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Efficacy of metformin on protein profile in breast tumor cells by assessment in vitro and in silico analysis

In vitro ve in silico analizi ile metforminin meme tümörü hücrelerinde protein profili üzerindeki etkinliği

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ABSTRACT

Aim*:* This study aimed to uncover the varieties in protein profiles of Met in breast tumor (BT) cells by assessment of in vitro and in silico analysis.

Materials and Methods*:* Here, the cells obtained from mastectomy patients were cultured, the effective Met-dose was determined as 25 mM through cell viability and BrdU tests. Protein identification in the breast tumor cells was implemented by employing LC-MS/MS technology.

Results*:* The expression of SSR3, THAP3, FTH1, NEFM, ANP32A, ANP32B, KRT7 proteins was significantly decreased whereas the GARS protein increased in the 25 mM Met group compared to the Non-Met (0 mM) control group. In silico analysis, we analyzed the probable interactions of all these proteins with each other and other proteins, to evaluate the analysis of the larger protein network, and which metabolic pathway proteins are involved in.

Conclusion*:* The stated proteomics analysis in our study proposes a better understanding of the prognosis of breast cancer and future studies to investigate the effect of metformin in this field on proteomic pathways in other sorts of cancer.

Keywords*:* Breast tumor cell, mass spectrometry, metformin, network analysis, protein networks.

ÖZ

Amaç: *Bu çalışmada, meme tümörü (BT) hücrelerinde Met'in protein profillerindeki çeşitlerin in vitro ve in siliko analizleri değerlendirilerek ortaya çıkarılması amaçlanmıştır.*

Gereç ve Yöntem: *Burada mastektomi hastalarından elde edilen hücreler kültürlendi, hücre canlılığı ve BrdU testleri ile etkin Met-doz 25 mM olarak belirlendi. Göğüs tümörü hücrelerinde protein tanımlaması, LC-MS/MS teknolojisi kullanılarak gerçekleştirilmiştir.*

Bulgular: *Proteomik analiz sonuçlarına göre, Non-Met (0 mM) kontrol grubuna kıyasla 25 mM Met grubunda SSR3, THAP3, FTH1, NEFM, ANP32A, ANP32B, KRT7 proteinlerinin ekspresyonu önemli ölçüde azalırken GARS proteininin ekpresyonu arttı. Silico analizde tüm bu proteinlerin birbirleriyle ve diğer proteinlerle olası etkileşimlerini analiz ederek daha büyük protein ağının analizini ve hangi metabolik yolak proteinlerinin rol oynadığını değerlendirdik.*

Sonuç: *Çalışmamızda belirtilen proteomik analizler, meme kanserinin prognozunun daha iyi anlaşılmasını ve metforminin diğer kanser türlerinde proteomik yolaklar üzerindeki etkisini araştırmak için gelecekteki çalışmaları önermektedir.*

Anahtar Sözcükler: *Meme tümör hücresi, kütle spektrometrisi, metformin, ağ analizi, protein ağları.*

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INTRODUCTION

Breast cancer is the most commonly diagnosed cancer following lung cancer with a rate of 11.6% among women and is categorized as the leading cause of cancer death globally with 2,088,849 new cases and 626,679 deaths in 185 countries (1). Despite having several therapy protocols, the patients are prone to greater risk of recurrence or secondary disease development during the progression of the disease due to the multifactorial nature of the disease, thus, an early diagnosis of the disease and avoidance of risk factors are the main strategies for preventing breast cancer (2). Hormonal therapy, mastectomy, and radiation, and chemotherapy are the most widely preferred therapy strategies to improve the survival of breast cancer patients (3).

Metformin (Met), one of the oral antidiabetics biguanides known to lower the risk of breast cancer, has an advantage in diabetic breast cancer patients if periodically used, compared to the MET-free diabetes treatments (4). Although several studies have underlined the association between diabetes and the increased risk of breast cancer, epidemiologic studies suggest that Met elevates the incidence of cancer-related survival in patients with type 2 diabetes. Libby et al. (5) in 2009 and Currie et al. (6) in 2012, two independent research groups, reported that the patients having both diabetes and cancer, had higher survival rates if treated with MET compared to other antidiabetic drugs.

