Olive Oil in Cancer Prevention and Progression

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Epidemiological studies have shown the potential health benefits of olive oil, specifically in relation to cancer incidence. The negative modulating effect, probably protective, of high virgin olive oil diets on carcinogenesis have been experimentally demonstrated. There is evidence that olive oil influences different stages of carcinogenesis, hormonal levels, cell membrane composition, signal transduction pathways, gene expression, and the immune system. Either its main monounsaturated fatty acid, oleic acid, or the minor compounds may be responsible for its chemoprotective effects. Its bioactive compounds are emerging as potential agents in the treatment of cancer.

Key words: cancer, minor compounds, oleic acid, olive oil, prevention

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EPIDEMIOLOGY

Cancer is one of the major causes of mortality in developed countries, where, given its increasing incidence, it is now a serious public health problem. It is believed that 25% of men and 20% of women will have a cancer-related process in the course of their lives.[1] Several epidemiological studies and animal experiments have implicated certain dietary compounds in the higher incidence rates of several types of cancer. It is now known that one-third of all human cancers may be related to specific compounds of the diet.[2] For this reason, many research groups are studying the effects of various dietary compounds on cancer processes, and dietary fats have been described as increasing the risk of colon, breast, and prostate cancers.[3] Moreover, incidences of cardiovascular disease and of cancers such as that of the colon and breast have been confirmed as being lower in the Mediterranean region than in the rest of Europe.[4]

Incidence and Prevalence Data

Ecological and Correlation Studies

There are wide international variations in the incidence of cancer, and although diagnostic differences may account for some of the variability, it is unlikely to account for the greater than 10-fold difference in incidence that has been observed between many countries.[5] Studies of migrant populations, such as Chinese migrants to the United States, clearly indicate that international variations primarily result from environmental influences rather than genetic background.[6] Japanese migrants to the United Sates also show a definite shift toward the colorectal cancer rates of their adopted country within the first generation. Examination of temporal trends in colorectal cancer incidence also suggests major contributions from environmental and lifestyle factors. The role of diet and nutrition in the production of initiators and promoters, and in modulating the sensitivity of the host to these agents, has led to the generation of a number of hypotheses. The diets most frequently associated with increased risk of colorectal cancer have several characteristics in common: rich in total fat, rich in total protein, rich in meat products, a high proportion of saturated fats, low in fruits and vegetables, and low in estimated fiber.[7]

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Breast cancer is common in North America and western Europe and much less common in most of Asia and Africa. Breast cancer rates have been observed to increase significantly in populations migrating from low-risk areas such as Japan, where diets are low in fat, to high-risk areas, such as the United States, where the population consumes diets high in fat. Time-trend studies also support the fat-breast cancer association.[8] Within Japan, estimates of per capita daily fat intake rose from 23 to 52 g/d over the 15-year period from 1959 to 1973.[9] During this period, breast cancer mortality increased in Japan by over 30%. However, not all ecological studies support a strong effect of dietary fat. A study from 65 Chinese counties showed only a weak positive association between fat intake and breast cancer mortality, in spite of a striking range of fat intake from 6% to 45% of energy. Correlation does not prove cause and effect, and many investigators have argued that fat intake may be an indicator of some other unidentified combination of diet and environmental components that are actually the critical factors. The strong correlation may indicate the overall effect of many dietary factors that change simultaneously. This observation suggests that nutritional or other environmental factors that are active during youth and adolescence may have a long-term and major impact on the subsequent risk of breast cancer.[10]

In relation to European cancer incidences, a gradient distribution from north to south has been observed. This information comes from the Cancer Incidence in Five Continents study, which includes data from 186 cancer registries all over the world, and from the cancer incidence estimates performed by IARC for European Union countries. Spain, Portugal, and Greece have the lowest rate of all cancers in women.[11] However, Spain is fifth in the rate of men's more prevalent cancers (i.e. lung, colorectal, and prostate). This is a negative change compared with a 1990 estimation in which Spain was in one of the last positions.[4]

Data on cancer prevalence in the European Union[12] show a heterogeneous distribution. Table 1 shows the data of prevalence for colorectal and breast cancers (those most related to diet). Spain, Italy, Greece, and Portugal have the lowest prevalences of these cancers. Spain presents rates that are 28% (colon) and 42% (breast) lower than the European Union average. According to these data, the Mediterranean strip of the European Union presents prevalence values for cancer that are significantly lower than for the rest of the European region. Diet may thus be an important factor in improving the quality of life and decreasing these incidences.

