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Review

Molecular mechanisms of the effects of olive oil and other dietary lipids on cancer

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Cancer is one of the main causes of mortality worldwide. Geographical differences in incidence rates suggest a key effect of environmental factors, especially diet, in its aetiology. Epidemiologic and experimental studies have found a role of dietary lipids in cancer, particularly breast, colorectal, and prostate cancers. Their incidence in the Mediterranean countries, where the main source of fat is olive oil, is lower than in other areas of the world. Human studies about the effects of dietary lipids are little conclusive, probably due to methodological issues. On the other hand, experimental data have clearly demonstrated that the influence of dietary fats on cancer depends on the quantity and the type of lipids. Whereas a high intake of n-6 PUFA and saturated fat has tumor-enhancing effects, n-3 PUFA, conjugated linoleic acid and γ-linolenic acid have inhibitory effects. Data regarding MUFA have not always been conclusive, but high olive oil diets seem to have protective effects. Such effects can be due to oleic acid, the main MUFA in olive oil, and to certain minor compounds such as squalene and phenolic compounds. This work aims to review the current knowledge about the relationship between dietary lipids and cancer, with a special emphasis on olive oil, and the molecular mechanisms underlying these effects: modifications on the carcinogenesis stages, hormonal status, cell membrane structure and function, signal transduction pathways, gene expression, and immune system.

Keywords: Cancer / Dietary lipids / Mediterranean diet / Oleic acid / Olive oil

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1 State of the art of the relationship between dietary lipids and cancer

Every year approximately ten million people are newly diagnosed with cancer and more than six million people die from this disease worldwide. In general the most common cancer site in men is lung, followed by prostate, stomach, colon and rectum and liver, while in women the most frequent is breast and then *cervix uteri*, colon and rectum, lung and stomach [1, 2]. Geographical differences in cancer incedence rates indicate a role of environment in the aetiology of this disease, nutrition being one of the most relevant environmental factors involved [3]. It has been estimated that a third of all cancers can be associated to diet and that they could be reduced through individual and social actions [4]. Among the numerous dietary compounds that have been related to cancer, dietary lipids have been revealed as significant ones. Epidemiologic and especially experimental studies have established a relationship between dietary fat and cancers of the breast, colon and rectum and, to a lesser extent, prostate. Scanty studies have also related non-melanoma skin cancer, and cancers of the oral cavity, upper digestive tract, stomach, pancreas, liver, pharynx, larynges, lung, endometrium and ovary with dietary lipids [3, 5, 6].

Human data regarding the association between dietary lipids and cancer is controversial. Descriptive epidemiologic studies (correlation and migration studies) have suggested a positive association between the total fat intake and the risk of breast, colorectal and prostate cancers [7]. Interestingly, countries with a Mediterranean diet, characterized by a high consumption of fibre, fish, fruits, vegeta-

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Abbreviations: AA, arachidonic acid; **CLA,** conjugated linoleic acid; **COX2**, cyclooxygenase-2; **DMBA,** 7,12-dimethylbenz(α)anthracene; **DHA,** docosahexaenoic acid; **EGFR,** epidermal growth factor receptor; **EPA,** eicosapentaenoic acid; **FFA**, free fatty acids; **GJIC**, gap junction-mediated intercellular communication; **GLA**, γ-linolenic acid; **LA**, linoleic acid; **LNA,** α-linolenic acid; **OA,** oleic acid; **PE**, phosphatidylethanolamine; **PGE_2**, prostaglandin E_2; **PGE_3**, prostaglandin E_3; **PL,** phospholipase; **PKC,** protein kinase C; **SHBG**, sex hormone-binding globulin

bles and olives and olive oil, show middle values for these cancers. An expanding number of retrospective case-control investigations have also found an increase in cancer risk with increasing fat intake, especially with animal and saturated fat intake [7]. However, it is difficult to draw clear conclusions from these studies because of the recall bias and confounding [8]. Regarding the prospective cohort studies, more properly conducted, taken together they do not support a strong association between total fat or individual fatty acid intake during adult life and cancer [9, 10]. Several pooled analyses have examined a wide range of fat intake and, overall, no association between intake of total, saturated, monounsaturated or polyunsaturated fat and risk of cancer has been observed [9, 10]. Interventional trials are needed to answer the question whether an increased consumption of certain fatty acids influences cancer risk [7, 8, 11]. Recently, the results obtained by The European Prospective Investigation into Cancer and Nutrition (EPIC) have been published. This multicenter prospective study was specifically designed to investigate the relationship between diet and cancer. To date, results do not support a strong association between fat or individual fatty acid intake during adult life and cancer, although some data suggest an increased risk of breast cancer by a high intake of saturated fat [12]. Therefore, the association between dietary lipids and cancer in human populations remains largely unresolved.

Animal studies have provided strong data supporting a relationship between dietary lipids and cancer, especially breast cancer. Experimental results have demonstrated that the effect of the lipids of the diet on cancer depends on the quality and amount of fat consumed, in addition to the stage of the carcinogenesis where they act. Carcinogenesis models have also provided abundant evidence for a lipid specific action beyond their caloric supply [3, 5, 6, 13, 14].

n-6 PUFAs, especially linoleic acid (LA) (18:2n-6), have shown a stimulating effect on breast, colorectal and prostate cancers in animal models. These PUFAs, found in vegetable oils, are highly consumed in Northern Europe. Diets with a high content in saturated fat, mainly from animal origin, have also a tumor-enhancing effect, although such effect is lower than the one exerted by high LA diets. On the other hand, high levels of n-3 PUFA inhibit breast and colon tumor growth and metastasis. This inhibitory effect is mainly exerted by the long chain n-3 PUFAs, eicosapentaenoic acid (EPA) (20:5n-3) and docosahexaenoic acid (DHA) (22:6n-3), from oily fish and fish oils and also derived from α-linolenic acid (LNA) (18:3n-3), found in vegetable oils, red meat and dairy products. The role of n-3 PUFA in prostate cancer still remains unclear, but experimental studies suggest that long chain n-3 PUFA have a tumor-suppression effect while high LNA diets promote tumor development. Conjugated linoleic acid (CLA), a naturally occurring compound found in ruminants products, has shown an inhibitory effect on cancer. Furthermore, antiproliferative effects of γ-linolenic acid (GLA) (18:3n-6), found in evening primrose oil, have also been reported [5, 6, 15 – 19].

