Review Article

Anticancer effects of oleuropein

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Abstract

Cancer cells exhibit enhanced proliferation rate and a resistance to apoptosis. Epidemiological studies suggest that olive oil intake is associated with a reduced risk of cancer. Olive oil, olives, and olive leaves contain many polyphenols, including oleuropein. Recently, several studies have demonstrated that oleuropein inhibits proliferation and induces apoptosis in different cancer cell lines. In addition, anticancer effects of oleuropein have been seen in animal studies. These effects are associated with oleuropein’s ability to modulate gene expression and activity of a variety of different signaling proteins that play a role in proliferation and apoptosis. This article summarizes the existing in vitro and in vivo studies focusing on the anticancer effects of oleuropein and its effects on key signaling molecules.

Keywords: olive tree; oleuropein; cancer; proliferation; survival; apoptosis; cell signaling

1. Introduction

Cancer is a complex multifactorial disease, in which normal cells are transformed into malignant cells acquiring several properties including enhanced proliferative and decreased apoptotic capabilities. In a healthy cell, the replication process is highly regulated with several checkpoints that control production of growth factors and the regulation of key signaling molecules, ensuring normal proliferation of cells. In contrast, in cancer cells, such regulatory signals are abolished due to the accumulation of mutations leading to alteration in gene expression as to amplify the production of growth factors (GF) and GF receptors (GFR) on the surface of the cell and influence/modulate downstream key signaling cascades [1]. Elevation in expression of either of GF or GFR, such as epidermal GFR (EGFR) [2], plasma membrane proteins with intrinsic tyrosine kinase (TK) activity, results in enhanced proliferation of these cells and produce signals influencing neighboring cells to promote their own proliferation [1]. GF binding enhances the tyrosine kinase activity of the receptor leading to autophosphorylation of the tyrosine residues. The phosphorylated tyrosine residues of the receptor act as docking sites for intracellular proteins containing Src-homology 2 (SH2) domains, leading to a downstream activation of intracellular signaling cascades such as the phosphatidylinositol 3-kinase (PI3K)-Akt [3–7] and the Ras-mitogen-activated protein kinase (MAPK) [8–10] cascades, that result in enhanced proliferation and inhibition of apoptosis. Enhanced cell proliferation demands an increased rate of protein synthesis. Mammalian target of rapamycin (mTOR) is a serine/threonine kinase that is responsible for sensing and integrating numerous environmental and nutritional signals and when activated leads to increased protein synthesis, proliferation, and survival [11,12].

In a normal cell, when damage to DNA occurs, specific checkpoints are put in place and monitor any damage leading to arrest in the progression of the cell in the replication cycle [13]. The tumor suppressor protein, p53, is responsible for monitoring cell damage [14–18]. Once cell damage is detected, p53 arrests the cell’s replication until the DNA damage can be repaired. However, if the damage to the cell is beyond the repair capacity of the cell then p53 will initiate apoptosis (programmed cell death) [14–18]. Stress such as ultraviolet and ionizing radiation, oxidative stress, chemical insults to DNA,
Anticancer Effects of Oleuropein

The main phenolic profile of virgin olive oil consists of phenolic acids and derivatives, phenolic alcohols, secoiridoids, lignans, and flavonoids [24]. The olive fruit contains all of the aforementioned phenolic compounds except lignans [24,25]. Secoiridoids are the most abundant compounds found in olive oil, and are exclusively present in Oleaceae family. Secoiridoids found in olive oil consist of the dialdehydic form of decarboxymethyl elenolic acid linked to p-HPEA (p-HPEA-EDA), oleuropein aglycone (3,4 DHPEA-EA), ligstroside aglycone, oleuropein, p-HPEA-derivative, dialdehydic form of oleuropein aglycon, and dialdehydic form of ligstroside aglycon. These compounds are classified as aglycone derivatives of secoiridoid glucosides. They are formed during the mechanical process of oil extraction, by hydrolysis of oleuropein, demethyleoleuropein, and ligstroside [25]. Olives contain high quantity of phenolic compounds (1–3% fresh pulp/weight) [24]. Oleuropein, demethyleoleuropein, ligstroside, and nühenide are the most abundant secoiridoid glucosides in olives [24]. In brief, oleuropein is present in all constituent parts of the plant but accumulates at higher levels in the olives and leaves.