The potential anti-cancer and anti-tumoral effects of Met on breast cancer have been discussed in several studies. Met has been reported to inhibit lipogenesis (7) and hyperinsulinemia, which have pivotal roles in the development of numerous types of cancer, including breast and prostate cancers (8). There is also some evidence suggesting that Met could induce apoptosis in many cancer cell lines, such as endometrial and triple-negative breast cancers (TNBC) (9).

To better understand the mechanisms of Met in molecular cancer studies, the effect of Met on breast cancer is examined under two headings: AMPK-independent and AMPK-dependent (10).

Met activates the p53 through the AMPK-dependent pathway, thus, prevents cell growth and induces apoptosis (11). Moreover, it co-activates p53 and BAX (Bcl-2-associated X) proteins, triggering apoptosis via the ERK signaling pathway in MCF-7 cells (12). In the other approach, namely the AMPK-independent mechanism, Met suppresses inflammation by hindering several mediators including COX-2, TNF-alfa, IL-6, IL-17, NF-kB (10). IL-6 was reported to take part in the growth and invasion of breast cancer through STAT3 and JAK pathways (13). Interestingly enough, Met has been shown to inhibit the pro-inflammatory mediators such as IL-17 and IL-6, blocking the activation of NF-kB, thus reducing tumor development (14), and similarly, the inflammatory response associated with the suppression of NF-kB activation in breast cancer cells. Recently, we reported that Met reduces the expression of MMP-2 and MMP-9 by blocking-translocating NF-kB from the cytosol to the nucleus and showing the anti-proliferative effect in MCF-7 cancer cells (15), and Met induces cell cycle arrest in primary breast cancer cells through upregulation of P53 whereas downregulation of cyclin D1 in an AMPK-independent pathway (16).

Exploring protein-protein interaction networks unveils and gives a solution to understand the molecular mechanisms and systems biology (17). The proteomics approaches based on mass spectrometry take a significant part in the visualization of these network studies through statistical quantification using bioinformatics tools, and elucidating drug pharmacokinetics, and identifying protein targets. The protein profile investigation is critical in revealing the function and interaction of proteins through the discovery of nascent proteins, drug development, and comparison with the previously identified information in databases to help recognize new target proteins.

In this paper, we explored the effect of Met on protein expression profiles in breast tumor cells using LC-MS/MS technology and provided the outcomes visually understandable by using the current proteomic databases and algorithms as shown workflow in Figure-1. Our results have offered a proteomic variability of Met, which also demonstrates therapeutic mechanisms and target proteins when applied to breast tumor cells and normal breast cells.

MATERIALS and METHODS

Experimental Medication and Dose Measurements

Metformin (1,1-Dimethylbiguanide hydrochloride) Molecular weight: 165.62 g/mol, H10000691. 100 mM metformin was prepared using 50 mL DMEM/F-12. The stock solution was stored at 4°C. Experimental doses (5, 10, 25 mM) were diluted from the prepared stock solution and measured in 24-well plates.

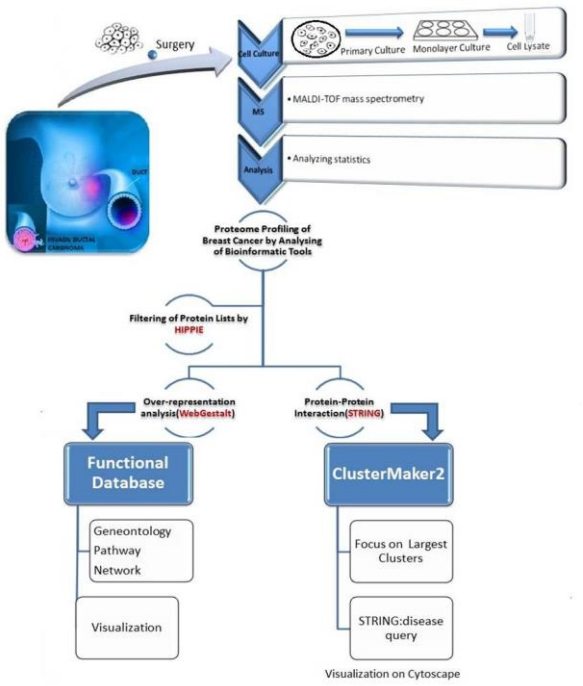

Figure-1. The study was composed of two main parts as in vitro and in silico analysis.

