REVIEW

Nutri-Epigenetic Effects of Phenolic Compounds from Extra Virgin Olive Oil: A Systematic Review

Andrea del Saz-Lara,[1,2] María-Carmen López de las Hazas,[1] Francesco Visioli,[2,3] and Alberto Dávalos[1]

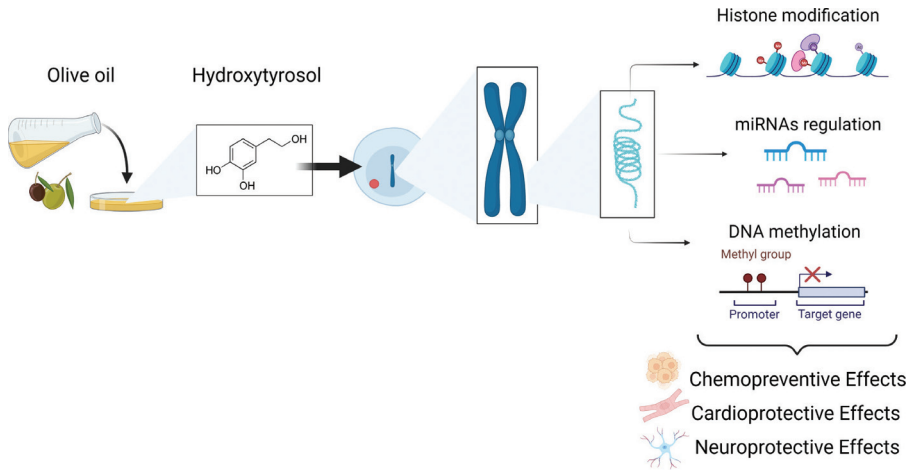
[1] Laboratory of Epigenetics of Lipid Metabolism, Madrid Institute for Advanced Studies (IMDEA)-Food, Campus de Excelencia Internacional de la Universidad Autónoma de Madrid y el Consejo Superior de Investigaciones Científicas (CEI UAM + CSIC), Madrid, Spain; [2] Laboratory of Functional Foods, Madrid Institute for Advanced Studies (IMDEA)-Food, CEI UAM + CSIC, Madrid, Spain; and [3] Department of Molecular Medicine, University of Padova, Padova, Italy

ABSTRACT

Dietary components can induce epigenetic changes through DNA methylation, histone modification, and regulation of microRNAs (miRNAs). Studies of diet-induced epigenetic regulation can inform anticipatory trials and fine-tune public health guidelines. We systematically reviewed data on the effect of extra virgin olive oil (EVOO) and its phenolic compounds (OOPCs) on the epigenetic landscape. We conducted a literature search using PubMed, Scopus, and Web of Science databases and scrutinized published evidence. After applying selection criteria (e.g., inclusion of in vitro, animal, or human studies supplemented with EVOO or its OOPCs), we thoroughly reviewed 51 articles, and the quality assessment was performed using the revised Cochrane risk of bias tool. The results show that both EVOO and its OOPCs can promote epigenetic changes capable of regulating the expression of genes and molecular targets involved in different metabolic processes. For example, oleuropein (OL) may be an epigenetic regulator in cancer, and hydroxytyrosol (HT) modulates the expression of miRNAs involved in the development of cancer, cardiovascular, and neurodegenerative diseases. We conclude that EVOO and its OOPCs can regulate gene expression by modifying epigenetic mechanisms that impact human pathophysiology. A full elucidation of the epigenetic effects of EVOO and its OOPCs may contribute to developing different pharma-nutritional strategies that exploit them as epigenetic agents. This study was registered in the International Prospective Register of Systematic Reviews (PROSPERO) as CRD42022320316. *Adv Nutr* 2022;13:2039–2060.

Statement of Significance: This systematic review analyzes accumulated data on the effect of extra virgin olive oil (EVOO) and its phenolic compounds (OOPCs) on the epigenetic landscape. Evidence suggests that EVOO and its phenolic components can regulate gene expression by modifying epigenetic mechanisms and, consequently, impacting human pathophysiology.

GRAPHICAL ABSTRACT



Keywords: hydroxytyrosol, oleuropein, phenolic compounds, miRNA, DNA methylation, histone modification

Introduction

The Mediterranean diet (MD) is very healthy and sustainable (1). Higher adherence to the MD is associated with increased longevity and prevention of many age-associated noncommunicable diseases such as cardiovascular and neurodegenerative diseases (2–4). The MD includes many healthy components, but extra virgin olive oil (EVOO) stands out because it is the main source of fat in the MD (5). The soluble fraction of EVOO mainly contains phenolic compounds (OOPCs), including phenolic acids, phenolic alcohols [hydroxytyrosol (HT) and tyrosol], and their secoiridoid precursors such as oleuropein (OL), its aglycone [oleuropein-aglycone mono-aldehyde (3,4-DHPEA-EA)], and the dialdehydic form of deacetoxy [oleuropein-aglycone di-aldehyde (3,4-DHPEA-EDA)]. In addition, EVOO also contains other types of polyphenols, such as flavonoids, lignans, and hydroxy-isocromans (6).

Several studies indicate that the consumption of EVOO and its OOPCs, especially HT, has healthful effects (5, 7). The mechanisms of action of OOPCs are manifold and might be mediated by the gut microbiota (8, 9). Examples of proposed mechanisms of action for OOPCs include: increased expression and activity of glutathione (GSH)-related enzymes, induction of nuclear factor erythroid 2-related factor 2 (Nrf2), inhibition of the proinflammatory activity of enzymes such as cyclooxygenase-2 (COX-2), or modulation of different signaling pathways such as nuclear factor k-light-chain-enhancer of activated B cells (NF-kB) or mitogen-activated protein kinase (MAPK) among others (10–12). Thus, the modulation of such signaling pathways can affect inflammation, cell metabolism, cell cycle regulation, and cell signaling, among others (13).

New research has demonstrated epigenetic actions of EVOO and its OOPCs. Epigenetic mechanisms are processes that produce reversible heritable variations that are not attributable to changes in the DNA sequence but can regulate gene expression (14). Genetics is responsible for ∼30% of the known variability, with the remaining ∼70% depending on epigenetics modulated by environmental factors (such as diet). Indeed, numerous cellular processes are influenced by epigenetic modifications (15). Major mechanisms involved in epigenetic regulation are DNA methylation, histone modification, and regulation by noncoding RNAs [i.e., microRNAs (miRNAs)]. These epigenetic changes are relatively stable, tissue-specific, and can be inherited across several generations (16). Heritability failure of epigenetic marks may result in inappropriate initiation or inhibition of gene expression and lead to pathological conditions. In addition, these epigenetic modifications are modulated by environmental and lifestyle factors such as diet and physical activity (17).

On the one hand, genomic DNA is packaged into chromatin by the 4 core histones (H2A, H2B, H3, and H4) and the linker histone (H1) (18). Histone modifications include acetylation, methylation, phosphorylation, and others. Histone acetylation is a dynamic process that regulates gene expression by modifying the accessibility of the DNA to transcription enzymes [histone acetyltransferase (HAT) or histone deacetylases (HDACs)] without altering the DNA nucleotide sequence (19, 20). The balance between HAT and HDACs dictates gene expression; however, dysregulation in this balance is associated with developmental defects and the onset of many diseases (21). On the other hand, DNA methylation originates from the addition of a methyl group to the fifth carbon of cytosine (C), forming 5-methylcytosine (5mC) mediated by DNA methyltransferase enzymes (DNMTs) (22). DNA methylation predominantly takes place in CpG dinucleotides (CpG islands), which are often found in the promoter region (23). As a general rule, methylation in promoters correlates with gene silencing, whereas genetic bodies and intergenic regions are correlated positively with gene expression (24). In several pathologies, such as cancer, aberrant DNA methylation patterns have been observed, which may be involved in the onset and progression of these diseases.

Finally, recent advances in RNA sequencing techniques revealed that >70–90% of the genome is transcribed into noncoding RNA (ncRNA) molecules, among which miRNAs are gaining great traction owing to their roles as biomarkers or therapeutic targets. miRNAs are small single-stranded RNA molecules with regulatory function comprised of 18–22 nucleotides, which are not translated into proteins but participate in the regulation of gene expression in different ways (25, 26). More than 2000 miRNAs have been identified in humans involved in the regulation of >60% of protein-coding genes, suggesting that a single miRNA may regulate hundreds of messenger RNAs (mRNAs) (27, 28). Generally, miRNAs are expressed inside the cells; however, miRNAs are also found in the circulation and in other biological fluids, such as blood, saliva, and urine, associated with extracellular vesicles, argonaute RISC catalytic component 2 (Ago2) complex, or HDL (these are the so-called circulating miRNAs) (29, 30). Indeed, the dysregulation of specific miRNAs is being proposed as a biomarker of diagnosis and progression of specific diseases. Moreover, adherence

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Supplemental Figures 1 and 2 are available from the "Supplementary data" link in the online posting of the article and from the same link in the online table of contents at https://academic.oup.com/advances/.

AdS-L and M-CLdlH contributed equally to this work.

Address correspondence to AD (e-mail: alberto.davalos@imdea.org) or FV (e-mail: francesco.visioli@imdea.org).

Abbreviations used: C, cytosine; DNMT, DNA methyltransferase enzyme; EVOO, extra virgin olive oil; exog-miRNA, exogenous miRNA; GBM, glioblastoma multiforme; H1/H2A/H2B/H3/H4, histones; HAT, histone acetyltransferase; HDAC, histone deacetylase; HT, hydroxytyrosol; MD, Mediterranean diet; miRNA/miR, microRNA; mRNA, messenger RNA; Nf-kβ, nuclear factor k-light-chain-enhancer of activated B cells; Nrf2, nuclear factor erythroid 2-related factor 2; O_3, ozone; OA, oleacein; OL, oleuropein; OOPC, olive oil phenolic compound; PREDIMED, Prevention with Mediterranean Diet; SIRT-1, sirtuin-1; SGBS, Simpson–Golabi–Behmel syndrome.

to certain dietary habits or the consumption of different bioactive compounds influence the modulation of circulating and tissue-specific miRNAs (31–35).

