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Review

Cellular Basis of Adjuvant Role of n-3 Polyunsaturated Fatty Acids in Cancer Therapy: Molecular Insights and Therapeutic Potential against Human Melanoma

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Abstract: Human melanoma is a highly aggressive malignant tumor originating from epidermal melanocytes, characterized by intrinsic resistance to apoptosis and the reprogramming of proliferation and survival pathways during progression, leading to high morbidity and mortality rates. This malignancy displays a marked propensity for metastasis and often exhibits poor responsiveness to conventional therapies. Fatty acids, such as n-3 polyunsaturated fatty acids (PUFAs) docosahexaenoic and eicosapentaenoic acids, exert various physiological effects on melanoma, with increasing evidence highlighting the anti-tumorigenic, anti-inflammatory, and immunomodulatory properties. Additionally, n-3 PUFAs have demonstrated their ability to inhibit cancer metastatic dissemination. In the context of cancer treatment, n-3 PUFAs have been investigated in conjunction with chemotherapy as a potential strategy to mitigate severe chemotherapy-induced side effects, enhance treatment efficacy and improve safety profiles, while also enhancing the responsiveness of cancer cells to chemotherapy. Furthermore, dietary intake of n-3 PUFAs has been associated with numerous health benefits, including a decreased risk and improved prognosis in conditions such as heart disease, autoimmune disorders, depression and mood disorders, among others. However, the specific mechanisms underlying their anti-melanoma effects and outcomes remain controversial, particularly when comparing findings from in vivo or in vitro experimental studies to those from human trials. Thus, the objective of this review is to present data supporting the potential role of n-3 PUFA supplementation as a novel complementary approach in the treatment of malignant cancers such as melanoma.

Keywords: melanoma; skin cancer; n-3 polyunsaturated fatty acid; docosahexaenoic acid; eicosapentaenoic acid; metastasis

Citation: Rojas-Solé, C.; Torres-Herrera, B.; Gelerstein-Claro, S.; Medina-Pérez, D.; Gómez-Venegas, H.; Alzolay-Sepúlveda, J.; Chichiarelli, S.; Saso, L.; Rodrigo, R. Cellular Basis of Adjuvant Role of n-3 Polyunsaturated Fatty Acids in Cancer Therapy: Molecular Insights and Therapeutic Potential against Human Melanoma. *Appl. Sci.* **2024**, *14*, 4548. https://doi.org/10.3390/app14114548

Academic Editor: Viswas Raja Solomon

Received: 28 March 2024
Revised: 21 May 2024
Accepted: 23 May 2024
Published: 25 May 2024

1. Introduction

Skin cancer holds the top position as the most frequently diagnosed cancer globally, a logical outcome considering the skin's status as the body's largest organ. Furthermore, it has demonstrated a significant increase in the global incidence rate over the past few years [1].

It is typically categorized into two primary groups: melanoma and nonmelanoma skin cancer. Nonmelanoma skin cancer includes basal cell and squamous cell carcinomas.

Although the most prevalent of these malignancies seldom lead to mortality, it is imperative to acknowledge that the local morbidity resulting from the tumors and their extirpation should not be underestimated [2]. It is noteworthy to emphasize that the form of skin cancer known as melanoma is caused by a malignancy of melanocytes, being a major public health concern due to its increase in recent years, especially due to the modern lifestyle and global atmospheric changes, involving increased UV exposure [3]. The occurrence of melanoma highly depends on the geographic area, and has the highest incidence rates among males from Australia and New Zealand, followed by Western Europe [4].

It was estimated that at least half of the 200,000 new cases of melanoma reported in 2021 would be invasive malignant melanoma and the other half would be melanoma in situ [5].

Surgery to remove the tumor is the primary treatment of all stages of melanoma. A wide local excision is used to remove the melanoma and some of the normal tissue around it. For melanoma in situ, surgery is considered curative. In contrast, in the context of a known advanced or metastatic disease surgical treatment is not meant to be curative and will require other treatment options [6]. Radiotherapy and chemotherapy can also be used for palliative treatment and in particular circumstances such as using radiation at the site of a lymphadenectomy or when the patient cannot undergo surgery [7].

Using chemotherapy as the sole treatment modality in patients diagnosed with advanced melanoma typically exhibits a median overall survival of 5.1 months from diagnosis, with a less than 5% probability of survival at the three-year mark [8].

Nowadays, combinations of BRAF inhibitors and MEK inhibitors are used to treat melanoma [9]. Roughly half of melanoma patients carry a BRAF mutation, rendering them candidates for targeted therapy involving BRAF/MEK inhibitors. BRAF inhibition produces rapid tumor regression. The addition of MEK inhibitors reduces resistance and decreases cutaneous toxicity which has been seen with single BRAF inhibition. Simultaneous inhibition of BRAF and MEK also improves response rates and survival compared with BRAF inhibition alone [10].

Although the response rate approaches 70%, over half of these patients will encounter disease progression within a year, primarily due to tumor resistance [11].

This scenario has prompted the investigation of numerous substances for an alternative or adjunctive treatment to this cancer such as botanical agents, phytochemicals, herbal formulas, cannabinoids and vitamin c, among others [12].

Fatty acids can be categorized into two primary types: saturated and unsaturated. Saturated fatty acids lack double bonds, whereas unsaturated fatty acids possess double bonds. Among unsaturated fatty acids, those designated as polyunsaturated fatty acids (PUFAs) feature multiple double bonds. Specifically, PUFAs with a double bond positioned three carbons from the methyl end of the fatty acid chain are termed omega-3 or n-3 PUFAs (Figure 1) [13]. These include α-linolenic acid (ALA), stearidonic acid, eicosapentaenoic acid (EPA), docosapentaenoic acids (DPA) and docosahexaenoic acid (DHA), with EPA and DHA being the most available types in the human body. Another class of PUFAs features a double bond originating from the sixth carbon position counting from the methyl end of the molecule (Figure 2). Consequently, this group is referred to as n-6 PUFAs, encompassing compounds such as arachidonic acid (AA) and linoleic acid, among others [13].

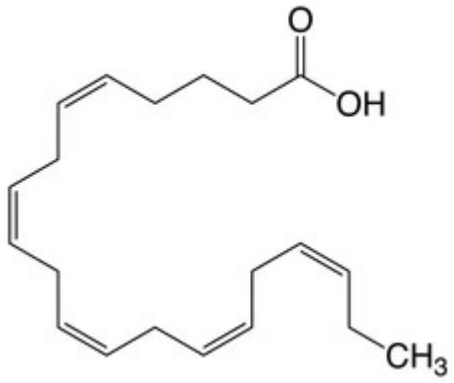

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Figure 1. Chemical structure of DHA.

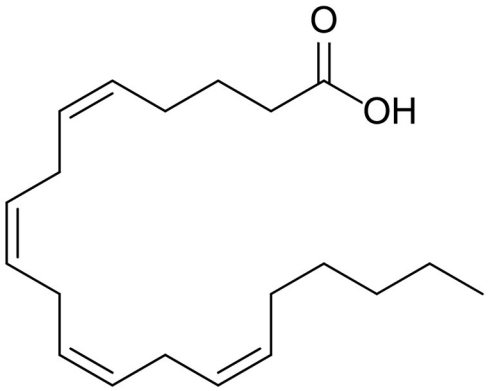

Figure 2. Chemical structure of AA.

The understanding of physicochemical properties is crucial for the application of DHA and other PUFAs in food science, pharmaceuticals and nutritional supplements due to their behavior in formulations and their biological effects being directly influenced by these properties.

Nowadays, the role of n-3 PUFAs has advanced significantly with the growing understanding of the unique specialized proresolving lipid mediators [14]. However, the extraction method also plays a role, as the quality of unconventional lipids largely depends on these extraction techniques [15].

The properties of DHA include physical properties in which the solubility is hydrophobic and insoluble in water. Density and viscosity, as typical for fatty acids, are influenced by their degree of unsaturation and temperature. More unsaturation usually correlates with lower viscosity. DHA has lower density and viscosity compared to saturated and monounsaturated fatty acids and has a melting point around −44 °C. The multiple double bonds lower the melting point compared to saturated fatty acids of similar molecular weight. The chemical properties include oxidation in which the reactivity can lead to the formation of lipid peroxides and other degradation products, which can be catalyzed by heat, light and metal ions, affecting the stability and shelf life of DHA-containing products. Moreover, DHA can undergo hydrogenation, a chemical reaction that adds hydrogen atoms to the double bonds, converting them into single bonds and producing more saturated fatty acids and also can undergo esterification and isomerization, potentially affecting its biological activity [14,16].

Furthermore, in DHA and EPA there are potential spectroscopic techniques and chemometric analyses for rapid measurement in algal oil. The best predictions for both were achieved via nuclear magnetic resonance spectroscopy, in which the determination coefficients of crossvalidation values were 0.963 and 0.967 [17].

PUFAs are described to be essential for the synthesis of eicosanoids, such as prostaglandins (PGs), prostacyclin, thromboxane, leukotrienes (LTs) and others. However, the activity of the eicosanoids depends on the kind of PUFA that it is derived from [18]. Those eicosanoids that come from n-3 PUFA are described to have an anti-inflammatory effect, which is why in recent years there has been a growing interest in possible therapeutic use on several diseases [19]. On the other hand, eicosanoids that come from n-6 PUFAs have a pro-inflammatory effect, which has been described as a predisposing factor for cancer and other diseases [20].

It is worth mentioning that in the last decades there has been an interesting change in the proportion of fatty acids present in the human diet. Historically, humans consumed 1:1 proportion of n-3 and n-6 fatty acids in the diet. However, in today's Western diet there is a 1:20 proportion of n-3/n-6 fatty acids, which is a substrate that leads to various inflammatory diseases [21].

There is a large amount of studies about the health benefits of n-3 PUFA supplementation, including its use for different diseases, such as the improvement of lipid profile and reduction of inflammation parameters such as tumor necrosis factor α (TNF-α) or high sensitivity C-Reactive Protein [22], the decrease in the severity of non-alcoholic fatty liver disease by reducing hepatic steatosis and liver injury [23], the enhancement of the neurocognitive function [24], the modulation of membrane-associated signal transduction involved in cancer pathogenesis and suppression of systemic inflammation [25] and many other diseases.

Despite the promising properties attributed to n-3 PUFAs, their translation into a clinical context encounters several challenges. One notable issue pertains to the potential adverse effects that n-3 PUFAs may pose to patients and the scarcity of evidence derived from human trials. Due to its anti-inflammatory effects, it has been theorized and described that n-3 PUFAs might be associated with immune function impairment [25]. Therefore, more clinical trials are needed to find the effective dose and formulas of n-3 PUFAs for different pathologies, including cancer [18].

The aim of this review is to provide data supporting the view that an n-3 PUFA treatment could serve as a complementary treatment in malignant cancers such as melanomaPUFAs, which are described to be essential for the synthesis of eicosanoids, such as prostaglandins (PGs), prostacyclin, thromboxane, leukotrienes (LTs) and others. However, the activity of the eicosanoids depends on the kind of PUFA that it is derived from [14]. Those eicosanoids that come from n-3 PUFA are described to have an anti-inflammatory effect, which is why in recent years there has been a growing interest in possible therapeutic use for several diseases [15]. On the other hand, eicosanoids that come from n-6 PUFAs have a pro-inflammatory effect, which has been described as a predisposing factor for cancer and other diseases [16].

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2. Omega-3 Polyunsaturated Fatty Acids

n-3 PUFAs represent integral constituents in human physiology, exhibiting a spectrum of favorable health attributes. Over the preceding two decades, n-3 PUFAs have been the subject of study to elucidate their multifaceted roles within biological frameworks. The accrued body of research has yielded promising findings elucidating the involvement across diverse biological functions and intricate biochemical pathways. Of particular significance is their discernible impact in orchestrating the mitigation and regulation of various disease processes, attenuating inflammation cascades and modulating antiangiogenic mechanisms. Such insights underscore the pivotal role of n-3 PUFAs as instrumental mediators in the dynamic interplay between cellular homeostasis and pathological states, thereby positioning them as promising candidates for therapeutic intervention and preventative strategies against a spectrum of afflictions.