Primer culture conditions

We acquired breast cancer cells from five human donors (aged 45–55) that underwent biopsies of breast tumors in the the Department of General Surgery of Cerrahpasa Faculty of medicine in Istanbul University. The acceptance criteria were being in the post-menopausal period and having breast cancer with estrogen and progesterone positivity but HER2 negativity. The patients with coexisting other cancers, any chronic diseases, or having any of the BRCA1 and BRCA2 gene mutations were discarded from the study. The protocol was approved by the Ethics Committee of Clinical Research Center of Cerrahpasa Faculty of medicine in Istanbul University, No:83045809- 604.01.02-257, 133). All of the experimental procedures were performed according to the Declaration of Helsinki. Informed consent was taken from all individual participants included in the study.

Breast tissues were cut in small slices, placed in cell culture flasks pre-coated with fetal calf serum, and incubated for 2 weeks in DMEM/F12 medium (Wisent Bioproducts, Quebec, Canada) supplemented with 10% fetal bovine serum (Wisent Bioproducts, Quebec, Canada), 100 U/mL penicillin (Wisent Bioproducts, Quebec, Canada), and 100 μg/mL streptomycin (Wisent Bioproducts, Quebec, Canada) at 37°C in 95% humidified air with 5% CO2. As the formation of monolayers of primary cancer was observed in flasks, cells were subcultured every 7th-day using trypsin. The number of cells from the 3th-8th passages was calculated as 300 000 cells/ml.

Determining the effective dose by cell viability and proliferation test

Proteomics Analysis

Sample preparation and protein extraction and for LC-MS/MS analysis

Cells planted for the protein analysis study were incubated for 24 h at 37°C by adding two doses of Met (0 mM and 25 mM). The dose optimization of Met was determined in our previous reports (16,18). At the end of 24 h, the medium on the cells was discarded, the cells were washed twice with PBS, trypsinized, re-washed 2 times with cold PBS, and the cell pellets were eventually transferred to + 4°C to be used within the study. For protein extraction, the cell pellet was mixed with the Universal Protein Extraction (UPX) Kit (Expedeon-44101) and the protease inhibitor cocktail (Thermo Sci.-87785). The samples were sonicated for 5x10 sec cycles and a 30-sec pause between these cycles. After sonication, the samples were kept on ice and cooled, and then boiled at 95 °C for 10 min through stirring at 1000 rpm. The samples were then centrifuged at 14,000 rpm for 10 min. The supernatants were discarded and the pellets were transferred to the new tubes. Peptide recovery was performed using FASP Protein Digestion Kit (Expedeon-44250) and the trypsin enzyme (Pierce-90057). Samples were then diluted with 0.1% formic acid so that the final concentration was 200 ng / µL. The samples were in turn transferred to the device-specific tubes.

LC-MS/MS analysis and data processing

Before the analysis, detector and calibration settings were conducted through the MassLynx program (V4.1-Waters), which is specific to the Xevo G2-XS Q-TOF (Waters) device on which the analysis was performed. The method was switched to SONAR and sensitivity mode and the tryptic peptides formed were fractionated with acetonitrile gradient on the HSS T3 column (Waters-186008818) according to their hydrophobicity. By increasing acetonitrile in the range of 5%-35%, the peptides were separated from the column and the result of electrospray ionization was analyzed in mass spectrometry. During the analysis, data were collected for peptides that could be identified in the m/z 50–1950. The amino acid sequence was gathered by implementing the MS and MS/MS functions over

0.7s periods. 100 fmol/ul Glu-1-fibrinopeptide B was operated as the lock mass calibrate.

