Tropical Journal of Pharmaceutical Research January 2023; 22 (1): 9-14
ISSN: 1596-5996 (print); 1596-9827 (electronic)

Available online at http://www.tjpr.org
http://dx.doi.org/10.4314/tjpr.v22i1.2

Original Research Article

Fucoxanthin inhibits the proliferation and stem cell formation of oral squamous cell carcinoma (OSCC) cells by regulating JAK/STAT3 pathway

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Sent for review: 28 September 2022 *Revised accepted: 25 December 2022*

Abstract

Purpose: *To investigate the effect and regulatory mechanism of fucoxanthin (FX) on oral squamous cell carcinoma (OSCC).*
Methods: *Human OSCC SCC9 and Cal27 cells were treated with different concentrations of FX (7.5, 15, and 30 μM) to determine cell viability, number of colonies, and apoptosis rate using methyl thiazolyl diphenyl-tetrazolium bromide (MTT) assay, crystal violet staining, and flow cytometry, respectively. In addition, the number of spheres of OSCC cells was determined by a cell sphere-forming assay. The stem cells and levels of pathway-related proteins were evaluated by western blotting.*
Results: *Fucoxanthin decreased SCC9 and Cal27 cell viability and the number of colonies, but increased apoptosis in a dose-dependent manner. The expression levels of SOX2 and POU5F1 were down-regulated, while the number of spheres was reduced in SCC9 and Cal27 cells treated with 7.5 or 15 μM of FX ($p < 0.05$). Moreover, FX attenuated p-JAK/JAK and p-STAT3/STAT3 expression levels in a dose-dependent manner ($p < 0.05$).*
Conclusion: *Fucoxanthin accelerates apoptosis and inhibits cell mobility and OSCC stem cell formation by suppressing JAK/STAT3 pathway in OSCC, thus providing an experimental basis for research on the anti-tumor effect of kelp extract and the development of new drugs from marine plants.*

Keywords: *Fucoxanthin (FX), Oral squamous cell carcinoma (OSCC), JAK/STAT3 pathway, Tumor stem cell, Apoptosis*

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INTRODUCTION

Oral squamous cell carcinoma (OSCC) is one of the most common head and neck cancers in humans, and OSCC patients have a low 5-year survival rate [1]. Smoking, alcohol consumption, betel nut chewing, and human papillomavirus infection are common risk factors for OSCC [2]. Currently, OSCC is treated with traditional surgical procedures which are supplemented with radiotherapy chemotherapy. High recurrence rates and insensitivity to treatment are the main reasons for the low survival rate of OSCC patients [3]. The cause of OSCC treatment failure is thought to be related to the presence of a subpopulation of cells called tumor

stem cells, within the tumor. The OSCC stem cells exhibit stem cell-like properties that enhanced the malignant biological behavior of OSCC [4,5]. Therefore, it is very important to study OSCC in-depth and to find new treatments for OSCC.

Although many anti-tumor drugs are derived from natural compounds, most are obtained from microbial or plant extracts. For example, the bioactive principle of fucoxanthin (FX), a carotenoid, is extracted from *Laminaria japonica* kelp [6]. Fucoxanthin has a variety of biological attributes, including anti-inflammatory, weight loss, and potent anti-tumor effects. In addition, FX inhibits the invasive ability of non-small cell lung cancer cells and it increases the sensitivity of tumor cells to the drug Gefitinib [6]. Fucoxanthin inhibits the proliferation of lymphatic vessel endothelial cells and the formation of tubular structures *in vitro* and *in vivo*, thereby suppressing the malignant phenotype of breast cancer cells [7]. Furthermore, FX promotes apoptosis of pharyngeal cancer cells by inducing an autophagic mechanism [8]. However, no studies related to the effects and mechanisms of FX action on OSCC have been published.

In the present study, OSCC cell lines SCC9 and Cal27 were used to study the *in vitro* effects of FX on cell growth to validate pathways that may be involved in this process. The results of this study will provide new strategies for the treatment of OSCC.

