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Anti-cancer properties of olive oil secoiridoid phenols: a systematic review of in vivo studies

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Running title: **Chemopreventive effects of secoiridoids polyphenols**

ABSTRACT

Epidemiological studies suggest that olive oil intake is associated to a reduced risk of cancer. Recently, the chemopreventive activity of olive oil has been attributed to its unique phenolic compounds represented by phenolic alcohols, hydroxytyrosol (3,4-dihydroxyphenylethanol: 3,4-DHPEA) and tyrosol (p-hydroxyphenylethanol: p-HPEA), and their secoiridoid derivatives 3,4-DHPEA-EA (oleuropein aglycon), *p*-HPEA-EA (ligstroside aglycon), 3,4-DHPEA-EDA, *p*-HPEA-EDA (oleocanthal), and oleuropein. Several studied have demonstrated that these compounds are able to inhibit proliferation and induce apoptosis in different tumor cell lines. These *in vitro* effects have been recently summarized in several reviews. The aim of this systematic review was to evaluate the *in vivo* anti-cancer activities of secoiridoids phenols as evidenced by either animal models of carcinogenesis or human intervention trials. From the literature research through "PubMed" and "Web of Science" 16 animal studies and 5 human intervention trials were identified and included in the review. Most of the animal studies have confirmed the ability of these compounds to inhibit the carcinogenesis process at both initiation and promotion/progression phases. All human intervention trials have investigated the effects of olive oil phenols on DNA damage. Among the five selected studies, three have shown a significant preventive effect on of oxidative DNA damage in terms of reduction of 8-oxo-7,8-dihydro-2′-deoxyguanosine in urine, in mitochondria DNA of mononuclear cells and in lymphocytes DNA. The other two studies failed to see an effect on urinary excretion of either etheno–DNA adducts or oxidation products of guanine. Further investigations are necessary to clarify the real chemopreventive potential of olive oil secoiridoids phenols on human performing intervention studies on populations at high cancer risk.

Key words: Olive oil; Secoiridoids; Phenols; Oleuropein; Hydroxytyrosol; Chemoprevention;

1. Introduction

Cancer is a complex chronic-degenerative disease characterized by a multistep process in which normal cells are transformed into malignant cells acquiring several properties such as abnormal proliferation and reduced apoptosis. It is actually responsible for major morbidity and mortality in Western countries, and although cancer mortality has decreased during the last 15–20 years, it has been estimated that it caused more than 8.2 million deaths in 2012.[1] Wide variations of cancer incidence have been observed in different geographical regions. These variations are certainly related to the human exposure to different modifiable factors which may either increase or decrease cancer risk. The two main cancer-causing factors are tobacco smoke and dietary habits. However, while cancers related to tobacco use could be prevented simply by stopping smoking, those associated to the diet are more difficult to manage since foods may contain both risk and preventive factors. In general, it has been estimated that over 30% of all cancers may be avoidable by changing food intake.[2] Therefore, identification and characterization of foods and food constituents that can prevent cancer is an important goal for modern nutritional research.

In this context, of particular relevance is the observation that populations living in the Mediterranean area have a lower cancer incidence compared to other regions. This phenomenon has been attributed to the traditional dietary pattern of this region referred as "Mediterranean diet" (MD).[3] Epidemiological data actually support the hypothesis that MD may have an important role in preventing several types of cancers. A recent meta-analysis showed that the highest adherence to MD was significantly associated to a 13% reduction of cancer mortality.[4] Furthermore, a reduction of risk was observed for cancer in different sites such as colorectal (17%), breast (7%), stomach (27%), prostate (4%), liver (42%), head and neck (60%), pancreas (52%) and respiratory system (90%).[4] Furthermore, a clinical trial in which the diet was modified toward an improved adherence to MD showed a reduced total mortality (56%) and cancer risk (61%) after 4-year of follow up.[5]

Typically, MD is characterized by a high intake of vegetables and fruits, plant proteins, whole grains, fish, low-fat dairy, moderate alcohol (red wine) intake and low red meat consumption. A

peculiar characteristic of the MD is that olive oil is the primary source of dietary lipids.[6] The regular consumption of olive oil has been related to a reduced risk of different chronic-diseases. The importance of olive oil in cancer prevention has been suggested by several epidemiological studies, began in the mid-nineties, which showed a decreased risk of cancer in different sites associated to the uptake of olive oil.[7] In addition, the chemopreventive potential of olive oil has been supported by several studies on animal models.[8,9] However, in these studies the contribution of phenols to the preventive activity of olive oil has not been investigated. In the past, the beneficial effect of olive oil has been traditionally attributed to the presence of oleic acid. More recently, it was hypothesized that the cancer preventive capacity of olive oil could be mediated, at least in part, by the presence of minor components which include more than 230 chemical compounds present in a small amount (about 2 % of oil weight).[10,11] Among these components, particular interest has been raised by the different classes of phenolic compounds represented by phenolic acids, phenolic alcohols, flavonoids, secoiridoids and lignans. In particular, the phenolic alcohols, hydroxytyrosol (3,4-dihydroxyphenylethanol: 3,4-DHPEA) and tyrosol (*p*-hydroxyphenylethanol: *p*-HPEA) are abundantly and exclusively present in olive, olive leaf and olive oil as both free compounds and linked to either elenolic acid (EA) or its dialdehydic form (EDA) giving rise to the following secoiridois derivatives: 3,4-DHPEA-EA (oleuropein aglycon), *p*-HPEA-EA (ligstroside aglycon), 3,4-DHPEA-EDA, *p*-HPEA-EDA (oleocanthal), and oleuropein (Figure 1).[11] These compounds are not generally present in other oils and in other foods of vegetable origin. Their concentration in olive oil is greatly variable (50-800 mg/kg) and depends upon several factors such as agronomic conditions and technological aspects of olive oil production.[11]

In the last few years, many *in vitro* and *in vivo* studies have clearly demonstrated that olive oil phenolic alcohols and their secoiridoids derivatives possess potent anti-oxidant and anti-inflammatory activities which may be at the basis of their positive effects on human health.[12-14] Nevertheless, some biological effects exerted by these phenols may be mediated by molecular mechanisms that are not directly related to their anti-oxidant activity. Indeed, several *in vitro* studies

have demonstrated that these compounds, in particular 3,4-DHPEA, are able to interfere with proliferation, apoptosis and differentiation of different tumor cells by acting on the expression of genes controlling these processes. The molecular mechanisms exerted *in vitro* and involved in these effects have in part been elucidated and summarized in a recent review.[15] However, up to now no revision has been published focusing on the anti-cancer activities of these compounds as evidenced *in vivo* either in animal model of carcinogenesis or in human intervention trials. Therefore, in this systematic review, the main studies describing the cancer preventive and/or therapeutic properties of secoiridoid phenols and their derivatives demonstrated in "*in vivo*" systems have been summarized and discussed.

2. Methods

A systematic electronic literature search, without restrictions, through PubMed (http://www.ncbi.nlm.nih.gov/pubmed/) and Web of Science (http://wokinfo.com/) was carried out. The original articles investigating the anti-cancer properties of olive oil secoiridoid phenols were identified using the following search key words: (3,4-dihydroxyphenyl-ethanol OR 3,4-DHPEA OR hydroxytyrosol OR p-hydroxyphenyl-ethanol OR p-HPEA OR tyrosol OR secoiridoid OR oleuropein OR oleocanthal OR "olive oil phenols") AND cancer. Furthermore, the reference lists of included articles and recent significant reviews were manually examined to identify additional relevant publications. Potential identified articles were included if they reported data on "*in vivo*" effects of olive oil phenols on different animal models of carcinogenesis. In addition, human intervention trials were also considered if they reported effects directly related to the carcinogenesis process, essentially referring to DNA damage. From the selected animal studies we extracted the following information: first author's last name, year of publication, animal model, damaging agent, phenol exposure, dose and duration, effects. In the case of intervention studies further information were as follow: study

design, population selection and subjects characteristics, type of intervention, washout period, dose and length of intervention.