Statistical analysis

Human protein sequence information in the UniProt protein database was used for protein identification. Statistical analysis was performed using Progenesis QI-P software (Waters-2018). The relative expressional rate of proteins was filtered by ANOVA p-value ≤ 0.05 and protein expression variation with statistical significance was evaluated as minimum fold change >2 in t-test statistical analysis. Glu-1-fibrinopeptide B peptide with m/z 785.8426 was set as a celebrant and normalization of the samples were conducted based on the total ion intensity. The power scores, which are calculated using Progenesis QI-P internal parameters, above 0.5 (50%) were admitted as a filter for multiple comparisons. For identified 8 proteins, please (see Table-1).

Bioinformatics Analysis

Databases and Preprocessing steps

A list of proteins was generated from the Uniprot database (19) following the statistical evaluation of mass spectrometry results (Table-2). These proteins were subjected to Human Integrated Protein-Protein Interaction rEference (HIPPIE) to enrich and detect a potential protein network. The interaction type of Association, Physical, and Direct, and Colocalization, tissue as Breast - Mammary Tissue are filtered, and confidence level was set up to medium (0.63). HIPPIE (20) is a very useful tool as it combines many experimentally approved databases including BIOGRID (21), MINT (22), and IntAct (23) databases.

Over-representation Analysis

We subjected the official gene names of the protein list from the HIPPIE into the WebGestalt (24) (WEB-based Gene Set Analysis Toolkit). As well known, the WebGestalt server is a common tool for the functional enrichment analysis of gene lists. We implemented an approach through geneontology and pathway analysis methods such as KEGG (25), Wikipathway (26), and Panther (27) which is namely the Over Representation Analysis (ORA) (28) to analyze the gene list in the WebGestalt. Illumina humanwg 6 v3 was selected as the Reference set, and all other advanced parameters were left at their default. Besides, GeneMANIA (29) was used to expand the gene list with functionally similar genes of the proteomics data.

String Database and Visualization on Cytoscape

We used the StringApp program (30), one of the most prominent data sources of networks being a Cytoscape application for both visualization and the analysis of protein networks on Cytoscape (31). Then, another Cytoscape application-the clusterMaker2 (32), that implements numerous clustering algorithms were used (clusterMaker2 version 1.3.1) to implement Markov clustering (MCL) (33), thus, to evaluate the protein network and determine the largest cluster. To run MCL on Cytoscape, the inflation value was set to 2.3, the array sources were adjusted to use the STRING confidence score, and MCL advance settings were left at their default. The *STRING: Disease query* tool was run to compare our result of protein network and output protein network of invasive ductal carcinoma. The *STRING Disease query* was set to the maximum of 240 proteins and a cut-off value of 0.50. Similarly, the confidence (score) cut-off value and maximum additional interactions were set to 0.50 and 0, respectively.

Table-1. Significantly changed protein description list.

Accession	Unique peptides	Anova (p)	Max fold change	Description
P41250	2	0.02256881	5.675406671	(GARS) Glycine tRNA ligase
Q9UNL2	1	0.03119226	15.91006341	(SSRG) Translocon-associated protein subunit gamma
Q8WTV1	1	0.03321887	9.442900878	(THAP3) THAP domain-containing protein 3
P02794	1	0.03579029	5.501142287	(FRIH) Ferritin heavy chain
P07197	4	0.04782681	41.2040797	(NFM)Neurofilament medium polypeptide
P39687;Q92688	3	0.04916017	15.86841797	(AN32A) Acidic leucine-rich nuclear phosphoprotein 32 family member A
P08729	10	0.04952918	6.640075817	(K2C7) Keratin type II cytoskeletal 7

Table-2. The list of the proteins retrieved information from the Uniprot database.