EXPERIMENTAL

Cell culture and treatment

The human OSCC cell lines, SCC-9 and Cal27, were purchased from Mingzhoubio (Ningbo, China) and identified by short tandem repeat authentication. The SCC-9 and Cal27 cells were cultured in Dulbecco's Modified Eagle Medium (DMEM; Regal, Shanghai, China) supplemented with 10 % fetal bovine serum (Solarbio, Beijing, China) and 1 % penicillin/streptomycin (Solarbio) at 37 °C until the logarithmic period. Different concentrations (7.5, 15, and 30 μM) of FX were used to treat SCC-9 and Cal27 cells for follow-up logarithmic experiments.

Cell viability

Methylthiazolyldiphenyl-tetrazolium bromide (MTT) assays were performed to determine cell viability. Fucoxanthin-treated cells were added to the MTT solution (10 mg/mL, Beyotime, Shanghai, China), incubated for 4 h, and then added together with dimethyl sulfoxide for 10 min. The absorbance at 450 nm was measured using a spectrophotometer (Laspec, China).

Cell cloning

The FX-treated cells were digested with 0.25 % trypsin (Beyotime) and suspended in DMEM. The cell suspension was diluted multiple times in gradient. Then, cells (< 100) were inoculated onto a culture dish containing a 37 °C pre-heated medium which was gently rotated to disperse the cells evenly, and then cultured at 37 °C for 2 weeks. The supernatant in the culture dish was discarded and the cells were carefully washed twice with phosphate-buffered saline (PBS). The washed cells were fixed with pure methanol for 15 min and stained with crystal violet for 15 min. The cells were photographed and counted under a microscope (Leica, Germany).

Apoptosis

The FX-treated cells were stained using a Hoechst Staining Kit (Beyotime), digested with 0.25 % trypsin (Beyotime). Then, cells were incubated with Annexin-V and Propidium solution (Beyotime) for 20 min. The apoptosis ratio was determined using flow cytometry (Beckman Coulter, USA). The results are expressed as the sum of early and late apoptotic.

Western blotting

The total protein was extracted from FX-treated cells via protein SDS-PAGE loading buffer (TaKaRa, Dalian, China), and then transferred onto polyvinylidene fluoride membranes. The proteins were incubated with primary antibodies, such as sex-determining region Y box 2 (SOX2, ab92494, Abcam, Cambridge, UK), POU class 5 homeobox 1 (POU5F1, ab230429), tyrosine kinase (JAK, ab108596), phosphorylated-JAK (p-JAK, ab32101), signal transducers and activators of transcription 3 (STAT3, ab68153), p-STAT3 (ab267373), and β-actin (ab8226). Then the proteins were incubated with horseradish peroxidase-labeled secondary antibody (ab150080, Abcam), rinsed in the blocking solution and imaged by using imaging system.

Cell sphere-forming assay

The FX-treated cells were digested with 0.25 % trypsin (Beyotime) and washed twice with PBS. Cells were re-suspended in stem cell culture medium and added to the 6-well plate for 10 days. Next, the spheroidization state of cells was examined, the number of spheres per 100 cells was counted, and sphere formation efficiency (SFE) was calculated.

Statistical analysis

The results obtained were analyzed using SPSS 22.0 software (SPSS Inc, USA) and the data presented as mean ± standard deviation (SD). Differences were considered statistically significant at $p < 0.05$.

RESULTS

Fucoxanthin inhibited cell growth and promoted apoptosis

The chemical structural formula of FX (Figure 1 A) contains acetoxy, two hydroxyl groups, and carbonyl groups. The influence of FX in OSCC cells was studied by MTT assay, crystal violet staining, and flow cytometry. The MTT assay results show that cell viability decreased gradually with increase of FX concentration in SCC9 and Cal27 cells ($p < 0.005$, Figure 1 B). Similarly, the number of colonies in groups treated with 7.5, 15, and 30 μM of FX was less than in groups without FX ($p < 0.005$, Figure 1 C). In addition, flow cytometry results (Figure 2) show that the apoptosis rate of SCC9 and Cal27 cells increased significantly after FX treatment ($p < 0.005$). These results indicate that FX suppressed cell viability and cloning and accelerated apoptosis.

