Mini-review

n-3 polyunsaturated fatty acids and HER2-positive breast cancer: Interest of the fat-1 transgenic mouse model over conventional dietary supplementation

Zuquan Zou a, b, 1, Célia Bidu a, b, Sandrine Bellenger a, b, Michel Narce a, b, Jérôme Bellenger a, b, *

a Université de Bourgogne, UFR Sciences de la Vie, de la Terre et de l’Environnement, 6 Boulevard Gabriel, 21000 Dijon, France
b INSERM UMR U866 Lipides Nutrition Cancer, Université de Bourgogne, 6 Boulevard Gabriel, 21000 Dijon, France

ABSTRACT

Overexpression of the tyrosine kinase receptor ErbB2/HER2/Neu, occurs in 25%–30% of invasive breast cancer (BC) with poor patient prognosis. Even if numerous studies have shown prevention of breast cancer by n-3 fatty acid intake, the experimental conditions under which n-3 fatty acids exert their protective effect have been variable from study to study, preventing unifying conclusions. Due to confounding factors, inconsistencies still remain regarding protective effects of n-3 polyunsaturated fatty acids (PUFA) on BC. When animals are fed with dietary supplementation in n-3 fatty acids (the traditional approach to modify tissue content and decrease the n-6/n-3 ratio) complex dietary interactions can occur among dietary lipids (antioxidants, vitamins...) that can modulate the activity of n-3 fatty acids. So, what are the specific roles of these n-3 PUFA in reducing breast cancer risk and particularly preventing HER2-positive breast cancer? In this review, we discuss crucial points that may account for discrepancies of results and provide a highly effective genetic approach that can eliminate confounding factors of diet for evaluating the molecular mechanisms of n-3 PUFA in HER2 signaling pathway regulation. The fat-1 transgenic mouse model is capable of converting n-6 to n-3 fatty acids leading to an increase in n-3 fatty acid content with a balanced n-6/n-3 fatty acid ratio in all tissues. The fat-1 mouse model allows well-controlled studies in HER2-positive breast cancer prevention to be performed, without the conflict of potential confounding factors of diet.

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kinase activity. HER2 amplification and overexpression have been reported in a number of human tumors, occurs in 25%–30% of invasive BCs [12] and is associated with a more aggressive phenotype and a poor patient prognosis [13] because of a high incidence of metastasis [14] and intrinsic resistance to endocrine and conventional chemotherapy [15,16]. HER3 is often expressed together with HER2 in this disease [17]. While both receptors are considered promising targets for therapy, the overemphasis on HER2 has shadowed the important role of HER3 in resistance to HER2-targeted therapies [18,19]. HER3 seems to be the preferred dimerization partner when signaling occurs through the PI3K pathway [3] and, as such, is emerging as a key target for inhibition of Erbb signaling. The HER2/HER3 dimer is crucial for HER2-mediated signaling in tumors containing amplifications of HER2 [8–10]. Moreover, HER3 is as crucial as HER2 in maintaining cell proliferation in BC cells overexpressing HER2 [20,21]. In three-dimensional culture and in xenograft BC models, loss of HER3 function results in a reduced Akt phosphorylation of 50% and a rapid tumor regression. Interfering with signaling through the HER2/HER3 dimer offers an alternative therapeutic strategy to targeting HER2 alone. Then, Anti-Erbb tyrosine kinase inhibitors might block HER3-dependent signaling through the PI3K-Akt pathway [22], requisite step in the ability of HER3 to interact with and activate downstream components of the PI3K-Akt pathway (Fig. 1). Xenograft studies with tumors generated using breast cancer cell lines or mouse embryonic fibroblasts transformed with HER2 indicated that targeting HER2 with murine monoclonal antibodies is a lasting approach for inhibiting HER2 and leading to anti-tumor activity [23,24]. The monoclonal antibody Trastuzumab binds to the extracellular membrane portion of HER2 and suppresses HER2 signaling activity, resulting in inhibition of downstream signaling pathways, cell arrest and a reduction in angiogenesis [25–27]. It has been suggested that the anti-signaling effect of trastuzumab might be mediated through the inhibition of PI3K-Akt pathway activation [28,29].

