Fucoxanthin: a marine carotenoid has anticancer activities and apoptosis-inducing effect (a review)

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Abstract. Fucoxanthin, a natural xanthophyll carotenoid, is generally found in brown seaweeds, such as Sargassum duplicatum, Turbinaria turbinata, Padina australis, Undaria pinnatifida, and Laminaria japonica; and microalga or diatom such as Phaeodactylum tricornutum, Isochrysis galbana and Odontella sinensis. Fucoxanthin is a marine xanthophyll exhibiting several anticancer activities, such as anticancer activities against leukemia, prostate, cervical, hepatoma, colon, and lung cancer. Cancer disease is frequently considered to be a disease of the cell cycle. Then, apoptosis is a dominant form of cell death with particular relevance to cancer, characterized initially by a series of stereotypic morphological changes, such as condensation and fragmentation of chromatin shrinking of cytoplasmic (cell shrinkage), a decrease in cell volume and alterations to the plasma membrane, mitochondrial depolarization, membrane blebbing, and cell packaging into apoptotic bodies or formation of apoptotic bodies. In general, there are four techniques for the detection of apoptosis, namely: (1) morphological changes analysis using an inverted microscope, scanning electron microscope, fluorescent microscope, (2) gel electrophoresis, (3). immunohistochemistry (e.g., analysis of caspase-3), and (4) flow cytometry.

1. Introduction

Fucoxanthin, a natural xanthophyll carotenoid, is generally found in edible brown seaweeds. This compound, together with β-carotene, is considered as one of the most abundant carotenoids in nature. This compound is a marine carotenoid exhibiting several health benefits [1]. Fucoxanthin has remarkable biological properties for human health [2]. It is known that fucoxanthin has antioxidant activity [3-5], antiobesity [6], antidiabetic [7], anti-inflammatory [8], anti-acne [9], neuroprotective [10], and anticancer activities [11-14].

This review is mainly concerned with fucoxanthin sources, anticancer activity, and the apoptosis-inducing effect of fucoxanthin, especially the primary mechanism for apoptosis induction and the techniques detection of apoptosis.

2. Fucoxanthin and its sources

Fucoxanthin is a major marine carotenoid found in edible brown seaweeds such as Undaria pinnatifida [15], Hijkia fusiformis [16], Sargassum duplicatum and Sargassum binderi [17], Turbinaria
turbinata [18] Padina australis [12, 13], Sargassum fulvellum [19] Fucus evanescens [20], and microalga or diatom such as Phaeodactylum tricornutum, Isochrysis galbana and Odontella sinensis. Fucoxanthin is one of the most abundant carotenoids, and contributes more than 10% of the estimated total production of carotenoids in nature [21], especially in the marine environment [22]. Its structure which includes an allenic bond, two hydroxyl, keto and epoxy groups such as 5,6-monoepoxide [23] (figure 1), differs from that of common carotenoids, such as β-carotene and lycopene [24].

Figure 1. Chemical structure of fucoxanthin.

Currently, the most common method for extracting the compound from a natural source is by liquid solvent extraction using hexane, petroleum ether, or toluene. For example, fucoxanthin has been extracted from Laminaria japonica using dimethyl sulfoxide [25] and ethanol [26], Undaria pinnatifida using acetone [27], chloroform/methanol [28], and ethanol [29], Hijikia fusiformis [30], Eisenia bicyclus using acetone [31], Ishige okamurae using methanol/chloroform [32], Sargassum hemiphyllum using ethanol [33], Sargassum binderi, Sargassum playyofillum, Turbinaria turbinata and Padina australis using hexane/aceton [11,12,34].

