Máster en Biotecnología y biomedicina

Centro de Estudios de Postgrado

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Trabajo Fin de Máster

Cytotoxicity of the main compound of olive oil, Oleic acid in highly invasive cell line"MDA-MB-231"

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Abstract

Oleic acid is the main monounsaturated fatty acid found in olive oil. Several studies have shown the effect of oleic acid on breast cancer, for this reason the Mediterranean diet is based on the olive oil and specifically on oleic acid. The effect of oleic acid on the risk of cancer differs from one study to another. Several Studies has been shown the beneficial effect of oleic acid against human breast cancer, it acts in different proteins activity that are in relation with breast cancer such as the AMPK protein, it can also acts on the receptors of human epidermal growth factors which is HER2. On the other hand, other investigators have shown that oleic acid promotes the proliferation and survival of metastatic breast cancer cells by the activation of the protein Kinase C. Thus, we sought to elucidate whether this compound has a cytotoxic activity or not. A line of highly invasive breast cancer cells MDA-MB-231 was used to determine the effects of oleic acid. The results showed that OA does not possess cytotoxic activity on this breast cancer cell line.

DEDICATION

I first want to thank the Almighty God,

For the courage, the health, the love of knowledge and the patience that gave me to realize this modest work that I dedicate:

To my dear parents:

As many expressive sentences as they are cannot express my gratitude and my gratitude. you have instilled in me the sense of responsibility, optimism and self-confidence in the face of life's difficulties. your advice has always guided my steps towards success. your endless patience, your understanding and your encouragement are for me the indispensable support that you have always been able to bring me.

To my dear Sisters Zaineb and Zina

In memory of a childhood of which we shared the best and the most pleasant moments. For all the complicity and understanding that unite us, this work is a testimony of my attachment and my love.

To my dear uncle Ammar

For all the atmosphere you have surrounded me, for the moral support for all the spontaneity and your warmth, I dedicate this work to you. May God the Almighty exalt all your wishes.

ACKNOWLEDGEMENTS

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Special thanks to Mrs. **Cristina Sanchez Quesada**, the research staff at the University of Jaen for her kindness and sympathy, her invaluable advice, her encouragement, her help and assistance during all the work period, and especially for her patience during the correction of this memory.

I want to express my deep gratitude to **Mr. BEN SELMA Walid** for framing me. His encouragement and his patience helped me a lot to overcome all the difficulties. I thank him very much for following and guiding this work.

I would also like to thank:

Mrs. or Mr. ..... for the honor she gave me to chair the jury and evaluate this work.

Mr. or Mrs. ..... for agreeing to examine this work.

Finally, I thank all those who contributed directly or indirectly to the development of this modest work, and thus express my deep gratitude and respect.

CONTENTS

LIST OF ABBREVIATIONS

BC: Breast Cancer

OA: Olive Oil

CPT: Camptothecin

AMPK: Adenosine Monophosphate-Activated Protein Kinase

PCK: Protein Kinase

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Introduction

I. Cancer

1. General

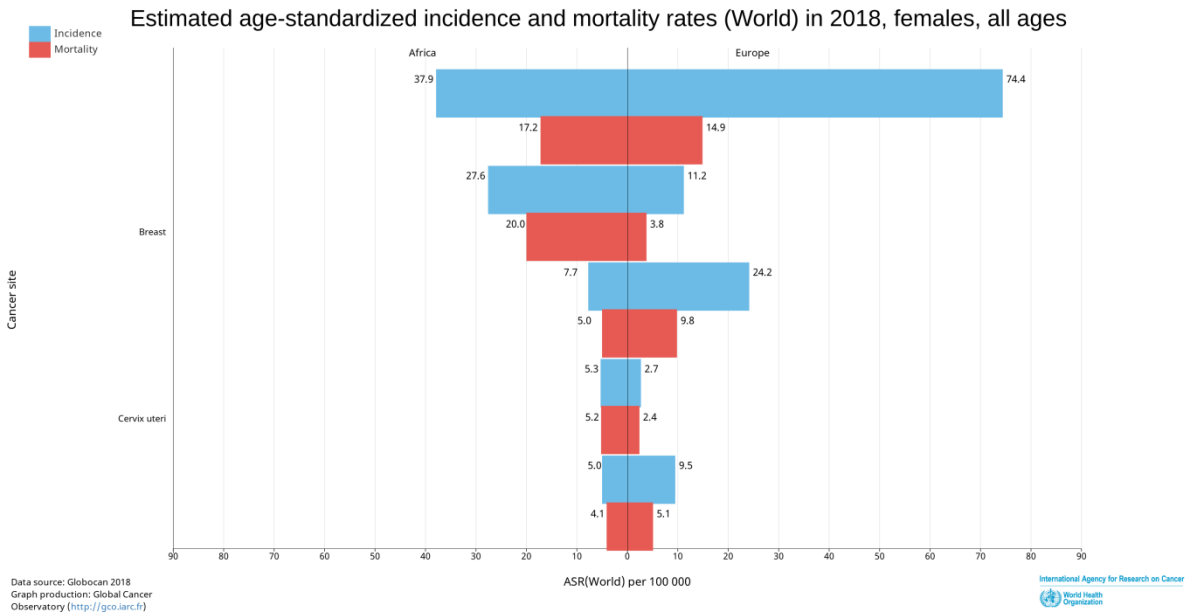
The cancer was in accordance with the World Health Organization (WHO) one of the most causes of death who attacks human live (more than 200 diseases). Inspite the technical and scientific research on cancer, the effectiveness of diagnosis and treatment was low by comparing the percentage of deaths yearly (Henriksen et al., 2014). How is cancer development process? In normal cells, division and development have been organized in a programmed way during disturbances or cell damage. This process is the secret for the development and functioning of a healthy cell. In the absence of this alternative, some cells become unable to respond to received intracellular signals and messengers and lose their ability to maintain this regulatory system (Almeida et al., 2011). This causes an anarchic multiplication and differentiation and uncontrolled abnormal cells that invade not only adjacent tissues, but also distant organs of the primary tumor (Stratton, Campbell and Futreal, 2009). This type of dysfunction may have appeared in any body tissue and may induce several types of cancer (Almeida and Barry, 2011).

2. Breast cancer and tumoral development:

Breast cancer is the first cancer of women in the world that cause a large health problem (Curado et al., 2011).Presently, breast cancer is the most spreading disease among women in the world around 2 million new cases in 2018 (World cancer research fund). Incidence rates are more important (80 per 100,000) in developed countries around the world and low (less than 40 per 100,000) in most developing countries (Ferlay et al., 2010).

3. Epidemiology: current situation

Breast cancer is the leading cause of death among women with a higher annual percentage. Incidence and mortality differed between African and European countries. Incidence and mortality rates vary geographically. In African countries, the incidence was lower than that of European countries (37.9 per 100,000) but higher than that of European countries (74.4 per 100,000). Compared to the breast cancer mortality rate, we find that the percentage was higher in African countries (17.2 per 100,000).