3. Results and discussion

From the primary literature research through PubMed (n=181) and Web of Science (n=281) databases and after removing duplicate (n=157), 305 records were identified for title and abstract revision (Figure 2). From them, 63 items were excluded because they did not investigate biological effects of olive oil phenols, 51 were review articles and 155 items were excluded because they tested different biological effects exerted by phenols *in vitro*. Therefore, 36 *in vivo* studies (32 on animal models and 4 human intervention trials) were selected for the full text revision, from them 20 articles were excluded since they did not considered the direct anti-cancer effects of olive oil phenols but some others activities such as anti-oxidant and anti-inflammatory. Four studies were found in the reference lists of included articles and recent significant reviews, and one additional paper has been recently submitted for publication by our group. At the end, 16 animal studies[16-31] and 5 human intervention trials were included in the systematic review (Figure 2).[32-36] The main characteristics of animal studies and human intervention trials are summarized in Table 1 and Table 2, respectively. In some animal studies single compounds were tested i.e. Oleuropein, hydroxytyrosol and oleocanthal. In other cases complex phenolic extracts derived from either olive leaf (particularly rich in Oleuropein) or olive oil have been tested. Only one investigation compared the effect of olive oil either deprived (low level of phenols) or enriched (high level of phenols) with phenolic compounds in comparison with corn oil (0 level of phenols).[17]

3.1 Oleuropein

Oleuropein is the most abundant phenolic secoiridoid present in leaves and unprocessed olive drupes of *Olea europaea* (Oleaceae), while in the olive oil it is mainly present as oleuropein aglycon (3,4-DHPEA-EA) (Figure 1). Oleuropein has been extensively studied both *in vitro* and *in vivo* with the

aim to demonstrate its anti-oxidant and anti-inflammatory activities. In the case of anti-cancer effects, 8 out of 16 animal studies selected in the present review investigated the effects of exposure to Oleuropein[16,18,20,24,27,28,31] and 1 study was carried out on a Oleuropein rich extract (Table 1).[22] The first *in vivo* study investigating the anti-cancer effect of Oleuropein was carried out using an house-established inbred strain of Swiss albino mice that spontaneously develops soft tissue sarcomas.[16] In this system, about 25% of mice, both male and female, develop tumors around 1 year of age. When the visible tumors reached the diameter of at least 2 cm, the animals were treated with Oleuropein, 1% in drinking water. Within 9-12 day of treatment Oleuropein induced complete tumor regression in 10/11 mice and partial regression in one animal, whereas all untreated animals die within 2 weeks of tumor appearance.[16] All Oleuropein treated mice were tumor-free for the remainder of their life-span. To give some mechanism explanation of these effects, tumor samples were stained and hystologically examined. It was observed that Oleuropein treatment induced cell rounding within the tumor without any evident effect on the vascular system. The *in vivo* data were in accordance with several effects demonstrated *in vitro* showing that Oleuropein was able to inhibit tumor cells growth, migration and invasion. In addition, it was observed that Oleuropein treatment of breast tumor cells (MCF-7) caused an evident disruption of the actin filaments organization within the cells. However, these effects could be simply due to the cytotoxic effects exerted *in vitro* by high dose of Oleuropein sometime used in these experiments (0.1 %, 1.85 mM). The *in vivo* results reported in this first study on the Oleuropein were particularly impressive and they have surely evoked the interest of researcher for further investigations on this compound. However, it should be noted that in subsequent studies other models of animal carcinogenesis have been used, so that up to now these results have not been reproduced using the same animal model. Instead, Oleuropein was tested in other carcinogenesis models in which tumors were induced by different treatments such as UVB irradiation[18,20] and azoxymethane (AOM).[28,31]

Two investigations have demonstrated the cancer preventive effect of Oleuropein on UVB induced skin damage and cancer on mice.[18,20] Early data demonstrated that topical application of

olive oil after UVB exposure could effectively reduce skin tumors in mice. However, in these studies the presence and the concentration of phenols in olive oil was not reported.[37,38] The first evidence suggesting that Oleuropein may have a skin protective activity was obtained in human healthy volunteers exposed to UVB irradiation. It was reported that the topical application of Oleuropein reduced irradiation induced erythema.[39] Successively, Kimura and Sumiyoshi demonstrated for the first time the preventive effect of orally administrated both olive leaf extract (containing 15% of Oleuropein) and purified Oleuropein on the radiation-induced skin damage and carcinogenesis in hairless mice.[18] Treatment with Oleuropein at two different doses (10 and 25 mg/Kg), administered via gavage twice daily, prevented the UVB-induced both increment of skin thickness and decrease of skin elasticity over a time period of 30 months. At the same time, Oleuropein significantly reduced both tumor incidence and tumor volume which, after 30 months of follow up, was reduced by 96% at the highest concentration tested (25 mg/Kg).[18] These chemopreventive effects were further supported by demonstrating that Oleuropein was able to prevent the radiation-induced increment of the expression of both the nuclear cellular proliferation marker Ki-67 and the angiogenic marker CD31. In addition, Oleuropein treatment efficiently prevented the increment of the UV irradiation induced skin expression of different matrix metalloproteinases (MMP-2, MMP-9 and MMP-13), vascular endothelial growth factor (VEGF) and cyclooxygenase-2 (COX-2). Similar effects were also obtained when animals were treated with the olive leaf extract.[18] MMP are zinc-dependent proteolytic endopeptidases which stimulate growth, migration, invasion, angiogenesis, and metastasis of tumor cells. Therefore the reduction of MMP expression induced by Oleuropein could be a mechanism by which this compound inhibit the skin carcinogenesis. Indeed, in vitro studies have confirmed the ability of Oleuropein to decrease the expression and/or the activity of MMP-9 in human breast cancer cells and THP-1 cells.[40,41] The inhibitory effects of Oleuropein on COX-2 expression is of particular interest because it may be a link between the anti-inflammatory and chemopreventive properties of this compound. The role of Oleuropein as anti-inflammatory agent has been observed in several studies both *in vivo* on different models of inflammation, and *in vitro*.[42,43] In

particular, it has been demonstrated that Oleuropein protects mice colon from chronic inflammation induced by dextran sodium sulfate (DSS).[44] This may be an important mechanisms by which this compound is able to prevent colitis-associated colorectal cancer, as will be described later in this section.

