

Review Article

The Roles of Dietary PPAR γ Ligands for Metastasis in Colorectal Cancer

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Dietary peroxisome proliferator-activated receptor (PPAR) γ ligands, linoleic acid (LA) and conjugated linoleic acid (CLA), showed anticancer effects in colorectal carcinoma cells. LA is metabolized by two pathways. Cyclooxygenase (COX)-2 produces procarcinogenic prostaglandin E₂, whereas 15-lipoxygenase (LOX)-1 produces PPAR γ ligands. The 15LOX-1 pathway, which is dominant in colorectal adenomas, was downregulated and inversely COX-2 was upregulated in colorectal cancer. LA and CLA inhibited peritoneal metastasis of colorectal cancer cells in nude mice. The inhibitory effect was abrogated by PPAR γ antisense treatment. A continuous LA treatment provided cancer cells quiescence. These quiescent cells formed dormant nests in nude mice administrated LA. The quiescent and dormant cells showed downregulated PPAR γ and upregulated nucleostemin. Thus, short-term exposure to dietary PPAR γ ligands inhibits cancer metastasis, whereas consistent exposure to LA provides quiescent/dormant status with possible induction of cancer stem and/or progenitor phenotype. The complicated roles of dietary PPAR γ ligands are needed to examine further.

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1. INTRODUCTION

Colorectal cancer is the third most common malignant neoplasm worldwide and the third leading cause of cancer deaths in Japan [1]. The frequency of colorectal cancer in Japan doubled in the last three decades according to the alteration of life style from Japanese to Western [2]. Especially, the increase in fat intake and decrease in fiber intake have been regarded as the most important nutritional influence on colon cancer development [3, 4]. In this review, we focused on the fatty acids, which possess ligand activity for peroxisome proliferator-activated receptor (PPAR) γ in foods. PPAR γ is activated by endogenous secreted prostaglandins and fatty acids. 15-deoxy- δ (12,14)-prostaglandin J₂ is a strong endogenous ligand of PPAR γ [5]. Linoleic acid (LA) is one of the essential fatty acids, which we must intake from food. Metabolic products of LA, such as 9-hydroxyoctadecadienoic acid (9-HODE), 13-hydroxyoctadecadienoic acid (13-HODE), and 13-oxooctadecadienoic acid (13-OXO), are known as PPAR γ ligands [6]. Conjugated linoleic acid is a stereoisomer of LA [7]. CLA is contained in beef, lamb, and also in vegetable oils

[8]. Roles of these dietary PPAR γ ligands on PPAR γ activation are not still unclear. Colorectal cancer is a good model for influence of nutritional factors to cancer development and progression [9, 10]. In this review, roles of LA and CLA for colorectal cancer progression and therapeutic possibility are discussed as dietary PPAR γ ligands.

2. METABOLIC PATHWAYS OF LINOLEIC ACID

Prostaglandins (PGs) are bioactive lipids derived from the metabolites of membrane polyunsaturated fatty acids (PUFAs), and play important roles in a number of biological processes [11]. Cyclooxygenases-2 (COX-2)-dependent overproduction of PGE₂ is hypothesized to be an important part of sustained proliferative and chronic inflammatory conditions [12, 13]. Several *in vivo* studies hypothesize that a high amount of ω -6 PUFA such as LA might enhance colorectal carcinogenesis via stimulation of colonic epithelial cell proliferation [14–17]. In fact, rats treated with a genotoxic agent, azoxymethane (AOM), and fed a diet supplemented with LA develop more tumors than those treated with AOM alone [18].

The oxidative metabolites of LA, in particular, 9-HODE, 13-HODE, and 13-OXO, have biological effects as a PPAR γ ligand [6]. CLA, a strong ligand for PPAR γ , has a substantial anticarcinogenic effect [8, 19]. Synthesized PPAR γ ligands including troglitazon have been shown to be effective chemopreventive agents in a rat model of carcinogenesis and in AOM-induced colon cancer in mice [20]. If LA provides PPAR γ ligands, LA might act as an anticarcinogenic agent.

Arachidonic acid is a fatty acid and a component of lipid membranes, and also a major substrate for lipoxygenase enzymes. 15lipoxygenase-1 (15LOX-1) is known for its anti-inflammatory properties and has a profound influence on the development and progression of cancers [21, 22]. Recent studies show that ligand activation of PPAR γ in colorectal cancer cells attenuates colonic inflammation and causes a reduction growth via the induction of apoptosis [23, 24]. Furthermore, it has been reported that a number of metabolites generated by 15LOX-1 can function as endogenous activators and ligands for PPAR γ [25, 26]. We confirmed the anticarcinogenic effect of LA by in vitro transformation assay using a rat intestinal cell line, IEC6, which expressed 15LOX-1, but not COX2. LA treatment inhibited AOM-induced transformation in IEC6 cells [27]. Many literatures reported that PPAR γ possesses an anticarcinogenic effect in colorectal cancer [20]. Moreover, a decrease in PPAR γ expression is associated with cancer metastasis [28, 29]. Inhibitory effect of PPAR γ to cancer metastasis is reported in several cancers, such as nonsmall cell lung cancer, colon cancer, thyroid cancer, and breast cancer [30–33].

3. SWITCHING OF LA METABOLIC PATHWAYS FROM 15LOX-1 TO COX-2 IN COLORECTAL CANCER DEVELOPMENT

Inhibitory effect of LA on intestinal epithelial cell transformation elucidated above suggests that 15LOX-1 LA metabolism suppresses colon carcinogenesis [27]. We next focused on the dual roles of LA in human colon cancer development. Expression of 15LOX-1 and COX-2 was examined in human colon adenoma and carcinoma to elucidate the balance of the two LA metabolic pathways in malignant transformation of human colon epithelium.

