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Effects of Oleuropein on tumor cell growth and bone remodeling: Potential implications for the prevention and treatment of malignant bone diseases

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Prof. L.E. Wold
Editor-in –Chief
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Palermo 10/19/2020

Dear Professor Wold,

Enclosed, please find the last revised versions of our review article "***Effects of Oleuropein on tumor cell growth and bone remodelling….***" (Your ref Ms.No. LFS-D-20-04499R1) which is being submitted for consideration for publication in Life Sciences.

Enclosed also find :

1. An additional file of the revised paper ("marked version") reporting the changes made throughout the text, and which are highlighted in yellow according to the editorial and reviews' suggestions.
2. A file containing the Response to Reviewers

As suggested by the Publisher, we have included the names of up to 5 chemical compounds which recur in the article.

Please let me know if you need any further assistance.

Thank you for your time and consideration in this matter.

Best regards

Sincerely Yours

Gaetano Leto

Response to Reviewers

REVIEWERS' COMMENTS:

Reviewer #1

The authors made the corrections, which increased the clarity of the manuscript. The tables and Figure 3 integrate the information about Ole's studies very well.

The observations are minimal to the format:

Q. *Correctly write the name of the species: it says Lactobacillus Plantarum must be Lactobacillus plantarum)*

Section 4:

a) ... tumor cell in the bone tissue [20,103,104] (Fig. 3) .. On the other hand ...

There is a point left

b) emodelling processes (Fig. 2, Fig. 3). the point is in bold

Format:

4.2. Oleuropein and Epithelial to Mesenchymal transition (EMT).

Use the same format for the arrows in the tables (1, 2 and 3)

In table 1:

In vivo instead of In vivo (in italics)

Says OLe instead of Ole

Explain the symbology (arrows)

In table 2:

In vitro instead of In vitro (in italics)

Some references are in red letters

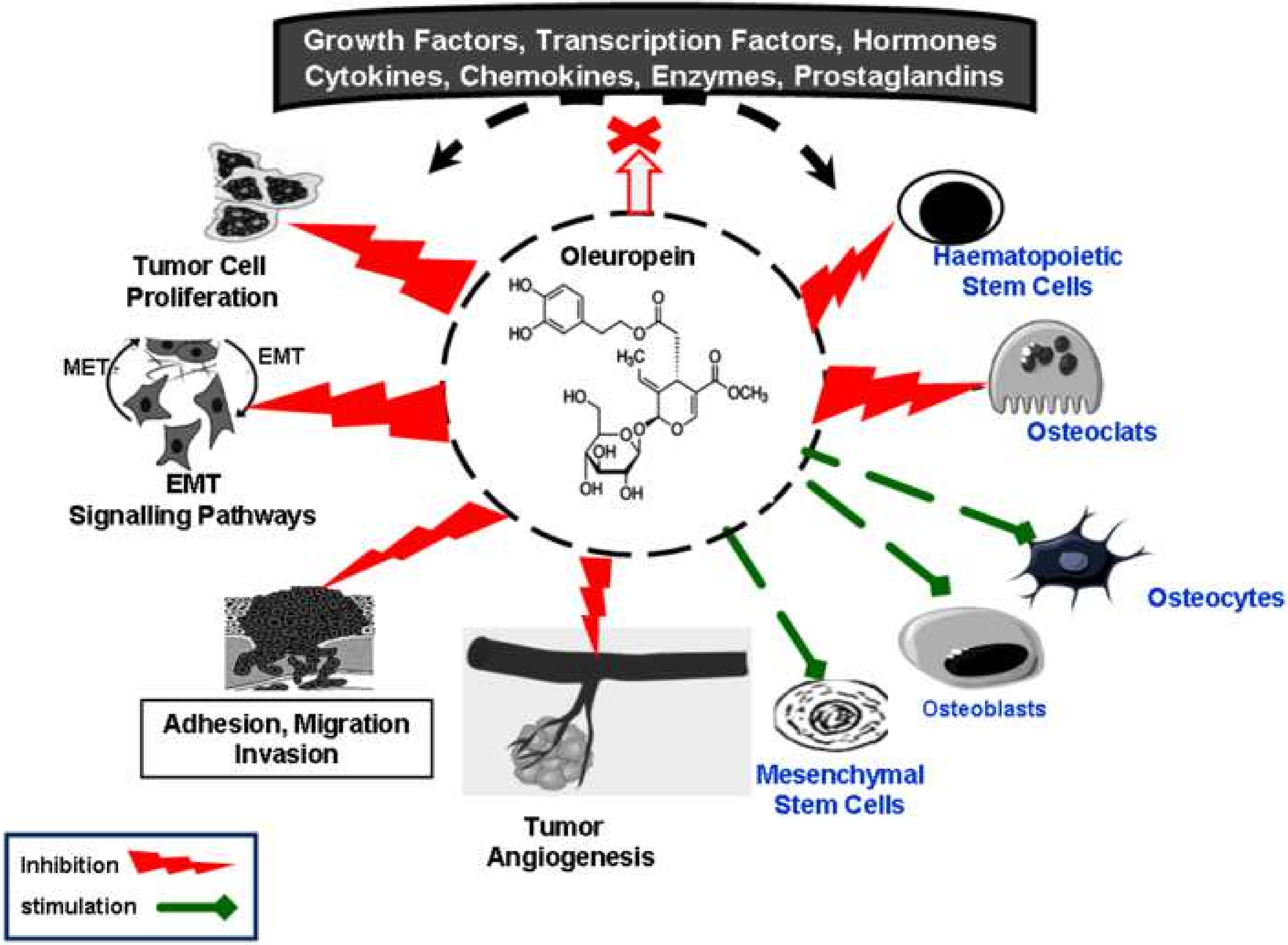
Explain the symbology (arrows)

R. We provided to correct typo and to make the appropriate changes indicated by the review where required. These corrections have been highlighted in yellow in the marked version.

Tables and figures have been revised accordingly

As suggested by the Publisher, we have enriched the text by including the names of up to 5 chemical compounds (highlighted in yellow in the marked version) which recur in the article

Graphical Abstract



Word count 10967 ; Figures 3; Tables 3.;

Effects of Oleuropein on tumor cell growth and bone remodeling: Potential clinical implications for the prevention and treatment of malignant bone diseases

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Abstract

Oleuropein (Ole) is the main bioactive phenolic compound present in olive leaves, fruits and olive oil. This molecule has been shown to exert beneficial effects on several human pathological conditions. In particular, recent preclinical and observational studies have provided evidence that Ole exhibits chemo-preventive effects on different types of human tumors. Studies undertaken to elucidate the specific mechanisms underlying these effects have shown that this molecule may thwart several key steps of malignant progression, including tumor cell proliferation, survival, angiogenesis, invasion and metastasis, by modulating the expression and activity of several growth factors, cytokines, adhesion molecules and enzymes involved in these processes. Interestingly, experimental observations have highlighted the fact that most of these signalling molecules also appear to be actively involved in the homing and growth of disseminating cancer cells in bones and, ultimately, in the development of metastatic bone diseases. These findings, and the experimental and clinical data reporting the preventive activity of Ole on various pathological conditions associated with a bone loss, are indicative of a potential therapeutic role of this molecule in the prevention and treatment of cancer-related bone diseases. This paper provides a current overview regarding the molecular mechanisms and the experimental findings underpinning a possible clinical role of Ole in the prevention and development of cancer-related bone diseases.

Keywords: Bone; Cancer; Chemoprevention; Metastasis; Oleuropein; Polyphenols; Tumor progression

*Chemical compounds***:** Elenolic Acid (PubChem CID: 169607); Hydroxytyrosol (PubChem CID: 82755); Oleuropein (PubChem CID: 5281544); Oleuropein aglycone (Pubchem CID: 56842347); Tyrosol (PubChem CID: 10393)

Abbreviations

BCa, Breast Cancer

BMSCs, Bone Marrow Mesenchymal Stem Cells

BMP, Bone Morphogenic Protein

BPH, Benign prostate hyperplasia epithelial cells

COX-2, Ciclooxygenase-2

CSC, Cancer Stem Cells

CXCRs, CXC Chemokine receptors

Elac, Elenolic Acid

ELAM-1 endothelial-leukocyte adhesion molecule 1

ECM, Extracellular matrix

EMT, Epithelial Mesenchimal Transition

EVOO, Extra Virgin Olive Oil

γ-GCS, γ-Glutamylcysteine Synthetase

Glo2, Mitochondrial Glyoxalase 2

HDAC2, Histone deacetylase 2

HDAC3, Histone deacetylase 3

HIF, Hypoxia Inducibile Factor

HO-1, Heme Oxygenase-1

Hyt, Hydroxytirosol

ICAM-1, Intercellular Adhesion Molecule-1

i-NOS, Inducible Form Of Nitric Oxide Synthase

IL-1β, Interleukin-1β

IL-6, Interleukin-6

IL-8, Interleukin-8

MAPK, Mitogen Activated Protein-Kinase

MCP-1, Monocyte Chemo-Attractant Protein-1

MMPS, Matrix Metalloproteinases

MMP-2, Matrix Metalloproteinases

MMP-9, Matrix Metalloproteinases

MM, Multiple Myeloma

mTOR, Mammalian Target Of Rapamycin

NF-kB, Nuclear Factor kB

NSLC, Non Small Cell Lung Cancer

OLE, Oleuropein.

OLE-A, Oleuropein Aglycone

OB, Osteoblast

OCL, Osteoclast

OPG/RANKL, Osteoprotegerin/Receptor Activator Of Nuclear Factor Kappa-B Ligand Expression Ratio

OS, Osteosarcoma

PARP, Poly (ADP-Ribose) Polymerase
PCa, Prostate Cancer
PTPB1, Protein Tyrosine Phosphatase B1
PTHrP, Parathyroid hormone-related protein
PGs, Prostaglandins
PI3K/AKT, Phosphatidylinositol-3 Kinase/ Protein Kinase B
PPARγ, Perixosomal Proliferator–Activated Receptor
RANK-L, Receptor Activator of NF- kB Ligand
RNI, Reactive Nitrogen Intermediates
ROS, Reactive Oxygen Species
RUNX2, Runt-Related Transcription Factor 2
SOD2, Superoxide Dismutase 2
STAT3, Signal Transducer and Activators of Transcription 3
TIMP, Tissue Inhibitor of Metallproteinase
TNF-α, Tumor Necrosis Factor-α
TGF-ß, Transforming Growth Factor ß,
TRAP, Tartrate-Resistant Acid Phosphatase
VEGF, Vascular Endothelial Growth Factor;
VEGFR, VEGF Receptor
VGSCs blocking voltage-gated sodium channels
V-CAM-1, Vascular Cellular Adhesion Molecule-1
Wnt/β-Catenin, Wingless-Related Integration Site/ B-Catenin

