Apoptosis Comparison Effects Between Synthetic and Natural B-carotene from Dunaliella salina on MDA-MB-231 Breast Cancer Cells

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Abstract

Dunaliella salina is genus most important species for β-carotene production. Several investigations have demonstrated that D. salina produces more than 10% of its dry mass. β-carotene is an important pro-vitamin A source and can also act as a lipid radical scavenger and as a singlet oxygen quencher. Vitamin A Deficiency (VAD) has been related with cancer, for this reason synthetic and natural β-carotene has been used for prevention and treatment of the disease. Synthetic β-carotene is cheaper than natural molecule but only contains all-trans-β-carotene (ATβC), while 9-cis-β-carotene (9CβC) and ATβC are both produce by D. salina. Meta-analysis of controlled trials using high levels of synthetic β-carotene supplementation in smoker’s individuals, mention that instead to prevent and control lung cancer, treatment increases risk and percentage of positive cases. Results obtained in cancer cell lines and animal models using β-carotene from Dunaliella, prevented and controlled diseases proliferation. In this work, effects of synthetic and natural β-carotene from D. salina were evaluated on MDA-MB-231 breast cancer cells, and even when apoptosis induction results were obtained with both sources, natural β-carotene generates considerable higher cell rates mortality.

Keywords: D. salina; β-carotene; ATβA; 9CβA; MDA-MB-231

Introduction

Breast cancer is most frequently diagnosed cancer globally and main cause of death among women [1,2]. Breast cancer is a complex disorder that can occur because of vitamin deficiencies and due process including oxidative stress and lipid peroxidation. Biological mechanisms exist to support anticancer properties of natural antioxidant pro-vitamin A molecule (β-carotene) in animals and overall impression is that intake of it, play a substantial role in prevention of cancer [3-5]. However, anticancer effects are not so positive in humans using synthetic β-carotene molecule [6-8].

Dunaliella genus includes a reduced number of species that when exposed to elevated luminous intensities, high saline concentrations and limited conditions of oxygen and nitrogen accumulate great amounts of β-carotene [9,10]. D. salina and D. bardawil are the most important species of the genus for β-carotene production. Several investigations have demonstrated that both species produces more than 10% of its dry mass [11,12]. The commercial production of β-carotene that comes from Dunaliella is the third most important microalga industry since 1986 [13]. β-carotene products derived from Dunaliella are; 1) Dunaliella powder for animal feed production, 2) Dunaliella powder for human food elaboration and 3) extracts of pure β-carotene for medical and pharmaceutical use, with prices oscillating between US $ 300 to US $ 3000 per kg, respectively [13]. Synthetic β-carotene is easier to obtain and cheaper than β-carotene from Dunaliella, reason why synthetic molecule dominates almost 95% of the market.

All-trans-retinoic-acid (ATRA) β-carotene derived molecule from synthetic and natural sources are 100% equals in structure and function [14]. ATRA is been considered the most potent biologically active metabolite derived from vitamin A, because prevent and rescue cancer anomalies induced by VAD in adult animals [15,16]. Subsequent studies anticipated a strong rationale for use of retinoids in treatment and prevention of cancer [17]. β-carotene from D. salina contains 47% of 9CβC and 53% ATβC (Figure 1) [18]. Antiperoxidative 9-cis-retinoic-acid (9CRA) activity is greater compared to that shown by ATRA, preventing further malignant cells development and cardiovascular diseases [19,20]. Cytochrome P450RAI (CYP26) metabolizes ATRA but not 9CRA isomer increasing its half-life [21,22]. ATRA and 9CRA ligands interact with nuclear retinoic acid receptors (RARs) and retinoic X receptors (RXRs); ATRA binds to RARs while 9CRA can bind to both RARs and RXRs [23,24]. DNA promoter retinoic acid respond element (RAREs) combinations tightly regulate gene expression, through interaction with homo and heterodimers constituted by RARs and RXRs previously bound to ATRA and 9CRA [25].

In this work D. Salina strain isolated from Baja California peninsula of México was identified both morphologically and molecularly via 18S rDNAconserved and specific primers [26,27]. Natural β-carotene from optimally grown cells was solvent extracted and quantified. β-carotene was assessed for its anticancer property in MDA-MB-231 human breast cancer cell line, showing 70% of effective apoptosis with respect to synthetic β-carotene that only induced 30% in a MTT analysis.