We examined the expressions of COX-2 and 15LOX-1 in 54 adenomas, 21 carcinoma-in-adenoma lesions, and 36 serosa-invading advanced carcinomas in the colon [34]. We examined 15LOX-1 mRNA and COX-2 protein by in situ hybridization and immunohistochemistry, respectively. In the nonpathological colon mucosa, which expressed 15LOX-1 but not COX-2, proliferation of colon epithelial cells was controlled at constitutive levels. 15LOX-1 mRNA was found in 96% of adenomas, 43% of adenoma in carcinoma-in-adenoma lesions, and 10% of carcinoma in carcinoma-in-adenoma lesions, but not in advanced carcinoma ($P < .0001$). In contrast, COX-2 production was found in 11% of adenomas, 52% of adenoma in carcinoma-in-adenoma lesions, 71% of carcinoma in carcinoma-in-adenoma lesions, and 92% of advanced carcinoma ($P < .0001$). Concurrence of COX-2 induction with 15LOX-1 downregulation was

found in 6% adenomas, in 33% adenoma components, and 71% carcinoma components of carcinoma-in-adenoma lesions (all mucosal cancer), in 89% cases in nonmetastatic serosa-invading carcinomas, and in 100% cases of nodal metastasized carcinomas. Our data showed that induction of COX-2 expression and downregulation of 15LOX-1 were sequentially increased from adenomas, adenoma components, and carcinoma components in carcinoma-in-adenoma lesions, to invasive carcinomas. Interestingly, low grade-adenoma components with in carcinoma-in-adenoma lesions showed COX-2 expression and 15LOX-1 downregulation at more frequency than low grade-adenomas, which suggests that the biological property is different in the same histological atypia. In contrast, high grade-adenoma components showed no difference from high grade-adenomas in expressions of COX-2 and 15LOX-1, which suggests that high grade-adenomas might already possess malignant potential as high as adenoma components in carcinoma-in-adenoma lesions. This sequential alteration of concurrence of COX-2 induction with 15LOX-1 downregulation possibly shows close association of the switching of LA-metabolizing pathways with colon cancer development and progression.

15LOX-1 is revealed as an apoptosis inducer in human cancers and inhibits cancer progression. The reduction of 15LOX-1 and the isoform 15LOX-2 is correlated with the disease progression of breast cancer and the poor clinical outcome [35]. Induction of 15LOX-1 provides apoptosis in oral cancer [36]. 15LOX-1 expression is downregulated in colon adenomas, and ectopic expression of 15LOX-1 induces apoptosis in Caco-2 colon cancer cells [37].

In expression of 15LOX-1, inflammatory cytokines play an important role. Interleukin (IL)-4 and IL-13 induce 15LOX-1 expression via Jak2/Tyk2/Stats pathway [36, 38–40]. In prostate cancer, ratio of ω -3/ ω -6 fatty acids is associated with expressions of 15LOX-1 and COX-2 [41]. 15LOX-1 induction by nonsteroidal anti-inflammatory drugs (NSAIDs), such as sulindac sulfone, provides apoptosis in SW480 colon cancer cells. Sulindac sulfone inhibits GMP (cGMP)-phosphodiesterases and activates protein kinase G, which enhances 15LOX-1 expression transcriptionally [42]. GATA-6 transcriptional factor is involved in NSAID-induced 15LOX-1 induction in RKO and DLD-1 colon cancer cells [43]. GATA-6 activates 15LOX-1 promoter in Caco-2 colon cancer cells but not in the cells induced differentiation by sodium-butyrate [44]. These are supported by conventional NSAIDs activates PPAR γ [45]. COX-2 expression also involves Jak2/Stats pathway and nuclear factor (NF κ B) [46–48]. Activation of PPAR γ downregulates COX-2 expression by inhibition of NF κ B and activator protein (AP)-1 [49]. This negative regulation of COX-2 expression by PPAR γ activation might be one of the mechanisms of reversal expressions between 15LOX-1 and COX-2. Furthermore, promoter DNA methylation is responsible for silencing 15LOX-1 expression, which is reversed by 5-aza-2-deoxycytidine treatment [50, 51]. The epigenetic alteration might be a trigger to switch 15LOX-1 repression and COX-2 upregulation along with malignant transformation and disease progression in colorectal cancer. Thus, switching of LA metabolism from 15LOX-1 to COX-2 is thought to be

a common mechanism to escape from antitumoral effect of LA.