Table 1. Cancer Incidence in Different Countries in Relation to Spain[12]

	Cancer	
Country	**Colon**	**Breast**
European Union	+28.8%	+42.1%
United Kingdom	+33.6%	+59.2%
Sweden	+36.8%	+80.0%
Holland	+30.2%	+75.6%
Luxembourg	+44.0%	+68.5%
Ireland	+20.5%	+37.5%
Finland	−13.2%	+50.4%
Denmark	+55.4%	+70.3%
Austria	+54.4%	+42.9%
Belgium	+43.5%	+85.9%
France	+20.8%	+33.5%
Germany	+62.9%	+60.2%
Italy	+12.1%	+24.8%
Greece	−41.9%	−4.9%
Portugal	+11.2%	+8.2%

Case-Control Studies

Many case-control studies on the effects of diet have been performed. Trichopoulos et al.[13] found that the vegetable consumption (including olive oil) of a group of 2368 women was associated with a reduction in breast cancer risk. A population-based case-control study suggested that dietary fat is responsible for 60% of colorectal cancer risk among Chinese migrants to the United States. Similar conclusions have been reported by Stoneham et al.[14], who found a reduction in desoxycholic acid in colonic mucosa with olive oil consumption.

Although they are too numerous to examine in detail here, a recent meta-analysis of 17 case-control studies comprising 6831 cases and 7105 controls only found four studies that identified a statistically significant positive association between fat intake and breast cancer. When all 17 studies were combined, however, a modest but statistically significant 21% increase in risk was found when the highest and lowest levels of intake from each study were compared. It is interesting that no association between fat intake and risk of breast cancer was seen in a case-control study in Japan, where the lower-fat-consumption groups were likely to have obtained less than 20% of their energy from fat.[15]

Prospective or Cohort Studies

Data from cohort studies assessing the relationship between dietary fat intake and breast cancer have become available in recent years. Ten prospective studies failed to provide compelling evidence for the high dietary fat-breast cancer association. In the largest cohort study, the Nurse's Health Study, 1439 cases of breast

cancer were documented among 89,494 women who were followed for 8 years. The relative risk, when the highest and lowest deciles of fat intake as a percent of energy intake at baseline were compared, was 0.86 (95% confidence interval, 0.67–1.08). The estimated range of total fat intake expressed as percent of calories from fat was 29% to over 49% in this population.[16] In contrast, a cohort study of Canadian women reported[17] that an increased risk of dying from breast cancer was associated with a greater saturated fat intake but not with greater total fat intake.

Some other cohort studies report that fat from red meats, rather than total fat, may be more important. A recent prospective study in a cohort of 88,000 US nurses supports a role for animal fat in colon cancer. An increased risk of 1.89 (95% confidence interval, 1.13–3.15) was observed for the highest quintile (>65 g/d) compared with the lowest quintile (<39 g/d) of animal fat intake.[18] Other cohort studies have confirmed a positive association with red meat, particularly processed meats, but not with total fat, animal fat, or saturated fat.[19,20]

Influence of Olive Oil Compounds on Epidemiological Data

Almost all case-control and cohort studies have related the influence of current dietary intake of adults to short-term breast cancer risk (of approximately 10 years). It is still possible that dietary fat intake during childhood and adolescence may influence the risk of breast cancer decades later. Furthermore, it may take even greater reductions in fat intake (e.g., to less than 20% of calories from fat) to reduce risk. Most case-control and cohort studies do not include significant numbers of participants with energy intakes of 20% or less derived from fat, and other studies do not make this relationship entirely clear. Investigations carried out by Smith-Warner[21] on 351,825 women demonstrated that a high consumption of fruits and vegetables did not decrease the risk of breast cancer.

These contradictory results could be due to erroneous diet evaluations from the subjects included in the studies, because the dietary fat intake must be analyzed together with fruit and vegetable consumption. It is important to mention that fat consumption in the Mediterranean area is higher than in the rest of Europe. However, the quality of the fat intake must be taken into account, because most fat intake in the Mediterranean area is in the form of olive oil, which is rich in monounsaturated fatty acids (MUFA) and antioxidants, whereas in the north of Europe dietary fat consists for the most part of polyunsaturated fatty acids (PUFA) from the n-6 series (which is more susceptible to oxidization).

The possibility of a moderate but significant reduction in the risk of colorectal and breast cancers through olive oil intake has been pointed out in at least two studies.[22,23] In addition, attention has been paid to the possible role of olive oil in the prevention of breast cancer, and two studies from Spain,[24,25] one from Greece[13] and another from Italy,[26] all showed reductions in risk of around 25% when women who were classified as relatively high consumers of olive oil were compared with those who consumed other types of oil or fat but not olive oil.

A number of different experimental approaches in animal and other models provide evidence of the relationship between olive oil consumption and inhibition of tumor growth, especially for breast, colon, and prostate cancers (see below).

Finally, we can conclude that in Mediterranean countries, olive oil is one of the major constituents of the diet. Consumption of olives or olive oil is regarded as important for preserving a healthy and relatively disease-free population. Epidemiological data show that the Mediterranean diet has significant protective effects against cancer and coronary heart disease.