Data source: GLOBOCAN 2018

Fig1.Estimated age-standardized incidence and mortality rates (world) in 2008, female

4. Incidence, mortality and evolution

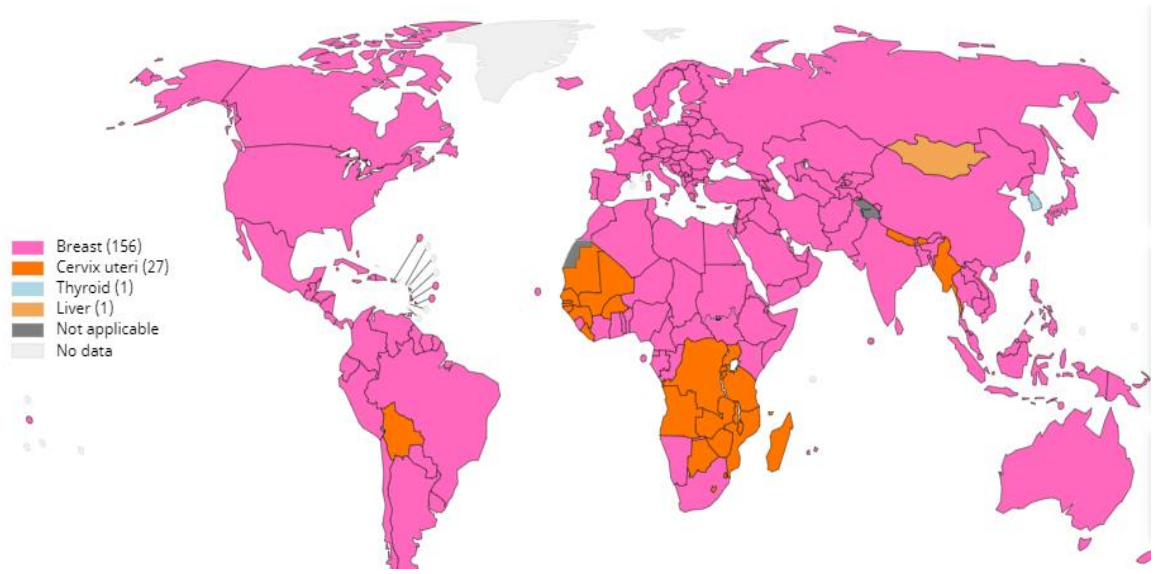


Fig2. Top cancer per country, estimated age-standardized incidence rates (World) in 2018, females.

Data source: GLOBOCAN 2018

5. Risk factors

Breast cancer is a major disease in the world, characterized by multiple factors and important stages. It was characterized by an uncontrolled proliferation of breast cells (Belkacem, 2011). This type of disease has more than one cause to develop in the human body. Several risk factors contribute to the development of breast cancer. The fact that the cancer appears only several years after the exposure may increase the difficulties of establishing the causal link between the agent and the cancer (Cochaud et al., 2004). According to the predictions required for risk factors, it was difficult to confirm the development of one specific cancer, depending on the risks to which it is exposed. However, knowledge of these risk factors allows the search of appropriate cancer prevention measures.

- **Age**:

One of the most common risk factors of the BC was the age. Incidence of this type of cancer was higher according to the age; the disease is more or less low in women who are less than 30 years old and increases among 45 and 70 years, then progressively decreases (Nam et al., 2008).

- **Familial and genetic factors**:

In addition of the age, familiar and genetic factor are also an important risk for the increase of BC. Different factors can identify an important cause of breast cancer. On the one hand, there is family history, which is defined by the existence of a specific breast cancer in the same family or the appearance of spontaneous cancer in one family (Hopper, 2001). On the other hand, we find gene mutations that are able to increase the risk of breast cancer. Three important genes are involved in the appearance of this type of cancer which are BRCA1, BRCA2 and TP53 (Olopade., 2001)

- **Overweight and lifestyle**:

In the other side, we find other risk factors associated to environmental conditions. The most important risk was the exposure to estrogen hormone that may be in two different way endogenous or exogenous.

* **Obesity**: Obesity is an important factor in the development of breast carcinoma. It is associated with the hormonal profile that promotes the risk of breast cancer. Obesity increases the risk of developing breast cancer by approximately 50% (Key., 2001). Excess adipose tissue leads to increased production and exposure to steroid hormones. As the majority of breast cancer types appear mostly in postmenopausal women, obesity becomes a remarkable factor.

* **Diabetes:** is the major universal problem of public health. According to Wolf et al., Diabetes can increase the relative risk of developing breast cancer. This may be due in particular to an overproduction of the insulin receptor caused by a higher insulin concentration. The complex formed by insulin and receptor can induce with different reactions an increase in cell proliferation and a decrease in apoptosis (Zhu et al., 2008).

* **Alcohol:** is a nutritional determinant of the risk of breast cancer. Several studies have shown that high consumption alcohol may increase the risk of developing breast carcinoma (Chen et al 2008).

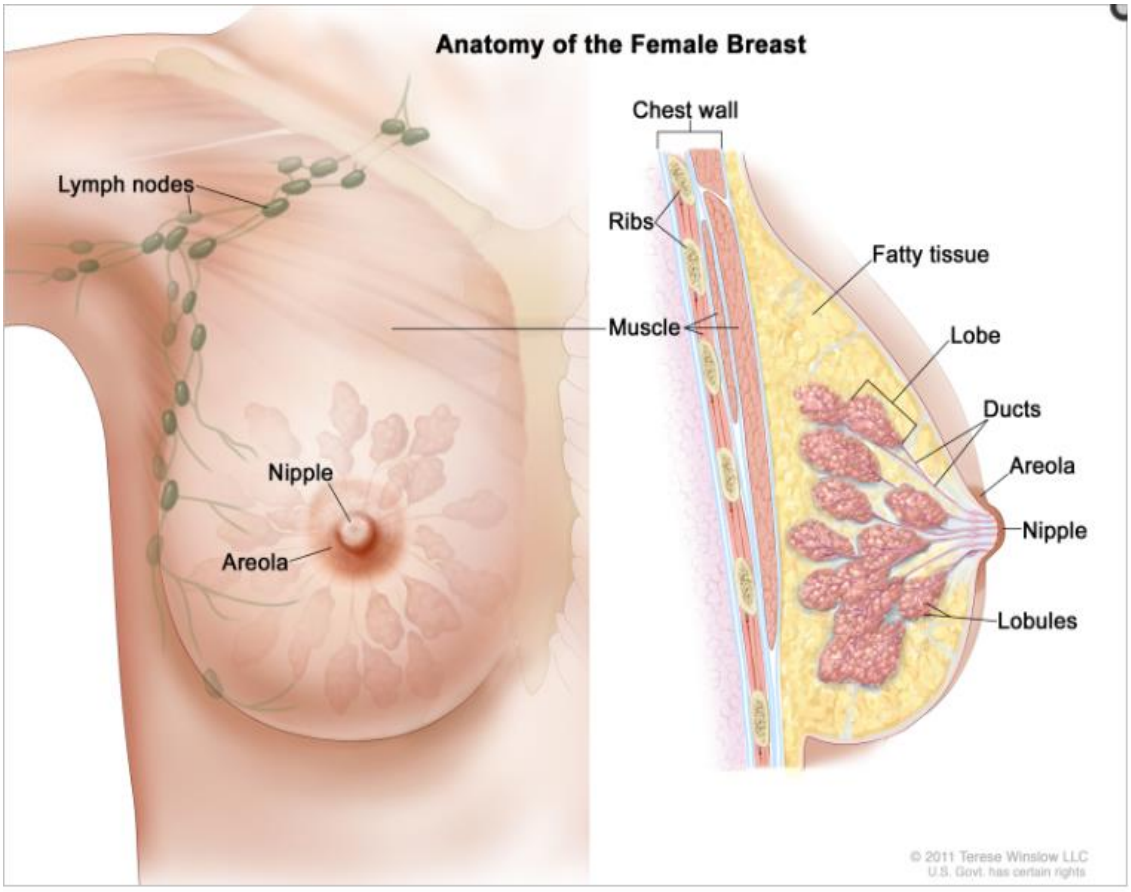
* **Smoking:** smoking has been identified as a major component containing carcinogens. It causes several types of diseases (Numasak et al., 2003). However, smoking is not considered a significant risk factor in breast cancer, but may have an effect on postmenopausal women (Kropp., 2002).