Preventive effects of Oleuropein were also observed in C57BL/6J mice exposed to UVB irradiation in acute conditions.[20] In this experimental approach, it was shown that Oleuropein efficiently inhibited the UVB induced increment of oxidative DNA damage measured by immunohistochemestry using anti- 8- hydroxy-2′-deoxyguanosine (OhdG) antibodies.[20] These anti-oxidant and DNA damage preventive activities of Oleuropein have also been demonstrated on human blood mononuclear cells exposed *ex vivo* to hydrogen peroxide.[45] Therefore, by preventing the DNA damage Oleuropein may inhibit the initiation phase of carcinogenesis. Further data supporting this point were recently reported *in vivo* on AOM-induced leukocytes DNA damage in A/J mice.[31] In this model, AOM was used as a specific colon carcinogen which acts on both initiation and promotion/progression phases of carcinogenesis. Nevertheless, it was observed that AOM was able to increased the mice peripheral leukocytes DNA damage, as evaluated by the "Comet assay". Inclusion of Oleuropein into the basal diet at a dose of 125mg/kg almost completely prevented the AOM-induced DNA damage.[31] In this study, it was also showed that Oleuropein enriched diet prevented the AOM-induced preneoplastic lesions in different colon segments reducing the severity of crypts dysplasia and tumor incidence in the medial colon segment.[31] Additional evidences suggesting that Oleuropein may prevent the initiation phase of carcinogenesis came from the study carried out on rats where the tongue tumors were induced by treatment with 4-nitroquinoline 1-oxide (4-NQO).[22] By using this animal model, it was demonstrated that Oleuropein was more effective to prevent cancer lesions in the rat tongue when administered concomitantly with 4-NQO rather than when it was given after the stoppage of the carcinogen.[22] The mechanisms by which Oleuropein prevent the DNA damage and the cancer initiation *in vivo* are actually not known but, in addition to its anti-oxidant potential, it may interfere with other cellular systems involved in this phenomenon. For instance, it

should be underlined that both AOM and 4-NQO initiate the carcinogenesis process after metabolic transformation to highly reactive intermediates that form adducts to DNA. Therefore, Oleuropein could prevent the initiation phase by inhibiting either the formation of reactive intermediates of the carcinogen metabolism or their interaction with DNA. Indeed, Oleuropein was able to inhibit the hepatic xenobiotic metabolism in human microsomes in vitro.[46] Further studies are needed to shed light on these aspects.

The inhibitory effects of Oleuropein on carcinogenic promotion/progression phases have been studied on animal models in which cancer cells are transplanted subcutaneously into susceptible animals and then the xenograft growth is followed over time. By using these experimental strategy, two studies have investigated the effect of Oleuropein on tumor development with opposite results.[24,27] In one case, using a breast cancer model in which MCF-7 cells (human breast tumor cells) were injected in the mammary fat pads of ovariectomised nude mice, it was observed a clear inhibitory effect of Oleuropein on xenograft tumor growth.[24] At the same time, Oleuropein efficiently prevented both the mice body weight loss and the peri-pulmonary dissemination of tumor masses which infiltrated from the mammary fat pad injection site. Furthermore, Oleuropein was able to reduce the intrapulmonary metastases dissemination.[24] In the other case, no effect of Oleuropein was reported on tumors growth derived by subcutaneous injection of C6 glioma cells on dorsal flanks of rats.[27] In addition, in this study it was observed that while hydroxytyrosol treatment efficiently reduced the tumor volume, the combination of hydroxytyrosol with Oleuropein nullified this effect. Furthermore, although the results were not reported, the authors argued that the prolongation of Oleuropein treatment and the increment of its concentration caused a stimulation of tumor growth.[27] These results are in sharp contrast with those reported in previous studies, therefore they deserve more in-depth research to be clarified.

Finally, a recent research has strongly supported the ability of Oleuropein to prevent both intestinal inflammation and colitis associated tumorigenesis in mice.[28] In this model, mice were co-exposed to AOM and DSS to induce both colon pro-inflammatory effects and cancer. The

inflammatory response was evidenced by the increment of several markers such as IL-6, IFN-γ, TNF-α, IL-17A and COX-2, all of which were efficiently reduced by including Oleuropein in the drinking water. Associated to these effects, AOM/DSS treatment caused also colon tumors development in all animals with an incidence of 100% in control group. This figure was reduced to 64% and 16% by treatment with Oleuropein at doses of 50 and 100 mg/kg, respectively. Oleuropein reduced also the number and dimension of tumors, inhibited the proliferation of neoplastic cells as assessed by the reduced expression of Ki-67 and influenced the cell death by up-regulating the pro-apoptotic factor Bax.[28] Furthermore, regarding the possible molecular mechanisms involved, it was observed that Oleuropein inhibited different pathways implicated in colorectal cancer upset such as activation of nuclear factor-κB (NF-κB) and Wnt/β-catenin, and phosphorylation of Akt and STAT-3.[28] The inhibitory effect of Oleuropein on Wnt signaling is of particular interest since an activating mutation of the Wnt/b-catenin pathway, mostly involving the adenomatous polyposis coli (APC) gene, is the first step in almost all colorectal cancers.[47] However, it is important to underline that in contrast with the above reported data, other studies have shown that Oleuropein stimulated the canonical Wnt signaling pathway which culminate in the increase of the β-catenin level in the nucleus.[48,49] This effect was correlated in one case to adipogenesis[48] and in the other to the hair follicle regeneration.[49] In any case, whether exist a relationship between these different effects and the identification of precise molecular mechanism by which Oleuropein interfere with these systems remain to be elucidate.

3.2 Hydroxytyrosol

From the systematic search of the literature, 5 studies were identified which tested the chemopreventive activity of hydroxytyrosol on animal models (Table 1).[19,21,23,27,29] The first study was carried out by investigating the effect of hydroxytyrosol on the human colon tumor xenograft growth after subcutaneous injection of HT-29 cells in the right flank of athymic nude mice.[19] Treatment with hydroxytyrosol at the dose of 10 mg/Kg body weight was started 4 days after implantation of HT-29 cells and continued for the following 14 days. In these experimental

conditions, hydroxytyrosol inhibited tumor growth by 50% and caused a reduction of the expression of Ki-67 and an activation of the apoptotic signal caspase-3. These effects were associated to an inhibition of tumor angiogenesis as evidenced by a clear reduction of vessels size, and a variation of vessels morphology and maturation. From the molecular point of view, it was found that hydroxytyrosol *in vivo* was able to inhibit the expression of hypoxia inducible factor-1α (HIF-1α), vascular endothelial growth factor (VEGF), and microsomal prostaglandin-E synthase-1 (mPGEs-1).[19] Further experiments carried out *in vitro* further support the hypothesis that hydroxytyrosol, by inhibiting ERK1/2 phosphorylation, suppressed the PGE-2/ERK1/2/HIF-1α signaling pathway with the consequent anti-inflammatory and anti-angiogenic effects.[19] In a subsequent investigation by the same authors using the same experimental model, the inhibitory effects of hydroxytyrosol on human colon tumor xenograft growth and Ki-67 expression were confirmed.[29] In addition, it was also found that hydroxytyrosol significantly reduced the epidermal growth factor receptor (EGFR) expression and enhanced its phosphorylation at tyrosine 1045. Furthermore, *in vitro* experiments showed that hydroxytyrosol accelerated EGFR degradation by reducing its half-life in different colon cancer cell lines. The molecular mechanisms involved in the EGFR processing were cell type dependent. Indeed, using specific inhibitors for either the lysosomal or the proteasome systems it was observed that the hydroxytyrosol induced degradation of EGFR in HT-29 and WiDr cells was reversed by the proteasome inhibitor, while in the CaCo2 cells it was affected by the lysosomal inhibitor.[29] Two more studies investigated the effect of hydroxytyrosol on tumor xenograft growth derived from transplantation of either cholangiocarcinoma or glioma cells.[23,27] In the first study, hydroxytyrosol was able to inhibit the growth of cholangiocarcinoma TFK-1 cells xenografts in nude mice.[23] Tumors were allowed to grow up to 0.5 cm^3 of size and then hydroxytyrosol (500 mg/kg/day) was administered by intraperitoneal injection every day for 3 weeks. At the end of treatment, animals exposed to hydroxytyrosol had a significantly reduced tumor size and weight as compared to controls. At the same time, no apparent change in liver, spleen and body weight were observed, so suggesting the hydroxytyrosol was not toxic to animals even when used at such high doses. The

