Olive Oil Polyphenols Differentially Inhibit Smooth Muscle Cell Proliferation through a G1/S Cell Cycle Block Regulated by ERK1/2

Rei Abe, M.D.¹ Joel Beckett, B.A.¹ Ryuzo Abe, M.D., Ph.D.¹ Alexander Nixon, M.D.¹
Adrienne Rochier, M.D.¹ Norio Yamashita, M.D.¹ Bauer Sumpio, M.D., Ph.D.¹

¹ Section of Vascular Surgery, Department of Surgery, Yale University School of Medicine, New Haven, Connecticut

Address for correspondence and reprint requests Bauer E. Sumpio, M.D., Ph.D., F.I.C.A., Section of Vascular Surgery, Department of Surgery, Yale University School of Medicine, 333 Cedar Street, BB 204, New Haven, CT 06520-8062 (e-mail: bauer.sumpio@yale.edu).

Int J Angiol 2012;21:69–76.

Abstract

We hypothesized that polyphenols contained in olive oil play a role in reducing the risk of atherosclerosis. The aim of this study was to determine if the polyphenols in olive oil, oleuropein (Ole), hydroxytyrosol (HT), and tyrosol (Tyr) could inhibit smooth muscle cell (SMC) proliferation through its influence on cell cycle regulation. Bovine vascular SMC were cultured in the presence of Ole, HT, or Tyr at concentration of 1, 10, or 100 $\mu\text{mol/L}$. On days 1, 3, and 5, numbers of cells were counted. Cell cycle analysis was performed by flow cytometry on day 1 after SMC were stained with propidium iodide. Cell populations grown in the presence of Ole or HT at 100 $\mu\text{mol/L}$ concentration were significantly inhibited after 5 days of exposure. Tyr had a similar tendency but it did not attain significance. Cell cycle analysis revealed that 66% of cells were in G1 phase in Ole group, compared with 48% in control group. To examine the cell cycle block between G1 and S phases, we performed Western blotting and found that ERK1/2 activation was inhibited by Ole or HT. We conclude that olive oil polyphenols could inhibit SMC proliferation through a cell cycle block between G1 and S phases which may be regulated by ERK1/2. These results demonstrate a mechanism by which olive oil consumption may be atheroprotective by inhibiting SMC proliferation.

Keywords

- ▶ olive oil
- ▶ oleuropein
- ▶ hydroxytyrosol
- ▶ tyrosol
- ▶ smooth muscle cell proliferation
- ▶ ERK1/2

The Mediterranean diet is associated with a reduced risk of coronary artery disease and it has been postulated that the high olive oil consumption in Mediterranean countries may be responsible for this phenomenon.^{1,2} This concept is analogous to the “French paradox,” where the unexpected low coronary heart disease mortality in France, despite a diet rich in fat, is thought to be due, in part, to the beneficial effects of red wine polyphenols.³

The major component of olive oil is the saponifiable or glyceride fraction. Minor components (present in ~2% of oil weight) include >230 chemical compound rich in hydrocarbons, nonglyceride esters, tocopherols, flavonoids, sterols, and phenolic constituents. Considerable research has centered on these minor components with the premise that they induce important cardiovascular protective effects.² A variety

of polyphenols have been shown to have several antiatherogenic activities, such as an increase in nitric oxide (NO), inhibition of low-density lipoprotein (LDL) oxidation, and suppression of platelet aggregation.² Since vascular smooth muscle cell (SMC) subintimal proliferation is the sine qua non of atherogenesis, the aim of this study was to characterize the action of olive oil polyphenols on SMC proliferation in vitro.

