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Effect of Fucoxanthinol on Pancreatic Ductal Adenocarcinoma Cells from an *N*-Nitrosobis(2-oxopropyl)amine-initiated Syrian Golden Hamster Pancreatic Carcinogenesis Model

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Abstract. *Background/Aim: Fucoxanthinol (FxOH) is a marine carotenoid metabolite with potent anti-cancer activity. However, little is known about the efficacy of FxOH in pancreatic cancer. In the present study, we investigated the inhibitory effect of FxOH on six types of cells cloned from N-nitrosobis(2-oxopropyl)amine (BOP)-induced hamster pancreatic cancer (HaPC) cells. Materials and Methods: FxOH action and its molecular mechanisms were investigated in HaPC cells using flow-cytometry, comprehensive gene array, and western blotting analyses. Results: FxOH (5.0 μM) significantly suppressed the growth of four out of six types of HaPC cells. Moreover, FxOH significantly suppressed cell cycle, chemokine, integrin, actin polymerization, microtubule organization and PI3K/AKT and TGF-β signals, and activated caspase-3 followed by apoptosis and anoikis induction in HaPC-5 cells. Conclusion: FxOH may have a high potential as a cancer chemopreventive agent in a hamster pancreatic carcinogenesis model.*

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Pancreatic cancer is one of the most lethal cancers worldwide because it is treatment resistant and has aggressive potential for metastasis/invasion with resultant poor prognosis. From the GLOBOCAN 2018 estimates, 432,242 pancreatic cancer deaths occur per year (4.5% of total) (1), and the 5-year survival rate remains poor at 10% (2). Accumulating evidence suggests that aberrant network integrity of gene mutation, gene methylation, transcriptome, microRNA, non-coding RNA, proteome, tumor microenvironment, and immune cells are crucial for human pancreatic cancer development. In particular, highly carcinogenic point mutations in driver genes, such as *KRAS*, *CDKN2A*, *TP53*, and *SMAD4*, are observed in many specimens (3-7). Pancreatic intraepithelial neoplasia (PanIN) is a premalignant lesion in pancreatic carcinogenesis and has the stepwise progress graded as four types from mild to severe. *KRAS*, *CDKN2A*, *TP53* and *SMAD4* are somatically mutated in turn along with the malignant progression of PanIN, followed by the cancer progression (8).

N-nitrosobis(2-oxopropyl)amine (BOP)-treated Syrian golden hamsters are a chemical carcinogenesis model that represents human pancreatic cancer because it induces PanIN, and pancreatic ductal adenocarcinoma resembles human pancreatic cancer, which also includes similar genetic mutations such as in *K-ras*, *CDKN2A*, and *SMAD4* (9). Therefore, the BOP-induced hamster pancreatic cancer

model is a useful model to investigate the mechanism of carcinogenesis and in the identification of chemopreventive agents against pancreatic cancer. Several cancer prevention experiments using BOP-treated hamsters revealed that natural dietary materials, such as fermented brown rice, 4-methylthio-3-butenyl isothiocyanate, benzyl isothiocyanate, sulforaphane, green tea polyphenols, and β-carotene may be candidate cancer chemopreventive agents; however, the anti-cancer mechanisms involved remain elusive (10-13).

Fucoxanthin (Fx) is a highly polar carotenoid that has a distinctive allene and a 5,6-monoepoxide. Fx predominantly accumulates in marine brown algae, some of which are used in foods. Dietary Fx is converted to its deacetylated form fucoxanthinol (FxOH) mainly in the intestine of humans as well as in mice (14, 15).

To date, human interventional studies aimed at preventing cancer with Fx or FxOH have been limited. On the other hand, many reports have shown that Fx has anti-cancer activity in various cancers *in vitro* and *in vivo* (16-23). Regarding FxOH, it suppressed tumorigenesis in immunodeficient NOD-SCID mice (24). It also induced apoptosis in colon cancer cells and colon cancer stem-like spheroids through attenuation of integrin, mitogen-activated protein kinase (MAPK), nuclear factor-κB, phosphatidylinositol-3 kinase/protein kinase B (PI3K/AKT), peroxisome proliferator-activated receptor, signal transducers and activators of transcription (STAT), chloride intracellular channel 4 (CLIC4), and caspase signaling (25-28). These molecules are also involved in migration, invasion, epithelial-mesenchymal transition, and cell-cycle arrest. However, little information in available on the anti-cancer function of FxOH in pancreatic cancer.

Herein, we showed the apoptosis-inducing effect of FxOH on a cell line cloned from pancreatic ductal adenocarcinoma in a BOP-treated hamster and elucidated its molecular mechanisms.

Materials and Methods

Chemicals. All-*trans*-FxOH (purity, ≥98%) was extracted and purified from algal lipids by Dr. Hayato Maeda (Hirosaki University, Japan). Anti-C-X-C chemokine receptor type 4 (CXCR4) and anti-CXCR7 antibodies were purchased from BioVision (Milpitas, CA, USA) and Novus Biologicals (Littleton, CO, USA), respectively. Anti-Akt (pan), anti-cyclin B1, anti-phosphorylated focal adhesion kinase [pFAK(Tyr^{397})], anti-integrin α5, anti-integrin β1, anti-integrin β4, and anti-caspase-3 antibodies were obtained from GeneTex (Irvine, CA, USA). Anti-cyclin D1, anti-pMEK1/2($Ser^{217/221}$), anti-pERK1/2(Thr^{202}/Tyr^{204}), anti-pAkt(Ser^{473}), and anti-pAkt(Thr^{308}) antibodies were from Cell Signaling Technology (Danvers, MA, USA). Anti-cyclin D2 and anti-integrin β8 antibodies were purchased from Bioss Antibodies (Beijing, PR China) and R&D Systems (Minneapolis, MN, USA), respectively. Anti-pPaxillin (Tyr^{31}) and anti-p53 antibodies were obtained from Novex (San Diego, CA, USA) and Thermo Scientific (Waltham, MA, USA), respectively. The cells were routinely maintained in Dulbecco's modified Eagle medium (DMEM, Wako Pure Chemicals, Osaka, Japan) containing 10% heat-inactivated fetal bovine serum (FBS), 4 mM L-glutamine, 40,000 U/l penicillin, and 40 mg/l streptomycin. All other reagents and solvents used were of analytical grade.

Establishment of cell lines. Female Syrian golden hamsters (5-week-old, Japan SLC, Shizuoka, Japan) were acclimated for a week and injected subcutaneously with BOP (Nacalai Tesque, Kyoto, Japan) 4 times (on days 1, 3, 5, and 7) at a dose of 10 mg/kg body weight. CE-2 pellets (CLEA Japan, Shizuoka, Japan) were used as a standard diet, and 1 group of hamsters was fed Quick Fat pellets (QF) (CLEA Japan, Shizuoka, Japan). Hamsters were sacrificed with deep anesthesia at 38-90 weeks of age, and then the pancreas from each hamster was taken and part of the tumor collected. Most pancreatic tissue was placed in 10% formalin/phosphate-buffered saline for 2-3 days. Histopathologic diagnosis of pancreatic tissue in the hamster was performed by a highly proficient pathologist. The animal experiments were approved by the Institutional Guidelines for Animal Care and Use in the National Cancer Center Research Institute.

The tumor sections were minced using scissor and cultured in 5% FBS/RPMI-1640 (Wako Pure Chemicals, Osaka, Japan) medium in 24-well plates. When the cultured cells reached confluence, they were seeded in another dish (6-well plates), and then in a larger dish (100-mm in diameter). The established cell lines were designated hamster pancreatic cancer (HaPC)-1, -2, -3, -4, and -5 derived from a hamster given CE-2, and HaPC-6 from a hamster given QF.

Cell viability assay. HaPC-1–6 cells were adhered at a density of 5×10^4 cells/ml into a 24-well plate in 10% FBS/DMEM medium for 3.5 h. Cells were then incubated in 1% FBS/DMEM medium with FxOH (final concentrations, 1.0 and 5.0 μM) or vehicle alone [dimethylsulfoxide (DMSO)] for 1 day. Cell viability was determined using a WST-1 reagent assay. The absorbance was monitored using an ELISA reader at 450 nm (TECAN Japan, Tokyo, Japan).

Cell cycle analysis. HaPC-5 cells were adhered at a density of 5×10^4 cells/ml into 100-mm dishes in 10% FBS/DMEM medium for 3.5 h. Cells were then incubated in 1% FBS/DMEM medium with FxOH (final concentration, 5.0 μM) or vehicle alone (DMSO) for 2 days. The cells were trypsinized, fixed with 70% ethanol, and then treated with ribonuclease A (Nacalai Tesque, Kyoto, Japan). Nuclei in the cells were stained with propidium iodide (Sigma-Aldrich, St Louis, MO, USA), and the cells were suspended with 0.1% bovine serum albumin (BSA)/phosphate-buffered saline. The ratios of Sub-G_1 (apoptosis-like cells), G_1, S, and G_2/M phases were determined using a FACSAria-III flow cytometer (BD Biosciences).