EXPERIMENTAL APPROACHES

Role in Cancer Initiation

As pointed out above, diet is believed to be one of the most important contributory factors to cancer risk, while also being linked to metabolic and genetic factors.[27] Nevertheless, although several decades of epidemiological research have reported links between diet and cancer, comparatively few specific nutrition-related factors have been unequivocally shown to contribute to pathogenesis. Among the dietary components that have been suggested as risk factors for cancer, perhaps none has attracted as much attention as fat intake.[28]

The process of conversion of a normal cell to the malignant state is called carcinogenesis, and agents that induce such changes are called carcinogens. Carcinogenesis is a complicated, multi-stage process. Essentially, a small population of abnormal cells is generated and then increases in abnormality as a result of a series of mutations and changes in the patterns of gene expression. The development of cancer has three stages: initiation, promotion, and progression. Initiation is a cellular phenomenon characterized by irreversible genetic changes. However, the promotion stage is a reversible process of gene activation that is the result of the action of xenobiotics or endogenous substances involving entire tissues and producing a benign tumor from initiated cells. Finally, the progression stage is the conversion of benign tumors to malignant forms, usually accompanied by more rapid

growth, invasiveness, metastasis, and an increase in genetic instability that is associated with further irreversible genetic change.[29]

The initiation stage of cancer is caused by irreversible DNA damage or alteration. Thus, a major source of protection against cancer initiation resides in efficient carcinogen detoxification by phase I/II enzymes, DNA repair, and the elimination of cells with badly damaged DNA (apoptosis). Radical oxygen species (ROS) and radical nitrogen species (RNS) are capable of chemically modifying DNA, so they are also considered to be carcinogens. The mechanism of action of these agents in the initiation stage included the following.

Direct DNA Damage

Several studies have indicated that genomic DNA derived from cancerous or precancerous human tissue contains elevated amounts of certain modified bases compared with normal tissues. Using gas chromatography/mass spectrometry with selected ion monitoring to assay damage products, Jaruga et al.[30] observed elevated amounts of 11 different oxidized DNA base modifications in chromatin derived from colon, stomach, ovary, brain, and lung carcinomas compared with histologically normal tissue removed at surgery. Malins et al.[31] were the first to report elevation of modified purines, including 8-oxoguanine and 8-oxoadenine, in DNA derived from human breast cancer specimens and adjacent non-malignant tissue compared with DNA from normal breasts. This evidence indicates that the increased production of ROS and RNS can cause DNA damage in cells.

Mutagenesis

A potential mechanism of carcinogenesis initiated by oxidatively modified DNA is via the induction of mutations in critical target genes. A number of ROS- and RNS-generating reagents are mutagenic in models of human mutagenesis systems.

Other Mechanisms of ROS/RNS Carcinogenicity

Oxidative damage to lipids and to proteins (e.g., DNA repair enzymes) may also have mutagenic effects. In addition, high ROS levels decrease cell proliferation, can increase net protein phosphorylation, and help to promote proliferation and the expression of immediate early genes such as *c-fos* and *c-myc*.[32]

Another way in which ROS/RNS could affect the behavior of tumor cells is by altering cell-cell communication. Communication through gap junctions is generally decreased in tumor cells, and this is thought to be involved in their excessive proliferation.[33]

Olive Oil in the Development of Cancer Initiation: Oxidative Stress. Olive oil has two important fractions: a saponificable fraction rich in MUFA (oleic acid [OA], 18:1n-9) and a minor compounds fraction with a high content of phenolic substances. There is growing evidence to suggest that the beneficial effects of olive oil intake on human health can be ascribed to elevated OA content,[34] as well as to the antioxidant properties of its minor components, including phenolic compounds.[35]

Oleic Acid. There is some evidence that MUFA have cancer-chemopreventive effects. As mentioned previously, the molecular pathways to cancer involve multiple genetic changes whereby extensive oxyradical damage causes mutations in cancer-related genes and leads to a recurrent cycle of cell death and regeneration. In addition to direct oxidative DNA damage, reactive oxygen and nitrogen species can induce DNA strand breakage, mainly via trans-4 hidroxy-2-nonenal, generated as the major aldehydes by lipid peroxidation of PUFA.[36] However, some studies[37,38] of OA show that levels of biomarkers for oxidative stress and lipid peroxidation decrease compared with those of n-6 PUFA, suggesting that they might have a favorable effect on the prevention and development of cancer, mainly in cancer types affected by diet (breast, colon, and prostate). On the basis of these studies, it can be suggested that there is a close correlation between the rate of lipid peroxidation and the degree of malignancy of the tumor cell on the one hand, and the susceptibility of the tumor cell to free radical-induced cytotoxicity on the other.

