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UNIVERSITÀ DEGLI STUDI DE L'AQUILA

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

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Introduction

It has been showed that the Mediterranean diet can importantly increase life expectancy, reducing the risk of developing cancer and other major chronic diseases and improving quality of life and well-being. Several studies assigned a highest reduction in tumour incidence to monounsaturated and saturated vegetable lipids, such olive oil.
On these bases, we focused on the effects of *Olea europaea* compounds on the growth and proliferation of breast cancer cells.

Breast cancer is the most frequently diagnosed cancer (23% of the total) and the main reason of tumour death among females (14%). Olea europaea leaves, oil and fruits have a potential effect to inhibit proliferation and to induce apoptosis in different cancer cell lines. Furthermore, epidemiological studies have recently demonstrated a decrease of the risk of cancer with high olive oil intake, showing even a 38% reduction of breast cancer development thanks to adherence to Mediterranean diet. The main mechanisms presumably contributing to these properties primarily entail anti-inflammatory and antioxidant actions, related to their ability to scavenge free radicals and prevent cellular injury.

Among *Olea europaea* compounds, olive polyphenols received great attention, particularly a major one called Oleuropein (OL) -present at higher levels in the olives and leaves- as well as its antioxidant metabolite, Hydroxytyrosol (HT). In this respect, our research focuses in the analysis of Olive leaf extracts rich in OL (~50%) as a potential cell viability reducing agent on a malignant TNBC cell line, **MDA-MB-231**.

Methods & Materials

The cellular model used in this study was the triple negative **MDA-MB-231**, which is highly aggressive and does not undergo to apoptosis via Fas-ligand upon paclitaxel challenge, as described for other breast cancer cell lines. This model represents the claudin-low/mesenchymal subtype, which overexpresses stem cell-enriched genes and has a natural tendency to metastasize to brain and lungs.

Cell viability was measured by MTS assay after 24, 48 and 72h of treatment with [100-400] µg/mL of Olive extract. The treatment was prepared from a stock solution with 2 mg of leaf extract diluted in 2 mL of DMEM complete medium supplemented with 10% FBS, 1% Glut and 1% P/S. From this stock we obtained 4 serial dilutions (400-300-200-100 µg/mL) that were replicated in 5 wells/each. Later, MDA cells seeded at 10.000 cells/w in a 96-well plate were incubated at 37ºC in a humidified 95% air-5% CO2 atmosphere for 24 h.

Once optimal concentrations were assessed, we studied the cell cycle by Flow Cytofluorimetry after 24 and 72h of treatment with [200 µg/mL] of Olive leaf extract. Then, we examined the oncogenic markers p21, p27 (oncosuppressors) and Cyclin D1 (invasiveness marker) by Western Blotting, after previous protein extraction and dosage under the same conditions of time and dose.

Results

The effects of leaf olive extracts were assayed in **MDA-MB-231** cell line. In Fig.1 dose and time MTS assay on cells treated with Olive extract is reported. It is possible to observe a significant effect of *Olea europaea* extract in decreasing **MDA-MB-231** cell viability when used at high concentrations (200-400 µg/mL), more evident after 72 hours.
Furthermore, the Flow Cytofluorimetry preliminary results indicate a block in the cell cycle at the S/G-2 phase (Fig. 2A) which was then evaluated by Western Blotting analysis (Fig. 3). In Fig. 2B the apoptosis profile for the same conditions is also shown.

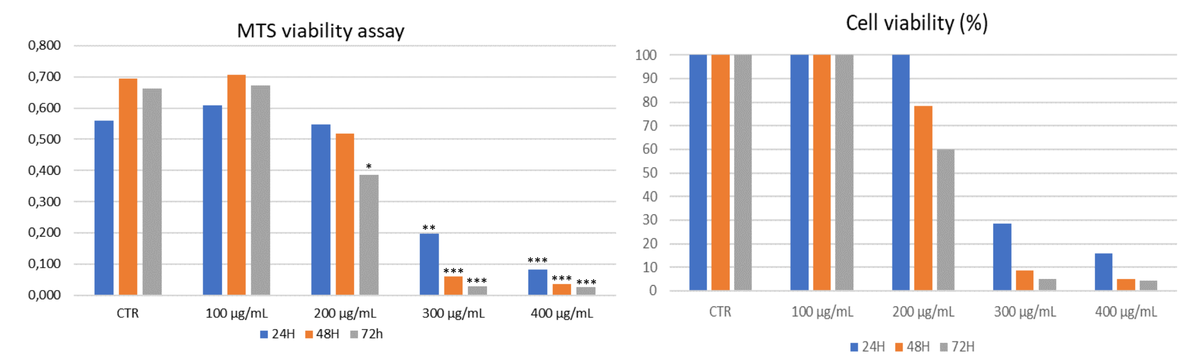

Fig 1 . MTS viability assay 24, 48 and 72h after the MDA-MB-231 cells treatment with different concentrations of the Olive leaf extract. Absorbance measured at 492 nm. Data are mean ±SE of 3 experiments. *,p<0,05; **,p<0,005; ***,p<0,0001