Total RNA preparation. HaPC-5 cells were adhered at a density of 5×10^4 cells/ml into 100-mm dishes in 10% FBS/DMEM medium for 3.5 h. Cells were then incubated in 1% FBS/DMEM medium with FxOH (final concentration, 5.0 μM) or vehicle alone (DMSO) for 1 day. Total RNA from HaPC-5 cells with or without 5.0 μM FxOH treatment was isolated using an RNeasy Mini Kit with RNase-Free DNase Set and QIAshredder (QIAGEN, Valencia, CA, USA) in accordance with the manufacturer's instructions. The concentration of total RNA was measured using Nanodrop ND-1000 (NanoDrop, Wilmington, DE, USA). Subsequently, quantitation of total RNA was determined using an Agilent 2100 bioanalyzer

(Agilent, Santa Clara, USA). Total RNA from FxOH-treated HaPC-5 cells or control cells was prepared as a sample with equivalently mixed mRNAs with triplicate experiments and then subjected to next-generation sequencing.

Transcriptome analysis. Whole transcriptome analysis was performed in accordance with the TruSeq Standard mRNA Reference Guide Document #1000000040498 v00 and the next generation sequencer NovaSeq 6000 System User Guide Document #1000000019358 v02. In brief, total RNA was purified into mRNA, fragmented, and then prepared as double-stranded cDNA. Subsequently, libraries from the cDNA template were prepared using a TruSeq standard mRNA LT Sample Prep kit (Illumina, San Diego, CA, USA) and sequenced using a NovaSeq 6000 S4 Reagent kit (Illumina, San Diego, CA, USA). The sequencing was carried out using a NovaSeq 6000 system (Illumina, San Diego, CA, USA) equipped with sequencing control software (version 1.4.0). Differentially expressed genes between FxOH-treated HaPC5 cells and the control cells were detected using ≥2.0- and ≤–2.0-fold cutoff and *p*-values <0.05 (exact test using edgeR). Gene profiles were displayed using volcano plots and hierarchy clustering maps. Gene annotation was performed using the NCBI reference sequence database *Mesocricetus auratus* Annotation Release 102 (ncbi.nlm.nih.gov/genome/annotation_euk/Mesocricetus_auratus/102/). Functional interpretation analysis was performed using the g:Profiler tool (https://biit.cs.ut.ee/gprofiler), based on the gene ontology (GO) database (http://www.geneontology.org/).

Quantitative-polymerase chain reaction (qPCR). The cDNA was synthesized from total RNA using a High Capacity cDNA Reverse Transcription kit (Thermo Fisher Scientific, Waltham, MA). Gene primer and probe sequences were as follows: *Ackr3*-forward (5'-AGG TAG GTA TCA GGC AGA G-3'), *Ackr3*-reverse (5'-CAG CAC CTC CAG CTA TAA GAA G-3'), glyceraldehyde-3-phosphate dehydrogenase (*GAPDH*)-forward (5'-GTT GGA ACC CAG TGC ATA GA-3'), *GAPDH*-reverse (5'-GGG TGT GAA CCA TGA CAA GTA-3'), *Ackr3* probe (5'-/56-FAM/TGT TGT CTG/ZEN/CAT CTT GGT GTG GCT/3lABkFQ/-3') and *GAPDH* probe (5'-/56-FAM/CTG CAC CAC/ZEN/CAA CTG GCT GAA ATG/3lABkFQ/-3') (Integrated DNA Technologies, Coralville, IA).

The cDNA template (10 ng), primers (final 500 pM)/probe (final 250 pM) sets (Integrated DNA Technologies, Coralville, IA, USA), PrimeTime Gene Expression Master Mix (Integrated DNA Technologies), and distilled water were mixed (total volume, 20 μl). qPCR was performed as follows: initial denaturation for 5 min at 95˚C, followed by 40 cycles of 15 s at 95˚C and 45 s at 60˚C. qPCR was performed using a LightCycler®Nano real-time PCR system (Roche Diagnostics, Mannheim, Germany).

Western blotting. HaPC-5 cells were adhered at a density of 5×10^4 cells/ml in 100-mm dishes in 10% FBS/DMEM medium for 3.5 h. Cells were then incubated in 1% FBS/DMEM medium with FxOH (final concentration, 5.0 μM) or vehicle alone (DMSO) for 1 day. HaPC-5 cells with or without 5.0 μM FxOH treatment were harvested and lysed in lysis buffer. The protein concentration in whole cell lysates was determined using the Bradford assay (Bio-Rad, Hercules, CA, USA). Ten μg of protein was separated using a sodium dodecyl sulfate-10% polyacrylamide gel. Gels were electroblotted onto a PVDF membrane (Amersham Bioscience, Chalfont St. Giles, UK). The membrane was incubated in Tris-buffered saline containing 0.1% polyoxyethylene (20) sorbitan monolaurate with 1% BSA (1% BSA/Tris-buffered saline containing 0.1% Tween 20 [TBS-T]) at room temperature for 1 h, and probed with each of the primary antibodies (1:1,000 dilution) in 1% BSA/TBS-T at 4˚C overnight. The membranes were then probed with horseradish peroxidase-conjugated anti-mouse or anti-rabbit secondary antibody (1:2,000 dilution) in 1% BSA/TBS-T at room temperature for 1 h. Protein bands were visualized using a chemiluminescence reagent (Millipore, Billerica, MA, USA).

Statistics analysis. All the values are expressed as the mean±standard error (SE). All differences were examined using the Student's *t*-test or exact test with edgeR between two groups, and one-way ANOVA with Tukey–Kramer *post-hoc* tests for multiple comparisons. Significant differences were presented at $*p<0.05$, $**p<0.01$ or exact *p*-values.

Results

Characterization of six types of pancreatic ductal adenocarcinoma cancers in BOP-treated hamster. Pathological findings revealed that several types of pancreatic cancers exist in BOP-treated hamsters. These were pathologically diagnosed as papillary adenocarcinoma, well differentiated tubular adenocarcinoma, moderately differentiated tubular adenocarcinoma, and poorly differentiated adenocarcinoma. These cells cloned from the six types of pancreatic adenocarcinomas were designated as HaPC-1–6 (Figure 1 and Table I).

Effect of FxOH on cell growth in HaPC-1–6 cells. The growth of HaPC-1, -4, -5, and -6 cells was significantly decreased in a dose-dependent manner by FxOH treatment. Little significant difference in the cell growth of HaPC-2 and -3 cells was observed with FxOH treatment. The percentages of cell growth (control 100%) were as follows: 1.0 μM FxOH, 89.8±2.8%; 5.0 μM FxOH, 88.3±2.0% in HaPC-1 cells; 1.0 μM FxOH, 97.4±6.0%; 5.0 μM FxOH, 85.6±1.7% in HaPC-4 cells; 1.0 μM FxOH, 82.5±2.8%; 5.0 μM FxOH, 76.6±2.2% in HaPC-5 cells; and 1.0 μM FxOH, 98.0±1.1%; 5.0 μM FxOH, 93.1±2.0% in HaPC-6 cells (Figure 2).

Effect of FxOH on apoptosis induction and cell-cycle arrest in HaPC-5 cells. Treatment of HaPC-5 cells with 5.0 μM FxOH showed drastic morphological changes from an elongated cell form to a thin spindle form (Figure 3A). Treatment with 5.0 μM FxOH significantly augmented the ratio of Sub-G_1 (control cells, 16.4±0.3% and FxOH-treated cells, 32.3±2.0%) in HaPC-5 cells. The ratio of HaPC-5 cells in each cell cycle phase was significantly changed by 5.0 μM FxOH treatment: G_0/G_1 phase, control cells, 65.8±0.1% and FxOH-treated cells, 71.7±0.4%; G_2/M phase, control cells, 15.0±0.2% and FxOH-treated cells, 10.0±0.4%. The proportion of HaPC-5 cells in S phase did not significantly differ between control and FxOH-treated cells (Figure 3B).