4. ANTITUMOR EFFECT OF CLA

We next argue the effect of CLA, which is an isomer of linoleic acid and is established as a PPAR γ ligand without metabolism by 15LOX-1. CLA is a natural content of some foods, such as beef, lamb, and also vegetable oils [8]. CLA is one of essential fatty acids, which possesses characteristic bioactive properties [8]. CLA is composed of positional- and stereoisomers of octadecadienoate (18:2). Chemoprotective properties of CLA are reported in experimental cancer models and in vitro examinations [8, 19]. Differently from LA, CLA is not a precursor of prostaglandines. CLA activates PPAR γ as a ligand [7, 52]. Through activation of PPAR γ , CLA might act as an antimetastatic agent as well as an anticarcinogenic agent. We examined the antimetastatic effect of CLA on peritoneal dissemination [53]. Cell growth of MKN28 human gastric cancer cells and Colo320 human colon cancer cells was suppressed by CLA in a dose-dependent manner with an increment in apoptosis. CLA significantly inhibited invasion into type IV collagen-coated membrane of MKN28 and Colo320 cells. CLA-induced growth inhibition was recovered by the exposure to antisense S-oligodeoxynucleotides (ODN) for PPAR γ in both cell lines. BALB/c nu-nu mice were inoculated with MKN28 and Colo320 cells into their peritoneal cavity, and administrated with CLA intraperitoneally (weekly, 4 times). CLA treatment did not affect food intake or weight gain of mice. CLA treatment significantly decreased metastatic foci of both cells in the peritoneal cavity. Survival rate in mice inoculated with MKN28 or Colo320 cells was significantly recovered by CLA treatment. PPAR γ initiates transcription of genes associated with energy homeostasis, cell growth, and anti-/proinflammatory effect [24, 54–57]. Protein production in MKN28 and Colo320 cells treated with CLA showed a decrease in epidermal growth factor receptor (EGFR) and transforming growth factor (TGF)- α and an increase in Bax. Our results showed that CLA inhibits cell growth and invasion, and induces apoptosis in cancer cells. Our data are supported with the reports, which indicate that PPAR γ downregulates EGFR, and upregulates Bax, p21Waf-1, and E-cadherin, which are associated with antiproliferative, proapoptotic, and prodifferentiation effects [33, 58–60].

We also reported the tumor suppressive effect of CLA on established peritoneal tumors using a syngeneic mouse peritoneal metastasis model [61]. C57BL6 mice were inoculated with LL2 cells into their peritoneal cavity. Two weeks after the inoculation, colonized peritoneal cancer foci (2.2 ± 0.4 mm in diameter) were treated with CLA administrated intraperitoneally (600 pmol/mouse, weekly, twice). CLA treatment decreased the number of peritoneal tumors to 26% of that in untreated mice ($P < .0001$). CLA treatment also decreased size of peritoneal tumors to 27% of that in untreated mice ($P < .0001$). In CLA-treated tumors, proliferating cells were decreased ($P < .0001$), whereas apoptotic cells were increased ($P < .0010$). CLA-treated

LL2 tumors showed decrease in PPAR γ and EGFR proteins, and increase in Bax protein in comparison with untreated tumors.

5. ANTITUMOR EFFECT OF LINOLEIC ACID

We confirmed antimetastatic effect of PPAR γ by CLA in gastric and colon cancer cells. We next examined antimetastatic effect of LA.

The effect of LA on peritoneal metastasis was examined using in vitro treatment of cancer cells and mouse peritoneal metastasis models as well as CLA examination. Firstly, cell growth of MKN28 human gastric cancer cells and Colo320 human colon cancer cells were suppressed by LA in a dose-dependent manner with increment of apoptosis. LA significantly inhibited invasion into type IV collagen-coated membrane of MKN28 and Colo320 cells ($P < .05$). The inhibition of growth and invasion and the induction of apoptosis by LA were abrogated by exposure to antisense S-ODN for PPAR γ . Moreover, the decrease in 15LOX-1 expression by exposure to antisense S-ODN for 15LOX-1 suppressed the inhibition of growth and invasion and the induction of apoptosis by LA. LA-induced growth inhibition was recovered by the exposure to antisense S-ODN for PPAR γ or 15LOX-1. BALB/c nu-nu mice inoculated with MKN28 and Colo320 cells into their peritoneal cavities were administrated IP with LA (weekly, 4 times). The LA treatment significantly diminished the number of metastatic foci of both cells in the peritoneal cavity ($P < .05$). Protein production in MKN28 and Colo320 cells treated with LA showed a decrease in EGFR and an increase in Bax. PPAR γ activation is reported to decrease EGFR expression and increase Bax expression [58, 60]. Our data suggest that LA possesses the same mechanism to CLA of PPAR γ ligand; however, its efficacy was 10^3 times weaker than CLA. LA-metabolites thought to be weaker agonists of PPAR γ . MKN28 and Colo320 cells expressed both COX-2 and 15LOX-1. At least, relative high concentration of LA might be metabolized dominantly by 15LOX-1, which consequently provides antimetastatic effect in these cells.

Thus, LA and CLA suppress occurrence of cancer metastasis and reduce existing metastatic tumors in animal models. These findings encourage clinical usage of LA and CLA for treatment of cancer metastasis.

6. EFFECT OF LA ON CANCER DORMANCY

A short-term treatment with LA or CLA induced apoptosis in a dose-dependent manner through PPAR γ activation as described above. On the contrary, in a long-term continuous treatment with adequate concentrations, LA induced reversible cell growth-arrest in cancer cells that escaped from apoptosis [62].

Cancer cell tumorigenicity in nude mice depends on several factors in cancer cells themselves and their host. To form macroscopical tumors, cancer cells must survive in their host tissue against host immunity, and proliferate with utilization of the host endothelial cells to make tumor