The role of MUFA, primarily oleic acid (OA) (18:1n-9), has attracted much attention, especially in the last years. Mediterranean diet, whose hallmark is the high consumption of olive oil, rich in this MUFA, has been traditionally linked to a protective effect on cardiovascular disease and cancer [20]. Considerable amounts of OA are also contained in animal fats (30 – 55%) and other plant oils (25 – 75%). As it was discussed above, epidemiologic studies about the relationship between dietary lipids and cancer are inconclusive, and the particular case of OA is not different to the rest of fatty acids. In this case, in addition to the methodological issues already pointed out, this fact can be due to the difference between single chemically defined nutrients (OA) and foodstuff (olive oil) [21]. Thus, the later contains not only this specific fatty acid as nutrient but also many minor compounds in the unsaponifiable fraction of the virgin olive oil, some of which have been defined as "bioactive compounds" [22] and as such exert chemopreventive effects on cancer [23]. In Table 1 the chemical composition of olive oil is shown [24].

Extra-virgin olive oil, the first-pressed olive oil, contains an abundance of squalene and phenolic antioxidants including simple phenols (hydroxytyrosol, tyrosol), aldehydic secoiridoids, flavonoids and lignans (acetoxypinoresinol, pinoresinol). Interestingly, it contains significantly higher concentrations of phenolic antioxidants and squalene than refined virgin oil and seed oils. In addition, seed oils, which contain very low amounts of squalene, have none of the phenolic antioxidants that are present in virgin and refined olive oils [23, 25]. The exact composition of olive oil does not only depend on the growth conditions in the respective year preceding the harvest but also on the degree of ripeness of the fruit and the technical processing (*e.g.* cold pressing, refining) [21].

There are few experimenal studies addressing the role of OA and olive oil on cancer if compared to the investigations about the role of other dietary lipids. Such studies have been mainly conducted in breast cancer, where it has been described a potential protective effect of olive oil and OA. However, other inconsistent results have also been obtained, including non-promoting, weak-promoting, and even promoting effects on tumor growth [3, 5, 19, 26 – 31]. The effects of high extra virgin olive oil diet on 7,12-dimethylbenz(α)anthracene (DMBA)-induced mammary carcinogenesis have been compared to the effects of high and low corn oil diets. High olive oil diet increased tumor latency, decreased tumor incidence (compared to the high corn oil diet), and reduced the tumor content and volume (in comparison to both high and low corn oil diets). The study of tumor regression, as a proposed protective effect for this high olive oil diet, showed a slow progression of tumors in the animals fed that diet rather than a real regression [32].

Table 1. Chemical composition of olive oil

Saponifiable fraction (98 – 99%)	Unsaponifiable fraction (about 2%)
Main fatty acids present in triacylglicerols:	Non-glyceride esters (alcoholic and sterol compounds, waxes)
Oleic acid (18:1n-9)	Aliphatic alcohols
Palmitic acid (16:0)	Triterpene alcohols
Linoleic acid (18:2n-6)	Sterols (B-sitosterol, campesterol, estigmasterol, …)
Estearic acid (18:0)	Hydrocarbons (squalene, B-carotene, lycopene, …)
Palmitoleic acid (16:1n-9)	Pigments (chlorophylls, …)
Linolenic acid (18:3n-3)	Lipophilic phenolics (tocopherols and tocotrienols)
Miristic acid (14:0)	Hydrophilic phenolics (phenolic acids, phenolic alcohols, secoiridoids, lignans and flavones)
	Volatile compounds

Table 2. Main mechanisms of action of olive oil on cancer and component involved in such mechanism when known

Mechanism	Component involved	References
Influence on the carcinogenesis stages		
Influence on carcinogen metabolism	Phenolic compounds	[38]
Protective effect from oxidative DNA damage	OA and antioxidants	[41], [42], [45]
Modulatory action of biosynthesis of the colon cancer promoters bile acids	Squalene	[52]
Alteration of the hormonal status		
Decreased estrogen synthesis in adipose tissue (aromatization)	OA	[14], [40]
Antiestrogenic effects by structural similarity with estrogens	Lignans	[42]
Decrease in free oestradiol by stimulating SHBG synthesis	Lignans	[42]
Modification of cell membranes structure and function		
Functional changes in integral and membrane-bound proteins by changes in membrane fluidity	OA	[73], [75]
Influence on membrane structural properties (increase in propensity of PE membranes to form HII phases)	OA	[80]
Changes in the degree of membrane peroxidation	OA and antioxidants	[41], [42]
Modulation of signal transduction pathways		
Decrease in biosynthesis of AA-derived eicosanoids by competition for Δ6 desaturase with LA	OA	[5], [29], [88]
Inhibition of lipooxygenase, enzyme responsible for leukotriene synthesis	Hydroxytyrosol	[41]
Modulation of prenylation of certain proteins by strong inhibitory activity of HMG-CoA[a)] reductase	Squalene	[52]
Decrease in Ras activation		[93]
Regulation of gene expression		
Modulation of genes involved in cell proliferation	OA, minor compounds?	[93], [100], [101]
Alteration of the immune system		
Antiimflammatory and immunomodulatory effects	OA and phenolic compounds	[123] – [125]

a) 3-hydroxy-3-methylglutaryl coenzyme A

In addition, tumors from animals fed the high olive oil diet, besides being less aggressive biologically, displayed features of low morphological malignancy, such as a low histopathological grade, unusual invasive growth and few necrotic areas, similar to the tumors from the low corn oil control diet group, as well as extensive papillary areas [33].