2. Oleuropein structure and biosynthesis

Oleuropein is an ester of hydroxytyrosol containing an oleosidic skeleton and a carbohydrate group (Fig. 1). The oleosidic skeleton is a common characteristic of secoiridoid glucosides of the Oleaceae family [26]. Oleuropein biosynthesis in O. europaea L. is complex and not yet well understood. The carbon skeleton is derived from mevalonic acid. The substrate intermediates are geraniol, 8-oxogeralanial, iridodial, 7-deoxylignanic acid, 7-epiloganic acid, 7-ketologanic acid, 7-ketologanin, oleoside-11-methyl ester, and 7-ß-1-p-glucopyranosyl-11-methyl oleoside. The final step of oleuropein synthesis involves the attachment of tyrosol to 7-b-1-p-glucopyranosyl-11-methyl oleoside, which leads to the formation of ligstroside and 3,4 DHPEA-EDA. Ligstroside is a direct intermediate to oleuropein, whereas 3,4 DHPEA-EDA is converted to oleuropein aglycone then to oleuropein [27,28]. Studies have shown that oleuropein can reach a
concentration of up to 140 mg g⁻¹ of a dry matter in olive fruit and 60–90 mg g⁻¹ (6–9%) of a dry matter in olive leaves [29], while other reports indicate that oleuropein concentration in the leaves is as high as 19% (w/w) [30].

Oleuropein has been reported to have antioxidant, antitumor, anti-inflammatory, cardioprotective, neuroprotective, and hepatoprotective effects [30–34]. The anticancer properties of olive oil secoiridoids have been summarized in a recent review [35]. In the following sections, in vitro and in vivo studies examining the effects of oleuropein have been summarized and sorted by cancer cell type. All published studies relating to the topic of interest were included in this article. The information presented in the text is also presented in a table format to allow the reader to easily extract the information available in the published literature.

3. Anticancer effects of oleuropein: in vitro studies

3.1. Breast cancer

Breast cancer is the most frequent cancer and is a major cause of death among women in the United States [36,37]. It is clearly established that activation of estrogen, and progesterone receptor and human epidermal growth factor receptor 2 signaling plays a role in the development of breast cancer [38,39]. There are several established subtypes of breast cancer that are classified based on sensitivity to chemotherapeutic agents: (1) estrogen receptor positive (ER+); (2) overexpressing human epidermal growth factor receptor 2 (HER2+) which can be ER+ or ER−; (3) triple negative (TN) subtype which is lacking expression of estrogen, progesterone, and HER2 receptors [38]. These different breast cancer cell lines have been used extensively in in vitro studies and enhanced our understanding of breast cancer cell biology. Exposure of HER2 gene-amplified SKBR3, MCF-7, and HER2-negative MCF-7 breast cancer cells to oleuropein aglycone (6.25 – 100 µM, 72 h) resulted in decreased cell viability and apoptosis [40,41] (Table 1). The SKBR3 breast cancer cells were more sensitive toward the effects of oleuropein (by ~5 times) than the HER2-negative MCF-7 breast cancer cells [40]. MCF-7 cells transfected with HER2 (MCF-7/HER2) had a response to oleuropein aglycone treatment that was similar to the response of SKBR3/HER2 cells indicating a potential of oleuropein to be used in HER2+ breast cancer. Importantly, treatment with oleuropein aglycone (100 µM) was found to reverse resistance toward the chemotherapeutic agent, trastuzumab, in SKBR3/Tzb100 cells [40]. This inhibition was associated with reduced HER2 extracellular domain cleavage, HER2 autophosphorylation, and reduced HER2 expression [40,41]. In HER2 overexpressing SKBR3 and MCF-7/HER2, breast cancer cells treated with deacetoxyoleuropein aglycone, ligstroside aglycone, oleuropein glycoside, and oleuropein aglycone (50 µM, 48 h) were significantly more effective (50–55%, ~85–90%, ~90–95%, and ~100% reduction, respectively) than trastuzumab (~50% reduction) and as effective as lapatinib (~95% reduction) at suppressing the levels of fatty acid synthase (FASN), a key enzyme involved in the anabolic conversion of dietary carbohydrates to fat [42]. Inhibitors of FASN have gained considerable attention as they have the potential to be used for the treatment of human malignancies and therefore these effects of oleuropein are of importance.