2.1. Diet

The recommended daily intake of n-3 PUFAs stands at 1.6 g for men and 1.1 g for women, serving as a primary dietary source. Noteworthy dietary contributors encompass a spectrum of plant-derived sources, prominently featuring vegetable oils such as flaxseed oil, chia seeds and canola oil, heralded for their enriched content of n-3 PUFAs. Concurrently, marine-derived offerings, including fish and various aquatic fauna, harbor substantial concentrations of n-3 PUFAs, predominantly in the form of EPA and DHA [26]. Of particular importance, in the context of maternal nutrition, a targeted augmentation in DHA intake by 200 milligrams per day is recommended for pregnant women [27].

2.2. Biological Functions

The principal biological functions attributed to n-3 PUFAs encompass their pivotal roles as structural constituents within cellular membranes, providers of metabolic energy, and as regulatory agents orchestrating the modulation of intricate biochemical pathways through the generation of "bioactive lipid" molecules [28]. These multifaceted functionalities are integral to sustaining cellular integrity, facilitating energy homeostasis and governing a spectrum of physiological processes critical for our organism.

In the context of cellular membranes, particularly within the vasculature, EPA assumes a notable role, exhibiting a stable conformation that contributes to the maintenance of cholesterol homeostasis and membrane fluidity. Conversely, within specialized neuronal and retinal tissues, DHA undergoes isomerization, thereby enhancing membrane fluidity and facilitating the sequestration of cholesterol into lipid rafts, characterized by an abundance of sphingolipids. Notably, a fraction of dietary ALA undergoes enzymatic conversion to EPA and DHA in adults, albeit with varying degrees of efficiency. Importantly, this conversion process is particularly augmented in women of reproductive age compared to men, attributable in part to the influence of plasma estrogen. However, notwithstanding endogenous enzymatic mechanisms, the primary source of EPA and DHA remains dietary intake, underscoring the imperative of dietary supplementation to meet optimal physiological requirements [29]. Other functions are related with intestinal immune tolerance and gut microbiota maintenance [30], pregnancy

and lactation [27], cardiovascular health [31], cognitive function [32], skeletal muscle health [33], sarcopenia [34] and osteoarthritis [35], among others [18].

In the realm of inflammation regulation, PUFAs undergo enzymatic oxygenation, leading to the formation of oxylipins, a class of molecules integral to mediating inflammatory processes. Among these oxylipins, particular attention is directed towards pro-resolving mediators, encompassing resolvins, protectins and maresins, which function to expedite the resolution of inflammatory responses. Broadly, n-3 PUFAs serve as precursors for the synthesis of anti-inflammatory oxylipins, whereas n-6 PUFAs act as progenitors of pro-inflammatory mediators, thus influencing the initiation and termination phases of inflammation. Consequently, the balance between n-6 and n-3 PUFAs, reflected in the n-6/n-3 ratio, delineates a critical determinant of inflammatory modulation [36].

Furthermore, n-3 PUFAs exert regulatory effects on transcriptional processes, notably enhancing peroxisome proliferator-activated receptor alpha expression while concurrently attenuating the transcription of nuclear factor kappa B (NF-κB) and the NLR family pyrin domain containing (NLRP) 3 inflammasome, thereby exerting a suppressive influence on inflammatory cascades. Additionally, oxylipins are implicated in the modulation of ion channels, particularly within neuronal and muscular tissues, although the precise physiological implications of these interactions remain incompletely understood, warranting further investigation [37].

The n-3 PUFAs, in conjunction with their n-6 counterparts, serve as substrates contributing to the biosynthesis of compounds within the endocannabinoid system, comprising neurotransmitters, receptors and associated enzymes. These bioactive compounds exert far-reaching effects across various cerebral processes and peripheral tissues, prominently including the regulation of appetite. Notably, enzymatic metabolism of EPA and DHA culminates in the formation of N-eicosapentanoyl ethanolamine (EPEA) and N-docosahexaenoyl ethanolamine (DHEA), both of which serve as endocannabinoids upon binding to their receptors, thereby orchestrating their biological functions [38]. EPEA and DHEA can be metabolized by cytochrome P450 to produce endocannabinoid epoxides, which have been shown to reduce inflammation and neoangiogenesis in animal models [39].

The active sites of DHA in brain tissue with the brain fatty acid binding protein (B-FABP) (Figure 3) are well known. However, to elucidate the active sites in other tissues further studies are needed [40].

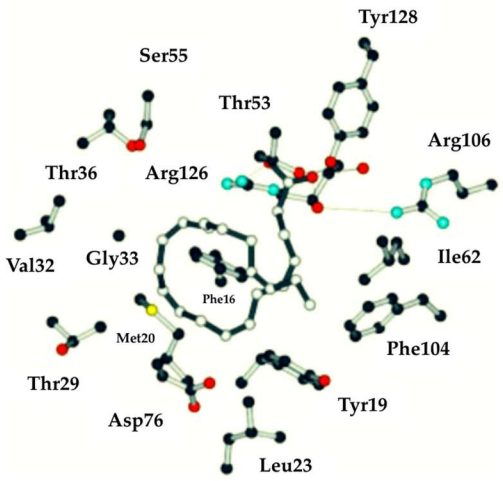

Figure 3. Active sites of DHA are shown as found with the complexes with human B-FABP. Red circles are for oxygen, black circles are for carbon, yellow circles are for sulfur and blue circles are for nitrogen (Adapted with permission from Balendiran et al. [40], Journal of Biological Chemistry; published by Elsevier, 2000).

2.3. Clinical Benefits

In the realm of clinical settings, positive outcomes have been proposed and substantiated concerning the consumption of n-3 PUFAs, with observed impacts on lipid metabolism, chronic inflammation, oxidative stress, immune response, cardiovascular risk and tumor suppression. Nevertheless, discordant findings have also emerged, attributable in part to the heterogeneity across studies in the administration protocols of n-3 PUFAs. Consequently, while the consumption of foods rich in n-3 PUFAs has demonstrated a reduction in cardiovascular risk and other related factors, the efficacy of EPA and DHA supplements in this regard has exhibited inconsistency. Furthermore, a clinical distinction arises between the utilization of EPA in isolation and the consumption of combined EPA and DHA supplements, the latter demonstrating comparatively diminished beneficial effects on cardiovascular health. Such disparities can be ascribed to inherent molecular variances and disparities in binding sites [29].

2.4. Cancer Research

In the context of cancer, accumulating evidence suggests that the consumption of n-3 PUFAs, such as EPA and DHA, has been associated with a decreased risk of several malignancies including colon, prostate and breast cancer, as well as leukemia and melanoma, along with a reduction in their respective metastases. This effect is mediated through several mechanisms, with a significant factor being the inhibition of inflammatory, proangiogenic and cell migration processes. These mechanisms are intricately linked to the downregulation of molecules such as angiopoietin 2, vascular endothelial growth factor (VEGF), matrix metalloproteinases (MMP) and chemokines. Notably, both in vitro and animal studies have demonstrated that DHA and its metabolites exhibit a particularly heightened efficacy in suppressing tumor neoangiogenesis compared to EPA. Conversely, treatment with AA appears to elicit contrasting effects, with evidence suggesting that n-6 PUFAs may promote a carcinogenic environment and enhance metastasis. This dual role of n-3 and n-6 PUFAs underscores the complexity of fatty acid metabolism and lipidomics in cancer pathogenesis and highlights the potential therapeutic implications of modulating PUFA intake for cancer prevention and management [36].

2.5. Characteristics of n-3 PUFA In Silico

Important n-3 PUFAs have been extensively studied using in silico methods to understand their various properties and potential therapeutic applications, which constitutes a significant contribution to drug design and development.

In silico pharmacokinetics predictions suggest that DHA can enhance the overall permeability of drugs, such as doxorubicin, in cancer cells. Doxorubicin, a chemotherapeutic agent known for its high cardiotoxicity and drug-resistance issues, may benefit from being conjugated with other molecules to optimize its potency. DHA has been shown to improve the absorption of doxorubicin in cancer cells. Computational methods based on structure-based design were used to explore the potential of a conjugated drug, predicting the pharmacokinetic properties of each component and identifying the best binding mode and energy to enhance the selectivity of the doxorubicin-DHA conjugate. These in silico approaches indicate that DHA might significantly improve the overall permeability and efficacy of doxorubicin in targeting cancer cells [41]. Some of the other drug interactions will be discussed in Section 4.

Moreover, in silico studies related to COVID-19 have shown that DHA has moderate binding affinities and interactions with the active sites of interleukin-6 (IL-6) and high binding affinity with optimal interactions at the active sites of angiotensin-converting enzyme 2 (ACE2). These findings suggest that DHA could play potential roles in modulating inflammatory responses and possibly influencing viral entry mechanisms, acting as a potent inhibitor of both ACE2 and IL-6 [42].

During the metabolic process, ALA is biotransformed into EPA and DHA. In silico ADME-Tox and pharmacokinetic profiling using artificial intelligence and machine learning approaches have shown that ALA is safe as a dietary ingredient, as it does not produce serious health problems. This essential fatty acid could be used as a nutraceutical and pharmacological food ingredient. However, the overall evidence on the association of ALA with health risks remains inconclusive [43].

To fully understand the potential and application of DHA in the treatment of melanoma, further in silico research is essential. Current studies indicate that DHA can modulate various molecular pathways and immune responses, which are crucial in the context of melanoma. For instance, DHA has been shown to induce apoptosis in cancer cells, regulate the cell cycle, and enhance the effectiveness of the immune system against tumor cells. Additionally, DHA can impact signaling pathways such as PI3K/Akt and modulate the expression of immune checkpoints [44–47]. However, while these findings are promising, comprehensive in silico analyses and simulations are needed to explore the specific mechanisms on melanoma cells.

3. Molecular Mechanisms of Omega-3 Fatty Acids in Cancer

In recent decades, there has been a rise in the interest for n-3 PUFA and its mechanisms involved in the inhibition of tumor cell growth. Indeed, these compounds, such as DHA and EPA, have shown to be involved in cancer prevention, installation and development as they act in many crucial cancer hallmarks including cell cycle progression, inflammation, oxidative stress, apoptosis, angiogenesis and immune system modulation, among others [48–50]. At this point, these main roles of n-3 PUFA will be reviewed with a broader perspective on cancers and will be further discussed with a focus on melanoma in Section 5.

However, it is important to discuss which pathways may be crucial for the antineoplastic effects of n-3 PUFAs. Numerous studies have demonstrated that n-3 PUFAs increase the production of prostaglandin E3 (PGE3) while reducing the levels of prostaglandin E2 (PGE2), which is involved in inflammatory and pro-carcinogenic pathways. Additionally, there is substantial information regarding the effects of these fatty acids on the cell cycle, specifically in regulating the expression of key proteins such as cyclin-dependent kinases (CDKs) and cyclin-dependent kinase inhibitors (CDKIs). These interactions between n-3 PUFAs and various proteins occur in separate pathways. Therefore, it is likely that the crucial molecular pathway involved in the anticarcinogenic effects of n-3 PUFAs is related to the cyclooxygenase-2 (COX-2) enzyme and the production of PGE3, which leads to a reduction in PGE2 levels.

3.1. Cell Cycle and Cell Death in Cancer Mediated by n-3 PUFA

The regulation of the cell cycle involves intricate mechanisms governed by checkpoints and a diverse array of CDK complexes. Transcriptional modulation of these complexes occurs in accordance with the specific phase of the cell cycle and may be upregulated or downregulated. Additionally, there are regulatory proteins known as CDKIs, which are classified into two main families; the CDK interacting protein/Kinase inhibitory protein family, which includes p21, p27 and p57, targeting cyclin-CDK1 and -CDK2 complexes, and the inhibitors of CDK4 and CDK6 family, known as the INK4 family, comprising p15, p16, p18 and p19, which interact primarily with cyclin-CDK4 and -CDK6 complexes [51]. This cell cycle machinery is often dysregulated in cancer, which can lead to uncontrolled growth [52].

Wei et al. studied the effects of n-3 PUFAs in transgenic embryonic mouse stem cells exhibiting a prolonged G0/G1 phase with an upregulation of p21 and a consequent downregulation of CDK4; this was demonstrated once the inhibitory effect in CDK4 was reduced in a p21 inhibitor pretreatment group [53]. Similar results were found in a neuroblastoma cell line study that showed that DHA and EPA had growth-inhibitory effects, producing a prolonged G0/G1 phase accompanied by a decrease in CKD2 and

cyclin E and activation of the intrinsic apoptosis pathway as well as almost no toxicity in non-tumorigenic cells both in murine rat and human cells [54]. Sun et al. reported antiproliferative effects of DHA that induced a G1 phase cell cycle arrest and cellular senescence in three human gastric cancer cell lines with upregulation of p21 and p27, and downregulation of proliferating cell nuclear antigen, cyclin E and cyclin D1 in a dose-dependent manner [55] (Figure 4).