II. Breast cancer development:

1. Architecture and breast development:

The breast is a glandular tissue consisting of an areola and a nipple (Figure 1). The mammary gland formed by two cellular compartments: the mesenchymal compartment, perfused by the blood vessels and nerves, and the epithelial compartment articulated around a network of galactophore ducts and lobules surrounding the alveoli. These two compartments are divided by a basal membrane of collagen, laminin and glycosaminoglycans, but there is strong collaboration between them during the development of mammary gland (Bear et al., 2003).

The architecture of the mammary gland develops over time, and depends on several factors such as age and stage of reproductive life (Wolmark et al., 2001) (Figure 2) and formed under the influence of ovarian sex hormones (estrogen and progesterone) and ordinary growth (Wolmark et al., 2001).



From: Breast Cancer Treatment During Pregnancy (PDQ®)

Fig. 3. Schematic representation of a breast in section

2. Evolution of the architecture of the mammary gland:

The mammary glands develop in several stages which begin at birth. This gland evolves following different stages. The mammary galleon remains at the repot from birth until puberty (A). At the stage of puberty, this gland begins to develop by budding which are multilayer thanks to the puberty hormone (B). Then, at the virginity stage, the mammary gland branches out and forms a two-layer epithelial structure (C). The mammary gland have an hormonal changes in the stage of the pregnancy and lactation, at this level there is a secretion of milk by the alveolar cells that is formed (D). Finally, we find the stages of involution at which a cell death occurs and the mammary gland gets in period of relaxation (E).

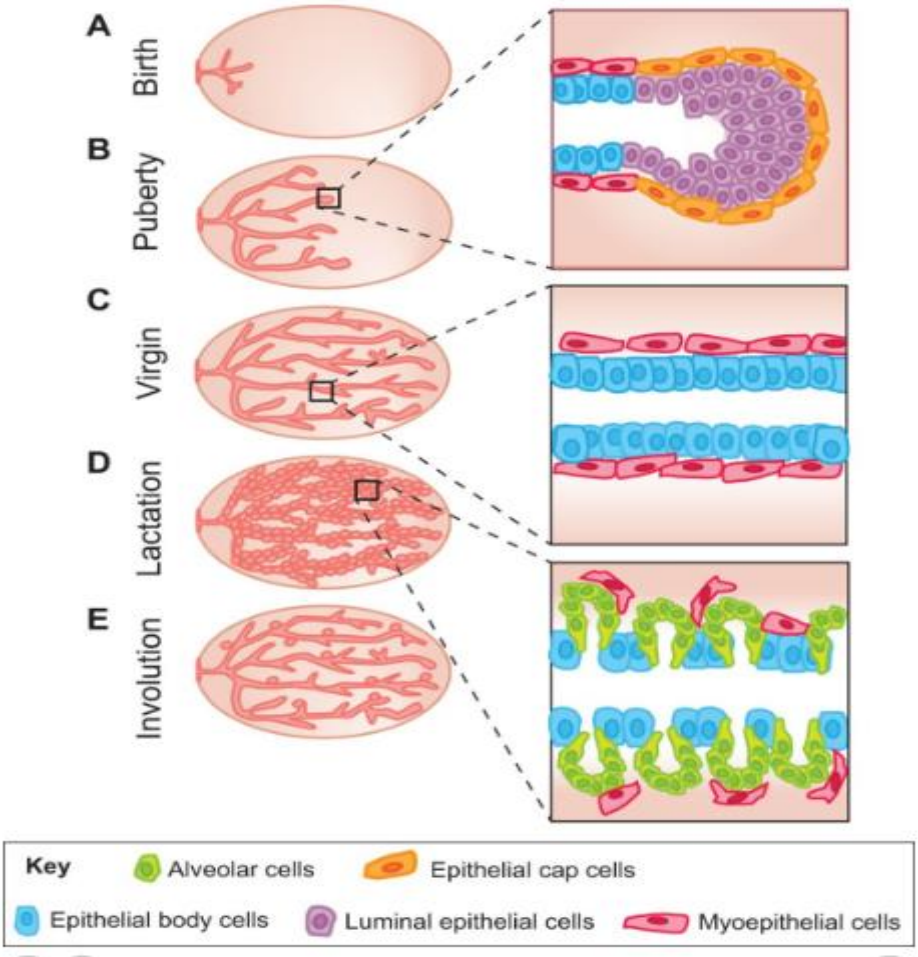


Fig.4. Evolution of the architecture of the mammary gland (Jamie et al 2015).

3. Carcinogenesis process:

The mammary gland as it was mentioned before is an organ in frequent evolution under the influence of hormones and growth factors (McNally et al., 2011). This makes it a specific organ from the point of view of the number of cells in growth and differentiation, and thus makes it suitable for cancerous transformations.

Cancer can be discovered in clinical term when it encompasses more than 109 cells (Cotterchio et al., 2014). All the cancer cells come from a single cell that has become cancerous or transformed (mother cell), during cell division control mechanisms, these cells become detached and escape and therefore an anarchic multiplication is formed by clonal expansion (Weinberg et al., 1996).

The carcinogenesis process is composed of four phases: initiation, promotion, progression and invasion (Fig 3).

