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REPUBLIC OF TURKEY

HACETTEPE UNIVERSITY

INSTITUTE OF HEALTH SCIENCES

EVALUATION OF METADHERIN EXPRESSION IN BREAST CANCER

PhD Mohammad Azim Ebrahimi

Tumor Biology and Immunology Program

DOCTOR OF PHILOSOPHY THESIS

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APPROVAL PAGE

Evaluation of Metadherin Expression in Breast Cancer

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This thesis study has been approved and accepted as a PhD dissertation in "Tumor Biology and Immunology Program" by the assesment committee, whose members are listed below, on 29 May 2018.

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In this thesis study, I declare that all the information and documents have been obtained in the base of the academic rules and all audio-visual and written information and results have been presented according to the rules of scientific ethics. I did not do any distortion in data set. In case of using other works, related studies have been fully cited in accordance with the scientific standards. I also declare that my thesis study is original except cited references. It was produced by myself in consultation with supervisor (Prof. Dr. A. Lale Doğan) and written according to the rules of thesis writing of Hacettepe University Institute of Health Sciences .

Mohammad Azim Ebrahimi

ACKNOWLEDGEMENTS

On the way of this study, my thesis supervisor Prof. Dr. Lale Dogan has devoted great efforts and provide me with her valuable consultations and advices due to that I am indebted to deeply appreciate her for the offered help and encouragement she had given me.

I would like to express my deep appreciation to Prof. Dr. Emin Kansu who has provide me the facilities to complete my post graduate studies in the Department of Oncology and obtain the degree of PhD and also he provide me with his valuable advices and consultations along this study and guide me in very smooth manner due to that I am indebted to highly appreciate him.

I am really grateful to Prof. Dr. Dicle Guc, Dr. Hande Canpinar, Prof. Dr. Gunes Esendagli, Assoc. Prof. Dr. Fusun Ozmen, and Prof. Dr. Aysegul Uner for all their valuable lectures and support to me.

Dr. Burcu Sirin has helped and directed me in case of experiment and laboratory affairs and I thank to her for such valuable help and sympathy. Dr. Altug Kesikli has also given me valuable advices at the beginning of my studies and due to that I thank him so much.

I thank to all the members of the Basic Oncology Department for their friendship, support, share of knowledge and help.

The other smooth and well-behaved members of the Department as Semra Solmaz and Canib Ulubey were so cooperative and friendly and helped me in any case according to their authority and so I am indebted to appreciate them.

Lastly in top of the above excellency members of the Basic Oncology Department, Mustafa Emre Gedik was highly appreciated due to his valuable assistance in case of technical issue of my experimental studies. He made him always available for my experiment in the laboratory and helped me technically I am much indebted to thank him a lot.

Last but not least, I would like to thank my spouse, Bi Bi Noor, who had taken care of my family in the absence of myself. She had looked after our childrens' education and supported them in any hard condition. I would like to dedicate this thesis to her.

ABSTRACT

Ebrahimi, MA. Evaluation of Metadherin Expression in Breast Cancer. Hacettepe University Institute of Health Sciences, Ph.D. Thesis in Tumor Biology and Immunology, Ankara, 2018. *Metadherin* gene (*MTDH*/ *AEG-1*/ *Lyric*) encodes a 582- aminoacid protein, metadherin (MTDH), which is almost undetectable by immunohistochemistry in normal breast tissue. MTDH expression is markedly increased in breast cancer cell lines as well as breast cancer patients which is found to be positively correlated with poor prognosis. The constitutive activation of PI3K/Akt/mTOR pathway triggers MTDH expression via GSK3β inactivation. In this thesis, the effect of PI-103 (dual PI3K/mTOR inhibitor) on MTDH expression in HER2+ breast cancer cell lines was investigated using qPCR and Western Blot. The results indicated different levels of inhibiton for MTDH expression in HER2 overexpressing cells. In MDA-MB-453 cells, incubation with PI-103 decreased MTDH mRNA and protein level significantly ($p<0.05$) which was considered in quite accordance with Akt dependency of these cells. MTDH mRNA expression decreased in SKBR-3 cells after 8 hours following a slight increase in 24 hours. The protein expression level was also affected moderately from PI-103. Taking into consideration the remarkable PTEN expression in these cells, we may speculate that decreased response to drug inhibitory effect of PI-103 may be attributed to the inverse corrrelation between MTDH protein level and PTEN activity. Finally, BT474 cells did not show significant change for MTDH at mRNA and protein level. In addition to cell lines, serum MTDH level of breast cancer patients (n=80) was evaluated by ELISA. Among molecular subtypes, HER2+ patients (n=9) had significantly high level of MTDH compared to patients diagnosed as Luminal A (n=20), and TNBC (n=32) ($p<0.05$) . There was no significant difference between HER2+ and Luminal B (n=19) subtypes. MTDH level was found significantly high in metastatic breast cancer (n=13) compared to nonmetastatic group (n=66) ($p<0.01$). In conclusion, PI3K/Akt targeting therapeutic strategies in HER2+ breast cancer may be involved in suppression of invasion and metastasis through regulation of MTDH expression.

Key Words: Metadherin, breast cancer, PI3K, mTOR, PI-103

ÖZET

Ebrahimi MA. Meme Kanserinde Metadherin Ekspresyonunun İncelenmesi. Hacettepe Üniversitesi Sağlık Bilimleri Enstitüsü, Tümör Biyolojisi ve İmmünolojisi Doktora Tezi, Ankara, 2018. *Metadherin* geni (*MTDH/ AEG-1*/ *Lyric*) 582 aminoasit uzunluğunda bir protein olan metadherini (MTDH) kodlamaktadır. Normal meme dokusunda immünohistokimya yöntemiyle ölçülemeyecek kadar az olan MTDH ekspresyonu meme kanseri hücre hatlarında ve meme kanseri hastalarında belirgin olarak artmaktadır ve MTDH düzeyi kötü prognoz ile doğru orantılıdır. Bu tezde, HER2+ meme kanseri hücre hatlarında dual PI3K ve mTOR inhibitörü olan PI-103'ün MTDH ekspresyonuna etkisi qPCR ve Western Blot yöntemleri ile incelendi. Sonuçlarımız, HER2 ekspresyonu artmış olan meme kanseri hücrelerinde farklı düzeylerde MTDH inhibisyonu olduğunu ortaya koydu. MDA-MB-453 hücrelerinde, PI-103 inkübasyonu MTDH mRNA ve protein düzeyini anlamlı olarak azalttı ($p<0.05$). Bu sonuç, hücrelerin Akt bağımlılığı ile oldukça uyumlu olarak değerlendirildi. SKBR-3 hücrelerinde, MTDH mRNA ekspresyonunda 8 saat sonra gözlenen azalmayı 24 saat sonunda hafif bir artış takip etti. Protein ekspresyonu da PI-103'ten orta düzeyde etkilendi. Bu hücrelerdeki belirgin PTEN ekspresyonunu dikkate aldığımızda, PI-103'ün inhibitör etkisine karşı düşük yanıt alınmasının, MTDH protein düzeyi ile PTEN aktivitesi arasında ters yönde korelasyonun bulunması ile bağlantılı olabileceğini öne sürebiliriz. Son olarak, BT474 hücreleri, mRNA ve protein düzeyinde anlamlı bir değişiklik göstermedi. Hücre hatlarına ilave olarak, meme kanseri hastalarının serumlarındaki (n=80) MTDH düzeyi ELISA yöntemiyle incelendi. Moleküler alttipler arasında, HER2+ hastaların (n=9) MTDH düzeyi, diğer alttipler olan Luminal A (n=20), ve TNBC (n=32) tanısı alan hastalarla kıyaslandığında anlamlı olarak yüksek bulundu ($p<0.05$). HER2+ ve Luminal B (n=19) alttipleri arasında anlamlı fark bulunmadı. MTDH düzeyi, metastatik meme kanseri grubunda (n=13), nonmetastatik grup (n=66) ile karşılaştırıldığında, anlamlı olarak yüksek saptandı ($p<0.01$). Sonuç olarak, HER2+ meme kanserinde PI3K/Akt yolağını hedefleyen terapötik stratejiler MTDH ekspresyonunun regülasyonu aracılığıyla invazyon ve metastazın baskılanmasında rol oynayabilirler.

Anahtar kelimeler: Metadherin, meme kanseri, PI3K, mTOR, PI-103

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LIST OF ABBREVIATIONS

ACR	Adaptor Connector Region
AEG-1	Astrocyte elevated gene-1
AML	Acute Myeloid Leukemia
AR	Amfiregulin
ATP	Adenosine Triphosphate
BAD	Bcl-2 Associated death promoter
BAX	Bcl-2 Associated x protein
Bcl-2	B-cell lymphoma-2
Bcl-XL	B-cell lymphoma extra large
BH3	Bcl-2 Homologous domain3
BRAF	B-Raf proto-oncogene, serine/threonine kinase
BTC	Betacelluline
CDC42	Cell division cycle 42
CDK4	Cycline depending kinase4
Chk	Checkpoint kinases
CK5/6	Cytokeratin5/6
Crk	Adaptor molecule crk
CTMP	Carboxy 3 modulator protein
EGF	Epidermal growth factor
EGFR	Epidermal growth factor receptor
elF4E	Eukaryotic translation initiation factor 4E
EPR	Epiregulin
EPG	Epigen
ErbB	Avian erythroblastosis oncogene B
ErbB1	Avian erythroblastosis oncogene B1
ErbB2	Avian erythroblastosis oncogene B2
ErbB3	Avian erythroblastosis oncogene B3
ErbB4	Avian erythroblastosis oncogene B4
ER	Estrogen receptor
FDA	Food and Drug Administration
FGFR	Fibroblast Growth Factor Receptor

FKBP12	Peptidyl-prolyl cis trans isomerase12
FOXO	Fork head box protein
FYVE	Fab 1,YOTB, Vac 1, and EEA 1 zinc finger domain
Gab1	Grb2-related binding protein-1
GFR	Growth factor receptor
GPCR	G protein couple receptor
Grb2	Growth factor receptor binding protein 2
GSK-3β	Glycogen synthase kinase -3β
GTP	Guanosin triphosphate
HB-EGF	Heparin binding epidermal growth factor
HER1	Human epidermal growth factor receptor 1
HER2	Human epidermal growth factor receptor 2
HER3	Human epidermal growth factor receptor 3
HER4	Human epidermal growth factor receptor 4
HRAS	Harvey sarcoma virus oncogene homologue
iGF-1R	Insulin like growth factor 1 receptor
IgG1	Immunoglobulin G1
IR	Insulin receptor
IRS	Insulin receptor substrate
JAK	Janus kinase
KRAS	Kirsten sarcoma virus oncogen homolog
LRR	Lucien rich repeat
LYRIC	Lysine-rich CEACAM1 co-isolated protein
MAPK	Mitogen-activated protein kinase
Mcl-1	Myeloid leukemia cell differentiation protein-1
MDM2	Mouse double minute 2 homolog
MTDH	Metadherin
mTOR	Molecular target of mammalian / rapamycin
mTORC1	Molecular target of mammalian / rapamycin complex-1
mTORC2	Molecular target of mammalian / rapamycin complex-2
NF-kB	Nuclear factor kappa-light-chain-enhancer of activated B cells
NGF	Nerve growth factor

NRAS	Neuroblastoma viral –ras oncogene homolog
NRG	Neurogulin
PDK1	Phospho inositoted-binding kinase-1
PH	Pleckstrin homolog
PHLPP	PH domain and Lucien rich repeat protein phosphatases
PI-103	4-morpholinopyrido[3',2':4,5]furo[3,2-d]pyrimidin-2-yl)phenol
PI3K	Phosphotidil inositol-3-kinase
PIK3CA	Phosphotidil inositol-3-kinase Catalytic subunit alpha
PIP2	phosphatidil inositol 4,5-bisphosphate
PIP3	phosphatidil inositol 3, 4,5-bisphosphate
PKB	Protein kinase B (Akt)
PKC	Protein kinase C
PLCY	Phospholipase CY
PR	Progesterone receptor
PTB	Phosphotyrosine binding region
PtdIns	Phosphotidil Inositol
PTEN	Phosphatase and tension homolog defeated on chromosome 10
PX	Phox homology
Rac1	Ras-related C3 botulinum toxin substrate 1
Ras	Rat Sarcoma
Rheb	Ras homolog enriched in brain
RICTOR	Rapamycin-insensitive mTOR
RTK	Receptor Tyrosine kinase
S6K	Ribosomal protein S6 kinase
SGK	Serum and glucocorticoid inducible kinase
SH2	Src homology 2 domain
Shc	Src homology 2 domain inside protein
SHIP1/2	SH2 domain inside splitting phosphatase
Src	Sarcoma
STAT	Signal transducer and activator of transcription
TACE	TNF-α converting enzyme
TGF-α	Transforming growth factor alpha

TKi	Tyrosine kinase inhibitor
TSC2	Tuberous sclerosis protein 2
VEGF	Vascular endothelial growth factor
VEGFR	Vascular endothelial growth factor receptor

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1. INTRODUCTION

Breast cancer is the most frequent cancer among women and the second among all types of cancer (1). According to 2012 data, 1.67 million breast cancer cases have been observed worldwide. This situation constitutes 25% of all new cancer cases. Approximately 522 000 deaths has been determined due to breast cancer (2). Accordingly, breast cancer is the most common type of cancer that results in death among women (3).

The transformation of normal cells into malign phenotype is a multi-step process in all cancer types. This conversion is mediated mainly via signal transduction. This provides survival and growth advantage for malignant cells (4).

Signal transduction occurs with signals from outside of the cell. These signals are transported from outside to the interior via various receptor tyrosine kinases (RTKs) on the cell surface. Thus, by activating the signal pathways inside the cell, it regulates amplification, differentiation, development, survival and migration of the cells. Alterations in expression levels and/or regulation of pathways of receptor tyrosine kinases and molecules involved in signaling pathways contribute to the development of cancer (5).

Deregulation of PI3K signaling pathway is common in various human cancer types (6). The PI3K (phosphatidylinositol 3-kinase) protein family plays an important role in cell growth, survival, proliferation and metabolism control of cell (7). After binding of specific ligands (Growth Factors, Insulin, etc.) to the receptor tyrosine kinases located in the cell membrane, signal transduction mechanism becomes active. As a result of the receptor-ligand interaction, specific tyrosine residues are phosphorylated through the catalytic activity of tyrosine kinase domains of RTKs. This mechanism is called autophosphorylation. p85, the regulatory subunit of PI3K, binds to phosphotyrosine residues of actived RTKs. Consequently, inhibition of the catalytic subunit of PI3K known as p110 is terminated. In this respect, PI3K phosphorylates PIP2 (Phosphatidylinositol 4,5-biphosphate) into PIP3 (Phosphatidylinositol 3,4,5-triphosphate). PIP3 maintains intracellular signal

transduction by binding directly to PH (Plekstrin Homoly) domains of Akt and PDK1 (Phosphoinositide Dependent Kinase 1) molecules. PDK1 phosphorylates threonine 308 residue and mTORC2 (Mammalian Rapamycin Target Complex 2) phosphorylates the serine 473 residue of Akt for Akt activation (8, 9).

Genetic alterations of PI3K pathway in breast cancer cells can be sorted as EGFR, HER2 and HER3 RTK amplifications, PTEN mutation/loss and PI3K catalytic subunit (p110a) mutations. These changes lead to increased PI3K/Akt pathway activation and an oncogenic potential (10, 11). The PI3K/Akt/mTOR cascade controls the metabolism, proliferation, growth, survival and motility in the cell by transmitting the signal from the RTKs to the effector molecules. RTK mutation (eg EGFR), gene amplification (eg HER2), PI3K mutation and/or PTEN deletion are frequently seen in tumor cells. These changes lead to uncontrolled activation of the signaling pathway (12, 13). Preclinical studies in the literature show that continuous activation of PI3K/Akt/mTOR pathway is an important cellular signal pathway that plays a role in metadherin expression (14). Metadherin expression is known to be increased in many different types of cancer such as breast, prostate and liver (15). Like in other types of cancer, breast cancer is accompanied by an increase in metadherin expression and cells gain metastatic character and drug resistance (16, 17). It is also known that increased expression of metadherin has a correlation with poor prognosis (18, 19).

These changes in the cell allow to progress carcinogenesis and the escape of cells from the physiological control mechanisms. Various therapeutic strategies have been developed to intervene in the dysfunctions of the signaling pathway (20).

The use of therapeutic agents against to HER (Human Epidermal Growth Factor) receptors such as trastuzumab and lapatinib in breast cancer is quite common. Rapalog class inhibitors such as Rapamycin, Temsirolimus, Everolimus inhibit mTOR while MK-2206 is an Akt inhibitor. Buparlisib is agent used as PI3K inhibitor, and PI-103 is agent used as PI3K/mTOR dual inhibitor (20, 21). Targeted therapy aims to inhibit the effects of PI3K/Akt/mTOR pathway molecules, which cause cell proliferation, survival and motility. At this stage, it is necessary to develop different treatment strategies by combining more than one therapeutic agent in

situations that the cells exhibit poor sensitivity and high drug toxicity, which reduces drug efficacy (22, 23).

Dual PI3K and mTOR inhibitor PI-103 inhibit the activation of PI3K and mTOR complexes. PI-103 interacts with p110a, the catalytic subunit of PI3K, inactivating PI3K, while also blocking the activation of mTORC1 and mTORC2, which are complexes of mTOR (24-26).

Due to the lack of a targeted agent for metadherin, different signaling pathway inhibitors have been tested to suppress the increased level of metadherin. Clinical trials in various cancer patient tissue samples suggest that increased expression of cellular metadherin may be a prognostic factor (27-29).

The aim of this study is to evaluate the effect of PI3K/Akt/mTOR pathway inhibitor PI-103 on methaderin expression in HER2 overexpressing human breast cancer cell lines. The selected cell lines are, SKBR3 cells (PI3K and PTEN wildtype), BT-474 cells (PI3K wildtype, PTEN mutant) and MDA-MB-453 cells (PI3K and PTEN mutant) breast cancer cell lines.

In addition, the level of metadherin will be determined in the serum samples of 80 breast cancer patients via ELISA assay Methaderin level of patients will be analyzed for the correlation between protein level and breast cancer subtype, lymph node involvement and metastasis status. We aim to point out the importance of HER2 targeting strategies with respect to possible methaderin supression that may lead to benefit for therapy through decreasing invasion and metastatic potential of HER2 + breast cancer cells.

2. GENERAL INFORMATION

2.1 Breast Cancer

Breast cancer is morphologically homogeneous, but has a heterogeneous structure in terms of biological character and clinical course (30). In terms of gene expression profile, breast cancer is divided into four subtypes; Luminal A, Luminal B, HER2 + and Basal-like (Triple Negative Breast Cancer (TNBC)) (Table 2.1). These subtypes are determined according to the expression of ER (estrogen receptor), PR (progesterone receptor) and HER2 (Human Epidermal Growth Factor Receptor) results of immunohistochemical staining of pathological tissues. Luminal A and B subtypes, also called hormone receptor-positive breast cancer type, have a better prognosis than the other subtypes and the 5-year survival rate is 80-85%. Basal-like or Triple-negative breast cancer types are negative for hormone receptors and HER2, positive for CK5 / 6 (cytokeratin 5/6) or EGFR (31). It is generally associated with excessive PI3K activation and PTEN loss and it shows poor prognosis. HER2 + breast cancer type is an aggressive phenotype with poor prognosis. The mean survival time is shorter than the other groups (4, 32). Addition to conventional classifications, recent studies have shown that mutated genes in breast cancer cases have 10 different subtypes in terms of genomic and transcriptomic expression (33, 34).

Table 2.1. Molecular subtypes of breast cancer

No	Subtypes of Breast cancer	Most common profile	Prevalence (Approximate)
1	Luminal A	ER-positive and/or PR-positive • HER2-negative Low Ki67	30-70%
2	Luminal B	ER-positive and/or PR-positive HER2-positive (or HER2-negative with high Ki67)	10-20%
3	Triple negative/ basal like	• ER-negative • PR-negative HER2-negative	15-20%
4	HER2 type	• ER-negative • PR-negative HER2-positive	5-15%

As in all types of cancer, as in breast cancer, the transformation of normal cells into cancerous cells is a multi-step process (4). As a result of this process, the genetic changes that occur in the normal cell and give rise to malignant character to the cell (35, 36). Disruption or disappearance of the control of signaling mechanisms associated with growth, proliferation and apoptosis, which regulate cellular functions, is the basis of neoplastic cell transformation. The PI3K/Akt/mTOR

(Phosphatidylinositol 3-kinase/ Protein Kinase B/ Mammalian Target of Rapamycin*)* cascade, which is involved in the carcinogenic process and is a signaling pathway through growth factor receptors (GFR), plays an important role in breast cancer development (37).

2.2 Receptor Tyrosine Kinase (RTK)

Cells are stimulated directly with cell-to-cell interaction or secretion by signal molecules presented in the paracrine/autocrine model in all multicellular organisms. These signaling molecules, called ligands, are recognized by receptors located on the cell surface as transmembrane proteins. Intracellular signal transduction occurs as a consequence of specific ligand binding to the receptor. The signaling pathway activates the phosphorylation of intracellular proteins. In this way, gene expression change and biological response occur by the stimulation of the signaling molecule.

Receptor tyrosine kinases (RTKs), one of the cell surface receptor proteins, play an important role in the regulation of biological responses such as cellular survival, proliferation, differentiation, migration and cell cycle (38). RTK activations, cell surface distribution and regulation are shifted because of genetic changes and anomalies resulting from the exposure of the cell to various factors. Overactivation of intracellular signaling pathway through mutations that occur in RTKs creates various anomalies. At the same time, this situation triggers the carcinogenic process (39).

All RTKs have a ligand binding domain outside the cell membrane, a transmembrane helix in the region near the membrane (juxtramembrane), a tyrosine kinase domain in the cytoplasmic region, and a carboxy (C-) terminal region (5). All known receptor tyrosine kinase groups, except for the insulin receptor (IR) group, are found in the monomeric and inactive form on the cell surface (40) (Figure 2.1).

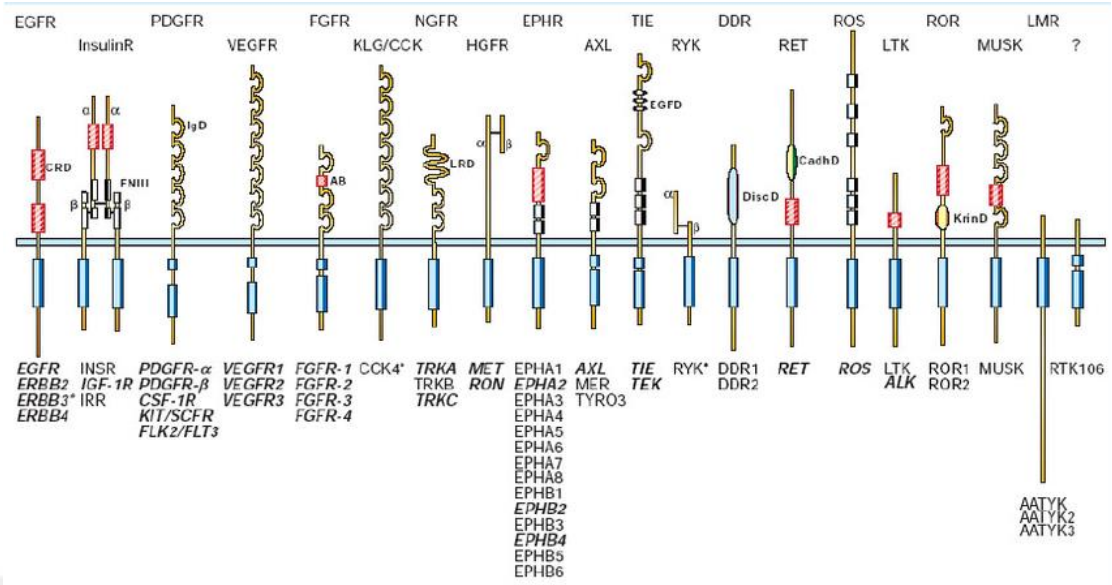


Figure 2.1. Subfamilies of Receptor Tyrosine Kinase (Blume-Jensen Hunter, 2001)

The presence of different modules such as immunoglobin-like domains, cysteine-rich domains, fibronectin type III-like domains and EGF-like (Epidermal Growth Factor-like) domains of RTKs outside the cell provides inter-group variability. This diversity allows specific binding of specific ligands to RTKs. Under favour of the transmembrane helix domain, RTKs are found attached to the cell membrane. The tyrosine kinase domain found in the cytoplasmic region constitutes catalytic activity and provides signaling pathway activation (41).