In view of the above, we systematically reviewed the effects of EVOO and its OOPCs on the modulation of epigenetic landmarks in relation to the development of chronic, degenerative diseases.

Methods

This systematic review was performed in accordance with the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) (36) and was conducted following the recommendations of the Cochrane Handbook of Systematic Reviews of Interventions (37). This study was registered in PROSPERO as CRD42022320316.

Search strategy

To carry out this systematic review, a literature search was performed using PubMed, Scopus, and Web of Science databases from their inception to 7 February, 2022. The following search terms were combined with Boolean operators, following the PICO strategy (population, intervention/exposure, comparison, and outcome): ("olive oil" OR hydroxytyrosol OR oleuropein OR oleocanthal OR oleacein OR secoiridoids) AND ("DNA methylation" OR "histone modifications" OR miRNA OR epigenetic). Furthermore, the reference lists of the included articles were searched, as well as previous systematic reviews or meta-analyses.

Study selection

All studies that examined epigenetic changes induced by supplementation with EVOO or its OOPCs were included in this systematic review. The inclusion criteria were: *1*) population: human line cells, animal models, and healthy volunteers or patients with different cardiometabolic diseases; *2*) intervention: supplementation with EVOO or its main phenolic compounds; *3*) comparison: control diet compared with diet supplemented with EVOO or its phenolic compounds; *4*) outcome: epigenetic changes (DNA methylation, histone modification, and modulation of miRNAs). We excluded: *1*) review articles or editorials; *2*) studies using olive oil as a control; and *3*) articles that were not written in English. Two independent investigators (AdS-L and M-CLdlH) screened titles and abstracts for relevant studies. Disagreements between the 2 were resolved by a third or fourth reviewer (AD or FV).

Data extraction and quality assessment

The main characteristics of the included studies are shown in **Tables 1–4**. They summarize information on: 1) type of experiment (in vivo/in vitro); *2*) study model and tissue (cells, animals, or humans); *3*) treatment and administration (EVOO or OOPCs); *4*) epigenetic change induced (DNA methylation, histone modification, or miRNA modulation); *5*) fold change value; *6*) quantification method; *7*) metabolic outcomes; and *8*) reference.

Study quality was assessed using the Cochrane Collaboration's tool for assessing risk of bias (Rob2) (38). This tool assesses risk of bias according to 5 domains: selection bias, performance bias, detection bias, attrition bias, and reporting bias. Moreover, it assesses whether a study has a low, unclear, or high risk of bias. Differences in opinion were resolved by group consultation (AdS-L, M-CLdlH, AD, and FV) until consensus was reached.

Results

Flow and characteristics of included studies

Figure 1 shows the flow chart of the studies in the review process. After removing duplicates, 209 records were identified through the initial literature search. By reviewing titles and abstracts, 120 potentially relevant articles were selected for full-text assessment. Subsequently, 51 eligible studies met the inclusion criteria (33, 35, 39–47, 49–88). All studies included are assays, conducted both in vitro and in vivo, and their records were published between 2012 and 2021. **Figure 2** summarizes the epigenetic mechanisms modulated by EVOO and/or its OOPCs according to the studies analyzed in this review.

Histone modifications induced by EVOO or OOPCs

Nine studies included in this systematic review reported different changes in histone modification (39–47) (Table 1). Accordingly, Juli et al. (39) observed a decrease in HDAC levels and a consequent increase in acetylated histone H3 and acetylated histone H4 after treatment of multiple myeloma cell lines with oleacein (OA). In addition, Cuyàs et al. (41) detected a decrease in lysine-specific histone demethylase 1A (LDS1) activity after treatment of breast cancer cell lines with this compound. Similarly, supplementation of MCF-7 breast cancers with OL produced a decrease in HDAC2 and HDAC3 (40). In the same MCF-7 model, OL was also able to modulate HDAC1 and HDAC4 (45). Furthermore, an effect of EVOO-rich diets on epigenetic patterns with possible roles in neoplastic transformation has also been observed in a rat 7,12-dimethylbenz (a)anthracene (DMBA)-induced breast cancer model (46).

In other lines of research, EVOO has also been implicated in the regulation of inflammation by, e.g., decreasing HDAC1 expression in macrophages (44), either by inducing – through one of its phenolic compounds, i.e., ((-)-methyl-oleocanthal) – H3K18 acetylation or increasing H3K9 and H3K27 methylation in murine macrophages (47). Furthermore, ArunSundar et al. observed that treatment with HT can modulate dysregulated epigenetic mechanisms in an Alzheimer's disease mouse model (43). In healthy humans, the mother's intake of olive oil during pregnancy can affect placental histone acetylation in immune regulatory genes (42).

TABLE 1 Effects of EVOO and its OOPCs on metabolic outcomes mediated by histone modifications in in vitro, animal, and human studies

In vivo/in vitro	Study model and tissue	Treatment and administration	Histone modification	Fold change	Quantification method	Reference
In vitro	Myeloma multiple (MM) cell lines NCI-H929, RPMI-8226, U266, MM1s, and JJN3 (models of human multiple myeloma)	Oleacein 2.5, 5, and 10 μM (24 h)	Oleacein can modulate the acetylome of MM cells	↑ acetylated histone H4: NCI-H929 (3.3/4.5/9.5) JJN3 (1.6/2.8/4.5) ↑ acetylated histone H3: NCI-H929 (1.5/1.4/4.5) 2.5/5/10 (1/1.3/3.8) ↓ HDAC1 (0.8/0.7/0.75) ↓ HDAC2 (0.85/0.6/0.8) ↓ HDAC3 (0.8/0.7/0.75) ↓ HDAC4 (0.8/0.7/0.75) ↓ HDAC6 (0.8/0.6/0.6)	Western blot + qRT-PCR	(39)
In vitro	Human breast adenocarcinoma MCF-7 cell line	OL 600 μg/mL (24/48/72 h)	Dose-dependent decrease in the level of HDAC2 and HDAC3 expression in the OLE-treated MCF-7 cells	↓ HDAC2 (4.5/3/2)∗ ↓ HDAC3 (5/3/0.5)∗	qRT-PCR	(40)
In vitro	MCF-7 and BT-474 breast carcinoma cell lines	Oleacein 0–100 μmol/L	Oleacein treatment suppressed the demethylase activity of LSD1	LDS1 activity ↓∗ (100% → 0%)	α-screen assay	(41)
In vivo	Individuals belonging to the prospective birth cohort Assessment of Lifestyle and Allergic Disease During INfancy (ALADDIN)	Regular use of olive oil as the main cooking fat during pregnancy	Regular olive oil usage during pregnancy was associated with increased H3 acetylation of several gene promoters	↑ FOXP3 (0.1/0.3)∗ ↑ IL10RA (0.1/0.4)∗ ↑ IL7R (0.1/0.4)∗	Chromatin immune precipitation + qRT-PCR	(42)
In vivo	Adult male C57BL/6 mice (induced Alzheimer's disease mouse model)	—	Epigenetic mechanism is dysregulated in induced Alzheimer's disease mice whereas modulated by HT treatment	↓ HDAC6 (2.5)∗	Western blot	(43)
In vitro	HP-1 cells (human macrophages)	EVOO 0.5–100 μM (24, 48, 72 h)	EVOO restore global normal expression levels of HDAC1, which was altered under inflammatory conditions	EVOO: ↓ HDAC1 (0.5)∗ ↓ HDAC2 (0.1) ↓ HDAC3 (0.5)∗	qRT-PCR	(44)
In vitro	Human breast adenocarcinoma MCF-7 cell line	OLE 0–2400 μg/mL (2, 48, 72 h)	OLE can decrease the expression of HDAC2 and HDAC3	↓ HDAC1 (0.1/0.25/0.4) ↓ HDAC4 (0.1/0.6/0.9)	qRT-PCR	(45)
In vivo	Female Sprague-Dawley rats (different ages)	High EVOO diet	The EVOO-enriched diet changed histone modification patterns	↓ H4K20me3 (0.25)∗ ↓ H4K16a (0.1)	Western blot	(46)
In vitro	LPS-induced peritoneal macrophages extracted from Swiss mice	(-)-methyl-oleocanthal: 12.5, 25, 50 μM (30 min)	(-)-methyl-oleocanthal prevented H3K18 acetylation or H3K9 and H3K27 demethylation	↓ H3K18 acetylation (0.5) ↑ H3K9 methylation (0.5) ↑ H3K27 methylation (0.5)	Western blot	(47)

ALADDIN, Assessment of Lifestyle and Allergic Disease During Infancy; DOPET, 3,4-dihydroxyphenyl ethanol (synonym of HT); EVOO, extra virgin olive oil; HDAC, histone deacetylase; HT, hydroxytyrosol; LSD1, lysine-specific demethylase 1A; MM, myeloma multiple; OLE, *Olea europea* leaf extract; OL, oleuropein; OOPC, olive oil phenolic compound; qRT-PCR, quantitative real time PCR. Up arrows indicate increase, down arrows indicate decrease. These changes are not always significant. The values separated by bars in the fold change column correspond to the different treatment concentrations used in the study (in the same order as mentioned in the respective column). Values marked with an asterisk showed significant differences.

