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Farklı Meme Kanseri Hücre Hatlarında HMGCR Gen Ekspresyonu

Başak Şentürk[1], Ahsen Merve Bayrak[2], Burcu Yücel[3]

İletişim / *Correspondence:*

[1] İstanbul Medeniyet Üniversitesi,
basaksenturk1999@gmail.com

[2] İstanbul Medeniyet Üniversitesi,
ahsen.bayrak.ab@gmail.com

[3] Doç. Dr./ *Assoc Prof.*
İstanbul Medeniyet Üniversitesi
burcu_yucel@msn.com
ORCID: 0000-0002-6599-4558

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Özet

Meme kanseri, dünyada kadınlar arasında en sık görülen kanser türlerinden biridir. Meme hücreleri, lobüllerde veya kanallarda kontrolsüz bir şekilde bölünebilir. Meme kanseri, türüne göre in situ veya invaziv olabilir. Kolesterol homeostazı kanser progresyonu için önemli bir parametredir. Normal hücrelerde kolesterol mevalonat yolağı olarak adlandırılan, De novo sentezi ile sıkı bir şekilde düzenlenir. Bu yolakta, kolesterol üretimi için önemli ara ürünler üretilir. Yolağın ilk aşamasında, HMG-CoA olarak bilinen 3-hidroksi-3-metilglutaril koenzim A, hız sınırlayıcı enzim HMGCR tarafından mevalonata dönüştürülür ve son ürün olarak kolesterolün yapı taşı olan FPP adı verilen farnesil pirofosfat üretilir. Bu çalışmada, gerçek zamanlı PCR aracılığıyla farklı meme kanseri hücre hatlarında, MDA-MB 231, MDA-MB 435, MDA-MB 453, UACC 2080 ve MCF-7, 3-hidroksi-3-metilglutaril-koenzim A redüktaz enzimini kodlayan HMGCR geninin ekspresyon seviyelerini belirlemeyi amaçladık. Sonuç olarak, MCF7 hücre hattının en düşük HMGCR mRNA seviyesine sahip olduğunu ve MDA-MB-453 hücre hattının ise en yüksek HMGCR mRNA seviyesine sahip olduğunu bulduk. Ekspresyon seviyelerindeki farklılıklar hormon reseptörlerine bağlı olabilir. Ayrıca, diğer üçlü negatif hücre hatları MCF-7'den daha fazla HMGCR ekprese etmelerine rağmen, kendi aralarında farklı ekspresyon seviyeleri göstermiştir. Bunun nedeninin kanser oluşumunun yeri veya hastanın yaşı ile ilgili olabileceği düşünülmüştür.

HMGCR Gene Expression in Different Breast Cancer Cell Lines

Abstract

Breast cancer is one of the most common cancer types among women in the world. Breast cells can be divided uncontrollably in either lobules or ducts. Breast cancer can be in situ or invasive, depending on its type. Cholesterol homeostasis is an important parameter for cancer progression. Mevalonate pathway, also known as the de novo cholesterol synthesis pathway, is the essential part for cholesterol synthesis. In this pathway, important intermediates are produced for production of cholesterol. In the first step of the pathway, 3-hydroxy-3-methylglutaryl coenzyme A, known as HMG-CoA, is converted to mevalonate by the rate-limiting enzyme HMGCR and the final product is farnesyl pyrophosphate called FPP, which is the building block of cholesterol. In this study, we aimed to determine the expression level of the HMGCR gene, which encodes 3-hydroxy-3-methylglutaryl-coenzyme A reductase enzyme, in different breast cancer cell lines: MDA-MB 231, MDA-MB 435, MDA-MB 453, UACC 2080, MCF-7, HCC 1938 by real-time PCR. As a result, we found that the MCF7 cell line has the lowest level of HMGCR mRNA, and the MDA-MB-453 cell line has the highest level of HMGCR mRNA. Differences in expression levels may be due to hormone receptors. Furthermore, other triple negative cell lines showed different expression levels among themselves, although they expressed more HMGCR than MCF-7. The reason for this was thought to be related to the location of cancer formation or the age of the patient.

Introduction

Breast cancer is uncontrolled proliferation of breast cells. There are different breast cancer types according to cells affected, such as duct cells or lobules. Breast cancer is the second most common cancer type among women. Almost 1.5 million women fight against breast cancer all over the world (Demircan, Yucel and Radosevich, 2019). According to data from the World Health Organization (WHO), there are 2.3 million women diagnosed with breast cancer worldwide in 2020 and, in the same year, there were 685,000 deaths related to breast cancer (World Health Organization [WHO], 2021).