marker of cell proliferation Ki-67 was decreased while apoptosis was increased in the tumor tissue of

hydroxytyrosol treated mice.[23] The expression of several other proteins was also measured by

western blot analysis on tumor homogenates. The data indicated that p-ERK, Pro-PARP, Bcl-2,

cyclin B1 and, p-Cdc2 (Thr15) were decreased in the tumors of hydroxytyrosol treated mice while

cleaved PARP, caspase-9, caspase-3, Bax and p-Cdc2 (Tyr161) were increased by hydroxytyrosol

treatment.[23] Similarly, hydroxytyrosol inhibited the xenograft tumor growth derived from

subcutaneous injection of C6 glioma cells in Wistar rats.[27] As above reported, unexpectedly the

cancer suppressive activity of hydroxytyrosol was almost completely abolished by treating the

animals in combination with Oleuropein. In this study several oxidative stress markers were

investigated in serum including both non-enzymatic (reduced glutathione "GSH" and oxidized

glutathione "GSSG") and enzymatic (superoxide dismutase "SOD", catalase "CAT" and glutathione

peroxidase "GPx") antioxidant defense systems. Curiously, also in this case Oleuropein reduced the

anti-oxidant properties of hydroxytyrosol.[27]

Further evidences supporting the chemopreventive activity of hydroxytyrosol *in vivo* were

obtained in a chemically (DMBA: 7,12-dimethylbenz[a]anthracene) induced mammary tumor model

in female Sprague–Dawley rats.[21] Due to the high similarity with breast cancer occurring in women,

this animal model has been particularly useful to study the effects of dietary components on this

neoplasia. Mammary tumors were induced by exposure of rats to DMBA, and then, when the tumor

volume reached 2 cm^3, the intervention was started by providing intragastric hydroxytyrosol at 0.5

mg/kg b.w., 5 days/week for 6wk. At sacrifice it was found that hydroxytyrosol reduced breast

tumors volume, its histopathological grade and Ki-67 expression. In addition, by using a microarray

analysis of cDNA from 595 sequences it was found that 99 and 74 genes were significantly up- and

down-regulated by hydroxytyrosol, respectively.[21] RP-PCR validation of the microarray data

confirmed that the expression of 13 genes was influenced by hydroxytyrosol, 6 genes were up-

regulated and 7 genes were down-regulated. The most impressive effect of hydroxytyrosol treatment

was observed on the expression of the gene coding for the secreted frizzled-related protein 4 (SFRP4)

13

which was increased by 12 times.[21] This is an important effect on SFRP mRNA which needs to be confirmed at the protein level. The SFRP (1-5) are a family of glycoproteins that acting as extracellular Wnt antagonist ligands have been implicated in the oncogenesis process. Down-regulation of SFRPs has been found in different cancers, including breast cancer. In particular, SFRP4 inhibits the canonical Wnt pathway with a consequent effects on cell proliferation and apoptosis.[50] It is interesting to note that, recently, the expression of SFRP4 has also been correlated to other diseases, in particular with type 2 diabetes (T2D). Is has been found that SFRP4 was highly over expressed in human pancreatic islets of T2D patients and it seems to be responsible for the reduced insulin secretion.[50] Moreover, it was shown that the serum level of SFRP4 was elevated several years before clinical diagnosis of T2D, so suggesting its potential use as early diabetes marker.[50] Therefore, in contrast to the cancer suppressive activity of SFRP4, in the case of T2D the SFRP4 expression seems to be positively associated to the disease. In any case, it is important to underline that the anti-diabetic properties of hydroxytyrosol have been demonstrated on a Wistar rat model where the diabetes was induced by intraperitoneal injections of alloxan.[51]

3.3 Oleocanthal

Only 2 studies were found to investigate the *in vivo* anti-cancer effect of oleocanthal.[25,30] In the first carcinogenic model, MDA-MB-231/GFP human breast cancer cells were inoculated subcutaneously into the mammary gland fat pad of each animal to generate orthotopic breast tumors. Five days post-injection mice were divided in two groups one of which was treated by intraperitoneal injection of 5 mg/kg body weight of oleocanthal, 3 time a week for 33 days. The mice were monitored by measuring tumor volume and body weight. At sacrifice tumors were excised, weighed and used to measure by immunohistochemistry the expression of Ki-67 and CD31, and by western blot analysis the activation of cMet and PARP.[25] The results demonstrated that Oleocanthal caused a significant reduction of tumor growth starting from the twenty-fourth day until the end of the experiment. The tumor weight at sacrifice was reduced by 60% in the treated group. No effect was observed on animal body weight so suggesting that oleocanthal lacks potential systemic toxicity in athymic nude mice.[25]

Furthermore, it was observed that oleocanthal treatment inhibited mitosis and new vessel formation in the tumor tissue as demonstrated by the reduction of the expression of their markers Ki-67 and CD31, respectively. On the other hand, the marker of apoptotic activation, cleaved PARP, was not affected by oleocanthal. These results indicate, that differently from what has been observed *in vitro* on different cell lines,[52] the *in vivo* anti-cancer activity of oleocanthal in this experimental system was not mediated by apoptosis induction. Instead, it was found that oleocanthal was able to reduce the phosphorylation of c-Met, a receptor with tyrosine kinase activity which seems to be involved in the tumor growth, survival and angiogenesis.[25] The effects of oleocanthal on c-Met signaling pathway has been supported also by *in vitro* experiments showing that this compound inhibited the "Hepatocyte Growth Factor" HGF-induced c-Met activation and its downstream mitogenic signals resulting in a reduction of cell growth, epithelial-to-mesenchymal transition and motility.[25] The anti-cancer activity of oleocanthal has been very recently demonstrated in a mouse model of human hepatocellular carcinoma (HCC).[30] In this model, primarily tumors were growth into the flanks of mice after injection of either HCCLM3 (a HCC tumor cell line) or HCC cells (directly derived from tumor of patients). After 1 week, tumors were excised, diced into 1 mm^3 cubes and then implanted into the left lobes of the mouse livers. To follow the tumor growth in the liver, the HCCLM3 were transfected with a lentiviral vector encoding the luciferase gene (HCCLM3-luc). Once tumors start to growth (as indicated by increment of bioluminiscence signals) the animal were treated with oleocanthal (5 mg/kg/d or 10 mg/kg/d, i.p.) for five weeks. Tumor growth, monitored non-invasively using bioluminescence imaging, resulted strongly inhibited by oleocanthal.[30] In addition, at sacrifice it was observed that oleocanthal treatment caused a reduction of proliferation index (Ki-67-positive cells) and an induction of apoptosis (TUNEL-positive cells). Similarly, an inhibition of tumor growth was also observed in the case HCC patient-derived xenografts expressing high level of p-STAT3. The involvement of STAT3 in the anti-cancer potential of oleocanthal was confirmed by *in vitro* experiments on HCC cell lines where it was shown that oleocanthal reduced STAT3 nuclear translocation and DNA binding, and down regulated its main signals including Cyclin D1, Bcl-2,

survivin and MMP 2.[30] Interestingly, the anti-metastatic potential of oleocanthal has also been

investigated *in vivo* by injecting into the circulation, through the tail veins, the HCCLM3-luc cells.