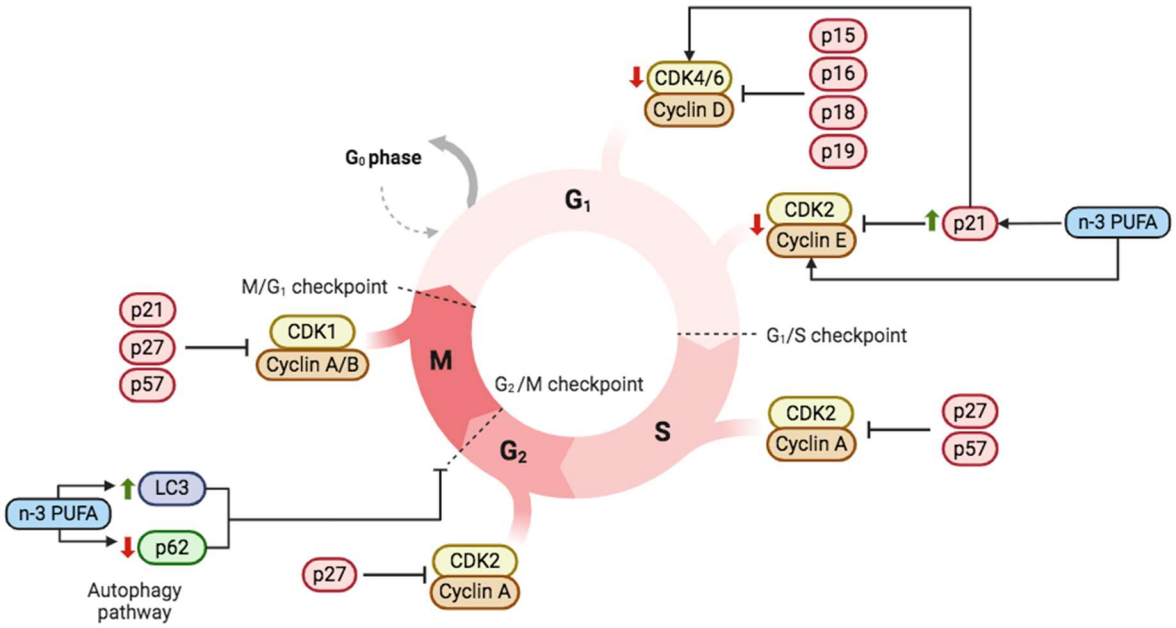

Figure 4. General mechanism by which n-3 affects the cell cycle and cell death. ↓, decrease; ↑, increase; CDK1, Cyclin-dependent kinase 1; CDK2, Cyclin-dependent kinase 2; CDK4, Cyclin-dependent kinase 4; CDK6, Cyclin-dependent kinase 6; LC3, Microtubule-associated protein 1 light chain 3; n-3 PUFA, n-3 polyunsaturated fatty acids. Numerous studies have demonstrated that n-3 PUFAs activate both endoplasmic reticulum (ER) and mitochondrial-dependent pathways of apoptosis. Jakobsen et al. showed that DHA produced ER stress and also induced unfolded protein response [56]. In SW620 cells, this process is initiated by the dissociation of phosphorylated extracellular signal-regulated kinases (p-ERK) from the ER-resident chaperone BiP/GRP78, which participates in various complexes [57]. The combined application of DHA and EPA proved to be more efficacious in inducing apoptosis in cancer stem-like cells SW620 than either DHA or EPA administered individually [58].

3.2. Cell Cycle and Cell Death in Melanoma Mediated by n-3 PUFA

In a previous investigation, Albino et al. conducted experiments utilizing human melanoma cell lines cultured with DHA, which exhibited growth-inhibitory effects and increased apoptosis in 7 out of 12 cell lines. To elucidate the mechanisms underlying these outcomes, the researchers assessed the levels of p21 and p27 CDKIs, revealing an upregulation of p27 in tumor cells, a cell line in which DHA demonstrated growth-inhibitory effects.

There are other mechanisms that may cause apoptosis in these tumor cells when they are exposed to n-3 PUFAs. The ability of the fatty acids to induce apoptosis has also been attributed to the increased susceptibility of these cells to lipid peroxidation. Zajdel et al. proposed that the main mechanism underlying the anti-tumor effects of n-3 PUFAs is the formation of cytostatic and cytotoxic compounds as well as reactive oxygen species (ROS) after the lipid peroxidation of these n-3 PUFAs [59].

3.3. DHA and Autophagy in Crosstalk with Cell Cycle Modulation

Other studies have demonstrated an alternative mechanism through which PUFAs, particularly DHA, induce cell cycle arrest in cancer cell lines concomitant with autophagy. An investigation examined the interplay between these two biological processes: cell cycle modulators, such as CKDIs—notably p16, p21, p27 and p57—and cyclin-dependent kinases were revealed to additionally regulate autophagy in addition to their primary roles in cell cycle regulation [60]. Similarly, autophagy has been demonstrated to regulate cell cycle progression and play a role in cancer development, adaptation and survival, exhibiting context-dependent effects.

This dual role of autophagy in cancer was also demonstrated by Li et al., wherein it was implicated in both the development of multidrug-resistant (MDR) cancer and the activation of the apoptosis pathway in MDR cancer, contingent upon contextual factors [61]. Continuing along this line of investigation, a study revealed the inhibitory effects of DHA in a human esophageal cancer cell line, leading to a G2/M cell cycle arrest concomitant with a significant upregulation of microtubule-associated protein light chain (LC3) and a slight downregulation of p62, both recognized as autophagy protein markers (Figure 3). These findings suggest that DHA may have induced autophagy in this particular cell line [62]. Additionally, this phenomenon was accompanied by the production of ROS. It was observed that the induction of autophagy by DHA was diminished upon the addition of N-acetylcysteine NAC, an antioxidant, suggesting that ROS was primarily responsible for this effect.

A study conducted in human epithelial ovarian cancer cells yielded comparable findings, demonstrating growth-inhibitory effects of DHA accompanied by induction of cell cycle arrest at the G2/M phase, alongside upregulation of LC3 levels (Figure 4), with effects observed in a dose-dependent manner. Notably, this effect was markedly attenuated when cells were co-treated with CQ and Bafilomycin A1, both autophagy inhibitors. Additionally, DHA exhibited anti-inflammatory properties, mechanistically attributed to its inhibition of the NF-κB signaling pathway [63].

Regarding the mechanism by which DHA induces autophagy in cancer cells, Wang et al. investigated this phenomenon in the HeLa cell line. They observed upregulation of phosphorylation of Beclin (Bcl)-2 at Ser70 (p-Bcl-2) without altering its expression level within the cell [63]. Usually, Bcl-2 is bound to Bcl-1, whose function is linked to the initiation of autophagy in mammalian cells and is hindered when bound to Bcl-2. However, p-Bcl-2 may dissociate from Bcl-1. This dissociation occurs independently of c-Jun N-terminal kinase (JNK) 1/2, a kinase protein involved in Bcl-2 phosphorylation at Ser70 in response to increased levels of ROS, which is one of the reported effects of DHA. Additionally, it was observed that DHA inhibited mTOR, an autophagy inhibitor, suggesting these two pathways through which DHA could promote autophagy in cancer cells [63].

DHA and Autophagy in Crosstalk with Melanoma Cell Cycle Modulation

In the context of melanoma, the interplay between cell cycle progression and autophagy has not been extensively investigated. However, evidence suggests an association between autophagy and other cancer-related processes in melanoma. For instance, studies have demonstrated anti-inflammatory effects through the inhibition of AIM2 (known as absent in melanoma 2), an interferon-inducible protein, as well as modulation of the NF-κB signaling pathways following n-3 PUFA treatment [64]. Moreover, n-3 PUFA has been associated with anti-metastatic effects, accompanied by an increase in LC3 levels and a decrease in p62 levels in in vivo studies [65].

3.4. n-3 PUFA Inhibits Angiogenesis

The vascular homeostasis is maintained by a balance between pro- and anti-angiogenic factors. The process where new blood vessels are formed is called angiogenesis, and happens when the pro-angiogenic signals are predominant [66].

For a tumor to proliferate uncontrollably and transition from a dormant state to an invasive one, it must acquire the ability to stimulate angiogenesis, thereby ensuring a sufficient blood supply to meet its escalating metabolic demands. This phenomenon, commonly referred to as the "angiogenic switch", involves the heightened activation of pro-angiogenic signaling pathways [67].

Since its identification as a viable target for cancer therapy, numerous angiogenic treatments have been formulated, primarily focusing on anti-VEGF modalities. However, their efficacy remains limited and unclear, failing to significantly enhance life expectancy and even demonstrating a propensity to promote metastasis, particularly evidenced in preclinical investigations. This phenomenon may be attributed to the varied mechanisms through which tumors develop resistance to such therapies, including compensatory processes, co-option of adjacent normal vasculature or induction of pro-angiogenic factors due to the hypoxic conditions induced by anti-angiogenic interventions. Given this context, it has been advocated that novel anti-angiogenic cancer therapies should target alternative angiogenic pathways and serve as adjuncts to other molecular targets rather than focusing solely on angiogenesis inhibition [67,68].

n-3 PUFA have been implicated in the inhibition of angiogenesis by virtue of their lipid metabolites, which compete with alternative substrates to yield metabolites of lesser harmfulness or potential benefit. For example, the enzyme COX-2 can utilize AA to generate PGE2 and, alternatively, can employ EPA as a less favorable substrate, resulting in the formation of PGE3 [69]. While PGE2 is widely known for inducing angiogenesis and inflammation as well as promoting tumor growth and metastasis, PGE3 has shown to have significantly reduced detrimental effects and has even shown to inhibit proliferation and invasion in cancer cell lines [58,70–72] including melanoma [73].

Furthermore, Groeger et al. demonstrated that DHA can be converted into hydroxyl DHA, whose lipid metabolites exhibit anti-angiogenic properties [74]. However, Sapieha et al. found that 4-hydroxy-docosahexaenoic acid, a product of DHA metabolism by 5-lipoxygenase (5-LOX), was crucial for these effects, rather than its metabolism by COX-2. [75].

5-LOX is also responsible for metabolizing AA, generating 5-Hydroxyeicosatetraenoic acid (HETE), which has been demonstrated to induce inflammation and angiogenesis and promote tumor progression. Furthermore, 15-Lipoxygenase can metabolize both EPA and DHA to produce HETE and 17-hydroxy-docosahexaenoic acid (17-HDHA), respectively. These metabolites have been shown to reduce the activity of 5-LOX, thereby mitigating inflammation and angiogenesis. Additionally, 17-HDHA has demonstrated other anti-inflammatory mechanisms by downregulating pro-inflammatory cytokines and modulating the immune response [69].

Notably, within both anti-angiogenic and anti-inflammatory mechanisms, there exist counterparts among n-6 and n-3 PUFAs, with n-3 derivatives being linked to anti-inflammatory, anti-angiogenic and anti-tumor proliferation effects. Kang et al. investigated this phenomenon and underscored the significance of maintaining a low tissue n-6/n-3 PUFA ratio as a novel strategy for cancer treatment [76].

3.5. Regulation of Cancer Metastasis through n-3 PUFA

The spread and proliferation of cancer cells in distant organs from the site of the primary tumor, known as metastasis, represents the most lethal aspect of cancer, accounting for a majority of cancer-related deaths and complications. Metastasis poses a significant threat to organ function and disrupts metabolic processes [77]. Despite its profound impact, the intricate and multi-step nature of metastasis presents a formidable

challenge, with no existing drugs or treatments demonstrating efficient curative potential [77,78]. Consequently, there is an imperative need for the development of novel drugs and therapies targeting metastasis.

Considering their inhibitor growth, anti-inflammatory and anti-angiogenic effects, n-3 PUFAs may have potential anti-metastatic effects as well. Chen et al. exhibited anti-metastatic effects in a dose-dependent manner of DHA in association with engaged autophagy in a human esophageal cancer cell line. This was measured by the ability of the cells to migrate in order to heal a wound, finding that DHA-treated cells had a significantly larger wound width [79].

Similar findings were observed by Sun et al., where DHA induced cell cycle arrest at the G1 phase and decreased motility and migration in human gastric cancer cell lines. These effects were assessed also using a scratch wound healing assay, [55].

