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A review on fucoidan antitumor strategies: From a biological active agent to a structural component of fucoidan-based systems

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ABSTRACT

Due to the severe side-effects and the toxicity to healthy tissues, cancer treatments based in chemotherapy have not fully achieved the desire outcomes so far. The use of natural compound may be of great value to develop better tolerated therapies. Fucoidan is a marine sulfated polysaccharide extracted from brown algae that, besides other biological activities, has been reported to present interesting anti-cancer potential. This review briefly introduces fucoidan chemical structure, physicochemical properties and the above-mentioned biological feature. Fucoidan usage as soluble agent presents promising results herein described for different types of cancer. Trying to enhance and optimize fucoidan usage in the cancer field, different systems, namely drug delivery, have been recently developed to target different types of cancers. This aspect will be presented in detail, highlighting the role of fucoidan on their reported or envisaged performance.

1. Introduction

More than 70 % of the world's surface is covered by oceans, being plants, animals, and microorganisms from the marine environment a significant source of natural products (Penesyan, Kjelleberg, & Egan, 2010). Among marine organisms, marine algae are rich sources of structurally diverse bioactive compounds with relevant biological activities (Khalid, Abbas, & Saeed, 2018). Marine algae are a good source of nutrients, being of particular interest their sulfated polysaccharides (Silva et al., 2012). The growing interest in marine polysaccharides extracted from brown algae is due to their therapeutic effects against different types of disorders and pathological conditions. In addition, the greater availability and lower production costs are other advantages of these algae polysaccharides, enabling their use at industrial scale (Khan, Shin, & Kim, 2018). Indeed, seaweeds have been exploited as supplements in functional food or the extracted compounds used in the development of new pharmaceutical agents and medical oriented products (Khalid et al., 2018). In this regard, the biomedical market represents an enormous opportunity for many of these bioactive compounds and materials. The exploitation of natural resources may present a potential added value that justify the potential risks related with the development and approval of such products (Silva et al., 2012).

Fucoidans are a class of sulfated polysaccharides, mainly composed by fucose, that can be extracted from different species of brown algae (Cunha & Grenha, 2016). Fucoidans are already available as food/dietary supplements and in cosmetics. However, they are not yet approved for biomedical applications, neither by direct administration nor by its incorporation in biomaterials, despite showing promising outcomes (Fitton, Stringer, & Karpiniec, 2015; Wells et al., 2017). In fact, fucoidan has been described to present distinct biological activities, namely antitumor, anti-viral and anti-inflammatory, as well as immunoregulatory and (anti-)angiogenic potential (Li, Lu, Wei, & Zhao, 2008; Wang, Kankala et al., 2019; Wang, Xing et al., 2019). Despite these achievements, variable and contradictory results compromise fucoidan usage in the clinic (Atashrazm, Lowenthal, Woods, Holloway, & Dickinson, 2015). The huge variety of algae fucoidan sources, allied to various extraction and purification methods, may influence fucoidan's physicochemical properties and bioactivity (Cunha & Grenha, 2016; Fitton et al., 2015) (Fig. 1).

In this review, a general introduction to fucoidan composition, chemical structure and physicochemical properties is addressed. The

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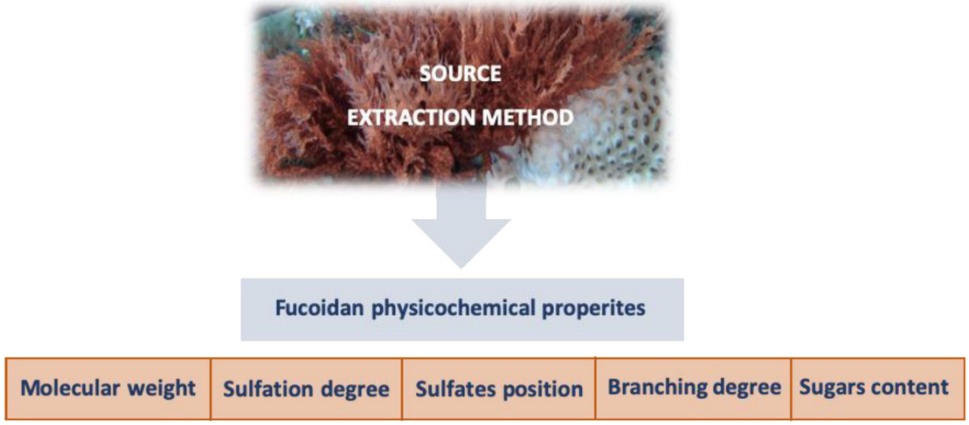

Fig. 1. The source and extraction method may influence fucoidan physicochemical properties and, consequently, the biological outcomes.

recent advances of fucoidan as an antitumor bioactive agent, divided by different types of cancer (*i.e.* colon, lung, breast and melanoma) is reviewed. In addition, the development and biological outcomes of fucoidan-based systems (mainly nanoparticles) is addressed in detail.

2. Fucoidan composition and chemical structure

Fucoidan may be extracted from different seaweed species, presenting different chemical compositions. In general, fucoidans from brown algae present a backbone of α(1 → 3) L-fucopyranose residues or of alternating α(1 → 3) and α(1 → 4)-linked L-fucopyranosyls, that can be replaced by sulfate or acetate and/or have side branches containing fucopyranoses or other glycosyl units, *e.g.* glucuronic acid. Fucoidan structures may also be composed of other monosaccharides as glucose, xylose, galactose and mannose (Ale & Meyer, 2013; Ale, Mikkelsen, & Meyer, 2011).

Fucus vesiculosus, that belongs to the order of Fucales, is the most common fucoidan's species source, being mainly composed of 1,2-α-fucose often sulfated. Most sulfate groups are located at position C-4 of the fucose units. Despite the relatively simpler composition of fucoidan from *Fucus vesiculosus*, most fucoidans present more complex structures. In the case of fucoidan extracted from *Ascophyllum nodosum* (Fucales), an α(1 → 3)-linked fucose backbone and a high proportion of α(1 → 4) linkages mainly sulfated at O-2, to a lesser extent at O-3, and only slightly at O-4, and fucose 2, 3-*O*-disulfate residues were observed. In the same order of Fucales, *Fucus evanescens* and *Fucus serratus* give rise to fucoidans presenting similar structures, with a large proportion of both *α*(1 → 3)- and *α*(1 → 4)-linked L-fucopyranose residues, which may be substituted with sulfate (SO_3^-) at C-2 and C-4. Fucoidans in the order of Laminariales present a chemical structure that is mainly composed of a backbone of α(1 → 3)-linked L-fucopyranose residues with sulfates at the C-2 position. Fucoidans isolated from *Laminari saccharina* consist of α(1 → 3)-linked L-fucopyranose units, however they can present sulfates at C-4 alone or at both C-2 and C-4. *Chorda filum* (Laminariales) present a similar structure, consisting of a backbone structure of poly-α(1 → 3)-linked L-fucopyranoses sulfated mainly at C-4. However, it can also be sulfated at the C-2 position, and with some of the α(1 → 3)-linked fucose residues being 2-*O*-acetylated (Ale & Meyer, 2013; Ale, Mikkelsen et al., 2011; Mulloy & Berteau, 2003). This information on chemical structures of different fucoidan sources is collected in Fig. 2.

Despite the backbone structure be mainly the same, (1 → 3)-linked α-L-fucopyranose, there might be different sulfates substitutions, molecular weights, branching and sugar residues, which may lead to different biological outcomes. Thus, although all receive the same designation as fucoidan, each extract is an individual entity.

