T.C.
REPUBLIC OF TURKEY
HACETTEPE UNIVERSITY
INSTITUTE OF HEALTH SCIENCES

INHIBITION OF SERUM AND GLUCOCORTICOID INDUCIBLE KINASE 1 (SGK1) IN TRIPLE NEGATIVE BREAST CANCER

Elif HAZNEDAROĞLU, MD

Tumor Biology and Immunology

DOCTOR OF PHILOSOPHY THESIS

ANKARA

2018

T.C.
REPUBLIC OF TURKEY
HACETTEPE UNIVERSITY
INSTITUTE OF HEALTH SCIENCES

INHIBITION OF SERUM AND GLUCOCORTICOID INDUCIBLE KINASE 1 (SGK1) IN TRIPLE NEGATIVE BREAST CANCER

Elif HAZNEDAROĞLU, MD

Tumor Biology and Immunology

DOCTOR OF PHILOSOPHY THESIS

ADVISOR OF THE THESIS

Prof. Dr. A. Lale DOĞAN

ANKARA

2018

INHIBITION OF SERUM AND GLUCOCORTICOID INDUCIBLE KINASE 1 (SGK1) IN TRIPLE NEGATIVE BREAST CANCER

Elif HAZNEDAROĞLU

Prof. Dr. A. Lale Doğan

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 25.06.2018.

Chairman of the Committee :	*Prof. Dr. Dicle Güç* *(Hacettepe University)*
Member :	*Prof. Dr. Bilkay Baştürk* *(Başkent University)*
Member :	*Prof. Dr. Mayda Gürsel* *(Middle East Technical University)*
Member :	Assoc. Prof. Dr. Füsun Özmen *(Hacettepe University)*
Member :	*Asst. Prof. Dr. Gürcan Günaydın* *(Hacettepe University)*

This dissertation has been approved by the above committee in conformity to the related issues of Hacettepe University Graduate Education and Examination Regulation.

17 Temmuz 2018

Prof. Diclehan ORHAN, MD, PhD

Institute Manager

YAYIMLAMA VE FİKRİ MÜLKİYET HAKLARI BEYANI

Enstitü tarafından onaylanan lisansüstü tezimin/raporumun tamamını veya herhangi bir kısmını, basılı (kağıt) ve elektronik formatta arşivleme ve aşağıda verilen koşullarla kullanıma açma iznini Hacettepe Üniversitesine verdiğimi bildiririm. Bu izinle Üniversiteye verilen kullanım hakları dışındaki tüm fikri mülkiyet haklarım bende kalacak, tezimin tamamının ya da bir bölümünün gelecekteki çalışmalarda (makale, kitap, lisans ve patent vb.) kullanım hakları bana ait olacaktır.

Tezin kendi orijinal çalışmam olduğunu, başkalarının haklarını ihlal etmediğimi ve tezimin tek yetkili sahibi olduğumu beyan ve taahhüt ederim. Tezimde yer alan telif hakkı bulunan ve sahiplerinden yazılı izin alınarak kullanılması zorunlu metinlerin yazılı izin alınarak kullandığımı ve istenildiğinde suretlerini Üniversiteye teslim etmeyi taahhüt ederim.

Tezimin/Raporumun 25.06.2023 tarihine kadar erişime açılmasını ve fotokopi alınmasını (İç kapak, Özet, İçindekiler ve Kaynakça hariç) istemiyorum.

25 /06/2018

Elif HAZNEDAROĞLU

ETHICAL DECLARATION

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.

Elif HAZNEDAROĞLU

ACKNOWLEDGEMENTS

I would like to thank my advisor Prof. Dr. A. Lale Doğan, for guiding my path through the difficulties of research with the light of her experience and knowledge, not only teaching but also supporting and motivating me at all times, and setting an example of excellence as a researcher and mentor.