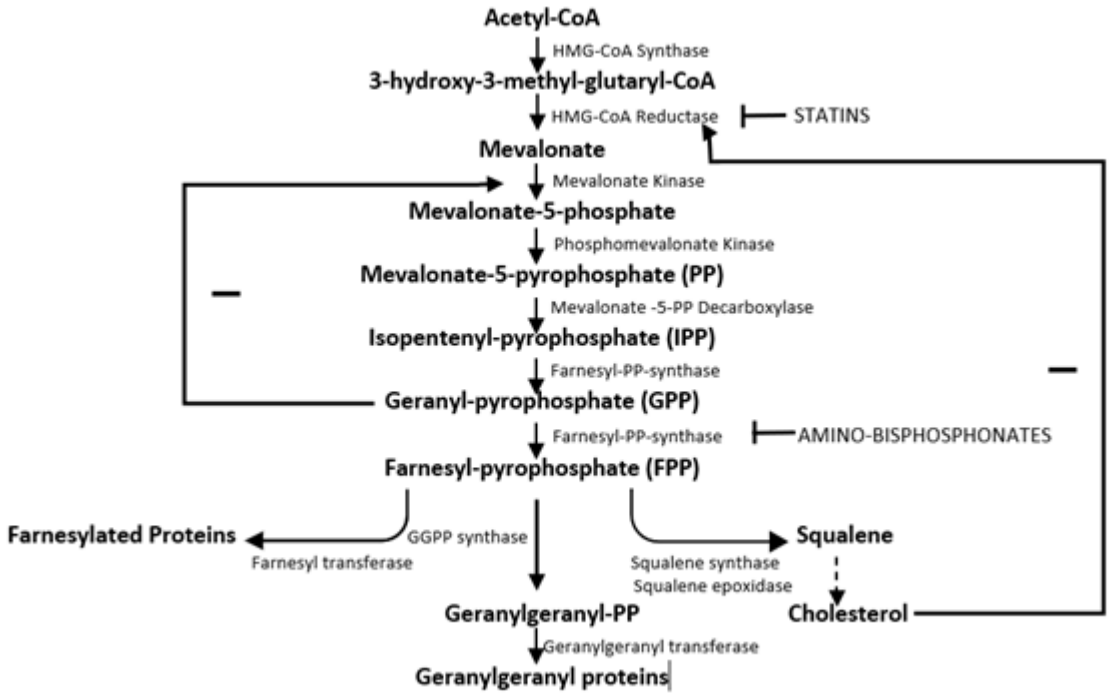
Breast cancer can be either in situ or invasive (Burstein, Polyak, Wong, Lester and Kaelin, 2004). Carcinoma in situ is made of non-spreadable abnormal cells. Abnormal cells stay where they first occur in the tissue. These cells do not have the ability to metastasize (Posner and Wolmark, 1992). After the formation of carcinoma in situ, cells can form cancer and spread nearby tissues later. This type is also considered as earliest form of cancer (Frykberg and Bland, 1993). Carcinoma in situ can be seen in milk glands or ducts of the breast. In invasive carcinoma, cancer cells disrupt the boundaries of the tissue. Cells metastasize farther from the tissues where cancer formation begins (Turashvili, Bouchal, Burkadze and Kolar, 2005). They create new tumor formation in new places. Invasive carcinoma can be seen in lobules or ducts.

Breast cancer is divided into groups according to presence of molecular markers. These markers are estrogen receptor (ER), progesterone receptor (PR) and human epidermal growth factor receptor 2 (HER2). Estrogen hormones have important role in regulation of differentiation and growth of mammary gland. Also, it has role in progression of breast carcinoma (Ross et al., 2009). In addition to estrogen, progesterone that is an ovarian hormone, play important role in development of breast during puberty. HER-2 is important for cell to cell and cell to stroma communication. Transcription of various genes are dependent on its signal transduction. Cell proliferation, survival of cells and mortality and adhesion can be affected by or via these receptors. Based on these markers there are four subtypes of breast cancer. In luminal types, estrogen receptors are positive. Luminal has also subtyped according to presence of HER2 and PR (McGuire, Horwitz, Pearson and Segaloff, 1977). In Luminal A breast cancer type, tumor formation tends to grow slowly. PR can be either positive or negative and HER2 is negative (Desai et al., 2000). In luminal B PR can be either positive or negative and HER2 is positive (Creighton, 2012). Other type is triple negative that is also known as Basal-like. In this type, estrogen, PR, and HER2 are negative (Gruvberger et al., 2001). Triple negative cancer cells grow rapidly and metastasize to another part of the body. Last type is HER2 enriched in which HER2 is highly expressed and ER/PR are negative.

