

3

Cellular and Molecular Effects of Bioactive Phenolic Compounds in Olives and Olive Oil

Nancy B. Ray ■ *McCord Research, Coralville, IA, USA*

Nicholas T. Lam ■ *Epigenomic Medicine, Baker IDI Heart and Diabetes Institute, VIC, AUS; Heart Failure Research Group, Baker IDI Heart and Diabetes Institute, VIC, AUS*

Raymond Luc, Natalie P. Bonvino, and Tom C. Karagiannis ■ *Epigenomic Medicine, Baker IDI Heart and Diabetes Institute, VIC, AUS; Department of Pathology, The University of Melbourne, VIC, AUS*

Introduction

Olea europaea belongs to the family Oleaceae of the genus *Olea*, and it is known commonly as the olive tree, bearing the widely consumed and studied olive fruit. The cultivated olive tree can be found distributed across the globe, from Greece and the Levant, southward to Africa and Australia, west into Italy and Spain, and east reaching across to China. The Oleaceae family encompasses 600 different species, and the genus *Olea* includes approximately 40 taxa of shrubs and trees divided into the subgenera *Olea*, *Paniculatae*, and *Tetrapilus*. The subgenus *Olea* includes the subspecies *Olea europaea* and included also within the subgenus is the section *ligustroides*.

The olive tree is a hardy plant that can survive in a wide range of environments, but it is found prominently distributed in tropical and subtropical areas; the forests of the Mediterranean, the Sahara, and lowland tropical forests are some indications of its habitat (Besnard et al., 2009). The olive tree has adapted its growth to a semi-arid environment including calcareous and sandy, sunny slopes. It is tolerant of hot summers and can be sustained despite limited water supplies (Wahbi et al., 2005).

In this chapter we explore the traditional medicinal properties and early uses of olives and olive oil. Further, we discuss the evolution and health benefits of the Mediterranean diet. In particular, we focus on the cellular and molecular effects of bioactive phenolic compounds in olives and olive oil. Wound healing, cardiovascular effects, and anticancer properties of the major phenolics, hydroxytyrosol and oleuropein, are also considered.

Traditional Medicinal Applications of Olives and Olive Oil

Historical Applications of Olives and Olive Oil

The scientific denomination of the cultivated olive is *Olea europaea L.sativa*, which distinguishes it from the wild olive, *Olea oleaster*. Used most commonly now in its

table oil form, mainly for cooking, the olive and its oil are preceded by a long and revered history. Unlike its debated geographical origins, it is known that *O. europaea* and its fruit find their historical homes in the Mediterranean: from the west in Italy, Greece, and Crete, to the east in the Levant, and particularly in the countries of Syria, Lebanon, and Palestine. Although it is speculated that the use of the wild olive extends as far back in history as the Neolithic Period, 6000 years before the birth of Christ, the recorded cultivation of olives can be traced to it beginning in the Chalcolithic period, early Bronze Age. From that point on, olives, derived from the Greek name *Elaia* (also termed *Oleum* in Latin and *Zayit* in Hebrew) became entwined in the mythology, history, religion, and lives of the Mediterranean people.


In the west, archaeological evidence indicates that the olive tree was central to Greek mythology, religion, and the livelihoods and sustenance of its people. Beyond the mythological, olive oil can often be traced to many facets of ancient Mediterranean life; it was used in cooking, as a cosmetic, and as a fuel source. The importance of olive oil has reached much further than local domestic use and has formed a significant part of Mediterranean commerce. The eastern Mediterranean presents a similar central role for the olive. Here it is also intertwined with religion, cited many times in the texts of the Babylonian Talmud, the Bible, and the Quran.

However, in describing a brief history of the olive and its uses, the final application for olive oil that has survived mythology and that is garnering increasing interest is its use in medicine. This use finds its roots in the traditional remedies of antiquity. For example, the leaves of *O. europaea* formed the basis of the olive-leaf tea, which provided health benefits that now are recognized as anti-inflammatory, antioxidant, and anti-atherogenic (Caramia et al., 2012). In turn, the ubiquitous use of olive oil by the ancient cultures has naturally alluded to its potential medicinal properties. Given its reference as liquid gold in Homeric texts, oil derived from the olive fruit was highly prized as a remedy for burned skin wounds and stomach and liver pains in both Western and Eastern cultures.

In alleviating sores and chills and in strengthening the skin and muscles, Pliny the Elder, in his encyclopedic works the *Historia Naturalis*, extensively documented the medicinal uses of olives during the rule of Vespasian, during which olives were also applied to ward off headaches, and the juice produced by olives was found efficacious in the treatment of ulcers. In progression to the Middle Ages, olives continued to be used in medicinal ointments and in applications against infections. This trend continued into the Renaissance, during which the health properties of the olive were recognized and used in a broader range against pathologies including heart conditions and diabetes.

Based on largely anecdotal evidence, numerous traditional cultures continually used olive oil and crude olive extracts for their medicinal properties. In more recent times, accumulating epidemiological and clinical evidence has provided the scientific basis for the health benefits of olives and olive oil.

Importance of Olives and Olive Oil in the Mediterranean Diet

The original stimulus for the current interest in olive-derived health benefits stems from the differential mortality rates observed among populations from different regions of the world, elucidated by the pioneering epidemiological study by Ancel Keys and his collaborators (Keys, 1966). The study addressed factors associated with the incidence and mortality of coronary heart disease (CHD), and the efforts of these investigators and participating countries produced what is now the enduring title of the investigation: the Seven Countries Study. Bringing together participant data from complying nations—Finland, Italy, Greece, Japan, the United States, slavia, and Serbia—Ancel Keys's research aimed to satisfy the queries that surrounded disparities of CHD incidence between populations under a common and unifying set of protocols and methods (Keys et al., 1984).

The results accumulated in the Seven Countries Study indicated the highest coronary death rate belonged to the northern Europeans, with the U.S. closely trailing behind (Mariotti et al., 1982). In contrast, southern Europeans and the Japanese yielded a much lower coronary death rate. Further analysis of the study indicated several critical risk factors associated with the higher incidence of coronary death rate; age, serum cholesterol, systolic blood pressure, and cigarette smoking.

This difference in coronary death rates prompted the analysis of dietary intakes in light of lacking significance between cohorts for the factors of age, body weight, fat composition, and habitual physical activity (Keys et al., 1986). From dietary analyses, Keys draws a direct comparison between the rural European cohorts, highlighting the variation of dietary fat intakes in relation to CHD incidences. Though having very similar lifestyles and physical activity levels, the dietary composition of the cohorts varied greatly with respect to their fat consumption. Southern Europeans favored a Mediterranean diet rich in fruits and vegetables, and fat consumption was primarily through olive oil, making up 15–30% of total dietary energy, whereas the contrasting European cohorts derived their dietary fats from milk and milk products containing saturated fats.

From these observations, subsequent interest in the Mediterranean diet was stirred and much emphasis has been placed on olive oil, one of the defining differences in diet in Keys's study and believed to be a prime contributor to the lower mortality rates shared by the populace of the Mediterranean. The Mediterranean diet existed far before its recent prominence as a model for good dietary habit. Though seemingly vague, because the term *Mediterranean* can refer to any or all of the countries that border the Mediterranean, the title of the Mediterranean diet often refers to the diet consumed by the populace of the island of Crete and the people of Naples and southern Italy. This view came about primarily from the earlier observations of Keys in his investigations of how diet contributes to coronary disease (Keys, 1966). The original

postulations by Keys linked a low-fat diet, the opposite of the prevailing eating habits, to a decreased risk of heart disease. The subsequent publishing of his Seven Countries Study seemingly confirmed his theories of diet and disease correlation. The positive health effects derived from the diet were by themselves worthy of elucidation. The diet prominently featured plant produce, moderate consumption of seafood, limited amounts of red meat, and the critical ingredient of the Mediterranean diet, olive oil (Hu, 2003). Despite being a culinary staple, the benefits of olive oil extend beyond gustatory satisfaction into the medicinal and scientific sphere.

Previously, the parallels drawn between olive oil and subsequent health benefits against coronary disease and cancer were attributed to the high concentration of monounsaturated fatty acid (MUFA) in olive oil composition. The persisting interest in MUFAs has drawn the focus on olive studies toward oleic acid (18:1n-9). As the predominant MUFA in olive oil, investigations into its properties have unveiled its potential to reduce the LDL/HDL ratio, which is associated with cardiovascular risk (Martinez-Gonzalez and Sanchez-Villegas, 2004). In addition, olive oil has hypertension-preventative properties, whereby its use is associated with a decrease in systolic and diastolic blood pressure, along with an inverse association with developing hypertension in males (Álvarez et al., 2006). To expound upon the benefits of olive oil, oleic acid has also been implicated in anti-oncogenic activity as well as lower incidences in and decreased risk of breast cancer (Menendez and Lupu, 2006).

Although not exclusive to olives, the extensive health benefits conferred by MUFA consumption cannot be understated. The MUFA content within olives strengthens the evidence that advocates for the health and medicinal properties that have been long observed throughout its use since prebiblical times. Yet despite its age old prevalence, human understanding of the olive fruit is still incomplete. An indication of this is the recent paradigm shift concerning the focus on the compounds that define the olive's inherent properties. Although MUFAs and oleic acid were at the forefront of these studies in the past, more recent research has revealed phenolic compounds as the prime contributors to the protective and medicinal characteristics of olives.

Phenolic Compounds in Olives and Olive Oil

Overview

Accumulating evidence suggests the emerging value of phenolic compounds as potent anti-inflammatory, antimicrobial, and antioxidant factors that contribute substantially to the health advantages of an olive oil–dominant diet (Cicerale et al., 2012). The abundance of these naturally occurring phenols and the phenolic profile of the olive and its oil are produced as a result of natural external factors and the developmental stages of the fruit (Charoenprasert and Mitchell, 2012) (see also Chapters 4 and

5). The translation of these phenolic compounds to the human taste of olive fruit is interpreted as sensations of bitterness/pungency and governs the overall palatability of the olive and its oil. These traits, however, are ancillary to the broadening appreciation toward the medicinal value of phenolic compounds found in the olive, which have become the primary interest of scientific study.

The phenol constituents in olives, and by extension olive oil, are many, numbering 36 distinct phenols that show considerable variation in both presence and concentration as determined by the olive variant, ripeness, and location of cultivation.

The assessment of structural components of phenolic molecules has enabled the determination of relationships between individual phenolic constituents as well as the identification of the potential origins of the biological effects stimulated by phenolic compounds. This is exemplified by the compound and structural component, elenolic acid. A defining structural component of the secoiridoid group of olive phenolic compounds, elenolic acid finds itself integrated within oleuropein, dimethyleuropein, ligstroside, and elenolic acid–linked hydroxytyrosol and tyrosol. Its presence within certain phenolic compounds allows differentiation of biological activity within otherwise identical or similar compounds that are devoid of its presence. This is true in the instance of hydroxytyrosol (3, 4-DHPEA) and elenolic acid–linked hydroxytyrosol (3, 4-DHPEA-EDA), for which a comparison study indicated a greater than 50% favor of elenolic acid–linked hydroxytyrosol in cell apoptotic percentage, comparable to catalase-induced apoptosis (Fabiani et al., 2009b).

Elenolic acid participation in the biological activity of olives is noted as a significant component that confers antimicrobial activity (Brenes et al., 2007). Its location within olive phenol structures may provide an explanation of the bactericidal and bacterial inhibitory activity of olive phenols against food-borne pathogens (*Listeria monocytogenes*, *Staphylococcus aureus*, *Salmonella enterica*), respiratory tract infection agents (*Haemophilus influenzae*, *Moraxella catarrhalis*, *Salmonella typhi*), as well as probiotic bacterium (*Lactobacillus acidophilus* and *Bifidobacterium bifidum*) (Bisignano et al., 1999; Medina et al., 2006). A specific study targeting *Lactobacillus pentosus*, *Escherichia coli*, and *Helicobacter pylori* using free elenolic acid and hydroxytyrosol placed significant weight on elenolic acid as the responsible agent for olive bactericidal activity (Medina et al., 2009).

A similar example of biological activity conferred by a structural component can be shown with homovanillic alcohol (Figure 3.1). However, in contrast to elenolic acid, homovanillic alcohol is studied and observed primarily as a metabolite of hydroxytyrosol within the renal system. Nonetheless, as a metabolite of hydroxytyrosol, homovanillic alcohol is known as a potent antioxidant similar to its parent molecule (Tuck et al., 2002). As a hydroxytyrosol metabolite, homovanillic alcohol directly affects the renal system, where it is thought to have protective effects against cell injury. Within the renal context, hydrogen peroxide (H₂O₂) oxidative damage of tubular

epithelial cells results in acute tubular cell injury via lipid peroxidation (Sheridan et al., 1996). Pretreatment with hydroxytyrosol and the subsequent generation of homovanillic alcohol, led to reductions in lipid peroxidation, as measured by malondialdehyde (MDA), a lipid peroxidation marker in renal tubular epithelial cells. Although not as potent as its hydroxytyrosol parent molecule, homovanillic alcohol reduced MDA concentrations significantly and established its position as an important renal cell protective metabolite (Deiana et al., 2008). Using a comparable concentration of homovanillic alcohol (10 μ M) to the previously described study, Incani et al. (2010) found protection of renal cells via several intracellular signaling pathways. Along with the reported protection against H₂O₂-induced cytotoxicity, homovanillic alcohol pretreatment also inhibited H₂O₂-mediated ERK (extracellular signal-regulated kinase) phosphorylation (Incani et al., 2010). Such modulation of the MAPK (mitogen-activated protein kinase) family has significant pathogenic implications due to its influence on cell survival regulation and cellular responses to toxic substances (Sinha et al., 2004; Wang et al., 2000).

In relation to the minor phenolic constituents that contribute to the antioxidative activity of olives and olive oil, oleocanthal plays a major role in the anti-inflammatory effects of olive-derived phenols. A contributor to pharyngeal pungency stimulated by high-quality olive oils, oleocanthal prompts a sensation of irritation by engaging ion channel activation in sensory neurons within the upper airways (Peyrot des Gachons et al., 2011). This sensation is likened to ibuprofen oral irritation, with which oleocanthal shares similar anti-inflammatory activity mediated via the inhibition of cyclooxygenase enzymes COX-1 and COX-2 (Beauchamp et al., 2005). Other oleocanthal signaling pathways include inducible nitric oxide synthase (iNOS) suppression in chondrocytes in arthritis pathogenesis and tau protein interaction inhibition in neurodegenerative disorders (Lucas et al., 2011). Despite the wealth of therapeutic benefits derived from phenolic compounds, olive phenol composition is dominated by the presence of oleuropein and hydroxytyrosol (see Figure 3.1).

Identification of Hydroxytyrosol and Oleuropein as Key Antioxidants in Olives and Olive Oil

Although the various olive compositions each include distinct phenolic profiles spanning a number of compounds, oleuropein and hydroxytyrosol are among the most prevalent compounds in high-phenolic olive cultivars, having 2- to 10-fold higher concentrations than other phenolic compounds (Alagna et al., 2012; Charoenprasert and Mitchell, 2012). Originally an undesirable compound due to its bitterness/pungency, oleuropein is a primary antioxidant that is noted for its benefits to human health (Esti et al., 1998). It is a major compound in olive fruit and a secoiridoid ester,

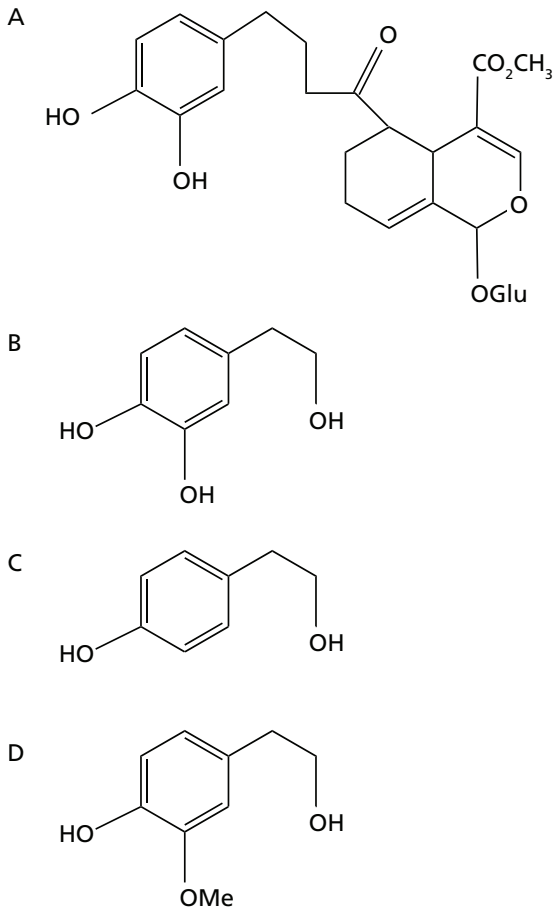


Figure 3.1 Chemical structures of major bioactive phenolics in olives and olive oil: (A) oleuropein, (B) hydroxytyrosol, (C) tyrosol, (D) p-coumaroyl vanillin alcohol.

derived from secologanin and secoxyglanin precursors, which include hydroxytyrosol and elenolic acid (Omar, 2010). Its hydrolysis metabolite, hydroxytyrosol, incorporates a similar chemical structure and is synthesized from esterase or acidic catalysis reactions (Bernini et al., 2008).