Furthermore, fucoxanthin isolated from brown seaweed had characteristics such as the TLC results using n-hexane: acetone as the mobile phase with ratio 70/30 and 60/40 % (v/v) have retardation of flow (Rf) values between 0.27 – 0.54 [12,18]. Moreover, fucoxanthin had maximum adsorption on the UV spectrum at 450 nm [12,18,35], IR absorption of fucoxanthin at 1030, 1646, and 3407 cm⁻¹ that indicator of ketone, allenic bond, and hydroxyl, respectively [12,36], and XRD spectrum of fucoxanthin exhibited several characteristic intense peaks at 2θ = 37.9, 38.5, 44.2, 44.6, 64.5, and 65.1°. All these peaks are ascribed to its crystalline nature (a crystalline solid) [12,36].

3. Anticancer Activities and Apoptosis-Inducing Effect of Fucoxanthin

Fucoxanthin is a major carotenoid in brown seaweed [37]. This compound exhibits inhibitory property on colon cancer cells, and this effect was associated with growth arrest [38], inhibited the growth of human neuroblastoma GOTO cells [39]. Furthermore, fucoxanthin exhibited anticancer activities against leukemia [40], colon [38], prostate [41], hepatoma [37], cervical [42], lung [11,12], bladder [43], gastric [44], glioma [45], and osteosarcoma [46]. Hence, fucoxanthin shows excellent promise as chemotherapeutic agents in cancer [1], and this compound could be useful for the treatment and prevention of cancer [47].

3.1. Primary Mechanism for Apoptosis Induction

Apoptosis is a cellular self-destruction mechanism involved in various biological events that play a critical role in development, tissue homeostasis [48], and immune defense of multicellular animals. It is characterized by distinct energy-dependent biochemical mechanisms and morphological characteristics [49] such as cell volume shrinkage, DNA fragmentation, chromatin condensation, and plasma membrane blebbing [50].

There are two primary mechanisms for the induction of apoptosis [48], namely the death receptor-mediated extrinsic pathway (regulated receptor-mediated) and the mitochondria-mediated intrinsic pathway (regulated at the level of the mitochondria) [49]. The intrinsic pathway occurs following the loss of mitochondrial transmembrane potential (MTP), resulting in the release of cytochrome-c into the cytoplasm [51]. The released cytochrome-c binds to apoptotic protease activating factor-1 (Apaf-1) and activates the nucleotide exchange activity of Apaf-1 [52]. Cytochrome-c made complexes with the
cytoplasmic protein Apaf-1 and pro-caspase-9 forming the apoptosome that results in activation of caspase-3 [51] (Figure 2). This caspase-3 is a member of the caspase family and is one of the key executioners of apoptosis [53].

**Figure 2.** Schematic of convergent extrinsic and intrinsic apoptosis signaling to caspase-3 [51]

3.2. Techniques for the detection of apoptosis

In general, there are four techniques for the detection of apoptosis can be summarized as following [54]:

3.2.1. Morphology Analysis.

Apoptosis is a dominant form of cell death, characterized initially by a series of stereotypic morphological changes [55], such as condensation and fragmentation of chromatin [56], shrinking of cytoplasmic (cell shrinkage) [57] and nuclear compartments [58], a decrease in cell volume and alterations to the plasma membrane [11], mitochondrial depolarization [51], membrane blebbing [58], and cell packaging into apoptotic bodies or formation of apoptotic bodies [12,58]. Moreover, Andrade et al. [60] have reported that apoptosis is initiated by cell shrinkage, followed by an intense blebbing activity. Extensive plasma membrane blebbing occurs followed by karyorrhexis and separation of cell fragments into apoptotic bodies during a process called ‘budding’ [49]. Blebbing is a useful marker of apoptosis independently of the apoptogenic stimulus. It has been found associated with apoptotic ladder formation and nuclear fragmentation [61].

Furthermore, different morphological techniques have been developed for detecting apoptosis. Morphological changes in the apoptotic cell might be easily detected under the microscope [62]. The morphological changes in apoptosis are best seen with an electron microscope [12,54]. Apoptotic cells can also be detected with a light microscope using stains or specific dye [62], which bind nucleic acids such as hematoxylin and the Schiff reagent used in the Feulgen reaction [54]. Apoptosis can also be detected by fluorescence microscopy [12,62].