2. Breast cancer prevention by n-3 polyunsaturated fatty acids

As tumors overexpressing HER2 are generally resistant to therapeutic agents, nutrition interventions might be promising therapeutic strategies in preventing and treating this aggressive subtype of cancer, by ablating HER2/HER3 expression and/or interfering with the interaction of HER2/HER3 heterodimers. Among other factors, changes in food patterns are more often associated with an increased incidence of cancers, as illustrated in migrant studies [30] and nutritional recommendations are followed by a decreased incidence of BC [31]. The contribution to mammary carcinogenesis of the specific fatty acid (FA) composition of the diet has received considerable attention in the literature. Among the FA, n-3 and n-6 FA have been suggested to respectively decrease and increase breast cancer risk [32]. Sources of n-3 FA include cold-water fish [rich in eicosapentaenoic, EPA (20:5) and docosahexaenoic acid, DHA (22:6)], seeds (flax) and nuts, and some vegetable oils (soy bean) [rich in α-linolenic acid (ALA, 18:3)]. Sources of n-6 FA are vegetable oils (corn or safflower oils) containing linoleic acid (LA, 18:2). Epidemiological and preclinical studies suggest a protective effect of fish oils towards breast cancer [33,34]. Numerous experimental studies indicate that n-3 FA may exert an antitumor action by altering the cell-membrane phospholipid composition and, consequently, affecting the expression and function of numerous receptors, proteins, and lipid-derived signaling molecules. In addition, in vivo studies demonstrate that n-3 fatty acids or their metabolites are able to reduce cellular proliferation and increase apoptosis in BC models [35]. Several of them show that animals treated with n-3 or its metabolites exhibit mammary tumor prevention [36–38], with increments of reactive oxygen species formation [38] and lipid peroxidation [39]. Akt is able to protect from apoptosis by activating antiapoptotic genes [40] and directly promoting cell survival by phosphorylating and inactivating components of the apoptotic machinery. Akt also can activate transcription factors such as NF-κB. In line of this, in vivo studies show an increased apoptotic index of MCF-7 cells injected into flaxseed oil-fed nude mice. This effect was probably due to the downregulation of tyrosine kinase receptors such as EGFR and HER2, and the subsequent downregulation of Akt [41]. It has also been reported that n-3 FA suppressed HER2/neu signaling pathways involved in the pathogenesis of BC [42,43]. Moreover, a very recent report showed that mice expressing MMTV-neu(ndl)-YD5 (Mouse Mammary Tumor Virus) an aggressive HER2-positive BC model, and endogenously synthesizing n-3 PUFA from n-6 can mitigate tumor development [44]. Nevertheless, when the lifelong tumor development has been investigated in this mammary tumorigenesis model over-expressing HER2, mechanisms underlying such anti-cancer role of n-3 PUFA have not been elucidated. Then, the relevance of HER2 pathway involvement remains to be explored, as inhibitory dietary effects of eicosapentaenoic and docosahexaenoic acids — the two main n-3 polyunsaturated fatty acids (PUFAs) found in fish oils — have been reported on HER family members [45,46].

3. Conflicting data and discrepancies regarding n-3-related studies in breast cancer

Despite research providing evidence that dietary or exogenously derived fatty acids may play a beneficial role in the etiology, evolution and/or progression of BC, many inconsistencies and discrepancies preclude definitive conclusions [47]. So, epidemiologic
studies have been inconclusive relative to the protective effects of n-3 FA against breast cancer development [33]. In regards to these observations, no recommendation can be proposed to women about n-3 FA ingestion for reduction of BC risk. Preclinical studies tend to support a protective effect of n-3 fatty acids in breast cancer prevention [39,43], but other studies showed no protection with increasing n-3 fatty acid intake [48,49], and some reported promotion of mammary carcinogenesis by fish oil [39,50–52]. Moreover, Holmes MD et al. [53] found an increased risk of BC associated with higher dietary marine n-3 polyunsaturated fatty acids (PUFA) in a cohort study with 88,795 women.

