 200 μmol/L on the growth of MCF-7 cells. c9, t11-CLA did not inhibit cell growth; for the remaining isomers, the potency for growth inhibition was: *c*9, *c*11-CLA > *t*10 *c*12-CLA > *t*9, *t*11-CLA > *c*11, *t*13-CLA. Another study showed that the growth inhibitory potency of 200 μmol/L CLA for MCF-7 breast tumor cells was: *t*9, *t*11-CLA > *c*11, *t*13-CLA > *c*9, *c*11-CLA, *t*7, *c*9-CLA > *c*9, *t*11-CLA > *t*10, *c*12-CLA (27). Inhibitory potencies of the CLA isomers varied when other cell lines were assessed. Thus, in most studies with mouse and human mammary tumor cell lines, *c*9, *t*11-CLA did not inhibit tumor cell growth.

The *t*10, *c*12-CLA isomer inhibited cell growth of colon, colorectal, and gastric cancer cell lines in all studies when assessed, whereas *c*9, *t*11-CLA inhibited cell growth in only a fraction (Table 2); *t*10, *c*12-CLA was more growth inhibitory than *c*9, *t*11-CLA in most of the studies. The *c*9, *t*11-CLA isomer was more potent than *t*10, *c*12-CLA in inhibiting the growth of colon cell lines (28,29). Most of the studies that did not detect inhibition of cell growth by *c*9, *t*11-CLA used a concentration of <50 μmol/L; however, 200 μmol/L failed to inhibit growth of HT-29 cells in 1 study (28). In addition, 50 and 100 μmol/L *c*9, *t*11-CLA inhibited the growth of HT-29 cells when the cells were cultured for 11 d (29) compared with 3 d (25). At 100 μmol/L, *c*9, *t*11-CLA inhibited the growth of the human gastric adenocarcinoma cell line, SGC-7901 and 50 μmol/L did not (30). Culture conditions including the concentration of CLA, duration of the treatment, and tumor type as well as the cell lines used seem to determine whether CLA isomers would affect cell growth.

In most studies with prostate cell lines, both isomers inhibited cell growth, with effects of *t*10, *c*12-CLA greater than *c*9, *t*11-CLA (27,29,31,32). In contrast, growth of a rat hepatoma cell line, dRLh-84, was inhibited by 10 μmol/L *t*10, *c*12-CLA or a mixture of CLA isomers, whereas *c*9, *t*12-CLA stimulated cell growth (33). This is the only study in which *c*9, *t*11-CLA stimulated tumor cell growth, suggesting something unique to this cell line or to the culture conditions used.

Summary of in vivo and in vitro studies. Overall, the growth inhibitory effects of *c*9, *t*11-CLA and *t*10, *c*12-CLA varied with the model used. MNU-induced mammary tumors were reduced by both isomers in rats and mice and *t*10, *c*12-CLA increased mammary tumorigenesis in 1 study and decreased it in 2 studies. Small intestine tumors were increased in 1 study and *c*9, *t*11-CLA had no effect in some of those studies. The *c*9, *t*11-CLA isomer did not inhibit the growth of mammary and colon tumor cell lines but did inhibit growth in prostate tumor cell lines and increased the growth of a hepatoma cell line. The *t*10, *c*12-CLA isomer inhibited growth of all the cell lines tested, including breast, colon, and prostate tumors. Results from 2 studies indicated that *c*9, *t*11-CLA was even more potent in inhibiting cell growth than *t*10, *c*12-CLA. Although results appear to be divergent, even tumors from a similar anatomical site may vary in their response to different chemotherapeutic agents and, thus, it could be expected that they would also differ in their response to lipids.

It can be difficult to extrapolate concentrations used in vitro to doses or dietary concentrations in vivo; the studies above tend to use pharmacological concentrations of CLA isomers. Additional studies are needed with appropriate animal models that represent the stages of human cancers to test the efficacy and safety of different CLA isomers. Most human tumors are not chemically induced, whereas transplanted cells grow as an expansive mass generally with a central necrotic core. Neither model resembles primary human tumors. To interpret the results in a meaningful manner it will also be important to use models where a number of the regulatory pathways and molecular signatures are characterized. An appropriate animal model for human breast cancer may be the mouse mammary intraepithelial neoplastic outgrowth, which has been shown to nearly recapitulate human ductal carcinoma in situ (34,35). With this model, investigators could assess the effects of CLA isomers not only on tumor growth and metastasis but also on transition of preneoplastic lesions to full malignancy. Because these issues need to be addressed, clear conclusions about CLA isomers and alterations of tumorigenesis and applicability to humans are difficult to make at this time.