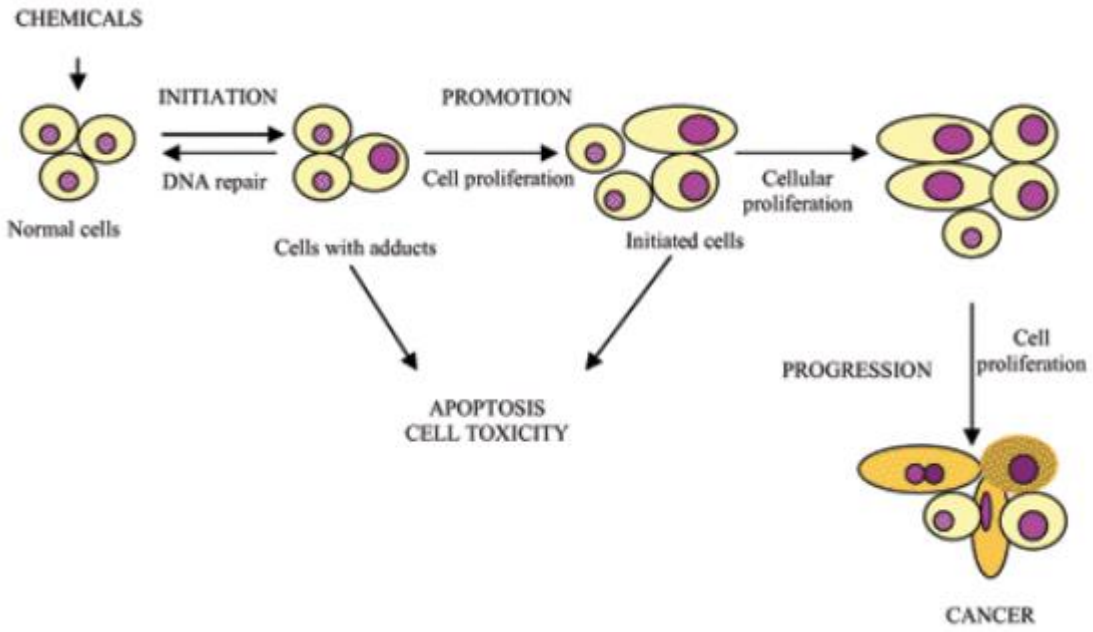


Fig.5. Carcinogenesis process (Paula et al., 2007)

Carcinogenesis is based on four successive phases. Initiation is the fastest phase in which the cell is affected by important mutations in the genome and will be transmitted to the child cells. The promotion is characterized by a strong proliferation of mutated cells during the first phase, which induces the formation of a clone of tumor cells. Conversion is the most sensitive step that corresponds to the transformation of a normal cell into a malignant cell and finally to the phase of progression during which the malignant cell will enter the tissues to form a metastasis (Mlika et al., 2014).

III. Mediterranean diet and olive oil

1. Mediterranean diet

The Mediterranean diet is a type of diet that encompasses several foods that are necessary for the health for which it is classified among the diets most advised for its good quality. This diet is found specifically in Mediterranean countries (Gardener et al., 2011). In addition, there is a link between the Mediterranean diet and the prevention of certain diseases such as brain and cardiovascular diseases, as well as certain types of cancer (Cicerale et al., 2009). This diet is based on considerable frequent consumption. fruit, vegetables, cereals and fish (high in fatty acids) and low consumption of red meats (Mena et al., 2009). The Mediterranean diet differs from one region to another and each of these regions has a specific diet but the only common element is olive oil which is characterized by a high composition of monounsaturated fatty acids (Rietjens et al., 2007). Several investigators have conducted research on olive oil and have shown the preventive and the beneficial effects on health, such as antioxidant and anti-inflammatory effects (Domitrovic et al., 2012).

2. Virgin olive oil:

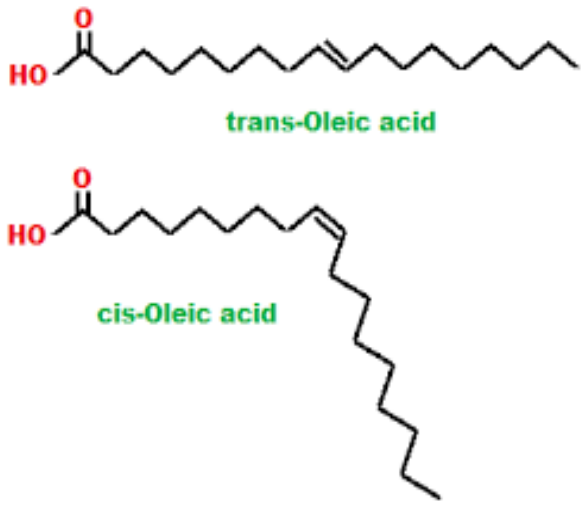
Virgin olive oil (VOO) is essentially composed of triglycerides (between 98% and 99%) which constitute the saponifiable fraction. The unsaponifiable fraction is represented by the rest of the elements of olive oil (between 1% and 2%), which represent about 230 very diverse substances (Sanchez, 2015). A process of transformations was carried out to obtain a virgin olive oil by physical methods without the addition of any type of additive elements. VOO is obtained from olives according to a process of transformation realized by physical methods. Olive oil is the only natural juice extracted from the olive that can be consumed. Once refined in VOO, the minor components of olive oil will be decreased or disappeared (Sanchez, 2015).

3. The beneficial effects of olive oil:

Several studies have shown the benefits of olive oil on human health. It has also been shown that olive oil can act as an anti-tumor agent thanks to the phenolic fractions it contains (Jiang et al., 2010). The anti-tumor effect has been studied thanks to its capacity to inhibit the proliferation of tumor cells and to induce the apoptosis of various metastatic cancer cells. In the Mediterranean countries, olive oil is considered as a fundamental constituent of the diet and is also known for its protective effect against certain diseases. In Mediterranean countries, the consumption of olive oil is the most recommended in the diet, especially to reduce the risk of cancer. Monounsaturated oleic acid is the main component of olive oil. Indeed, the protective effect of olive oil comes from the effect of oleic acid (Owen et al.,2000). Extra virgin olive oil also contains several elements such as phenolic, antioxidants, oleuropein, tyrosol and oleic acid, all of which are important components of cancer protection. Therefore, high consumption of olive oil reduces the risk of cancer.

IV. Oleic Acid- The Anti-Breast Cancer Component in Olive Oil

Oleic acid is known as the main monounsaturated fatty acid in olive oil (Cicerale et al., 2009), so it is considered an essential compound that acts against breast cancer. Thanks to this compound, the Mediterranean diet is known as a healthy diet and the most recommended (Rietjens et al., 2007). Also, other studies showed that the consumption of olive oil which is rich in oleic acid at the same time with the anti-cancer drugs makes it possible to increase the antitumor effect (Domitrovic et al., 2012), as the case of herceptin which has been improved by an association with oleic acid, in order to increase its effect against the carcinoma (Rietjens et al., 2007). Indeed, oleic acid acts on the enzymes responsible for energy metabolism by stimulating its activities (Yang., 1992). Oleic acid is found in various compounds such as mainly in olive oil also in almonds, macadamia, peanuts, sesame oil, etc. it is an important compound in terms of prevention and protection against cancer.



Chemistry Tutor Vista

Fig.6. The chemical structure of oleic acid

Hypothesis

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Objectives

The scientific evidence published by different authors suggests that certain types of tumors, breast cancer among them, are less frequent in those countries where virgin olive oil is usually consumed**.**

Certain monounsaturated fatty acids such as oleic acid present in virgin olive oils may be those which confer, at least partially, a protective role against the appearance of the breast cancer tumor in humans.

According to this hypothesis, the purpose of this work was:

To determine the cytotoxic effect of oleic acid on human breast cancer cells of a highly metastatic cell line (MDA-MB-231) in vitro.

Materials

&

Methods

In this section we describe the experiments that were used to determine the cytotoxicity caused by oleic acid and camptothecin, this last as a positive control of antitumor chemotherapy.

