Is STARD3 A New Biomarker for Breast Cancer?

Almila Nazli Korucu¹, Nihal Inandiklioglu²
¹Yozgat Bozok University Faculty of Medicine, Yozgat, Turkey
²Department of Medical Biology, Yozgat Bozok University Faculty of Medicine, Yozgat, Turkey

ABSTRACT
Despite advances in diagnosis and treatment, breast cancer is still one of the three most common cancers in the world and a significant cause of morbidity and mortality. Lipids play a role in many basic physiological pathways in cells, from regulating cell homeostasis to energy expenditure. As in many types of cancer, changes in lipid metabolism and their relationship have been reported in breast cancer. The STARD3 gene encodes a member of the subfamily of lipid trafficking proteins. It is a sterol-binding protein that mediates the transport of cholesterol from the endoplasmic reticulum to endosomes. It has been shown that STARD3 is correlated with human epidermal growth factor receptor 2 (HER2) amplification since it has the same localization as HER2 in the chromosome. In this review, we aimed to emphasize that investigating lipid metabolism together with the STARD3 biomarker has great potential not only for subtype-specific strategies but also for patient-specific strategies.

Keywords: Breast; cancer; lipid; STARD3

Cite this article as: Korucu AN, Inandiklioglu N. Is STARD3 A New Biomarker for Breast Cancer?. Eur J Breast Health 2024; 20(2): 89-93

Key Points
• Breast
• Cancer
• Lipid
• STARD3

Introduction
Breast cancer is the most common malignancy in woman. According to the Global Cancer Observatory (GLOBOCAN) 2020 datas there are 2.26 million new cases and more than 680,000 deaths due to breast cancer globally, which is very concerning (1). Breast cancer can be classified into 4 molecular subtypes; Luminal A [estrogen receptor (ER) and progesterone receptor (PR) positive, human epidermal growth factor receptor 2 (HER2) negative], Luminal B (ER and HER2 positive, PR negative), Basal-Like (ER, PR, HER2 negative) and HER2-enriched (ER and PR negative, HER2 positive) (2). However, some researchers classify breast cancer into 5 subtypes by adding antijen Kiel 67 (Ki67) proliferative markers to the classification. These subtypes; Luminal A (ER and/or PR positive/HER2 negative/low Ki67), Luminal B (ER and/or PR positive/HER2 negative/high Ki67), HER2 positive Luminal B (ER and/or PR positive/HER2 overexpression/any Ki67), non-Luminal HER2 positive (ER and PR amplified/HER2 overexpression), Triple Negative (ER/PR/HER2 negative) (3). Molecular classification is vital for predicting prognosis and clinical outcome, as well as designing treatment strategy based on patients’ condition.

According to histological classification, invasive ductal carcinoma accounts for approximately 85% of invasive breast cancers. Breast carcinomas originate from the same part of the terminal duct lobular unit. Invasive ductal carcinoma is the most common type of invasive breast cancer. It accounts for 55% of the incidence of breast cancer at diagnosis (4). Invasive breast carcinomas subtypes and histological variants are known well. In general, breast neoplasias can be classified as carcinoma in situ (CIS) and invasive carcinoma. Ductal CIS is a noninvasive and potentially malignant intraductal proliferation of epithelial cells confined to ducts and lobules. Invasive or infiltrative carcinoma is the malignant abnormal proliferation of neoplastic cells that penetrate the stroma trough the duct walls in the breast tissue. Invasive carcinoma and CIS are classified as ductal and lobular depending on the site of tumor origin. Cancers arising from the ducts are called ductal carcinoma, arising from the lobules are called lobular carcinoma. However, it has now been found that such tumor growth variation doesn’t correlate with the site or cell of origin but with whether the tumor cells express E-cadherin (5).

