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OLIVE LEAF AND DERIVED COMPOUNDS EFFECTS ON TRIPLE NEGATIVE BREAST CANCER MODEL

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Introduction

Olea europaea → Oil, Leaves, Fruits

have shown notable effects in inhibiting proliferation and inducing apoptosis, mainly by anti-inflammatory actions

Breast Cancer→ most frequently diagnosed cancer (23% of total)
main reason of death among females (14%)

Several studies→ Assigned high reduction in tumour incidence to monounsaturated/saturated vegetable lipids → **Olive** oil and **polyphenols**
Nevertheless, **Olive leaves** still remain a non-edible source rich in polyphenols that may play an interesting role in cancer. These polyphenols have been reported to interfere with initiation, promotion and progression of cancer by affecting tumorigenic cell transformation, particularly **Oleuropein (OL)** and its metabolite **Hydroxytyrosol (HT).**
Our research focus: Analysis of an **Olive leaf extract (OLE)** rich in Oleuropein (~50%) as a potential antitumor agent on a malignant TNBC cell line, **MDA-MB-231**, that overexpresses stem cell-enriched genes and has a natural tendency to metastasize to bone, brain and lungs.

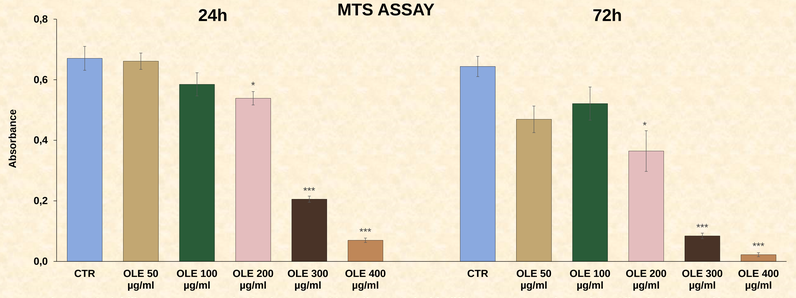

Fig 1. MTS viability assay at 24 and 72h of MDA-MB-231 cells treated with different concentrations of the Olive leaf extract (OLE, 50-400 µg/ml). IC50 and working concentration was settled at 200 µg/ml for the following experiments. Absorbance was measured at 492 nm. Data are means ±SEM of 3 different experiments run in quintuplicate. Statistical significance vs control: * p<0,05, ***p<0,0001

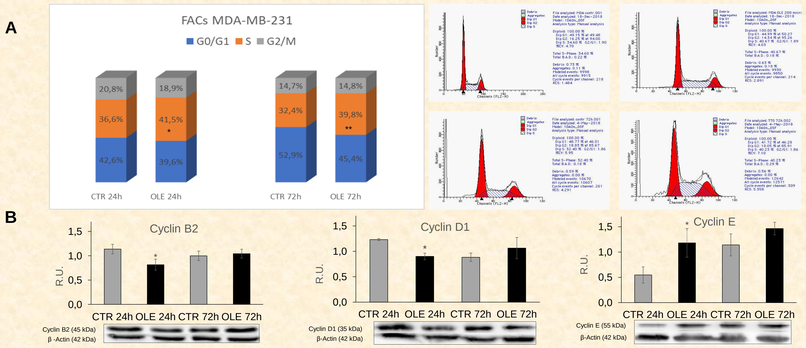

Fig 2. (A) Cell cycle analysis by Flow Cytometry in Control and OLE-treated MDA-MB-231 cells after 24 and 72 h. Representative plots are shown at right. (B) Representative Western Blotting and relative densitometric analysis of cell cycle proteins Cyclins B2, D1 and E in cell lysates. Data are the means of ±SEM of 4 experiments. *p<0.05, ** p<0.005.

