The effects of omega-3 PUFA (ALA) on WT1 gene expression in pancreatic cancer cell line (MIA PaCa-2)

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Abstract

Background: Cancer starts by cells acquiring a number of characteristic alterations especially abnormal cell growth. These properties reflect faults in signalling pathways in cancer cells. Wilms' tumor 1(WT1) acts as a tumor suppressor by negative regulation of WNT/beta-catenin signaling pathway. The role of WT1 in many cancers has been studied.

Aims: To evaluate the WT1 gene expression alterations in response to different concentrations of omega-3 PUFA alpha linolenic acid (ALA) in pancreatic cancer cell line (MIA PaCa-2) in a time dependent manner.

Methods: MiaPaca-2cell line was cultured in monolayers. After that began the cells treatment with omega-3 fatty acid (ALA) using different concentrations of 25, 50, 100, 250, 500 and 1000 µM for 24, 48 and 72 hours. The RNA was extracted from both control (untreated) and treated cells. Viability was checked by MTT assay and WT1 expression was evaluated by RT-PCR.

Results: Real-time RT-PCR analysis showed that the level of WT1 mRNA, was decreased after 24, 48, 72 hours treatment with omega-3 fatty acid (ALA) for 100, 250, 500 and 1000 µM concentrations but not 25 and 50 µM. Data obtained from MTT revealed antiproliferative effects of omega-3 fatty acid (ALA) for 100-1000 µM concentrations but not 25 and 50 µM.

Conclusion: Our findings indicated that the cell viability and level of WT1 mRNA was decreased during Omega 3 ALA Treatment. So, it a potential role of Omega 3 in prevention or as a supplementary for pancreatic cancer treatment has been suggested.

Key words: omega-3 PUFA (ALA), pancreatic cancer, MIA PaCa-2, WT1

Please cite this article as: Herati E. et al. The effects of omega-3 PUFA (ALA) on WT1 gene expression in pancreatic cancer cell line (MIA PaCa-2).
2018;16(2):275-281. World Family Medicine.
DOI: 10.5742/MEWFM.2018.93287

Introduction

Cancer is one of the world's biggest challenges. It is the cause of every four deaths in the United States. [1] Pancreatic cancer is a fatal disorder with the 5-year survival of lower than 5%, and the fourth leading cause of cancer-related deaths in the world. Pancreatic cancer is aggressive with few symptoms until the cancer is advanced. Symptoms may include abdominal pain, weight loss, diarrhea, and jaundice. Treatments include surgery, chemotherapy, and radiation. [2] Because of high fatality rates, pancreatic cancer incidence rates are almost equal to mortality rates. [3]

Risk factors for pancreatic cancer are summarized as follows: age, sex, race, smoking, obesity and low physical activity, family history, certain jobs and some genetic diseases like ovarian cancer and breast cancer, gastric disorders (H. pylori infection). [4]

Wnt proteins are extracellular signaling molecules. Canonical Wnt signaling has a great impact on embryonic development, tissue homeostasis, cell division and cancer. Thus deregulation of Wnt signaling causes uncontrolled proliferation and unstable chromosomes duplication. [5]
The Wilms' tumor gene (WT1) provides information for making a zinc finger domain that is crucial for the organogenesis and development of the kidney. Furthermore this gene encodes a transcription factor and a RNA-binding protein and was revealed to be connected with kidney disorder in children and gonadal dysgenesis. The expression of WT1 gene is tissue specific and has been indicated to occur in kidney stem cells, gonads, adult spleen, heart, lung, thymus and mesothelium perdurable. [5, 6]