HER2 overexpression is present in approximately 15% to 20% of breast cancers. It has generally been associated with an increased risk of the development of systemic metastases and poor survival intratumoral heterogeneity of HER2 expression has been described in 16–36% of HER2-positive BC patients and it is defined as the presence of varying degrees of HER2 overexpression in different areas of the same
tumor (6). In the HER2-positive group, targeted therapies have been studied mostly in metastatic cases. It has been shown that HER2 overexpression is associated with short disease-free survival and overall survival in node-positive cases. Although the biological consequences of HER2 overexpression on the prognosis of HER2-positive breast cancer and its predictive significance for anti-HER2 therapy are widely understood, the evidence regarding HER2-low breast cancers has been relatively ambiguous, particularly regarding whether it constitutes a separate biological or clinical subtype (7).

Despite the large amount of funding and personnel devoted to breast cancer research over many years, the etiology of breast cancer is not yet fully understood. Treatment resistance and recurrence in breast cancer patients remain unresolved. Because it is a disease with high heterogeneity, the treatment and prognosis of patients are quite different.

**Lipid Metabolism-Breast Cancer Correlation**

Lipids are in the class of water-insoluble metabolites. Lipid types vary from 10,000 to millions depending on molecular classification (8). Despite this heterogeneity, most lipid molecules consist of fatty acids and cholesterol. The numerous studies to date identifying proteins and genes expressed in cancer cells have almost always identified lipid metabolism as one of the main processes affected. One of the most important characteristics of this condition is that cancer cells depend on the source of fatty acids and cholesterol. This requirement is linked to the increasing need for membranes that support cell growth and division and provide energy to fuel cellular processes such as metastasis (9).

Early studies on lipid metabolism and cancer revealed increasing cholesterol levels and changes on phospholipids in tumor tissues. Studies with radioactive substrates in the early 1960s has shown that cancer cells exhibit a dynamic lipid metabolism and actively synthesize and uptake lipids (10, 11). It’s known that lipid metabolism is reprogrammed in tumors. Lipids are can be used on hormonal therapy in the future due to this reprogrammed cholesterol metabolism is association with migration, invasion and cell proliferation on endocrine related cancers (12, 13).

Recent studies have shown characteristic changes in lipid parameters between patients with invasive breast cancer and patients with benign breast tumors. Moreover, these changes were also seen in patients with different molecular types of breast cancer. In addition, postoperative chemotherapy has been found to cause abnormal plasma lipid metabolism changes in breast cancer patients (14). Similarly, it has been thought lipid transduction may be necessary for carcinogenesis and survival in breast cancer (15, 16). However, although many studies have shown the effect of lipid metabolism on carcinogenesis, data on its effects on breast cancer recurrence and survival are limited (17-19).

Some studies have shown that low-density lipoprotein cholesterol (LDL-c) is not associated with breast cancer risk, but serum LDL-c levels may be marker of breast cancer progression (20-22). However, it was demonstrated that significant up-regulation of LDL receptor increased LDL uptake in cancer cells because of the demand of rapid proliferation (23, 24). One recent meta-analysis, based mostly on case-control studies, concluded that high triglyceride levels increase the risk of breast cancer by 8% and low high-density lipoprotein cholesterol (HDL-c) levels increase the risk of breast cancer by 38% (25). Bhat et al. (26) in their study, they found that there was no significant change in HDL-c levels when they compared breast cancer patients with the control group. Borrelli et al. (27) have found that revers relation between HDL-c and breast cancer risk and argued that HDL-c is a biochemical marker that may be associated with an increased risk of breast cancer. In another study, it was observed that oxidized LDL-c levels were also high in breast cancer patients with high blood LDL-c levels, and it was concluded that oxidized LDL-c was associated with the risk of breast cancer. However, it has been suggested that HDL-c is less sensitive to peroxidation due to its lipid and Apo protein content, and therefore acts as an anti-oxidant because it cannot produce reactive oxygen species (28).

