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Bioactive peptides from microalgae: Focus on anti-cancer and immunomodulating activity

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Abstract

In addition to the rapidly expanding field of using microalgae for food and feed, microalgae represent a tremendous potential for new bioactive compounds with health-promoting effects. One field where new therapeutics is needed is cancer therapy. As cancer therapy often cause severe side effects and loose effect due to development of drug resistance, new therapeutic agents are needed. Treating cancer by modulating the immune response using peptides has led to unprecedented responses in patients. In this review, we want to elucidate the potential for microalgae as a source of new peptides for possible use in cancer management. Among the limited studies on anti-cancer effects of peptides, positive results were found in a total of six different forms of cancer. The majority of studies have been performed with different strains of *Chlorella*, but effects have also been found using peptides from other species. This is also the case for peptides with immunomodulating effects and peptides with other health-promoting effects (e.g., role in cardiovascular diseases). However, the active peptide sequence has been determined in only half of the studies. In many cases, the microalga strain and the cultivation conditions used for producing the algae have not been reported. The low number of species that have been explored, as opposed to the large number of species available, is a clear indication that the potential for new discoveries is large. Additionally, the availability and cost-effectiveness of microalgae make them attractive in the search for bioactive peptides to prevent cancer.

1 | INTRODUCTION

Microalgae are microscopic, photosynthetic eukaryotic organisms that are considered a good source of proteins and other nutrients. These microorganisms additionally produce a broad range of bioactive compounds and are potential sources of anti-microbial, anti-cancerous, anti-inflammatory as well as immunomodulatory molecules and may also have other health-promoting effects. Moreover, there is an increasing request for microalgae to be used as nutraceuticals and food supplements. In this review, we look into bioactive peptides from microalgae with a focus on anti-cancer and immunomodulatory activities. Cyanobacteria are in some cases also referred to as microalgae, but these prokaryotic organisms will not be included in this review.

Microalgae represent a vast and essentially untapped source of new structures and biologically active molecules. The pharmacological properties of microalgae are generally based on their phytochemical

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components, especially the secondary metabolites, which are outstanding sources of value-added bioactive compounds. However, peptides are an insufficiently investigated group of compounds with a high potential for use in pharmaceuticals. Peptides are short amino acid chains, usually ranging from 2 to 20 units, with a molecular weight under 3 kDa. In plants, bioactive peptides are involved in defence response, as well as in cellular signalling and development regulation (Schaller, 2001). Microalgae produce peptides with active functions, but bioactive peptides can also be produced in vitro by enzymatic hydrolysis of extracted proteins or by digestion (in the digestive tract) of proteins from ingested algae biomass. There is thus a greater potential for peptide production than what is immediately extracted from microalgae.

Cancer is, along with cardiovascular disease, the main cause of death in industrialised countries worldwide. Although the treatment and prevention of cancer are gradually improving, the current therapy options often only prolong survival and do not give the wanted complete and lasting response and often cause severe side effects in patients. Conventional cancer treatments are losing their therapeutic uses due to drug resistance, lack of tumour selectivity and solubility; therefore, there is a need to develop new therapeutic agents.

Therapeutic peptides are a promising approach to treat many diseases, including cancer. They have several advantages over proteins or antibodies. They are easy to synthesise, have a high target specificity and selectivity, high potency of action, low accumulation in tissues and have low toxicity. On the other hand, therapeutic peptides have some significant drawbacks related to their stability (short half-life) and high manufacturing costs (Craik et al., 2013). Therapeutic peptides aimed at cancer treatment may be classified into three main groups: (1) anti-microbial/pore-forming peptides that are part of the innate immune defence system and occur naturally in all living organisms, (2) cell-permeable peptides that provide a promising mechanism for drug delivery and (3) tumour targeting peptides that target markers such as receptors expressed on tumour cell membranes (Boohaker et al., 2012). Marqus et al. (2017) give an overview of different peptides shown to have anti-cancer effects from each group.

The aim of this review is to explore the potential for bioactive peptides from microalgae for health-promoting purposes. The main focus is on cancer-inhibiting peptides and the interactions with immunomodulating effects. The potential and requirements for new discoveries are also discussed.

2 | POTENTIAL USE OF MICROALGAE IN CANCER TREATMENT

Several studies have demonstrated the anti-cancer effects of extracts from microalgae. Both from salt and fresh water sources, microalgae extracts have been found to inhibit the growth of human cancer cells. Marine diatoms contain water-soluble compounds that induce cell death in leukaemia cells by apoptosis and autophagy (Prestegard et al., 2009), and ethanol extracts of the Antarctic freshwater microalgae *Micractinium sp.* and *Chloromonas reticulata* were found to suppress the growth of human colon cancer cells as well as the expression of proinflammatory mediators in macrophages (Suh et al., 2018; Suh et al., 2019). Altering the culturing conditions is shown to influence the production of metabolites (Prestegard et al., 2014) and may also affect the outcome of microalgae extracts on cancer growth. *Skeletonema marinoi* showed anti-cancer activity towards human melanoma cells only under nitrogen-starved culturing conditions (Lauritano et al., 2016), whereas altering light and temperature conditions affected the anti-cancer effect of extracts of marine diatoms (Ingebrigtsen et al., 2016). In both cases, the metabolites in these microalgae extracts may prevent cancer development and progression, for example by inhibition of the innate immune system. Although the bioactive compounds were not identified, these studies demonstrate the outstanding potential of microalgae as sources of anti-cancer compounds. Several other groups have isolated and identified bioactive compounds with anti-cancer activities from microalgae. Examples are carotenoids and other pigments, polyunsaturated aldehydes (PUAs), polysaccharides, polyunsaturated fatty acids (PUFAs), polyphenols, vitamins and amphipathic lipids like phytosterols. These substrates have recently been reviewed thoroughly; please see Andrade et al. (2018) and Galasso et al. (2019).

3 | CANCER AND IMMUNOMODULATION

The tumour microenvironment consists of a heterogeneous population of cells, not only cancer cells but also stromal cells that may additionally be infiltrated by inflammatory cells of the immune system, such as macrophages, natural killer (NK) cells, neutrophils, and lymphocytes (Quail & Joyce, 2013). Macrophages are classically considered vital for immune defence; however, tumour-associated macrophages (TAMs) are supporting multiple aspects of tumour progression and metastasis (Qian & Pollard, 2010). TAMs secret a variety of cytokines and immune-suppressive factors into the tumour environment inhibiting cytotoxic T lymphocytes and NK cells. Thus, inflammation plays a pivotal role in cancer initiation and progression, as well as in metastasis, and may also affect therapy response and resistance (for a recent review, see Munn (2017)). Key mediators for the link between inflammation and cancer are tumour necrosis factor (TNF-α) and nuclear factor-κB (NF-κB); targeting the signalling pathways involving these molecules may be an effective anti-cancer therapy (Yu et al., 2020). Changes in DNA through mutations and chromosomal rearrangement cause tumour cells to present aberrant cell surface antigens that may be recognised by the immune system of the host, which may eliminate the cancer cells. Such elimination is dependent on a functional adaptive immune system involving T and B lymphocytes, antigen-presenting cells, cytokines and the MHC system. Specific activation of the adaptive immune system represents a powerful strategy in cancer therapy (He & Xu, 2020), and blocking immune checkpoints has in recent years been used with great success in the clinic (Farkona et al., 2016); however, side-effects may occur (Li et al., 2021). Other strategies activating the immune system with the aim to defeat cancer are cancer vaccines or engineered T cells (Jeanbart & Swartz, 2015).

4 | MICROALGAE SPECIES PRODUCING PEPTIDES WITH IMMUNOMODULATING OR CANCER-INHIBITING EFFECTS

The number of reports on cancer-inhibiting or immunomodulating effects from bioactive microalgae peptides is very limited (Table 1). In all of these studies, strains from either the genera *Chlorella*, *Dunaliella* or *Pavlova* have been investigated. Several species have considerable economic potential as food, feed and cosmoceuticals, as specified below (Nethravathy et al., 2019).

