Mediterranean diet, olive oil and cancer

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Olive oil is an integral ingredient of the «Mediterranean diet» and accumulating evidence suggests that it may have a potential role in lowering the risk of several types of cancers. The mechanisms by which the carcer-preventing effects of olive oil can be performed, however, are not known. We recently hypothesized that a novel molecular explanation concerning the anti-cancer actions of olive oil may relate to the ability of its monounsaturated fatty acid (MUFA) oleic acid (OA; 18:1n-9) to specifically regulate cancer-related oncogenes. Supporting our hypothesis, exogenous supplementation of cultured breast cancer cells with physiological concentrations of OA was found to suppress the overexpression of HER2 (Her-2/*neu*, *erb*B-2), a well-characterized oncogene playing a key role in the etiology, progression and response to chemotherapy and endocrine therapy in approximately 20% of breast carcinomas. OA treatment was also found to synergistically enhance the efficacy of trastuzumab, a humanized monoclonal antibody binding with high affinity to the ectodomain (ECD) of the Her2-coded p185^{HER2} oncoprotein. Moreover, OA exposure significantly diminished the proteolytic cleavage of the ECD of HER2 and, consequently, its activation status, a crucial molecular event that determines both the aggressive behavior and the response to trastuzumab of Her2-overexpressing breast carcinomas. Our most recent findings further reveal that OA exposure may suppresses HER2 at the transcriptional level by up-regulating the expression of the *Ets* protein PEA3 -a DNA-binding protein that specifically blocks HER2 promoter activity- in breast, ovarian and stomach cancer cell lines. This anti-HER2 property of OA offers a previously unrecognized molecular mechanism by which olive oil may regulate the malignant behavior of cancer cells. From a clinical perspective, it could provide an effective means of influencing the outcome of Her-2/*neu*-overexpressing human carcinomas with poor prognosis. Indeed, OA-induced transcriptional repression of HER2 oncogene may represent a novel genomic explanation linking «Mediterranean diet», olive oil and cancer as it seems to equally operate in various types of Her-2/*neu*-related carcinomas.

Key words: olive oil, oleic acid, Her2, *erb*B-2, mediterranean diet, cancer.

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INTRODUCTION

Cancer incidence, geography and nutrition

Several epidemiological studies in humans and animal experiments have implicated directly specific dietary components with a higher incidence of several human cancers. It is currently accepted that more than one third of human cancers can be related directly with a component of the diet[1,2].

European Union cancer data highlight the heterogeneous distribution of cancer among countries. The incidence of cancers of the colon and the breast (which are more sensitive to dietary changes) is lower in countries such as Italy, Greece, Portugal and Spain. In particular, in relation with the median of the European Union, Spain presents a reduction of 28% (breast cancer) and 42% (colon cancer)[3]. This might be related, among other reasons, with some of the components of the so-called Mediterranean diet[4].

Although total fat has shown an association with an increased risk to develops cancers of the breast, colon and prostate, it is now known that rather than the total amount, it is the type of fat that has a major influence on cancer incidence[5]. This is specially significant when we consider that, in absolute terms, the mean fat intake is often higher in the mediterranean countries. In southern Europe, the major fat is predominantly monounsaturate (olive oil), and has a high content in antioxidants, contrasting with the pre-

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dominance of polyunsaturate fat, essentially n-6 (seed oils), in northern European countries.

There are important differences in cancer mortality even among different Spanish populations. In the years 1978-1992, the adjusted breast cancer mortality rate per 100,000 (european standard population) was 24.39 in Girona and 15.28 in Jaén, respectively, according to data from the Centro Nacional de Epidemiología (http://www2.uca.es/hospital/atlas92/www/Mama.pdf). This significant trend can be observed very graphically in figure 1. The differences in mortality can be related to different reasons, among which are possibly differences in nutrition and lifestyle but not treatment differences, since medical and surgical procedures are very similar thoughout Spain. In relation to nutritional contrasts, it is interesting to state two nutritional studies from Catalonia and Andalusia[6,7]. In these studies, the consumption of monoinsaturate fatty acids was 49.65 g/day for andalusian population, while it was 38.6 g/day in Catalonia (20% less, approximately). Nutrition can have an important role not only in cancer risk but also in cancer progression[8].