Olive Oil Minor Compounds. It has become increasingly apparent that the putative health benefits of dietary olive oil in the classical "Mediterranean diet" may not be entirely due to the lipid component of the oil and that minor components such as monophenolics may play an important role.[35,39] Several studies have attempted to elucidate the contribution of the phenolic components in virgin olive oil to the positive health effects attributed to the oil per se. Compelling data from in vitro and in vivo laboratory studies, epidemiological investigations, and human clinical trials indicate that antioxidants present in the unsaponificable fraction of olive oil have important effects on cancer chemoprevention and therapy. These compounds may interfere in several of the steps that lead to the development of malignant tumors, including protecting DNA from oxidative damage, inhibiting carcinogen activation, and activating carcinogen-detoxifying systems. Studies conducted to understand the effects of minor constituents of virgin olive oil on cancer development are scarcer and somewhat contradictory. The anticarcinogenic activity of olive oil phenols may be due not only to their antioxidant properties but also to their ability to reduce the bioavailability of food carcinogens and to inhibit their metabolic activation.[40]

Quiles et al.[41] have studied the effects of three olive

oil phenolic derivatives (hydroxytyrosol, tyrosol, and caffeic acid) that have a similar structure but differ in the number and position of their hydroxyl groups on modulating the oxidative DNA damage and oxidant-induced gene expression of redox enzymes in human prostate cells elicited by hydrogen peroxide. The results of this study suggest that hydroxytyrosol and caffeic acid, two phenolic compounds found in the non-glyceride fraction of virgin olive oil, may act as antioxidants, protecting DNA and lipids against oxidative damage. However, tyrosol appears to be a prooxidant. Concerning the antioxidant effectiveness of these two active phenolic molecules, hydroxytyrosol is more efficient than caffeic acid. Since hydroxytyrosol is the major phenolic component of virgin olive oil (70%–80% of the total), it is conceivable that its antioxidant effects predominate. This would explain the reported beneficial health effects of virgin olive oil consumption in Mediterranean countries.

Finally, these phenolics are able to modify the gene expression of cytosolic glutathione peroxidase (cGPx) more than of phospholipid hydroperoxide glutathione peroxidase (PHGPx), suggesting that they are more effectve in a hydrophilic atmosphere than in membrane structures, which may be an important clue concerning the subcellular distribution of virgin olive oil phenolics.

Squalene, another minor compound present in olive oil, displays anti-tumor promoter activity against colon cancer. Rao et al.[42] have demonstrated that the dietary administration of squalene inhibits the formation of preneoplasic lesions in the colon, with no significant effect on serum cholesterol levels. This anti-tumorigenic activity of squalene has also been described by several investigators, although mainly with regard to skin cancer.

The antioxidant vitamin E is another component present in the unsaponificable fraction of virgin olive oil (at a level of about 300 to 400 ppm). It is known that phenolic compounds in the diet can lower the DNA damage provoked by ROS/NOS, thus preventing mutations and cancer development. Vitamin E as an antioxidant has chemopreventive effects. It has been demonstrated that vitamin E-depleted rats have more lipid peroxidation and DNA damage than rats fed vitamin E. Supplementation with vitamin E thus protects against DNA damage and prevents carcinogenesis.[43]

Effects on Tumor Clinical Behavior and Molecular Mechanisms of Action

Experimental models have demonstrated that the effect of dietary lipids on cancer depends on the type and quantity of fat consumed, as well as on the particular critical phases of the carcinogenesis where they act.[28,44,45]

Main Types of Cancer Affected by Dietary Lipids

Breast Cancer. To date, breast cancer has been the more exhaustively studied cancer in relation to dietary lipids. In general, the n-6 PUFA, mainly linoleic acid (LA; 18:2n-6) from vegetable oils, are the main promoters of carcinogenesis.[44] Thus, in the 7,12-dimethylbenz[a] anthracene (DMBA)-induced mammary cancer model, it has been shown that in animals fed a high n-6 fat diet, the tumors appear earlier and the incidence, tumor content, and volume are higher than in the other dietary groups.[46-49] γ-linolenic acid (GLA; 18:3n-6), which is found in evening primrose oil, is an exception within the n-6 PUFA family because it has antiproliferative properties.[50] Moreover, the conjugated LA found in meat and in ruminant-derived dairy products could also have an inhibitory effect on breast cancer.[51] On the other hand, the n-3 PUFA α-linoleic acid (ALA; 18:3n-3), found in low quantities in vegetable oils, red meat, and dairy products, and eicosapentaenoic acid (EPA; 20:5n-3) and docosaexahenoic acid (DHA; 22:6n-3), from fish and fish oils, inhibit mammary tumor growth.[52] Saturated fats, mainly of animal origin, also promote experimental mammary cancer, but they are less potent than the vegetable-origin n-6 PUFA.[53]

While epidemiological studies suggest that MUFA (especially OA, the main component of olive oil) exert a protective effect on breast cancer, experimental studies have found a non-promoting effect, a weak promoting effect, and even a promoting effect.[51,54] Interestingly, a negative modulatory role of a high-virgin olive oil diet in the appearance and progression of experimental breast cancer has been described.[49] Moreover, mammary tumors from animals fed this kind of diet not only show a more benign clinical behavior but also a lower degree of morphological malignancy compared with control and high n-6 fat diets.[55,56] In any case, components other than OA need to be considered in analyzing the beneficial effects of virgin olive oil on breast cancer.[42,57,58]