1. Introduction

The skeleton is the third most common site of metastatic disease after lung and liver [1]. Many types solid cancers, in particular, breast, prostate, thyroid, lung, and kidney tumors preferentially metastasize to bone [1,2]. The incidence of bone metastasis by tumor type, reaches 65–90% in prostate cancer (PCa), about 65–75% in breast cancer (BCa), 60% in thyroid cancer, 30-40% in lung cancer, 40% in bladder, cancer, 20-25% in renal cell carcinoma. Skeletal involvement is less frequent in other malignancies such as melanoma (11-17%) and colorectal tumors (10%) [2,3]. The median-survival from diagnosis of bone metastasis from breast cancer, prostate cancer, thyroid cancer are 27 months, 25 months, and 23 months, respectively while, in patients with renal, bladder, lung, and melanoma median survival is lower (12 months, 8 months, 7-9 months and 6 months respectively) [2-4]. Furthermore, in some haematological malignancies such as in multiple myeloma (MM) the rate of incidence reaches 70–95% [4,5]. Interestingly, MM patients diagnosed after 2010 experienced improved clinical outcomes, the 2-year survival rate having increased from 69.9% in 2006 to 87.1% in 2012 [5,6]. On the other hand, primary malignant bone tumors are relatively rare, occurring at a rate of about one to 100,000 [4,5]. In adults, over 40% of primary bone cancers are chondrosarcomas. This is followed by osteosarcomas (28%), chordomas (10%), Ewing tumors (8%), and malignant fibrous histiocytoma and fibrosarcomas (4%). The remainder of cases are several rare types of bone cancer [4,5]. In children and teenagers (those younger than 20 year), osteosarcoma (56%) and Ewing's sarcoma (34%) are much more common than chondrosarcoma (6%) [4,5]. The clinical treatment of primary malignant bone tumors or bone metastasis is currently based on therapeutic approaches encompassing systemic treatments, including chemotherapy, hormone therapy, immunotherapy, bone modifying agents, radiopharmaceuticals, and local treatments such as radiation therapy, radiofrequency ablation and surgery [7,8]. Unfortunately, to date, none of these therapeutic options have shown to exert a positive clinical impact on patients' survival [7,8]. Furthermore, the systemic administration of antitumor drugs and/or bone modifying agents and/or radiopharmaceuticals may negatively affect the normal metabolic turnover of bone tissue with detrimental consequences for cancer patients [9,10]. Hence, the need to identify new compounds that may be effective in preventing the growth and dissemination of malignant cells in the bone and, at the same time, are endowed with low toxicity and/or limited side effects [7,8,11]. In recent years an increasing number of preclinical studies have been directed towards the discovery and the development of new drugs based on natural products from plants environment [12-17]. The advantages of using natural products for therapeutic purposes in cancer treatment are manifold, as these compounds appear, in general, to be *i)* readily available, *ii*) non-toxic to normal human cells, *iii)* may act as multi-target agents since they may affect different signalling pathways that control cancer progression [12-18]. Among the natural compounds, phytochemicals such as polyphenols, terpenoids, alkaloids, phytosterols, and organosulfur compounds have been reported to produce beneficial effects on human health and, in particular, in pathological conditions such as inflammation and cancer [12-14]. In this setting, there is growing experimental evidence highlighting the fact that several extra virgin olive oil (EVOO)-derived phenolic compounds are endowed with anti-proliferative, anti-invasive and anti-metastatic properties [14,17-20]. Therefore, these molecules seem to be promising as potential chemo-preventive agents. In particular, oleuropein (OLe) (Fig.1), one of the main bioactive phenolic compounds present in olive leaves (*Olea europaea L*., Oleaceae), in unprocessed olive drupes and, in the aglycone form, in olive-oil exhibits a broad range of pharmacological properties [17,20-22], which may account for the therapeutic effects of this molecule observed in various human pathological conditions [14,18,20-22] including chronic inflammatory diseases associated with bone loss [20-22] and human cancers [17-19,23-25]. The finding that OLe may thwart the dissemination of cancer cells to distant organs [18,21-23] and exercises protective effects against the onset of various pathological conditions associated with bone loss [20-22] suggests the potential clinical usefulness of this molecule in the prevention and treatment of cancer-related bone diseases. In this review we discuss and critically appraise, in particular, the results from emergent data suggesting a potential role for OLe in the chemoprevention and clinical management of malignant bone tumors.

2. Oleuropein in human tumors

An increasing number of *vitro* studies have shown that OLe may inhibit the growth and survival of cancer cells from different human solid tumors including breast cancer [18,20,22-25] colorectal cancer [20,23-25], genito-urinary cancer [20,23-26], lung cancer [19,23-25], central nervous system tumors [19,20,23-25] melanoma [19,27,28], and haematological malignancies such as leukemias [19,20,29,30]. In line with these observations, *in vivo* studies have highlighted the fact that the administration of OLe to experimental animals may hinder the development of tumors such as skin cancer [31], soft tissue sarcoma [32], melanoma [27,28] or breast cancer [33-36] (Table 1) In particular, the administration of OLe to mice transplanted with B16F10 melanoma or MCF-7 human breast cancer cells, resulted in a significant inhibition of lymph-node or lung metastasis respectively [33-36] (Table 1). The numerous studies carried out in order to elucidate the specific mechanisms underlying the antitumor effects of OLe have highlighted the fact that this molecule, besides its inhibiting activity on tumor cell proliferation and survival [23-26], may also hinder other key steps of malignant progression such as cell migration [16,19,20,24,32,37-40], invasion[19,20,24,32,39,40,43], angiogenesis [20,24, 32,33,44,45] and dissemination of malignant cells to distant organs [15,20,24-26,28,32-36,46,47]. (Fig. 2) In this setting, molecular studies have revealed that OLe can modulate the expression and activity of several growth factors, hormones, cytokines, adhesion molecules and enzymes, which regulate different cellular processes involved in cancer progression including apoptosis [20,24,33,39,42,43,49,50], cell cycle progression [20,26,43,44,50], cell differentiation [20,24,30,51] and epithelial-to-mesenchimal transition (EMT) [20,37,52-56] (Fig. 2). These findings indicate the therapeutic potential of OLe as a chemo-preventive drug that might thwart early events in the metastatic process [24-28,35,36,44-48] (Fig. 2). However, at least *in vivo*, the probable contribution of a few active metabolites of OLe, endowed with anticancer activity such as hydroxytirosol (Hyt), and OLe-aglycone (Ole A) (Fig. 1) may further account for the anticancer activity of this molecule [19,20,23-25,51,57]. In fact, experimental and clinical pharmacokinetic studies have shown that following its absorption in the intestinal tract, Ole undergoes phase I and phase II metabolic processes that generate several metabolites endowed with various pharmacological effects including OLe-A and Hyt [58-61]. In this setting, Mosele et al. [62] have provided evidence on the involvement of some lactic acid bacteria which can be found in human gastrointestinal tract (in particular *Lactobacillus plantarum*) in the metabolic conversion of OLe in OLe-A and then into Hyt.

3. Oleuropein and the bone microenvironment

Experimental and observational studies have provided evidence on the effectiveness of OLe in the prevention and treatment of various pathological conditions associated with bone loss [36,63-72]. These effects appear to be related to the modulating activity of this phenolic compound on multiple common signalling molecules underlying the pathogenesis of malignant and non-malignant bone diseases [7,47,68,71]. On the basis of these observations it appears conceivable to speculate about a potential clinical role of OLe as a drug that might prevent the growth and dissemination of cancer cells in the bone [18,20,38,45-47,54,55,63] **(**Fig 2). In this context, compelling evidence shows that the protective effects exhibited by OLe on bone health appear to be due to the antioxidant [20,21,57-60] anti-inflammatory and immune-modulating properties of this molecule [20-22,67-71]. The antioxidant effects of OLe have been attributed to its ability to prevent the production of reactive oxygen species (ROS) and/or to act as radical chain breakers, and/or as a metal ion chelator [20,21,72]. Instead, the anti-inflammatory and immune-modulatory activities of OLe recognize multiple mechanisms and involve the capability of this compound to modulate the expression and activity of signalling molecules, such as growth factors [20,41,66,67,72-75], transcription factors [18,20,38,71,75-80], hormones [76,81,81], cytokines, chemokines [20,21,55,69,71,73,75] and enzymes [20,43,44,71,75,77], which, in physiological conditions, regulate the normal metabolic turnover of bone tissue while their expression proves to be deregulated in various pathological conditions associated with altered bone remodelling processes [20,44,45,71,82-84]. In particular, preclinical studies have highlighted the fact that the anti-inflammatory and immunomodulatory effects of OLe are the result of its inhibitory activity on nuclear factor-kB (NF-κB) and mitogen activated protein-kinase (MAPk) signalling pathways [20,66,71,79,84,85]. These phenomena eventuate in an attenuated production of various downstream molecular effectors modulating the immune-inflammatory response, such as tumor necrosis factor-α (TNF-α)[20,68,79,80,85,86], interleukin-1β(IL-1β)[20-22,71,85,86], interleukin-6 (IL-6) [20-22,71,86-89], interleukin-8 (IL-8) [8,20,21,47,68,72,88], monocyte chemo-attractant protein-1 (MCP-1) [89], prostaglandins (PGs), in particular prostaglandin E_2 (PGE_2), and enzymes such as cyclooxigenase-2 (COX-2) [20,24,44,70,71,85,86,90], matrix metalloproteinases (MMPs) [20,21,44,45,51,71] and the inducible form of nitric oxide synthase (iNOS) [20,65,71,80,85]. On the other hand, these findings are in line with the results from experimental studies indicating that the bone-protective effects of OLe appear to be related mainly to its modulating activity on inflammatory signalling pathways rather than directly on bone metabolism [63-68]. Nevertheless, recent findings have shown that OLe may facilitate osteoblast (OB) proliferation and differentiation [66,82,91-93], whilst it suppresses osteoclastogenesis [64,65,82,89-91]. These phenomena were associated with an increase in the expression levels of osteogenic transcription factors such as runt-related transcription factor 2 (Runx2) and osterix, with an increased osteoprotegerin/receptor activator of nuclear factor kappa-B ligand (OPG/RANKL) expression ratio [91,94] and the up-regulation of other osteoblastic markers such as type I collagen, osteocalcin or alkaline phosphatase [67,77,94,95]. In particular, OLe elicits osteoprotective effects by fostering the differentiation of human bone marrow mesenchymal stem cells into osteoblasts [91,96] and by reducing tartrate-resistant acid phosphatase (TRAP) activity in osteoclasts formed from mouse spleen cells [65]. Furthermore, recent *in vitro* studies have shown that OLe modulates osteoblastogenesis by suppressing the expression and activity of perixosome proliferator–activated receptor γ (PPARγ),which functions as a transcriptional regulator of adipocyte differentiation and inhibitor of OB [91,96-98].

4. Oleuropein and malignant bone disease progression

Most of the molecular pathways involved in pathological conditions associated with disorders of bone remodeling and which may be targeted by Ole, have also been shown to contribute to the homing and growth of cancer cells in the bone [20,75,99-103] (Fig 3). The findings that various chronic inflammatory bone diseases and bone-related cancers share common molecular mechanisms underlying their pathogenesis [20,75,83,99-103] may, in part, explain the reason why chronic inflammation is actually considered as one of the main pathological processes that may foster the homing and growth of tumor cell in the bone tissue [20,103,104] (Fig. 3). On the other hand, these observations are consistent with the results from several experimental studies showing that chronic inflammation may function as a promoting factor in the early stages of a malignant progression [101, 103-107] (Fig. 3). These data may also, partially, explain the findings that disturbances of the bone microenvironment, induced by osteoporosis and/or other immuno-inflammatory bone diseases, appear to facilitate the growth and dissemination of some types of cancer cells in the bone tissue [106-113]. Ultimately, these observations indicate that OLe might well exert its potential chemo-preventive and therapeutic effects on cancer-related bone disease through multiple mechanisms involved in early events of cancer progression and in the regulation of bone remodelling processes (Fig. 2, Fig. 3).