Chlorella species are currently among the most studied microalgae, and they are also the most extensively used commercially (Barsanti & Gualtieri, 2018; de la Jara et al., 2016). This is mostly due to high production rates, uncomplicated cultivation requirements and its status as EFSA/FDA-approved for human consumption. *Chlorella vulgaris* is a temperate freshwater green alga known for its high protein content combined with rapid growth. It is presently used commercially for food, feed, pharmaceuticals and cosmeceuticals. Different strains of this species show significant differences in cultivation efficiency and biomass composition (Přibyl et al., 2012). *Chlorella pyrenoidosa* has many similarities with *C. vulgaris*: extensively used, shows rapid growth, and among all the species mentioned in Tables 1 and 2, these two are the only ones with current EFSA/FDA food approval status. *Dunaliella salina* is a marine halophilic green alga known for its ability to thrive in high saline environments, and its ability to accumulate β-carotene as a secondary metabolite (Lamers et al., 2012). *Pavlova lutheri* is a haptophyte marine alga known to be rich in LC-PUFAs such as DHA and EPA. It is, among many other species, commonly used as feed in aquaculture due to the valuable nutrient content (Patil et al., 2007).

There are large amounts of information available on how the biomass composition changes according to cultivation conditions for the species mentioned in Table 1 (Ma et al., 2020; Paliwal et al., 2017). Yet, most of the studies of peptides with cancer-inhibiting or immunomodulating effects have paid no attention to the importance of the algae cultivation conditions. This challenge is discussed further in the chapter describing the potential for new discoveries.

5 | MODE OF ACTION OF BIOACTIVE PEPTIDES

5.1 | Mechanisms of action of cancer inhibitors from microalgae

Claiming cancer inhibitory properties of bioactive compounds or developing anti-cancer drugs requires an understanding of the mechanism of action of the drug in question and/or to show that the effect is selective for cancer cells. For example, concentrated water extracts from *Chlorella sorokiniana* were shown to induce mitochondrial-mediated apoptosis in non-small cell lung cancer (NSCLC) cell lines by downregulation of the anti-apoptotic factors Bcl-2 and XIAP. In addition, the tumour growth of subcutaneous

TABLE 1 Microalgae species with anti-cancer and immunomodulating effects

Species	Strain	Cultivation conditions provided?	Pept seq known	Cancer type/immune response	Target and mechanism	Reference
Chlorella pyrenoidosa	Not reported	Not reported	N	Liver cancer, HepG2 cells	Apoptosis (morphology)	Wang and Zhang (2013)
Chlorella vulgaris	Not reported	Not reported	Y	Gastric cancer, AGS cells	Cell cycle regulation	Sheih et al. (2010)
C. vulgaris	211/11B	L, T, IO, G, M	N	Breast cancer, MCF-7 cells	Antiproliferation/cytotoxicity	Sedighi et al. (2016)
C. vulgaris	87/1	IO, M	N	Immune stimulation, Balb/c mice	Multiple immune functions	Morris et al. (2007)
Chlorella sp.	Not reported	Not reported	Y	Anti-inflammation, Wistar rats	Nitric oxide production inhibition	Cherng et al. (2010)
Chlorella sp.	Not reported	Not reported	Y	Anti-inflammation	Suppression of E-selectin, ICAM, VCAM, MCP-1, ET-1	Shih et al. (2013)
Dunaliella salina	Own isolate	L/IO/G/M	N	Colon cancer, SW480 cells	Antiproliferation	Darvish et al. (2018)
Pavlova lutheri	KMCC H-006	Not reported	Y	Fibrosarcoma, HT1080 cells	Metastasis inhibition, MMP-9 inhibition	Ko et al. (2018)
P. lutheri	KMCC H-006	Not reported	Y	Skin cancer/melanogenesis, B16F10 cells	Microphthalmia-associated transcription factor (MITF) and TYR protein expression, activation of extracellular signal regulated kinase	Oh et al. (2015)

Note: Information provided on cultivation conditions: L = light intensity, T = temperature, IO = indoor closed systems or outdoor open systems, G = growth phase, M = nutrient medium composition. N = no, Y = yes. If not specifically stated, molecular mechanism is not reported.

TABLE 2 Bioactive peptides from microalgae with other health effects

Species	Strain	Cultivation conditions provided	Peptide sequence known?	Health effect	Mechanism	Reference
Bellerochae sp.	5 own isolates	T/IO/M	N	Antihypertensive	ACE inhibition	Barkia et al. (2019)
Chlorella elipsoidea	Not reported	Not reported	Y	Antihypertensive	ACE inhibition	Ko et al. (2012)
Chlorella pyrenoidosa	Not reported	Not reported	N	Bone health	Calcium uptake	Hua et al. (2019)
Chlorella sorokiniana	Not reported	Not reported	N	Antihypertensive	ACE inhibition	Tejano et al. (2019)
C. sorokiniana	Not reported	Not reported	N	Antibiotic	Bacterial growth inhibition, 2 sp.	Tejano et al. (2019)
Chlorella vulgaris	Not reported	Not reported	Y	Antihypertensive	ACE inhibition	Suetsuna and Chen (2001)
Chlorella sp.	Not reported	Not reported	N	Anti UV-effects	Inhibit MMP-1 activity	Chen et al. (2011)
Chlorella sp.	Not reported	Not reported	N	Anti UV-effects, fibroblasts	Cytotoxic	Shih and Cherng (2012)
Dunaliella salina	Not reported	Not reported	Y	Anti-osteoporosis	Osteoblast inhibition	Chen et al. (2021)
D. salina	Own isolate	L/IO/G/M	N	Antibiotic	Bacterial growth inhibition 3 sp.	Darvish et al. (2018)
Isochrysis galbana	3011	Not reported	Y	Antihypertensive	ACE inhibition	Wu et al. (2015)
Nannochloropsis oculata	KMMCC-16	L/IO/G/M	Y	Antihypertensive	ACE inhibition	Samarakoon et al. (2013)
N. oculata	Not reported	Not reported	Y	Anti-osteoporosis	Osteoblastic differentiation	Nguyen et al. (2013)
Navicula incerta	Not reported	Not reported	Y	Alcohol protection of liver	HepG2 cytotoxicity inhibition	Kang et al. (2012)
Nitzschia sp.	SP70	T/IO/M	N	Antihypertensive	ACE inhibition	Barkia et al. (2019)
Pavlova lutheri	Not reported	Not reported	Y	Anti-osteoporosis	Increase in ALP and OCN via p38/p65 pathway	Qian et al. (2018)
Phaeodactylum tricornutum	UTEX 646	L/T/IO/M	N	Anti-diabetes	DPP IV inhibition	Stack et al. (2018)
Porphyridium purpureum	PLY 539	L/T/IO/M	N	Anti-diabetes	DPP IV inhibition	Stack et al. (2018)
Scendesmus obliqus	Not reported	L/T/IO/G/M	N	Antiviral	CVB3 inhibition	Afify et al. (2018)
Tetraselmis suecica	CCAP904	T/M	Y	Antibiotic	Bacterial mortality of 6 sp.	Guzman et al. (2019)

Note: Information provided on cultivation conditions: L = light intensity, T = temperature, IO = indoor closed systems or outdoor open systems, G = growth phase, M = nutrient medium composition.
N = no, Y = yes.

xenografts in vivo was markedly inhibited after oral intake of *C. sorokiniana* (Lin et al., 2017).

A limited number of microalgae have been found to harbour bioactive peptides with cancer inhibitory effects, see Table 1. A potential anti-cancer effect of enzymatic protein hydrolysates from *C. vulgaris* has been found; however, neither a mechanism of action nor a selective effect on cancer cells were described (Sedighi et al., 2016). A protein hydrolysate from *D. salina* has been found to inhibit colon cancer (SW480) cell viability and have anti-microbial effects. However, the peptides were not tested on non-cancerous cells and the authors emphasise that further testing, like in vivo experiments, is necessary (Darvish et al., 2018).