Activation of inactive form of receptor tyrosine kinases occurs in two different ways. Dimerization of RTKs occurs when polypeptide ligands bind spontaneously to the extracellular ligand binding domain of monomeric RTKs (5). After this phase, there are two basic requirements for RTK activation. The first is the catalytic activation of the receptor, and the second is the phosphorylation of the tyrosine residues, which will stimulate the pathway proteins. Conformational change occurs in the transmembrane helix and cytoplasmic region of the RTK construct with ligand stimulation. At this point, the Y-phosphate present in the ATP structure is catalyzed and transfer to the hydroxyl groups present in the tyrosine residue of the receptor (activation site) takes place. This is called autophosphorylation. Phosphorylated tyrosine residues allow binding of proteins with residues recognizing phosphotyrosine, such as SH2 (Src homology 2), SH3 (Src homology 3) and PTB

(Phosphotyrosine-binding). In this regard, receptor activation results in the phosphorylation of RTK specific signal transduction pathway cascade proteins (38, 40-44).

RTK is overexpressed as a result of over-activation and gene amplifications, which results in oncogenic fusion proteins, mutations or deletions. Excessive expression of RTKs is observed in cancer (45, 46). The most common RTK irregulations in breast cancer are observed in the EGFR tyrosine kinase family (47).

2.3 Epidermal Growth Factor Receptors (EGFR) and Regulation

EGFR (Epidermal Growth Factor Receptor), the first defined family of RTK superfamily, is also a prototypic receptor family that exhibits intrinsic protein kinase activity (48). Nobel prize-winning Rita Levi-Montalcini and Stanley Cohen in 1957, isolated from the tumor cell, showed that physiological development of the neonatal mice was accelerated and the eyes opened more quickly than usual with injection of NGF (49, 50). After these studies, Cohen and his team have managed to isolate EGF, the second polypeptide discovered. They have defined the EGFR with their work with Graham Carpenter (51, 52).

The ErbB (Avian erythroblastosis oncogene B, EGFR) group consists of 4 receptor tyrosine kinases. These are Epidermal Growth Factor Receptor (EGFR, ErbB1), HER2 (Human Epidermal Growth Factor Receptor 2, ErbB2), HER3 (ErbB3) and HER4 (ErbB4) (53). ErbB ligands can not bind directly to HER2. However, HER2 provides kinase activity through dimerization with other ErbB receptor family members. HER3 is capable of only activating the receptor in heterodimer form as it lacks kinase activity (54-56) (Figure 2.2).

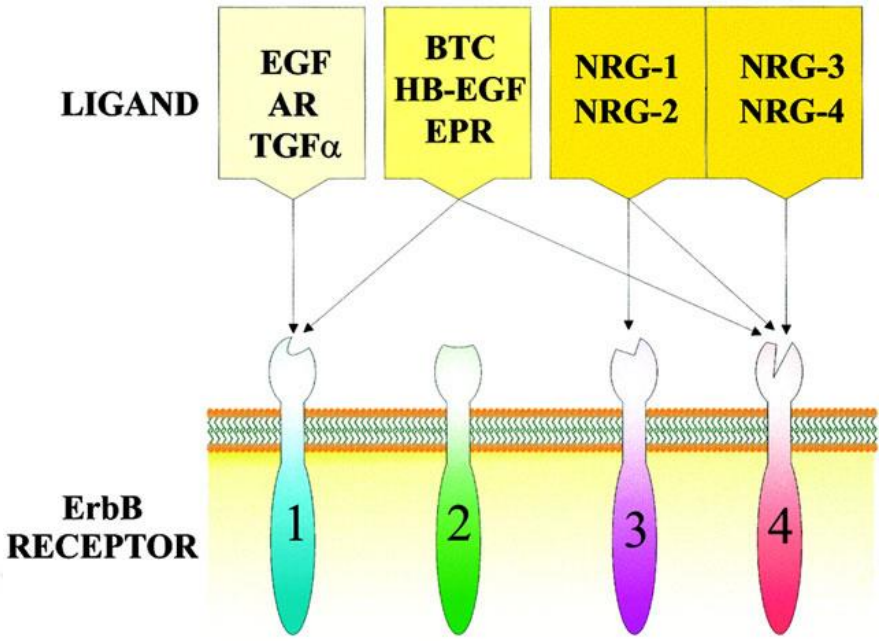


Figure 2.2 ErbB receptors (Olayiyole M., 2000)

There are 13 ligands identified up to now that can specifically bind to the EGFR family (57). They are divided into 3 groups. The first group of ligands is EGF, TGF-α (Transforming Growth Factor), AR (Amphiregulin) and EPG (Epigen) specifically to EGFR. The second group is BTC (Beta-cellulin), HB-EGF (Heparin-binding EGF) and EPR (Epiregulin) are dual specific ligands binding to both EGFR and ErbB4. The latter group is NRGs (Neuregulins) are ligands specific for both ErbB3 and ErbB4 (58, 59).

There are 4 domains (I-IV) in the extracellular domain of ErbB receptors. I and III domains of receptor are known as ligand binding domains. II and IV domains are known as the dimerization arm, which is provided by receptor-receptor interaction. The binding of specific ligands to the extracellular domain of EGFR, HER3 or HER4 triggers the formation of a hetero- or homo-oligomer kinase active form (54). Unlike the other ErbB receptor family members of HER2, there is no known ligand. I and III domains of HER2 region is close to each other makes it impossible to bind an EGF-related ligand. Furthermore, homodimerization of HER2 due to this particular structural form leads to electrostatic repulsion. This explains why HER2 should induce heterodimers for tyrosine activation (58, 60).

The ErbB receptor family activation model is the ligand-dependent activation model. After ligand binding, ErbB receptors form a homo or heterodimer. After the dimerization step, autophosphorylation of the tyrosine kinase domains of the cytoplasmic regions of the receptors takes place. Phosphorylated C-terminal tyrosine residues are specifically phosphorylated for attachment of adapter proteins, protein kinases or protein tyrosine phosphatases, which have SH2, SH3 and PTB domains. Activation of signal pathways takes place together with phosphorylation of intracellular mediator molecules. PI3K (phosphatidylinosithol-3 kinase) and Ras/MAPK (Rat sarcoma/mitogen activated protein kinase) pathways might be activated with all ErbB receptors. In addition to these, activations of JAK/STAT and PKC pathways may also occur (56, 60, 61).

2.4 Epidermal Growth Factor (EGFR) and Its Relation to Cancer

ErbB, which is an oncogenic retrovirus is known to be a family of receptors associated with cancer pathogenesis for more than 30 years (54, 62). Oncogenic transformation was observed both in the cell lines and primary human tumors resulting from increasing dimerization of ErbB receptors (56).

ErbB1 is the primary growth factor receptor responsible for the development of skin and squamous epithelium under normal physiological conditions. The other 3 members of the family play an important role in the development of the breast tissue as well as in the development of the cardiovascular system and the nervous system (57).

The role of ErbB2 or HER2 in cancer development was first found by isolating the mutant cDNA of mouse ortholog Neu oncogene from carcinogen-induced neuroblastoma. HER2/neu gene amplification is seen in about 30% of breast cancer casesa and is known to be associated with a poor prognosis. It is also known to play a role in the development of stomach, esophagus and over cancer (50, 57, 63). The HER2/neu gene locates in the 17q chromosome and is coded as transmembrane tyrosine kinase growth factor receptor (64). Approximately 100,000 HER2 receptors are found in a normal cell, whereas in a cancerous cell this number reaches approximately 2 million receptors (53, 57). High rates of aneuploidy,

somatic mutations, TP53 mutations, FGFR (Fibroblast Growth Factor Receptor), EGFR, CDK4 (cyclin dependent kinase-4) and cyclin D1 amplifications are seen in HER2+ tumors (53). The overexpression of ErbB2 triggers breast and over cancer formation and has a role on poor prognosis (59).

ErbB3 (HER3) differs from the other members of the ErbB family in two different discrepancies. First, HER3 has the defect of kinase domain. HER3 can not catalyzed the phospho-transfer reaction despite the ATP binding. Second, HER3 has 6 phospho-tyrosine domains in its C-terminal fragment, which is the strongest activator of the PI3K / AKT pathway (65). Because of the lack of kinase activation of HER3, HER3 homodimerization can not perform signal pathway activation. Therefore, it becomes active by heterodimerizing with other RTK family members (66).

ErbB4 mutations are usually encountered in melanoma, lung adenocarcinoma and medullablastoma cases. ErbB4 has known seven different ligands. Studies show that they play an important role in myocardial expression and hence in ventricular differentiation. Because of this, it is known that arrhythmias develop in ErbB4 mutant mice (53, 67).

ErbB3 and ErbB2, which play a role in tumor development of breast cancer, increase the cell proliferation by spontaneous heterodimer formation. ErbB3 expression in NIH 3T3 fibroblast cell lines has shown to increase ErbB2 mediated transformation and tumorigenic growth. Such synergistic effects are accompanied by tumor progression with irregularity of overexpressing endogenous cell proliferation (56, 68). Mutations in the extracellular domain of HER3 as a result of increase the oncogenic potency of the heterodimer and ligand-independent tyrosine kinase activation with overexpression of HER2. This disables the inhibitor effects of some conventional therapies (53, 59, 69).

In the light of all these results, the uncontrolled expression of the ErbB receptor family, spontaneous dimerization and excessive tyrosine kinase activation resulted in continuous activation of the mitogenic signaling pathways, which together with the proliferation of the tumor cell gain speed.

2.5 PI3K/Akt Signaling Pathway

PI3K (phosphatidylinosithol-3 kinase) is a lipid kinase that is heterodimeric structure, composed of regulatory and catalytic units coded by different genes. The PI3K signaling pathway plays an important role in the regulation of many cellular processes such as cell proliferation and growth, apoptosis, and regulation of the cytoskeleton structure (10, 70).

The PI3K protein is divided into three classes. PI3K class I consists of two subunits, the p85 regulator and the p110 catalytic domain. PI3K class I is divided into two subsections A and B in itself. PI3K class IA is directly activated by receptor tyrosine kinases. PI3K class IB is activated by GPCR (G Protein Coupled Receptor) (71, 72). PI3K class II does not have membrane localization and the Adb (Adaptor-binding site) domain. It is known that activation of the catechin molecule is responsible for the endocytosis of surface receptors (71, 73, 74). PI3K class III is coded by the Vps34p gene. Initially, PtdIns (phosphatidylinositols) in yeasts were discovered to be the only protein that phosphorylates to convert to PtdIns-3-P (PIP3). It can only be recognized by proteins, which have FYVE and PX (Phox homology) domains (75, 76).

RTK, which is autophosphorylated by the ligand, phosphorylates PI3K in two different way as directly or indirectly. In indirect phosphorylation, the activated RTK phosphorylates the adapter protein IRS 1/2 (Insulin Receptor Substrate). Subsequently, the adapter protein phosphorylates the SH2 (SRC Homology 2) domain of p85, the regulator subunit of PI3K, which is localized to the membrane. Direct phosphorylation activates the SH2 domain of the p53 subunit of PI3K, which is directly localized to the membrane, without the need for an activated RTK adapter protein. In both cases, p110, the catalytic subunit of PI3K, liberates the inhibitory effect of p85 and converts it from PIP2 (Phosphatidylinositol 4,5-diphosphate) to PIP3 (Phosphatidylinositol 3,4,5-triphosphate) with the catalytic domain (10, 77). Under normal physiological conditions, heterodimeric p85 suppresses p110 activation (78).

PI3K pathway is associated with cell growth, apoptosis resistance, invasion and migration (Figure 2.3). After the conversion of PIP2 to PIP3, PIP3 is localized to the membrane and forms binding and activation regions for proteins, which have PH (Pleckstrin homology) domain (73). Activation of the Akt, which has the PH domain and the serine/threonine protein kinase, is initiated by membrane translocation (77, 79). Under conditions where the pathway is inactive, CTMP (Carboxy-terminal Modulator Protein) inhibits phosphorylation of Akt. Following membrane translocation of Akt, the CTMP becomes phosphorylated and loses its inhibitory effect on Akt and Akt phosphorylation is occurred (10).

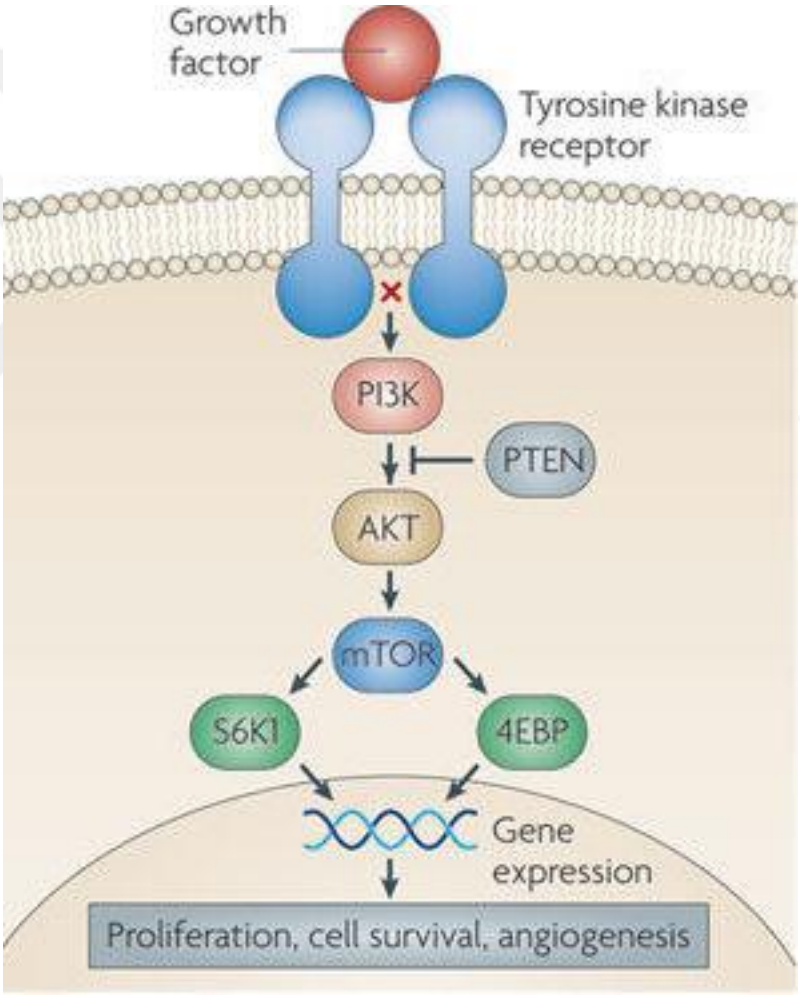


Figure 2.3. PI3K/Akt/mTOR pathway and biological function (Holmes D., 2011)

Akt protein acts as a key regulator in biological processes such as cell proliferation, metabolism, survival, invasion, migration, apoptosis and DNA repair (4). Akt or c-Akt is a 57 kDa Ser / Thr kinase that is the cellular homolog of v-Akt, a viral oncoprotein that causes leukemia in mice (77, 80). For full activation of Akt1 / PKB-a, phosphorylation of two regions is required. By binding of Akt and PDK1 to PIP3, PDK1 phosphorylates the activation loop of Akt into the T308 residue. Then mTORC2 (Mammalian Target of Rapamycin Complex 2), which is activated by the RTK, phosphorylates residue of Akt from the S473 residue of the regulator lobe (81). This provides the full activation of Akt. By allowing the S473 residue to be dephosphorylated by the PHLPP phosphatase protein, Akt activation is stopped (82-84).

PIP3 phosphatases regulate the PI3K mitogenic signaling pathway by converting PIP3 to PIP2. PTEN (Phosphotase and Tensin Homology) is the phosphatase, which is dephosphorylated to phosphate in the 3' position. As for SHIP 1/2 (Phosphotase and Tensin Homology) is the phosphatase, which is dephosphorylate to phosphate in the 5' position. PTEN has an important phosphatase property in terms of controlling mitogenic activity, as it inhibits the activation of proteins that bind to the D3 position, such as the Akt protein possesses PH domain, which is a messenger property (10). Studies have shown that PTEN is an important tumor suppressor gene and that PTEN mutation increases risk of glioblastoma, breast and prostate cancer (85, 86).

PI3K class IA is activated by RTKs and it is known that physiological function is deregulated in some types of cancer (10). PI3K/Akt signaling pathway is deregulated in consequence of mutations of pathway proteins in many cancers. Particularly, p110α mutation is seen in 20-25% of breast cancer cases. There is usually no mutation of Akt in HER2+ breast cancer cases. In addition, PTEN expression is low in many cases of breast cancer (87, 88).

2.6 PI3K/Akt Signaling Pathway and Breast Cancer

As a result of mutation of PI3K/Akt/mTOR pathway proteins in many cancers, the regulation of the signal pathway is impaired. The PI3K signal is

amplified in some situations such cases as the loss of tumor suppressors (eg, PTEN), resulting in the amplification of genes coding pathway proteins (eg. PIK3CA and Akt1) and mutation of responsible for pathway activation related proteins (eg. RTK and Ras) (75, 89-91).

The most common genetic change in cancer cases is PTEN tumor suppressor gene inactivation. This causes PIP3 accumulation. A mutation in PTEN or inactivation as a result of gene deletion is seen in many cancers (86, 92). Mutations of PIK3CA, Akt, PTEN KRAS, HRAS, NRAS and BRAF genes are frequently observed in breast cancer cases (91), with at least one of these mutations occurring with 70% probability in breast cancer cases (4, 93).

In breast cancer, mutations of p110α, the catalytic subunit of PI3K, are seen in 20-25% of cases. As a result, PI3K gains oncogenic potential. The studies showed that, the mice with p85α deficiency, the regulator subunit of PI3K, showed impaired B cell development and decreased serum immunoglobulins (71, 94-96). PIK3CA mutation in breast cancer is showed positive correlation with estrogen and progesterone receptor (ER/PR) expression, lymph node metastasis and ErbB2 overexpression (97). Because of overexpression of the receptor in HER2+ breast cancer, continuous activation of PI3K has a worse prognosis than other types of breast cancer (59, 98). Studies show that HER2 dimerization is a potential activator of cell growth, anti-apoptosis and invasion pathways. In addition, signaling can be compensated by different RTKs due to oncogene addiction (99-101).

2.7 Metadherin

The metadherin gene, also known as AEG-1 (Astrocyte elevated gene-1) or LYRIC (lysine-rich CEACAM coisolated), was first discovered in HIV-infected human primary fetal astrocytes, which are highly expressed (102). Primary studies on metadherin gene expression has shown that cells overexpressed the metadherin protein in the 4T1 mouse breast cancer cells, which could metastasize to the lungs and engraft into the pulmonary vasculature. The metadherin protein, that has a lung adhession domain, is homologous with the LYRIC protein, which is expressed in rats and is associated with cellular adhesion. For this reason, the metadherin name is

formed by abbreviation of metastasis adhesion protein (metastasis adhesion protein) (15, 103). With the increase of functional studies and the publication of data for the enhancement of metastasis to lung, the common nomenclature of AEG-1/MTDH/LYRIC proteins has been identified as metadherin (104).

Metadherin gene is localized in the second band (8q22) of the long arm of the 8th chromosome. Metadherin protein is approximately 84 kDa and human metadherin cDNA sequence shows 90% homology with mouse and 89% nucleotide homology with rat. This situation suggests that metadherin is a gene with conserved sequence inter species. The metadherin protein is structurally composed of one transmembrane domain (TMD), three nuclear localization signal domains (NLS-1,2,3), one SND1/NF-KB interaction domain (SND1/NF-kB) and one lung homing domain (LHD) (104). 47 known residues of the human metadherin protein that consist of 582 amino acid sequence undergo numerous posttranslational modification (phosphorylation, acetylation, ubiquitination). However, sufficient literature is not available on the biological significance of these modifications (105, 106).

The studies on metadherin over the last 10 years show that metadherin is an important oncogene. In many types of cancer, such as breast, stomach, liver, lung, colorectal, urinary and central nervous system cancers, metadherin overexpression results in increased tumor growth, invasion, angiogenesis and metastasis, leading to a more aggressive character and poor prognosis (107-112). In a recent study on breast cancer, by genetic suppression of metadherin via shRNA, inhibition of angiogenesis was demonstrated (28). Along with the increase of the metadherin expression, the cells also acquire chemoresistance (17, 107, 113). It has been shown that increased metadherin level led to resistance to doxorubicin, which is used as a first-line anthracycline drug in breast cancer. Additionally, metadherin suppression with siRNA transfection allowed cells become more sensitive to therapy (114). In another study, it was indicated that the sensitivity of cells to cisplatin was increased by MTDH siRNA transfection in prostate cancer cells and in this case apoptosis was triggered by loss of invasive potential of cells (29).

Metadherin expression is important in the development of neural tubules in the brain and spinal cord in the embryonic period. However, in normal physiological conditions, metadherin mRNA expression decreases with the slowing of embryonic development (115). Metadherin levels are increasing again in the course of the conversion of differentiated cells into neoplastic cells. This can be explained by the reactivity of various cellular signaling pathways involved as in embryonic development. Preclinical studies in breast and hepatocellular carcinoma cells have indicated that metadherin overexpression led cells to lose their contact inhibition property to gain spindle morphology as well as to decrease epithelial surface marker expression, and increase mesenchymal surface markers to enlarge cell population with stem cell phenotype (18, 116). This suggests a link between metadherin expression and the process of epithelial-mesenchymal transformation (EMT). At this rate, cancer cells are becoming more aggressive. An up-to-date study in breast cancer shows that the expression of metadherin increases in parallel to the the mesenchymal cell marker TWIST1 protein, and that the cancer stem cell (CSC) population is expanded (117). Different miRNA expressions such as miR-153, miR-26a and miR-375, may target methaderin thereby suppressing the EMT process and making the tumor cells more sensitive to therapy in breast cancer (118-120).

In accordance with increase of metadherin expression, over-activation of various cellular signal transduction pathways such as survival and proliferation signals occur. The studies of breast cancer, malignant glioma and melanoma cells put out that, Ha-ras and metadherin synergistically increase the invasive potential of cells and tumor progression (121). In another study with human astrocytes and mouse embryonic fibroblast cells, this synergistic effect is enhanced by NF-κB expression due to the Ha-ras-mediated PI3K/Akt pathway activation. It is also indicated that PI3K pathway activation increases cell survival and cells can escape from stress-mediated apoptotic activity (14). The relation between metadherin expression and PI3K/Akt pathway is also shown in head and neck squamous cell carcinoma. In addition, it is indicated that vascular endothelial growth factor (VEGF) expression is increased by pathway activation and angiogenesis is induced in cells (122). Studies in glioma cells show that the cells upregulate hypoxia-inducible factor-1α expression through PI3K/Akt pathway to protect the metabolic stress

associated with metadherin-mediated VEGF uptake and on this count, cancer cells can proliferate while escaping from cellular stress and apoptosis (123). In the proteomic study of HER2+ breast cancer cells, HER2 and metadherin expressions are found to be positively correlated (124). In another study, it was shown that the level of PTEN, which is the negative regulator of PI3K/Akt pathway was decreased when there was an increase of metadherin expression in breast cancer cells. In this respect, it is stated that the p-Akt level is high and the cells develop tamoxifen resistance (16). All these results demonstrate that metadherin expression plays an important role in the PI3K/Akt signaling pathway regulation in progression of various types of cancers, especially in breast cancer (125, 126) (Figure 2.4).