Mechanisms by which CLA isomers may inhibit tumor growth

Mixtures of CLA isomers can alter initiation, promotion, progression, and metastasis of malignant tumors. Those effects can

TABLE 2 Effect of individual CLA isomers on the in vitro growth of tumor cell lines

Cell line	CLA	Results	Comments	Reference
Breast, MCF-7 (ER^+)	10 μmol/L each of *c*9, *t*11 and *t*10, *c*12-CLA; mixed CLA, 4 d	Potency for growth inhibition: *c*9, *t*11 > t10, *c*12 > mix-CLA	*c*9, *t*11-CLA more inhibitory than *t*10, *c*12-CLA	22
MCF-7; MDA-MB-231 (ER^-)	Mix and 5 different purified isomers, 100 or 200 μmol/L, 2 d	CLA mix inhibited growth of ER^+ but not ER^- cells; growth inhibition potency for MCF-7: *c*9, *c*11 > *t*10, *c*12 > *t*9, *t*11 > *c*11, *t*13-CLA	*c*9, *t*11-CLA did not inhibit cell growth; potency varied for other preparations	23
MCF-7, T47D, A-549 DLD-1, M4beu, PC-3	6 purified isomers, 200 μmol/L, 2 d	For MCF-7, potency of growth inhibition: *t*9, *t*11 > *c*11, *t*13 > *c*9, *c*11 > *t*7, *c*9 > *c*9, *t*11 > *t*10, *c*12-CLA; varied for other cell lines	Inhibitory potency varied with cell line and CLA isomer	24
Breast, line 4526	10–100 μmol/L *c*9, *t*11-CLA; 10–100 μmol/L *t*10, *c*12-CLA	*t*10, *c*12-CLA but not *c*9, *t*11-CLA decreased viability	*t*10, *c*12-CLA reduced growth may involve suppression of the 5-LOX metabolite 5-HETE	40
Breast, MDA-MB-231	10–100 μmol/L *c*9, *t*11-CLA; 10–100 μmol/L *t*10, *c*12-CLA	Viability decreased with increasing t10, c12-CLA and c9, t11-CLA concentration	*t*10, *c*12-CLA inhibited 5-HETE production and FLAP expression	41
Breast, MCF-7	10–160 μmol/L each of *c*9, *t*11-CLA; *t*10, *c*12-CLA; CLA mix	Growth inhibited with increasing concentration of *t*10, *c*12-CLA or CLA mix but not *c*9, *t*11-CLA	*c*9, *t*11-CLA did not inhibit growth at 160 μmol/L	39
Colon, HT-29	3 CLA isomers, 50 or 100 μmol/L, 5–9 d	Potency for growth inhibition: *t*10, *c*12 > *c*9, *t*11 > *c*9, *c*11-CLA	Inhibitory potency varied with CLA isomer	26
Colon, HT-29	*c*9, *t*11-CLA, 4 μmol/L; *t*10, *c*12-CLA, 4 μmol/L	*t*10, *c*12-CLA but not *c*9, *t*11- CLA inhibited cell growth	At low concentration *t*10, *c*12-CLA but not *c*9, *t*11-CLA inhibited growth	48