Fucoxanthin inhibited the characteristics of OSCC stem cells

It has been said that OSCC produces stem cells and that OSCC stem cells are highly tumorigenic. Therefore, the effect of FX on OSCC stem cells were investigated. The relative expression levels of SOX2 and POU5F1, (transcription factors associated with stem cells) were significantly decreased ($p < 0.005$) in FX-treated SCC9 and Cal27 cells (Figure 3 A). The higher the concentration of FX, the expression level of SOX2 and POU5F1 was down-regulated. In addition, cell sphere-forming assay results show that the number of spheres were reduced in SCC9 and Cal27 cells in a dose-dependent manner ($p < 0.005$, Figure 3 B). Overall, the data demonstrated that FX suppressed the formation of OSCC stem cells.

Fucoxanthin inhibited JAK/STAT3 pathway

A protein level associated with JAK/STAT3 signaling pathway was also determined in this study (Figure 4). The results show that p-JAK/JAK and p-STAT3/STAT3 expression levels were significantly inhibited in stimulated cell concentration of FX in SCC9 and Cal27 cells ($p < 0.005$), demonstrating that FX attenuated the activation of the JAK/STAT3 pathway.

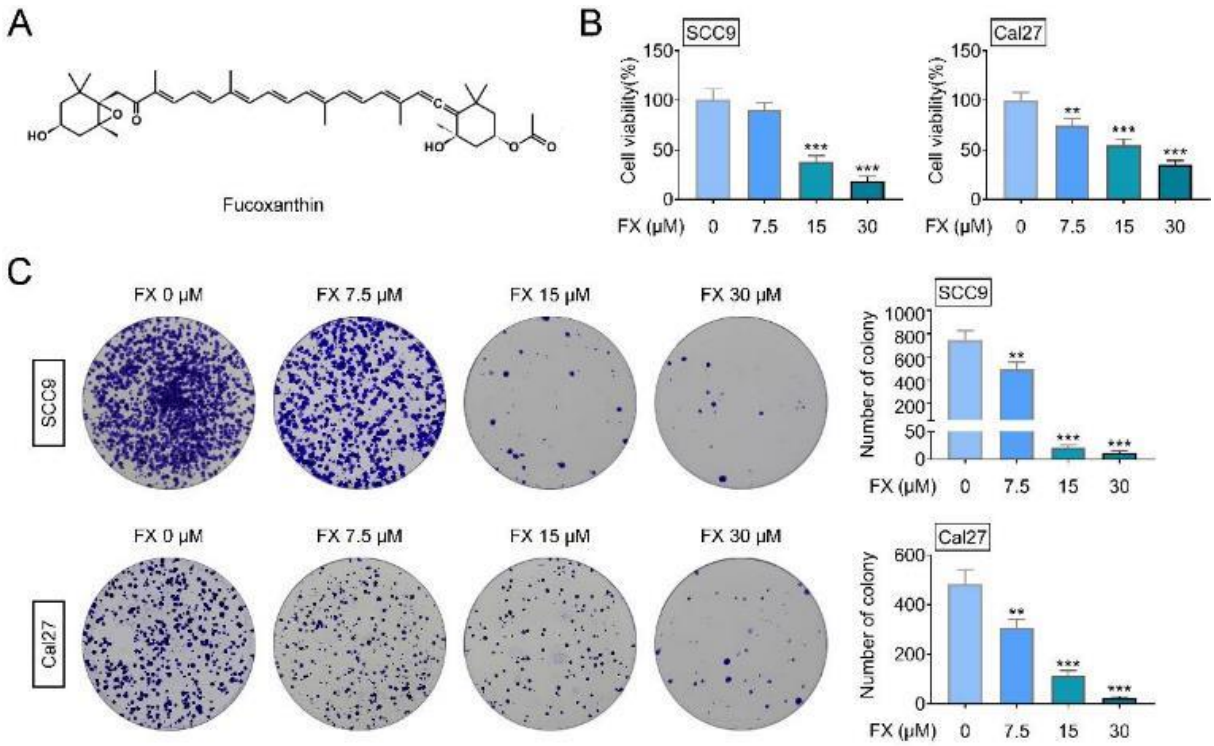

Figure 1: Fucoxanthin (FX) inhibited cell growth. SCC9 and Cal27 cells were treated with different concentrations (7.5, 15, and 30 μM) of FX. (A) The chemical structural formula of FX. (B) Cell viability was determined using MTT assay. (C) The number of colonies was determined using crystal violet staining. ** $P < 0.01$; ***$p < 0.005$

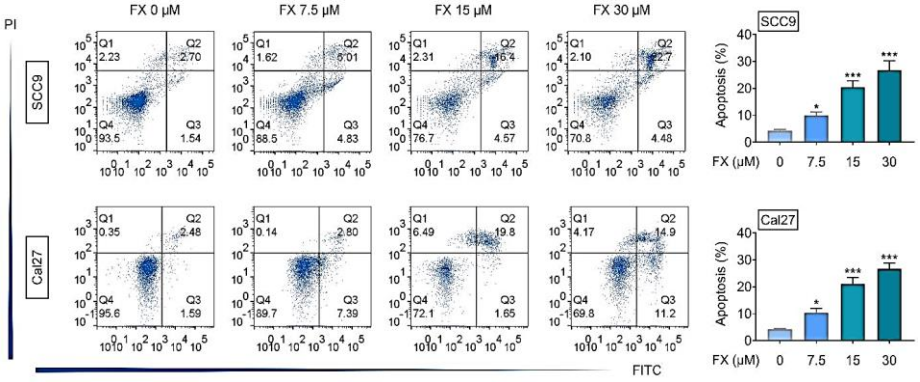

Figure 2: Fucoxanthin (FX) enhanced apoptosis. SCC9 and Cal27 cells were treated with different concentrations (7.5, 15, and 30 μM) of FX. Apoptosis was determined using flow cytometry. *P < 0.05; ** p < 0.01; ***p < 0.005; * represents the comparison with the group without FX

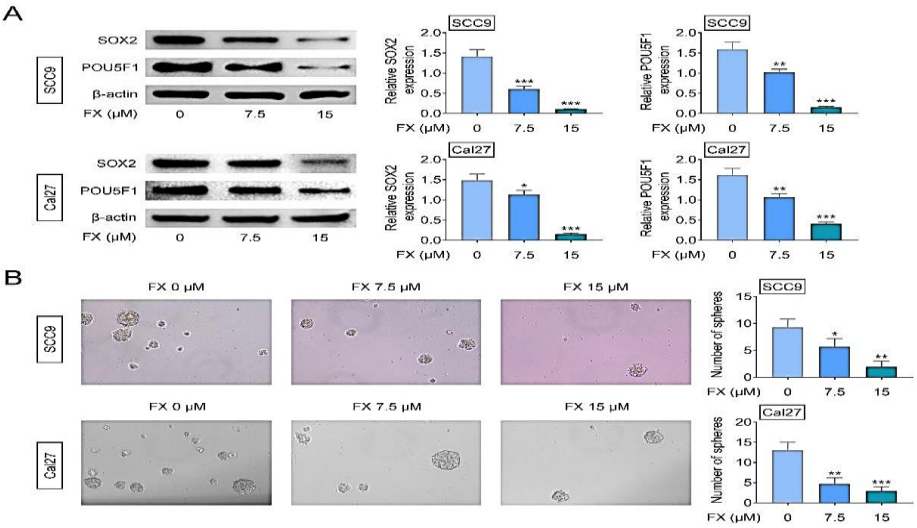

Figure 3: Fucoxanthin (FX) inhibited the characteristics of OSCC stem cells. SCC9 and Cal27 cells were treated with different concentrations (7.5 and 15 μM) of FX. (A) The protein levels of SOX2 and POU5F1 were determined using western blotting. (B) The number of spheres was determined using a cell sphere-forming assay. *P < 0.05, **p < 0.01, ***p < 0.005; * represents the comparison with the group without FX