3. Fucoidan physicochemical properties

As previously stated, the extraction conditions, algae species and life cycle state, among others, have a huge influence over fucoidan composition and chemical structure, providing extracts with different molecular weights and sulfate patterns (Mak, Hamid, Liu, Lu, & White, 2013). The molecular weight is one of the main properties described to influence the biological activity of fucoidans. Nevertheless, it is difficult to establish a direct correlation based in one property alone (Álvarez-Viñas, Flórez-Fernández, González-Muñoz, & Domínguez, 2019). Specifically, low molecular weight fucoidan extracts have gained most attention due to their bioavailability, being explored in several biological conditions. For example, the synergistic effect of low molecular weight fucoidan (0.8 kDa) and fucoxanthin was studied in a diabetic mouse model (Lin, Tsou, Chen, Lu, & Hwang, 2017). This combination was able to maintain glucose at homeostatic levels, improving the outcomes as compared to separate administration. In a clinical trial, low molecular weight fucoidan (0.8 kDa) increased the disease control rate by 24 % in patients with metastatic colorectal cancer (Tsai, Tai, Huang, Chang, & Wang, 2017). In another attempt, fucoidan was hydrolyzed in fractions with different molecular weights, being observed increased cytotoxic effects over cancer cells when using lower molecular weight fractions (< 30 kDa). It was suggested that low molecular weight fractions present greater mobility and diffusivity than high molecular weight fucoidan, improving the interaction with cancer cell components (You, Yang, Lee, & Lee, 2010). The bioactivity of low molecular weight fucoidan fractions (10–37 kDa) obtained by gamma irradiation also demonstrated increased antioxidant effects (Choi et al., 2009). Controversially, high molecular weight fucoidan was also reported to present anticancer and anti-inflammatory effects, which might indicate the involvement of other factors on the determination of biological activity (Kang et al., 2011; Synytsya et al., 2010).

Besides the molecular weight, the sulfate content has been deeply studied regarding fucoidan biological responses. From previously reported works, higher sulfation degree has been related with enhanced bioactivity responses, namely anti-angiogenic and anticancer activities, which have encouraged the production of over-sulfated fucoidans (Koyanagi, Tanigawa, Nakagawa, Soeda, & Shimeno, 2003; Soeda, Shibata, & Shimeno, 1997). Over-sulfation leads to molecules with high negative charge, which enables the formation of fucoidan-protein complexes. This may hinder cells' proliferation and thus, indirectly, exerts the referred biological activities (Zorofchian Moghadamtousi et al., 2014). Besides the sulfate content, the position of the sulfate groups along the fucoidan backbone, as well as the branching degree, may also be related with fucoidan biological activities. In a previous paper, a fucoidan with effective anti-tumor activity presented higher percentage of fully branched chains (in both 3-O and 4-O positions)

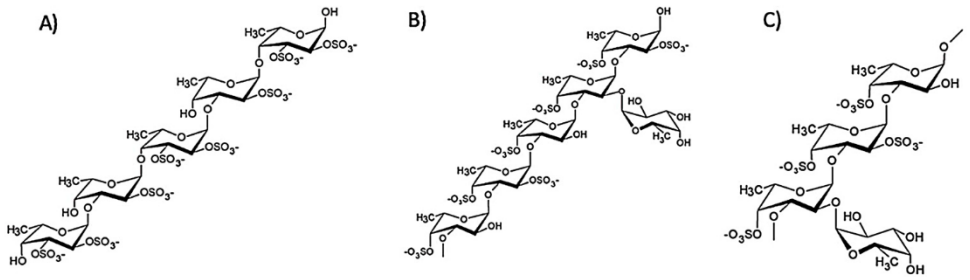

Fig. 2. Chemical structure of fucoidans obtained from: A) *Fucus vesiculosus* and *Ascophyllum nodosum*, B) *Laminaria saccharina* or C) *Chorda filum*.

than other fucoidan extracts that did not presented this biological activity (Oliveira et al., 2017).

The carbohydrate composition is another property that should be analyzed. As previously stated, fucoidans are mainly composed of fucose residues, often sulfated, but they also present other carbohydrates in its composition, as neutral sugars: xylose, galactose, mannose and glucose (Nagaoka et al., 1999; Nishino, Nishioka, Ura, & Nagumo, 1994). They may also present uronic acids, which may be associated with the biological response of fucoidan (Li et al., 2008). The absence of fucoidan carbohydrate composition determination and its relation to the biological activities may be the cause of some contradictory results, being structure-activity relationship studies of utmost importance to reach more elucidative and consistent findings.

4. Anti-cancer activity

Cancer is characterized by an uncontrolled cell growth and can start at any tissue of the body. Cancer cells can spread to tissues other than where they started proliferating, causing metastasis (Cooper, 2000). Although more people than ever have survived different types of cancer after treatment, cancer is still one of the main causes of death worldwide (Siegel, Miller, & Jemal, 2019). The type of cancer, size and location will influence the treatment options which may include chemotherapy, surgery and radiotherapy (Arruebo et al., 2011). Surgery is the most common treatment modality which is used to remove the tumor. Chemotherapy uses cytotoxic drugs with a systemic action, which may affect healthy tissues, leading to severe side effects (Sak, 2012). Radiation therapy uses high energy beams to destroy cancer cells and may be used before surgery (Baskar, Lee, Yeo, & Yeoh, 2012). Target therapies rely on specific markers, leading to fewer side effects (Testa, Pelosi, & Castelli, 2018). These treatments may be applied in a single or combined way.

Depending on the drugs and treatment duration, severe side effects may be observed, the reason why more effective anti-cancer drugs are needed. Fucoidan anti-cancer capability has gained tremendous interest in the last years and has been studied over different types of cancer. Indeed, fucoidan may regulate and mediate its anti-cancer potential through different mechanisms and cancer-related pathways, namely cell cycle arrest, apoptosis and immune system activation, as reviewed by others (Atashrazm et al., 2015; van Weelden et al., 2019). Herein, the action of fucoidan over breast, lung and colorectal cancer, as well as melanoma will be discussed.

4.1. Lung cancer

Lung cancer is the second most common (both in man and woman) and the deadliest, comprising about 13 % of all new diagnosed cancers (Miller et al., 2016; Siegel et al., 2019). There are different types of lung cancer: carcinoid tumors, small cell and non-small cell, with the latest being the most common, accounting for 85 % of all lung cancers. Like in most cancers, the possible treatments include surgery, radiation therapy, chemotherapy and targeted therapies, which may be used as single or combined treatments (Ellouali, Boisson-Vidal, Durand, & Jozefonvicz, 1993; Lemjabbar-Alaoui, Hassan, Yang, & Buchanan, 2015; Zappa & Mousa, 2016). Native fucoidan from *Turbinaria conoides* presented increased inhibition of lung cancer cells proliferation when compared with native fucoidan from *Undaria pinnatifida* (Marudhupandi, Ajith Kumar, Lakshmanasenthil, Suja, & Vinothkumar, 2015). In a different approach, fucoidan exhibited anti-metastatic effects over lung cancer cells (A549 cell line) *via* the down-regulation of different signaling pathways, resulting in decreased cell migration and invasion (Lee, Kim, & Kim, 2012). Furthermore, typical apoptotic characteristics, such as chromatin condensation and an increase in the population of sub-G1 hypodiploid cells, were observed by the addition of fucoidan, with an effective antiproliferative activity (Boo et al., 2011). The effects of molecular weight were also analyzed in A549 cells, showing that hydrolysis in boiling water with HCl significantly increased anticancer activity when compared with microwave hydrolysis of native fucoidan (Yang et al., 2008). The simultaneous administration of fucoidan and cisplatin presented a synergistic effect by inhibiting lung cancer cells viability, inducing apoptosis trough upregulation of cleaved caspase-3 and poly (ADP ribose) polymerase expression (Hsu et al., 2017). This dual drug approach allowed a decrease in cisplatin concentration, leading to diminished side effects. In another study, fucoidan activated the TLR4/ROS/ER signaling pathway, resulting in apoptotic cell death, both *in vitro* and *in vivo,* resulting in tumor shrinkage (Hsu, Lin, Hu, Shu, & Lu, 2018).