Exposure of MCF-7 human breast cancer cells to oleuropein (370 µM, for 3, 6, and 12 h) decreased cell viability, inhibited cell proliferation, induced apoptosis, and these effects were associated with increased cell number in G0/G1 phase [43] (Table 1). In another study, exposure of MCF-7 breast cancer cells to oleuropein (10–75 µM, 48 h) inhibited 17β-estradiol (E2)-induced cell proliferation which was associated with inhibition of E2–induced Erk 1/2 activation [44]. Furthermore, oleuropein (10 µM) showed similar effects observed with PD98089, a specific Erk 1/2 inhibitor [44] (Table 1). Exposure of ER-negative MDA-MB-231 (TN) (basal) and MCF-7 (luminal) breast cancer cells to oleuropein (200 µM, 72 h) resulted in a significant inhibition of cell proliferation, an effect associated with cell cycle arrest (S phase) and upregulation of p21, a tumor suppressor gene [45]. In addition, oleuropein induced apoptosis in both breast cancer cell lines which was associated with upregulation of the active form of caspase-3, increased levels of proapoptotic Bax protein and decreased levels of antiapoptotic Bcl-2 and survivin proteins [45]. The levels of the NF-κB downstream effector cyclin D1, an important protein involved in cell cycle progression, were significantly reduced by oleuropein treatment [45]. Similarly, the oleuropein-induced apoptosis in MCF-7 cells was associated with a significant upregulation of the proapoptotic gene p53 and Bax while the levels of the antiapoptotic gene Bcl2 were downregulated [46] (Table 1). Matrix Metalloproteinases (MMP) are enzymes that play a role in promoting metastasis of carcinogenic cells into different tissue, while the tissue inhibitors of metalloproteinases (TIMPS) induce apoptosis. In MDA-MB-231 (TN) human breast cancer cells, oleuropein (370 µM and 24, 48, and 72 h) was able to induce antimitastatic effects associated with an increase of TIMP1, −3, −4 and a downregulation of MMP2 and −9 gene expression [47].

Oleuropein (50, 100, and 200 µM, 72 h) triggered apoptosis in SKBR3 (estrogen receptor (ER)-negative and G-protein coupled receptor (GPER) positive) breast cancer cells [48]. Oleuropein was found to bind and activate GPER resulting in the upregulation of the proapoptotic Bax protein and decreased expression of the antiapoptotic protein Bcl-2 [48] (Table 1). Furthermore, oleuropein activated the mitochondrial apoptotic pathway, which resulted in cleavage of caspase-9, caspase-3, and PARP-1. In addition, oleuropein increased p21 protein content and p53 expression [48]. On the other hand, oleuropein reduced the expression of cyclin D1 [48]. These effects were associated with continued Erk 1, and 2 activation [48]. Although, Erk activation usually suggests increased cell proliferation and inhibition of apoptosis, a few studies have found that Erk induces apoptosis through phosphorylation of...
its downstream effectors such as nuclear factor erythroid 2-related factor 2 (Nrf2)/sestrin-2 pathway [49,50]. Therefore, the increase of Erk activation may be the mechanism involved in the increase of apoptosis induced by oleuropein [48]. It should be noted that the rosemary polyphenol carnosic acid has been shown to have a similar effect in activating Erk [51,52] and inducing apoptosis in prostate cancer cells [52].

### 3.2. Colon cancer

Exposure of human colon HT29 and SW620 cancer cells to oleuropein (10–100 μM, 72 h) resulted in a significant inhibition of SW620 cell proliferation [53] (Table 2). In addition, oleuropein induced apoptosis in both colon cancer cells, effects that were associated with cell cycle arrest (S phase) [53]. Similarly, oleuropein (10–100 μM, 72 h) inhibited cell proliferation and increased apoptosis in HT29 and SW480 human colon adenocarcinoma cell lines that was associated with G2M cell cycle arrest [54] (Table 2). Exposure of HT29, human colon adenocarcinoma grade II cells, to oleuropein (200–800 μM and 24, 48, and 72 h) resulted in a significant inhibition of cell proliferation, an effect associated with cell cycle arrest (S phase). In addition, oleuropein induced apoptosis which was associated with upregulation of p53, and decreased levels of hypoxia-inducible factor-1α (HIF-1α) protein expression [55].

### 3.3. Liver cancer

Oleuropein (20–80 μM, 24 h) significantly decreased cell viability, inhibited proliferation, and induced apoptosis in HepG2
## TABLE 2  

Anticancer effects of oleuropein (OP). In vitro studies: colon, liver, prostate, pancreatic, thyroid, osteosarcoma, mesothelioma, and pancreatic cancer