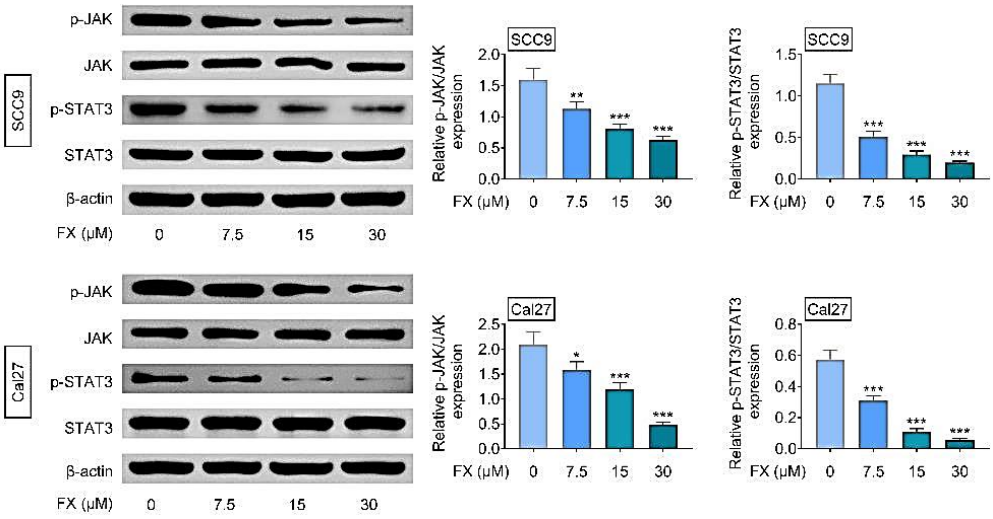

Figure 4: Fucoxanthin (FX) inhibited the JAK/STAT3 pathway. SCC9 and Cal27 cells were treated with different concentrations (7.5, 15, and 30 μM) of FX. The protein levels of JAK, p-JAK, STAT3, and p-STAT3 were determined using western blotting. *P < 0.05; **p < 0.01; ***p < 0.005; * represents the comparison with the group without FX

DISCUSSION

Oral squamous cell carcinoma (OSCC) is characterized by strong aggressiveness, early recurrence, and metastasis [10]. Currently, there are no effective treatments for OSCC. It was first found that the anti-tumor effect of FX reduce the proliferation of neuroblastoma cells by more than 60 % after incubation and blocked the neuroblastoma cell cycle in G0/G1 phase, thus showing the anti-tumor effect of FX [11]. It is shown that FX inhibited the growth of human hepatocellular carcinoma HepG2 cells, thereby blocking the cells in G0/G1 phase and inhibiting phosphorylation of the Ser780 site of Rb protein [12]. The study reported that the apoptosis-inducing effect of FX on an acute myeloid leukemia HL-60 cell line reached a plateau at 24 h after activation, suggesting that the mechanism by which FX exerts its tumor suppressive effect involves induction of apoptosis [13]. Overall, these previous studies demonstrate that FX achieves its anti-tumor effect by inhibiting cell proliferation and promoting apoptosis. The results of the present study are identical to those of previous studies in that FX inhibited the viability and cell cloning of OSCC cell lines SCC-9 and Cal27 and boosted their apoptosis. The emergence of the tumor stem cell theory holds promise for discovering ways to eradicate OSCC. It has been shown that OSCC produces stem cells and that OSCC stem cells are highly tumorigenic and play a role in tumor differentiation, treatment resistance, recurrence, and metastasis [10]. These stem cells are capable of self-renewal growth and produce heterogeneous tumor cells that sustain tumor development. In addition, cancer stem cells are highly tolerant to chemotherapeutic drugs. Interestingly, FX has been found to suppress stem cell-like cell tumorigenicity and tumorsphere formation in colon cancer [14]. Similarly, this study shows that FX reduced the number of spheres of OSCC cells. In addition, stem cell markers are important ways to target OSCC. Meanwhile, SOX2 is a potential therapeutic target for stem cell identification, isolation, and treatment of OSCC [15]. The POU5F1, also known as Oct4, is a transcription factor present in embryonic stem cells, and is associated with the pluripotency, proliferative potential, and self-renewal ability of germ cells and embryonic stem cells, and also maintains embryonic stem cells in an undifferentiated state [16]. In recent years, POU5F1 was found to be differentially expressed in malignant tumors. In this study, POU5F1 and SOX2 expressions was depressed after the stimulation of FX in OSCC cells, indicating that FX inhibited the formation of OSCC stem cells. Another study found that FX blocked the activation of the JAK/STAT3 signal pathway in gastric cancer [9]. Moreover, miR-769-5p inhibits OSCC cell development by targeting the JAK1/STAT3 pathway [17]. Activation of the JAK/STAT3 pathway led to an OSCC malignant process [18]. The JAK/STAT3 pathway is closely related to the characteristics of tumor stem cells [19]. The results of the present experiments show that FX inhibited the level of p-JAK/JAK and p-STAT3/STAT3, indicating that the JAK/STAT3 pathway is important in the process of FX inhibiting OSCC.