1. Cell culture and treatments

Cells from the highly invasive MDA-MB-231 human breast cancer cell line (ATCC® number: HTB-26 ™) (estrogen receptor and negative progesterone receptor), were obtained from American Type Culture Collection (ATCC, Manassas, VA, USA). Cells (MDA-MB-231) were cultured as monolayer cultures in MEM supplemented with 10% FBS, 1% Hepes buffer, 1% sodium pyruvate and 1% NEAA. Cells were maintained at 37 ° C in a humidified atmosphere containing 5% CO2. Cells were regularly under-grown using the TrypLE Express solution. These cells were used for all experiments after acquire an exponential growth state. Except for the tests specifying the opposite, the cells were treated with 0.1µM, 1µM and 10 µM oleic acid (OA) and camptothecin for 4 h.

2. Cytotoxicity Assay

Cell survival as measured by growth of treated versus untreated control cells. It was performed in MDA-MB-231cells using the Cell Titer Blue based assay according to Scudiero et al.(1988) with some modifications. Briefly, the cells were inoculated into 96-well culture plates in a total volume of 100 µL per well (5×10^3 cells). After 24h to allow cell attachment, 100µL of fresh medium with increased the concentrations from 0.001µM to 100µM of OA and CTP (as positive control) was added to samples. After 24h cells were incubated with XTT in RPMI medium free of phenol-red for 3 h at 37 °C with 5% CO_2 and the absorbance measured at a wavelength of 450 nm (620 nm) in a flat reader (TECAN GENIOS Plus). Viability was calculated using the formula:

$$\% \text{ of viable cells} = [(\text{cells treated}) / (\text{control A})] \times 100$$

Where A is the difference in absorbance between optical density units ($A = OD_{450} - OD_{620}$).

All measurements were performed in quintuplicate and each experiment was repeated at least three times. As a control vehicle, the cells were treated with Et-OH at the highest concentration of OA and CTP used.

	1	2	3	4	5	6	7	8	9	10
A	Control MEM	Control MEM	Control MEM	Control Cell titter blue	Control Cell titter blue	Control Cell titter blue	Control DMSO	Control DMSO	Control DMSO	
B	100	100	100	100	100	100	100	100	100	100
C	10	10	10	10	10	10	10	10	10	10
D	1	1	1	1	1	1	1	1	1	1
E	0,1	0,1	0,1	0,1	0,1	0,1	0,1	0,1	0,1	0,1
F	0,01	0,01	0,01	0,01	0,01	0,01	0,01	0,01	0,01	0,01
G	0,001	0,001	0,001	0,001	0,001	0,001	0,001	0,001	0,001	0,001
H	0,0001	0,0001	0,0001	0,0001	0,0001	0,0001	0,0001	0,0001	0,0001	0,0001

Camptothecin

Oleic acid

3. Statistical Analysis

The results are displayed as an average of at least three independent experiments (± SEM). The results are expressed as a percentage relative to the untreated control, defined at 100%.

Statistical analysis was performed using STATGRAPHICS Plus 5.1 statistical software (Stat point Technologies, Inc., Warrenton, Va., USA). Values of $p<0.05$ were considered significant.

Results

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Discussion

I. Results

1. Structures of the two components studied

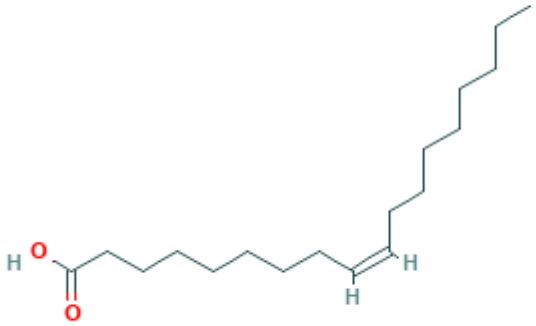

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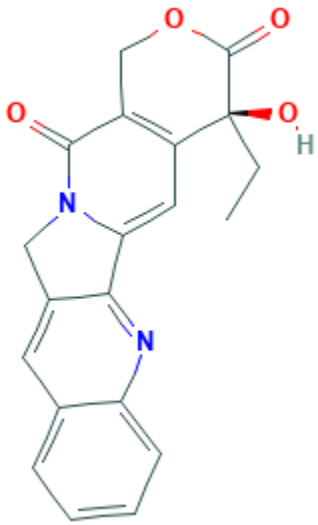
Fig 7 : Oleic acid



Source : PubChem

Fig 8 : Camptothecin

2. Citotoxicity effects:

2.1. **Oleic Acid:**

The results are expressed as the percentage of cell survival with respect to the untreated control, which was set as 100%. Cells from MDA-MB-231cell line treated with OA does not show a marked cytotoxic effect with all concentrations assayed. The difference observed between averages is not significant (Fig.9).

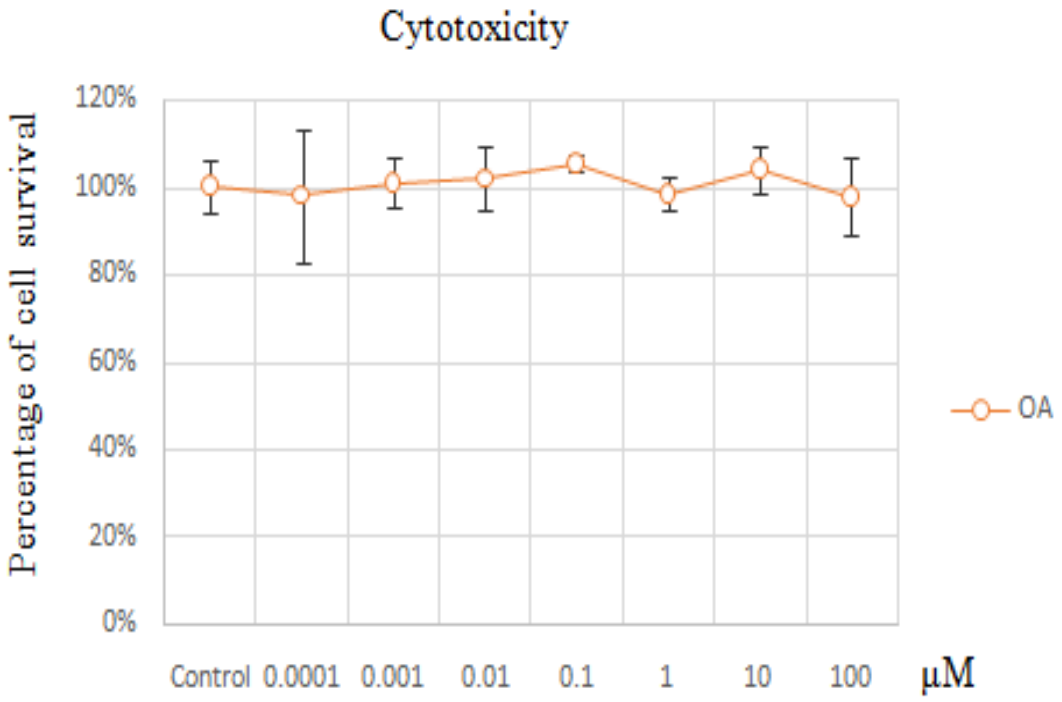


Fig.9 Cytotoxicity of OA proteins from 0.001 µM to 100 µM in MDA-MB-231 cells.