TABLE 2 Effects of EVOO and its OOPCs on metabolic outcomes mediated by changes in DNA methylation in in vitro, animal, and human studies

In vivo/in vitro	Study model and tissue	Treatment and administration	Results	Methylation level	Quantification method	Reference
In vitro & in vivo	3 T3-L1 cells (human adipose cells; 10 d postdifferentiation) 24 male Sprague rats aged 4 wk	100 μmol/L palmitic, oleic, or linoleic acids (24 h) with or without 5′-aza-cytidine 10 μM. Commercial diets like IN-93 M diet: coconut oil/olive oil/sunflower oil (4 wk)	TNF-α promoter methylation levels were different according to the diets and fatty acid treatments	Olive diet: 6%	Bisulfite-pyrosequencing method	(49)
In vitro & in vivo & in silico	HMLER cells, MCF10DCIS.com, and SUM-159 cells, MCF-7/HER2 cells Female athymic nude mice (aged 4–5 wk) and female SCID/Beige mice	Secoiridoid decarboxymethyl oleuropein aglycone (DOA) 20 μmol/L (2 h)	Phenol-conjugated oleoside DOA as a dual mTOR/DNMT inhibitor that suppresses CSC-like states responsible for maintaining tumor initiating cell properties	DNMT activity: ∼30% (↓)	ELISA-based DNMT activity/inhibition assay	(50)
In vivo	36 individuals of the PREDIMED-Navarra trial (men and women aged between 60 and 70 y)	MD + EVOO MD + nuts Low-fat control group	EVOO induced methylation changes in CpG sites related to intermediate metabolism, diabetes, inflammation, and signal transduction 2 CpGs selected: *cg01081346* and *cg17071192*	MD + EVOO: *cg01081346* ≈ 5% (↑) *cg17071192* ≈ 5% (↓)	Bisulfite treatment + Infinium Human Methylation 450 K BeadChip	(51)
In vivo	8 trained male cyclists	n–3 PUFA and EVOO supplementation (4 wk; 6 capsules per day)	A CpG of IL6 showed decreased methylation following EVOO supplementation	EVOO: IL-6 CpG3 ≈ 0.5% (↓)∗ DNMT1 ≈ 0.5% (↓)∗	Bisulfite pyrosequencing	(52)
In vivo	Female, virgin Sprague-Dawley rats (aged ∼7 wk)	AIN-93 G diet AIN-based high-fat olive oil diet, high-fat butter diet, or high-fat safflower oil diet	The diet induced aberrant expression of Dnmt3a, Mbd1, and Mbd3, consistent with potential epigenetic disruption	High-fat olive diet: ↓: *Lrrn1* (0.1)∗ ↑; *Tmem45b* (1,8)∗ and *Btn1a1* (6)∗	Real-time PCR	(53)
In vivo	13 primipara Iberian sows	HT: 1.5 mg/kg (from day 35 to day 100 of pregnancy)	HT prevented hypomethylation of DNA associated with oxidative stress	% of methylated to total cytosine (mCyt/tCyt): 4.3 ± 0.1∗	Ratio of serine-to-glycine in fetal plasma	(54)

(*Continued*)

TABLE 2 (*Continued*)

In vivo/in vitro	Study model and tissue	Treatment and administration	Results	Methylation level	Quantification method	Reference
In vivo	600 large yellow croakers	Fish oil, palmitic acid, olive oil (OO), sunflower oil, or perilla oil as the dietary lipid source	Mitochondrial arginine transfer RNA and NAD(H) dehydrogenase 4 L encoding region methylation in the liver was higher in the OO; 12S ribosomal RNA (rRNA) methylation in the liver was lower in the OO group	OO: 9.5% (↑)∗ 2.7% (↓)	Pyrosequencing	(55)
In vivo	Men and women with renal disease (aged 53 to 63 y) and healthy adults	4 g daily of OO for 8 wk	Both OO and n–3 LCPUFA induced significant changes compared to baseline in the level of methylation of individual CpG loci in specific genes involved in PUFA metabolism in PBMCs	OO → FADS2 Males: ↑ CpG-2806 (13%) CpG2775 (24%) Females: ↑ CpG 21119 (6.3%) 21101 (14.6%) 2871 (13.1%) 2869 (16.5%) 2855 (13.3%) 2817 (17.5%) 2806 (13.9%) CpG 2775 (8.9%) OO → ELOVL5 Females: ↓ CpG 2686 (3.1%) ↑ CpG 2269 (7.6%) ↑ CpG 2259 (4.6%)	Sodium bisulfite pyrosequencing	(56)
In vivo	PREDIMED-Valencia participants (aged 67 ± 7 y)	MD supplemented with EVOO	The results show inverse association between adherence to MD and methylation levels in the TCF7L2	*P* <0.01∗: CpG site 7 in amplicon B of the TCF7L2-rs7903146 CpG site 8A of the FTO	MALDI-TOF mass spectrometry	(57)
In vitro	HP-1 cells (human macrophages)	EVOO 0,5–100 μM (24, 48, 72 h)	EVOO restored global DNA methylation, which was altered under inflammatory conditions	EVOO: ↓ Global methylation (0.15%)∗	ELISA colorimetric assays	(44)
In vivo	Female Sprague-Dawley rats (different ages)	EVOO diet	The EVOO diet increased the levels of global DNA methylation in mammary gland and tumor	↑ Global methylation (10%)∗	LUMA and bisulfite pyrosequencing assays	(46)

(*Continued*)

TABLE 2 (*Continued*)

In vivo/in vitro	Study model and tissue	Treatment and administration	Results	Methylation level	Quantification method	Reference
In vivo	Healthy male Sprague-Dawley rats	EVOO (thrice a week at a dose of 1 g/kg body weight)	EVOO induces: Hypermethylation of NF-κB, VEGF, and MMP-9 Demethylation of caspase-3 and caspase-9 Demethylation of miR-143 y miR-143	NF-κB ↑ (55%/65%)* MMP-9 ↑ (61%/81%)* VEGF ↑ (95%/98%) Caspase-3 ↓ (40,87%)* Caspase-9 ↓ (57%) miR-143 ↓ (70%) miR-145 ↓ (30%)*	Bisulfite modification and methylation-sensitive high-resolution melt analysis (MS-HRM)	(58)
In vitro	C-28/I2 human cell line (representative of primary chondrocytes)	+/- 100 μmol/L H_2O_2 (2 h) + 100 μmol/L HT (30 min later)	Promoter methylation levels of miR-9-1, miR-9-2, and miR-9-3 changed in response to HT and/or H_2O_2	miR-9 promoter methylation: ↓ H_2O_2 ↑ HT	Bisulfite conversion and methylation- specific PCR	(59)
In vitro & in vivo	Caco-2 (human colon cancer cells) 18 female Sprague-Dawley rats	Short- and long-term dietary EVOO (100 ppm), OPE: 50 μM, HT: 50 μM (24 h)	Selective upregulation of CNR1 gene-encoding for type 1 cannabinoid receptor (CB_1), which was inversely correlated to DNA methylation at CNR1 promoter	↓ CpG methylation of rat Cnr1 promoter (50%)*	Methylation-specific PCR (MSP)-qPCR and pyrosequencing	(60)

AIN, American Institute of Nutrition Rodent Diet; CpG, cytosine-guanine dinucleotide; CSC, cancer stem cells; DOA, secoiridoid decarboxymethyl oleuropein aglycone; DNMT, DNA methyltransferase; EVOO, extra virgin olive oil; FTO, gene encoding the fat mass and obesity-associated protein; HFD, high-fat diet; HT, hydroxytyrosol; LUMA, Luminometric Methylation Assay; MALDI-TOF, matrix-assisted laser desorption/ionization-time-of-flight; mCyt, methylated cytosine; MD, Mediterranean diet; miR, microRNA; mTOR, mammalian target of rapamycin; OO, olive oil; OOPC, olive oil phenolic compounds; OPE, EVOO phenolic extracts; PBMC, peripheral blood mononuclear cell; PREDIMED, Prevención con Dieta Mediterránea (Prevention with Mediterranean Diet); SCID, severe combined immunodeficient; tCyt, total cytosine; VEGF, vascular endothelial growth factor. Up arrows indicate increase, down arrows indicate decrease. The different values separated by bars in the fold change column correspond to the different treatment concentrations or different times used in the study (in the same order as mentioned in the respective column). Values marked with an asterisk showed significant differences.

TABLE 3 Effects of EVOO and its OOPCs on metabolic outcomes mediated by miRNA modulation in cells and animal models

In vivo/in vitro	Study model and tissue	Treatment and administration	miRNA and fold change	Quantification method	Metabolic outcomes	Reference
In vitro	Human Simpson–Golabi–Behmel syndrome (SGBS) adipocytes	HT (1 and 10 μmol/L)	miR-155–5p ↓ (0.1)∗ miR-34a–5p ↓ (2)∗ let-7c–5p↑ (2.5)∗	RT-qPCR	Reduced macrophage recruitment and improved inflammation	(61)
In vitro	Human primary C-28/I2 chondrocytes	HT (100 μM, 30 min) + H_2O_2 (100 μM)	miR-9 ↓ (4)∗	RT-qPCR	Inhibition of H_2O_2-induced cell death and modulation of osteoarthritis-related gene	(62)
In vitro	Human immortalized HaCat keratinocyte cell line	HT oleate (HtyOle) (5 μM, 24 h)	hsa-miR-21 ↑ (6.5)∗ hsa-miR-29a ↑ (5)∗ hsa-miR-34a ↑ (2)	RT-qPCR	Control of cellular redox status and skin regenerative processes	(63)
In vitro	Glioblastoma multiforme (GBM) cell lines: 98 G, U-138MG and U-87MG GBM	OLE: 1 or 2 mg/mL of OLE/450 μM TMZ/a combination of OLE and 450 μM TMZ (24 h)	miR-153 ↑ (25)∗ miR-145 ↑ (9)∗ miR-137 ↑ (24)∗	RT-qPCR	Antiproliferative effects via apoptosis and necrosis	(64)
In vitro	Murine macrophage cell line: RAW264.7	10 μM resveratrol, HT, or OL In presence of LPS (1 μg/mL; 18 h)	miR-146a: HT: ↓ (0.5)∗ OL: ↓ (0.25)	RT-qPCR	Anti-inflammatory effects at relatively low concentrations	(65)
In vitro	Human GBM cell line T98G	OL: 277, 5, and 555 μM (alone or in combination with TMZ 325 μM)	Let-7d ↑ (125.94/269.97)∗ miR-181b ↑ (1.09/17.34)∗ miR-137 ↑ (6.69/10.33) miR-153 ↑ (3.07/1.24)	RT-qPCR	Decrease of cell viability antitumor effect anticancer roles Caveat: nonphysiological concentrations	(66)
In vitro	Hepatocellular carcinoma cell line: Huh-7	OL 80 μmol/L	miR-155 ↓ (0.33)∗ miR-194–5p ↑ (3)∗	RT-qPCR	↓ tumorigenic properties ↑ tumor suppression	(67)
In vitro	Breast cancer cell line: MDA-MB-231	200 μM of OL for 72 h	miR-194 ↓ (1.3)∗	TaqMan Real-Time Q-PCR	Control of miR-194/XIST/PD-L1 loop in triple negative breast cancer. Caveat: nonphysiological concentrations	(68)
In vitro	Human melanoma cells: 501Mel	Oleacein (OA) 20 μM for 72 h	miR-193a–3p/5p ↑ (3)∗ miR-34a–5p ↑ (5)∗ miR-16–5p ↑ (13)∗ miR-214–3p ↓ (0.5)∗	RT-qPCR	Decrease of proliferation of cutaneous melanoma cells. Possible medical food for cancer disease cotherapy	(69)