Results

The antiproliferative and proapoptotic effects of OLE were assayed in the **MDA-MB-231** cell line. In **Fig. 1** the cell viability of these TNBC cells treated with different concentrations of Olive extract (50-400 µg/mL) at 24 and 72h is reported. The dose–effect curve showed a IC50 value of OLE next to **200 µg/mL,** which lead us to choose this concentration as the best working concentration for all the following experiments.
In **Fig. 2** the cell cycle analysis is reported. As shown in the Flow Cytometry experiment (**Fig. 2A)**, OLE induces a block of cell cycle at the S phase (DNA synthesis phase). These data were further confirmed by Western Blotting analysis showing a significant drop in the protein expression of Cyclins D1 and B2 at 24h, as well as the overexpression of Cyclin E -specific of S phase- at both 24 and 72h (**Fig. 2B**).
Fig. 3 shows the apoptosis assay. In **Fig. 3A** the apoptotic profile obtained by FACs with the same experimental conditions (200 µg/ml OLE, 24 and 72h) is reported, confirmed by Annexin V Live-cell IncuCyte® analysis (**Fig. 3B)** and by WB showing the increase of the apoptotic markers Caspase-3 and p27 (**Fig.3C**).
Additional studies on mitochondrial damage were performed by confocal laser microscopy. **Fig.4A** shows a marked decrease in mitochondria number and changes in morphology in OLE-treated MDA cells, evaluated by MitoTracker, events that presumably lead to cell death. In **Fig. 4B** an increase of nuclear PPAR-γ immunolocalization is observed upon treatment suggesting a anti-proliferative effects of OLE.

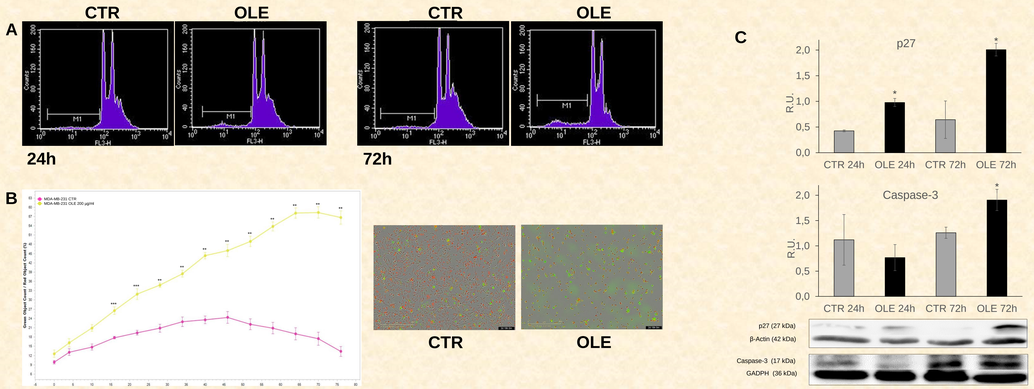

Fig 3. (A) Apoptosis profile by FACs in control and treated cells. (B) Annexin V live-cell assay by IncuCyte © in Control (pink) and treated (yellow) MDA-MB-231 from 0 to 72h. Representative images at 67h are exposed, where green dots represent apoptotic cells and red ones the nuclear staining with NucLight. (C) Western blotting and relative densitometric analysis for apoptotic markers p27 and Caspase-3. Representative blotting are also shown. Data are the means of ±SEM of 4 experiments. *p<0.05, ** p<0.005, p<0.0001.

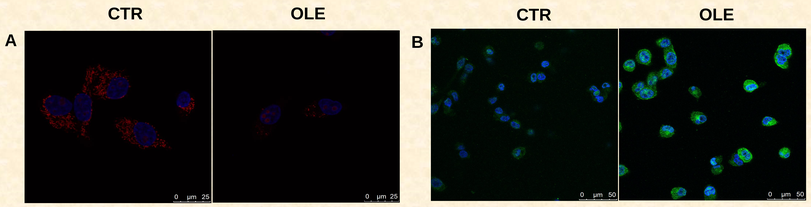

Fig 4. Confocal laser microscopy images of MDA-MB-231 control and OLE-treated. Cells were stained with mitochondrial and nuclear probes: (A) MitoTracker Orange CMTMRos (Red) and DAPI (Blue) 24h after treatment (B) IF with PPAR-γ and Alexa Fluor ® 488 1h after treatment

Conclusion & Future Perspective

- OLE compromises MDA-MB-231 cell viability, blocking their cell cycle at the S phase and inducing apoptosis.
- Caspase-3 and p27 significantly increase at 72h accompanied by a marked decrease of mitochondria number and morphology changes, suggesting a mitochondrial impairment upon treatment. Our present aim is to find out the molecular pathways modulated by OLE as well as to study the mitochondrial membrane potential changes and Cyt C localization and release.
- Since the nuclear receptor PPAR-γ is strongly involved in mitochondrial biogenesis and function, we hypothesize that Oleuropein and its metabolites may modulate PPAR-γ transcriptional activity leading to anti-proliferative events. Preliminary confocal images showing a marked increase in PPAR-γ signal in treated cells bring us to point on PPAR-γ as a potential target of the OLE pathway, to be confirmed in further experiments.