

## Effect of Docosahexaenoic Acid (DHA) on Breast Cancer Cells

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### DHA의 유방암 세포에 영향

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Today, many materials as drug are developed having various prominent function in order to treatment of disease or cancer. Among these materials, especially docosahexaenoic acid (DHA), main constituents of omega-3 fatty acid, has a lot of beneficial and natural effects, so it has been known as anticancer material especially breast cancer. Breast cancer is disease taking high occurrence level among feminine diseases. DHA has anticancer effects on breast cancer cell, representatively inducing apoptosis, inhibiting proliferation or metastasis. Main effect of DHA on breast cancer cell is apoptosis inducing, which has mechanism that treated DHA causes lipid peroxidation increasing reactive oxygen species (ROS) level and it activates caspase 8 and caspase 9 so activated caspase occurs apoptosis. Cell lines of breast cancer are MDA-MB-231, MCF-7, SK-BR-3, T47D and ZR75. Especially this article uses the MCF-7 cell line at experiment of anti-proliferation by DHA, the MDA-MB-231 cell line at experiment of anti-metastasis by DHA, because that cell line has specialized metastasis activity. Therefore, this paper discusses the effects of natural material DHA as drug of breast cancer.

**Key Words:** Apoptosis, breast neoplasms, docosahexaenoic acid, metastasis, proliferation

Omega-3 fatty acid is material playing a beneficial inhibition action at the progress of various postmenopausal diseases and cancer.<sup>1</sup> Omega-3 fatty acid component of docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA).<sup>2,3</sup> In terms of inhibition activity of cancer cell, DHA has more inhibiting effect than EPA.<sup>4,5</sup> According to have being reported, DHA

clinic-beneficial effects are known constantly, which plays a role of inhibiting cell growth or angiogenesis in various diseases such as breast cancer.<sup>6,7</sup> It is considered a good dietary supplement in female's health, and the well-studied anti-cancer drug in vitro and in vivo.<sup>8</sup> Today many feminine diseases have been known and treatments or drugs on these diseases are

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developed constantly. We considered among many drugs, what more natural material plays a role in cancer cell, and found that DHA has anti-cancer effect against many cancers, and has been developed constantly. So we investigate that DHA effects against breast cancer, disease taking high occurrence level among feminine diseases. At breast cancer, effects of DHA have been uncovered that it attenuates metastasis and inhibits proliferation cancer cell, and induces the apoptosis and anti-angiogenesis.<sup>4,9</sup> This review article focuses on anti-cancer effects of DHA on breast cancer cell.

### **1. Research of breast cancer**

The breast is made up of glands called lobules that can make milk and thin tubes called ducts. Breast tissue also contains fat and connective tissue, lymph nodes, and blood vessels. The most general type of breast cancer is carcinoma of ductal. Breast cancer can also begin in the cells of the lobules and in other tissues lobules to surrounding tissue. Breast cancer is the most common cancer among women worldwide.<sup>10</sup> It is also classified with variety of characteristics and marker for breast cancer patients (HER2, progesterone receptor, estrogen receptor,) and p53 mutation.<sup>11,12</sup> In recent study, targeted therapies have improved patient survival for advanced Breast Cancer. These tumors frequently relapse due to drug resistance mechanisms. Metastasis is a process characterized by local invasion, transport of tumor cells to the parenchyma of other organs, extravasation and establishment of secondary lesions.<sup>13,14</sup>

### **2. DHA and breast cancer; anti-proliferation**

Breast cancer cell has proliferation activity and anti-proliferation material is required, for example DHA. In order to confirm this, many experiment has been progressed, relationship of cell's optical density