<table>
<thead>
<tr>
<th>Reference</th>
<th>Cell type</th>
<th>Oleuropein dose/duration</th>
<th>Findings</th>
<th>Mechanism</th>
</tr>
</thead>
<tbody>
<tr>
<td>Notarnicola et al., 2011 [53]</td>
<td>Colon cancer cells: HT29, SW620</td>
<td>10–100 µM and 72 h</td>
<td>↓ proliferation</td>
<td>cycle arrest (S phase)</td>
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<td></td>
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<td>↑ apoptosis</td>
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<tr>
<td>Fernández-Arroyo et al., 2012 [54]</td>
<td>Colon cancer cells: HT29, SW480</td>
<td>10–100 µM and 72 h</td>
<td>↓ proliferation</td>
<td>cycle arrest (G2M)</td>
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<td></td>
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<td></td>
<td>↑ apoptosis</td>
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<tr>
<td>Cárdeno et al., 2013 [55]</td>
<td>Colon cancer cells: HT29</td>
<td>200–800 µM and 24 and 48 h</td>
<td>↓ proliferation</td>
<td>cycle arrest (S phase)</td>
</tr>
<tr>
<td>Yan et al., 2015 [56]</td>
<td>Liver cancer cells: HCC, HepG2, Huh7</td>
<td>20–80 µM and 24 h</td>
<td>↓ viability</td>
<td>p53</td>
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<tr>
<td></td>
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<td></td>
<td>↑ proliferation</td>
<td>HIF-1α</td>
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<td></td>
<td>↑ apoptosis</td>
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<tr>
<td>Acquaviva et al., 2012 [57]</td>
<td>Prostate cancer cells: LNCaP, DU145</td>
<td>100 and 500 µM and 72 h</td>
<td>↓ viability</td>
<td>thiol group modifications</td>
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<td>↑ PARP</td>
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<td>↑ Bax</td>
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<td>↑ Bcl-2</td>
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<td>↑ p-Akt</td>
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<td>↑ heme oxygenase1</td>
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<tr>
<td>Goldsmith et al., 2015 [58]</td>
<td>Pancreatic cancer cells: MiaPaCa-2</td>
<td>Olive leaf extract 200 µg/kg containing 20 nM oleuropein, 98 h</td>
<td>↓ viability</td>
<td></td>
</tr>
<tr>
<td>Bulotta et al., 2013 [59]</td>
<td>Thyroid cancer cells: Tpc-1, BCPAP</td>
<td>10, 50, and 100 µM and 48 h</td>
<td>↓ proliferation</td>
<td>p-Akt</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td>↓ H2O2</td>
<td></td>
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<tr>
<td>Morana et al., 2016 [60]</td>
<td>Osteosarcoma cells: MG-63, Saos2</td>
<td>↑ cytotoxicity</td>
<td>MG-63 and Saos2</td>
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<td></td>
<td></td>
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<td>(IC50 247.4-475.0 and 798.7-359.9 µM respectively)</td>
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<tr>
<td>Anter et al., 2011 [61]</td>
<td>Leukemia: HL60</td>
<td>40–640 µM and 72 h</td>
<td>↓ viability (IC50</td>
<td>p-Akt</td>
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<td>170 µM)</td>
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<td>↑ DNA fragmentation</td>
<td>p-Erk</td>
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<td></td>
<td>↑ apoptosis</td>
<td>H2O2 -induced ROS</td>
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<td></td>
<td></td>
<td></td>
<td>cycle arrest</td>
<td>CCND1, −2, −3</td>
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<td></td>
<td></td>
<td></td>
<td>↑ CDK4, −6</td>
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<td>↑ p53</td>
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<td></td>
<td></td>
<td></td>
<td>↑ CDK inhibitors</td>
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<tr>
<td>Segme et al., 2016 [62]</td>
<td>Neuroblastoma cells: SH-SY5Y</td>
<td>25–800 µM and 72 h</td>
<td>↓ proliferation</td>
<td>cytosolic Ca2+</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td>↑ apoptosis</td>
<td></td>
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<tr>
<td>Marchetti et al., 2015 [63]</td>
<td>Mesothelioma cells: REN</td>
<td>1–100 µg/mL and 48 h</td>
<td>↓ proliferation</td>
<td>cytosolic Ca2+</td>
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<td></td>
<td>cycle arrest</td>
<td>CCND1, −2, −3</td>
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<td></td>
<td>↑ CDK4, −6</td>
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<td></td>
<td>↑ p53</td>
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<td></td>
<td></td>
<td></td>
<td>↑ CDK inhibitors</td>
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<tr>
<td>Lamy et al., 2016 [64]</td>
<td>Glioblastoma cells: U-87 MG</td>
<td>100 µM and 24 h</td>
<td>No change in inflammation</td>
<td>No effect on TNF-α induced (COX)-2</td>
</tr>
</tbody>
</table>
and Huh7, hepatocellular carcinoma (HCC) cell lines [56]. This apoptotic activity of oleuropein was associated with the upregulation of activated caspase-3, caspase-8, caspase-9, PARP, Bax protein and with the downregulation of antiapoptotic Bcl-2 protein in HepG2 liver cancer cells [56]. Furthermore, treatment of HepG2 cells with oleuropein (60 μM, 24 h) decreased the levels of phosphorylated Akt (S473) and increased intracellular ROS levels [56].