Experimental Section

Dunaliella environmental sampling and growth conditions

Locations in Baja California peninsula of México were selected for water-sample collection [11]. Environmental samples were obtained using 50 ml plastic tubes from red hypersaline waters between May
was discarded and pellets were homogenized and disrupted in one ml of 100% cold methanol. Samples were incubated on ice for one hour in the dark, centrifuged at 10000 rpm 5 min, filtered and kept at -20°C. 20 µl methanol β-carotene extracted sample was injected into a Hewlett Packard 1100 model HPLC apparatus, using a reverse phase YWDAC 201 TP52 C-18 column of 4 mm internal diameter and 150 mm length, with 5 µm particle size. The detector was set at 340 nm and 450 nm for identification of 9CβC and ATRA respectively. Elution was performed with an isocratic mixture of acetonitrile:methanol:tetrahydrofuran:water (70:15:10:5) at 0.5 ml/min. Synthetic ATβC was obtained from Sigma and used as standard.

**Purification of β-Carotene from D. salina**

Thirty days old D. salina grown under laboratory condition was centrifuged at 2000 rpm for 5 min and pellet was collected. Five gram pellets were taken, washed with distilled water and centrifuged again to remove salt. Collected pellets were dissolved with 10 ml of methanol and macerated with mortar and pestle in an ice bath until the pellet became colorless, sample was centrifuged again, pellet was discharged and methanol was evaporated at room temperature. β-carotene resultant amorphous dry powder was dissolved in olive oil and concentrations of 10 µg/ml were used for following anticancer studies.

**Effect of Natural β-Carotene from D. salina on MDA-MB-231**

Human keratinocytes cells (HaCat) and human breast cancer cells (MDA-MB-231), were cultured in RPMI-1640 medium supplemented with 10% inactivated fetal bovine serum (v/v) (GIBCO), and 1% anti-commercial antibiotic (invitrogen) in a humidified incubator with 5% CO₂ at 37°C. The MTT method described by Mosmann was used to quantitatively detect living cells [29]. In brief, 5x10⁵ cells/well were charged onto 96 well plates. After 24 h, supplemented medium was discarded, non-supplemented medium was added and cells were incubated 24 h more. 10 µg/ml solution of synthetic and purified β-carotene from D. salina, were added to HaCat and MDA-MB-231 cells that were incubated 2 h at the same conditions. Medium was discarded and 100 µl of fresh medium was added with 10 µl of MTT (5 mg/ml). After 2 h of incubation medium was eliminated and 100 µl of cold isopropanol was added to dissolve crystals formed. Absorbance was quantified with a microplate reader at 570 nm. Growth inhibition percentage was calculated using DMSO cells treated as positive control and as negative control RPMI medium was used.

**DNA Purification and PCR Amplification**

Isolation of chromosomal DNA from Dunaliella strains was carried out and species identification of isolates was developed using MA1 [5'-CGGGATCCGTAGTCATATGCTTGTCTC-3'] and MA2 [5'-GGATTCTTCTGGAGGTTACAC-3'] conserved and DSs (D. salina) [5'-GCAGGAGGCTAATAGGA-3'] specific oligonucleotide reported by Olmos and coworkers [5, 11]. MA1 and MA2 conserved oligonucleotides amplify 18S rDNA complete sequence. DSs oligonucleotide is specific for β-carotene "D. salina var Teod" hyper producer strain [11,12]. PCR reactions were carried out in a total volume of 100 µl containing 50 ng of chromosomal DNA in TE (Tris-EDTA) buffer, pH 8 and 200 ng MA1 and MA2 conserved primers. The amplification was carried out using 25 cycles with a Tm of 52°C to all reactions. One cycle consisted of 1 minute at 95°C, 1 minute at 52°C and 2 minute at 72°C. PCR with specific primer was combined with MA2 conserved primer (DSs-MA2) and reactions were carried out under same conditions.