CONCLUSION

Fucoxanthin is a new and effective inhibitory component of OSCC which accelerates apoptosis while inhibiting cell mobility and OSCC stem cell formation by suppressing JAK/STAT3 pathway in OSCC. The findings of this study not only open up new avenues of treatment for patients with OSCC but also provide an experimental basis for research on the anti-tumor effect of kelp extract, and the development of new drugs from marine plants.

DECLARATIONS

Acknowledgements

None provided.

Funding

This work was supported by the Graduate Innovative Research Project of the Heilongjiang University of Chinese Medicine. (Grant no. 2022yjscx018).

Ethical approval

None provided.

Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Conflict of Interest

No conflict of interest associated with this work.

Contribution of Authors

We declare that this work was done by the authors named in this article and all liabilities pertaining to claims relating to the content of this article will be borne by the authors. Xue Feng

and Yu Li designed the study and carried out the experiments. Xue Feng, Yu Li, Lijia Zhang, and Heng Sun supervised the collection, analysis, and interpretation of the data. Xue Feng, Yu Li, and Songjiang Liu prepared the manuscript for publication and reviewed the draft of the manuscript. Xue Feng and Yu Li contributed equally to the work and should be considered co-first authors. All authors read and approved the manuscript.

REFERENCES

1. *Liang Y, Li F, Huang J, Zhang Z. Efficacy of some selected neo-adjuvant chemotherapy regimens in the treatment of advanced oral squamous cell carcinoma, and their effects on immune function. Trop J Pharm Res 2021; 20(11): 2419-2424.*
2. *Singh V, Husain N, Akhtar N, Khan MY, Sonkar AA, Kumar V. p16 and p53 in HPV-positive versus HPV-negative oral squamous cell carcinoma: do pathways differ? J Oral Pathol Med 2017; 46(9): 744-751.*
3. *Naruse T, Yanamoto S, Okuyama K, Yamashita K, Omori K, Nakao Y, Yamada SI, Umeda M. Therapeutic implication of mTORC2 in oral squamous cell carcinoma. Oral Oncol 2017; 65: 23-32.*
4. *Liu X, Fu Y, Huang J, Wu M, Zhang Z, Xu R, Zhang P, Zhao S, Liu L, Jiang H. ADAR1 promotes the epithelial-to-mesenchymal transition and stem-like cell phenotype of oral cancer by facilitating oncogenic microRNA maturation. J E Clin Cancer Res CR 2019; 38(1): 315-315.*
5. *Hu J, Mirshahidi S, Simental A, Lee SC, De Andrade Filho PA, Peterson NR, Duerksen-Hughes P, Yuan X. Cancer stem cell self-renewal as a therapeutic target in human oral cancer. Oncogene 2019; 38(27): 5440-5456.*
6. *Ming JX, Wang ZC, Huang Y, Ohishi H, Wu RJ, Shao Y, Wang H, Qin MY, Wu ZL, Li YY, et al. Fucoxanthin extracted from Laminaria Japonica inhibits metastasis and enhances the sensitivity of lung cancer to Gefitinib. J Ethnopharmacol 2021; 265: 113302.*
