Dietary fatty acids regulate the activation status of Her-2/neu (c-erb B-2) oncogene in breast cancer cells

Research from several sources provides strong evidence that dietary or exogenously derived fatty acids (FAs) may play an important role in the etiology, evolution and/or progression of breast cancer [1]. However, the type of individual FAs in a diet, rather than the amount of total dietary fat, may be more important in breast cancer disease. In this regard, epidemiological, experimental and mechanistic data implicate ω-9 oleic acid (OA; 18:1n-9), the main FA of olive oil, α-linolenic acid (ALA; 18:3n-3), the ω-3 FA of vegetable oils, and eicosapentaenoic (EPA; 22:5n-3) and docosahexaenoic (DHA; 22:6n-3) FAs, the two main ω-3 FAs of fish oils, as inhibitors of the development and progression of human breast cancer, and ω-6 FAs such as linoleic acid (LA; 18:2n-6), the main FA of corn and sunflower oils, as stimulators of the disease. Moreover, one of the most interesting yet controversial dietary approaches has been the possible role of dietary FAs in treating breast cancer. However, little is know about the ultimate mechanisms underlying FA-regulated breast cancer risk and progression. Interestingly, we previously reported that exogenous supplementation with FAs such as γ-linolenic acid (GLA; 18:3n-6), an essential FA found in the plant-seed oils of evening primrose, blackcurrant and borage oils, synergically sensitizes cultured breast cancer cells to anti-mitotic drugs such as paclitaxel (Taxol™) [2], vinorelbine (Navelbine™) [3] and docetaxel (Taxotere™) [4]. Moreover, this GLA-induced sensitization was also synergic with the anti-estrogens tamoxifen and ICI 182,780 (Faslodex™) [5]. Since the human Her-2/neu (c-erb-B-2) oncogene and its p185Her-2/neu orphan receptor oncoprotein, which are overexpressed in 25–30% of invasive breast cancers, have been associated with cytotoxic and endocrine therapy resistance [6], we recently envisioned that FA-induced breast cancer chemosensitization may involve regulation of Her-2/neu-dependent signaling.

In the last few years it has become clearer that 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 the breast cancer response to chemotherapy, anti-estrogens and the anti-Her-2/neu antibody trastuzumab (Herceptin™) [7, 8]. Although the mechanism of activation of the Her-2/neu oncoprotein is not completely understood, in vitro and in vivo Her-2/neu activation has been demonstrated 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 downstream signaling. In this regard, we recently reported that Her-2/neu ECD expression correlates with a diminished efficacy of the combination chemotherapy of biweekly gemcitabine plus paclitaxel [9]. This study validated our previous results, showing that both the probability of obtaining a complete response and the duration of clinical response to a paclitaxel–dorxorubicin chemotherapy regimen were significantly lower and shorter, respectively, in metastatic breast cancer patients with increased Her-2/neu ECD concentrations compared with the cases with non-increased concentrations [10].

The present investigation was performed to evaluate the effects of exogenous supplementation with dietary FAs on Her-2/neu ECD concentrations in cultured breast cancer cells. First, the Oncogene Science Her-2/neu Microtiter ELISA (Cambridge, MA, USA) was used to compare the baseline expression of Her-2/neu ECD in BT-474, SK-Br3, MCF-7 and MDA-MB-231 human breast cancer cell lines. This sandwich-type enzyme immunoassay utilizes two monoclonal antibodies, NB-3 and TA-1, that recognize the extracellular, ligand-binding domain of the Her-2/neu oncoprotein, and quantifies the Her-2/neu ECD in cell cultures. As expected, very high levels of Her-2/neu ECD [expressed as human Neu units (HNU) per μg of protein] were found in BT-474 and SK-Br3 breast cancer cells (105±12 and 95±11 HNU per μg protein, respectively), whereas MCF-7 (1.6 HNU/μg protein) and MDA-MB-231 (0.5 HNU/μg protein) cells contained physiological and low levels of Her-2/neu ECD, respectively. Thus, Her-2/neu ECD levels in Her-2/neu-overexpressing SK-Br3 and BT-474 breast cancer cells were ~200-fold higher than in Her-2/neu-negative MDA-MB-231 cells.