### 3.4. Prostate cancer

Treatment with oleuropein (100 and 500 μM, 72 h) reduced cell viability, proliferation, and induced necrotic cell death in LNCaP and DU145 prostate cancer cells [57] (Table 2). Furthermore, oleuropein reduced the phosphorylation of Akt and increased thiol group modifications, γ-glutamylcysteine synthetase, reactive oxygen species, and upregulation of heme oxygenase-1 (HO-1) protein expression [57].

### 3.5. Pancreatic cancer

Exposure of MiaPaCa-2 human pancreatic cancer cells to 200 μg/mL (98 h) of crude olive leaf extract containing 20 nM of oleuropein resulted in the reduction of cell viability [58]. Despite this very low concentration of oleuropein, the crude leaf extract was still able to significantly reduce the viability of human pancreatic cancer cells (<1% of control) and was significantly more toxic compared to the chemotherapeutic drug, gemcitabine (47.8% of control) [58].

### 3.6. Thyroid cancer

Exposure of Tpc-1 and BCPAP thyroid cancer cell lines to oleuropein (10, 50, and 100 μM, 48 h) and its acetylated form significantly inhibited proliferation of both cell lines, and reduced Akt and Erk phosphorylation levels and H2O2-induced ROS levels [59].

### 3.7. Osteosarcoma

Exposure of MG-63 and Saos2 human osteosarcoma cells to oleuropein resulted in cytotoxic effects on both MG-63 (IC50 247.4–475.0 μM) and Saos2 (IC50 798.7–359.9 μM) cells [60].

### 3.8. Leukemia

Exposure of HL60 human promyelocytic leukemia cells to oleuropein (40–640 μM, 72 h) reduced cell viability in a dose-dependent manner with an IC50 value of 170 μM. These effects were associated with induction of the apoptotic pathway, with DNA fragmentation [61] (Table 2).

### 3.9. Neuroblastoma

Exposure of SH-SY5Y neuroblastoma cells to oleuropein (25–800 μM, 72 h) resulted in reduced cell proliferation, induction of apoptosis, and cell cycle arrest with an IC50 of 350 μM [62]. These effects were associated with downregulation of cyclin D1 (CCND1), CCND2, CCND3, cyclin-dependent kinase (CDK)-4, CDK6 gene expression, and upregulation of p53 and CDK inhibitor (CDKN1A, CDKN2A, and CDKN2B) gene expression. Furthermore, oleuropein induced apoptosis by inhibiting Bcl2 and activating Bax, caspase-9, and caspase-3 gene expression [62]. Also, oleuropein decreased invasion, migration, and colony formation in SH-SYSY cells [62].

### 3.10. Mesothelioma

Exposure of mesothelioma REN cells to oleuropein (1–100 μg/mL for 48 h) inhibited their proliferation (IC50 22 μg/mL). Additionally, oleuropein treatment increased cytosolic Ca2+ concentration by targeting T-type Ca2+ channels which are involved in tumor cell progression and proliferation [63].

### 3.11. Glioblastoma

Treatment with oleuropein (100 μM, 24 h) did not significantly prevent TNF-α induced expression of cyclooxygenase (COX)-2 in human U-87 glioblastoma cells (U-87 MG) [64]. COX-2 is a contributor to the inflammatory process and evidence indicate that it promotes tumor formation and glioma recurrence and this study indicates COX-2-independent effects of oleuropein [64].

### 4. Anticancer effects of oleuropein: in vivo animal studies

#### 4.1. Breast cancer and glioma

A limited number of studies have examined the effects of oleuropein administration on tumor growth in animals in vivo (Table 3). Administration of oleuropein (125 mg/kg b.w) to ovariectomised nude mice xenografted with MCF-7 human breast cancer cells resulted in inhibition of xenograft tumor growth and metastases and efficiently prevented both the mouse body weight loss and the peripulmonary dissemination of tumor masses [65] (Table 3). In contrast to the above studies, administration of oleuropein (0.3 mg/kg b.w) to male adult Wistar rats xenografted with C6 glioma cells did not result in inhibition of xenograft tumor growth [66] (Table 3).