In addition to the different relative content of the minor compounds in the different types and varieties of olive oil, to explain the apparent inconsistency among the studies, some authors have proposed that the null or the cancer-suppressive response of olive oil is not a function of the high 18:1 content in this oil but it is due to the lower content of 18:2n-6 [19]. A certain amount of this fatty acid, by virtue of its role as a precursor of eicosanoids, would be necessary for optimal promotion of mammary carcinogenesis [13]. Related to this hypothesis, a competitive inhibition by OA of the Δ6-desaturase, the first step of the eicosanoid biosynthesis leading from LA to arachidonic acid (AA), could explain, in part, that the tumor growth progresses slower with high olive oil feeding [14]. However, other authors that

have found a tumor promoting effect of diets enriched with OA have attributed such effect to the development of essential fatty acid deficiency [34, 35].

At present, there is an increasing understanding of the specific mechanisms by which dietary fat in general and high olive oil diets in particular may exert their modulatory effects on cancer. Those that stand out are: influence on the stages of the carcinogenesis process, alteration of the hormonal status, modification of the structure and function of cell membranes, modulation of cell signaling transduction pathways, regulation of gene expression and influence on the immune system. It is probable that, *in vivo*, lipids may act through all these mechanisms in an integrated, simultaneous and/or sequential way. Next, the scientific evidence on these mechanisms, with a special emphasis in the scarce available olive oil data, will be reviewed. In Table 2 the most important mechanisms that have been experimentally demonstrated for the olive oil are shown.

2 Mechanisms of action of dietary lipids on cancer

2.1 Influence on the carcinogenesis stages

Tumorigenesis is a multi-step process whose steps reflect structural or epigenetic alterations in genes controlling proliferation, differentiation and programmed cell death or apoptosis. This drives the progressive transformation and evolution of normal cells into highly malignant derivates [36, 37]. Dietary lipids may affect the different stages of the carcinogenesis, mainly the promotion although they can also act during the initiation. Firstly, it must be taken into account the possible initiating action of substances that accompany the dietary fats, such as food pollutants, additives and hormones. In this sense, it has been noted that the anticarcinogenic activity of olive oil phenols in the first stages of the carcinogenesis may be due to their ability to reduce the bioavailability of food carcinogens and to inhibit their metabolic activation [38]. But the main mechanism described by which dietary lipids may act stimulating the initiation of human malignancies is lipid peroxidation and the subsequent oxidative DNA damage. Peroxidation of n-6 PUFA by free radicals and reactive oxygen and nitrogen species produced in cells generates reactive α,β-unsaturated aldehydes, such as *trans*-4-hydroxy-2-nonenal and malondialdehyde. These aldehydes can form exocyclic DNA adducts, highly miscoding lesions that initiate the carcinogenic process through specific point mutations [5]. However, the role of lipid peroxidation in the modulation of tumor development is currently controversial, especially in the case of n-3 PUFA, which seem to inhibit cell growth even being highly unsaturated. Nevertheless this effect of n-3 PUFA has been related to the induction of growth arrest and apoptosis by the formation of oxidation products [13, 39, 40]. On the other hand, CLA has been reported to suppress peroxidation of unsaturated fatty acids, thus reducing oxidative damage [19]. The anti-tumor properties proposed for the virgin olive oil have been related to its protective effect from oxidative DNA damage because of both its high content of OA, which is far less susceptible to oxidation than n-6 PUFA, and its richness of minor antioxidant components, such as hydroxytyrosol, oleuropein and caffeic acid, which are potent scavengers of reactive species [23, 41, 42]. However, Yu *et al.,* [43] have proposed that OA could be a potential breast initiating carcinogen after the 17beta-oestradiol (E2) epoxidation, being its effect stronger than that of palmitoleic acid and LNA. Hydroxyl radical-induced DNA damage has also been linked to the progression of human cancers to the metastatic stage, notably because it results in loss of cell adhesion, which is a prerequisite for cellular detachment and invasion of host tissues [44]. Phenols extracted from virgin olive oil have been demonstrated to significantly decrease the invasiveness of colon cancer cells [45].

Dietary lipids can also have an indirect role in the initiation stage of the carcinogenesis, acting as co-carcinogens by enhancing the genotoxic effect of other compounds [46]. Actually, lipids may alter the structure of the chromatin, and thus affect the accessibility to specific genes of carcinogens, DNA repair factors and/or transcription complexes. *In vitro* studies have shown that the electrostatic interactions between histones and DNA can be affected by membranes containing acidic phospholipids and sphingosine, suggesting an effect of specific lipids in the regulation of chromatin structure and function [47]. Moreover, there is evidence that the lipid component of chromatin, mostly sphingomyelin and phosphatidylserine, plasmalogens and cholesterol, may play crucial roles in transcriptional regulation and modulation of cell proliferation, differentiation and apoptosis [48].

Dietary lipids can also have a role in the development of colon cancer, specifically modifying bile acid action. Dietary lipids have been reported to induce cell proliferation in the colonic mucosa and act as tumor promoters. The concentration of bile acids in the colonic lumen is increased by the effect of diets rich in lard, beef tallow or corn oil, but not by high fish oil diets [49, 50]. Interestingly, the secretion of bile acids does not increase due to high olive oil diets [51]. Moreover, squalene, a constituent of olive oil, has a chemopreventive effect on colon carcinogenesis itself, which can be partially exerted by a modulatory action on bile acid biosynthesis [52].