Methods

SMC Cultures

Bovine SMC procured by the aortic explant method⁴ were obtained from Dr. Joseph Madri (Dept. of Pathology, Yale University). SMC were positively identified by positive staining for α -SMC actin and failure to uptake acetylated LDL. Cells

were grown in Dulbecco modified Eagle's medium/F12 (Gibco, Carlsbad, CA) with 10% penicillin-streptomycin and 10% fetal bovine serum (FBS) at 37°C, 5% carbon dioxide. Experiments were conducted on SMC between passages 6 and 10. Hydroxytyrosol (HT), Oleuropein (Ole; INDOFINE Chemical Company Inc., Hillsborough, NJ), and Tyrosol (Tyr; SigmaAldrich company, St. Louis, MO) were each dissolved in dimethyl sulfoxide (DMSO) at a stock concentration of 100 mmol/L and stored at -20°C.

Cell Proliferation

SMC were seeded in six well plates (3000 cells/cm²) and incubated for 24 hours at 37°C in a medium containing 10% FBS. In parallel experiments, HT, Ole, or Tyr was added after the incubation period to a final concentration of 1, 10, or 100 µmol/L using DMSO to dilute the stock solution. The control group was SMC with DMSO added but without any polyphenol. The medium was changed every 48 hours with a new dose of the respective phenols or DMSO added at that time. On days 1, 3, and 5 cells were harvested by trypsinization, stained by trypan blue and an aliquot counted with a hemocytometer. The average cell number of three wells was calculated for each experiment. Experiments were repeated at least in triplicate.

To examine the reversibility of SMC growth inhibition mediated by the phenolic compounds, SMC were seeded, incubated, and initially exposed to 100 µM of the respective phenols, as described above. After 1 day of phenol exposure, the medium was changed without any polyphenol added, and subsequent medium changes were spaced at 48-hour intervals. On days 1, 3, and 5 cells were harvested by trypsinization, and an aliquot counted with a hemocytometer. The number of cells was expressed as the ratio compared with day 0.

Cell Cycle Analysis

SMC were seeded in culture flasks, incubated with 10% FBS medium for 24 hours at 37°C and synchronized overnight with serum-free medium (SFM). HT, Ole, or Tyr were added after the incubation period to a final concentration of 100 µmol/L. After 24 hours, SMC were trypsinized, washed three times with PBS, and fixed in 70% ethanol overnight at 4°C. The cells were then centrifuged and incubated with 1 mg/mL RNase (SigmaAldrich, St. Louis, MO) for 45 minutes at room temperature before staining in the dark with 50 µg/mL propidium iodide (Sigma, St. Louis, MO). Cell cycle analysis was performed by using BD FACSCalibur (Dept. of Laboratory Medicine, Yale University) and analyzed with a FlowJo software (Tree Star, Inc., Ashland, OR).

Western Blot Analysis

SMC were seeded in six well plates, incubated with 10% FBS medium for 24 hours at 37°C and then placed in SFM for 24 hours. Cells were exposed to 1, 10, or 100 µmol/L of HT, Ole, or Tyr for 60 minutes for assessment of extracellular signal-regulated kinase (ERK)1/2 phosphorylation or for up to 24 hours for determination of retinoblastoma protein (Rb) phosphorylation and cyclinD1, p21/WAF1, and p27 expres-

sion. We chose these time points because in pilot time-course studies phosphorylation of ERK1/2 peaked at 60 minutes, and that of Rb and expression of cyclinD1 and p27 peaked at 24 hours (data are not shown).

After experimentation, SMC were washed twice in ice-cold phosphate buffered saline and lysed by scraping in lysis buffer containing radioimmunoprecipitation assay, complete protein inhibitor (Roche, Mannheim, Germany), phosphatase inhibitor set 1 and 2, 1 mmol/L phenylmethylsulfonyl fluoride, 2 mmol/L Na₃VO₄, and 1% sodium dodecyl sulfate. Lysed cells were centrifuged for 20 minutes at 15,000 xg, and the supernatants were collected. Protein content was determined using the BioRad protein assay system according to the manufacturer's protocol (Bio-Rad Laboratories, Hercules, CA). Cell lysates containing 15 µg protein were loaded in each lane and separated in 10% Tris-HCL gels (Bio-Rad) and then transferred to polyvinylidene fluoride membrane (Millipore, Billerica, MA). Membranes were incubated with antiphospho-Rb Ab, anti-total-Rb Ab, anti-cyclinD Ab, anti-p21 Ab, and anti-p27 Ab (Cell Signaling Technology, Danvers, MA) in a 5% milk Tris-buffered saline with Tween-20 blocking solution. Band densities were measured with ImageJ software (NIH, Bethesda, MD). Band densities were normalized against the control lane (untreated cells).