De novo cholesterol synthesis pathway is also known as mevalonate pathway (Figure 1) (Göbel, Rauner, Hofbauer and Rachner, 2020). Mevalonate pathway is anabolic pathway that responsible for providing metabolites for multiple cellular processes in eukaryotes, archaea and some bacteria (Karlic, and Varga, 2017). This pathway starts with acetyl-CoA, the product of glycolysis. Three acetyl-CoA molecules are combined with each other and create 3-hydroxy-3-methylglutaryl coenzyme A known as HMG-CoA. HMG-CoA is converted into mevalonate via 3-Hydroxy-3-Methylglutaryl-CoA Reductase enzyme that is also known as HMGCR (Göbel, Breining, Rauner, Hofbauer, and Rachner, 2019). HMGCR has role for rate limiting of mevalonate pathway. HMG-CoA is reduced to mevalonate by NADPH. Mevalonate undergoes phosphorylation by mevalonate kinase enzyme. After phosphorylation, it is metabolized into isopentenyl pyrophosphate which is IPP. FPP synthase catalyses two important reactions, first converting IPP to geranyl-pyrophosphate, then catalysing the reaction of the conversion of geranyl-pyrophosphate to farnesyl

pyrophosphate. FPP is building block of production of squalene. At the final step cholesterol is synthesized by the squalene epoxidase and squalene synthase. Mevalonate pathway can be blocked by statins and amino bisphosphonates (Goldstein and Brown, 1990). Statins target HMGCR, and amino bisphosphonates target FPP synthase enzyme.

Figure 1. Mevalonate Pathway



Cholesterol has important role in cancer development. Some evidence supports that changing regulation of cholesterol metabolism is comprised in cancer development (Silvente-Poirot and Poirot, 2012). Dysregulation of cholesterol levels in blood is associated with cancer incidence. External cholesterol leads to activation of oncogenic Hedgehog pathway. Cholesterol is related with other membrane receptor in cell membrane. They bind Smoothened receptor that is important receptor for Hedgehog pathway. Dysregulation of Hedgehog pathway result in cell differentiation, cell proliferation and tumorigenesis (Ding, Zhang, Li and Yang, 2019). In addition, lysosomal cholesterol that is internal could change mTORC1 signaling. When the signals become higher, it results in increased cell proliferation, invasion, and metastasis. During cancer development, cholesterol metabolism is also rewired. Cholesterol biosynthesis and uptake of the cholesterol into cell is upregulated. Mevalonate pathway enzymes are relevant with dysregulation of cholesterol synthesis. Altered cholesterol metabolism due to expression of the pathway related genes create a potential risk factor for tumor growth and poor diagnosis in different cell types such as breast, prostate, and brain.

In this study, it is aimed to determine the expression levels of HMGCR gene which have important role of de novo synthesis of cholesterol in different breast cancer cell lines, MDA-MB 231, MDA-MB 435, MDA-MB 453, UACC 2080, MCF-7, HCC 1938 via quantitative Real-Time PCR (qRT-PCR).

Materials and Methods

Cell Culture

MDA-MB 231, MDA-MB 435, MDA-MB 453, UACC2087, MCF-7 and HCC-1937 cell lines were cultured in DMEM medium (Gibco, Thermo Fisher, USA) complemented with 10% fetal bovine serum (FBS), 1% pen/strep and 1% l-glutamine. Cells were grown at 37°C with 5% CO2 in atmospheric oxygen.

Table 1. Cell lines used in the experiment and their hormone receptors status

Cell Lines	Hormone Receptors
MCF7	Luminal A ER+, PR+, Her2-
UACC 2087	Triple negative ER-, PR-, Her2-
MDA-MB 231	Triple negative ER-, PR-, Her2-
MDA-MB 435	Triple negative ER-, PR-, Her2-
MDA-MB 453	Triple negative ER-, PR-, Her2-
HCC 1927	Triple negative ER-, PR-, Her2-

RNA Isolation and cDNA Synthesis

RNA was isolated using the NucleoSpin RNA Isolation Kit (Macherey-Nagel, Germany) and concentrations were measured. One microgram of RNA was reverse-transcribed with using the High-Capacity RNA-to-cDNA Synthesis Kit (Applied Biosystems, USA) according to the manufacturer's protocol.