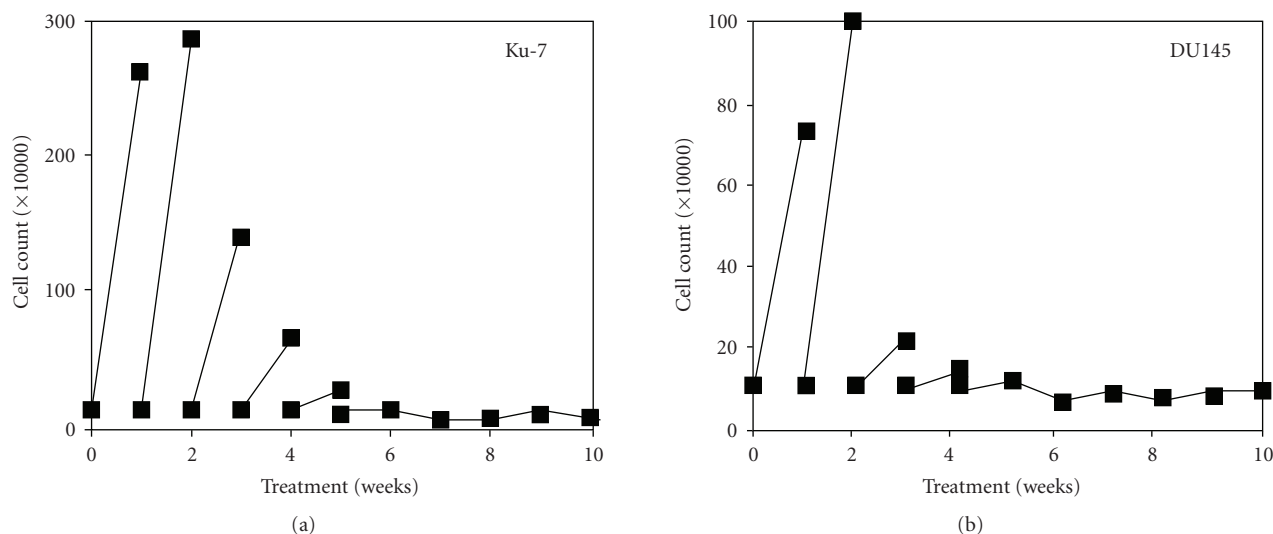


FIGURE 1: Effect of LA on growth inhibition in Ku-1 bladder cancer cells and DU145 prostate cancer cells by long-term treatment. Ku-7 cells and DU145 cells were continuously treated with LA (100 µg/mL) for the indicated period with weekly reseeding by 1×10^5 cells per well. If cells were less than 1×10^5 cells per well, all cells were reseeded. Standard deviation of each cell number was less than 10% of the value.

vasculature. These steps are similar to those in metastasized cancer cells on the metastasis targets [63]. If cancer cells do not endure the attacks by host immunity, they cannot form tumor. When they survive but not proliferate, the condition resembles quiescent or static dormancy. When they survive and proliferate but do not generate tumor vasculature, the cancer cells stay microscopical cell aggregation; the condition is to be an equivalent condition to tumor dormancy [64].

In vitro cell growth was suppressed by 48-hour treatment with LA in a dose-dependent manner in MKN28 and Colo320 cells. Continuous treatment with LA induced quiescence in both cells at 5 to 7 weeks after the LA treatment. The finding was also observed in Ku-7 bladder cancer cells and DU145 prostate cancer cells (Figure 1). In LA-induced quiescent cancer cells, protein production of Bcl-2 was increased, whereas Bak, EGFR, and vascular endothelial growth factor (VEGF) levels were decreased [62]. These alterations might be associated with inhibition of cell growth, angiogenesis, and apoptosis [65, 66], which explained well the characteristics of LA-treated cancer cell aggregation. These alterations of protein levels were the same as those in cells treated with PPAR γ ligands, troglitazon, and CLA [62]. The PPAR γ expression was also decreased in quiescent MKN28 and Colo320 cells by continuous treatment with LA. The LA metabolites by 15LOX-1 activate mitogen-activated protein kinase (MAPK) and increase PPAR γ phosphorylation, but downregulate PPAR γ activity [67]. Continuous PPAR γ phosphorylation might decrease PPAR γ expression by long-term LA treatment.

When MKN28 and Colo320 cells were inoculated to nude mice subcutaneous tissue, LA-induced quiescent MKN28 and Colo320 cells showed higher tumorigenicity than nontreated cells in nude mice. In the contrary to the tumorigenicity, LA-induced quiescent cancer cells showed 1/10

slower tumor growth than nontreated cells. LA withdrawal after the inoculation provided regrowth in the cancer cells, which subsequently grew into macroscopic tumors. LA-induced quiescent cells were different from growth-arrested cells by aging or senescence, which are irreversible, and refractory to growth factors [68].

In mice treated with LA weekly administration after the inoculation, inoculated quiescent cancer cells did not form macroscopical tumors. Histological examination revealed less than 500 µm-sized cancer-cell aggregations in the inoculation site. These cancer cell nests showed no proliferative activity, no vascularity, and no immune cell infiltration [62]. These features of the cancer cell nests were similar to those in tumor dormancy status [64]. The dormant cells expressed undetectable levels of PPAR γ protein, which suggests that inactivation of PPAR γ might be associated with tumor dormancy formation. In contrast, withdrawal of LA might break the dormancy status in cancer cells. Moreover, PPAR γ -negative dormant cells expressed increased levels of nucleostemin. Nucleostemin possesses a role for maintaining stemness [69, 70]. PPAR γ inhibits Wnt and LIF (leukemia inhibitory factor) signaling pathways, which maintain pluripotency and self-renewal of stem cells [5, 71]. Downregulation of PPAR γ might induce dedifferentiation and stem cell/progenitor phenotype in cancer cells, which might be associated with cancer dormancy and metastasis.

In this story, several possibilities are considered. Firstly, LA-induced PPAR γ downregulation provides stemness in cancer cells. Secondly, LA-induced apoptosis abolishes PPAR γ -positive cancer cells and PPAR γ -negative cancer stem cell/progenitor cells remain. Thirdly, LA possesses direct effect on cancer stem niche. To confirm the mechanism underlying LA-induced cancer dormancy, further examination is requested to focus on the relation of PPAR γ with cancer stem cells.