Lung metastases were evidenced at sacrifice by hematoxylin and eosin staining. The oleocanthal-

treated group had fewer and smaller lung metastases compared to the control group.[30]

3.4 Olive oil phenolic extract and phenolic rich olive oil

Differently from the above reported investigations testing purified single compounds, two more

studies were included in this systematic review, in which animals were treated with a complex

mixture of phenols. In one case, the phenols were derived from olive oil and used as such (olive oil

phenolic extract).[26] In the other case, animals were fed with either an extra-virgin olive oil rich in

phenolic compounds or a rectified olive oil derived from the rich oil deprived of phenols but having

the same fatty acid composition.[17] In the first study, a crude phenolic fraction was obtained from

virgin olive oil by methanol/water extraction. The amount of phenols in the extract was 65.4%

including also lignans (acetoxypinoresinol and pinoresinol). In this model, human adenocarcinoma

HT115 cells were transfected with a luciferase expression vector before to be implanted

subcutaneously in the rear dorsum of mice. Animals were treated by gavage with the phenol extract

at a dose of 25 mg/Kg/day in tree different regimes in which the phenols were given as follow: just 2

weeks before the cells implantation (pre-implantation), 8 weeks after cells implantation (post-

implantation) and 10 weeks for the entire experiment (pre+post). The results demonstrated a

significant reduction of tumor growth in mice treated with all different regimes, even if most evident

effect was obtained in animal treated for the entire experimental time. Similarly, there was a clear

reduction of metastatic deposits measure after 70 days.[26] In the second study, colon carcinogenesis

was induced in F344 rats by treatment with 1,2-dimethylhydrazine (DMH). Animals were fed diets

containing 230 g/kg of oil as follow: i) extra virgin olive oil rich of phenols (EV, 668.6 mg/kg of

secoiridoids), ii) extra virgin olive oil devoid of phenolic compounds (ROO, 5.8 mg/kg of

secoiridoids), iii) corn oil (CO).[17] Part of the animals were sacrificed thirteen weeks after the first

DMH injection to determine early events of colon carcinogenesis, aberrant crypt foci (ACF) and

mucin depleted foci (MDF) while the rest rats were followed for thirty-two weeks to measure the number e dimension of tumors. No significant differences were observed in the three groups of animal for multiplicity of both ACF and MDF. Similarly, the incidence of tumors was not different among the different groups.[17] In addition, no differences were observed regarding both the gravity of dysplasia of the adenomas and the grading of cancers in the three different groups.[17] These results are in contrast with those reported above where oleuropein was able to prevent cancer in mice colon carcinogenic models.[28,31] These different responses may be due to the different species of rodent used, rats in one case and mice in the other. In addition, it should be considered the different types and dose of phenols. Indeed, in the olive oil extract was present the oleuropein aglycone (3,4-DHPEA-EA) and not oleuropein. Further research will be necessary to clarify these points.

3.5 Human intervention trials

While several human intervention trials have been carried out to investigate the effects of olive oil phenols on different markers correlated to cardiovascular and metabolic diseases, very little is known about their effects on parameters more directly correlated to the carcinogenesis process.[12] Indeed, various pre-clinical experimental models have demonstrated that secoiridoids phenols possess anti-oxidant, anti-inflammatory, vasodilatatory, anti-platelet aggregation, anti-ipertensive and anti-atherogenic effects. Although, the systemic anti-oxidant and anti-inflammatory action may be mechanisms by which olive oil phenols exert their cancer preventive activity,[14] the effects on more direct cancer related biomarkers such as DNA damage is certainly more predictive of cancer risk and therefore more indicative of the chemopreventive potential. In this respect, 5 human intervention trials have so far been published investigating the DNA damage preventive activity of olive oil phenols (Table 2).[32-36] These studies were carried out using a randomized double-blinded cross-over design and the intervention was done by exposing the subjects to olive oils containing different amounts of phenols. In the first study, 12 healthy, male, nonsmoking volunteers were recruited and asked to consume 25 mL/d of 3 different olive oils containing increasing doses of phenols over 4 consecutive days.[32] The washout period between the treatments was 10 days. After each treatment,

the oxidative DNA damage was investigated by measuring the presence of 8-oxo-7,8-dihydro-2'-deoxyguanosine (8-oxo-dG) in mitochondrial DNA of mononuclear cells and its excretion in the urine. The results showed a dose-dependent reduction of 8-oxo-dG in both mitochondrial DNA and urine in regard to the increasing doses of phenols contained in the olive oils administered. The intervention with the olive oil containing the highest amount of phenols (486 mg/kg) caused a decrease of 8-oxo-dG in mitochondrial DNA and in urine by 49% and 52%, respectively.[32] A reduction of oxidative DNA damage in lymphocytes from peripheral blood was also observed in healthy postmenopausal women after consumption of a high-phenol extra-virgin olive oil.[33] In this study, participants were asked to substitute all fats and oils generally used with the selected extra-virgin olive oils (EVOO) containing either a low (147 mg/kg) or an high (592 mg/kg) amount of phenols. It was recommended to consume at least 50 g of olive oil per day. The total uptake of olive oil phenols, quantified by a 24 h diet recall interview, resulted approximately four times higher during the high-EVOO consumption as compared with the low-EVOO treatment. The effect of intervention was evaluated on both basal DNA damage (single and double strand breaks) and oxidative DNA damage (oxidized bases) by the single-cell gel electrophoresis (Comet assay). The oxidative damage was quantified by a DNA digestion step using the enzyme formamidopyrimidine DNA glycosylase (FPG), which introduces breaks at sites of oxidized purines (8-oxo-dG). The results demonstrated that basal DNA damage was not affected by treatment whereas a 30% significant reduction of oxidative DNA damage was observed after intervention with high-EVOO.[33] In addition, no differences between high-EVOO and low-EVOO treatment were found on DNA strand breaks induced by exposure *ex vivo* of lymphocytes to hydrogen peroxide.[33] The preventive effects of olive oil phenols on oxidative DNA damage was further supported by a recent intervention study in which virgin olive oil was enriched with its own phenolic compounds.[36] In this case the intervention was carried out on 33 hypercholesterolemic volunteers and the effect was evidenced by measuring the urinary excretion of 8-oxo-dG which was found significantly lower after intervention with the phenol enriched olive oil.[36]

In contrast with the above reported data, another study showed that the urinary excretion of the oxidation products of guanine (8-oxo-guanine, 8-oxo-guanosine and 8-oxo-deoxyguanosine) were not affected by 2 weeks consumption of different olive oils with low, medium, and high phenolic content.[35] This was a large multicenter study carried out on 182 nonsmoking men enrolled in 5 European countries divided into Northern (n=54), Central (n=70) and Southern (n=58) Europe. Curiously, this study showed a significant decrease of 8-oxo-dG in all three populations after olive oil consumption regardless of its phenols content. These results suggest that other components in the olive oil may be effective in reducing DNA oxidation. However, the nature of these compounds have not been invesigated.[33] Furthermore, no effect has been observed on the urinary excretion of etheno–DNA adducts after 3 weeks intervention with olive oils containing different amounts of phenols in 28 healthy volunteers.[34] Etheno–DNA adducts (1,N6-ethenoadenine; 1,N6-etheno-2′-deoxyadenosine; 3,N4-etheno-2′-deoxycytidine) are formed by reaction of DNA bases with intermediates originated from the PUFAs peroxidation during oxidative stress and have been used as a biomarkers for lipid peroxidation-derived DNA damage.[34]