7. *Wang J, Ma Y, Yang J, Jin L, Gao Z, Xue L, Hou L, Sui L, Liu J, Zou X. Fucoxanthin inhibits tumour-related lymphangiogenesis and growth of breast cancer. J Cell Mol Med 2019; 23(3): 2219-2229.*
8. *Long Y, Cao X, Zhao R, Gong S, Jin L, Feng C. Fucoxanthin treatment inhibits nasopharyngeal carcinoma cell proliferation through induction of autophagy mechanism. Environ Toxicol 2020; 35(10): 1082-1090.*
9. *Yu RX, Yu RT, Liu Z. Inhibition of two gastric cancer cell lines induced by fucoxanthin involves downregulation of Mcl-1 and STAT3. Hum Cell 2018; 31(1): 50-63.*
10. *Costea DE, Tsinkalovsky O, Vintermyr OK, Johannessen AC, Mackenzie IC. Cancer stem cells - new and potentially important targets for the therapy of oral squamous cell carcinoma. Oral Dis 2006; 12(5): 443-454.*
11. *Okuzumi J, Nishino H, Murakoshi M, Iwashima A, Tanaka Y, Yamane T, Fujita Y, Takahashi T. Inhibitory effects of fucoxanthin, a natural carotenoid, on N-myc expression and cell cycle progression in human malignant tumor cells. Cancer Lett 1990; 55(1): 75-81.*
12. *Liu CL, Huang YS, Hosokawa M, Miyashita K, Hu ML. Inhibition of proliferation of a hepatoma cell line by fucoxanthin in relation to cell cycle arrest and enhanced gap junctional intercellular communication. Chem Biol Interact 2009; 182(2-3): 165-172.*
13. *Hosokawa M, Wanezaki S, Miyauchi K, Kurihara H, Kohno H, Kawabata J, Odashima S, Takahashi K. Apoptosis-inducing effect of fucoxanthin on human leukemia cell line HL-60. Food Sci Technol Res 1999; 5(3): 243-246.*
14. *Terasaki M, Maeda H, Miyashita K, Tanaka T, Miyamoto S, Mutoh M. A marine bio-functional lipid, fucoxanthinol, attenuates human colorectal cancer stem-like cell tumorigenicity and sphere formation. J Clin Biochem Nutr 2017; 61(1): 25-32.*
15. *Zhou C, Yang X, Sun Y, Yu H, Zhang Y, Jin Y. Comprehensive profiling reveals mechanisms of SOX2-mediated cell fate specification in human ESCs and NPCs. Cell Res 2016; 26(2): 171-189.*
16. *Familari M, Au PC, de Iongh RU, Cruz Y, Selwood L. Expression analysis of Cdx2 and Pou5f1 in a marsupial, the stripe-faced dunnart, during early development. Mol Reprod Dev 2016; 83(2): 108-123.*
17. *Zhou Y, Xu XM, Feng Y. MiR-769-5p inhibits cancer progression in oral squamous cell carcinoma by directly targeting JAK1/STAT3 pathway. Neoplasma 2020.*
18. *Zheng M, Cao MX, Yu XH, Li L, Wang K, Wang SS, Wang HF, Tang YJ, Tang YL, Liang XH. STAT3 Promotes Invasion and Aerobic Glycolysis of Human Oral Squamous Cell Carcinoma via Inhibiting FoxO1. Front Oncol 2019; 9: 1175.*
19. *Park SY, Lee CJ, Choi JH, Kim JH, Kim JW, Kim JY, Nam JS. The JAK2/STAT3/CCND2 Axis promotes colorectal Cancer stem cell persistence and radioresistance. J Exp Clin Cancer Res 2019; 38(1): 399.*