Both oleuropein and hydroxytyrosol confer antioxidant properties from an underlying orthodiphenolic structure that contributes to their protective role against

oxidation damage–induced disease, including cardiovascular disease and cancers. The antioxidant action of oleuropein and hydroxytyrosol is demonstrated by their capacity to prevent hydroxylation reactions on salicylic acid, through competition as a substrate or classical proton donation. Although similar in structure, hydroxytyrosol is the stronger of the two compounds (Carrasco-Pancorbo et al., 2005).

Focusing on cardiovascular pathology, oleuropein and hydroxytyrosol's influence is illustrated. The extension of their oxidation prevention on low-density lipoproteins, via inhibition of copper sulfate–induced oxidation and hypochlorous acid scavenging, represents a mechanism for preventing atherosclerotic lesion development (Visioli and Galli, 1994). Yet, their utility against cardiovascular disease is not solely dependent on their antioxidant interference but is complemented by their activity in anti-inflammatory processes through the attenuation of nitric oxide (NO) production resulting from decreased iNOS activity in macrophages (Visioli et al., 1998b).

The significance of olive phenolic efficacy in the prevention of human disease is not exclusively related to cardiovascular pathology, but is shared by its involvement in anti-carcinogenic activities in both *in vitro* and *in vivo* contexts (Carrera-González et al., 2013). The study of olive phenols has revealed positive effects in inducing apoptosis and inhibiting cell proliferation primarily in leukemia, breast cancer, and colorectal cancers.

Though very briefly reviewed at this point, the current literature and mechanisms concerning oleuropein and hydroxytyrosol contribution to human pathology prevention will now be given greater focus, and the importance of olive phenols in the areas of wound healing, cardiovascular pathology, and carcinogenesis will be discussed.

Olive and Olive Oil Products in Cosmetics

Since ancient times, plant oils have traditionally been used to treat a range of cutaneous conditions from rashes to tumors (Brussell, 2004), and recently, their derivatives have elicited a revived popularity in modern cosmetic and skincare formulations. The ethnobotanical applications of plant-derived compounds in relation to skin care have formed the basis of their use in cosmetics products. Specifically, the olive and its obtained oils and extracts have long been used in the ancient Mediterranean cultures in skin protectants, soaps, bath oils, and cosmetics (Boardman et al., 1976). Olive oil was also used by the ancient Egyptians to make creams and perfumes (Brun, 2000), and the Romans kept their skin elastic by applying olive oil during bathing.

There are numerous attributes that have contributed to the timeless appeal and suitability of olive oil for its usage in a range of skin-care products. The beneficial properties of olive oil as a topical agent include its function as a solvent, surfactant, emollient, antimicrobial, fragrance enhancer, and skin conditioner. Considered an

“occlusive hydrophobic ingredient” (Weber et al., 2010), olive oil is instrumental in protecting the skin from external elements. It is efficient at removing the ashen appearance of dry skin, since the squalene and triacylglycerol content of the oil resembles human sebum (Nikkari, 1974; Picardo et al., 2009). Its ability to retard the growth of a range of microorganisms, as well as to inhibit the oxidation of fats, allows olive oil to have a long storage life, and hence enables its capacity to function as an epidermal antimicrobial and preservative for perishable goods.

The fatty acids that comprise the saponifiable fraction of extra virgin olive oil constitute almost 99% of the oil by weight, and of this fraction, 70–80% is oleic acid (Dugo et al., 2004; Gomez-Alonso et al., 2002; Ollivier et al., 2006). These fatty acids form an occlusive layer on the skin that maintains the integrity of the epidermal barrier in a process involving epidermal keratinocyte metabolism of lipids derived from topically applied emollients, due to a fatty acid transporter on their surface, permitting the cells to form a functional epidermal barrier through the imported lipids (Schurer et al., 1995). It has also been shown that ingestion of olive oil fatty acid constituents, including oleic acid and linoleic acid, accelerates the wound healing process (Rodrigues et al., 2012).

Oleic acid in particular has also been found to function as a penetration enhancer (Walker and Hadgraft, 1991), wherein it facilitates transdermal drug delivery through the stratum corneum (Naik et al., 1995; Wang et al., 2004), thereby raising epidermal penetrability. Through the temporary and reversible disordering of the stratum corneum, oleic acid increases the fluidization of the intercellular lipid medium, making the epidermis more permissible to a range of compounds (Larrucea et al., 2001). However, because of the increased permeability of the skin barrier, there is the concern of excessive transepidermal water loss, which may result in the impairment of skin barrier function.

The polar phenolic fraction of the olive provides considerable delivery of antioxidant and anti-inflammatory properties to the skin, primarily derived from oleuropein and hydroxytyrosol. These properties have proven to be beneficial in the mitigation of photo damage and skin aging (Ichihashi et al., 2000; Perugini et al., 2008) as well as in improving the course of cutaneous wound healing (Mehraein et al., 2014). The antioxidant activity mitigates the oxidizing effects of external aggressors, including UVA-B light damage and ambient particulate matter (D’Angelo et al., 2005; Guo et al., 2010; Mehraein et al., 2014) through the activation of enzyme p53, which prevents and repairs skin damage following UV exposure (Budiyanto et al., 2000).

The repairing capacity of its high fatty acid and antioxidant content endows olive oil with a unique ability to afford protection and hydration when applied topically to the skin. Its enduring use in cosmetic concoctions and its subsequent reemergence as a key ingredient in cleansers, moisturizers, and body oils solidify its popularity and ubiquitous use as a cosmetic product throughout the world.

Cellular Effects of Olives and Olive Oil in Relation to Dermatology and Wound Healing

There are many applications for the use of the olive and its oil in the dermatological arena. Olive oil is used extensively to improve a range of skin conditions in neonates and infants (Kiechl-Kohlendorfer et al., 2008), as well as in the treatment of wounds and burns (Sakazaki et al., 2007), through both oral administration and topical application. The treatment objective of many cutaneous ailments is the restoration of integrity, tensile strength, and barrier function of the skin (Singer et al., 2000), a process that invariably draws on the wound healing cascade. It has been shown that the leaf extract and the extra virgin oil fraction of the olive can improve dermatitis and accelerate the course of wound healing (Koca et al., 2011). The wound healing process encompasses a multifaceted series of interactions between existing keratinocytes, the extracellular matrix (ECM), immune cells, cytokine mediators, and growth factors (Singer and Clark, 1999). The phases consist of: inflammation, proliferation, and re-epithelialization, leading to scar formation (Werner and Grose, 2003). The spatial and temporal levels of a range of cytokines, growth factors, and reactive oxygen species (ROS) within the wound remain in flux, and this dynamic balance between stimulators and inhibitors can lose its equilibrium and lead to a pathologic state.

Normal wound healing processes are reliant on the categorical transition of the aforementioned phases to achieve partial reconstruction of the wound area. Initially, platelets release eicosanoids, including COX-1 and COX-2, that convert arachidonic acid into proinflammatory prostaglandins such as PGE₂, producing dilated and porous blood vessels and thereby facilitating inflammatory migration. The pool of cytokines and growth factors released upon platelet degranulation are among other mediators involved, including PDGF, bFGF, TGFβ, EGF, and VEGF, which attract neutrophils and monocytes (Bahou and Gnatenko, 2004; Singer and Clark, 1999). Subsequently, resident immune cells such as mast cells, T-cells, and Langerhans cells (Noli and Miolo, 2001) are activated, releasing a range of cytokines including tumor necrosis factor-α (TNF-α) and interleukin-1 (IL-1) (Szpadarska et al., 2003).

Early on in the wound healing process, the onset of acute inflammation ensues, characterized by the dense level of neutrophils that constitute around 50% of cells at the wound site (Engelhardt et al., 1998). Accordingly, neutrophils perform their function of cleansing the wound bed of foreign pathogens (Gillitzer and Goebeler, 2001), mainly through bursts of ROS, which serve as signaling molecules and are involved in the destruction of foreign microbes and regulating the activation of transcription factors (Roy et al., 2006). However, the perpetuation of oxidant activity is detrimental to the wound healing process because it contributes to fibrotic scarring, damages tissue architecture (van der Vliet and Janssen-Heininger, 2014), and induces oxidative stress in adjacent normal tissue, which enhance pathologic processes. The

destructive capacity of the inflammatory phase may convey impaired healing ability upon prolonged tissue inflammation (Ashcroft et al., 1999) through the exaggerated activity of macrophages and neutrophils (Chen et al., 2014; Cooper et al., 2005). It is seen that excessive platelet activation, generation of arachidonic acid derivatives, and the subsequent secretion of proinflammatory cytokines have been correlated with an augmented inflammatory response (Diegelmann, 2003; Muscara et al., 2000). Previous studies have found improved wound healing in the setting of decreased inflammation (Ponrasu et al., 2014). Particularly, neutrophil infiltration has been attributed to the damaging effects of inflammation within the skin (Henson and Johnston, 1987).

It has been demonstrated that hydroxytyrosol and oleuropein can offset the deleterious effects of extreme inflammatory responses through their capacity to inhibit the respiratory burst of neutrophils (Visioli et al., 1998a), which reduces the oxidative load to surrounding keratinocytes and promotes wound closure and connective tissue formation (Loo et al., 2012). Oleuropein and hydroxytyrosol have also exhibited protection against genotoxic damage induced by ROS in the DNA of several cell lines (Fabiani et al., 2008a; Grasso et al., 2007; Nouis et al., 2005), limiting damage-associated molecular pattern molecules (DAMPs) (Bianchi, 2007) and further curbing the inflammatory response in light of their potent antioxidant activity (Figure 3.2). Furthermore, hydroxytyrosol has been shown to abrogate the secretion of several proinflammatory cytokines within macrophages including TNF- α , NO, and COX-2 (Maiuri et al., 2005), mediated through the attenuation of deleterious signalling cascades, such as NF- κ B (Scoditti et al., 2012). Moreover, it has been elucidated that olive phenols possess antithrombotic and antiplatelet properties (see also Chapter 2). This can be attributed to the reduction in expression levels of adhesion molecules involved in leukocyte diapedis within endothelial cells, including intercellular and vascular cell-adhesion molecules (ICAM-1 and VCAM-1) and E-selectin (Manna et al., 2009), leading to the mitigation of the coagulation response and platelet-released proinflammatory cytokines and growth factors (Singer and Clark, 1999).

The proliferative and re-epithelialization phases of wound repair are dependent upon the activity of fibroblasts that produce granulation tissue (Fernandes et al., 2004; Rajkumar et al., 2006) and the subsequent synthesis, bundling, and alignment of collagen fibers, which eventually allow for wound closure. The dense ECM that facilitates cellular migration must undergo restoration to normal histoarchitecture, which is achieved through the action of MMPs that degrade ECM components and are secreted by a range of dermal cells (Singer and Clark, 1999).

A distinguishing trait of granulation tissue is the formation of blood vessels, which are necessary to deliver nutrients and oxygen to the new tissue. This neo-vascularization is prompted by several growth factors including VEGF, TGF β , and bFGF (Folkman and D'Amore, 1996) and is facilitated by MMP activity (Haas et al., 2000) and hypoxic conditions (Detmar et al., 1997). However, in nonhealing

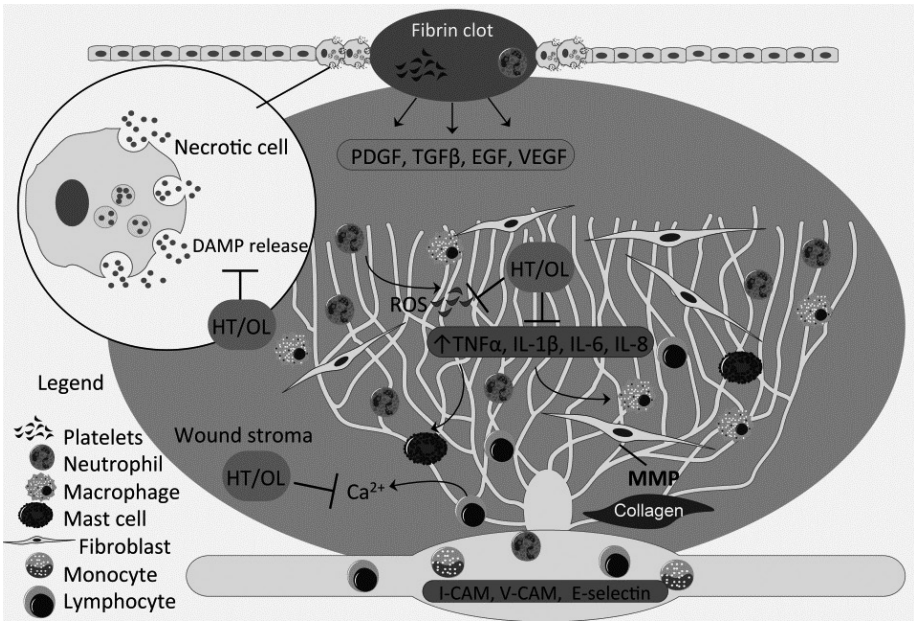


Figure 3.2 Schematic representation of wound repair mechanisms. Growth factors necessary for cell motility and proliferation are shown, as are the effects of hydroxytyrosol (HT) and oleuropein (OL) on key cytokines and DAMPs involved in the inflammatory response of wound healing.

wounds, reduced levels of these proangiogenic factors combined with endothelial cell dysfunction (Sluimer and Daemen, 2009) contribute to impaired angiogenesis and healing. Thus, therapeutic strategies are centered on mechanisms related to the cytoprotection and proliferation of the vasculature within the wound.

Olive phenols including oleuropein and hydroxytyrosol have been discovered to impart protective effects on vascular endothelial cells through limiting oxidative injury (Palmieri et al., 2012; Zrelli et al., 2011b) and inflammatory damage mediated by TNF- α (Zrelli et al., 2013), thereby supporting vascular cell proliferation. In contrast, these phenols have also been shown to abrogate angiogenesis through the inhibition of MMP-2 (Fortes et al., 2012) and MMP-9 activity (Scoditti et al., 2012), as well as the down regulation of VEGF expression (Lamy et al., 2014). However, these inhibitory effects on neovascularization have generally been found *in vitro* using high concentrations of hydroxytyrosol ($\geq 100 \mu\text{m}$) (Fortes et al., 2012) as well as in neoplastic settings (Zhao et al., 2014). It has also been shown that abnormal vessel growth, which promotes tumorigenesis, is attributed to aberrant VEGF expression (Kerbel

and Folkman, 2002) and numerous signaling pathways unique to cancer cells, which differ from the processes within normal wounds, where the dynamic reciprocity between cellular components, matrix proteins, and bioactive molecules propel conserved angiogenic mechanisms that allow for adequate tissue repair (Greaves et al., 2013). However, within impaired wound healing, decreased VEGF expression is observed, which is credited to DNA damage and lipid peroxidation (Warleta et al., 2011) induced by the wound environment. Interestingly, oleuropein has been shown to increase VEGF expression during *in vivo* wound healing studies, which was correlated with the observed acceleration of re-epithelialization (Mehraein et al., 2014). Thus, olive phenols may serve as suitable proangiogenic compounds in the context of inflammatory environments that are associated with wound healing.