Khadge et al. conducted a comparative analysis of cancer progression in mice inoculated with a murine mammary adenocarcinoma cell line, distinguishing between groups fed n-3 PUFA and n-6 PUFA diets. Their findings revealed that the n-3 PUFA group exhibited decreased tumor volume and weight, improved survival rates and reduced metastasis in various organs including the lung, liver, spleen, heart, kidney, ovary and bone. Additionally, enhanced apoptosis and decreased angiogenesis were observed in this group [80].

Another important signaling pathway which is described to trigger metastasis and is involved in different types of cancer, including conjunctival malignant melanoma, is the epidermal growth factor and epidermal growth factor receptor (EGFR) pathway [81]. Some studies on other cancer types [82,83] have found that DHA treatment might alter the EGFR localization in the lipid rafts, thus inhibiting its activation and having an anti-metastasis effect.

Recent investigations have revealed that metastasis and tumor growth is often associated with the upregulation of microRNA (miRNA), small noncoding RNAs pivotal in regulating gene expression post-transcriptionally. Among the pioneering oncogenic miRNAs identified, microRNA-21 (miR-21) has garnered attention for its significant involvement in various cancers [84] such as breast cancer and melanoma metastasis [25,85,86]. Interestingly, n-3 PUFAs have shown to downregulate miR-21 expression in breast and colon cell cancer lines [87,88]. Several mechanisms have been elucidated through which miR-21 can facilitate metastasis in cancer, including its promotion of mesothelial-to-mesenchymal transition [89,90] and upregulation of pro-inflammatory mediators such as TNF-α [88].

The mechanisms underlying how n-3 PUFAs downregulate the expression of miR-21 remain somewhat elusive. Nevertheless, Fluckiger et al. demonstrated that DHA induced nuclear accumulation of Forkhead box O3 (FOXO3) in a colon cancer cell line. FOXO3 was found to bind to the promoter region of miR-21, resulting in its suppression [88].

Regulation of Melanoma Metastasis through n-3 PUFA

The role of n-3 PUFA and melanoma metastasis has been studied. Mannini et al. showed significantly fewer lung colonizations of a highly metastatic murine melanoma cell line in association with enhanced apoptosis and reduced angiogenesis in an n-3 PUFA diet group when compared to the control group [91].

Li et al. demonstrated the anti-metastatic effects of DHA in a murine melanoma cell line. The DHA-treated group exhibited reduced lung colonization, which was associated with the downregulation of the chemokine receptor gene 4 (CXCR4). CXCR4 is known to exert pro-inflammatory, proliferative, angiogenic, metastatic and cell adhesion effects in cancer [89]. It was further elucidated that the primary mediator of this effect was 18-HEPE, an anti-inflammatory lipid metabolite derived from EPA [92].

3.6. Oxidative Stress and n-3 PUFA

Oxidative stress arises from a disruption in the equilibrium between the generation of ROS and the protective mechanisms of the antioxidant defense system. ROS possess the capability to inflict damage upon lipids, nucleic acids and proteins, thereby perturbing their respective functionalities. This phenomenon is implicated in the onset and/or progression of numerous diseases, rendering it a prospective focal point in conditions such as atherosclerosis, chronic obstructive pulmonary disease, Alzheimer's disease, cancer and dermatological disorders, among others. Despite the promising therapeutic potential demonstrated by numerous small molecules acting as antioxidants in preclinical investigations, outcomes from clinical trials have been underwhelming. This discrepancy is primarily attributed to the complexity of pharmacokinetics and the multifaceted pathways through which ROS operate, which remain unaffected by the employment of singular antioxidant agents [93–96].

PUFAs may act as antioxidants by regulating the antioxidant signaling pathway and modulate inflammatory processes. The influence is through the hepatic lipid metabolism [97], physiological responses of other organs such as the heart [98], regulation of platelet homeostasis and lower risk of thrombosis [99,100], modulation of neurodegenerative diseases [101] and skin damage [102,103], among others.

Sakai et al. studied the effect of EPA and DHA on hydrogen peroxide (H_2O_2)-induced DNA damage response in human aortic endothelial cells. Through immunofluorescence staining, they showed that a marker of DNA damage was significantly reduced in the cells treated with EPA and DHA. The H_2O_2-induced activation of a kinase orchestrating the DNA damage response was significantly reduced with EPA and DHA treatment. Both compounds attenuated DNA damage regardless of the DNA damage response. Furthermore, n-3 PUFA reduced intracellular ROS levels under both basal conditions and H_2O_2 stimulation. Additionally, mRNA levels of antioxidant molecules were significantly elevated with EPA and DHA treatment. Silencing of nuclear factor erythroid 2-related factor 2 (Nrf2) notably abolished the increases in mRNA levels of antioxidant molecules and the reduction in intracellular ROS [104].

3.7. Inflammation and Immunomodulation

As previously mentioned, oxylipins formed from the metabolization of n-3 PUFAs interfere with the conversion of AA to inflammatory PGs and LTs [105]. Specifically, DHA is a precursor to the D-series resolvins, protectins, maresin MaR1 and the E-series resolvins.

On the other hand, EPA and DHA are direct precursors of anti-inflammatory 3-series prostanoids (PGE3 and thromboxane A3), 5-series LTs (LTB5, LTC5, LTD5 and LTE5) and the E-series resolvins [106].

In addition, n-3 PUFAs have been shown to reduce inflammation by downregulating NF-κB and NLRP3 pathways. DHA has been shown to prevent NLRP3 and NLRP1b inflammasome activation and inhibit caspase-1 activation and IL-1b maturation against various damage-associated molecular patterns in a dose-dependent manner via G protein-coupled receptors (GPR) 40 and GPR120. The 2013 study by Yan et al. demonstrated that the scaffolding protein β-arrestin-2 (ARRB2) binds to GPR120 and GPR40 and is a major contributor to the inhibition of DHA in NLRP3 or NLRP1. However, the study suggests that there is another mechanism of inhibition independent of ARRB2, as DHA retains some of its anti-inflammatory properties through the same pathway in the absence of ARRB2 [107].

Moreover, EPA and DHA cause immunomodulation by increasing circulating IL-10 and IL-22, diminishing the inflammation in the cancer response [108]. This point has been demonstrated in cells cultured in high concentrations of EPA and DHA in human nonmelanoma skin cells [109].

Referring to other mechanisms, it has been found in prospective cohort epidemiological studies that high intake of n-3 PUFAs decreases the risk of colorectal cancer with a high density of FOXP3+ T cells. Subsequent in vitro investigations have elucidated that DHA exerts a suppressive effect on regulatory T cells, thereby alleviating the inhibition on the proliferation of effector T cells. This immunomodulatory mechanism facilitates the mitigation of certain forms of colorectal cancer, thus indicating a potential avenue for risk reduction through DHA-mediated processes [110]. Although the experiment was performed with colorectal cancer cells, it will be necessary to test whether this mechanism that enhances the antitumor response is shared in other types of cancer.

3.8. Nutrigenomics

Nutrigenomics, the science examining gene–nutrient interactions, facilitates the creation of personalized dietary recommendations to promote optimal health and prevent disease. This science integrates multiple disciplines, including nutrition, bioinformatics, genomics, molecular biology, molecular medicine and epidemiology [111]. Bioactive food components and nutrients influence gene expression. In this sense, PUFAs play a crucial role in modulating gene expression, and have a significant relationship with nutrigenomics such as inflammation, lipid metabolism and cellular growth and they can influence the activity of various transcription factors [47]. Moreover, PUFAs are capable of regulating gene expression by modifying epigenetic mechanisms and consequently result in positive or negative impacts on metabolic outcomes [112]. This can lead to dietary recommendations tailored to individual genetic profiles and nutrigenomic insights by targeting specific genes and pathways influenced by PUFAs.

3.9. Multidrug-Resistant Cancer

The efficacy of chemotherapy is significantly contingent upon the susceptibility of cancerous cells within tumors to drugs, a characteristic that may manifest as either inherent or acquired resistance during the course of treatment. Consequently, the emergence of MDR cancer poses a formidable obstacle in cancer therapy, given its propensity to resist a diverse array of drug compounds and therapeutic interventions [113].

Hence, surmounting drug resistance in cancer assumes paramount importance to enhance treatment outcomes. Numerous investigations have demonstrated the chemosensitizing properties of n-3 PUFAs, especially DHA. Menendez et al. showed that DHA enhanced the cytotoxicity of taxane in a highly metastatic human breast cancer cell line in a dose-dependent manner, a downregulation was also noted of up to 78% of Her-2/neu (known as c-erbB-2) gene and its oncoprotein [114]. DHA was also shown to enhance the cytotoxicity of cisplatin in two human ovarian epithelial cancer cell lines in a dose-dependent manner, along with induction of apoptosis and anti-proliferative effects [115].

A study revealed that DHA increased the tumor sensitivity to docetaxel in human mammary cancer cell lines by downregulating the phosphoinositide 3-kinase (PI3K)/protein kinase B(AKT) and ERK signaling pathways, survival pathways involucrated in cancer cell proliferation [116].

Moreover, Sturlan et al. showed that DHA dramatically increased arsenic trioxide (As_2O_3)-mediated apoptosis in As_2O_3-resistant cells, which was found to be associated with increased production of intracellular ROS and toxic lipid peroxidation products. This was demonstrated as these results were abolished by vitamin E (VitE) treatment, an antioxidant, or when combining As_2O_3 with a non-peroxidizable fatty acid such as oleic acid (OA) [117]. Similar results were exhibited by Lindskog et al., who found that DHA chemosensitized human neuroblastoma cell lines to cisplatin, doxorubicin and irinotecan both in chemosensitive and in MDR neuroblastoma cells, and enhanced the cytotoxicity of As_2O_3 via ROS generation. These results were also significantly decreased when also treated with VitE or OA [118].

Furthermore, Corsetto et al. reviewed the mechanisms underlying the chemosensitizer effects of n-3 PUFAs, highlighting the generation of ROS and lipid peroxidation, as well as increasing drug transport across the cell membrane and intracellular concentration [119]. For instance, a study showed that DHA chemosensitized a breast cancer cell line to anthracyclines, such as doxorubicin, in association with post-transcriptional downregulation of glutathione peroxidase, a protector enzyme against hydrogen and lipid peroxides, suggesting an ROS-enhancing effect of DHA [120].

A prominently identified phenotype in MDR involves the heightened expression of ATP-binding cassette, known as ABC transporters, notably including MDR-related proteins such as P-glycoprotein (Pgp) and breast cancer-resistance protein. These transporters function to confer resistance to cancer cells via the efflux of therapeutic agents, thereby diminishing intracellular drug concentrations [121].

The functionality of many of these transporters has been demonstrated to be substantially reliant on the composition of the cellular membrane as well as its physicochemical properties [122,123]. Interestingly, it has been observed that n-3 PUFAs may chemosensitize MDR cancer cell lines by integrating into the cellular membrane, thereby potentially altering its composition and fluidity. Moreover, these PUFAs have been implicated in enhancing ROS production and lipid peroxidation, further contributing to the sensitization of MDR cancer cells to chemotherapy [119].

For instance, the activity of Pgp has been linked to its localization within lipid rafts and membrane cholesterol [124,125]. Lipid rafts represent lipid-driven subcompartments of the cell membrane comprising sphingolipids, cholesterol and specific proteins. These specialized microdomains are hypothesized to concentrate membrane-associated activities [126].

The study by Gelsomino et al. investigated a human MDR colon cancer cell line and revealed that DHA and EPA were incorporated into the membrane, thereby influencing its composition. This led to a reduction in cholesterol synthesis and membrane integration, consequently resulting in diminished activity of Pgp and its presence in the cell membrane. However, substantial evidence supporting the notion that n-3 PUFAs can modulate endogenous cholesterol synthesis remains elusive, while the role of an ROS-mediated mechanism in the chemosensitizing effects of n-3 PUFAs persists as the primary consideration [127].