Omega-3 (n-3) fatty acids are long-chain polyunsaturated fatty acids (LCPUFA) with a first double carbon bond at the third carbon atom counting from the methyl end of the chain. Several studies have recently reported the cancer preventing effects of polyunsaturated fatty acids (PUFAs) in vitro. Indeed n-3PUFAs including α-linolenic acid (ALAor18:3n-3), eicosapentaenoic acid (EPA or 20:5n-3), docosapentaenoic acid (n-3 DPA or 20:5n-3), and docosahexaenoic acid (DHAor22:6n-3) are the most important for human nutrition. [7] Moreover the hypothesis that PUFAs can reduce tumorigenesis has been supported by many scientists using animal models worldwide. In any case epidemiologic studies have suggested the cancer preventing effects for n-3 PUFA and demonstrated the tumor inducing role of n-6 PUFA contrarily. [8, 9]Some studies showed that pretreatment of colon cancer cells (COLO 320 DM) with 25 μM Ecosapantanoic acid (EPA), increases sensitivity of total cell population to chemotherapy in both 24, 48, 72 and 96 hours after treatment. [10] Treatment of MDA-MB-231 breast cancer cell line with 100 μM LA (60 μM EPA+ 40 μM DHA), decreased cell growth about 40%. [11] Apoptotic activity of omega-3 PUFAs (ALA) alone [12] or in combination with other anticancer therapies [13, 14] are illustrated. Although potential of omega-3 PUFAs for target protein alterations in chemotherapy resistant SW620 colon cancer cells are demonstrated, the mechanisms by which these PUFAs assert their effects are not fully understood. [15, 16]

Materials and methods

MiaPaca-2، cell line was purchased from Pasteur Institute of Iran and cultured in monolayers using RPMI 1640 Biosera supplemented with 10% fetal bovine serum and 1% antibiotic (100 U/ml of penicillin, 10 mg/ml of streptomycin) at 37°C under a humidified atmosphere of 5% CO2. The cells were seeded in 6 well cell culture plates, each well containing 8x105 cells for all concentration and control. After treatment of the cells with omega-3 fatty acid (ALA) using concentration of 99% ethanol we prepared dilutions 25, 50, 100, 250, 500 and 1000 μM. At intervals of 24, 48 and 72 hours, cells were harvested and isolated from medium. RNA was extracted using total RNA purification kit (GeneAll RibospinTM), according to the manufacturers. For quantitative analysis of RNA extraction, we used Nanodrop techniques. For cDNA synthesis we used RT reagent kit Revert A-L, Extraction Kit from AmpliSens®. Real-time RT-PCR was performed using Rotor-Gene Q instrument (QIAGENE co.) and analyzed by Rest 2009 software.

Primers used for WT1 expression were 5′-GCGGCGCAGTTCCCCAACCA-3′ and 5′-ATGGTTTCTCACCAGTGTGCTT-3′ for forward and reverse respectively. [17]

Glyceraldehyde 3-phosphate dehydrogenase (GAPDH) was used as a RNA integrity control and amplified using primers 5′-CAATGACCCCTTCATTGACC-3′ and 5′-TGGAAGATGGTGATGGGATT-3′ for forward and reverse respectively. [18] The cycles were as follows: 95°C for 5 minutes, followed by 35 cycles of denaturing at 95°C for 30 seconds, annealing at 56°C for 40 seconds and extension at 72°C for 40 seconds. This was followed by the final extension at 72°C for 10 minutes.

For MTT, cells were cultured in 96-well tissue culture plate (2x103 cell/200μl). 24 hours after the first culture, we replaced ambient with 0.1% ethanol as control and concentrations of 25, 50, 100, 250, 500 and 1000 μM omega-3 fatty acid (ALA). Each concentration was performed as a quadruplet. After 24, 48, 72 hours of treatment, cells were washed with PBS and MTT solution (Sigma) was added at a concentration of 0.05 mg/ml diluted in PBS. Cells were incubated at 37°C for 4 hours to allow the formation of purple formazan crystals due to mitochondrial dehydrogenase activity. Then 100 μl of filtered DMSO was added to each well followed by 25 μl glycine buffer. The color intensity was then measured by spectrophotometric at 570 nm using a micro plate reader (ELISA reader, BioTek ELX800, USA). Data were analyzed by one-way analysis of variance ANOVA followed by Dunnett's multiple comparison test using graphpad prism 7 software where $P < 0.05$ was considered to be statistically significant.

Results

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As shown in Figure 1, real-time RT-PCR analysis revealed that the level of WT1 mRNA, was decreased after 24, 48, 72 hours treatment with omega-3 fatty acid (ALA) in a time dependent and concentration manner for all concentrations but 25 and 50 μM of omega-3 fatty acid (ALA). (Data for 48 hours are shown here).