qRT-PCR

qRT-PCR was applied to investigate HMGCR mRNA expression. qPCR was performed on the QIAGEN RotorGene Q system using SYBR Green (Applied Biosystems) with the following primers: HMGCR-Forward: GCCATTTTGCCCGAGTTTTAG, Reverse: TGCCAGAGGGAAACACTTG; RPLP0-Forward: AGCATCTACAACCCTGAAGTG, Reverse: AGCAAGTGGGAAGGTGTAATC. Ct results were normalized to RPLP0 housekeeping gene. Relative mRNA fold changes were calculated using ΔΔCt method.

Results

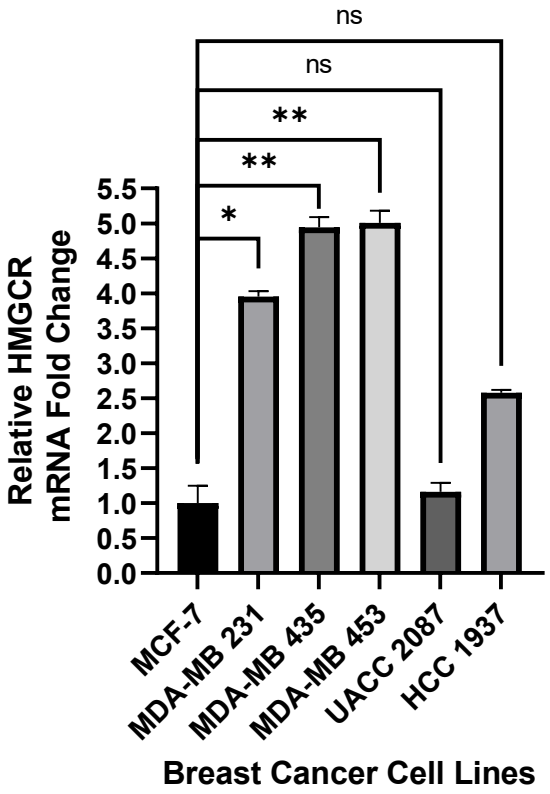
HMGCR mRNA Expression Is Lower in The MCF7 Cell Line

As shown in Figure 2, MCF7 cell line has the lowest level of HMGCR mRNA (ΔΔCt 0,0067), as compared to other cell lines in the study. MCF-7 mRNA level was considered as 1-fold and other cell lines were calculated as in fold changes. MDA-MB-453 cell line expressed the highest level of HMGCR mRNA (ΔΔCt 0,0336) and it was found that the mRNA fold change increased by 5 times compared to MCF7 (p=0.0047). Also, it was determined that the mRNA fold change of MDA-MB-435 (ΔΔCt 0,0331) was significantly higher

than MCF7 which was approximately 5 times (p=0.0066).

In MDA-MB-231 (ΔΔCt 0,0265) cell line, the mRNA fold change was detected 4 times higher than MCF7, which was statistically significant (p=0.0241). HMGCR mRNA expression in the HCC1937 (ΔΔCt 0,0173) cell line was also found higher than MCF7 cell line. HMGCR expression was 2.5 times higher compared to MCF7, however, this was not statistically significant (p=0.0647). Also, HMGCR mRNA expression was slightly increased in UACC2087 (ΔΔCt 0,0078) cell line compared to MCF7, which was not significant (p=0.5184).

Figure 2. Graph of relative change of HMGCR mRNA expression levels in breast cancer cell lines (Data presented as mean ± SD. *p<0.05, **p<0.01; ns means nonsense).



Discussion

Cholesterol, the main structural component of cell membranes, is synthesized by enzymatic reactions known as the mevalonate pathway. Cholesterol is involved in key cellular functions such as cellular signalling, cell motility, membrane fluidity, and membrane protein or receptor exchange. Mevalonate pathway genes are mostly upregulated in cancers and abnormal production of cholesterol is associated with tumor growth and survival (Yang, Y. 2014; Ding, X. 2019; Vona, R. 2021). Based on this information, overexpression of the HMGCR gene, a rate-limiting enzyme of the mevalonate pathway, can cause abnormal cholesterol production in cancer, resulting in disruption of cell membrane structure and cellular signalling. Also, altered cholesterol mechanism leads to create oncogenic signals that increase invasion and metastasis in cancer.