(*Continued*)

TABLE 3 (*Continued*)

In vivo/in vitro	Study model and tissue	Treatment and administration	miRNA and fold change	Quantification method	Metabolic outcomes	Reference
In vitro	Endothelial cells: HREC and HUVEC	IL-1β (10 ng/mM) with or without HT-3Os (10 μM/24 h for 7 d)	miRNA let-7 ↑ (1)∗	RT-qPCR	Reversal of endothelial dysfunction and inflammation phenotypes	(70)
In vitro	Breast cancer cell lines: MCF7 and MDA-MB-231	OL Cell viability: increasing concentrations (100–1400 μg/mL); 24/48 h Apoptosis: 200, 275, and 312 μg/mM; 48 h	miR-125b ↑ (5/40)∗ miR-16 ↑ (4/60)∗ miR-34a ↑ (1.5/80)∗ miR-221 ↓ (5/8)∗ miR-29a ↓ (15/2)∗ miR-21 ↓ (1.5/2)	RT-qPCR	Decrease in cell viability induction of apoptosis	(71)
In vitro	Breast cancer cell line: MCF-7	OL 600 μg/mL (24, 48, 72 h)	miR-21 ↓ (0.7/0.3/0.1)∗ miR-155 ↓ (0.5/0.3/0.2)∗	qRT-PCR	Anticancer effect by modulation of tumor suppressor gene expression, targeted by oncomiRs	(72)
In vitro	Differentiated Simpson–Golabi–Behmel syndrome adipocytes	25 μmol/L oleocanthal (OC) or oleacein (OA) 6 h + Stimulation with TNF-α for 18 h	miR-155–5p ↓ (0.2)∗ miR-34a–5p ↓ (2)∗ let-7c–5p ↑ (3)∗	RT-qPCR	Attenuation of NF-κB activation reduction of adipocyte inflammation	(73)
In vitro	Human GBM cell line T98G	1 or 2 mg/mL of OLE, 325 or 450 μM of TMZ, or a combination of 325 μM TMZ and 1 mg/mL OLE	miR-181b ↑ (555)∗ miR-153 ↑ (17.5)∗ miR-145 ↑ (250)∗ miR-137 ↑ (140)∗ let-7d ↑ (10)∗	RT-qPCR	OLE modulates the expression of some miRNAs related to anticancer activity in GBM	(74)
In vitro	C-28/I2 human cell line, representative of primary chondrocytes	100 μmol/L HT (30 min) Absence or presence of 100 μmol/L H_2O_2 (2 h)	miR-9	TaqMan microRNA assays	↑ miR-9 promoters' methylation (H_2O_2) ↓ methylation (HT)	(59)
In vitro	Tumor-derived human colon cancer cell line Caco-2	EVOO phenolic extracts and HT 50 μM	miR-23a ↓ (0.5)∗ miR-301a ↓ (0.6)∗	RT-qPCR	Possible new nutraceutical for cotreatment and/or prevention of colon cancer	(60)
In vitro	Ovarian cancer cell lines: A2780S and A2780/CP	OL: 0–400 μg/mL (48/72 h) + Cisplatin 100 μM	miR-34a ↑ (7/100)∗ miR-16 ↑ (15)∗ miR-125b ↑ (5/25)∗ miR-21 ↓ (15)∗	RT-qPCR	Increased sensitivity to cisplatin and apoptosis	(75)
In vivo	Male Wistar Kyoto (WKY) rats aged 3 wk	Diet enriched with 6% by weight fish oil or olive oil (8 wk)	miR-150–5p ↓ (0.7) miR- 29c–5p ↑ (1.4) miR-21 ↑ (1.75) miR-486 ↑ (1.2)	TaqMan + RT-qPCR	Possible amelioration of endothelial dysfunction	(76)

(*Continued*)

TABLE 3 (*Continued*)

In vivo/in vitro	Study model and tissue	Treatment and administration	miRNA and fold change	Quantification method	Metabolic outcomes	Reference
In vivo	Young C57BL/6 mice Homozygous apoE KO mice Female Wistar rats	45 mg HT/kg bw/d (8 wk) 15 mg HT + H_2O 10 mg HT/kg/d (aqueous solution) 5 mg secoiridoids/kg/d (21 d)	miR-802–5p ↑ (0.8) miR-30a–5p ↑ (0.5) miR-146b–5p ↑ (0.5) miR-423–3p ↓ (0.5)	Microarray + RT-qPCR	Endothelin signaling pathway, metabolic processes, cellular component organization, etc.	(77)
In vivo	Adult male C57BL/6 mice, an A42i-induced AD mouse model	100 μM DOPET (3,4-dihydroxyphenylethanol)	miR-124 ↑ (0.7)*	RT-qPCR	Regulation of various behavioral facets	(43)
In vivo	Balb/C male mice (aged 6 wk) with CCl4-induced liver fibrosis	Oleocanthal 10 mg/kg for 3 wk	miR-29b–3p ↑ (0.5)* miR-101b–3p ↑ (0.8)* miR-221–3p ↓ (4)* miR-181–5p ↓ (2.5)*	RT-qPCR	Reduction of oxidative stress and inflammation, suggesting potential antifibrotic effects	(78)
In vivo	Wistar rats aged 24 mo for 21 d	OLE 100 mg/kg	miR-21 ↓ miR-146a ↓	qRT-PCR	Attenuation of the cardiometabolic and muscular disorders associated with aging	(79)
In vivo	Middle-aged male C57B1/6 J mice	H-EVOO: 718.8 mg of total phenols/kg of EVOO L-EVOO: 9.3 mg of total phenols/kg of EVOO (6 mo)	miR-101a ↓ (18.8) miR-30a ↓ (12.1)* miR-34a-b-c/5p ↓ (9)* miR-124–3p ↓ (14.7) miR-27 ↓ miR-137–3p ↓ miR-30c-b/5p ↓ miR-484 ↓ miR-340 ↓	Microarray + RT-qPCR	Cognitive and motor improvement	(80)
In vivo	Female Sprague-Dawley rats	EVOO diet: 9% EVOO (first 12 d of pregnancy)	miR-449c–5p ↑ (4/2)* miR-134–5p ↑ (3/2)* miR-130a–3p ↑ (0.5/1)* miR-431 ↑ (1.8/2)* miR-188–5p ↑ (2/1)* miR-32–5p ↑ (2.5) miR-144–3p ↑ (2.5)* miR-142–5p ↑ (1.9) miR-33–5p ↑ (3) miR-340–5p ↑ (2)* miR-301a–3p ↑ (2) miR-26b–3p ↑ (0.8)	RT-qPCR	Maternal consumption of different fatty acids during early pregnancy modulates miRNAs in maternal and off-spring tissues	(81)

(*Continued*)

TABLE 3 (*Continued*)

In vivo/in vitro	Study model and tissue	Treatment and administration	miRNA and fold change	Quantification method	Metabolic outcomes	Reference
			miR-30a–5p ↑ (1)∗ miR-106–5p ↑ (1.3)∗ miR-136–5p ↑ (1)∗ miR-761 ↑ (1.5) miR-668↑ (1.5) miR-325–5p ↓ (2) miR-380–5p ↑ (0.5)∗ miR-27a-3p ↑ (0.5)∗ miR-296–3p ↓ (1)∗ miR-708 ↑ (1.8) miR-500 ↑ (1.3)∗ miR-382 ↓ (1) miR-181c ↑ (1) miR-199a–5p ↑ (1) miR-22–5p ↑ (0.5) miR-674–5p ↑ (0.5) miR-215 ↓ (1/0.5)∗ miR-10b–5p ↓ (1)∗ miR-26b–5p ↑ (3)∗ miR-9a–5p ↓ (0.3)∗ miR-383–5p ↓ (2)∗ miR-377–3p ↓ (0.5)∗ miR-192–5p ↓ (0.2)∗			
In vivo	Healthy male Sprague-Dawley rats	Single dose of 1 g/kg body weight of EVOO (thrice a week)	miR-143 ↑ (1/3)∗ miR-145 ↑ (1/1.5)∗	TaqMan microRNA assay + RT-qPCR	↓ Tumor development ↓ angiogenic markers ↑ expression of proapoptotic markers	(58)
In vitro & in vivo	Human nasopharyngeal carcinoma (NPC) cell line: HNE1 and HONE1 Xenograft mouse model	OL 200 μM (24 h) OL 1% + H_2O (14 d)	miR-519d ↑ (0.3)∗	RT-qPCR	Significant enhancement of the radiosensitivity of NPC cells both in vitro and in vivo. Caveat: nonphysiological concentrations	(82)
In vitro & in vivo	Human ovarian cancer cell lines: Caov3 and Skov3 Xenograft mouse model	OL 200 μM (24 h)	miR-299 ↑ (3)∗ miR-186 ↑ miR-219a ↑ miR-516a/b ↑ miR-577 ↑ miR-624 ↑ miR-1271 ↑ miR-1323 ↑ miR-1468 ↑	Microarray + RT-qPCR	Potential synergistic effect of OL with radiotherapy for ovarian cancer treatment. Caveat: nonphysiological concentrations	(83)

