



Advances in the development of anticancer drugs from *Euglena*

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Abstract

Microalgae are genuine cell factories for the biological synthesis of value-added metabolites with abundant biomedical applications. Among them, bioactive molecules with anticancer activities are one major group of targeted compounds. The unicellular protist genus *Euglena* comprises a vastly untapped pharmacological source that can be developed for better and safe cancer therapeutics. Primary and secondary metabolites from *Euglena* spp. are the leading focus of biotechnological research for novel anticancer drugs in the last decade. The major metabolites from *Euglena* with potent use in cancer drug research are lipids, polysaccharides, carotenoids and toxins, and their derivatives. Nevertheless, the commercialization of these potent molecules urgently requires a further therapeutic rationale for human application, besides the ongoing quest for novel bioactive compounds.

Keywords: *Euglena*; metabolites; anticancer; PUFAs, astaxanthin

Abbreviations: PUFA, polyunsaturated fatty acids; ACF, aberrant crypt foci; VEGF, vascular endothelial growth factor; ALA, α -linolenic acid; ARA, arachidonic acid; EPA, eicosapentaenoic acid; DHA, docosahexaenoic acid; NSCLC, non-small cell lung cancer; DMBA, dimethylbenz-(a)anthracene; MAPK, mitogen-activated protein kinase.

Introduction

More than half of the drugs in clinical use are made from natural products or their derivatives (Sithranga Boopathy & Kathiresan, 2010) and the scientific interest on natural drug formulations has been momentarily growing in recent years. Microalgal metabolites are widely explored since these indispensable organisms thrive in a competitive environment and, therefore, a significant level of chemical structural diversity exists in their various metabolic pathways. A series of bioactive metabolites derived from algae has been recently characterized for their potential application in medicine as anti-inflammatory, antibiotic, and anti-disease agents (de Morais et al., 2015; de Vera et al., 2018; Encarnação et al., 2015).

Cancer is one of the dreadful pathological conditions and the highest death-causing disease in the world. Several compounds of natural origin such as flavonoids, phenolic acids, carotenoids are potent cancer inhibitors by antioxidative effects, immunomodulation, and inhibition of critical enzymatic process if cancer cell growth (Russo et al., 2015). The development of novel anti-cancer drugs and formulations is the most significant aspect of contemporary therapeutic cancer management. Recent advances in microalgal biotechnology have initiated the extraction of bioactive substances which are effective in killing cancer cells by inducing apoptotic death (Bajpai et al., 2018; Lauritano et al., 2016), therefore, are potentially useful as molecular models in anticancer drug research.

Euglena is a flagellate genus, of mostly freshwater unicellular organisms, thriving on a variety of habitats. The plastids of *Euglena* are active cellular factories of complex synthetic pathways for bioactive metabolites, making them unique organisms that simultaneously produce antioxidants, wax esters, Polyunsaturated Fatty Acids (PUFAs) and phytotoxins (Kottuparambil et al., 2019). Value-added metabolites from *Euglena* are widely studied in recent years for potential applications in pharmaceutical, nutraceutical and cosmetic industries (Kottuparambil et al., 2019). This brief review summarizes up-to-date progress in the detection of bioactive compounds from *Euglena* spp. with proven anticancer properties. The feasibility and prospects of these compounds to foster the ongoing efforts for anticancer drug discovery are emphasized.

Anti-cancer properties of *Euglena* metabolites

The large biodiversity and the consequent variability in the respective biochemical composition make microalgae a promising resource for novel chemically and biologically active formulations with high commercial values (Encarnação et al., 2015). *Euglena* produces several value-added compounds with high potential for biotechnological exploitation. Paramylon, a β -(1 \rightarrow 3)-D-glucan, isolated from *Euglena gracilis*, is an attractive polysaccharide due to its dietary

qualities and various benefits on human health. The pioneering study on the bioactivity of *Euglena* was the identification of an anti-tumor effect of paramylon on the transplantable sarcoma-180 in mice (Quesada et al., 1976). Apart from the antibacterial (Miyatake et al., 1995), antiviral (Nakashima et al., 2017), and anti-inflammatory (Choi et al., 2013) properties of paramylon, a preventive effect against the formation of preneoplastic Aberrant Crypt Foci (ACF) in the colon has been reported recently (Watanabe et al., 2013). The authors reported that in the weaning male mice, feeding by β -glucans in the form of *Euglena* for 11 weeks resulted in a lower number of ACF, lower liver weight, and an increased lactic acid levels in the cecal contents, suggesting the preventive effects of β -glucans such as paramylon found in *Euglena* against colon cancer in humans. This confirms that β -glucans, such as paramylon and its isomer amorphous paramylon, have preventive effects against colon cancer and are more effective against the condition than *Euglena* in the raw form (Zhu et al., 2016).

Euglenophycin, an alkaloid toxin found in several *Euglena* species (Zimba et al., 2017) has shown antitumor and anti-colorectal cancer potentials and its likely use as an anti-cancer agent, targeting multiple cancer-promoting processes is promising (Cabang et al., 2017). Euglenophycin demonstrated cytotoxic, anti-proliferative, anti-clonogenic, and anti-migration effects against HCT116, HT29, and SW620 CRC cells over a concentration range of 49.1–114.6 μM through G1 cell cycle arrest and cell type-dependent modulation of autophagy (Cabang et al., 2017). Recently, the angiogenic inhibition properties of Euglenophycin have been reported, including metabolic retardation of Vascular Endothelial Growth Factor (VEGF) and Angiopoietin 2 (Ang-2) production and inhibition of cell proliferation against human leukemia (K562, THP-1, and Jurkat) and murine endothelial (SVR) and epithelial (IEC-6) cell lines (Zimba et al., 2016). The property of *Euglena* toxin to reduce the number of viable leukemia cells and reduce *in vitro* leukemic metabolic activity confirms its use in future cancer therapeutics. This phycotoxin also showed selective cytotoxicity against mouse neuroblastoma *in vitro* cell lines (N2a), with an estimated 50% reduction in cell viability at a concentration of 25 $\mu\text{g mL}^{-1}$ (Wahome et al., 2015). This result emphasizes the potential of *Euglena* toxin against neuroblastoma, an embryonal malignancy of the sympathetic nervous system that forms in certain types of nerve tissues (Brodeur, 2003).