Inhibitor Studies

Cell proliferation, cell cycle analysis, and Western blot analysis were performed as described above using a mitogen-activated protein kinase (MAPK)/ERK kinase 1 (MEK1) inhibitor, PD98059 (Promega Corporation, Madison, WI) instead of the polyphenols. PD98059 was also dissolved in DMSO to achieve the desired concentration.

Statistics

Data are expressed as mean ± standard error (SE). Results from the proliferation study were statistically evaluated by one-way ANOVA followed by a Scheffe post hoc test using SPSS version 16.0 (SPSS Inc., Chicago, IL). Two-group comparisons of data obtained from cell proliferation studies or Western blot analyses were performed by the Student *t* test for unpaired value. Pearson chi-square (χ^2) test was used to analyze data from the cell cycle analysis.

Results

Polyphenols Inhibited SMC Proliferation in a Dose-Dependent but Reversible Manner

We first examined SMC proliferation in the presence or absence of 1, 10, or 100 µmol/L of polyphenols (→Fig. 1). There was no difference in proliferation of SMC treated with 1 µM HT, Ole, or Tyr compared with control. HT and Ole (10 µmol/L) had a slight, but not significant, inhibition of SMC proliferation. SMC proliferation was significantly inhibited ($p < 0.001$) in the presence of 100 µmol/L HT or Ole, but not in the presence of 100 µM Tyr compared with control. The results suggest that Ole and HT inhibited SMC proliferation in a dose-dependent manner.

Although the growth of cells exposed to 100 µM HT or Ole was inhibited, removal of these phenols on day 1 resulted in

Figure 1 Smooth muscle cell (SMC) proliferation in the presence of hydroxytyrosol (HT) (A), oleuropein (Ole) (B), or tyrosol (Tyr) (C). SMC were exposed to different concentration of HT, Ole, or Tyr for 5 days (left graphs), or exposed to phenols for 1 day and then the phenol removed (small inset right graphs). The x-axis represents the time course of the experiment, and the y-axis represents the ratio of cell number compare with day 0 control. Data are presented as mean $\pm$ standard error from three separate experiments. There is a dose-dependent but reversible effect of polyphenols on SMC proliferation. +, $p < 0.001$ compared with control on day 5.

cell recovery ($\rightarrow$ Fig. 1 inset). Tyr and HT group had a normal growth pattern by day 3 that was indistinguishable from control while the Ole group had a gradual recovery in cell numbers by day 5. This suggests that the polyphenols have different potencies on SMC proliferation with Ole > HT >> Tyr.

Polyphenols Induced a Block between G1 and S Phases of the Cell Cycle

Cell cycle analysis using propidium iodide was performed on SMC treated with 100 μ mol/L HT, Ole, or Tyr for 1 day ($\rightarrow$ Fig. 2). In the control nontreated group, the cell population

in G1 phase was 48% and that in S phase was 40%. In the Ole group, the cell population in G1 phase was 66% and that in S phase was 23%. There was a significant increase ($p < 0.05$) in Ole-treated cells in G1 phase compared with control, with a corresponding decrease in S phase. This result demonstrates a block between G1 and S phases of the cell cycle in SMC treated with Ole compared with control. HT and Tyr tended to have similar but less striking effects than Ole.