Colon, HT-29	4 isomers, 200 μmol/L, 3 d	*t*9, *t*11 > *t*10, *c*12-CLA, no inhibition caused by *c*9, *c*11- and *c*9, *t*11-CLA	Inhibitory potency varied with CLA isomer	25
Colon, HT-29	*c*9, *t*11-CLA, 4 μmol/L; *t*10, *c*12-CLA, 4 μmol/L	*t*10 *c*12-CLA, but not *c*9, *t*11-CLA caused cell cycle arrest	*c*9, *t*11-CLA did not inhibit growth at low concentrations	44
Colon, DCD-1	4 isomers, 200 μmol/L, 3 d	Potency for growth inhibition: *t*9, *t*11 > *t*10, *c*12 > *c*9, *t*11 > *c*9, *c*11-CLA	Inhibitory potency varied with CLA isomer	25
Colon, Caco-2	*c*9, *t*11-CLA, 1–5 μmol/L; *t*10, *c*12-CLA, 1–5 μmol/L, 4 d	Growth inhibition increased as concentration of *t*10, *c*12-CLA increased from 1 to 5 μmol/L, but not by *c*9, *t*11-CLA	At low concentration *t*10, *c*12-CLA but not *c*9, *t*11-CLA inhibited growth	49
Colon, PC-3	25–150 μmol/L each of *c*9, *t*11-CLA; *t*10, *c*12-CLA; CLA mix	All inhibited cell growth at concentration > 100 μmol/L; potency: *t*10, *c*12 > mix > *c*9, *t*11-CLA	*t*10, *c*12-CLA was more growth inhibitory than *c*9, *t*11-CLA	29
Colorectal, MIP-101	3 CLA isomers, 50 or 100 μmol/L, 5–9 d	Potency for inhibition of growth: *t*10, *c*12 > *c*9, *t*11 > *c*9, *c*11-CLA	Inhibitory potency varied with CLA isomer	26
Colorectal cells	*c*9, *t*11-CLA, 50 μmol/L; *t*10, *c*12-CLA, 50 μmol/L	*t*10, *c*12-CLA but not *c*9, *t*11-CLA inhibited growth	*t*10, *c*12-CLA inhibited cell growth	50
Stomach, SGC-7901	*c*9, *t*11-CLA, 25–200 μmol/L, 8 d	Growth inhibition: 82,76, 20, and 6% by 200, 100, 50, and 25 μmol/L CLA	Increasing *c*9, *t*11-CLA increased growth inhibition	27
Hepatoma, dRLh-84	1–25 μmol/L each of *c*9, *t*11-CLA; *t*10, *c*12-CLA; CLA mix, 1–5 μmol/L	Growth decreased with increasing concentration of *t*10, *c*12-CLA and mixture but increased with *c*9, *t*11-CLA	Opposing effects of *c*9, *t*11-CLA and *t*10, *c*12-CLA on growth	30
Prostate, PC-3	3 CLA isomers, 50 or 100 μmol/L, 11 d	Potency for growth inhibition: *t*10, *c*12 > *c*9, *t*11 > *c*9, *c*11-CLA	Inhibitory potency varied with CLA isomer	26
Prostate, DU 145	*c*9, *t*11-CLA, 2.5–10 μmol/L; *t*10, *c*12-CLA, 2.5–10 μmol/L	Growth inhibition with increasing concentration of *t*10, *c*12-CLA, but no inhibited cell growth by *c*9, *t*11-CLA	At physiological concentrations, *t*10, *c*12-CLA, but not *c*9, *t*11-CLA had effect	49