7. CONCLUSION

In this article, we described that the dietary PPAR γ ligands, LA and CLA, are deeply involved in colorectal cancer development and progression through PPAR γ activation. LA and CLA provide remarkable anticancer effects by short-term treatment. In contrast, long-term continuous treatment with LA induces quiescent and dormancy in cancer cells with PPAR γ downregulation. These conditions might be associated with phenotypes of cancer stem cells/progenitor cells. LA is a dietary factor to be taken from food everyday. Cancer cells might be continuously exposed to various concentrations of LA in human body. Taken together, short-term administration of LA and CLA is an effective therapeutic tool for cancer metastasis. We are requested to evaluate the effect of dietary LA on cancer metastasis for prevention of cancer metastasis and cancer dormancy.

NOMENCLATURE

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| PPAR: | Peroxisome proliferator-activated receptor |
| LA: | Linoleic acid |
| CLA: | Conjugated linoleic acid |
| COX: | Cyclooxygenases |
| LOX: | Lipoxygenase |
| HODE: | Hydroxyoctadecadienoic acid |
| OXO: | Oxoctadecadienoic acid |
| PG: | Prostaglandin |
| AOM: | Azoxymethane |
| IL: | Interleukin |
| NSAID: | Nonsteroidal anti-inflammatory drug |
| NF: | Nuclear factor |
| AP: | Activator protein |
| ODN: | Oligodeoxynucleotides |
| EGFR: | Epidermal growth factor receptor |
| TGF: | Transforming growth factor |
| VEGF: | Vascular endothelial growth factor |
| MAPK: | Mitogen-activated protein kinase |
| LIF: | Leukemia inhibitory factor. |

REFERENCES

- [1] M. Shike, S. J. Winawer, P. H. Greenwald, A. Bloch, M. J. Hill, and S. V. Swaroop, "Primary prevention of colorectal cancer. The WHO Collaborating Centre for the Prevention of Colorectal Cancer," *Bulletin of the World Health Organization*, vol. 68, no. 3, pp. 377–385, 1990.
- [2] "Cancer Statistics in Japan," Editorial Board (ed.) Cancer Statistics in Japan, 2005 edition, http://ganjoho.ncc.go.jp/pro/statistics/en/graph_db_index.html.
- [3] A. B. Miller, G. R. Howe, M. Jain, K. J. P. Craib, and L. Harrison, "Food items and food groups as risk factors in a case-control study of diet and colo-rectal cancer," *International Journal of Cancer*, vol. 32, no. 2, pp. 155–161, 1983.
- [4] E. Giovannucci and W. C. Willett, "Dietary factors and risk of colon cancer," *Annals of Medicine*, vol. 26, no. 6, pp. 443–452, 1994.
- [5] J. Rajasingh and J. J. Bright, "15-deoxy- $\Delta^{12,14}$ -prostaglandin J₂ regulates leukemia inhibitory factor signaling through JAK-STAT pathway in mouse embryonic stem cells," *Experimental Cell Research*, vol. 312, no. 13, pp. 2538–2546, 2006.
- [6] L. Nagy, P. Tontonoz, J. G. A. Alvarez, H. Chen, and R. M. Evans, "Oxidized LDL regulates macrophage gene expression through ligand activation of PPAR γ ," *Cell*, vol. 93, no. 2, pp. 229–240, 1998.
- [7] S. Y. Moya-Camarena and M. A. Belury, "CLA and PPAR γ activation," *Journal of Nutrition*, vol. 129, no. 11, p. 2106, 1999.
- [8] M. A. Belury, "Inhibition of carcinogenesis by conjugated linoleic acid: potential mechanisms of action," *Journal of Nutrition*, vol. 132, no. 10, pp. 2995–2998, 2002.
- [9] M. Lipkin, B. Reddy, H. Newmark, and S. A. Lamprecht, "Dietary factors in human colorectal cancer," *Annual Review of Nutrition*, vol. 19, pp. 545–586, 1999.
- [10] J. A. Meyerhardt, D. Niedzwiecki, D. Hollis, et al., "Association of dietary patterns with cancer recurrence and survival in patients with stage III colon cancer," *Journal of the American Medical Association*, vol. 298, no. 7, pp. 754–764, 2007.
- [11] H. R. Herschman, W. Xie, and S. Reddy, "Inflammation, reproduction, cancer and all that... The regulation and role of the inducible prostaglandin synthase," *BioEssays*, vol. 17, no. 12, pp. 1031–1037, 1995.
- [12] R. A. Gupta and R. N. DuBois, "Colorectal cancer prevention and treatment by inhibition of cyclooxygenase-2," *Nature Reviews Cancer*, vol. 1, no. 1, pp. 11–21, 2001.
- [13] J. Singh, R. Hamid, and B. S. Reddy, "Dietary fat and colon cancer: modulation of cyclooxygenase-2 by types and amount of dietary fat during the postinitiation stage of colon carcinogenesis," *Cancer Research*, vol. 57, no. 16, pp. 3465–3470, 1997.
- [14] W. C. Chang, R. S. Chapkin, and J. R. Lupton, "Predictive value of proliferation, differentiation and apoptosis as intermediate markers for colon tumorigenesis," *Carcinogenesis*, vol. 18, no. 4, pp. 721–730, 1997.
- [15] B. S. Reddy, C. Burill, and J. Rigotty, "Effect of diets high in ω -3 and ω -6 fatty acids on initiation and postinitiation stages of colon carcinogenesis," *Cancer Research*, vol. 51, no. 2, pp. 487–491, 1991.
- [16] S. Zhou, G. Wang, B. Chen, and P. Wang, "Effect of dietary fatty acids on tumorigenesis of colon cancer induced by methyl nitrosourea in rats," *Journal of Environmental Pathology, Toxicology and Oncology*, vol. 19, no. 1-2, pp. 81–86, 2000.
- [17] C. V. Rao, Y. Hirose, C. Indranie, and B. S. Reddy, "Modulation of experimental colon tumorigenesis by types and amounts of dietary fatty acids," *Cancer Research*, vol. 61, no. 5, pp. 1927–1933, 2001.
- [18] M. Morotomi, Y. Sakaitani, M. Satou, T. Takahashi, A. Takagi, and M. Onoue, "Effects of a high-fat diet on azoxymethane-induced aberrant crypt foci and fecal biochemistry and microbial activity in rats," *Nutrition and Cancer*, vol. 27, no. 1, pp. 84–91, 1997.
- [19] Á. Miller, C. Stanton, and R. Devery, "Cis 9, trans 11- and trans 10, cis 12-conjugated linoleic acid isomers induce apoptosis in cultured SW480 cells," *Anticancer Research*, vol. 22, no. 6C, pp. 3879–3887, 2002.
- [20] E. Osawa, A. Nakajima, K. Wada, et al., "Peroxisome proliferator-activated receptor γ ligands suppress colon carcinogenesis induced by azoxymethane in mice," *Gastroenterology*, vol. 124, no. 2, pp. 361–367, 2003.
- [21] C. D. Funk, "The molecular biology of mammalian lipoxygenases and the quest for eicosanoid functions using lipoxygenase-deficient mice," *Biochimica et Biophysica Acta*, vol. 1304, no. 1, pp. 65–84, 1996.

