Research Article

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Biochemistry & Molecular Biology Journal
ISSN 2471-8084

2020
Vol.6 No.3:11

DOI: 10.36648/2471-8084.6.2.11

Comparison of the Effect of Breast Tumor Tissue Proteome on PI3K/AKT and Ras/ERK Signaling Pathways Activity in Luminal Patients with Higher and Lower than 30 BMI

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Citation: Hosseini v, AwsatMellati A, Mousavi SS, Moghimi MH (2020) Comparison of the Effect of Breast Tumor Tissue Proteome on PI3K/AKT and Ras/ERK Signaling Pathways Activity in Luminal Patients with Higher and Lower than 30 BMI. Biochem Mol Biol Vol.6 No.3:11

Abstract

Introduction: Studies have found that overactivity of mitogenic signaling pathways and suppression of tumor suppressors' expression play the most important role in breast cancer development and pathogenesis. In this study, we compare the breast tissue proteome of women with a BMI higher than 30 with those with a BMI lower than 30.

Methods: In this descriptive-analytical study, tumor tissue biopsy was done on 10 women diagnosed with invasive ductal breast cancer. Subjects were grouped into two groups, high BMI (33.41 on average) and low BMI (26.04 on average). Proteomics analysis was done on homogenized proteins of the breast tumor biopsy. Data analysis was done using ImageMaster 2D Platinum 6.0 and t-test with 95% confidence interval. Protein identification was done using MALDI-TOF MS/MS mass spectrophotometry.

Results and Discussion: Glyceraldehyde 3-phosphate, hemoglobin alpha chain and calreticulin expression levels were higher in high BMI group in comparison to low BMI group ($P<0.05$). On the contrary, cytokratin 19, collagen (I) alpha-1 chain and superoxide dismutase expression levels were lower in in high BMI group in comparison to low BMI group ($P<0.05$). Apo A-1 protein and albumin were only expressed in low BMI group and constant region of the immunoglobulin gamma chain, galectin-1 and HSP27 were exclusively expressed in high BMI group.

Conclusion: Among the wide range of protein expression changes, the changes of several proteins mentioned in the results may affect the metabolic changes in obese individuals, which requires further studies.

Keywords: Breast cancer; Proteome; BMI; Proteomics

Received: July 11, 2020; **Accepted:** October 07, 2020; **Published:** October 14, 2020

Introduction

Immunohistochemistry (IHC) is an important technique for defining protein biomarkers in the classification of breast cancer. Based on this method, breast cancer cases can be divided into two major groups: ER + and ER-. Among different subtypes of ER+ cancers, luminal A and B and among ER- cancers, Basal-like and HER2+/ER- are worth mentioning [1]. Luminal A is the most common subtype of breast cancer making up nearly 50 to 60 percent of the all breast cancer incidents. In Luminal A cancers, expression levels of the progesterone receptor (PR) and Estrogen Receptor (ER) genes are high, whereas expression of the human epidermal growth factor receptor (HER2) is low (ER +, PR +, HER2-) and the *TP53* gene undergoes the least amount of mutations in comparison to other subtypes of cancer. Cytokeratin biomarkers (CK8, 18) are also present. In nearly 90 percent of patients diagnosed with Luminal A cancer the prognosis time extends up to 5 years. Lymph nodes involvement is minimum. Additionally, Ki67 levels, a non-histone nuclear protein and an important marker of cell proliferation, is low [2]. Statistics on breast cancer incidence and its association with body fat percentage indicate that those with a high calorie diet are at higher risk for breast

cancer [3]. Additionally, studies show that women with a BMI of 30 compared to women with a BMI of 20 to 24.9, have up to 80% greater risk of advanced breast cancer and 22% more of for topical cancer. Chronic inflammation and overexpression of estrogen appear to be one of the factors causing the increased risk in obese individuals. With an increase in adipose tissue mass, active macrophages infiltrate into the breast and produce proinflammatory cytokines, aromatase activity increases, estrogen levels and subsequently proliferation of the breast epithelial cells increase, and finally the PI3 kinase and AKT/mTOR pathways activate [4,5]. As is generally the case in cancer, growth factor and angiogenesis receptors play a key role in initiating cell proliferation pathways and in survival of cells. Moreover, insulin-like growth factor-1receptors (IGF-1R) also play an important role in the survival of cancer cells in breast cancer. Growth receptors can be continuously activated by overexpression of the ligand or receptor, activating mutations and an increase in the number of epidermal growth factor receptor (EGFR) gene copies, ultimately leading to undesired kinase activity and activation of growth signals. The IGF-1R pathway is recognized as an effective therapeutic intervention point in breast cancer, as there is a direct relationship between IGF-1R expression levels and the recovery and survival rates in patients diagnosed with breast cancer [6].