(*Continued*)

TABLE 3 (*Continued*)

In vivo/in vitro	Study model and tissue	Treatment and administration	miRNA and fold change	Quantification method	Metabolic outcomes	Reference
In vitro & in vivo	Young C57BL/6 mice Human colonic adenocarcinoma cell line (Caco-2) Human primary epithelial intestinal cells (InEpCells) Mouse primary organoids	Long-term: 0.03 g% HT (8 wk) 25 mg/d HT or placebo (1 wk) HT (10 μM) or vehicle (24 h)	miR-483–3p ↑ liver (2) miR-196b–3p ↓ small intestine (1.2) miR-483–3p ↑ small intestine (1.4) miR-1247–5p ↑ all points miR-196b–5p ↓ Caco-2 cells (0.3) miR-193a–5p ↑ "miniguts" (1.4) miR-1247–5p ↑ "miniguts" (1.3)	RT-qPCR	Hypertension apoptosis proliferation inflammation cancer calcification etc.	(33)

AD, Alzheimer's disease; CAD, coronary artery disease; DOPET, 3,4-dihydroxyphenylethanol (synonym of HT); EVOO, extra virgin olive oil; GBM, glioblastoma multiforme; H_2O_2, hydrogen peroxide; H-EVOO, extra virgin olive oil rich in phenolics; HIF1α, hypoxia-inducible factor 1-α; HT, hydroxytyrosol; HtyOle, hydroxytyrosol oleate; HT-3Os, hydroxytyrosol sulfate metabolite; KO, knock-out; L-EVOO, extra virgin olive oil deprived of phenolics; MD, Mediterranean diet; miR/miRNA, microRNA; NF-κB, nuclear factor κ-light-chain-enhancer of activated B cells; NPC, human nasopharyngeal carcinoma; OA, oleacein; OC, oleocanthal; OL, oleuropein; OLE, *Olea europaea* leaf extract; OO, olive oil; OOPC, olive oil phenolic compounds; PDRG1, DNA damage-regulated gene protein 1; PREDIMED, Prevención con Dieta Mediterránea (Prevention with Mediterranean Diet); RT-qPCR, real time qPCR; SGBS, Simpson–Golabi–Behmel syndrome; TMZ, temozolomide; WKY, Wistar Kyoto. Up arrows indicate increase, down arrows indicate decrease. These changes are not always significant. The different values separated by bars in the fold change column correspond to the different treatment concentrations used in the study (in the same order as mentioned in the respective column). The right column for each miRNA indicates whether it increases or decreases and the organ where the variation occurs. Values marked with an asterisk showed significant differences.

Changes in DNA methylation induced by EVOO or OOPCs

Fourteen studies included in this systematic review reported changes in DNA methylation (44, 46, 49–60) (Table 2).

Current evidence shows the effect of MD and EVOO supplementation on DNA methylation patterns in relation to various pathologies in humans and animal models (48). In this context, EVOO or its OOPCs modulates the aberrant methylation patterns that appear during cancer progression, in both breast tumors (46, 53) and in colon carcinogenesis (58, 60). Furthermore, Bordoni et al. (44) observed an altered global DNA methylation turnover under inflammatory conditions, suggesting that EVOO could play a major role in the modulation of low-grade inflammation and metabolic syndrome prevention.

In addition, HT can prevent the hypomethylation of DNA associated with oxidative stress in primipara Iberian sows, which could be useful in nutraceutical research on the treatment of different pathologies (54). Other OOPCs affect DNMT enzymes, for instance, it has been shown that decarboxymethyl oleuropein aglycone (DOA) potently blocks the formation of multicellular tumorspheres by reducing DNMT activity in cells and animal models (50). A dietary fatty acid regulation of adipocyte TNF-α concentrations, through changes in the methylation of its promoter, has been reported and could be exploited in cardiometabolic prevention in rats (49). Finally, Arpón et al. (51) demonstrated that the changes observed in the methylation of CpG sites of patients following an MD are related to intermediary metabolism, diabetes, inflammation, and different transduction signals.

Modulation of miRNAs by EVOO or OOPCs

Regarding the modulation of miRNAs after treatment with EVOO or its OOPCs, 34 studies were included (33, 35, 43, 58–88) (Tables 3 and 4).

In vitro modulation of miRNAs by OOPCs.

Various in vitro studies suggest that HT induces epigenetic changes via the modulation of miRNAs, for example, HT can counteract the hydrogen peroxide (H_2O_2)-induced increase in miR-9 expression in human chondrocytes. This suggests that HT could act as a preventive or therapeutic agent for osteoarthritis, and that its mechanism of action could be related to the modulation of miR-9 expression (59, 62). The anti-inflammatory actions of EVOO and its OOPCs have been actively investigated and their mechanisms of action are being elucidated. Related to this review, Bigagli et al. (65) observed an anti-inflammatory effect of HT at nutritionally relevant concentrations for humans, which was mediated by the induction of nuclear translocation of Nrf2 and reduction of miR-146a expression in LPS-stimulated macrophages. Also, HT sulfate (one of the main circulating metabolites of HT) decreases pathological phenotypes in inflamed endothelial cells and maintains an elevated expression of let-7 miRNA (70). Moreover, treatment of Caco-2

TABLE 4 Effects of EVOO and its OOPCs on metabolic outcomes mediated by miRNA modulation in humans

Study model and tissue	Treatment and administration	miRNA and fold change	Quantification method	Metabolic outcomes	Reference
Healthy participants	2 diets: + 25 mL EVOO + 25 mL sunflower oil	miR-216a–5p ↑ (5/4/3)∗ miR-20a–5p ↑ (2/2/1)∗ hsa-miR-126–5p ↑ (2/1.5/1)∗ miR-19b–3p ↑ (2/1/0.5)∗ miR-485–3p ↓ (2/2/1)∗ hsa-miR-204–5p ↓ (2.5/3/1)∗	RT-qPCR	Protective role of EVOO against the atherogenic process through microRNA regulation in endothelial cells	(84)
Healthy participants	30 mL: Low-phenols EVOO (250 mg kg – 1 of oil) Medium- phenols EVOO (500 mg kg –1 of oil) High-phenols EVOO (750 mg kg – 1 of oil)	let-7e–5p ↓ (2) miR-328a–3p ↓ (1.3) miR-17–5p ↑ (1) miR-20a–5p ↑ (0.9) let-7e–5p miR-17–5p ↑ (0.5) miR-20a–5p miR-192–5p ↑ (1) let-7e–5p ↓ (0.5) miR-10a–5p miR-21–5p miR-26b–5p	RT-qPCR	Potential mechanism behind the cardiovascular benefits associated with EVOO intake	(85)
Participants of PREDIMED randomized trial	MD vs. control diet	miR-410	TaqMan	Novel association between a microRNA target site variant and stroke incidence	(86)
Coronary heart disease patients	Mediterranean diet or low-fat diet	miR181c-5p ↓ let-7e–5p ↓ miR-939–5p ↓ miR-188 ↑ miR-25–5p ↑	Next-generation sequencing	Better modulation of endothelial function and balance of vascular homeostasis	(87)
Plasma from participants of PREDIMED study (1 y)	MD + EVOO MD + Nuts Low-fat diet (LFD)	miR-222–3p ↓ miR-185–5p ↓ miR-27a–3p ↓ miR-21–5p ↓ miR-29c–3p ↓ miR-34b–5p ↓ miR-320b ↓ miR-107 ↓ miR-20a/b–5p ↓ miR-1246 ↓ miR-106a-5p ↓ miR-23a–3p ↓ miR-28–5p ↑ miR-215–5p ↑	RT-qPCR	Data showing that exosome transported lncRNAs and miRNAs can be modulated by specific dietary patterns (MD), which may help develop therapeutic strategies for human diseases	(35)
PBMCs of healthy subjects and patients with metabolic syndrome	EVOO (high polyphenols) EVOO (low polyphenols) 50 mL (single dose)	miR-146b–5p ↓ (1.5) miR-19a–3p ↓ (1.5) miR-181b–5p ↓ (2.5) miR-107 ↓ (1.4) miR-769–5p ↓ (1.5) miR-192–5p ↓ (1.5) miR-15b–3p ↓ (1.5) miR-548c–5p ↓ (1.6) miR-23b–3p ↑ (1.3) miR-519b-3p ↑ (1.5) miR-614 ↑ (1.4) miR-302c–5p ↑ (1.6) miR-619–3p ↑ (1.6) miR-1286 ↑ (1.7)	Microarray + RT-qPCR	Modulation of genes and miRNAs involved in metabolism, inflammation, and cancer	(88)
21 healthy volunteers	25 mg/d HT or placebo (1 wk)	miR-193a–5p ↑ (0.3)∗	RT-qPCR	Hypertension, apoptosis, proliferation, inflammation, cancer, calcification, etc.	(33)

CAD, coronary artery disease; EVOO, extra virgin olive oil; HT, hydroxytyrosol; lncRNA, long noncoding RNA; MD, Mediterranean diet; miR/miRNA, microRNA; NGS, next generation sequencing; OOPC, olive oil phenolic compounds; PBMC, peripheral blood mononuclear cell; PREDIMED, Prevención con Dieta Mediterránea (Prevention with Mediterranean Diet); RT-qPCR, quantitative real time PCR. Up arrows indicate increase, down arrows indicate decrease. These changes are not always significant. The different values separated by bars in the fold change column correspond to the different treatment concentrations used in the study (in the same order as mentioned in the respective column). Values marked with an asterisk showed significant differences.