in some cases be attributed to alteration of lipid peroxidation, tissue fatty acid composition, eicosanoid metabolism, gene expression, cell cycle regulation, cell proliferation, and apoptosis. With respect to tissue fatty acid composition, levels of incorporation of *c*9, *t*11-CLA into mouse liver lipids were more pronounced compared with *t*10, *c*12-CLA (36). It is not known whether that phenomenon was due to the increased uptake of *c*9, *t*11-CLA or increased metabolism of *t*10, *c*12-CLA. Furthermore, the greatest concentration of *c*9, *t*11-CLA in mouse liver lipids was in the cholesterol esters and triglycerides, whereas that of *t*10, *c*12-CLA was in the phospholipids (36). Results from those studies with purified *c*9, *t*11-CLA and *t*10,

*c*12-CLA showed that the 2 isomers had differential effects on fatty acid composition of several tissues, including liver, adipose tissue, heart, spleen, and mammary gland (18,37–39).

Recent studies have examined the effects of separate CLA isomers on gene expression in mouse liver and adipose tissue. Feeding *t*10, *c*12-CLA for 8 wk caused a >1-fold increase in the expression of 278 genes and a decrease in 121 genes in mouse liver, whereas *c*9, *t*11-CLA increased the expression of only 22 genes and decreased that of 9 genes (40). In another study, feeding *t*10, *c*12-CLA for 14 d caused a >1-fold increase in the expression of 125 genes in adipose tissue (41). Because of those differences between the 2 CLA isomers, it is more appropriate to study their health effects individually rather than as a mixture. Here, we will discuss only the mechanisms investigated using purified isomers. A diagram of possible effects are summarized in **Figure 1**.

Effects of CLA isomers and eicosanoids

Prostaglandins (PG), eicosanoid metabolites of arachidonic acid (AA), have been implicated in tumorigenesis. Results from several studies discussed above showed that *c*9, *t*11-CLA and *t*10, *c*12-CLA have differential effects on tissue fatty acid composition that can lead to alterations in AA availability. Similar differential effects were obtained in tumor cell lines cultured with individual CLA isomers. For example, *c*9, *t*11-CLA decreased the incorporation of AA into phosphatidylcholine and increased AA uptake into phosphatidylethanolamine in human breast MCF-7 and human colon SW480 cell lines. In contrast, *t*10 *c*12-CLA had no effect on AA uptake into MCF-7 cells but did increase AA uptake into phosphatidylserine by SW 480 cells (42). Thus, the 2 isomers had differential effects on cellular partitioning dependent on the cell type. In that study, *c*9, *t*11-CLA and *t*10, *c*12-CLA were equally effective inhibiting cell growth, but only *c*9, *t*11-CLA decreased PGE_2. It is possible that PG could be involved in growth inhibition by *c*9, *t*11-CLA and that other mechanism(s), including other eicosanoid metabolites are involved for *t*10, *c*12-CLA. Another study examined the effects of CLA isomers on 12-O-tetradecanoylphorbol-13-acetate (TPA)-induced expression of the enzyme system responsible for conversion of AA to PG, cyclooxygenase-2 (COX-2), in MCF-7 cells (43). Either as a mixture or individually, *c*9, *t*11-CLA and *t*10, *c*12-CLA were equally effective in blocking the TPA-induced expression of COX-2 compared with cultures treated with LA. However, *t*10, *c*12-CLA was more effective than *c*9, *t*11-CLA in reducing the binding of *c*-Jun to either COX-2, cyclic AMP-responsive element, or collagenase-1 TPA-responsive element. Two other studies reported that *c*9, *t*11-CLA did not suppress growth of MCF-7 cells (44,45); 1 study indicated that *c*9, *t*11-CLA was more inhibitory than *t*10, *c*12-CLA (25). It is difficult to rationalize that altered COX-2 expression was the common mechanism for inhibition of cell growth by both CLA isomers, but that growth inhibition may be due to CLA alteration of AP-1-regulated COX-2 transcription (43). Inhibition of TPA-induced transcription of COX-2 by *c*9, *t*11-CLA was recently confirmed in the hairless skin mouse (46). CLA treatment blocked NF-kB driven-COX-2 expression by blocking the IkB kinase and PI3-Akt signaling pathway. Cell growth was not monitored in this study. Also, *c*9, *t*11-CLA but not *t*10, *c*12-CLA reduced nuclear factor-kB transcriptional activity and increased TNFα-induced apoptosis in a human prostate cancer cell line (47). In contrast, neither of the 2 CLA isomers inhibited COX-2 messenger RNA (mRNA) expression in a human prostate cancer cell line, PC-3 (32); both isomers inhibited cell growth. Thus, results from most of the studies showed that *c*9, *t*11-CLA inhibited COX-2 expression. Growth was inhibited in some studies but not examined in others. Alternatively, *t*10, *c*12-CLA reduced COX-2 expression and growth in a small percentage of studies. Collectively, these results suggest that changes in COX-2 expression may mediate the inhibitory effects of *c*9, *t*11-CLA and alternative mechanisms may mediate the effects of *t*10, *c*12-CLA.