4.1. Oleuropein and tumor cell proliferation and survival

A consistent body of *in vitro* studies have demonstrated that OLe may hinder the proliferation, survival and migration of malignant cells from human primary bone tumor, such as osteosarcoma [22,38,63,114], and from human tumors which preferentially metastasize to the bone, such as breast cancer [20,23-25,38-40,45,49,115], prostate cancer [20,23-25,116,117] and lung cancer [23-25,117-120]. The mechanisms underlying the anti-proliferative effects of OLe on osteostarcoma cells still need to be fully understood [37,63,114,116]. Conversely, accumulating evidence shows that OLe can inhibit the growth and survival of human breast, prostate or lung cancer cell lines by *i)*delaying or halting different phases of cell cycle progression [20,26,119-123], *ii)*by activating different pro-apoptotic molecular signalling pathways [20,26,40,45,76,113,116-118,123-127] and/or *iii)*by inhibiting the expression of the transcription factor NF-kB which regulates many genes driving cancer growth and progression (Table 2).

4.2. Oleuropein and Epithelial to Mesenchymal transition (EMT) .

OLe has been demonstrated to modulate *in vitro* multiple signalling pathways associated with the different types of EMT, including type 3 EMT [36,46,51-54,119], which has been recognized as an initial, critical event for metastasis formation by individually-invading carcinoma cells [119,122,124,128-131]. Almost all the key regulators of EMT are expressed in the bone microenvironment where their cross-talk contributes to favouring the homing and growth of cancer cells [37,47,51-54,129-131] (Fig. 2, Table 3)**.** In particular, these studies have highlighted the promoting role of signalling pathways such as TGF-β/BMP[52,74,132-134], MAP/ERK[52,120,125,134-136], PI3K/AKT [52,105,137], Wnt/β-catenin [54-56,74,125-131], NF-kB [128,133,137,140,141], HIF [36,142], and PTHrP [142,143] on the growth and dissemination of cancer cells in the bone (Table 3). OLe has been reported to preserve bone health by interfering with various cellular processes triggered by these molecular pathways [20,66,68,71,85,87,92-94,135]. On the basis of these findings, numerous preclinical investigations have been undertaken to assess the therapeutic effectiveness of this molecule, alone or in association with antitumor drugs in the prevention and treatment of several human solid tumors [13,15,43,45,50-53,71,111-113,134].

4.3. Oleuropein and tumor angiogenesis.

Tumor angiogenesis is a crucial event for the growth and dissemination of circulating tumor cells to distant organs including the bones [128-130]. Several experimental observations highlight the fact that OLe appears to thwart tumor neovascularization by decreasing the expression levels of various signaling molecules involved in this process [32,33,36,44, 89,118,123-129,144] (Fig. 2, Table 3). In particular, these investigations have shown that the treatment of endothelial cells [75,76,89,90,143,144] and/or human cancer cells from different tumors [34,35,76,124,125,143,145], with OLe results in a decrease in the expression level of specific growth factors and receptors such as VEGF and VEGFR2 [31,33,36,75,90,118,129], pro-inflammatory cytokines such as IL-6, IL-8, IL-1β TNF-α [19,20,22,48,80,90,143-146], adhesion molecules such as vascular cellular adhesion molecule-1 (VCAM-1) intercellular adhesion molecule-1 (ICAM-1) and E-selectin [20,22,39,52,71,75,145,146], inflammatory and proteolytic enzymes such as COX-2 [31,39,44,71,75,98] and MMPs [31,37,44,45,52,71,75,118,130] (Table 3). Most of these effects can also be attributed to the direct up-stream inhibiting activity of OLe on NF-kB, a transcription factor that modulates the expression of various genes and related signalling pathways involved in almost all the hallmarks of malignant progression [20,39,79,131,132, 143,147].

4.4. Oleuropein and tumor cell migration, adhesion and invasion.

Besides tumor neo-vascularization, some of the molecules mentioned above, such as pro-inflammatory cytokines, adhesion molecules or COX-2 and MMPs, also modulate the adhesion, migration and invasion of tumor cells [44,147-153] (Table 3). For instance, *in vitro* studies have demonstrated that adhesion molecules such as VCAM-1, ICAM-1 and E-selectin contribute to the escaping of tumor cells from dormancy and bone metastasis formation [146,150,152-154]. Moreover, emerging evidence has shown that OLe may indirectly affect the adhesive properties of tumor cells by inhibiting protein tyrosine phosphatase B1 (PTPB1), a regulator of cell adhesion and migration in normal and cancer cells [155,156]. Furthermore, MMP-2 and MMP-9 have been reported to actively contribute to the migratory activity of tumor cells, to the degradation of the extracellular matrix (ECM) and to regulating the expression of cytoskeletal proteins, growth factors, cytokines and chemokines, which favour the preferential adhesion of disseminating cancer cells to the ECM underlying epithelial cells and vascular endothelial cells [44,134,135,150,152]. Consistent with these observations, recent *in vitro* studies demonstrated that the and anti-invasive activity induced by OLe on tumor cells paralleled the down-regulation of MMP-9 and MMP-2 expression and the up-regulation of their intracellular inhibitors TIMP-1,/-3,/-4 [23,31,44,45,55,71,132,143,152] (Table 3)

4.5. Oleuropein and tumor-bone niche

Most of the signalling molecules mentioned above have also been shown to contribute to the formation of a permissive microenvironment, the so-called “(pre)metastatic niche”, which supports the homing, colonization and growth of cancer cells in the bone and, their subsequent survival, dormancy and resistance to clinical treatments [157-159] (Fig. 2, Fig.3, Table 3)**.** Therefore, the targeting of these signalling molecules may be regarded as a novel potential therapeutic tool in the prevention and, eventually, eradication of the establishment of malignant cells in the bone. In this regard, preclinical evidence has suggested the possibility that OLe might thwart the formation of the (pre)metastatic bone niche (Fig. 2). For instance, pro-inflammatory interleukins (ILs) such as IL-6, IL-8, IL-1β, TNF-α have been shown actively to contribute to the homing of malignant cells in the bone [19,20,90,143,147,160-163] (Fig. 3) Ole can down-regulate the expression of these cytokines in several pathological conditions associated with an altered bone resorption and in early events of the metastatic process, namely EMT transition [37,52-55,130,147] and angiogenesis [20,36,75,143,148] (Fig. 3, Table 3). Moreover, proteolytic enzymes such as MMP-2 and MMP-9 have also been shown to contribute to the formation of the “(pre)metastatic niche” by modifying the ECM structure and by promoting the release growth factors and chemokines which fuel the “vicious cycle” of bone metastasis [44,90,130-133,147,152] (Table 3). As described above, OLe has been proven to inhibit the expression levels of MMP-2 and MMP-9 in human metastatic tumor cells while it may increase the expression of intracellular inhibitors of these enzymes, namely TIMP-1, TIM,-3 and TIMP-4 [45,51,54,75,132,152]. Moreover, OLe has been reported to indirectly affect the expression and activity of these proteolytic enzymes through the up-stream inhibition NF- κB [39,51,54,71,148,149,165] (Fig. 3). Furthermore, experimental evidence has shown that, the osteo-protective effects of OLe also appear to be due to its inhibiting activity on RANKL-induced NF-κB activation, a phenomenon which facilitates osteoclast differentiation and bone remodeling [68,70,93,94,161,166]. On the other hand, *in vitro* studies have highlighted the fact that the RANKL/RANK/OPG system appears to exert an active role in the formation of the pre-metastatic niche and in fostering the metastatic spread to bone [7,100,149,153,157,165,166]. On the basis of these observations it is conceivable to hypothesize a potential clinical role of OLe as a novel drug that may thwart the formation of a permissive microenvironment enabling tumor cells to grow and disseminate in the bone [160,165].

5. Discussion

Despite the therapeutic advances registered in recent years, the current treatments for bone-related cancers based on the systemic administration of anti-resorptive drugs and/or anti-tumour agents and/or radiopharmaceutical, still experience several drawbacks, including the negative effects elicited by these agents on the normal bone metabolism. These effects may result in detrimental consequences for cancer patients, such as increased risk of osteoporosis and fractures [7,8,9]. Furthermore, none of the currently available clinical treatments have been shown to exert a positive impact on patients' survival [7,8,9]. Hence, the need to develop new molecules endowed with both antitumor activity and low toxicity and/or limited side effects [12-17,24]. In this setting, an increasing number of preclinical studies are currently aimed at identifying and/or developing new molecules based on natural products from plants [12-17]. In particular, a notable number of preclinical investigations have provided convincing evidence on the chemo-preventive effects of several extra virgin olive oil (EVOO)-derived phenolic compounds, including oleuropein, in combatting the growth of several human solid tumors [17-20,22,23,35,36]. Furthermore, experimental and observational studies have shown that OLe exhibits protective effects on bone health due to its property of modulating various molecular signalling pathways involved in the regulation of bone resorption in normal and pathological conditions, including bone–related tumors [7,16,65-68,75,107-109,143]. Therefore, the results emerging from these studies make this molecule an attractive candidate as a novel potential agent in the prevention and treatment of bone-related cancers [17-20,54,45,53,65,66,143]. Interestingly, as OLe appears to be endowed with both anti-tumor [19,20,23,24,25,35,53,142] and bone-anabolic effects [20,65,66,81,82,91-94,98,143], this molecule might be regarded as a potential, additional therapeutic option, in the treatment of specific tumors such as multiple myeloma (MM), which is known to cause severe osteolytic bone loss due to hyper-activation of osteoclasts and suppression of the differentiation of bone marrow mesenchymal stem cells (BMSCs) into functional osteoblasts [91,96,97,167,168] (Table 3). Furthermore these findings also indicate the administration of OLe may be effective in the prevention of bone loss induced by the systemic administration of antitumor drugs and/or bone modifying agents and/or radiopharmaceuticals [8,9,10,19]. Although, the data from *in vitro* studies are promising in this regard, unfortunately, there is still a lack of *in vivo* studies with the specific aim to evaluate the therapeutic effectiveness of this compound on primary bone tumors or metastatic bone diseases by using suitable experimental animal tumor models. Nevertheless, the accumulating *in vitro* evidence on the capability of OLe to target different molecular pathways involved in the pathogenesis of cancer- related bone diseases, prompts the need for extensive future experimental and clinical investigations.