2.2 Alteration of the hormonal status

It has long been known that sex hormones have proliferative effects on tissues sensitive to their actions, and that high levels of such hormones can be related to increased risk of hormone-dependent cancers. Dietary lipids could induce modifications in the availability of sex hormones, such as

estrogens and testosterone, and thus influence the development of breast and prostate cancers.

Alteration of estrogen metabolism is a potential mechanism whereby dietary lipids may modify the mammary carcinogenesis process. The augmented risk of breast cancer among post-menopausal obese women [53, 54] support that a high fat intake can increase the adipocyte number and, as a consequence, increase peripheral estrogen synthesis through cytochrome P450 aromatase, which forms aromatic C18 estrogens from C19 androgens [55]. Prostaglandin E_2 (PGE_2), an AA metabolite, has been shown to directly enhance aromatization by stimulating the activity and expression of aromatase P450 in human adipose stromal cells. In contrast, PGE_3, a product of EPA metabolism, does not activate aromatase P450. Hence, an increased intake of EPA, which leads to increased production of PGE_3 and decreased production of PGE_2, is expected to decrease local estrogen production and thus reduce estrogen-estimulated cell growth [40]. In the case of the olive oil, the competitive inhibition by OA of the Δ6-desaturase, already mentioned above, could also act decreasing the PGE_2 production thus reducing the hormone-stimulated tumor growth. However, this issue has not been yet examined directly in humans.

Dietary lipids have also been described to increase estrogenicity at other levels. For instance, high intake of n-6 PUFA can displace estrogens from sex hormone-binding globulin (SHBG) and thus increase free estrogens level, it can enhance the estrogen affinity for its receptor, and it can also inhibit the activity of 17β-dehydrogenase avoiding in this way the conversion of oestradiol (the most active estrogen) in estrone [5, 14, 56]. In addition, a high fat intake has been described to cause an increase in the secretion of bile acid, which together with cholesterol derivatives can be transformed into estrogens by certain bacteria of the intestinal flora [57]. Recently, LA has also been shown to increase expression of the estrogen receptor α mRNA and decrease expression of the androgen receptor in human mammary tumor cells T47D [58]. On the other hand, lignans, which are minor components of olive oil, seem to have antiestrogenic effects, because of their structural similarities with oestradiol and the synthetic antiestrogen tamoxifen. Thus, in MCF-7 human breast carcinoma cells, lignans have been shown to inhibit oestradiol-induced proliferation and stimulate SHBG synthesis, with a subsequent decrease in free oestradiol [42]. Despite the relationship between dietary lipids and sex hormones, the data reported on the literature about the effect of high fat intake on plasma levels of gonadal steroid levels or their receptors and breast cancer risk is inconclusive [14]. Few studies have addressed the role of other hormones, like prolactin, insulin, thyroxine, growth hormone and corticosterone in the relation of dietary lipids and breast cancer, with inconsistent results [59 – 61].

The effects of dietary fat on prostate cancer are unclear, although a potential mechanism through the regulation of androgen actions has been proposed [62]. In this regard, a rat experimental model has shown that a high dietary fat and caloric intake (from corn oil) increases the conversion of testosterone to dihydrotestosterone, a more potent androgen, through the induction of the 5α-reductase-2 gene expression, thus stimulating prostate growth in neonatal, but not adult rats [63]. This effect was also observed in a transplantable prostate cancer model in rats, in which a high corn oil diet slightly increased serum testosterone levels [64]. On the other hand, in athymic nude mice bearing human prostate cancer xenografts the administration of a low fat diet did not modify serum testosterone levels [13]. Two studies in humans reported that a high fat diet with an elevated ratio of saturated to polyunsaturated fat increased total urinary androgens [65] and total plasma testosterone respectively [66]. Another trial concluded that a reduction in intake of dietary fat led to a decrease in serum testosterone and androstenedione levels [67]. Interestingly, a prospective cohort study found a strong association between increasing plasma testosterone levels and risk of prostate cancer after adjustment for SHBG levels [6]. On the other hand, a study in adolescent boys reported that modest reductions in total fat, saturated fat, and possibly energy intake did not alter serum sex hormone concentrations [68].

Finally, leptin has also been suggested to have a role in the effects of high fat diets on carcinogenesis. Results in the DMH-induced colon cancer model in rats hinted that high corn oil diet might enhance colon carcinogenesis by elevating cell proliferation through higher serum leptin [69]. Other studies also reported that this hormone controls the proliferation of both normal and malignant breast epithelial cells, providing a new level in the association of diet-induced obesity, mammary gland development and the risk for breast cancer [70]. There is also evidence for a relationship between leptin, dietary fat and prostate cancer [71].

2.3 Modification of cell membranes structure and function

The intake of a particular type of dietary fat can induce changes in the lipid profile of cell membranes, modifying cell behavior. The influence of dietary lipids on tumor fatty acid composition and growth has been studied in the DMBA-induced mammary cancer model. Adenocarcinomas from rats fed a high corn oil diet, more aggressive clinically and histopathologically in comparison with control diet tumors, displayed a significant increase in the LA relative content and a decrease in that of OA in the phosphatidylcholine, phosphatidylethanolamine (PE) and free fatty acids (FFA) lipid fractions. Since the hydrolysis products of these phospholipids and FFA have been linked to tumor cell proliferation, these results are in accordance with experimental and epidemiologic studies showing the stimulating role of high LA diets and the likely protective role of high virgin olive oil diets, rich in OA, on mammary carcinogenesis [72].