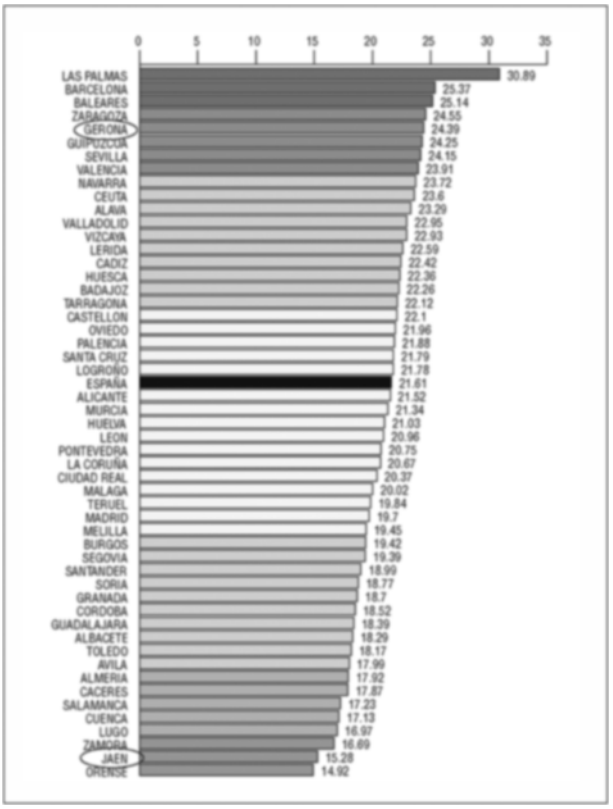

Fig. 1. Breast cancer mortality in women by province.

Nutrition in patients with cancer

A recent review of the nutrition-related issues to the breast cancer survivor[9] highlights the scarcity of research exploring diet composition and treatment endpoints such as recurrence or survival. Remarkably, none of the published studies has been conducted in Mediterranean countries. Although there exists a certain perception that a correct nutrition can ameliorate the quality of life or the outcomes of cancer patients, the role of chemotherapy or surgery have not been evaluated on oxidative stress and diet, two parameters that are highly related. Among cancer treatments, chemotherapy plays a pivotal role. Drugs such as doxorubicin, cisplatin, oxaliplatin, or paclitaxel are widely used for a wide variety of neoplasms. In general, chemotherapy agents induce a substantial oxidative stress[10].

Since many anticancer agents can produce oxidative stress in biological systems, the reactive oxygen species generation during chemotherapy might interfere with the efficacy of treatment[11]. For example, oxidative stress inhibits chemotherapy-induced apoptosis[12,13].

Obesity and cancer

Obesity is a risk factor for breast cancer in post-menopausal women, and obese women regardless of their menopausal status are particularly likely to have metastatic breast cancer when they are first diagnosed, and to have a disease with a poor outcome. Increased production of estrogens associated with obesity has been claimed to mediate part of these associations. Besides estrogens, hyperinsulinemia, as seen in obesity, has been shown to be a risk factor for breast cancer regardless of estrogen receptor status. Insulin exerts a mitogenic effect on both normal and neoplastic breast epithelial cells, and the hyperinsulinemia and the insulin resistance syndrome are likely candidates for a mechanism by which obesity affects breast cancer risk and prognosis.

A recent study has shown that overweight and underweight children and adolescents with acute myeloid leukemia were less likely to survive than patients with BMI in the 11th through 94th percentiles. Inferior survival in both extreme BMI groups was attributable to early treatment-related mortality, and treatment-related mortality was mostly from infection. This was the first study to show excess mortality in overweight pediatric cancer patients[14]. In most adult cancers, overweight patients have excess cancer-related death rather than death from excessive toxicity[15,16].

