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FOOD HABITS, LIFE STYLE, GENETIC BACKGROUND IN TUMOUR INITIATION AND PROGRESSION OF REPRODUCTIVE SYSTEM

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Introduction

The relationship existing between diet and health is really engaging, in that has been showed that modifications in dietary intake, and specifically the benefits of the Mediterranean diet, can importantly increase life expectancy, reducing the risk of major chronic disease and improve quality of life and well-being.

It has been suggested an association between dietary fats, obesity and a higher risk of developing cancer. In this way, several studies assigned a highest reduction in tumor incidence to monosaturated and saturated lipids present in vegetables, such olive oil. On these bases, this study will be focused on the comprehension and understanding of initiation and progression phases linked to environmental stressors and food habits in the tumors of the reproductive system (breast and ovarian cancer).

Breast cancer is not only the most frequently diagnosed (23% of the total) but also the main reason of tumor death among females (14%). Recently it has been described the potential effect of the olive tree (*Olea europaea*) leaves, oil and fruits to inhibit proliferation and to induce apoptosis in different cancer cell lines.
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 all these compounds, the phenolic fraction of Olive extract becomes specially interesting, including a prominent polyphenol called Oleuropein (OL) -present at higher levels in the olives and leaves- as well as its hydrolysis metabolite, Hydroxytyrosol (HT). 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.
Taking this background into account, we have focused our research in the analysis of Olive leaf extracts with a high content in OL (48,6%) as a potential cell viability reducing agent on a malignant triple negative breast cancer-derived cell line, **MDA-MB-231**.

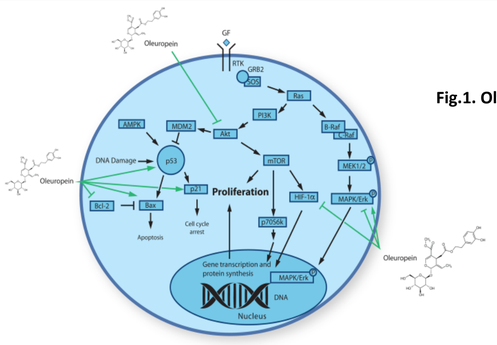

Fig.1. Oleuropein mechanisms of of action to block Proliferation in injured cells.

Methods & Materials

The cellular model used in this study was the triple negative **MDA-MB231** cells, which is highly aggressive and does not undergo to apoptosis via Fas-ligand upon paclitaxel challenge, as described for other breast cancer cell lines. **MDA-MB231** cells represents the claudin-low/mesenchymal subtype, which overexpresses stem cell-enriched genes and has a natural tendency to metastasize to brain and lungs. In addition, this is the breast cancer cell line with the highest ALDH1 expression.

For this reason, cell viability was measured with a MTS assay 24 and 48 hours after the treatment with the Olive extract. This 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% Glutamine and 1% Penicillin/Streptomycin. From this stock we obtained 6 serial dilutions (400-200-100-50-25-10 µg/mL) that were replicated in 5 wells/each. Later, MDA cells were seeded at 5.000 cells/well in a 96-well plate and incubated at 37ºC in a humidified 95% air-5% CO2 atmosphere for 24 hours.

Since **MDA-MB 231** cells express high levels of the stemness marker ALDHA1, Breast Cancer Stem cells were isolated from this cell line (Fig.3) In order to isolate and characterize the MDA-derived stem cells (mammospheres) we have examined the stemness markers ABCG2, CD44+ and ALDHA1 , by cytofluorimetry (FACS).

References

1. Brandolini L, Cristiano L, Fidoamore A, De Pizzol M, Cimini A; et al; "Targeting CXCR1 on breast cancer stem cells: signaling pathways and clinical application modelling"
2. Antic Shamshoum H, Vlavcheski F, Tsiani E; "Anticancer effects of oleuropein"
3. Schwingshackl L and Hoffmann G; "Does a Mediterranean-Type Diet Reduce Cancer Risk?
4. Manzanares MÁ, de Miguel C, Ruiz de Villa MC, Santella RM, Escrich E, Solanas M
"Dietary lipids differentially modulate the initiation of experimental breast carcinogenesis through their influence on hepatic xenobiotic metabolism and DNA damage in the mammary gland."

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Results

In the first 3 months of my project, I have optimized the triple negative **MDA-MB-231** cell culture model of Breast Cancer. On these cells, I assayed the effects of leaf olive extracts. In Fig.2 dose and time graphs for MTS assay on MDA cells treated with different extracts is reported. It is possible to observe a significate effect of the *Olea europaea* extract in decreasing the **MDA-MB-231** cell viability at higher concentrations (200-400 µg/mL), more evident after 48 hours of treatment.