(OD) level and DHA concentration or relationship of glycerol-3-phosphate dehydrogenase (GPDH) activity and DHA concentration.<sup>15</sup> Cell's OD level indicates proliferation of cell number, so this level is increased according to cell's proliferation progression and the higher DHA concentration, the lower OD level.<sup>15</sup> GPDH is indicator of cell proliferation and its activity causes the differentiation of cell.<sup>15</sup> DHA-treated sample caused decrease of GPDH activity and the higher concentration of DHA, the lower GPDH activity.<sup>15</sup> Using MDA-MB-231, malignant breast cancer cell, absorbance of cell and number of cell is affected DHA concentration; when the DHA concentration was higher, the cell's absorbance and number was lower.<sup>9</sup> Another breast cancer cell MCF-7, doxorubicin-resistant cell, is used for other studies, so doxorubicin, one of the anti-cancer drugs, can't inhibit this cancer cell proliferation well. But when DHA is combined, could inhibit the growth of MCF-7 cell.<sup>9</sup> Doxorubicin alone can't lower breast cancer cell proliferation but DHA improves doxorubicin's inhibit proliferation activity.<sup>9</sup> Analyzing previous all results of experiment, DHA has pathways leading to cell anti-proliferation activity and specific growth inhibitory activity.<sup>16</sup> DHA has potential effects of anti-proliferation not only other cells but also especially breast cancer cells.

### **3. DHA and breast cancer; anti-metastasis**

Breast cancer cell often metastases other organs, for example to bone or brain or lung or lymph node. Among metastasis to various organs, metastasis to bone takes 15 percent of breast cancer patients and it especially causes another disease, osteolysis. Breast cancer cell migrates to bone from breast and develops osteolysis which is form of bone predated by cancer cell results in bone fracture severe and pain. First of all, to confirm effect of DHA on bone metastasis, in H&E staining, DHA-treated cancer cell has the lowest

level of staining in bone.<sup>9</sup> Also using MDA-MB-231, one of breast cancer cells especially resistant-drugs in different organ metastasis and specialize metastasis, treated-DHA bone sample has the lowest level of luciferase expression compared to nothing-treated sample or EPA-treated sample.<sup>9</sup> In the level of MDA-MB-231 proliferation in the bone, DHA-treated cell group significantly lower proliferates in bone compared to other cell groups (nothing-treated, EPA-treated).<sup>9</sup> Breast cancer cells migrated to bone significantly and DHA or EPA attenuated migration of cells in many studies.<sup>17</sup> Because bone metastasis causes the osteolysis, according to X-ray of mice bone, injected breast cancer cell, DHA-treated mice bone has less osteolytic lesion compared nothing-treated mice bone.<sup>9</sup> Therefore, according to many experiments, DHA act as specific inhibitor against breast cancer cell metastasis to bone and it also prevents the formation of osteolytic lesions in bone.

#### **4. DHA and breast cancer; apoptosis**

Apoptosis, defined only one form of programmed cell death, is regarded as important mechanism for anticancer action and DHA also has been studied as target for inducing apoptosis.<sup>18</sup> DHA is known essential material of occurrence apoptosis in various cancer cell and to find out DHA effect on cancer cell, according to experiment using colon cell lines, HT-29 and HCT116 and SW480, when DHA treatment concentration is higher, colon cancer cell lines all have higher apoptosis level, and using various materials as drug each colon cell line, all cell lines had the highest level of apoptosis in case of DHA treated sample.<sup>19</sup> In recently many studies, it has been reported constantly that apoptosis level increases according to DHA concentration, and especially experiment of breast cancer cell apoptosis has been reported constantly, n-3 PUFA, especially DHA, has activity of inducing