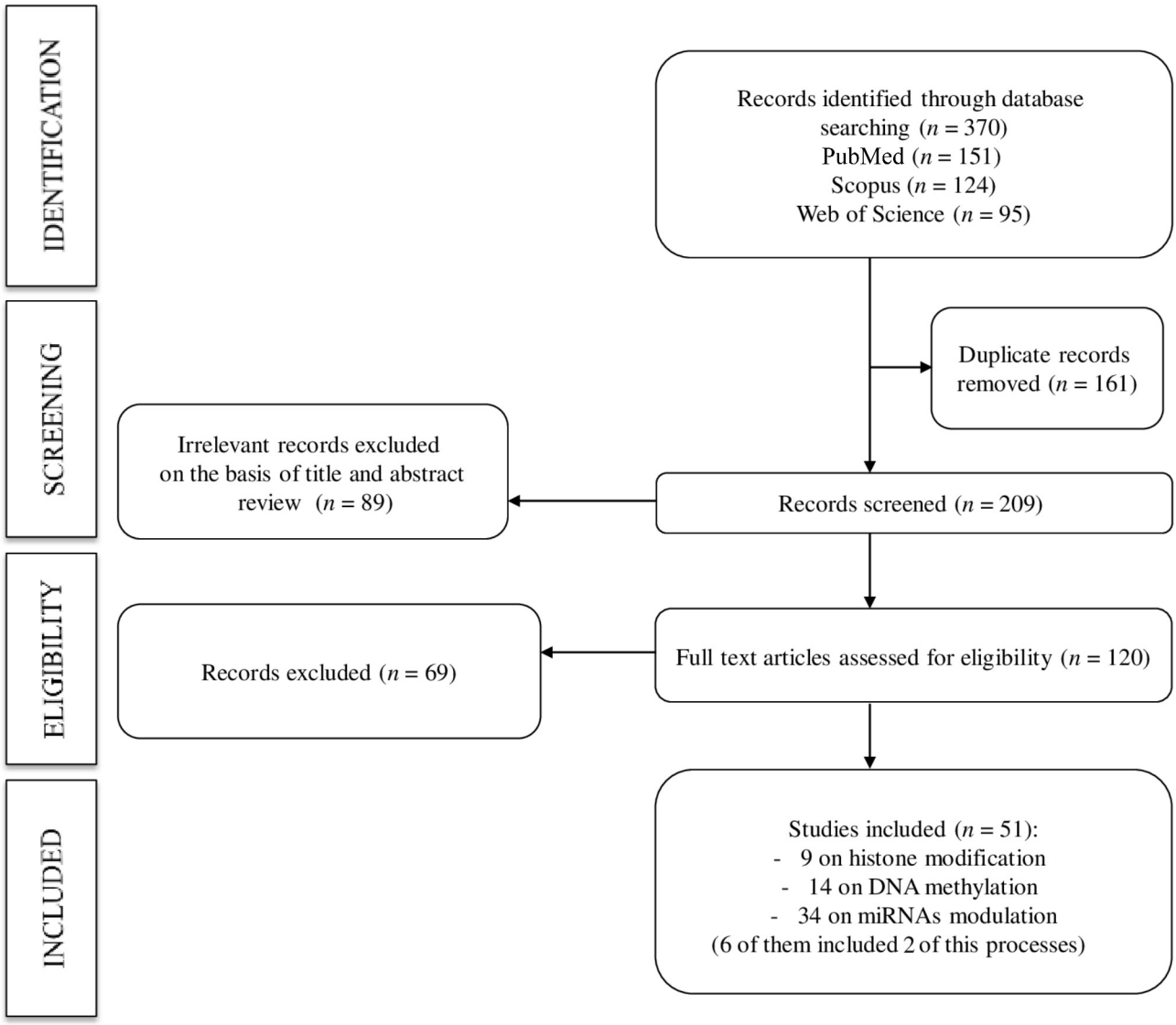

FIGURE 1 Flow diagram of the screened studies on EVOO and its OOPCs according to Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) 2020. EVOO, extra virgin olive oil; OOPC, olive oil phenolic compound.

cells with HT for 24 h repressed the expression of miR-196b-5p; the same treatment performed in human primary epithelial intestinal cells significantly induced the expression of miR-1247–5p and miR-483–3p. Furthermore, miR-193a-5p and miR-1247–5p were induced in HT-treated intestine organoids (33).

Some research is focusing on elucidating the anticarcinogenic properties of HT and its (poly)phenolic precursors as mediated by miRNAs. For example, an olive leaf extract can inhibit the pluripotency of glioblastoma cells (GSCs) by inducing the expression of miR-153, miR-145, and miR-137 and downregulating the expression of their target genes (64). In addition, the exposure to OL of glioblastoma multiforme (GBM) T98G cells significantly increased the expression of some miRNAs, i.e., let-7d, compared with a whole *Olea europaea* leaf extract (66). Also, *Olea europaea* leaf extracts showed an anticancer effect in T98G cells via upregulating miR-181b, miR-153, miR-145, miR-137, and let-7d, i.e., miRNA target genes that participate in cell cycle and apoptotic pathways (74). In addition, OL pretreatment of ovarian cancer cells (Caov3 and Skov3) made them more sensitive to radiation and altered the expression profile of miRNAs. Specifically, OL was able to alleviate the repression of miR-299, which is involved in hypoxia and its sequelae (83). Furthermore, Xu et al. found that OL strongly enhanced the radiosensitivity of nasopharyngeal carcinoma cells both in vitro (HNE1 and HONE1) and in xenograft mouse models, concomitantly reducing the activity of the miR-519d pathway (82). Regarding the potential effects of EVOO, it was observed that EVOO-derived triglycerides exert higher atheroprotective effects in endothelial cells of healthy participants than sunflower oil-derived ones,

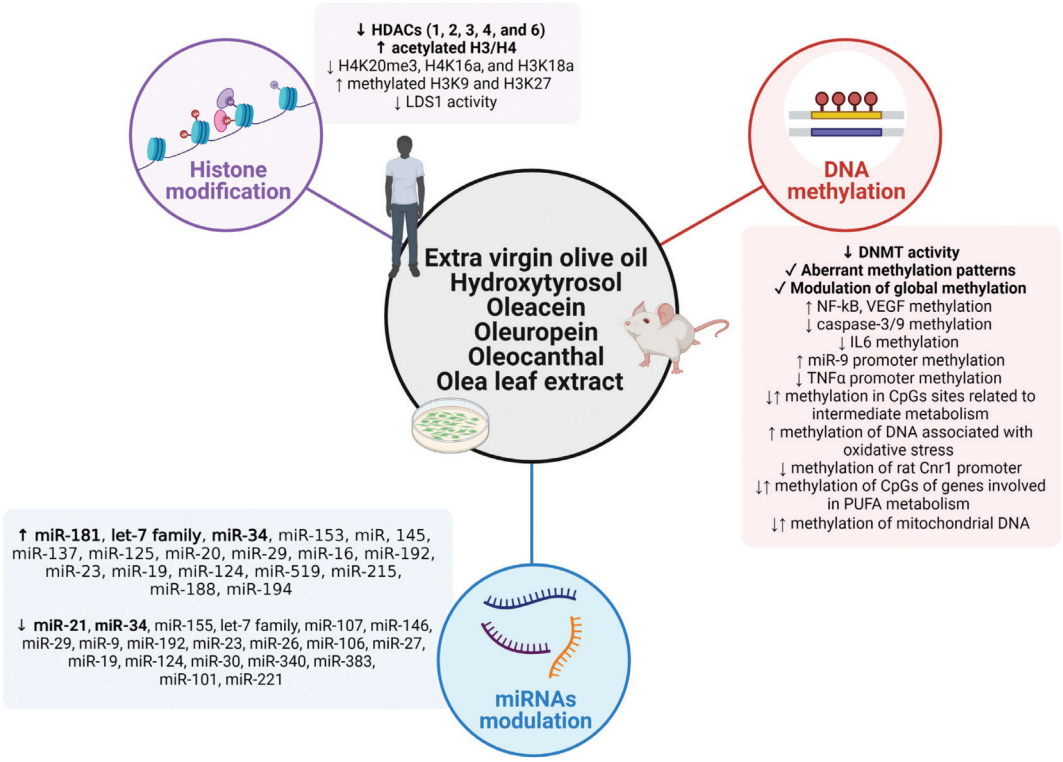

FIGURE 2 Diagram of the epigenetic mechanisms modulated by EVOO and/or its OOPCs according to the studies summarized in this review. CpG, cytosine-guanine dinucleotide; DNMT, DNA methyltransferase enzyme; EVOO, extra virgin olive oil; HDAC, histone deacetylases; LDS1, lysine-specific histone demethylase 1A; OOPC, olive oil phenolic compound; VEGF, vascular endothelial growth factor.

by upregulating 28 miRNAs which, in turn, regulate 22 atherosclerosis-related genes (84).

In vivo modulation of miRNAs by olive oil, EVOO, or its OOPCs.

HT induces the modulation of certain miRNAs that contribute to the regulation of genes involved in oxidative stress, lipid metabolism, and other metabolic processes in animals (33). Specific miRNA expression profiles have been identified in different diseases. Like circulating miRNAs, tissue-specific miRNAs can also be modulated by environmental factors such as diet (27); hence, HT could theoretically be used to regulate endogenous miRNAs. As a matter of fact, the sustained consumption of HT by mice increases the expression of miR-483–3p in the liver and miR-1982–5p in the spleen; conversely, the small intestine expression of miR-196b-3p and miR-483–3p is reduced (33).