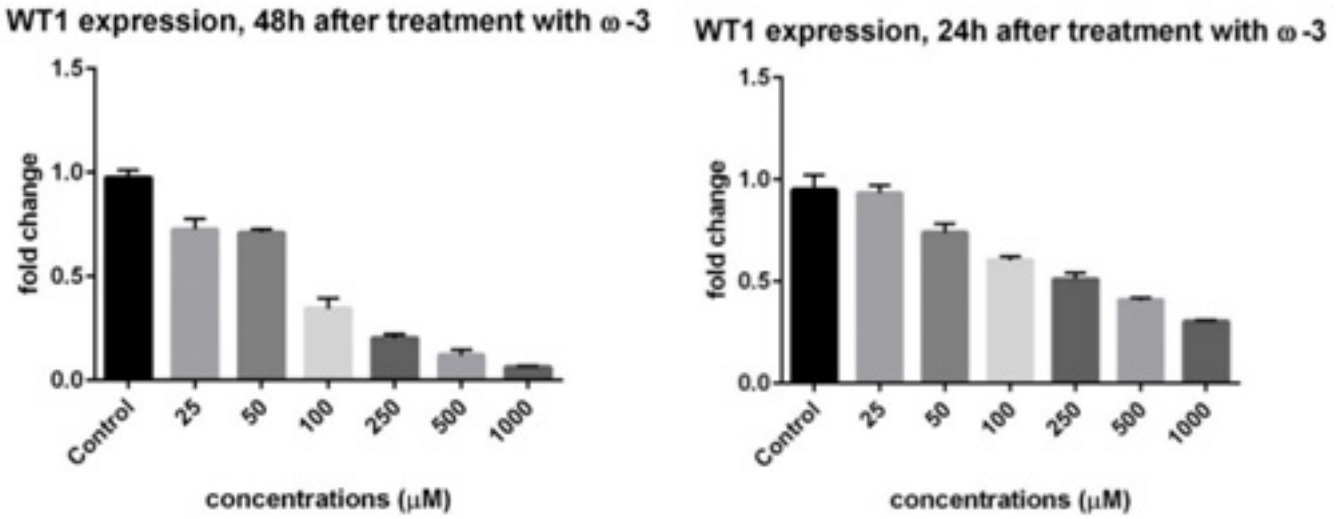

Figure 1: Omega-3 fatty acid (ALA) induced downregulation of WT1 expression in a time and concentration manner. Detecting the transcription of WIF mRNA using real-time RT-PCR. MiaPaca-2 cells were treated with 25, 50, 100, 250, 500 and 1000 μM ALA for 24, 48, 72 hours. The levels of WT1 mRNA were analyzed by REST 2009 software (P< 0.05) (only data for 24 and 48 hours are shown here).

To confirm the anti-proliferative activity of omega-3 fatty acid (ALA) in pancreatic cancer cells, we performed a cell viability assay (MTT) in both concentration and time dependent manner as described in Material and methods section. As shown in Figure 2 (only data for 48 and 72 hours are shown here) the anchorage-dependent cell viability of MiaPaca-2 cells after exposure to omega-3 fatty acid (ALA) was decreased significantly. These data demonstrate that omega-3 fatty acid (ALA) have antiproliferative activity in pancreatic cancer cell lines.

