OLEA EUROPAEA COMPOUNDS IN TUMOUR INITIATION AND PROGRESSION OF BREAST CANCER CELLS

R.Benot, M.Catanesi, A.Antonosante, M.d'Angelo, V.Castelli, E.Benedetti, A.Giordano, A.Cimini

Department of Life, Health and Environmental Sciences, Università degli Studi dell'Aquila (Italy)

Introduction

Breast cancer (BC) is the most frequently diagnosed cancer (23% of total) and the main reason of death among females (14%). *Olea europaea* leaves, oil and fruits have demonstrated a notable effect to inhibit proliferation and induce apoptosis in different cell lines, throughout anti-inflammatory actions. Among these compounds, polyphenols have been reported to interfere with the initiation, promotion and progression of cancer by affecting tumorigenic cell transformation. Particularly Oleuropein (OL) -present at higher levels in the olives and leaves- and its antioxidant metabolite, Hydroxytyrosol (HT). Previous studies showed a high reduction in tumour incidence after the intake of dietary monounsaturated and saturated vegetable lipids, such olive oil. However, Olive leaves still remain a non-edible source rich in polyphenols that can play an interesting role in cancer treatment.

Our research focuses in the analysis of an Olea europaea leaf extract (OLE), rich in Oleuropein (~50%), as a potential antitumor agent on a Triple negative breast cancer (TNBC) malignant tumor cell line **MDA-MB-231**, that overexpresses stem cell-enriched genes and has a natural tendency to metastasize to bone, brain and lungs.

Results

The antiproliferative and proapoptotic effects of OLE were assayed in TNBC cell line **MDA-MB-231**. **Fig.1** shows the cell viability of these cells treated with different concentrations [100-400] µg/mL of Olive extract. Dose–response curve showed an IC50 of OLE on MDA-MB-231 equal to 200 µg/mL. For all the other experiments, OLE was used at the final concentration of 200 µg/mL. In **Fig.2** the cell cycle analysis by FACs is reported. The OLE is able to induce a block of cell cycle at the S phase (DNA Synthesis phase of cell cycle) (**Fig. 2A**). This data was further confirmed in Western Blotting (WB) analysis by a drop in Cyclins D1 and B2 protein expression at 24h mainly, as well as by the increase in Cyclin E -specific of S phase- at both 24 and 72h (**Fig. 3A**). In **Fig. 2B** we show the apoptosis profile got by FACs for the same experimental conditions, the apoptosis was also confirmed by WB throughout the increase of the apoptotic markers Caspase-9, Caspase-3 and p27 (**Fig.3B**).

Additional experiments of mitochondrial damage were performed by Immunofluorescence assay. **Fig. 4** shows a marked decrease in the mitochondria number and morphological changes in treated vs control cells, which presumably leads to cell death.

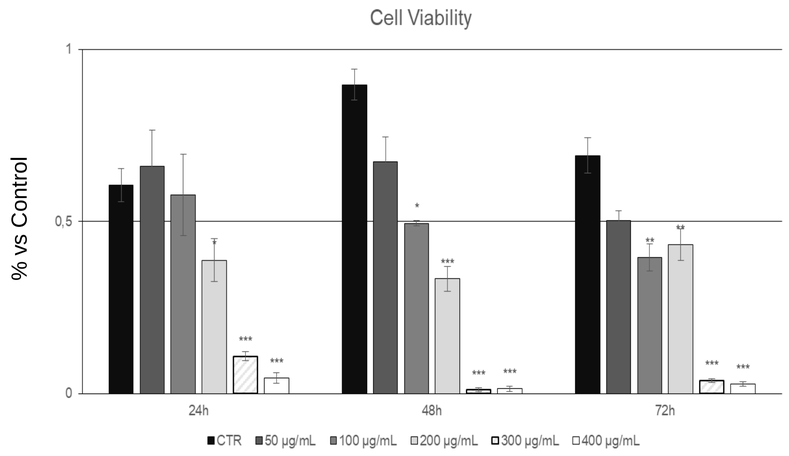


Fig 1. MTS assay measured after 24, 48 and 72 h in MDA-MB-231 cells treated with different concentrations of the Olive leaf extract. Absorbance was measured at 492 nm. Data are the means ±SEM of 2 experiments. * P<0,05, **P<0,005, ***P<0,0001.