4. Interactions of n-3 PUFA with Other Antineoplastic Drugs

The management of melanoma poses considerable challenges owing to the intricate nature of the disease, characterized by the emergence of diverse cancer cell subtypes that demonstrate resistance to conventional chemotherapeutic interventions, exemplified by cisplatin administration. Over time, melanoma cells acquire resistance mechanisms against cisplatin, exacerbating therapeutic complexities. Nevertheless, the incorporation of n-3 PUFAs presents a promising adjuvant therapeutic strategy, potentially reinstating the sensitivity of melanoma cells to cisplatin. Notably, findings from an in vitro investigation revealed a synergistic interaction between DHA and cisplatin, accentuating growth inhibition within melanoma cell lines. In the context of wound healing assays, the combined application of these constituents exhibited a synergistic effect, notably attenuating the invasive potential of melanoma cells. Cisplatin-induced reduction in dual specificity phosphatase 6 (DUSP6) levels elicits heightened p-ERK ½, thereby augmenting the DNA-repair machinery within melanoma cells, thus elucidating the incremental resistance observed in melanoma progression. In contrast, DHA intervenes in this DNA-repair pathway by upregulating DUSP6 phosphatase expression, thereby engendering the synergistic therapeutic outcome delineated previously [128]. Similar outcomes are observed upon the administration of EPA concurrent with 5-fluorouracil (5-FU) treatment, whereby connexin 43 (Cx43) modulation is implicated. Notably, cell viability decreases in EPA-treated cells compared to untreated counterparts, and these cells exhibit enhanced sensitivity to chemotherapy [129].

Furthermore, emerging evidence suggests that n-3 PUFAs may serve as a plausible adjunctive therapeutic modality to potentiate the efficacy of doxorubicin. An in vitro investigation has demonstrated that DHA exhibits a synergistic interplay with doxorubicin in MCF7 cells, augmenting intracellular drug accumulation [130], thereby corroborating the synergistic therapeutic outcome mentioned earlier. Notably, the downregulation of Pgp and Transglutaminase 2 emerges as a putative mechanism underlying the sensitization of drug-resistant cancer cells to chemotherapy [131–133]. Elevated expression levels of these proteins in drug-resistant cancer phenotypes precipitate heightened drug efflux and concomitant attenuation of drug accumulation, hence highlighting their pivotal roles in chemotherapeutic resistance mechanisms [134,135]. However, to understand completely the mechanisms, further studies are needed.

In addition, an n-3 PUFA supplementation has been shown to be effective in reducing kidney and liver doxorubicin-induced damage in mice [136], and has even been observed to be effective in reducing the early doxorubicin-induced cardiac toxicity in children with acute lymphoblastic leukemia [137]. These effects are suspected to be related to the inhibition of NADPH oxidase 4, oxidative stress and apoptosis [138].

Subsequent investigations by other researchers have unveiled a synergistic relationship between DHA and docetaxel. Specifically, isoforms of protein kinase C (PKC), PKCε and PKCδ, have garnered attention in this context. PKCε is known to promote cell proliferation and differentiation, while PKCδ is implicated in apoptotic processes. Notably, both isoforms have been implicated in conferring chemoresistance to docetaxel through activation of the ERK pathway precipitated by the therapeutic intervention [139,140]. A study has reported that administration of docetaxel resulted in elevated expression levels of PKCε and PKCδ within both membrane and nuclear subcellular fractions. Furthermore, supplementation with DHA was observed to mitigate the membrane and nuclear localization of PKCε and PKCδ in cells subjected to docetaxel treatment, thereby culminating in the downregulation of pERK1/2, and therefore sensitized doxacetel in cancer cells [116]. Moreover, in investigations concerning breast cancer, it has been found that supplementation with n-3 PUFAs may ameliorate tumor vasculature abnormalities by diminishing vessel diameter, a phenomenon also observed with docetaxel administration. This effect stems from the independent downregulation induced by both therapeutic agents on epiregulin (EREG), an epithelial growth factor. Furthermore, emerging evidence suggests that the combined administration of n-3 PUFAs and docetaxel may potentiate this modulation of tumor vasculature, as evidenced by the concurrent downregulation of EREG and amphiregulin (AREG), another epithelial growth factor. It is important to underscore that while the individual administration of docetaxel or n-3 PUFAs does not lead to a downregulation of AREG expression, the co-administration of these agents accentuates this effect on tumor vasculature [141]. However, to describe the exact mechanisms by which this synergistic effect on AREG occurs, more studies are needed.

Given the significant issue of drug resistance in melanoma and other cancers, these studies demonstrate that n-3 PUFAs can be a cost-effective and readily available complement to various chemotherapy treatments.

5. Studies and Mechanism about n-3 PUFAs and Melanoma

Melanoma demonstrates a high tendency for metastasis and shows limited responsiveness to traditional treatments. The dysregulation of various chemokine receptor genes and different drug sensitivities and resistances is linked to the progression of melanoma [89,128]. For this reason, researchers have been prompted to develop alternative treatment options for resistant metastatic melanoma.

There are some mechanisms of n-3 PUFA in melanoma that overlap with other types of cancers. However, there are studies demonstrating specific mechanisms of n-3 PUFAs in melanoma, which will be described below. Some of them include changes in the PG

profile; different types of cell death; and interactions with signaling pathways, melanogenesis and downregulation or upregulation of certain proteins.

Both epidemiological and experimental studies indicate that dietary n-3 PUFA inhibits carcinogenesis and tumor growth. Metastatic diffusion has also been found to be affected in animals fed diets containing purified n-3 PUFA or fish oil [91]. While n-3 PUFAs are recognized for their benefits in melanoma prevention, the underlying mechanism of this effect is not completely elucidated and varies depending on the study, as we reviewed previously.

5.1. In Vitro Studies

One of the earliest pieces of evidence regarding n-3 PUFA in melanoma dates back to the beginning of the century, where Albino et al. demonstrated that DHA was associated with decreased retinoblastoma protein (Rb) phosphorylation and cell cycle arrest (Figure 5). In this study, p27 has an inhibitory effect in the progression of the cell cycle which is mediated by CDK inhibition; therefore, the cells cannot progress from the G1 to S phase and apoptosis of melanoma cells occurs [142].

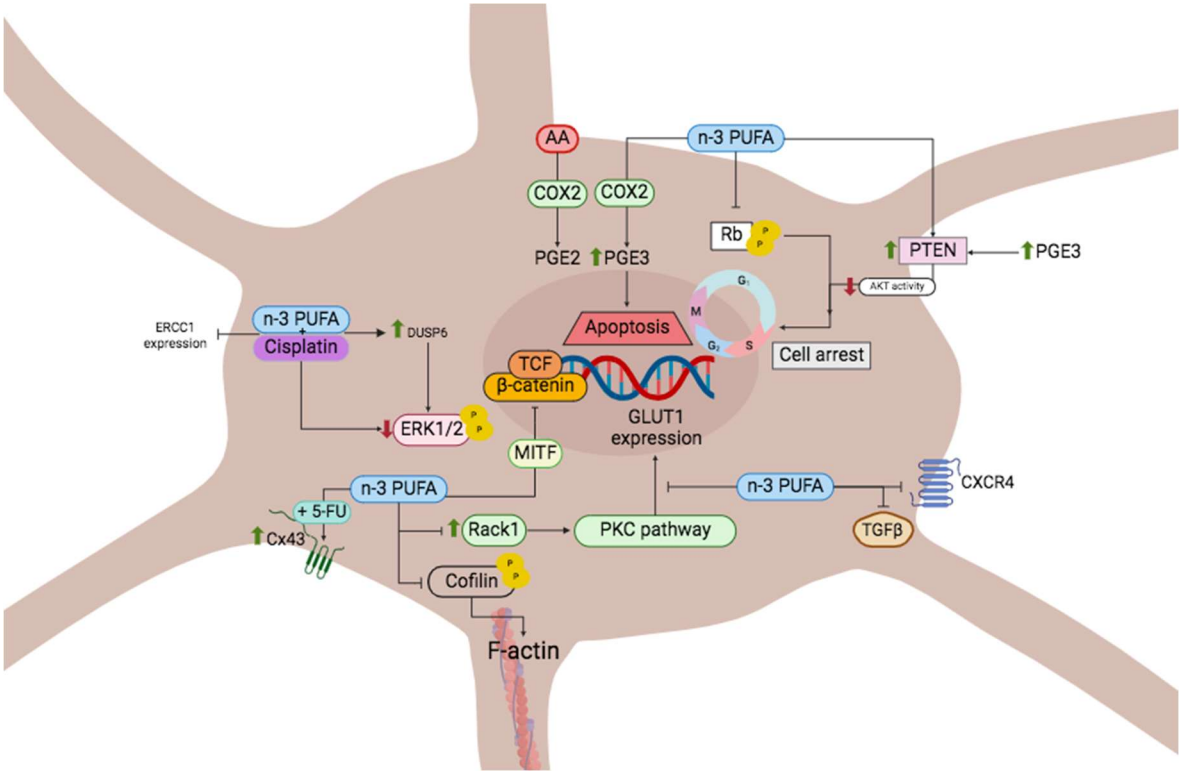

Figure 5. Molecular mechanisms of n-3 PUFAs in melanoma cells. Yellow circle with letter P means phosphorylation; ↓, decrease; ↑, increase; 5-FU, 5-fluorouracil; AA, arachidonic acid; AKT, protein kinase B; COX-2, cyclooxygenase-2; CXCR4, C-X-C chemokine receptor type 4; Cx43, connexin 43; DUSP6, dual specificity phosphatase 6; ERCC1, excision repair cross-complementation group 1 enzyme; ERK1/2, extracellular signal-regulated kinase 1/2; GLUT1, glucose transporter 1; MITF, melanocyte inducing transcription factor; n-3 PUFA, n-3 polyunsaturated fatty acids; PGE-2, prostaglandin E2; PGE-3, prostaglandin E3; PKC, protein kinase C; PTEN, phosphatase and tensin homolog; Rack1, receptor for activated C kinase 1; Rb, retinoblastoma protein; TCF, T-cell factor; TGF-β, transforming growth factor beta.

In most studies, in vitro models with various cell cultures are proposed. In the case of in vivo models, the n-3 fatty acid desaturase (Fat-1) transgenic mouse model is more commonly used, which can produce endogenous n-3 PUFA from n-6 PUFA in all tissues. In this section, we summarize the main studies regarding n-3 PUFA and melanoma, emphasizing some of the mechanisms proposed above.

Regarding melanoma resistance to drugs such as cisplatin, Ottes et al. considered the use of new combinatorial strategies with n-3 PUFA, due to its antineoplastic properties, as an enhancing therapy for the treatment of this cancer [128].

This study demonstrated that DHA and EPA sensitize the cells to the cisplatin-induced inhibition of cell growth and migration by reverting cisplatin effects on DNA damage and protein excision repair cross-complementation group 1 (ERCC1) expression [128] (Figure 4). ERCC1, a crucial element in DNA repair vital for the repair of DNA adducts, has recently garnered significant attention in the context of this cancer. This is attributed to the fact that elevated expression of DNA repair genes plays a role in the heightened resistance exhibited by melanoma towards traditional DNA-damaging chemotherapeutic agents [143]. DHA and EPA also reverted the DUSP6 and pERK expressions, which regulate ERCC1 activation upward (Figure 5). In line with this, DUSP6 gene silencing prevented the effect of DHA, confirming that DHA acted on the DUSP6/p-ERK/ERCC1 repair pathways to sensitize melanoma cells to the anticancer effect of cisplatin [128] (Table 1).

Inflammation stands as a crucial hallmark of cancer, capable of initiating and sustaining tumor development. The carcinogenic effects of COX-2 are supported by studies that show a growth-inhibitory effect in melanoma cells treated with COX-2 inhibitors [144,145]. Indeed, growing evidence supports the notion that the constant expression of COX-2 plays a pivotal role in the progression of malignant epithelial tumors, including melanoma. This is evident from the positive COX-2 status observed in oral and cutaneous melanomas, contrasting with the negative status found in all oral and cutaneous melanocytic nevi [146]. COX-2 expression in malignant melanoma is linked to the regulation of various factors, including tumor invasion, number of mitoses, tumor thickness, melanoma subtype, lymph node involvement and metastases [147]. Studies indicate that the overexpression of PGE2 is associated with skin cancer and correlates with a poor prognosis. On the other hand, PGE3 has been found to have anticancer effects (Figure 4). Denkis et al. and Serini et al. demonstrated that n-3 PUFA regulate COX-2 mRNA expression and PGE2 production in brain metastatic melanoma. Notably, COX-2 mRNA expression decreased in cells incubated with EPA or DHA [73,148]. In contrast, n-6 PUFA increased cell invasion, whereas EPA or DHA did not, highlighting the potential of n-3 PUFA over other PUFAs. These findings demonstrate that incubation with EPA or DHA led to the downregulation of both COX-2 mRNA and protein expression, resulting in a subsequent decrease in invasion in brain-metastatic melanoma [73]. Chiu and Ooi studied the cytostatic and cytotoxic effects of different non-steroidal anti-inflammatory drugs (NSAIDs) in human skin melanoma A-375 cells, such as celecoxib, indomethacin, aspirin and piroxicam. The study revealed that both celecoxib and indomethacin demonstrated inhibitory properties against cell proliferation, with additive effects observed when cells were co-incubated with DHA. Nonetheless, the anticancer mechanisms of NSAIDs cannot be solely attributed to their COX-inhibitory activities, as only celecoxib and indomethacin exhibited cytostatic or cytotoxic effects on the cancer cells. However, aspirin enhanced the DHA-induced growth inhibition as well as piroxicam [149] (Table 1).