6. Conclusions

Oleuropein has been reported to target several signaling pathways involved in the modulation of bone remodelling processes in normal and pathological conditions including malignant bone diseases [45,52,53,68,144,161]. These observations suggest the potential usefulness of OLe, administered alone and/or in association with cytotoxic drugs and/ or inhibitors of bone metabolism and/or radiopharmaceuticals to prevent the growth and spreading of cancer cells in the bone [19,20,29,37,38,168]. These drug associations might also result in improved therapeutic activity, in a reduction of side effects and in a decreasing probability of developing drug resistance [19,20,24,28,37,76,169,170]. However, there are several drawbacks in transferring these experimental in clinic data. One of the major drawbacks regards the bioavailabilty and, more in general, the pharmacokinetic properties of OLe [171-174]. The rate of absorption and bioavailability of this molecule appears to be influenced by several factors, such a the route and form of administration, interaction with food, age, sex, different extraction and analytical methods used [171-174]. Experimental and clinical studies have shown that the oral administration of OLe results in rapid absorption, metabolism and renal clearance [117,171-174]. Furthermore, following absorption this compound undergoes extensive phase I and phase II metabolic processes [56,169,173,174]. The potential therapeutic effectiveness of OLe is closely related the possibility that this molecule may reach in adequate concentrations its specific molecular targets in human tissues [20,143]. Therefore, these phenomena may affect the bioavailability of OLe and its systemic transfer at adequate concentrations to the target tissues, ultimately blunting the therapeutic effectiveness of this compound. However, in this context, data on the effective organ distribution of OLe in human tissues including the bone are currently lacking. Nevertheless, a promising strategy to overcome these hurdles may rely on the development of new semi-synthetic OLe derivatives endowed with better bioavailability and possibly, improved biological activity and spectrum of activity [19,20,160,175-178] and by the use of novel drug delivery systems based on of nanotechnology [179-184]. In this latter case, preclinical *in vivo* studies have reported that the nano-encapsulation of OLe could prolong circulation, improve localization, enhance efficacy and reduce the chances of multidrug resistance [117,183-

185]. In conclusion, the increasing number of preclinical *in vitro* studies suggesting a potential chemo-preventive role of OLe in cancer-related bone disease, warrant future studies to assess the impact of this phenolic compound on the clinical management and outcome of patients with primary tumors that reside in, or form metastasis in the bone [13,15,36,43,54,51,67,134,151,156].

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Conflict of Interest statement

The authors declare that there are no conflicts of interest.

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Figure 1.Chemical structure of Oleuropein and its hydrolysis products

Figure 2. **Pleiotropic effects of Oleuropein on cellular and molecular components involved in pre-metastatic niche formation**.

OLe may inhibit ( ) the expression and activity of growth factors, hormones, proinflammatory cytokines, adhesion molecules and enzymes, which regulate different cellular processes involved in cancer progression, including ***i*)**apoptosis, ***ii)***cell cycle progression, ***iii*)**cell differentiation and ***iv***) epithelial-to-mesenchimaltransition (EMT). Most of these signalling molecules have been shown to be deregulated in several pathological conditions associated with bone loss and to contribute to the formation of the "*bone pre-metastatic niche*". On the other hand, OLe has been shown to exerts osteo-protective effects by fostering( ) the differentiation of human bone marrow mesenchymal stem cells into osteoblasts and by inhibiting ( ) osteoclastogenesis.

Figure3 Schematic representation of the effects of Oleuropein on molecular signalling pathways linking chronic inflammation and cancer progression

OLe may modulate the expression and activity of various signalling molecules, such as ***1)*** cytokines, chemokines [20,21,55,69,71,73,75], ***2)*** transcription factors [18,20,38,71,75-80], and ***3***) enzymes [20,43,44,71,75,77] which, in physiological conditions, regulate several biological functions including the normal metabolic turnover of bone tissue while, their expression proves to be deregulated in various pathological conditions associated with altered bone remodelling such as chronic inflammatory processes and malignant bone disorders [7,20,47,68,71,75,83, 99-103,106].

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Effects of Oleuropein on tumor cell growth and bone remodeling: Potential clinical implications for the prevention and treatment of malignant bone diseases

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Abstract

Oleuropein (Ole) is the main bioactive phenolic compound present in olive leaves, fruits and olive oil. This molecule has been shown to exert beneficial effects on several human pathological conditions. In particular, recent preclinical and observational studies have provided evidence that Ole exhibits chemo-preventive effects on different types of human tumors. Studies undertaken to elucidate the specific mechanisms underlying these effects have shown that this molecule may thwart several key steps of malignant progression, including tumor cell proliferation, survival, angiogenesis, invasion and metastasis, by modulating the expression and activity of several growth factors, cytokines, adhesion molecules and enzymes involved in these processes. Interestingly, experimental observations have highlighted the fact that most of these signalling molecules also appear to be actively involved in the homing and growth of disseminating cancer cells in bones and, ultimately, in the development of metastatic bone diseases. These findings, and the experimental and clinical data reporting the preventive activity of Ole on various pathological conditions associated with a bone loss, are indicative of a potential therapeutic role of this molecule in the prevention and treatment of cancer-related bone diseases. This paper provides a current overview regarding the molecular mechanisms and the experimental findings underpinning a possible clinical role of Ole in the prevention and development of cancer-related bone diseases.

Keywords: Bone; Cancer; Chemoprevention; Metastasis; Oleuropein; Polyphenols; Tumor progression

*Chemical compounds***:** Elenolic Acid (PubChem CID: 169607); Hydroxytyrosol (PubChem CID: 82755); Oleuropein (PubChem CID: 5281544); Oleuropein aglycone (Pubchem CID: 56842347); Tyrosol (PubChem CID: 10393)

Abbreviations

BCa, Breast Cancer

BMSCs, Bone Marrow Mesenchymal Stem Cells

BMP, Bone Morphogenic Protein

BPH, Benign prostate hyperplasia epithelial cells

COX-2, Ciclooxygenase-2

CSC, Cancer Stem Cells

CXCRs, CXC Chemokine receptors

Elac, Elenolic Acid

ELAM-1 endothelial-leukocyte adhesion molecule 1

ECM, Extracellular matrix

EMT, Epithelial Mesenchimal Transition

EVOO, Extra Virgin Olive Oil

γ-GCS, γ-Glutamylcysteine Synthetase

Glo2, Mitochondrial Glyoxalase 2

HDAC2, Histone deacetylase 2

HDAC3, Histone deacetylase 3

HIF, Hypoxia Inducibile Factor

HO-1, Heme Oxygenase-1

Hyt, Hydroxytirosol

ICAM-1, Intercellular Adhesion Molecule-1

i-NOS, Inducible Form Of Nitric Oxide Synthase

IL-1β, Interleukin-1β

IL-6, Interleukin-6

IL-8, Interleukin-8

MAPK, Mitogen Activated Protein-Kinase

MCP-1, Monocyte Chemo-Attractant Protein-1

MMPS, Matrix Metalloproteinases

MMP-2, Matrix Metalloproteinases

MMP-9, Matrix Metalloproteinases

MM, Multiple Myeloma

mTOR, Mammalian Target Of Rapamycin

NF-kB, Nuclear Factor kB

NSLC, Non Small Cell Lung Cancer

OLE, Oleuropein.

OLE-A, Oleuropein Aglycone

OB, Osteoblast

OCL, Osteoclast

OPG/RANKL, Osteoprotegerin/Receptor Activator Of Nuclear Factor Kappa-B Ligand Expression Ratio

OS, Osteosarcoma

PARP, Poly (ADP-Ribose) Polymerase

PCa, Prostate Cancer

PTPB1, Protein Tyrosine Phosphatase B1

PTHrP, Parathyroid hormone-related protein

PGs, Prostaglandins

PI3K/AKT, Phosphatidylinositol-3 Kinase/ Protein Kinase B

PPARγ, Perixosomal Proliferator–Activated Receptor

RANK-L, Receptor Activator of NF- kB Ligand

RNI, Reactive Nitrogen Intermediates

ROS, Reactive Oxygen Species

RUNX2, Runt-Related Transcription Factor 2

SOD2, Superoxide Dismutase 2

STAT3, Signal Transducer and Activators of Transcription 3

TIMP, Tissue Inhibitor of Metallproteinase

TNF-α, Tumor Necrosis Factor-α

TGF-ß, Transforming Growth Factor ß,

TRAP, Tartrate-Resistant Acid Phosphatase

VEGF, Vascular Endothelial Growth Factor;

VEGFR, VEGF Receptor

VGSCs blocking voltage-gated sodium channels

V-CAM-1, Vascular Cellular Adhesion Molecule-1

Wnt/β-Catenin, Wingless-Related Integration Site/ B-Catenin

1. Introduction

The skeleton is the third most common site of metastatic disease after lung and liver [1]. Many types solid cancers, in particular, breast, prostate, thyroid, lung, and kidney tumors preferentially metastasize to bone [1,2]. The incidence of bone metastasis by tumor type, reaches 65–90% in prostate cancer (PCa), about 65–75% in breast cancer (BCa), 60% in thyroid cancer, 30-40% in lung cancer, 40% in bladder, cancer, 20-25% in renal cell carcinoma. Skeletal involvement is less frequent in other malignancies such as melanoma (11-17%) and colorectal tumors (10%) [2,3]. The median-survival from diagnosis of bone metastasis from breast cancer, prostate cancer, thyroid cancer are 27 months, 25 months, and 23 months, respectively while, in patients with renal, bladder, lung, and melanoma median survival is lower (12 months, 8 months, 7-9 months and 6 months respectively) [2-4]. Furthermore, in some haematological malignancies such as in multiple myeloma (MM) the rate of incidence reaches 70–95% [4,5]. Interestingly, MM patients diagnosed after 2010 experienced improved clinical outcomes, the 2-year survival rate having increased from 69.9% in 2006 to 87.1% in 2012 [5,6]. On the other hand, primary malignant bone tumors are relatively rare, occurring at a rate of about one to 100,000 [4,5]. In adults, over 40% of primary bone cancers are chondrosarcomas. This is followed by osteosarcomas (28%), chordomas (10%), Ewing tumors (8%), and malignant fibrous histiocytoma and fibrosarcomas (4%). The remainder of cases are several rare types of bone cancer [4,5]. In children and teenagers (those younger than 20 year), osteosarcoma (56%) and Ewing's sarcoma (34%) are much more common than chondrosarcoma (6%) [4,5]. The clinical treatment of primary malignant bone tumors or bone metastasis is currently based on therapeutic approaches encompassing systemic treatments, including chemotherapy, hormone therapy, immunotherapy, bone modifying agents, radiopharmaceuticals, and local treatments such as radiation therapy, radiofrequency ablation and surgery [7,8]. Unfortunately, to date, none of these therapeutic options have shown to exert a positive clinical impact on patients' survival [7,8]. Furthermore, the systemic administration of antitumor drugs and/or bone modifying agents and/or radiopharmaceuticals may negatively affect the normal metabolic turnover of bone tissue with detrimental consequences for cancer patients [9,10]. Hence, the need to identify new compounds that may be effective in preventing the growth and dissemination of malignant cells in the bone and, at the same time, are endowed with low toxicity and/or limited side effects [7,8,11]. In recent years an increasing number of preclinical studies have been directed towards the discovery and the development of new drugs based on natural products from plants environment [12-17]. The advantages of using natural products for therapeutic purposes in cancer treatment are manifold, as these compounds appear, in general, to be *i)* readily available, *ii*) non-toxic to normal human cells, *iii)* may act as multi-target agents since they may affect different signalling pathways that control cancer progression [12-18]. Among the natural compounds, phytochemicals such as polyphenols, terpenoids, alkaloids, phytosterols, and organosulfur compounds have been reported to produce beneficial effects on human health and, in particular, in pathological conditions such as inflammation and cancer [12-14]. In this setting, there is growing experimental evidence highlighting the fact that several extra virgin olive oil (EVOO)-derived phenolic compounds are endowed with anti-proliferative, anti-invasive and anti-metastatic properties [14,17-20]. Therefore, these molecules seem to be promising as potential chemo-preventive agents. In particular, oleuropein (OLe) (Fig.1), one of the main bioactive phenolic compounds present in olive leaves (*Olea europaea L.*, Oleaceae), in unprocessed olive drupes and, in the aglycone form, in olive-oil exhibits a broad range of pharmacological properties [17,20-22], which may account for the therapeutic effects of this molecule observed in various human pathological conditions [14,18,20-22] including chronic inflammatory diseases associated with bone loss [20-22] and human cancers [17-19,23-25]. The finding that OLe may thwart the dissemination of cancer cells to distant organs [18,21-23] and exercises protective effects against the onset of various pathological conditions associated with bone loss [20-22] suggests the potential clinical usefulness of this molecule in the prevention and treatment of cancer-related bone diseases. In this review we discuss and critically appraise, in particular, the results from emergent data suggesting a potential role for OLe in the chemoprevention and clinical management of malignant bone tumors.