- [22] V. E. Steele, C. A. Holmes, E. T. Hawk, et al., "Potential use of lipoxygenase inhibitors for cancer chemoprevention," *Expert Opinion on Investigational Drugs*, vol. 9, no. 9, pp. 2121–2138, 2000.
- [23] W.-L. Yang and H. Frucht, "Activation of the PPAR pathway induces apoptosis and COX-2 inhibition in HT-29 human colon cancer cells," *Carcinogenesis*, vol. 22, no. 9, pp. 1379–1383, 2001.
- [24] P. Desreumaux, L. Dubuquoy, S. Nutten, et al., "Attenuation of colon inflammation through activators of the retinoid X receptor (RXR)/peroxisome proliferator-activated receptor γ (PPAR γ) heterodimer: a basis for new therapeutic strategies," *Journal of Experimental Medicine*, vol. 193, no. 7, pp. 827–838, 2001.
- [25] R. L. Schild, W. T. Schaiff, M. G. Carlson, E. J. Cronbach, D. M. Nelson, and Y. Sadosky, "The activity of PPAR γ in primary human trophoblasts is enhanced by oxidized lipids," *Journal of Clinical Endocrinology and Metabolism*, vol. 87, no. 3, pp. 1105–1110, 2002.
- [26] G. G. Chen, H. Xu, J. F. Y. Lee, et al., "15-hydroxy-eicosatetraenoic acid arrests growth of colorectal cancer cells via a peroxisome proliferator-activated receptor gamma-dependent pathway," *International Journal of Cancer*, vol. 107, no. 5, pp. 837–843, 2003.
- [27] T. Sasaki, K. Yoshida, H. Shimura, et al., "Inhibitory effect of linoleic acid on transformation of IEC6 intestinal cells by in vitro azoxymethane treatment," *International Journal of Cancer*, vol. 118, no. 3, pp. 593–599, 2006.
- [28] A. F. Badawi, M. B. Eldeen, Y. Liu, E. A. Ross, and M. Z. Badr, "Inhibition of rat mammary gland carcinogenesis by simultaneous targeting of cyclooxygenase-2 and peroxisome proliferator-activated receptor γ ," *Cancer Research*, vol. 64, no. 3, pp. 1181–1189, 2004.
- [29] Y. Terashita, H. Sasaki, N. Haruki, et al., "Decreased peroxisome proliferator-activated receptor gamma gene expression is correlated with poor prognosis in patients with esophageal cancer," *Japanese Journal of Clinical Oncology*, vol. 32, no. 7, pp. 238–243, 2002.
- [30] Y. Bren-Mattison, V. Van Putten, D. Chan, R. Winn, M. W. Geraci, and R. A. Nemenoff, "Peroxisome proliferator-activated receptor- γ (PPAR γ) inhibits tumorigenesis by reversing the undifferentiated phenotype of metastatic non-small-cell lung cancer cells (NSCLC)," *Oncogene*, vol. 24, no. 8, pp. 1412–1422, 2005.
- [31] B. Galusca, J. M. Dumollard, M. L. Chambonniere, et al., "Peroxisome proliferator activated receptor gamma immunohistochemical expression in human papillary thyroid carcinoma tissues. Possible relationship to lymph node metastasis," *Anticancer Research*, vol. 24, no. 3b, pp. 1993–1997, 2004.
- [32] H. Liu, C. Zang, M. H. Fenner, K. Possinger, and E. Elstner, "PPAR γ ligands and ATRA inhibit the invasion of human breast cancer cells in vitro," *Breast Cancer Research and Treatment*, vol. 79, no. 1, pp. 63–74, 2003.
- [33] T. Yoshizumi, T. Ohta, I. Ninomiya, et al., "Thiazolidinedione, a peroxisome proliferator-activated receptor-gamma ligand, inhibits growth and metastasis of HT-29 human colon cancer cells through differentiation-promoting effects," *International Journal of Oncology*, vol. 25, no. 3, pp. 631–639, 2004.
- [34] M. Yuri, T. Sasahira, K. Nakai, S. Ishimaru, H. Ohmori, and H. Kuniyasu, "Inverse expression of cyclooxygenase-2 to 15-lipoxygenase-1 is associated with colon cancer development," *Histopathol*, vol. 51, no. 4, pp. 520–527, 2007.