Changes in the lipid profile of cell membranes caused by dietary lipids can affect the membrane structure and fluidity, the signalling transduction pathways mediated by lipids, and the degree of lipid peroxidation in the cell membranes [73 – 75]. Thus, changes in membrane fluidity due to PUFA enrichment may produce functional changes of specific integral and membrane-bound protein, since their lateral mobility, conformation and interaction with other components can be altered. For instance, PUFA enrichment has been shown to reduce the number of sodium channels, whereas saturated and *trans*-unsaturated fatty acids have the opposite effect and OA does not produce any change [73]. Unsutarated fatty acids can also increase adenylate cyclase and Na+-K+-ATPase activities, and change the binding of insulin receptor to its ligand [74, 75]. In all these cases, PUFA have a greater effect than MUFA [75]. Proteins with a role in signaling pathways from cell membranes are commonly regulated by amphitropism. Such proteins, as Small GTPases Ras, Src-family protein tyrosine kinases, Ras-guanine nucleotide exchange factor, CTP:phosphocholine cytidylyltransferase, Protein kinase C (PKC), phospholipase C (PLC), several extracellular matrix and cytoskeletal-related proteins, can be regulated by specific lipids which modify their assembly, folding or topological organization [76]. Lipids can influence, moreover, the organization of the plasma membrane into microdomains. These domains are involved in important cell functions, like the sphingolipid- and cholesterol-rich membrane microdomains named lipid rafts, that recruit and regulate specific proteins with a role in signal transduction, protein sorting in polarized cells, endocytosis and cell adhesion [77]. In this way, a colon cancer model in mouse showed that dietary n-3 PUFA, in comparison to n-6 PUFA, strongly altered the microenvironment of a subtype of lipid raft (caveolae), thus modifying cell signaling pathways [78].

Furthermore, modulation of the membrane lipid structure by dietary lipids has also been shown to regulate the protein membrane interactions and mobilization upon activation. Thus, the presence of lipids such as PE modulates the localization of peripheral proteins, like PKC, G proteins and PL A_2 among others, which are capable of translocating from the membrane to the cytosol and propagate intracellular signals [79]. Model systems have shown that OA enhances these properties of PE membranes, whereas trans-monounsaturated and saturated fatty acids have little influence. This effect of OA has been suggested to be one of the mechanisms of the modulation of this fatty acid on membrane and cell functions. In this sense OA, but not other fatty acids, has been reported to regulate G protein localization and function [80].

The composition of dietary fat can also induce changes in phospholipid fatty acid profile of the nuclear membrane, altering its function. Phospholipids of this membrane, especially if they contain unsaturated fatty acids, can regulate *in vitro* the activity of some DNA binding proteins with which they interact, like DNA replication, transcription and recombination proteins [81]. In addition, diets with a high content in LA, compared with a low LA diet, increased the activity of the nucleosidetriphosphatase in rat liver, and consequently the RNA export from the nucleus to the cytoplasm [74]. An increase of unsaturated fatty acids in the nuclear membrane, resulting in changes in its fluidity, has been suggested to affect the phosphorylation, and consequently the activity and function, of the nuclear membrane-associated enzymes involved in this RNA transport [82].

Dietary lipids can also modify, as already mentioned, the degree of lipid peroxidation in cell membranes. Persistent cellular oxidative stress leads to macromolecular damage and disruption of signaling pathways stimulating the development of cancer. The degree of unsaturation of the fatty acids found in the membrane phospholipids determines the susceptibility to peroxidation. Therefore, high unsaturation levels lead to an increase in cell oxidative stress. In this way, a source of polyunsaturated fat will lead to membranes more susceptible to oxidation, compared to sources of saturated or monounsaturated fats [39 – 42].

Finally, dietary lipids could also influence the carcinogenesis at the membrane level by modulating the gap junction-mediated intercellular communication (GJIC). The reduced GJIC in tumor cells is associated with excessive proliferation, increased cell motility, invasion and metastasis. n-6 PUFA have been described to inhibit this kind of cell-to-cell communication in an experimental breast cancer model [83], whereas GLA has been shown to reduce *in vitro* adhesion of human breast and colon cancer cells to the endothelium partly by improving GJIC [84].

2.4 Modulation of signal transduction pathways

Changes in the physicochemical properties of the membrane due to dietary fat intake may affect the production and the composition of bioactive molecules generated from membrane lipids, such as inositol triphosphate, diacylglycerol, FFA and phosphatidic acid, by the action of PL A_2, C and D. These molecules play important roles as second messengers or as modulators in the intracellular signaling network and, consequently, these changes will affect gene expression and cell function [85, 86]. Unsaturated fatty acids removed from the sn-2 position of the membrane phospholipids also serve as substrates for the eicosanoid synthesis. Eicosanoids derived from the LA, via AA, and those derived from the LNA, via EPA, are synthesized using the same enzymes but they have different and, in some cases, contrary effects. Thus, the first ones have been linked to increased growth, inhibited apoptosis, angiogenesis and metastasis [39, 40]. In fact, the tumor promoting effect of high n-6 PUFA diets on breast cancer has been correlated with a greater production of eicosanoids [5]. In contrast, the protective effect of n-3 fatty acids on cancer could be exerted, at least partially, through their suppressing effect

on the biosynthesis of AA-derived eicosanoids, in favor of EPA-derived eicosanoids. This suppression is achieved at different levels: decreasing the availability of AA precursors into membrane phospholipids; by the competition of n-3 PUFA with n-6 PUFA for desaturases and elongases, for which n-3 fatty acids have greater affinities; and suppressing the cyclooxygenase-2 (COX-2) [40]. Likewise, the effect that CLA can have in cancer risk reduction has been associated with a decrease in LA metabolites that are substrates for eicosanoid biosynthesis [87]. In relation to this, the possible tumor protective effect of olive oil can also be related, at least in part, to the competition between OA and LA for desaturation enzymes. Although OA has lower affinity for Δ6 desaturase than LA, relatively high concentrations of OA can reduce the conversion of LA to GLA and consequently its entry into the eicosanoid biosynthetic pathways [5]. In addition, the minor component of olive oil hydroxytyrosol has been reported to inhibit lipooxygenase, enzyme responsible for leukotriene synthesis [41]. An experimental model of carcinogenesis in colon showed that the modulation of AA metabolism is one of the mechanisms of the preventive effect that olive oil based diets exerts [29]. The inhibition of PGE_2 production, together with an inactivation of the Erk cascade, have been also proposed to account for the suppressive effect of a high OA oil on chemically-induced lung tumorigenesis in mice [88].