In our study, we found that HMGCR mRNA expression is lowest in the MCF7 cell line, while highest in the MDA-MB-453 cell line. Considering the classification of cell lines according to hormone receptors, only MCF7 is found in Luminal class A within the cell lines we were

investigated. Other cell lines are classified as triple negative breast cancer. In this case, it can be concluded that the expression level of HMGCR in triple negative cell lines may be higher than that of Luminal A cell lines. Differences between cell line is ER receptor that is present only in MCF-7. Previous studies showed that extrinsic and intrinsic pathways of apoptosis are associated with lipid rafts because changes in cholesterol content within specific membrane regions regulate apoptotic signalling (Li, et al., 2006; Chimento et al., 2019).

One study found that high levels of HMGCR tumor expression in breast cancer was associated with lower histological grade, ER and PR positivity, HER2 negativity, and less axillary lymph node involvement. In this study suggested that there might be an association between age, HMGCR expression, and ER-positive tumors because patients with tumors that expressed strong HMGCR were older (Gustbée et al., 2015). In contrast to this study, Bjarnadottir et al. reported that patients with strong HMGCR expression had tumors of high histological grade, ER-negative, and high Ki67. However, breast cancer mortality is not associated with HMGCR expression level. Also, statistical data indicates in same article that, diagnosis age of patients are higher in high HMGCR expression than low HMGCR expression breast cancer cell lines. It was speculated as this opposition might be due to the use of a different antibody and breast cancer heterogeneity (Bjarnadottir et al., 2020). Based on this information, it can be hypothesized that the increased expression level of HMGCR in breast cancer has an important role in the diagnosis of the disease. Further studies are also required to understand the correct relationship between ER receptor and HMGCR expression. There may different correlation with ER receptor that influence HMGCR expression level. For instance, MCF-7 cells not only have ER but also PR. Presence of both receptors may affect expression of HMGCR gene. To understand this, classification of cells according to hormone receptors can be done.

In another study on HMGCR expression, Clendening et al. showed that high mRNA HMGCR levels are associated with poor prognosis in breast cancer and suggested that HMGCR might be a candidate as a metabolic oncogene (Clendening et al., 2010). New treatment strategies may be developed for dysregulated HMGCR expression in breast cancer. In normal cells, when intracellular cholesterol levels are low, HMGCR activity is increases. Higher expression of HMGCR in tumor cells might be explained as a resistance against the feedback system of the mevalonate pathway. Uncontrolled mevalonate pathway can affect the oncogenic activity of transformation (Clendening and Penn, 2012; Gustbée et al., 2015).

It has been speculated that the role of cholesterol and its carriers, has a great impact on in the development and progression of breast cancer (Danilo and Frank, 2012). Cholesterol influences the growth of cancer cells. Also, it affects the metastatic ability of breast cancer. In addition, cholesterol has effect on proliferation of cells and potential of migration. Recent evidence suggests that blockage of De novo synthesis of cholesterol reduces the adhesion ability and migration of cancer cells (Murai, 2012). Furthermore, a study in 2022 showed that genetically proxied inhibition of HMGCR is associated with decreased ER-positive breast cancer risk whereas no significant association was found for the development of ER-negative breast cancer (Sun et al., 2022). Considering the data obtained from this experiment and previous studies, it can be speculated that cholesterol production of cancer cells, which will occur as a result of disruption of the mevalonate pathway, may influence adhesion and migration in cancer cells with different HMGCR expression levels.

Conclusion

Our data suggest that MCF-7 has the lowest HMGCR expression and MDA-MB 453 has the highest expression of HMGCR. It is estimated that lowest HMGCR expression might be related to hormone receptor status of cells. The main limitation of this study is the lack of healthy/normal cells in the experiment. In future studies, more breast cancer cell lines with normal breast cells can be used to elucidate the effects of the HMGCR gene on breast cancer, Moreover, the relation of hormone receptors and HMGCR expressions shall be analysed. During the cancer progression, understanding effect of the dysregulation of cholesterol metabolism on the cells may not only rely on HMGCR expression but also correlate the other enzymes. Therefore, the expression levels of other enzymes such as FPP synthase and mevalonate kinase, which are responsible for cholesterol metabolism, can also be investigated. Additionally, studies can be carried out in future on which properties of cancer cells are affected by hormone receptors. In these studies, cell morphology, vitality and metabolic activities can be observed by inactivating the hormone receptors in cell line that are member of different hormone receptor classes.

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