The carcinogenic process often involves mutations of cancer-critical genes, either gain-of-function mutations that can drive a cell toward cancer (proto-oncogenes) or loss-of-function mutations that can contribute to cancer (tumour suppressor genes). Many of these genes are central in regulating the cell cycle and constitute checkpoints whereby cell division may be arrested as part of maintaining the normal cell homeostasis. Regulation of the cell cycle is an effective way of inhibiting cancerous cell growth. Algae protein waste is a byproduct from production of algae extracts such as lipids, pigments or carbohydrates for food/feed, pharmaceutics and cosmetics industry. A peptide fraction isolated from pepsin hydrolysate of algae protein waste from *C. vulgaris* was shown to have a strong dose-dependent anti-proliferation effect and induced a post-G1 cell cycle arrest in gastric adenocarcinoma cells. In this case, no cytotoxicity was observed in the WI-38, normal lung fibroblasts cells in vitro (Sheih et al., 2010). They also showed that lipopolysaccharide (LPS)-induced NO production in macrophages was inhibited by a peptide (Sheih et al., 2010), suggesting that the peptide may prevent inflammation. Two additional publications with this 11-amino acid peptide have further shown that it may act anti-inflammatory and might be useful in chronic inflammatory-related diseases, and thus also has potential in cancer therapy (Cherng et al., 2010; Shih et al., 2013).

Both increased cell proliferation and inhibition of apoptosis (programmed cell death) can contribute to tumorigenesis. A polypeptide (*C. pyrenoidosa* antitumor polypeptide, CPAP) isolated from *C. pyrenoidosa* after enzymatic hydrolysis exhibited inhibitory activity on human liver cancer HepG2 cells. Phase-contrast microscopy studies of morphological changes revealed that some cells exhibited the characteristics of apoptosis such as cell membrane shrinkage, condensation and fragmentation of nuclear chromatin, as well as the formation of black apoptotic bodies. Together, these results suggested that CPAP could induce apoptosis and necrotic death of the HepG2 cells (Wang & Zhang, 2013).

Another critical factor in the carcinogenic process is metastasis. Malignant cells acquire the ability to invade local tissues and vessels, move through the circulation, leave the vessels and then establish new cellular colonies at distant sites. This is the deadliest and least understood aspect of cancer, responsible for 90% of cancer-associated deaths. Proteins like metalloproteinases play a critical role in the induction of tumour migration and invasion and are therefore considered an important target for the prevention of cancer metastasis (Gonzalez-Avila et al., 2019). HT1080 fibrosarcoma cells are widely used as a model system to study matrix metalloproteinase (MMP) activity and expression. The marine microalga *P. lutheri* showed an inhibitory effect on human fibrosarcoma HT1080 cells by inhibition of MMP-9. A potent MMP-9 inhibitory peptide composed of seven amino acids was isolated. This peptide reduced mRNA and protein expression levels of MMP-9. (Ko et al., 2018).

Melanoma is a common and severe type of skin cancer usually caused by DNA damage upon exposure to ultraviolet (UV) radiation from sunlight or other artificial sources. Although the production of melanin pigment through melanogenesis protects against the development of melanoma, excessive production of melanin may cause diverse problematic skin conditions such as melasma and pigmented acne scars as well as cancer. The inhibition of melanogenesis by inhibiting tyrosinase activity increases the sensitivity of melanoma cells to chemo- or immunotherapy (Slominski et al., 2009). Recently, Oh et al. (2015) isolated a peptide of four amino acids from fermented *P. lutheri* with inhibitory effects on H_2O_2-induced intracellular reactive oxygen species (ROS) production in mouse melanoma B16F10 cells. Moreover, melanocyte-stimulating hormone (MOS)-induced expression of melanin and microphthalmia-associated transcription factor and activation of tyrosinase were inhibited in a dose-dependent manner by the peptide. Looking into the molecular mechanisms behind the observed peptide-effect on melanogenesis, the effect was found to depend on ERK phosphorylation in the B16F10 cells (Oh et al., 2015). Thus, as an ERK stimulator, this peptide may inhibit tyrosinase and melanogenesis as well as decrease ROS production and thereby prevent the development of malignant melanoma formation.

5.2 | Mechanisms of action of immunomodulating agents from microalgae

Immunomodulation is a regulatory adjustment of the immune system that will normally act to fight any diseases and maintain homeostasis in the body. For immunotherapy, the immune system may be artificially activated or suppressed depending on the desired outcome. Some studies have shown that microalgae biomass, either in its crude form or as an extract, may activate the immune system. Chuang et al. (2014) showed that oral administration of *D. salina* prolonged the survival of BALB/c mice injected with WEHI-3 leukaemia cells. Consumption of *D. salina* by the mice affected the immune response by (1) increasing the population and proliferation of T- and B- cells, (2) increasing the phagocytosis by macrophages, and (3) enhancing the cytotoxicity of NK cells (Chuang et al., 2014). Similar immunomodulatory mechanisms, with increased activity of NK cells and a significant rise in serum concentrations of the cytokines interferon-γ and interleukin-1β (IL1-β, were observed in healthy subjects upon intake of *C. vulgaris* after a short 8-week trial (Kwak et al., 2012).

Different pure compounds from microalgae act in an immunomodulatory way, for example, sulphate polysaccharides, sulpholipids, PUFAs and the carotenoid astaxantin. Riccio and Lauritano (2019)

have recently written an informative review on the subject of the immunomodulatory activities of microalgae, in which they also include some cyanobacteria. As mentioned, scientists from Taiwan have identified an 11-amino acid long peptide with the potential to have cancer-inhibiting effects through its anti-inflammatory activity (Cherng et al., 2010; Sheih et al., 2010; Shih et al., 2013). To the best of our knowledge, no other peptides from microalgae have been identified with immunomodulatory activities yet; however, the potential is high. Morris et al. (2007) found that a protein hydrolysate from *C. vulgaris*, orally administrated to undernourished Balb/c mice, activated immune responses, such as a marked increase of lymphocytes, production of T-cell-dependent antibody responses and reconstitution of delayed-type hypersensitivity (DTH) response as a measurement of immunisation. Moreover, peptides from many other natural sources have immunomodulatory properties. Immunomodulating protein hydrolysates and peptides have, for example, been identified in soybeans, honey and milk. Some of these have been shown to have anti-cancer effects. Chen et al. found that extract of fermented milk decreased the growth of human breast cancer cells while leaving normal mammary epithelial cells unaffected (Chen et al., 2007). The effect might be caused by a change in peptide content upon milk fermentation, resulting in immunomodulating peptides (Chen et al., 2007). Recently, a case report of one patient showed that treatment with a lytic peptide resulted in an upregulation of immune genes and T-cell infiltration, causing tumour regression (Jebsen et al., 2019). These results emphasise the potential of naturally occurring peptides as immunomodulators.

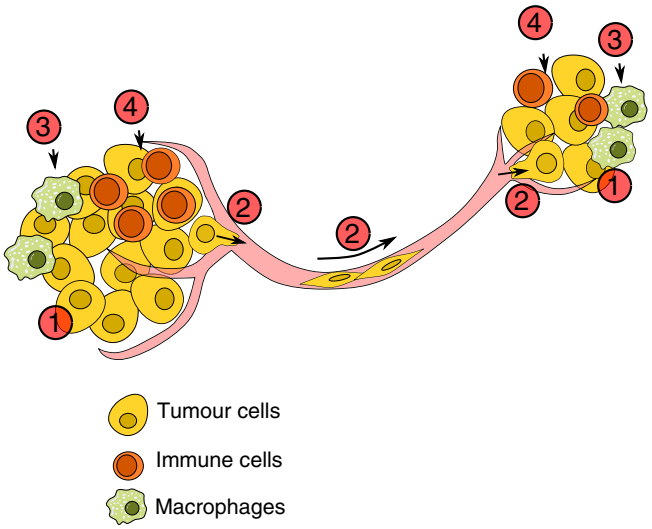

FIGURE 1 Simplified illustration of a solid tumour and its microenvironment, with putative points of attack for anti-cancer drugs indicated in numbers. The response to cancer therapy depends on the composition of the tumour microenvironment. This abnormal microenvironment consists of stromal cells, inflammatory cells and vasculature in addition to tumour cells. Anti-cancer peptides may act by attacking the cancer cells directly (1), for example, by disruption of the cell membrane. Anti-cancer peptides may also prevent metastasis by inhibition of, for example, matrix metalloproteases, ability to invade blood or lymph or to extravasate into the interstitium and form new tumours (2). Peptides can also disturb the interaction between tumour cells and tumour-associated macrophages (3) or other immune cells (4) and as such promote anti-cancer immune responses or attenuate tumourigenic signalling

Figure 1 summarises the different targets where one can expect that microalgal peptides can exert anti-cancer effects on, either by direct action on the cancer cells or their ability to metastasise or by modulating the interplay between cancer cells and the different members of the cellular immune system. In many cases, a combination of drugs targeted towards several of the elements depicted in Figure 1 will produce an enhanced anti-cancer effect compared to mono-therapy directed towards only one of the factors.