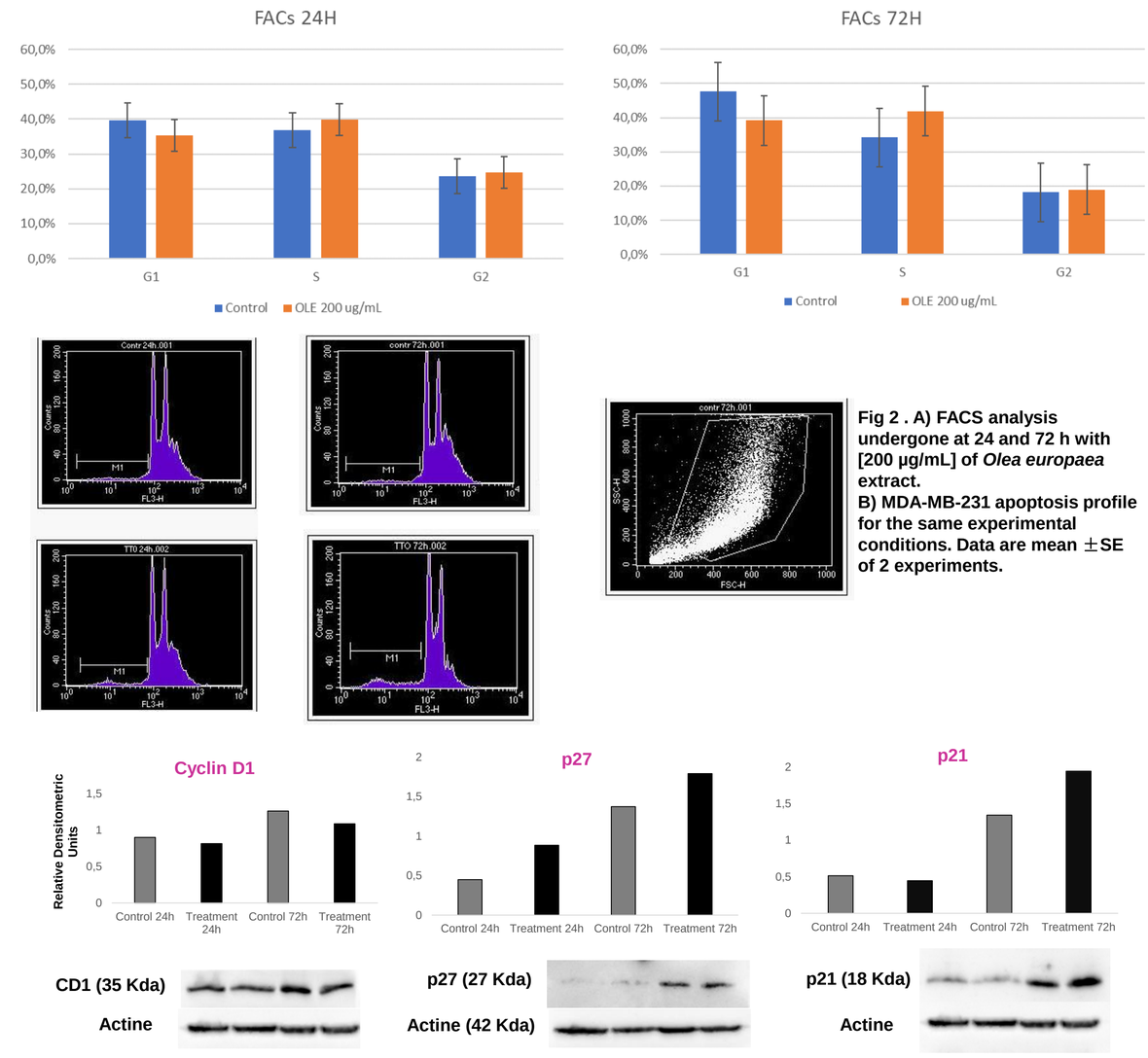

Fig 2 . A) FACS analysis undergone at 24 and 72 h with [200 µg/mL] of *Olea europaea* extract.
B) MDA-MB-231 apoptosis profile for the same experimental conditions. Data are mean ±SE of 2 experiments.

Fig 3. Western Blotting assay performed in MDA-MB-231 tumor cells. Oncogenic markers p21, p27 and CD1 are reported.

Conclusions & Future Prospectives

The reduction in the Cyclin D1 expression with the rise of p21 and p27 oncosuppressor protein in MDA-MB-231 treated cells confirm that Olive leaf extract is able to reduce breast cancer cell proliferation and to induce apoptosis by blocking the cell cycle in the G2/S phase –as shown by Flow Cytofluorimetric analysis- , reducing the overall MDA cancer cells viability.

Once the model has been optimized, we will compare the whole leaf extract effect with purified OL/HT polyphenols, in order to understand if the single compounds may be more effective than the crude extract.

Subsequently, we also intend to carry out the same study in the Breast cancer stem cells model (Mammospheres) with both the OL, HT and the whole pure Olive extract. By using the same parameters and treatment conditions, we will also extrapolate the experiment to Ovarian cancer cells (OVCAR) in order to better understand the effect of the Olive extracts in other cancers of the reproductive system.

At a later stage, in collaboration with my external tutor -Prof. Antonio Giordano-, the same experimental models underexposed to tomato extract will be investigated, as an essential ingredient of the Mediterranean diet. Finally, the project will also study the impact of endocrine disruptors in normal human mammary epithelial cells in order to clarify whether pollutants may induce tumorigenesis and by which mechanisms.

References

1. Barak Y et al. Cancer Genomics Proteomics. 2017,14(6):403-408
2. Navarro-Tito N et al. Int J Biochem Cell Biol. 2010,42(2):306-17
3. Ayoub NM et al. Eur J Pharmacol. 2017,810:100-111
4. Rafehi H et al. J Diet Suppl. 2012,9(2):96-109
5. Brandolini L et al. Oncotarget 2015,6(41):43375-94

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