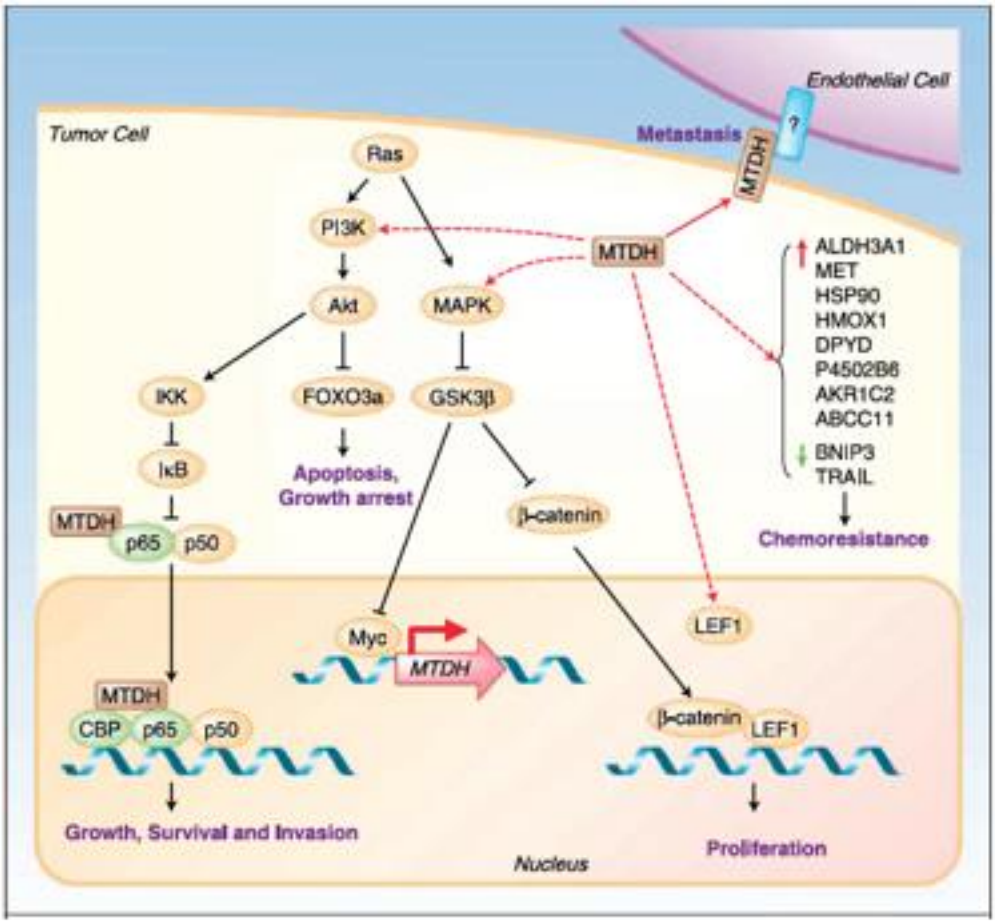


Figure 2.4. The role of metadherin on PI3K/Akt/mTOR signaling pathway (Hu G., 2009)

A limited number of clinical data confirms the positive correlation of increased level of metadherin with poor prognosis of cancer. Patients with invasive

characteristics (high nuclear grade, vascular invasion, negative ER/PR, high Ki67 index) were found to have significantly higher levels of metadherin compared to non-invasive specimens which was determined by immunohistochemical staining of breast cancer pathological tissue specimens. It has been shown that metadherin expression is correlated with low mean survival time (127, 128). In studies conducted with triple negative breast carcinoma patient samples, it was stated that the metadherin levels were correlated positively with VEGF expression and decreased survival and also it is expressed that the risk of recurrence is significantly increased in patients with high VEGF-metadherin levels (129). In another clinical trial with hepatocellular cancer patient tissue samples, it was shown that the increase in metadherin positively correlated with the increase of the vimentin expression, which is the mesenchymal marker that triggers EMT, and negatively correlated with the decrease of the E-cadherin expression, which is the epithelial marker (27).

2.8 Therapeutic Approach to PI3K/Akt/mTOR Pathway

PI3K pathway is continuously active in cancer cases due to the mutant form of RTKs or Ras oncogenes, loss of PTEN or mutant endogenous inactivation and/or gain-of-function mutations of the PI3KCA gene coding p110α. In this context, inhibition of PI3K activation is a potent target in anti-tumor strategies (130, 131).

Dual PI3K/mTOR inhibitor molecules are inhibitors that target the ATP binding sites of mTOR and PI3K with the same potency. In contrast to monotarget inhibitors, inhibition of at least three proteins (PI3K, Akt and mTOR) is one of the advantages of this group of inhibitors(132). Due to the mTORC1 protein inhibition alone by the rapalogs, mTOR-S6K-IRS1 negative feedback loop is inactivated and therefore PI3K activation is increased(133). In this context, the use of dual inhibitors to target PI3K/mTOR can more effectively block PI3K pathway reactivation than monotarget therapies. Preclinical studies show that the dual PI3K/mTOR inhibitor NVP-BEZ235 has an anti-proliferative effect on HER2+ breast cancer cells that are resistant to lapatinib and trastuzumab due to PI3KCA mutation. Furthermore, dual PI3K/mTOR inhibitors have shown a suppressive effect on tumor growth in human cancer xenograft models (134-136).

PI-103 (pyrido[3',2':4,5]furo[3,2-d]pyrimidine), is another effective dual PI3K/mTOR inhibitor that cause inhibition of PI3K-mTOR without serious side effects. Studies show that PI-103, when used at low concentrations, inhibits the growth of glioma cells by capturing G1 phase in the cell cycle. Ongoing phase studies demonstrate the direct anti-proliferative activity of PI-103 interfering with invasion, angiogenesis and metastasis progression, enhancement of p27 and reduction of cyclin D1 expression (137).

The initial studies performed by Hayakawa et al in PI-103 on four different cell lines including cervix, lung, breast cancer and melanoma showed that the anti-tumoral effect of PI-103 in different cancer types (138). Studies in human leukemic cell lines show that PI-103 captures the cell in the G1 phase and suppresses growth and proliferation of the cell. In blast cells isolated from AML (Acute Myeloid Leukemia) patients, it is shown that it inhibits AML progenitor clonogenesis by triggering apoptosis. It is also known that PI-103 enhances the efficacy of radiotherapy and chemotherapy-mediated apoptosis (139).

2.9 Aim of the Study

The aim of this study is to evaluate the effect of PI3K/Akt/mTOR pathway inhibitor PI-103 on methaderin expression in HER2 overexpressing human breast cancer cell lines. The selected cell lines are, SKBR3 cells (PI3K and PTEN wildtype), BT-474 cells (PI3K wildtype, PTEN mutant) and MDA-MB-453 cells (PI3K and PTEN mutant) breast cancer cell lines.

In addition, the level of metadherin will be determined in the serum samples of 80 breast cancer patients via ELISA assay Methaderin level of patients will be analyzed for the correlation between protein level and breast cancer subtype, lymph node involvement and metastasis status.

3. MATERIAL AND METHODS

All of the required equipment and materials are utilized in this study is provided by the department of basic oncology of the cancer institute of the Hacettepe University. This study is carried out in the research laboratory of the basic oncology that provided all the necessary facilities for the smooth running of this study.

3.1 Chemicals, Kits and Buffer Solutions

The chemicals, kits and buffer solutions are used in this study are listed as their name and manufactures as in Table 3.1

Table 3.1. Chemicals, kits and buffer solutions

No	Product	Product origin
01	Lyric/Metadherin(D5Y8R) xp Rabbit mAb#14065	Cell signaling technology USA
02	Polyclonal Goat Anti-rabbit Ig/HRP	Bio legend USA
03	HRP Donkey anti-rabbit IgG(minimal X-reactivity) antibody)	Biolegend USA
04	β-actin Rabbit Anti-human primer antibody	Cell signaling technology USA
05	PI-103 (pridofuroprimidin)	Chyman Chemical USA
06	Acrylamide (1.29 acrylamide-bisacrylamide)	Applichem Germany

07	Tris	Applichem Germany
08	Ammonium Per sulfate (APS)	Riedel-de Haen Germany
09	Tetramethyl ethylenediamine (TEMED)	Amresco -USA
10	Dulbecco & apos's modified Eagle medium (DMEM)	Gibco-USA
11	FBS (Fetal Bovine serum)	Lonza- Swetzerland
12	L-glutamine	Biochem- Germany
13	Pencillin/streptomycin	Biochem-Germany
14	PBS (phosphate Buffer Saline)	Sigma Aldrech-Germany
15	Tween 20	Applichem-Germany
16	Super Signal West-femto Maximum sensitivity Substrate	Thermo Scientific-USA
17	Bromophenil blue	Sigma -USA
18	B-Mercapto ethanol	Sigma-USA
19	Glycerol	Girbu-Germany

20	NP-40	Sigma -USA
21	Tris-Hcl	Invitrogen-USA
22	Sodium chlorure	Carlo Erba-Italy
23	Ethylene diamin tetra acetic acid (EDTA)	Sigma -USA
24	Sodium dodecyl sulfate (SDS)	Sigma Aldrich-Germany
25	Butyl alcohol (buthanol)	Sigma Aldrich-Germany
26	Glycine	Sigma-USA
27	Sodium florure	Merck-Germany
28	Protease inhibitor Coktail(PIC)	Roche diagnostic-Germany
29	Free fat milk powder	Pinar Turkey
30	Dimethyl sulfoxide(DMSO)	WAK-Chemie Medical-Germany
31	Trypan blue	Sigma-USA
32	Coomassie plus protein Assay reagent kit	Thermo scientific-USA
33	Bovine Serum albumin standard set	Thermo scientific-USA

34	Methanol	Merck-Germany
35	Protein transfer system	Biorad -USA
36	Full range rainbow molecular weight marker	GE Healthcare-USA
37	Sodium pyrophosphate	Sigma-USA
38	Calyculin	Cell signaling technology USA
39	Trypsin 0.05%(1X)	Lonza-Switzerland
40	Ethylene glycol tetra acetic acid (EGTA)	Ambresco-USA
41	Isopropanol	Sigma-USA
42	Ethydium bromide	Sigma-USA
43	RNA isolation kit	Qiagen- Germany
44	Conventional PCR kit	Fermentas-Lithuania
45	RNA concentration kit	Zymo Research-USA
46	PCR primer (metadherin)	Iontek -Turkey

47	PCR primer (β-actin)	Iontek -Turkey
48	Agarose	Amresco-USA
49	Real time PCR kit (SYBR-Green)	Qiagen- Germany
50	cDNA synthesis kit	Fermentas-Lithuania
51	TBE (Tris-Boric acid-EDTA)	Dr.Zeydanli Turkey
52	Real time PCR mycoplasma kit	Biological industries Israil
53	DNA free RNA kit	Zymo Research- USA
54	Human Metadherin ELISA Kit	Abbexa - United Kingdom

3.2 Equipments and Devices

Equipment and devices used in the current study are listed in the following list as their name and their manufacturers as in Table 3.2

Table 3.2. Equipments and devices

No	**Product name**	**Manufacturer**
01	Kodak Gel Logic 1500 Imaging System	Care stream Health Inc.-USA
02	Phase contrast microscope	Olympus England

03	Incubator or oven	Heraeus-Germany
04	Automatic Shaker	Heidolph-Germany
05	Refrigerated Microcentrifuge	Eppendorf-Germany
06	Mini Protean Western System	Biorad- USA
07	Spectrophotometer	Spectromax-USA
08	-80 ° C Deep Freezer	Thermo Electron-USA
09	Centrifuge	Heraeus-Germany
10	-196 Liquid Nitrogen Tank	Taylor Wharton- USA
11	Ice machine	Scotsman AF200-UK
12	Electrophorese Power Supply	Thermo Scientific
13	Electrophorese Power Supply	Amerscham
14	Gel Execution Tank	Thermo Scientific
15	Gel Execution Tank	Amerscham
16	Precision Balance	Denver Instrument-USA

17	Vortex	Clofton Cycline-England
18	Distiller water system	GFL-Germany
19	Shocker or vibrator	GFL-Germany
20	Heating Block	Techne Dri Bloc-UK
21	Magnetic Mixer	Ika RH Basic-Germany
22	Drawer hood	Unitest-Turkey
23	Vertical Flow Air Cabinet	Thermo-Electron-USA
24	Water bath	GFL-Germany
25	+4 ^{o}C Cold Room	Alarko Carrier-Turkey
26	+4 ^{o}C Refrigerator	Bosch-Germany
27	-20 ^{o}C Deep Freezer	Bosch-Germany
28	Fuchs Resenthal Counting Chamber	Fuchs Rosenthal-USA
29	Cell Counting with Neubauer Chamber Basic Hemocytometer	Neubauer-USA
30	SpectraMax microplate reader	Molecular Devices - USA

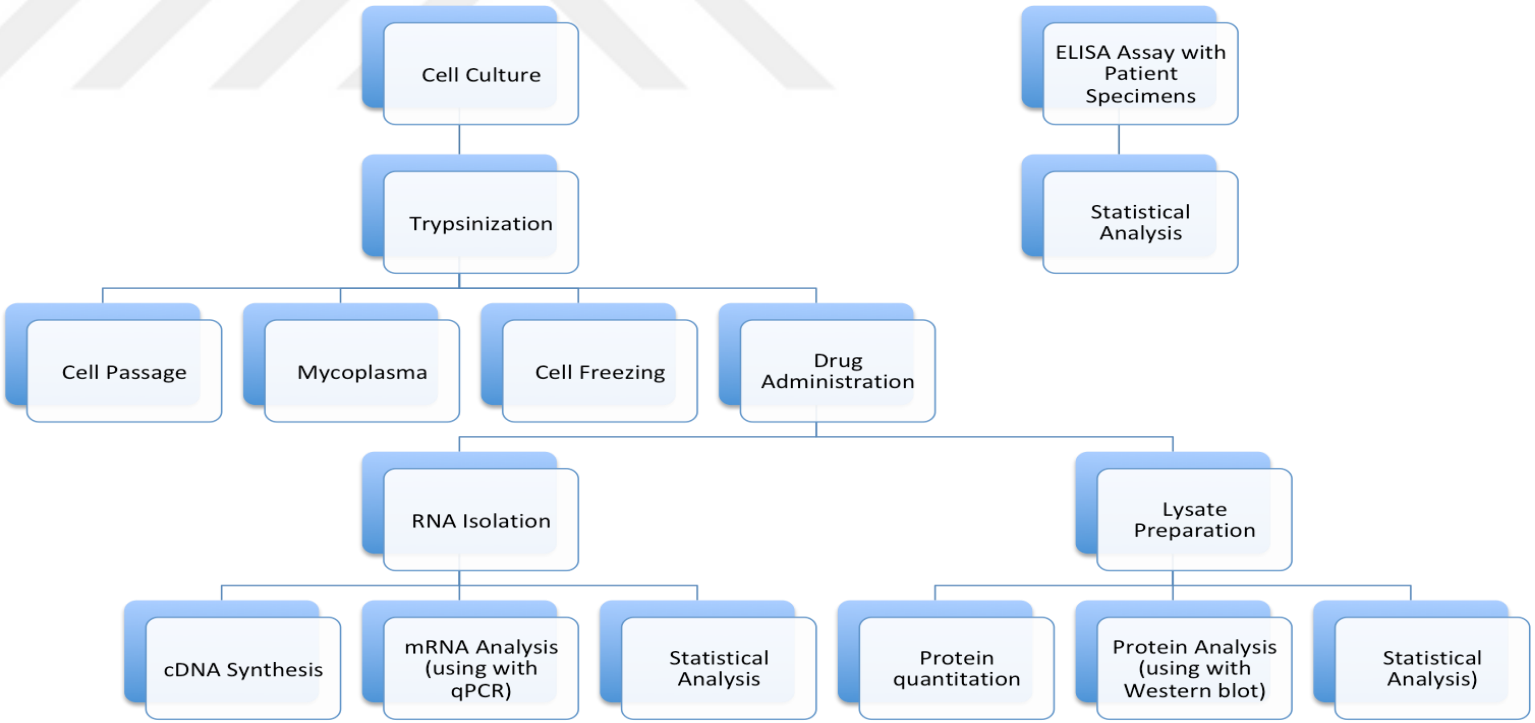


Figure 3.1. Experimental process schematic plan

3.3 Cell Culture

MDA-MB-453, BT-474, SKBR-3 human breast cancer cell lines were used in the study. These cell lines are purchased from ATCC (American Type Culture Collection) and cultured according to the ATCC specified term and conditioned method. These cell lines were maintained in 25 cm2 flasks containing a complete medium of DMEM (Dulbecco's Modified Eagle Medium) 10% fetal bovine serum (FBS), antibiotic (Penicillin/ Streptomycin) and L-glutamine and RPMI-1640 (Roswell Park Memorial Institute-1640) at 37 ^{o}C and 5% CO_2 for replication. The medium was changed every 3 days.

The growth of the cells was allowed to occupy 90 to 100% area of the flasks. In the first passage of the culture $1x10^6$ cells/ml were transferred to crayovial tube and kept in liquid nitrogen for further usage of the experiment. Three to 11 passages were produced. After the appropriate amount of the cells are produced for the experiment trypan blue is used for detection of dead cells and the cells are counted by counting chamber (Thoma counter). Before we incubate the cells with the considered drug in the experiment attempt was made to use at least 90% liable cells.

3.4 Trypsinization

The cell lines were passaged by trypsinization. In this case the cells were removed from the flasks by the method of trypsinization. For this purpose the nutrient medium full DMEM was withdrawal by serologic pipettes. Then 0.5ml trypsin/EDTA was added to the 25ml flasks and 1.5ml of the same was added to the 75ml flasks. To homogenize and suspend the flasks cells with trypsin/EDTA the flasks are gently shacked for a while. The flasks are incubated for 3-5 minutes at 37^{o}C and 5% CO_2. Then the flasks are checked under the phase contrast microscope to ensure homogenization and separation of the adherent cells to the flask the flasks are gently shaken and leave them for some more minutes in incubator. To stop further effects of the trypsin/EDTA on the cells 2-4ml full DMEM is added. Then the cell suspension is transferred from flask to 50ml falcon tube and centrifuged it 2000rpm at 4^{o}C for 5 minutes. Then the

supernatant of the tube is discorded and the pellet at the bottom of the tube is carefully handled. 2-6ml full DMEM is added to the pellet of the falcon tube and made it as a suspension. To determine the number of cells in the falcon tube take 10 microliter cell suspension and mix with the same amount of trypan blue, for rolling out the dead cells, then count them by Thomas counting chamber slide under the microscope. In this process the dead cells, which looks blue are excluded and the viable cells looking plane are considered to counted. The cells are counted in 4 large squares (each include 16 small squares). The total number of the cells in 4 squares are multiplied by dilution and trypan blue factors as following:

Number of counted cells × 10000 ×10×2/4=number of cell/falcon tube Thoma counting chember slide measurement is as 0.1mm depth×0.1mm width×0.1mm length=0.001mm^3(0.0001ml)=10000 dilution factors. 2 and 10 are trypan blue factor as the cell suspension is diluted with equal amount of trypan blue.

3.5 Cell Passage

Following the counting process of the cells in case of finding that the cells number is not enough for the experiment the cells should be passaged. In this case SKBR-3 cell line is undergone a new passage. Two size culture flasks are selected as 25ml and 75ml to every one of them 500 000 cells plus full DMEM final volume 5ml and 1000000-12500000 cells plus full DMEM final volume 15ml were added respectively. The flasks were incubated at 37 ºC and 5% CO_2 . The cultured flasks were checked under the microscope on daily base to keep control of the cells normal growth. In case of finding that the PH of the medium is dropped and its color is changed and the number of dead cells are increased in this case for better nourishment and growth of the cells the medium is renewed.

3.6 Cell Freezing

Following utilization of the cultured cells SKBR-3 between passage 1 and 3 after counting of the cells 1 X 10^6 cells/tube are transferred to cryovial tubes. Adding 10%

DMSO to the cell suspension to reduce the risk of cell lysis due to crystallization of the intracellular water. The cryovial tubes in propanol are placed in gradually cooling system, which could reach -86 ºC within 24 hours. After 24 hours the tubes transferred to the liquid nitrogen capsules and save and stored them for longer time.

3.7 Mycoplasma Test For The Culture

This test is done to find out whether the cultured cells are contaminated with intracellular bacteria (mycoplasma). Along the growing process of the cultured cells there are possibility of the contamination of the cells with mycoplasma. This test is sensitive for some mycoplasma species as M.fermentas, M,hayorhinis, M.arginini and some other species as Acholeplasma and Spiroplasma. For applying of this test 1ml of the SKBR-3 and BT-474 cultured cell line are taken and put in an eppendorf tube (1.5ml capacity tube) then the sample is centrifuged at +4ºC 2000rpm for 90 sec. in mini span centrifuge machine. Then the supernatant is transferred to a tube and the pellet is discarded. Then the collected supernatant is centrifuged at 14000 rpm for 10 minutes and after centrifugation the supernatant is discarded and 25µl mycoplasma buffer solution (from kit) is added to the pellet and made it as a suspension by gently pipetting. Then this suspension is incubated at 95 ºC for 3 minutes in heating block. After this step we run the PCR process as following:

We have prepared three tubes as sample, positive control and negative control tubes.

1. Sample tube includes: 2.5 µl sample + 17.5 µl water + 5 µl reaction mixture (from mycoplasma kit).
2. Positive control tube: 19 µl water + 5 µl reaction mixture (from kit) + 1 µl positive control template(from kit)
3. Negative control tube: 20 µl water + 5 µl reaction mixture (from kit)

PCR Steps:

i. Denaturation: 95^{o}C for 30 sec

ii. Annealing: 94 ^{o}C --> 30sec

60 ^{o}C --> 120 sec

72 ^{o}C --> 60 sec (totally 35 cycles)

iii. Amplifying: 94 ^{o}C --> 30 sec

60 ^{o}C --> 120 sec

72 ^{o}C --> 5 min

At the end of the PCR process we have made 2% agarose gel electrophoresis and add 20 µl to each well of the electrophoresis and normally we insert loading dye for determining of the running sample but in this experiment we don't because the reaction mixture already includes the loading dye. The mycoplasma DNA fragment gives a band in 270 base pair. This band appeared in positive control but not in our samples. It means that fortunately the test result was negative for our samples and approved free of mycoplasma contamination.

3.8 Drug Administration

Following the process of trypsinization to analyze mRNA we have prepared 6 well plates and from our cell suspension $1X10^{6}$ cells are added to each well of the plate. In order to enable the cells to adhere the floor of the well, the 6 well plates are incubated at 37^{o}C and 5% CO_2 for 24 hours.

PI-103 a dual inhibitor of PI3K and mTOR was prepared under sterile condition inside the hood cabin and then administered to the considered cells (SKBR-3 and BT-474 and MDA-MB-453). Water crystallization protective agent, DMSO was prepared as solution in 10mM concentration. The drug (PI-103) was also prepared as 500µl in

divided volumes and stored in -20ºC. Before withdrawal of the medium from wells the serial dilution of the drug aliquots is prepared with 10mM DMSO. After 24 hours the full medium is removed from the wells then the drug together with the fresh full medium is added to the wells. 1 µl PI-103, dual inhibitor of PI3K/mTOR is added to the wells of 3 cell lines (SKBR-3, BT-474 and MDA-MB-453) and incubated them for 8 and 24 hours and free of drug wells were considered as control group.