Acridine orange and Hoechst are two fluorochromes used to demonstrate condensed DNA in apoptotic cells [63]. The DNA in the acridine orange staining is yellow-green in healthy cells, while apoptotic DNA stains brightly green and condensed [63,64]. In Hoechst staining, apoptotic cells showed strong blue fluorescence microscope, healthy cells did with only weak fluorescence, while dead cells were not stained in the fluorescence microscopy [65].

3.2.2. Gel electrophoresis.

A series of hallmarks characterize apoptosis and one of them is DNA fragmentation [66]. Apoptosis is a physiological model with characteristic biochemical and cytological features, including DNA digestion into high and low molecular weight fragments [67]. In this case, electrophoretic gel analysis
of DNA has demonstrated that apoptosis is linked to internucleosomal DNA fragmentation [68]. Agarose gel electrophoresis was used to detect DNA fragmentation [69]. However, not all apoptotic cells have this characteristic DNA ladder pattern and some oncotic cells also show this feature [70].

3.2.3. Immunohistochemistry.
A new immunohistochemical technique has been used for the detection of caspase-3 activity [71]. In this case, caspase-3 plays a central role in the apoptotic process and has so far not been detected in oncosis, which makes this technique very specific [72]. Furthermore, Promega Corporation has designed a kit for the detection of caspase-3 and -7 activities. The activities of caspase-3 and -7 can be detected by a Caspase-Glo® 3/7 Assay [73]. The assay provides a luminesogenic caspase-3/7 substrate, containing the tetrapeptide sequence DEVD, a reagent optimized for caspase activity, luciferase activity, and cell lysis. Adding a single Caspase-3/7® 3/7 Reagent in an ‘add-mix-measure’ format results in cell lysis, followed by caspase cleavage of substrate and generation of a ‘glow-type’ luminescent signal, produced by the luciferase enzyme. The luminescence is proportional to the amount of caspase activity present [73].

3.2.4. Flow cytometry.
Flow cytometry is a technique that can be used for detecting diverse parameters of the apoptotic process. Flow cytometry is primarily used to detect phosphatidylserine (PS) located on the inner leaflet of the plasma membrane of living cells' inner leaflet. PS is located on the inner surface of the plasma membrane [75]. Annexin V can identify extracellular phosphatidylserine (PS) and can be used to detect apoptotic cells [76]. The protein annexin V binds PS that is exposed to the outside of the membrane of apoptotic cells [77]. Therefore, the annexin V binding assay provides an excellent indicator of apoptosis’s early stages [75].

Currently, there are some techniques for the detection of the induction-apoptosis effect of fucoxanthin on human cancer cell lines. However, this review discusses four techniques only, such as morphology analysis [10-13], DNA fragmentation by using gel electrophoresis [45], immunohistochemical analysis [12,42,43,45], and flowcytometry analysis [12]. The summary of the techniques for the detection of the induction-apoptosis effect of fucoxanthin on human cancer cell lines is shown in Table 1.

Table 1. The techniques for the detection of the induction-apoptosis effect of fucoxanthin on human cancer cell lines

| Type of Cancer | Human Cell Lines | Techniques for the Detection of Apoptosis | Ref |
|----------------|------------------|----------------------------------------|-----|
| Bladder        | EJ-1 T24         | + + + + +                              | [78]|
| Breast         | MCF-7 HeLa       | + + - + -                              | [79]|
| Cervical       | Caco-2 SGC-7901  | + - + + +                              | [80]|
| Colon          | U251 HL-60       | + + - + +                              | [45]|
| Gastric        |                  | + - - + -                              | [81]|
| Lung           |                  | + - - - -                              | [11]|

Phosphatidylserine (PS) is an aminophospholipid that resides in the plasma membrane of living cells. Therefore, the annexin V binding assay provides an excellent indicator of apoptosis’s early stages [75].
4. Conclusion

Fucoxanthin is a major marine carotenoid found in brown seaweeds. This carotenoid shows excellent promise as chemotherapeutic agents in cancer, and this compound could be useful for the treatment and prevention of cancer.