Regulation of gene expression mediated by fatty acids

Although it has been assumed that gene expression is not affected directly by nutrients, in contrast to hor-

mones, growth factors and cytokines[17], it has recently been described that lipids can regulate gene expression[18], and also the expression of oncogenes[19]. These direct effects of dietary fats on gene expression open the door to a whole new line of research. Fatty acids interact with the human genome by regulating the activity or the amount of transcription factors such as PPAR alfa, beta and gamma, or the sterol response element binding protein (SREBP)[20-22].

A recent report describes that n-3 PUFAs can improve the efficacy of treatment and ameliorate the quality of life of cancer patients[23]. We have reported several *in vitro* studies in which the efficacy of chemotherapy can be synergistically improved experimentally by adding fatty acids[24-27]. Oleic acid can decrease the expression of c-fos and cox-2[28], PGE^{2}[29], can enhance apoptosis[30] and increase phosfatidylinositol 3 kinase in cancer cell lines. Recently published experiments from our laboratory show that oleic acid reduces the levels of the HER2 oncogene and increases the anticancer effect of the anti-HER2 monoclonal antibody trastuzumab[18].

Olive oil, oleic acid and cancer

The relationship between the olive oil intake and cancer risk has become an issue for human health. Different studies have shown that the consumption of olive oil may have a potential role in lowering the risk of malignant neoplasms, especially breast cancer (stomach, ovary, colon and endometrium cancer too)[31-40]. However, the mechanisms by which the effects of olive oil can be performed are not well understood. One of the remaining concerns, before going for a causal interpretation of the inverse relation between olive oil intake and cancer risk, is to definitely establish whether the olive oil-related anti-cancer effects can be explained through either the monounsaturated fatty acid (MUFA) content (i.e., high levels of the ω-9 MUFA oleic acid) or the antioxidant components of the unsaponifiable fraction[41].

Since cancer development and progression is believed to be a multi-step process involving the expression of several oncogenes, we recently hypothesized that a novel molecular explanation concerning the anti-cancer actions of olive oil may relate to the ability of oleic acid (OA; 18:1n-9) to specifically regulate cancer-related oncogenes such as HER2[18,42].

HER2(*erb*B-2) oncogene and cancer

At present, the HER2 oncogene (also called *neu* and *erb*B-2) represents one of the most commonly analyzed oncogenes in breast cancer studies. HER2 codes for a transmembrane tyrosine kinase orphan receptor $p185^{HER2}$ that regulates biological functions as diverse as cellular proliferation, transformation, differentiation, motility and apoptosis[44-46]. Therefore, modulation of HER2 levels must be tightly regulated for normal cellular function. Accordingly, *in vitro* and animal studies clearly demonstrate that deregulated HER2 overexpression plays a pivotal role in malignant transformation, tumorigenesis and metastasis. For instance, HER2 gene amplification and overexpression occurs in ~ 30% of breast carcinomas and is associated with unfavorable clinical outcome and resistance to some chemo- and endocrine-therapies[47-55].

On the other hand, HER2 also represents a successful therapeutic target of the biotechnology era as exemplified by the drug trastuzumab (Herceptin™)[56]. Trastuzumab is a humanized monoclonal antibody binding with high affinity to the ECD of $p185^{HER2}$ that has clinical activity in a subset of Her-2/*neu*-overexpressing breast cancer patients, thus confirming the active role of HER2 in the aggressive behavior of breast cancer disease[57-59]. However, the benefits of trastuzumab are modest and usually do not represent a cure. Moreover, not all Her-2/*neu*-overexpressing cancer tumors respond to treatment with trastuzumab and its clinical benefit is limited by the fact that resistance develops rapidly in virtually all treated patients[60-62]. Unfortunately, there are no data concerning strategies able to sensitize breast cancer cells to the growth-inhibitory activity of trastuzumab.