I would like to thank the academic members of the Department of Basic Oncology, Prof. Dr. Dicle Güç, Prof. Dr. Güneş Esendağlı, Assoc. Prof. Dr. Füsun Özmen, Asst. Prof. Dr. Gürcan Günaydın, for giving me their support and motivation whenever needed. I would like to thank Dr. Hande Canpınar for helping me with Cell Cycle Analysis experiments, which were an important part of my thesis.

I would like to thank Prof. Dr. Turgay Dalkara and Prof. Dr. Alev Özön for their coordination of the Hacettepe University MD-PhD Dual Degree Program.

I would like to thank M. Emre Gedik, for being like a brother I never had. I would like to thank Burcu Şirin for helping me with everything especially when I first started. I would like to thank fellow graduate students for always helping me and making everything more fun during my PhD by being my friends. I would like to thank the delightful members of our department.

I would like to thank my best friend Barış Alten, for always being there on this and every road that we've walked together. I would like to thank fellow MD-PhD students and close friends Betül Gök Yavuz and Özlem Hökelekli, for their emotional support.

I would like to express my infinite gratitude for my family, to whom I can't thank enough, especially my mother and father, who are the ones who generated my love for research and medicine, and are the most important role models in my life.

This study was supported by Hacettepe University Scientific Research Projects Coordination Unit (Project No: THD-2016-12969).

ABSTRACT

Haznedaroğlu, E., Inhibition of Serum and Glucocorticoid Inducible Kinase 1 (SGK1) in Triple Negative Breast Cancer, Hacettepe University Institute of Health Sciences Tumor Biology and Immunology Doctor of Philosophy Thesis, Ankara, 2018. Glucocorticoid receptor overexpression leads to poor prognosis in breast cancer, particularly in triple negative phenotype. This poor prognosis has been shown to be due to the activation of SGK1 (serum and glucocorticoid inducible kinase 1). In this study, we analyzed SGK1 levels and sensitivity of a panel of TNBC (triple negative breast cancer) cell lines towards SGK1 inhibitor GSK650394. Among these cell lines, MDA-MB-436 cells, displaying markedly elevated SGK1 and showing high phosphorylation of the SGK1 substrate NDRG1 (N-Myc downstream regulated gene 1), was unresponsive to the SGK1 inhibitor. The other cell lines with varying SGK1 levels (MDA MB 231, HCC1937 and BT549) showed marked decrease of NDGR1 phosphorylation due to kinase activity inhibition. Intriguingly, despite GSK650394 sensitivity in these cells, pharmacological SGK1 inhibition did not decrease GSK3β phosphorylation, exhibiting no effect on GSK3β reactivation. In addition, SGK1 inhibition did not change E-cadherin and vimentin expression showing that epithelial-mesenchymal transition (EMT) phenotype was not suppressed. Accordingly, Slug, Snail and Twist mRNA levels were not affected from SGK1 inhibition. GSK650394 treatment suppressed proliferation in MDA-MB-231 cells and led to a slight decrease in S-phase. The results of this study support the idea that SGK1 inhibition strategies may have therapeutic potential in triple negative breast cancer.

Keywords: triple negative breast cancer, SGK1, GSK650394

This study was supported by Hacettepe University Scientific Research Projects Coordination Unit (Project No: THD-2016-12969).