#### 4.2. Colon cancer

A study done on azoxymethane (AOM)-induced leukocyte DNA damage in A/J mice showed that inclusion of oleuropein (125 mg/kg and 7 and 17 weeks) into the basal diet almost completely prevented the AOM-induced DNA damage, and preneoplastic lesions in different colon segments reducing the severity of crypt dysplasia and tumor incidence in the medial colon segment [67] (Table 3).

In a recent study, mice (C57BL/6) were co-exposed to dextran sulfate sodium (DSS) and AOM to induce proinflammatory effects and cancer [68]. The inflammatory response was shown by the increase in markers of inflammation such as IL-6, IFN-γ, TNF-α, IL-17A, and COX-2 levels in colon tissue [68]. Administration of oleuropein (50 mg/kg or 100 mg/kg b.w) to these animals resulted in a decrease of markers of inflammation and reduction in colon tumor development (50 mg/kg: 64% and 100 mg/kg: 16%) [68]. In addition, oleuropein also reduced the number and dimension of tumors, and inhibited the proliferation of neoplastic cells [68]. These effects of oleuropein were associated with reduced expression of Ki-67 and
4.3. Sarcoma and skin cancer

Hamdi and Castellon found that administration of oleuropein (1% in drinking water, 9–12 days) to Swiss albino mice with soft tissue sarcomas resulted in a complete tumor regression [69]. These effects were associated with induction of cell rounding within the tumor without any effects on the vascular system [69] (Table 3).

Kimura and Sumiyoshi examined the preventative effect of orally administered oleuropein (10 and 25 mg/kg, 30 weeks) on the UVB-induced skin damage and carcinogenesis in male hairless mice (HR-1 mice) [70] (Table 3). Treatment of male HR-1 mice with oleuropein (10 and 25 mg/kg, 30 weeks), administered via gavage twice daily, prevented both the UVB-induced increase in skin thickness and decrease of skin elasticity over a time period of 30 months. In addition, treatment with oleuropein reduced both tumor volume and incidence of histopathological lesions in the tongue mucosa [69].

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**Table 3. Anticancer effects of oleuropein. In vivo animal studies**

<table>
<thead>
<tr>
<th>Reference</th>
<th>Animal cancer model/tumor site</th>
<th>Oleuropein dose/duration</th>
<th>Findings</th>
</tr>
</thead>
<tbody>
<tr>
<td>Seppota et al., 2014 [65]</td>
<td>Female nu/nu athymic mice MCF-7 cells xenografted into the mammary fat pads</td>
<td>125 mg/kg for 35 days</td>
<td>↓ volume of tumor</td>
</tr>
<tr>
<td>Martinez-Martos et al., 2014 [66]</td>
<td>Male Wistar rats C6 glioma cells xenografted into the dorsal flank</td>
<td>100 μg (≈ 0.3 mg/kg b.w.) for 5 days</td>
<td>No effects on tumor volume</td>
</tr>
<tr>
<td>Seppota et al., 2016 [67]</td>
<td>AOM-induced colon cancer A/J mice</td>
<td>125 mg/kg (7 and 17 weeks)</td>
<td>Completely prevented the AOM-induced DNA damage Prevented pre-neoplastic lesions Reduced the severity of crypt dysplasia and tumor incidence</td>
</tr>
<tr>
<td>Giner et al., 2016 [68]</td>
<td>Female C57BL/6 mice Colon</td>
<td>50–100 mg/kg for 63 days</td>
<td>↓ incidence of tumors ↓ multiplicity of tumors ↓ Ki-67 ↓ IL-6, IL-17A, INF-γ, TNF-α, COX-2 ↓ nuclear p65 NF-κB, nuclear B-catenin, p-STAT3, p-Akt ↑ Bax</td>
</tr>
<tr>
<td>Hamdi et al., 2005 [69]</td>
<td>Swiss albino mice Soft tissue sarcomas</td>
<td>1% in drinking water (9–12 days)</td>
<td>Complete tumor regression Cell rounding within the tumor No effect on the vasculature</td>
</tr>
<tr>
<td>Kimura et al., 2009 [70]</td>
<td>Albino hairless HR-1 mice Skin</td>
<td>25 or 85 mg/kg (30 weeks)</td>
<td>↓ Tumor volume, incidence ↓ VEGF ↓ MMP-2, −9, −13 ↓ COX-2</td>
</tr>
<tr>
<td>Sumiyoshi et al., 2010 [71]</td>
<td>C57BL/6J mice Skin</td>
<td>25 or 85 mg/kg, for 14 days</td>
<td>↓ Ki67 ↓ melanin granule area ↓ MMP-13 ↓ 8-Oxo-dG positive cell</td>
</tr>
<tr>
<td>Grawish et al., 2011 [72]</td>
<td>Male F344 rats Tongue</td>
<td>oleuropein-rich extract 3 mg/kg b.w. for 31 weeks</td>
<td>↓ tumor incidence ↓ tumor volume ↓ tumor burden ↓ incidence of histopathological lesions in the tongue mucosa ↓ C-Met and Ki-67 expression</td>
</tr>
</tbody>
</table>
with greater effects (96%) achieved with the highest concentration tested (25 mg/kg) [70]. These effects were associated with inhibition of the expression of VEGF, MMP-2, MMP-9, and MMP-13 and a reduction in COX-2 levels [70]. Another study showed that administration of oleuropein (25–85 mg/kg, 14 days) to C57BL/6J mice exposed to UVB irradiation resulted in inhibition of the UVB-induced skin damage that was associated with decreased in Ki-67 (a cellular marker of proliferation of keratinocytes) and 8-hydroxy-2′-deoxyguanosine-positive cell number (indicator of oxidative DNA damage) and decreased melanin granule area, and MMP-13 expression [71] (Table 3).