We recently observed, to the best of our knowledge for the first time, that OA, the main MUFA of olive oil, specifically suppresses HER2 overexpression, which, in turn, interacts synergistically with anti-HER2 immunotherapy by promoting cell death of cancer cells with amplification of the HER2 gene[18]. We now summarize our observations.

EXOGENOUS SUPPLEMENTATION WITH OA DOWN-REGULATES HER2 ONCOGENE EXPRESSION

To assess the effects of OA on HER2 expression we used BT-474 and SK-Br3 human breast cancer cells, two *in vitro* models that naturally exhibit HER2 gene amplification and are Her-2/*neu*-dependent[63]. Using flow cytometry, immunoblotting and immunofluorescence microscopy techniques we found a significant decrease of $p185^{HER2}$ expression levels (36% to 46% reduction when compared to untreated control cells) following a 48 h incubation period with physiological concentrations (10 μM) of exogenously supplemented OA (fig. 2A). Remarkably, this down-regulatory effect was comparable to that found following exposure to optimal concentrations of the anti-HER2 antibody trastuzumab (up to 48% reduction at 20 μg/ml trastuzumab; fig. 2A). Moreover, the concurrent combination of OA and trastuzumab reduced $p185^{HER2}$ expression more than when either agent was administered

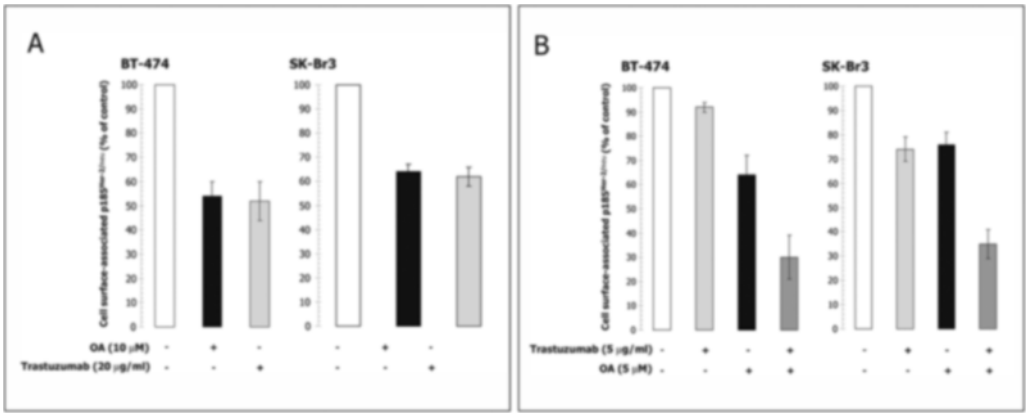

Fig. 2. Effects of exogenous supplementation with OA on p185$^{Her2/neu}$ concentrations in human breast cancer cells bearing Her-2/*neu* gene amplification. A) To assess the effects of OA treatment on Her-2/*neu* expression, BT-474 (*left panel*) and SK-Br3 (*right panel*) breast cancer cells, following a 24 h starvation period in media without serum, were incubated for 48 h with 0.5% BSA FA-free BSA (controls = 100%) or 10 μM OA complexed to BSA in low-serum (0.1% FBS) conditions. The cell surface-associated expression of Her-2/*neu*-coded p185$^{Her-2/neu}$ oncoprotein was then determined by measuring the binding of a mouse monoclonal antibody directed against p185$^{Her-2/neu}$ using flow cytometry techniques as described elsewhere[13]. B) To assess whether OA co-treatment may enhance the well-known down-regulatory actions of trastuzumab on Her-2/*neu* expression[42,43], cell surface-associated p185$^{Her-2/neu}$ was measured by flow cytometry following treatment of BT-474 (*left panel*) and SK-Br3 (*right panel*) cells with sub-optimal doses of trastuzumab (5 μg/ml) and/or OA (5 μM).

alone (fig. 2B). These findings clearly demonstrate that OA, similarly to trastuzumab, selectively down-regulates p185^{HER2} overexpression in breast cancer cells harboring amplification of the HER2 oncogene.

