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Knockdown of delta-5-desaturase promotes the anti-cancer activity of dihomo-γ-linolenic acid and enhances the efficacy of chemotherapy in colon cancer cells expressing COX-2

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ABSTRACT

Cyclooxygenase (COX), commonly overexpressed in cancer cells, is a major lipid peroxidizing enzyme that metabolizes polyunsaturated fatty acids (ω-3s and ω-6s). The COX-catalyzed free radical peroxidation of arachidonic acid (ω-6) can produce deleterious metabolites (e.g. 2-series prostaglandins) that are implicated in cancer development. Thus, COX inhibition has been intensively investigated as a complementary therapeutic strategy for cancer. However, our previous study has demonstrated that a free radical-derived byproduct (8-hydroxyoctanoic acid) formed from COX-catalyzed peroxidation of dihomo-γ-linolenic acid (DGLA, the precursor of arachidonic acid) can inhibit colon cancer cell growth. We thus hypothesize that the commonly overexpressed COX in cancer (~90% of colon cancer patients) can be taken advantage to suppress cell growth by knocking down delta-5-desaturase (D5D, a key enzyme that converts DGLA to arachidonic acid). In addition, D5D knockdown along with DGLA supplement may enhance the efficacy of chemotherapeutic drugs. After knocking down D5D in HCA-7 colony 29 cells and HT-29 cells (human colon cancer cell lines with high and low COX levels, respectively), the antitumor activity of DGLA was significantly enhanced along with the formation of a threshold range (~0.5–1.0 μM) of 8-hydroxyoctanoic acid. In contrast, DGLA treatment did not inhibit cell growth when D5D was not knocked down and only limited amount of 8-hydroxyoctanoic acid was formed. D5D knockdown along with DGLA treatment also enhanced the cytotoxicities of various chemotherapeutic drugs, including 5-fluorouracil, regorafenib, and irinotecan, potentially through the activation of pro-apoptotic proteins, e.g. p53 and caspase 9. For the first time, we have demonstrated that the overexpressed COX in cancer cells can be utilized in suppressing cancer cell growth. This finding may provide a new option besides COX inhibition to optimize cancer therapy. The outcome of this translational research will guide us to develop a novel ω-6-based diet-care strategy in combination with current chemotherapy for colon cancer prevention and treatment.

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1. Introduction

Colon cancer is the third most prevalent cancer and the second leading cause of cancer deaths in the United States. A variety of chemotherapy drugs as well as nutritional supplements, including polyunsaturated fatty acids (PUFAs), have been studied and applied to colon cancer treatment [1–9]. There are two different types of PUFAs (ω-3s and ω-6s) that are both essential for human health and must be provided from dietary sources. ω-3s, mainly found in marine products, have been reported to have many beneficial effects for human health and are commonly used in complementary therapeutic strategies for cancer treatment [7–13]. However, as the more abundant dietary source (available from meat, cereals and plant oils), the consumption of ω-6s has been shown to correlate with cancer development [14–19]. Increasing evidence suggests that the pro-cancer effect of ω-6s may mainly

Abbreviations: AA, arachidonic acid; COX, Cyclooxygenase; DGLA, dihomo-γ-linolenic acid; D5D, Delta-5-desaturase; ESR, electron spin resonance; GLA, γ-linolenic acid; GC, gas chromatography; HPLC, high performance liquid chromatography; LA, linoleic acid; MS, mass spectrometry; NC-si, negative control siRNA transfection; PBS, phosphate buffered saline; PFB, pentafluorobenzyl; PI, propidium iodide; PGE1, prostaglandin E1; PGE2, prostaglandin E2; PUFAs, polyunsaturated fatty acids; *wt*-, wild type; 5-FU, 5-Fluorouracil; 8-HOA, 8-hydroxyoctanoic acid

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be attributed to the formation deleterious metabolites (e.g., prostaglandin E2, PGE2) from COX-catalyzed peroxidation of arachidonic acid (AA) [20–26]. On the other hand, the precursors of AA, e.g., dihomo-γ-linolenic acid (DGLA) as well as γ-linolenic acid (GLA), were reported to possess certain anti-proliferative activities towards cancer cells [27–33]. DGLA might therefore represent a promising dietary source for cancer prevention and therapy, but the molecular mechanisms of its anti-cancer activities remain unclear.

COX is a membrane-bound bi-functional enzyme that peroxidizes ω-6s (e.g. DGLA and AA) as well as ω-3s (e.g. docosahexaenoic acid and eicosapentaenoic acid) into various bioactive products. Two isoforms of COX have been identified, e.g. COX-1, the constitute form, and COX-2, the inducible form. COX-2 can be readily induced by various pro-inflammatory stimuli, such as lipopolysaccharide and cytokines [34–37], and is commonly overexpressed in human adenocarcinomas (80–90% [38–40]). High expression levels of COX-2 are implicated in inflammatory disorders and cancer, mainly due to formation of 2-series prostaglandins from COX-catalyzed AA peroxidation [38–45]. Therefore, COX inhibition strategies aiming at limiting COX-catalyzed AA peroxidation has been extensively studied and applied as a complementary therapeutic approach for cancer as well as inflammatory conditions [1–3,41–45].