Antioxidants are highly beneficial in cancer healing as a therapeutic supplement and several studies have suggested that some antioxidants selectively inhibit the growth of tumor cells and improve cytotoxic therapy by inducing cellular differentiation and changing intracellular redox state, (Ladas et al., 2004). Phenolic compounds found in certain algae are cytotoxic against several human cancer cells by apoptosis, antioxidant, and antiangiogenesis effects (Namvar et al., 2013). *Euglena cantabrica* showed high concentrations of phenolic compounds, particularly gallic and protocatechuic acids, estimated up to 5.87 and 2.97 mg G^{-1} dried biomass, respectively (Jerez-Martel et al., 2017). The high levels of phenolic compounds enhance the antioxidant activity of *E. cantabrica* extract and their use in cancer treatment is likely feasible.

The fatty acid profiles of *Euglena* spp. reveal high content of polyunsaturated fatty acids (PUFAs), including the highly desired α -linolenic (ALA), arachidonic (ARA), eicosapentaenoic (EPA) and docosahexaenoic (DHA) acids (Wang et al., 2018), whose potential use for human health have been extensively studied. The *in vitro* inhibitory effect of EPA and DHA on MDA-MB-231 breast cancer cells (by 20–25% at concentrations as low as 25 μM) with a simultaneous increase in the neutral sphingomyelinase (N-SMYase) activity in the tumors has been recently reported (Wu et al., 2005). Although DHA represents only 2% of the total fatty acids in *Euglena*, it contains a wide array of molecules ranging from C16 to C22 PUFAs (Korn, 1964). The exploitation of these potent metabolites in cancer drug development would be highly valuable for safe and efficient therapeutics.

Astaxanthin (3,3'-dihydroxy- β ,SbT-carotene-4,4'-dione) is the dominant xanthophyll carotenoid present in *Euglena sanguinea*, representing up to 80% of the total carotenoid pool (Laza-Martínez et al., 2019). The anticancer property of astaxanthin has been widely documented in animal cancer models. Astaxanthin shows great potential as a chemotherapeutic agent in cancer therapy through anti-proliferative, anti-invasion, and anti-apoptosis effects via various pathways including signal transducer and activator of transcription 3 (STAT3), nuclear factor kappa-light-chain-enhancer of activated B cells (NF- κ B) and peroxisome proliferator-activated receptor gamma (PPAR γ), etc. (Zhang & Wang, 2015). Liao et al. (Liao et al., 2016) demonstrated the down-regulation of thymidylate synthase (TS) expression in two non-small cell lung cancer (NSCLC) cell lines, human lung adenocarcinoma H1650 and squamous cell carcinoma H1703 cells.

Astaxanthin and its isomers have shown inhibitory effects on HCT116 and HT29 human colon cancer cells (Liu et al., 2016), MCF7 and MDA-MB-231 breast cancer cells (McCall et al., 2018), and MCF-7 human breast carcinoma cells (Atalay et al., 2019) through G2/M cell cycle arrest and cellular apoptosis in cancer cells. Similarly, microalgal astaxanthin showed high anticancer potency against UV-7,12 dimethylbenz(a)-anthracene (DMBA)-induced skin

cancer model in rats through its characteristic antioxidant and immunomodulatory actions (Rao et al., 2013). *E. sanguinea* is a carotenoid-rich and bloom-forming species (Zimba et al., 2010), and the extraction of bioactive metabolites from *Euglena* blooms would be a natural source of anticancer formulation based on astaxanthin.

Anticancer properties of extracts of several Euglenoids have been reported. The methanolic extract of *Euglena tuba* showed significant inhibition of human lung (A549) and breast cancer (MCF-7) cells *in vitro*, through induction of apoptosis, with respective IC₅₀ values of 92.14 µg mL⁻¹ and 50.27 µg mL⁻¹ (Panja et al., 2016). Additionally, the *E. tuba* extract elevated the ROS levels in cancer cells which regulate mitogen-activated protein kinase (MAPK) pathways, thereby inducing the apoptosis and suppresses metastasis including cell migration and cell invasion (Panja et al., 2016). Another study on the ethanol fractionated extract of *Euglena viridis* exhibited an *in vitro* cytotoxic activity against two prostate cancer cell lines, PC3, Du145 and colon cancer cell line, HCT-116 (Das et al., 2012).

Conclusion

Certain metabolites produced by various *Euglena* species are excellent molecular models for the development of chemopreventive drugs for cancer by the induction of apoptosis cell death in cancer cells. The latest trends in biotechnology have made tremendous progress in exploiting algal metabolites in cancer/tumor research. However, the feasibility of these phytochemicals is limited by the availability of molecules in larger amounts, cost of purification, and lack of adequate information on their efficacy in human models. Moreover, specific targets of these compounds and mechanisms behind the cancer cell cytotoxicity are yet to be identified. Therefore, further extensive scientific efforts focusing on the potential of these metabolites would help delivering safe and better cancer therapeutics for human applications.

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