ÖZET

Haznedaroğlu, E., Triple Negatif Meme Kanserinde Serum ve Glukokortikoid İle İndüklenebilen Kinaz 1'in (SGK1) İnhibisyonu, Hacettepe Üniversitesi Sağlık Bilimleri Enstitüsü Tümör Biyolojisi ve İmmünolojisi Programı Doktora Tezi, Ankara, 2018. Glukokortikoid reseptörünün ekspresyonunda artış özellikle triple negatif meme kanserinde kötü prognoza yol açmaktadır. Bu kötü prognozun SGK1 (Serum ve Glukokortikoid ile İndüklenebilen Kinaz 1) aktivasyonuna bağlı olduğu ortaya konulmuştur. Bu çalışmada, TNBC (triple negatif meme kanseri) hücre hatlarında SGK1 düzeyi ve SGK1 inhibitörü olan GSK650394'e duyarlılıkları incelendi. Bu hücreler arasında, SGK1 ekspresyonu belirgin olarak yüksek bulunan MDA-MB-436 hücreleri, SGK1 inhibisyonuna yanıt vermedi ve SGK1'in substratı olan NDRG1'in (N-Myc downstream regulated gene 1) fosforilasyonu yüksek düzeyde bulundu. SGK1 düzeyleri farklılık gösteren diğer hücre hatlarında (MDA-MB-231, HCC1937 ve BT549) ise kinaz aktivitesinin inhibisyonuna bağlı olarak NDRG1'in fosforilasyonunda belirgin azalma gözlendi. İlginç olarak, bu hücreler GSK650394'e duyarlı olmalarına karşın SGK1'in farmakolojik inhibisyonu GSK3β'nın fosforilasyonunda azalmaya yol açmadı ve GSK3β 'nın yeniden aktivasyonu gözlenmedi. Buna ilave olarak, SGK1 inhibisyonu E-kaderin ve vimentin ekspresyonlarını değiştirmedi ve epitelyal-mezenkimal dönüşüm (EMT) ile ilgili baskılanma saptanmadı. Slug, Snail ve Twist mRNA düzeyleri de SGK1 inhibisyonundan etkilenmedi. GSK650394 ile inkübasyon sonucu, MDA-MB-231 hücrelerinin proliferasyonunda baskılanma ve S- fazında hafif azalma gözlendi. Bu çalışmanın sonuçları, SGK1 inhibisyonuna yönelik stratejilerin triple negatif meme kanserinde terapötik potansiyeli olabileceği görüşünü desteklemektedir.

Anahtar kelimeler: triple negatif meme kanseri, SGK1, GSK650394

Bu çalışma Hacettepe Üniversitesi Bilimsel Araştırmalar Projeleri Koordinasyon Birimi tarafından desteklenmiştir. (Proje Kodu: THD-2016-12969)