4.4. Tongue cancer

Another study shines the light on oleuropein-rich extract ability to prevent the initiation phase of carcinogenesis induced by 4-nitroquinoline 1-oxide (4-NQO) in male F344 rats. Administration of oleuropein-rich extract (3 mg/kg b.w, 31 weeks) to a rat model of 4-NQO-induced tongue cancer resulted in reduced tumor incidence, volume, and burden [72]. The mechanisms by which oleuropein prevented DNA damage and the cancer initiation was associated with decreased C-Met and Ki67 expression [72]. These data showed a preventative effect of oleuropein to reduce cancer lesions in the rat tongue.

5. Conclusions and perspectives

The primary metabolite of oleuropein is hydroxytyrosol, a compound reported to exert anticancer effects [44,73–77], and therefore it is possible that some of the anticancer effects of oleuropein are due to hydroxytyrosol. Exposure of HL60 promyelocytic leukemia cells [73], PC-3 prostate cancer cells [74], MCF-7 breast cancer cells [44,75], and CaCo2 colon cancer cells [76] to hydroxytyrosol (10–100 μM) resulted in a significant inhibition of cell proliferation and induction of apoptosis. In addition, in vitro animal studies have shown that administration of hydroxytyrosol (10 mg/kg b.w) for 14 days to female athymic nude mice resulted in a significant (50%) reduction in tumor size and tumor angiogenesis [78]. In female Sprague-Dawley rats, hydroxytyrosol administration (0.5 mg/kg bw) for 6 weeks inhibited mammary tumor growth [79]. Furthermore, hydroxytyrosol administration (500 mg/kg/day) for 3 weeks resulted in inhibition of cholangiocarcinoma tumor growth [77]. Overall, these studies provide evidence that hydroxytyrosol has anticancer properties, and therefore, it is possible that oleuropein is metabolized/converted to hydroxytyrosol which then induces the anticancer effects.

Administration of a liquid form of olive leaf extract containing 76.6 mg oleuropein and 14.5 mg hydroxytyrosol to healthy volunteers resulted in plasma oleuropein levels of 3.55 ng/mL [80] and based on the molecular weight of oleuropein (540.51 g/mol), this corresponds to 6.56 nM. The in vitro studies included in this review, used oleuropein in the range of 10–100 μM, which is highly unlikely to be achieved in vivo from olive oil consumption. However, as mentioned previously epidemiological studies clearly have shown a reduced cancer risk with olive oil consumption [21,22,81]. Therefore, the anticancer effects of olive oil consumption could be attributed to many different polyphenols/components of olive oil acting in a synergistic, additive manner exerting a potent chemopreventive effect.

Recently, the focus for cancer treatment has been shifted toward finding chemicals/strategies that target specific signaling molecules/pathways that are altered in cancer. The existing studies up to now indicate that oleuropein inhibits the Akt signaling cascade involved in proliferation and survival [56,57,59,68] (Fig. 2). The effect of oleuropein on Erk is not clear, as both inhibition [44,59] and stimulation [48] has been observed in different studies. It is possible that oleuropein’s effect on Erk may be cell specific. In addition, oleuropein has been shown to inhibit HIF-1α [55] (Fig. 2). The current studies available, although limited, indicate that oleuropein has been found to increase the levels of proapoptotic proteins p53 [46,48,55,62] and Bax [45,46,48,56,68] while decreasing the levels of the antiapoptotic protein Bcl-2 [45,46,48,56] (Fig. 2). Therefore, oleuropein may be used to target specific pathways leading to decreased proliferation, decreased cell survival, and induction of programmed cell death (apoptosis).