**STARD3**

STARD3 (StAR Related Lipid Transfer Domain Containing 3), also known as metastatic lymph node protein 64 (MLN64), is a sterol-binding protein that forms endoplasmic reticulum-endosome contact areas. The STARD3 gene located in the q12-q21 zone on chromosome 17 and encodes a protein containing two separate domains. The N-terminus of this protein has the feature of a potential trans membrane region, C-terminus has the same homology to a protein involved in steroid hormone synthesis. Some studies have suggested that the second domain is present in proteins involved in various cell functions and has 37% similarity to STAR (29-31). STARD3 gene encodes a cholesterol-binding membrane protein [51, 32] and two different cell culture studies conducted in 2005 and 2010 it was shown that this protein may be involved in the actin-dependent movement of late endocytic organelles and cholesterol transfer between this organelles and other membrane-dependent organelles such as mitochondria (33, 34). Due to its activity in lipid metabolism, it has been a matter of curiosity whether STARD3 has any activity in cancer types that may be related to lipid-based hormones. Based on this idea, when its relationship with androgen-dependent breast cancer was investigated, it was seen that STARD3 was closely related to HER2 and it was suggested that STARD3 contributed to proliferation in HER2+ cell lines (35). In a study conducted using the quantitative polymerase chain reaction method with DNA samples obtained from frozen tumor samples, the amplification of genes co-localized with HER2 [mediator complex subunit 1 (MED1), STARD3, HER2, growth factor receptor bound protein 7 (GRB7), thyroid hormone receptor alpha (THRA), retinoic acid receptor alpha (RARα), DNA topoisomerase II alpha (TOP2A), insulin like growth factor binding protein 4 (IGFBP4), C-C motif chemokine receptor 7 (CCR7), keratin 20 (KRT20), keratin 19 (KRT19) and gastrin (GAS)] in HER2+ cell lines was evaluated. As a result of the study, it was shown that HER2 amplification and STARD3 were correlated (36). To determine the functional interactions of the STARD3 protein in cellular processes, STRING network analysis was applied. Protein-protein interactions with the top 5 proteins in the shell [MOSPD2 (motile sperm domain-containing protein 2), PGAP3 (post-GPI attachment to proteins factor 3), STARD3NL (STARD3 N-terminal-like protein), VAPA (vesicle-associated membrane protein-associated protein A), VAPB (vesicle-associated membrane protein-associated protein B/C)] fell within a homology score range of 0.990-0.940 and were statistically highly significant (p<0.05) (Figure 1). Various mechanisms have been proposed regarding STARD3′s ability to increase plasma membrane cholesterol. One of these mechanisms involves the newly synthesized STARD3 protein moving to late endocytic organelles via the plasma membrane, thereby increasing cholesterol accumulation in the organelles. The increased cholesterol in late endocytic organelles containing STARD3 can become transportable...
to the plasma membrane. Finally, the accumulated cholesterol inside the cell is transferred from the endoplasmic reticulum to the plasma membrane (37). In this way, the increased biosynthetic activity of cholesterol observed in cells overexpressing STARD3 also facilitates enrichment of plasma membrane cholesterol. In cells overexpressing STARD3, an increase in mRNA levels encoding HMGR, the rate-limiting cholesterol biosynthesis enzyme, has been observed. Increased cholesterol biosynthesis, especially under low nutrient conditions where membrane biogenesis is limited, allows cells to continue survival and division (38).

STARD3 has been determined to play a role in the development of various types of cancer, such as colorectal, prostate and stomach cancers (39). However, STARD3 was found to have the highest expression levels in breast cancer tissues compared to other types of cancer such as prostate and liver cancers (40).

**STARD3 - Breast Cancer Correlation**

In recent years, considering the effect of STARD3 on cells, it has been a matter of curiosity whether it is effective in the lipid metabolism changes seen in breast cancer patients.