Since the average oxygen levels in tumors is significantly lower than in normal tissues, tumor cells have to survive through adaptation to low oxygen pressure and angiogenesis. During hypoxia, Hypoxia-inducible factor 1-alpha (HIF-1-alpha) plays an important activating role in vascular endothelial growth factor (VEGF) transcription. In addition to hypoxia, IGF-1 and EGF can also lead to more HIF-1-alpha expression [7]. HIF mediates the activity of angiogenesis-related genes that are crucial for tumor growth, metastasis and tumor invasion. VEGF plays a key role in this and activates the signaling pathway by binding to VEGF-2 [8]. Numerous studies suggest that the majority of signaling pathways in breast cancer are PI3K/AKT and Ras/ERK [9]. In the PI3K/AKT pathway, after the ligands bind to the respective tyrosine kinase receptors and receptor activation, phosphatidylinositol 3-kinase (PI3K) is activated and converts PIP2 to PIP3. PIP3 binds to and activates PDK1, a protein kinase. In the next step, mTOR induces phosphorylation and activation of AKT. Activated AKT phosphorylates the Bad complex, which has two domains. Part of the BAD complex, which is an inactive apoptosis inhibitor protein, is activated through phosphorylation by the AKT and prevents apoptosis, which eventually leads to the cells not undergoing apoptosis and an abnormal cell proliferation [10]. The PTEN tumor suppressor acts as a phosphatase regulates the phosphoinositide 3-kinase (PI3-K) signal by dephosphorylating the phosphatidylinositol triphosphate position and preventing the conversion of PIP2 to PIP3 resulting in disruption of survival signaling cascades of cancer cells. This tumor suppressor is either mutated or expressed in lower levels in most cases of breast cancer [11]. The Ras/ERK signaling pathway is another important pathway in breast cancer. After the ligand binds to the respective tyrosine kinase receptor and activating it, GRB2/SOS complex activates and affects the inactive GDP-bound Ras. With GTP replacing GDP with the help of GRB2/SOS complex, Ras protein activates, and in turn activates Raf protein, which is a MAP kinase. In the next step, through phosphorylation of Mek, Erk is activated. The activated Erk causes changes in gene expression and protein function [12,13]. PI3K/AKT and Ras/ERK signaling pathways ultimately promote cell cycle progression, cell proliferation, increased gene transcription, cell migration and invasion, and increased survival [14]. Determining the clinical course, especially the decision to treat breast cancer, requires the prognosis of the disease, which may include some clinicopathologic characteristics such as the tumor size, histologic grade, and the extent of the axillary lymph nodes' involvement. One of the most effective and powerful ways to assess cancer is tumor markers and protein profiles, which are very effective in determining the invasive power of the disease and consequently the expected response to adjuvant therapies [15]. Therefore, the aim of this study was to compare the protein profile of breast tumor tissue in Luminal A patients in two groups with BMIs higher and lower than 30 and discuss its possible effects on PI3K/AKT and Ras/ERK signaling pathways.

Material and Methods

Subjects

In this descriptive research study, tumor biopsy was performed on 10 women with invasive ductal carcinoma who underwent surgery in Zanjan hospitals between March 2013 and February 2014. Part of the tissue (about 500 mg) was selected to study and the remainder were sent to the laboratory for pathological examination. After receiving pathologic results, patients diagnosed with stage 3 invasive ductal carcinoma who did not receive any medication or radiotherapy and also had no family history of breast cancer, were enrolled in the study in two groups of high BMI (averagely 33.41) and low BMI (averagely 26.04).

Sample preparation

After being washed by phosphate-buffered saline (PBS), each sample was moved into sterile microtubes and stored at 80°C until use.

Protein extraction

After bringing the samples out of the lab freezer (-80°C) and each sample was thoroughly broken down in mortar with liquid nitrogen. 400 µL of lysis buffer (6 M urea, 2 M thiourea, 50 mM Tris, 2% CHAPS, 50 mM DTT) was added to 150 mg of broken down samples. Additionally 10 mM PMSF and P8340 were added to the mix at a ratio of 1:50 and 1:20, respectively. The samples were incubated at room temperature for one hour until cell lysis processes are all completed. The samples were then centrifuged at 14000 g for 15 minutes at 4°C. The supernatant was collected and the Bradford protein assay was used to measure the concentration of total protein in samples. In order to confirm the accuracy of the concentration measurement results, SDS-PAGE electrophoresis was also performed [16].