Colorectal Cancer. While epidemiological studies have failed to show a consistent association between intake of n-6 PUFA and colorectal cancer risk, most experimental studies have shown an increased risk of chemically induced colon carcinogenesis associated with high n-6 PUFA diets. On the other hand, some epidemiological and animal studies have found a protective effect of n-3 PUFA. Although n-9 MUFA from olive oil have been studied less, they have been shown to have either no effect or a protective effect on the development of colorectal cancer.[59] Recently, experimental evidence has demonstrated the efficacy of the olive oil-pharmacological combination in reducing colon cancer incidence.[60]

Prostate Cancer. Studies relating dietary lipids and prostate cancer are not so abundant. The results of epidemiological studies of dietary lipids and prostate cancer are controversial,[61] and the experimental data limited, especially in the case of olive oil, due to the lack of suitable animal models. However, the experimental evidence suggests that dietary fatty acids influence the biological behavior of prostatic cancer cells once neoplastic transformation has taken place. Generally, fish oils containing high levels of long-chain n-3 PUFA suppress tumor growth, whereas oils high in PUFA such as LA or ALA promote tumor growth.[62] Recently, it has been shown that the increase in the incidence of prostate cancer induced by a high-fat diet in a transgenic mouse model is blocked by the addition of antioxidants at an achievable dose for humans.[63]

Mechanisms of the Modulating Action of Dietary Lipids on Cancer

Although the mechanisms of the modulating action of dietary lipids on cancer are not well understood, their influence could be exerted at several levels.

Influence on Stages of Carcinogenesis. The influence of lipids on carcinogenesis seems to be exerted mainly during the promotion stage, although there does exist some evidence of their possible role in the initiation stage as co-carcinogens, favoring the genotoxic action of several agents. Moreover, the possible initiating action of substances that accompany dietary fats, such as food pollutants, additives, and hormones, needs to be taken into account. Likewise, as was described in the previous section, PUFA metabolites resulting from peroxidation could have a stimulating effect on cancer development. A mechanism of DNA-adduct formation associated with a high intake of n-6 PUFA has been proposed.[44] On the other hand, virgin olive oil better protects the cell structures from oxidative damage, as its main compound, OA, is far less susceptible to oxidation than n-6 PUFA, and it also contains some antioxidant minor components.[36,64]

In colon tumorigenesis, bile acids secreted in response to high saturated or n-6 fat diets, but not high fish or olive oil diets, could act as tumor promoters. Moreover, squalene itself modulates the biosynthesis of biliary acids.[14,42]

Effects on Hormonal Levels. It has been suggested that dietary lipids may influence the development of the hormone-dependent cancers, breast and prostate cancer, through modifications in the concentrations of circulating sex hormones, such as estrogens and testosterone, respectively. With respect to breast cancer, the n-6 PUFA would be expected to increase estrogenicity at several levels.[44,65] On the other hand, long-chain n-3 fatty acids would lead to a decrease in the local production of estrogens, and the minor components of olive oil lignans would have an anti-estrogenic effect.[64] Other hormones such as prolactin and insulin have also been studied in relation to lipids and breast cancer, but with inconsistent results.

In spite of the relationships suggested above, no modifications in the plasma levels of the main hormones regulating mammary development, the tumou content of steroidal receptors, or even in other plasmatic biochemical parameters have been found. In the case of prostate cancer, little is known about the possible influence of dietary fats on male sex hormone levels.[62]

Modification of the Cell Membranes. Lipids modulate the biological activity of cell membranes because they are essential components of their structure. Membrane composition could be altered mainly by n-3 PUFA and the n-3/n-6 ratio of the diet, because these fatty acids cannot be synthesized de novo by higher animals.[66] Changes in the lipid profile of cell membranes produced by dietary lipids modify cell behavior by influencing membrane fluidity, lipid-mediated cell-signaling transduction pathways, and the degree of lipid peroxidation in the cell membranes.[67] A high content of PUFA increases membrane fluidity, whereas MUFA have been reported to have a lesser effect.[66] These changes affect specific integral and membrane-bound proteins, which may undergo functional changes, thus modifying fundamental cell processes. It has been demonstrated that particular types of fat can affect the composition of the cell membrane microdomains, altering important elements of signaling cascades.[68] Moreover, dietary fat, mainly from polyunsaturated sources, is capable of modifying the degree of lipid peroxidation in the cell membranes, eventually disrupting the signaling pathways and stimulating the development of cancer.