3.6 Bio-availability of olive oil secoiridoids

The above reported studies have used different doses of phenols to evaluate their anti-cancer activity. In particular, human trials were performed with olive oils enriched with phenols at doses which ranged from 2.7 mg/kg to 592 mg/kg (Table 1). These amounts may be considered physiological and near the intake of Mediterranean populations.[53] Bioavailability studies in humans demonstrated that olive oil phenols are dose-dependently absorbed (over 50%) and undergo a complex metabolism represented by hydrolysis and conjugation reactions giving rise to their glucuronidated and sulphated derivative which are excreted in urine.[12,54]A further metabolic pathway of hydroxytyrosol catalyzed by catecholmethyltransferases which results in the formation of homovanyl alcohol has also been described.[54,55] Hydroxytyrosol and its main metabolites has been found both in plasma and urine.[12,54] It has been shown that administration of 40 mL of olive oil with a high phenolic content (366 mg/kg) resulted in a plasma concentration of hydroxytyrosol higher than 15 μM.[55] A dose which was

sufficient to prevent DNA damage on peripheral blood mononuclear cells pre-incubated with hydroxytyrosol and then stressed with hydrogen peroxide.[56] Similar to human intervention trials, only in one animal study the phenols were added to the diet as virgin olive oil (668.6 mg/Kg of secoiridoids) at doses which may be representative of human exposure (Table 1).[17] In some other studies, the phenols were included in the animal diet but at doses somewhat higher than those of human consumption.[22,24,31] In the other cases, the exposure was performed through either intragastric gavage or intraperitoneal injection with pharmacological doses of phenols which ranged from 0.3 mg/Kg up to 1000 mg/Kg. The bio-availability and the pharmacokinetic of both oleuropein and hydroxytyrosol has been deeply investigated in animals. In particular, recently the tissue and plasma distribution of hydroxytyrosol and its metabolites in rats in relation to the uptake doses (1, 10, and 100 mg/kg) has been reported.[57] Hydroxytyrosol and its metabolites were found in the plasma in the μmolar concentrations and they accumulated mainly in liver, kidney, and brain at nutritionally relevant human doses.[57] Regarding the preventive effects of Oleuropein toward the colon carcinogenesis[28,31], it is noteworthy that this compound is not efficiently absorbed in the small intestine.[58] Therefore, in the large intestine Oleuropein may reach elevated concentrations, interacts with the gut microbiota and exerts different healthy effects.

4. Conclusions

Most of the studies carried out on different animal models have confirmed the *in vitro* results showing that olive oil phenolic compounds are able to inhibit both initiation and promotion/progression phases of carcinogenesis. The anti-initiation activity is suggested by studies showing that these compounds may prevent DNA damage induced by oxidative stress and other carcinogens, and their anti-cancer effects are more evident whenever they are present during the carcinogen exposure. In addition, the *in vivo* anti-proliferative and pro-apoptotic activities have also been demonstrated by investigating the effects of olive oil phenols on the expression of different molecular markers. On the other hand, human intervention studies have essentially investigated the effects of phenols on DNA damage (initiation phase). Among the five human trials found in the

literature, three have shown a significant preventive effect of phenols on oxidative DNA damage as evidenced by a reduction of urine 8-oxo-dG excretion, of oxidative altered bases in mitochondria DNA of mononuclear cells and in lymphocytes DNA. The other two studies failed to see any effect on urinary excretion of either etheno–DNA adducts or oxidation products of guanine. It is curious to note that all human studies were carried out on non-smokers subjects. It is possible that a more evident protective effects will be observed in subjects who are usually heavily exposed to environmental carcinogens or at workplace or to tobacco smoke. Further research is suggested carrying out intervention studies on human subjects at high cancer risk and investigating the effects of olive oil phenols on other important intermediate cancer biomarkers such as DNA-adducts, micronuclei, sister chromatid exchange and chromosome aberration. These studies will clarify the real chemopreventive potential of olive oil secoiridoids on human populations.

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Legend to the figures

Fig. 1. Chemical structures of phenolic alcohols (hydroxytyrosol and tyrosol) and their secoiridoid derivatives present in olive oil.

Fig. 2. Flowchart of the study selection process

Hydroxytyrosol (3,4-DHPEA)
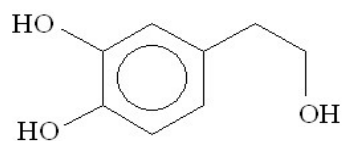
Tyrosol (*p*-HPEA)
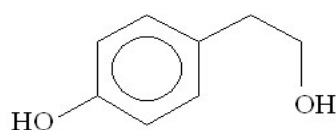
3,4-DHPEA-EA (Oleuropein aglycon)

***p*-HPEA-EA (Ligstroside aglycon)**

3,4-DHPEA-EDA

p-HPEA-EDA (Oleocanthal)