Using a novel HPLC/ESR/MS combined technique coupled with spin trapping method, our lab has demonstrated that there are different free radical mechanisms to generate distinct free radical metabolites from COX-catalyzed peroxidation of DGLA vs. AA, and that 8-hydroxyoctanoic acid (8-HOA) is an exclusive free radical-derived byproduct from DGLA peroxidation [46–52]. We also observed that 8-HOA inhibits cell growth, causes cell cycle arrest, and promotes apoptosis in colon cancer cells, while similar concentration of exogenous or endogenous prostaglandin E1 and E2 (commonly viewed as bioactive products of DGLA vs. AA) has no effect on cancer cell growth [48–49]. Thus, 8-HOA might be the beneficial bio-product that is responsible for DGLA's anti-cancer activity. Here we tested the hypothesis that the overexpression of COX-2 in cancer cells can be targeted to suppress cancer cell growth through the formation of 8-HOA, and that knocking-down of delta-5-desaturase (D5D, a key enzyme converting DGLA to AA) to limit the conversion of DGLA to AA and to maintain 8-HOA at a threshold range can be used to elicit DGLA's anti-cancer effect.

A large body of work including ours has showed that supplementation of ω-3s/ω-6s and 8-HOA could enhance the cytotoxicity of chemo-drugs in cancer cells [12,33,49,53]. In this study, we have further demonstrated that DGLA supplement along with D5D knockdown could be used to sensitize colon cancer cells to various chemo-drugs, including 5-fluorouracil (5-FU), regorafenib, and irinotecan, potentially through promoting chemotherapy-induced cell cycle arrest and apoptosis (e.g., activation of p53, caspase 9, and PARP). The results from this work could provide a biochemical rationale for the development of novel ω-6-based diet care strategies to combine with chemo-drugs for colon cancer treatment, by taking advantage of the wide availability of dietary ω-6s as well as the overexpression of COX-2 in colon cancer cells.

2. Materials and methods

2.1. Cell lines and materials

The human colon cancer cell lines HCA-7 colony 29 (high COX-2 expression, European Collection of Cell Cultures, Salisbury, UK) and HT-29 (low COX-2 expression, ATCC) were grown in Dulbecco's Modified Eagle's Medium and McCoy's 5A Medium (with 10% fetal bovine serum, Thermo Fisher Scientific, UT, USA), respectively. Cells were cultured in an incubator containing a 95% humidified atmosphere with 5% CO_2 at 37 °C.

DGLA was purchased from Nu-Chek-Prep (MN, USA); 8-HOA and 5-FU were purchased from Sigma-Aldrich (MO, USA); regorafenib was obtained from Adooq Biosciences (CA, USA); irinotecan, PGE1, DGLA-d_6 and PGE1-d_4 were purchased from Cayman Chemicals (MI, USA).

2.2. SiRNA transfection

Negative control siRNA (NC-si), D5D-targeting siRNA (catalog # 4390825) and Lipofectamine™ RNAiMAX transfection reagent were purchased from Life Technologies (Grand Island, NY, USA). GlutaMAX™ Opti-MEM reduced serum medium was purchased from Thermo Fisher Scientific (MA, USA). Briefly, colon cancer cell lines (HCA-7 and HT-29) were seeded at 3.0×10^5 cells per well in a 6-well plate or 8000 cells per well in a 96-well plate for different experiments. After overnight incubation and removing culture medium, cells were washed by phosphate buffered saline (PBS) and treated with siRNA transfection mixture containing D5D siRNA (final concentration at 150 nM) and Lipofectamine™ RNAiMAX transfection reagent (both diluted in GlutaMAX™ Opti-MEM reduced serum medium). After 6 h transfection, the reduced serum medium was replaced by Dulbecco's Modified Eagle's Medium (for HCA-7) or McCoy's 5A Medium (for HT-29) with 10% fetal bovine serum. After 48 h, the transfected cells were ready for further treatments (e.g. 8-HOA, ω-6s, chemo-drugs) and other experiments, e.g. western blot, MTS assay, colony formation assay, LC/MS analysis, GC/MS analysis, cell cycle distribution and apoptosis analysis. Cells transfected with a non-target control siRNA were used as controls.

2.3. MTS assay

Cell proliferation of D5D-*KD* colon cancer cell lines and negative control cells upon treatments (e.g. 8-HOA, ω-6s and/or chemo-drugs) was assessed using CellTiter® 96 Aqueous One Solution Reagent (Promega, Madison, WI, USA). Briefly, cells were seeded at 8000 cells (in 100 μL medium) per well into 96-well plates, incubated overnight and transfected with D5D siRNA or negative control siRNA for 48 h. Upon 48 h treatments of ω-6s and/or chemo-drugs, 20 μL per well of CellTiter® 96 Aqueous One Solution Reagent was added. After up to 4 h incubation, the quantity of formazan product was assessed by recording the absorbance at 490 nm with a 96-well plate reader (SpectraMax M5; Molecular Devices). Cell viability was calculated as a percentage of the control group (treated with vehicle).

2.4. Clonogenic cell survival assay (colony formation assay)

Colony formation of D5D-*KD* HCA-7 colony 29 cells and negative control cells upon treatments (e.g. ω-6s and/or chemo-drugs) was assessed for cell survival study. Briefly, cells were seeded at 3.0×10^5 cells per well into a 6-well plate, incubated overnight, and transfected with D5D siRNA or negative control siRNA. After 24 h transfection, the cells were trypsinized, collected, seeded at 2000 cells per well into a 6-well plate, and then exposed to 48 h treatments of ω-6s, chemo-drugs, or their combination. The cells were then washed with PBS and incubated with fresh medium for 10 days. After incubation, the cells were washed with PBS, fixed with 10% neutral buffered formalin, and stained with 0.05% crystal violet solution. Cell colonies (more than 30 cells) formed in each well were counted and plate efficiency was calculated as number of colonies divided by number of cells seeded; surviving fraction was calculated as the plate efficiency of treatment group vs. the plate efficiency of control groups (e.g., vehicle treatment).