Moreover, some evidence obtained in animal models indicates that the anticarcinogenic properties of EVOO are at least, in part, mediated by miRNA modulation. For instance, the (single and sustained) administration of EVOO to rats modulates the expression of miR-23a and miR-301a, which are targets of the type 1 cannabinoid receptor (CB1) involved in the pathogenesis of colorectal cancer (60). Interestingly, the use of 1 g olive oil/kg body weight in a preclinical model of 2-dimethylhydrazine-induced colon cancer rats reduced tumor incidence and inhibited tumor development along with modulation of miR-143 and miR-145, whose promoters are hypermethylated during colon cancer (58).

In other research, Tong et al. (76) aimed to examine the cardiovascular effects of exposing rats to ozone (O_3) and the protective effects of diets enriched with fish and olive oils. The authors observed that the increase in the expression of miR-150–5p and miR-208-5p induced by O_3 was attenuated by fish oil and/or olive oil. Also, López de las Hazas et al. (77) analyzed, via small RNA sequencing, modulated miRNAs that respond to HT supplementation with 45 mg HT per kg body weight per day for 8 wk, i.e., miR-802–5p, miR-30a-5p, miR-146b-5p (upregulated), and miR-423–3p (downregulated). Interestingly, miR-802–5p was regulated in the liver and intestine as previously described by Tomé-Carneiro et al. (33). In addition, the consumption of olive oil induces transgenerational effects in female Sprague-Dawley rats; Casas-Agustench et al. (81) observed that maternal consumption of different types of fatty acids during early pregnancy influences the expression of miRNAs in both parental and offspring tissues. Treatment with olive oil induced the expression of miR-500, miR-449c–5p, miR-134–5p, miR-130a–3p, and miR-431 in the liver and adipose tissue of pregnant rats, whereas a decrease in miR-383–5p was noted in the liver tissue of newborn and adult pups.

Modulation of miRNAs by EVOO and its OOPCs in human studies.

As mentioned, modulation of certain miRNAs could partially explain the cardiovascular effects associated with EVOO intake. Along this line, several studies evaluated circulating miRNAs after EVOO intake, for instance, Corella et al. examined the relation between rs13702 polymorphism and coronary artery disease (CAD) incidence in subjects enrolled in the Prevention with Mediterranean Diet (PREDIMED) trial (those who consumed an MD supplemented with EVOO). The authors found a novel association between miR-410 and stroke incidence that could be modulated by EVOO consumption. In addition, the MD supplemented with EVOO produced an increase of miR-188 and miR-25–5p expression accompanied by a reduction in miR-181c–5p, let-7e–5p, and miR-939–5p expression compared with patients who were given a low-fat diet (86). Furthermore, according to a study by Yubero-Serrano et al., the alterations of miRNAs induced by EVOO might translate to an improvement in endothelial function (87). Indeed, Mantilla-Escalante et al. analyzed exosomal long noncoding RNA (lncRNAs), mRNA, and miRNAs modulation in plasma samples from participants of the PREDIMED after 1 y of dietary interventions. The authors noted that the group supplemented with EVOO exhibited 15 differently expressed miRNAs, of which 14 were downregulated (hsa-miR-222–3p, hsa-miR-185–5p, hsa-miR-27a-3p, hsa-miR-21–5p, hsa-miR-29c–3p, hsa-miR-34b–5p, hsa-miR–320b, hsa-miR–107, hsa-miR-20b–5p, hsa-miR-20a–5p, hsa-miR–1246, hsa-miR-106a–5p, hsa-miR-23a–3p, hsa-miR-28–5p) and 1 (hsa-miR-215–5p) was upregulated (35).

Other human studies have addressed the modulation of miRNAs during the postprandial state. For instance, Daimiel et al. studied the concentrations of circulating miRNAs after the intake of EVOOs with various concentrations of OOPCs. The authors reported that EVOO intake alters the miR-17–92 cluster, which is proposed to be involved in fatty acid metabolism and nutrient sensing (89).

miRNAs most frequently modulated by OOPC.

As the above-mentioned studies describe the modulation of several miRNAs in response to OOPCs, we reasoned that bone fide candidates should respond in similar ways under different experimental settings. Thus, based on the literature search on in vitro, in vivo, and human studies involving miRNAs and OOPCs we performed a global analysis of modulated miRNAs. The Venn diagram we created shows the modulation of miRNAs in animals compared with humans, animals compared with in vitro, and humans compared with in vitro, and among all models (**Figure 3**A). The literature also shows that the regulation of some miRNAs has been consistently described in several studies (Figure 3B); the most frequently reported are miR-181, let-7 family, miR-34, and miR-21.

Risk of bias

The overall risk of bias of the included studies assessed using the revised Cochrane risk of bias tool (38) showed low risk of bias in 15.7% of studies, moderate risk of bias in 39.2% of studies, and high risk of bias in 45.1% of studies. Regarding the specific domains, in missing outcome data almost 100% of the studies were rated as low risk of bias; in deviations from intended interventions, around 50% of the studies were rated as some concerns; in selection of the report results, the studies were divided among the 3 categories; and finally, in the randomization process and measurement of outcome domains, close to 100% of the studies were rated as low bias (**Supplemental Figures 1** and **2**).

Discussion

This systematic review aimed to analyze the epigenetic changes induced by supplementation with EVOO or its OOPCs. Our findings provide a synthesis of the evidence supporting that these compounds could induce different histone modifications, changes in DNA methylation, as well as modifications in the modulation of miRNAs, both in vitro and in vivo (summarized in Figure 2).

Previous evidence has shown a possible role of dietary compounds in the regulation of histone modification mechanisms. More specifically, there are several polyphenols from different foods that exert effects like those induced by OOPCs present in EVOO. A good example of this is the study carried out by Dani et al. (91), in which they analyzed the impact of red grape juice consumption on the global levels of histone H3 and H4 acetylation in healthy elderly women. Another interesting example is the study by Bo et al. (92), in which they observed a significant reduction of histone H3 acetylation at lysine residue 56 (H3K56ac) following sirtuin-1 (SIRT-1) induction by resveratrol supplementation in patients with type 2 diabetes mellitus.

Likewise, several studies have shown that some bioactive compounds of food can also modulate different changes in DNA methylation. An example of this is the study of Crescenti et al. (93), which shows that cocoa consumption (with a high polyphenol content) decreases global DNA methylation of peripheral leukocytes in humans with cardiovascular risk factors. It also suggests the possibility that cocoa may exert this effect, in part, through downregulation of key genes involved in this epigenetic process, such as DNMT. Another study worth mentioning is that of Zhu et al. (94), which examined whether *trans*-resveratrol supplementation in women at high risk of breast cancer resulted in changes in the methylation of 4 cancer-related genes (*p16*, *RASSF-1α*, *APC*, *CCND2*).

Finally, the increasing interest in miRNAs and their regulatory mechanisms has led to the discovery that this epigenetic mechanism can be modulated by external agents, such as diet. Many studies have analyzed the changes produced in miRNAs by different bioactive compounds in food. For example, Tomé-Carneiro et al. (95) observed that supplementation with grape extract containing resveratrol in patients with type 2 diabetes and hypertension could

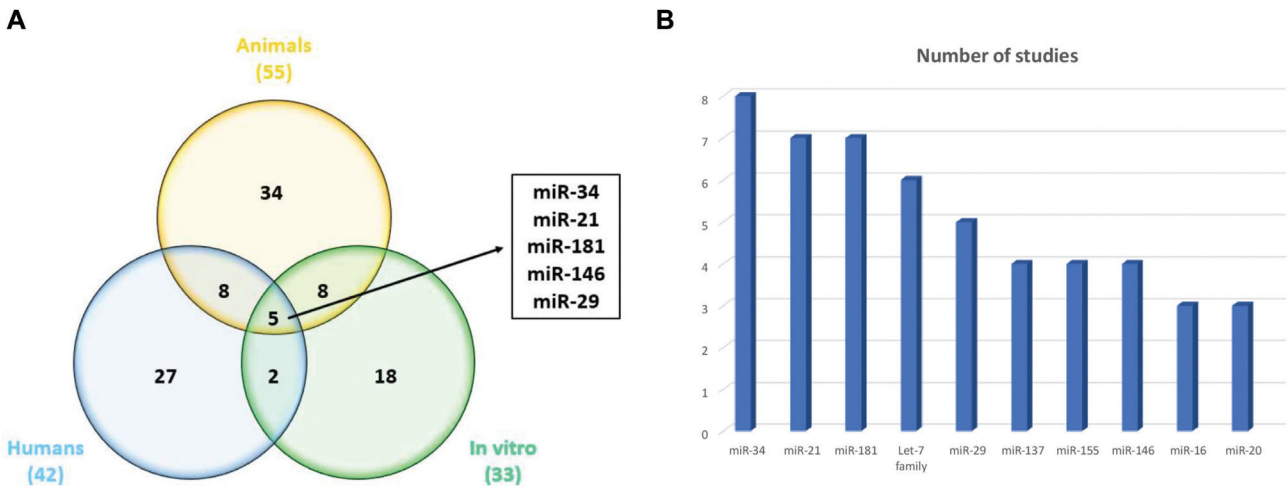

FIGURE 3 A: Venn diagram of the miRNAs studied in the different experimental models. B: Number of studies including each miRNA.

modulate inflammation-related miRNAs in peripheral blood mononuclear cells.

miRNAs most frequently modulated by OOPCs

miR-34.

The miR-34 family is known to inhibit tumorogenesis and to exert a tumor suppressor role. For these reasons, the deregulation of miR-34 in different types of cancer is becoming the focus of current research with the aim of using it in cancer therapy (96, 97). In fact, re-expression of miR-34a and the use of miRNA mimics have been investigated in clinical trials as a potential treatment of advanced cancers (98).