Oleuropein (OLE)
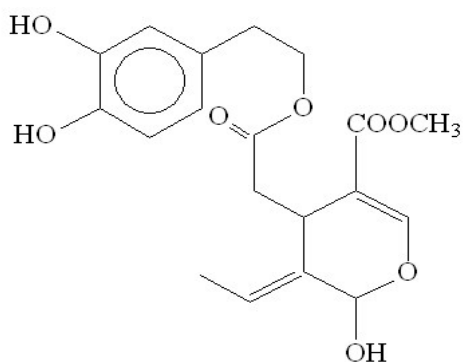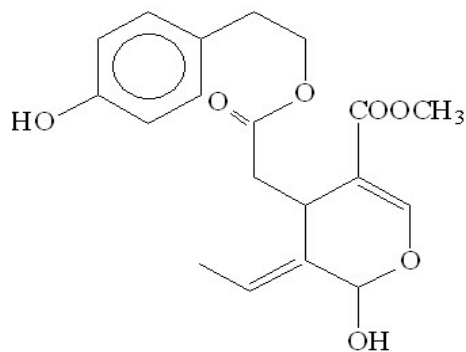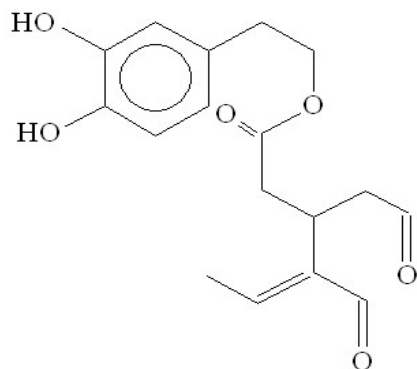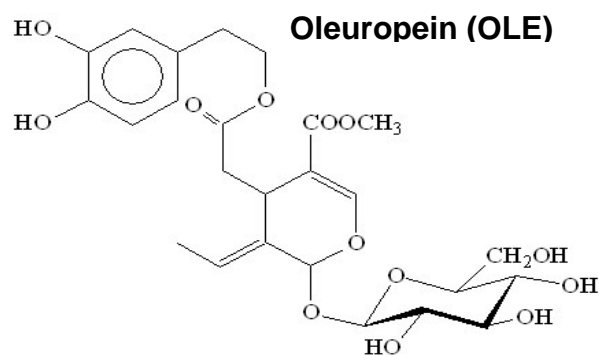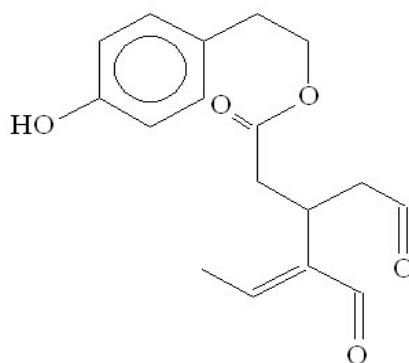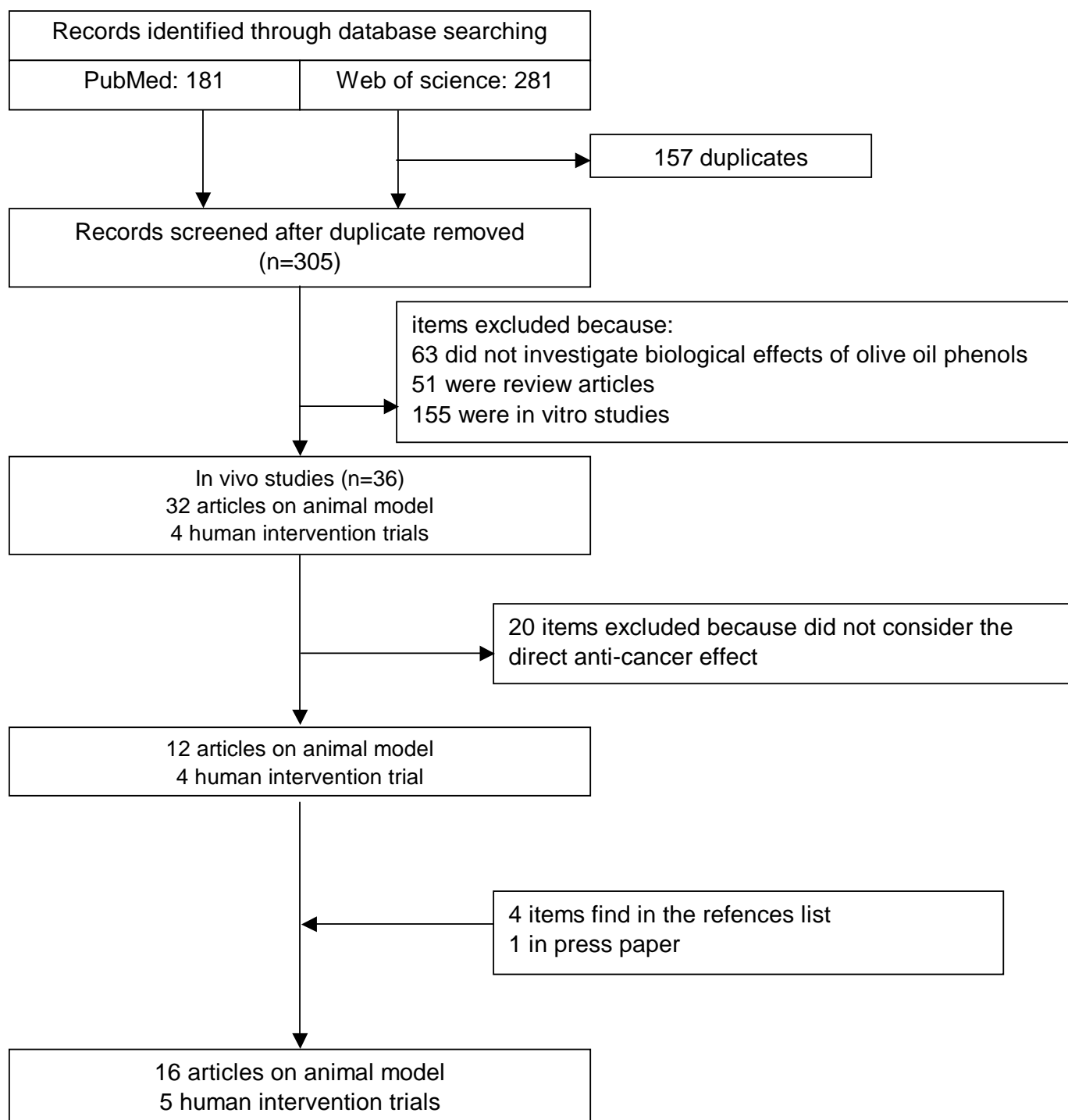
Figure 1

Figure 2

Table 1. Characteristics of selected studies showing direct anti-cancer effects of olive oil secoiridoid phenols on animal models.

Author, year	Animal model/ tumor site	Damaging Agent/Dose/ Treatment regime/ Xenograft origin	Compound/ Treatment/ Number of animals	Exposure	Dose/Time	Effects
Hamdi et al, 2005[16]	Swiss albino mice Soft tissue sarcomas	Spontaneously develop tumors	Oleuropein n=5 control group n=15 treatment group	Drinking water (consumed *ad libitum*)	1%, 9-12 days	Complete tumor regression Cell rounding within the tumor No effect on the vasculature
Femia et al., 2008[17]	Male F344 rats Colon	1,2-dimethylhydrazine:DMH 150 mg/kg x 2 times s.c, 1 week apart	-Extra virgin olive oil (n=32) -Refined olive oil (n=32) -Corn oil (n=33)	23% in the diet	5.8 mg/kg vs. 668.6 mg/Kg (secoiridoids in olive oil) 13 weeks (ACF-MDF) 32 weeks (tumors)	[1]ACF=, [2]MDF= Number of tumors= Gravity of dysplasia of the adenomas= Grading of tumors=
Kimura and Sumiyoshi 2009[18]	Albino hairless HR-1 mice Skin	Long-term UVB irradiation Dose increasing over time from 36 to 180 mJ/cm^2 3 times/wk	-Olive leaf extract (15% Oleuropein) (n=7 in each group) -Oleuropein (n=7 in each group)	Gavage (intragastric) twice daily	300 or 1000 mg/kg 25 or 85 mg/kg 30 weeks	Skin thickness⇓ Skin elasticity⇑ [3]MMP-2, MMP-9, MMP-13⇓ [4]VEGF⇓ [5]COX-2 ⇓ Incidence and volume of skin tumors⇓
Terzuoli et al., 2010[19]	Female athymic nude mice	Subcutaneous injection of 10^{10} HT-29 cells into the right flank Human colorectal cancer	Hydroxytyrosol (n=14 in both control and treatment groups)	i.p. daily	200 μg/mouse, (10 mg/kg b.w./day) 14 days	Tumor volume⇓ Ki-67⇓, CD40⇓, cleaved caspase-3⇑ Vessel number = Vessel size in tumors⇓ [6]mPGEs-1, VEGF, [7]HIF-1α protein⇓
Sumiyoshi and Kimura 2010[20]	C57BL/6J mice Skin	Strong UVB radiation daily at a dose of 120 mJ/cm^2 for the first 5 days and then every other day for 9 days	-Olive leaf extract (15% Oleuropein) (n=6 in each group) -Oleuropein (n=6 in each group)	Gavage (intragastric) twice daily	300 or 1000 mg/kg 25 or 85 mg/kg 14 days	Skin thickness⇓ Ki-67⇓ [8]8-oxo-dG positive cell⇓ Melanin granule area⇓ MMP-13⇓
Granados-Principal et al., 2011[21]	Female Sprague–Dawley rats Breast	7,12-Dimethyl benz[a]anthracene (DMBA) 100 mg/kg body weight, intragastric	Hydroxytyrosol (n=10 in each group)	Gavage (intragastric)	0.5 mg/kg b.w. 5 days/week 6 weeks	Tumor volume⇓, Histopathological grade⇓, Ki-67⇓, [9]SFRP4⇑
Grawish et al., 2011[22]	Male F344 rats Tongue	4-nitroquinoline 1-oxide (4-NQO) solution (20 ppm) in drinking water for 8 weeks	Oleuropein-rich extract (ORE) n=10 control group n=20 treatment group	Diet	3 mg/kg b.w. 31 weeks	Tumor incidence⇓, tumor volume⇓ tumor burden⇓ Incidence of histopathological lesions in the tongue mucosa⇓