4.2. Colorectal cancer

Colon cancer is the second most common cause of cancer deaths, considering both man and woman (Siegel et al., 2019). Surgery is the most common treatment, where the tumor and adjacent lymph nodes are removed. Chemotherapy is often used in colon cancer treatments, which may also help to shrink the tumor before surgery. Fucoidan from *Sargassum feldmannii* and*S. duplicatum* at different concentrations were tested over different types of colon cancer cell lines (DLD-1, HT-29 and HCT-116) and did not show any cytotoxicity (Usoltseva et al., 2019). However, in a similar study, native and deacetylated fucoidan at 200 μg/ml inhibited colony formation of colon cancer cells (Usoltseva et al., 2019). Fucoidan inhibited HT-29 cells growth, migration and sphere formation by suppressing the PI3K/Akt/mTOR pathway (Han, Lee, & Lee, 2015; Han, Lee, & Lee, 2015). A study that comprises *in vitro* and *in vivo* assays showed that fucoidan inhibited the growth and angiogenesis of colon cancer cells (Han et al., 2015a, 2015b). Specifically, the intraperitoneal injection of fucoidan reduced tumor volume which was associated with induction of apoptosis and decreased angiogenesis mediated by Akt signaling. In another *in vivo* study with 26 colon cancer cell lines, the oral administration of fucoidan suppressed tumor growth and prolonged survival times of tumor-bearing mice (Azuma et al., 2012). The molecular weight was defined as determinant of fucoidan anti-tumor effects and an increase of natural killer cells population may be the possible mechanism of action. In another attempt, the activation of caspases (*via* both the death receptor-mediated and mitochondria-mediated apoptotic pathways) was associated to fucoidan growth inhibition and induction of apoptosis in human colon cancer cells (Kim,

Park, Lee, & Park, 2010). Complementary, fucoidan administration led to sub-G1 phase cell cycle arrest and inhibited cell growth and increased apoptotic cell death (Kim, Kwon, & Nam, 2017). Since the human intestine does not have enzymes able to hydrolyze fucoidan, the administration of fucoidan may result in an increased concentration of luminal fucoidan within the large intestine (Bilan et al., 2004; Han et al., 2015a, 2015b). Having this in consideration, fucoidan seems to be an interesting alternative for the prevention of colon cancer. Besides this preventive effect, these studies showed that fucoidan has a potent anti-cancer effect over colon cancer both *in vitro* and *in vivo*.

4.3. Breast cancer

Breast cancer is the most common and the second cause of deaths in women. The chance of a woman dying of breast cancer is 1 in 37 (DeSantis, Ma, Bryan, & Jemal, 2014; Siegel et al., 2019). This type of cancer is characterized by a lump or thickening of the breast. Surgery, chemotherapy and radiation are the common treatment options. Chemotherapeutics such as doxorubicin (DOX), paclitaxel (PAX) and gemcitabine (Gem) have been used in breast cancer treatments (Nounou et al., 2015; Tong, Wu, Cho, & To, 2018). However, these chemotherapeutic agents often present severe side effects, namely toxicity to healthy tissues. Fucoidan decreased the viability of MCF-7 human breast cancer cells in a dose-dependent manner and arrested the cell cycle in G1 phase which was associated with a decrease in the expression of specific markers (Banafa et al., 2013). Using a different human breast cancer cell type (MDA-MB-231), low molecular weight fucoidan inhibited cancer cells proliferation and promoted apoptosis, which was associated with caspases activation and mitochondrial malfunction (Zhang, Teruya, Eto, & Shirahata, 2013). The critical effect of molecular weight was analyzed by comparing the effects of low and high molecular weight fucoidan over two breast cancer cell lines, MCF-7 and MDA-MB-231(Lu et al., 2018). Low molecular weight fucoidan presented significant cytotoxicity over both cell types. Fucoidan presented a time-dependent action over MCF-7, whereas induction of caspase-dependent apoptosis was observed in the MDA-MB-231 cells. Fucoidan at 400 mg/kg body weight per day inhibited the proliferation of MCF-7 cells implanted in rats, inducing cancer cells apoptosis and suppressing cell migration by modulating *E*-cadherin and MMP-9 expression (He et al., 2019). Proliferation of MDA-MB-231 and 4T1 (mouse breast cancer cells) was inhibited *in vitro,* and tumorigenesis and metastasis of the 4T1 cells was inhibited in mice, showing a decreased tumor volume and metastatic nodules in the lungs (Chen et al., 2012). Fucoidan from *Fucus vesiculosus* presented toxicity over 4T1 cells, inducing apoptosis and down-regulation of VEGF. The tumor size and weight, as well as the lung metastasis were reduced when fucoidan was injected in mice breast cancer cell models (Xue et al., 2012). These *in vivo* results corroborate the *in vitro* observations, since this response was associated with decreased in angiogenesis and enhanced apoptosis. The combination of fucoidan with chemotherapeutic drugs (*i.e.* cisplatin, tamoxifen and paclitaxel) was also evaluated over breast cancer cell lines. This combination increased the therapeutic efficacy of the treatments, enhancing apoptosis in cancer cells and showing an increased level of ROS, suggesting that induction of oxidative stress can lead to cancer cells death (Zhang, Teruya, Yoshida, Eto, & Shirahata, 2013).

4.4. Melanoma

Melanoma is not the most common skin cancer, but it is the most aggressive since it tends to spread and metastasize. It is one of the most diagnosed cancer in young adults (age group 25–30) (Siegel et al., 2019). Melanoma is mostly found in the skin, although it can also be found in other parts of the body such as eyes, mouth, genitals and anal area. This cancer appears when melanocytes mutate and become cancerous. It has been demonstrated that melanoma occurs by the activation of ERBB signaling, specifically through the ERBB3/ERBB2 cascade (Thakur et al., 2017). In the particular case of melanoma, surgery is the most common treatment since, unlike many internal cancers, it is easier to access and remove. Less common treatments may include chemotherapy and biological therapy (Apalla, Nashan, Weller, & Castellsagué, 2017; Linares, Zakaria, & Nizran, 2015).

Fucoidan from *Undaria pinnatifida* was evaluated over different melanoma cells lines (Thakur et al., 2017). Results demonstrated that the addition of fucoidan to a currently clinical approved inhibitor of ERBB2/EGFR (lapatinib), inhibited melanoma cells' viability in 85 %. Besides tumor growth inhibition, fucoidan also reduced the morbidity associated with prolonged use of lapatinib. In another attempt, fucoidan from two different sources presented toxicity over B16 cells (murine melanoma cells) in a dose-dependent manner (Ale, Maruyama, Tamauchi, Mikkelsen, & Meyer, 2011). Complementary results showed that the addition of fucoidan led to morphological changes in melanoma cells, indicating apoptosis. Furthermore, in a mice model was observed an enhanced natural killer cells' activity when fucoidan was present, acting as the key mediators of tumor cell death. In a similar study, fucoidan from *Sargassumhenslowianum* C. Agardh (FSAR) and *Fucus vesiculosus* (FVES) were evaluated also over B16 cells, indicating a decreased cell proliferation (Ale, Maruyama, Tamauchi, Mikkelsen, & Meyer, 2011). FSAR presented more effective anti-cancer effects at lower doses than FVES, whereas the latter presented higher toxicities at higher. Annexin V studies showed that both polymers induced apoptosis which was also achieved by the activation of caspase-3. In a recent study, fucoidan from *Fucus vesiculosus* showed increased cytotoxicity over melanoma cells in a dose-dependent fashion. Cells morphological changes and apoptosis were also observed (Wang, Xu, Liang, Wang, & Kang, 2017). Despite the use of fucoidan is rarely studied for melanoma, it has shown interesting and effective results that should be further explored to treat this aggressive type of skin cancer.

5. Fucoidan-based systems for anti-cancer therapies

Most of the studies that explore the potential use of fucoidan to treat different types of cancer use fucoidan in its soluble form. However, in recent years, fucoidan-based systems have been developed aiming to develop more effective anti-cancer strategies. Most of them are nanosystems that comprise the use of nanoparticles (NPs), micelles or liposomes, playing an important role in biological systems (Chollet et al., 2016). Indeed, fucoidan has been included in nanosystems for diagnostic, drug delivery systems (DDS) and regenerative medicine. These fucoidan-based systems have been summarized in Table 1.

The majority of the fucoidan-based systems have been developed for breast cancer. Fucoidan has been electrostatically assembled with positively charged polyethylenimine, and loaded with doxorubicin (DOX), leading to the production of NPs systems (Pawar et al., 2018). These NPs presented a size range from 41 to 160 nm (an increase in fucoidan ratio lead to bigger NPs), a polydispersive index (Pdi) of 0.153 and were negatively charged. The release of DOX was evaluated at different pH, and around 30 % of the drug was released in the first 24 h. NPs arrested the cell cycle progression in G1-S phase, followed by apoptosis. This NPs system presented increased cytotoxicity when compared to the free drug, both *in vitro* and *in vivo*, where tumor shrinkage was observed. DOX has also been encapsulated into protamine/fucoidan NPs (Lu et al., 2017). These two compounds were able to produce stable NPs only when weight ratios were lower than 1.0, indicating that the surface of NPs was covered by fucoidan. Therefore, negatively charged NPs were produced with a size around 180 nm. Approximately 70 % of DOX was released in the first 24 h at pH 7.4, whereas at pH 4.5 the release was considerably faster, with 80 % of DOX being released in 12 h. This result was attributed to the deionized chemical groups and consequent weakness of electrostatic interactions between the NPs and the drug. The developed NPs presented increased cytotoxicity over MDA-MB-231 human breast cancer cells when compared to the same concentration of

Table 1
Fucoidan-based systems for anti-cancer therapies.