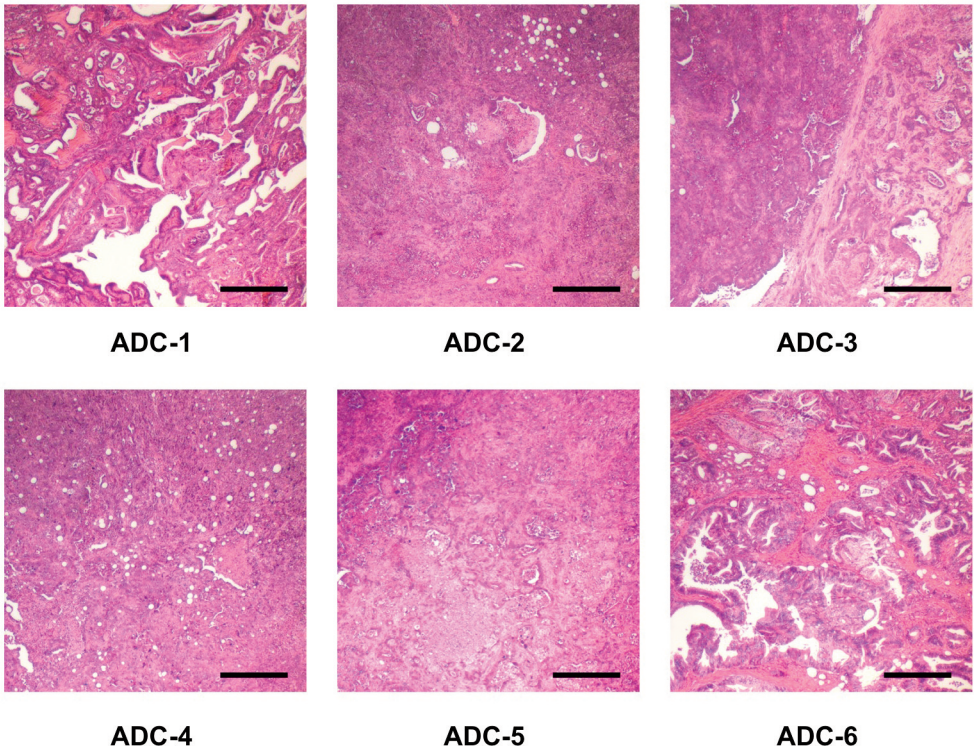

Figure 1. *Representative histopathology of six pancreatic ductal adenocarcinomas developed in BOP-treated hamsters. ADC, adenocarcinoma. Bar, 400 μm.*

Effect of FxOH on the transcriptome in HaPC-5 cells. Transcriptome alterations in HaPC-5 cells after 5.0 μM FxOH treatment for 1 day were investigated. As a result, volcano plots showed that the number of down-regulated genes was greater than that of up-regulated genes in both fold-change and *p*-value (Figure 4A). Heat maps were used to display one-way hierarchical clustering of the 1,213 genes that showed differences between the two groups (Figure 4B). Overall, 344 up-regulated and 869 down-regulated genes (total 1,213 genes) were altered in FxOH-treated HaPC-5 cells compared to control cells (Figure 4C). The top 16 GO terms for biological processes and 1 GO term for cellular components were significantly enriched in the 344 up-regulated genes. The genes in the GO terms on response to hormone (15 genes), taxis (13 genes), muscle tissue development (11 genes), and euchromatin (4 genes) were mainly associated with growth and inflammation, although few up-regulated genes involved in apoptosis induction were observed (Figure 5 and Table II). The genes for cellular response to hormone stimulus and chemotaxis are not shown in Table II, because the genes contained in these were similar for responses to hormone and chemotaxis, respectively. The top 20 GO terms for biological processes and cellular components and the top 12 GO terms for molecular function were significantly enriched in the 869 down-regulated genes. The GO terms for mitotic cell-cycle process (53 genes), cell surface (33 genes), supramolecular polymer (30 genes), supramolecular complex (30 genes), supramolecular fiber organization (33 genes), tubulin binding (21 genes), and microtubule binding

Table I. *Pathological findings for six cell lines cloned from pancreatic ductal adenocarcinoma of BOP-initiated Hamster.*

Hamsterno.	Adenocarcinoma	Feature	Cell line[a]
1	1	Pap ADC[b] >>> Tub2[c] >> Por[d]	HaPC-1
1	2	Por	HaPC-2
1	3	Por >> Pap ADC	HaPC-3
1	4	Por >> Tub2	HaPC-4
1	5	Tub2 >> Por	HaPC-5
2	6	Tub2 >>> Tub[e]	HaPC-6

BOP, *N*-Nitrosobis(2-oxopropyl)amine; HaPC, Hamster pancreatic cancer. [a]Name of cell lines cloned from each pancreatic tumor. [b]Pap ADC, papillary adenocarcinoma. [c]Tub2, moderately differentiated tubular adenocarcinoma. [d]Por, poorly differentiated adenocarcinoma. [e]Tub1, well differentiated tubular adenocarcinoma.

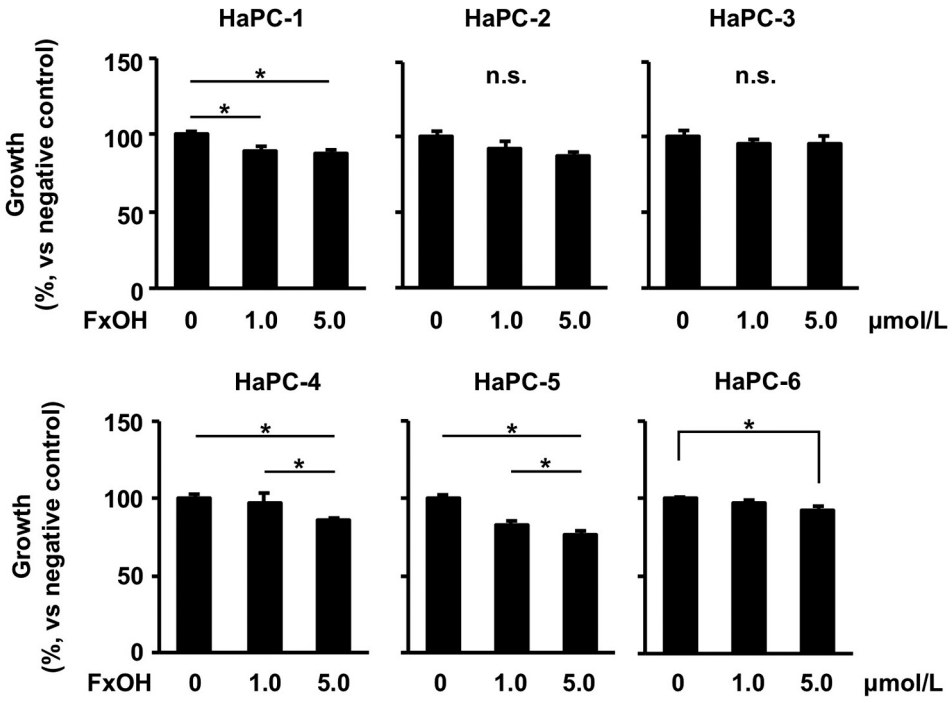

Figure 2. *Effects of fucoxanthinol (FxOH) on cell growth in pancreatic cancer HaPC-1–6 cells. HaPC-1-6 cells were treated with 1.0 and 5.0 μM FxOH for 1 day. Cell viability was measured using WST-1 reagent assay. The cell viability of control cells was set as 100%. Means±SE (n=6). *p<0.05 vs. control cells (vehicle only).*

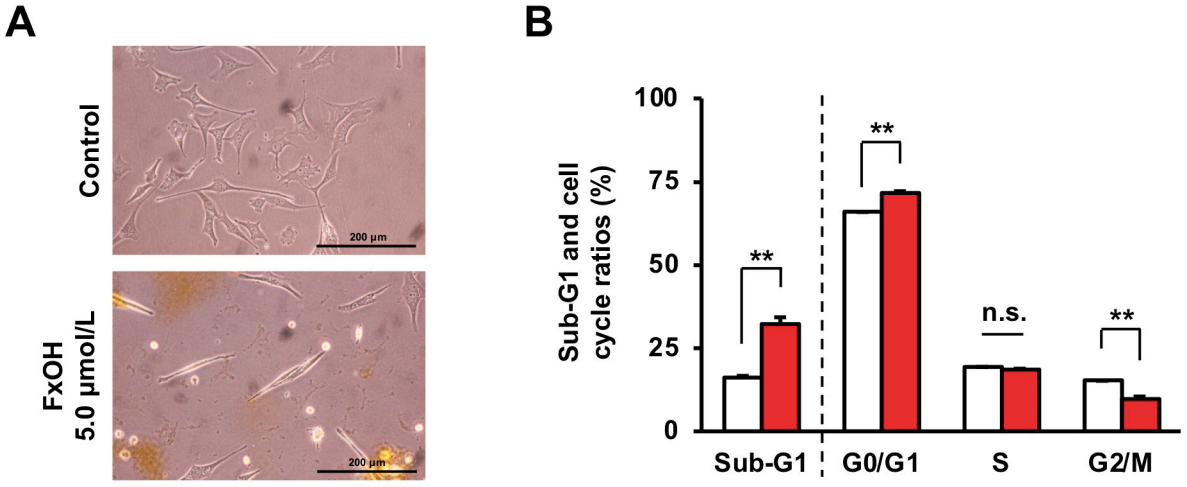

Figure 3. *Effects of fucoxanthinol (FxOH) on apoptosis induction in pancreatic cancer HaPC-5 cells. HaPC-5 cells were treated with 5.0 μM FxOH for 2 days. (A) Phase contrast microscopy images. Bar, 200 μm. (B) Proportion of sub-G_1 phase (apoptotic-like cells) and cells in each cell-cycle phase (G_1, S and G_2/M) in FxOH-treated and control HaPC-5 cells, which were evaluated using a FACSAria-III flow cytometer are shown. Means±SE (n=3). **p<0.01.*

(19 genes) were mainly correlated with many signals as follows: cell cycle, cell division, chemokine, cadherin, extracellular matrix, integrin, actin polymerization, microtubule organization, Ras, transforming growth factor beta (TGF-β) and wingless/integrated (Wnt). Moreover, a GO term for regulation of the MAPK cascade for biological processes was decreased by FxOH treatment (Figure 6, Tables III and IV).