Re-epithelialization of wounds involves both the migration and proliferation of keratinocytes within the epidermal parameters of the wound. Ultimately, the outcome of mammalian wound healing is the formation of a relatively acellular scar with refined vasculature (Adams and Alitalo, 2007). The inflammatory response completely resolves with the apoptosis of neutrophils (Haslett, 1992), and some leukocytes return to the vasculature (Mathias et al., 2006) or emigrate through the lymph vessels (Schwab et al., 2007). The mitigation of inflammation is achieved through the deactivation of macrophages by anti-inflammatory cytokines (Ma et al., 2003), sequestration of proinflammatory chemokines (D'Amico et al., 2000), and production of endogenous anti-inflammatory molecules (Schwab et al., 2007). Failure to resolve inflammation results in nonhealing wounds, increased scar formation, and reduced tensile strength.

Oleuropein and hydroxytyrosol assert their anti-inflammatory capabilities via several mechanisms. Concurrently, oleuropein and hydroxytyrosol have been shown to influence cytosolic Ca^{2+} levels, augmenting the activation of T and B lymphocytes (Palmerini et al., 2005; Zbidi et al., 2009). *In vivo*, oleuropein and hydroxytyrosol have been found to reduce the level of acute inflammation via the reduction of cytokines IL- β and TNF- α (Gong et al., 2009), as well as by decreasing the number of infiltrating neutrophils (de la Puerta et al., 2000).

These anti-inflammatory effects are demonstrated with topical application to the skin such that the olive phenols reduce oedematous tissue by almost 50% (de la Puerta et al., 2000), effectively accelerating the contraction of the wound area *in vivo* (Mehraein et al., 2014). In conjunction, the antifibrotic value and scar minimization of hydroxytyrosol may be mediated through its effects in up-regulating keratinocyte SMAD7 (Rafehi et al., 2012), which is known to be highly expressed in the wound stroma (Han et al., 2011). SMAD7 acts as a TGF- β antagonist and thereby limits fibrosis through blocking its chief facilitator (Flanders et al., 2003). The healing value of olive oil is diverse in its cellular effects on the epidermis and underlying dermis. As antioxidants, the scavenging abilities of the olive phenolic compounds oleuropein and hydroxytyrosol contribute to its therapeutic capabilities

(Manna et al., 2002; Owen et al., 2000). Because excessive ROS production during the proliferative and re-epithelialization phases is implicated in perturbed wound healing, olive oil is a suitable restorative product. Moreover, the therapeutic qualities conveyed by the olive phenols include their preventative capacities to ameliorate excessive inflammatory responses and tissue damage during impaired wound healing.

Molecular Mechanisms Accounting for the Wound Healing Properties of Phenolic Compounds from Olives and Olive Oil

The typical epidermal wound involves multiple cell types of several distinct lineages, each responding to and generating different signals at different times. The underlying basis for these marked responses is the activation of conserved signal transduction pathways leading to the transcription of wound-response genes expressed at the wound site that orchestrate the repair process (Cole et al., 2001). However, as previously mentioned, an excessive and prolonged inflammatory response can impair wound healing due to the exacerbation of cytokine production (Martin and Leibovich, 2005). Specifically, olive phenolic compounds have the capacity to suppress cytokine signaling through the regulation of gene expression, in which perturbations of gene transcription have been implicated in nonhealing chronic wounds (Tregrove et al., 1999) as well as hypertrophic scarring (Russell et al., 1989).

Receptor tyrosine kinases such as EGFR, PDGF (Campos et al., 2010; Wang et al., 2009), VEGF, and FGF (Komi-Kuramochi et al., 2005) bind ligands that initiate from damaged cells within the wound. The subsequent signaling via cytosolic phosphorylation in several pathways potentiates the nuclear activation of transcription factors, which modulate gene expression in cells near the wound site. Hydroxytyrosol has been found to promote such signaling activity, which selectively modulates the phosphorylation of kinases implicated in wound repair, including PI3K/AKT (Zou et al., 2012) and ERK1/2 pathways (Zrelli et al., 2011b). It has been discovered that these pathways are partly responsible for the activation of Nrf2, which is essential for the transcriptional activation of antioxidant and phase II detoxifying genes (Mann et al., 2007; Motohashi and Yamamoto, 2004; Nguyen et al., 2009), including glutathione peroxidase, superoxide dismutase-1, catalase, and heme-oxygenase (HO-1) (Surh et al., 2008). Within keratinocytes, hydroxytyrosol has been shown to up-regulate several of these antioxidant defense enzymes, especially HO-1 (Rafehi et al., 2012), which also promotes proliferation and inhibits apoptosis (Clark et al., 1997; Petrache et al., 2000). The transcriptional activation of Nrf2 by hydroxytyrosol has been demonstrated to stimulate and protect vascular endothelial cells (Zrelli et al., 2011b) from oxidative stress through the phosphorylation of PI3K/AKT and

ERK1/2 and the downstream induction of HO-1 (Zrelli et al., 2011b), which has been implicated in promoting neovascularization (Florczyk et al., 2014) and tissue repair (Bussolati and Mason, 2006). Upon phosphorylation, these kinases induce COX-2 and subsequent secretion of MMP-9 (Zhang et al., 2009a), for which elevated levels of MMP-9 have been associated with detrimental protease activity leading to growth factor degradation and the obstruction of cellular proliferation (Jang et al., 2011). Thus, hydroxytyrosol may prove to be a valuable therapeutic against destructive eicosanoids and proteases.

Notably, olive phenols have been shown to prevent the stimulation of redox-sensitive transcription factors such as NF- κ B (Figure 3.3). Bound to its inhibitory subunit I κ B within the cytosol, NF- κ B may be activated by several initiators, including ROS, TNF- α , IL-1 β , and ionizing radiation (Basu et al., 1998; Chandel et al., 2000; Renard et al., 1997), via the phosphorylation and subsequent degradation

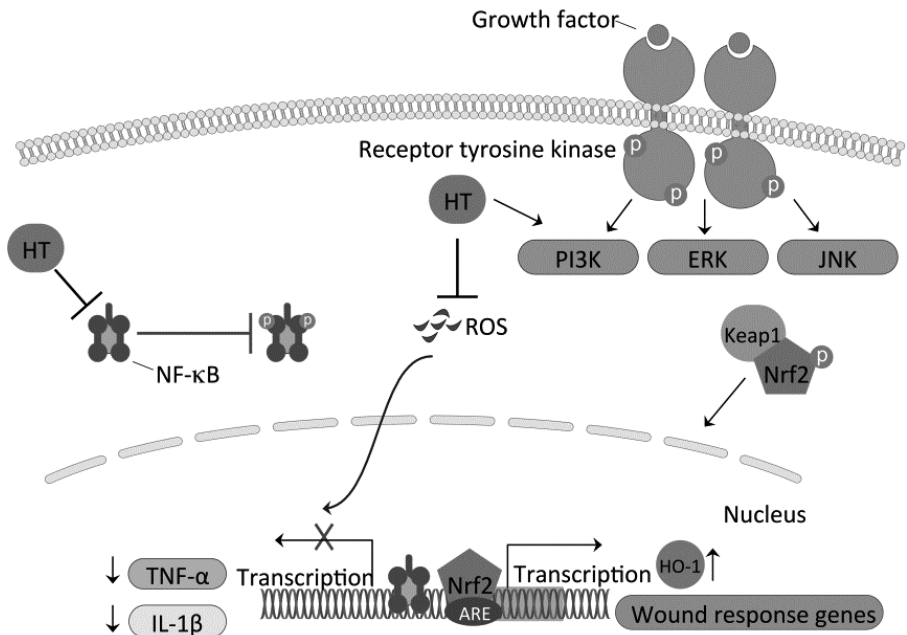


Figure 3.3 Molecular mechanisms of damage-signaling pathways in wound healing. The transcriptional modulating effects of hydroxytyrosol on NF- and Nrf-2 are depicted, mitigating inflammation and promoting cellular proliferation during wound repair.

of $\text{I}\kappa\beta$, which allows $\text{NF-}\kappa\beta$ to translocate into the nucleus. Hydroxytyrosol has been shown to interfere with this nuclear translocation (Scoditti et al., 2012), reducing the expression of $\text{NF-}\kappa\beta$ -governed pro-inflammatory genes that mediate $\text{TNF-}\alpha$, $\text{IL-1}\beta$, ICAM-1 , and MCP-1 secretion (Basu et al., 1998; Chandel et al., 2000; Renard et al., 1997). This supports the hypothesis that hydroxytyrosol induces $\text{NF-}\kappa\beta$ inhibition activity and the subsequent production of key inflammatory cytokines, which have been implicated in aberrant wound healing. Furthermore, the enzyme p38 MAPK, found upstream in the $\text{NF-}\kappa\beta$ signaling pathway, has been shown to attenuate neutrophil and monocyte release of $\text{TNF-}\alpha$ and $\text{IL-1}\beta$ when inhibited (Song et al., 2001). In line with this, hydroxytyrosol has been shown to reduce p38 MAPK activity (Sánchez-Fidalgo et al., 2012) and to exert chemopreventative effects through the same mechanism.

In parallel, the transcription of wound response genes may also be modulated through various epigenetic mechanisms, which appear to be uniquely related to the stage of repair. Although limited research has been conducted on the specific effects of olive phenols on epigenetic factors, it is known that phenolic compounds regulate the epigenome through their antioxidant activity.

DNA methylation, histone modifications, and posttranscriptional regulation by microRNAs are involved in the regulation of many biological processes through gene expression modulation (Jaenisch and Bird, 2003; Lin and Hannon, 2004), and numerous natural compounds have been identified as histone deacetylase (HDAC) inhibitors, histone acetyltransferase (HAT) activators, and HAT inhibitors. The generation of ROS and hypoxic conditions have been found to increase DNA methylation, in which the reduced expression of $\text{PKC}\epsilon$ gene was observed; however, treatment with ROS scavengers blocked hypoxia-induced CpG methylation. Because $\text{PKC}\epsilon$ expression is pertinent to cutaneous wound closure and limiting fibrosis (Leask et al., 2008; Mesquita et al., 2014), the introduction of antioxidants such as olive phenols to the wound environment may convey therapeutic effects. Similarly, in the case of histone acetylation, several thiol antioxidants were discovered to inhibit acetylation induced by hydrogen peroxide (Gilmour et al., 2003), thereby limiting the secretion of the proinflammatory cytokine IL-8 (Tomita et al., 2003).

Furthermore, it is seen that under proinflammatory conditions, human monocytes are susceptible to acetylation of $\text{NF-}\kappa\beta$ and subsequent cytokine gene expression; however, the inhibition of $\text{NF-}\kappa\beta$ target genes, including IL-6 and $\text{TNF-}\alpha$, in THP-1 monocytes cells was achieved through the up-regulation of HDAC activity and inhibition of HAT activity, preventing $\text{NF-}\kappa\beta$ -mediated chromatin acetylation and subsequent transcription of cytokines (Hye Joo et al., 2012). Although olive phenols demonstrate significant anti-inflammatory effects, their structurally related cousins elicit HDAC activity in a proinflammatory setting, which suggests oleuropein and hydroxytyrosol may operate in a similar manner.

Antioxidant and Anti-Inflammatory Effects of Phenolic Compounds in Olives and Olive Oil

Conventional Antioxidant Mechanisms of Phenolic Compounds in Olives and Olive Oil

Since its discovery in 1960, when the phenolic compound oleuropein was isolated from olive leaves (Panizzi, 1960), it has been elucidated that oleuropein and its derivative, hydroxytyrosol, possess powerful scavenging abilities through reducing a range of ROS, including peroxy radicals, HClO, superoxide radical, H₂O₂, and synthetic radicals that contribute to the olive's health promoting effects.

In particular, oleuropein's antioxidant activity is characterized by its ability to scavenge aqueous and chain-propagating lipid peroxy radicals. This ability is substantiated and complemented by its concurrent indirect inhibitory effect on xanthine oxidase, an ROS-generating enzyme; following its inhibition, there is a compounding loss of super-oxide generation (Visioli et al., 1998a). As previously mentioned, the potent antioxidant activity of oleuropein and hydroxytyrosol may be attributed to the orthodiphenolic group found in their molecular structures and the high electron donating effect of the second hydroxyl group. Together these olive phenols convey protection through regulating antioxidant/pro-oxidant enzymes (Martinez-Gonzalez and Sanchez-Villegas, 2004). The activation of phase II detoxifying enzyme systems, including glutathione (GSH), SOD, HO-1, and NAD (P)H quinone oxidoreductase-1 (Copple et al., 2008), may be induced through activation of the transcription factors Nrf2 and AMPK-FOXO3a. Thus, activating alternate antioxidant expression at the gene level contributes to their antioxidant activities. In addition, the activation of peroxisome proliferator-activated receptor coactivator 1 α (PPARGC1 α) in retinal epithelial cells has resulted in increased protein expression of mitochondrial transcription factors, leading to enhanced mitochondrial biogenesis. This further contributes to its protective effects against damage resulting from oxidative stress.

In congruence with the antioxidant effects of olive phenols are their anti-inflammatory activities. It has been demonstrated that hydroxytyrosol attenuates oxidative stress-associated inflammation. These effects are facilitated by the abrogation of intracellular redox-sensitive gene expression, including expression of the gene encoding COX-2 (Barbieri et al., 2003), and is exemplified with human monocytes in which hydroxytyrosol diminished ROS-mediated COX-2 expression (Zhang et al., 2009b). It is also through the inhibition of proinflammatory transcription factors, such as PKC α /PKC β 1, NF- κ β (Scoditti et al., 2012), STAT-1 α , and IRF-1 (Maiuri et al., 2005), that olive phenol compounds convey their anti-inflammatory action. PKC α and PKC β 1 induce COX-2 secretion, whereas hydroxytyrosol has been shown to obstruct their phosphorylation (Scoditti et al., 2012; Zhang et al., 2009a). Through NF- κ β inhibition, hydroxytyrosol causes a reduction in the expression of proinflammatory genes that facilitate TNF- α ,

IL-1 β , ICAM-1, and MCP-1 secretion (Basu et al., 1998; Chandel et al., 2000; Renard et al., 1997), thereby mitigating the ROS-induced inflammation.

The conventional antioxidant activities together with their transcriptional anti-inflammatory effects support the therapeutic utility of olive phenol compounds.

Emerging Insights from Genome-Wide Transcription Studies Relating to the Antioxidant and Anti-Inflammatory Effects of Phenolic Compounds in Olives and Olive Oil

Insights into the molecular mechanisms of olive phenolic antioxidant and anti-inflammatory activities have been revealed through study of their modulation of gene expression. Recent identification of specific antioxidant and anti-inflammatory pathways of olive phenols have reinforced previous epidemiological studies that highlighted the chemopreventive and cardioprotective properties of olive oil.

Modulation of inflammation-associated gene expression has been documented following treatment with hydroxytyrosol. For example, mRNA sequencing analysis demonstrated the up-regulation of the anti-inflammatory receptor IFN- γ and down-regulation of the cytokine IL-11 (Rafehi et al., 2012). The same study also identified genes associated with the antioxidant activity of hydroxytyrosol, including the gene-encoding HO-1, which is up-regulated in response to oxidative stress via the Nrf-2/ARE pathway (Chen et al., 2011; Kang et al., 2005; Park et al., 2011), and which was expressed in treated keratinocytes, along with the antioxidant enzyme glutathione peroxidase 3 (Rafehi et al., 2012).

It was discovered that the phenol fraction of the olive repressed the expression of several genes related to inflammation pathways *in vivo*. Consumption of phenol-rich virgin olive oil in humans was associated with decreased expression in PBMCs of both postprandial *IL1B* and *PTGS2*, which are up-regulated in chronic inflammatory conditions (FitzGerald, 2003). This finding is analogous to previous *in vitro* studies that demonstrated olive oil-induced attenuation of LPS-induced *PTGS2* transcription (Zhang et al., 2009b). *In vivo* reduction of NF- κ B activation following olive oil consumption was found (Bellido et al., 2004; Perez-Martinez et al., 2007) and is supported by the reported under-expression of *NFKB1A*, which encodes I κ B α , a member of an inhibitory I κ B family that retains NF κ B in the cytoplasm (Aggarwal, 2004). Furthermore, *in vitro* studies showing reduced NF- κ B activation by resveratrol support the hypothesis that phenols use similar pathway mechanisms involving the mitigation of genes encoding inflammatory cytokines and adhesion molecules (Zern and Luz-Fernandez, 2005).