FFA are also known to modify the activity of PL A_2, PL C, PL D, PKC, AMPc-dependent protein kinase, Ca^{2+}/Calmoduline-dependent protein kinase II, G proteins, adenilate and guanylate cyclases, as well as ionic channels and calcium mobilization [73 – 75]. PKC, for example, is a pivotal enzyme in the intracellular signaling which is regulated by many different lipids, including diacylglycerol, posphatidylserine, FFA, lysophospholipids, and phosphatidylinositol. Lipid- and calcium-mediated control of PKC activity is subtype specific [89]. n-6 PUFA, but not OA, have been described to induce changes in cell adhesion to collagen IV in human breast cancer cells. This effect was exerted by selectively activating PKCϵ and PKCμ isozymes, which leads to the activation of β1 integrins [90]. However, dietary n-3 fatty acids inhibit PKCβII activity and suppress PKCßII-mediated hyperproliferation and colon carcinogenesis *in vivo* [91].

Fatty acids can also have an influence on membrane receptors involved in the mitogenic signaling pathways. However, their effects on such proteins, irrespective of those derived from changes in the structure of cell membranes mentioned above, are not well known. Unsaturated FFAs including oleate but not saturated FFAs have been shown to trigger tyrosine phosphorylation and epidermal growth factor receptor (EGFR) activation in an endothelial cell line [92]. However, EGFR is not activated in experimental mammary adenocarcinomas and either a high n-6 PUFA diet (corn oil) or a high MUFA diet (virgin olive oil) do not modify this state. In contrast, Neu is activated in these tumors, although its activity does not seem to be modified by these dietary lipids [93]. In MDA-MB-231 breast cancer cells, EGFR is not activated by oleate either although, interestingly, this fatty acid stimulates proliferation in these cells whereas palmitate induces apoptosis. This proliferative effect of oleate are mediated at least in part via the G protein-coupled receptor GPR40, and many downstream signal transduction pathways, including PL C, Src, MEK1/2, PI3K, and PKCξ may participate in this effect [94].

Dietary lipids can also have an effect on the activation of Ras proteins, which are critical regulators of cell function, including growth, differentiation and apoptosis. The mechanisms of this modulation are unknown. A high fish oil diet decreased membrane-bound Ras in the AOM-induced colon cancer model, probably by interfering with posttranslational modifications of the protein. On the contrary, in the same model a high corn oil diet increased the membrane localization of Ras, being one possible mechanism of the promoting effect on colon carcinogenesis of this diet [95]. However, in colonocytes and colon tumors other authors have demonstrated that n-3 PUFA, in comparison to n-6 PUFA, decreased the Ras membrane-to-cytosol ratio, but not Ras farnesylation and palmitoylation [96]. Furthermore, the tumor-inhibitory effect of squalene on rat AOM-induced colonic aberrant crypt foci has been mainly attributed to its strong inhibitory activity of 3-hydroxy-3-methylglutaryl coenzyme A reductase, the rate-limiting enzyme in the mevalonate pathway, a precursor of nonsterol isoprenoids which are required for Ras prenylation [52]. In experimental breast cancer, a high virgin olive oil diet, in comparison with high and low corn oil diets, has also been shown to regulate Ras activation, decreasing the GTP-bound (activated) Ras levels, but not Ras localization. This suggests that the modulating effect of the olive oil-based diet on this cancer may be exerted by modifying Ras activation and Ras-dependent signaling [93].

2.5 Regulation of gene expression

Dietary lipids can also have an influence on cancer development by modulating the expression of genes potentially involved in cell transformation and tumorigenesis. Actually, it has been widely described that regulation of transcription, RNA processing and stability of genes with a role in metabolism are done by dietary lipids and other dietary components. The effect of those lipids on gene expression can be due, at least in part, to the already mentioned changes in cell membranes and signal transduction pathways to the nucleus. However, a direct, fast and acute control of gene transcription has also been considered [97]. In this last case, the effect of fatty acids or their metabolites may be directly mediated by binding to various nuclear receptors (PPAR, LXR, RXR, HNF-4α) and activating their transcription factor action, or indirectly mediated as the

result of changes in the abundance of regulatory transcription factors (SREBP-1c, NFκB, c/EBPβ, HIF-1α). Some of these molecules have been described to regulate cell proliferation, differentiation and survival [86].

Dietary lipids and their metabolites may exert some of their effects on carcinogenesis by modifications in gene expression or activities of molecules involved in signaling pathways, like the ErbB-Ras-MAPK one. Supporting this hypothesis, the treatment of *v-Ha-ras*-transfected MCF-7 cells with high concentrations of EPA resulted in an increase in c-erbB2/neu mRNA levels, but no significant effect was found when non transfected cells were treated with LNA or EPA. These data suggested that the effect of EPA on HER-2/neu expression was enhanced by the presence of high levels of another oncogene [98]. Other studies reported a decrease in c-Ha-ras protein levels in MCF-7 mammary tumors grown in nude mice fed fish oil [99]. In experimental breast cancer, high corn oil and high olive oil diets had a different effect on the modulation of the gene expression of the ErbB family. High corn oil diet had a stimulating effect on mammary carcinogenesis that was associated with an increase in the ratio between the levels of the 9.5 kb transcript and the 2.7 kb transcript of c-erbB1. On the contrary, high olive oil diet exerted a protective effect on carcinogenesis, concomitantly with a decrease in that ratio. The 9.5 kb mRNA is translated to the 170 kDa functional EGF receptor, whereas the 2.7 kb mRNA codes for a truncated form without enzymatic activity that can be secreted and act as a negative regulator of different members of the ErbB family. In addition, the high olive oil diet had a trend to decrease c-erbB2/neu mRNA and p185ErbB2/Neu protein levels, whereas the high corn oil diet did not modify the expression of this receptor [93, 100]. Other authors have later described in breast cancer cells with *neu* amplification that OA treatment decreased c-erbB2/Neu expression [101]. In the former model of DMBA-induced mammary cancer, c-Ha-ras1 mRNA levels were not modified by high corn or olive oil diets. In contrast, as mentioned above, these diets have different effects on Ras activity [93, 102]. Furthermore, the analysis of the gene expression of PCPH, a protooncogene that acts synergistically with Ras, showed an association between its expression and the cell differentiation degree, and it also suggested a down-regulation of PCPH expression in response to a high n-6 lipid diet [93, 103].