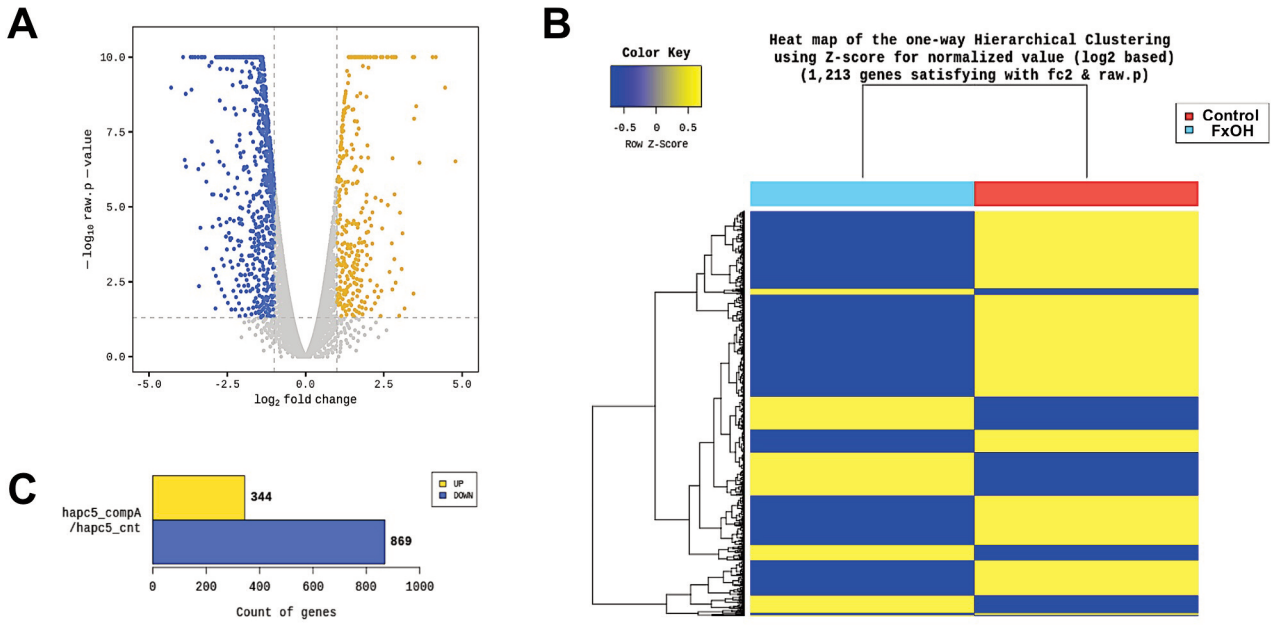

Figure 4. *Effects of fucoxanthinol (FxOH) on the transcriptome profile in HaPC-5 cells. HaPC-5 cells were treated with 5.0 μM FxOH for 1 day. Gene alterations between FxOH-treated HaPC-5 cells and control cells were analyzed using a next-generation sequencer NovaSeq 6000 system and sequencing control software (version 1.4.0). Levels of gene expression with ≥2.0 and ≤–2.0 -fold with cutoff p-value <0.05 in FxOH-treated HaPC-5 cells and control cells are presented as a sample with equivalently mixed mRNAs with triplicate experiments. (A) Volcano plots between the two groups. (B) Hierarchical clustering analysis for 1,213 genes with significant expression level differences between the two groups. (C) Number of up- (≥2.0-fold), and down-regulated (≤-2.0-fold) genes between the two groups. Yellow, up-regulated genes. Blue, down-regulated genes.*

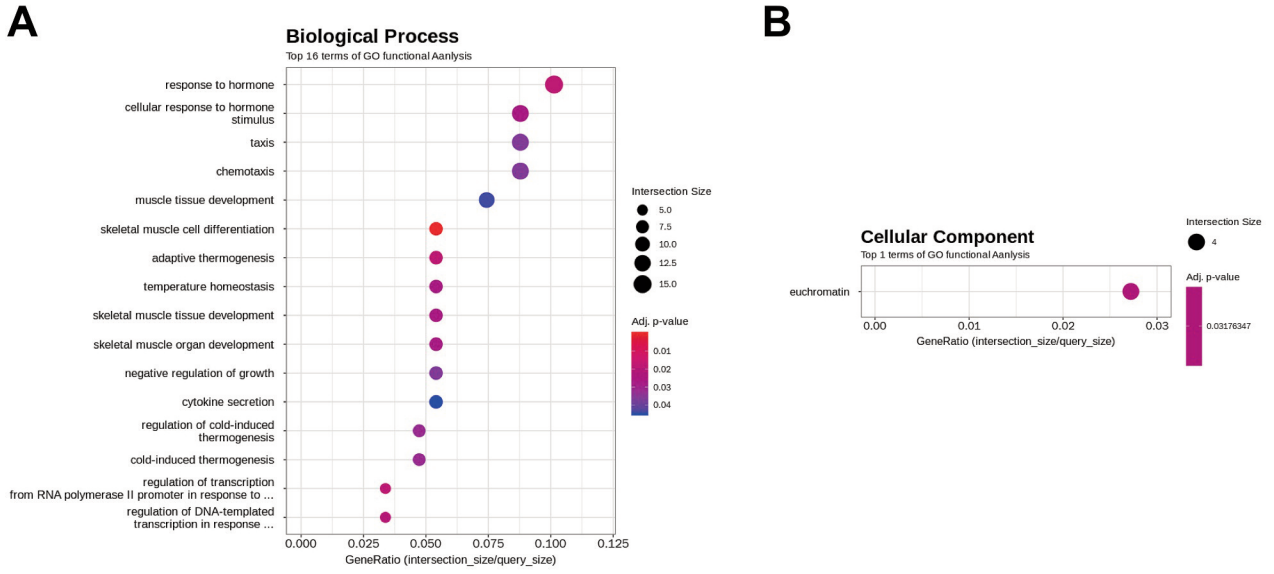

Figure 5. *Gene ontology (GO) enrichment profiles of genes up-regulated by fucoxanthinol (FxOH) treatment in HaPC-5 cells. The functional interpretation of genes up-regulated by ≥2.0-fold and cutoff p-value <0.05 were performed using g:Profiler. The top 16 GO terms in more than four gene sizes are shown. (A) Sixteen GO terms in a biological process category. (B) One GO term in a cellular component category.*

Table II. *Up-regulated genes in HaPC-5 cells treated with FxOH*[a].

Gene symbol	Description	Fold[b]	*p*-Value[c]
Response to hormone			
Prkcq	Protein kinase C theta	8.6	0.001
Notch1	Notch 1	7.4	0.026
Socs2	Suppressor of cytokine signaling 2	5.3	<0.001
Chrm1	Cholinergic receptor muscarinic 1	5.0	0.061
Gdf15	Growth differentiation factor 15	4.8	<0.001
Ly6g6d	Lymphocyte antigen 6 family member G6D	4.3	0.122
Slit3	Slit guidance ligand 3	3.8	<0.001
Mef2c	Myocyte enhancer factor 2C	3.3	0.148
Nr1h4	Nuclear receptor subfamily 1 group H member 4	3.1	0.011
Fibin	Fin bud initiation factor homolog (zebrafish)	2.9	0.014
Nr4a1	Nuclear receptor subfamily 4 group A member 1	2.8	0.016
Areg	Amphiregulin	2.3	0.012
Rorb	RAR related orphan receptor B	2.3	<0.001
Spp1	Secreted phosphoprotein 1	2.1	<0.001
Ddit4	DNA damage inducible transcript 4	2.1	<0.001
Taxis			
Hoxb9	Homeobox B9	11.1	<0.001
Prkcq	Protein kinase C theta	8.6	0.001
Notch1	Notch 1	7.4	0.026
LOC101840973	Ephrin type-A receptor 7	4.2	0.022
Tnfsf18	TNF superfamily member 18	4.1	<0.001
Dysf	Dysferlin	3.9	<0.001
Slit3	Slit guidance ligand 3	3.8	<0.001
Ch25h	Cholesterol 25-hydroxylase	3.3	0.104
LOC101827575	C-X-C motif chemokine 2-like	3.1	<0.001
Hsd3b7	Hydroxy-delta-5-steroid dehydrogenase, 3 beta- and steroid delta-isomerase 7	2.4	<0.001
Tbr1	T-box, brain 1	2.3	0.068
Il17ra	Interleukin 17 receptor A	2.3	<0.001
Dcc	DCC netrin 1 receptor	2.2	0.176
Muscle tissue development			
Notch1	Notch 1	7.4	0.026
LOC101840643	Cytochrome P450 26B1	3.8	<0.001
Mef2c	Myocyte enhancer factor 2C	3.3	0.148
Nupr1	Nuclear protein 1, transcriptional regulator	3.2	<0.001
Nr4a1	Nuclear receptor subfamily 4 group A member 1	2.8	0.016
Atf3	Activating transcription factor 3	2.3	<0.001
Ifrd1	Interferon related developmental regulator 1	2.3	<0.001
Maff	MAF bZIP transcription factor F	2.3	<0.001
Ppargc1a	PPARG coactivator 1 alpha	2.2	0.004
Ankrd2	Ankyrin repeat domain 2	2.0	0.068
Kdm6b	Lysine demethylase 6B	2.0	<0.001
Euchromatin			
Nr1h4	Nuclear receptor subfamily 1 group H member 4	3.1	0.011
LOC101829141	Histone H1.3	2.5	0.021
LOC101826763	Histone H1.2	2.4	0.095
Ankrd2	Ankyrin repeat domain 2	2.0	0.069