6 | PHARMACOLOGICAL ASPECTS OF PEPTIDES

Peptides and proteins are similar to other polymeric biomolecules such as polysaccharides and oligonucleotides, already used as drugs to treat a number of diseases. The perhaps best-known peptide-based drug is insulin, which has been used to treat diabetes for several decades. Common for all these biomolecule-based drugs is that they must be administered parenterally, that is, as injections, usually subcutaneous or intravenous. These drugs do not tolerate the harsh environment in the stomach, and enterocapsules, ensuring the release of the drug after the capsules have entered the small intestine, do not prevent degradation of the drugs. Furthermore, large molecules are not easily absorbed through the intestinal mucosa and the bioavailability is very low and variable (for a recent review on oral delivery of peptides, see Tyagi et al., 2018).

In treatments of severe diseases like cancer, the threshold for parenteral administration of drugs is lower than for less severe conditions. This is partly due to the poor bioavailability of anti-cancer drugs but also to ensure that the plasma-concentration of the drug is within the therapeutic range. The intra-individual, and sometimes inter-individual pharmacokinetics, can cause plasma-concentrations over the toxic limits. Thus, therapeutic drug monitoring (TDM) is advised for several oral anti-cancer drugs (Mueller-Schoell et al., 2020). The fact that potential anti-cancer or immunomodulatory peptides from microalgae may have poor oral bioavailability is therefore not necessarily a problem as long as they are relatively stable after parenteral injection.

The advantages of bioactive peptides in cancer treatment are reflected by the number of clinical trials already done. When searching Clinicaltrials.gov (https://clinicaltrials.gov/ct2/home) using the term peptide, 6088 clinical trials appear (January 2021). Combining the terms peptide and cancer gives 1080 hits, and although some of these (158 studies) have been withdrawn or terminated before completion, most have been completed, and several are actively recruiting. One example is the previously mentioned lytic peptide LTX-315, and a few more examples of anti-cancer peptides in clinical trials are given by Kurrikoff et al. (2019). In 2012, Thundimadathil reviewed anti-cancer peptides, which were already approved or in clinical trials

(Thundimadathil, 2012). The author concluded that the use of peptides (not including monoclonal antibodies) will become more common in future cancer therapy because of their chemical versatility and their target specificity to receptors in a tumour, such as the RGD moiety in receptors found in tumour neovasculature (Sani et al., 2021).

Still, the rapid degradation of peptides that are not naturally present in human blood can be problematic. Several of the peptides already tested or used as drugs are peptide hormones, which are naturally present in the blood. A strategy to increase stability is to develop peptidomimetics based on the physio-chemical properties of the bioactive peptide (see Lenci and Trabocchi (2020) for a tutorial review article on peptidomimetic drug discovery). Peptidomimetics will have the same functional moieties and the three-dimensional structure as the peptide they mimic (Laxio Arenas et al., 2019), but the backbone will not be prone to enzymatic degradation by proteases. One can therefore anticipate that the anti-cancer or immunomodulatory peptides from microalgae also can be mimicked and that drug candidates based on these can be developed.

7 | COMMERCIAL USE OF MICROALGAE PEPTIDES IN CANCER TREATMENT

Patenting to protect possible intellectual properties and thereby rights for commercial exploitation of the product is surprisingly not common in the microalgal biotechnology sector (de la Jara et al., 2016). In a simple search of the European Patent database, Espacenet, (https://www.epo.org/searching-for-patents/technical/espacenet.html, search date 11.01.2021) for the terms cancer, peptide and microalgae in title, abstract or claims, seven results were found. Of these, only one refers to a peptide isolated from microalgae with intended use as a cancer inhibitor (https://worldwide.espacenet.com/patent/search?q=pn%3DKR101637640B1). Likewise, when searching the US Patent and Trademark Office (the USPTO, search date 11.01.2021), no patents claiming the use of peptides from microalgae for the treatment of cancer were found. This may reflect a possible tendency of postponing filing patent applications in the particular sector of microalgae-based industry—or it may suggest an unexplored potential of peptide utilisation in the fight against cancer.

8 | MICROALGAE SPECIES PRODUCING PEPTIDES WITH OTHER HEALTH-PROMOTING EFFECTS

Production of health-promoting compounds from microalgae is a large research field (Barkia et al., 2019; Hamidi et al., 2020; Hunt & Vincent, 2006; Raposo et al., 2013), but the health-promoting effects of peptides from microalgae have been studied less extensively. Table 2 lists the main algae shown to produce peptides with potential health-improving effects, including anti-hypertensive, anti-diabetes, anti-UV damages, anti-osteoperotic, antibiotic and antiviral effects. The anti-hypertensive effect through ACE inhibition is an example where there is potential for discovery of active peptides from a number of species, illustrated in this overview by finding this effect in peptides isolated from seven species from five genera. In half of the cases reported in Table 2, the bioactive effect is found in a protein hydrolysate where the active peptide is not yet identified. As discussed below, there is reason to believe that the algae proteome profile changes according to the cultivation conditions used for the algae biomass production; still, in many cases, this important fact is ignored when the results are reported.

9 | POTENTIAL FOR NEW DISCOVERIES

Properties of microalgae differ significantly between species. The total number of algae species has been estimated from ~30,000 to several million unique species, and these numbers are continuously debated (Guiry, 2012). Different strains of the same species may have immensely differing properties, often depending on the environment from which the strain was isolated.

Microalgae are found in a wide range of environmental conditions, and some algae can thrive in environments that are challenging or deadly for others. For instance, temperature ranges from tropical to arctic environments, salinities ranging from freshwater to highly saline ponds, UV light exposure, different day lengths, drought and pressure (Barsanti et al., 2008; Seckbach, 2007). Although most microalgae are aqueous, they can also be found in soil, on rocks and on ice. To survive and thrive in these highly diverse environments, different species of algae have developed significantly divergent metabolic properties. Although many algae show optimal growth at conditions that are detrimental for others, most algae can survive at conditions that diverge from their optimal environments, using stress response mechanisms. Some of these stress responses can be induced and utilised to produce valuable secondary metabolites, including discoveries of bioactive metabolites, and this topic has been previously reviewed (Ma et al., 2020; Paliwal et al., 2017; Skjånes et al., 2013).

Screening for cancer-inhibiting effects from microalgae is often performed using general extracts from algae biomass without identifying the active component. Most of these studies do not explore the impact of cultivation conditions on the presence of bioactive compounds. However, in a study using general extracts from 32 species of microalgae, it was shown that a strain of *Skeletonema marinoi* exhibited anti-cancer activity on human melanoma cells, but only when the alga was cultured under nitrogen-starvation (Lauritano et al., 2016). Another strain of the same species did not show this trait. Both were isolated from the Mediterranean Sea, but testing was limited to hydrophobic extracts. Another study exploring five diatoms showed that changing cultivation light intensity and temperature resulted in algae biomass from which extracts from polar compounds inhibited the activity on melanoma cells (Ingebrigtsen et al., 2016).

Cells exposed to high light intensity employ defence mechanisms against photoinhibition, that is, damaging effects of light-induced stress. Over-excitation of the photosynthetic apparatus causes direct degradation of photosystem II (PSII) and increased production of ROS.

ROS causes cell damage and inhibition of important metabolic processes, leading to changes in the synthesis of proteins involved in the affected processes (Nishiyama et al., 2006). Photoinhibition increases when the cell is exposed to stress conditions that limit growth, such as nutrient limitation, pH-, salt- or temperature stress, since the formation of biomass represents an electron sink. Any loss of electron sinks leads to a reductive state of the electron transport chains in the chloroplast, thereby increasing oxidative stress. Combinations of stress conditions can be considered an efficient tool to change the proteome profile in the cells. Adaptation mechanisms to high light are essential for survival and can be solved by balancing the energy input with the energy output. Balance is obtained through increased CO_2 assimilation creating an energy sink not related to cell division and growth. This energy sink is often expressed as lipids or carbohydrates (Bránykiová et al., 2011; Hu et al., 2008).