**β-Carotene HPLC identification and quantification**

One ml sample was centrifuged at 2000 rpm for 5 min, supernatant and September 2006. Red flagellated microalgae strains contained in samples were morphologically and molecularly identified as Dunaliella, before purification and growth. Samples containing D. salina were serially diluted up to 10⁻¹ and 0.1 ml spread on 2% De Walne’s agar before purification and growth. Samples containing samples were morphologically and molecularly identified as Dunaliella, and September 2006. Red flagellated microalgae strains contained in concentrated brines of Montpellier on Mediterranean coast of France was reported in 1838 by Dunali, who described occurrence of...
the organism we know today as *Dunaliella salina* [31]. Descriptions of *Dunaliella* (*Chlorophyta, Chlorophyceae, Chlamydomonadales, Dunaliellaceae*) as a new genus, was presented in year 1905 by Teodoresco from Bucharest using Romanian salt lake samples and by Clara Hamburger from Heidelberg using samples from Cagliari, Sardinia [32, 33]. *Dunaliella* do not have a cell wall only is enclosed by a thin elastic plasma membrane, in this sense species of the genus present a vast morphological variability with respect to environmental conditions were algae growth [34-36]. *Dunaliella* can be ellipsoid, ovoid to almost spherical, pyriform or fusiform (Figure 2).

Motile cells are biflagellate, with flagella inserted at anterior end of the cell with length varying between species. There is a single large posterior chloroplast occupying most of the cell volume. It is either cup-, dish or bell-shaped and contains a pyrenoid in the thickened basal part in all species except some of the freshwater species [37].

Taxonomic studies among *Dunaliella* have identified several new species since Teodorescos identification in 1905 [36]. However, high plasticity in green stage and almost indistinguishable differences in red phase make identification and differentiation of species very difficult and time consuming [26]. *Dunaliella* genus includes a reduced number of species that when exposed to elevated luminous intensities, high saline concentrations, oxygen and nitrogen limitation accumulates great amounts of β-carotene [9,10]. *D. salina* and *D. bardawil* are most important species for β-carotene production; several investigations have demonstrated that both produce more than 10% of its dry mass [27]. In this sense, *Dunaliella* isolates were morphological and molecularly identified. Figure 2, shown *Dunaliella* strain isolated from locations reported by Olmos and coworkers [11], picture presents a microalga growing on a brine lagoon containing high levels of β-carotene and two hyperproducer strain. Thus, Intron-Sizing-Method was applied to make an easy, fast and precise identification of the isolates [11,12]. In natural samples "*D. salina var Teod" was identified due its 18S rDNA fingerprinting profile presented by Sheu and coworkers shown a *D. salina* strain producing β-carotene under conditions established (Figure 5a). Other factors involved in 9CβC:ATβC ratio differences obtained between Sheu and this work maybe was due by culture medium, culture conditions and/or timing of evaluation. Other factors involved in 9CβC:ATβC ratio differences could be HPLC methodology and/or *Dunaliella* strain used.

Once isolated strain was identified, it was grown using an inducer medium formulated with addition of three NaCl concentrations (Table 1), to evaluate its influence in growth and β-carotene production [19]. Figure 3, shown *D. salina* specific identification. Lane 1, Molecular Weight Marker. Lane 2, PCR product using MA1-MA2 conserved primers. Lane 3, PCR product using DSs-MA2 specific-conserved primers.

![Figure 3: D. salina specific identification. Lane 1, Molecular Weight Marker. Lane 2, PCR product using MA1-MA2 conserved primers. Lane 3, PCR product using DSs-MA2 specific-conserved primers.](image)

**Table 1:** Culture medium with 1, 1.5 and 2 M of NaCl, respectively.

| Component    | Concentration |
|--------------|---------------|
| CaCl₂        | 0.10 µM       |
| MgSO₄        | 1.67 mM       |
| NaH₂PO₄      | 0.48 mM       |
| NaNO₃        | 1.17 mM       |
| NaCl         | 1.17 mM       |
| FeCl₃        | 1.33 µM       |
| EDTA         | 13.3 µM       |
| ZnSO₄        | 100 mM        |
| Na₃MoO₄      | 33 nM         |
| MnSO₄        | 1.21 µM       |
| CoCl₂        | 53 nM         |
| CuSO₄        | 46.67 nM      |
| Thiamine     | 3.5221 µM     |
| Cyanocobalamin | 4.38 µM    |
| NaCl         | 1.0-1.5-2.0 M |

9CβC and ATβC production in green (15 days) and red (30 days) phases were evaluated with an HPLC methodology developed. In this sense, *D. salina* isolated strain produces 9CβC:ATβC ratio of 70:30 under conditions established (Figure 5a). However, results obtained by Sheu and coworkers showed a *D. salina* strain producing β-carotene 9CβC:ATβC ratio of 47:53 [18]. Additionally, it is important to point out that 9CβC is preferentially produced in red phase, due in green phase 9CβC:ATβC ratio was inversely proportional (Figure 5b). Results differences obtained between Sheu and this work maybe was due by culture medium, culture conditions and/or timing of evaluation. Other factors involved in 9CβC:ATβC ratio differences could be HPLC methodology and/or *Dunaliella* strain used.