Cancer type	Species	System	Drug	Preparation method	Cell type/animal model	Biological outcomes	Refs.
Breast	*F. vesiculosus*	Fucoidan/ polyethylenimine NPS	DOX	Polyelectrolyte complexation	MDA-MB-231 J774.1A 4T1 tumor bearing mice	Caspase dependent apoptosis; IL-12 driven Th1 immune response	Pawar et al. (2018)
	F. vesiculosus	Fucoidan/chitosan NPs	Gem	Polyelectrolyte complexation	MDA-MB-231 EA.hy926	Increased cytotoxicity of loaded NPs when compared with free drug over cancer cells, without further affecting endothelial cells	Oliveira et al. (2018)
	F. vesiculosus	Fucoidan capped gold NPs	DOX	Addition of fucoidan to Gold (III) chloride trihydrate, under magnetic heater stirrer.	MDA-MB-231	Increased toxicity for Drug-loaded NPs; Induce cell death trough the reactive oxygen species (ROS)-mediated apoptotic process.	Manivasagan et al. (2016)
	F. vesiculosus	Fucoidan-drug based NPs (FiPAX, FiMEK, FiDOX)	PAX DOX MEK	FiPAX and FiMEK co-encapsulation and a near-infrared fluorophore. FiDOX: layer-by-layer assembly.	MDA-MB-231 Lung metastatic mice model	Reduction of tumor and prolonged survival with FiDOX	Shamay et al. (2016)
	L. japonica	Fucoidan/ protamine NPs	DOX	Self-assembled colloidal nanocomplex formed by electrostatic interactions.	MDA-MB-231	Cellular uptake of NPs is not only affected by endocytosis, but also by p-selectin-mediated internalization	Lu et al. (2017)
	n.d	Poly-allylamine hydrochloride and fucoidan NPs (PAH/F)	MTX	Self-assembly of two polyelectrolytes	MCF-7 L929	PAH/F NPs allowed MTX release more efficiently, increasing the inhibition MCF-7 cells' proliferation when compared with free drug	Wang, Kankala et al. (2019), Wang, Xing et al. (2019)
	n.d	Calcium carbonate microparticles ($CaCO_3$MPs) coated with fucoidan and poly-L-ornithine (PLO)	DOX	$CaCO_3$ MPs were prepared by co-precipitation. PLO and fucoidan were added by layer by layer.	MCF-7 cells C2C12	Drug-loaded NPs exhibited stronger cell inhibition effect when compared to pure DOX, which was attributed to cell internalization and the intracellular delivery efficiency.	Wang et al. (2018)
Cervical	*F. vesiculosus*	Rutin-fucoidan complex	Rutin	Fucoidan was added dropwise to a completely dissolved solution of rutin	HaCaT HeLa	Ru-Fu NPs presented anti-proliferative effects, arrested cell cycle in S-phase and showed higher percentages of apoptotic HeLa cells when compared to rutin and fucoidan alone.	Deepika et al. (2019)
	F. vesiculosus	Silver NPs with chitosan/fucoidan coating	–	Silver nitrate was added to fucoidan solution, followed by the addition of fucoidan.	HeLa	Cytotoxicity in HeLa cells is associated with changes in morphology; Flow cytometry indicates increased apoptosis for NPs condition.	Venkatesan et al. (2018)
	F. vesiculosus	Fucoidan-coated copper sulfide NPs (F-CuS)	–	Synthesis of sodium citrate-stabilized copper sulfide NPs (CuS) and coating using the layer-by-layer (LbL) technique (PAH and Fu)	HeLa Nude mice	Laser irradiation (LI) of NPs reduced HeLa cells viability; NPs and LI promoted caspase-3 activation; NPs and LI decreased the tumor size in mice model.	Jang et al. (2018)
	n.d	Poly-allylamine hydrochloride and fucoidan NPs (PAH/F)	MTX	Self-assembly of two polyelectrolytes	HeLa	PAH/F NPs allowed the drug accumulation in cytoplasm to release MTX over an extended period of time, inhibiting the proliferation of Hela cells more efficiently.	Wang, Kankala et al. (2019), Wang, Xing et al. (2019)
Colon	*F. vesiculosus*	Acetylated fucoidan (AcFu) NPs	DOX	Fucoidan was dissolved in formamide solution, followed by addition of pyridine acetic anhydride. AcFu NPs were added into Dox solution.	HCT-116 HCT-8 Raw264.7	Immunomodulatory effects of AcFu were similar to native fucoidan (increased TNF-α and GM-CSF); AcFu increased internalization and cytotoxic effects over HCT-8 cells when compared with free DOX.	Lee et al. (2013)
	F. vesiculosus	Fucoidan-drug based NPs (FiPAX, FiMEK, FiDOX)	PAX DOX MEK	FiPAX and FiMEK co-encapsulation and a near-infrared fluorophore. FiDOX: layer-by-layer assembly.	HCT-116 SW620 Mice model	FiMEK treatment resulted in enhanced efficacy as compared to free MEK; Caspase 3 cleavage significantly higher in the tumors treated with FiMEK.	Shamay et al. (2016)
	n.d	Fucoidan coated gold NPs (FMG–Au–NPs)	–	Synthesis of Fucoidan-mimetic (FM)-glycopolymers *via* a free-radical chain transfer polymerization reaction. Coating gold NPs with FM-glycopolymers.	HCT-116 NIH3T3	The developed NPs preferentially triggered apoptosis in the HCT-116 cells (dose-dependent cytotoxicity) than in NIH3T3 cells (no cytotoxicity)	Tengdelius et al. (2015)
Eye	*F. vesiculosus*	Gold NPs coated with fucoidan	DOX	Fucoidan was poured into a gold(iii) chloride hydrate solution to obtain Fu-coated Au-NPs	VX2 Raw 264.7	Photothermal treatment on the NPs caused cell death by damaging intracellular biomolecules; The combination of the laser with the NPs removed the tumors.	Kim, Nguyen et al. (2017)

(*continued on next page*)

Table 1 (*continued*)

Cancer type	Species	System	Drug	Preparation method	Cell type/animal model	Biological outcomes	Refs.
Glioma	*F. vesiculosus*	Dextran-coated superparamagnetic iron oxide NPs (SPIONs) with fucoidan	–	Coating of SPIONs with fucoidan	RAW 264.7 Subcutaneous tumor implantation (GL261) in mice model	Longer circulatory half-life achieved with fucoidan was associated with a doubling in tumor SPIONs uptake.	Abdollah et al. (2018)
Leukemia	*F. vesiculosus*	Fucoidan-coated copper sulfide NPs (F-CuS)	–	Synthesis of sodium citrate-stabilized copper sulfide NPs (CuS) and coating using the layer-by-layer (LbL) technique (PAH and Fu)	K562 K562 xenograft in nude mice model	F-CuS increased apoptosis in K562 cells; F-CuS and laser irradiation promoted caspase-3 activation F-CuS and laser irradiation decreased the tumor size in mice model.	Jang et al. (2018)
Lung	*F. vesiculosus*	Fucoidan-coated copper sulfide NPs (F-CuS)	–	Synthesis of sodium citrate-stabilized copper sulfide NPs (CuS) and coating using the layer-by-layer (LbL) technique (PAH and Fu)	A549 A549 xenograft in nude mice model	F-CuS and LI increased early and late apoptosis; A549 cells increased caspase-3 activity after treatment with F-CuS combined with LI; F-CuS and LI decreased the tumor size in mice model.	Jang et al. (2018)
Melanoma	*F. vesiculosus*	Fucoidan-drug based NPs (FiPAX, FiMEK, FiDOX)	PAX DOX MEK	FiPAX and FiMEK co-encapsulation and a near-infrared fluorophore. FiDOX: layer-by-layer assembly.	A375 Nude mice B16F10 melanoma	FiDOX treatment resulted in smaller tumors and prolonged survival compared to free DOX.	Shamay et al. (2016)
Osteosarcoma	*C. okamuranus*	Liposome Encapsulating Fucoidan	–	Mechanochemical method	143B LM8 xenograft in mice model	Apoptosis trough the caspase pathway; Inhibition of tumor growth and spontaneous metastases in the lung in mice.	Kimura et al. (2013)
Pancreatic	*F. vesiculosus*	Fucoidan-coated manganese dioxide NPs (Fuco-MnO_2-NPs)	–	Manganese NPs were produced, followed by a coating with fucoidan.	AsPC-3 BxPC-3 Subcutaneously injection of BxPC-3 cells in mice model	NPs reversed hypoxia-induced radioresistance and increased apoptotic cell death in response to radiation therapy (RT); Combination treatment resulted in delayed tumor growth when compared with RT alone.	Shin et al. (2018)
Tongue carcinoma	*n.d*	3D ECM-like scaffold	–	Fucoidan was added on the surface of co-assembled hydrogel formation	SCC25	Fucoidan allows the hydrogel to act as a powerful inhibitor of cytokinesis and the uncontrolled cell proliferation.	Li et al. (2016)