Swiss-Prot Accession Number	Official gene names	Function	Sub-cellular localization	Pathology	Post-translasyonel modification	Structure
P41250	GARS	Catalyzes the ATP-dependent ligation of glycine to the 3'-end of its like tRNA through the creation of a Gly-AMP	Cytoplasm, axon	Neurodegeneration, Neuropathy, Charcot-Marie-Tooth	Acetylation, Phosphorylation	X-ray, PDB:2PME
Q9UNL2	SSR3	Regulation of the retention of ER-localized proteins by binding Calcium to the ER membrane	Endoplasmic reticulum membrane	Mutations involved in many sorts of cancer	Phosphorylation, Acetylation	None
Q8WTV1	THAP3	To regulate the transcriptional activity of RRM is needs to be a part of a THAP1/THAP3-HCFC1-OGT complex.	Nuclear chromatin	Mutations associated with many types of cancer	Phosphorylation	The experimental structure is unavailable
P02794	FTH1	Possesses ferroxidase activity, takes a part in the delivery of iron to cells as well, and is essential for iron homeostasis.	Cytosol, Lysosome, Nucleus	Hemochromatosis type 5, and mutations in many cancer types	Acetylation	X-ray, PDB:1FHA
P07197	NEFM	It is a neurofilament containing three intermediate filament proteins.	Cytoskeleton	Mutations associated with many types of cancer	Acetylation, Methylation	The experimental structure is unavailable
P39687	ANP32A	Involved in several cellular processions, repression of transformation, inhibition of phosphatase 2A, regulation of mRNA and takes a part in the suppression of E4F1-mediated transcriptional.	Cytoplasm, Endoplasmic reticulum, Nucleus	Mutations associated with many sorts of cancer	Phosphorylation	X-ray, PDB:2JE0
Q92688	ANP32B	Working as a cell cycle factor in G1 to S phase and cell survival factor, a caspase-3 inhibitor as anti-apoptotic protein.	Nucleus	Mutations linked to many sorts of cancer	Acetylation	NMR, PDB:2ELL
P08729	KRT7	Involved in blocking interferon-linked interphase and stimulating DNA synthesis.	Cytoplasm, Nucleus	Mutations linked to many types of cancer	Dimethylation, Acetylation	X-ray, PDB:4XIF

RESULTS

Our approaches in bioinformatics analysis are briefly presented in Figure-1. We expanded the protein list through statistically significant MS protein results, and 241 nodes and 270 edges with experimentally validated for functional enrichment analysis using the HIPPIE bioinformatics tool. Figure-2 represents the interaction through the source to the target protein. According to Figure-2, ANP32A, FTH1, THAP3, KRT7, ANP32B, NEFM, and SSR3 proteins were upregulated whereas GARS protein was downregulated under 25 mM Met-dose compared to Met-free breast tumor cells (see Figure-2).

We used MCL clustering to make the protein network easier to understand. In the largest cluster, String Functional analysis was acquired by Markov clustering. After performing clustering, we solely focused on determining whether there is interaction with the largest and small clusters of our downregulated proteins and GARS in Figure-3.

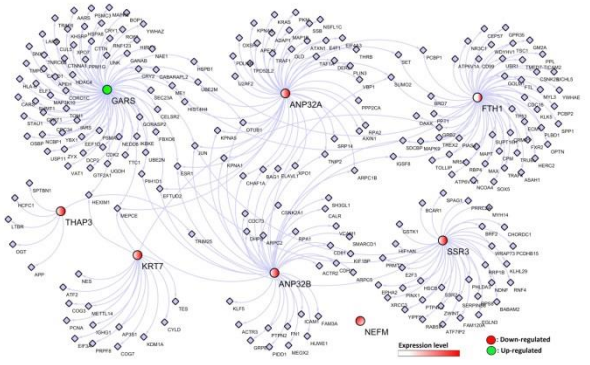

Figure-2. Enriched protein profiles in breast tumor cells with 25mM Met-dose compared to Met-free breast tumor cells. Only GARS protein (green node) was observed to be up-regulated, whereas the other red nodes show the down-regulated proteins (p<0,05).

Among the down-regulated proteins, ANP32A and THAP3 are in the largest cluster, while other proteins are in small clusters and indicated in larger font sizes (see Figure-3).