DHA increases the expression of the microphthalmia transcription factor (MITF), which prevents β-catenin/TCF from activating metalloproteinase genes such as membrane type 1-MMP, MMP-2 and MMP-13 (Table 1 and Figure 5). Another study reported that DHA not only hindered cell growth and prompted differentiation of melanoma cells in vitro but also demonstrated an anti-neoplastic role associated with the presence of MITF, an oncogene that is involved in melanoma and other malignancies and plays a major role in melanocytic differentiation and survival/proliferation, as well as in oncogenesis [150,151]. This MITF presence interfered with the transcription of pro-invasive target genes. Furthermore, the study revealed that DHA exhibited inhibitory effects on anchorage-independent cell growth, diminished migration and invasion in vitro, and led to the downregulation of various MMPs such as MMP-2, MT1-MMP, MMP-

9 and MMP-13, which are known to play a role in melanoma invasion. These MMPs are considered promising molecules for the management of melanoma patients due to their roles as biomarkers and therapeutic targets [150,152]. The role of β-catenin in the anti-cancer effect of n-3 PUFAs is complex and different between the types of cancer. The abnormal Wnt/β-catenin signaling pathway plays a pivotal role in tumor formation and response to therapy by promoting the renewal, proliferation and differentiation of cancer stem cells as well as advancement of malignant progression [153,154]. In the case of melanoma, these effects are related to heightened expression of MITF. DHA also reduces the expression of total β-catenin in metastatic melanoma cell line as well as decreases in cytosolic β-catenin and increases its translocation to the nuclei [148,150]. Serini et at. also showed that DHA and EPA induce apoptosis in melanoma cells through the caspase-3 pathway by increasing Bax proteins and decreasing Bcl-2 proteins, therefore augmenting the Bax/Bcl-2 ratio. In this study, a pro-apoptotic effect was observed for DHA and EPA and not observed with the other fatty oleic acid, suggesting that the pro-apoptotic effect was specific for n-3 PUFAs [148]. These effects on Bcl-2 family proteins have been documented in prior investigations, such as in vitro studies of colorectal cancer [155,156] (Table 1).

Oxidative stress plays a pivotal role across all stages of melanoma progression and is intricately involved in both melanin synthesis and the formation of melanoma. Melanoma exhibits heightened susceptibility to oxidative stress, attributed to the interplay of melanin synthesis and UV radiation in the generation of ROS. The impact of oxidative stress extends to melanoma immunity, the metastatic potential of melanoma cells, and their resistance to therapeutic interventions. Within malignant melanocytes, melanogenesis is frequently upregulated, offering potential therapeutic targets. Paradoxically, this process both promotes tumor initiation and impedes vertical growth and metastasis in later stages of the disease. Despite advancements in treatment modalities, such as targeted and immune therapies, the emergence of drug resistance poses a persistent challenge. Redox biology, encompassing ROS and reactive nitrogen species, assumes a central role in the entire spectrum of melanoma pathophysiology, ranging from initiation to progression and metastatic cell development. Redox metabolic rewiring has been implicated in acquired resistance to BRAF/MEK inhibitors. The consequences of this redox rebalance appear to be dual-faceted: on the one hand, cells may exhibit less aggressive behavior or undergo apoptosis; on the other hand, they may demonstrate enhanced survival upon dissemination into the circulating system or after exposure to drug treatment, thereby facilitating metastasis promotion or fostering additional drug resistance [157–159]. Furthermore, Nrf2 has been acknowledged as a pivotal regulator orchestrating cellular responses to oxidative fluctuations. Upon activation, this signaling cascade induces the expression of a diverse array of antioxidant enzymes. Conversely, Nrf2 has been implicated in a spectrum of activities, and sustained activation of Nrf2 in malignant cells may expedite processes such as metastasis and chemoresistance. This duality suggests that Nrf2 may assume distinct roles, either acting to prevent or promote cancer, adding complexity to its potential therapeutic implications and consequently there has been a growing interest in exploring this transcription factor as a potential target for cancer treatment [160]. However, to date, there are no studies that account for this modulation by PUFAs and Nrf2 in melanoma.

An in vitro investigation revealed a reduction in the proliferation of melanoma cells when exposed to varying concentrations of EPA and DHA (50 mM and 100 mM) across A375, A2058 and G361 human melanoma cell lines. This reduction was concomitant with observed oxidative damage to both protein and DNA [59] (Table 1).

5.2. In Vivo Studies

Nevertheless, in spite of the increasing evidence supporting the benefits of n-3 PUFA, experimental findings suggest that the ability of n-3 PUFAs to inhibit cancer growth is contingent not only on their quantity but also on the baseline levels of n-6 PUFAs. Additionally, research has demonstrated that the therapeutic advantage of dietary n-3 PUFAs is most pronounced when their proportion significantly surpasses that of n-6 PUFAs.

Studies showed that endogenously increased levels of n-3 PUFAs in the tumor tissues of Fat-1 transgenic mice was associated with a reduction in the growth rate of melanoma [161,162]. In these studies, the researchers implant melanoma cells with a higher metastatic potential in the Fat-1 and wild type (WT) mice and examine different outcomes. Xia et al. demonstrated reduction of melanoma formation and growth in Fat-1 transgenic mice. The level of n-3 PUFA and their metabolite prostaglandin PGE3 were much higher (but the n-6/n-3 ratio is much lower) in the tumor and surrounding tissues of Fat-1 mice than of WT animals and the amounts of PGE2 in the tumor and surrounding tissues of Fat-1 transgenic mice are lower than those of WT mice. Moreover, in this study the phosphatase and tensin (PTEN) homologue deleted on chromosome 10 was upregulated in the Fat-1 mice. PTEN is a classical tumor suppressor gene through its action as a negative regulator of the PI3K signaling pathway and plays a role in the homeostatic maintenance of the PI3K/AKT cascade by decreasing downstream AKT activity and cell cycle progression is arrested at the G1/S phase [163] (Figure 5). In in vitro experiments, it was observed that the introduction of n-3 PUFA or PGE3 suppressed the proliferation of melanoma cells and heightened the expression of PTEN (Figure 5). This effect was found to be partially diminished when the production of PGE3 was inhibited. These findings suggest an anti-melanoma impact of n-3 PUFA, potentially attributed to the activation of the PTEN pathway facilitated by PGE3. In this study, the incidence of tumor formation and growth rate was much slower in the Fat-1 transgenic mice when compared to the WT mice, showing that the modification of the tissue n-6/n-3 PUFA ratio by increasing n-3 PUFA and decreasing n-6 PUFA could potentially serve as an efficacious therapeutic strategy, as it has the potential to not only decrease the cancer-promoting eicosanoids derived from AA but also enhance the production of anti-tumor eicosanoids derived from n-3 PUFA, resulting in a dual effect [161]. Yin et al. reported comparable findings, revealing that the naturally elevated levels of n-3 PUFAs in tumor tissues of transgenic Fat-1 mice were linked to a decrease in the growth rate of melanoma xenografts. Moreover, they also showed that the protein expression levels of E-cadherin and N-cadherin were markedly upregulated and downregulated in the tumor tissues of at-1 mice compared with those of the WT controls, respectively. On the other hand, the levels of NF-κB and phosphorylated NF-κB protein expression levels were lower in Fat-1 mouse tumor tissues than those from WT mice as well as signal transducer and activator of transcription 3 levels, suggesting that both may be involving in modulating E-cadherin expression. Furthermore, Fat-1 tumor tissues exhibited a marked decrease in the expression levels of β-catenin and c-Myc, a known transcriptional target of β-catenin, compared with those from WT mice, and the study reveals that the EGFR/AKT/GSK-3β signaling pathway may be involved in controlling endogenous n-3 PUFA-induced β-catenin degradation and also shows that the tumor growth inhibition was not related to n-3 PUFA-induced oxidative stress after VitE administration. However, Fat-1 mice exhibited decreased tumor growth compared with WT-mice, suggesting that VitE enhances the antitumor effects of n-3 PUFAs [162] (Table 1).

There is also dysregulation of the chemokine receptor genes that are associated with melanoma progression such as CXCR4, which is expressed in a variety of immune cells and cancer tissues, producing ulceration, tumor thickness and lymph node metastasis and enhancing the progression of melanoma [164]. Li et al. conducted an in vivo experiment with Fat-1 transgenic mice and showed an inverse correlation between lung tissue n-3 PUFA levels with the frequency of melanoma pulmonary metastasis. The Fat-1 group had

fewer pulmonary tumor foci and smaller metastatic foci volume in both early and late stages of metastasis and the lung tissue levels of n-3 PUFAs, including EPA, DPA and DHA, were higher in Fat-1 mice than in WT mice. Moreover, pulmonary myeloperoxidase activity was higher in WT mice than in Fat-1 mice and serum levels of immune cell-expressed inflammatory factors with roles in tumor metastasis, such as IL-17 and TNF-α, were lower in Fat-1 mice than in WT mice as well as metastasis-related factors, including CXCR4 and transforming growth factor-β, suggesting that n-3 PUFAs that inhibit melanoma pulmonary metastasis involve suppression of pulmonary and systemic inflammation. It can be highlighted that n-6 and n-3 PUFAs exhibit differential effects on CXCR4 expression. They showed that levels of EPA and its derivatives were significantly higher in the serum of Fat-1 mice than in WT mice with a lower ratio of n-5/n-3 PUFAs in Fat-1 mice compared with WT mice and the cells either with EPA or 18-HEPE suppress CXCR4 expression although AA elicits the opposite. In synthesis, n-3 PUFAs can decrease lung metastasis of melanoma by increasing the production of 18-HEPE, which can inhibit CXCR4 expression and inflammatory response level [89] (Table 1 and Figure 5).

In vivo studies employing non-Fat-1 transgenic mice have also been conducted, yielding outcomes comparable to those previously described. Tan et al. showed that mice subjected to algal oil treatment, particularly rich in DHA, exhibited suppressed pulmonary metastases and inhibited outgrowth of melanoma cells. This effect was associated with the induction of autophagy, as evidenced by an elevation in LC3-II levels (Figure 3). The mechanism involved the modulation of mammalian target of rapamycin (mTOR) and p38 mitogen-activated protein kinase (MAPK), along with the activation of JNKs, resulting in reduced p62 accumulation and diminished secretion of the proinflammatory cytokine IL-1β [65] (Table 1), which are also related with oxidative stress pathways [165]. These outcomes suggest that the antitumorigenic activities of algal oil are mediated through autophagy-driven elimination of p62 and possess anti-inflammatory properties [65] (Figure 4).

Studies demonstrate that in tumor cells there are reduced expressions of Cx43 which are important for enhancing chemosensitivity that is suppressed in a tumor microenvironment [129,166,167]. Yang et al. carried out a study where B16-F10 murine melanoma cells were used. These cells were pretreated with various inhibitors for 1 h and then EPA was added. EPA-treated, non-treated or transfected cells were exposed to 5-FU. Moreover, they used mice that were inoculated with B16-F10 cells. Groups of tumor-bearing mice were orally administrated with EPA followed by 5-FU treatment, or with either treatment alone. Exposing cells to escalating concentrations of EPA led to a dose-dependent rise in Cx43 levels compared to controls. The observed correlation between the expression of Cx43 induced by EPA in melanoma cells and the degrees of gap junction intercellular communication implies that EPA may trigger Cx43 expression, potentially enhancing Cx43 functionality in intercellular communication through gap junctions (Figure 5). Moreover, the potential molecular mechanisms in EPA-induced Cx43 expression through the MAPK signaling pathways are described. In this study, the administration of EPA resulted in elevated phosphorylation levels of JNK and p38, while no observable phosphorylation of ERK was detected, in accordance with studies described above. In the presence of 5-FU treatment, the cell viability in EPA-treated cells exhibited a significant decrease compared to untreated cells. However, when the expression of Cx43 was suppressed, the combination therapy did not induce additional cytotoxic activity. These findings imply that Cx43 plays a crucial role in the susceptibility of cells to 5-FU (Figure 4). Likewise, the combined treatment of EPA and 5-FU effectively suppressed tumor growth, prolonged the survival of mice with tumors, and resulted in an elevated number of apoptotic cells in the EPA-treated tumors, as observed in sections from B16-F10-bearing mice [129] (Table 1).