2.2. Camptothecin:

The results are expressed as the percentage of cell survival with respect to the untreated control, which was set as 100%. Cells from MDA-MB-231 cell line treated with CPT shows a marked cytotoxic effect at higher concentrations (Fig.10). The difference observed between the averages is significant (p<0.05). At a concentration of 100 µM CPT induced a strong cytotoxic effect (58%).

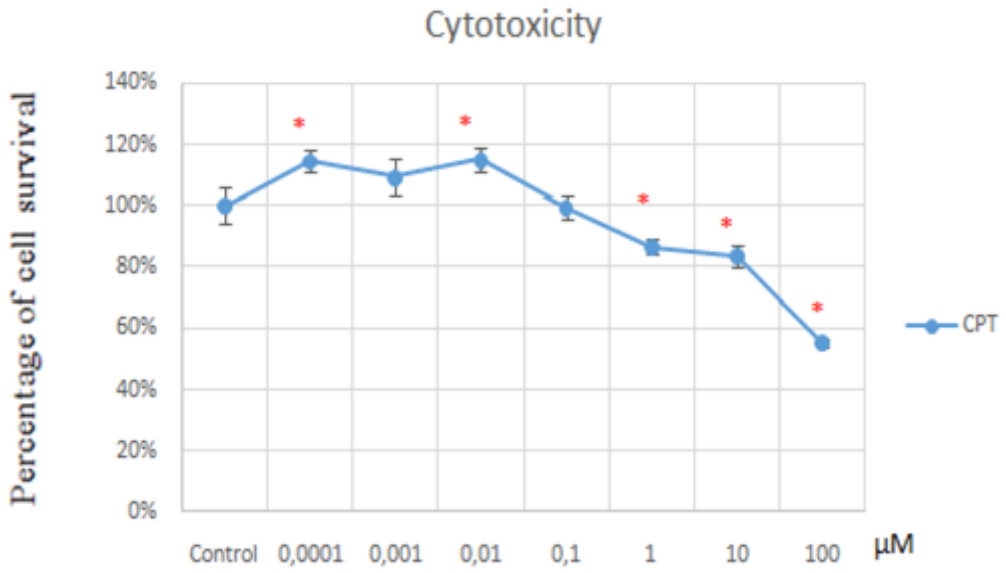


Fig 10: Cytotoxicity proteins from 0.001 µM to 100 µM in MDA-MB-231 cells.

2.3. Opposite Effects of oleic acid and camptothecin on the viability of Cancer Cell Line « MDA-MB-231 »

The MDA-MB-231 cells treated with the two different compounds: oleic acid did not show a cytotoxic effect of the cells. OA concentrations between 0.01 µM and 100 µM seemed to promote cell survival.

A chemical treatment was carried out with the camptothecin which induces a strong cytotoxic effect at 100µM (cell survival was 58%) (Fig.11).The values represent the mean ± SEM of three independent experiments. The statistically significant differences are represented by (*) for CPT at p <0.05 compared to the untreated control.

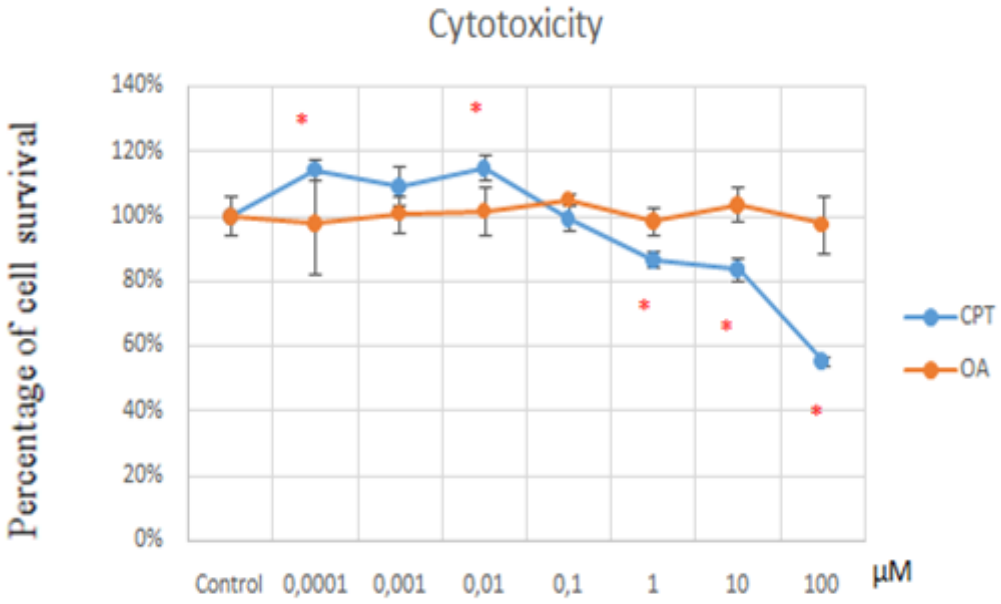


Fig11: Cytotoxicity of OA and CPT proteins from 0.001 µM to 100 µM in MDA-MB-231 cells.

II. Discussion

Our study is carried out in vitro on a human breast cancer epithelial cell line MDA-MB-231 which is a highly invasive, triple negative and highly aggressive cancer cell line. The purpose of this study is to show the effect of a natural compound, the oleic acid on these types of cells.

Oleic acid (OA) is a monounsaturated fatty acid, the main compound of olive oil. It is well known that the Mediterranean diet plays a role in the prevention of cancer, and this olive oil is usually found in this diet.

The present study focuses on the cytotoxicity effect that this natural compound found in olive oil, could have on breast cancer cells.

In our work, we realized the cytotoxicity test of oleic acid on metastatic cells of MDA-MB-231 breast cancer using camptothecin as a positive control. Contrary to our initial hypothesis, we found that oleic acid does not produce a cell death (100% cell survival), so it can be concluded from our results that oleic acid does not have a cytotoxic effect on the highly invasive cancerous cells.

Several studies have suggested the antitumor properties of OA, but so far, there is no scientific data on their cytotoxic activity against human breast cancer cell.

Many studies have been realized on the antitumor effect of oleic acid on highly metastatic cancer cells such as Shuai et al (2014), he has showed the link between oleic acid and the activity of AMPK which is a responsible enzyme in energetic metabolism. AMPK promotes the production of ATP which is necessary for cell survival and growth.

Indeed, Shuai has demonstrated that oleic acid inhibits the activity of the AMPK enzyme subsequently decreasing the level of ATP and therefore inhibiting the growth of highly metastatic breast cancer cells.

Other studies done by Mendez et al (2005), showed that oleic acid can act on the gene that codes for the human epidermal growth factor receptor that is HER2. And he concluded that an over expression of the HER2 protein promotes the proliferation of cancer cells. Therefore, oleic acid suppresses this expression in order to decrease the proliferation of these cancerous cells.