MDA-MB-231 and MCF-7 (human breast cancer cell line); 4T1 (mouse breast cancer cell line); J774.1A (mouse macrophage cells); EA.hy926 (human endothelial cells); L929 cells (mouse fibroblast cells); C2C12 cells (mouse myoblast cells); HaCaT (non-cancerous human keratinocyte cells); HeLa (human cervical cancer cells); HCT-116, HCT-8 and SW620 (human colon cancer cells); Raw264.7 (human macrophage cells); NIH3T3 (mouse fibroblast cells); VX2 (rabbit tumor cells); GL261 (murine glioma cells); K562 (human leukemia cells); A549 (human lung cancer cells); A375 (human melanoma cells); B16F10 (murine melanoma cells); 143B (human osteosarcoma cells); LM8(mouse osteossarcoma cells) LM8 (murine osteosarcoma cellw); AsPC-3 and BxPC-3 (human pancreatic cancer cells); SCC25 (tongue squamous cell carcinoma cells).

free DOX, which was attributed to different cellular trafficking pathways. Gold NPs coated with fucoidan and loaded with DOX were also developed, being stable for up to 6 months (Manivasagan et al., 2016). This system released the drug faster at the physiological pH, which may reduce the toxicity of the system over normal tissues. The IC50 of these NPs was 5 μg/ml, whereas for the drug was 30 μg/ml, corroborating the efficacy of the system over MDA-MB-231 breast cancer cells. In a similar approach, fucoidan coated gold NPsincorporating DOX were applied to remove eye tumors (Kim, Nguyen et al., 2017). NPs were stable at physiological conditions and the system did not present toxicity to normal cells, although presenting increased cytotoxicity over cancer cells. The combination of this system with laser irradiation (LI) was even more effective, inducing around 90 % of cell death (laser alone did not present significant toxicity). The combined therapy was also the most effective strategy *in vivo*, when compared with loaded NPs or laser alone. In a different attempt, calcium carbonate microparticles produced by layer-by-layer technique (fucoidan and poly-L-ornithine), and incubated with DOX, showed an efficient release of the drug and presented toxicity to cancer cells in a dose-dependent manner (Wang et al., 2018). Gemcitabine (Gem) was loaded into fucoidan/chitosan NPs with 115−140 nm in diameter, a Pdi < 0.2, a positive zeta potential of 29 mV and a maximum entrapment efficiency of 42 % (Oliveira, Neves, Reis, Martins, & Silva, 2018). These NPs presented increased toxicity over cancer cells (around 25 %) without increasing toxicity over normal cells, when compared to the free drug. Methotretaxe (MTX), a commonly used drug to treat osteosarcoma and breast cancer, present a short blood retention time, requiring high administration doses. Trying to circumvent this problem, self-assembled NPs comprising fucoidan and MTX were produced (Wang, Kankala et al., 2019). NPs' size increased from 130 nm to 163 nm with the incorporation of MTX, and around 60 % of the drug was released within 24 h. The developed system presented increased cytotoxicity effects over MCF-7 and HeLa cells when compared with free MTX. In another attempt, fucoidan NPs with affinity to P-selectin were developed (Shamay et al., 2016). These NPs encapsulated DOX, PAX or targeted MEK (mitogen-activated protein kinase kinase) inhibitor. The P-selectin was chosen because it presents increased expression on tumor cells and vasculature than in normal tissues. These NPs encapsulated different drugs (*i.e.* PAX, DOX and MEX), aiming to develop more efficient systems to treat melanoma and colon cancer. These targeting systems presented increased toxicity when compared with untargeted drugs and NPs.

Fucoidan-coated manganese dioxide NPs, in combination with radiation therapy (RT), resulted in significant pancreatic tumor shrinkage (by 46 %) when compared with the untreated control group, and by 22.5 % compared to RT alone (Shin et al., 2018). Two different fucoidan-based systems were developed aiming to treat cervical cancer: a novel complex of rutin-fucoidan presented higher toxicity over cancer cells than to normal cells, whereas silver NPs coated with chitosan/fucoidan presented toxicity to cancer cells at 50 μg/ml (Deepika et al., 2019; Venkatesan, Singh, Anil, Kim, & Shim, 2018). Fucoidan-coated sulfide NPs induced apoptosis in cancer cells, in combination with near-infrared induced hyperthermic effect. This combined approach was applied to different tumors (cervical, lung and leukemia), eliminating several tumors *in vivo* (Jang et al., 2018). In another attempt, superparamagnetic iron oxide NPs (SPIONs) were coated with fucoidan, which conferred a longer blood circulating half-time, presenting a 2X fold increase in glioma tumors uptake (Abdollah et al., 2018). Regarding colon cancer therapies, the internalization of DOX by the cancer cells were much higher for acetylated fucoidan NPs loaded with DOX when compared with fucoidan-DOX mixture or the free drug (Lee, Jeong, & Na, 2013). Fucoidan-coated gold NPs presented cytotoxicity to colon cancer cells with no toxicity to normal fibroblasts (Tengdelius et al., 2015). Liposomes encapsulating fucoidan were also developed, inducing apoptosis of osteosarcoma cells and reducing tumor volume and weight *in vivo*, comparing to native fucoidan (Kimura, Rokkaku, Takeda, Senba, & Mori, 2013). In a different approach, fucoidan was added at the surface of a hydrogel (fluorenylmethoxycarbonyl) (Li et al., 2016). This self-assembly process was able to maintain fucoidan bioactivity, so that the scaffold provides a non-toxic environment for fibroblasts, although presenting toxicity to cancer cells.

These systems presented interesting results regarding their effectiveness in different types of cancer. The use of fucoidan, either for the development of drug delivery system or as a coating, increased the toxicity over different cancer cells when compared to free drugs. However, due to the cytotoxic effects over non-cancer cells, it is also important to develop more effective therapies with fewer side effects, an aspect that is still missing in some studies.