References

[1] Kumar S R, Hosokawa M and Miyashita K 2013 *Mar. Drugs* **11** 5130
[2] Li Y, Liu Y, Wang Y, Yu Y, Zeng Y, Li L and Wang L *Am. J. Biochem. Biotechnol.* **12** 139
[3] Ha A W, Na S J and Kim W K. 2013 *Nutr. Res. Pract.* **7** 475
[4] Kawee-ai A, Kuntiya A and Kim S M 2013 *Nat. Prod. Commun.* **8** 1381
[5] Sujatha M, Suganya P and Pradeepa V 2017 *Int. J. Innov. Res. Sci. Eng. Technol.* **6** 16734
[6] Hito S and Shimoda H 2017 *Funct. Foods Health Dis.* **7** 246
[7] Maeda H, Kanno S, Kodate M, Hosokawa M, Miyashita K, 2015, *Mar. Drugs*, **13** 4799
[8] Choi J-H, Kim N-H, Kim S-J, Lee H-J and Kim S 2016 *J. Biochem. Mol. Toxicol.* **30** 111
[9] Renhoran M, Noviendri D, Setyaningsih I and Uju 2017 *JPHPI* **20** 370
[10] Zhang L, Wang H, Fan Y, Gao Y, Li X, Hu Z, Ding K, Wang Y and Wang X 2017 *Sci. Rep.* **7** 1
[11] Jaswir I, Noviendri D, Salleh M H and Miyashita K 2011 *Afr. J. Biotechnol.* **10** 18855
[12] Noviendri D 2014 *Isolation and Microencapsulation of Fucoxanthin for Drug Delivery System of Human Lung Cancer (H1299) Cell Line Ph.D. Thesis* (Faculty of Engineering, International Islamic University Malaysia, Malaysia) p 201
[13] Noviendri D 2014 *Squalen Bull. Mar. Fish. Postharvest Biotechnol.* **9** 137
[14] Nursid M, Noviendri D, Rahayu and Novelita V 2016 *JPBKP* **11** 83
[15] Wang S K, Li Y, White W L and Lu J 2014 *J. Funct. Biomater.* **5** 29
[16] Maeda H, Hosokawa M, Sashima T, Funayama K and Miyashita K 2007 *J. Oleo Sci.* **56** 615
[17] Noviendri D, Jaswir I, Salleh H M, Taher M, Miyashita K and Ramli N 2011 *J. Med. Plant Res.* **5** 2405
[18] Jaswir I, Noviendri D, Salleh H M, Taher M, Miyashita K and Ramli N 2013 *J. Liq. Chrom. Rel. Technol.* **36** 1340
[19] Urikura I, Sugawara T and Hirata T 2011 *Biosci. Biotechnol. Biochem.* **75** 757
[20] Imbs T I, Ermakova S P, Fedoreyev S A, Anastyuk S D and Zvyagintseva T N 2013 *Mar. Biotechnol.* **15** 606
[21] Matsuno T 2001 *Fish. Sci.* **67** 771
[22] Dembitsky V M and Maoka T 2007 *Prog. Lipid Res.* **46** 328
[23] Miyashita K 2013 *Obes. Control Ther.* **1** 1
[24] Tsukui T, Konno K, Hosokawa M, Maeda H, Sashima T and Miyashita K 2007 *J. Agric. Food Chem.* **55** 5025
[25] Wang W-J, Wang G-C, Zhang M and Tseng C K 2005 *J. Integr. Plant Biol.* **47** 1009
[26] Hashimoto T, Ozaki Y, Taminato M, Das S K, Mizuno M, Yoshimura K, Maoka T and Kanazawa K 2009 *British J. Nutr.* **102** 242
[27] Beppu F, Hosokawa M, Niwano Y and Miyashita K 2012 *Lipids Health Dis.* **11** 1
[28] Maeda H, Tsukui T, Sashima T, Hosokawa M and Miyashita K 2008 *Asia Pac. J. Clin. Nutr.* **17** 196
[29] Jeon S-M, Kim H-J, Woo M-N, Lee M-K, Shin Y C, Park Y B and Choi M-S 2010 *Biotechnol. J.* **5** 961
[30] Yan X, Chuda Y, Suzuki M and Nagata T 1999 *Biosci. Biotechnol. Bioeng.* **63** 605
[31] Kim S M, Shang Y F and Um B H 2011 *Phytochem. Anal.* **22** 322
[32] Kang M-C, Lee S-H, Lee W-W, Kang N, Kim E-A, Kim S Y, Lee D H, Kim D and Jeon Y-J 2014 *J. Funct. Foods* **11** 304
[33] Hwang P-A, Hung Y-L, Gau S-Y, Wu C-H and Chou Y-C 2014 *J. Taiwan Fish. Res.* **22** 93
[34] Jasiwir I, Noviendri D, Salleh H M and Miyashita K 2012 *Food Sci. Technol. Res.* **18** 251