3.9 RNA Isolation

For protein analyses following trypsinization, 2 X 10^6 cells in the form of suspension are added to each well of 6 well plates and in order to adhere the cell to the floor of the wells they are incubated for 24 hours at 37 ºC and 5% CO_2. After 24 hours the full feed medium is removed from the wells and free of serum medium is added to them and incubated again for 24 hours under similar condition as above. This process is called serum starvation following which all cells reach the G0 phase of the cell cycle. After the serum starvation the serum free medium is withdrawn from the wills then 4µM PI-103 in 500µl medium is added to each of the wells and followed by adding 1.5ml full medium to each well. In this way the final concentration of the drug is made 1µM. All of the above 3 cell lines are incubated with the drug for 8 and 24 hours and the control well were treated with only medium and was free of drug and incubated under the same condition of treated wells.

At the end of the 0, 8 and 24 hours of incubations, RNA isolation of the cells was performed according to the RNeasy Mini Kit 50 protocol. Quantities of RNA obtained were measured using 260/230 (inorganic contaminant) and 260/280 (organic solvent) values using a 1.5 µl sample in a nano-drop device. Whether or not the obtained RNAs were pure (presence of DNA contamination) was checked by GAPDH (148 bp) reverse transcriptase polymerase chain reaction (RT-PCR) method. The samples obtained from the result of PCR were observed by agarose gel electrophoresis. The presence of band in the wells in which the samples are loaded indicates DNA contamination in the samples (Figure 3.2).

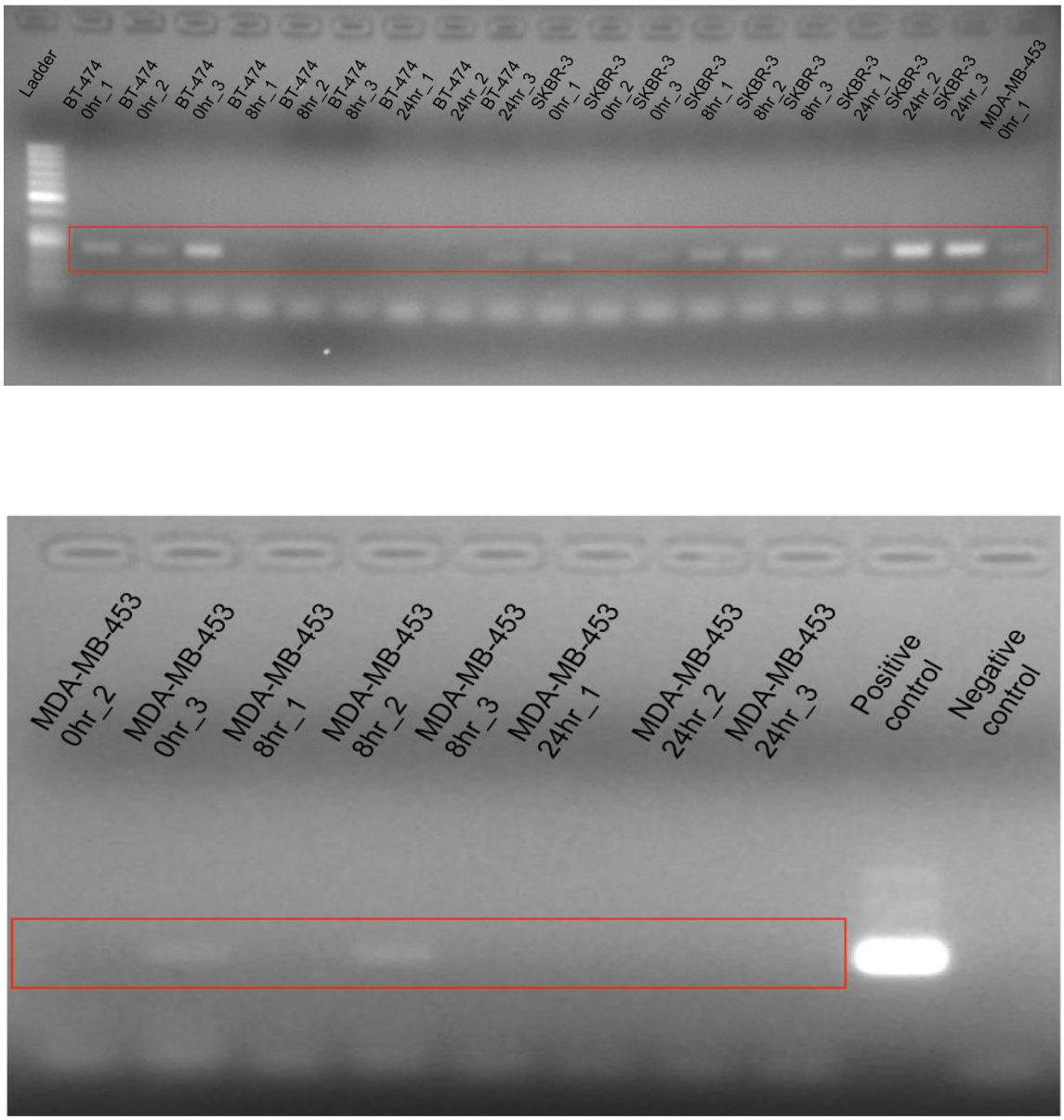


Figure 3.2. GAPDH gel electrophoresis image of RNA extracts obtained from BT-474, SKBR-3 and MDA-MB-453 cells after 8 and 24 h of 1 μM PI-103 drug and 0 hour drug-free incubation. The band images in the red box show DNA contaminations.

For this purpose, samples with DNA contamination were cleaned with DNAse enzyme according to Zymo RNA Clean & Concentration kit protocol (Figure 3.3). Due to that all samples were cleaned.

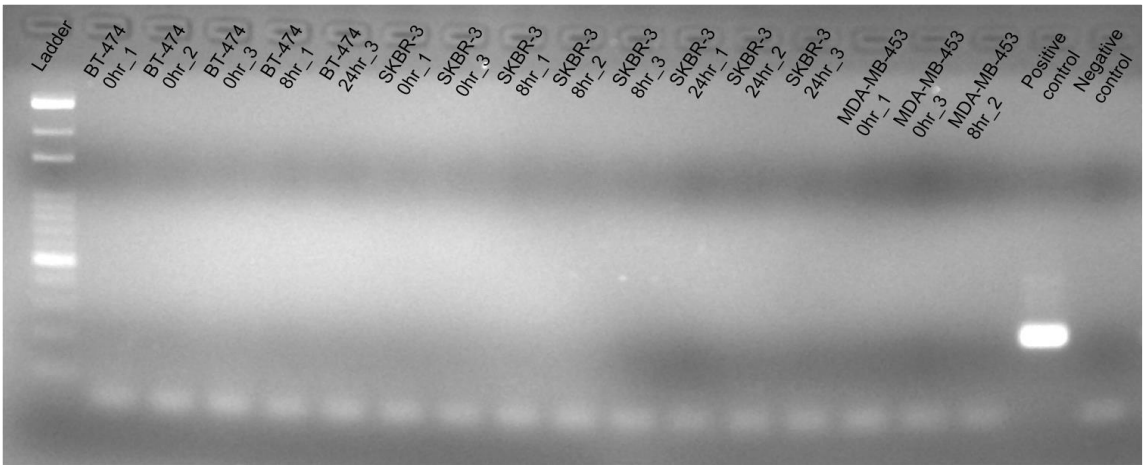


Figure 3.3. A total of 16 samples with DNA contamination were cleaned with kit containing DNase enzyme following which the GAPDH PCR gel electrophoresis image obtained.

3.10 cDNA Synthesis from Obtained RNAs

We need to synthesize cDNA from pure RNAs we have obtained before RT-PCR. cDNA samples should be 1000 ng/µl. Thus, the amount of RNA calculated by nano-drop device.

After calculation of RNAs from MDA-MB-453, BT-474, SKBR-3 cells for cDNA synthesis, Thermo Scientific™ RevertAid™ First Strand cDNA Kit was used. cDNA synthesis of all the samples was performed in accordance with the Synthesis Kit protocol. (OligodT = 1 µl, 5 × Reaction Buffer = 4 µl, Ribolock Inhibitor = 1 µl, 10 mM dNTP = 2 µl, M-MuLV (Moloney Murine Leukemia Virus Reverse Transcriptase) = 1 µl) was prepared according to the protocol of the cDNA synthesis kit protocol. In the result of reverse transcriptase of PCR 1000 ng/µl cDNA samples were obtained. The resulting cDNA samples were checked by GAPDH (148 bp) reverse transcriptase

polymerase chain reaction method. The samples obtained from the PCR were observed by agarose gel electrophoresis (Figure 3.4).

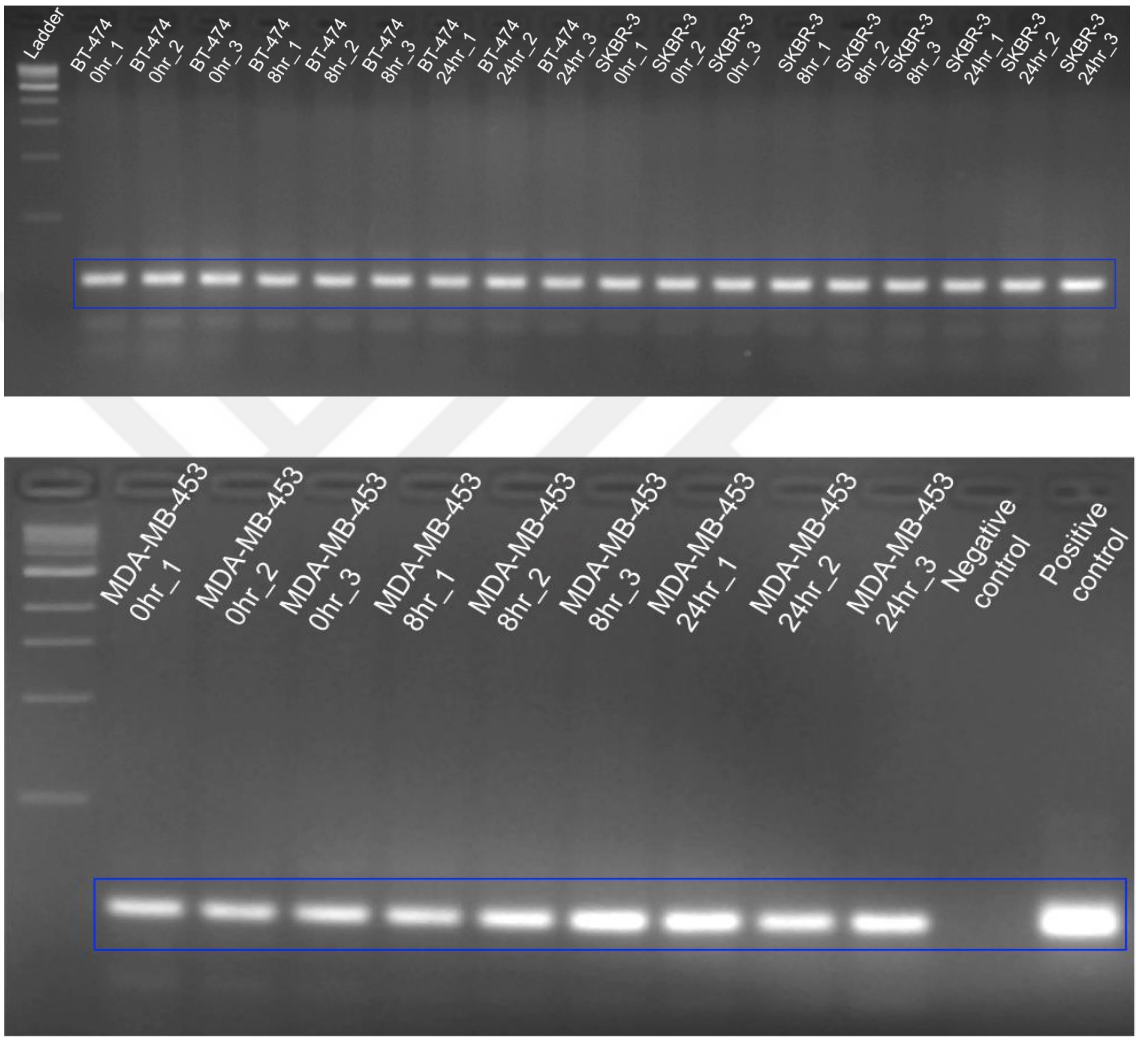


Figure 3.4. Gel electrophoresis image of GAPDH cDNAs of all samples obtained (27 samples in total)

3.11 Preparation of Drug Treatment and Lysate with PI-103 for Protein Assay

MDA-MB-453, BT-474, SKBR-3, 9 T25 flasks were incubated in 37 ºC, 5% CO_2 overnight to allow adhesion of adherent breast cancer cells to the flask floor. An

average of $4x10^5$ cells were plated per flask. After incubation for 24 hours, the medium of the cells was changed. Serum-free medium was added to capture the cells in the same proliferation phase. Cells in serum-free medium were incubated for 24 hours. After serum starvation, the serum free medium was removed. After this, the cells were treated with 1 M PI-103. Drug treatment times of MDA-MB-453, BT-474, SKBR-3 cells were set to 0-8-24 hours. Three independent samples (flasks) were used for each hour (n=3). Inactivation of the drugs was achieved after the drug treatment. Cells were treated with 400 µl of lysis buffer to prepare lysates.

3.12 Lyric/Metadherin Protein Detection and Analysis by Western Blot Method in All Cells

The protein lysates were quantitated by the Western Blot experiment. Protein standards used in quantitation and known protein quantities allow the determination of the protein in mg/ml of lysates. Protein quantitation determined the amount of protein to be added for each well of the SDS-PAGE gel in equal volumes. After protein quantitation, the lysates were allowed to stand for 5 minutes in a dry heating block at 95 °C to allow denaturation of the proteins. After the phase of protein denaturation, proteins with different weights (kDa) in the lysate were separated by SDS polyacrylamide gel electrophoresis. The samples were run at 200 volts for 30-40 minutes.

After completing this step, proteins separated by weight in gel were transferred to PVDF membrane by gel. The transfer process was carried out with a half-dry transfer system in 7 minutes at 25 volts. After the transfer step, the membrane was put on a shaker set at room temperature and 100 rpm for 1 hour in skimmed fat free milk powder or bovine serum albumin solution. After blocking, the membrane was washed with 1X PBS-T in a shaker at 100 rpm 3 times for 10 minutes each. After this step, the membrane was treated with the Lyric/metadherin primary antibody on a shaker at 100 rpm for 16 hours at +4 °C on a shaker. Monoclonal antibodies, which recognize a single determinant of denatured proteins with high affinity and specificity, were used to identify the targeted protein. Incubation of the membrane with the primary antibody was

allowed to bind to the target proteins. β-actin was used as the control protein. After primary antibody incubation, the HRP (Horseradish Peroxidase) conjugate secondary antibody was introduced into the incubation phase with the secondary antibody produced against the organism in order to be able to display the primary antibody. Polyclonal Goat anti-rabbit immunoglobulins/HRP was used as the secondary antibody. Ultimately, ECL (Enhanced chemiluminescence) solution containing luminol, the substrate of the peroxidase enzyme in the secondary antibody conjugate HRP, was used for the chemoluminescence emission of the proteins to which the secondary antibody was attached. Finally, images of the chemiluminescent radiating protein bands were obtained.

3.13 Patient Specimens and ELISA Assay

This study was performed with 80 breast cancer patient blood sample which were collected at the Hacettepe University Cancer Institute Department of Medical Oncology. Research ethics committee approval was obtained from Hacettepe University Ethics Board and Commissions. Prior to collect blood samples, the research purpose was explained and the consent of patients was taken. After blood samples were collected, sample tubes were centrifuged via ultracentrifuge at 2000 rpm for 10 minutes for isolation of serums from whole blood. Then each serum sample was aliquoted in microcentrifuge tubes. Serum samples were stored in freezer (-86 ^{o}C) until using on ELISA.

This analyze was performed according to manufacturer protocol (Abbexa Human Metadherin Kit). As a summary, before start to analyze, serum specimens and kit reagents take out from freezer for allowing to all of the products temperature become at room temperature. After that, protein standards were diluted in different concentration. Each standards, serum samples and quality controls were added duplicate in 96-well plate. Firstly for analysis of target protein, each well was incubated with biotin labeled Metadherin antibody for 60 minutes at room temperature. Then Streptavidin-HRP incubation was performed for 30 minutes at room temperature. Lastly, substrate solution

was added and incubated for 15 minutes at room temperature. Plate wells were washed 3 times right after entire incubation steps. After last incubation step, stop solution was added into each well and optical density of samples was measured under 450 nm wavelength with *SpectraMax® Plus* microplate reader device within 15 minutes according to concentration of protein standards.

3.14 Statistical Analysis

Real-time PCR results were obtained by statistical analysis of mRNA analysis data and western blot results obtained and statistically analyzed by student t test. Values of $p < 0.05$ were considered statistically significant. Statistical analyzes were performed using the IBM® SPSS® Statistics Version 20 program.

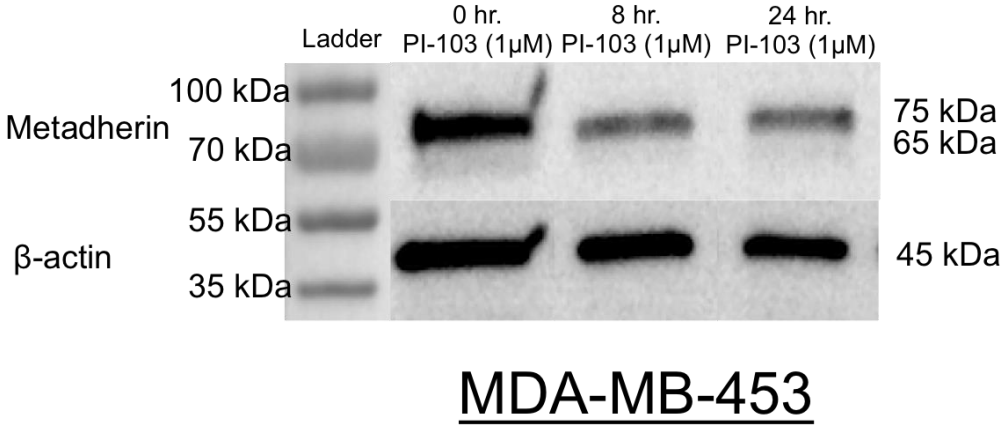
4. RESULTS

4.1 Effect of pharmacological inhibition of PI3K pathway with PI-103 dual inhibitor on protein expression of metadherin (MTDH) in breast cancer cell lines

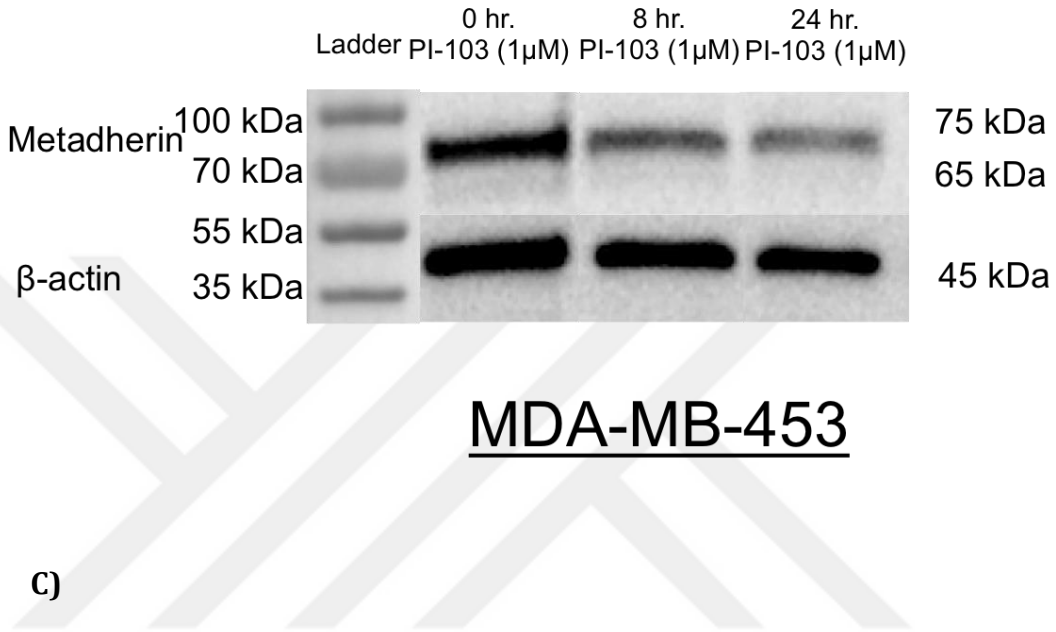
MDA-MB-453, BT-474 and SKBR-3 cells were incubated with 1 μM PI-103 for 8 and 24 hours. Metadherin expressions were compared with control cells (0 hour samples) with western blotting method. Protein bands normalized with β-actin, used as loading control. For each condition, 3 independent experiments were performed and relative bandwidth densities were obtained (n=3). The changes in the expression levels of metadherin in the cell were analyzed by independent groups student's t test.

As a result of the analysis in MDA-MB-453 cells, it was determined that there was a statistically significant difference in the levels of metadherin expression between the control cells and the cells incubated with PI-103 for 8 and 24 hours depending on the time (*p <0,05) (Figure 4.1 & 4.2).