Vassilev et al. (38) produced polyclonal rabbit antibodies against the START domain of human STARD3 for the role of the STARD3 protein in breast cancer cells and tissues. These antibodies have been used to suppress STARD3 expression in breast cancer patients and have shown reduced survival of tumor cells in patients given the antibody. In the same study, to gain insight into how STARD3 may support cell survival independent of HER2 amplification generated MCF-7 (HER2-negative) breast cancer cells stably overexpressing STARD3-green fluorescent protein (GFP) or soluble GFP as a control. Remarkably, the overall morphological features of STARD3-GFP high-expressing cells appeared to be strikingly different from those of control GFP cells. STARD3-GFP cell clusters appeared to have increased filipin density, especially at the plasma membrane, compared with control cells. This raises the possibility that STARD3-overexpressing cells may have high cholesterol content. However, based on biochemical cholesterol determination, the total amount of cellular free cholesterol in STARD3-GFP cells was not increased but rather slightly decreased compared with control cells. Based on this, it was concluded that overexpression of STARD3 causes changes in cellular cholesterol distribution and homeostatic control, increasing plasma membrane cholesterol but decreasing ER cholesterol (38).

In 2006, Kao and Pollack (41) investigated the impact of targeted disruption of various genes associated with HER2/neu on cell function. They demonstrated a significant correlation between the inactivation of STARD3 and GRB7 with decreased cell proliferation and progression of the cell cycle, suggesting that the amplification of these genes and the overexpression of their encoded proteins could play a role in cellular tumorigenesis. An immunohistochemical study in breast cancer patients revealed higher expression of STARD3 in malignant breast tissue compared to normal breast tissue, which was associated with tumor size and histological grade. The study concluded that STARD3 could be a potential marker in HER2+ breast cancer patients (42). Silencing STARD3 by siRNA in HER2+ breast cancer cell lines induced apoptosis, suggesting that STARD3 may be necessary for the growth and survival of these cells (43). Similarly, a study by Li et al. (44) found that patients with high STARD3 expression had a lower survival rate compared to those with low expression. The study also demonstrated that inhibiting STARD3 expression reduced PI3K/AKT/mTOR pathway activity and induced apoptosis in MCF-7 cell lines.

Using traditional qualitative PCR and various bioinformatics websites such as Oncomine, GEPIA (gene expression profiling interactive analysis), and Expression Atlas, the expression of STARD3 at mRNA and protein levels in breast cancer was examined. The impact of STARD3 as a prognostic and diagnostic biomarker was assessed. The study revealed that the mRNA expression of STARD3 was significantly higher in HER2+ cell lines compared to ER+ normal cell lines. Based on this, STARD3 was suggested to be a potential diagnostic and prognostic biomarker for HER2+ breast cancer (40). Lodi et al. (45)
investigated the relationship between STARD3 expression and breast cancer-specific survival, comparing it with other relevant patient and tumor characteristics in HER2+ series. In this study, STARD3 DNA copy number showed a strong positive correlation with HER2 DNA copy number. Both STARD3 DNA copy number and RNA expression were found to be strongly associated with HER2. Vinatzer et al. (46) based on their measurements using quantitative RT-PCR, concluded that the overexpression of STARD3 enhances the prognostic power of HER2 overexpression for disease-free survival in breast cancer patients. It has been shown that MLN64 and HER2 genes share common transcriptional controls along with a physical connection on chromosome 17q. Based on this, they hypothesized that, in addition to the oncogenic potential of HER2 overexpression, the unbalanced effect of MLN64 contributes to poor clinical outcomes in breast tumors carrying this amplified region (47). HER2 amplification is present in 13–15% of breast cancer cases and biologically leads to a more aggressive malignancy by increasing sensitivity to chemotherapy in cells (48). Furthermore, the evaluation of response to anti-HER2 agents used in chemotherapy is of great importance in treatment monitoring (49). In a study examining STARD3 expression in HER2+ breast cancer patients, a strong correlation was observed between STARD3 and HER2 DNA amplification and RNA expression. Based on these findings, it has been suggested that STARD3 could be evaluated as a subgroup for HER2+ breast cancer, potentially used in treatment planning and patient prognosis monitoring (45). In a similar study, it was observed that STARD3 expression is higher in HER2+ patients compared to HER2-. It has been suggested that STARD3 may have an impact on overall survival, recurrence-free survival, and non-metastatic survival (40). The STARD3 inhibitor has recently been developed and tested in various breast and colon cancer cell lines (50). The study results are promising, but it is a fact that further in vitro and in vivo research is needed.