[35] Komba S, Kotake-Nara E and Machida S 2015 *J. Oleo Sci.* **64** 1009
[36] Noviendri D, Jasiwir I, Taher M, Mohamed F, Salleh H M, Noorbatcha I A, Octavianti F, Lestari W, Hendri R, Ahmad H, et al. 2016 *J. Oleo Sci.* **65** 641
[37] Das S K, Hashimoto T and Kanazawa K 2008 *Biochim. Biophys. Acta* **1780** 743
[38] Ravi A, Kurrey N, Manabe Y, Sugawara T, Baskaran V, *Mater. Sci. Eng. C.* 2018
[39] Okuzumi J, Nishino H, Murakoshi M, Iwashima A, Tanaka Y, Yamane T, Fujita Y and Takahashi T 1990 *Cancer Lett.* **55** 75
[40] Kim S M, Shang Y F and Um B H 2011 *Phytochem. Anal.* **22** 322
[41] Satomi Y 2017 *Anticancer Res.* **37** 1557
[42] Ye G L, Jin L J, Wang L L, Du D L, 2018, *Biomed. J. Sci. Tech. Res.* **9** 7218
[43] Wang L, Zeng Y, Liu Y, Hu X, Li S, Wang Y, Li L, Lei Z, Zhang Z, 2014, *Acta Biochim. Biophys. Sin.* **46** 877
[44] Yu R-X, Hu X-M, Xu S-Q, Jiang Z-J, Yang W, 2011, *Eur. J. Pharmacol.* **657** 10
[45] Wu H-L, Fu X-Y, Cao W-Q, Xiang W-Z, Hou Y-J, Ma J-K, Wang I Y, Fan C-D, 2019, *J. Agric. Food Chem.* **67** 2212
[46] Rokkaku T, Kimura R, Ishikawa C, Yasumoto T, Senba M, Kanaya F, Mori N, 2013, *Int. J. Oncol.* **43** 1176
[47] Martin L J 2015 *Mar. Drugs* **13** 4784
[48] Hwang H S and Kim H A 2015 *Int. J. Mol. Sci.* **16** 26035
[49] Elmore S 2007 *Toxicol. Pathol.* **35** 495
[50] Zhang J-H, Zhang Y, Herman B, 2003, *Ageing Res. Rev.* **2** 357
[51] Fox R and Aubert M 2008 Flow Cytometric Detection of Activated Caspase-3. In: *Methods in Molecular Biology (apoptosis vol 414)* Eds. Mor G and Alvero A B (Humana Press Inc, Totowa, NJ) Ch. 5 pp 47-56.
[52] Xiong S, Mu T, Wang G and Jiang X 2014 *Protein Cell* **5** 737
[53] Prabhu A, Venkat P, Gagaraj B and Nadumane V K 2014 *Turk. J. Biol.* **38** 922
[54] Cruchten S V and Broeck W V D 2002 *Anat. Histol. Embryol.* **31** 214
[55] Cohen G M 1997 *Biochem. J.* **326** 1
[56] Wu R-C, Wang Z, Liu M-J, Chen D-F and Yue X-S 2004 *Cell. Mol. Life Sci.* **61** 2071
[57] Briglia M, Calabro S, Signoretto E, Alzoubi K, Laufer S, Faggio C and Lang F 2015 *Cell. Physiol. Biochem.* **37** 2464
[58] Ekshiyyan O and Aw T Y 2004 *Curr. Neurovasc. Res.* **1** 355
[59] Denault J B and Salvesen G S 2002 *Chem. Rev.* **102** 4489
[60] Andrade R, Crisol L, Prado R, Boyano M D, Arluzea J and Aréchaga J 2010 *Biol. Cell.* **102** 25
[61] Takano Y S, Harmon B V and Kerr J F 1991 *J. Pathol.* **163** 329
[62] Barisic K, Petrik J and Rumora L 2003 *Acta Pharmacuetic.* **53** 151
[63] Chakravarti B V S K, Sujay R, Kuriakose G C, Karande A A and Jayabaskaran C 2013 *Cancer Cell Int.* **13** 1
[64] McCarty N J and Evan G I 1998 *Curr. Top. Dev. Biol.* **36** 259
[65] Fu H, Wang Q-S, Luo Q, Tan S, Su H, Tang S-L, Zhao Z-L and Huang L-P 2014 *World J. Emerg.Med.* **5** 291
[66] Anthony M L, Zhao M and Brindle K M 1999 *J. Biol. Chem.* **274** 19686
[67] Khodarev N N, Sokolova I A and Vaughan A T 1998 *Int. J. Rad. Biol.* **73** 455
[68] Das S K, Ren R, Hashimoto T and Kanazawa K 2010 *J. Agric. Food Chem.* **58** 6090
[69] Hung T M, Dang N H and Dat N T 2014 *Biol. Res.* **47** 1
[70] Saikumar P, Dong Z, Mikhailov V, Denton M, Weinberg J M and Ventachalam M A 1999 *Am. J. Med.* 107 489