To assess the effects of FAs on Her-2/neu ECD concentration, SK-Br3 and BT-474 cells, after a 24 h starvation period in media without serum but with 0.5% FA-free bovine serum albumin (BSA), were incubated for 48 h with 0.5% FA-containing FAs (Table 1). Her-2/neu ECD levels in SK-Br3 cells were slightly decreased by 24% at 10 μM ALA, while Her-2/neu ECD expression dramatically decreased by 53% with 10 μM ALA in BT-474 cells. Culture of SK-Br3 cells with EPA significantly decreased Her-2/neu ECD expression by 47% at 10 μM EPA. A less marked, but significant, decrease in Her-2/neu ECD expression was observed in BT-474 cultured in the presence of 10 μM EPA (28% reduction). SK-Br3 cells treated with 10 μM DHA showed a 38% decrease in Her-2/neu ECD expression.
**Table 1.** Effects of exogenous supplementation with dietary FAs on Her-2/neu ECD concentrations in human breast cancer cells bearing Her-2/neu oncogene amplification

<table>
<thead>
<tr>
<th>Cell line treatment</th>
<th>ω-3 FAs</th>
<th>ω-6 FAs</th>
<th>ω-9 FAs</th>
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<tbody>
<tr>
<td>SK-Br3</td>
<td>ALA</td>
<td>EPA</td>
<td>DHA</td>
</tr>
<tr>
<td>95±11</td>
<td>72±4**</td>
<td>50±4*</td>
<td>59±10**</td>
</tr>
<tr>
<td>BT-474</td>
<td>105±12</td>
<td>49±11**</td>
<td>75±9*</td>
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<tr>
<td>49±13**</td>
<td>25±3**</td>
<td>54±10**</td>
<td></td>
</tr>
<tr>
<td>OA</td>
<td>51±6**</td>
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</tbody>
</table>

SK-Br3 and BT-474 cells were incubated in serial dilution of FAs for 48 h. Shown are Her-2/neu ECD concentrations using a 10 μM dose of FAs. The amount of Her-2/neu ECD is given in arbitrary human Neu units (HNU) per μg total protein. Values are means±SD of three independent experiments made in duplicate. Statistically significant differences in Her-2/neu ECD concentrations when compared with untreated cells are shown by asterisks (*P<0.05; **P<0.005; Student’s t-test). For quantitative determination of Her-2/neu ECD expression, a human Her-2/neu quantitative ELISA (Her-2/neu Microtiter ELISA; Oncogene Science, Cambridge, MA, USA) was used according to the manufacturer’s instructions. This immunoassay for detection and quantitation of Her-2/neu ECD is a sandwich assay utilizing a mouse monoclonal antibody (capture antibody coated onto microtiter wells) and a rabbit polyclonal serum (detector antibody). The amount of detector antibody bound to antigen was detected using a goat anti-rabbit IgG/horseradish peroxidase conjugate. After the addition of the chromogenic substrate tetramethylbenzidine, the color change was measured at 492 nm using a microplate reader. A standard curve was generated using standard solutions as per the manufacturer’s instructions. The concentration of Her-2/neu ECD in test samples was determined by interpolation of the sample absorbances from the standard curve.

FAs, fatty acids; ECD, extracellular domain; ALA, α-linolenic acid; EPA, eicosapentaenoic; DHA, docosahexaenoic; LA, linoleic acid; GLA, γ-linolenic acid; OA, oleic acid.

Remarkably, DHA profoundly reduced the downregulatory effect on the Her-2/neu ECD concentration in BT-474 cells, with diminutions ranging from 32% at 1.25 μM DHA to 78% at 10 μM DHA. Of the ω-6 polyunsaturated FAs tested, LA significantly increased Her-2/neu ECD concentrations by 31% and 47% in SK-Br3 and BT-474 cells, respectively. Conversely, the exposure of SK-Br3 cells to increasing concentrations of GLA significantly reduced Her-2/neu ECD levels from 17% to 50% relative to untreated controls. Similarly, GLA-treated BT-474 cells demonstrated 10–55% reductions in the basal expression of Her-2/neu ECD. Exposure of SK-Br3 cells to ω-9 OA at 10 μM decreased Her-2/neu ECD levels by 38% relative to untreated controls, while 10 μM OA reduced Her-2/neu ECD concentration by 51% in BT-474 cells.