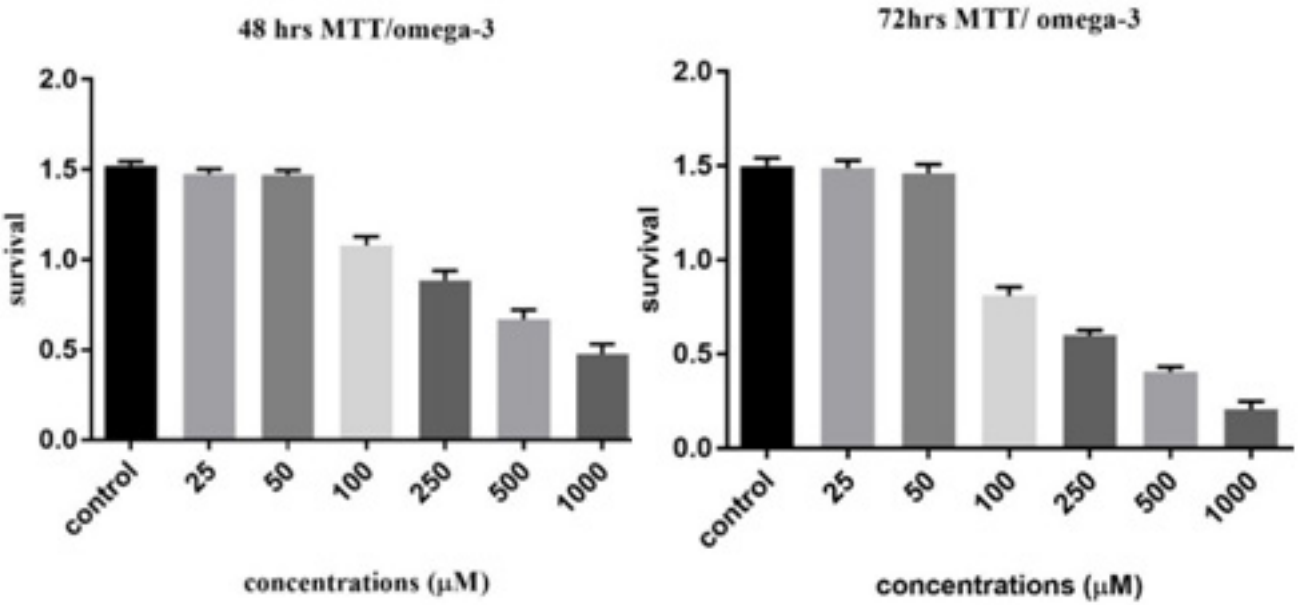

Figure 2: Anchorage-dependent cell viability of pancreatic cancer cell line (MiaPaca-2(, after treatment with different concentrations of omega-3 fatty acid (ALA) as described in material and methods. Antiproliferative effects were seen at 100 to 1000 μM of ALA with a time- and concentration-dependent manner (P<0.05).

Discussion

In line with our study anticancer potential of omega-3 rich foods that have been reported in previous studies. [19, 20] As illustrated in Figure 1 and 2 the antiproliferative and downregulation of WT1 expression effects of ALA at 100 to 1000 µM treatment concentrations were seen in pancreatic cancer cell line (MiaPaca-2), respectively ($P<0.05$). The first WT1, has been identified as the tumor suppressor gene in Wilms' tumor, a kidney cancer with an incidence of 1 in 10,000 children. Its target genes are involved in cell growth, metabolism, extracellular matrix components and growth factors. [21, 22] During embryogenesis in mammals, WT1 has firmly been indicated in the kidneys, gonads, spleen and mesothelioma. After birth, its expression in renal glomerular epithelial cells it continues in this area probably to maintain skin performance [23] WT1 expression in lung cancer, colon cancer and glioblastoma cell lines has been detected. [24] Recently, oncogenic properties of WT1 in both various hematological malignancies and solid tumors have been documented. [25] So, this study emphasizes the oncogene suppression role of omega-3 PUFA (ALA) particularly in pancreatic cancer cell line (MiaPaca-2). Many studies have established global and/or local toxicity of anticancer drugs on normal cells. [26] Some chronic diseases such as cancer and inflammatory disease can be affected by omega-3 PUFAs . [27] Also EPA, in human colon cancer cell lines, HT-29 and Caco-2, decreases proliferation. . [28] In fact either PUFAs or its purified EPA and DHA form have proven anti-neoplastic functions which enable them to play an important role in cancer prevention or therapy. [29-31] EPA and DHA at concentrations of 40 and 60 µg/mL respectively increased apoptosis of SW1990 cells in a concentration-dependent manner. [32] Many biological functions including cancer cells invasion, metastasis, apoptosis and proliferation are shown to be affected by omega-3 PUFAs . [33, 34] In summary, in confirmation of the above studies pancreatic cancer cells proliferation was inhibited in 100, 250, 500 and 1000 µM ALA in all three times studied. Besides, WT1 was downregulated by omega-3 PUFA (ALA) at the same concentrations in a time- and concentration- dependent manner. Overall, these findings are consistent with others, proposing a potential role for omega-3 PUFA (ALA) as an anticancer dietary/drug supplement that affects on WT1 gene as a therapeutic target in pancreatic cancer. Further studies are suggested for investigating the mechanisms by which omega-3 PUFAs (ALA) assert their anticancer effects. Investigations for clearing omega-3 PUFA (ALA) functions as a supplementary combined with anticancer drugs in pancreatic drug resistant cells remains to be shown.

Acknowledgements

We are grateful to the Qazvin University of Medical Sciences, deputy of research and technology for financial support.

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