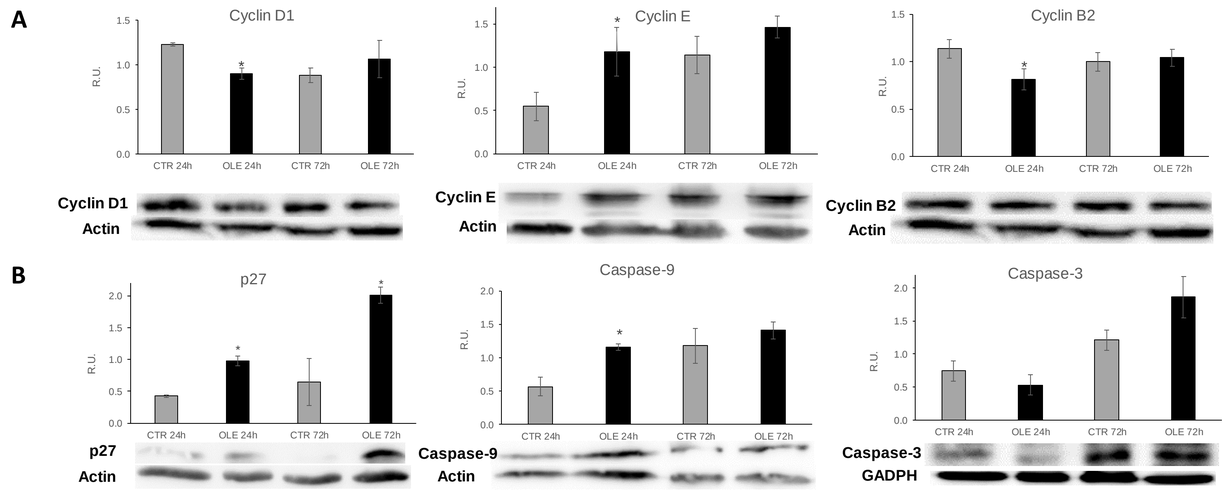
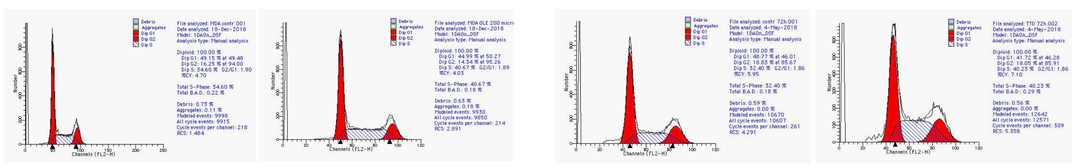
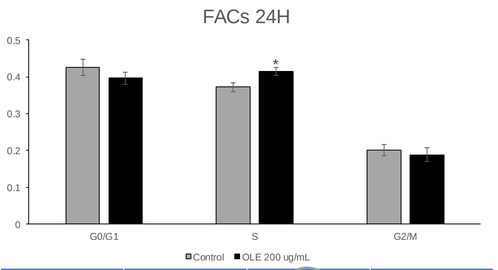


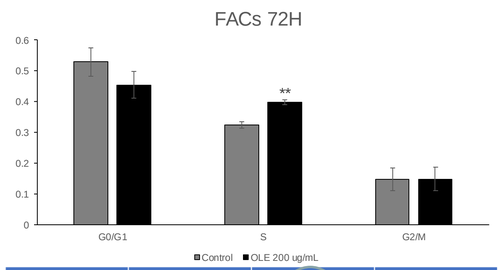
Fig 3. Western Blotting and relative densitometric analysis of cell cycle proteins Cyclins D1, E and B2 (A) and apoptotic markers p27 and Caspases-9 and -3 (B) in cell lysates from control and treated MDA-MB-231 cell line at 24 and 72 hours. A representative blotting is shown. Data are means ±SEM of 3 experiments. * P<0,05.