EXOGENOUS SUPPLEMENTATION WITH OA REGULATES THE ACTIVATION STATUS OF P185$^{HER-2/neu}$

The activation status of Her-2/*neu*, and not just its overexpression, is a crucial event that determines both the aggressive biological behavior of breast carcinomas and their response to chemotherapy, anti-estrogens and the anti-HER2 antibody trastuzumab[64,65]. HER2 activation seems to occur as a consequence of proteolytic cleavage of its extracellular domain (ECD), thereby resulting in the production of truncated membrane-bound fragment with kinase activity, a key event for down-stream activation of the proliferative and anti-apoptotic-transduction cascades Ras → Raf → MEK1/2 → MAPK ERK1/2 and PI-3'K → AKT, respectively. In this regard, exogenous supplementation with OA was found to significantly enhance the ability of trastuzumab to inhibit Her-2/*neu*-dependent hyperactivation of MAPK ERK1/2 and AKT[18]. Moreover, when we assessed the effects of OA in HER2 ECD concentration, exposure of BT-474 cells to 10 μM OA reduced HER2 ECD levels by 51%, while OA-treated SK-Br3 cells demonstrated a less pronounced, but significant 38% reduction relative to untreated controls[42] (fig. 3).

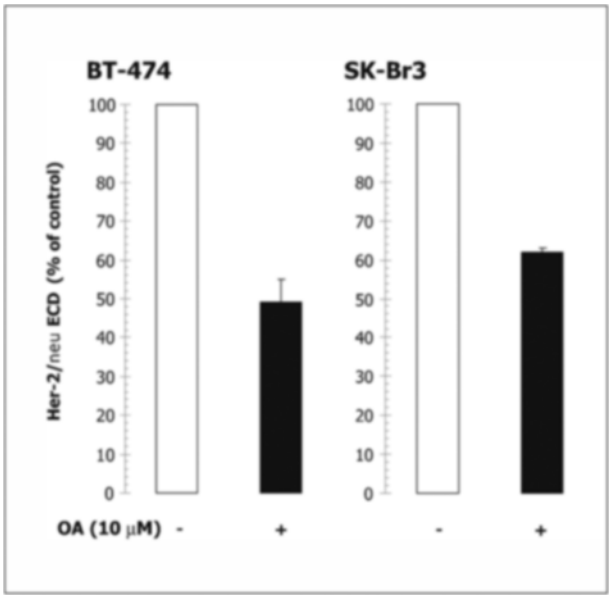

Fig. 3. Effects of exogenous supplementation with OA on the activation status (ECD concentrations) of p185$^{Her-2/neu}$ in human breast cancer cells bearing Her-2/*neu* gene amplification. To assess the effects of OA on Her-2/*neu* ECD concentration, BT-474 (left panel) and SK-Br3 (*right panel*) breast cancer cells, following a 24 h starvation period in media without serum, were incubated for 48 h with 0.5% BSA FA-free BSA (controls = 100%) or 10 μM OA complexed to BSA in low-serum (0.1% FBS) conditions. For determination of Her-2/*neu* ECD levels, a human Her-2/*neu* quantitative ELISA (Her-2/*neu* Microtiter ELISA; Oncogene Science) was used according to the manufacturer's instructions.