2D-PAGE

Based on the final concentration of the samples, 100 µl of the

samples (containing 155 µg of protein) were mixed with 150µl of aqueous buffer (6 M Urea, 4% CHAPS, 0.5% Biolyt 3-10, 0.0002% bromophenol blue, 50 mM DTT) bringing the mixture volume to 250 µl. The resulting mixture was centrifuged at 14000 g for 10 minutes at 4°C. The sample and aqueous buffer mixture was transferred to tray and the IPG tapes (11 cm non-linear pH 3-10) were placed in the mixture. After one hour, the surface of the IPG tapes were covered with mineral oil and they theb were stored at room temperature for approximately 16 hours. After 17 hours the IPG tapes were removed from the tray and placed in IEF tray and Ettan IPGphor 3. After IEF completion, the tapes were placed in a equilibration buffer (50 mM Tris-HCl, 4% w/v Idoacetamide, 1% w/v DTT, 6M Urea, 30% w/v Glycerol, 2% w/v SDS) twice, each time for 20 minutes. Next, the IPG tapes were placed on 12% SDS-PAGE vertical gel, then they were embedded in an electrophoresis tank (16 cm, SE 600 Ruby, Amersham) and connected to a Power supply device. Electrophoresis was performed in the following manner [17]:

- 15 mA current for each gel for one hour
- 25 mA current until the bromophenol blue dye reaches the bottom of the gel

Gel staining

The gels were immersed in coomassie blue G250 staining solution for 3-4 hours. They were then rinsed with bleach solution (twice, 20 minutes each time) and left for one night in bleach solution (7% acetic acid, 30% methanol, final volume was brought to 100 ml with distilled water) [17].

Stain identification

The gels were scanned by Image scanner IV and the images were analyzed using ImageMaster 2D Platinum 6.0 software (stain identifying, editing and matching). After analyzing the gels and identifying the proteins that had undergone altered gene expression, these specific protein blots were cut from the gels and sent in microtubes to York University, England, for identification with MALDI TOF MS/MS technique **(Figure 1).**

Statistical analysis

Student's t-test was used for data analysis. Qualitative data were reported in frequency and percentage, and quantitative data were reported in mean and standard deviation. The confidence interval was 95% and differences in expression with P<0.05 was considered statistically significant. It should be noted that in order to ensure repeatability, the experiments were performed in triplicate.

Ethical considerations

The ethics of the research was approved by the Ethics Committee of Zanjan University of Medical Sciences. All patients were entered into the study after giving written consent with complete knowledge. During the study and afterwards the patients' personal information has been kept confidential. The study did not impose any financial costs on patients.

Results

In this study, breast tumor tissue in 10 women with ductal carcinoma with a mean age of 49.3 ± 9.82 years was studied in two groups with high BMI (mean 33.41) and low BMI (mean10 26.04). Proteins separated with two-dimensional electrophoresis (which was performed in triplicate) were analyzed using ImageMaster 2D Platinum 6.0 software at two intergroup and intragroup levels. The number of proteins expressed in the high BMI group was 1007 and in the low BMI group was 822 stains. In the intergroup analysis, t-test with 95% confidence interval revealed 45 significant protein stains between the two groups (P <0.05). Due to project limitations, among the 45 stains identified by the software, 11 protein stains were selected for identification by MS spectrometry. The results, including gel images and identified proteins are presented in **Figure 2** and **Table 1**.

According to spectrophotometry results, stain number 87 corresponds to collagen (I) alpha-1 chain, which was expressed about 8 times less in the BMI>30 group than the BMI<30 group (P<0.01). Stain number 202 corresponds to superoxide dismutase protein which was expressed about 5.6 times less in the BMI>30 group than the BMI<30 group (P<0.05). And stain number 264 corresponds to Cytokeratin 19 which was expressed about 1.25 times less in the BMI>30 group than the BMI<30 group (P<0.01).