A)



B)



C)

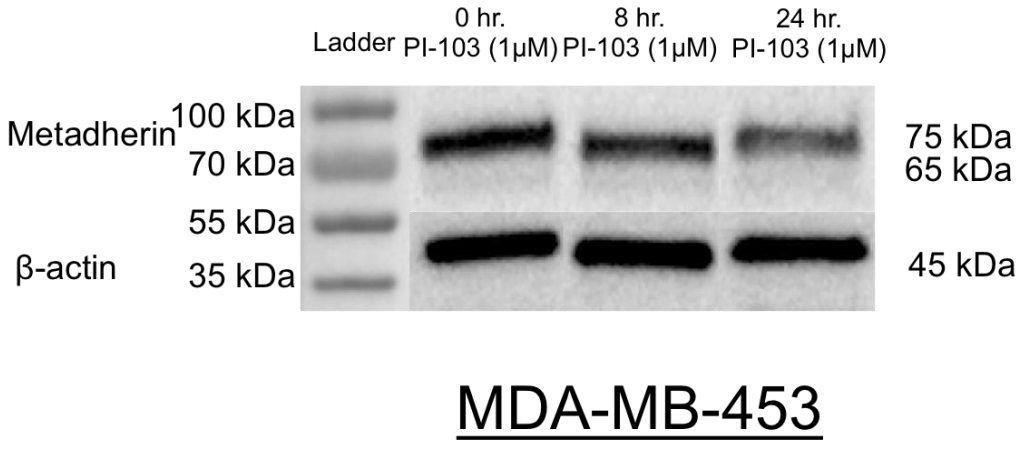


Figure 4.1. A) Metadherin and β-actin protein band images from western blot results of MDA-MB-453 cell line (n=3), demonstrative replicate, **B)** Second independent replicate, **C)** Third independent replicate

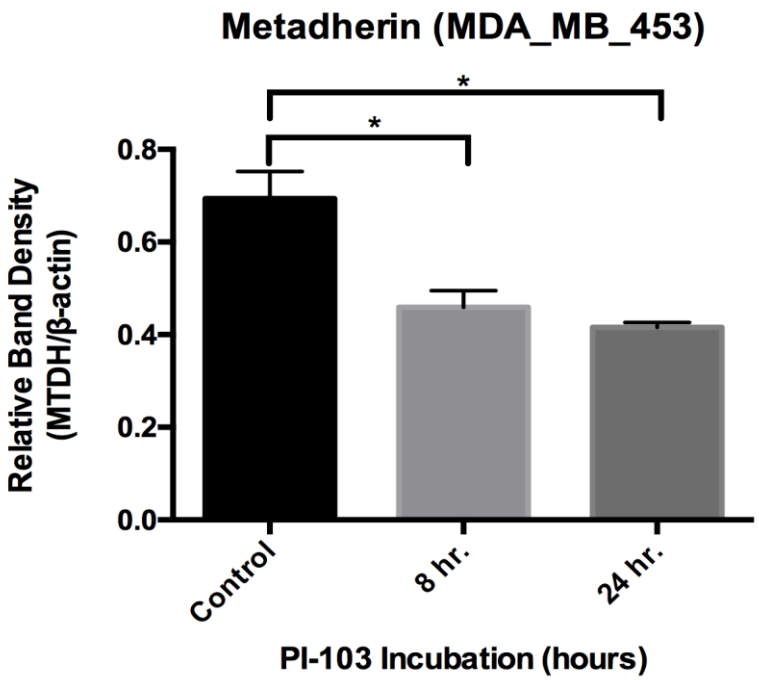
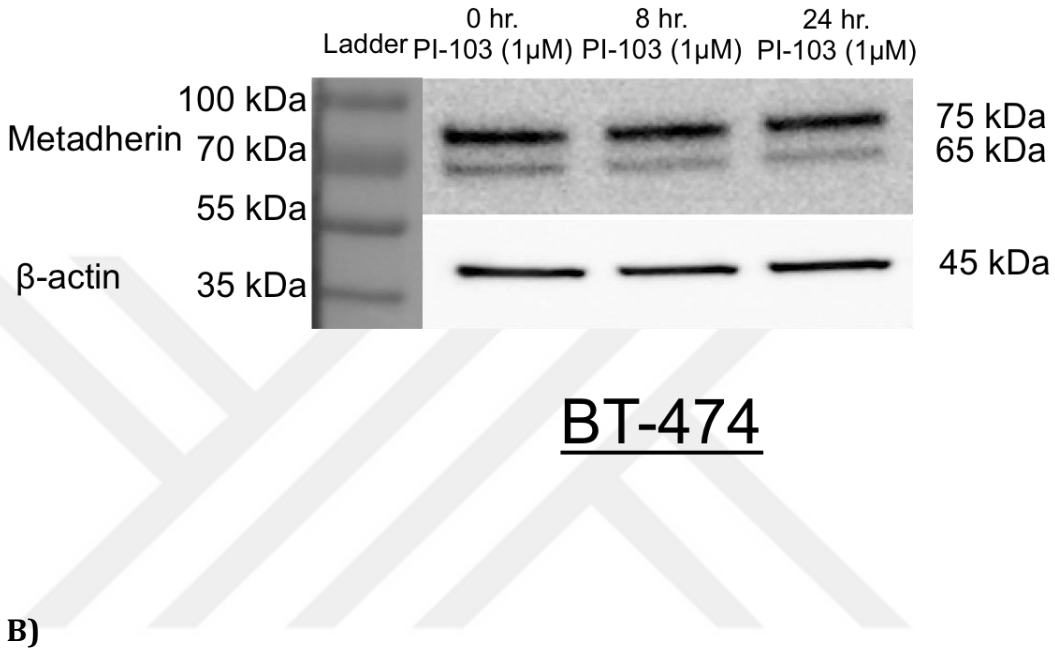


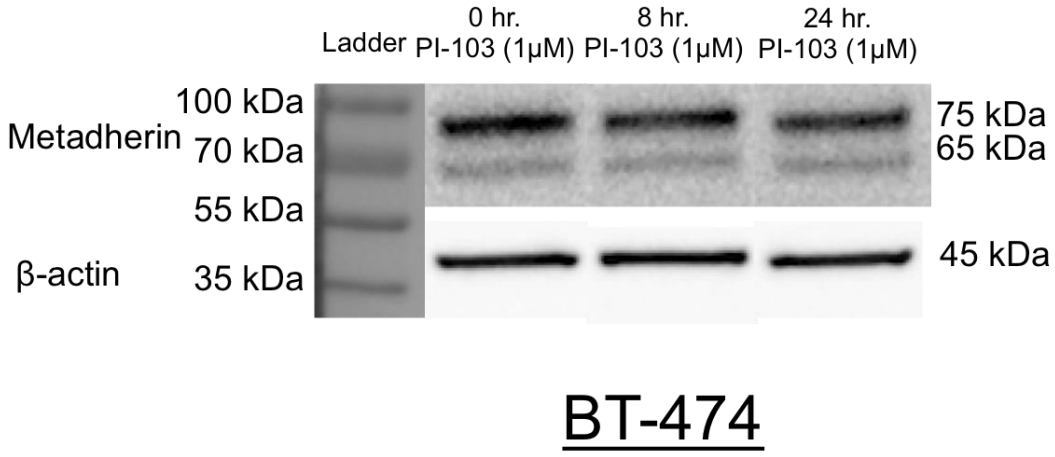
Figure 4.2. Relative band intensities of metadherin protein of MDA-MB-453 cell line (n=3) *p <0.05

As a result of the analysisin BT-474 cells, it was determined that there was not statistically significant difference in the levels of metadherin expression between the control cells and the cells incubated with PI-103 for 8 and 24 hours depending on the time (Figure 4.3 & 4.4).

A)


Ladder
0 hr. PI-103 (1μM)
8 hr. PI-103 (1μM)
24 hr. PI-103 (1μM)
100 kDa
Metadherin
70 kDa
55 kDa
β-actin
35 kDa
75 kDa
65 kDa
45 kDa
BT-474

B)


Ladder
0 hr. PI-103 (1μM)
8 hr. PI-103 (1μM)
24 hr. PI-103 (1μM)
100 kDa
Metadherin
70 kDa
55 kDa
β-actin
35 kDa
75 kDa
65 kDa
45 kDa
BT-474

C)

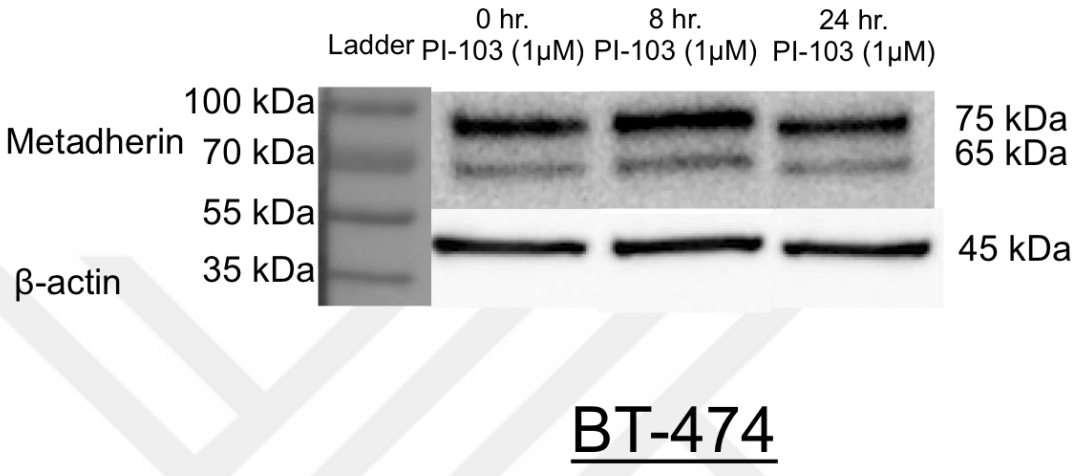


Figure 4.3. A) Metadherin and β-actin protein band images from western blot results of BT-474 cell line (n=3), demonstrative replicate, **B)** Second independent replicate, **C)** Third independent replicate

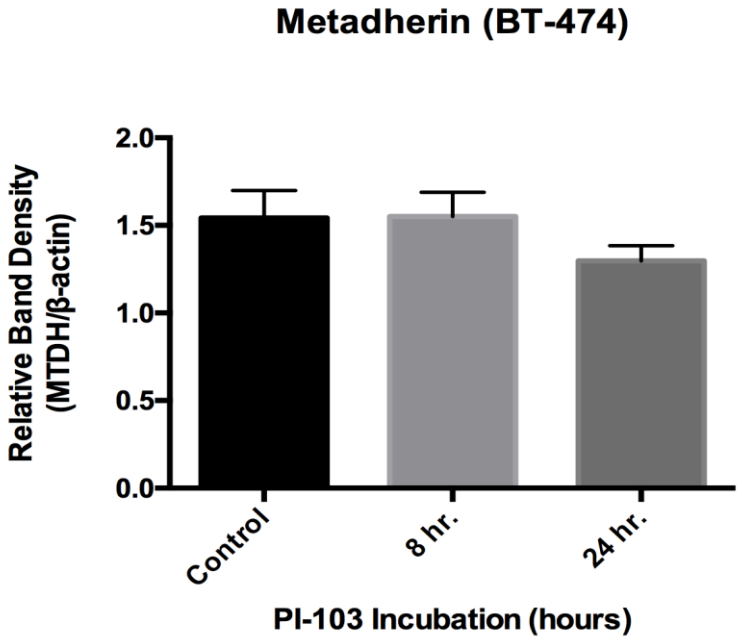
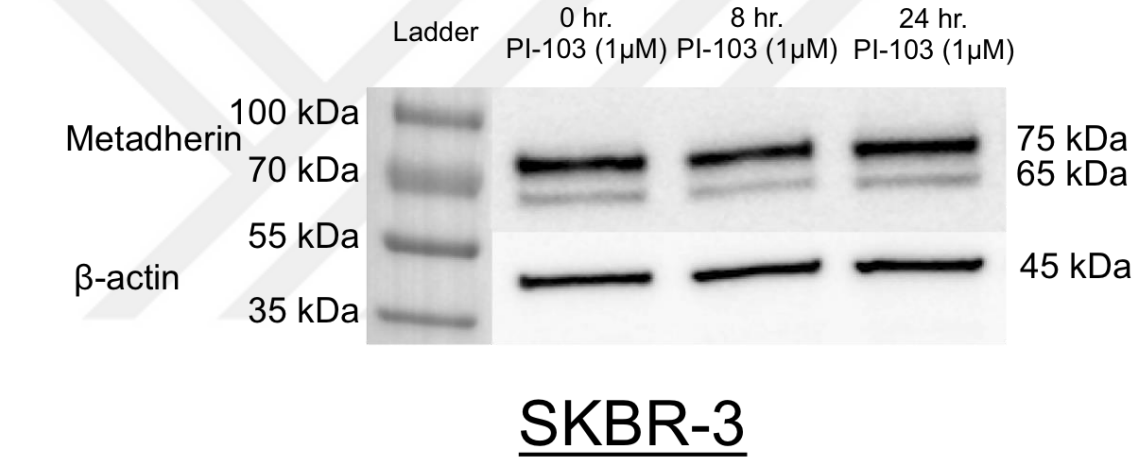


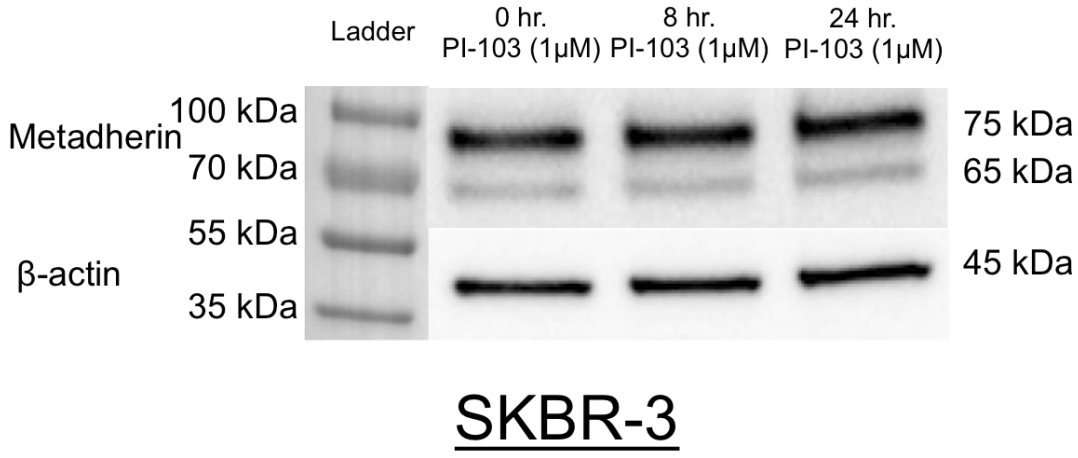
Figure 4.4. Relative band intensities of metadherin protein of BT-474 cell line (n=3)

As a result of the analysis in SKBR-3 cells, it was determined that there was statistically significant difference in the levels of metadherin expression between the control cells and the cells incubated with PI-103 for 24 hours (*p <0,05) (Figure 4.5 & 4.6). There was not statistically significant difference in the levels of metadherin expression between the control cells and the cells incubated with PI-103 for 8 hours.

A)



B)



C)

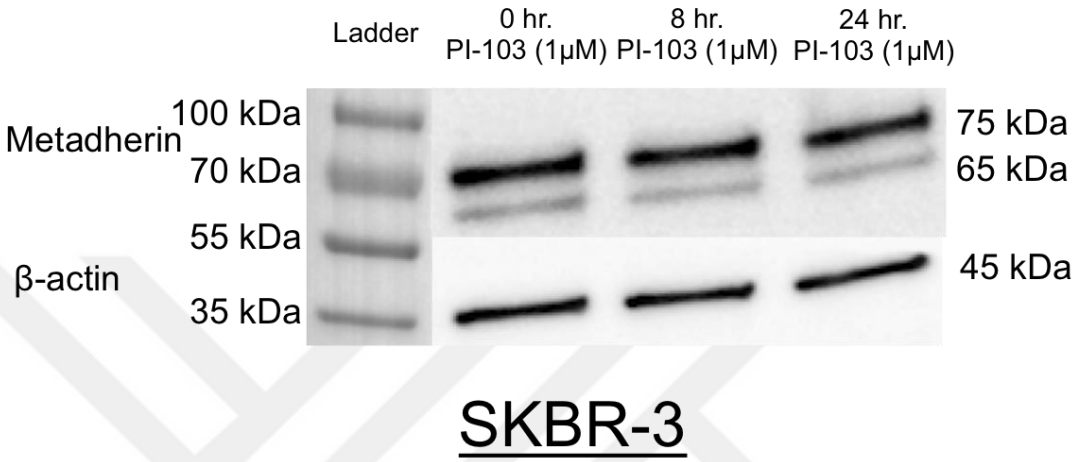


Figure 4.5. Metadherin and β-actin protein band images from western blot results of SKBR-3 cell line (n=3), demonstrative replicate, B) Second independent replicate, C) Third independent replicate

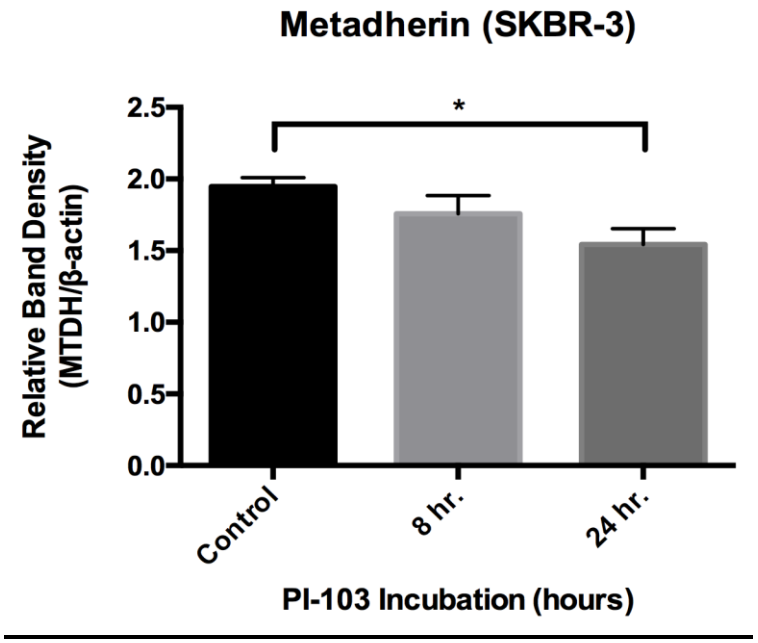
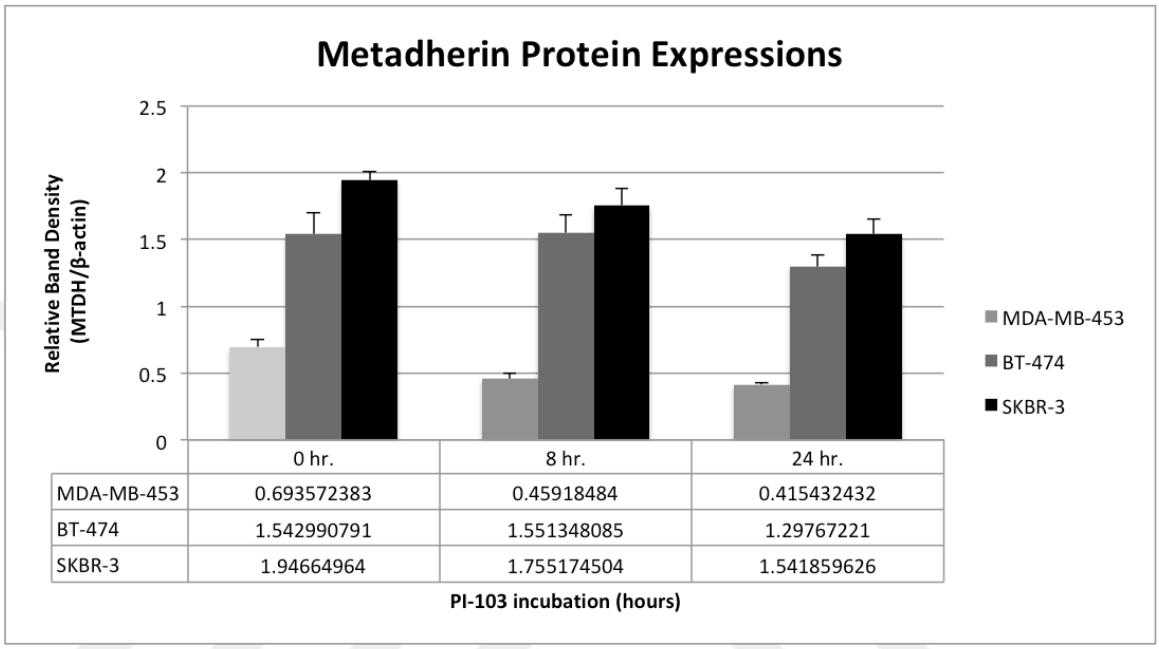


Figure 4.6. Relative band intensities of metadherin protein of SKBR-3 cell line (n=3) *p <0.05

Comparison of the expression of metadherin protein expression in all cell lines is shown in Figure 4.7.



	0 hr.	8 hr.	24 hr.
MDA-MB-453	0.693572383	0.45918484	0.415432432
BT-474	1.542990791	1.551348085	1.29767221
SKBR-3	1.94664964	1.755174504	1.541859626

Figure 4.7. Comparison of the expression of metadherin in all cell lines (n=3)

4.2 Effect of pharmacological inhibition of PI3K pathway with PI-103 on metadherin mRNA expressions in MDA-MB-453, BT-474 and SKBR-3 cell lines

MDA-MB-453, BT-474 and SKBR-3 cells were incubated with 1 μM PI-103 for 8 and 24 hours. HER2 and HER3 mRNA expressions were compared with control cells using the qPCR method. β-actin was used for housekeeping gene as a control gene. For each condition, 3 independent experiments were performed (n=3). Changes in the metadherin mRNA expressions of the cells were statistically analyzed using independent student t test.

4.2.1 Effect of time-dependent incubation of PI-103 on metadherin mRNA expression in the MDA-MB-453 cell line

The effect of PI-103 used in MDA-MB-453 cells on time-dependent expression of metadherin mRNA was examined by qPCR method. $2^{-\Delta\Delta Ct}$ values were obtained by calculating the difference between the Ct values of the control group and the Ct values of the time-dependent Ct values (140). Statistically analyzed results with $2^{-\Delta\Delta Ct}$ values showed significant difference between control cells and cells treated with PI-103 for 8 hours (n=3) (*p<0,05) (Figure 4.8).

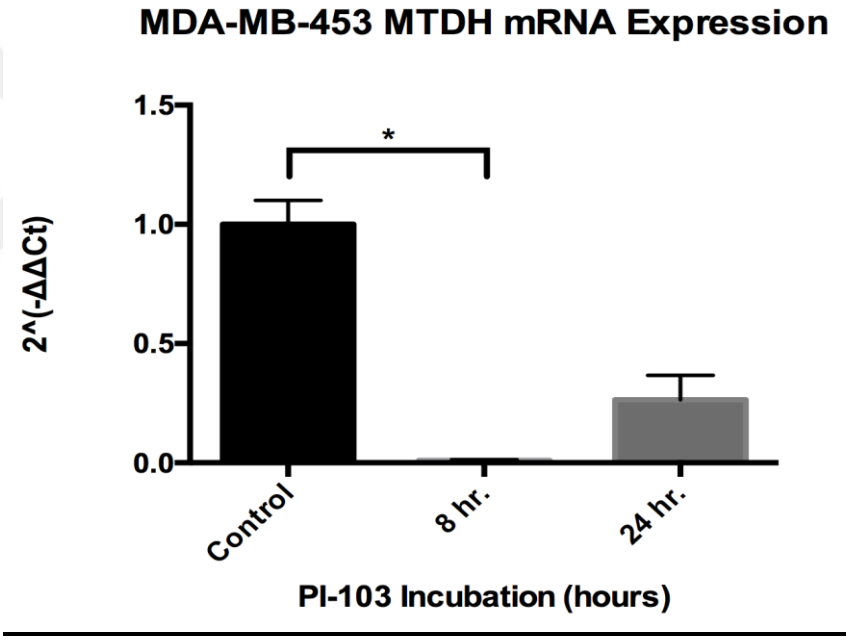


Figure 4.8. Time-dependent effect of PI-103 on metadherin mRNA expression in MDA-MB-453 cell line (n=3) (*p<0.05)

4.2.2. Effect of time-dependent incubation of PI-103 on metadherin mRNA expression in the BT-474 cell line

The effect of PI-103 used in BT-474 cells on time-dependent expression of metadherin mRNA was examined by qPCR method. $2^{-\Delta\Delta Ct}$ values were obtained by calculating the difference between the Ct values of the control group and the Ct values

of the time-dependent Ct values. Statistically analyzed results with $2^{-\Delta\Delta Ct}$ values showed significant difference between control cells and cells treated with PI-103 for 24 hours (n=3) (*p<0,05) (Figure 4.9).