apoptosis too. According to MTT assay result, using MCF-7, MDA-MB-231 and MDA-MB435, breast cancer cells, between DHA treatment concentration and cell viability has inverse proportion, the higher DHA treatment, the lower cell viability.<sup>18</sup> And to confirm apoptosis in DHA-treated cell, Annexin V / PI double staining is used. Annexin V is calcium-dependent dye attached negative charged phospholipid, when phosphatidyl serine (PS) moves to outside of cell membrane, annexin V attaches to PS selectively and fluoresces apoptosis cell. According to this, in annexin V staining, following DHA-treated in concentration or in time, its single positive cells were increased, meaning that apoptotic cell death is increased.<sup>18</sup> Also, TUNEL assay was performed and TUNEL is terminal deoxynucleotidyl transferase-mediated dUTP nick end-labeling and that assay is experimented to choice for rapid quantification and identification of the apoptotic cell. According to this experiment, the TUNEL-positive cells had increasing level following increased DHA-treatment level, so DHA predominantly induced MCF-7 breast cancer cell apoptosis.<sup>18</sup> Mechanism of apoptosis is that after treatment DHA, lipid peroxidation is caused, increasing reactive oxygen species (ROS) level and it activates caspase 8, causing activation of caspase 9 so apoptosis occurs.<sup>18</sup> Focusing this mechanism, many related-studies have been known, according to one study, caspase 8 or caspase 9 expressions cultured MCF-7 was proportional to treated DHA concentration and it induced MCF-7 apoptosis.<sup>18</sup> Mechanistically in apoptosis, caspase 8 activation contribute to the induction of apoptosis, and caspase 8 is a caspase protein and it is encoded by the CASP8 gene and caspase 8 sequential activation plays a role in inducing cell apoptosis.<sup>18</sup> Treatment DHA affects to activation of caspase 8, so when treated DHA, caspase 8 is activated and it causes apoptosis. Furthermore from breast cancer, triple-negative breast

cancers (TNBCs) comprise 15~20% of breast cancer and it defined lack of estrogen and progesterone receptor, human epidermal growth factor receptor-2 (Her-2).<sup>4</sup> It has poor prognosis and it is resistance at drug and toxicity so many materials especially DHA has been studied constantly as anti-TNBC drug. In western blotting using MDA-MB-231 and SUM159, representative TNBC cells, DHA acted caspases level high so it caused inducing apoptosis of TNBC cells.<sup>4</sup> DHA induces apoptosis in breast cancer as the main progression of inhibition against breast cancer.

This review confirmed clinical effect of DHA on breast cancer cell. In effect of DHA on breast cancer cell anti-proliferation, using MCF-7, resistant-doxorubicin breast cancer cell, also using MDA-MB-231, in case of treatment DHA, proliferation of cell could be inhibited. So it was concluded that DHA inhibits breast cancer cell proliferation and differentiation. In effect of DHA on breast cancer cell anti-metastasis, using breast cancer cell MDA-MB-231, specialized cell at metastasis, luciferase expression of DHA-treated sample resulted in lower expression than DHA-not treated sample in bone injected MDA-MB-231. Si it was concluded DHA inhibits breast cancer cell metastasis, especially to bone. In effect of DHA on breast cancer cell inducing apoptosis, in MCF-7, DHA activated caspase and it induced apoptosis and it was concluded that DHA induces apoptosis on breast cancer and it is important mechanism for anticancer action. Therefore, DHA has anti-proliferation, anti-metastasis and inducing apoptosis effects on breast cancer cell.

## FUTURE DIRECTION

Today, although many drugs are developed and prescribed in order to care of anticancer, many patients experience drug tolerance, various side effects. So in order to mitigate these, many studies of natural

materials as drug has been gone along constantly especially DHA. DHA is main natural material of omega-3 fatty acid, derived from fish oil, so it will be drug having both good anti-cancer function and resistant-side effect drug if its beneficial effects of anticancer is developed well.

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Competing interests

The authors declare no conflict of interest.

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#### Peer Reviewer's Commentary

Breast cancer is the most common cancer among women worldwide. Many drugs are developed to prevent recurrence and metastasis for breast cancer. Docosahexaenoic acid (DHA), one of the components of omega-3 fatty acid, has been found to have anti-cancer effect on breast cancer. This article discussed the effect of DHA on breast cancer by three different mechanisms; 1) anti-proliferation 2) anti-metastasis and 3) inducing apoptosis. According to this review, DHA could be considered as one of a new strategy of treatment of breast cancer because of its good anti-cancer effect and tolerability.

(Editorial Board)