The antineoplastic activity of olive phenol compounds has also been attributed to their gene-modulating abilities. Olive oil consumption with high phenol content has resulted in decreased PBMC expression of *DUSP1* and *DUSP2*, which are known

to regulate members of the MAPK pathway, including p38, MAPK/ERK, and SAPK/JNK (Lang et al., 2006). *In vitro*, it has been demonstrated that the STAT family of transcription factors, which are associated with the survival of a variety of different tumors (Das et al., 2007; Pedranzini et al., 2004; Zhang et al., 2006), were down-regulated in human chronic myelogenous leukemia K562 cells following treatment with hydroxytyrosol (Rafehi et al., 2012). Moreover, it has been revealed that hydroxytyrosol and oleuropein induce antiproliferative and proapoptotic effects in certain human colorectal cancer cell types, mediated by the down-regulation of FAS gene expression. Thus, the modulation of the aforementioned genes may prove to be a molecular target for the antiproliferative activity of olive oil phenols in a subset of cancers. Other possible mechanisms through which olive phenols convey chemopreventive activity include their anti-inflammatory action against processes previously described, involving the ability of proinflammatory cytokines to promote the tumor microenvironment (Colotta et al., 2009).

Although olive phenols may act solely as conventional antioxidants, they also promote cellular defense against oxidative stress, conveying anti-inflammatory and antitumorigenic properties.

Cardiovascular Effects of Hydroxytyrosol and Oleuropein

Anti-Atherogenic Effects of Hydroxytyrosol and Oleuropein

Of the phenolic compounds found in olives, hydroxytyrosol and oleuropein are some of the most potent antioxidants and radical scavengers that protect against cardiovascular disease. These phenols protect the heart by decreasing oxidative stress, atherosclerosis risk factors, and cardiotoxicity and by promoting high-density lipoprotein (HDL) cholesterol and angiogenesis (see Chapter 2).

Given that atherosclerosis and cardiovascular disease are dependent on accumulative oxidative damage to biomolecules, it is reasonable to link phenolic compounds to their prevention. Hydroxytyrosol was shown to prevent atherosclerotic plaque formation by inhibiting the oxidation of LDL cholesterol in macrophage-like J774 A.1 cells (Di Benedetto et al., 2007). In rats, a cholesterol-rich diet induced hypercholesterolemia associated with elevated total cholesterol, triacylglycerols, and LDL cholesterol (Jemai et al., 2008). Rats fed a cholesterol-rich diet supplemented with hydroxytyrosol in drinking water had decreased total cholesterol, triacylglycerol, and LDL cholesterol and increased HDL cholesterol in their serum, in contrast to rats that consumed only cholesterol-rich diets (Jemai et al., 2008). Furthermore, following human consumption, hydroxytyrosol was detected in the plasma gradient fractions containing LDL, suggesting that the phenol has a transient association with LDL, revealing a physiological relevance to atherosclerosis development (Gonzalez-Santiago et al., 2010). In

vitro, hydroxytyrosol has also been implicated in the inhibition of platelet aggregation and prevention of eicosanoid formation (Petroni et al., 1995). Hydroxytyrosol diet supplementation in hyperlipemic rabbits improved blood lipids and antioxidant status and decreased atherosclerotic lesion size (Gonzalez-Santiago et al., 2006). In addition, hydroxytyrosol protected the aorta against oxidative stress in rats (Rietjens et al., 2007).

Monocyte adhesion to vascular endothelium is a known contributor to atherosclerosis development. ICAM-1, VCAM-1, and E-selectin have been found to play a role in endothelial activation. Extra virgin olive oil was shown to reduce ICAM-1 and VCAM-1 expression in human umbilical vascular endothelial cells, which was primarily mediated by oleuropein and hydroxytyrosol (Dell'Agli et al., 2006). Specifically, hydroxytyrosol and oleuropein treatment has also decreased VCAM-1 in endothelial cells (Carluccio et al., 2003; Manna et al., 2009). In addition, hydroxytyrosol has been shown to have anti-atherogenic and antioxidant effects on porcine pulmonary artery endothelial cells mediated by an increase in catalase and activation of the AMP-FOXO3a signaling pathway (Zrelli et al., 2011c).

Excessive vascular smooth muscle cell (VSMC) proliferation and migration also contribute to atherosclerosis development, and hydroxytyrosol was found to induce apoptosis of rat VSMCs mediated by NO production and inactivation of Akt (Zrelli et al., 2011a). Furthermore, oleuropein was shown to inhibit bovine VSMCs by blocking the G1 to S cell transition, possibly mediated by ERK1/2 (Abe et al., 2011). Together these studies suggest hydroxytyrosol and oleuropein may decrease the onset of atherosclerosis by preventing excessive VSMCs.

Hydroxytyrosol and Oleuropein Protect the Heart

The anti-atherogenic effects of olive phenols rest on their ability to attenuate the damage associated with oxidative stress. Because LDL oxidation and its downstream effects, including ischaemia, may be inhibited by olive phenols, these compounds serve as both a prophylactic and treatment for cardiac pathologies.

For example, pretreatment with oleuropein in isolated rat hearts with sustained ischemia-reperfusion induced ROS (oxidized glutathione and membrane lipid peroxidation) afforded protection from oxidative stress (Manna et al., 2004). In a separate study, rabbits fed a normal or hypercholesterolemic diet were subjected to ischemia, and it was seen that ischemia-treated rabbits having diets supplemented with oleuropein had a reduction in infarct size, total cholesterol, and triacylglycerols (Andreadou et al., 2006).

In ischemic tissues, endothelial progenitor cells (EPCs) play an important role in neovascularisation and cardiac repair. Arterial hypertension affects cardiovascular disease, during which angiotensin II damages vascular endothelium (through oxidative stress) and inhibits the regenerative functions of EPCs. An interesting study by

Parzonko (2013) and colleagues investigated the effects of oleuropein on EPCs. Pretreatment of EPCs with oleuropein partially prevented angiotensin II-mediated decreased proliferation and telomerase activity. Angiotensin II impaired EPC migration and adhesion; however, pretreatment with oleuropein abrogated this effect. Oleuropein also induced EPCs to undergo increased tube formation. Collectively, oleuropein promoted angiogenesis in a process possibly mediated by Nrf2 activation and increased HO-1 expression. In addition, dietary supplementation of hydroxytyrosol and oleuropein was able to reverse high-fat diet induced cardiac stiffness and reduce high-fat diet induced cardiac collagen deposition in rats (Poudyal et al., 2010).

As a cancer chemotherapeutic drug with damaging cardiac side effects, doxorubicin-associated cardiotoxicity is defined by the increased oxidative stress and mitochondrial dysfunction it induces. Hydroxytyrosol has been shown to reduce oxidative damage and the percentage of altered mitochondria in the heart following doxorubicin administration (Granados-Principal et al., 2014). Oleuropein has also been demonstrated to reduce doxorubicin-induced cardiotoxicity. Doxorubicin-treated rats that had cardiotoxicity as characterized by increased lipid peroxidation and iNOS in cardiomyocytes (which lead to higher NO production and subsequent oxidative apoptosis/necrosis) were protected by oleuropein, which was able to prevent these increases (Andreadou et al., 2007). In the same study, doxorubicin changed the metabolic profile of rat hearts by increasing myocardial acetate and succinate; however, pre- and post-treatment with oleuropein abrogated these doxorubicin-induced changes.

Although there are no reports on the effects of hydroxytyrosol on cardiomyocytes, recent work revealed a decrease in mitochondrial-dependent oxidative stress, which favors cardiomyocyte proliferation (Puente et al., 2014). Therefore, it is possible that the antioxidant effects of hydroxytyrosol and oleuropein could potentially promote cardiomyocyte proliferation by decreasing oxidative stress and DNA damage. In light of recent literature supporting adult mammalian cardiomyocyte renewal (Bergmann et al., 2009; Mollova et al., 2013; Senyo et al., 2013), and given the favorable effect of olive oil on the heart, future studies should be performed to determine whether hydroxytyrosol and oleuropein might possibly promote cardiomyocyte proliferation to favor cardiac regeneration or increase the low homeostatic levels of cardiomyocyte renewal.

Anticancer Effects of Phenolic Compounds in Olives and Olive Oil

In Vitro and in Vivo Evidence

The proposal of olive antagonism against cancer is founded on epidemiological studies correlating a lower incidence of certain cancers within the Mediterranean region. In these studies, in addition to the observed reduced death rates, the prevalence of

cancer also saw a marked decrease of 61% in the case of the Lyon Heart Study, due to the adherence to a Mediterranean diet (de Lorgeril et al., 1998). These lower relative mortality rates were seen particularly in breast and colorectal cancers, as well as leukemia (Casaburi et al., 2013). The association between olive consumption and its anti-inflammatory and antioxidant activities forms the basis for the proposed effects against tumorigenesis. The phenolic compounds of the olive demonstrated a number of actions that counter the classical hallmarks of cancer: apoptosis evasion, angiogenesis, growth signal self-sufficiency, invasion of tissue, and insensitivity antigrowth signals (Hanahan and Weinberg, 2000). Here, we discuss some insights into phenolic compound interactions with cancer and oncogenesis.

When discussing classical cancer characteristics, tumor formation is typified by uninhibited cellular proliferation. This proliferation is derived from an accumulation of cellular abnormalities that enable cancerous cells to grow uncontrollably and is achieved via the dysregulation of growth promoting signals, evasion of apoptosis, and a proangiogenic environment.

The antitumoral potential of oleuropein has been investigated *in vitro* on the cancer cell lines TF-1 glioblastoma, 786-O renal cell adenocarcinoma, RPMI7951 skin lymph node melanoma, and LoVo colorectal adenocarcinoma (Hamdi and Castellon, 2005). The findings indicate growth inhibition induced by oleuropein treatment in a dose-dependent manner in all cell lines. However, this effect was considerably diminished in LoVo colorectal adenocarcinoma (Hamdi and Castellon, 2005). The varying efficacy of oleuropein treatment on these cancer cell lines is partially explained by glucose transporter (GLUT)-mediated oleuropein diffusion. Variable expression of GLUT in cancer cell lines leads to varying concentrations of oleuropein delivered to different cell lines. This manifests as differential sensitivity toward oleuropein, whereby cervical, thyroid, breast, and colon cancers up-regulate GLUT transporters and are therefore more susceptible to treatment. In a subsequent *in vivo* investigation, partial to complete tumor regression was observed in Swiss albino mice that consumed 1% oleuropein in their drinking water (Hamdi and Castellon, 2005).

Comparable observations in which oleuropein induced inhibition of tumor proliferation were made in a Mediterranean study that linked colorectal and breast cancers. In the instance of human colorectal cancer, the down-regulation of HIF-1 α , a regulator of angiogenic cytokine and cell motility adhesion genes, was identified as the mechanism of olive phenolic action in the inhibition of HT-29 grade II adenocarcinoma cell line proliferation (Cardeno et al., 2013). The same study revealed that the induction of apoptotic events in HT-29 cells was time dependent, where hydroxytyrosol induced significant dose-dependent early and late apoptotic populations. This was correspondingly linked to the proapoptotic tumor suppressor p53 protein, which was significantly up-regulated 48 hours post-hydroxytyrosol treatment, such that a greater than threefold increase of p53 expression comparative to

controls was found. This olive phenol stimulation of apoptosis via the p53 pathway was corroborated in MCF-7 breast cancer cells that were treated with oleuropein, in which 3.5-fold up-regulation of p53 expression was found (Hassan et al., 2013). This result was correlated with an increase in observed Bax gene expression that promoted the disruption of mitochondrial integrity and initiated the apoptotic pathway. A related breast cancer study investigated the variant SKBR3, which has a type-1 receptor tyrosine kinase HER-2 overexpressing phenotype, where HER-2 regulates cellular proliferation, differentiation, and apoptosis (Yarden, 2001). Olive oil phenolic fractions, including oleuropein aglycone, played a multifaceted role in diminishing SKBR3 cell viability. The first observation included reduced cell viability of SKBR3 cancer cells through the induction of cytotoxic activity (Menendez et al., 2008). In addition, overall down-regulation of the HER-2 oncoprotein was found. In the same study, a synergistic activity of oleuropein aglycone and ligitroside aglycone, caused a 68–85% reduction of HER-2 expression. In single phenol treatments, the compound hydroxytyrosol alone reduced HER-2 expression by 35% (Menendez et al., 2008).

The most interesting aspect of oleuropein's cellular effects is its interaction with drug therapy. A complementary study of HER-2 breast cancer presents additional data that suggest that a group of 30 olive phenolic compounds may reverse auto resistance to Trastuzumab, a HER-2 breast cancer monoclonal antibody therapy (Menendez et al., 2007). While supporting oleuropein aglycone dose-dependent inhibition of SKBR3, the results suggested a correlation between the levels of HER-2 expression and the efficacy of oleuropein aglycone treatment. Co-exposure of oleuropein aglycone with Trastuzumab in HER-2 overexpressing breast cancer cells indicated a synergistic enhancement of Trastuzumab activity, with a reported 50-fold increase in Trastuzumab efficacy in reducing cell viability, hinting at the reversal of Trastuzumab autoresistance (Menendez et al., 2007).

In an alternative model utilizing breast cancer cell lines derived from estrogen receptor alpha (ER- α) negative SKBR-3 cells, which have 25–30% prevalence among breast cancers, proliferation inhibition by oleuropein and hydroxytyrosol involved GPER30 dependent pathways. ER- α negative breast cancer cell proliferation is activated upon binding to the G-protein coupled receptor, GPER30, and cell proliferation is initiated via triggering ERK1/2 signalling (Patani et al., 2013).

Illumination of oleuropein, hydroxytyrosol, and GPER binding pocket biochemistry indicated a degree of binding affinity of phenols to the GPER binding site. In contrast to tamoxifen, an antagonist to ER- α positive breast cancer proliferation and an agonist of GPER that stimulates growth in ER- α negative breast cancer, oleuropein and hydroxytyrosol are growth inhibitors that reduce cyclin D1 protein expression and enhance expression of the p53 tumor suppressor. Cyclin D1 is a component of cell cycle progression that is up-regulated in cases of human neoplasia (Weinstat-Saslow et al., 1995). Apoptosis stimulation resulted from GPER binding to oleuropein

and hydroxytyrosol and subsequent sustained ERK phosphorylation and nuclear accumulation (Chimento et al., 2014).

As mentioned previously, besides the more commonly studied breast and colorectal cancers that are less prevalent in the Mediterranean region, there has been some interest in phenolic interactions with human leukemia. In experiments using HL-60 cells as part of a broader investigation, concentrated efforts were limited to human leukemia as the focal point of study in relation to olive phenol fractions (Hamdi and Castellon, 2005). K562 cells stand as an important target for therapeutics due to their stem cell–like properties, such as pluripotency, as well as their resistance against chemical inducers. Application of olive leaf extract, which is rich in oleuropein, inhibited the proliferation of K562 in a dose-dependent manner. Effects were particularly evident at 72 hours post treatment, in which olive extract treatment reduced cell proliferation levels to below 20% relative to controls.

Although cell viability and proliferation assays feature prominently in assessing treatment efficacy, genotypic expression alterations are also correlated with olive leaf extract treatment (Samet et al., 2014). Oleuropein and hydroxytyrosol have also been shown to up-regulate CHEK2 gene expression (Frazer and Young, 2012), which is involved in cell cycle arrest. In addition, observed apoptotic effects can be linked to the up-regulation of CASP6, CASP8, DFFA, and BID proapoptotic genes by olive leaf extract (Samet et al., 2014).

As knowledge of the biological activity and influences of phenolic compounds, such as oleuropein and hydroxytyrosol, become increasingly clear and extensive, it should be appreciated that their therapeutic benefits extend beyond classical anti-inflammatory and antioxidant activities. It has been demonstrated that phenolic compounds possess the potential to modulate both protein expression and interfere with cell signaling pathways to exert their effects. With increasing research, however, it appears that among the results garnered, anomalies have surfaced that present an almost paradoxical model of phenolic compound influence on the instigation of cell death in tumors.