Blot No. 126 corresponds to the calreticulin protein, which was expressed approximately 2 times higher in BMI>30 group than BMI<30 group (P<0.01). Blot No. 227 corresponds to the hemoglobin alpha chain which was expressed about 3 times more in the BMI>30 group than BMI<30 group (P<0.01) and stain number 359 corresponds to glyceraldehyde 3-phosphate dehydrogenase which was expressed about 40 times more in the BMI>30 group than the BMI<30 group (P <0.01) **(Table 2).**

Blots 150 and 391 are apolipoprotein A-1 and albumin, respectively, which were expressed only in the BMI<30 group. These two stains have no expression in the other group and have

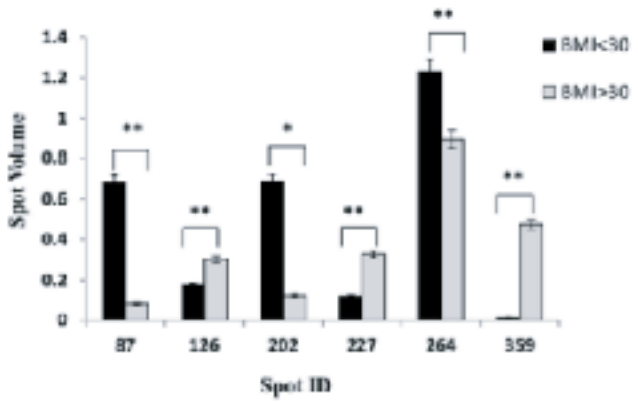

Figure 1 Expression ratio of identified proteins in the BMI<30 and BMI>30 groups. Protein blots 87 and 264 (P<0.01) and 202 (P<0.05) were expressed significantly less in BMI>30 group than BMI<30 group. Protein blots 126, 227 and 359 had a significant increase in expression in the BMI>30 group compared to BMI<30 group (P<0.01).

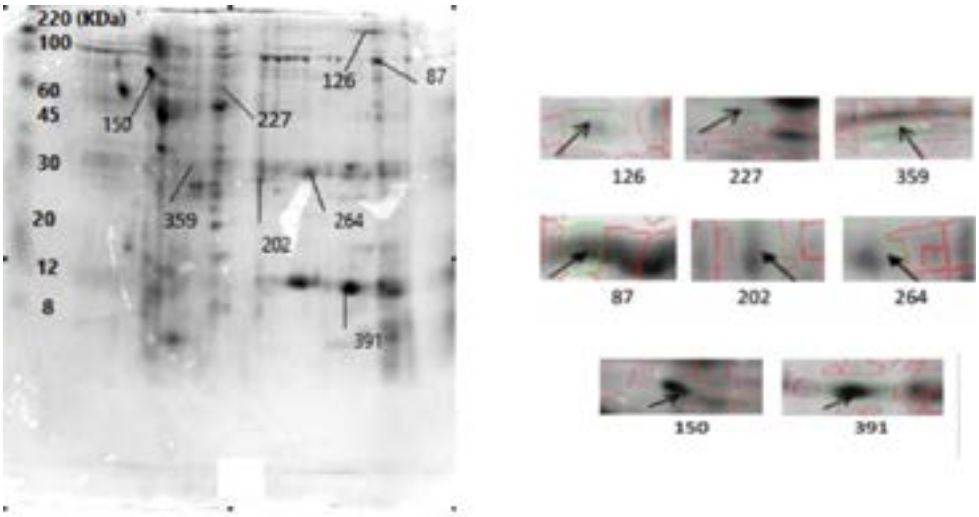

Figure 2a Two-dimensional gel image and protein identified in breast cancer samples of Gel A belongs to the group with BMI<30. The arrows and numbers show the proteins identified in each gel.

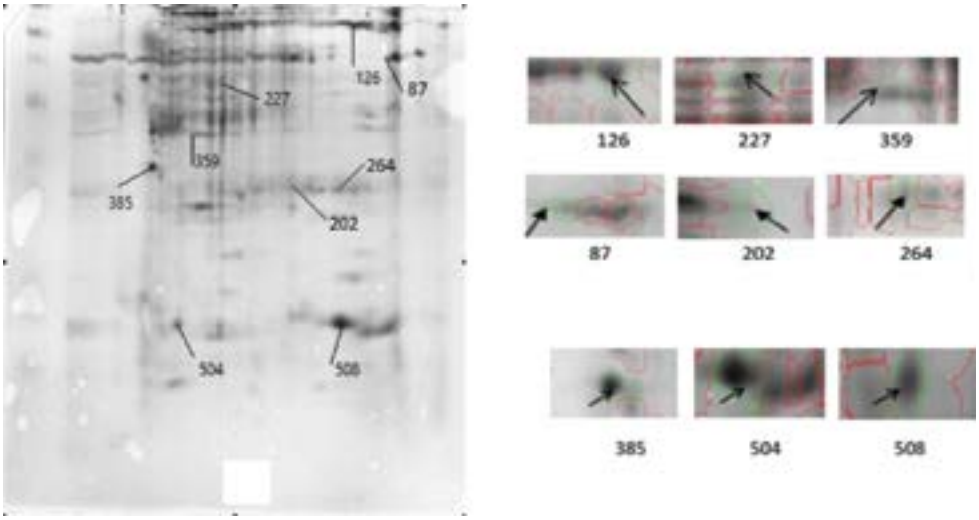

Figure 2b Two-dimensional gel image and protein identified in breast cancer samples gel B belongs to the group with BMI>30. The arrows and numbers show the proteins identified in each gel.