[71] Saunber P A, Cooper J A, Roedell M M, Schroeder D A, Borchert C J, Isaacson A L, Schendel M J, Loegering R T, Gaylor H, Woyno I J, et al. 2000 *Anal. Biochem.* 282 114

[72] Saengkhae C, Premsuriya Y, Srivibool R and Praiboon J 2015 *Walailak J. Sci. Technol.* 16 515

[73] Truman A, Hook B and Schagat T 2014 *Using a Real-Time Kinetic Cytotoxicity Assay to Determine when to Detect Apoptosis* (Promega Corporation) pp 1-9

[74] Zhu C, Zeng Z, Li H, Li F, Fan C, Zhang H, 2013, *J. Am. Chem. Soc.* 135 5998

[75] Zhang G, Slaski J J, Archambault D J, Taylor G J, 1997, *Physiol. Plant.* 99 302

[76] Krysko D V, Vanden B T, D’Harde K and Vandenabeele P 2008 *Methods* 44 205

[77] Vermes I, Haanen C, Steffens-Nakken H and Reutelingsperger C 1995 *J Immunol. Meth.* 184 39

[78] Zhang Z, Zhang P, Hamada M, Takahashi S, Xing G, Liu J and Sugiura N 2008a *Oncol. Rep.* 20 1099

[79] Rwigemera A, Mamelona J, Martin L J, 2015, *Anticancer Res.* 35 207

[80] Zhu Y, Cheng J, Min Z, Yin T, Zhang R, Zhang W, Hu L, Cui Z, Gao C, Xu S, Zhang C, Hu X, 2018, *J. Cell Biochem.* 1

[81] Kim K-N, Heo S-J, Kang S-M, Ahn G and Jeon Y-J 2010 *Toxicol. in Vitro.* 24 1648

[82] Kotake-Nara E, Asai A and Nagao A 2005 *Cancer Lett.* 220 75