Polyphenols Inhibited ERK1/2 Phosphorylation

To investigate the mechanism of SMC inhibition by polyphenols, we assessed several proteins which are known to

Figure 2 Cell cycle analysis. (A) Representative flow cytometric histograms of smooth muscle cell (SMC) (in the presence or absence of 100 μ M polyphenols for 24 hours. (B) Mean results in percentage of three experiments. *, $p < 0.05$ compared with control.

regulate the cell cycle at the G1 and S phases.^{5–8} Phosphorylated Rb (p-Rb), cyclinD, p27 showed no difference between control and the cells treated with 100 μ M phenols for up to 24 hours, and no band for p21 could be obtained (**Fig. 3**). Pilot studies showed that maximal activation of ERK1/2 activity in SMC occurred at 60 minutes, so we assessed polyphenol effects on SMC at this time point. HT and Ole

(100 μ M/L) inhibited ERK1/2 phosphorylation significantly, and although Tyr showed similar tendency it did not reach significance (**Fig. 4**).

PD98059 (ERK1/2 Inhibitor) Inhibited SMC Proliferation and Induced a Block between G1 and S Phases of the Cell Cycle

To assess the functional significance of the inhibition of ERK1/2 activity by the polyphenols we assessed SMC proliferation in the presence of a MEK1 inhibitor, PD98059 (**Fig. 5**). SMC grown in the presence of PD98059 at 10 and 100 μ M/L concentration were significantly inhibited after 5 days of exposure (65 and 32% of control, respectively). PD98059 (1 μ M/L) did not significantly inhibit cell proliferation. After removal of PD98059 on day 1, the cells recovered to a normal growth pattern; not different from control (**Fig. 5** inset). The SMC treated with PD98059 showed reversibility similar to the cells treated with polyphenols (**Fig. 1**). We also confirmed that these doses of PD98059 induced a dose-dependent inhibition of ERK1/2 phosphorylation (**Fig. 4**) similar to that induced by 100 μ M/L Ole.

SMC treated with 100 μ M/L PD98059 for 1 day also had a block between G1 and S phases of the cell cycle compared with control. In the control (nontreated) group, cell population in G1 phase was 49% and that in S phase was 40%. However, in the PD98059 group the cell population in G1 phase was increased to 66% and that in S phase correspondingly decreased to 23%. PD (1 and 10 μ M/L) tended to have similar effects, but these were less than 100 μ M/L PD98059 effect (**Fig. 6**).

Figure 3 Western blot for phospho-Rb, cyclin D, p21, and p27 of smooth muscle cells (SMC) exposed to 100 μ M hydroxytyrosol (HT), oleuropein (Ole), or tyrosol (Tyr). These blots are representative of at least two different experiments for each protein. No significant changes were observed.

Figure 4 ERK1/2 expression of smooth muscle cells (SMC) exposed to 100 μM hydroxytyrosol (HT), 100 μM oleuropein (Ole), 100 μM tyrosol (Tyr), or 1, 10, or 100 μM PD98059 for 60 minutes. Data are presented as mean ± standard error. *, $p < 0.05$; **, $p < 0.01$ compared with control; n, number for performed.

Discussion

There are several scientific studies that support the notion that the dietary pattern characteristics of Mediterranean countries is associated with an overall reduction in cardiovascular and cancer mortality and a decrease in the incidence of coronary heart disease, cancer, and neurodegenerative diseases.⁹ Extra virgin olive oil contains 50 to 800 mg/kg of polyphenols and there is an accumulating evidence, both in vitro and in vivo, for antiatherogenic activity of these phenolic compounds. Phenolic compounds have been reported to decrease the presence of cell-adhesion molecules, increase NO disposability, suppress platelet aggregation, in-

hibit endothelial expression of tissue factor, reduce inflammation, and inhibit oxygen use in neutrophils.^{2,10} In addition, certain phenolic compounds have antiproliferative effects on a variety of cells. For instance, HT has been demonstrated to inhibit proliferation of colon adenocarcinoma cells, lung cancer cells, and leukemia cells.^{5,11}