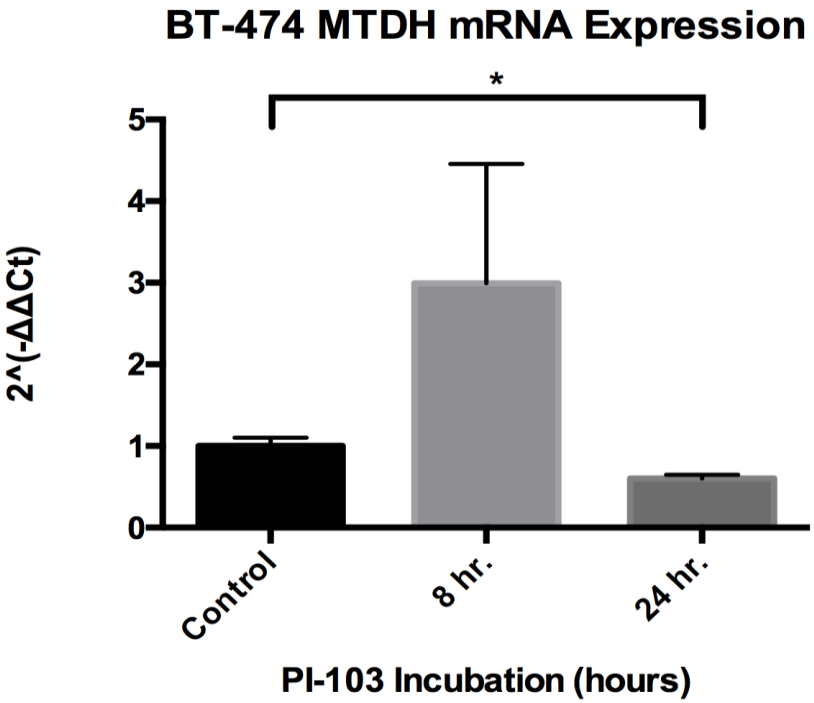


Figure 4.9. Time-dependent effect of PI-103 on metadherin mRNA expression in BT-474 cell line (n=3) (*p<0.05)

4.2.3. Effect of time-dependent incubation of PI-103 on metadherin mRNA expression in the SKBR-3 cell line

The effect of PI-103 used in SKBR-3 cells on time-dependent expression of metadherin mRNA was examined by qPCR method. $2^{-\Delta\Delta Ct}$ values were obtained by calculating the difference between the Ct values of the control group and the Ct values of the time-dependent Ct values. Statistically analyzed results with $2^{-\Delta\Delta Ct}$ values showed significant difference between control cells and cells treated with PI-103 for 8 hours and cells treated with control cells for 24 hours (n=3) (*p<0,05) (Figure 4.10).

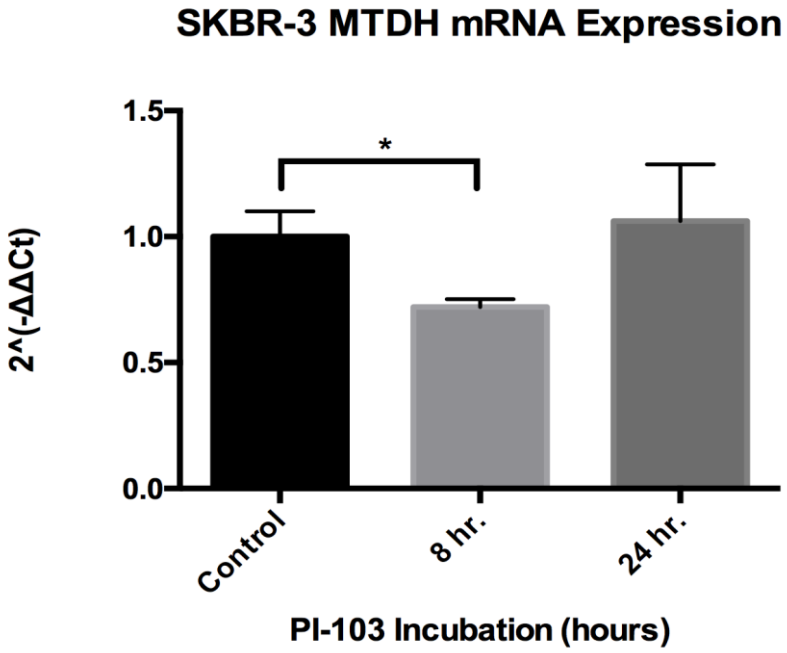
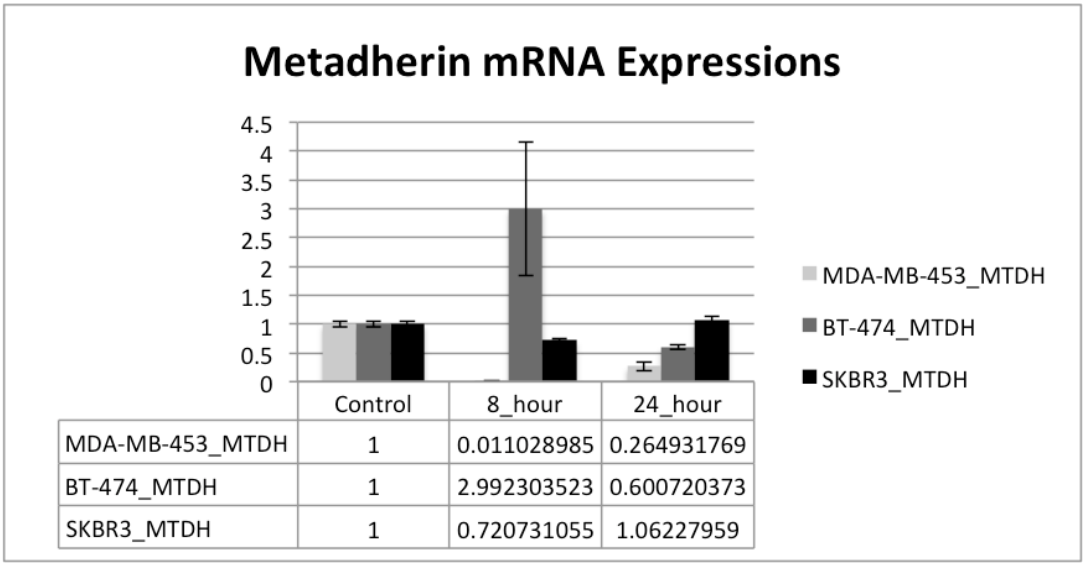


Figure 4.10. Time-dependent effect of PI-103 on metadherin mRNA expression in SKBR-3 cell line (n=3) (*p<0.05)

The comparison of the expression of metadherin mRNA expression in all cell lines is shown in Figure 4.11.



	Control	8_hour	24_hour
MDA-MB-453_MTDH	1	0.011028985	0.264931769
BT-474_MTDH	1	2.992303523	0.600720373
SKBR3_MTDH	1	0.720731055	1.06227959

Figure 4.11. Comparison of the mRNA expression of metadherin in all cell lines (n=3)

4.3 Evaluation of metadherin level in breast cancer patients

Metadherin ELISA kit was used with a total of 80 breast cancer patients serum. Molecular subtypes, HER2 immunohistochemical scoring, presence or absence of metastases, tumor grades and node positivities of 80 patients are indicated in the table below (Table 4.1).

Table 4.1. Demographic Feature of Patients

		N	%
Molecular Subtype	HER2+	9	11,3
	TNBC	32	40,0
	Luminal A	20	25,0
	Luminal B	19	23,8
HER2 IHC	2+	4	5,0
	3+	24	30,0
	Missing	52	65,0
Metastasis	Yes	13	16,3
	No	66	82,5
	Missing	1	1,3
Grade	1	7	8,8
	2	22	27,5
	3	42	52,5
	Missing	9	11,3
Node	N0	34	42,5
	N1, N2, N3	41	51,2
	Missing	5	6,3

4.3.1 Evaluation of metadherin level in different molecular subtypes of breast cancer

Metadherin levels were significantly higher in HER2+ (ER/PR-, HER2+) patients compared to molecular subtypes compared to TNBC (ER/PR-, HER2-) and Luminal A (ER/PR+, HER2-) ($p<0.05$). The difference between HER2+ and Luminal B (ER/PR+, HER2+) groups was not statistically significant (Figure 4.12).

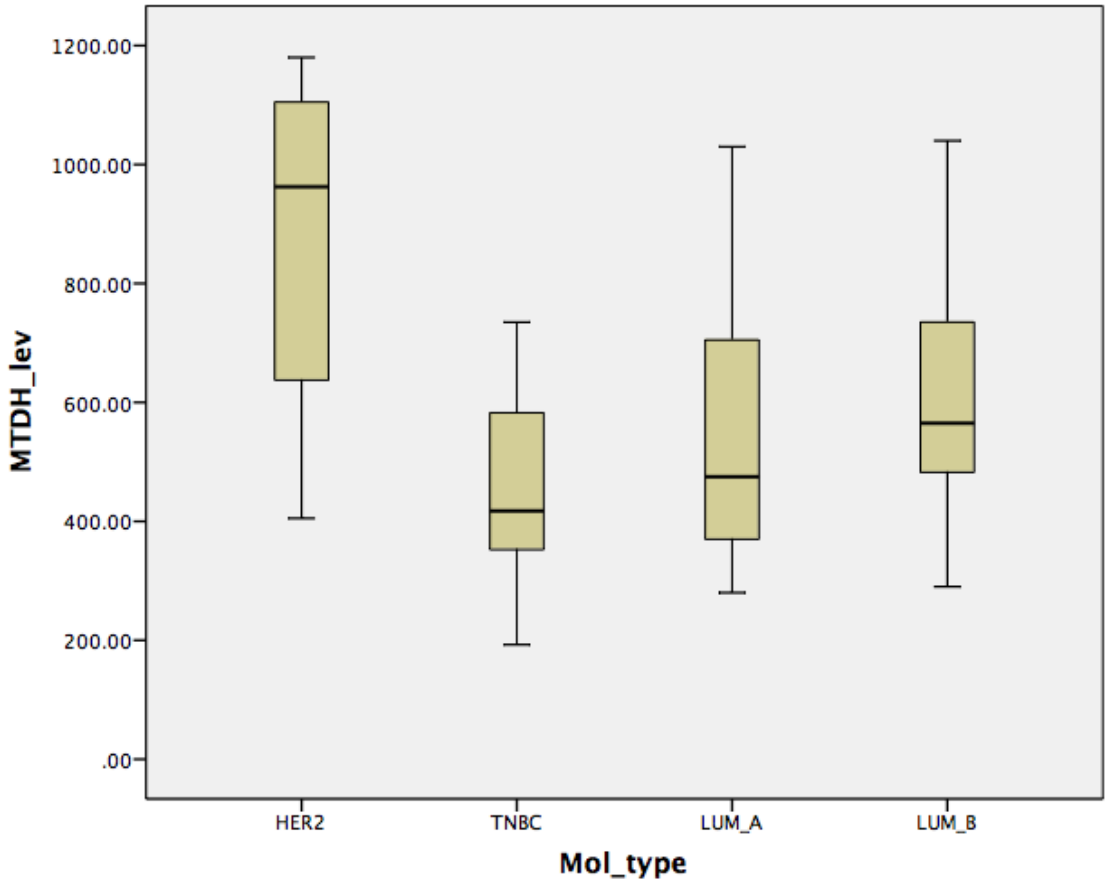


Figure 4.12. Metadherin levels according to the molecular subtype of breast cancer

4.3.2 Evaluation of metadherin level according to the immunostaining score of HER2 in breast cancer patients

According to the HER2 IHC score, the difference in the levels of metadherin between 3+ and 2+ patients was not statistically significant (Figure 4.13).

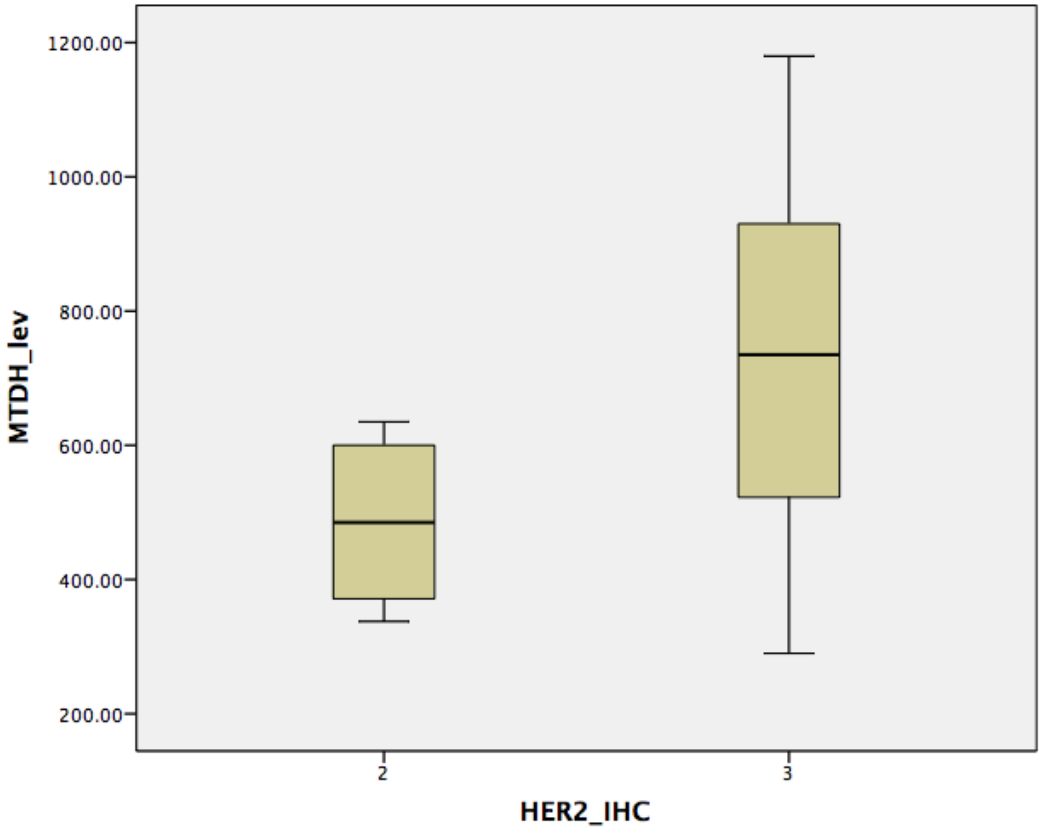


Figure 4.13. Comparison of metadherin level according to HER2 immunostaining

4.3.3 Comparison of metadherin level between metastatic or non-metastatic breast cancer patients

Metadherin levels of metastatic patients were found to be statistically higher than non-metastatic patients ($p<0.01$) (Figure 4.14).

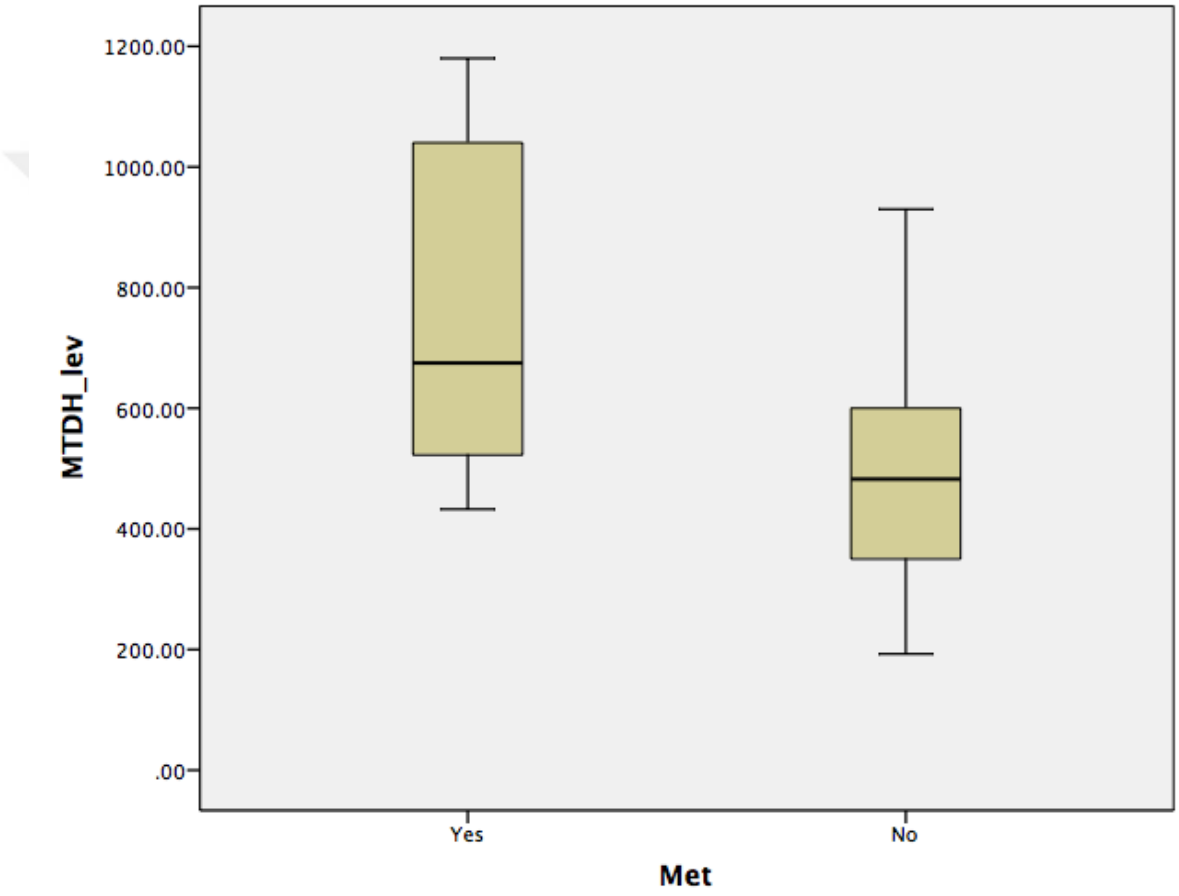


Figure 4.14. Comparison of metadherin level in metastatic and non-metastatic patient groups

4.3.4 Evaluation of metadherin level according to tumor grade of breast cancer patients

In grouping according to tumor grades, there was no statistically significant difference between the levels of metadherin in breast cancer patients (Figure 4.15).

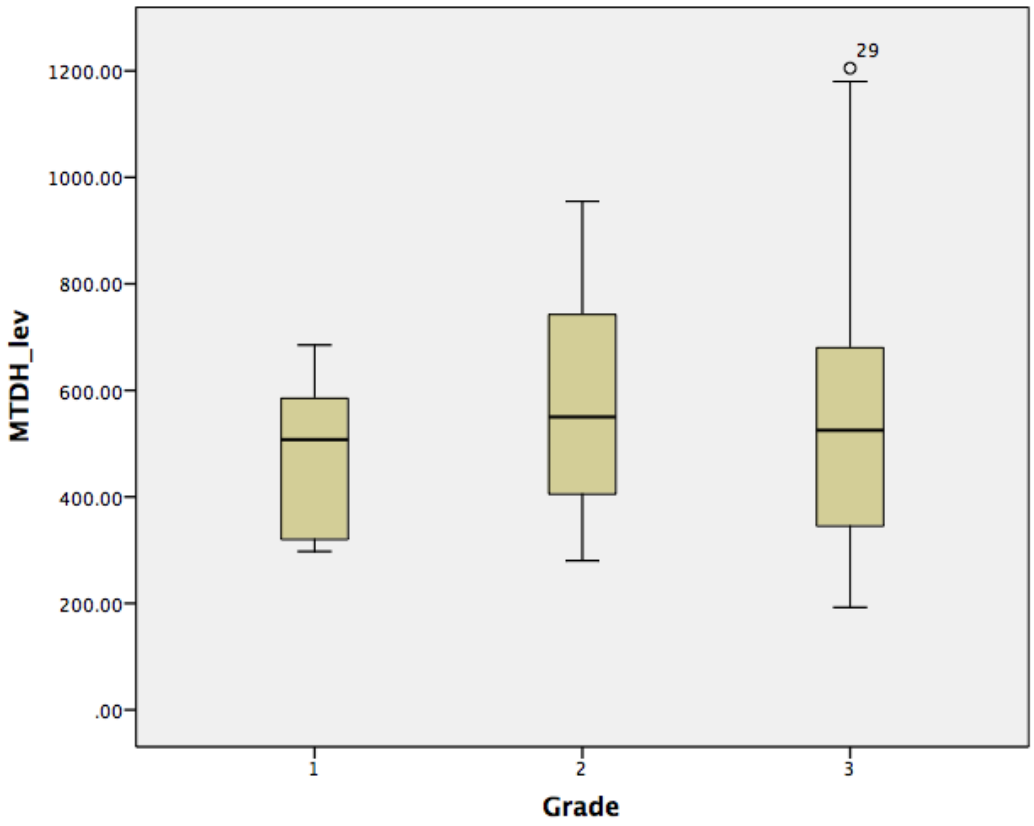


Figure 4.15. Metadherin levels in different tumor grade groups

4.3.5 Comparison of metadherin level in node positive or node negative in breast cancer patients

There was no statistically significant difference between the levels of metadherin in patients with lymph node positive (N1, N2 and N3) and negative (N0) grouping (Figure 4.16).

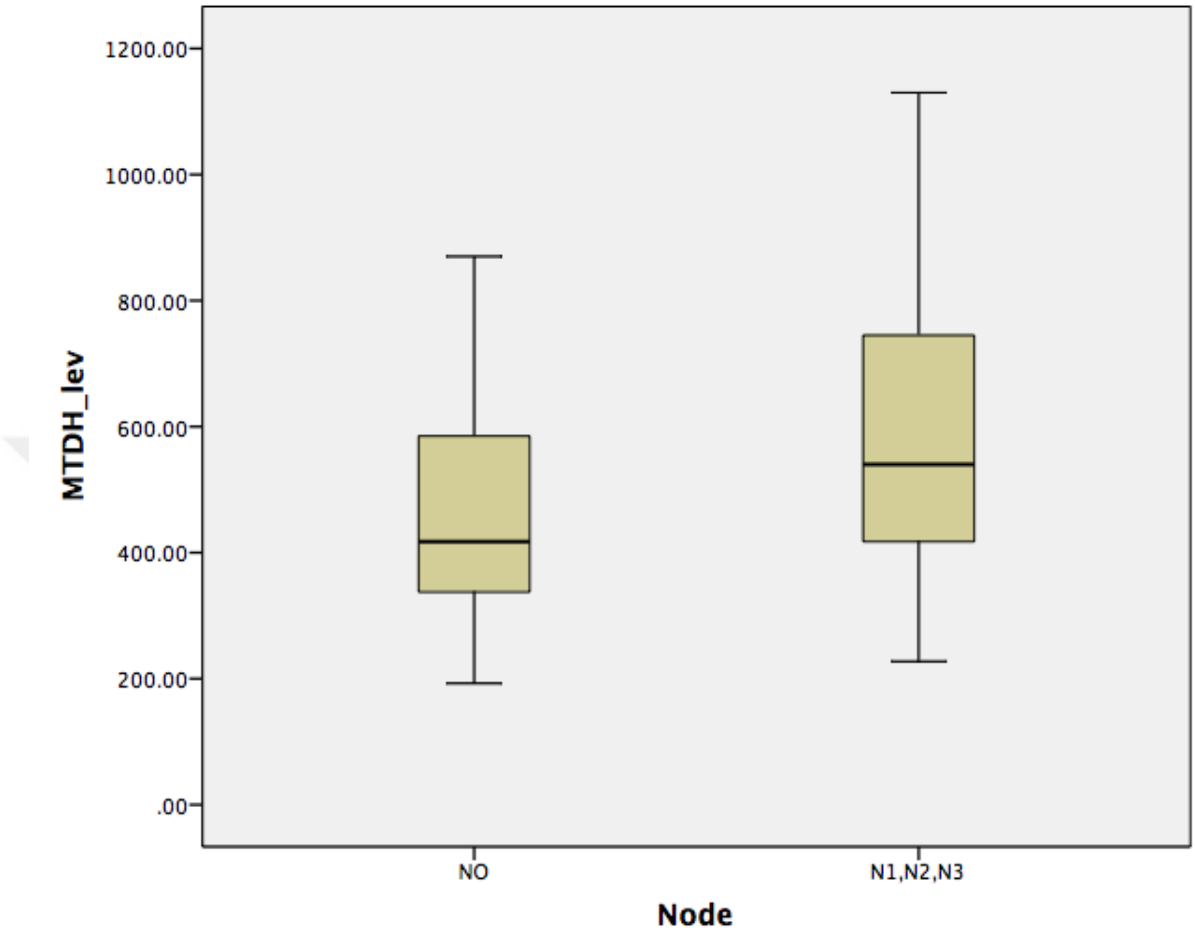


Figure 4.16. Comparison of metadherin level in node positive and node negative patients

5. DISCUSSION

Aberrant PI3K/Akt pathway signalling frequently contributes to the progression of breast cancer. HER2 amplification, activating mutation of PIK3CA that encodes catalytic subunit of PI3K and loss-of-function mutation of PTEN in breast cancer cells lead to constitutive activation. In breast cancer, PI3K/Akt signaling is integrated with MTDH overexpression. MTDH is an oncoprotein mediating initiation, progression and metastasis of cancer (110).