CONTENTS

PAGE OF APPROVAL iii

YAYINLAMA VE FİKRİ MÜLKİYET HAKLARI BEYANI iv

ETHICAL DECLARATION v

ACKNOWLEDGEMENTS vi

ABSTRACT vii

ÖZET viii

CONTENTS ix

SYMBOLS AND ABBREVATIONS xiii

INDEX OF FIGURES xv

INDEX OF TABLES xix

1. INTRODUCTION 1

2. REVIEW OF LITERATURE 3

2.1. Development of The Mammary Tissue 3

2.2. Breast Cancer 4

2.2.1. Important Signaling Pathways in Breast Cancer 5

2.2.2. Triple Negative Breast Cancer 9

2.3 Breast Cancer and Stress Receptors 12

2.3.1. Breast Cancer and SGK1 14

2.4. EMT 18

3. MATERIAL AND METHODS 22

3.1. Substances Used for This Study 22

3.2. Tools and Equipment 23

3.3. Study Plan 25

3.4. Cell Culture 26

3.4.1. Passaging and Counting of Cells with Trypsin-EDTA solution 26

3.4.2. Freezing of Cells 27

3.5. Drug Incubation 28

3.6. MTT Assay 28

3.7. mRNA Expression Analysis 28
3.7.1. RNA Isolation from Cells 29
3.7.2. One-step Real Time PCR (Polymerase Chain Reaction) 29
3.8. Western Blot and Protein Analysis 30
3.8.1. Preparation of Protein Lysates and Protein Quantification 30
3.8.2. Polyacrylamide Gel Electrophoresis 32
3.8.3. Semi-dry Transfer and Blotting with *Trans-Blot Turbo system* 33
3.8.4. Chemiluminescent Imaging 33
3.8.5. Reblotting for Loading Control Proteins 34
3.8.6. Densitometry Analysis 34
3.9. Crystal Violet Assay 34
3.10. Cell Cycle Analysis 35
3.11. Statistical Analysis 35
4. RESULTS **36**
4.1. SGK1 mRNA and protein expressions in triple negative breast cancer cells 36
4.1.1. SGK1 protein expressions in triple negative breast cancer cells 37
4.1.2. SGK1 mRNA expressions in triple negative breast cancer cells 37
4.2. Determination of subtoxic doses of GSK650394 with MTT Assay 38
4.2.1. Determination of subtoxic doses of GSK650394 with MTT Assay in MDA-MB-231 cells 39
4.2.2. Determination of subtoxic doses of GSK650394 with MTT Assay in MDA-MB-436 cells 41
4.2.3. Determination of subtoxic doses of GSK650394 with MTT Assay in HCC1937 cells 42
4.2.4. Determination of subtoxic doses of GSK650394 with MTT Assay in BT549 cells 44
4.2.5. Determination of subtoxic doses of GSK650394 with MTT Assay in MDA-MB-468 cells 45

4.3. Determination of SGK1 inhibition through phospho-NDRG1 protein expression levels in triple negative breast cancer cell lines 45
4.3.1. Determination of SGK1 inhibition through phospho-NDRG1 protein expression levels in MDA-MB-231 cells 45
4.3.2. Determination of SGK1 inhibition through phospho-NDRG1 protein expression levels in BT549 cells 46
4.3.3. Determination of SGK1 inhibition through phospho-NDRG1 protein expression levels in HCC1937 cells 47
4.3.4. Determination of SGK1 inhibition through phospho-NDRG1 protein expression levels in MDA-MB-436 cells 48
4.3.5. Determination of SGK1 inhibition through phospho-NDRG1 protein expression levels in MDA-MB-468 cells 51
4.4. Effect of SGK1 inhibition via GSK650394 on the proliferation of MDA-MB-231 cells 52
4.4.1. Effect of SGK1 inhibition via GSK650394 on the proliferation of MDA-MB-231 cells macroscopically 52
4.4.2. Effect of SGK1 inhibition via GSK650394 on the proliferation of MDA-MB-231 cells microscopically 53
4.5. Effect of SGK1 inhibition via GSK650394 on the cell cycle phases distribution in MDA-MB-231 cells 54
4.6. Effect of SGK1 inhibition on phospho-GSK3 beta expression in triple negative breast cancer cell lines 55
4.6.1. Effect of SGK1 inhibition on phospho-GSK3 beta expression in MDA-MB-231 cell line 56
4.6.2. Effect of SGK1 inhibition on phospho-GSK3 beta expression in HCC1937 cell line 57
4.6.3. Effect of SGK1 inhibition on phospho-GSK3 beta expression in BT549 cell line 58
4.6.4. Effect of SGK1 inhibition on phospho-GSK3 beta expression in MDA-MB-436 cell line 59

4.7. Effect of SGK1 inhibition via GSK650394 on EMT related proteins E-cadherin and vimentin protein expressions in triple negative breast cancer cell lines 59

4.7.1. Effect of SGK1 inhibition via GSK650394 on E-cadherin protein expressions in triple negative breast cancer cell lines 60

4.7.2. Effect of SGK1 inhibition via GSK650394 on vimentin protein expressions in triple negative breast cancer cell lines 61