HaPC, Hamster pancreatic cancer; FxOH, fucoxanthinol. [a]Among all 1,213 genes significantly changed, up-regulated 43 genes classified to response to hormone, taxis, muscle tissue development and euchromatin in Gene Ontology (GO) term analysis are showed. [b]Fold change of gene expression in FxOH-treated HaPC5 cells in comparison with that of control cells. [c]Significant difference between HaPC5 cells with and without FxOH treatments by an exact test on edgeR.

Effect of FxOH on Ackr3 (Cxcr7) gene expression in HaPC-5 cells. The gene expression of *Ackr3* (*Cxcr7*) in HaPC-5 cells after 5.0 μM FxOH treatment for 1 day was evaluated. The mRNA expression of *Ackr3* decreased significantly to 0.5-fold after 5.0 μM FxOH treatment in HaPC-5 cells compared with untreated control cells (Figure 7).

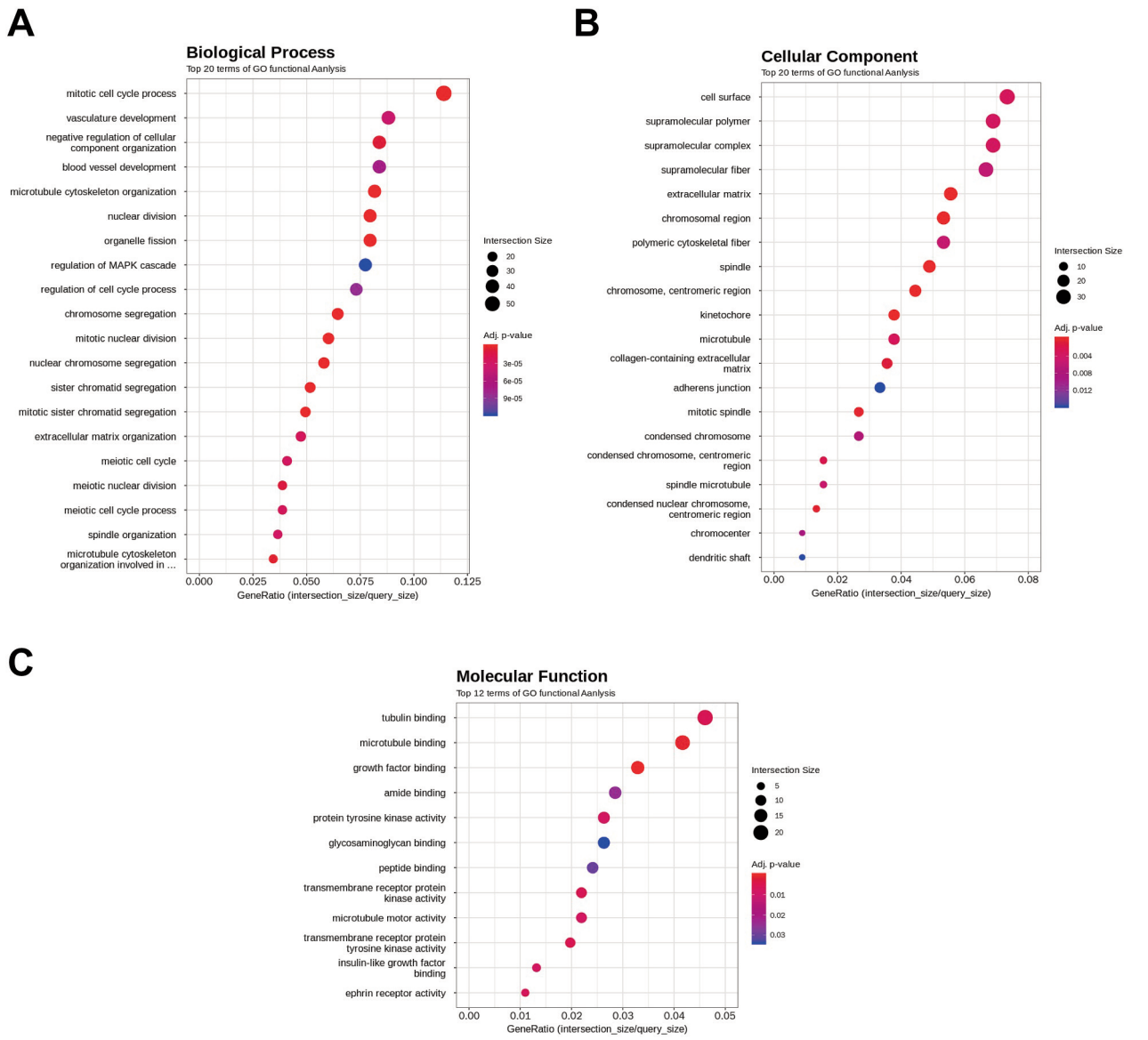

Figure 6. *Gene ontology (GO) enrichment profiles of genes down-regulated by fucoxanthinol (FxOH) treatment in HaPC-5 cells. The functional interpretation of genes down-regulated by ≤–2.0-fold and cutoff p-value <0.05 were performed using g:Profiler. The top 20 GO terms in more than five gene sizes are shown. (A) Twenty GO terms in a biological process category. (B) Twenty GO terms in a cellular component category. (C) Twelve GO terms in a molecular function.*

Effect of FxOH on protein expressions in HaPC-5 cells. Based on the cell-cycle arrest and transcriptome analysis, the effect of FxOH on protein expression and activation in HaPC-5 cells was determined. FxOH treatment decreased the expression levels of cyclin D1, cyclin B1, CXCR7, integrin α5, pFAK(Tyr^{397}), pPaxillin(Tyr^{31}), pAKT(Ser^{473}), and pSmad2(Ser$^{465/467}$) and increased that of pERK1/2(Thr^{202}/Tyr^{204}) in HaPC-5 cells. Expression of cleaved caspase-3 (p17/p19), the active form of caspase-3, was increased in HaPC-5 cells after FxOH treatment. Little difference between FxOH-treated HaPC-5 cells and control cells was observed for cyclin D2, CXCR4, integrin β1, integrin β4, integrin β8, pAKT(Thy^{308}), AKT(pan), pMEK1/2 (Ser$^{217/221}$), Smad2, pro-caspase-3, and p53 (Figure 8).

Discussion

The present study demonstrated that FxOH induced apoptosis in HaPC-5 cells through suppression of many

Table III. *Down-regulated genes in HaPC-5 cells treated with FxOH*[a].