The exact mechanism by which oleuropein affects the aforementioned signaling molecules is not known and it is not clear whether oleuropein is transported into the target cells by specific transporters or binds to specific receptors. Hamdi et al. found that removing the glucose moiety from oleuropein by β-glycosidase decreased its antiproliferative activity in normal fibroblasts [69]. In addition, co-incubation of human melanoma cells with excess D-glucose reduced the ability of oleuropein to induce cell rounding suggesting that both oleuropein and D-glucose compete for the facilitated diffusion glucose transporter proteins (GLUTs) [69]. It is therefore possible that GLUTs are involved in the transportation of oleuropein into the cell. It is already established that cancer cells display increased glucose uptake and utilization and GLUT isofrom [82,83] expression is enhanced in cancer cells. It is likely that oleuropein may bind to different GLUTs with different affinity which may explain why different cancer cells respond differently to oleuropein treatment. The response may be dependent on the GLUTs expressed in a specific cancer cell.

The structure of oleuropein and hydroxytyrosol involves an aromatic ring, which is a common characteristic of estrogen. The shared structure of oleuropein with estrogen may be the putative mechanism by which this polyphenol competes with estrogen for estrogen receptor (ER) binding. Exposure of MCF-7 (ER+) breast cancer cells to oleuropein (75 μM) resulted in inhibition of cell proliferation and inhibition of Erk 1/2 with no effect on ER gene expression [44]. In ER-negative SKBR3 breast cancer cells, oleuropein was found to activate the G-protein-coupled receptor GPER/GPR30 leading to sustained ERK1/2 activation and induction of apoptosis [48]. The above studies indicate that binding of oleuropein to GLUTs, estrogen receptor, and/or GPER/GPR30 may be some of the
mechanisms by which oleuropein affects downstream signaling cascades leading to anticancer effects.

Oleuropein has been shown by different studies to have antioxidant properties. Increased reactive oxygen species (ROS) production is associated with cancer and therefore it is possible that the anticancer effects of oleuropein are due to its antioxidant effects. In TPC-1 and BCPAP thyroid cancer cell lines, the inhibition of cell proliferation was associated with a reduction of H$_2$O$_2$-induced ROS levels [84]. On the other hand, some studies showed conflicting evidence in regards to the ability of oleuropein to reduce ROS. Exposure of BPH-1 non-malignant cells and LNCaP and DU145 prostate cancer cell lines to oleuropein showed a pro-oxidant effect in cancer cells and an antioxidant effect in nonmalignant prostate cells [57]. Exposure of HepG2 to ROS blockers followed by treatment with oleuropein significantly promoted cell survival indicating that the oleuropein anticancer effects are partially ROS dependent [56]. Based on this conflicting evidence in the literature, the effect of oleuropein on ROS levels remains unclear. It is possible that both a pro-oxidant and antioxidant effect can be exerted by oleuropein and the effect may be cell specific.

Different chemical components of the cell culture media may have scavenging activity against H$_2$O$_2$ or other ROS. Pyruvate, a component found in media, has been found to exert antioxidant effects toward H$_2$O$_2$ [85] and therefore, the presence of pyruvate in the media in all in vitro studies may influence oleuropein action.

The data from the in vitro and in vivo animal studies indicates that oleuropein is able to inhibit both initiation of carcinogenesis and metastasis. Unfortunately, no studies exist where oleuropein was administered in humans. The limited in vivo animal studies are pointing to a strong anticancer potential of oleuropein, and therefore more studies should be performed in the future. Future in vivo animal and human studies should determine effective dose and best route of oleuropein administration and any side effects related to chronic administration.

Overall, as seen in Fig. 2, oleuropein affects key signaling molecules involved in cancer cell proliferation and survival and therefore could be used as an agent to target them. Specifically, the inhibition of Akt and induction of p53 indicate a potential a chemotherapeutic agent. Future research could focus on oleuropein analogs development and investigate their specific effect on cellular signaling pathways.

Only one study had examined the effects of oleuropein on reversing chemotherapy-induced resistance [40], indicating...
that it is possible for oleuropein to be used as a nutraceutical to improve the anticancer effects of current chemotherapeutics. Oleuropein administration may act synergistically with the current traditional chemotherapy treatments and decrease the doses that often lead to toxicity and severe side-effects.

In conclusion, the current literature available provides supporting evidence for the use of oleuropein as an anticancer agent and more systematic in vivo animal and human studies are required to have a better understanding of the anticancer effects of oleuropein and the mechanisms involved.

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