EXOGENOUS SUPPLEMENTATION WITH OA SYNERGISTICALLY ENHANCES THE GROWTH-INHIBITORY ACTIVITY OF THE ANTI-HER2 ANTIBODY TRASTUZUMAB (HERCEPTIN™)

In the light of the above results, we hypothesized that exogenous supplementation with OA may enhance the efficacy of trastuzumab towards Her-2/*neu*-overexpressing breast cancer cells. As expected, the increase in trastuzumab-induced inhibition of cell proliferation with the concomitant addition of OA over that of trastuzumab itself was statistically significant. The most significant changes were seen in BT-474 cells, in which co-exposure to 10 μM OA increased by 40 times the cytotoxic activity of trastuzumab[18]. For SK-Br3 cells, OA co-treatment enhanced by 27 times the efficacy of trastuzumab[18]. Remarkably, Her-2/*neu*-induced anchorage-independent growth in soft-gar, which is considered to be an *in vitro* property closely associated with the ability of colonization of metastatic tumor cells at a distant site, was completely abolished following co-exposure to OA and trastuzumab[18]. When we finally assessed if the synergistic interaction between OA and trastuzumab represented cell death, there was an impressive increase in apoptosis when Her-2/*neu*-overexpressing breast cancer cells were treated simultaneously with both agents[18].

IMPLICATIONS

The strongest evidence that MUFAs such as OA may influence breast cancer risk comes from studies of southern European populations, in whom intake of OA sources, particularly olive oil, appears to be protective. However, little is known about the ultimate biochemical and cellular pathways through which OA may modulate breast cancer development and/or progression. Our recent findings demonstrating that OA can repress HER2 oncogene overexpression represent a previously unrecognized mechanism through which the main MUFA of olive oil may regulate both the etiology and the aggressive behavior of breast