4.8. Effect of SGK1 inhibition via GSK650394 on EMT related transcription factors in MDA-MB-231 and BT549 cell lines 64

4.8.1. Effect of SGK1 inhibition via GSK650394 on EMT related transcription factors in MDA-MB-231 cells 65

4.8.2. Effect of SGK1 inhibition via GSK650394 on EMT related transcription factors in BT549 cells 67

5. DISCUSSION 70

6. CONCLUSION AND RECOMMENDATIONS 75

7. REFERENCES 76

8. SUPPLEMENTS 83

Supp.1. Approval of the Ethics Committee 83

Supp.2. Abstracts Derived from This Thesis 84

9. CURRICULUM VITAE 86

ABBREVIATIONS

BSA : Bovine serum albumin

CCND1 : Cyclin D1

CCND2 : Cyclin D2

Cdk : Cyclin dependent kinase

DMEM : Dulbecco's Modified Eagle's Medium

DMF : Dimethyl formamide

ECL : Enhanced chemiluminescence

ECM : Extracellular matrix

EGF : Epidermal growth factor

EGFR : Epidermal growth factor receptor

EMT : Epithelial-mesenchymal transition

ER : Estrogen receptor

ERBB2 : avian erythroblastosis oncogene B2

ERBB3 : avian erythroblastosis oncogene B3

ERBB4 : avian erythroblastosis oncogene B4

ERK : Extracellular Signal-Regulated Kinase

FBS : Fetal bovine serum

FGF : Fibroblast growth factor

FGFR1 : Fibroblast growth factor receptor 1

FOXO3 : Forkhead box O3

GAPDH : Glyceraldehyde 3-phosphate dehydrogenase

GR : Glucocorticoid receptor

GSK3 : Glycogen synthase kinase-3

HDAC : Histon deacetylase

HER2 : Human EGF receptor 2

HGF : Hepatocyte growth factor

HIF1 : Hypoxia induced factor

IGF1R : Insulin like growth factor 1 receptor

INPP4B : PtdIns(3,4,5)trisphosphate

JNK : Jun N-terminal Kinase

MAPK : Mitogen activated protein kinase

mTOR : Mammalian target of rapamycin

Mtorc2 : Mammalian target of rapamycin complex 2

MTT : 3-(4,5-Dimethylthiazol-2-Yl)-2,5-Diphenyltetrazolium Bromide

NDRG1 : N-Myc Downstream Regulated 1

PARP : Poly ADP (Adenosine Diphosphate)-Ribose Polymerase

PBS : Phosphate buffered saline

PCR : Polymerase chain reaction

PDK1 : Phosphoinositide-dependent Protein Kinase-1

PI3K : Phosphatidylinositide 3-Kinase

PR : Progesteron receptor

PTEN : Phosphatase and Tensin Homolog

PVDF : Polyvinylidene difluoride

RB1 : Retinoblastoma

Rpm : Rate per minute

RPMI : Roswell Park Memorial Institute

SGK : Serum and Glucocorticoid Regulated Kinase

Smad : Mothers against decapentaplegic

TGF beta : Transforming growth factor beta

Thr : Threonine

TNBC : Triple negative breast cancer

TSC : Tuberous sclerosis complex

INDEX OF FIGURES

Figure

2.1. Changes in the mammary tissue in different stages of development. Adapted from (1). 4

2.2. Classification of breast cancer. Adapted from (2). 5

2.3. Effect of glucocorticoid receptor on hormone receptor positive and triple negative breast cancer chemotherapy sensitivity, schematically shown. Adapted from (3). 12

2.4. Expression of stress hormone receptors in breast cancer cell lines. Adapted from (4). 13

2.5. Activation of SGK1 by phosphorylation. Adapted from (5). 15

2.6. SGK1 mRNA expressions among breast cancer cell lines, compared with Akt inhibitor sensitivity. Adapted from (17). 16