Metadherin is not expressed in normal breast epithelial cells (141). On the other hand, its expression is increased in various tumors including invasive breast cancer. Noticeably, HER2 expression level was shown to be correlated with MTDH in breast cancer cell lines as well as breast cancer tissue samples (142). HER2 overexpression enhances PI3K/Akt signaling pathway in breast cancer mainly via HER2-HER3 activation. Increased activation of pathway triggers MTDH expression at the transcriptional level by c-myc transcription factor (143). MTDH expression is induced by Ha-Ras which in turn activates PI3K/Akt oncogenic signaling. In accordance with such integration, MTDH upregulation was found to be negatively correlated with PTEN expression in HER2 overexpressing breast cancer tissues (142). Taking into account the oncogenic potential of MTDH in HER+ breast cancer pathogenesis, we evaluated the effect of PI3K/Akt signaling inhibition on methaderin expression through PI-103 treatment of three breast cancer cell lines expressing high levels of HER2.

In invasive breast cancer, HER2-HER3 dimerization has highly potent effect. That is, in HER2+ breast cancer cells HER3 expression is required for growth of tumor (144). In our study, we tested PI-103 effect on oncogenic signaling in MDA-MB-453 cells, which express high levels of HER2 and HER3. Importantly, in these cells HER3 expression is 3-4 times higher than BT474 and SKBR3 cells according to flow cytometry analysis (145). HER3 is able to trans-phosphorylate HER2. Additionally, PIK3CA mutation is a remarkable feature of MDA MB 453 cells (7). As far as we know, there is no report concerning incubation of these cells with PI-103. Treatment of cells by

PI-103 led to decrease of MTDH expression significantly after 8 hours and duration of this effect was confirmed after 24 hours. Breast cancer cells with PIK3CA mutation and HER2 overexpression were found to be dependent to Akt signaling. It was shown that breast cancer cell lines carrying these anomalies were highly sensitive to Akt inhibitor (7). This was noteworthy because it led the strategy of targeting intracellular signaling molecules to be a therapeutic goal in tumors including Herceptin resistant phenotype. In accordance with Akt addiction in MDA-MB-453 cells, our results point out that, MDA-MB-453 cells are sensitive to dual inhibition of PI3K and mTOR. In the PI3K/Akt signaling dependent breast cancer cells, targeting pathway by a combination protocol may be a useful therapeutic strategy for attenuating MTDH expression and tumor progression as well.

In our study, in contrast with MDA-MB-453 cells, HER2 overexpressing BT474 cells MTDH expression level was found to be unaffected from PI-103 treatment. These cells have different pattern of metadherin protein expression with two bands, which may be attributed to alternative splicing and/or posttranslational modifications. The role of these modifications on protein function and localization is unknown.

According to our results, in HER2 overexpressing SKBR3 cells PI3K/mTOR inhibitor led much slower and slight decrease in MTDH expression compared to MDA MB 453 cells. These cells express remarkable amount of PTEN. Besides, trastuzumab resistance in SKBR3/R cell was shown to be mediated by MTDH through PTEN inhibition (142). As we used sensitive cells, we may speculate that decreased response to drug inhibitory effect of PI-103 may be attributed to the inverse correlation between MTDH protein level and PTEN activity. That is, negative regulatory effect of PTEN on PI3K/Akt may cause lower MTDH expression in naive cells, which in turn creates moderate MTDH inhibition due to the dual inhibitor treatment. It should certainly be confirmed by MTDH construct transfection in naive SKBR3 cells. We have previously shown that, PI-103 treatment interrupted PI3K/Akt signaling transiently in SKBR-3 cells with a reactivation after 12 hours. In the light of this fact, moderate decrease in MTDH

level in these cells may be due to transient signaling inhibition yet not excluding the interaction between PI3K/Akt pathway and MTDH (146).

Metadherin is known to be transcriptionally regulated through various miRNA upregulation or downregulation in breast cancer. MTDH is the target of miRs-30a,-375, -153,-630 (147). There is an inverse correlation between mRNA expression level of MTDH and miR-30a in SKBR3 cells (148). In our experiments, we only tested whether or not PI-103 would show any effect at transcriptional level. In MDA- MB-453 cells and SKBR-3 cells, MTDH was found to be regulated transcriptionally. It may be due to signaling inhibition, which in turn downregulates c-myc via GSK3β (113). This consequence needs to be shown by assessment of myc expression level in drug treated cells. Additionally, regulation of MTDH mRNA in each cell line through miRNA expression may also clarify breast cancer biology at molecular level.

Among molecular subtypes, HER2+ patients had significantly high level of MTDH. We can not exclude the fact that, we need to enlarge HER+ positive patient group. On the other hand, Luminal B group (ER/PR+, HER2+) in our study pointed out the positive correlation between HER2 expression and MTDH level as well. The abundance of MTDH is positively correlated with distant metastasis and poor prognosis (125). In accordance with this correlation, we found a positive relation between MTDH level and metastasis in breast cancer patients. MTDH level was not related to grade and lymph node status of disease.

As a result, MTDH is an oncogenic protein, which is involved in pathogenesis of breast cancer. Taking into account the impact of PI3K/Akt signaling especially in HER2 overexpressing breast cancer, we may conclude that targeted therapeutic strategies in HER2+ breast cancer may be involved in suppression of invasion and metastasis through regulation of MTDH expression.

6. CONCLUSIONS AND FUTURE DIRECTIONS

1.PI-103 (PI3K/mTOR dual inhibitor) treatment confirmed the regulatory role of PI3K/Akt pathway on methaderin expression in HER2 overexpressing cells.

2. In MDA-MB-453 cells, PI-103 treatment indicated noticable regulation on MTDH expression. The cellular consequences of MTDH inhibition should be tested via invasion and chemoresistance assays.

3. MTDH regulation in naive SKBR-3 cells was moderate compared to MDA-MB-453 cells. Since MTDH is known to mediate trastuzumab resistance in HER2+ breast cancer by decreasing PTEN expression, slightly decreased MTDH levels may be atrributed to PTEN activity in naive cells.

4. BT474 cells did not show significant change for MTDH at mRNA and protein level. The contribution of different expression pattern in these cells may be investigated.

5. Serum MTDH level of breast cancer patients confirmed the significant correlation between HER2+ subtype and MTDH. Apart from Luminal B group, we need to enlarge HER2+ patient group and re-evaluation will be better choice.

6. The positive correlation between MTDH level and metastasis is noteworthy for breast cancer biology and traetment approach.

7. PI3K/Akt pathway targeting drugs in HER2+ breast cancer may be involved in suppression of invasion and metastasis through regulation of MTDH expression.

8. Taking into consideration of high MTDH expression in breast cancer and the role in drug resistance, MTDH silencing should be kept in mind as an efficient goal.

7.REFERENCES

1. Ghayad SE, Cohen PA. Inhibitors of the PI3K/Akt/mTOR pathway: new hope for breast cancer patients. Recent patents on anti-cancer drug discovery. 2010;5(1):29-57.

2. Ferlay J, Soerjomataram II, Dikshit R, Eser S, Mathers C, Rebelo M, et al. Cancer incidence and mortality worldwide: sources, methods and major patterns in GLOBOCAN 2012. International journal of cancer Journal international du cancer. 2014.

3. Mohd Sharial MS, Crown J, Hennessy BT. Overcoming resistance and restoring sensitivity to HER2-targeted therapies in breast cancer. Ann Oncol. 2012;23(12):3007-16.

4. Hernandez-Aya LF, Gonzalez-Angulo AM. Targeting the phosphatidylinositol 3-kinase signaling pathway in breast cancer. Oncologist. 2011;16(4):404-14.

5. Lemmon MA, Schlessinger J. Cell signaling by receptor tyrosine kinases. Cell. 2010;141(7):1117-34.

6. Rodrik-Outmezguine VS, Chandarlapaty S, Pagano NC, Poulikakos PI, Scaltriti M, Moskatel E, et al. mTOR kinase inhibition causes feedback-dependent biphasic regulation of AKT signaling. Cancer discovery. 2011;1(3):248-59.

7. She QB, Chandarlapaty S, Ye Q, Lobo J, Haskell KM, Leander KR, et al. Breast tumor cells with PI3K mutation or HER2 amplification are selectively addicted to Akt signaling. PLoS One. 2008;3(8):e3065.

8. Carmona FJ, Montemurro F, Kannan S, Rossi V, Verma C, Baselga J, et al. AKT signaling in ERBB2-amplified breast cancer. Pharmacol Ther. 2016;158:63-70.

9. Sharma VR, Gupta GK, Sharma AK, Batra N, Sharma DK, Joshi A, et al. PI3K/Akt/mTOR Intracellular Pathway and Breast Cancer: Factors, Mechanism and Regulation. Current pharmaceutical design. 2017;23(11):1633-8.

10. Vivanco I, Sawyers CL. The phosphatidylinositol 3-Kinase AKT pathway in human cancer. Nature reviews Cancer. 2002;2(7):489-501.

11. Torbett NE, Luna-Moran A, Knight ZA, Houk A, Moasser M, Weiss W, et al. A chemical screen in diverse breast cancer cell lines reveals genetic enhancers and suppressors of sensitivity to PI3K isoform-selective inhibition. The Biochemical journal. 2008;415(1):97-110.

12. Chandarlapaty S, Sawai A, Scaltriti M, Rodrik-Outmezguine V, Grbovic-Huezo O, Serra V, et al. AKT inhibition relieves feedback suppression of receptor tyrosine kinase expression and activity. Cancer Cell. 2011;19(1):58-71.

13. Brana I, Pham NA, Kim L, Sakashita S, Li M, Ng C, et al. Novel combinations of PI3K-mTOR inhibitors with dacomitinib or chemotherapy in PTEN-deficient patient-derived tumor xenografts. Oncotarget. 2017;8(49):84659-70.

14. Lee SG, Su ZZ, Emdad L, Sarkar D, Franke TF, Fisher PB. Astrocyte elevated gene-1 activates cell survival pathways through PI3K-Akt signaling. Oncogene. 2008;27(8):1114-21.

15. Brown DM, Ruoslahti E. Metadherin, a cell surface protein in breast tumors that mediates lung metastasis. Cancer Cell. 2004;5(4):365-74.

16. Xu C, Kong X, Wang H, Zhang N, Kong X, Ding X, et al. MTDH mediates estrogen-independent growth and tamoxifen resistance by down-regulating PTEN in MCF-7 breast cancer cells. Cellular physiology and biochemistry : international journal of experimental cellular physiology, biochemistry, and pharmacology. 2014;33(5):1557-67.

17. Wei Y, Hu G, Kang Y. Metadherin as a link between metastasis and chemoresistance. Cell cycle. 2009;8(14):2131-7.

18. Li X, Kong X, Huo Q, Guo H, Yan S, Yuan C, et al. Metadherin enhances the invasiveness of breast cancer cells by inducing epithelial to mesenchymal transition. Cancer Sci. 2011;102(6):1151-7.

19. Li Y, Gonzalez Bosquet J, Yang S, Thiel KW, Zhang Y, Liu H, et al. Role of metadherin in estrogen-regulated gene expression. International journal of molecular medicine. 2017;40(2):303-10.

20. Hynes NE, Gullick W. Therapeutic targeting of signal transduction pathways and proteins in breast cancer. Journal of mammary gland biology and neoplasia. 2006;11(1):1-2.

21. Saini KS, Loi S, de Azambuja E, Metzger-Filho O, Saini ML, Ignatiadis M, et al. Targeting the PI3K/AKT/mTOR and Raf/MEK/ERK pathways in the treatment of breast cancer. Cancer Treat Rev. 2013;39(8):935-46.

22. Burris HA, 3rd. Overcoming acquired resistance to anticancer therapy: focus on the PI3K/AKT/mTOR pathway. Cancer Chemother Pharmacol. 2013;71(4):829-42.

23. Mayer IA, Arteaga CL. The PI3K/AKT Pathway as a Target for Cancer Treatment. Annu Rev Med. 2016;67:11-28.

24. Fan QW, Knight ZA, Goldenberg DD, Yu W, Mostov KE, Stokoe D, et al. A dual PI3 kinase/mTOR inhibitor reveals emergent efficacy in glioma. Cancer Cell. 2006;9(5):341-9.

25. Kong D, Yamori T. Phosphatidylinositol 3-kinase inhibitors: promising drug candidates for cancer therapy. Cancer Sci. 2008;99(9):1734-40.

26. Yi YW, Kang HJ, Kim HJ, Hwang JS, Wang A, Bae I. Inhibition of constitutively activated phosphoinositide 3-kinase/AKT pathway enhances antitumor activity of chemotherapeutic agents in breast cancer susceptibility gene 1-defective breast cancer cells. Molecular carcinogenesis. 2013;52(9):667-75.

27. Zheng J, Li C, Wu X, Yang Y, Hao M, Sheng S, et al. Astrocyte elevated gene-1 is a novel biomarker of epithelial-mesenchymal transition and progression of hepatocellular carcinoma in two China regions. Tumour Biol. 2014;35(3):2265-9.

28. Liu Y, Kong X, Li X, Li B, Yang Q. Knockdown of metadherin inhibits angiogenesis in breast cancer. Int J Oncol. 2015;46(6):2459-66.

29. Wei YB, Guo Q, Gao YL, Yan B, Wang Z, Yang JR, et al. Repression of metadherin inhibits biological behavior of prostate cancer cells and enhances their sensitivity to cisplatin. Molecular medicine reports. 2015;12(1):226-32.

30. Rakha EA, Ellis IO. Triple-negative/basal-like breast cancer: review. Pathology. 2009;41(1):40-7.

31. Cancer Genome Atlas N. Comprehensive molecular portraits of human breast tumours. Nature. 2012;490(7418):61-70.

32. Rupninder Sandhu M, Joel S. Parker, MS, Wendell D. Jones, PhD, Chad A. Livasy, MD, William B. Coleman, PhD. Microarray-Based Gene Expression Profiling for Molecular Classification of Breast Cancer and Identification of New Targets for Therapy. LABMEDICINE. 2010;41(6):364-72.

33. Dawson SJ, Rueda OM, Aparicio S, Caldas C. A new genome-driven integrated classification of breast cancer and its implications. The EMBO journal. 2013;32(5):617-28.

34. Wheler JJ, Atkins JT, Janku F, Moulder SL, Yelensky R, Stephens PJ, et al. Multiple gene aberrations and breast cancer: lessons from super-responders. BMC cancer. 2015;15:442.

35. Hanahan D, Weinberg RA. The hallmarks of cancer. Cell. 2000;100(1):57-70.

36. Hanahan D, Weinberg RA. Hallmarks of cancer: the next generation. Cell. 2011;144(5):646-74.

37. Castaneda CA, Cortes-Funes H, Gomez HL, Ciruelos EM. The phosphatidyl inositol 3-kinase/AKT signaling pathway in breast cancer. Cancer metastasis reviews. 2010;29(4):751-9.

38. Blume-Jensen P, Hunter T. Oncogenic kinase signalling. Nature. 2001;411(6835):355-65.

39. Hunter T. The Croonian Lecture 1997. The phosphorylation of proteins on tyrosine: its role in cell growth and disease. Philosophical transactions of the Royal Society of London Series B, Biological sciences. 1998;353(1368):583-605.

40. Schlessinger J. Cell signaling by receptor tyrosine kinases. Cell. 2000;103(2):211-25.

41. Hubbard SR. Structural analysis of receptor tyrosine kinases. Progress in biophysics and molecular biology. 1999;71(3-4):343-58.

42. Hubbard SR, Miller WT. Receptor tyrosine kinases: mechanisms of activation and signaling. Current opinion in cell biology. 2007;19(2):117-23.

43. Hubbard SR, Mohammadi M, Schlessinger J. Autoregulatory mechanisms in protein-tyrosine kinases. J Biol Chem. 1998;273(20):11987-90.

44. Jiang G, Hunter T. Receptor signaling: when dimerization is not enough. Current biology : CB. 1999;9(15):R568-71.

45. Li E, Hristova K. Role of receptor tyrosine kinase transmembrane domains in cell signaling and human pathologies. Biochemistry. 2006;45(20):6241-51.

46. Choura M, Rebai A. Receptor tyrosine kinases: from biology to pathology. J Recept Signal Transduct Res. 2011;31(6):387-94.

47. Casalini P, Iorio MV, Galmozzi E, Menard S. Role of HER receptors family in development and differentiation. Journal of cellular physiology. 2004;200(3):343-50.

48. Wells A. EGF receptor. The international journal of biochemistry & cell biology. 1999;31(6):637-43.

49. Cohen S, Levi-Montalcini R. Purification and properties of a nerve growth-promoting factor isolated from mouse sarcoma 180. Cancer Res. 1957;17(1):15-20.

50. Yarden Y, Pines G. The ERBB network: at last, cancer therapy meets systems biology. Nature reviews Cancer. 2012;12(8):553-63.

51. Savage CR, Jr., Inagami T, Cohen S. The primary structure of epidermal growth factor. J Biol Chem. 1972;247(23):7612-21.

52. Cohen S, Carpenter G. Human epidermal growth factor: isolation and chemical and biological properties. Proc Natl Acad Sci U S A. 1975;72(4):1317-21.

53. Arteaga CL, Engelman JA. ERBB receptors: from oncogene discovery to basic science to mechanism-based cancer therapeutics. Cancer Cell. 2014;25(3):282-303.

54. Yarden Y, Sliwkowski MX. Untangling the ErbB signalling network. Nature reviews Molecular cell biology. 2001;2(2):127-37.

55. Guy PM, Platko JV, Cantley LC, Cerione RA, Carraway KL, 3rd. Insect cell-expressed p180erbB3 possesses an impaired tyrosine kinase activity. Proc Natl Acad Sci U S A. 1994;91(17):8132-6.

56. Olayioye MA, Neve RM, Lane HA, Hynes NE. The ErbB signaling network: receptor heterodimerization in development and cancer. The EMBO journal. 2000;19(13):3159-67.

57. Eccles SA. The epidermal growth factor receptor/Erb-B/HER family in normal and malignant breast biology. The International journal of developmental biology. 2011;55(7-9):685-96.

58. Hynes NE, Lane HA. ERBB receptors and cancer: the complexity of targeted inhibitors. Nature reviews Cancer. 2005;5(5):341-54.

59. Hynes NE, MacDonald G. ErbB receptors and signaling pathways in cancer. Current opinion in cell biology. 2009;21(2):177-84.

60. Holbro T, Hynes NE. ErbB receptors: directing key signaling networks throughout life. Annual review of pharmacology and toxicology. 2004;44:195-217.

61. Wee P, Wang Z. Epidermal Growth Factor Receptor Cell Proliferation Signaling Pathways. Cancers. 2017;9(5).

62. Bassiri M, Privalsky ML. Mutagenesis of the avian erythroblastosis virus erbB coding region: an intact extracellular domain is not required for oncogenic transformation. Journal of virology. 1986;59(2):525-30.

63. Bacus SS, Ruby SG, Weinberg DS, Chin D, Ortiz R, Bacus JW. HER-2/neu oncogene expression and proliferation in breast cancers. The American journal of pathology. 1990;137(1):103-11.

64. Ross JS, Fletcher JA. The HER-2/neu Oncogene in Breast Cancer: Prognostic Factor, Predictive Factor, and Target for Therapy. Oncologist. 1998;3(4):237-52.

65. Gala K, Chandarlapaty S. Molecular pathways: HER3 targeted therapy. Clin Cancer Res. 2014;20(6):1410-6.

66. Amin DN, Campbell MR, Moasser MM. The role of HER3, the unpretentious member of the HER family, in cancer biology and cancer therapeutics. Seminars in cell & developmental biology. 2010;21(9):944-50.

67. Roskoski R, Jr. The ErbB/HER family of protein-tyrosine kinases and cancer. Pharmacological research : the official journal of the Italian Pharmacological Society. 2014;79:34-74.

68. Cao GD, Chen K, Chen B, Xiong MM. Positive prognostic value of HER2-HER3 co-expression and p-mTOR in gastric cancer patients. BMC cancer. 2017;17(1):841.

69. Jaiswal BS, Kljavin NM, Stawiski EW, Chan E, Parikh C, Durinck S, et al. Oncogenic ERBB3 mutations in human cancers. Cancer Cell. 2013;23(5):603-17.

70. Sun Y, Thapa N, Hedman AC, Anderson RA. Phosphatidylinositol 4,5-bisphosphate: targeted production and signaling. Bioessays. 2013;35(6):513-22.

71. Wymann MP, Zvelebil M, Laffargue M. Phosphoinositide 3-kinase signalling--which way to target? Trends in pharmacological sciences. 2003;24(7):366-76.

72. Fresno Vara JA, Casado E, de Castro J, Cejas P, Belda-Iniesta C, Gonzalez-Baron M. PI3K/Akt signalling pathway and cancer. Cancer Treat Rev. 2004;30(2):193-204.

73. Wymann MP, Marone R. Phosphoinositide 3-kinase in disease: timing, location, and scaffolding. Current opinion in cell biology. 2005;17(2):141-9.

74. Gaidarov I, Smith ME, Domin J, Keen JH. The class II phosphoinositide 3-kinase C2alpha is activated by clathrin and regulates clathrin-mediated membrane trafficking. Molecular cell. 2001;7(2):443-9.

75. Wymann MP, Pirola L. Structure and function of phosphoinositide 3-kinases. Biochim Biophys Acta. 1998;1436(1-2):127-50.

76. Schu PV, Takegawa K, Fry MJ, Stack JH, Waterfield MD, Emr SD. Phosphatidylinositol 3-kinase encoded by yeast VPS34 gene essential for protein sorting. Science. 1993;260(5104):88-91.

77. Osaki M, Oshimura M, Ito H. PI3K-Akt pathway: its functions and alterations in human cancer. Apoptosis. 2004;9(6):667-76.

78. Yu J, Wjasow C, Backer JM. Regulation of the p85/p110alpha phosphatidylinositol 3'-kinase. Distinct roles for the n-terminal and c-terminal SH2 domains. J Biol Chem. 1998;273(46):30199-203.

79. Scaltriti M, Baselga J. The epidermal growth factor receptor pathway: a model for targeted therapy. Clin Cancer Res. 2006;12(18):5268-72.

80. Staal SP. Molecular cloning of the akt oncogene and its human homologues AKT1 and AKT2: amplification of AKT1 in a primary human gastric adenocarcinoma. Proc Natl Acad Sci U S A. 1987;84(14):5034-7.

81. Morrison Joly M, Hicks DJ, Jones B, Sanchez V, Estrada MV, Young C, et al. Rictor/mTORC2 Drives Progression and Therapeutic Resistance of HER2-Amplified Breast Cancers. Cancer Res. 2016;76(16):4752-64.

82. Guertin DA, Sabatini DM. Defining the role of mTOR in cancer. Cancer Cell. 2007;12(1):9-22.

83. Manning BD, Cantley LC. AKT/PKB signaling: navigating downstream. Cell. 2007;129(7):1261-74.

84. Sarbassov DD, Guertin DA, Ali SM, Sabatini DM. Phosphorylation and regulation of Akt/PKB by the rictor-mTOR complex. Science. 2005;307(5712):1098-101.

85. Steck PA, Pershouse MA, Jasser SA, Yung WK, Lin H, Ligon AH, et al. Identification of a candidate tumour suppressor gene, MMAC1, at chromosome 10q23.3 that is mutated in multiple advanced cancers. Nature genetics. 1997;15(4):356-62.

86. Li J, Yen C, Liaw D, Podsypanina K, Bose S, Wang SI, et al. PTEN, a putative protein tyrosine phosphatase gene mutated in human brain, breast, and prostate cancer. Science. 1997;275(5308):1943-7.