On the other hand, various studies have been carried out concerning the antitumor effect of oleic acid and proposed mechanisms for which oleic acid may act on the appearance of breast cancer. In fact, oleic acid stimulates protein kinase C (PKC), which is a protein involved in different processes such as survival, proliferation and apoptosis of cells (Karam et al., 2012).

Indeed, several studies have shown that the over expression of PKC, which promotes the estrogen response for cell proliferation and the independent growth of MDA-MB-231 cells, such as the study by Karam et al. (2014) .PKC acts on estrogen receptors and concluded that an excess of PKC causes an increase in the expression of these receptors and results in an increase in cell proliferation, which is essential for the development of breast cancer.

According to some studies done by Uchida et al. (1997), The activity of protein kinase C (PKC) appears high in human mammary tumors due to the presence of oleic acid. Indeed, oleic acid can promote the proliferation of metastatic cancer cells by the activation of another protein phosphatidylinositol 3-kinase, that is implicated in the control cell process (Hardy et al., 2000).

Other studies show that in the Mediterranean region, oleic acid has a preventive effect on breast cancer, but until now these studies remain contradictory. Oleic acid exists in two types of configurations, cis oleic acid which reduces the risk of breast cancer, and trans oleic acid. From a chemical point of view, trans and cis oleic acids differ just in the form of the molecule and, from the point of view of health its differ in their effects specially against cancer.

Kohmeier et al (1997) showed in his studies that trans oleic acid can increase the risk of breast cancer development. In fact, he used different types of trans fatty acids such as oleic acid and he concluded that it has an increase in the growth and proliferation of cancer cells thanks to these trans fatty acids. When trans fatty acid enters the cell, it promotes the growth and proliferation of these cancer cells, therefore, the tumor effect of these cells becomes more aggressive (Kohmeier et al., 1997).

However, studies that have shown that oleic acid reduces the risk of breast cancer remain insufficient and unconvincing to be confirmed.

Conclusion
&
Perspectives

Oleic acid is an abundant monounsaturated fatty acid present in olive oil, the main fat of the Mediterranean diet. In the present work we are interested in studying of the cytotoxicity of oleic acid in a highly invasive cell line MDA-MB-231. According to our results, we were able to conclude that oleic acid (OA) appears not to be an antitumor agent in breast cancer cells in vitro. But our results are not sufficient to confirm the effect of oleic acid on the risk of breast cancer.

In perspective, it would be interesting to complete the studies of the effect of oleic acid against highly invasive cell line MDA-MB-231 by realizing complementary tests in vitro such as the cell proliferation test and the apoptosis test.

Bibliographic references

-Almeida, C.A. et Barry, S.A. (2011). Cancer basic science and clinical aspects. Willey-Blackwell, 1-3.

-Barbara, B., Toietta, G., Maggio, R., Arciello, M., Tarocchi, M., Galli, A. et Balsano, C. (2014). Effects of the olive-derived polyphenol oleuropein on human health. /nt J Mol Sei, 15(10), 18508-18524.

-Belkacem S.(2011). Research of recurrent mutations on the BRCA1 gene involved in the predisposition to hereditary breast cancer in young patients from western Algeria.

-Bear, H. D. et al.(2003). The effect on tumor response of adding sequential preoperative docetaxel to preoperative doxorubicin and cyclophosphamide: preliminary results from National Surgical Adjuvant Breast and Bowel Project.Protocol B-27. J. Clin. Oncol. 21, 4165Ŕ74.

-Chen WC, Lai YH, Chen HY, Guo HR, Su IJ, Chen HH: Interleukin-17-producing cell infiltration in the breast cancer tumour microenvironment is a poor prognostic factor. Histopathology, 63(2):225-233.

-Chuong, C.-M., Bhat, R., Widelitz, R. B. and Bissell, M. J. (2014). SnapShot:branching morphogenesis. Cell 158; 1212-1212.

-Cicerale, S., Conlim, X.A., Sinclair, A.J. et Keast, R.S. (2009). Chemistry and health of olive oil phenolics. Crit Rev Food Sei Nutr, 49(3), 218-236.

- Cochaud, S. (2013).IL-17A is produced by breast cancer TILs and promotes chemoresistance and proliferation through ERK1/2.Sci. Rep. 3, 3456.

-Cotterchio, M., Mirea, L., Ozcelik, H. & Kreiger, N.(2014). Active Cigarette Smoking, Variants in Carcinogen Metabolism Genes and Breast Cancer Risk among Pre- and Postmenopausal Women in Ontario, Canada. Breast J; 20, 468Ŕ80.

-Curado MP.(2011). Breast cancer in the world: incidence and mortality. Salud publica Mex (53);372-384.

-Domitrovic, R., Jak:ovac, H., Marchesi, V.V., Sain, I., Romic, Z. et Rahelic, D. (2012). Preventive and therapeutic effects of oleuropein against carbon tetrachloride-induced li ver damage in mi ce. Pharmacol Res ; 65(4), 451-464.

-Ferlay J., Héry C., Autier P., Sankaranarayanan R. (2010). Global Burden of Breast Cancer. In: Li C. (eds) Breast Cancer Epidemiology.New York, NY ;1-19.

-Gardener, H., Wright, C.B., Gu, Y., Demmer, R.T., Boden-AlbaJa, B., Elkind, M.S ., Sacco, R.L. et Scarmeas, N. (2011). Mediterranean-style diet and risk of ischemie stroke, myocardia1 infarction, and vascular death: the Northern Manhattan Study. Am J Clin Nutr; 94(6), 1458-1464.

-Hardy S, Langelier Y, Prentki M.(2000). Oleate activates phosphatidylinositol 3-kinase and promotes proliferation and reduces apoptosis of MDA-MB-23 1 breast cancer ceils, whereas palmitate has opposite effects. Cancer Res ;60:6353-58.

-Henriksen, M., Johnsen, K.B., Andersen, H.H., Pilgaard, L. et Duroux, M. (2014). MicroRNA Expression Signatures Determine Prognosis and Survival in Glioblastoma Multiforme-a Systematic Overview. Mol Neurobiol, 50(3), 896-913.

-Hopper JL.(2001). Genetic epidemiology of female breast cancer. $emin Cancer Biol ;1 1:367-74.

-Jamie L., Claire R., Joni D. Mott and Mina J.(2015). Mammary gland development: cell fate specification, stem cells and the microenvironment (142); 1028-1042.

-Jiang, H., Gautam, S.C., Jiang, F., Pu, P. et Chopp, M. (2010). Diatary polyphenols as a preventive and therapeutic agents in glioblastoma. Glioblastoma: Molecular mecanisms of oathogenesis and current therapeutic strategies; 325-335.

-Karam M., Legay C., Auclair C, Ricort J-M.(2012). Protein kinase D1 stimulates proliferation and enhances tumorigenesis of MCF-7 human breast cancer cells through a MEK/ERK-dependent signaling pathway. Exp Cell Res; 318: 558–69.

-Karam M., Bièche I., Legay C., Vacher S., Auclair C, Ricort J-M.(2014). Protein kinase D1 regulates ERα-positive breast cancer cell growth response to 17β-estradiol and contributes to poor prognosis in patients. J Cell Mol Med; 18: 2536–52.