6. Conclusion and future perspectives

In this review, a brief description of fucoidan structure and physicochemical properties that make it a promising and versatile polysaccharide are explained. Despite the enthusiastic results, and the fact that fucoidan products have been commercialized as dietary or nutritional supplements for different diseases, including cancer, the use of fucoidan in the clinic has not been approved so far. Some contradictory results regarding its biological outcomes may be one of the possible reasons. Trying to overcome this issue and achieve more consistent and reproducible results, some aspects need to be considered. Specifically, it is known that different extraction methods can lead to fucoidans with different chemical features that will have an impact in the final biological outcomes. Indeed, different molecular weight, sulfates position and degree, as well as the polymer branching had a huge influence over the final biological activity of fucoidan. Optimization and standardization of extraction methods, that retain the structural features of interest, should be addressed considering the most common and promising fucoidan species sources, namely *Fucus vesiculosus*. Another relevant aspect is the need to perform structure-activity relationship studies to elucidate the final chemical structure. Regarding the main focus of this review, fucoidan antitumor behavior can be due to different mechanisms of action, making it difficult to understand and get to a consensual pathway, which should be also elucidate. Another important aspect that is missing in some studies is the effects of fucoidan over non-cancer cells. Despite some clear evidences that soluble fucoidan inhibits different cancer cell proliferation and tumor growth, its effects over normal cells is not always stated. It is known that current anti-cancer treatments can present severe side effects and, in this sense, it is important that the tumor microenvironment (that comprises other normal cells besides the cancer cells) is taken into consideration when trying to develop more effective anti-cancer therapies. Having this in consideration, the mechanism by which some fucoidan extracts present a selective behavior (concentration where fucoidan presents toxicity to cancer cells without affecting normal cells) should be investigated. Trying to overcome the cytotoxic effects of current chemotherapeutic drugs, DDS have been proposed. In the case of fucoidan, most of the developed DDS are nanoparticle systems which presented increased cytotoxic effects over cancer cells when compared with the free form of the drug. A convincing chemical characterization, associated with knowledge on the mechanism of action and reduced cytotoxic effects of chemotherapeutic drugs, offer promising and reasonable hope on developing more effective fucoidan-based treatments and therapies to fight cancer.

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References

Abdollah, M. R. A., Carter, T. J., Jones, C., Kalber, T. L., Rajkumar, V., Tolner, B., ... Chester, K. A. (2018). Fucoidan prolongs the circulation time of dextran-coated iron oxide nanoparticles. *ACS Nano, 12*(2), 1156–1169.

Ale, M. T., & Meyer, A. S. (2013). Fucoidans from brown seaweeds: An update on structures, extraction techniques and use of enzymes as tools for structural elucidation. *RSC Advances, 3*(22), 8131–8141.

Ale, M. T., Maruyama, H., Tamauchi, H., Mikkelsen, J. D., & Meyer, A. S. (2011). Fucose-containing sulfated polysaccharides from brown seaweeds inhibit proliferation of melanoma cells and induce apoptosis by activation of caspase-3 in vitro. *Marine Drugs, 9*(12), 2605–2621.

Ale, M. T., Maruyama, H., Tamauchi, H., Mikkelsen, J. D., & Meyer, A. S. (2011). Fucoidan from Sargassum sp. and Fucus vesiculosus reduces cell viability of lung carcinoma and melanoma cells in vitro and activates natural killer cells in mice in vivo. *International Journal of Biological Macromolecules, 49*(3), 331–336.

Ale, M. T., Mikkelsen, J. D., & Meyer, A. S. (2011). Important determinants for fucoidan bioactivity: A critical review of structure-function relations and extraction methods for fucose-containing sulfated polysaccharides from brown seaweeds. *Marine Drugs, 9*(10), 2106–2130.

Álvarez-Viñas, M., Flórez-Fernández, N., González-Muñoz, M. J., & Domínguez, H. (2019). Influence of molecular weight on the properties of Sargassum muticum fucoidan. *Algal Research, 38*, 101393.

Apalla, Z., Nashan, D., Weller, R. B., & Castellsagué, X. (2017). Skin cancer: Epidemiology, disease burden, pathophysiology, diagnosis, and therapeutic approaches. *Dermatology and Therapy, 7*(Suppl 1), 5–19.

Arruebo, M., Vilaboa, N., Sáez-Gutierrez, B., Lambea, J., Tres, A., Valladares, M., ... González-Fernández, A. (2011). Assessment of the evolution of cancer treatment therapies. *Cancers, 3*(3), 3279–3330.

Atashrazm, F., Lowenthal, R. M., Woods, G. M., Holloway, A. F., & Dickinson, J. L. (2015). Fucoidan and cancer: A multifunctional molecule with anti-tumor potential. *Marine Drugs, 13*(4), 2327–2346.

Azuma, K., Ishihara, T., Nakamoto, H., Amaha, T., Osaki, T., Tsuka, T., ... Okamoto, Y. (2012). Effects of oral administration of fucoidan extracted from Cladosiphon okamuranus on tumor growth and survival time in a tumor-bearing mouse model. *Marine Drugs, 10*(10), 2337–2348.

Banafa, A. M., Roshan, S., Liu, Y.-y., Chen, H.-j., Chen, M.-j., Yang, G.-x., ... He, G.-y. (2013). Fucoidan induces G1 phase arrest and apoptosis through caspases-dependent pathway and ROS induction in human breast cancer MCF-7 cells. *Journal of Huazhong University of Science and Technology [Medical Sciences], 33*(5), 717–724.

Baskar, R., Lee, K. A., Yeo, R., & Yeoh, K.-W. (2012). Cancer and radiation therapy: Current advances and future directions. *International Journal of Medical Sciences, 9*(3), 193–199.

Bilan, M. I., Grachev, A. A., Ustuzhanina, N. E., Shashkov, A. S., Nifantiev, N. E., & Usov, A. I. (2004). A highly regular fraction of a fucoidan from the brown seaweed Fucus distichus L. *Carbohydrate Research, 339*(3), 511–517.

Boo, H.-J., Hyun, J.-H., Kim, S.-C., Kang, J.-I., Kim, M.-K., Kim, S.-Y., ... Kang, H.-K. (2011). Fucoidan from Undaria pinnatifida induces apoptosis in A549 human lung carcinoma cells. *Phytotherapy Research, 25*(7), 1082–1086.

Chen, R.-H., Tsao, S.-M., Hsu, H.-Y., Lin, T.-Y., Hwang, P.-A., Tseng, L.-M., ... Hsu, J. (2012). Fucoidan induces changes in the epithelial to mesenchymal transition and decreases metastasis by enhancing ubiquitin-dependent TGFβ receptor degradation in breast cancer. *Carcinogenesis, 34*(4), 874–884.

Choi, J.-i., Kim, H.-J., Kim, J.-H., Byun, M.-W., Soo Chun, B., Hyun Ahn, D., ... Lee, J.-W. (2009). Application of gamma irradiation for the enhanced physiological properties of polysaccharides from seaweeds. *Applied Radiation and Isotopes, 67*(7), 1277–1281.

Chollet, L., Sabboural, P., Chauvierre, C., Villemin, J.-N., Letourneur, D., & Chaubet, F. (2016). Fucoidans in nanomedicine. *Marine Drugs, 14*(8), 145.

Cooper, G. M. (2000). *The cell: A molecular approach* (2nd edition). Sunderland (MA): Sinauer Associates.

Cunha, L., & Grenha, A. (2016). Sulfated seaweed polysaccharides as multifunctional materials in drug delivery applications. *Marine Drugs, 14*(3), 42.

Deepika, M. S., Thangam, R., Sheena, T. S., Sasirekha, R., Sivasubramanian, S., Babu, M. D., ... Thirumurugan, R. (2019). A novel rutin-fucoidan complex based phytotherapy for cervical cancer through achieving enhanced bioavailability and cancer cell apoptosis. *Biomedecine & Pharmacotherapy, 109*, 1181–1195.

DeSantis, C., Ma, J., Bryan, L., & Jemal, A. (2014). Breast cancer statistics, 2013. *CA: A Cancer Journal for Clinicians, 64*(1), 52–62.

Ellouali, M., Boisson-Vidal, C., Durand, P., & Jozefonvicz, J. (1993). Antitumor activity of low molecular weight fucans extracted from brown seaweed Ascophyllum nodosum. *Anticancer Research, 13*(6a), 2011–2019.

Fitton, J. H., Stringer, D. N., & Karpiniec, S. S. (2015). Therapies from fucoidan: An update. *Marine Drugs, 13*(9), 5920–5946.

Han, Y.-S., Lee, J. H., & Lee, S. H. (2015a). Antitumor effects of fucoidan on human colon cancer cells via activation of Akt signaling. *Biomolecules & Therapeutics, 23*(3), 225–232.

Han, Y. S., Lee, J. H., & Lee, S. H. (2015b). Fucoidan inhibits the migration and proliferation of HT-29 human colon cancer cells via the phosphoinositide-3 kinase/Akt/mechanistic target of rapamycin pathways. *Molecular Medicine Reports, 12*(3), 3446–3452.

He, X., Xue, M., Jiang, S., Li, W., Yu, J., & Xiang, S. (2019). Fucoidan promotes apoptosis and inhibits EMT of breast cancer cells. *Biological & Pharmaceutical Bulletin, 42*(3), 442–447.