Different research demonstrates that n-3 PUFA can act as agonists of GPR, namely, GPR40/free fatty acid receptor (FFA) 1 and GPR120/FFA4, which is a promising target in several pathologies such as obesity, type 2 diabetes, inflammation-related diseases and

cardiovascular diseases, among others [18,168,169]. Particularly in melanoma, Nehra et al. investigated whether the G protein-coupled receptors such as GPR40, which is expressed at high levels in several human melanoma cell lines, are a potential therapeutic target in melanoma. They used human melanoma and control fibroblast cells for in vitro studies, which were treated with DHA and TAK-875 and GW9508 (selective GPR40 agonist and non-selective GPR agonist that stimulate GPR120 and GPR40, respectively). A murine subcutaneous xenograft model of human melanoma was employed to assess the impact of dietary intervention with an n-3 PUFA-enriched diet in comparison to an n-6 PUFA-enriched diet on the progression of human melanoma in vivo. Additionally, a comparable animal model was utilized to evaluate the efficacy of oral TAK-875 in inhibiting the growth of established melanoma tumors in vivo. Their findings revealed that DHA exhibits inhibitory effects on the proliferation of human melanoma cells both in vitro and in vivo. Tumors derived from subjects receiving the n-3 PUFA-rich diet exhibited a reduction in weight by 69% and in volume by 76% compared to those from subjects on the n-6 PUFA-rich diet. Furthermore, TAK-875 demonstrated inhibitory effects on human melanoma cell growth both in vitro and in vivo. Specifically, tumors from subjects treated with TAK-875 displayed reductions in weight by 46% and in volume by 62% and exhibited a 77% slower growth rate compared to the control group [170] (Table 1).

Another investigation revealed contrasting effects of fatty acids on melanogenesis in melanoma cells, indicating that saturated fatty acids augment melanogenesis, whereas PUFAs exert a suppressive influence. Specifically, EPA not only diminishes tumor growth but also mitigates F-actin levels by attenuating the phosphorylation of cofilin (Figure 5). This stands in stark contrast to the action of palmitic acid, which induces the formation of F-actin stress fibers through the activation of the RhoA protein [171] (Table 1). This study elucidated a direct interaction between DHA and Receptor for Activated C Kinase 1 (Rack1), prompting the activation of PKC and subsequent initiation of oncogenic signaling pathways (Figure 4). Rack1 is a conserved eukaryotic protein renowned for its multifaceted biological roles in humans, including the facilitation of cyclin binding activities. Notably, the upregulation of Rack1 has been associated with lymph node metastasis in cervical cancer, while PKC-mediated signaling pathways have been implicated across a spectrum of cancer types [172].

In melanoma cells cultured with DHA and PMA (utilized to activate PKC signaling), a decline in the antitumor effect of DHA was observed. Moreover, it is well established that PKC has been documented to upregulate the expression of glucose transporters such as GLUT-1 and GLUT-4 [173]. Indeed, Yamada et. al. observed that the gene expression of GLUT-1 was suppressed by EPA and DHA [171] (Figure 5).

Additional in vitro and in vivo studies can be referenced within Table 1 for further examination.

5.3. Discrepancy in Results and Approaches to Studies in Humans

On the other hand, other authors found contradictory results. Salem et al. showed that EPA treatment increased both the growth and metastasis of B16 melanoma cells by suppressing the cytolytic function of both T cells and macrophages [174]. Moreover, in human studies it is challenging to standardize the levels of n-3 PUFA intake based on the amounts and frequency of fish ingested and this may be an explanation for the conflicting results in human observational studies [175–177] (Table 1).

Despite contradictory results, this could be attributed to discrepancies in the doses of n-3 PUFA and in the different experimental protocols used in the respective studies, which renders these findings not yet successful in clinical practice. However, various clinical trials have demonstrated that the combination of DHA with other solid cancer chemotherapeutics such as paclitaxel is well tolerated by patients [178–180], justifying further studies about melanoma resistance to treatment and lipidomics due to the recent advances in the scientific field.

Another challenge is the scarcity of human studies on supplementation with n-3 PUFA in melanoma and other cancers. Over the past decades, there has been a shift in dietary patterns in the current human population compared to our ancestors, and modulating the n-6/n-3 ratio has been considered an intriguing point in the research field across various pathologies [181–184]. However, a randomized controlled trial showed that supplementation with n-3 PUFA did not result in a lower incidence of major cardiovascular events or cancer than placebo [185]. In contrast, another clinical trial demonstrated that dietary supplementation with n-3 PUFA led to a clinically important and statistically significant benefit about cardiovascular death [186] (Table 1).

Table 1. Main findings from different studies with n-3 PUFA in melanoma. 18-HEPE, 18-hydroxy eicosapentaenoic acid; 5-FU, 5-fluorouracil; AA, Arachidonic acid; c-Myc, MYC proto-oncogene; CDDP, Cisplatin; COX-2, Cyclooxygenase 2; Cx, connexin; CXCR, Chemokine receptor; DHA, Docosahexaenoic acid; DUSP6, Dual specificity phosphatase 6; EPA, Eicosapentaenoic acid; ERCC1, Excision repair cross-complementation group 1; Fat-1, n-3 fatty acid desaturase; IL, interleukin; JNK, c-Jun N-terminal kinases; MAPK, mitogen-activated protein kinase; MDA, Malonaldehyde; MITF, Microphthalmia-associated transcription factor; MMP, Matrix metalloproteinase; MPO, Myeloperoxidase; MT1-MMP, Membrane-type I matrix metalloproteinase; mTOR, Mammalian target of rapamycin; NF-κB, Nuclear factor kappa-light-chain-enhancer of activated B cells; NSAIDs, Non-steroidal anti-inflammatory drugs; LC3, Microtubule-associated protein 1 light chain 3; PA, Palmitic acid; PCB, Polychlorinated biphenyls; pERK, Phosphorylated extracellular signal-regulated kinases; PGE, prostaglandin; PKA, protein kinase A; PKC, Protein kinase C; PTEN, Phosphatase and tensin homolog; PUFA, Polyunsaturated fatty acids; Rab, Ras-associated binding; STAT3, Signal transducer and activator of transcription 3; TGFβ, Transforming growth factor beta; TNF-α, tumor necrosis factor alpha; WT, Wild type.

Model	Dose	Results	Reference
In vitro (metastatic B16F10 murine melanoma cells and metastatic WM266-4 human melanoma cells)	CDDP (1, 2.5, and 5 μmol/l), DHA or EPA (10–30 μmol/l) alone or in combination at different time points (24–72 h)	- Growth-inhibitory effect was enhanced with DHA + CDDP - Inhibit melanoma cell migration was enhanced with DHA + CDDP - Both DHA and EPA + CDDP revert ERCC1 expression. - Reduction of pERK1/2 was observed in both cell lines in a DHA dose-dependent manner - DHA or EPA, given alone significantly increased DUSP6 expression with respect to control cells	[128]
In vitro (70 W human melanoma cell line that metastasizes to the brain in nude mice)	50 μM each for 24 h of AA, EPA or DHA	- TNF-α upregulates the expression of both COX-2 mRNA and PGE2 - PGE3 incubation significantly decreased invasive values - DHA and EPA decreased COX-2 mRNA expression - EPA or DHA decreased the cell invasion, whereas AA caused invasion to increase by 2.4 times - EPA and DHA downregulated both COX-2 mRNA and protein expression, with a subsequent decrease in invasion.	[73]
In vitro (WM115 and WM266-4 human melanoma, and B16-	10–30 μM of DHA at 24 and 48 h	- Significantly suppressed the anchorage independent colony growth	[150]

F10 murine melanoma cell lines)		- Inhibits melanoma cell growth, migration and invasion - Inhibits the expression of several matrix metalloproteinases (MMP-2, MMP-13, and MT1-MMP) - These effects are related to the β-catenin increased nuclear expression and PKA-dependent phosphorylation, as well as to the increased expression of MITF.	
In vitro (A375, A2058, and G361 human melanoma cells)	AA, EPA and DHA (50 mM and 100 mM)	- Decrease of proliferation, associated by oxidative protein and DNA damage was observed for EPA and DHA (50 mM and 100 mM) in A375, A2058, and G361 cells - In C32 (amelanotic melanoma), EPA and DHA inhibited cell proliferation at 100 mM only and the effect of DHA was more pronounced, whereas AA did not show antiproliferative action in this cell line.	[59]
In vitro (human skin melanoma A-375 cells)	NSAIDs, at doses 10–480 μM, was incubated simultaneously with the melanoma cells and 160 μM of DHA for 72 h	- 50 and 100 μM of celecoxib reduced proliferation of the melanoma cells at 72-h incubations by 34% and 82.7%, respectively. - Indomethacin inhibited the cell proliferation by about 40% at 240 and 480 μM - Aspirin and piroxicam do not exhibited cytostatic or cytotoxic effect on the cancer cells. - Both celecoxib and indomethacin, starting from 20 μM, exhibited additive effects on the DHA-induced growth inhibition. - Aspirin enhanced the DHA-induced growth inhibition by 42.8% at 480 μM as well as piroxicam by 15.9–66.4% at 40–240 μM.	[149]
In vitro (Primary WM115 and WM266-4 metastatic melanoma cell line)	10–30 μM DHA, EPA, oleic acid or linoleic acid for 72 h	- The constitutive expression and mRNA-expression of COX-2 protein was inhibited in a dose-dependent manner by 10–30 μM DHA - Degradation of COX-2 mRNA was accelerated by the presence of 30 μM DHA in cells whose mRNA transcription was inhibited by 10 l g/mL actinomycin D. - DHA reduced the expression of cytosolic HuR (stabilizer of COX-2 mRNA) in a concentration- and time-dependent manner	[148]

		- DHA and EPA inhibited the growth and apoptosis of both the cell lines and the effect was concentration dependent - DHA reduced the expression of total β-catenin in both the cell lines studied - DHA and EPA induce apoptosis in melanoma cells through caspase-3 pathway by increasing Bax proteins and decreasing Bcl-2 proteins, therefore, augmenting the Bax/Bcl-2 ratio. - The pro-apoptotic effect was observed for DHA and EPA and not observed with the other fatty acids oleic acid.	
In vitro (B16-F10 mouse melanoma cells)	DHA (1–25 μmol/L) for 3 days	- Inhibits melanogenesis in cells through increasing tyrosinase degradation.	[187]
In vitro (Melanoma cell lines [B16F10, Colo679, G361, HOMM, and HTMM])	Cells were cultured with 50 μM of PA or EPA on culture for 24 h or 25 μM of DHA for 24 h	- EPA suppresses membrane-associated Tyr and Tyrp1 - PA induces formation of F-actin, while EPA decreases F-actin levels in melanoma cells - EPA and DHA suppressed gene expression of Glut-1 - PA increases and EPA decreases the level of Rab27a in B16F10 cells - DHA delayed the cell cycle of melanoma - DHA interacts with the receptor for activated C kinase 1, leading to the repression of melanoma cell proliferation through the inhibition of PKC signaling.	[171]
In vivo (Fat-1 C57BL/6 transgenic mice with implantation of B16 melanoma cells)	50 μM AA, 50 μM AA plus 50 μM indomethacin, 50 μM EPA, and 50 μM EPA plus 50 μM indomethacin	- Inhibition of melanoma formation and growth in Fat-1 transgenic mice - Upregulation of PTEN in the tumor and surrounding tissues of Fat-1 mice - AA (50 μM) did not affect cell growth - EPA (50 μM) exhibited an inhibitory effect on the growth of melanoma cells. This growth-inhibitory effect could be blocked by the presence of 50 μM indomethacin	[161]
In vivo (Fat-1 C57BL/6 transgenic mice with implantation of B16-F10 melanoma cells)	Their diets (per 100 g) consisted of 4.5 g sucrose, 18.6 g casein, 8.6 g cellulose, 50 g wheat starch, 0.3 g DL-methionine, 7 g mineral mix, 1 g vitamin mix and 10 g safflower oil. A group underwent 100 IU/kg VitE via oral gavage for 3 weeks treatment	- No significant difference in the levels of PGE2 - The protein expression levels of E-cadherin was upregulated and N-cadherin were downregulated in the tumor tissues of fat-1 mice - Fat-1 mouse tumor tissues exhibited a decrease in NF-κB and STAT3 than those from WT mice	[162]