Table 1 Information on proteins identified by MALDI-TOF/TOF MS.

Spot Number	Protein Name	Protein	PI	AA	Mw (DA)	Protein ID
87	Collagen (I) alpha-1 chain	435	5.60	1464	139883	P02452
126	Calreticulin	340	4.29	417	48283	P27797
150	ApoA-1	346	5.56	264	30759	P02647
202	Superoxide dismutase	93	5.70	154	16154	P00441
227	Hemoglobin alpha chain	599	6.75	147	16102	P68871
264	Cytokeratin 19	43	5.04	400	44079	P08727
359	GAPDH	110	8.57	335	36201	P04406
385	HSP27	94	5.43	367	40098	P02765
391	Serum albumin	206	5.92	609	71317	P02768
504	Ig gamma-1 chain C region	104	8.46	330	36596	P01857
508	Galectin-1	438	5.34	135	15048	P09382

pI: Isoelectric pH, AA: Amino Acid Number, Mw: Molecular Weight, Protein ID: Protein ID Number in NCBI Database

been removed. Blots 504, 385 and 508 are constant region (C) of gamma immunoglobulin chain, heat shock protein 27 and galectin-1, respectively, which are expressed only in the BMI>30 group **(Table 3).**

Discussion

In the present study, aimed to compare the effect of a breast turmeric tissue proteome on the activity of PI3K/AKT and Ras/ERK signaling pathways in luminal A patients with higher and lower than 30 BMI revealed that the collagen (I) alpha-1 chain, superoxide dismutase and cytokeratin 19 decreased in expression in the BMI>30 group compared to BMI<30 group. On the other hand, calreticulin, alpha-hemoglobin chain and glyceraldehyde 3-phosphate dehydrogenase increased in expression in the BMI>30 group compared to BMI<30 group. Apolipoprotein A- | and albumin were expressed only in BMI <30 and eliminated in the other group. The gamma immunoglobulin (C) constant chain region, heat shock protein 27 and galectin-1 are expressed only in BMI> 30 group. The results of this study indicate that there is a significant difference between the proteome of breast cancer tissue in individuals with high BMI compared to those with low BMI. Therefore, we will investigate the protein profile changes in women diagnosed with breast cancer presented in **Table 1** and discuss their relationship with biochemical pathways and the possible effects of adipose tissue mass on their expression levels.

Studies show that conditions governing tumor production play an important role in changing cell behavior. The stroma surrounding the epithelial cells of the breast also plays a key role in their deformation, tumor growth and metastasis [18]. Collagen is the most abundant protein in the extracellular matrix. The matrix has a dynamic nature in which the production and destruction of collagen is normal. Although tumor formation is a multi-stage process involving changes in epithelial cells, the interaction between the extracellular matrix and the epithelium plays an important role in causing these changes [19,20]. It is therefore likely that collagen (I) alpha-1 produced from adipose tissue around breast tumor cells could promote tumor growth. Additionally, it has been found that the expression of collagen-degrading enzymes increases as stages of tumor cell growth progress. Collagen-degrading matrix metalloproteinases (MMPs) contribute to the destruction of the extracellular matrix by collagen degradation and thus facilitate metastasis [21,22]. Provenzano et al. reported that collagen expression decreases in patients with breast cancer (P<0.05) and metastasis increases 3-fold [23], which is consistent with the results of our study. In our study, collagen (I) alpha-1 expression was decreased in the high BMI group, which can be attributed to the increase in tumor mass (T3) compared to the low BMI group with lower tumor growth and size (T2). It appears that alterations in collagen expression levels are probably an important factor contributing to the pathogenesis and progression of breast cancer. Ghosh et al. reported that interferon gamma decreases the expression level of collagen (I) alpha-1 through the JAK/STAT pathway [24]. Increased adipose tissue mass in the high BMI group is expected to increase the production of IFN-γ cytokine, a type of adipokine secreted from adipose tissue, and consequently decrease collagen (I) alpha-1 expression. This mechanism well confirms the results of our study. Studies have shown that active oxygen metabolites such as superoxide anion, hydrogen peroxide, and hydroxyl radicals can cause alterations in nucleic acids and proteins and have been identified as initiators of cancer [25]. Superoxide dismutase (SOD) is the most important antioxidant enzyme in all aerobic organisms that converts superoxide radical to hydrogen peroxide and oxygen [26]. Studies have shown that cancer cells produce a lower level of SOD than normal cells [27]. Increased expression of SOD can suppress the malignant phenotype of breast cancer and acts as a tumor suppressor. Studies show that there seems to be a strong relationship between expression levels of SOD and breast cancer development [28]. Increased BMI may exert its effect on expression levels of SOD through high levels of estrogen. One of the effects of estrogen is to increase gene expression and cell proliferation. As cell proliferation increases, the tumor microenvironment shifts to hypoxia, reducing the