![Figure 4: D. salina growth under 1, 1.5 and 2 M of NaCl, respectively.](image)

**Figure 2:** *Dunaliella salina* isolated strain.

**Figure 3:** *D. salina* specific identification. Lane 1, Molecular Weight Marker. Lane 2, PCR product using MA1-MA2 conserved primers. Lane 3, PCR product using DSs-MA2 specific-conserved primers.

**Figure 4:** *D. salina* growth under 1, 1.5 and 2 M of NaCl, respectively.
Moreover, β-carotene production level reached by our isolate was around 20 mg/l meaning that formulated medium succeed in its overproduction, as well as in 9CβC induction. Furthermore, β-carotene isomers identification was made by using 340 λ, due 9CβC presents a better absorption than ATβC at this wavelength [38]. The obtained results corroborate findings made by Briton and coworkers, where *D. salina* 9CβC peak showed higher levels than ATβC (Figure 6). Additionally, ATβC from *D. salina* presented same retention time as synthetic molecule obtained from Sigma-Aldrich (data not shown). Supporting these findings Grune and coworkers mention that both molecules presented the same structure-function and by consequence the same retention time [14]. Differences between 9CβC:ATβC ratio from synthetic and natural samples, was not possible to calculate due synthetic just contains ATβC molecule.

Metaanalysis of controlled trials using synthetic β-carotene in Finland (ATβC) and synthetic β-carotene and retinol in USA (CARET), showed that high levels (20-30 mg/day) of supplemental β-carotene instead to prevent and control lung cancer, increases risk and percentage of positive cases [6]. In this sense, Palozza and coworkers found that under low oxygen pressure (15 mmHg pO₂) β-carotene behaved as antioxidant, inhibiting cigarette smoke condensate (tar) to induced lipid peroxidation [8]. Nevertheless, β-carotene progressively acted as a prooxidant in a dose-dependent manner under 100-760 mmHg pO₂ range [8]. β-carotene auto-oxidation, measured as formation of 5,6-epoxy- β-carotene, was faster at high than at low pO₂ and carotenoid was more rapidly consumed in tar presence [39]. In contrast to ATβC and CARET studies, the Physicians’ Health Study was conducted among mainly nonsmokers; 50 mg of β-carotene was given every other day and no effect was found on lung cancer risk in either smokers or nonsmokers [40]. β-carotene serum concentration (210–300 µg/dL) in trials where lung cancer incidence increased was markedly higher than in Physicians’ Health Study (120 µg/dL), where no increase in lung cancer risk was seen from β-carotene supplementation [40]. Additionally, normal range of serum β-carotene derived from dietary intake is around 5–50 µg/dL [41]. In this sense, 10 µg/ml of synthetic and natural β-carotene were supplemented on experiments developed in this work, using MDA-MB-231 Brest cancer and HaCat cell lines (Figure 7a and 7b). Due β-carotene low levels used in these assays and low culture plates pO₂, pro-oxidant activity was discarded in these experiments. Obtained results in MDA-MB-231 Brest cancer cells shown that β-carotene from *Dunaliella* induced 70% cells death with respect to synthetic molecule that only presented 30% in MTT assays (Figure 7a). These results are in agreement with cell and animal model trials published, where induction of apoptosis at different levels was carried out by *D. salina* extracts [3-5,18]. Nevertheless, no one of the mentioned authors had worked with MDA-MB-231 Brest cancer cells neither HaCat, only Prakash and coworkers made some
β-carotene inhibition test in MDA-MB-231 cells, but non-results were reported [42]. Breast cancer is the most frequently diagnosed cancer globally and main cause of death among women [1,2]. For these reasons, Brest cancer is one of the most important targets to natural and synthetic molecules development. Major carotenoids in D. salina include ATβC and 9CβC; specifically 9-cis isomer had demonstrated a great antiperoxidative effect due to higher reactivity of cis bond compared to trans [5]. In addition, 70% MDA-MB-231 apoptotic induced cells using natural β-carotene was in accordance to higher 9CβC levels produced by D. salina isolate (Figure 7a and 5a). On the other hand, MDA-MB-231 synthetic β-carotene treated cells reached 30% mortality only (Figure 7a). Thus, obtained results suggest that 9CβC is the most influential isomer in apoptosis induction in MDA-MB-231 Brest cancer cells. Moreover, non-deleterious effects in HaCat cells were induced by β-carotene treatment, meaning that concentration was not harmful to tested cells (Figure 7b). Additionally, it is important to point out that failed human clinical trials in Finland and USA (ATβC and CARET), were developed using high levels of synthetic β-carotene and without inclusion of 9CβC [6]. In this sense, clinical trials development using natural β-carotene with high levels of 9-cis isomer are recommended, due results obtained in this work and other reports published [3-5,18]. For this reason, a culture medium specifically formulated to overproduce 9CβC in D. salina, as the one developed in this work is required. Additionally, timing to D. salina β-carotene harvesting is also of extreme importance to obtain the greatest 9CβC concentration (Figure 5a and 5b). In addition, D. salina species-specific identification is highly recommended to obtain desired results.