Most of the discrepancies described above (no protection, protection or promotion of BC by n-3 FA) may be explained by the fact that the experimental conditions (source of fatty acids, doses, duration of diet…) under which n-3 fatty acids exert their protective effect have been variable from study to study, preventing regular conclusions. Although feeding animals with dietary supplementation in n-3 PUFA is a traditional and safe approach to modify tissue nutrient composition and to establish an n-6/n-3 ratio close to 2–5:1, it has a number of limitations.

One of the first limitations is the variability in n-3 fatty acid content of the diet which is likely to have introduced significant experimental variability among studies, because n-3 FA levels differ among fish species as well as within same species, owing to their developmental age, the season, and their diet at time of harvest, thus making it difficult to compare them to another [54]. Such variations may be biologically important if the beneficial effect of fish oil is restricted to a narrow n-6/n-3 ratio or to specific FA. Therefore, it appears that DHA is more active than EPA in inhibiting mammary tumorigenesis by interfering with prostaglandin metabolism [55,56].

Feeding animals fish oil supplement by gavage instead of mixing it in the diet [57,58] could modify the biological efficiency of the n-3 fatty acids.

Feeding animals with different diets that consist of many components with different materials can bring in many variations between experimental groups. So, these variations act as confounding factors and may contribute to inconsistent or conflicting results. For example, fish oils and plant seed or vegetable oils are generally used to provide n-3 and n-6 FA, respectively. These FA are derived from different sources and probably contain other bioactive compounds that may interact and therefore add further complexity to their evaluation. Nevertheless, factors such as oil origin, preparation and storage of the diets, feeding procedures are not often discussed in n-3-related studies. Thus, the use of a mouse genetic model would allow to modify tissue essential fatty acid composition and particularly balanced the n-6/n-3 ratio under well-controlled conditions and be essential for identifying the specific roles of n-3 PUFA and addressing nutrient–gene interactions.

4. The fat-1 transgenic mouse model

Although mammals contain the enzymatic activity to convert LA (18:2n-6) and ALA (18:3n-3) to the longer-chain PUFA (where the rate of conversion is limiting), they lack the 12- and 15-desaturase activities necessary to synthesize the precursor (parent) PUFA, LA, and ALA [60,61]: so these last must be present in our diet. Furthermore, the n-3 and n-6 PUFA are not interconvertible in mammalian cells [62]. Thus, LA, ALA and their elongation and desaturation products are considered essential fatty acids in mammalian and particularly in humans [63]. In 1997, a fat-1 gene encoding a n-3 fatty acid desaturase has been cloned from the roundworm Caenorhabditis elegans [64]. Interestingly, this enzyme, when expressed in the plant Arabidopsis, can catalyze the conversion of n-6 PUFA to n-3 PUFA by introducing a n-3 double bond into their hydrocarbon chains. A study has demonstrated clearly that the fat-1 gene can be expressed functionally in mammalian cells, and its expression could confer cells capability of converting n-6 PUFA to corresponding n-3 PUFA, leading to a balanced n-6/n-3 ratio and a change in eicosanoid production [65]. Thus, this fat-1 gene has been used to create a transgenic mouse model [66] – the fat-1 mouse – able of converting n-6 to n-3 fatty acids (Fig. 2). By the use of a diet rich in n-6 PUFA and containing very little n-3 PUFA (similar to the western diet), fat-1 mice exhibit a tissue lipid profile very different from that of the wild-type mice: tissues of the wild-type mice are essentially constituted by n-6 PUFA (especially LA and AA) and have little n-3 PUFA in their tissues as the animals cannot naturally produce n-3 from n-6 FA, these n-3 FA resulting exclusively from food supply. On the other hand, tissues of the fat-1 mice are rich in n-3 PUFA (especially ALA, EPA and DHA derived from n-6 fatty acids). The n-6 PUFA tissue levels of these mice are strongly reduced compared to those observed in the wild type mice [67]. The fat-1 mice n-6 PUFA are massively converted into n-3 PUFA changing their n-6/n-3 ratio from 20 to 50:1 to approximately 1:1. This n-3-rich profile with drastic decrease of the n-6/n-3 ratio can be observed in all the organs and tissues such as liver, heart, kidneys, brain, muscles, and lungs [64] without changing the mass of tissue fatty acids [67]. These data clearly show that, contrary to the wild-type animals, the transgenic mice expressing the fat-1 gene are able to produce n-3 FA from n-6 FA leading to a n-3 tissue enrichment without the need of dietary n-3 supply, source of confounding factors.