Another defence strategy from photoinhibition is to quench oxidative stress by producing antioxidants before ROS damages the photosystems. All algae produce antioxidants scavenging free radicals, the most common group of antioxidants being carotenoids. Carotenoids also have an important function contributing to light absorption as part of the light-harvesting complex. Under light stress, many algae will accumulate carotenoids in large amounts, astaxanthin from *Haematococcus* (Del Campo et al., 2007; He et al., 2007) and β-carotene from *Dunaliella* (Lamers et al., 2008) being the two most commercially exploited. In addition to preventing oxidative damage, carotenoids accumulating in large amounts function as an energy sink. Some of the peptides mentioned in Tables 1 and 2 may also have antioxidant activities. Although in some cases the peptides reported in these studies are a result of hydrolysis of protein extracts, it is also likely that some peptides are produced to have an active role in the photo-protection of algae cells. Other antioxidants that can be accumulated in microalgae under light stress are, for example, polyphenols, flavonoids, butylated hydroxytoluene (BHT) and vitamins (Babu & Wu, 2008; Durmaz, 2007).

Most microalgae have a poor defence system for temperatures higher than their optimum temperatures for growth, likely due to irreversibly denaturation of proteins, but many algae are able to handle lower temperatures to some extent. A typical defence strategy for low temperatures is the production of PUFAs to maintain the fluidity of membrane systems (Jiang & Gao, 2004). Light absorption by the photosystems is independent of temperatures, but the energy consumption is not, and reduced temperatures will therefore cause an energy imbalance. Slower enzyme activities can cause oxidative stress, which can be compensated for by increased production of the enzymes. This means that when peptides from proteins' hydrolysates have the desired bioactive effect, cultivation at lower temperatures can be used as a tool for producing more of the desired peptide. Additional mitigation measures at low temperatures are the production of antioxidants and energy sinks such as starch and lipids. Psychrophilic strains adapted to life on snow and ice produce anti-freeze proteins that bind to ice crystals and prevent drought and cell damage (Morgan-Kiss et al., 2006). As far as we know, hydrolysation of these specific proteins has not been explored as a potential source of peptides with cancer-inhibiting or immunomodulating effects. Life in cold environments is often combined with very variable daylengths and exposure to UV light. The reason snow algae often cause red and yellow patches on snow is the production of UV-screening carotenoids, and many algae also produce UV-protectants in the form of phenols or mycosporine-like amino acids, all of which are commercially relevant (Duval et al., 2000; Shi et al., 2017).

Many algae can tolerate a wide range of salinities. The osmotic stress is compensated by using water flux, ion transport and production of osmolytes such as glycerol, mannitol, dimethylsulphoniopropionate (DMSP) or carbohydrates (Chen & Jiang, 2009). The most common method for inducing stress reactions leading to biomass composition with increased commercial potential is nutrient deprivation. By depriving the cells of one or more nutrients, cell division will inevitably stop. Cell components that are not essential during the deprivation are degraded to make the limiting nutrient(s) available, and it is likely that the extensive protein degradation inevitably occurring leads to the formation of peptides that were not present before. Another metabolic shift during nutrient deprivation is a

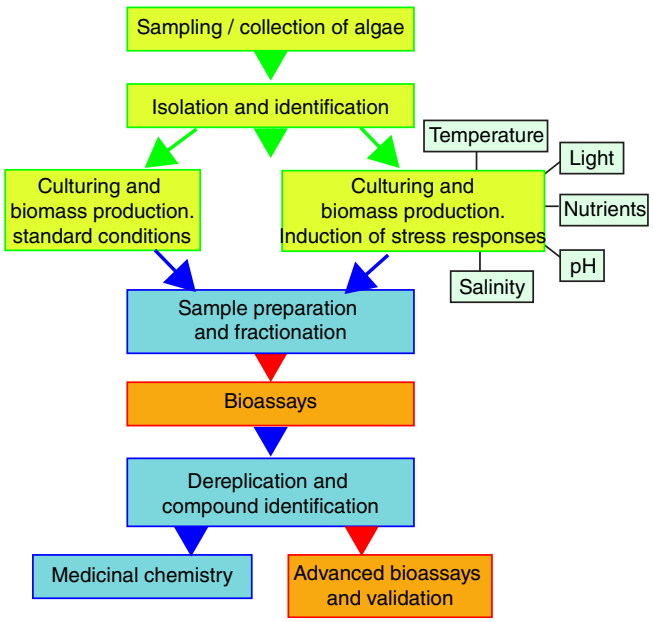

FIGURE 2 Procedure for the discovery of new bioactive compounds from microalgae. After collection of microalgae from the environment, the strains are isolated, identified at genus or species level, and cultivation procedures are established. To maximise the chance of new discoveries, at least two different cultivation lines could be performed. One production line cultivates the alga under semi-optimal conditions for growth, and the other(s) exposes the alga for environmental stress conditions to induce stress management mechanisms, resulting in the production of secondary metabolites. After algae biomass production and harvesting, the extracts from different extraction procedures are prepared, and the extracts are fractionated. The selected bioassays are performed, and fractions with a positive response are explored to identify the active compound. Colour coding represents the scientific discipline involved in the different steps. Green/yellow: Microalgae biology. Blue: Chemistry. Red/orange: Cell biology

decrease in photosynthetic activity. There is a correlation between the cells' reactions to nutrient deprivation and light intensity, and light saturation occurs at a lower light intensity in nutrient-deprived cultures. The reactions described as a result of photoinhibition will also occur during nutrient deprivation in light.

All the stress mechanisms described above represent metabolic shifts that changes the protein profile in the cells, but utilising cultivation conditions as a tool for changing the content of potential bioactive peptides in microalgae has been insufficiently investigated. In most cases, in bioactive peptide studies, the cultivation conditions are not even reported, as indicated in Tables 1 and 2. However, it has been shown that the amino acid profile can be changed in species from the genera *Chlorella*, *Scenedesmus* and *Dunaliella* using cultivation methods (Samek et al., 2013; Sui et al., 2019; Wild et al., 2019), which indicates an accompanied change of the proteome profile.

It is clear that the potential for discovering new peptides from microalgae with cancer-inhibiting effects is significant and mostly unexplored. In addition to the large number of species and the even larger number of strains available for exploration, the possibility for finding the desired bioactive effects can be significantly increased by using cultivation conditions to induce stress management mechanisms in the algae. Based on these observations, it is suggested that the procedure for identifying new bioactive compounds includes a step where the alga culture is exposed to environmental stress such as high light intensities, UV-light, low temperatures, high salinities and nutrient deprivation to induce the production of secondary metabolites. The suggested process is described in Figure 2. Furthermore, the underexplored possibility of utilising different proteolytic enzymes and enzyme combinations in the process of biorefinery adds to the potential of discovering new bioactive peptides. Finally, given the variation in both therapeutic groups (anti-microbial/pore-forming, cell-permeable peptides and tumour targeting) and the diversity of the carcinogenic processes (cell cycle regulation, apoptosis and metastasis), including a high number of possible targets within each group and process, there is a tremendous potential for new discoveries of peptides from microalgae for inhibiting tumour development.

10 | CONCLUSIONS

Along with the ageing population of Western countries, we expect that the demand for novel drugs will increase. This is particularly the case for cancer, where most cancer types are considered diseases of the elderly. The recent knowledge on the interplay between carcinogenesis and immunomodulation also makes drugs targeted to the immune system relevant in cancer therapy as well as prevention of cancer development. The newly developed CAR-T cell therapy is one example on how we can utilise the immune system to eradicate malignant cells.

Despite the vast number of microalgal species and their large variation in habitat and physiology, few bioactive peptides from these organisms are reported. This can partly be because terrestrial plants have been the main source for drug candidates for centuries, and also that the focus on bioactivities from microalgae has been on poisonous species. However, the easy access for sampling, the possibility to mass culture, together with the advances in genetic characterisation and manipulation make microalgae highly relevant in drug discovery. Based on this, we believe that the development and maintenance of culture collections are pivotal to fully exploit the potential of microalga as producers of bioactive compounds. Here, it will be possible to manipulate the algae to trigger the synthesis of a bioactive compound. If connected with a high-throughput screening platform that can be modified for different screening assays (see Figure 2 for a simplified scheme of drug discovery from microalgae), the chances for discovering novel bioactive compounds are, in our opinion, very high.