Table 2 Expression ratio of identified proteins in the BMI>30 group compared to BMI<30 group.

Identified Protein	Expression Ratio BMI>30/BMI<30	P-value
Collagen (I) alpha-1 chain	-8	P<0.01
Superoxide dismutase	-5.6	P<0.05
Cytokeratin 19	-1.25	P<0.01
Calreticulin	+2	P<0.01
Hemoglobin alpha chain	+3	P<0.01
GAPDH	+40	P<0.01
"-" sign Indicates decrease in expression and "+" sign indicates increased expression of the protein. Deleted and Emerged Proteins in BMI> 30 and BMI <30		

Table 3 Eliminated and emerged proteins in BMI>30 and BMI<30 groups.

Groups	Identified Protein	
	Presence (Emerging)	Absence (Eliminated)
BMI < 30	Apolipoprotein A-1 Albumin	Constant region (C) of gamma immunoglobulin chain heat shock protein 27 galectin-1
BMI > 30	Constant region (C) of gamma immunoglobulin chain heat shock protein 27 galectin-1	Apolipoprotein A-1 Albumin

production of free radicals, which in turn reduces the expression level of SOD. The results of our study and similar studies show that antioxidant defense plays an important role in breast cancer.

Creatines are a member of a large group of intermediate filaments that protect the cell against mechanical stress by forming extracellular scaffolds and are also actively involved in cancer invasion and metastasis [29]. Erg1 (early growth response-1) is a transcription factor that regulates the expression of several genes, such as PTEN. The PTEN tumor suppressor acts as a phosphatase that inversely regulates the phosphoinositide 3-kinase (PI3-K) signal by dephosphorylating phosphatidylinositol triphosphate position and prevents the conversion of PIP2 to PIP3 thus disrupting the PI3K/AKT signaling pathway resulting in disruption of cell migration and invasion, cell proliferation, cell cycle progression, increased gene transcription and cell survival [30]. Erg1 contains an NTS (nuclear translocation signal) domain in its C-terminal region that is required for its nuclear localization. IMP7 (Importin-7) binds to the NTS domain of Erg1 and phosphorylates it and activates Erg1. Cytokeratin 19 can bind to the Erg1-IMP7 complex and inhibit Erg1 activation, as a result PTEN won't be expressed and the PI3K/AKT signaling pathway which ultimately leads to cell proliferation, migration, and cell invasion, will resume [31]. Studies have shown the role of cytokeratin 19 in invasion and metastasis of cancer cells in breast cancer patients, which is associated with its increased expression [31], but the relationship between its expression and BMI has not been investigated. Decreased expression of this protein in the high BMI group may be associated with larger tumor size and higher rate of cell proliferation. Based on the results of our study, it is not possible to determine whether changes in the expression of cytokeratin 19 in the tumor tissue of obese individuals, can be considered as a marker of tumor development. Discussion in this area seems to require further research.

Calreticulin (CRT) is a multifunctional protein in the endoplasmic reticulum, involved in calcium homeostasis and cell adhesion. Additionally, CRT binds to abnormally folded proteins and prevents their transfer from the endoplasmic reticulum to the Golgi apparatus [32]. Many studies have shown that alterations in CRT expression in breast cancer have profound effects on tumor cell proliferation. Increased levels of CRT increases the rate of metastasis and cell invasion thus decreases survival [33]. Decreased CRT expression leads to the cell cycle stopping in the G_0/G_1 phase, leading to suppression of growth rate and growth-dependent colony formation capacity [34].