With respect to DNA fragmentation assays better degradation results were obtained in MDA-MB-231 Brest cancer cells treated with natural instead of synthetic β-carotene (data not shown), results expected due better apoptosis induction obtained using natural molecules (Figure 7a) and by previously reported works using D. salina extracts [3-5,18]. Evasion of apoptosis is considered a hallmark of human cancers; apoptosis cell death modality is executed by caspases, which are up-regulated by factors like retinoids [43-45]. Although, caspases activity is regulated primarily at post-translational level its overexpression sensitizes cells for apoptosis [43-45]. In addition, CYP26 expression is regulated by ATRA and 9CRA throughout RARs and RXRs nuclear receptors. Furthermore, an important function of RARs and RXRs receptors is auto-regulate their own activity by controlling CYP26 transcription that in turn, controls ATRA ligand concentration by its degradation but without affecting 9CRA [22,23,25,46]. In this sense, caspase and CYP genes expression is modulated by ATRA and 9CRA ligands; however, 9-cis activate both RARs and RXRs nuclear receptors, while ATRA only activate RARs regulators [23]. Therefore, data mentioned above could explain why 9CβC:ATβC (70:30) ratio obtained from D. salina, induces apoptosis more efficiently than ATβC from synthetic origin. Additionally, presence of both isomers seems to be imperative for a correct RXR:RAR heterodimer genes regulation and expression [25].

Conclusion

Retinoid regulates a wide variety of essential biological processes, such as vertebrate embryonic morphogenesis and organogenesis, cell growth arrest, differentiation and apoptosis, as well as, homeostasis and their disorders [15,47-49]. VAD is been related with cancer, although controlled trials using synthetic β-carotene increased the disease in smokers. 9CRA and ATRA are the most potent biologically active metabolites derived from natural β-carotene (9CβC:ATβC); a pro-vitamin A molecule isolated principally from Dunaliella species. However, synthetic β-carotene only generates ATRA and non-9CRA is detected in human bodies. Commercial price differences between both isomers are of great concern, reason why synthetic β-carotene (ATβA) is been used preferentially for a long period. 9CRA and ATRA regulates gene expression throughout interactions with nuclear retinoic acid receptors (RARs) and retinoic X receptors (RXRs), being RXRs the most important and represented in human bodies. DNA promoter retinoic acid respond elements (RAREs) combinations tightly regulate gene expression through homo and heterodimerization between RARs and RXRs, after interaction with 9CRA and ATRA. In this sense, presence of both retinoids is indispensable to an accurate gene expression and apoptosis induction as was demonstrated in this work. Pending tasks are related to know about precise ratio needed between 9CRA:ATRA and the concentration that must be apply, to prevent and/or to cure cancer in humans. Meta-analysis already demonstrated that application of high levels of synthetic ATRA one of the two components of the ratio, is not adequate to prevent neither to cure cancer. Utilization of natural sources of β-carotene like D. salina is more expensive at the beginning, but at the end could be cheaper preventing this kind of diseases.