Although this review focuses on peptides and anti-cancer and immunomodulatory effects, there are several other diseases that are in need of improved therapy. Multi-resistant bacteria are one example where clinicians are left without further tools to combat severe infections. We, therefore, urge algae researchers to collaborate with biomedical researchers to fully exploit the capacity of microalgae as drug producers. As the body's immune defence plays a pivotal role in preventing cancer development and progression, targeting the heterogeneity of cancers through a combination of immunomodulating and cancer-inhibiting peptides appears as a promising strategy for targeting malignant tumours.

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All authors contributed to the conceptualisation of the review article, the literature search and analyses, as well as writing. The final version of the article has been read and approved by all authors.

DATA AVAILABILITY STATEMENT

Data sharing is not applicable to this article as no new data were created or analyzed in this study.

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REFERENCES

Afify, A.E.-M.M.R., El Baroty, G.S., El Baz, F.K., Abd El Baky, H.H. & Murad, S.A. (2018) *Scenedesmus obliquus*: Antioxidant and antiviral activity of proteins hydrolyzed by three enzymes. *Journal, Genetic Engineering and Biotechnology*, 16(2), 399–408.

Andrade, K.A.M., Lauritano, C., Romano, G. & Ianora, A. (2018) Marine microalgae with anti-cancer properties. *Marine Drugs*, 16(165), 1–17.

Babu, B. & Wu, J.T. (2008) Production of natural butylated hydroxytoluene as an antioxidant by freshwater phytoplankton. *Journal of Phycology*, 44(6), 1447–1454.

Barkia, I., Al-Haj, L., Hamid, A.A., Zakaria, M., Saari, N. & Zadjali, F. (2019) Indigenous marine diatoms as novel sources of bioactive peptides with antihypertensive and antioxidant properties. *International Journal of Food Science and Technology*, 54(5), 1514–1522.

Barsanti, L., Coltelli, P., Evangelista, V., Frassanito, A.M., Passarelli, V., Vesentini, N. et al. (2008) Oddities and curiosities in the algal world. In: Evangelista, V., Barsanti, L., Frassanito, A.M., Passarelli, V. & Gualtieri, P. (Eds.) *Algal toxins: nature, occurrence, effect and detection*. Dordrecht: Springer, pp. 353–391.

Barsanti, L. & Gualtieri, P. (2018) Is exploitation of microalgae economically and energetically sustainable? *Algal Research*, 31, 107–115.

Boohaker, R.J., Lee, M.W., Vishnubhotla, P., Perez, J.L.M. & Khaled, A.R. (2012) The use of therapeutic peptides to target and to kill cancer cells. *Current Medicinal Chemistry*, 19(22), 3794–3804.

Brányiková, I., Maršálková, B., Doucha, J., Brányik, T., Bišová, K., Zachleder, V. et al. (2011) Microalgae-novel highly efficient starch producers. *Biotechnology and Bioengineering*, 108(4), 766–776.

Chen, C., Chan, H.M. & Kubow, S. (2007) Kefir extracts suppress in vitro proliferation of estrogen-dependent human breast cancer cells but not normal mammary epithelial cells. *Journal of Medicinal Food*, 10(3), 416–422.

Chen, C.L., Liou, S.F., Chen, S.J. & Shih, M.F. (2011) Protective effects of *Chlorella*-derived peptide on UVB-induced production of MMP-1 and degradation of procollagen genes in human skin fibroblasts. *Regulatory Toxicology and Pharmacology*, 60(1), 112–119.

Chen, H. & Jiang, J.G. (2009) Osmotic responses of *Dunaliella* to the changes of salinity. *Journal of Cellular Physiology*, 219(2), 251–258.

Chen, Y.X., Chen, J.C., Zheng, Y.F., Yu, H.L., Zhao, J.W., Chen, J. et al. (2021) *Dunaliella salina*-derived peptide protects from bone loss: Isolation, purification and identification. *LWT – Food Science and Technology*, 137, 10.

Cherng, J.Y., Liu, C.C., Shen, C.R., Lin, H.H. & Shih, M.F. (2010) Beneficial effects of *Chlorella*-11 peptide on blocking lps-induced macrophage activation and alleviating thermal injury-induced inflammation in rats. *International Journal of Immunopathology and Pharmacology*, 23(3), 811–820.

Chuang, W.-C., Ho, Y.-C., Liao, J.-W. & Lu, F.-J. (2014) *Dunaliella salina* exhibits an antileukemic immunity in a mouse model of WEHI-3 leukemia cells. *Journal of Agricultural and Food Chemistry*, 62(47), 11479–11487.

Craik, D.J., Fairlie, D.P., Liras, S. & Price, D. (2013) The future of peptide-based drugs. *Chemical Biology and Drug Design*, 81(1), 136–147.

Darvish, M., Jalili, H., Ranaei-Siadat, S.O. & Sedighi, M. (2018) Potential cytotoxic effects of peptide fractions from *Dunaliella salina* protein hydrolyzed by gastric proteases. *Journal of Aquatic Food Product Technology*, 27(2), 165–175.

de la Jara, A., Assuncao, P., Portillo, E., Freijanes, K. & Mendoza, H. (2016) Evolution of microalgal biotechnology: a survey of the European patent office database. *Journal of Applied Phycology*, 28(5), 2727–2740.

Del Campo, J.A., Garcia-Gonzalez, M. & Guerrero, M.G. (2007) Outdoor cultivation of microalgae for carotenoid production: current state and perspectives. *Applied Microbiology and Biotechnology*, 74(6), 1163–1174.

Durmaz, Y. (2007) Vitamin E (alpha-tocopherol) production by the marine microalgae *Nannochloropsis oculata* (Eustigmatophyceae) in nitrogen limitation. *Aquaculture*, 272(1–4), 717–722.

Duval, B., Shetty, K. & Thomas, W.H. (2000) Phenolic compounds and antioxidant properties in the snow alga *Chlamydomonas nivalis* after exposure to UV light. *Journal of Applied Phycology*, 11(6), 559–566.

Farkona, S., Diamandis, E.P. & Blasutig, I.M. (2016) Cancer immunotherapy: the beginning of the end of cancer? *BMC Medicine*, 14(73), 1–18.

Galasso, C., Gentile, A., Orefice, I., Ianora, A., Bruno, A., Noonan, D.M. et al. (2019) Microalgal derivatives as potential nutraceutical and food supplements for human health: a focus on cancer prevention and interception. *Nutrients*, 11(1226), 1–22.

Gonzalez-Avila, G., Sommer, B., Mendoza-Posada, D.A., Ramos, C., García-Hernández, A.A. & Falfán-Valencia, R. (2019) Matrix metalloproteinases participation in the metastatic process and their diagnostic and therapeutic applications in cancer. *Critical Reviews in Oncology/Hematology*, 137, 57–83.

Guiry, M.D. (2012) How many species of algae are there? *Journal of Phycology*, 48(5), 1057–1063.

Guzman, F., Wong, G., Roman, T., Cardenas, C., Alvarez, C., Schmitt, P. et al. (2019) Identification of antimicrobial peptides from the microalgae *Tetraselmis suecica* (Kylin) butcher and bactericidal activity improvement. *Marine Drugs*, 17(8), 19.

Hamidi, M., Kozani, P.S., Kozani, P.S., Pierre, G., Michaud, P. & Delattre, C. (2020) Marine Bacteria versus microalgae: who is the best for biotechnological production of bioactive compounds with antioxidant properties and other biological applications? *Marine Drugs*, 18(28), 1–38.

He, P., Duncan, J. & Barber, J. (2007) Astaxanthin accumulation in the green alga *Haematococcus pluvialis*: effects of cultivation parameters. *Journal of Integrative Plant Biology*, 49(4), 447–451.

He, X. & Xu, C. (2020) Immune checkpoint signaling and cancer immunotherapy. *Cell Research*, 30(8), 660–669.

Hu, Q., Sommerfeld, M., Jarvis, E., Ghirardi, M., Posewitz, M., Seibert, M. et al. (2008) Microalgal triacylglycerols as feedstocks for biofuel production: perspectives and advances. *The Plant Journal*, 54(4), 621–639.