The role of the 5-lipoxygenase (5-LOX) pathway in CLA inhibition of tumor growth has been examined. In PC-3 cells, *c*9, *t*11-CLA but not *t*10, *c*12-CLA decreased transcripts for 5-LOX (32). In breast cancer cells, the *t*10, *c*12-CLA isomer but not *c*9, *t*11-CLA decreased cell growth and the production of hydroxy-eicosatetraenoic acid (5-HETE) while increasing apoptosis (45,48). The inhibitory effect of *t*10, *c*12-CLA was reversed by the addition of 5-HETE (48). The growth inhibitory effect of *t*10, *c*12-CLA in the human breast tumor cell line MDA-MB-231 could be reversed by the overexpression of 5-LOX-activating protein (FLAP) (45). These authors concluded that 5-HETE may mediate the effects of *t*10, *c*12-CLA on breast tumor cell growth and apoptosis by CLA competition with the AA substrate as well as FLAP but not directly on 5-LOX. The *c*9, *t*11-CLA isomer increased production of 8-epi-$PGF_{2\alpha}$ in MCF-7 and SW480 tumor cells, whereas *t*10, *c*12-CLA increased it only in MCF-7 cells (42).

CLA isomers may also have their effects on tumorigenesis by altering lipid peroxidation. However, information regarding the effects of individual CLA isomers on lipid peroxidation is limited. Further studies are needed to confirm the role of the 5-LOX pathway and of lipid peroxidation in CLA isomer-specific effects on cell growth.

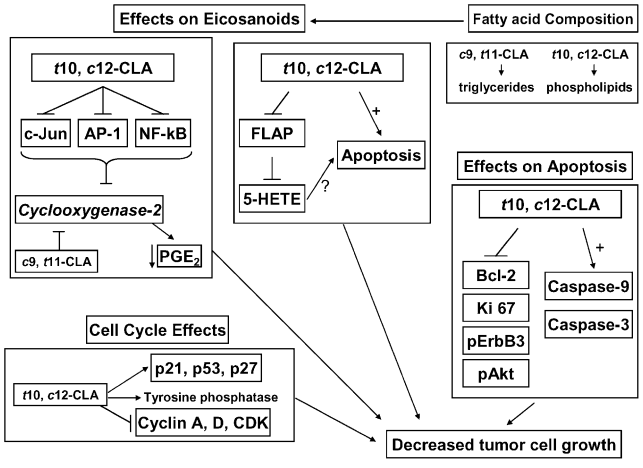

FIGURE 1 Possible mechanisms of CLA-altered tumorigenesis.

Effects of CLA isomers on the expression of genes regulating cell growth and apoptosis

Tumor burden may be reduced by inhibiting cell growth or by increasing apoptosis. CLA has been shown to stimulate the accumulation of tumor suppressive proteins such as p53, p27, and p21, which interrupt the G_1-S phase of the cell cycle. Six studies have examined the effects of purified CLA isomers on the expression of genes regulating the cell cycle (31,44,46,49–51). Results from 2 of these studies showed that *t*10, *c*12-CLA but

not *c*9, *t*11-CLA decreased cell growth and increased apoptosis in a prostate carcinoma cell line, DU-145, and a human colon adenocarcinoma cell line, HT-29 (31,49). This was associated with increased expression of p21 and decreased expression of cyclins A and D and cyclin-dependent kinases. In MCF-7 cells, both CLA isomers reduced cell growth and altered the expression of genes regulating cell growth, but *t*10, *c*12-CLA was more effective than *c*9, *t*11-CLA (44,52). Similarly, *t*10, *c*12-CLA was associated with a greater increase in the expression of protein tyrosine phosphatase-γ than *c*9, *t*11-CLA in human breast cancer cells (47). Also, *t*10, *c*12-CLA was more effective in increasing the accumulation of p53 and hypophosphorylated protein. p53 and hypophosphorylated protein are required for the progression of G_1 to the S phase and protein tyrosine phosphatase-γ counterbalances the growth-promoting effects of protein kinases. Two other studies examined the effects of purified *c*9, *t*11-CLA alone on the expression of genes regulating cell proliferation. *c*9, *t*11-CLA decreased the mRNA for *c*-myc, cyclin D1, *c*-jun, and β-catenin in HT-29 and Caco-2 cells (50); it decreased phosphorylation of ERK, P-38, MAPK, and Akt in a murine skin model (46). Based on the limited number of studies, *t*10, *c*12-CLA appears to be more inhibitory of the genes regulating cell cycle and growth than *c*9, *t*11-CLA.