References

1. Jemal A, Bray F, Center MM, Ferlay J, Ward E, et al. (2011) Global cancer statistics. CA Cancer J Clin 61: 69-90.
2. Mendoza G, Portillo A, Olmos-Soto J (2013) Accurate breast cancer diagnosis through real-time PCR her-2 gene quantification using immunochromotically-identified biopsies. Oncol Lett 5: 295-298.
3. Jayappriyana KR, Rajkumar R, Venkatakrishnanc V, Nagaraj S, Rengasamy R (2013) In vitro anticancer activity of natural β-carotene from Dunaliella salina EU5891199 in PC-3 cells. Biomed Prevent Nutr 9: 3-105.
4. Raja R, Hemaivarysara W, Balasubramanyam D, Rengasamy R (2007) Protective effect of Dunaliella salina (Volvocales, Chlorophyta) against experimentally induced fibrosarcoma on wistar rats. Microbiol Res 162: 177-184.
5. Tsai CF, Lu FJ, Hsu YW (2012) Protective effects of Dunaliella salina - a carotenoids-rich alga - against ultraviolet B-induced corneal oxidative damage in mice. Mol Vis 18: 1540-1547.
6. Druses-Pecolino N, Latino-Martel P, Norat T, Barrandon E, Bertris S, et al. (2010) Beta-carotene supplementation and cancer risk: a systematic review and metaanalysis of randomized controlled trials. Int J Cancer 127: 172-184.
7. Misotti AM, Gagnarella P (2013) Vitamin supplement consumption and breast cancer risk: a review. Ecancermedicalscience 7: 365.
8. Palozza P, Serini S, Trombino S, Lauriola L, Ranefeltti FO, et al. (2006) Dual role of beta-carotene in combination with cigarette smoke aqueous extract on the formation of mutagenic lipid peroxidation products in lung membranes: dependence on pO2. Carcinogenesis 27: 2383-2391.
9. Ben-Amott A, Katz A, Avron M (1982) Accumulation of β-carotene in halotolerant algae: purification and characterization of β-carotene-rich globules from Dunaliella bardawil (Chlorophyceae). J Phycol 18: 529-537.
10. Borowtika LJ. The microflora: Adaptations to life in extremely saline lakes. Hydrobiologia 1981, 81, 33-46.
11. Olmos J, Ochoa L, Paniagua-Michel J, Contreras R (2009) DNA fingerprinting differentiation between beta-carotene hyperproducer strains of Dunaliella from around the world. Saline Systems 5: 5.
12. Olmos SJ, Paniagua-Michel J, Contreras R, Ochoa L (2012) DNA fingerprinting intron-sensing, and sensitive identification of carotenogenic Dunaliella species. In Microrganisms. Carotenoids from Bacteria and Microalgae. 1st ed.; Barredo, J.L.; Ed. Human Press, c/o Springer Science + Bussines Media, LLC, New York, USA., pp 269-281.
13. Spolaore P, Joannis-Cassan C, Duran E, Isambert A (2006) Commercial applications of microalgae. J Biosci Bioeng 101: 87-96.
14. Gruen T, Lietz G, Palou A, Ross AC, Stah W, et al. (2010) Beta-carotene is an important vitamin A source for humans. J Nutr 140: 2268S-2285S.
15. Kastner P, Mark M, Chambon P (1995) Nonsteroid nuclear receptors: what are genetic studies telling us about their role in real life? Cell 83: 859-869.

16. Woltbach SB, Howe PR (1925) Tissue changes following deprivation of fat-soluble vitamin A. J Exp Med 42: 753-777.

17. Hong WK, Sporn MB (1997) Recent advances in chemoprevention of cancer. Science 278: 1073-1077.

18. Sheu MJ, Huang GJ, Wu CH, Chen JS, Chang HY, et al. (2008) Ethanol extract of Dunaliella salina induces cell cycle arrest and apoptosis in A549 human non-small cell lung cancer cells. In Vivo 22: 369-378.

19. Ben-Amotz A, Levy Y (1996) Bioavailability of a natural isomer mixture compared with synthetic all-trans beta-carotene in human serum. Am J Clin Nutr 63: 729-734.