High fat diets can exert a modulating effect on tumorigenesis through changes in the activity and/or expression of enzymes involved in eicosanoid production, as it has been already discussed. These diets can thus regulate the eicosanoid signaling. For example, high n-6 PUFA diets have a promoting effect on mammary and colon tumorigenesis that has been associated with overexpression of COX2 and, to some extent, with COX1 genes. On the contrary, high n-3 PUFA diets have an antitumoral effect that could be exerted through the inhibition of the COX2 expression [39, 40, 50, 104]. Furthermore, DHA has been shown to inhibit the inducible nitric oxide synthase and expression of related proinflammatory genes in CaCo-2 colon cancer cells [105]. However, in the *in vivo* rat AOM-induced colon cancer model, DHA did not influence nitric oxide synthase or COX2 expression [106]. Some hydroperoxides produced from LA have also been shown to upregulate COX2 expression in human colon adenoma and carcinoma cells [107].

Fatty acids have also been reported to regulate several nuclear proteins, either directly or indirectly. Results in MCF-7 breast tumors grown in nude mice showed that a high fish oil diet reduced c-myc expression [99], while in rat normal mammary gland and in PhIP-induced benign breast tumors diets rich in LA increased the expression of this oncogene [108]. In human prostate cancer PC-3 cells AA had a growth stimulating effect, not observed with EPA and OA. This stimulating influence of AA was related with an induction of c-fos and COX-2 mRNA in a dose-dependent fashion [31]. The gene function of tumor suppressor genes can also be regulated by fatty acids. Thus, in mammary tumor cells, the addition of LA increased cell proliferation and decreased p53 protein levels, whereas DHA induced further suppression of DNA synthesis and upregulated expression of p53 [109]. On the other hand, other studies *in vivo* concluded that a 10% corn oil diet did not modify p53 mRNA and protein levels in AOM-induced colon tumors, but this diet decreased mitochondrial localization of p53 and increased inactive cytosolic p53, reducing its activity [110]. Furthermore, dietary lipids have also been shown to affect the expression of the suppressor genes BRCA1 and BRCA2. Thus, LA and oestradiol exerted a synergistic effect on the gene expression of BRCA1 in MCF-7 tumor cells, as they decreased the mRNA levels of this gene compared with controls or with cells treated with LA or oestradiol alone [111]. On the contrary, in MCF-7 and MDA-MB231 tumor cell lines treatment with n-3 PUFA (EPA and DHA) increased the BRCA1 and BRCA2 mRNA expression, but no effects were observed with n-6 PUFA (AA) treatment [112].

Dietary fatty acids can also induce changes in the expression of cell-to-cell adhesion molecules, such as E-cadherin. In different cancer cell lines, like lung, colon, breast, melanoma, and liver tumor cells, GLA, but not LA and AA, has been associated with upregulation of E-cadherin. Higher levels of E-cadherin expression have been correlated with reduced *in vitro* invasion and increased aggregation, in accordance with the anticancer properties of GLA [113]. GLA can also regulate the expression of maspin and the motility of cancer cells. Hence, GLA upregulated maspin mRNA and protein levels, what was associated with a reduced motility in different human cell lines. In addition, LNA and AA had no significant effect whereas LA had an inhibitory influence on maspin levels [114].

With the development of powerful new methodologies for the high-throughput analysis of expression, even subtle

effects of lipids on gene expression can be studied. Thus, in DMBA-induced breast tumors, the use of cDNA microarrays allowed to identify four genes that were downmodulated by the effect of a high corn oil diet. These genes, identified and validated, were submaxillary gland α-2u globulin, vitamin D3-upregulated protein 1, the paternally imprinted gene H19 and the unknown expressed sequence tag Rn.32385. Tumors from high n-6 fat diet animals, showing downregulation of these genes, were associated with clinical behavior and anatomopathological features of higher malignancy than control tumors [115]. Moreover, the expression of these genes was not modified by the effect of a high virgin olive oil diet. Other studies have associated vitamin D3-upregulated protein 1, the riboregulator H19 and α-2u globulin with cell proliferation and differentiation, suggesting that the different effects that high corn oil and high virgin olive oil diets induce on experimental mammary carcinogenesis can be partly exerted by a differential modulation of the expression of such genes [93].

The effect of LA treatment on the human breast cancer cell line T47D has also been analyzed using microarrays. This treatment induced changes in the expression of estrogen receptor α, G13α G protein and p38 MAP kinase, and genes with a role in cell cycle regulation and RNA transcription [58]. A high beef fat diet has been also reported to modulate the expression of several genes in the ACI rat model for spontaneous, age-onset prostate cancer. Those genes were related to inflammation, glucose and fatty acid metabolism, androgen metabolism and tumor suppression, in addition to genes coding for proteins with kinase activity, intracellular and extracellular matrix molecules and growth factors, as well as androgen responsive genes [116]. Recently, in PC-3 human prostate cancer cell line it has been found that the ω-6 fatty acid AA induces eleven genes that are regulated by nuclear factor-κB, including *COX-2*, Iκ*Bα*, *NF-κB*, *GM-CSF*, *IL-1*β, *CXCL-1, TNF*-α, *IL-6, LTA, IL-8, PPAR*γ, and *ICAM-1* [117]. Furthermore, microarrays analysis evidenced specific changes in the gene expression profile at various stages of AOM-induced colon carcinogenesis by effect of a high n-3 diet. Such changes were related to the protective effect of this high fish-oil diet on initiation and promotion of the carcinogenesis. In this study n-9 MUFA did not exert a protective effect, probably due to an elevated content of highly fermentable fiber in this diet [106].