-Key TJ, Verkasalo PK, Banks E.(2001). Epidemiology of breast cancer. Lancet Oncot ;2: 133-40.

-Kohlmeier, L.,Simonsen, N., Veer,P., Strain, J., Martin-Moreno, J., Margolin,B., Huttunen, J., Navajas,J., Martin, B.C., Thamm,M., Kardinaal,A., Kok,F.J.(1997).Adipose Tissue Trans Fatty Acid and Breast Cancer in the European community Multicenter study on Antioxidants, Myocardial Infarction and Breast Cancer;6: 705-710.

-Kropp S, Chang-Claude J.(2002). Active and passive smoking and risk of breast cancer by age 50 years among German women. Am JEpiderniol;156:616-26.

-McNally, S. & Martin, F.(2011). Molecular regulators of pubertal mammary gland development. Ann. Med;43, 212Ŕ34.

-Mlika, M., Ayadi-Kaddour, A., Laabidi, S., Boudaya, S., Boussen, H., El Mezni, F. (2014) Carcinogenesis of non-small cell lung carcinoma and therapeutic implications (6); 92: 368-372.

-Mena, M.P., Sacanella, E., Vazquez-Agell, M., Morales, M., Fito, M., Escoda, R.,Serrano-Martinez, M., Salas-Salvado, J., Benages, N., Casas, R., LamuelaRaventos, R.M., Masanes, F., Ros, E. et Estruch, R. (2009). Inhibition of circulating immune cell activation: a molecular antiinflammatory effect of the Mediterranean diet. Am J Clin Nutr; 89(1), 248-256

- Menendez, J. A. ,Vellon1, L., Colomer, R and Lupu, R.(2005). Oleic acid, the main monounsaturated fatty acid of olive oil, suppresses Her-2/neu (erb B-2) expression and synergistically enhances the growth inhibitory effects of trastuzumab (Herceptine) in breast cancer cells with Her-2/neu oncogene amplification;16: 359–371.

- Nam, J .(2008). TGF-β subverts the immune system into directly promoting tumor growth through IL-17.Cancer Res 68, 3915Ŕ392

-Numasaki M, Fukushi J, Ono M, Narula SK, Zavodny PJ, Kudo T, Robbins PD, Tahara H, Lotze MT.(2003). Interleukin-17 promotes angiogenesis and tumor growth:2620-2627.

-Olopade 01, Pichert G.(2001).Cancer genetics in oncology practice. Ann Oncol;12:95-98.

-Ouyang W, Kolls JK, Zheng Y. (2008).The biological functions of T helper 17 cell effector cytokines in inflammation. Immunity;28(4):454-467.

- Owen R.W., Mier W., Giacosa A., Hule W.E., Spiegelhalder B., Bartsch H.(2000). Phenolic compounds and squalene in olive oils: the concentration and antioxidant potential of total phenols, simple phenols, secoroids, lignans and squalene.Food Chem Toxico ;38:647–59.

- Paula A., Aura C., Raquel C., Henrique G., Luis F., Carlos L.(2007). Chemical carcinogenesis. ISSN 0001-3765.

- Rietjens, S.J., Bast, A., de Vente, J. et Haenen, G.R. (2007). The olive oil antioxidant hydroxytyrosol efficiently protects against the oxidative stress-induced impairment of the NObullet response of isolated rat aorta. Am J Physiol HeartCirc Physiol ; 292(4), H1931-1936.

- Robinson., G.(2007). Cooperation of signalling pathways in embryonic mammary gland development. Nat. Rev. Genet. 8; 963-972.

- Sánchez-Quesada, C., López-Biedma,A and Juan-Gaforio,J .(2015). Oleanolic Acid, a Compound Present in Grapes and Olives,Protects against Genotoxicity in Human MammaryEpithelial Cells *20*:13670-13688

- Shuai Li., Ti Zhou., Cen Li., Zhiyu Dai., Di Che., Yachao Y., Lei Li., Jianxing Ma, Xia Yang.,Guoquan Gao.(2014) High Metastaticgastric and Breast Cancer Cells Consume Oleic Acid in an AMPK Dependent Manner. PLoS ONE 9(5); journal.pone.0097330.

-Stratton, M.R., Campbell, P.J. et Futreal, P.A. (2009). The cancer genome. Nature, 458(7239), 719-724.

- Weinberg, R.(1996). Molecular basis of carcinogenesis : understanding the cell cycle clock. Cytokines Mol. Ther.2(2):105-1.

- Wolmark, N., Wang, J., Mamounas, E., Bryant, J. & Fisher, B.(2001). Preoperative chemotherapy in patients with operable breast cancer: nine-year results from National Surgical Adjuvant Breast and Bowel Project. B-18. J. Natl. Cancer Inst. Monogr; 15212, 96Ŕ102.

- Yang, X., Hubbard, EJ, Carlson, M .(1992). A protein kinase substrate identified by the two-hybrid system. Science 257 (5070): 680-2.

-Zhu X, Mulcahy LA, Mohammed RA, Lee AH, Franks HA, Kilpatrick L, Yilmazer A, Paish EC, Ellis IO, Patel PM. (2008). IL-17 expression by breast-cancer-associated macrophages: IL-17 promotes invasiveness of breast cancer cell lines. Breast Cancer Res.10(6):R95.

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✓ Academic training

- 2018 - 2019 currently: Professional Master in Biotechnology and Biomedicine at the University of Jaen.
- 2017-2018: Professional Master in Bio analysis and Quality Control at the University of Mahdia (Tunisia)
- 2014-2017: Degree in Analytical and Experimental Biology, Bio analysis and Quality Control Course at the University of Sidi Thabet-Tunisia (ISBST).
- 2013-2014: Bachelor of Experimental Sciences, Institute of Bousalem (Tunisia).

✓ Internships and practicals

- January - May 2017: End of study internship of studies. Unit of investigation of Bio ecology and Evolutionary systematic of the Faculty of Sciences, Tunisia.
- August 2016: internship in the analysis laboratory at the LA RABTA hospital _Tunis, Tunisia.
- August 2015: internship in the Biomedical and Oncogenetic Genomics laboratory. Pasteur Institute, Tunisia.

- June 2015: internship in the Microbiological Analysis laboratory at the regional hospital, Bousalem-Tunisia.
- March 2015: internship in the analysis laboratory in alimentary food industry Bousalem-Tunisia.

✓ Skills

- Computer Science:

- Use of word processing software: Microsoft Word, Microsoft PowerPoint.
- Use of spreadsheets: Microsoft Excel.
- Database: Microsoft Access.
- Make image processing: Adobe Photoshop.

Software R: software of statistics and econometrics.

✓ Languages:

- Spanish (basic notions)
- Italian (basic notions)
- English (advanced level)
- French (advanced level)
- Arabic (native language)

✓ Interests

Italian training: training at the pilot vocational secondary school in Monastir-Tunisia.

Training in English for stewards: "smart business and consulting" training center in Tunis.

Leisure: travel, training ...

✓ Professional experiences

- Seasonal work in French call centers.