Hua, P.P., Xiong, Y., Yu, Z.Y., Liu, B. & Zhao, L.N. (2019) Effect of *Chlorella pyrenoidosa* protein hydrolysate-calcium chelate on calcium absorption metabolism and gut microbiota composition in low-calcium diet-fed rats. *Marine Drugs*, 17(6), 20.

Hunt, B. & Vincent, A.C.J. (2006) Scale and sustainability of marine bioprospecting for pharmaceuticals. *Ambio*, 35(2), 57–64.

Ingebrigtsen, R.A., Hansen, E., Andersen, J.H. & Eilertsen, H.C. (2016) Light and temperature effects on bioactivity in diatoms. *Journal of Applied Phycology*, 28(2), 939–950.

Jeanbart, L. & Swartz, M.A. (2015) Engineering opportunities in cancer immunotherapy. *Proceedings of the National Academy of Sciences*, 112(47), 14467–14472.

Jebsen, N.L., Apelseth TO, Haugland, H.K., Rekdal, Ø., Patel, H., Gjertsen, B.T. et al. (2019) Enhanced T-lymphocyte infiltration in a desmoid tumor of the thoracic wall in a young woman treated with intratumoral injections of the oncolytic peptide LTX-315: a case report. *Journal of Medical Case Reports*, 13(177), 1–9.

Jiang, H.M. & Gao, K.S. (2004) Effects of lowering temperature during culture on the production of polyunsaturated fatty acids in the marine diatom *Phaeodactylum tricornutum* (Bacillariophyceae). *Journal of Phycology*, 40(4), 651–654.

Kang, K.H., Qian, Z.J., Ryu, B., Karadeniz, F., Kim, D. & Kim, S.K. (2012) Antioxidant Peptides from Protein Hydrolysate of Microalgae *Navicula incerta* and their Protective Effects in Hepg2/CYP2E1 Cells Induced by Ethanol. *Phytotherapy Research*, 26(10), 1555–1563.

Ko, S.-C., Heo, S.-Y., Choi, S.-W., Qian, Z.-J., Heo, S.-J., Kang, D.-H. et al. (2018) A heptameric peptide isolated from the marine microalga *Pavlova lutheri* suppresses PMA-induced secretion of matrix metalloproteinase-9 through the inactivation of the JNK, p38, and NF-κB pathways in human fibrosarcoma cells. *Journal of Applied Phycology*, 30(4), 2367–2378.

Ko, S.C., Kang, N., Kim, E.A., Kang, M.C., Lee, S.H., Kang, S.M. et al. (2012) A novel angiotensin I-converting enzyme (ACE) inhibitory peptide from a marine *Chlorella ellipsoidea* and its antihypertensive effect in spontaneously hypertensive rats. *Process Biochemistry*, 47(12), 2005–2011.

Kurrikoff, K., Aphkhazava, D. & Langel, Ü. (2019) The future of peptides in cancer treatment. *Current Opinion in Pharmacology*, 47, 27–32.

Kwak, J.H., Baek, S.H., Woo, Y., Han, J.K., Kim, B.G., Kim, O.Y. et al. (2012) Beneficial immunostimulatory effect of short-term *Chlorella* supplementation: enhancement of natural killercell activity and early inflammatory response (randomized, double-blinded, placebo-controlled trial). *Nutrition Journal*, 11(53), 1–8.

Lamers, P.P., Janssen, M., De Vos, R.C.H., Bino, R.J. & Wijffels, R.H. (2008) Exploring and exploiting carotenoid accumulation in *Dunaliella salina* for cell-factory applications. *Trends in Biotechnology*, 26(11), 631–638.

Lamers, P.P., Janssen, M., De Vos, R.C.H., Bino, R.J. & Wijffels, R.H. (2012) Carotenoid and fatty acid metabolism in nitrogen-starved *Dunaliella salina*, a unicellular green microalga. *Journal of Biotechnology*, 162(1), 21–27.

Lauritano, C., Andersen, J.H., Hansen, E., Albrigtsen, M., Escalera, L., Esposito, F. et al. (2016) Bioactivity screening of microalgae for antioxidant, anti-inflammatory, anticancer, anti-diabetes, and antibacterial activities. *Frontiers in Marine Science*, 3(68), 1–12.

Laxio Arenas, J., Kaffy, J. & Ongeri, S. (2019) Peptides and peptidomimetics as inhibitors of protein-protein interactions involving beta-sheet secondary structures. *Current Opinion in Chemical Biology*, 52, 157–167.

Lenci, E. & Trabocchi, A. (2020) Peptidomimetic toolbox for drug discovery. *Chemical Society Reviews*, 49(11), 3262–3277.

Li, H., Xu, J., Bai, Y., Zhang, S., Cheng, M. & Jin, J. (2021) Nephrotoxicity in patients with solid tumors treated with anti-PD-1/PD-L1 monoclonal antibodies: a systematic review and meta-analysis. *Investigational New Drugs*, 39, 860–870.

Lin, P.-Y., Tsai, C.-T., Chuang, W.-L., Chao, Y.-H., Pan, I.H., Chen, Y.-K. et al. (2017) *Chlorella sorokiniana* induces mitochondrial-mediated apoptosis in human non-small cell lung cancer cells and inhibits xenograft tumor growth *in vivo*. *BMC Complementary and Alternative Medicine*, 17 (88), 1–8.

Ma, R.J., Wang, B.B., Chua, E.T., Zhao, X.R., Lu, K.Y., Ho, S.H. et al. (2020) Comprehensive utilization of marine microalgae for enhanced co-production of multiple compounds. *Marine Drugs*, 18(9), 26.

Marqus, S., Pirogova, E. & Piva, T.J. (2017) Evaluation of the use of therapeutic peptides for cancer treatment. *Journal of Biomedical Science*, 24 (21), 1–15.

Morgan-Kiss, R.M., Priscu, J.C., Pocock, T., Gudynaite-Savitch, L. & Huner, N.P.A. (2006) Adaptation and acclimation of photosynthetic microorganisms to permanently cold environments. *Microbiology and Molecular Biology Reviews*, 70(1), 222–252.

Morris, H.J., Carrillo, O., Almarales, A., Bermúdez, R.C., Lebeque, Y., Fontaine, R. et al. (2007) Immunostimulant activity of an enzymatic protein hydrolysate from green microalga *Chlorella vulgaris* on undernourished mice. *Enzyme and Microbial Technology*, 40(3), 456–460.

Mueller-Schoell, A., Groenland, S.L., Scherf-Clavel, O., van Dyk, M., Huisinga, W., Michelet, R. et al. (2020) Therapeutic drug monitoring of oral targeted antineoplastic drugs. *European Journal of Clinical Pharmacology*, 77, 441–464.

Munn, L.L. (2017) Cancer and inflammation. *WIREs Systems Biology and Medicine*, 9(e1370), 1–13.

Nethravathy, M.U., Mehar, J.G., Mudliar, S.N. & Shekh, A.Y. (2019) Recent advances in microalgal bioactives for food, feed, and healthcare products: commercial potential, market space, and sustainability. *Comprehensive Reviews in Food Science and Food Safety*, 18(6), 1882–1897.

Nguyen, M.H.T., Qian, Z.J., Nguyen, V.T., Choi, I.W., Heo, S.J., Oh, C.H. et al. (2013) Tetrameric peptide purified from hydrolysates of biodiesel byproducts of *Nannochloropsis oculata* induces osteoblastic differentiation through MAPK and Smad pathway on MG-63 and D1 cells. *Process Biochemistry*, 48(9), 1387–1394.

Nishiyama, Y., Allakhverdiev, S.I. & Murata, N. (2006) A new paradigm for the action of reactive oxygen species in the photoinhibition of photosystem II. *Biochimica et Biophysica Acta – Bioenergetics*, 1757(7), 742–749.

Oh, G.-W., Ko, S.-C., Heo, S.-Y., Nguyen, V.-T., Kim, G., Jang, C.H. et al. (2015) A novel peptide purified from the fermented microalga *Pavlova lutheri* attenuates oxidative stress and melanogenesis in B16F10 melanoma cells. *Process Biochemistry*, 50(8), 1318–1326.

Paliwal, C., Mitra, M., Bhayani, K., Bharadwaj, S.V.V., Ghosh, T., Dubey, S. et al. (2017) Abiotic stresses as tools for metabolites in microalgae. *Bioresource Technology*, 244, 1216–1226.