2. Oleuropein in human tumors

An increasing number of *vitro* studies have shown that OLe may inhibit the growth and survival of cancer cells from different human solid tumors including breast cancer [18,20,22-25] colorectal cancer [20,23-25], genito-urinary cancer [20,23-26], lung cancer [19,23-25], central nervous system tumors [19,20,23-25] melanoma [19,27,28], and haematological malignancies such as leukemias [19,20,29,30]. In line with these observations, *in vivo* studies have highlighted the fact that the administration of OLe to experimental animals may hinder the development of tumors such as skin cancer [31], soft tissue sarcoma [32], melanoma [27,28] or breast cancer [33-36] (Table 1) In particular, the administration of OLe to mice transplanted with B16F10 melanoma or MCF-7 human breast cancer cells, resulted in a significant inhibition of lymph-node or lung metastasis respectively [33-36] (Table 1). The numerous studies carried out in order to elucidate the specific mechanisms underlying the antitumor effects of OLe have highlighted the fact that this molecule, besides its inhibiting activity on tumor cell proliferation and survival [23-26], may also hinder other key steps of malignant progression such as cell migration [16,19,20,24,32,37-40], invasion[19,20,24,32,39,40,43], angiogenesis [20,24, 32,33,44,45] and dissemination of malignant cells to distant organs [15,20,24-26,28,32-36,46,47]. (Fig. 2) In this setting, molecular studies have revealed that OLe can modulate the expression and activity of several growth factors, hormones, cytokines, adhesion molecules and enzymes, which regulate different cellular processes involved in cancer progression including apoptosis [20,24,33,39,42,43,49,50], cell cycle progression [20,26,43,44,50], cell differentiation [20,24,30,51] and epithelial-to-mesenchimal transition (EMT) [20,37,52-56] (Fig. 2). These findings indicate the therapeutic potential of OLe as a chemo-preventive drug that might thwart early events in the metastatic process [24-28,35,36,44-48] (Fig. 2). However, at least *in vivo*, the probable contribution of a few active metabolites of OLe, endowed with anticancer activity such as hydroxytirosol (Hyt), and OLe-aglycone (Ole A) (Fig. 1) may further account for the anticancer activity of this molecule [19,20,23-25,51,57]. In fact, experimental and clinical pharmacokinetic studies have shown that following its absorption in the intestinal tract, Ole undergoes phase I and phase II metabolic processes that generate several metabolites endowed with various pharmacological effects including OLe-A and Hyt [58-61]. In this setting, Mosele et al. [62] have provided evidence on the involvement of some lactic acid bacteria which can be found in human gastrointestinal tract (in particular *Lactobacillus plantarum*) in the metabolic conversion of OLe in OLe-A and then into Hyt.

3. Oleuropein and the bone microenvironment

Experimental and observational studies have provided evidence on the effectiveness of OLe in the prevention and treatment of various pathological conditions associated with bone loss [36,63-72]. These effects appear to be related to the modulating activity of this phenolic compound on multiple common signalling molecules underlying the pathogenesis of malignant and non-malignant bone diseases [7,47,68,71]. On the basis of these observations it appears conceivable to speculate about a potential clinical role of OLe as a drug that might prevent the growth and dissemination of cancer cells in the bone [18,20,38,45-47,54,55,63] **(**Fig 2). In this context, compelling evidence shows that the protective effects exhibited by OLe on bone health appear to be due to the antioxidant [20,21,57-60] anti-inflammatory and immune-modulating properties of this molecule [20-22,67-71]. The antioxidant effects of OLe have been attributed to its ability to prevent the production of reactive oxygen species (ROS) and/or to act as radical chain breakers, and/or as a metal ion chelator [20,21,72]. Instead, the anti-inflammatory and immune-modulatory activities of OLe recognize multiple mechanisms and involve the capability of this compound to modulate the expression and activity of signalling molecules, such as growth factors [20,41,66,67,72-75], transcription factors [18,20,38,71,75-80], hormones [76,81,81], cytokines, chemokines [20,21,55,69,71,73,75] and enzymes [20,43,44,71,75,77], which, in physiological conditions, regulate the normal metabolic turnover of bone tissue while their expression proves to be deregulated in various pathological conditions associated with altered bone remodelling processes [20,44,45,71,82-84]. In particular, preclinical studies have highlighted the fact that the anti-inflammatory and immunomodulatory effects of OLe are the result of its inhibitory activity on nuclear factor-kB (NF-κB) and mitogen activated protein-kinase (MAPk) signalling pathways [20,66,71,79,84,85]. These phenomena eventuate in an attenuated production of various downstream molecular effectors modulating the immune-inflammatory response, such as tumor necrosis factor-α (TNF-α)[20,68,79,80,85,86], interleukin-1β(IL-1β)[20-22,71,85,86], interleukin-6 (IL-6) [20-22,71,86-89], interleukin-8 (IL-8) [8,20,21,47,68,72,88], monocyte chemo-attractant protein-1 (MCP-1) [89], prostaglandins (PGs), in particular prostaglandin E_2 (PGE_2), and enzymes such as cyclooxigenase-2 (COX-2) [20,24,44,70,71,85,86,90], matrix metalloproteinases (MMPs) [20,21,44,45,51,71] and the inducible form of nitric oxide synthase (iNOS) [20,65,71,80,85]. On the other hand, these findings are in line with the results from experimental studies indicating that the bone-protective effects of OLe appear to be related mainly to its modulating activity on inflammatory signalling pathways rather than directly on bone metabolism [63-68]. Nevertheless, recent findings have shown that OLe may facilitate osteoblast (OB) proliferation and differentiation [66,82,91-93], whilst it suppresses osteoclastogenesis [64,65,82,89-91]. These phenomena were associated with an increase in the expression levels of osteogenic transcription factors such as runt-related transcription factor 2 (Runx2) and osterix, with an increased osteoprotegerin/receptor activator of nuclear factor kappa-B ligand (OPG/RANKL) expression ratio [91,94] and the up-regulation of other osteoblastic markers such as type I collagen, osteocalcin or alkaline phosphatase [67,77,94,95]. In particular, OLe elicits osteoprotective effects by fostering the differentiation of human bone marrow mesenchymal stem cells into osteoblasts [91,96] and by reducing tartrate-resistant acid phosphatase (TRAP) activity in osteoclasts formed from mouse spleen cells [65]. Furthermore, recent *in vitro* studies have shown that OLe modulates osteoblastogenesis by suppressing the expression and activity of perixosome proliferator–activated receptor γ (PPARγ),which functions as a transcriptional regulator of adipocyte differentiation and inhibitor of OB [91,96-98].

4. Oleuropein and malignant bone disease progression

Most of the molecular pathways involved in pathological conditions associated with disorders of bone remodeling and which may be targeted by Ole, have also been shown to contribute to the homing and growth of cancer cells in the bone [20,75,99-103] (Fig 3). The findings that various chronic inflammatory bone diseases and bone-related cancers share common molecular mechanisms underlying their pathogenesis [20,75,83,99-103] may, in part, explain the reason why chronic inflammation is actually considered as one of the main pathological processes that may foster the homing and growth of tumor cell in the bone tissue [20,103,104] (Fig. 3). On the other hand, these observations are consistent with the results from several experimental studies showing that chronic inflammation may function as a promoting factor in the early stages of a malignant progression [101, 103-107] (Fig. 3). These data may also, partially, explain the findings that disturbances of the bone microenvironment, induced by osteoporosis and/or other immuno-inflammatory bone diseases, appear to facilitate the growth and dissemination of some types of cancer cells in the bone tissue [106-113]. Ultimately, these observations indicate that OLe might well exert its potential chemo-preventive and therapeutic effects on cancer-related bone disease through multiple mechanisms involved in early events of cancer progression and in the regulation of bone remodelling processes (Fig. 2, Fig. 3).

4.1. Oleuropein and tumor cell proliferation and survival

A consistent body of *in vitro* studies have demonstrated that OLe may hinder the proliferation, survival and migration of malignant cells from human primary bone tumor, such as osteosarcoma [22,38,63,114], and from human tumors which preferentially metastasize to the bone, such as breast cancer [20,23-25,38-40,45,49,115], prostate cancer [20,23-25,116,117] and lung cancer [23-25,117-120]. The mechanisms underlying the anti-proliferative effects of OLe on osteostarcoma cells still need to be fully understood [37,63,114,116]. Conversely, accumulating evidence shows that OLe can inhibit the growth and survival of human breast, prostate or lung cancer cell lines by *i)*delaying or halting different phases of cell cycle progression [20,26,119-123], *ii)*by activating different pro-apoptotic molecular signalling pathways [20,26,40,45,76,113,116-118,123-127] and/or *iii)*by inhibiting the expression of the transcription factor NF-kB which regulates many genes driving cancer growth and progression (Table 2).

4.2. Oleuropein and Epithelial to Mesenchymal transition (EMT) .

OLe has been demonstrated to modulate *in vitro* multiple signalling pathways associated with the different types of EMT, including type 3 EMT [36,46,51-54,119], which has been recognized as an initial, critical event for metastasis formation by individually-invading carcinoma cells [119,122,124,128-131]. Almost all the key regulators of EMT are expressed in the bone microenvironment where their cross-talk contributes to favouring the homing and growth of cancer cells [37,47,51-54,129-131] (Fig. 2, Table 3)**.** In particular, these studies have highlighted the promoting role of signalling pathways such as TGF-β/BMP[52,74,132-134], MAP/ERK[52,120,125,134-136], PI3K/AKT [52,105,137], Wnt/β-catenin [54-56,74,125-131], NF-kB [128,133,137,140,141], HIF [36,142], and PTHrP [142,143] on the growth and dissemination of cancer cells in the bone (Table 3). OLe has been reported to preserve bone health by interfering with various cellular processes triggered by these molecular pathways [20,66,68,71,85,87,92-94,135]. On the basis of these findings, numerous preclinical investigations have been undertaken to assess the therapeutic effectiveness of this molecule, alone or in association with antitumor drugs in the prevention and treatment of several human solid tumors [13,15,43,45,50-53,71,111-113,134].