20. Levin G, Yeshurun M, Mokady S (1997) In vivo antiperoxidative effect of 9-cis beta-carotene compared with that of the all-trans isomer. Nutr Cancer 27: 293-297.

21. Niederreither K, Abu-Abed S, Schuhbaur B, Pelkovich M, Chambon P, et al. (2002) Genetic evidence that oxidative derivatives of retinoic acid are not involved in retinoid signaling during mouse development. Nat Genet 31: 84-88.

22. Loudig O, Babichuk C, White J, Abu-Abed S, Mueller C, et al. (2000) Cytochrome P450RAI(CYP206) promoter: a distinct composite retinoic acid response element underlies the complex regulation of retinoic acid metabolism. Mol Endocrinol 14: 1483-1497.

23. Germain P, Chambon P, Eichele G, Evans R, Lazar M, et al. (2006) International Union of Pharmacology LX. Retinoic acid receptors. Pharmacol Rev 58: 712–725.

24. Lou X, Toresson G, Benod C, Suh JH, Philips KJ, et al. (2014) Structure of the retinoid X receptor 1α-liver X receptor 3 (RORα-LXR3) heterodimer on DNA. Nat Struct Mol Biol 21: 277-281.

25. Evans RM, Mangelsdorf DJ (2014) Nuclear Receptors, RXR, and the Big Bang. Cell 157: 215-232.

26. Olmos J, Gómez R, Ruiz VP (2015) Apoptosis Comparison Effects Between Synthetic and Natural B-Carotene from Dunaliella salina on MDA-MB-231 Breast Cancer Cells. J Micro Biochem Technol 7: 051-056. doi:10.4172/1948-5948.1000181

27. Massyuk NP (1973) Morphology, Taxonomy, Ecology and Geographic Distribution of the Genus Dunaliella Teod and Prospects for its Potential Utilization. Kieve, Naukova Dumka, Utilization. Kieve, Naukova Dumka, Distribution of the Genus Dunaliella Teod and Prospects for its Potential Utilization. Kieve, Naukova Dumka, Distribution of the Genus Dunaliella Teod and Prospects for its Potential Utilization. Kieve, Naukova Dumka, Distribution of the Genus Dunaliella Teod and Prospects for its Potential Utilization. Kieve, Naukova Dumka, Distribution of the Genus Dunaliella Teod and Prospects for its Potential Utilization. Kieve, Naukova Dumka, Distribution of the Genus Dunaliella Teod and Prospects for its Potential Utilization. Kieve, Naukova Dumka, Distribution of the Genus Dunaliella Teod and Prospects for its Potential Utilization. Kieve, Naukova Dumka, Distribution of the Genus Dunaliella Teod and Prospects for its Potential Utilization. Kieve, Naukova Dumka, Distribution of the Genus Dunaliella Teod and Prospects for its Potential Utilization. Kieve, Naukova Dumka, Distribution of the Genus Dunaliella Teod and Prospects for its Potential Utilization. Kieve, Naukova Dumka, Distribution of the Genus Dunaliella Teod and Prospects for its Potential Utilization. Kieve, Naukova Dumka, Distribution of the Genus Dunaliella Teod and Prospects for its Potential Utilization. Kieve, Naukova Dumka, Distribution of the Genus Dunaliella Teod and Prospects for its Potential Utilization. Kieve, Naukova Dumka, Distribution of the Genus Dunaliella Teod and Prospects for its Potential Utilization. Kieve, Naukova Dumka, Distribution of the Genus Dunaliella Teod and Prospects for its Potential Utilization. Kieve, Naukova Dumka, Distribution of the Genus Dunaliella Teod and Prospects for its Potential Utilization. Kieve, Naukova Dumka, Distribution of the Genus Dunaliella Teod and Prospects for its Potential Utilization. Kieve, Naukova Dumka, Distribution of the Genus Dunaliella Teod and Prospects for its Potential Utilization. Kieve, Naukova Dumka, Distribution of the Genus Dunaliella Teod and Prospects for its Potential Utilization. Kieve, Naukova Dumka, Distribution of the Genus Dunaliella Teod and Prospects for its Potential Utilization. Kieve, Naukova Dumka, Distribution of the Genus Dunaliella Teod and Prospects for its Potential Utilization. Kieve, Naukova Dumka, Distribution of the Genus Dunaliella Teod and Prospects for its Potential Utilization. Kieve, Naukova Dumka.