Studies conducted in recent years support that STARD3 may be a potential biomarker in the diagnosis of breast cancer, especially since it originates from the same gene region as HER2. However, the limitations of the data we have and the limited number of studies and patient population should also be taken into consideration. Elucidating the molecular mechanism of STARD3 function will provide new insights into its mechanism of action. Future studies will provide evidence on how to regulate the molecule’s lipid transfer activities and its role in breast cancer treatment.

Authorship Contributions

Conflict of Interest: No conflict of interest was declared by the authors.

Financial Disclosure: The authors declare that this study received no financial disclosure.

References
8. Lydic TA, Goo YH. Lipidomics unveils the complexity of the lipidome in metabolic diseases. Clin Transl Med 2018; 7: 4. (PMID: 29374337) [Crossref]
10. Spector A, Steinberg D. Relationship between fatty acid and glucose utilization in Ehrlich ascites tumor cells. J Lipid Res 1966; 7: 657-663. (PMID: 5971047) [Crossref]
15. Cruz PM, Mo H, McConathy WJ, Sabnis N, Lacko AG. The role of cholesterol metabolism and cholesterol transport in carcinogenesis: a review of scientific findings, relevant to future cancer therapeutics. Front Pharmacol 2013; 4: 119. (PMID: 24093019) [Crossref]
Korucu and Inandiklioglu. Is STARD3 A New Biomarker for Breast Cancer?

20. Rodrigues Dos Santos C, Fonseca I, Dias S, Mendes de Almeida JC. Plasma level of LDL-cholesterol at diagnosis is a predictor factor of breast tumor progression. BMC Cancer 2014; 14: 132. (PMID: 24571647) [Crossref]


30. Soccio RE, Breseow JL. StAR-related Lipid Transfer (START) Proteins: Mediators of Intracellular Lipid Metabolism. J Biol Chem 2003; 278: 22183-22186. (PMID: 12724317) [Crossref]


32. Alpy F, Tomasetto C. MLN64 and MENTHO, two mediators of endosomal cholesterol transport. Biochem Soc Trans 2006; 34: 343-345. (PMID: 16709157) [Crossref]


34. Charman M, Kennedy BE, Osborne N, Karten B. MLN64 mediates egress of cholesterol from endosomes to mitochondria in the absence of functional Niemann-Pick Type C1 protein. J Lipid Res 2010; 51: 1023-1034. (PMID: 19965586) [Crossref]


41. Kao J, Pollack JR. RNA interference-based functional dissection of the 17q12 amplicon in breast cancer reveals contribution of coamplified genes. Genes Chromosomes Cancer 2006; 45: 761-769. (PMID: 16708353) [Crossref]

42. Fararjeh A, Kaddumi E, Al-Khader A, Aburayyan W. The significance of StAR-related lipid transfer protein-3 expression in breast cancer. Pol J Pathol 2022; 73: 215-222. (PMID: 36734436) [Crossref]

43. Sahlberg KK, Hongisto V, Edgren H, Mäkelä R, Hellström K, Due EU, et al. The HER2 amplicon includes several genes required for the growth and survival of HER2 positive breast cancer cells. Mol Oncol 2013; 7: 392-401. (PMID: 23253899) [Crossref]

44. Li P, Zhang Z, Lv H, Sun P. Inhibiting the expression of STARD3 induced apoptosis via the inactivation of PI3K/AKT/mTOR pathway on ER+ breast cancer. Tissue Cell 2022; 79: 101971. (PMID: 36375355) [Crossref]