Several studies have demonstrated that supplementation with EVOO or its OOPCs modulates the expression of miR-34 (61, 63, 69, 73, 72, 79, 81). On the one hand, Carpi et al. studied the antimelanoma activity of OA and its mechanism of action in cutaneous melanoma cells, and found an increase in the concentrations of miR-34a–5p, after OA treatment (69). On the other hand, Asgharzade et al. studied the effect of OL on MCF-7 and MDA-MB-231 breast cancer cell lines. OL decreased cell viability and increased apoptosis, which could be mediated by the modulation of certain miRNAs. The authors observed an increase in the expression of miR-34a, miR-125b, and miR-16, and a decrease in the concentrations of miR-221, miR-29a, and miR-21 (71). OL also induced apoptosis, inhibited cell proliferation, and decreased cisplatin resistance in ovarian cancer cell lines via increased expression of both miR-34 and miR-16 (75).

Many studies have analyzed miR-34 expression in relation to other diseases. For instance, HT significantly counteracted the inflammation-induced increase in miR-34a–5p and miR-155–5p concentrations in human Simpson–Golabi–Behmel syndrome (SGBS) adipocytes (61). Similarly, Carpi et al. observed that EVOO counteracted the TNF-α-induced upregulation of miR-34a–5p and miR-155–5p expression levels, and at the same time counteracted the TNF-α-induced downregulation of let-7c–5p expression, suggesting that EVOO represses the activation of nuclear factor kappa-light-chain-enhancer of activated B cells (NF-kB)(73). Regarding neurodegenerative effects, Luceri et al. carried out a study with mice fed for 6 mo with EVOO rich in phenolic compounds, including HT. The authors observed cognitive and motor improvements compared with mice fed the same olive oil without phenolics. Downregulation of miR-34a-5p correlated with improved contextual memory (80).

miR-21.

miR-21 has been associated with cardiac functions. Its expression increases in response to OO intake after O_3 administration to rats, which induces alterations in miRNAs linked to inflammation, cardiac function, and endothelial dysfunction (76). However, the administration of virgin olive oil enriched with 750 mg (poly)phenols/kg olive oil reduces the expression of miR-21–5p in the postprandial state (88).

A high expression of miR-21 is associated with proliferation, invasion, and metastasis by targeting programmed cell death-4 (PCD4) and tropomyosin genes (TM1) (99, 100). OL prevents cell proliferation and produces antineoplastic actions via decreasing the expression of miR-21, which is accompanied by an increase in apoptosis of MCF-7 and MDA-MB-231 breast cancer cells (71) and in A2780S and A2780/CP ovarian cancer cells (79).

In addition, miR-21 expression is involved in inflammation and is associated with the release of prosenescence signals affecting DNA methylation and cell replication. Its concentrations remain low in healthy elderly patients (101) and the administration of a nutraceutical formulation based on an oil mixture (2.5 mL/kg) and OLE (100 mg/kg) to Wistar rats aged 24 mo for 21 d reduced miR-21 expression levels (86). Further, the expression of miR-21 increased in vitro after supplementation with HT oleate, promoting keratinocyte cell migration (63).

miR-181.

miR-181 expression is associated with neurodegenerative disorders and/or senescence. The administration of EVOO rich in phenolic compounds (6 mg/kg) – for 6 mo – to middle-age C57Bl/6 J mice mitigates the expression of this miRNA in the cortex (80). In line with these data, the CORDIOPREV (Coronary Diet Intervention With Olive Oil and Cardiovascular Prevention) trial (87) described that the circulating expression of this miRNA in elderly patients following an MD is lower than that in patients who adhered to a low-fat diet. Of interest, this miRNA is also involved in reactive oxygen species production and might be associated with endothelial dysfunction (87).

Regarding the involvement of miR-181 in carcinogenesis, supplementation with an *Olea europaea* extract (74) and OL (66) enhanced the expression of this miRNA in GBM cells. In addition, miR-181–5p expression is suggested to be increased in fibrosis (102). Furthermore, the daily administration of oleocanthal to Balb/C mice with CCl4-induced liver fibrosis, reduced the hepatic expression of miR-181–5p (78).

Let-7 family.

Numerous studies have described how EVOO, and its phenolic compounds, modulate the expression of the let-7 family. For instance, the CORDIOPREV study aims to compare the effect of low-fat versus MD on the incidence of cardiovascular events (103). This trial found low levels of miRNA let-7e–5p expression in patients who were given an MD compared with the low-fat diet. This miRNA appears to participate in the activation of NF-kB and the consequent activation of the inflammatory pathways (87). Also, in a cellular model of human adipocytes, the expression of let-7c–5p was upregulated after cellular pretreatment with oleocanthal and oleacein before stimulation with TNF-α (73). The same effects have been described for HT in human SGBS adipocytes (61). In addition, OL exhibited an antitumor effect in GBM cells, mainly via increasing let-7d expression (66, 74). Further, Terzuoli et al. demonstrated that HT sulfate (the main phase II metabolite produced after HT consumption, see above) restored the expression of let-7 in human umbilical vein endothelial cells (HUVEC) and human retinal endothelial cells (HREC) after exposure to IL-1b (70).

Finally, it has been suggested that let-7e–5p target genes are involved in different types of cancer and related processes, fatty acid biosynthesis, and FOXO and PI3K/AKT signaling. As Daimiel et al. demonstrated, the postprandial expression of let-7e–5p decreases dose dependently after the consumption of 30 mL of EVOO containing different concentrations of OOPCs (250, 500, and 750 mg total phenols/kg of oil) (85).

Exogenous miRNAs and their biological actions. *Olea europaea* as an example

Foods contain miRNAs [exogenous miRNAs (exog-miRNAs)] which can survive the harsh conditions of the gastrointestinal tract and, possibly, modulate host gene expression (104–107). The putative effects of exog-miRNAs might approximate those of the endogenous ones.

miRNAs from different kingdoms share a common ancestor that provides certain degrees of similarity, and in the specific case of plant miRNAs, the modification is 2′-O-methylation at the 3′-terminal nucleotide (108), which provides resistance against degradation. In addition, exog-miRNAs can be found encapsulated in vesicle carriers, which further contributes to protection during digestion and classifies exog-miRNAs as potentially bioactive food components (107). Despite their similarities and in contrast with mammals, most plant miRNAs regulate their targets by directing mRNA cleavage at single sites in the coding regions (109). Suggestive evidence of the therapeutic effects of plant miRNAs (106, 107) is available, with some evidence indicating that certain plant miRNAs may find functional homology in mammals and modulate the translation of mammalian miRNA genomic targets (110). Nevertheless, there is still controversy about the actual (if any) biological impact of exogenous miRNAs on host gene expression (111, 112).

Certain plant miRNAs have been shown to be stable under cooking conditions and during digestion (113). Given the above, miRNA mediation and the bioactive effects of *Olea europaea* miRNAs are plausibly connected, at least to some extent. Olive miRNA varies between fruit and olive leaves and during the developmental phase-transition process, with miR156 and miR166 appearing to be the 2 most abundant conserved miRNAs in the olive tree. In the plant, the miRNA targeted genes are involved in many different biochemical pathways such as those of carbohydrate metabolism (114). Surprisingly, certain *Olea europaea* miRNAs, oeu-sR20, oeu-sR27, and oeu-sR34, possess sequence homology with hsa-miR34a and have antitumoral potential modulated by protein expression of hsa-miR34a specific targets, e.g., SIRT-1, B-cell lymphoma 3-encoded protein (BCL-3), and zinc finger protein SNAI1 (SNAIL), as shown in some cell lines. An experimental approach in humans failed to identify plant miRNAs circulating in plasma 2 h after the acute intake of 40 mL EVOO. The authors hypothesize that fruit processing may reduce plant miRNA stability because it breaks the tissue structure, releasing different RNases that impair miRNA stability (115). Although EVOO may transport the above-mentioned and other miRNAs, their possible biological function, if any, on the human genome deserves further investigation.

Strengths and limitations of the study

As far as we know, this is the most comprehensive systematic review on the epigenetic effects induced by EVOO or its OOPCs (90). It also summarizes changes in DNA methylation, histone modification, and RNA modulation following supplementation with EVOO or one of its phenolic compounds. In addition, in this review we discuss the biological and physiological importance of the most widely studied miRNAs (miR-34, miR-21, miR-181, and let-7 family), as well

as the possibility that olive miRNAs may exert an effect on the regulation of epigenetic mechanisms in the host.

However, there are some limitations of this study that should be acknowledged. First, we must consider the variability (EVOO composition, outcomes, methodologies, etc.) that characterizes different studies, which makes it necessary to interpret our results with caution. Second, EVOOs have differing contents of phenolic compounds depending on the cultivar, which means that the beneficial effects on health may vary. Third, the effects differ among olive oil, virgin olive oil, and EVOO, because the more processed the oil is, the lower the phenolic compound content. Possibly, the more important limitation of the study is that most of the articles analyzed do not indicate the variety of olive oil used. Fourth, most in vitro studies use a supraphysiological concentration of phenolic compounds. Moreover, none of them include information on their metabolism or on the phase 2 metabolites that may be generated. For instance, OL is one of the main phenolic compounds in raw olive oil, but during processing various enzymatic reactions transform it into other compounds. Fifth, any study involving the participation of humans and/or animals raises various ethical issues; however, using foods rather than isolated compounds often facilitates in vivo studies. Therefore, more studies of higher quality, with a larger number of subjects, and greater diversity are needed to elucidate the molecular mechanisms involved in the epigenetic changes generated by supplementation with OOPCs and/or EVOO.

Conclusions

Elucidating the epigenetic effects of EVOO and its OOPCs may contribute to identifying and developing different pharma-nutritional strategies focused on the use of these compounds as epigenetic agents. Here, we summarize several studies showing the regulation of different epigenetic mechanisms involved in many biological fields. The results of this review show that adherence to the MD or a regular consumption of EVOO or its phenolic compounds could prevent the development of certain chronic pathologies, such as cancer or cardiovascular disease, through these epigenetic mechanisms. Of course, more research is required using appropriate in vitro and, especially, in vivo models to clarify the epigenetic effects of EVOO and its OOPCs and their potential future role as adjunct "therapeutic" agents.

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