Gene symbol	Description	Fold[b]	*p*-Value[c]
Biological process – mitotic cell cycle process			
Edn1	Endothelin 1	–6.9	<0.001
Dact1	Dishevelled binding antagonist of beta catenin 1	–4.5	<0.001
Iqgap3	IQ motif containing GTPase activating protein 3	–3.9	<0.001
Mastl	Microtubule associated serine/threonine kinase like	–3.6	<0.001
Cdkn2c	Cyclin dependent kinase inhibitor 2C	–3.5	<0.001
Apc	APC, WNT signaling pathway regulator	–3.4	<0.001
E2f7	E2F transcription factor 7	–3.4	<0.001
Ccna2	Cyclin A2	–3.3	<0.001
Nusap1	Nucleolar and spindle associated protein 1	–3.3	<0.001
Cenpa	Centromere protein A	–3.0	<0.001
Anln	Anillin actin binding protein	–2.9	<0.001
Pkia	cAMP-dependent protein kinase inhibitor alpha	–2.9	<0.001
Psrc1	Proline and serine rich coiled-coil 1	–2.8	0.001
Fbxo5	F-box protein 5	–2.8	<0.001
Aurkb	Aurora kinase B	–2.8	<0.001
Chek2	Checkpoint kinase 2	–2.8	<0.001
Ckap2	Cytoskeleton associated protein 2	–2.8	<0.001
Bub1b	BUB1 mitotic checkpoint serine/threonine kinase B	–2.8	<0.001
Ect2	Epithelial cell transforming 2	–2.7	<0.001
Espl1	Extra spindle pole bodies like 1, separase	–2.7	<0.001
Plk1	Polo like kinase 1	–2.7	<0.001
Kif2c	Kinesin family member 2C	–2.7	<0.001
Sapcd2	Suppressor APC domain containing 2	–2.6	<0.001
Zwint	ZW10 interacting kinetochore protein	–2.5	<0.001
Kif20b	Kinesin family member 20B	–2.5	<0.001
Rad51c	RAD51 paralog C	–2.5	<0.001
Cdk1	Cyclin dependent kinase 1	–2.5	<0.001
Knstrn	Kinetochore localized astrin/SPAG5 binding protein	–2.5	<0.001
Foxm1	Forkhead box M1	–2.4	<0.001
Trip13	Thyroid hormone receptor interactor 13	–2.4	<0.001
Tacc3	Transforming acidic coiled-coil containing protein 3	–2.4	<0.001
Sgo1	Shugoshin 1	–2.4	<0.001
Spag5	Sperm associated antigen 5	–2.3	<0.001
Cdca8	Cell division cycle associated 8	–2.3	<0.001
Cdc20	Cell division cycle 20	–2.3	<0.001
Wdr62	WD repeat domain 62	–2.3	<0.001
Gpnmb	Glycoprotein nmb	–2.3	0.063
Skp2	S-phase kinase associated protein 2	–2.3	<0.001
Ccsap	Centriole, cilia and spindle associated protein	–2.2	<0.001
Ndc80	NDC80, kinetochore complex component	–2.2	<0.001
Cdca5	Cell division cycle associated 5	–2.2	<0.001
Pttg1	Pituitary tumor-transforming 1	–2.2	<0.001
Smc4	Structural maintenance of chromosomes 4	–2.1	<0.001
Mad2l1	Mitotic arrest deficient 2 like 1	–2.1	<0.001
Kif20a	Kinesin family member 20A	–2.1	<0.001
Nek2	NIMA related kinase 2	–2.1	<0.001
Ccne2	Cyclin E2	–2.1	<0.001
Kif18a	Kinesin family member 18A	–2.1	<0.001
Ncapg	Non-SMC condensin I complex subunit G	–2.1	<0.001
Cdkn2d	Cyclin dependent kinase inhibitor 2D	–2.1	<0.001
Spry1	Sprouty RTK signaling antagonist 1	–2.1	<0.001
Tpx2	TPX2, microtubule nucleation factor	–2.0	<0.001
Kif22	Kinesin family member 22	–2.0	<0.001
Cellular component – supramolecular polymer			
Col1a1	Collagen type I alpha 1 chain	–15.0	<0.001
Thsd4	Thrombospondin type 1 domain containing 4	–11.5	<0.001
Pgm5	Phosphoglucomutase 5	–8.4	<0.001

Table III. *Continued*

Table III. *Continued*

Gene symbol	Description	Fold[b]	*p*-Value[c]
Fkbp1b	FK506 binding protein 1B	–6.7	<0.001
Fbn1	Fibrillin 1	–3.4	<0.001
Nusap1	Nucleolar and spindle associated protein 1	–3.3	<0.001
Cryab	Crystallin alpha B	–3.2	<0.001
Col4a6	Collagen type IV alpha 6 chain	–3.2	<0.001
Tubb3	Tubulin beta 3 class III	–3.1	<0.001
Psrc1	Proline and serine rich coiled-coil 1	–2.8	0.001
Ckap2	Cytoskeleton associated protein 2	–2.8	<0.001
Plk1	Polo like kinase 1	–2.7	<0.001
Kif2c	Kinesin family member 2C	–2.7	<0.001
Cdk1	Cyclin dependent kinase 1	–2.5	<0.001
Gtse1	G2 and S-phase expressed 1	–2.5	<0.001
Knstrn	Kinetochore localized astrin/SPAG5 binding protein	–2.5	<0.0017
Rac3	Ras-related C3 botulinum toxin substrate 3 (rho family, small GTP binding protein Rac3)	–2.5	0.070
Bfsp2	Beaded filament structural protein 2	–2.5	<0.001
Lmnb1	Lamin B1	–2.4	<0.001
Krt80	Keratin 80	–2.4	0.095
Synm	Synemin	–2.4	<0.001
Spag5	Sperm associated antigen 5	–2.3	<0.001
Tube1	Tubulin epsilon 1	–2.2	<0.001
Ccsap	Centriole, cilia and spindle associated protein	–2.2	<0.001
Kifc1	Kinesin family member C1	–2.2	<0.001
Carmil1	Capping protein regulator and myosin 1 linker 1	–2.1	<0.001
Kif20a	Kinesin family member 20A	–2.1	<0.001
Kif18a	Kinesin family member 18A	–2.1	<0.001
Tpx2	TPX2, microtubule nucleation factor	–2.0	<0.001
Krt25	Keratin 25	–2.0	<0.001
Kif22	Kinesin family member 22	–2.0	<0.001
Cellular component – supramolecular complex			
Col1a1	Collagen type I alpha 1 chain	–15.0	<0.001
Thsd4	Thrombospondin type 1 domain containing 4	–11.5	<0.001
Pgm5	Phosphoglucomutase 5	–8.4	<0.001
Fkbp1b	FK506 binding protein 1B	–6.7	<0.001
Fbn1	Fibrillin 1	–3.4	<0.001
Nusap1	Nucleolar and spindle associated protein 1	–3.3	<0.001
Cryab	Crystallin alpha B	–3.2	<0.001
Col4a6	Collagen type IV alpha 6 chain	–3.2	<0.001
Tubb3	Tubulin beta 3 class III	–3.1	<0.001
Psrc1	Proline and serine rich coiled-coil 1	–2.8	<0.001
Ckap2	Cytoskeleton associated protein 2	–2.8	<0.001
Plk1	Polo like kinase 1	–2.7	<0.001
Kif2c	Kinesin family member 2C	–2.7	<0.001
Cdk1	Cyclin dependent kinase 1	–2.5	<0.001
Gtse1	G2 and S-phase expressed 1	–2.5	<0.001
Knstrn	Kinetochore localized astrin/SPAG5 binding protein	–2.5	<0.001
Rac3	Ras-related C3 botulinum toxin substrate 3 (rho family, small GTP binding protein Rac3)	–2.5	0.070
Bfsp2	Beaded filament structural protein 2	–2.5	<0.001
Lmnb1	Lamin B1	–2.4	<0.001
Krt80	Keratin 80	–2.4	0.095
Synm	Synemin	–2.4	<0.001
Spag5	Sperm associated antigen 5	–2.3	<0.001
Tube1	Tubulin epsilon 1	–2.2	<0.001
Ccsap	Centriole, cilia and spindle associated protein	–2.2	<0.001
Kifc1	Kinesin family member C1	–2.2	<0.001
Carmil1	Capping protein regulator and myosin 1 linker 1	–2.1	<0.001
Kif20a	Kinesin family member 20A	–2.1	<0.001
Kif18a	Kinesin family member 18A	–2.1	<0.001