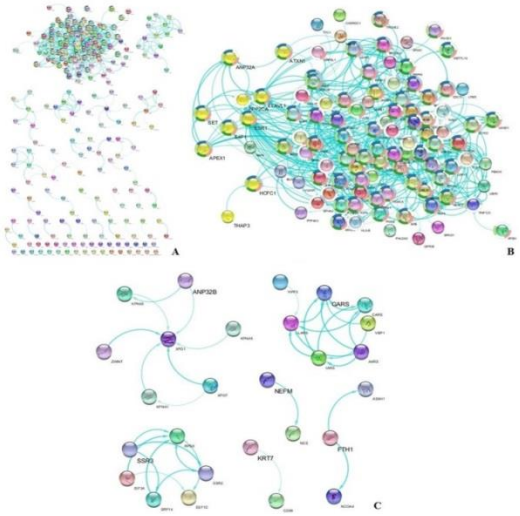

Figure-3. A. The visualization of the protein network related to breast cancer cells using Markov (MCL) in Cytoscape.

B. The largest Cluster in the Network. C. The small clusters in the Network after performing the MCL. The names of proteins from the MS output are represented in larger font sizes (GARS, SSR3, THAP3, FTH1, NEFM, ANP32A, ANP32B, KRT7).

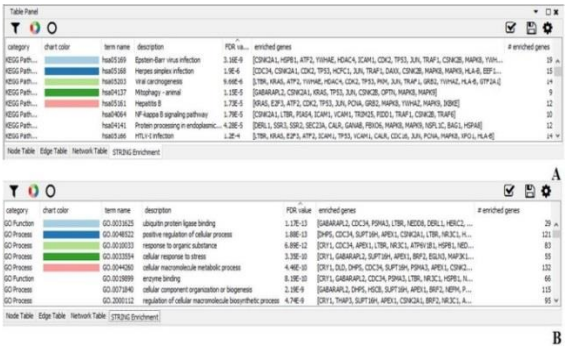

Figure-4. Functional enrichment analysis of the small and largest clusters acquired by using MCL clustering. A. KEGG pathway, B. Gene Ontology (GO Function, GO Process). FDR values are ranked significantly. The results were filtered by a redundancy cut-off value of 0.05 in the STRING enrichment table.

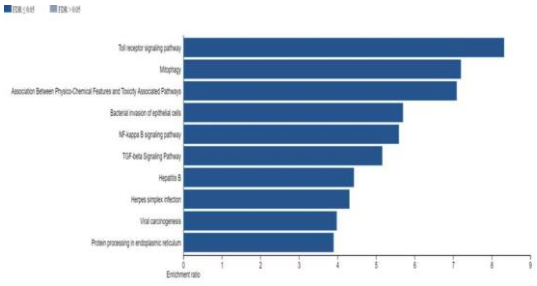

Figure-5. The WebGestalt result of GARS protein and the down-regulated proteins and their relationships with the pathways(KEGG, Wikipathway, Panther) in breast tumor cell at 25 mM Met-dose (FDR≤0.05).

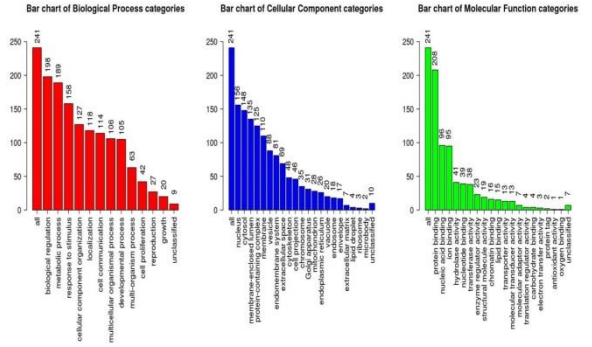

Figure-6. Each Biological Process, Cellular Component, and Molecular Function category is represented by a red, blue, and green bar, respectively. The height of the bar represents the number of IDs in the user list and the category.

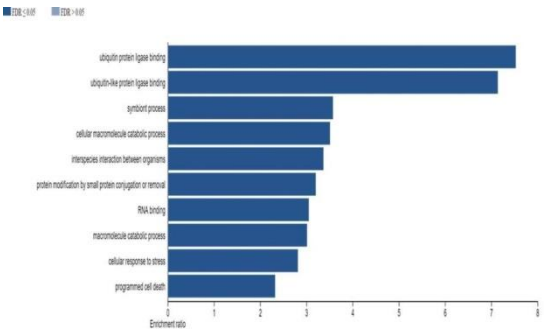

Figure-7. The WebGestalt result of GARS protein and the down-regulated proteins and their relationships with the Geneontology (Biological process and Molecular Function) in breast tumor cell at 25 mM Met-dose (FDR≤0.05).