Chen et al. reported that higher levels of CRT promote cell proliferation and positive regulation of vascular endothelial growth factor (VEGF) in gastric cancer [33]. VEGF activates mitogen signaling pathways such as Ras/ERK and increases proliferation and cell migration by increasing angiogenesis. CRT is an important apoptosis regulator and protects the cell against calcium-induced apoptosis. CRT prevents cell apoptosis by using an ionophore called A23187 by increasing the buffering activity towards calcium inside the cell. The results of our study indicate that CRT expression is increased in patients with high BMI compared to the other group, which is consistent with the results of previous studies. Increased adipose mass appears to be associated with an increase in estrogen hormone, resulting in increased cell proliferation and increased CRT production. The cellular buffering capacity is maximized towards intracellular calcium, preventing apoptosis. In patients with low BMI, however, estrogen decreased as the adipose tissue decreased and the cells were more likely to be exposed to apoptosis.

Hemoglobin accounts for 96% of the weight of red blood cells and its main role is to transfer oxygen from the lungs to various tissues of the body [35]. As a result of the growth and proliferation of tumor cells, the oxygen pressure in the tumor tissue is reduced compared to other tissues. Oxygen depletion is associated with induction of HIF expression. HIF, through affecting the cells, produces vascular endothelial growth factor (VEGF). VEGF affects the blood vessels around the tumor tissue and induces the sprouting of the veins towards the tumor tissue to support and supply the tumor's need. The findings of our study indicate that hemoglobin increases in the high BMI group compared to the low BMI group, which is consistent with the results of Deng et al. [36]. Increased hemoglobin in the high BMI group may be justified by the larger tumor size and more severe hypoxic conditions and consequently an increase in angiogenesis in comparison to the other group.

Glyceraldehyde 3-phosphate dehydrogenase is one of the enzymes in the glycolysis pathway that converts glyceraldehyde 3-phosphate to 1,3-bisphosphoglycerate. Regardless of the molecular changes and the initial position of the tumor in the body, many metabolic changes occur within the tumor cells, including changes in glucose metabolism. The glycolysis pathway is typically more active in tumor cells (Warberg Effect). Glycolysis is likely to be adapted to the hypoxic conditions in the tumor to provide the optimum conditions for growth by producing the required compounds of the cell. As glycolysis increases, lactic acid level increases and as the environment becomes acidic, the extracellular matrix breaks down and invasion of tumor cells occur [37]. Graven et al. reported that tumor size plays a role in increasing the amount of glyceraldehyde 3-phosphate dehydrogenase (GAPDH) [38]. This finding is consistent with the results of our study. In our study in the high BMI group, the tumor mass was larger than that of the other group, with the glycolysis rate likely to increase, subsequently, GAPDH levels will increase too. Additionally, as the tumor mass increases, hypoxia occurs more severely, which stimulates increased GAPDH expression. Through activating PI3K /AKT signaling pathway, GAPDH, increases cell proliferation, tumor formation and drug resistance [39].

Apolipoprotein A-1 and albumin are two proteins expressed in BMI<30 and absent in the other group. In the case of apolipoprotein A-1, it should be noted that high-density lipoprotein (HDL) and cholesterol levels are prognostic factors in breast and lung cancer [40]. Apolipoprotein A-1, the main polypeptide in HDL, plays a key role in cholesterol homeostasis and induces reverse cholesterol transfer from the tissues to the liver [41]. Bertucci and Goncalves reported apolipoprotein A-1 expression in 83% of patients diagnosed with invasive ductal breast cancer [42]. It seems that in the low BMI group due to the decrease in adipose

tissue mass compared to the other group, HDL levels were higher thus Apo A-1 production was induced.

Albumin is makes up the majority of the total protein in the blood and plays an important role in regulating the colloid osmotic pressure of the blood and the intra-blood transfers. Previous studies have suggested that serum albumin depletion can possibly be an independent prognostic factor for survival in people with breast cancer [43]. According to previous studies, serum albumin is reduced in cancer [44]. In our study, albumin is only expressed in the low BMI group. It seems that with the increase in tumor mass in the high BMI group (T3) the production of proinflammatory cytokines is increased, which consequently decreases the expression of negative phase proteins such as albumin, whereas in the low BMI group it is due to the small tumor size (T2), this process is much less prominent hence the lower expression levels of albumin. Since albumin is the most abundant protein in the blood (60%), its presence in the sample may be due to angiogenesis in the tumor tissue to supply blood to the tumor. Therefore, it seems that albumin cannot be a biomarker in the early diagnosis of the disease.