4.3. Oleuropein and tumor angiogenesis.

Tumor angiogenesis is a crucial event for the growth and dissemination of circulating tumor cells to distant organs including the bones [128-130]. Several experimental observations highlight the fact that OLe appears to thwart tumor neovascularization by decreasing the expression levels of various signaling molecules involved in this process [32,33,36,44, 89,118,123-129,144] (Fig. 2, Table 3). In particular, these investigations have shown that the treatment of endothelial cells [75,76,89,90,143,144] and/or human cancer cells from different tumors [34,35,76,124,125,143,145], with OLe results in a decrease in the expression level of specific growth factors and receptors such as VEGF and VEGFR2 [31,33,36,75,90,118,129], pro-inflammatory cytokines such as IL-6, IL-8, IL-1β TNF-α [19,20,22,48,80,90,143-146], adhesion molecules such as vascular cellular adhesion molecule-1 (VCAM-1) intercellular adhesion molecule-1 (ICAM-1) and E-selectin [20,22,39,52,71,75,145,146], inflammatory and proteolytic enzymes such as COX-2 [31,39,44,71,75,98] and MMPs [31,37,44,45,52,71,75,118,130] (Table 3). Most of these effects can also be attributed to the direct up-stream inhibiting activity of OLe on NF-kB, a transcription factor that modulates the expression of various genes and related signalling pathways involved in almost all the hallmarks of malignant progression [20,39,79,131,132, 143,147].

4.4. Oleuropein and tumor cell migration, adhesion and invasion.

Besides tumor neo-vascularization, some of the molecules mentioned above, such as pro-inflammatory cytokines, adhesion molecules or COX-2 and MMPs, also modulate the adhesion, migration and invasion of tumor cells [44,147-153] (Table 3). For instance, *in vitro* studies have demonstrated that adhesion molecules such as VCAM-1, ICAM-1 and E-selectin contribute to the escaping of tumor cells from dormancy and bone metastasis formation [146,150,152-154]. Moreover, emerging evidence has shown that OLe may indirectly affect the adhesive properties of tumor cells by inhibiting protein tyrosine phosphatase B1 (PTPB1), a regulator of cell adhesion and migration in normal and cancer cells [155,156]. Furthermore, MMP-2 and MMP-9 have been reported to actively contribute to the migratory activity of tumor cells, to the degradation of the extracellular matrix (ECM) and to regulating the expression of cytoskeletal proteins, growth factors, cytokines and chemokines, which favour the preferential adhesion of disseminating cancer cells to the ECM underlying epithelial cells and vascular endothelial cells [44,134,135,150,152]. Consistent with these observations, recent *in vitro* studies demonstrated that the and anti-invasive activity induced by OLe on tumor cells paralleled the down-regulation of MMP-9 and MMP-2 expression and the up-regulation of their intracellular inhibitors TIMP-1,/-3,/-4 [23,31,44,45,55,71,132,143,152] (Table 3)

4.5. Oleuropein and tumor-bone niche

Most of the signalling molecules mentioned above have also been shown to contribute to the formation of a permissive microenvironment, the so-called "(pre)metastatic niche", which supports the homing, colonization and growth of cancer cells in the bone and, their subsequent survival, dormancy and resistance to clinical treatments [157-159] (Fig. 2, Fig.3, Table 3)**.** Therefore, the targeting of these signalling molecules may be regarded as a novel potential therapeutic tool in the prevention and, eventually, eradication of the establishment of malignant cells in the bone. In this regard, preclinical evidence has suggested the possibility that OLe might thwart the formation of the (pre)metastatic bone niche (Fig. 2). For instance, pro-inflammatory interleukins (ILs) such as IL-6, IL-8, IL-1β, TNF-α have been shown actively to contribute to the homing of malignant cells in the bone [19,20,90,143,147,160-163] (Fig. 3) Ole can down-regulate the expression of these cytokines in several pathological conditions associated with an altered bone resorption and in early events of the metastatic process, namely EMT transition [37,52-55,130,147] and angiogenesis [20,36,75,143,148] (Fig. 3, Table 3). Moreover, proteolytic enzymes such as MMP-2 and MMP-9 have also been shown to contribute to the formation of the "(pre)metastatic niche" by modifying the ECM structure and by promoting the release growth factors and chemokines which fuel the "vicious cycle" of bone metastasis [44,90,130-133,147,152] (Table 3). As described above, OLe has been proven to inhibit the expression levels of MMP-2 and MMP-9 in human metastatic tumor cells while it may increase the expression of intracellular inhibitors of these enzymes, namely TIMP-1, TIM,-3 and TIMP-4 [45,51,54,75,132,152]. Moreover, OLe has been reported to indirectly affect the expression and activity of these proteolytic enzymes through the up-stream inhibition NF- κB [39,51,54,71,148,149,165] (Fig. 3). Furthermore, experimental evidence has shown that, the osteo-protective effects of OLe also appear to be due to its inhibiting activity on RANKL-induced NF-κB activation, a phenomenon which facilitates osteoclast differentiation and bone remodeling [68,70,93,94,161,166]. On the other hand, *in vitro* studies have highlighted the fact that the RANKL/RANK/OPG system appears to exert an active role in the formation of the pre-metastatic niche and in fostering the metastatic spread to bone [7,100,149,153,157,165,166]. On the basis of these observations it is conceivable to hypothesize a potential clinical role of OLe as a novel drug that may thwart the formation of a permissive microenvironment enabling tumor cells to grow and disseminate in the bone [160,165].

5. Discussion

Despite the therapeutic advances registered in recent years, the current treatments for bone-related cancers based on the systemic administration of anti-resorptive drugs and/or anti-tumour agents and/or radiopharmaceutical, still experience several drawbacks, including the negative effects elicited by these agents on the normal bone metabolism. These effects may result in detrimental consequences for cancer patients, such as increased risk of osteoporosis and fractures [7,8,9]. Furthermore, none of the currently available clinical treatments have been shown to exert a positive impact on patients' survival [7,8,9]. Hence, the need to develop new molecules endowed with both antitumor activity and low toxicity and/or limited side effects [12-17,24]. In this setting, an increasing number of preclinical studies are currently aimed at identifying and/or developing new molecules based on natural products from plants [12-17]. In particular, a notable number of preclinical investigations have provided convincing evidence on the chemo-preventive effects of several extra virgin olive oil (EVOO)-derived phenolic compounds, including oleuropein, in combatting the growth of several human solid tumors [17-20,22,23,35,36]. Furthermore, experimental and observational studies have shown that OLe exhibits protective effects on bone health due to its property of modulating various molecular signalling pathways involved in the regulation of bone resorption in normal and pathological conditions, including bone–related tumors [7,16,65-68,75,107-109,143]. Therefore, the results emerging from these studies make this molecule an attractive candidate as a novel potential agent in the prevention and treatment of bone-related cancers [17-20,54,45,53,65,66,143]. Interestingly, as OLe appears to be endowed with both anti-tumor [19,20,23,24,25,35,53,142] and bone-anabolic effects [20,65,66,81,82,91-94,98,143], this molecule might be regarded as a potential, additional therapeutic option, in the treatment of specific tumors such as multiple myeloma (MM), which is known to cause severe osteolytic bone loss due to hyper-activation of osteoclasts and suppression of the differentiation of bone marrow mesenchymal stem cells (BMSCs) into functional osteoblasts [91,96,97,167,168] (Table 3). Furthermore these findings also indicate the administration of OLe may be effective in the prevention of bone loss induced by the systemic administration of antitumor drugs and/or bone modifying agents and/or radiopharmaceuticals [8,9,10,19]. Although, the data from *in vitro* studies are promising in this regard, unfortunately, there is still a lack of *in vivo* studies with the specific aim to evaluate the therapeutic effectiveness of this compound on primary bone tumors or metastatic bone diseases by using suitable experimental animal tumor models. Nevertheless, the accumulating *in vitro* evidence on the capability of OLe to target different molecular pathways involved in the pathogenesis of cancer- related bone diseases, prompts the need for extensive future experimental and clinical investigations.

6. Conclusions

Oleuropein has been reported to target several signaling pathways involved in the modulation of bone remodelling processes in normal and pathological conditions including malignant bone diseases [45,52,53,68,144,161]. These observations suggest the potential usefulness of OLe, administered alone and/or in association with cytotoxic drugs and/ or inhibitors of bone metabolism and/or radiopharmaceuticals to prevent the growth and spreading of cancer cells in the bone [19,20,29,37,38,168]. These drug associations might also result in improved therapeutic activity, in a reduction of side effects and in a decreasing probability of developing drug resistance [19,20,24,28,37,76,169,170]. However, there are several drawbacks in transferring these experimental in clinic data. One of the major drawbacks regards the bioavailabilty and, more in general, the pharmacokinetic properties of OLe [171-174]. The rate of absorption and bioavailability of this molecule appears to be influenced by several factors, such a the route and form of administration, interaction with food, age, sex, different extraction and analytical methods used [171-174]. Experimental and clinical studies have shown that the oral administration of OLe results in rapid absorption, metabolism and renal clearance [117,171-174]. Furthermore, following absorption this compound undergoes extensive phase I and phase II metabolic processes [56,169,173,174]. The potential therapeutic effectiveness of OLe is closely related the possibility that this molecule may reach in adequate concentrations its specific molecular targets in human tissues [20,143]. Therefore, these phenomena may affect the bioavailability of OLe and its systemic transfer at adequate concentrations to the target tissues, ultimately blunting the therapeutic effectiveness of this compound. However, in this context, data on the effective organ distribution of OLe in human tissues including the bone are currently lacking. Nevertheless, a promising strategy to overcome these hurdles may rely on the development of new semi-synthetic OLe derivatives endowed with better bioavailability and possibly, improved biological activity and spectrum of activity [19,20,160,175-178] and by the use of novel drug delivery systems based on of nanotechnology [179-184]. In this latter case, preclinical *in vivo* studies have reported that the nano-encapsulation of OLe could prolong circulation, improve localization, enhance efficacy and reduce the chances of multidrug resistance [117,183-185]. In conclusion, the increasing number of preclinical *in vitro* studies suggesting a potential chemo-preventive role of OLe in cancer-related bone disease, warrant future studies to assess the impact of this phenolic compound on the clinical management and outcome of patients with primary tumors that reside in, or form metastasis in the bone [13,15,36,43,54,51,67,134,151,156].

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Conflict of Interest statement

The authors declare that there are no conflicts of interest.

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Figure 1.Chemical structure of Oleuropein and its hydrolysis products

Figure 2. **Pleiotropic effects of Oleuropein on cellular and molecular components involved in pre-metastatic niche formation**.