87. Hynes NE, Dey JH. PI3K inhibition overcomes trastuzumab resistance: blockade of ErbB2/ErbB3 is not always enough. Cancer Cell. 2009;15(5):353-5.

88. Lux MP, Fasching PA, Schrauder MG, Hein A, Jud SM, Rauh C, et al. The PI3K Pathway: Background and Treatment Approaches. Breast Care (Basel). 2016;11(6):398-404.

89. Courtney KD, Corcoran RB, Engelman JA. The PI3K pathway as drug target in human cancer. Journal of clinical oncology : official journal of the American Society of Clinical Oncology. 2010;28(6):1075-83.

90. Miled N, Yan Y, Hon WC, Perisic O, Zvelebil M, Inbar Y, et al. Mechanism of two classes of cancer mutations in the phosphoinositide 3-kinase catalytic subunit. Science. 2007;317(5835):239-42.

91. Hollestelle A, Elstrodt F, Nagel JH, Kallemeijn WW, Schutte M. Phosphatidylinositol-3-OH kinase or RAS pathway mutations in human breast cancer cell lines. Molecular cancer research : MCR. 2007;5(2):195-201.

92. Chalhoub N, Baker SJ. PTEN and the PI3-kinase pathway in cancer. Annual review of pathology. 2009;4:127-50.

93. Xie Y, Naizabekov S, Chen Z, Tokay T. Power of PTEN/AKT: Molecular switch between tumor suppressors and oncogenes. Oncol Lett. 2016;12(1):375-8.

94. Carnero A. The PKB/AKT pathway in cancer. Current pharmaceutical design. 2010;16(1):34-44.

95. Fruman DA, Snapper SB, Yballe CM, Davidson L, Yu JY, Alt FW, et al. Impaired B cell development and proliferation in absence of phosphoinositide 3-kinase p85alpha. Science. 1999;283(5400):393-7.

96. Samuels Y, Wang Z, Bardelli A, Silliman N, Ptak J, Szabo S, et al. High frequency of mutations of the PIK3CA gene in human cancers. Science. 2004;304(5670):554.

97. Saal LH, Holm K, Maurer M, Memeo L, Su T, Wang X, et al. PIK3CA mutations correlate with hormone receptors, node metastasis, and ERBB2, and are mutually exclusive with PTEN loss in human breast carcinoma. Cancer Res. 2005;65(7):2554-9.

98. Bachman KE, Argani P, Samuels Y, Silliman N, Ptak J, Szabo S, et al. The PIK3CA gene is mutated with high frequency in human breast cancers. Cancer biology & therapy. 2004;3(8):772-5.

99. Engelman JA. Targeting PI3K signalling in cancer: opportunities, challenges and limitations. Nature reviews Cancer. 2009;9(8):550-62.

100. Guerrero-Zotano A, Mayer IA, Arteaga CL. PI3K/AKT/mTOR: role in breast cancer progression, drug resistance, and treatment. Cancer metastasis reviews. 2016;35(4):515-24.

101. Wilks ST. Potential of overcoming resistance to HER2-targeted therapies through the PI3K/Akt/mTOR pathway. Breast. 2015;24(5):548-55.

102. Su ZZ, Kang DC, Chen Y, Pekarskaya O, Chao W, Volsky DJ, et al. Identification and cloning of human astrocyte genes displaying elevated expression after infection with HIV-1 or exposure to HIV-1 envelope glycoprotein by rapid subtraction hybridization, RaSH. Oncogene. 2002;21(22):3592-602.

103. Britt DE, Yang DF, Yang DQ, Flanagan D, Callanan H, Lim YP, et al. Identification of a novel protein, LYRIC, localized to tight junctions of polarized epithelial cells. Experimental cell research. 2004;300(1):134-48.

104. Lee SG, Kang DC, DeSalle R, Sarkar D, Fisher PB. AEG-1/MTDH/LYRIC, the beginning: initial cloning, structure, expression profile, and regulation of expression. Adv Cancer Res. 2013;120:1-38.

105. Safran M, Chalifa-Caspi V, Shmueli O, Olender T, Lapidot M, Rosen N, et al. Human Gene-Centric Databases at the Weizmann Institute of Science: GeneCards, UDB, CroW 21 and HORDE. Nucleic acids research. 2003;31(1):142-6.

106. Hornbeck PV, Kornhauser JM, Tkachev S, Zhang B, Skrzypek E, Murray B, et al. PhosphoSitePlus: a comprehensive resource for investigating the structure and function of experimentally determined post-translational modifications in man and mouse. Nucleic acids research. 2012;40(Database issue):D261-70.

107. Yoo BK, Emdad L, Lee SG, Su ZZ, Santhekadur P, Chen D, et al. Astrocyte elevated gene-1 (AEG-1): A multifunctional regulator of normal and abnormal physiology. Pharmacol Ther. 2011;130(1):1-8.

108. Zhou J, Li J, Wang Z, Yin C, Zhang W. Metadherin is a novel prognostic marker for bladder cancer progression and overall patient survival. Asia-Pacific journal of clinical oncology. 2012;8(3):e42-8.

109. Xia Z, Zhang N, Jin H, Yu Z, Xu G, Huang Z. Clinical significance of astrocyte elevated gene-1 expression in human oligodendrogliomas. Clinical neurology and neurosurgery. 2010;112(5):413-9.

110. Huang Y, Li LP. Progress of cancer research on astrocyte elevated gene-1/Metadherin (Review). Oncol Lett. 2014;8(2):493-501.

111. Chen X, Dong K, Long M, Lin F, Wang X, Wei J, et al. Serum anti-AEG-1 auto-antibody is a potential novel biomarker for malignant tumors. Oncol Lett. 2012;4(2):319-23.

112. Srivastava J, Siddiq A, Emdad L, Santhekadur PK, Chen D, Gredler R, et al. Astrocyte elevated gene-1 promotes hepatocarcinogenesis: novel insights from a mouse model. Hepatology. 2012;56(5):1782-91.

113. Hu G, Chong RA, Yang Q, Wei Y, Blanco MA, Li F, et al. MTDH activation by 8q22 genomic gain promotes chemoresistance and metastasis of poor-prognosis breast cancer. Cancer Cell. 2009;15(1):9-20.

114. Song Z, Wang Y, Li C, Zhang D, Wang X. Molecular Modification of Metadherin/MTDH Impacts the Sensitivity of Breast Cancer to Doxorubicin. PLoS One. 2015;10(5):e0127599.

115. Jeon HY, Choi M, Howlett EL, Vozhilla N, Yoo BK, Lloyd JA, et al. Expression patterns of astrocyte elevated gene-1 (AEG-1) during development of the mouse embryo. Gene expression patterns : GEP. 2010;10(7-8):361-7.

116. Zhu K, Dai Z, Pan Q, Wang Z, Yang GH, Yu L, et al. Metadherin promotes hepatocellular carcinoma metastasis through induction of epithelial-mesenchymal transition. Clin Cancer Res. 2011;17(23):7294-302.

117. Liang Y, Hu J, Li J, Liu Y, Yu J, Zhuang X, et al. Epigenetic Activation of TWIST1 by MTDH Promotes Cancer Stem-like Cell Traits in Breast Cancer. Cancer Res. 2015;75(17):3672-80.

118. Li W, Zhai L, Zhao C, Lv S. MiR-153 inhibits epithelial-mesenchymal transition by targeting metadherin in human breast cancer. Breast cancer research and treatment. 2015;150(3):501-9.

119. Liu P, Tang H, Chen B, He Z, Deng M, Wu M, et al. miR-26a suppresses tumour proliferation and metastasis by targeting metadherin in triple negative breast cancer. Cancer letters. 2015;357(1):384-92.

120. Ward A, Balwierz A, Zhang JD, Kublbeck M, Pawitan Y, Hielscher T, et al. Re-expression of microRNA-375 reverses both tamoxifen resistance and accompanying EMT-like properties in breast cancer. Oncogene. 2013;32(9):1173-82.

121. Kang DC, Su ZZ, Sarkar D, Emdad L, Volsky DJ, Fisher PB. Cloning and characterization of HIV-1-inducible astrocyte elevated gene-1, AEG-1. Gene. 2005;353(1):8-15.

122. Zhu GC, Yu CY, She L, Tan HL, Li G, Ren SL, et al. Metadherin regulation of vascular endothelial growth factor expression is dependent upon the PI3K/Akt pathway in squamous cell carcinoma of the head and neck. Medicine (Baltimore). 2015;94(6):e502.

123. Noch E, Bookland M, Khalili K. Astrocyte-elevated gene-1 (AEG-1) induction by hypoxia and glucose deprivation in glioblastoma. Cancer biology & therapy. 2011;11(1):32-9.

124. Zhang EY, Cristofanilli M, Robertson F, Reuben JM, Mu Z, Beavis RC, et al. Genome wide proteomics of ERBB2 and EGFR and other oncogenic pathways in inflammatory breast cancer. Journal of proteome research. 2013;12(6):2805-17.

125. Wan L, Kang Y. Pleiotropic roles of AEG-1/MTDH/LYRIC in breast cancer. Adv Cancer Res. 2013;120:113-34.

126. Shi X, Wang X. The role of MTDH/AEG-1 in the progression of cancer. International journal of clinical and experimental medicine. 2015;8(4):4795-807.

127. Tokunaga E, Nakashima Y, Yamashita N, Hisamatsu Y, Okada S, Akiyoshi S, et al. Overexpression of metadherin/MTDH is associated with an aggressive phenotype and a poor prognosis in invasive breast cancer. Breast Cancer. 2014;21(3):341-9.

128. Erdem H. OM, Uzunlar A.K., Yıldırım Ü., Ankaralı H., Yaşar M. AEG-1 expression in invasive ductal and lobular breast cancers and its relationship with prognostic parameters. Turk J Med Sci. 2013;43(1):93-8.

129. Li C, Li R, Song H, Wang D, Feng T, Yu X, et al. Significance of AEG-1 expression in correlation with VEGF, microvessel density and clinicopathological characteristics in triple-negative breast cancer. Journal of surgical oncology. 2011;103(2):184-92.

130. Brachmann S, Fritsch C, Maira SM, Garcia-Echeverria C. PI3K and mTOR inhibitors: a new generation of targeted anticancer agents. Current opinion in cell biology. 2009;21(2):194-8.

131. Holmes D. PI3K pathway inhibitors approach junction. Nature reviews Drug discovery. 2011;10(8):563-4.

132. Bahrami A, Khazaei M, Shahidsales S, Hassanian SM, Hasanzadeh M, Maftouh M, et al. The Therapeutic Potential of PI3K/Akt/mTOR Inhibitors in Breast Cancer: Rational and Progress. Journal of cellular biochemistry. 2018;119(1):213-22.

133. Schettini F, Buono G, Trivedi MV, De Placido S, Arpino G, Giuliano M. PI3K/mTOR Inhibitors in the Treatment of Luminal Breast Cancer. Why, When and to Whom? Breast Care (Basel). 2017;12(5):290-4.

134. Azab SS. Targeting the mTOR Signaling Pathways in Breast Cancer: More Than the Rapalogs. Journal of biochemical and pharmacological research. 2013;1(2):75-83.

135. Eichhorn PJ, Gili M, Scaltriti M, Serra V, Guzman M, Nijkamp W, et al. Phosphatidylinositol 3-kinase hyperactivation results in lapatinib resistance that is reversed by the mTOR/phosphatidylinositol 3-kinase inhibitor NVP-BEZ235. Cancer Res. 2008;68(22):9221-30.

136. O'Reilly KE, Rojo F, She QB, Solit D, Mills GB, Smith D, et al. mTOR inhibition induces upstream receptor tyrosine kinase signaling and activates Akt. Cancer Res. 2006;66(3):1500-8.

137. Workman P, Clarke PA, Raynaud FI, van Montfort RL. Drugging the PI3 kinome: from chemical tools to drugs in the clinic. Cancer Res. 2010;70(6):2146-57.

138. Hayakawa M, Kawaguchi K, Kaizawa H, Koizumi T, Ohishi T, Yamano M, et al. Synthesis and biological evaluation of sulfonylhydrazone-substituted imidazo[1,2-a]pyridines as novel PI3 kinase p110alpha inhibitors. Bioorganic & medicinal chemistry. 2007;15(17):5837-44.

139. Park S, Chapuis N, Bardet V, Tamburini J, Gallay N, Willems L, et al. PI-103, a dual inhibitor of Class IA phosphatidylinositide 3-kinase and mTOR, has antileukemic activity in AML. Leukemia. 2008;22(9):1698-706.

140. Livak KJ, Schmittgen TD. Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)) Method. Methods. 2001;25(4):402-8.

141. Li J, Zhang N, Song LB, Liao WT, Jiang LL, Gong LY, et al. Astrocyte elevated gene-1 is a novel prognostic marker for breast cancer progression and overall patient survival. Clin Cancer Res. 2008;14(11):3319-26.

142. Du C, Yi X, Liu W, Han T, Liu Z, Ding Z, et al. MTDH mediates trastuzumab resistance in HER2 positive breast cancer by decreasing PTEN expression through an NFkappaB-dependent pathway. BMC cancer. 2014;14:869.

143. Lee SG, Su ZZ, Emdad L, Sarkar D, Fisher PB. Astrocyte elevated gene-1 (AEG-1) is a target gene of oncogenic Ha-ras requiring phosphatidylinositol 3-kinase and c-Myc. Proc Natl Acad Sci U S A. 2006;103(46):17390-5.

144. Amin DN, Sergina N, Lim L, Goga A, Moasser MM. HER3 signalling is regulated through a multitude of redundant mechanisms in HER2-driven tumour cells. The Biochemical journal. 2012;447(3):417-25.

145. Stanley A, Ashrafi GH, Seddon AM, Modjtahedi H. Synergistic effects of various Her inhibitors in combination with IGF-1R, C-MET and Src targeting agents in breast cancer cell lines. Sci Rep. 2017;7(1):3964.

146. Tunalı G. Meme kanseri hücre dizilerinde PI3K/Akt/mTOR sinyal yolağı inhibisyonunun reseptör tirozin kinazların ekspresyonuna etkisi. Ankara: Hacettepe Üniversitesi; 2012.

147. Emdad L, Das SK, Hu B, Kegelman T, Kang DC, Lee SG, et al. AEG-1/MTDH/LYRIC: A Promiscuous Protein Partner Critical in Cancer, Obesity, and CNS Diseases. Adv Cancer Res. 2016;131:97-132.

148. Zhang N, Wang X, Huo Q, Sun M, Cai C, Liu Z, et al. MicroRNA-30a suppresses breast tumor growth and metastasis by targeting metadherin. Oncogene. 2014;33(24):3119-28.

8.APPENDIX

Appendix-1: Ethics committee approval of cell lines

T.C.
HACETTEPE ÜNİVERSİTESİ
Girişimsel Olmayan Klinik Araştırmalar Etik Kurulu

Sayı : 16969557 -255
Konu :

07.02.2017

Prof. Dr. Ayşe Lale DOĞAN
Kanser Enstitüsü
Temel Onkoloji Anabilim Dalı
Öğretim Üyesi

Sayın Prof. Dr. DOĞAN,

Kurulumuza değerlendirilmek üzere sunduğunuz GO 17/109 kayıt numaralı ve ***"Meme Kanserinde Metadherin Ekspresyonunun İncelenmesi"*** başlıklı proje Kurulumuzun 07.02.2017 tarihli toplantısında değerlendirilmiş olup, çalışma materyalinin ticari olarak satın alınmış hücrelerde yapılacağı insandan elde edilen primer kültürlerin kullanılmayacağı görülmüştür Klinik Araştırmalar Yönetmeliği gereği gönüllü insanlar üzerinde gerçekleştirilecek nitelikte olmayan bu tip çalışmalar Etik Kurulların kapsamı dışında kalmaktadır.

Bu yazı Etik Kurul kararı yerine geçmek üzere hazırlanmıştır.

Prof. Dr. Nurten AKARSU
Başkan

EK :
Toplantı Katılım Tutanağı.

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Ayrıntılı Bilgi için:

Appendix-2: Ethics committee approval of patient materials

T.C.
HACETTEPE ÜNİVERSİTESİ
Girişimsel Olmayan Klinik Araştırmalar Etik Kurulu

Sayı : 16969557 – 219
Konu :

ARAŞTIRMA PROJESİ DEĞERLENDİRME RAPORU

Toplantı Tarihi	: 31 OCAK 2018 ÇARŞAMBA
Toplantı No	: 2018/03
Proje No	: GO 18/122 (Değerlendirme Tarihi : 31.01.2018)
Karar No	: GO 18/122 – 05

Üniversitemiz Kanser Enstitüsü Temel Onkoloji Anabilim Dalı öğretim üyelerinden Prof. Dr. A. Lale DOĞAN' ın sorumlu araştırmacı olduğu, Mustafa Emre GEDİK ile birlikte çalışacakları, GO 18/122 kayıt numaralı, ***"Meme Kanserli Hastaların Serumlarında Metadherin Ekspresyon Düzeylerinin İncelenmesi"*** başlıklı proje önerisi araştırmanın gerekçe, amaç, yaklaşım ve yöntemleri dikkate alınarak incelenmiş olup, etik açıdan uygun bulunmuştur.

1.Prof. Dr. Nurten AKARSU	(Başkan)	10 Prof. Dr. Oya Nuran EMİROĞLU	(Üye)
2. Prof. Dr. Sevda F. MÜFTÜOĞLU	(Üye)	11 Yrd. Doç. Dr. Özay GÖKÖZ	(Üye)
İZİNLİ			
3. Prof. Dr. M. Yıldırım SARA	(Üye)	12. Doç. Dr. Gözde GİRGİN	(Üye)
4. Prof. Dr. Necdet SAĞLAM	(Üye)	13. Doç. Dr. Fatma Visal OKUR	(Üye)
5. Prof. Dr. Hatice Doğan BUZOĞLU	(Üye)	14. Doç. Dr. Can Ebru KURT	(Üye)
6. Prof. Dr. R. Köksal ÖZGÜL	(Üye)	15. Yrd. Doç. Dr. H. Hüsrev TURNAGÖL	(Üye)
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7. Prof. Dr. Ayşe Lale DOĞAN	(Üye)	16. Öğr. Gör. Dr. Müge DEMİR	(Üye)
İZİNLİ			
8. Prof. Dr. Elmas Ebru YALÇIN	(Üye)	17. Öğr.Gör.Dr. Meltem ŞENGELEN	(Üye)
		İZİNLİ	
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Ayrıntılı Bilgi için:

Appendix-3: Poster presentation related to this thesis

1. TEMEL ONKOLOJİ SEMPOZYUMU
09-11 Mayıs 2018 / İzmir

PS-14

HER2+ MEME KANSERİNDE METADHERIN EKSPRESYONUNUN REGÜLASYONU

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AMAÇ: Metadherin (MTDH) normal meme dokusunda bulunmayan ancak meme kanserinde ekspresyonu artan bir proteindir. Metadherin ifadesi kötü prognoz ile ilişkilidir. PI3K/Akt/mTOR yolağının sürekli aktivasyonu metadherin ekspresyonunun artışında rol oynamaktadır. Bu çalışmada, PI3K/Akt/mTOR sinyal yolağı inhibisyonunun metadherin ekspresyonuna etkisinin incelenmesi amaçlandı.

GEREÇ-YÖNTEM: HER2+ meme kanseri hücre hatları olan MDA-MB-453, BT-474 ve SKBR-3 hücreleri 1 µM PI-103 (PI3K/mTOR inhibitörü) ile 8 ve 24 saat inkübe edildi. PI-103'ün hücrelerde MTDH ekspresyonuna etkisi qPCR ve Western Blot yöntemi ile incelendi. Meme kanseri tanısı alan 80 hastanın serumlarında MTDH düzeyi ELISA yöntemiyle ölçüldü ve histopatolojik alttipler arasında metadherin düzeyi karşılaştırıldı.

BULGULAR:

1. MDA-MB-453 hücrelerinde, PI-103 inkübasyonu MTDH mRNA ve protein düzeyini anlamlı olarak azalttı ($p<0.05$). Bu sonuç, hücrelerin Akt bağımlılığı ile oldukça uyumlu olarak değerlendirildi.

2. SKBR-3 hücrelerinde, MTDH mRNA ekspresyonunda 8 saat sonra gözlenen azalmayı 24 saat sonunda hafif bir artış izledi. Protein ekspresyonu da PI-103'ten orta düzeyde etkilendi. Bu sonucun, MTDH protein düzeyi ile PTEN aktivitesi arasında ters yönde korelasyonun bulunması ile bağlantılı olabileceği düşünüldü.

3. BT474 hücreleri, mRNA ve protein düzeyinde anlamlı değişiklik göstermedi.

4. Moleküler alttiplere göre, HER2+ hastaların (n=9) MTDH düzeyi, diğer alttipler olan Luminal A (n=20), ve TNBC (n=32) tanısı alan hastalarla kıyaslandığında anlamlı olarak yüksek bulundu ($p<0.05$). HER2+ ve Luminal B (n=19) alttipleri arasında anlamlı fark bulunmadı. MTDH düzeyi, metastatik meme kanseri grubunda (n=13), nonmetastatik grup (n=66) ile karşılaştırıldığında, anlamlı olarak yüksek saptandı ($p<0.01$).

SONUÇ: HER2+ meme kanserinde PI3K/Akt yolağını hedefleyen terapötik stratejiler MTDH ekspresyonunun regülasyonu aracılığıyla invazyon ve metastazın baskılanmasında rol oynayabilir.

Anahtar Kelimeler: HER2, meme kanseri, metadherin, PI3K, PI-103


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ONKOLOJİ ENSTİTÜSÜ
HACETTEPE ÜNİVERSİTESİ
KANSER ENSTİTÜSÜ
1.
TEMEL ONKOLOJİ SEMPOZYUMU
Tümör Biyolojisi ve Genetiği
Sempozyum Kitabı
09 -11 Mayıs 2018
Dokuz Eylül Üniversitesi
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CURRICILUM VITAE

1. KİŞİSEL BİLGİLER

ADI, SOYADI: DOĞUM TARİHİ ve YERİ:	MOHAMMAD AZIM EBRAHIMI 15.12.1961, KABUL
HALEN GÖREVİ: DOKTORA ÖĞRENCİSİ YAZIŞMA ADRESİ: HACETTEPE KANSER ENSTİTÜSÜ, TEMEL ONKOLOJİ ABD TELEFON: 312-3054407-3054423 E-MAIL: azimebrahimi56@gmail.com, ebrahimi@hacettepe.edu.tr	

2. EĞİTİM

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1982-1985	Y.LİSANS	PERADENIYA ÜNİVERSİTESİ (KANDY/SRI-LANKA)	VETERİNERLİK VE HAYVAN ÇALIŞMALARI FAKÜLTESİ
2012-.....	DOKTORA	HACETTEPE ÜNİVERSİTESİ	KANSER ENSTİTÜSÜ

Yüksek Lisans Tez Başlığı ve Tez Danışmanı:

Effects of Androgen and Anti-androgens on the Epididymis of the Rat

Tez Danışmanı. Prof. Dr. V. K. Gunawardena

3. ÇALIŞMA ALANLARI

ÇALIŞMA ALANI	ANAHTAR SÖZCÜKLER
Epididimiste androjen etkileri,	Androjen, Histokimya, Kanser, Sinyal

tümör hücrelerinde sinyal iletimi mekanizmaları	iletimi, Metadherin

4. ESERLER

B. <u>Uluslararası bilimsel toplantılarda sunulan ve bildiri kitabında (*Proceedings*) basılan bildiriler :</u>

B1. Gunawardena, V. K., **Ebrahimi, M. A.** "Effects of Cyproterone acetate and flutimade on Male Genital System", *Annual Academic Sessions of The Kandy Medical Society of Medicine, University of Peradeniya*, Kandy/Sri-Lanka, 1985.