There are scant reports on the effect of olive oil on vascular cell proliferation, which is important in atherogenesis. Although resveratrol, which is a predominant red wine polyphenol, has been reported to inhibit SMC proliferation,⁴ there are no studies to date on the effect of olive oil polyphenols. One of the main factors responsible for delayed research on polyphenols is the diversity and the complexity of their

Figure 5 Smooth muscle cell (SMC) proliferation in the presence of PD98059. SMC were exposed to different concentration of PD98059 for 5 days (left graphs), or exposed to PD98059 for 1 day and then the inhibitor removed (inset right graphs). The x-axis represents the time course of the experiment and the y-axis represents the ratio of cell number. Data are presented as mean ± standard error from three experiments. +, $p < 0.001$ compared with control on day 5.

Figure 6 Cell cycle analysis. (A) Representative flow cytometric histograms of smooth muscle cells (SMC) in the presence or absence of 1, 10, or 100 μ M of PD98059 for 24 hours. (B) Mean results in percentage of three experiments. *, $p < 0.05$ compared with control.

chemical structure, which influences their antioxidant power.¹² In this study, we evaluated the effect of three different polyphenols in olive oil on SMC proliferation and our results are the first to document that Ole and HT could inhibit SMC proliferation in a dose-dependent manner. Our flow cytometry findings (**Fig. 3**) indicate that this inhibition was the result of a cell cycle block between the G1 and S phases. Further support for the cell arrest mechanism is shown in **Fig. 2** whereby removal of the polyphenol reversed the inhibition of SMC proliferation. In addition, there was no difference between phenol-treated cells and the control DMSO group using trypan blue exclusion (data are not shown), which effectively excludes the possibility of cytotoxicity. Although some reports indicate that resveratrol induced apoptosis of cancer cells,¹³ little is known about this with other types of polyphenols, and further studies will be required to investigate this possibility with the olive oil polyphenols we used in our study.

Interference with the cell cycle has been previously reported with olive oil phenolic compounds and may be a common effect of phenols on different cell types. Although the reports uniformly suggest an arrest of cell progression before the M phase, the precise location of the block is not clear. Depending on the cell type studied, different investigators demonstrate either a G1-S or S-G2 phase block. For example, Fabiani et al found that HT induced a G1-S block in leukemia cells HL60,⁵ and Han et al showed that Ole resulted in a G1-S block in breast cancer MCF-7 cells.¹⁴ However, Corona et al described that a G2-M block caused by HT in colon cancer cells.¹⁵ In our previous study we determined that resveratrol induced a G1-S block in vascular SMC⁴ and

this was confirmed by Lee et al who reported G1-S block by piceatannol.⁶ Taken together, these reports are consistent with the findings of our present study that Ole induced a G1-S block in SMC.

The molecular mechanisms by which polyphenols exert their antiproliferative effects are poorly understood. As cell arrest occurred between G1 and S phases in our study, we investigated the effect of the olive oil phenols on regulators of the G1 and S phases. CyclinD, p21, p27, and cyclin-dependent kinases (CDKs) and Rb are known to regulate progression of G1 to S phases but did not appear to be affected by the polyphenols we tested, even at high doses. This appears to be in contrast to the reports of the effect of olive oil polyphenols on different cell types but not on SMC. For instance, HT induces p21 and p27, and then inhibits cyclinD3 and CDK6 on leukemia cells.⁵ Resveratrol, which is one of the major red wine polyphenols have also been reported to activate p21 and downregulate cyclinD, CDK2, CDK4, or p-Rb.^{6,16} In our study, although p21, p27, cyclinD, or Rb were not affected by HT, Ole, or Tyr, we demonstrated that another G1 regulator, ERK1/2, was affected by Ole and HT (**Figs. 3 and 4**). During the preparation of this article, we noted that ERK1/2 has also been recently reported to be affected by Ole or HT in MCF-7 breast cancer cell at 10 to 75 μ M/L.¹⁷ The ERK MAPK signaling pathway has long been known to play an important role in cell proliferation.⁸ Although various mechanisms have been reported to explain how the ERK1/2 MAPK pathway regulates G1 phase progression, a complete picture is yet to emerge. In normal cells, sustained activation of ERK1/2 is necessary for G1-to-S-phase progression and is associated with induction of positive regulators of the cell cycle and inactivation of