We detected that functional enrichment analysis of Geneontology and Pathway from STRING and WebGestalt are the same description in the string enrichment table in Figure-4 and bar graphs in Figure-5 and Figure-7. Furthermore, we retrieved 100 proteins in the network associated with invasive ductal carcinoma by importing them from the STRING: disease database. Then, we identified both networks of enrichment protein in string query (240 nodes) and the disease network (240 nodes) in STRING by using the Merge Networks tool in Cytoscape. As a result of the network (not shown here), 6 proteins (ESR1, KRT7, TP53, JUN, KRAS, MAPK8) were found to be associated with the Disease network. As a last one, we summarized bar graphs in Figure-6 from our results as Geneontology categories in WebGestalt.

ESR1 and JUN have four edges (interaction) by GARS, ANP32B, ANP32A, and SSR3 proteins, whereas KRT7 has three edges by CYLD, ATF2, KDM1A; and THAP protein has three edges by APP, OGT, and LTBR. All of these proteins (ESR1, JUN, KRT7, CYLD, ATF2, KDM1A, APP, OGT, LTBR) are known to have roles in programmed cell death (see Figure-7). Besides, a total of 62 nodes from the network is related to programmed cell death (p-value: 1.1314e-10 in Figure-7). TRIM25 has two edges by SSR3 and THAP domain-containing 3 (THAP3), and is related to NF-kappa B signaling pathway. 10 nodes are involved in NF-kappa B signaling pathway (p-value: 0.000010805 in Figure-5). No interaction of NEFM was determined in the enriched proteomic profile of the Breast tumor cells although in vitro analysis NEFM was observed to be down-regulated in Met treated breast cancer group. Moreover, it takes part in Association Between Physico-Chemical Features and Toxicity Associated Pathways via Wikipathway database by nodes of ACTR2, ACTR3, AXIN1, FN1, GRB2, JUN, MAPK, PPP2CA in Figure-5 (p-value: 0.0000041950).

In the pathway analysis, we found mitophagy-associated proteins such as CSNK2A1, CSNK2B, GABARAPL2, JUNE, MAPK8, MAPK9, OPTN, TP53, and KRAS (GTPase KRas) nodes through KEGG (Figure-5) (p-value: 0.000003). According to the in vitro outcomes, down-regulated FTH1, and upregulated protein GARS had the most interactor nodes with these mitophagy-associated proteins. As summarized in Table-2, ANP32A is involved in several processes in the cell in addition to interacting physically with KRAS.

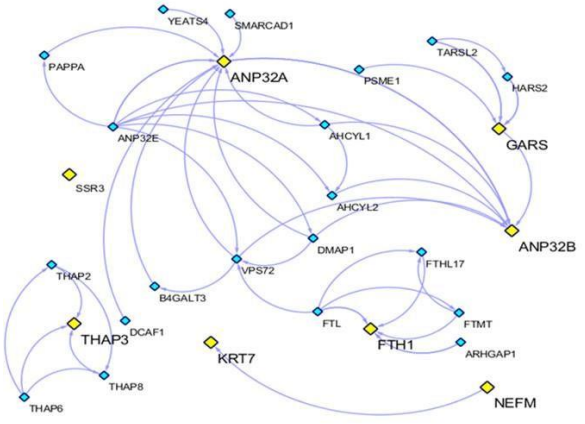

Figure-8. The functional enrichment (physical interaction, co-expression, shared protein domains) analysis in GeneMANIA. Yellow nodes in the network are the gene lists.

Moreover, as shown in Figure-8, the interaction of NEFM and KRT7 shared protein domains from PFAM. Figure 8 represents the expanded gene list with functionally similar genes of the proteomics data obtained using GeneMANIA (29). GARS and ANP32B proteins and KRT7 and NEFM proteins have direct interactions with each other. Similarly, ANP32A and ANP32B have not only a direct interaction but also shared protein domains. While SSR3 has no interactions or shared domains with these proteins, THAP3 is shown to have interactions with THAP family proteins but no other type of proteins (Figure-8).