Patil, V., Källqvist, T., Olsen, E., Vogt, G. & Gislerød, H.R. (2007) Fatty acid composition of 12 microalgae for possible use in aquaculture feed. *Aquaculture International*, 15, 1–9.

Prestegard, S.K., Knutsen, G. & Herfindal, L. (2014) Adenosine content and growth in the diatom *Phaeodactylum tricornutum* (Bacillariophyceae): effect of salinity, light, temperature and nitrate. *Diatom Research: The Journal of the International Society for Diatom Research*, 29(4), 361–369.

Prestegard, S.K., Oftedal, L., Coyne, R.T., Nygaard, G., Skjaerven, K.H., Knutsen, G. et al. (2009) Marine benthic diatoms contain compounds able to induce leukemia cell death and modulate blood platelet activity. *Marine Drugs*, 7(4), 605–623.

Přibyl, P., Cepák, V. & Zachleder, V. (2012) Production of lipids in 10 strains of *Chlorella* and *Parachlorella*, and enhanced lipid productivity in *Chlorella vulgaris*. *Applied Microbiology and Biotechnology*, 94 (2), 549–561.

Qian, B.Z. & Pollard, J.W. (2010) Macrophage diversity enhances tumor progression and metastasis. *Cell*, 141(1), 39–51.

Qian, Z.J., Ryu, B., Kang, K.H., Heo, S.J., Kang, D.H., Bae, S.Y. et al. (2018) Cellular properties of the fermented microalgae *Pavlova lutheri* and its isolated active peptide in osteoblastic differentiation of MG-63 cells. *Molecular Medicine Reports*, 17(1), 2044–2050.

Quail, D.F. & Joyce, J.A. (2013) Microenvironmental regulation of tumor progression and metastasis. *Nature Medicine*, 19(11), 1423–1437.

Raposo, M.F.D., de Morais, R. & de Morais, A. (2013) Health applications of bioactive compounds from marine microalgae. *Life Sciences*, 93(15), 479–486.

Riccio, G. & Lauritano, C. (2019) Microalgae with immunomodulatory activities. *Marine Drugs*, 18(2), 1–18.

Samarakoon, K.W., O-Nam, K., Ko, J.Y., Lee, J.H., Kang, M.C., Kim, D. et al. (2013) Purification and identification of novel angiotensin-I converting enzyme (ACE) inhibitory peptides from cultured marine microalgae (*Nannochloropsis oculata*) protein hydrolysate. *Journal of Applied Phycology*, 25(5), 1595–1606.

Samek, D., Misurcova, L., Machu, L., Bunka, F. & Fisera, M. (2013) Influencing of amino acid composition of green freshwater algae and cyanobacterium by methods of cultivation. *Turkish Journal of Biochemistry*, 38(4), 360–368.

Sani, S., Messe, M., Fuchs, Q., Pierrevelcin, M., Laquerriere, P., Entz-Werle, N. et al. (2021) Biological relevance of RGD-integrin subtype-specific ligands in cancer. *ChemBioChem*, 22, 1151–1160.

Schaller, A. (2001) Bioactive peptides as signal molecules in plant defense, growth, and development. In: Attaur, R. (Ed.) *Studies in Natural Products Chemistry*. Elsevier, pp. 367–411.

Seckbach, J. (2007) *Algae and cyanobacteria in extreme environments*, Vol. 11. Dordrecht: Springer, p. 814.

Sedighi, M., Jalili, H., Ranaei-Siadat, S.O. & Amrane, A. (2016) Potential health effects of enzymatic protein hydrolysates from *Chlorella vulgaris*. *Applied Food Biotechnology*, 3(3), 160–169.

Sheih, I.C., Fang, T.J., Wu, T.-K. & Lin, P.-H. (2010) Anticancer and antioxidant activities of the peptide fraction from algae protein waste. *Journal of Agricultural and Food Chemistry*, 58(2), 1202–1207.

Shi, K.P., Yu, J., Liu, C.Y., Xu, Z.M. & Tang, X.X. (2017) Impacts of enhanced UVB radiation on photosynthetic characteristics of the marine diatom *Phaeodactylum tricornutum* (Bacillariophyceae, Heterokontophyta). *Journal of Applied Phycology*, 29(3), 1287–1296.

Shih, M.F., Chen, L.C. & Cherng, J.Y. (2013) *Chlorella* 11-peptide inhibits the production of macrophage-induced adhesion molecules and reduces endothelin-1 expression and endothelial permeability. *Marine Drugs*, 11(10), 3861–3874.

Shih, M.F. & Cherng, J.Y. (2012) Protective effects of *Chlorella*-derived peptide against UVC-induced cytotoxicity through inhibition of caspase-3 activity and reduction of the expression of phosphorylated FADD and cleaved PARP-1 in skin fibroblasts. *Molecules*, 17(8), 9116–9128.

Skjånes, K., Rebours, C. & Lindblad, P. (2013) Potential for green microalgae to produce hydrogen, pharmaceuticals and other high value products in a combined process. *Critical Reviews in Biotechnology*, 33 (2), 172–215.

Slominski, A., Zbytek, B. & Slominski, R. (2009) Inhibitors of melanogenesis increase toxicity of cyclophosphamide and lymphocytes against melanoma cells. *International Journal of Cancer*, 124(6), 1470–1477.

Stack, J., Le Gouic, A.V., Tobin, P.R., Guihéneuf, F., Stengel, D.B. & RJ, F.G. (2018) Protein extraction and bioactive hydrolysate generation from two microalgae, *Porphyridium purpureum* and *Phaeodactylum tricornutum*. *Journal of Food Bioactives*, 1(1), 153–165.

Suetsuna, K. & Chen, J.R. (2001) Identification of antihypertensive peptides from peptic digest of two microalgae, *Chlorella vulgaris* and *Spirulina platensis*. *Marine Biotechnology*, 3(4), 305–309.

Suh, S.S., Hong, J.M., Kim, E.J., Jung, S.W., Chae, H., Kim, J.E. et al. (2019) Antarctic freshwater microalga, *Chloromonas reticulata*, suppresses inflammation and carcinogenesis. *International Journal of Medical Sciences*, 16(2), 189–197.

Suh, S.S., Hong, J.M., Kim, E.J., Jung, S.W., Kim, S.M., Kim, J.E. et al. (2018) Anti-inflammation and anti-cancer activity of ethanol extract of Antarctic freshwater microalga, *Micractinium* sp. *International Journal of Medical Sciences*, 15(9), 929–936.

Sui, Y., Muys, M., Vermeir, P., D'Adamo, S. & Vlaeminck, S.E. (2019) Light regime and growth phase affect the microalgal production of protein quantity and quality with *Dunaliella salina*. *Bioresource Technology*, 275, 145–152.

Tejano, L.A., Peralta, J.P., EES, Y. & Chang, Y.W. (2019) Bioactivities of enzymatic protein hydrolysates derived from *Chlorella sorokiniana*. *Food Science & Nutrition*, 7(7), 2381–2390.

Thundimadathil, J. (2012) Cancer treatment using peptides: current therapies and future prospects. *Journal of Amino Acids*, 2012(967347), 1–13.

Tyagi, P., Pechenov, S. & Anand Subramony, J. (2018) Oral peptide delivery: translational challenges due to physiological effects. *Journal of Controlled Release*, 287, 167–176.

Wang, X. & Zhang, X. (2013) Separation, antitumor activities, and encapsulation of polypeptide from *Chlorella pyrenoidosa*. *Biotechnology Progress*, 29(3), 681–687.

Wild, K.J., Trautmann, A., Katzenmeyer, M., Steingaß, H., Posten, C. & Rodehutscord, M. (2019) Chemical composition and nutritional characteristics for ruminants of the microalgae *Chlorella vulgaris* obtained using different cultivation conditions. *Algal Research*, 38, 101385.

Wu, H., Xu, N.J., Sun, X., Yu, H. & Zhou, C.X. (2015) Hydrolysis and purification of ACE inhibitory peptides from the marine microalga *Isochrysis galbana*. *Journal of Applied Phycology*, 27(1), 351–361.

Yu, H., Lin, L., Zhang, Z., Zhang, H. & Hu, H. (2020) Targeting NF-κB pathway for the therapy of diseases: mechanism and clinical study. *Signal Transduction and Targeted Therapy*, 5, 209.

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