The Radical-Mediated Hypothesis: Controversies Surrounding in Vitro Evidence for the Anticancer Effects of Phenolic Compounds in Olives and Olive Oil

The broad and extensive cellular modulatory effects of phenolic compounds from olives and olive oil on cancer cells have produced conflicting evidence of their mechanism of action. This controversy stems from the varying results of in vitro studies on different cell lines using similar phenolic treatment doses. A 100 μM dose of hydroxytyrosol was sufficient to inhibit growth and induce apoptosis in HL-60 leukaemia cells; however, in MCF-7 and SKBR3, cell resistance was found with the same treatment (Fabiani et al., 2008b; Han et al., 2009). Yet, in an opposing study, a 12.5 μM treatment dose of

hydroxytyrosol was sufficient to attenuate MCF-7 cell growth (Goulas et al., 2009). This variation has been considered to be an anomaly resulting from the accumulation of H_2O_2 within the culture medium, wherein H_2O_2 encourages antiproliferative and proapoptotic reactions as a pro-oxidant (Fabiani et al., 2009a). Hydroxytyrosol generation of H_2O_2 was identified as a partial explanation of the proapoptotic conditions instigated by the phenol. Contrary to its known antioxidant activity, it was observed that oxidative stress from H_2O_2 generated by hydroxytyrosol resulted in the apoptosis of HL-60 cells. In support of this, detoxification of H_2O_2 prevented cell apoptosis, which was further supported by the finding that the structurally similar compounds tyrosol and caffeic acid, which are known to generate H_2O_2 , were ineffective at increasing the percentage of apoptotic cells (Fabiani et al., 2009b).

A further study of olive phenolic compounds clarified the significance of the contribution of H_2O_2 generation to their cellular effects (Fabiani et al., 2009b). The results confirmed that H_2O_2 -generating phenolic compounds were particularly capable in promoting apoptosis, an effect that was reduced following catalase treatment. Despite this, it was shown concurrently that oleuropein and ligstroside aglycone were able to induce apoptosis with minimal production of H_2O_2 (Fabiani et al., 2009b).

In an additional study that sought to further explain the effect of hydroxytyrosol on a number of tumor cell lines, it was found that, H_2O_2 -induced tumor cell apoptosis was not a complete explanation (Fabiani et al., 2012). An aspect of the radical mediated hypothesis proposes culture media influence on H_2O_2 accumulation and apoptosis instigation. Although H_2O_2 release is observed with hydroxytyrosol, simple phosphate buffered saline solution also produced this effect, indicating a dependence on experimental conditions. More importantly, apoptosis of tumor cells following hydroxytyrosol application is still greater than the direct application of H_2O_2 (Fabiani et al., 2012).

In addressing the controversial aspects of phenol anticancer properties, the current literature cannot adequately elaborate and amalgamate the mechanisms of phenolic activity against cancers. Although the radical-mediated hypothesis presents a viable explanation of the experimental results of apoptosis induced by phenolic compounds, it is not uniformly observed with a single compound, let alone all olive phenols, as shown with oleuropein and ligstroside aglycones. This controversy stems from an *in vitro* setting in which the cell culture medium may produce adverse effects that are unlikely to occur *in vivo*. With more rigorous and extensive research, the biological outcomes of olive phenols may be elucidated.

Acknowledgments

Tom C. Karagiannis, a future fellow, and the Epigenomic Medicine Laboratory are supported by the Australian Research Council, the Victorian Government's Operational Infrastructure Support Program and McCord Research. Tom C. Karagiannis

and Nancy B. Ray are directly supported by McCord Research, and the authors would like to acknowledge the intellectual contribution of Dr. Darlene McCord.

References

- Abe, R.; Beckett, J.; Nixon, A.; Rochier, A.; Yamashita, N.; Sumpio, B. Olive Oil Polyphenol Oleuropein Inhibits Smooth Muscle Cell Proliferation. *Eur. J. Vasc. Endovas. Surg.* **2011**, *41*, 814–820.
- Adams, R. H.; Alitalo, K. Molecular Regulation of Angiogenesis and Lymphangiogenesis. Reviews Molecular Cell Biology, *Nat. Rev. Mole. Cell Biol.* **2007**, 464.
- Aggarwal, B. B. Nuclear Factor- κ B: The Enemy Within. *Cancer Cell* **2004**, *6*, 203–208.
- Alagna, F.; Mariotti, R.; Panara, F.; Caporali, S.; Urbani, S.; Veneziani, G.; Esposito, S.; Taticchi, A.; Rosati, A.; Rao, R.; et al. Olive Phenolic Compounds: Metabolic and Transcriptional Profiling During Fruit Development. *BMC Plant Biol.* **2012**, *12*, 162.
- Álvaro, A.; Valentina, R.-G.; Miguel Ángel, M.-G. Monounsaturated Fatty Acids, Olive Oil and Blood Pressure: Epidemiological, Clinical and Experimental Evidence. *Public Health Nutr.* **2006**, *9*, 251–257.
- Andreadou, I.; Iliodromitis, E. K.; Mikros, E.; Constantinou, M.; Agalias, A.; Magiatis, P.; Skaltsounis, A. L.; Kamber, E.; Tsantili-Kakoulidou, A.; Kremastinos, D. T. The Olive Constituent Oleuropein Exhibits Anti-Ischemic, Antioxidative, and Hypolipidemic Effects in Anesthetized Rabbits. *J. Nutr.* **2006**, *136*, 2213–2219.
- Andreadou, I.; Sigala, F.; Iliodromitis, E. K.; Papaefthimiou, M.; Sigalas, C.; Aliagiannis, N.; Savvari, P.; Gorgoulis, V.; Papalabros, E.; Kremastinos, D. T. Acute Doxorubicin Cardiotoxicity Is Successfully Treated with the Phytochemical Oleuropein through Suppression of Oxidative and Nitrosative Stress. *J. Mol. Cell Cardiol.* **2007**, *42*, 549–558.
- Ashcroft, G. S.; Yang, X.; Glick, A. B.; Weinstein, M.; Letterio, J. J.; Mizel, D. E.; Anzano, M.; Greenwell-Wild, T.; Wahl, S. M.; Deng, C.; et al. Mice Lacking Smad3 Show Accelerated Wound Healing and an Impaired Local Inflammatory Response. *Nature Cell Biol.* **1999**, *1*, 260.
- Bahou, W. F.; Gnatenko, D. V. Platelet Transcriptome: The Application of Microarray Analysis to Platelets. *Sem. Thrombosis Hemostasis.* **2004**, *30*, 473–484.
- Barbieri, S. S.; Eligini, S.; Brambilla, M.; Tremoli, E.; Colli, S. Reactive Oxygen Species Mediate Cyclooxygenase-2 Induction during Monocyte to Macrophage Differentiation: Critical Role of NADPH Oxidase. *Cardio. Res.* **2003**, *60*, 193–197.
- Basu, S.; Rosenzweig, K. R.; Youmell, M.; Price, B. D. The DNA-Dependent Protein Kinase Participates in the Activation of NF kappa B following DNA Damage. *Biochem. Biophys. Res. Commun.* **1998**, *247*, 79–83.
- Beauchamp, G. K.; Keast, R. S.; Morel, D.; Lin, J.; Pika, J.; Han, Q.; Lee, C. H.; Smith, A. B.; Breslin, P. A. Phytochemistry: Ibuprofen-Like Activity in Extra-Virgin Olive Oil. *Nature* **2005**, *437*, 45–46.
- Bellido, C.; Lopez-Miranda, J.; Blanco-Colio, L. M.; Perez-Martinez, P.; Muriana, F. J.; Martin-Ventura, J. L.; Marin, C.; Gomez, P.; Fuentes, F.; Egido, J.; et al. Butter and Walnuts, But Not Olive Oil, Elicit Postprandial Activation of Nuclear Transcription Factor $\text{I}\kappa\text{B}$ in

- Peripheral Blood Mononuclear Cells From Healthy Men. *Am. J. Clin. Nutr.* **2004**, *80*, 1487–1491.
- Bergmann, O.; Bhardwaj, R. D.; Bernard, S.; Zdunek, S.; Barnabe-Heider, F.; Walsh, S.; Zupicich, J.; Alkass, K.; Buchholz, B. A.; Druid, H.; et al. Evidence for Cardiomyocyte Renewal in Humans. *Science* **2009**, *324*, 98–102.
- Bernini, R.; Mincione, E.; Barontini, M.; Crisante, F. Convenient Synthesis of Hydroxytyrosol and Its Lipophilic Derivatives from Tyrosol or Homovanillyl Alcohol. *J. Agric. Food Chem.* **2008**, *56*, 8897–8904.
- Besnard, G.; Rubio de Casas, R.; Christin, P.A.; Vargas, P. Phylogenetics of Olea (Oleaceae) Based on Plastid and Nuclear Ribosomal DNA Sequences: Tertiary Climatic Shifts and Lineage Differentiation Times. *Ann. Botany* **2009**, *104*, 143–160.
- Bianchi, M. E. DAMPs, PAMPs and Alarmins: All We Need to Know About Danger. *J. Leukocyte Biol.* **2007**, *81*, 1–5.
- Bisignano, G.; Tomaino, A.; Lo Cascio, R.; Crisafi, G.; Uccella, N.; Saija, A. On the In-Vitro Antimicrobial Activity of Oleuropein and Hydroxytyrosol. *J. Pharm. Pharmacol.* **1999**, *51*, 971–974.
- Boardman, J.; Kenyon, K. M.; Moynahan, E. J.; Evans, J. D. The Olive in the Mediterranean: Its Culture and Use [and Discussion]. *Philosophical Transactions of the Royal Society of London Series B, Biol. Sci.* **1976**, *275*, 187–196.
- Brenes, M.; Medina, E.; Romero, C.; De Castro, A. Antimicrobial Activity of Olive Oil. *Agro Food Industry Hi-Tech* **2007**, *18*, 6–8.
- Brun, J.-P. The Production of Perfumes in Antiquity: The Cases of Delos and Paestum. *Am. J. Arch.* **2000**, *104*, 277–308.
- Brussell, D. E. Medicinal Plants of Mt. Pelion, Greece. *Econ. Botany* **2004**, *58*, S174–S202.
- Budiyanto, A.; Ahmed, N. U.; Wu, A.; Bito, T.; Nikaido, O.; Osawa, T.; Ueda, M.; Ichihashi, M. Protective Effect of Topically Applied Olive Oil against Photocarcinogenesis Following UVB Exposure of Mice. *Carcinogenesis* **2000**, *21*, 2090.
- Bussolati, B.; Mason, J. C. Dual Role of VEGF-induced Heme-oxygenase-1 in Angiogenesis. *Antiox. Redox Signalling* **2006**, *8*, 1163.
- Campos, I.; Geiger, J. A.; Santos, A. C.; Carlos, V.; Jacinto, A. Genetic Screen in *Drosophila Melanogaster* Uncover a Novel Set of Genes Required for Embryonic Epithelial Repair. *Genetics* **2010**, *184*, 129–140.
- Caramia, G.; Gori, A.; Valli, E.; Cerretani, L. Virgin Olive Oil in Preventive Medicine: From Legend to Epigenetics. *Eur. J. Lipid Sci. Tech.* **2012**, *114*, 375–388.
- Cardeno, A.; Sanchez-Hidalgo, M.; Alarcon-de-la-Lastra, C. An Update of Olive Oil Phenols in Inflammation and Cancer: Molecular Mechanisms and Clinical Implications. *Curr. Med. Chem.* **2013**, *20*, 4758–4776.
- Carluccio, M. A.; Scialella, L.; Ancora, M. A.; Massaro, M.; Scoditti, E.; Storelli, C.; Visioli, F.; Distanto, A.; De Caterina, R. Olive Oil and Red Wine Antioxidant Polyphenols Inhibit Endothelial Activation: Antiatherogenic Properties of Mediterranean Diet Phytochemicals. *Art. Thromb. Vasc. Biol.* **2003**, *23*, 622–629.
- Carrasco-Pancorbo, A.; Cerretani, L.; Bendini, A.; Segura-Carretero, A.; Del Carlo, M.; Gallina-Toschi, T.; Lercker, G.; Compagnone, D.; Fernandez-Gutierrez, A. Evaluation of the

- Antioxidant Capacity of Individual Phenolic Compounds in Virgin Olive Oil. *J. Agric. Food Chem.* **2005**, *53*, 8918–8925.
- Carrera-González, M. P.; Ramírez-Expósito, M. J.; Mayas, M. D.; Martínez-Martos, J. M. Protective Role of Oleuropein and Its Metabolite Hydroxytyrosol on Cancer. *Trends Food Sci. Tech.* **2013**, *31*, 92–99.
- Casaburi, I.; Puoci, F.; Chimento, A.; Sirianni, R.; Ruggiero, C.; Avena, P.; Pezzi, V. Potential of Olive Oil Phenols as Chemopreventive and Therapeutic Agents against Cancer: A Review of in Vitro Studies. *Mol. Nutr. Food Res.* **2013**, *57*, 71–83.
- Chandel, N. S.; Trzyna, W. C.; McClintock, D. S.; Schumacker, P. T. Role of Oxidants in NF-kappa B Activation and TNF-alpha Gene Transcription Induced by Hypoxia and Endotoxin. *J. Immun.* **2000**, *165*, 1013–1021.
- Charoenprasert, S.; Mitchell, A. Factors Influencing Phenolic Compounds in Table Olives (*Olea Europaea*). *J. Agric. Food Chem.* **2012**, *60*, 7081–7095.
- Chen, J. S.; Huang, P. H.; Wang, C. H.; Ling, F. Y.; Tsai, H. Y.; Wu, T. C.; Lin, S. J.; Chen, J. W. Nrf-2 Mediated Heme Oxygenase-1 Expression, an Antioxidant-independent Mechanism, Contributes to Anti-atherogenesis and Vascular Protective Effects of Ginkgo Biloba Extract. *Atherosclerosis.* **2011**, *214*, 301–309.
- Chen, L.; Mehta, N. D.; Zhao, Y.; DiPietro, L. A. Absence of CD4 or CD8 Lymphocytes Changes Infiltration of Inflammatory Cells and Profiles of Cytokine Expression in Skin Wounds, But Does Not Impair Healing. *Exp. Derm.* **2014**, *23*, 189–194.
- Chimento, A.; Casaburi, I.; Rosano, C.; Avena, P.; De Luca, A.; Campana, C.; Martire, E.; Santolla, M. F.; Maggiolini, M.; Pezzi, V.; et al. Oleuropein and Hydroxytyrosol Activate GPER/ GPR30-dependent Pathways Leading to Apoptosis of ER-negative SKBR3 Breast Cancer Cells. *Mol. Nutr. Food Res.* **2014**, *58*, 478–489.
- Cicerale, S.; Lucas, L. J.; Keast, R. S. J. Antimicrobial, Antioxidant and Anti-inflammatory Phenolic Activities in Extra Virgin Olive Oil. *Curr. Opin. Biotech.* **2012**, *23*, 129–135.
- Clark, J. E.; Green, C. J.; Motterlini, R. Involvement of the Heme Oxygenase-carbon Monoxide Pathway in Keratinocyte Proliferation. *Biochem. Biophys. Res. Comm.* **1997**, *241*, 215–220.
- Cole, J.; Tsou, R.; Wallace, K.; Gibran, N.; Isik, F. Early Gene Expression Profile of Human Skin to Injury Using High-density cDNA Microarrays. *Wound Rep. Regen.* **2001**, *9*, 360–370.
- Colotta, F.; Allavena, P.; Sica, A.; Garlanda, C.; Mantovani, A. Cancer-related Inflammation, The Seventh Hallmark of Cancer: Links to Genetic Instability. *Carcinogenesis.* **2009**, *30*, 1073–1081.
- Cooper, L.; Johnson, C.; Burslem, F.; Martin, P. Wound Healing and Inflammation Genes Revealed by Array Analysis of “Macrophageless” PU.1 Null Mice. *Genome Biol.* **2005**, *6*, 5–21.
- Copple, I. M.; Goldring, C. E.; Kitteringham, N. R.; Park, B. K. The Nrf2-Keap1 Defence Pathway: Role in Protection against Drug-Induced Toxicity. *Toxicology* **2008**, *246*, 24–33.
- D’Amico, G.; Frascaroli, G.; Bianchi, G.; Transidico, P.; Doni, A.; Vecchi, A.; Sozzani, S.; Allavena, P.; Mantovani, A. Uncoupling of Inflammatory Chemokine Receptors by IL-10: Generation of Functional Decoys. *Nature Imm.* **2000**, *1*, 387–391.
- D’Angelo, S.; Ingrosso, D.; Migliardi, V.; Sorrentino, A.; Donnarumma, G.; Baroni, A.; Masella, L.; Antonietta Tufano, M.; Zappia, M.; Galletti, P. Hydroxytyrosol, a Natural An-