OLe may inhibit ( ) the expression and activity of growth factors, hormones, proinflammatory cytokines, adhesion molecules and enzymes, which regulate different cellular processes involved in cancer progression, including ***i*)**apoptosis, ***ii)***cell cycle progression, ***iii*)**cell differentiation and ***iv***) epithelial-to-mesenchimaltransition (EMT). Most of these signalling molecules have been shown to be deregulated in several pathological conditions associated with bone loss and to contribute to the formation of the "*bone pre-metastatic niche*". On the other hand, OLe has been shown to exerts osteo-protective effects by fostering( ) the differentiation of human bone marrow mesenchymal stem cells into osteoblasts and by inhibiting ( ) osteoclastogenesis.

Figure3 Schematic representation of the effects of Oleuropein on molecular signalling pathways linking chronic inflammation and cancer progression

OLe may modulate the expression and activity of various signalling molecules, such as ***1)*** cytokines, chemokines [20,21,55,69,71,73,75], ***2)*** transcription factors [18,20,38,71,75-80], and ***3***) enzymes [20,43,44,71,75,77] which, in physiological conditions, regulate several biological functions including the normal metabolic turnover of bone tissue while, their expression proves to be deregulated in various pathological conditions associated with altered bone remodelling such as chronic inflammatory processes and malignant bone disorders [7,20,47,68,71,75,83, 99-103,106].

Table

Table 1
In vivo effects of OLe on some human tumors which metastasize to the bone

Tumor model	Treatment	Effects		Mechanisms		References
Nu/nu athymic ovariectomised mice MCF7 human breast cancer	OLe enriched diet (125mg OLe/kg of diet up to 35 days)	tumor growth lung metastasis	▼ ▼	cell proliferation cell migration cell invasion	▼ ▼ ▼	[35]
BALB/c OlaHsd-foxn1 nude mice MDA-MB-231 human breast cancer	OLe 50 mg/kg i.p. once a week for 4 weeks	tumor growth	▼	NF-kB Bcl-2 Cyclin D1	▼ ▼ ▼	[34]
BALB/c nude mice 22Rv1 Human prostate cancer	OLe, 25 mg/kg/ every24h/30 days	tumor growth apoptosis	▼ ▼	cell proliferation	▼	[117]
Swiss albino mice Soft tissue sarcoma	1% OLe in drinking water, consumed ad libitum for 12 days	tumor growth	▼	cell proliferation cell migration cell invasion	▼ ▼ ▼	[32]
C57BL/6N mice BF16F10 melanoma	High Fat Diet + OLe 0.02% or 0.04%	tumor growth lymph-node metastasis	▼ ▼	Angiogenesis Lymphangiogenesis	▼ ▼	[36]

▼, inhibition

Table

Table 2.
In vitro effects of OLe on the growth and survival of tumor cells from human neoplasms which preferentially metastasize to the bone

Tumor	Cell line	Effects	Mechanisms	References
Breast Cancer	MCF-7	cell proliferation ▼ apoptosis ▲	DNA damage PARP levels ▲	[33]
	MCF-7	cell proliferation ▼ apoptosis ▲	block of G_1 to S phase transition	[121,122]
	MCF-7 MDA-MB-231	cell proliferation ▼ apoptosis ▲	cell cycle arrest in the G2M cell cycle delaying the at S-phase cyclin D1 ▼ Erk1/2 ▼ Bax ▲ Bcl-2 ▲	[34,115,124]
	MCF-7 MDA-MB-231	apoptosis ▲	NF-kB ▼	[34,37,115]
	MCF-7	apoptosis ▲	P53, Bax ▲ BCl-2 ▼	[126]
	SKBR3 (ER-)	apoptosis ▲	Activation GPER/GPR30 dependent pathway ▲	[123]
	MCF7	apoptosis ▲	HDAC2 , HDAC3 ▼	[50]
	MCF7	cell proliferation ▼	PTP1B ▼	[155]
	MCF-7 MDA-MD-231	cell cycle ▼ apoptosis ▲	miR-125b, miR-16, miR-34a, p53, p21, and TNFRS10B ▲	[42]
			bcl-2, mcl1, miR- 21 and miR- 155 ▼ miR-221, miR-29a and miR-21	[127]
Prostate cancer	LNCaP, DU145 and BPH-1	apoptosis ▲ autophagy ▲	pAkt, ▼ γ-GCS, ▼ HO-1 ▼ pro-oxidant activity in cancer cells but not in normal cells ▲	[26]

	22Rv1	apoptosis	▲	mitochondrial membrane potential caspase-3	▼ ▲	[117]
	Highly metastatic Dunning rat prostate cancer	cell motility	▼	blocking voltage-gated sodium channels (VGSCs).	▲	[41]
Thyroid cancer	TPC-1 and BCPAP	cell proliferation	▼	block in S phase AKT and ERK phosphorylation H_2O_2-induced ROS levels ROS levels	▼ ▼ ▼	[176]
Lung Cancer	A549 NSLC	apoptosis	▲	phosphorylation of p38MAPK protein mitochondrial membrane potential mithocondrial cytochrome C release Bax/Bcl2 ratio caspase 9 and caspase 3 SOD2 upregulation Akt signaling pathway mitochondrial Glo2 expression	▲ ▼ ▲ ▲ ▲ ▲ ▼ ▼	[110,111,125]
	H1299 lung cancer	apoptosis	▲	Activation of p38MAPK		[109]
Osteosarcoma	143B OS, Saos2, p53-null MG-63	cell proliferation apoptosis autophagy	▼ ▲ ▲	Bax/Bcl-2, ratio Caspase -3	▲ ▲	[38,63, 114]

▼inhibition; ▲ promotion

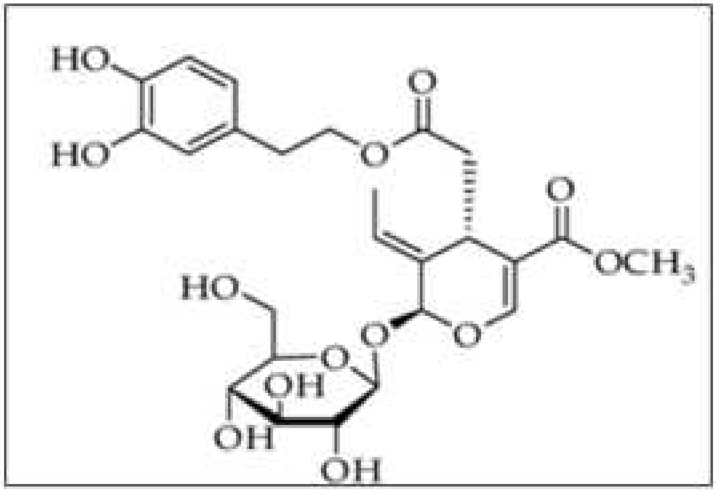
Table

Table 3
Effects of OLe on the key events and signalling pathways underlying the dissemination of cancer cells in the bone microenvironment

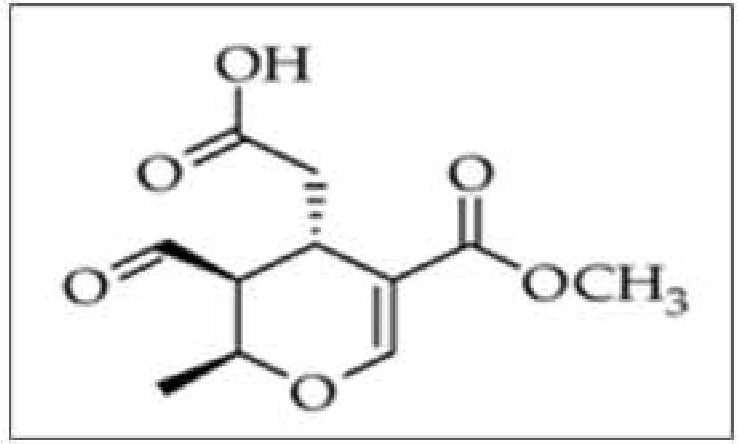
Event	Mechanisms	Effects	References
EMT	TGF-β/	▼	[54,74,132-134],
	MAP/ERK	▼	[55,120,125,134-136]
	PI3K/AKT	▼	[54,105,137]
	Wnt/β-catenin,	▼	[54-56,74,131,138-140]
	NF-kB	▼	[127,133,138,140,141]
	HIF and	▼	[36,142]
	PTHrP	▼	[143]
Migration /Adhesion	EMT	▼	[37]
	PTPB1	▼	[155,156]
Invasion	MMPs	▼	[23,31,45,46,55,71,132,143,152]
	AKT	▼	
Angiogenesis	VEGF, VEGFR-2	▼	[33, 90]
	HIF-1α, VEGF-A VEGF-D	▼	[36]
	NF-kB,	▼	[20,39,79,131,132, 143,147]
	IL-6, IL-8, IL-1β TNF-α	▼	[19,20,22,48,80,90,143-146]
	VCAM-1, ICAM-1, E-selectin	▼	[20,22,39,52,71,75,145,146]
	COX-2	▼	[31,39,45,71,75,98]
	MMPs	▼	[31,37,45,46,51,71,75,118,130]
Bone micro-environment (pre-metastatic niche)	IL-6, IL-8, IL-1β, TNF-α	▼	[19,20,90,143,147,160-164].
	EMT transition	▼	[37,53-56,130-147]
	Angiogenesis	▼	[20, 36, 75,143,148]
	MMP2, MMP9	▼	[45,90,130-134, 147,152]
	TIMP-1, TIM,-3 and TIMP-4	▲	[46,51,71,75,131,152]
	NF- κB	▼	[20,39,51,55,71,148,149,165]
	Osteoclast	▼	[20, 64-68, 89,91,143,160]
	Osteoblast	▲	[20, 64-68, 91-93,143]
	HSC	▼	[20,92 97,143]
	MSC	▲	[20, 92,96,143]
	RANKL-induced NF-κB activation	▼	[68,70,93,94,161,166].
	RANKL/RANK/OPG system	▼/▲	[100,149,153,157,165,166].
	PPARγ	▼	[91,96-98]

▼inhibition ; ▲promotion

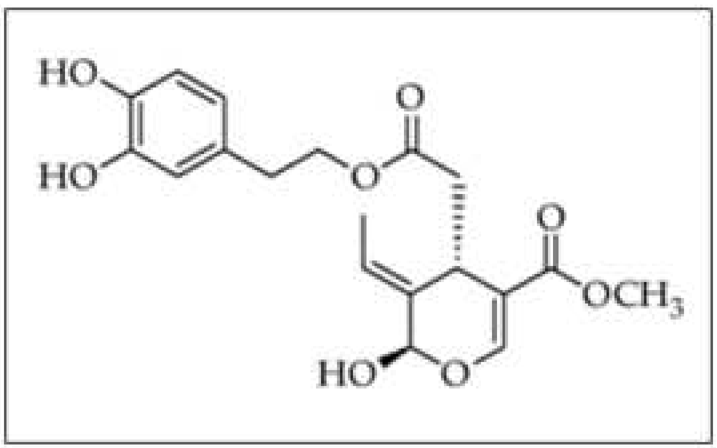
Fig.1



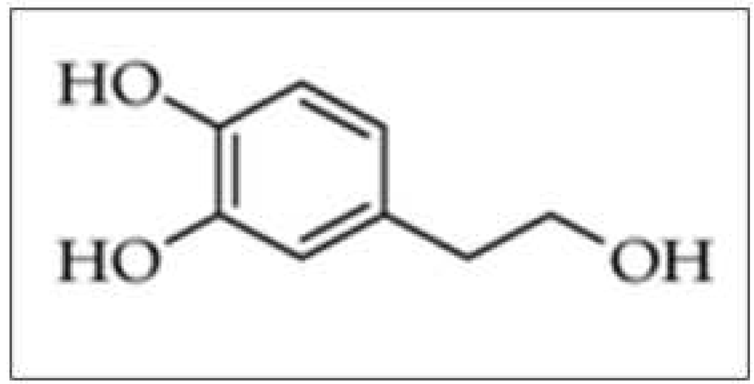
Oleuropein (Ole)