- [35] W. G. Jiang, G. Watkins, A. Douglas-Jones, and R. E. Mansel, "Reduction of isoforms of 15-lipoxygenase (15-LOX)-1 and 15-LOX-2 in human breast cancer," *Prostaglandins, Leukotrienes and Essential Fatty Acids*, vol. 74, no. 4, pp. 235–245, 2006.
- [36] J. H. Kim, J. H. Chang, J.-H. Yoon, J. G. Lee, J. H. Bae, and K.-S. Kim, "15-lipoxygenase-1 induced by interleukin-4 mediates apoptosis in oral cavity cancer cells," *Oral Oncology*, vol. 42, no. 8, pp. 825–830, 2006.
- [37] I. Shureiqi, Y. Wu, D. Chen, et al., "The critical role of 15-lipoxygenase-1 in colorectal epithelial cell terminal differentiation and tumorigenesis," *Cancer Research*, vol. 65, no. 24, pp. 11486–11492, 2005.
- [38] B. Chen, S. Tsui, W. E. Boeglin, R. S. Douglas, A. R. Brash, and T. J. Smith, "Interleukin-4 induces 15-lipoxygenase-1 expression in human orbital fibroblasts from patients with graves disease: evidence for anatomic site-selective actions of Th2 cytokines," *Journal of Biological Chemistry*, vol. 281, no. 27, pp. 18296–18306, 2006.
- [39] B. Xu, A. Bhattacharjee, B. Roy, et al., "Interleukin-13 induction of 15-lipoxygenase gene expression requires p38 mitogen-activated protein kinase-mediated serine 727 phosphorylation of Stat1 and Stat3," *Molecular and Cellular Biology*, vol. 23, no. 11, pp. 3918–3928, 2003.
- [40] Y. W. Lee, H. Kühn, S. Kaiser, B. Hennig, A. Daugherty, and M. Toborek, "Interleukin 4 induces transcription of the 15-lipoxygenase I gene in human endothelial cells," *Journal of Lipid Research*, vol. 42, no. 5, pp. 783–791, 2001.
- [41] U. P. Kelavkar, J. Hutzley, R. Dhir, P. Kim, K. G. D. Allen, and K. McHugh, "Prostate tumor growth and recurrence can be modulated by the ω -6: ω -3 ratio in diet: athymic mouse xenograft model simulating radical prostatectomy," *Neoplasia*, vol. 8, no. 2, pp. 112–124, 2006.
- [42] A. Deguchi, S. W. Xing, I. Shureiqi, et al., "Activation of protein kinase G up-regulates expression of 15-lipoxygenase-1 in human colon cancer cells," *Cancer Research*, vol. 65, no. 18, pp. 8442–8447, 2005.
- [43] I. Shureiqi, W. Jiang, S. M. Fischer, et al., "GATA-6 transcriptional regulation of 15-lipoxygenase-1 during NSAID-induced apoptosis in colorectal cancer cells," *Cancer Research*, vol. 62, no. 4, pp. 1178–1183, 2002.
- [44] H. Kamitani, H. Kameda, U. P. Kelavkar, and T. E. Eling, "A GATA binding site is involved in the regulation of 15-lipoxygenase-1 expression in human colorectal carcinoma cell line, Caco-2," *FEBS Letters*, vol. 467, no. 2-3, pp. 341–347, 2000.
- [45] J. B. Nixon, H. Kamitani, S. J. Baek, and T. E. Eling, "Evaluation of eicosanoids and NSAIDs as PPAR γ ligands in colorectal carcinoma cells," *Prostaglandins, Leukotrienes and Essential Fatty Acids*, vol. 68, no. 5, pp. 323–330, 2003.
- [46] H.-W. Koon, D. Zhao, Y. Zhan, S. H. Rhee, M. P. Moyer, and C. Pothoulakis, "Substance P stimulates cyclooxygenase-2 and prostaglandin E₂ expression through JAK-STAT activation in human colonic epithelial cells," *Journal of Immunology*, vol. 176, no. 8, pp. 5050–5059, 2006.
- [47] V. Benoit, E. de Moraes, N. A. Dar, et al., "Transcriptional activation of cyclooxygenase-2 by tumor suppressor p53 requires nuclear factor- κ B," *Oncogene*, vol. 25, no. 42, pp. 5708–5718, 2006.
- [48] J. Duque, M. D. Díaz-Muñoz, M. Fresno, and M. A. Iñiguez, "Up-regulation of cyclooxygenase-2 by interleukin-1 β in colon carcinoma cells," *Cellular Signalling*, vol. 18, no. 8, pp. 1262–1269, 2006.