Heat shock protein 27, galectin-1, and the constant region of the IG gamma-1 chain are proteins expressed only in the BMI>30 group and are referred to as emerging stains. HSP proteins as molecular chaperones are involved in many processes such as folding or 3D rearrangement of proteins, their accumulation and transport, peptide transport and antigen processing under physiological and stress conditions. HSP protein expression is induced by several types of stressors such as fever, inflammation, oxidative stress, as well as conditions that cause injury and necrosis. HSP27 interacts with the mitochondrial outer membrane and interferes with the activation of cytochrome c/ Apaf-1/dATP, which in turn inhibits procaspase-9 activation. Caspases are inducers of apoptosis and their inhibition leads to uncontrolled cell growth and proliferation. HSP27 also interacts with actin and intermediate filaments and prevents degradation of actin filaments [45]. Another function of HSP27 is to activate the proteasome. Degradation of denatured proteins occurs by binding to ubiquitinated proteins and to the proteasome. HSP27 controls many processes through the NF-kB pathway, such as cell growth and inflammatory and stress responses. Gusev et al. reported that some proinflammatory cytokines such as TNFα and IL-1, which are involved in inflammation and cell destruction, induce HSP27 production [46]. Kabbage et al. Reported high expression of HSP27 in invasive ductal carcinoma patients with larger tumor size [47], which is consistent with the results of our study. This protein was only expressed in the high BMI group, whereas in the low BMI group its expression is inhibited, which can be attributed to the increased tumor size and possibly to higher thermal and hypoxic stress conditions in the high BMI group. Additionally, with the increase in adipose tissue mass, production of cytokines such as TNFα and IL-1 increased, which in turn increased the expression of heat shock proteins such as HSP27.

Galectin-1 is one of the most important lectins known to date that plays a role in the development of malignant tumors. Galectin-1 is expressed in many different cancers [48]. Galectin-1 induces angiogenesis and facilitates metastasis. It also induces apoptosis in activated T cells [49]. In fact, cancer cells protect themselves from the immune system by expressing galectin-1 [50]. Metastatic breast adenocarcinoma has been found to increase expression of galectin-1 in response to TGFβ, a type of tumor growth factor [51]. There is also a relationship between increased expression of galectin-1 in cancer-associated tumor stroma cells and the extent of tumor invasion. Imai et al. by studying and comparing seven metastatic breast cancer cell line proteome to seven normal human breast epithelial cell line proteomes, showed that galectin-1 expression was increased in metastatic cells compared to normal cells [52].

Zhou et al. showed that galectin-1 expression was correlated with androgens [53]. Thijssen et al. reported that galectin-1 expression was positively controlled by FSH and TNFα [54].

The findings of our study are consistent with previous findings that galectin-1 increases in tumor tissue and also the role it plays in tumorigenesis and tumor progression. Given that galectin-1 was only expressed in the high BMI group, it may be possible that in this group, with increased adipose tissue mass, estrogen production on the one hand and adipokines production on the other hand, such as TNFα inflammatory cytokines, increased. Results from previous studies have shown that increased levels of estrogen and TNFα increase the expression of galectin-1. Thus, galectin-1 may be considered as a biomarker and therapeutic target. Further research is needed on this matter.

Huber et al. showed that the presence of IgG in the serum of breast adenocarcinoma patients induces a type of suppressor cells that attenuate the antitumor activity [55]. Carcinomas and cancer cells have also been shown to be able to produce IgG, which contributes to increased cell proliferation, disease progression and metastasis [56]. Eric et al. found that in humans, the presence of serum IgG that responds to tumor stroma is associated with increased metastasis in breast cancer, along with increased expression of calreticulin by cancer cells [57]. Cho et al. found that the constant region of the Ig gamma-1 chain peptide expressed in tumor tissue is one of the major constituents of the IgG molecule and its increase in expression reflects the accumulation of IgG in the tumor microenvironment [58]. We found that the constant region of the Ig gamma-1 chain is expressed only in the high BMI group. It is likely that this increase in expression occurred in response to high expression of calreticulin (2-fold) in the high BMI group. Thus, the results of our study are consistent with those of Eric et al.

Conclusion

In summary, according to the above reports, changes in expression of some of the mentioned proteins can be used as biomarkers for early detection of disease and design of therapeutic targets. However, the association of these proteins with each other and the protein profile of breast cancer as well as their association as contributors to altered metabolic pathways require further research.

Acknowledgments

This article is the result of the MA thesis in clinical biochemistry of Masoumeh Hosseini. I would like to extend my sincere thanks to the Biochemistry Department, the Metabolic Disease Research Center, and the Vice Chancellor for Research, Zanjan University of Medical Sciences, for their support and assistance in carrying out this dissertation.

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