- tiioxidant from Olive Oil, Prevents Protein Damage Induced by Long-wave Ultraviolet Radiation in Melanoma Cells. *Free Rad. Biol. Med.* **2005**, *38*, 908–919.
- Das, S.; Roth, C. P.; Wasson, L. M.; Vishwanatha, J. K. Signal Transducer and Activator of Transcription-6 (STAT6) is a Constitutively Expressed Survival Factor in Human Prostate Cancer. *Prostate*. **2007**, *67*, 1550–1564.
- de la Puerta, R.; Martinez-Dominguez, E.; Ruiz-Gutierrez, V. Effect of Minor Components of Virgin Olive Oil on Topical Anti-inflammatory Assays. *J. Biosci.* **2000**, *55*, 814–819.
- Deiana, M.; Incani, A.; Rosa, A.; Corona, G.; Atzeri, A.; Loru, D.; Paola Melis, M.; Assunta Dessi, M. Protective Effect of Hydroxytyrosol and Its Metabolite Homovanillic Alcohol on H₂O₂ Induced Lipid Peroxidation in Renal Tubular Epithelial Cells. *Food Chem. Toxicol.* **2008**, *46*, 2984–2990.
- de Lorgeril, M.; Salen, P.; Martin, J. L.; Monjaud, I.; Boucher, P.; Mamelle, N. Mediterranean Dietary Pattern in a Randomized Trial: Prolonged Survival and Possible Reduced Cancer Rate. *Arch. Int. Med.* **1998**, *158*, 1181–1187.
- Dell'Agli, M.; Fagnani, R.; Mitro, N.; Scurati, S.; Masciadri, M.; Mussoni, L.; Galli, G. V.; Bosisio, E.; Crestani, M.; De Fabiani, E.; et al. Minor Components of Olive Oil Modulate Proatherogenic Adhesion Molecules Involved in Endothelial Activation. *J. Agric. Food Chem.* **2006**, *54*, 3259–3264.
- Detmar, M.; Brown, L. F.; Berse, B.; Jackman, R. W.; Elicker, B. M.; Dvorak, H. F.; Claffey, K. P. Hypoxia Regulates the Expression of Vascular Permeability Factor/Vascular Endothelial Growth Factor (VPF/VEGF) and Its Receptors in Human Skin. *J. Invest. Derm.* **1997**, *108*, 263–268.
- Di Benedetto, R.; Vari, R.; Scazzocchio, B.; Filesi, C.; Santangelo, C.; Giovannini, C.; Matarrese, P.; D'Archivio, M.; Masella, R. Tyrosol, The Major Extra Virgin Olive Oil Compound, Restored Intracellular Antioxidant Defences in Spite of Its Weak Antioxidative Effectiveness. *Nutr. Metab. Card. Dis.* **2007**, *17*, 535–545.
- Diegelmann, R. F. Excessive Neutrophils Characterize Chronic Pressure Ulcers. *Wound Rep. Regen.* **2003**, *11*, 484–490.
- Dugo, G.; Alfa, M.; Pera, L. L.; Mavrogeni, E.; Pollicino, D.; Maisano, R.; Pizzimenti, G. Characterization of Sicilian Olive Oils. Note X. A Comparison between Cerasuola and Nocellara del Belice varieties. *Grasas y Aceites* **2004**, *55*, 415–422.
- Engelhardt, E.; Toksoy, A.; Goebeler, M.; Debus, S.; Broecker, E. B.; Gillitzer, R. Chemokines IL-8, GRO α , MCP-1, IP-10, and Mig Are Sequentially and Differentially Expressed During Phase-Specific Infiltration of Leukocyte Subsets in Human Wound Healing. *Am. J. Path.* **1998**, *153*, 1849–1860.
- Esti, M.; Cinquanta, L.; La Notte, E. Phenolic Compounds in Different Olive Varieties. *J. Agric. Food Chem.* **1998**, *46*, 32–35.
- Fabiani, R.; Rosignoli, P.; Bartolomeo, A.; Fuccelli, R.; Servili, M.; Montedoro, G. F.; Morozzi, G. Oxidative DNA Damage Is Prevented by Extracts of Olive Oil, Hydroxytyrosol, and Other Olive Phenolic Compounds in Human Blood Mononuclear Cells and HL60 cells. *J. Nut.* **2008a**, *138*, 1411–1416.
- Fabiani, R.; Rosignoli, P.; De Bartolomeo, A.; Fuccelli, R.; Morozzi, G. Inhibition of Cell Cycle Progression by Hydroxytyrosol is Associated with Upregulation of Cyclin-Dependent

- Protein Kinase Inhibitors p21 (WAF1/Cip1) and p27 (Kip1) and with Induction of Differentiation in HL60 cells. *J. Nutr.* **2008b**, *138*, 42–48.
- Fabiani, R.; Fuccelli, R.; Pieravanti, F.; De Bartolomeo, A.; Morozzi, G. Production of Hydrogen Peroxide Is Responsible for the Induction of Apoptosis by Hydroxytyrosol on HL60 Cells. *Mol. Nutr. Food Res.* **2009a**, *53*, 887–896.
- Fabiani, R.; Rosignoli, R.; Fuccelli, R.; Pieravanti, F.; De Bartolomeo, A.; Morozzi, G. Involvement of Hydrogen Peroxide Formation on Apoptosis Induction by Olive Oil Phenolic Compounds. *Czech J. Food Sci.* **2009b**, *27*, S197–S199.
- Fabiani, R.; Sepporta, M.; Rosignoli, P.; Bartolomeo, A.; Crescimanno, M.; Morozzi, G. Anti-proliferative and Pro-Apoptotic Activities of Hydroxytyrosol on Different Tumour Cells: The Role of Extracellular Production of Hydrogen Peroxide. *Eur. J. Nutr.* **2012**, *51*, 455–464.
- Fernandes, K. J. L.; McKenzie, I. A.; Mill, P.; Smith, K. M.; Akhavan, M.; Barnabe-Heider, F.; Biernaskie, J.; Junek, A.; Kobayashi, N. R.; Toma, J. G.; et al. A Dermal Niche for Multipotent Adult Skin-Derived Precursor Cells. *Nat. Cell Biol.* **2004**, *6*, 1082–1093.
- FitzGerald, G. A. COX-2 and Beyond: Approaches to Prostaglandin Inhibition in Human Disease. *Nat. Rev Drug Disc.* **2003**, *2*, 879–891.
- Flanders, K. C.; Major, C. D.; Arabshahi, A.; Aburime, E. E.; Okada, M. H.; Fujii, M.; Blalock, T. D.; Schultz, G. S.; Sowers, A.; Anzano, M. A.; et al. Interference with Transforming Growth Factor- β /Smad3 Signaling Results in Accelerated Healing of Wounds in Previously Irradiated Skin. *Am. J. Path.* **2003**, *163*, 2247–2257.
- Florczyk, U.; Jazwa, A.; Maleszewska, M.; Mendel, M.; Szade, K.; Kozakowska, M.; Grochot-Przeczek, A.; Viscardi, M.; Czauderna, S.; Bukowska-Strakova, K.; et al. Nrf2 Regulates Angiogenesis: Effect on Endothelial Cells, Bone Marrow-Derived Proangiogenic Cells and Hind Limb Ischemia. *Antiox. Redox Signalling.* **2014**, *20*, 1693–1708.
- Folkman, J.; D'Amore, P. A. Blood Vessel Formation: What Is Its Molecular Basis? *Cell*, **1996**, *87*, 1153–1156.
- Fortes, C.; García-Vilas, J. A.; Quesada, A. R.; Medina, M. Á. Evaluation of the Anti-Angiogenic Potential of Hydroxytyrosol and Tyrosol, Two Bio-Active Phenolic Compounds of Extra Virgin Olive Oil, in Endothelial Cell Cultures. *Food Chem.* **2012**, *134*, 134–140.
- Frazer, C.; Young, P. G. Carboxy-terminal Phosphorylation Sites in Cdc25 Contribute to Enforcement of the DNA Damage and Replication Checkpoints in Fission Yeast. *Curr. Gen.* **2012**, *58*, 217–234.
- Gillitzer, R.; Goebeler, M. Chemokines in Cutaneous Wound Healing. *J. Leuk. Biol.* **2001**, *69*, 513–521.
- Gilmour, P. S.; Rahman, I.; Donaldson, K.; MacNee, W. Histone Acetylation Regulates Epithelial IL-8 Release Mediated by Oxidative Stress from Environmental Particles. *Am. J. Phys. Lung Cell. Mol. Phys.* **2003**, *284*, L533–L540.
- Gomez-Alonso, S.; Desamparados-Salvador, M.; Fregapane, G. Phenolic Compounds Profile of Cornicabra Virgin Olive Oil. *J. Agric. Food Chem.* **2002**, *50*, 6812–6817.
- Gong, D.; Geng, C.; Jiang, L.; Cao, J.; Yoshimura, H.; Zhong, L. Effects of Hydroxytyrosol-20 on Carrageenan-Induced Acute Inflammation and Hyperalgesia in Rats. *Phyto. Res.* **2009**, *23*, 646–650.

- Gonzalez-Santiago, M.; Martin-Bautista, E.; Carrero, J. J.; Fonolla, J.; Baro, L.; Bartolome, M. V.; Gil-Loyzaga, P.; Lopez-Huertas, E. One-Month Administration of Hydroxytyrosol, a Phenolic Antioxidant Present in Olive Oil, to Hyperlipemic Rabbits Improves Blood Lipid Profile, Antioxidant Status and Reduces Atherosclerosis Development. *Atherosclerosis*. **2006**, *188*, 35–42.
- Gonzalez-Santiago, M.; Fonolla, J.; Lopez-Huertas, E. Human Absorption of a Supplement Containing Purified Hydroxytyrosol, A Natural Antioxidant From Olive Oil, and Evidence For its Transient Association with Low-Density Lipoproteins. *Pharm. Res.* **2010**, *61*, 364–370.
- Goulas, V.; Exarchou, V.; Troganis, A. N.; Psomiadou, E.; Fotsis, T.; Briasoulis, E.; Gerotheranassis, I. P. Phytochemicals in Olive-Leaf Extracts and Their Antiproliferative Activity against Cancer and Endothelial Cells. *Mol. Nutr. Food Res.* **2009**, *53*, 600–608.
- Granados-Principal, S.; El-Azem, N.; Pamplona, R.; Ramirez-Tortosa, C.; Pulido-Moran, M.; Vera-Ramirez, L.; Quiles, J. L.; Sanchez-Rovira, P.; Naudi, A.; Portero-Otin, M.; et al. Hydroxytyrosol Ameliorates Oxidative Stress and Mitochondrial Dysfunction in Doxorubicin-induced Cardiotoxicity in Rats with Breast Cancer. *Biochem. Pharm.* **2014**, *90*, 25–33.
- Grasso, S.; Siracusa, L.; Spatafora, C.; Renis, M.; Tringali, C. Hydroxytyrosol Lipophilic Analogues: Enzymatic Synthesis, Radical Scavenging Activity and DNA Oxidative Damage Protection. *Bioorganic Chemistry* **2007**, *35*, 137–152.
- Greaves, N. S.; Ashcroft, K. J.; Baguneid, M.; Bayat, A. Current Understanding of Molecular and Cellular Mechanisms in Fibroplasia and Angiogenesis during Acute Wound Healing. *J. Derm. Sci.* **2013**, 206.
- Guo, W.; An, Y.; Jiang, L.; Geng, C.; Zhong, L. The Protective Effects of Hydroxytyrosol Against UVB-induced DNA Damage in HaCaT Cells. *Phyto. Res.* **2010**, *PTR 24*, 352–359.
- Haas, T. L.; Milkiewicz, M.; Davis, S. J.; Zhou, A. L.; Egginton, S.; Brown, M. D.; Madri, J. A.; Hudlicka, O. Matrix Metalloproteinase Activity Is Required for Activity-Induced Angiogenesis in Rat Skeletal Muscle. *Am. J. Phys. Heart Circ. Phys.* **2000**, *279*, H1540–H1547.
- Hamdi, H. K.; Castellon, R. Oleuropein, A Non-Toxic Olive Iridoid, Is an Anti-Tumor Agent and Cytoskeleton Disruptor. *Biochem. Biophys. Res. Comm.* **2005**, *334*, 769–778.
- Han, G.; Li, F.; Ten Dijke, P.; Wang, X.-J. Temporal Smad7 Transgene Induction in Mouse Epidermis Accelerates Skin Wound Healing. *Am. J. Path.* **2011**, *179*, 1768–1779.
- Han, J.; Talorete, T. P.; Yamada, P.; Isoda, H. Anti-Proliferative and Apoptotic Effects of Oleuropein and Hydroxytyrosol on Human Breast Cancer MCF-7 Cells. *Cytotechnology* **2009**, *59*, 45–53.
- Hanahan, D.; Weinberg, R. A. The Hallmarks of Cancer. *Cell* **2000**, *100*, 57–70.
- Haslett, C. Resolution of Acute-Inflammation and the Role of Apoptosis in the Tissue Fate of Granulocytes. *Clin. Sci.* **1992**, *83*, 639–648.
- Hassan, Z. K.; Elamin, M. H.; Omer, S. A.; Daghestani, M. H.; Al-Olayan, E. S.; Elobeid, M. A.; Virk, P. Oleuropein Induces Apoptosis Via the p53 Pathway in Breast Cancer cells. *Asian Pac. J. Canc. Prevention.* **2013**, *14*, 6739–6742.
- Henson, P. M.; Johnston, R. B., Jr. Tissue x. Oxidants, Proteinases, and Cationic Proteins. *J. Clin. Invest.* **1987**, *79*, 669–674.

- Hu, F. B. The Mediterranean Diet and Mortality—Olive Oil and Beyond. *New Eng. J. Med.* **2003**, *348*, 2595–2596.
- Hye Joo, K.; Seong Hwan, K.; Jung-Mi, Y. Fisetin Inhibits Hyperglycemia-Induced Proinflammatory Cytokine Production by Epigenetic Mechanisms. *Evidence-Based Compl. Alt. Med.* **2012**, *1*, 1–10.
- Ichihashi, M.; Ahmed, N. U.; Budiyo, A.; Wu, A.; Bito, T.; Ueda, M.; Osawa, T. Preventive Effect of Antioxidant on Ultraviolet-Induced Skin Cancer in Mice. *J. Derm. Sci.* **2000**, *23*, 45–50.
- Incana, A.; Deiana, M.; Corona, G.; Vafeiadou, K.; Vauzour, D.; Dessi, M. A.; Spencer, J. P. Involvement of ERK, Akt and JNK Signalling in H₂O₂-Induced Cell Injury and Protection by Hydroxytyrosol and Its Metabolite Homovanillic Alcohol. *Mol. Nutr. Food Res.* **2010**, *54*, 788–796.
- Jaenisch, R.; Bird, A. Epigenetic Regulation of Gene Expression: How the Genome Integrates Intrinsic and Environmental Signals. *Nat. Gen.* **2003**, *33* (Suppl.), 245–254.
- Jang, Y. H.; Sim, J. H.; Kang, H. Y.; Kim, Y. C.; Lee, E. S. Immunohistochemical Expression of Matrix Metalloproteinases in the Granulomatous Rosacea Compared with the Non-Granulomatous Rosacea. *J. Eur. Acad. Derm. Vener.* **2011**, *25*, 544–548.
- Jemai, H.; Fki, I.; Bouaziz, M.; Bouallagui, Z.; El Feki, A.; Isoda, H.; Sayadi, S. Lipid-Lowering and Antioxidant Effects of Hydroxytyrosol and Its Triacetylated Derivative Recovered from Olive Tree Leaves in Cholesterol-Fed Rats. *J. Agric. Food Chem.* **2008**, *56*, 2630–2636.
- Kang, K. W.; Lee, S. J.; Kim, S. G. Molecular Mechanism of Nrf2 Activation by Oxidative Stress. *Antiox. Redox Signalling.* **2005**, *7*, 1664–1673.
- Kerbel, R.; Folkman, J. Clinical Translation of Angiogenesis Inhibitors. *Nat. Rev. Cancer* **2002**, *2*, 727.
- Keys, A. Epidemiological Studies Related to Coronary Heart Disease: Characteristics of Men Aged 40–59 in Seven Countries. *Acta Medica Scandinavica.* **1966**, *1*, 4–5.
- Keys, A.; Menotti, A.; Aravanis, C.; Blackburn, H.; Djordjevic, B. S.; Buzina, R.; Dontas, A. S.; Fidanza, F.; Karvonen, M. J.; Kimura, N.; et al. The Seven Countries Study: 2,289 Deaths in 15 Years. *Prev. Med.* **1984**, *13*, 141–154.
- Keys, A.; Menotti, A.; Karvonen, M. J.; Aravanis, C.; Blackburn, H.; Buzina, R.; Djordjevic, B. S.; Dontas, A. S.; Fidanza, F.; Keys, M. H.; et al. The Diet and 15-Year Death Rate in the Seven Countries Study. *Am. J. Epidim.* **1986**, *124*, 903–915.
- Kiechl-Kohlendorfer, U.; Berger, C.; Inzinger, R. The Effect of Daily Treatment with an Olive Oil/Lanolin Emollient on Skin Integrity in Preterm Infants: A Randomized Controlled Trial. *Pediatric Derm.* **2008**, *25*, 174–178.
- Koca, U.; Suntar, I.; Akkol, E. K.; Yilmazer, D.; Alper, M. Wound Repair Potential of *Olea europaea* L. Leaf Extracts Revealed by in Vivo Experimental Models and Comparative Evaluation of the Extracts' Antioxidant Activity. *J. Medicinal Food.* **2011**, *14*, 140–146.
- Komi-Kuramochi, A.; Kawano, M.; Oda, Y.; Asada, M.; Suzuki, M.; Oki, J.; Imamura, T. Expression of Fibroblast Growth Factors and Their Receptors During Full-Thickness Skin Wound Healing in Young and Aged Mice. *J. Endocrin.* **2005**, *186*, 273–289.
- Lamy, S.; Ouanouki, A.; Béliveau, R.; Desrosiers, R. R. Olive Oil Compounds Inhibit Vascular Endothelial Growth Factor Receptor-2 Phosphorylation. *Exp. Cell Res.* **2014**, *322*, 89–98.