2.6 Alteration of the immune system

Fatty acids might be expected to have some impact on immune function because of several reasons. First, changes in dietary lipids can modulate the fatty acid composition of cells of the immune system, specifically their cell membrane phospholipids, thus altering the actions of intracellular second messengers produced. Second, alteration of the fatty acid composition of these phospholipids may influence eicosanoids synthesis, which are important immunoregulatory molecules. Finally, fatty acids may alter cell function by direct interaction with intracellular targets, including transcription factors, which in turn could alter gene expression.

Dietary lipids have been shown to modulate the immune response and modify inflammatory cytokine production [30]. The suppressive effect of dietary PUFA on lymphocyte proliferation, natural killer cell activity and expression of adhesion molecules has been demonstrated in several studies. Eicosanoids could be involved in this immunosuppressive effect [118 – 120]. They modulate the inflammatory and immune response, besides playing a critical role in platelet aggregation, and cell growth and differentiation. In general, EPA-derived eicosanoids have antiinflammatory effects, whereas AA-derived eicosanoids have proinflammatory effects (although PGE_2 has been suggested to also have anti-inflammatory properties) [40, 121]. Consequently, diets with a high content of n-6 PUFA in relation with the content of n-3 PUFA would increase synthesis of inflammatory eicosanoids. On the contrary, diets with a low ratio of n-6:n-3 would decrease the production of eicosanoids [86]. It has been reported that PGE_2 inhibits immune responses through the suppression of macrophages, T- and B-lymphocytes, and stimulating immunosuppressor cells. PGE_2 can also inhibit the production of T-helper type 1 cytokines such as interleukin (IL)-2, interferon-γ, and IL-12, whereas it can increase the production of T-helper type 2 cytokines (IL-4, IL-5 and IL-10). *In vitro* and *in vivo* studies have also shown a reduction in the production of IL-1, IL-2 and tumor necrosis factor a by effect of n-3 PUFA [120, 121]. Since n-3 PUFA can suppress the tissue levels of AA and thus the synthesis of PGE_2, increased intake of n-3 PUFA would lead to enhancement instead of suppression of immune responses. This suggests that the antiinflamatory and immunomodulatory actions of n-3 PUFA may be mediated by eicosanoids-independent mechanisms, like inducing changes in antioxidant status, transcription activity and intracellular signaling pathways [120 – 122]. Some nuclear receptors can be involved in the actions that PUFA have in the immune system. As an example, PPAR activation by fatty acids can suppress the expression of cytokines and other molecules involved in the inflammatory response [121].

Although the effects of olive oil on the immune system are less known and some results are disparate, there is enough evidence that it is capable of modulating functions of cells of the immune system. The effects appear to be similar to, albeit weaker than, those seen following feeding diets containing fish oils. The animal studies have demonstrated that the suppressive effects of olive oil on immune function are due to OA, but they have also contributed with data showing that the polyphenolic components of olive oil have some anti-inflammatory and immunomodulatory properties [123, 124]. In contrast, consumption of a MUFA-

rich diet by humans does not appear to bring about a general suppression of immune cell functions. The lack of a clear effect of MUFA in humans may be attributed to the higher level of these fatty acids in the animal studies. Moreover, it is extremely difficult to determine conclusively whether the effects observed are indeed due to an increased level of OA or to a concomitant decrease in the level of saturated fatty acids. Furthermore, potential effects of non-lipid components of olive oil on immune and inflammatory responses cannot be excluded, particularly since olive oil-derived phenolic compounds are associated with potent antioxidant activity and inhibition of lipooxygenase activity [125].

3 Concluding remarks

Epidemiology and especially experimental research support that there is an influence of dietary lipids on cancer development. Although further studies in humans are needed, the current available data show that distinct dietary lipids may modulate differentially the multistep process of carcinogenesis. Moreover, it has been clearly demonstrated experimentally that the effect of dietary fats depends on the quantity and type of lipid. Olive oil, highly consumed in Mediterranean countries, is the principal source of OA. This MUFA has shown protective effects in the development of several cancers, although some inconsistent results have also been reported. This can be probably related to the great diversity of types and varieties of commercially available olive oil, with a variable relative content of LA and OA and also with different content of its minor bioactive compounds. Different mechanisms for the modulatory actions of olive oil and other dietary lipids on cancer have been proposed. Among them, there is experimental evidence about an influence on the hormonal status, cell membranes structure and function, signal transduction pathways, gene expression and the immune system.

The current knowledge about the link between dietary lipids and cancer allow characterizing dietary lipids as functional foods. Functional foods have a biological activity beyond their nutritional value, and, therefore, have a potential impact on the population health and the risk of disease. Accordingly, from a public health focus, studies supporting a protective role for certain dietary lipids in cancer would be placed in the field of primary and secondary cancer prevention, and should allow us to specify scientifically healthy dietary advices to the population. For instance, the scientific data available regarding the beneficial effects of virgin olive oil intake on health may allow designing future prevention strategies based on the regular intake of this oil in the diet [126]. The protective effect of the intake of virgin olive oil in the development of cancer should be contextualized within the concept of the dietary and lifestyle habits, especially from the early decades of life with a special attention to the adolescence. Moreover, it must be taken into account that human diet involves the interaction between its multiple components along with other environmental as well as genetic factors.

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