antiproliferative genes.⁷ How this is affected by cell type and duration and dose of polyphenol treatment is unclear.

To establish a casual association of Ole and HT inhibition of SMC proliferation and Ole and HT inhibition of ERK1/2 activity, we show that the MEK1 inhibitor PD98059 could inhibit both SMC ERK1/2 activity (**Fig. 4**) and SMC proliferation (**Fig. 5**) in a dose-dependent fashion. PD98059 (100 $\mu\text{mol/L}$) significantly inhibited cell proliferation to the same degree as Ole or HT. These results strongly suggest that at least the olive oil polyphenols we tested could work as ERK1/2 inhibitor resulting in an inhibition of SMC proliferation. Ole (100 $\mu\text{mol/L}$) inhibited SMC proliferation to the same level as 100 $\mu\text{mol/L}$ PD98059, although Ole appeared to inhibit phosphorylation of ERK1/2 only to the same level as 10 $\mu\text{mol/L}$ PD98059, suggesting there may be other pathways involved in the inhibition of SMC proliferation. In fact, resveratrol has been reported to suppress PI3K pathway or JAK/STAT pathway in cancer cells.^{18,19} Further studies are needed to clarify these mechanisms.

We acknowledge that the design of our in vitro studies has some limitations. For instance, it has been estimated that inhabitants of Mediterranean countries ingest 30 to 50 g of olive oil per day.²⁰ Maximal plasma concentration of HT after ingestion of 40 mL of virgin olive oil with a phenolic content of 366 mg/kg olive oil is $\sim 0.3 \mu\text{mol/L}$.²⁰ Although the amount of olive oil consumed in Mediterranean countries is much less than that in our short-term experimental studies, it is probable that daily ingestion of olive oil could have cumulative effects over the human life span. Nonetheless, the dose of 100 $\mu\text{mol/L}$ HT, Ole, or Tyr that we used in our experiments is similar to that of previous in vitro studies where up to 370 $\mu\text{mol/L}$ concentration was used.^{14,15,17,21}

In summary, olive oil polyphenols such as Ole and HT inhibited SMC proliferation through a G1/S cell cycle block, which may be regulated by ERK1/2 and the inhibitions were dose-dependent. These results demonstrate a potential mechanism by which olive oil consumption may have beneficial effects on cardiovascular mortality by inhibiting SMC proliferation.

Note

This study was supported in part by NIH R01 HL-47345.