- Lang, R.; Hammer, M.; Mages, J. DUSP Meet Immunology: Dual Specificity MAPK Phosphatases in Control of the Inflammatory Response. *J. Imm.* **2006**, *177*, 7497–7504.
- Larrucea, E.; Arellano, A.; Santoyo, S.; Ygartua, P. Combined Effect of Oleic Acid and Propylene Glycol on the Percutaneous Penetration of Tenoxicam and Its Retention in the Skin. *Euro. J. Pharmaceutics. Biopharmaceutics.* **2001**, *52*, 113–119.
- Leask, A.; Shi-Wen, X.; Khan, K.; Chen, Y.; Holmes, A.; Eastwood, M.; Denton, C. P.; Black, C. M.; Abraham, D. J. Loss of Protein Kinase Cepsilon Results in Impaired Cutaneous Wound Closure and Myofibroblast Function. *J. Cell. Sci.* **2008**, *121*, 3459–3467.
- Lin, H.; Hannon, G. J. MicroRNAs: Small RNAs with a Big Role in Gene Regulation. *Nat. Rev. Gen.* **2004**, *5*, 522–531.
- Loo, A. E. K.; Yee Ting, W.; Rongjian, H.; Wasser, M.; Tiehua, D.; Wee Thong, N.; Halliwell, B. Effects of Hydrogen Peroxide on Wound Healing in Mice in Relation to Oxidative Damage. *PLoS ONE* **2012**, *7*, 1–13.
- Lucas, L.; Russell, A.; Keast, R. Molecular Mechanisms of Inflammation. Anti-Inflammatory Benefits of Virgin Olive Oil and the Phenolic Compound Oleocanthal. *Curr. Pharm. Des.* **2011**, *17*, 754–768.
- Ma, J.; Chen, T.; Mandelin, J.; Ceponis, A.; Miller, N. E.; Hukkanen, M.; Ma, G. F.; Konttinen, Y. T. Regulation of Macrophage Activation. *Cell. Mol. Life Sci.* **2003**, *60*, 2334–2346.
- Maiuri, M. C.; De Stefano, D.; Di Meglio, P.; Irace, C.; Savarese, M.; Sacchi, R.; Cinelli, M. P.; Carnuccio, R. Hydroxytyrosol, a Phenolic Compound from Virgin Olive Oil, Prevents Macrophage Activation. *Naunyn-Schmiedeberg's Archives of Pharmacology* **2005**, *371*, 457–465.
- Mann, G. E.; Niehueser-Saran, J.; Watson, A.; Gao, L.; Ishii, T.; de Winter, P.; Siow, R. C. Nrf2/ARE Regulated Antioxidant Gene Expression in Endothelial and Smooth Muscle Cells in Oxidative Stress: Implications for Atherosclerosis and Preeclampsia. *Acta Physiologica Sinica* **2007**, *59*, 117–127.
- Manna, C.; D'Angelo, S.; Migliardi, V.; Loffredi, E.; Mazzoni, O.; Morrica, P.; Galletti, P.; Zappia, V. Protective Effect of the Phenolic Fraction from Virgin Olive Oils against Oxidative Stress in Human Cells. *J. Agri. Food. Chem.* **2002**, *50*, 6521–6526.
- Manna, C.; Migliardi, V.; Golino, P.; Scognamiglio, A.; Galletti, P.; Chiariello, M.; Zappia, V. Oleuropein Prevents Oxidative Myocardial Injury Induced by Ischemia and Reperfusion. *J. Nutr. Biochem.* **2004**, *15*, 461–466.
- Manna, C.; Napoli, D.; Cacciapuoti, G.; Porcelli, M.; Zappia, V. Olive Oil Phenolic Compounds Inhibit Homocysteine-Induced Endothelial Cell Adhesion Regardless of Their Different Antioxidant Activity. *J. Agric. Food Chem.* **2009**, *57*, 3478–3482.
- Mariotti, S.; Capocaccia, R.; Farchi, G.; Menotti, A.; Verdecchia, A.; Keys, A. Differences in the Incidence Rate of Coronary Heart Disease Between North and South European Cohorts of the Seven Countries Study as Partially Explained By Risk Factors. *Eur. Heart J.* **1982**, *3*, 481–487.
- Martin, P.; Leibovich, S. J. Inflammatory Cells during Wound, Repair: The Good, The Bad and the Ugly. *Trends in Cell Biol.* **2005**, *15*, 599–607.
- Martinez-Gonzalez, M. A.; Sanchez-Villegas, A. The Emerging Role of Mediterranean Diets in Cardiovascular Epidemiology: Monounsaturated Fats, Olive Oil, Red Wine or the Whole Pattern? *Eur. J. Epidem.* **2004**, *19*, 9–13.

- Mathias, J. R.; Perrin, B. J.; Liu, T. X.; Kanki, J.; Look, A. T.; Huttenlocher, A. Resolution of Inflammation By Retrograde Chemotaxis of Neutrophils in Transgenic Zebrafish. *J. Leukoc. Biol.* **2006**, *80*, 1281–1288.
- Medina, E.; De Castro, A.; Romero, C.; Brenes, M. Comparison of the Concentrations of Phenolic Compounds in Olive Oils and Other Plant Oils: Correlation with Antimicrobial Activity. *J. Agric. Food Chem.* **2006**, *54*, 4954–4961.
- Medina, E.; Brenes, M.; Garcia, A.; Romero, C.; de Castro, A. Bactericidal Activity of Glutaraldehyde-Like Compounds from Olive Products. *J. Food Protect.* **2009**, *72*, 2611–2614.
- Mehraein, F.; Sarbishegi, M.; Aslani, A. Evaluation of Effect of Oleuropein on Skin Wound Healing in Aged Male Balb/c Mice. *Cell J.* **2014**, *16*, 25–30.
- Menendez, J. A.; Lupu, R. Mediterranean Dietary Traditions for the Molecular Treatment of Human Cancer: Anti-Oncogenic Actions of the Main Olive Oil's Monounsaturated Fatty Acid Oleic Acid (18:1n-9). *Curr. Pharm. Biotech.* **2006**, *7*, 495–502.
- Menendez, J. A.; Vazquez-Martin, A.; Colomer, R.; Brunet, J.; Carrasco-Pancorbo, A.; Garcia-Villalba, R.; Fernandez-Gutierrez, A.; Segura-Carretero, A. Olive Oil's Bitter Principle Reverses Acquired Autoresistance to Trastuzumab (Herceptin) in HER2-Overexpressing Breast Cancer Cells. *BMC Cancer* **2007**, *7*, 80.
- Menendez, J. A.; Vazquez-Martin, A.; Garcia-Villalba, R.; Carrasco-Pancorbo, A.; Oliveras-Ferreros, C.; Fernandez-Gutierrez, A.; Segura-Carretero, A. TabAnti-HER2 (erbB-2) Oncogene Effects of Phenolic Compounds Directly Isolated from Commercial Extra-Virgin Olive Oil (EVOO). *BMC Cancer* **2008**, *8*, 377.
- Mesquita, R. F. D. S.; Paul, M. A.; Valmaseda, A.; Francois, A.; Jabr, R.; Anjum, S.; Marber, M. S.; Budhram-Mahadeo, V.; Heads, R. J. Protein Kinase Ce-calcineurin Cosignaling Downstream of Toll-Like Receptor 4 Downregulates Fibrosis and Induces Wound Healing Gene Expression in Cardiac Myofibroblasts. *Mol. Cell. Biol.* **2014**, *34*, 574–594.
- Mollova, M.; Bersell, K.; Walsh, S.; Savla, J.; Das, L. T.; Park, S.-Y.; Silberstein, L. E.; dos Remedios, C. G.; Graham, D.; Colan, S.; et al. Cardiomyocyte Proliferation Contributes to Heart Growth in Young Humans. *Proceedings of the National Academy of Sciences* **2013**, *110*, 1446–1451.
- Motohashi, H.; Yamamoto, M. Nrf2-Keap1 Defines A Physiologically Important Stress Response Mechanism. *Trends Mol. Med.* **2004**, *10*, 549–557.
- Muscara, M. N.; McKnight, W.; Asfaha, S.; Wallace, J. L. Wound Collagen Deposition in Rats: Effects of an NO-NSAID and a Selective COX-2 Inhibitor. *Brit. J. Phar.* **2000**, *129*, 681–686.
- Naik, A.; Pechtold, L.; Potts, R. O.; Guy, R. H. Mechanism of Oleic Acid-Induced Skin Penetration Enhancement in Vivo in Humans. *J. Controlled Release.* **1995**, *37*, 299–306.
- Nguyen, T.; Nioi, P.; Pickett, C. B. The Nrf2-Antioxidant Response Element Signaling Pathway and Its Activation by Oxidative Stress. *J. Biol. Chem* **2009**, *284*, 13291–13295.
- Nikkari, T. Comparative Chemistry of Sebum. *J. Invest. Derm.* **1974**, *62*, 257–267.
- Noli, C.; Miolo, A. The Mast Cell in Wound Healing. *Vet. Derm.* **2001**, *12*, 303–313.
- Nousis, L.; Doulias, P.-T.; Aligiannis, N.; Bazios, D.; Agalias, A.; Galaris, D.; Mitakou, S. DNA Protecting and Genotoxic Effects of Olive Oil Related Components in Cells Exposed to Hydrogen Peroxide. *Free Rad. Res.* **2005**, *39*, 787–795.

- Ollivier, D.; Artaud, J.; Pinatel, C.; Durbec, J. P.; Guerere, M. Differentiation of French Virgin Olive Oil RDOs by Sensory Characteristics, Fatty Acid and Triacylglycerol Compositions and Chemometrics. *Food Chem.* **2006**, *97*, 382–393.
- Omar, S. H. Oleuropein in Olive and Its Pharmacological Effects. *Scientia Pharmaceutica* **2010**, *78*, 133–154.
- Owen, R. W.; Giacosa, A.; Hull, W. E.; Haubner, R.; Würtele, G.; Spiegelhalter, B.; Bartsch, H. Olive-oil Consumption and Health: The Possible Role of Antioxidants. *Lancet Onc.* **2000**, *1*, 107–112.
- Palmerini, C. A.; Carlini, E.; Saccardi, C.; Servili, M.; Montedoro, G.; Arienti, G. Activity of Olive Oil Phenols on Lymphomonocyte Cytosolic Calcium. *J. Nutr. Biochem.* **2005**, *16*, 109–113.
- Palmieri, D.; Aliakbarian, B.; Casazza, A. A.; Ferrari, N.; Spinella, G.; Pane, B.; Cafueri, G.; Perego, P.; Palombo, D. Effects of Polyphenol Extract From Olive Pomace On Anoxia-Induced Endothelial Dysfunction. *Microvas. Res.* **2012**, *83*, 281–289.
- Panizzi, L. S.; Oriente, E. G. Structure of the Bitter Glucoside Oleuropein. *Gazzetta Chimica Italiana* **1960**, *1*, 1449–1485.
- Park, S. Y.; Park, D. J.; Kim, Y. H.; Kim, Y.; Kim, S. G.; Shon, K. J.; Choi, Y.-W.; Lee, S.-J. Upregulation of Heme Oxygenase-1 via PI3K/Akt and Nrf-2 Signaling Pathways Mediates the Anti-Inflammatory Activity of Schisandrin in Porphyromonas Gingivalis LPS-Stimulated Macrophages. *Immun. Lett.* **2011**, *1*, 93–101.
- Parzonko, A.; Czerwinska, M. E.; Kiss, A. K.; Naruszewicz, M. Oleuropein and Oleacein May Restore Biological Functions of Endothelial Progenitor Cells Impaired by Angiotensin II via Activation of Nrf2/heme Oxygenase-1 Pathway. *Phytomedicine* **2013**, *20*, 1088–1094.
- Patani, N.; Martin, L. A.; Dowsett, M. Biomarkers for the Clinical Management of Breast Cancer: International Perspective. *Int. J. Cancer* **2013**, *133*, 1–13.
- Pedranzini, L.; Leitch, A.; Bromberg, J. Stat3 Is Required for the Development of Skin Cancer. *J. Clin. Invest.* **2004**, *114*, 619–622.
- Perez-Martinez, P.; Lopez-Miranda, J.; Blanco-Colio, L.; Bellido, C.; Jimenez, Y.; Moreno, J. A.; Delgado-Lista, J.; Egido, J.; Perez-Jimenez, F. The Chronic Intake of a Mediterranean Diet Enriched in Virgin Olive Oil, Decreases Nuclear Transcription Factor kappaB Activation in Peripheral Blood Mononuclear Cells From Healthy Men. *Atherosclerosis* **2007**, *194*, e141–e146.
- Perugini, P.; Vettor, M.; Rona, C.; Troisi, L.; Villanova, L.; Genta, I.; Conti, B.; Pavanetto, F. Efficacy of Oleuropein against UVB Irradiation: Preliminary Evaluation. *International Journal of Cosmetic Science* **2008**, *30*, 113–120.
- Petrache, I.; Otterbein, L. E.; Alam, J.; Wiegand, G. W.; Choi, A. M. Heme Oxygenase-1 inhibits TNF-alpha-induced Apoptosis in Cultured Fibroblasts. *Am. J. Phys. Lung Cell. Mol. Phys.* **2000**, *278*, L312–L319.
- Petroni, A.; Blasevich, M.; Salami, M.; Papini, N.; Montedoro, G. F.; Galli, C. Inhibition of Platelet Aggregation and Eicosanoid Production By Phenolic Components of Olive Oil. *Thromb. Res.* **1995**, *78*, 151–160.
- Peyrot des Gachons, C.; Uchida, K.; Bryant, B.; Shima, A.; Sperry, J. B.; Dankulich-Nagrudny, L.; Tominaga, M.; Smith, A. B., III; Beauchamp, G. K.; Breslin, P. A. Unusual Pungency