On the other hand, the relative content of membrane n-6 PUFA has been associated with a higher rate of cell proliferation.[69] Accordingly, chemically induced breast cancer tumors in rats fed a high corn oil diet, which displayed more aggressive clinical behavior and a higher histopathological degree, were characterized by an increase in the relative content of LA and a decrease in the relative level of OA.[46]

Actions on Signal Transduction Pathways. Dietary lipids can modify the activity of phospholipases A2, C and D, PKC, PKA, CaM-K II, G proteins, adenylate and guanylate cyclases, as well as ionic channels and calcium mobilization.[70,71] In this way, they modify the production and composition of the second messengers within the intracellular signal cascades.[67] Moreover, in vitro studies of several types of cells have shown

that dietary fats can also modulate the activation of membrane receptors that are mitogenic signal cascade effectors, such as the the epidermal growth factor receptor (EGFR). However, in an in vivo experimental mammary cancer model, EGFR has been described as not being involved in the lipid effects.[72] Furthermore, the tumor-promoting effect of high-fat diets on breast cancer has been correlated with greater production of prostaglandins. Other kinds of eicosanoids, molecules derived from fatty acids released from the membrane phospholipids, have also been related to the adhesion and metastatic potential of tumor cells.[69,73] The tumor-protective effect of virgin olive oil has been partly attributed to the modulation, both by OA and some minor components such as hydroxytyrosol, of eicosanoid biosynthetic pathways.[44] Finally, dietary lipids, including olive oil, have been shown to affect the activation of Ras proteins.[42,58,72,74]

Effects on Gene Expression and Protein Activity. It has been widely demonstrated that dietary PUFA can specifically modulate the expression of genes related to hepatic metabolism. The mechanisms of the fatty acid regulation of gene transcription are just beginning to be discovered.[67]

However, the regulation by dietary lipids of the expression of cancer genes is much less understood. Dietary lipids have been shown to affect the expression of genes potentially involved in cell transformation and tumorigenesis,[70, 71] such as c-*erbB2/neu* and *c-Ha*-ras.[47,48,72,75] Some transcription factor genes, such as *c-myc*, *NKκB*, and *SREBP*, as well as some tumor suppressor genes, such as *p53* and *BRCA1*, have also been described as being modulated by dietary lipids.[75,76]

Today's microarray technology is enabling us to detect new differentially expressed genes by the effects of dietary lipids in different animal experimental models.[77,78]

Immunosuppressor Effect. Dietary lipids are able to modulate the immune response and modify inflammatory cytokine production.[79] The PUFA immunosuppressor effect has been observed in several studies, and it has been reported for both n-6 and n-3 PUFA[73] as being related to the type of synthesized eicosanoids involved. Oleic acid, a component of olive oil, has also been demonstrated to have antiinflammatory effects,[79] and some extra virgin olive oil phenolics have been shown to inhibit the production of inflammatory eicosanoids and cytokines by animal and human cells in vitro.[80] However, in healthy human subjects, the consumption of a high-OA diet did not appear to bring about general suppression of immune cell functions.[81]

BIOACTIVE COMPOUNDS AS POTENTIALLY USEFUL THERAPEUTIC AGENTS IN CANCER

Minor Compounds

It is not yet clear which components or combination of components in olive oil are responsible for its protective effects, what their mechanisms of action are. For this reason, it is important to establish which components of olive oil contribute to its potential anticancer activity. The first important question to answer is, is the protective effect derived from the MUFA content or is it related to the antioxidant components of the unsaponificable fraction?

A multinational study carried out by five European centres has shown that the protective effect reported for olive oil intake may be due at least in part to components contained in the unsaponificable fraction of the oil.[82]

Extra-virgin 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.[64] These compounds, defined by Kitts[83] as "bioactive compounds," are extra-nutritional constituents that vary widely in chemical structure and function and typically occur in small quantities in foods. In this section, the role played by squalene and polyphenolic compounds as useful therapeutic agents in the management of cancer patients will be emphasized.

As mentioned above, scientific evidence has shown that free radicals or ROS play an important role in the initiation and progression of cancer. Antioxidants protect body tissues against oxidative stress and associated pathologies such as cancers. Olive oil has greater potency as an antioxidant than other vegetable oils. Consequently, compounds with antioxidant potential present in olive oil should be studied as possible useful agents in cancer therapy.

In this regard, gastric cancer is an interesting tumor model. It is widely accepted that gastric cancer is positively associated with *Helicobacter pylori* infection. The precancerous process usually takes decades to develop, and its different precancerous stages have been well characterized. The well-recognized steps in this process are: gastritis, atrophy, intestinal metaplasia, and dysplasia. Recently, some reports have looked at the possibility that oxidative stress may be a crucial mechanism in the chain of events that may finally result in neoplastic cell transformation and progression.[84] Some results provide

support to the hypothesis that oxidative stress may represent the final common path of *H. pylori* carcinogenesis. Indeed, it would appear that the damage could be prevented by the use of antioxidant agents. Further studies are needed to determine whether some of the antioxidant compounds found in olive oil could be useful in the treatment of gastric cancer.