Hsu, H.-Y., Lin, T.-Y., Hu, C.-H., Shu, D. T. F., & Lu, M.-K. (2018). Fucoidan upregulates TLR4/CHOP-mediated caspase-3 and PARP activation to enhance cisplatin-induced cytotoxicity in human lung cancer cells. *Cancer Letters, 432*, 112–120.

Hsu, H.-Y., Lin, T.-Y., Lu, M.-K., Leng, P.-J., Tsao, S.-M., & Wu, Y.-C. (2017). Fucoidan induces Toll-like receptor 4-regulated reactive oxygen species and promotes endoplasmic reticulum stress-mediated apoptosis in lung cancer. *Scientific Reports, 7* 44990-44990.

Jang, B., Moorthy, M. S., Manivasagan, P., Xu, L., Song, K., Lee, K. D., ... Jin, J.-O. (2018). Fucoidan-coated CuS nanoparticles for chemo-and photothermal therapy against cancer. *Oncotarget, 9*(16), 12649–12661.

Kang, S.-M., Kim, K.-N., Lee, S.-H., Ahn, G., Cha, S.-H., Kim, A.-D., ... Jeon, Y.-J. (2011). Anti-inflammatory activity of polysaccharide purified from AMG-assistant extract of Ecklonia cava in LPS-stimulated RAW 264.7 macrophages. *Carbohydrate Polymers, 85*(1), 80–85.

Khalid, S., Abbas, M., & Saeed, F. (2018). Huma Bader-Ul-Ain and Hafiz Ansar Rasul Suleria. In S. Maiti (Ed.). *Therapeutic potential of seaweed bioactive compounds*. Seaweed Biomaterials.

Khan, M. I., Shin, J. H., & Kim, J. D. (2018). The promising future of microalgae: Current status, challenges, and optimization of a sustainable and renewable industry for biofuels, feed, and other products. *Microbial Cell Factories, 17*(1) 36-36.

Kim, E. J., Park, S. Y., Lee, J.-Y., & Park, J. H. Y. (2010). Fucoidan present in brown algae induces apoptosis of human colon cancer cells. *BMC Gastroenterology, 10*(1), 96.

Kim, I.-H., Kwon, M.-J., & Nam, T.-J. (2017). Differences in cell death and cell cycle following fucoidan treatment in high-density HT-29 colon cancer cells. *Molecular Medicine Reports, 15*(6), 4116–4122.

Kim, H., Nguyen, V. P., Manivasagan, P., Jung, M. J., Kim, S. W., Oh, J., ... Kang, H. W. (2017). Doxorubicin-fucoidan-gold nanoparticles composite for dual-chemo-photothermal treatment on eye tumors. *Oncotarget, 8*(69), 113719–113733.

Kimura, R., Rokkaku, T., Takeda, S., Senba, M., & Mori, N. (2013). Cytotoxic effects of fucoidan nanoparticles against osteosarcoma. *Marine Drugs, 11*(11), 4267–4278.

Koyanagi, S., Tanigawa, N., Nakagawa, H., Soeda, S., & Shimeno, H. (2003). Oversulfation of fucoidan enhances its anti-angiogenic and antitumor activities. *Biochemical Pharmacology, 65*(2), 173–179.

Lee, H., Kim, J.-S., & Kim, E. (2012). Fucoidan from seaweed Fucus vesiculosus inhibits migration and invasion of human lung cancer cell via PI3K-Akt-mTOR pathways. *PLoS One, 7*(11), e50624.

Lee, K. W., Jeong, D., & Na, K. (2013). Doxorubicin loading fucoidan acetate nanoparticles for immune and chemotherapy in cancer treatment. *Carbohydrate Polymers, 94*(2), 850–856.

Lemjabbar-Alaoui, H., Hassan, O. U., Yang, Y.-W., & Buchanan, P. (2015). Lung cancer: Biology and treatment options. *Biochimica et Biophysica Acta, 1856*(2), 189–210.

Li, B., Lu, F., Wei, X., & Zhao, R. (2008). Fucoidan: Structure and bioactivity. *Molecules, 13*(8), 1671–1695.

Li, R., Pavuluri, S., Bruggeman, K., Long, B. M., Parnell, A. J., Martel, A., ... Williams, R. J. (2016). Coassembled nanostructured bioscaffold reduces the expression of proinflammatory cytokines to induce apoptosis in epithelial cancer cells. *Nanomedicine Nanotechnology Biology and Medicine, 12*(5), 1397–1407.

Lin, V. H.-T., Tsou, Y.-C., Chen, Y.-T., Lu, W.-J., & Hwang, P.-A. (2017). Effects of low-molecular-weight fucoidan and high stability fucoxanthin on glucose homeostasis, lipid metabolism, and liver function in a mouse model of type II diabetes. *Marine Drugs, 15*(4), 113.

Linares, M. A., Zakaria, A., & Nizran, P. (2015). Skin Cancer. *Primary Care: Clinics in Office Practice, 42*(4), 645–659.

Lu, J., Shi, K. K., Chen, S., Wang, J., Hassouna, A., White, L. N., ... Nie, S. (2018). Fucoidan extracted from the New Zealand undaria pinnatifida-physicochemical comparison against five other fucoidans: Unique low molecular weight fraction bioactivity in breast cancer cell lines. *Marine Drugs, 16*(12), 461.

Lu, K.-Y., Li, R., Hsu, C.-H., Lin, C.-W., Chou, S.-C., Tsai, M.-L., ... Mi, F.-L. (2017). Development of a new type of multifunctional fucoidan-based nanoparticles for anticancer drug delivery. *Carbohydrate Polymers, 165*, 410–420.

Mak, W., Hamid, N., Liu, T., Lu, J., & White, W. L. (2013). Fucoidan from New Zealand Undaria pinnatifida: Monthly variations and determination of antioxidant activities. *Carbohydrate Polymers, 95*(1), 606–614.

Manivasagan, P., Bharathiraja, S., Bui, N. Q., Jang, B., Oh, Y.-O., Lim, I. G., ... Oh, J. (2016). Doxorubicin-loaded fucoidan capped gold nanoparticles for drug delivery and photoacoustic imaging. *International Journal of Biological Macromolecules, 91*, 578–588.

Marudhupandi, T., Ajith Kumar, T. T., Lakshmanasenthil, S., Suja, G., & Vinothkumar, T. (2015). In vitro anticancer activity of fucoidan from Turbinaria conoides against A549 cell lines. *International Journal of Biological Macromolecules, 72*, 919–923.

Miller, K. D., Siegel, R. L., Lin, C. C., Mariotto, A. B., Kramer, J. L., Rowland, J. H., ... Jemal, A. (2016). Cancer treatment and survivorship statistics, 2016. *CA: A Cancer Journal for Clinicians, 66*(4), 271–289.

Mulloy, B., & Berteau, O. (2003). Sulfated fucans, fresh perspectives: Structures, functions, and biological properties of sulfated fucans and an overview of enzymes active toward this class of polysaccharide. *Glycobiology, 13*(6), 29R–40R.

Nagaoka, M., Shibata, H., Kimura-Takagi, I., Hashimoto, S., Kimura, K., Makino, T., ... Yokokura, T. (1999). Structural study of fucoidan from Cladosiphon okamuranus tokida. *Glycoconjugate Journal, 16*(1), 19–26.

Nishino, T., Nishioka, C., Ura, H., & Nagumo, T. (1994). Isolation and partial characterization of a noval amino sugar-containing fucan sulfate from commercial Fucus vesiculosus fucoidan. *Carbohydrate Research, 255*, 213–224.

Nounou, M. I., ElAmrawy, F., Ahmed, N., Abdelraouf, K., Goda, S., & Syed-Sha-Qhattal, H. (2015). Breast cancer: Conventional diagnosis and treatment modalities and recent patents and technologies. *Breast Cancer: Basic and Clinical Research, 9*(Suppl 2), 17–34.

Oliveira, C., Ferreira, A. S., Novoa-Carballal, R., Nunes, C., Pashkuleva, I., Neves, N. M., ... Silva, T. H. (2017). The key role of sulfation and branching on fucoidan antitumor activity. *Macromolecular Bioscience, 17*(5), 1600340.