						[10]C-Met⇓ and Ki-67⇓ expression
Li et al., 2014[23]	Male nude BALB/c mice	Subcutaneous injection of 5x10^6 TFK-1 cells into the flanks Human cholangiocarcinoma	Hydroxytyrosol (n=3 in each group)	i.p.	500 mg/kg/day every day 3 weeks	Tumor volume⇓ Ki-67⇓ Apoptosis (TUNEL)⇑ P-ERK, Pro-PARP, Bcl-2, cyclin B1, p-Cdc2, (Thr15)⇓ PARP, caspase-9, caspase 3, Bax, p-Cdc2 (Tyr161), ⇑ T-ERK=
Sepporta et al., 2014[24]	Female nu/nu athymic mice Ovariectomised Subcutaneous implant of 17-β E2	Injection of 1x10^6 MCF-7 cells into the mammary fat pads Human breast cancer	Oleuropein (n=10 in each group)	Diet	125 mg/kg of diet 35 days	Tumor growth (volume)⇓ Intra- and peri- pulmonary metastases⇓
Akl et al., 2014[25]	Athymic nude mice	Injection of 1x10^6 MDA-MB-231/GFP cells into the mammary fat pads Human breast cancer	Oleocanthal (n=5 in each group)	i.p.	5 mg/kg b.w. 3 days a week 33 days	Tumor growth (volume and weight)⇓ Phospho c-Met⇓ Cleaved PARP= Ki-67 ⇓, CD31⇓
Hashim et al., 2014[26]	Severe Combined Immuno Deficiency (SCID) Balb-c mice	Subcutaneous injection of 5x10^6 HT115 cells Human colorectal cancer	Olive oil phenolic extract (n=5 in each group)	Gavage	25mg/Kg b.w.	Tumor volume⇓ Metastatic deposits⇓
Martínez-Martos et al., 2014[27]	Male adult Wistar rats	Injection of 5x10^6 C6 cells into the dorsa flanks Rat glioma	-Hydroxytyrosol -Oleuropein -Ole. + Hydr. (n=8 in each group)	Subcutaneous injections	100 µg oleuropein (~0.3 mg/kg b.w.) 100 µg hydroxytyrosol 100 µg Ole. + 100 µg Hydr. 5 days	Tumor growth (volume) - hydroxytyrosol⇓ - oleuropein= - Ole. + Hydr.= Oxidative stress marker in serum: [11]TBARS, carbonyl groups, GSH, GSSG, SOD, CAT, GPx
Giner et al., 2016[28]	Female C57BL/6 mice Colon	Azoxymethane (AOM), a single i.p. injection (7.5 mg/kg) at day –7 followed by Dextran sulfate sodium (DSS), three cycles started at day 0 (7 days of 1.5% DSS and 14 days of fresh tap Water)	Oleuropein (n=10 in each group)	Drinking water	50-100 mg/Kg 63 days	Incidence of tumors⇓ Multiplicity of tumors⇓ Ki-67 ⇓ IL-6, IFN-γ, TNF-α, IL-17A, COX-2⇓ Bax⇑ Nuclear p65 NF-κB, nuclear β-catenin⇓ p-STAT3, p-Akt⇓

Terzuoli et al., 2016[29]	Female athymic nude mice	Subcutaneous injection of 10^{10} HT-29 cells into the right flank Human colorectal cancer	Hydroxytyrosol (n=3 in each group)	i.p. daily	200 µg/mouse, (10 mg/kg b.w./day) 14 days	Tumor volume⇓ Ki-67⇓ EGFR⇓, p-EGPR tyr1045⇑
Pei et al., 2016[30]	Male BALB/c athymic nude mice	4×10^6 HCCLM3-luc cells injected into the flanks After 1 week - 1 mm^3 cubes, implanted into the left lobes of the livers Orthotopic HCC patient derived xenografts Metastasis model 3×10^6 HCCLM3-luc cells injected into tail vein	Oleocanthal (n=6 in each group)	i.p.	5 mg/kg or 10 mg/kg b.w. five weeks	Tumor growth (volume and photon counts)⇓ Apoptosis (TUNEL)⇑ Ki-67⇓ Tumor growth on [12]HCC patient-derived xenografts expressing high level of p-STAT3⇓ Number and dimension of metastatic lung foci⇓
Sepporta et al., 2016[31]	Female A/J mice Colon	Azoxymethane (AOM) 10 mg/kg body weight (prepared in 0.9% saline just before use) via an i.p. injection once a week for 6 weeks	Oleuropein n=10 control group n=12 treatment group	Diet	125 mg/kg of diet 7 and 17 weeks	Severity of colon crypts dysplasia⇓ Tumor incidence⇓ Leukocytes DNA damage⇓

[1]Aberrant crypt foci; [2]Mucin depleted foci, [3]Matrix metalloproteinases; [4]Vascular endothelial growth factor; [5]Cyclooxygenase-2; [6]Microsomal prostaglandin-E synthase-1; [7]Hypoxia inducible factor-1α; [8]8-oxo-7,8-dihydro-2'-deoxyguanosine; [9]Secreted frizzled-related protein 4; [10]Hepatocyte growth factor receptor; [11]Thiobarbituric acid-reactive substances; [12]Human hepatocellular carcinoma

Table 2. Characteristics of human intervention trials on DNA damage preventive activity of olive oil secoiridoid phenols.

Author, year, region	Study design	Population	Subjects characteristics	Intervention	Washout	Dose/Time	Effects
Weinbrenner et al, 2004,[32] Spain	Randomised double-blinded cross-over trial	Healthy men (n=12)	Nonsmoking Age (years)= 21.1 [2]BMI=22.9	3 olive oils phenolic content: - low: 10 mg/kg - moderate: 133 mg/kg - high: 486 mg/kg	10 weeks	25 mL/d 4 days	[1]8-oxo-dG in mitochondrial DNA of mononuclear cells ⇓ 8-oxo-dG in urine⇓
Salvini et al., 2006,[33] Italy	Randomised cross-over trial	Healthy postmenopausal women (n=10)	Nonsmoking Age (years)= 57.1 BMI=25.1	2 olive oils phenolic content: - low 147 mg/kg - high 592 mg/kg	8 weeks	50 g/d 8 weeks	Oxidative DNA damage in lymphocytes⇓
Hillestrøm et al., 2006,[34] Denmark	Randomized, double-blinded, cross-over trial	Healthy men (n=28)	Nonsmoking Age (years)= 32.1 BMI=23.3	3 olive oils phenolic content: - low 2.7 mg/kg - moderate 164 mg/kg - high 366 mg/kg	2 weeks	25 mL/d 3 weeks	Urinary excretion of the etheno–DNA adducts: [3]εAde= [4]εdA= [5]εdC=
Machowetz et al., 2007[35] Denmark Finland Germany Italy Spain	Multicenter, randomized, double-blind, cross-over trial	Healthy male volunteers Southern (n=58) Central (n=70) Northern (n=54)	Nonsmoking Age (years)= 32 BMI=24.8 Age (years)= 33 BMI=23.7 Age (years)= 29 BMI=23.2	3 olive oils phenolic content: - low 2.7 mg/kg - moderate 164 mg/kg - high 366 mg/kg	2 weeks	25 mL/d 3 weeks	Urinary oxidation products of guanine: 8-oxo-guanine= 8-oxo-guanosine= 8-oxo-dG=
Romeu et al., 2016[36]	Randomized, double-blinded, cross-over, trial	Hyperlipidemic subjects (n=33, 19 men, 14 women)	Nonsmoking <7 cigarettes/week	2 olive oils phenolic content: - low 2.88 mg total phenol - high 12.59 mg total phenol	2 weeks	25 mL/d 3 weeks	8-oxo-dG⇓

[1]8-oxo-7,8-dihydro-2′-deoxyguanosine; [2]Body Mass Index; [3]1,N6-ethenoadenine; [4]1,N6-etheno-2′-deoxyadenosine; [5]3,N4-etheno-2′-deoxycytidine