A recent study by Gill et al.[85] showed that phenols extracted from virgin olive oil are capable of inhibiting several stages (initiation, promotion, and metastasis) of colon carcinogenesis in vitro. Interestingly, this study showed that the extract of olive oil phenols significantly decreased the invasiveness of colon cancer cells. Another interesting study reported that some polyphenolic compounds have been found to inhibit the lung metastasis induced by B16F10 melanomas in mice.[86] These results indicate a possible role for these compounds in arresting the metastatic growth of tumor cells.

On the other hand, the inhibition of angiogenesis in vivo can attenuate tumor growth and metastasis. Interestingly, it has been found that some polyphenolic compounds inhibit angiogenesis in vitro.[87] Further studies are needed to confirm the presence of anti-angiogenic polyphenolic compounds in olive oil, as these could represent a new class of antitumor drugs acting as angiogenesis inhibitors.

The flavonoids are typical phenolic compounds found in olive oil, which have long been recognized as exhibiting anti-inflammatory, antioxidant, and anticarcinogenic activities. Caltagirone et al.[88] reported that some flavonoids (apigenin and quercetin) inhibit melanoma growth and invasive and metastatic potential. In addition, quercetin was found to down-regulate expression of mutant p53 protein to nearly undetectable levels in human breast cancer cell lines.[89] The inhibition of p53 was found to arrest the cells in the G2/M phase of the cell cycle. Mutations of p53 are among the most common genetic abnormalities in human cancers. Moreover, Ranelletti et al.[90] have reported that quercetin reduces steady-state levels of p21Ras proteins in both colon cancer cell lines and primary colorectal tumors. Pouget et al.[91] have also reported that some flavonoids have an antiproliferative activity against MCF-7 human breast cancer cells.

Flavonoids and tocopherols (vitamin E) share a common structure, i.e., the chromane ring. Recently, Tomasetti et al.[92] reported that a vitamin E analog suppresses malignant mesothelioma in a preclinical model. Interestingly, Hakimuddin et al.[93] have reported that some flavonoids show selective cytotoxicity toward breast cancer cells, whereas they are only marginally cytostatic to normal cells. In summary, these results suggest that certain flavonoids have anticancer properties and these compounds may be useful in cancer therapy.

Olive oil contains 0.2 to 0.7% squalene. Experimental studies have shown that squalene can effectively inhibit chemically induced colon, lung, and skin tumorigenesis in rodents.[58] A mechanism is proposed for tumor-inhibitory activity of squalene based on its known strong inhibitory effect on 3-hydroxy-3-methylglutaryl coenzyme A reductase catalytic activity in vivo, thus reducing farnesylation of Ras oncoproteins. Mutation of Ras, especially K-Ras, occurs in approximately 20% to 30% of all human cancer, particularly in pancreatic (90%), colon (50%), and lung carcinomas (30%). Oncogenic Ras proteins are in a perpetual "on" state, leading to uncontrolled cell division in the absence of growth signals. Indeed, reduction of mutated Ras oncogene activation may be useful in cancers that are strongly associated with Ras oncogenes.[74]

Another interesting biological effect of squalene was reported by Das et al[94], whose results suggest that squalene has a selective in vitro cytoprotective effect on bone marrow-derived hematopoietic stem cells, but does not protect the neuroblastoma cell lines from cisplatin-induced toxicity. Thus, squalene allows a selective cytoprotective activity on normal tissues from chemotherapy toxicity, without protecting malignant tissues. These results suggest a possible use for squalene in the management of cancer chemotherapy.

Kampa et al.[95] reported that caffeic acid (the phenolic acid found in olive oil) exerts a direct antiproliferative action on T47D human breast cancer cells. This action is evident at low concentrations comparable to those found in biological fluids after the ingestion of foods rich in phenolic acids. The direct interaction with the aryl hydrocarbon receptor and the pro-apoptotic effect could explain its biological mode of action.

Hydroxytyrosol has been shown to inhibit proliferation of both human promyelocytic leukemia cells HL60 and colon adenocarcinoma cells HT29.[96] Hydroxytyrosol induced an appreciable apoptosis in HL60 cells after 24 h of incubation associated with early release of cytochrome *c* from mitochondria, which precedes caspase 8 activation. Interestingly, no effect on apoptosis was observed after similar treatment of freshly isolated human lymphocytes and polymorphonuclear cells. Hidroxytyrosol arrested the cells in the G0/G1 phase, with a concomitant decrease in the cell percentage in the S and G2/M phases. These findings provide experimental support for the putative anticancer activity of this compound.

Finally, some studies have reported that antioxidants enhance the apoptosis induced by standard chemotherapeutic agents employed for the management of some cancers.[97] Thus, chemotherapeutic agents administered in the presence of antioxidants could be a novel therapy for cancer. Nevertheless, larger trials are needed to dem-

onstrate whether bioactive compounds from olive oil individually or in conjunction with chemotherapy increase the response rates and/or survival time in cancer patients. On the other hand, a recent meta-analysis examining the effects of vitamin E supplementation noted a possible increase in all-cause mortality.[98]

Fatty Acids in Cytotoxicity of Chemotherapeutic Agents

In addition to the modulating effect of dietary fatty acids on the appearance and progression of cancer, which is supported by epidemiologic and experimental studies as described above, some fatty acids have been shown to be cytotoxic for cancer cells in vitro and in vivo without affecting normal cells.[99,100] In this context, the administration of certain fatty acids is arousing interest as a novel, relatively nontoxic anticancer agent.