Apoptosis is executed through a series of biochemical reactions involving numerous apoptotic and survival genes as well as associated cell proteins. Eight publications have reported the effects of individual CLA isomers on cell apoptosis (28–30,32,33,53–55). CLA used in these studies varied from 1 to 200 μmol/L. Results from studies with Caco-2 cells have been inconsistent; *t*10, *c*12-CLA increased apoptosis in 1 study (31), while both isomers increased apoptosis in another (28). Results from a study with HT-29 cells showed that 4 μmol/L *t*10, *c*12-CLA but not *c*9, *t*11-CLA induced apoptosis (53). These investigators attributed increased apoptosis to decreased phosphorylation of ErbB3 and Akt. In PC-3 cells, at least 25 μmol/L *t*10, *c*12-CLA increased apoptosis by increasing caspase-3 activity and p21 mRNA but decreased bcl-2 mRNA (29,32). The *c*9, *t*11-CLA isomer did not alter the expression of bcl-2 or p21 (32). In HCT-116 cells, 50 μmol/L *t*10, *c*12-CLA but not *c*9, *t*11-CLA induced apoptosis, which was associated with an increase in the expression of a pro-apoptotic gene, nonsteroidal antiinflammatory drug-activated gene-1, and activating transcription factor-3 (55). In the rat hepatoma cell line dRLH-84, 1 μmol/L *t*10, *c*12-CLA but not *c*9, *t*11-CLA induced apoptosis by activating caspases-3 and 9 (33). In a human gastric adenocarcinoma cell line, SGC-7901, *c*9, *t*11-CLA decreased the expression of bcl-2, *c*-myc, and Ki 67 and increased Fas (30). Thus, in most of the studies, *t*10, *c*12-CLA increased apoptosis by either increasing expression of apoptotic genes or by decreasing expression of antiapoptotic genes or both. *c*9, *t*11-CLA did not alter apoptosis in a majority of the studies and increased it in only a fraction. In studies that observed increased apoptosis with *c*9, *t*11-CLA, concentration was higher compared with studies that failed to detect an effect. Concentrations ranged from 25 to 200 μmol/L, whereas for studies with negative results the concentrations of this isomer were 1 to 50 μmol/L. These data suggest that pharmacological but not physiological concentrations of c9, t11-CLA induced apoptosis.

Results regarding the antitumorigenic effects of purified *c*9, *t*11-CLA and *t*10, *c*12-CLA isomers were dependent on the tumor type as well as the organ or cellular site. In the aggregate for all tumors tested, *t*10, *c*12-CLA tended to reduce tumorigenesis in a majority of studies but increased it in some. *c*9, *t*11-CLA also reduced tumorigenesis in most of the studies and had no effect in others. The amount of CLA used in these studies varied from 0.1 to 1.0 weight % of the diet, which would equate to 5–50 g/d for a 70-kg human. That will only be nutritionally attainable with supplements; the risk-benefit ratio of using CLA as an adjuvant or chemopreventive agent for humans remains to be determined.

*c*9, *t*11-CLA did not have any noticeable adverse health effects in human and animal studies and it inhibited tumorigenesis in most of the animal studies where it was assessed. Of note is the recent assessment of minor isomers like *t*9, *t*11-CLA that may be more potent than *t*10, *c*12-CLA. Studies with mixtures of CLA isomers seem now to lack a scientific basis, because the results reviewed here indicate that different CLA isomers act through different mechanisms and have potentially opposing effects on several metabolic pathways. There is an urgent need to have standardized preparations highly enriched in individual CLA isomers. Controlled studies with purified isomers of CLA need to be conducted to determine which isomer(s) may be responsible for benefits as well as risks to human health (56,57). Studies need to be conducted to determine the minimum concentration of CLA necessary to produce the desired effects. To avoid risks associated with high concentrations and long duration of CLA intake, it will be preferable to conduct initial studies first with nonhuman primates.

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