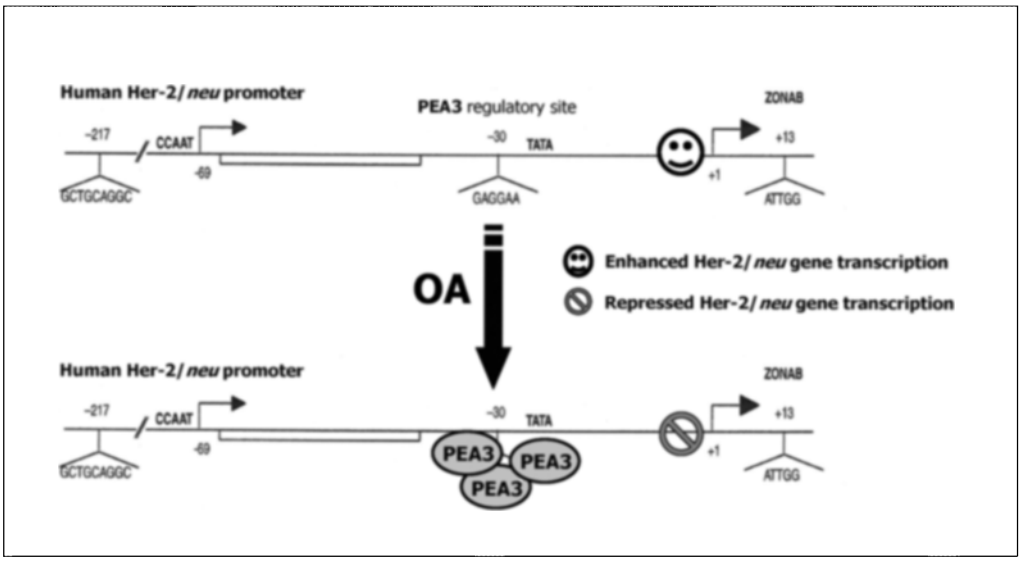

Fig. 4. Working model of OA-regulated Her-2/*neu* gene expression *via* up-regulation of the Her-2/*neu* transcriptional repressor PEA3. Although overexpression of Her-2/*neu* both in tumors and in derived cell lines was originally attributed solely to amplification of the *erb*B-2 gene (usually 2- to 10-fold), an elevation of Her-2/*neu* mRNA levels per gene copy is also observed in all the cell lines examined exhibiting gene amplification[44]. Indeed, an increase in transcription rate sufficient to account for the degree of overexpression has been shown in a number of Her-2/*neu*-overexpressing breast cancer cell lines. Recent experiments in our laboratory established the ability of OA to specifically repress the transcriptional activity of the human Her-2/*neu* gene promoter in tumor-derived cell lines naturally exhibiting Her-2/*neu* gene amplification and overexpression (SK-Br3 breast, SK-OV3 ovarian and NCI-N87 stomach cancer cells) but not in cancer cells expressing physiological levels of Her-2/*neu* (data not shown). OA treatment was also found to induce the up-regulation of the *Ets* protein PEA3 (a transcriptional repressor of Her-2/*neu* promoter), solely in Her-2/*neu*-overexpressing cancer cells, whereas a Her-2/*neu* promoter bearing a PEA3 site-mutated sequence was not subjected to negative regulation by OA (data not shown). These findings, altogether, strongly suggest that OA-induced formation of inhibitory «PEA3 protein-PEA3 DNA binding site» complexes at the human Her-2/*neu* promoter may represent a novel genomic explanation linking «Mediterranean diet», olive oil and cancer as it seems to equally operate in various types of Her-2/*neu*-related human cancer cells including breast, ovarian and stomach.

cancer. We found that exogenous supplementation with physiological concentrations of OA significantly down-regulates the expression levels of Her-2/*neu*-coded $p185^{HER2}$ oncoprotein in BT-474 and SK-Br3 cells, two human breast cancer models naturally bearing amplification of the HER2 oncogene. Since no toxicities have been reported or suspected with OA, it is reasonable to suggest that supplementation with OA may represent a promising dietary intervention for the prevention and/or management of Her-2/*neu*-overexpressing breast carcinomas. Moreover, our current findings suggest further that dietary interventions based on OA may be even more beneficial when given in combination with novel therapies directed against HER2 such as the monoclonal antibody trastuzumab (Herceptin™).

The findings that we have shown have generated an intense public interest as they could be helpful in the design of future epidemiological studies and, eventually, dietary counseling to delay or prevent trastuzumab resistance in Her-2/*neu*-positive breast cancer patients[66,67]. Two main issues, however, remain to be addressed: which is the ultimate molecular mechanism linking tumor cells' response to OA and HER2 oncogene expression, and whether the ability of OA to down-regulate HER2 is a common mechanism of OA's action towards other types of cancer or it is restricted to breast cancer. The specific mechanism through which OA molecularly modulates HER2 expression certainly merits further investigation. Preliminary results in our laboratory strongly suggest that OA exposure significantly reduces the transcriptional activity of HER2 gene by up-regulating the expression of the *Ets* protein Polyomavirus Enhancer Activator 3 (PEA3) –a DNA-binding protein that has been shown to inhibit Her-2/*neu*-promoted tumorigenesis by blocking HER2 promoter activity[68,69]– in SK-Br3, SK-OV3 and NCI-N87 breast, ovarian and stomach cancer cell lines, respectively (data not shown; fig. 4).

CONCLUSION

The previously unrecognized anti-HER2 properties of OA will help us to understand the ultimate molecular mechanisms by which individual FAs such as OA may regulate the malignant behavior of cancer cells. Moreover, they may be helpful in the design of future epidemiological studies and, eventually, dietary counseling to manage Her-2/*neu*-overexpressing carcinomas. Although caution must be applied when extrapolating *in vitro* results into clinical practice, our current findings also reveal a valuable approach to delay or prevent trastuzumab resistance in Her-2/*neu*-positive cancer because: a) Exogenous supplementation with OA diminishes the proteolytic cleavage of the HER2 ECD, a crucial event determining cancer cell response to trastuzumab[42, 65], and b) OA appears to mitigate HER2 overexpression *via* PEA3 binding to the HER2 promoter (fig. 4), a mechanism of action that should not be affected by the mechanisms of resistance recently described for trastuzumab-based anti-HER2 immunotherapy[61,70]. Nevertheless, it is reasonable to suggest that OA-induced transcriptional repression of HER2 oncogene may represent a novel genomic explanation linking Mediterranean diet, olive oil and cancer as it seems to equally operate in various types of Her-2/*neu*-related human cancer cells including breast, ovarian and stomach.

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