Several pilot clinical trials have been performed on GLA, the most promising PUFA in the treatment of human tumors, and the results suggest that this fatty acid could be a valuable new agent in cancer treatment, although this still requires confirmation in randomized controlled trials.[101,102] Recent experimental studies have also indicated that exogenous supplementation with certain fatty acids may modulate tumor cell chemosensitivity. However, questions about which types or amounts of fatty acids improve chemotherapeutic effectiveness and the mechanisms of the fatty acid action are still controversial.

In vitro cancer cell line studies have demonstrated that PUFAs potentiate the action of radiotherapy and cytotoxic drugs and are capable of reversing multi-drug resistance.[100] In human ovarian cancer cell lines, GLA and DHA have been shown to enhance the efficacy of oxyradical-producing drugs such as anthracyclines (cisplatin and doxorubicin).[103] When the effect of DHA on doxorubicin activity was tested in the human breast cancer cell line MDA-MB-231, results indicated that DHA may increase the efficacy of the drug through a mechanism involving generation of lipoperoxides, since the DHA effect was abolished by a lipid peroxidation inhibitor (dl-α-tocopherol) or when OA (a non-peroxidizable fatty acid) was used in place of DHA.[104] Other authors have attributed the enhanced chemosensitivity of the NIH3T3 cell line and its transformants resulting from EPA supplementation to the modification of membrane fatty acid composition, leading to changes in physical properties such as membrane fluidity and drug transport.[105] Interestingly, the efficacy of cytotoxic drugs has been shown to correlate with levels of DHA in adipose tissue in locally advanced breast carcinomas.[106]

DHA and GLA have also been shown to be capable of enhancing synergistically the anticancer actions of microtubule-interfering agents such as paclitaxel (Taxol), docetaxel (Taxotere), and vinorelbine (Navelbine) in metastatic and non-metastatic human breast cancer cell lines in a dose-dependent manner.[107,108] Simultaneous exposure to these fatty acids and chemotherapeutic drugs in the presence of the antioxidant vitamin E also resulted in synergism, suggesting a limited influence of the oxidative status of GLA or DHA in achieving potentiating drug-induced cytotoxicity, and that mechanisms other than lipoperoxidation might exist. Thus, exogenous supplementation with DHA or GLA markedly decreased the expression of *Her-2/neu* (*c-erbB-2*) oncogene expression in human breast cancer cells, suggesting that the fatty acid-induced transcriptional repression of this oncogene might be one mechanism of the interaction of the fatty acid and the drug.[109,110] It has also been suggested that this mechanism could account for the synergistic interaction between OA and the growth-inhibitory effects of trastuzumab (Herceptin) in breast cancer cell lines with *Her-2/neu* oncogene amplification.[111]

The modulation of steroid hormone receptors has also been suggested as a mechanism of GLA action. Thus, the effects of GLA with primary hormone therapy based on tamoxifen administration in endocrine-sensitive breast cancer patients has been assessed in a phase II study. GLA plus tamoxifen achieved a significantly faster clinical response with a significant reduction in estrogen receptor expression compared with tamoxifen controls. These results suggest that the additive or synergistic inhibitory effect of GLA with tamoxifen in breast cancer may involve enhanced down-regulation of estrogen receptor-mediated growth.[102] More recently, in an in vitro study, GLA was shown to synergistically enhance the ability of tamoxifen and the pure antiestrogen ICI 182,780 (Faslodex) to inhibit estrogen receptor-dependent transcriptional activity.[112]

In experimental colon cancer induced in male F344 rats with azoxymethane, it has been demonstrated that a low dose of celecoxib, a COX-2 inhibitor, administered in diet high in fish oil produced a significant inhibition of COX-2 activity and expression and tumor incidence compared with a low dose of celecoxib in a diet high in mixed lipids (saturated and unsaturated fats). This suggests a degree of synergism between dietary n-3 PUFA and celecoxib in the suppression of colon carcinogenesis, and is a new mechanism of interaction between fatty acids and cancer drugs.[113]

SUMMARY

In conclusion, from epidemiological and, in particular, experimental studies, compelling evidence exists about the protective effect of olive oil consumption on

the appearance and progression of some cancers, mainly those of the breast, colon, and prostate. Both its main monounsaturated fatty acid, OA, and some specific minor components could account for the biological effects of olive oil on the distinct stages of carcinogenesis through different molecular mechanisms of action. Although some important questions remain to be answered, the possibility of the administration of the bioactive compounds from olive oil, alone or in combination with anticancer drugs, opens a new dimension in the treatment of cancer and in dietary counseling.

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