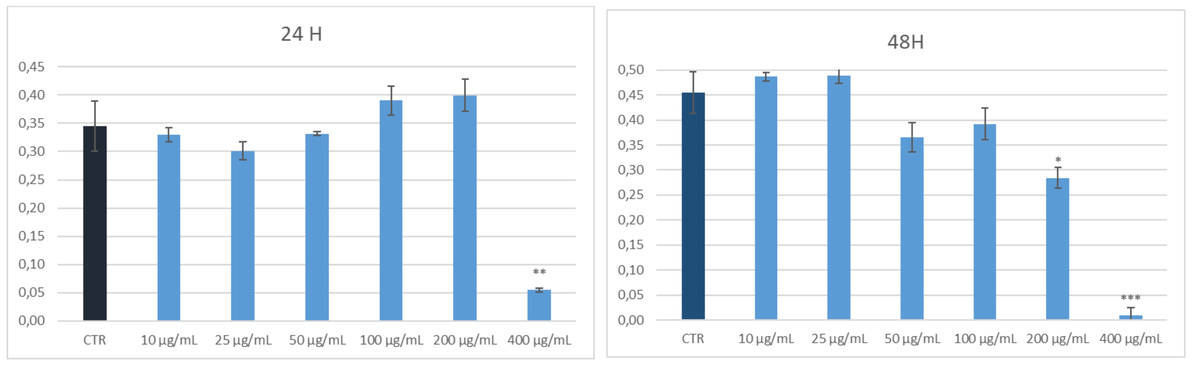

Fig 2 . MTS viability assay 24 and 48h after the MDA-MB-231 cells treatment with the Olive extract. Absorbance measured at 492 nm. Data are mean ±SE of 3 experiments.*,p<0.05; **,p<0.005 ***,p<0.0001.

In parallel, I isolated and characterized Breast cancer stem cells (BCSC, mammospheres, Fig. 3B) by cytofluorimetry (Fig. 4) by assaying the expression of stem cell markers such as CD44, ATP-binding cassette sub-family G member 2 (ABCG2) and aldehyde dehydrogenase A1 (ALDHA1) and by the immunolocalization of ALDHA1 (Fig.5)

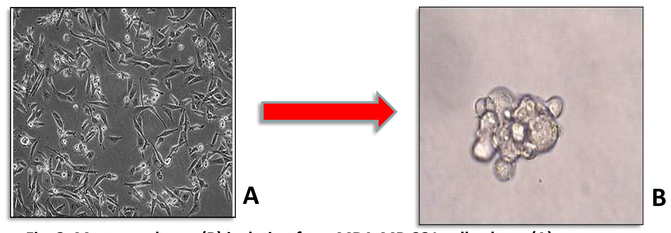

Fig. 3. Mammospheres (B) isolation from MDA-MB-231 cell culture (A).

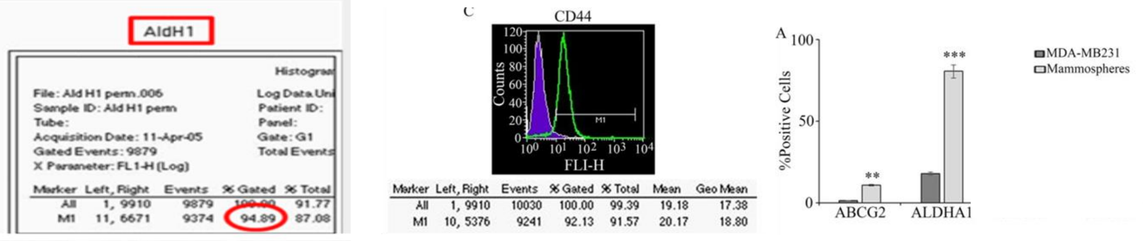

Fig. 4 . Mammospheres formation and characterization from MDA-MB-231: breast tumor stem cell marker enrichment, with respect to the starting cell line, such as ABCG2, ALDHA1 and CDC44+, evaluated by cytofluorimetry, is reported.

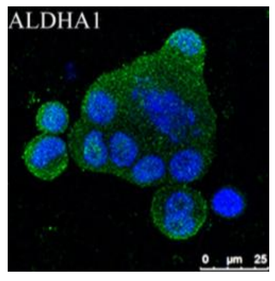

Fig. 5. Immunofluorescence of ALDHA1 stem cell marker in purified mammospheres. Bar = 25 µm.

Conclusions & Future Prospectives

This preliminary results seem to indicate that Olive leaf extract at high concentrations (200-400 µg/mL) determines a reduction in the **MDA-MB-231** cell viability at 24 and 48 hours. Once the model has been optimized and the optimal concentrations assessed, we will look to elucidate the treatment impact on the cell cycle (blocking) and/or death (apoptosis or autophagy).

Secondly, we will develop a comparison between this whole leaf extract effect and its main polyphenol compound with demonstrated anticancer activity: Oleuropein; in order to understand if the purified compound may be more effective than the crude extracts. Subsequently, we also intend to carry out the same study in the Breast cancer stem cells model 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 will be investigated under treatment with the tomato extract, as an essential ingredient of the Mediterranean diet.
Finally, the project will also study the impact of endocrine disruptors in normal human epithelial mammary in order to clarify whether pollutants may induce tumorigenesis and by which mechanisms.