Hydroxytyrosol (Hytr)

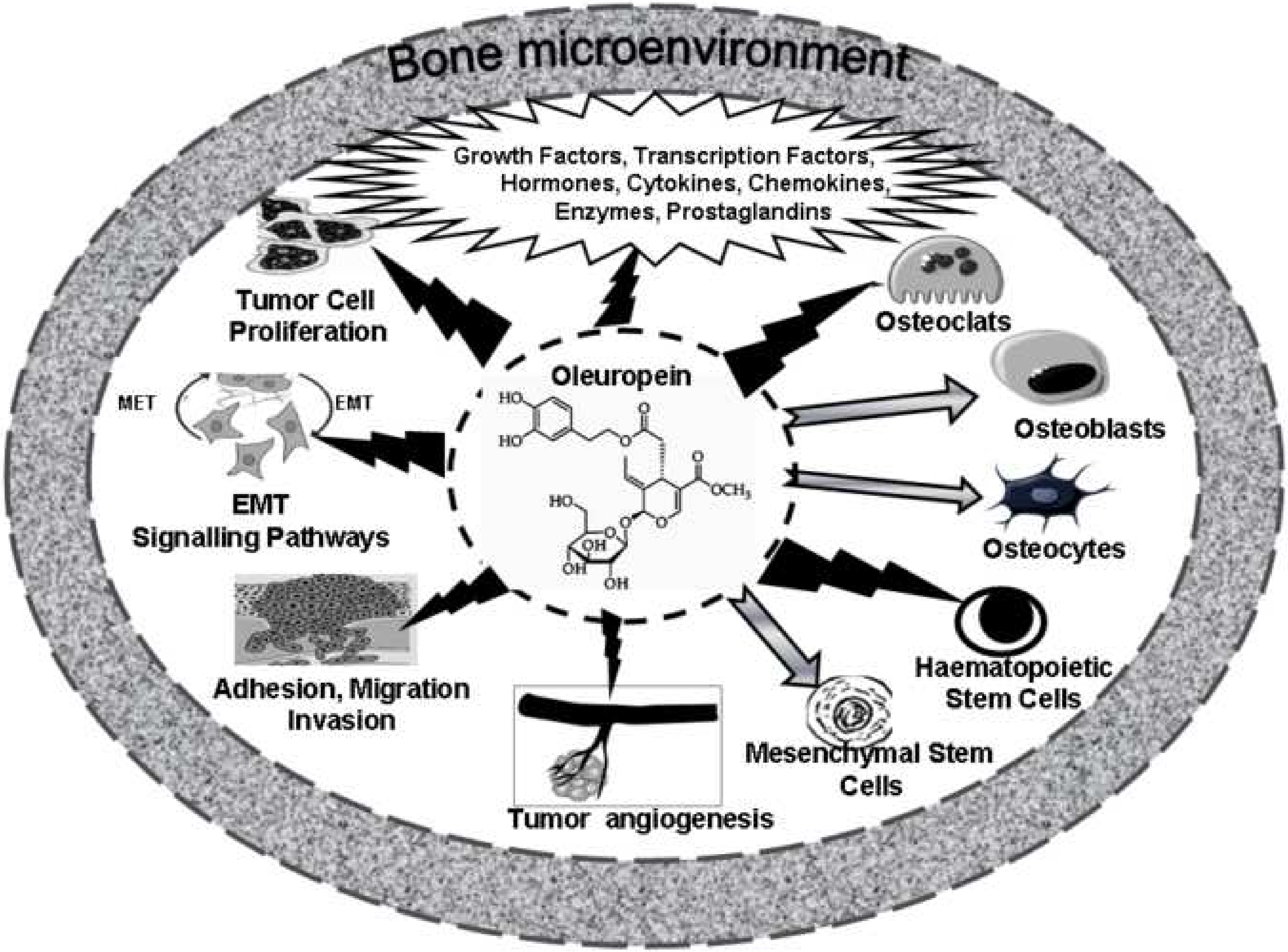


Oleuropein Aglycone (Ole-A)



Elenolic Acid (ElAc)

Figure 2


Bone microenvironment
Growth Factors, Transcription Factors, Hormones, Cytokines, Chemokines, Enzymes, Prostaglandins
Tumor Cell Proliferation
Osteoclats
Oleuropein
MET
EMT
EMT Signalling Pathways
Osteoblasts
Osteocytes
Adhesion, Migration Invasion
Haematopoietic Stem Cells
Mesenchymal Stem Cells
Tumor angiogenesis

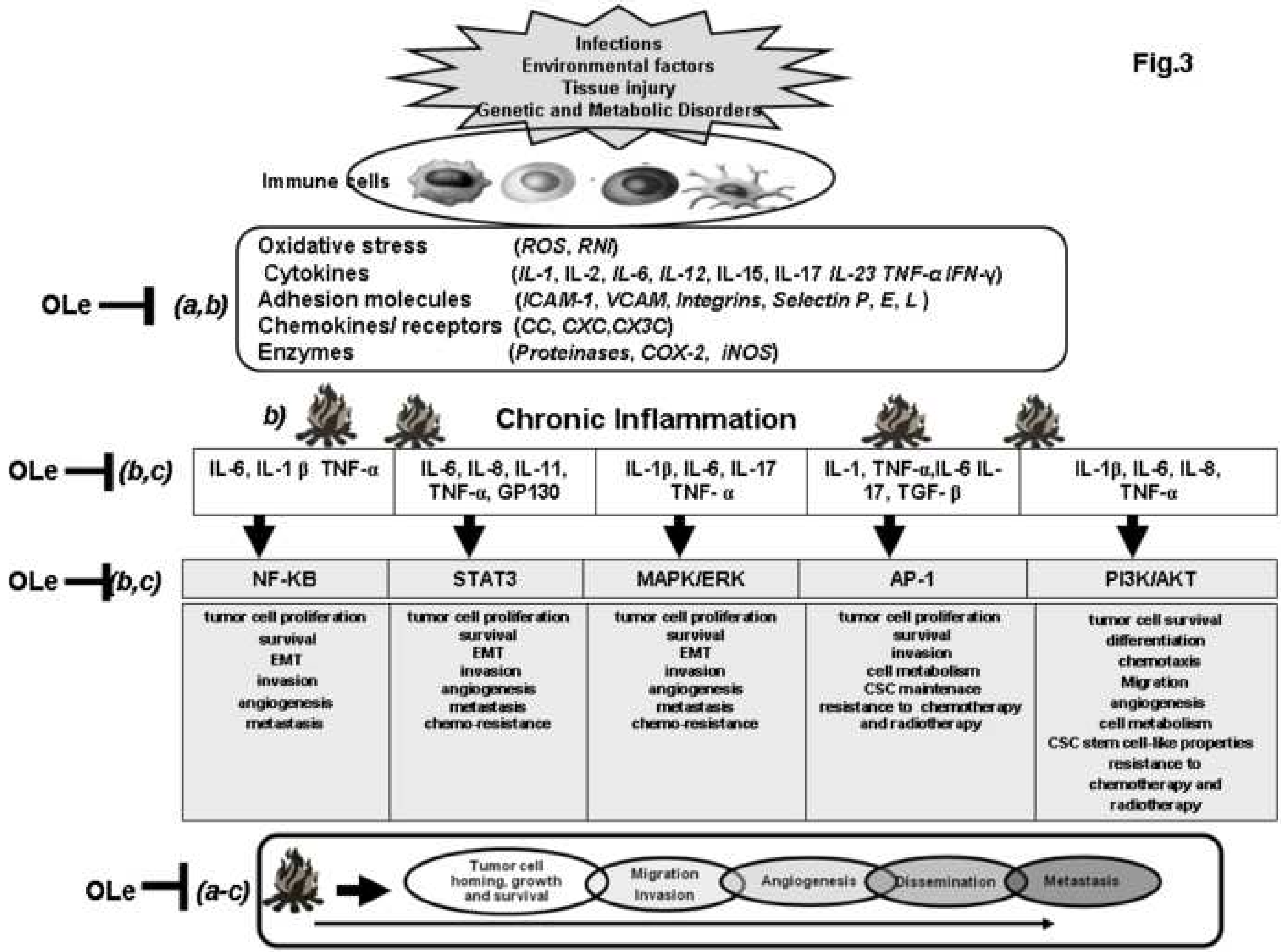


Fig.3
Infections
Environmental factors
Tissue injury
Genetic and Metabolic Disorders
Immune cells
Oxidative stress (ROS, RNI)
Cytokines (IL-1, IL-2, IL-6, IL-12, IL-15, IL-17 IL-23 TNF-α IFN-γ)
Adhesion molecules (ICAM-1, VCAM, Integrins, Selectin P, E, L)
Chemokines/ receptors (CC, CXC,CX3C)
Enzymes (Proteinases, COX-2, iNOS)
OLe (a,b)
b)
Chronic Inflammation
OLe (b,c)
IL-6, IL-1 β TNF-α
IL-6, IL-8, IL-11, TNF-α, GP130
IL-1β, IL-6, IL-17 TNF- α
IL-1, TNF-α,IL-6 IL-17, TGF- β
IL-1β, IL-6, IL-8, TNF-α
OLe (b,c)
NF-KB
STAT3
MAPK/ERK
AP-1
PI3K/AKT
tumor cell proliferation survival EMT invasion angiogenesis metastasis
tumor cell proliferation survival EMT invasion angiogenesis metastasis chemo-resistance
tumor cell proliferation survival EMT invasion angiogenesis metastasis chemo-resistance
tumor cell proliferation survival invasion cell metabolism CSC maintenace resistance to chemotherapy and radiotherapy
tumor cell survival differentiation chemotaxis Migration angiogenesis cell metabolism CSC stem cell-like properties resistance to chemotherapy and radiotherapy
OLe (a-c)
Tumor cell homing, growth and survival
Migration Invasion
Angiogenesis
Dissemination
Metastasis

Highlights

- Oleuropein, a phenolic compound present in olive leaves and fruits, exerts beneficial effects against various human diseases including cancer.
- This compounds may thwart cancer progression by modulating the expression and activity of several molecular pathways fostering this process.
- Most of these pathways also appear to favour the homing and growth of disseminating cancer cells in bones.
- These findings suggest a potential therapeutic role of this molecule in the prevention and treatment of malignant bone diseases.
- This paper provides a current overview regarding the results of recent experimental studies underpinning a possible clinical role of Ole in the prevention and development of cancer-related bone diseases.