References

- Owen RW, Giacosa A, Hull WE, et al. Olive-oil consumption and health: the possible role of antioxidants. *Lancet Oncol* 2000;1: 107–112
- Huang CL, Sumpio BE. Olive oil, the mediterranean diet, and cardiovascular health. *J Am Coll Surg* 2008;207(3):407–416
- Cordova AC, Jackson LS, Berke-Schlessel DW, Sumpio BE. The cardiovascular protective effect of red wine. *J Am Coll Surg* 2005;200(3):428–439
- Poussier B, Cordova AC, Becquemin JP, Sumpio BE. Resveratrol inhibits vascular smooth muscle cell proliferation and induces apoptosis. *J Vasc Surg* 2005;42(6):1190–1197
- 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* 2008;138(1):42–48
- Lee B, Lee EJ, Kim DI, Park SK, Kim WJ, Moon SK. Inhibition of proliferation and migration by piceatannol in vascular smooth muscle cells. *Toxicol In Vitro* 2009;23(7):1284–1291
- Meloche S, Pouyssegur J. The ERK1/2 mitogen-activated protein kinase pathway as a master regulator of the G1- to S-phase transition. *Oncogene* 2007;26(22):3227–3239
- Torii S, Yamamoto T, Tsuchiya Y, Nishida E. ERK MAP kinase in G cell cycle progression and cancer. *Cancer Sci* 2006;97(8): 697–702
- Lopez-Miranda J, Delgado-Lista J, Perez-Martinez P, et al. Olive oil and the haemostatic system. *Mol Nutr Food Res* 2007;51(10): 1249–1259
- Carluccio MA, Siculella L, Ancora MA, et al. Olive oil and red wine antioxidant polyphenols inhibit endothelial activation: antiatherogenic properties of Mediterranean diet phytochemicals. *Arterioscler Thromb Vasc Biol* 2003;23(4):622–629
- Corona G, Deiana M, Incani A, Vauzour D, Dessì MA, Spencer JP. Hydroxytyrosol inhibits the proliferation of human colon adenocarcinoma cells through inhibition of ERK1/2 and cyclin D1. *Mol Nutr Food Res* 2009;53(7):897–903
- D'Archivio M, Filesi C, Di Benedetto R, Gargiulo R, Giovannini C, Masella R. Polyphenols, dietary sources and bioavailability. *Ann Ist Super Sanita* 2007;43(4):348–361
- Singh N, Nigam M, Ranjan V, Zaidi D, Garg VK, Sharma S, et al. Resveratrol as an adjunct therapy in cyclophosphamide-treated MCF-7 cells and breast tumor explants. *Cancer Sci* 2011;102(5): 1059–1067
- Han J, Talorete TP, Yamada P, Isoda H. Anti-proliferative and apoptotic effects of oleuropein and hydroxytyrosol on human breast cancer MCF-7 cells. *Cytotechnology* 2009;59(1): 45–53
- Corona G, Deiana M, Incani A, Vauzour D, Dessì MA, Spencer JP. Inhibition of p38/CREB phosphorylation and COX-2 expression by olive oil polyphenols underlies their anti-proliferative effects. *Biochem Biophys Res Commun* 2007;362(3):606–611
- Bai Y, Mao QQ, Qin J, et al. Resveratrol induces apoptosis and cell cycle arrest of human T24 bladder cancer cells in vitro and inhibits tumor growth in vivo. *Cancer Sci* 2010;101(2):488–493
- Sirianni R, Chimento A, De Luca A, et al. Oleuropein and hydroxytyrosol inhibit MCF-7 breast cancer cell proliferation interfering with ERK1/2 activation. *Mol Nutr Food Res* 2010;54(6):833–840
- Roy P, Kalra N, Prasad S, George J, Shukla Y. Chemopreventive potential of resveratrol in mouse skin tumors through regulation of mitochondrial and PI3K/AKT signaling pathways. *Pharm Res* 2009;26(1):211–217
- Madan E, Prasad S, Roy P, George J, Shukla Y. Regulation of apoptosis by resveratrol through JAK/STAT and mitochondria mediated pathway in human epidermoid carcinoma A431 cells. *Biochem Biophys Res Commun* 2008;377(4):1232–1237
- Fitó M, de la Torre R, Farré-Albaladejo M, Khymenetz O, Marrugat J, Covas MI. Bioavailability and antioxidant effects of olive oil phenolic compounds in humans: a review. *Ann Ist Super Sanita* 2007; 43(4):375–381
- Menendez JA, Vazquez-Martin A, Garcia-Villalba R, et al. Anti-HER2 (erbB-2) oncogene effects of phenolic compounds directly isolated from commercial Extra-Virgin Olive Oil (EVOO). *BMC Cancer* 2008;8:377