		- After VitE supplementation, no significant difference in the levels of MDA was observed between fat-1 and WT mice, however, fat-1 mice exhibited decreased tumor growth compared with WT-mice - Fat-1 tumor tissues exhibited a marked decrease in the expression levels of β-catenin and c-Myc compared with those from WT mice	
In vivo (Fat-1 C57BL/6 transgenic mice with implantation of B16-F0 melanoma cells)	400 pg/mL 18-HEPE (a n-3 EPA-derived lipid mediator) in cells for 48 h and then 100 pg and 1 μg of 18-HEPE to each mouse through i.v. and intraperitoneal (i.p.) injection every other day, respectively.	- Pulmonary metastasis of melanoma B16-F0 cells is significantly reduced in Fat-1 transgenic mice - 18-HEPE directly repressed CXCR4 expression of B16-F0 cells - Higher lung TGFβ expression in WT mice than Fat-1 mice - Pulmonary MPO activity was higher in WT mice than in Fat-1 mice as well as inflammatory cytokines	[89]
In vivo (C57BL/6 mice with implantation of B16-F10 melanoma cells)	Daily intragastrically administered with 50 μL algal oil for four weeks prior to tumor cell injection. The control group were daily intragastrically administered with equivoluminal safflower oil (deficient in n-3 PUFAs but high in n-6 PUFA)	- Algal oil treatment inhibited lung metastases of B16-F10 melanoma cells - Algal oil treatment produced significant increases in n-3 PUFA levels (EPA, DPA and DHA) and the corresponding decreases in AA in lung tumor tissues - The endogenous n-6/n-3 PUFA ratio in lung tumor tissues was significantly lower - Algal oil treatment induced autophagy (measured by ↑ LC3-II protein and ↓ p62) through inactivation of mTOR and p38 MAPK and activation of JNK MAPK in lung tumor tissues from the mice treated with algal oil - Significant decrease in cleaved IL-1β and slight decrease in pro-IL-1β proteins levels in lung tumor tissues from the algal oil-treated mice.	[65]
In vitro/In vivo (B16-F10 murine melanoma cells & C57BL/6 mice with B16-F10 incoulation)	Cells with EPA (0–100 μM) for 24 h and then EPA-treated, non-treated, or transfected cells were exposed to 0–40 μM of 5-FU under for 48 h. Groups of tumor-bearing mice were orally administrated with	- Treatment of cells with 0, 50, 100 μM of EPA induced a dose-dependent increase in Cx43 levels compared to controls. - The levels of intercellular communication through gap junctions exhibited a correlation with the increased expression of Cx43 induced by EPA in melanoma cells.	[129]

	EPA (25 mg/kg) at day 7 followed by 5-FU (40 mg/kg) treatment on days 9, 11, and 13, or with either treatment alone.	- EPA increased the susceptibility of cells to 5-FU. - EPA in combination with 5-FU enhanced the antitumor activity	
In vitro/In vivo (human melanoma cells [A2058, A375, SK-Mel 3]/murine subcutaneous xenograft model of human melanoma)	Cells with DHA at 25–250 μM for 144 h, GW9508 at 25–150 μM and TAK-875 at 0–0.4 μM for 72 h. Animal study group were undergo to a 20:1 ratio of DHA:AA diet for 3 weeks prior to tumor cell inoculation and continued throughout the study period. Animals with established tumors underwent with once daily oral TAK-875 at 100 mg/kg.	- DHA had a profound, selective inhibitory effect on the growth of all human melanoma cell lines - GW9508 and TAK-875 had a profound inhibitory effect on the growth of all human melanoma cell lines - Growth of human melanoma in vivo is inhibited by a diet rich in DHA. - Tumors were smaller in weight and smaller in volume in the TAK-875 animal group	[170]
In vivo (C57Bl/6 mice with implantation of a highly metastatic F10-SR melanoma cells)	5% maize oil diet for 1 week, and then was switched to the 5% fish oil diet for 5 weeks.	- Cells reproduced a lower number of metastatic colonies in the lungs of mice fed with fish oil diet - Reduced lung colonization of melanoma cells in animals fed a fish oil diet is associated with an increased apoptotic activity - There was a reduction of von Willebrand factor immunoreactivity in pulmonary colonies of cells grown in fish oil-fed animals which indicates a decrease of angiogenesis effects.	[91]
Human (Observational study)	Not applicable	- No association between the consumption of PUFAs and melanoma risk - Only VitE from food and zinc from food and supplements were found to be associated with melanoma	[177]
Human (Observational study)	Not applicable	- EPA-DHA intake showed to have a substantial protective association between dietary PCB exposure and risk of melanoma	[176]
Human (Observational study)	Not applicable	- Patients who have high consumption of meat, fish, and fats, leading to relatively elevated levels of omega-3 and omega-6 fatty acids, are at a higher risk of being diagnosed with thick melanomas rather than thin ones.	[175]
Human Phase 1 Clinical Trial (DHA-paclitaxel in resistant solid tumor malignancies)	DHA-paclitaxel 200 mg/m^2, dose increased from 100 mg/m^2 per cohort to 600	- Administration of DHA-paclitaxel for 3 weeks is well tolerated, providing consistent prodrug exposure and its	[178]

	mg/m² (200–300–400–500–600 mg/m²), weekly infusion at i.v. of 2 h.	metabolite exposure, while minimizing the dose-limiting side effects of the drug.	

5.4. Safety and Side Effects of n-3 PUFA

Intakes of up to 1 g/day of n-3 PUFA from dietary fish are generally regarded as having very low risk. However, higher intakes can increase the risk of side effects. High intakes of n-3 PUFA can lead to gastrointestinal upset, increased blood glucose levels and elevated concentrations of low-density lipoprotein (LDL) cholesterol. Additionally, n-3 PUFA can decrease the synthesis of the eicosanoid thromboxane A_2, thereby increasing the risk of bleeding [188]. However, this was not generally observed in a randomized clinical trial of fish oil supplementation [189].

The US Food and Drug Administration has confirmed the general safety of fish oil containing DHA and EPA, deeming them generally recognized as safe at levels up to 3 g/day (substances affirmed as generally recognized as safe: menhaden oil). Regarding DHA, the toxicological profile of DHA from single-cell organisms indicates that it is safe in rats up to a consumption of 3290 mg/kg/day. DHA supplementation studies in adults have employed doses ranging from less than 1 to 7.5 g/day without resulting in any consistent side effects [190].

6. Future Directions

The future directions for the implementation of dietary n-3 PUFAs in the treatment of various diseases, particularly cancer and melanoma, are promising and multifaceted. Currently, they constitute ingredients in functional food development. However, further research is needed to elucidate the precise molecular mechanisms through which PUFAs exert their therapeutic effects, including their role in modulating inflammation, gene expression, cell signaling pathways, lipomics and apoptosis. Investigating how PUFAs interact with other cancer treatments such as chemotherapy, immunotherapy and radiotherapy could enhance their effectiveness and reduce side effects. Moreover, nowadays the majority of the results are based on in vitro and in vivo models. Indeed, long-term, large-scale clinical trials are necessary to confirm the benefits of PUFAs in cancer prevention and treatment and establish optimal dosages, treatment durations and the most effective forms of PUFAs.

Currently, medicine is changing its approach to treating people due to scientific and biotechnological advances. Personalized medicine is now taking on a highly important role, where treatments are tailored to the patient's metabolism, optimizing results. Exploring the potential of PUFAs as part of personalized medicine, where dietary interventions are based on an individual genetic makeup, type of cancer and metabolic and lipomics profile, is also critical.

It is known that PUFAs interact with other bioactive compounds. The integration of PUFAs into broader dietary interventions and lifestyle modifications could enhance overall health and reduce cancer risk. For this reason, it is also important to develop predictive biomarkers to identify which patients are most likely to benefit from PUFA supplementation, and using biomarkers to monitor treatment effectiveness and adjust protocols is another important direction which could involve new inflammation or oxidation markers.

Research should also focus on the role of PUFAs in various types of cancer, including both common and rare cancers, and their potential in prevention through epidemiological studies and clinical trials. Essential future directions include developing improved formulations of PUFAs that enhance bioavailability, stability and targeted delivery to cancer cells, as well as comparing the efficacy of dietary sources versus supplements. Additionally, promoting awareness of the potential benefits of PUFAs in cancer prevention and treatment among healthcare providers and the public, and informing

public health policies to include recommendations for PUFA consumption as part of cancer-prevention strategies, will be crucial for leveraging the therapeutic potential of PUFAs.

7. Conclusions

In summary, the prevalence of melanoma presents a significant and pressing concern within clinical contexts, with projections indicating a likely escalation in its incidence in the foreseeable future. Nowadays, a definitive cure remains elusive, and the intricacies of treatment modalities are compounded by the multifaceted mutational landscape and inherent resistance to chemotherapeutic agents exhibited by this malignancy. A burgeoning body of literature underscores the potential therapeutic role of n-3 PUFAs in conjunction with other antineoplastic agents. Notably, this combined approach has exhibited enhanced sensitivity of tumor cells to basic treatments, mediated through various mechanisms. Furthermore, independent treatment with n-3 PUFAs has been associated with favorable outcomes across diverse physiological pathways, including but not limited to cell cycle progression, inflammation, oxidative stress mitigation, apoptosis induction, angiogenesis suppression and modulation of the immune system.

The combination of treatments offers an intriguing opportunity in the realm of personalized medicine, where the integration of metabolomics poses a significant challenge. This approach holds promise for a deeper understanding of disease processes, including skin cancer, and for identifying biomarkers essential for diagnosis, tracking progression and evaluating responses to fatty acid-based therapies.

However, it has become evident that there exists a considerable amount of controversy surrounding the interpretation of clinical study findings and their implications for the purported beneficial role of n-3 PUFA. Additionally, the ongoing debate regarding the optimal n-6/n-3 PUFA ratio remains unresolved. However, emerging evidence suggests that a ratio of 1:1 is potentially more balanced and favorable for health outcomes and a high one may elevate the risk of developing diseases.

Meta-analyses of randomized controlled trials demonstrated that n-3 PUFA supplementation has been associated with improvements in various clinical outcomes, yet conflicting study results have emerged, with some indicating no effect or even negative consequences. These discrepancies can be attributed to several factors, including inadequate study protocols lacking robust inclusion and exclusion criteria, small sample sizes, absence of a run-in period before baseline assessment, variations in study populations, short duration of the studies, discrepancies in supplement dosages, variations in the actual content of molecules within interventions across trials and differences in the ratio of active molecules utilized.

Finally, a variety of experimental therapeutic approaches combining n-3 PUFA administration with different antineoplastic agents has been designed, and plenty of encouraging results have been obtained both in vitro and in vivo. Based on findings from experimental studies and in the few clinical trials carried out with n-3 PUFAs, further intervention studies are needed to evaluate the effects of combined treatments with n-3 PUFAs alongside conventional antineoplastic drugs and should be warranted in the future as well as integration of personalized metabolomics treatments for developing evidence-based recommendations for the use of fatty acid supplementation as a therapeutic intervention.

Author Contributions: Conceptualization, C.R.-S., B.T.-H., D.M.-P., J.A.-S., S.G.-C. and H.G.-V.; writing—original draftpreparation, C.R.-S., B.T.-H., D.M.-P., J.A.-S., S.G.-C., H.G.-V. and R.R.; writing—review and editing, C.R.-S., B.T.-H., D.M.-P., J.A.-S., S.G.-C., H.G.-V. and R.R.; visualization and figures elaborations, C.R.-S. and D.M.-P.; supervision, R.R., L.S. and S.C.; funding acquisition, R.R. All authors have read and agreed to the published version of the manuscript.

Funding: This manuscript was funded by the Agencia Nacional de Investigación y Desarrollo (ANID-FONDECYT), grant number 1211850. This institution did not participate in the design, literature review, or writing of the manuscript.

Data Availability Statement: Data sharing is not applicable to this article as no new data were createdor analyzed in this study.

Conflicts of Interest: The authors declare no conflicts of interest.

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