Oliveira, C., Neves, N. M., Reis, R. L., Martins, A., & Silva, T. H. (2018). Gemcitabine delivered by fucoidan/chitosan nanoparticles presents increased toxicity over human breast cancer cells. *Nanomedicine, 13*(16), 2037–2050.

Pawar, V. K., Singh, Y., Sharma, K., Shrivastav, A., Sharma, A., Singh, A., ... Chourasia, M. K. (2018). Improved chemotherapy against breast cancer through immunotherapeutic activity of fucoidan decorated electrostatically assembled nanoparticles bearing doxorubicin. *International Journal of Biological Macromolecules, 122*, 1100–1114.

Penesyan, A., Kjelleberg, S., & Egan, S. (2010). Development of novel drugs from marine surface associated microorganisms. *Marine Drugs, 8*(3), 438–459.

Sak, K. (2012). Chemotherapy and dietary phytochemical agents. *Chemotherapy Research and Practice, 2012*, 282570.

Shamay, Y., Elkabets, M., Li, H., Shah, J., Brook, S., Wang, F., ... Heller, D. A. (2016). P-selectin is a nanotherapeutic delivery target in the tumor microenvironment. *Science Translational Medicine, 8*(345), 345ra387.

Shin, S.-W., Jung, W., Choi, C., Kim, S.-Y., Son, A., Kim, H., ... Park, C. H. (2018). Fucoidan-manganese dioxide nanoparticles potentiate radiation therapy by co-targeting tumor hypoxia and angiogenesis. *Marine Drugs, 16*(12), 510.

Siegel, R. L., Miller, K. D., & Jemal, A. (2019). Cancer statistics, 2019. *CA: A Cancer Journal for Clinicians, 69*(1), 7–34.

Silva, T. H., Alves, A., Popa, E. G., Reys, L. L., Gomes, M. E., Sousa, R. A., ... Reis, R. L. (2012). Marine algae sulfated polysaccharides for tissue engineering and drug delivery approaches. *Biomatter, 2*(4), 278–289.

Soeda, S., Shibata, Y., & Shimeno, H. (1997). Inhibitory effect of oversulfated fucoidan on tube formation by human vascular endothelial cells. *Biological & Pharmaceutical Bulletin, 20*(11), 1131–1135.

Synytsya, A., Kim, W.-J., Kim, S.-M., Pohl, R., Synytsya, A., Kvasnička, F., & Il Park, Y. (2010). Structure and antitumour activity of fucoidan isolated from sporophyll of Korean brown seaweed Undaria pinnatifida. *Carbohydrate Polymers, 81*(1), 41–48.

Tengdelius, M., Gurav, D., Konradsson, P., Påhlsson, P., Griffith, M., & Oommen, O. P. (2015). Synthesis and anticancer properties of fucoidan-mimetic glycopolymer coated gold nanoparticles. *Chemical Communications, 51*(40), 8532–8535.

Testa, U., Pelosi, E., & Castelli, G. (2018). Colorectal cancer: Genetic abnormalities, tumor progression, tumor heterogeneity, clonal evolution and tumor-initiating cells. *Medical Sciences (Basel), 6*(2), 31.

Thakur, V., Lu, J., Roscilli, G., Aurisicchio, L., Cappelletti, M., Pavoni, E., ... Bedogni, B. (2017). The natural compound fucoidan from New Zealand Undaria pinnatifida synergizes with the ERBB inhibitor lapatinib enhancing melanoma growth inhibition. *Oncotarget, 8*(11), 17887–17896.

Tong, C. W. S., Wu, M., Cho, W. C. S., & To, K. K. W. (2018). Recent advances in the treatment of breast cancer. *Frontiers in Oncology, 8* 227-227.

Tsai, H.-L., Tai, C.-J., Huang, C.-W., Chang, F.-R., & Wang, J.-Y. (2017). Efficacy of low-molecular-weight fucoidan as a supplemental therapy in metastatic colorectal cancer patients: A double-blind randomized controlled trial. *Marine Drugs, 15*(4), 122.

Usoltseva, R. V., Anastyuk, S. D., Surits, V. V., Shevchenko, N. M., Thinh, P. D., Zadorozhny, P. A., ... Ermakova, S. P. (2019). Comparison of structure and in vitro anticancer activity of native and modified fucoidans from Sargassum feldmannii and S. duplicatum. *International Journal of Biological Macromolecules, 124*, 220–228.

van Weelden, G., Bobiński, M., Okła, K., van Weelden, W. J., Romano, A., & Pijnenborg, J. M. A. (2019). Fucoidan structure and activity in relation to anti-cancer mechanisms. *Marine Drugs, 17*(1), 32.

Venkatesan, J., Singh, K. S., Anil, S., Kim, S.-K., & Shim, S. M. (2018). Preparation, characterization and biological applications of biosynthesized silver nanoparticles with chitosan-fucoidan coating. *Molecules, 23*(6), 1429.

Wang, P., Kankala, R. K., Fan, J., Long, R., Liu, Y., & Wang, S. (2018). Poly-L-ornithine/fucoidan-coated calcium carbonate microparticles by layer-by-layer self-assembly technique for cancer theranostics. *Journal of Materials Science: Materials in Medicine, 29*(5), 68.

Wang, Z.-J., Xu, W., Liang, J.-W., Wang, C.-S., & Kang, Y. (2017). Effect of fucoidan on B16 murine melanoma cell melanin formation and apoptosis. *African Journal of Traditional Complementary and Alternative Medicines, 14*(4), 149–155.

Wang, P., Kankala, R. K., Chen, B., Long, R., Cai, D., Liu, Y., ... Wang, S. (2019). Polyallylamine hydrochloride and fucoidan-based self-assembled polyelectrolyte complex nanoparticles for cancer therapeutics. *Journal of Biomedical Materials Research Part A, 107*(2), 339–347.

Wang, Y., Xing, M., Cao, Q., Ji, A., Liang, H., & Song, S. (2019). Biological activities of fucoidan and the factors mediating its therapeutic effects: A review of recent studies. *Marine Drugs, 17*(3), 183.

Wells, M. L., Potin, P., Craigie, J. S., Raven, J. A., Merchant, S. S., Helliwell, K. E., ... Brawley, S. H. (2017). Algae as nutritional and functional food sources: Revisiting our understanding. *Journal of Applied Phycology, 29*(2), 949–982.

Xue, M., Ge, Y., Zhang, J., Wang, Q., Hou, L., Liu, Y., ... Li, Q. (2012). Anticancer properties and mechanisms of fucoidan on mouse breast cancer in vitro and in vivo. *PLoS One, 7*(8), e43483.

Yang, C., Chung, D., Shin, I.-S., Lee, H., Kim, J., Lee, Y., ... You, S. (2008). Effects of molecular weight and hydrolysis conditions on anticancer activity of fucoidans from sporophyll of Undaria pinnatifida. *International Journal of Biological Macromolecules, 43*(5), 433–437.

You, S., Yang, C., Lee, H., & Lee, B.-Y. (2010). Molecular characteristics of partially hydrolyzed fucoidans from sporophyll of Undaria Pinnatifida and their in vitro anticancer activity. *Food Chemistry, 119*(2), 554–559.

Zappa, C., & Mousa, S. A. (2016). Non-small cell lung cancer: Current treatment and future advances. *Translational Lung Cancer Research, 5*(3), 288–300.

Zhang, Z., Teruya, K., Eto, H., & Shirahata, S. (2013). Induction of apoptosis by low-molecular-weight fucoidan through calcium- and caspase-dependent mitochondrial pathways in MDA-MB-231 breast cancer cells. *Bioscience, Biotechnology, and Biochemistry, 77*(2), 235–242.

Zhang, Z., Teruya, K., Yoshida, T., Eto, H., & Shirahata, S. (2013). Fucoidan extract enhances the anti-cancer activity of chemotherapeutic agents in MDA-MB-231 and MCF-7 breast cancer cells. *Marine Drugs, 11*(1), 81–98.

Zorofchian Moghadamtousi, S., Karimian, H., Khanabdali, R., Razavi, M., Firoozinia, M., Zandi, K., ... Abdul Kadir, H. (2014). Anticancer and antitumor potential of fucoidan and fucoxanthin, two main metabolites isolated from brown algae. *The Scientific World Journal, 2014*, 10.