DISCUSSION

The widespread use of metformin worldwide and its efficiency on individuals need to be extensively figured out. Met has been accepted to have a relative safety profile compared to other antidiabetic drugs (6). Met possesses its cell growth inhibition ability through triggering apoptosis in cancer cells such as glioma and triple-negative breast cancer cells (34, 35). However, the full mechanism by which Met is beneficial to cancer treatment is not thoroughly understood yet. In this study, we employed primary breast cancer cell culture and protein extracts for proteomics processes to elucidate previously unreleased results behind metformin's beneficial effect on invasive ductal carcinoma treatment and protein-targeted studies in future studies.

Protein network theory is a pivotal method of choice to figure out the protein interaction and function, subcellular components in the cell to analyze the proteomics data. THAP3 is a subunit of a THAP1/THAP3-HCFC1-OGT complex that regulates the transcriptional activity of RRM1 which was approved to be a drug target in human protein atlas by the FDA (36).

Concerning the in vitro results, FTH1 and GARS proteins have the most interactor nodes with mitophagy-associated proteins. Mitophagy is specific autophagy, targeted for the degradation of mitochondria (37). Surprisingly, it is thought to play two roles in carcinogenesis depending on the tumor type and stage (38). In general, mitophagy targets to remove malfunctioning mitochondria to cope with oxidative stress and block carcinogenesis. Nevertheless, it can preserve cells from cell death and support the survival of tumor cells under unfavorable conditions such as insufficient nutrients or hypoxia (38). That is, as the level of mitophagy in

a cancer cell increases, the ROS ratio decreases and it becomes resistant to treatment with healthy mitochondria. During anti-cancer treatment, therefore, the decrease in the level of mitophagy makes it more sensitive to the treatment in the cancer cell. Thus, mitophagy appears to be a featured quality control factor in target-based prevention of cancer (39).

Down-regulated ANP32A protein, for example, is known to be part of various processes including physical interaction with the KRAS gene. KRAS considering the main target in anticancer drug discovery (40), takes a significant part in the regulation of cell proliferation and oncogenic events (41).

NEFM (Neurofilament medium polypeptide) is mostly a cytoplasmic protein expressed in CNS and peripheral nerves. Interestingly enough, although NEFM is known to be expressed in brain cells, it has the most value in fold change compared to the control (Met-free) group in our statistical conclusion. Tyanova et al. similarly reported high NEFM expression levels in Breast cancer proteomic data in Expression Atlas (42).

We need to determine the interactions of expressed proteins with each other to understand cellular function, thus, the system biology. The STRING database integrates known and predicted protein-protein interactions physically and functionally, collecting proteomics data from the public and visualizing them by scoring. STRING utilizes famous categorizing systems including Gene Ontology and KEGG for enrichment analysis (43).

CONCLUSION

The molecular characterization of breast cancer has become revolutionary for therapeutic approaches. The fact that breast cancer has a multifactorial feature requires RNA and DNA analysis with its microarray and sequencing techniques almost constantly in molecular size. The obvious advances in proteomic technologies have made substantial progress compared to the past and have now increased the profiling of clinical specimens and their accuracy in identification and quantification. Molecular characterization, such as proteomic profiling, will be of great importance for breast cancer person-oriented treatment, as it is target-oriented in developing existing therapy options. Although the mRNA profile is predominant in this characterization, analysis of high-tech proteome MS data provides a versatile approach to the protein profile of the disease, the classification of subtypes, application of protein-targeted therapies under system biology, and the effective treatment of the findings.

Here, we analyzed the change of metformin, which is relatively safe and effective compared to chemotropic agents, economically appropriate, to the protein profile in breast tumor cells. Our analysis supports the fact that metformin possesses anti-cancer features with changes in proteomic pathways on breast cancer. With the study findings we designed, metformin may contribute to its potential therapeutic effect in breast cancer treatment. However, metformin as a cancer-targeted agent and its mechanism of action in proteomic pathways requires further supportive investigations to be fully understood.

Conflict of interest: The authors declare that no conflict of interest.

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