Table III. *Continued*

Table III. *Continued*

Gene symbol	Description	Fold[b]	*p*-Value[c]
Tpx2	TPX2, microtubule nucleation factor	–2.0	<0.001
Krt25	Keratin 25	–2.0	<0.001
Kif22	Kinesin family member 22	–2.0	<0.001
Cellular component – supramolecular fiber			
Col1a1	Collagen type I alpha 1 chain	–15.0	<0.001
Thsd4	Thrombospondin type 1 domain containing 4	–11.5	<0.001
Sorbs2	Sorbin and SH3 domain containing 2	–10.0	<0.001
Edn1	Endothelin 1	–6.9	<0.001
Adamts14	ADAM metallopeptidase with thrombospondin type 1 motif 14	–5.5	<0.001
Wnt4	Wnt family member 4	–4.8	<0.001
Cdh5	Cadherin 5	–4.1	<0.001
Apc	APC, WNT signaling pathway regulator	–3.4	<0.001
Ltbp2	Latent transforming growth factor beta binding protein 2	–3.3	<0.001
Cryab	Crystallin alpha B	–3.2	<0.001
Ctgf	Connective tissue growth factor	–3.1	<0.001
Tgfb2	Transforming growth factor beta 2	–3.1	<0.001
Efemp2	EGF containing fibulin like extracellular matrix protein 2	–3.0	0.001
Psrc1	Proline and serine rich coiled-coil 1	–2.8	0.001
Fbxo5	F-box protein 5	–2.8	<0.001
Ckap2	Cytoskeleton associated protein 2	–2.8	<0.001
Ptger4	Prostaglandin E receptor 4	–2.7	<0.001
Kif2c	Kinesin family member 2C	–2.7	<0.001
Pdgfra	Platelet derived growth factor receptor alpha	–2.6	<0.001
Phldb2	Pleckstrin homology like domain family B member 2	–2.6	<0.001
Cdc42ep2	CDC42 effector protein 2	–2.5	<0.001
Ldlr	Low density lipoprotein receptor	–2.5	<0.001
Pde2a	Phosphodiesterase 2A	–2.4	<0.001
Loxl3	Lysyl oxidase like 3	–2.3	<0.001
Kiaa1211	KIAA1211 ortholog	–2.2	<0.001
Coro2b	Coronin 2B	–2.2	<0.001
Ccsap	Centriole, cilia and spindle associated protein	–2.2	<0.001
P3h4	Prolyl 3-hydroxylase family member 4 (non-enzymatic)	–2.2	<0.001
Carmil1	Capping protein regulator and myosin 1 linker 1	–2.1	<0.001
Kif18a	Kinesin family member 18A	–2.1	<0.001
LOC101839568	Cytochrome P450 1B1	–2.1	0.039
Krt25	Keratin 25	–2.0	<0.001
Kif24	Kinesin family member 24	–2.0	<0.001
Molecular function – tubulin binding			
Kif26a	Kinesin family member 26A	–5.7	<0.001
Gas2	Growth arrest specific 2	–3.6	0.031
Nusap1	Nucleolar and spindle associated protein 1	–3.3	<0.001
Cep70	Centrosomal protein 70	–3.0	<0.001
Psrc1	Proline and serine rich coiled-coil 1	–2.8	0.001
Prc1	Protein regulator of cytokinesis 1	–2.8	<0.001
Plk1	Polo like kinase 1	–2.7	<0.001
Kif2c	Kinesin family member 2C	–2.7	<0.001
Kif20b	Kinesin family member 20B	–2.5	<0.001
Kif15	Kinesin family member 15	–2.4	<0.001
Pde4b	Phosphodiesterase 4B	–2.4	<0.001
Spag5	Sperm associated antigen 5	–2.3	<0.001
Mdm1	Mdm1 nuclear protein	–2.3	<0.001
Ccsap	Centriole, cilia and spindle associated protein	–2.2	<0.001
Kif18b	Kinesin family member 18B	–2.2	<0.001
Kifc1	Kinesin family member C1	–2.2	<0.001
Kif20a	Kinesin family member 20A	–2.1	<0.001
Kif18a	Kinesin family member 18A	–2.1	<0.001
Dpysl2	Dihydropyrimidinase like 2	–2.0	<0.001
Kif24	Kinesin family member 24	–2.0	<0.001
Kif22	Kinesin family member 22	–2.0	<0.001

Table III. *Continued*

Table III. *Continued*

Gene symbol	Description	Fold[b]	*p*-Value[c]
Molecular function – microtubule binding			
Kif26a	Kinesin family member 26A	–5.7	<0.001
Gas2	Growth arrest specific 2	–3.6	0.031
Nusap1	Nucleolar and spindle associated protein 1	–3.3	<0.001
Psrc1	Proline and serine rich coiled-coil 1	–2.8	0.001
Prc1	Protein regulator of cytokinesis 1	–2.8	<0.001
Plk1	Polo like kinase 1	–2.7	<0.001
Kif2c	Kinesin family member 2C	–2.7	<0.001
Kif20b	Kinesin family member 20B	–2.5	<0.001
Kif15	Kinesin family member 15	–2.4	<0.001
Spag5	Sperm associated antigen 5	–2.3	<0.001
Mdm1	Mdm1 nuclear protein	–2.3	<0.001
Ccsap	Centriole, cilia and spindle associated protein	–2.2	<0.001
Kif18b	Kinesin family member 18B	–2.2	<0.001
Kifc1	Kinesin family member C1	–2.2	<0.001
Kif20a	Kinesin family member 20A	–2.1	<0.001
Kif18a	Kinesin family member 18A	–2.1	<0.001
Dpysl2	Dihydropyrimidinase like 2	–2.0	<0.001
Kif24	Kinesin family member 24	–2.0	<0.001
Kif22	Kinesin family member 22	–2.0	<0.001

HaPC, Hamster pancreatic cancer; FxOH, fucoxanthinol. [a]Among all 1,213 genes significantly changed, down-regulated genes classified to biological process [1 gene ontology (GO) term], cellular component (3 GO terms) and molecular function (2 GO terms) in GO term analysis are showed. [b]Fold change of gene expression in FxOH-treated HaPC5 cells in comparison with that of control cells. [c]Significant difference between HaPC5 cells with and without FxOH treatments by an exact test on edgeR.

genes and signal transduction pathways. This is the first study suggesting the anti-proliferating effect of FxOH on cell lines from a hamster pancreatic cancer model.

No associations between pathological findings and growth inhibition in FxOH-treated HaPC-1–6 were observed (Table I and Figure 2). However, FxOH significantly suppressed the growth of HaPC-1, -4, -5, and -6 cells among the six types of HaPC cells (Figure 2). Thus, we decided to elucidate the molecular mechanisms of the growth inhibitory effects using FxOH-treated HaPC-5 cells, which showed the highest growth inhibition. For example, 5.0 μM FxOH treatment significantly induced apoptosis in HaPC-5 cells with G_1 phase arrest (Figure 3B).

Transcriptome analysis revealed that FxOH treatment significantly inhibited gene sets for cell cycle, cell division, chemokine, cadherin, extracellular matrix, integrin, actin polymerization, microtubule organization, and the pathways of the renin–angiotensin system, MAPK, TGF-β, and Wnt in HaPC-5 cells (Figure 6, Tables III and IV). On the other hand, few up-regulated genes involved in apoptosis induction were found (Figure 5 and Table II). Based on these transcriptome profiles, we confirmed alterations in the levels of proteins related to cell cycle, chemokines, adhesion, apoptosis, and pathways for PI3K/AKT, MAPK, and TGF-β by western blotting analysis. Western blotting analysis showed that the levels of cyclin D1, cyclin B1, CXCR7, integrin α5, pFAK(Tyr^{397}), pPaxillin(Tyr^{31}), pAKT(Ser^{473}), and pSmad2 ($Ser^{465/467}$) were decreased, and pERK1/2(Thr^{202}/Tyr^{204}) and cleaved caspase-3(p17/p19) levels were increased in FxOH-treated HaPC-5 cells (Figure 8).

In the present study, FxOH treatment inhibited the cell cycle in HaPC-5 cells (Figures 3B and 8). The effects of cell cycle arrest by FxOH or Fx in cancer cells were previously found in various cancer cell lines (20, 28, 29). As was observed in pancreatic cancer cells, cell-cycle arrest followed by apoptosis induction may be a common feature of various cancer cells.

In the present experiments, we especially focused on the expression levels of CXC motif chemokine receptor 4 (*Cxcr4*) and atypical chemokine receptor 3 (*Ackr3*, also called as *Cxcr7*) with the GO term for cell surface in cellular component. Regarding CXCR7, a significant decrease in *Ackr3* (*Cxcr7*) gene expression was also identified (Figure 7). FxOH treatment also decreased CXCR7 expression but not CXCR4 in HaPC-5 cells (Figure 8). CXCR7, a member of the chemokine receptors, is known to interact with homeostatic and inflammatory chemokines, although CXCR4 interacts only with homeostatic chemokines (30). CXCR7 has been reported to enhance cell growth, migration and metastasis, induction of angiogenesis, homing of immune cells, recruitment of β-arrestin, release of interleukin, and activation of the vascular endothelial growth

Table IV. *Down-regulated genes in HaPC-5 cells treated with FxOH*[a].

Gene symbol	Description	Fold[b]	*p*-Value[c]
Cellular component – cell surface			
Lipg	Lipase G, endothelial type	–12.7	<0.001
Fut4	Fucosyltransferase 4	–10.3	<0.001
Anpep	Alanyl aminopeptidase, membrane	–9.0	0.003
Lbp	Lipopolysaccharide binding protein	–7.0	<0.001
Itgb8	Integrin subunit beta 8	–5.8	<0.001
Folh1b	Folate hydrolase 1B	–5.0	<0.001
Adamts9	ADAM metallopeptidase with thrombospondin type 1 motif 9	–4.9	0.003
Bgn	Biglycan	–4.2	<0.001
Cdh5	Cadherin 5	–4.1	<0.001
Mxra8	Matrix remodeling associated 8	–3.7	<0.001
Cxcr4	C-X-C motif chemokine receptor 4	–3.5	<0.001
Abcc2	ATP binding cassette subfamily C member 2	–3.5	<0.001
Pcsk6	Proprotein convertase subtilisin/kexin type 6	–3.4	<0.001
Adamts7	ADAM metallopeptidase with thrombospondin type 1 motif 7	–3.3	<0.001
Ephb6	EPH receptor B6	–3.2	<0.001
Aqp11	Aquaporin 11	–3.2	<0.001
Sulf2	Sulfatase 2	–3.2	<0.001
Gpc4	Glypican 4	–2.7	<0.001
Itgb4	Integrin subunit beta 4	–2.7	<0.001
Pdgfra	Platelet derived growth factor receptor alpha	–2.6	<0.001
Efna5	Ephrin A5	–2.6	<0.001
Rtn4r	Reticulon 4 receptor	–2.6	<0.001
Ldlr	Low density lipoprotein receptor	–2.5	<0.001
Ptprj	Protein tyrosine phosphatase, receptor type J	–2.3	<0.001
Vasn	Vasorin	–2.3	<0.001
Rgma	Repulsive guidance molecule family member a	–2.2	<0.001
Ackr3	Atypical chemokine receptor 3	–2.1	<0.001
Sparc	Secreted protein acidic and cysteine rich	–2.1	<0.001
Csf1r	Colony stimulating factor 1 receptor	–2.0	<0.001
Ramp2	Receptor activity modifying protein 2	–2.0	0.017
Rtn4rl1	Reticulon 4 receptor like 1	–2.0	<0.001

HaPC, Hamster pancreatic cancer; FxOH, fucoxanthinol. [a]Among 1,213 genes with significantly changed in expression levels, 32 down-regulated genes classified as cell surface among cellular components in the gene ontology term analysis are shown. [b]Fold change in gene expression in FxOH-treated HaPC-5 cells in comparison with that of control cells. [c]Significant difference between HaPC-5 cells with and without FxOH treatments using an exact test in edgeR.

factor, PI3K/AKT, mammalian target or rapamycin, and MAPK pathways (31, 32). CXCR7 is highly expressed in both pancreatic cancer tissue and pancreatic cancer cell lines (33). This protein expression is positively associated with poor prognosis in pancreatic cancer patients (32, 34-36).