A





	G1	S	G2/M
CTR	42,6%	37,2%	20,2%
OLE 200 ug/ml	39,6%	41,5%	18,9%



	G1	S	G2/M
CTR	52,9%	32,4%	14,7%
OLE 200 ug/ml	45,4%	39,8%	14,8%

B

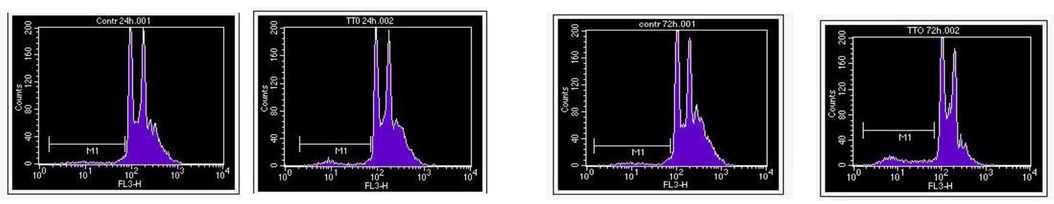


Fig 2. A) Cell cycle analysis by Flow Cytometry in Control and OLE-treated MDA-MB-231 cells after 24 and 72 h. (A) Representative histograms of Cell cycle and (B) apoptosis profile. Data are the means of ±SEM of 4 experiments. *P<0.05, ** P<0.005.

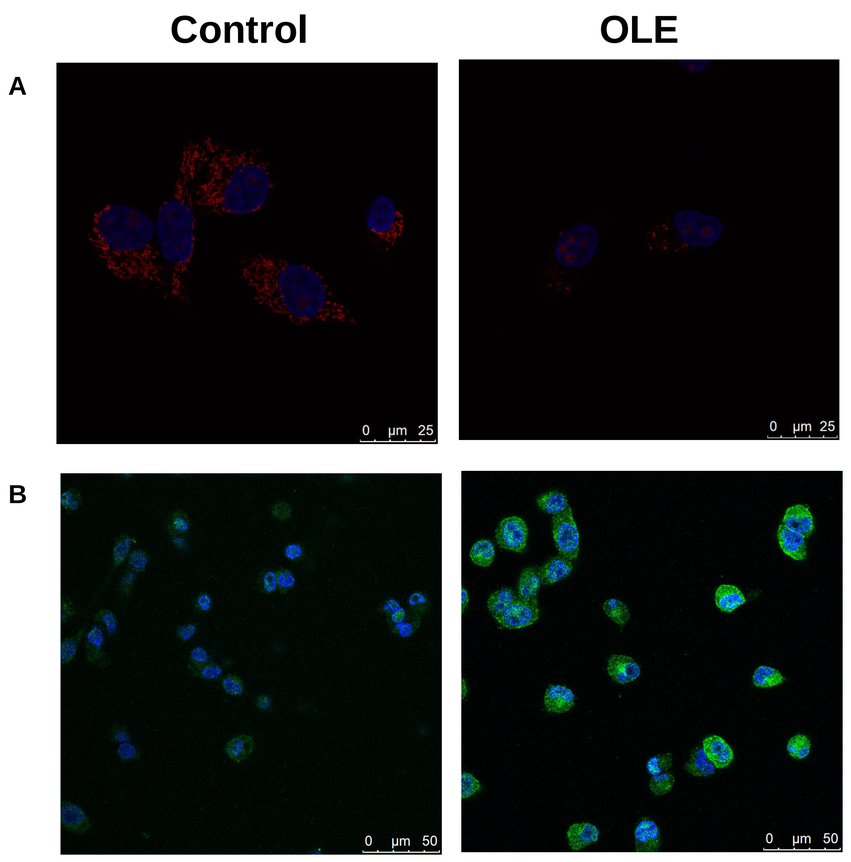


Fig 4. Confocal images of MDA-MB-231 control and treated cells stained with mitochondrial and nuclear probes:
A) MitoTracker Orange CMTMRos (Red) and DAPI (Blue) staining 24h after the treatment.
B) IF with PPAR-γ and Alexafluor 488 (Green) and DAPI (Blue) in 1h-treated cells

Conclusion & Future Perspective

- Olive leaf extract induces a block in the S phase and Caspase-9 dependent apoptosis in MDA-MB-231. This is similar to studies carried out in different tumor cell lines.
- Caspase-9 activation with a marked decrease in the mitochondria number and its morphological changes lead us to deep in the study of mitochondrial function. Since Oleuropein and other metabolites contained in OLE may modulate PPAR-γ activity and due to this nuclear receptor is strongly involved in both mitochondrial biogenesis and function, we will focus our attention in this transcriptional factor. Preliminary confocal images results showed an increase in PPAR-γ signalling in treated vs control cells. This transcriptional factor could be a potential target of the OLE pathway, which will be later tested by PPAR-γ silencing experiments. In addition, further functional mitochondria studies and Cyt C localization will be performed.
- After the optimization of the model, we will develop a comparison between this whole leaf extract effect and OL/HT polyphenols; in order to understand if purified compounds may be more effective than the crude leaf extract.

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