- from Extra-Virgin Olive Oil Is Attributable to Restricted Spatial Expression of the Receptor of Oleocanthal. *J. Neurosci.* **2011**, *31*, 999–1009.
- Picardo, M.; Ottaviani, M.; Camera, E.; Mastrofrancesco, A. Sebaceous Gland Lipids. *Dermato-Endocrinology*, **2009**, *1*, 68–71.
- Ponrasu, T.; Natarajan Madhukumar, K.; Ganeshkumar, M.; Iyappan, K.; Sangeethapriya, V.; Subramani Gayathri, V.; Suguna, L. Efficacy of Acorus Calamus on Collagen Maturation on Full Thickness Cutaneous Wounds in Rats. *Pharm. Mag.* **2014**, *10*, S299–S305.
- Poudyal, H.; Campbell, F.; Brown, L. Olive Leaf Extract Attenuates Cardiac, Hepatic, and Metabolic Changes in High Carbohydrate-, High Fat-Fed Rats. *J. Nutr.* **2010**, *140*, 946–953.
- Puente, B. N.; Kimura, W.; Muralidhar, Shalini A.; Moon, J.; Amatruda, James F.; Phelps, Kate L.; Grinsfelder, D.; Roethermel, Beverly A.; Chen, R.; Garcia, Joseph A.; et al. The Oxygen-Rich Postnatal Environment Induces Cardiomyocyte Cell-Cycle Arrest through DNA Damage Response. *Cell* **2014**, *157*, 565–579.
- Rafehi, H.; Smith, A. J.; Balcerzyk, A.; Ziemann, M.; Ooi, J.; Loveridge, S. J.; Baker, E. K.; El-Osta, A.; Karagiannis, T. C. Investigation into the Biological Properties of the Olive Polyphenol, Hydroxytyrosol: Mechanistic Insights By Genome-Wide mRNA-Seq Analysis. *Genes and Nutr.* **2012**, *7*, 343–355.
- Rajkumar, V. S.; Xu, S. W.; Bostrom, M.; Leoni, P.; Muddle, J.; Ivarsson, M.; Gerdin, B.; Denton, C. P.; Bou-Gharios, G.; Black, C. M.; et al. Platelet-Derived Growth Factor-Beta Receptor Activation Is Essential for Fibroblast and Pericyte Recruitment during Cutaneous Wound Healing. *Amer. J. Path.* **2006**, *169*, 2254–2265.
- Renard, P.; Zachary, M. D.; Bougelet, C.; Mirault, M. E.; Haegeman, G.; Remacle, J.; Raes, M. Effects of Antioxidant Enzyme Modulations On Interleukin-1-Induced Nuclear Factor Kappa B Activation. *Biochem. Pharm.* **1997**, *53*, 149–160.
- Rietjens, S. J.; Bast, A.; de Vente, J.; Haenen, G. R. M. M. The Olive Oil Antioxidant Hydroxytyrosol Efficiently Protects Against The Oxidative Stress-Induced Impairment of the NO Response of Isolated Rat Aorta. *Am. J. Phys. Heart Circ. Phys.* **2007**, *292*, H1931–H1936.
- Rodrigues, H. G.; Vinolo, M. A. R.; Magdalon, J.; Vitzel, K.; Nachbar, R. T.; Pessoa, A. F.M.; dos Santos, M. F.; Hatanaka, E.; Calder, P. C.; Curi, R. Oral Administration of Oleic or Linoleic Acid Accelerates the Inflammatory Phase of Wound Healing. *J. Invest. Derm.* **2012**, *132*, 208–215.
- Roy, S.; Khanna, S.; Nallu, K.; Hunt, T. K.; Sen, C. K. Dermal Wound Healing Is Subject to Redox Control. *Mol. Ther.* **2006**, *13*, 211–221.
- Russell, S. B.; Trupin, J. S.; Myers, J. C.; Broquist, A. H.; Smith, J. C.; Myles, M. E.; Russell, J. D. Differential Glucocorticoid Regulation of Collagen MRNAs in Human Dermal Fibroblasts. Keloid-Derived and Fetal Fibroblasts Are Refractory to Down-Regulation. *J. Biol. Chem.* **1989**, *264*, 13730–13735.
- Sakazaki, F.; Kataoka, H.; Okuno, T.; Ueno, H.; Semma, M.; Ichikawa, A.; Nakamuro, K. Ozonated Olive Oil Enhances the Growth of Granulation Tissue in a Mouse Model of Pressure Ulcer. *Ozone: Sci. Eng.* **2007**, *29*, 503–507.
- Samet, I.; Han, J.; Jlaiei, L.; Sayadi, S.; Isoda, H. Olive (*Olea europaea*) Leaf Extract Induces Apoptosis and Monocyte/Macrophage Differentiation in Human Chronic Myelogenous Leukemia K562 Cells: Insight Into The Underlying Mechanism. *Oxidative Medicine and Cellular Longevity* **2014**, *2014*, 1–16.

- Sánchez-Fidalgo, S.; Sánchez de Ibarguén, L.; Cárdeno, A.; Alarcón de la Lastra, C. Influence of Extra Virgin Olive Oil Diet Enriched with Hydroxytyrosol in A Chronic DSS Colitis Model. *Eur. J. Nutr.* **2012**, *51*, 497–506.
- Schurer, N.; Schliep, V.; Williams, M. L. Differential Utilization of Linoleic and Arachidonic Acid by Cultured Human Keratinocytes. *Skin Pharmacol.* **1995**, *8*, 30–40.
- Schwab, J. M.; Chiang, N.; Arita, M.; Serhan, C. N. Resolvin E1 and Protectin D1 Activate Inflammation-Resolution Programmes. *Nature*, **2007**, 869.
- Scoditti, E.; Calabriso, N.; Massaro, M.; Pellegrino, M.; Storelli, C.; Martines, G.; De Caterina, R.; Carluccio, M. A. Mediterranean Diet Polyphenols Reduce Inflammatory Angiogenesis through MMP-9 and COX-2 Inhibition in Human Vascular Endothelial Cells: A Potentially Protective Mechanism in Atherosclerotic Vascular Disease and Cancer. *Arch. Biochem. and Biophys.* **2012**, *527*, 81–89.
- Senyo, S. E.; Steinhäuser, M. L.; Pizzimenti, C. L.; Yang, V. K.; Cai, L.; Wang, M.; Wu, T.-D.; Guerquin-Kern, J.-L.; Lechene, C. P.; Lee, R. T. Mammalian Heart Renewal by Pre-Existing Cardiomyocytes. *Nature* **2013**, *493*, 433–436.
- Sheridan, A. M.; Fitzpatrick, S.; Wang, C.; Wheeler, D. C.; Lieberthal, W. Lipid Peroxidation Contributes to Hydrogen Peroxide Induced Cytotoxicity in Renal Epithelial Cells. *Kidney Int.* **1996**, *49*, 88–93.
- Singer, A. J.; Clark, R. A. F. Mechanisms of Disease: Cutaneous Wound Healing. *New Eng. J. Med.* **1999**, *341*, 738–746.
- Singer, A. J.; Thode, H. C.; McClain, S. A. Development of a Histomorphologic Scale to Quantify Cutaneous Scars after Burns. *Acad. Emer. Med.* **2000**, *7*, 1083–1088.
- Sinha, D.; Bannerjee, S.; Schwartz, J. H.; Lieberthal, W.; Levine, J. S. Inhibition of Ligand-Independent ERK1/2 Activity in Kidney Proximal Tubular Cells Deprived of Soluble Survival Factors Up-Regulates Akt and Prevents Apoptosis. *J. Biol. Chem.* **2004**, *279*, 10962–10972.
- Sluimer, J. C.; Daemen, M. J. Novel Concepts in Atherogenesis: Angiogenesis and Hypoxia in Atherosclerosis. *J. Path.* **2009**, *218*, 7–29.
- Song, G. Y.; Chung, C. S.; Chaudry, I. H.; Ayala, A. MAPK p38 Antagonism As a Novel Method of Inhibiting Lymphoid Immune Suppression in Polymicrobial Sepsis. *Am. J. Cell. Phys.* **2001**, *281*, C662–C669.
- Surh, Y. J.; Kundu, J. K.; Na, H. K. Nrf2 as a Master Redox Switch in Turning on the Cellular Signaling Involved in the Induction of Cytoprotective Genes by Some Chemopreventive Phytochemicals. *Planta Medica.* **2008**, *74*, 1526–1539.
- Szpadarska, A. M.; Egozi, E. I.; Gamelli, R. L.; DiPietro, L. A. The Effect of Thrombocytopenia on Dermal Wound Healing. *J. Invest. Derm.* **2003**, *120*, 1130–1137.
- Tomita, K.; Barnes, P. J.; Adcock, I. M. The Effect of Oxidative Stress on Histone Acetylation and IL-8 Release. *Biochem. Biophys. Res. Comm.* **2003**, *301*, 572–577.
- Trengove, M. K.; Stacey, M. C.; McCauley, S.; Bennett, N.; Gibson, J.; Burslem, F.; Murphy, G.; Schultz, G. Analysis of The Acute and Chronic Wound Environments: The Role of Proteases and Their Inhibitors. *Wound Rep. Regen.* **1999**, *7*, 442–452.
- Tuck, K. L.; Hayball, P. J.; Stupans, I. Structural Characterization of the Metabolites of Hydroxytyrosol, the Principal Phenolic Component in Olive Oil, in Rats. *J. Agric. Food Chem.* **2002**, *50*, 2404–2409.

- van der Vliet, A.; Janssen-Heininger, Y. M.W. Hydrogen Peroxide as a Damage Signal in Tissue Injury and Inflammation: Murderer, Mediator, or Messenger? *J. Cellular Biochem.* **2014**, *115*, 427–435.
- Visioli, F.; Galli, C. Oleuropein Protects Low Density Lipoprotein from Oxidation. *Life Sci.* **1994**, *55*, 1965–1971.
- Visioli, F.; Bellomo, G.; Galli, C. Free Radical-Scavenging Properties of Olive Oil Polyphenols. *Biochem. Biophys. Res. Comm.* **1998a**, *247*, 60–64.
- Visioli, F.; Bellostà, S.; Galli, C. Oleuropein, the Bitter Principle of Olives, Enhances Nitric Oxide Production By Mouse Macrophages. *Life Sci.* **1998b**, *62*, 541–546.
- Wahbi, S.; Wakrim, R.; Aganchich, B.; Tahi, H.; Serraj, R. Effects of Partial Rootzone Drying (PRD) on Adult Olive Tree (*Olea Europaea*) in Field Conditions Under Arid Climate: I. Physiological and Agronomic Responses. *Agric. Ecosys. Envir.* **2005**, *106*, 289–301.
- Walker, M.; Hadgraft, J. Oleic-Acid a Membrane Fluidizer or Fluid within the Membrane. *Int. J. Pharmaceutics.* **1991**, *71*, R1–R4.
- Wang, M. Y.; Yang, Y. Y.; Heng, P. W. S. Role of Solvent in Interactions between Fatty Acids-Based Formulations and Lipids in Porcine Stratum Corneum. *J. Controlled Release.* **2004**, *94*, 207–216.
- Wang, S.; Tsarouhas, V.; Xylourgidis, N.; Sabri, N.; Tiklová, K.; Nautiyal, N.; Gallio, M.; Samakovlis, C. The Tyrosine Kinase Src Activates Grainy Head and Epidermal Wound Healing in *Drosophila*. *Nat. Cell. Biol.* **2009**, *11*, 890–895.
- Wang, X.; McCullough, K. D.; Franke, T. F.; Holbrook, N. J. Epidermal Growth Factor Receptor-Dependent Akt Activation By Oxidative Stress Enhances Cell Survival. *J. Biol. Chem.* **2000**, *275*, 14624–14631.
- Warleta, F.; Quesada, C. S.; Campos, M.; Allouche, Y.; Beltran, G.; Gaforio, J. J. Hydroxytyrosol Protects against Oxidative DNA Damage in Human Breast Cells. *Nutrients.* **2011**, *3*, 839–857.
- Weber, T. M.; Schoelermann, A. M.; Breitenbach, U.; Scherdin, U.; Kowcz, A. Hand and Foot Moisturizers. In *Cosmetic Dermatology*; Ed.; Wiley-Blackwell: New York, 2010; pp 130–138.
- Weinstat-Saslow, D.; Merino, M. J.; Manrow, R. E.; Lawrence, J. A.; Bluth, R. F.; Wittenbel, K. D.; Simpson, J. F.; Page, D. L.; Steeg, P. S. Overexpression of Cyclin D mRNA Distinguishes Invasive and in Situ Breast Carcinomas From Non-Malignant Lesions. *Nat. Med.* **1995**, *1*, 1257–1260.
- Werner, S.; Grose, R. Regulation of Wound Healing by Growth Factors and Cytokines. *Phys. Rev.* **2003**, *83*, 835–871.
- Yarden, Y. Biology of HER2 and its Importance in Breast Cancer. *Oncology* **2001**, *61* Suppl. 2, 1–13.
- Zbidi, H.; Salido, S.; Altarejos, J.; Perez-Bonilla, M.; Bartegi, A.; Rosado, J. A.; Salido, G. M. Olive Tree Wood Phenolic Compounds with Human Platelet Antiaggregant Properties. *Blood Cells, Molecules and Diseases.* **2009**, *42*, 279–285.
- Zern, T. L.; Luz-Fernandez, M. Cardioprotective Effects of Dietary Polyphenols. *J. Nutr.* **2005**, *135*, 2291–2294.
- Zhang, M. S.; Zhou, Y. F.; Zhang, W. J.; Zhang, X. L.; Pan, Q.; Ji, X. M.; Luo, Z. G.; Wu, J. P. Apoptosis Induced By Short Hairpin RNA-Mediated STAT6 Gene Silencing in Human Colon Cancer Cells. *Chinese Med. J.* **2006**, *119*, 801–808.

- Zhang, X.; Cao, J.; Zhong, L. Hydroxytyrosol Inhibits Pro-Inflammatory Cytokines, iNOS, and COX-2 Expression in Human Monocytic Cells. *Naunyn-Schmiedeberg's Archives of Pharmacology* **2009a**, *379*, 581–586.
- Zhang, X. M.; Cao, J.; Jiang, L. P.; Zhong, L. F. Suppressive Effects of Hydroxytyrosol on Oxidative Stress and Nuclear Factor-kappa B Activation in THP-1 Cells. *Biological and Pharmaceutical Bulletin*. **2009b**, *32*, 578–582.
- Zhao, B.; Ma, Y.; Xu, Z.; Wang, J.; Wang, F.; Wang, D.; Pan, S.; Wu, Y.; Pan, H.; Xu, D.; et al. Hydroxytyrosol, a Natural Molecule From Olive Oil, Suppresses the Growth of Human Hepatocellular Carcinoma Cells Via Inactivating AKT and Nuclear Factor-Kappa B Pathways. *Cancer Lett.* **2014**, *347*, 79–87.
- Zou, X.; Feng, Z.; Li, Y.; Wang, Y.; Wertz, K.; Weber, P.; Fu, Y.; Liu, J. Stimulation of GSH Synthesis to Prevent Oxidative Stress-Induced Apoptosis by Hydroxytyrosol in Human Retinal Pigment Epithelial Cells: Activation of Nrf2 and JNK-p62/SQSTM1 Pathways. *J. Nutr. Biochem.* **2012**, *23*, 994–1006.
- Zrelli, H.; Matsuka, M.; Araki, M.; Zarrouk, M.; Miyazaki, H. Hydroxytyrosol Induces Vascular Smooth Muscle Cells Apoptosis through NO Production and PP2A Activation with Subsequent Inactivation of Akt. *Planta Med* **2011a**, *77*, 1680–1686.
- Zrelli, H.; Matsuoka, M.; Kitazaki, S.; Araki, M.; Kusunoki, M.; Zarrouk, M.; Miyazaki, H. Hydroxytyrosol Induces Proliferation and Cytoprotection against Oxidative Injury in Vascular Endothelial Cells: Role of Nrf2 Activation and HO-1 Induction. *J. Agri. Food Chem.* **2011b**, *59*, 4473–4482.
- Zrelli, H.; Matsuoka, M.; Kitazaki, S.; Zarrouk, M.; Miyazaki, H. Hydroxytyrosol Reduces Intracellular Reactive Oxygen Species Levels in Vascular Endothelial Cells by Upregulating Catalase Expression Through the AMPK-FOXO3a Pathway. *Eur. J. Pharm.* **2011c**, *660*, 282–286.
- Zrelli, H.; Wu, C. W.; Zghonda, N.; Shimizu, H.; Miyazaki, H. Combined Treatment of Hydroxytyrosol with Carbon Monoxide-Releasing Molecule-2 Prevents TNF α -Induced Vascular Endothelial Cell Dysfunction through NO Production with Subsequent NF κ B Inactivation. *Biomed. Res. Int.* **2013**, *2013*, 912–916.