- [49] P. A. Konstantinopoulos, G. P. Vantoros, G. Sotiropoulou-Bonikou, A. Kominea, and A. G. Papavassiliou, "NF- κ B/PPAR γ and/or AP-1/PPAR γ 'on/off' switches and induction of CBP in colon adenocarcinomas: correlation with COX-2 expression," *International Journal of Colorectal Disease*, vol. 22, no. 1, pp. 57–68, 2007.
- [50] C. Liu, D. Xu, J. Sjöberg, P. Forsell, M. Björkholm, and H.-E. Claesson, "Transcriptional regulation of 15-lipoxygenase expression by promoter methylation," *Experimental Cell Research*, vol. 297, no. 1, pp. 61–67, 2004.
- [51] L. C. Hsi, X. Xi, Y. Wu, and S. M. Lippman, "The methyltransferase inhibitor 5-aza-2-deoxycytidine induces apoptosis via induction of 15-lipoxygenase-1 in colorectal cancer cells," *Molecular Cancer Therapeutics*, vol. 4, no. 11, pp. 1740–1746, 2005.
- [52] M. F. McCarty, "Activation of PPAR γ may mediate a portion of the anticancer activity of conjugated linoleic acid," *Medical Hypotheses*, vol. 55, no. 3, pp. 187–188, 2000.
- [53] H. Kuniyasu, K. Yoshida, T. Sasaki, T. Sasahira, K. Fujii, and H. Ohmori, "Conjugated linoleic acid inhibits peritoneal metastasis in human gastrointestinal cancer cells," *International Journal of Cancer*, vol. 118, no. 3, pp. 571–576, 2006.
- [54] S. L. Gray, E. Dalla Nora, and A. J. Vidal-Puig, "Mouse models of PPAR- γ deficiency: dissecting PPAR- γ 's role in metabolic homeostasis," *Biochemical Society Transactions*, vol. 33, no. 5, pp. 1053–1058, 2005.
- [55] J. Bassaganya-Riera, R. Hontecillas, and D. C. Beitz, "Colonic anti-inflammatory mechanisms of conjugated linoleic acid," *Clinical Nutrition*, vol. 21, no. 6, pp. 451–459, 2002.
- [56] T. Tanaka, H. Kohno, S.-I. Yoshitani, et al., "Ligands for peroxisome proliferator-activated receptors α and γ inhibit chemically induced colitis and formation of aberrant crypt foci in rats," *Cancer Research*, vol. 61, no. 6, pp. 2424–2428, 2001.
- [57] J. Berger and J. A. Wagner, "Physiological and therapeutic roles of peroxisome proliferator-activated receptors," *Diabetes Technology and Therapeutics*, vol. 4, no. 2, pp. 163–174, 2002.
- [58] A. Chen and J. Xu, "Activation of PPAR γ by curcumin inhibits Moser cell growth and mediates suppression of gene expression of cyclin D1 and EGFR," *American Journal of Physiology*, vol. 288, no. 3, pp. G447–G456, 2005.
- [59] J. J. Liu, R. W. Huang, D. J. Lin, et al., "Expression of survivin and bax/bcl-2 in peroxisome proliferator activated receptor- γ ligands induces apoptosis on human myeloid leukemia cells in vitro," *Annals of Oncology*, vol. 16, no. 3, pp. 455–459, 2005.
- [60] B. Majumder, K. W. Wahle, S. Moir, et al., "Conjugated linoleic acids (CLAs) regulate the expression of key apoptotic genes in human breast cancer cells," *The FASEB Journal*, vol. 16, no. 11, pp. 1447–1449, 2002.
- [61] Y. Sakai, T. Sasahira, H. Ohmori, K. Yoshida, and H. Kuniyasu, "Conjugated linoleic acid reduced metastasized LL2 tumors in mouse peritoneum," *Virchows Archiv*, vol. 449, no. 3, pp. 341–347, 2006.
- [62] H. Ohmori, T. Sasahira, K. Fujii, T. Shimomoto, and H. Kuniyasu, "Linoleic acid-induced growth suppression induces quiescent cancer cell nests in nude mice," *Pathobiology*. In press.
- [63] I. J. Fidler, "Critical factors in the biology of human cancer metastasis: twenty-eighth G. H. A. Clowes Memorial Award Lecture," *Cancer Research*, vol. 50, no. 19, pp. 6130–6138, 1990.
- [64] G. N. Naumov, I. C. MacDonald, P. M. Weinmeister, et al., "Persistence of solitary mammary carcinoma cells in a secondary site: a possible contributor to dormancy," *Cancer Research*, vol. 62, no. 7, pp. 2162–2168, 2002.
- [65] T. Sasaki, K. Fujii, K. Yoshida, et al., "Peritoneal metastasis inhibition by linoleic acid with activation of PPAR γ in human gastrointestinal cancer cells," *Virchows Archiv*, vol. 448, no. 4, pp. 422–427, 2006.
- [66] D. Panigrahy, S. Singer, L. Q. Shen, et al., "PPAR γ ligands inhibit primary tumor growth and metastasis by inhibiting angiogenesis," *Journal of Clinical Investigation*, vol. 110, no. 7, pp. 923–932, 2002.
- [67] L. C. Hsi, L. Wilson, J. Nixon, and T. E. Eling, "15-lipoxygenase-1 metabolites down-regulate peroxisome proliferator-activated receptor γ via the MAPK signaling pathway," *Journal of Biological Chemistry*, vol. 276, no. 37, pp. 34545–34552, 2001.
- [68] J. S. Vande Berg and M. C. Robson, "Arresting cell cycles and the effect on wound healing," *Surgical Clinics of North America*, vol. 83, no. 3, pp. 509–520, 2003.
- [69] R. Y. L. Tsai and R. D. G. McKay, "A nucleolar mechanism controlling cell proliferation in stem cells and cancer cells," *Genes and Development*, vol. 16, no. 23, pp. 2991–3003, 2002.
- [70] T. G. Natarajan and K. T. FitzGerald, "Markers in normal and cancer stem cells," *Cancer Biomarkers*, vol. 3, no. 4-5, pp. 211–231, 2007.
- [71] M. Katoh and M. Katoh, "WNT signaling pathway and stem cell signaling network," *Clinical Cancer Research*, vol. 13, no. 14, pp. 4042–4045, 2007.