Although albumin is an effective scavenger of reactive oxygen species, FAs bound to albumin can undergo auto-oxidation to yield reactive lipid peroxides and free radicals. Thus, it could be argued that FA-induced modulation of Her-2/neu is not specific, and is probably due to cell toxicity caused by lipid peroxidation. However, the strong inhibitory effects of FAs such as GLA, ALA, EPA and DHA on Her-2/neu ECD accumulation were not significantly prevented by vitamin E, a potent inhibitor of lipid peroxidation (data not shown).

Finally, to confirm further that FA-induced regulation of Her-2/neu ECD is indeed associated with changes in active Her-2/neu tyrosine kinase signaling we evaluated the phosphorylation status of the 1248 tyrosine residue (Tyr1248), which constitutes the main autophosphorylation site of Her-2/neu, in FA-treated breast cancer cells. When western blotting analyses were performed in ALA-, EPA-, DHA-, GLA- and OA-treated SK-Br3 and BT-474 cells using a monoclonal c-erb B-2/Her-2/neu (phospho-specific) Ab-18 (clone PN2A; NeoMarkers, Fremont, CA, USA), which specifically recognizes the activated, tyrosine phosphorylated (p-Tyr1248) form of Her-2/neu, decreased tyrosine kinase activities (smaller PN2A bands) were observed (data not shown).

This report shows, to the best of our knowledge for the first time, that dietary FAs previously characterized for either their breast cancer protective effect (ALA, EPA, DHA and OA) or its tumoricidal actions (GLA) significantly downregulate Her-2/neu ECD concentration and, consequently, the activation status of Her-2/neu in SK-Br3 and BT-474 human breast cancer cell lines, which contain Her-2/neu oncogene amplification. Remarkably, LA, a ω-6 FA with a strong tumorigenesis stimulating effect, significantly increased Her-2/neu ECD concentration. Our current results using human breast cancer cell lines are in concordance with our previous findings demonstrating that dietary lipids influence DMBA-induced experimental mammary tumorigenesis in female rats through modulation of Her-2/neu expression [11]. Although much remains to be learned about the ultimate molecular mechanisms of FAs in relation to Her-2/neu, the recent characterization of a molecular link between Her-2/neu and the proinflammatory prostaglandin biosynthesis catalyzed by the enzyme cyclooxygenase-2 (COX-2) suggest an original working model in which dietary FAs would regulate either the expression and/or the activation status of Her-2/neu oncogene via COX-2 [12–15]. Nonetheless, it is reasonable to suggest that some types of dietary FAs not only represent promising therapies for prevention and/or management of Her-2/neu-overexpressing breast carcinomas, but also may be even more beneficial when given in combination with novel therapies directed against Her-2/neu. We are currently investigating whether these findings will be helpful in the design of novel approaches to delay or prevent trastuzumab (Herceptin™) resistance.

**Acknowledgements**

This study was supported by Grants from the Fondo de Investigación Sanitaria (FIS 96/0226 and FIS 00/0271), and the Sociedad Española de Oncologia Medica (SEOM).

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Expression of Cox-2 protein in radioresistant laryngeal cancer

Nix et al. [1] concluded that their study demonstrates that Cox-2 expression may have prognostic value in predicting response to radiotherapy in T1 or T2 laryngeal cancer. However, in examining results of the article (May 5 issue), we find that there was no Cox-2 overexpression in 33% of the patients with radioresistant tumours, as compared with 59% of the patients with radiosensitive tumours [1], and we have a comment on the study.

Many patients experience throat pain during radiotherapy-induced mucositis. Is there any use of Cox-1 or -2 inhibitors for analgesic means during radiotherapy in either group, especially in Cox-2-positive patients? On the contrary, Ranelletti et al. [2] demonstrated that Cox-2 was overexpressed in less aggressive, low-grade laryngeal cancers, whereas its expression was lost when tumours progressed to a more malignant phenotype. It is somewhat difficult to say that radiosensitivity only correlated with Cox-2 expression in tumour tissue in these patients. Also, the relationships between Cox-2 expression and other molecular targets, including the epidermal growth factor receptor (EGFR) pathway, and angiogenesis should be studied in well-designed preclinical and clinical studies in head and neck cancers.

References


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Cox-2 inhibitors are not routinely used for analgesia in head and neck cancer patients treated with radiotherapy. In vitro and in vivo experiments suggest that Cox-2 inhibitors may improve radiation response in Cox-2-positive tumours [1], and this would be an exciting area for head and neck cancer research. We did not find a correlation between histological grade (well/moderate/poor differentiation) and Cox-2