Thuc, the use of fat-1 transgenic mice is such a desirable animal model that can quickly and effectively evidence therapeutic and disease-preventive effects of n-3 fatty acids in breast cancer, without the need of ingestion of supplements or change in dietary habits. In comparison with conventional dietary intervention, this approach is more effective in balancing the n-6 to n-3 ratio not only because it does elevate tissue concentrations of n-3 PUFA, but also because it does decrease the levels of excessive endogenous n-6 PUFA [66] that is ideal for identifying the specific roles of n-3 PUFA and addressing nutrient–gene interactions. Then, this mouse model would represent a useful in vivo system for giving new insights of the role of n-6/n-3 fatty acid ratio in breast cancer tumorigenesis. We very recently examined the impact of enhanced n-3 PUFA production, by inducing xenografts in the fat-1

To achieve a significant increase of n-3 PUFA in tissue concentration in vivo requires a chronic intake of high doses of n-3 PUFA for a period of several weeks. It is well recognized that the polyunsaturated fatty acids are highly unstable and susceptible to oxidation. Therefore many variables arising from the diet and feeding procedure can potentially impose confounding factors. Moreover, dietary fatty acid bioavailability to cells involves a series of physiological processes including fatty acid digestion, absorption, transport, and metabolism which are altered in cancer. It looks clear that diets and particularly n-3 FA supplementations bring many other components that may interfere and also add further complexity to their evaluation.
transgenic mice, toward the development of breast cancer and observed that the tumors totally disappeared by day 15 in fat-1 mice when they continued to grow up in the wild-type. Our results indicate that modulation of breast cancer development by n-3 fatty acids seems to be mediated in part through HER2 signaling pathway downregulation and formation of significant levels of n-3 PUFAs derived bioactive mediators in the tumor of fat-1 mice compared to wild-type (unpublished results). Altogether, these data provide encouraging preclinical evidence and molecular mechanisms by which n-3 PUFAs may regulate the malignant behavior of BC cells.

5. Conclusion

The use of the fat-1 transgenic mice in the study of n-3 fatty acids on breast cancer risk can eliminate the need of feeding animals a diet supplemented with n-3 fatty acids and thereby avoid the potential confounding factors (as type and bioavailability of n-3 fatty acids, n-6/n-3 fatty acid ratio, timing of diet) derived from dietary supplementation. Thus, this mouse model can provide a well-controlled experimental condition for addressing the roles of n-3 fatty acids in HER2 signaling pathway regulation, and would be an important addition to the conventional methods used in this field.

In combination with standard treatments, supplementing the diet with n-3 fatty acids may be a nontoxic means to synergistically improve cancer treatment outcomes for breast cancer in which HER2 is overexpressed and may slow or prevent recurrence of cancer. Moreover, used alone, a n-3-supplement may be a useful dietary alternative for patients who are not candidates for standard toxic cancer therapies.

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References