FxOH treatment also decreased the expression of integrin α5, and the activation of FAK and Paxillin, which are downstream regulators of integrins, in HaPC-5 cells (Figure 8). Anoikis is caspase-dependent apoptosis that happens after the detachment of cancer cells from the extracellular matrix *via* attenuation of integrin signaling along with the suppression of PI3K/AKT, MAPK, and TGF-β signals (37-39). Our previous studies demonstrated that Fx and FxOH induce anoikis in murine colorectal tissue and in colon cancer DLD-1 cells, respectively, through suppression of

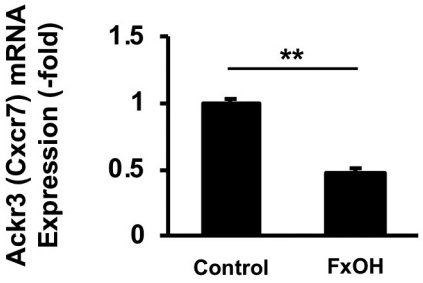

Figure 7. *Effect of fucoxanthinol (FxOH) on Ackr3 gene expression in HaPC-5 cells. HaPC-5 cells were treated with 5.0 μM FxOH for 1 day. Ackr3 mRNA expression was determined using quantitative PCR. Expression was normalized to Gapdh expression levels. Ackr3 mRNA expression in the control cells was set as 1.0. Means±SE (n=3). **p<0.01.*

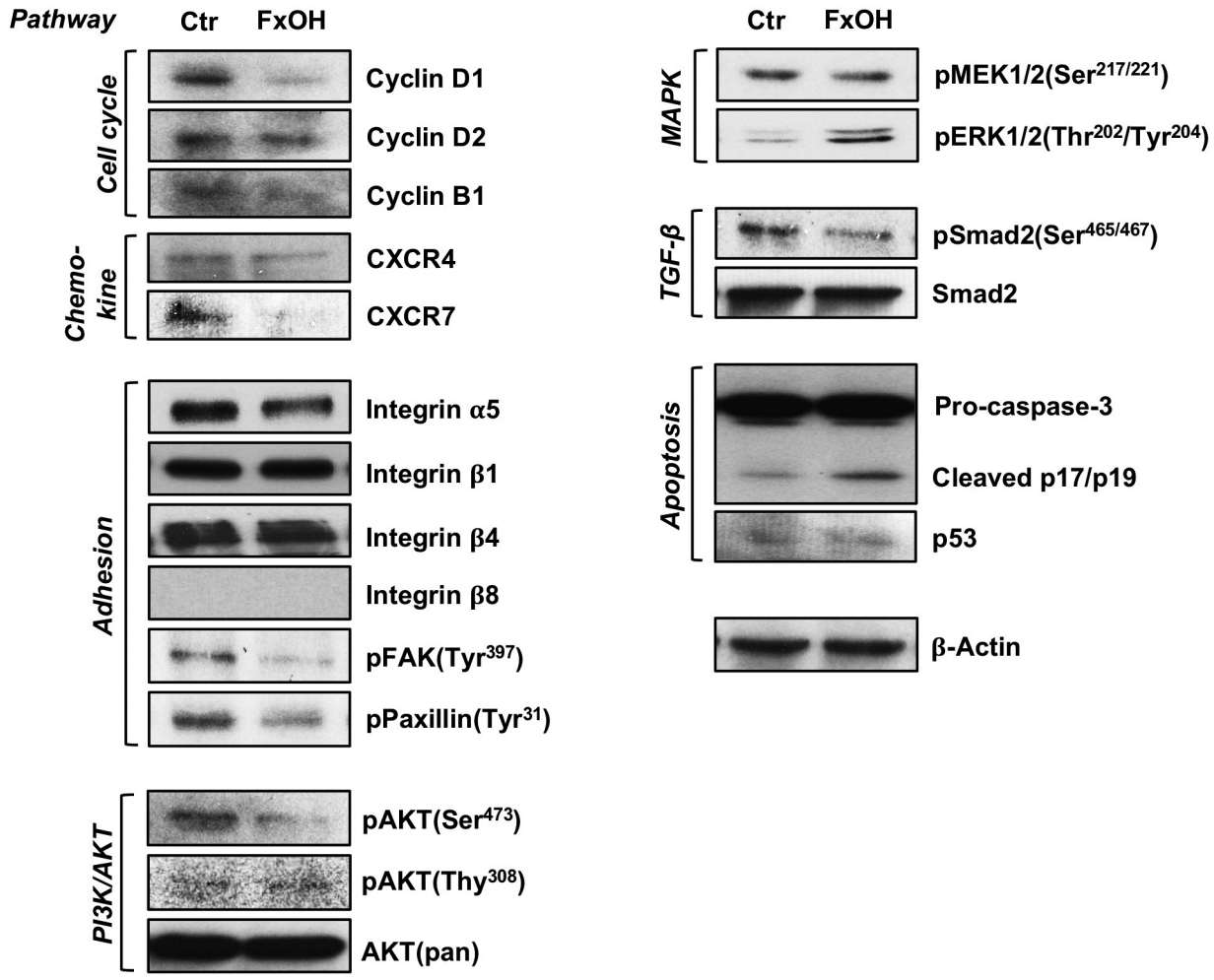

Figure 8. *Effect of fucoxanthinol (FxOH) on protein expression levels in HaPC-5 cells. HaPC5 cells were treated with 5.0 μM FxOH for 1 day, and protein expression levels were evaluated using western blotting. Levels of indicated protein are shown with β-actin (internal control).*

integrin signals (25, 26). FxOH is also known to down-regulate many cytoskeletal genes in HaPC-5 cells (Figure 6, Tables III and IV). Taking into consideration that integrins interact with the extracellular matrix and intracellular cytoskeleton, and promote migration/metastasis in cancer cells (40-42), it was suggested that FxOH may suppress firstly CXCR7 and integrin α5 on cellular membrane, and then alter the down-streams of PI3K/AKT, FAK/Paxillin, TGF-β and cell cycle signals, actin polymerization and microtubule organization in HaPC-5 cells, followed by apoptosis and anoikis inductions.

A previous study revealed the growth inhibition by Fx in a human pancreatic cancer MIA PaCa-2 cell (43). On the other hands, crocetinic acid, a carotenoid having two carboxylic acids, induced apoptosis in human pancreatic cancer MIA PaCa-2 cells and suppressed a tumorigenesis in the xenograft model mice by inhibiting EGFR and AKT pathways (44). Lycopene, a hydrophobic carotenoid, could induce apoptosis in human pancreatic cancer PANC-1 cells by inhibiting the activation of NF-κB signals through suppression of reactive oxygen species (45). Further studies are needed to elucidate the effects of FxOH in pancreatic cancer cells.

In conclusion, FxOH modified the expression levels of 1,213 genes and induced apoptosis in a pancreatic ductal adenocarcinoma HaPC-5 cell cloned from a pancreatic cancer hamster model. Moreover, the protein expression and activation levels of cyclin D1, cyclin B1, CXCR7, integrin α5, pFAK(Tyr^{397}), pPaxillin(Tyr^{31}), pAKT(Ser^{473}), and pSmad2(Ser$^{465/467}$), which play central roles in cell cycle, chemokine, adhesion, and TGF-β signals were significantly suppressed. Our findings suggested that FxOH may have

high potential as a cancer chemopreventive agent in a hamster pancreatic carcinogenesis model.

Conflicts of Interest

No conflicts of interest.

Authors' Contributions

M. Terasaki conceived and designed the study and wrote the paper. M. Terasaki, Y.N., W. M., T. T. and M. Takahashi performed the experiments. A. K., H. K., M. K., H. M., K. M., M. M. and M. Takahashi reviewed and edited the manuscript. All Authors read and approved the final manuscript.

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