2.7. Schematic presentation of EMT. 18

2.8. Important signaling pathways in EMT. Adapted from (6). 20

3.1. Study plan schematized. 25

4.1. Protein levels of SGK1 in triple negative breast cancer cell lines. (n=3) 36

4.2. mRNA levels of SGK1 in triple negative breast cancer cell lines. (n=3) 37

4.3. Percentage of viable MDA-MB-231 cells, after treatment with GSK650394 for 48 hours. (n=4) 38

4.4. Percentage of viable MDA-MB-231 cells, after treatment with GSK650394 for 72 hours. (n=4) 39

4.5. Percentage of viable MDA-MB-436 cells, after treatment with GSK650394 for 48 hours. (n=4) 40

4.6. Percentage of viable MDA-MB-436 cells, after treatment with GSK650394 for 72 hours. (n=4) 40

4.7. Percentage of viable HCC1937 cells, after treatment with GSK650394 for 48 hours. (n=4) 41

4.8. Percentage of viable HCC1937 cells, after treatment with GSK650394 for 72 hours. (n=4) 42

4.9. Percentage of viable BT549 cells, after treatment with GSK650394 for 48 hours. (n=4) 43

4.10. Percentage of viable BT549 cells, after treatment with GSK650394 for 72 hours. (n=4) 43

4.11. Percentage of viable MDA-MB-468 cells, after treatment with GSK650394 for 48 hours. (n=4) 44

4.12. Percentage of viable MDA-MB-468 cells, after treatment with GSK650394 for 72 hours. (n=4) 44

4.13. Change in the protein level of phosphorylated NDRG1 after treatment with GSK650394 in MDA-MB-231 cell line. (n=3, **p<0.05**) 45

4.14. Change in the protein level of phosphorylated NDRG1 after treatment with GSK650394 in BT549 cell line. (n=3, **p<0.05**) 46

4.15. Change in the protein level of phosphorylated NDRG1 after treatment with GSK650394 in HCC1937 cell line. (n=3, **p<0.05**) 47

4.16. Change in the protein level of phosphorylated NDRG1 after treatment with GSK650394 in MDA-MB-436 cell line. (n=3, p>0.05) 48

4.17. Change in the protein level of phosphorylated NDRG1 after treatment with different doses of GSK650394 for 6 hours in MDA-MB-436 cell line. (n=3) 49

4.18. Change in the protein level of phosphorylated NDRG1 after treatment with different doses of GSK650394 for 6 hours in MDA-MB-231 cell line. (n=3) 50

4.19. Change in the protein level of phosphorylated NDRG1 after treatment with GSK650394 in MDA-MB-468 cell line. (n=3, p>0.05) 51

4.20. Inhibition of proliferation of MDA-MB-231 cells after treatment with GSK650394 for indicated time and doses, macroscopically imaged. (n=3) 52

4.21. Inhibition of proliferation of MDA-MB-231 cells after treatment with GSK650394 for indicated time and doses, microscopically imaged (10x). (n=3) 53

4.22. Effect of GSK650394 on the distribution of cell cycle phases. (n=3) 54

4.23. Changes in the protein level of phosphorylated GSK3ß after treatment with GSK650394 in triple negative breast cancer cell lines. (n=3) 55

4.24. Change in the protein level of phosphorylated GSK3ß after treatment with GSK650394 in MDA-MB-231 cell line. (n=3, p>0.05) 56

4.25. Change in the protein level of phosphorylated GSK3ß after treatment with GSK650394 in HCC1937 cell line. (n=3, p>0.05) 57

4.26. Change in the protein level of phosphorylated GSK3ß after treatment with GSK650394 in BT549 cell line. (n=3, p>0.05) 58

4.27. Change in the protein level of phosphorylated GSK3ß after treatment with GSK650394 in MDA-MB-436 cell line. (n=3, p>0.05) 59

4.28. Changes in the protein levels of E-cadherin after treatment with GSK650394 in triple negative breast cancer cell lines. (n=3) 60

4.29. Changes in the protein levels of E-cadherin after treatment with GSK650394 in HCC1937 cell line. (n=3, p>0.05) 61

4.30. Changes in the protein levels of vimentin in response to GSK650394 in triple negative breast cancer cell lines. (n=3) 62

4.31. Changes in the protein levels of vimentin after treatment with GSK650394 in MDA-MB-231 cell line. (n=3, p>0.05) 62

4.32. Changes in the protein levels of vimentin after treatment with GSK650394 in BT549 cell line. (n=3, p>0.05) 63

4.33. Changes in the protein levels of vimentin after treatment with GSK650394 in MDA-MB-436 cell line. (n=3, p>0.05) 64

4.34. Effect of GSK650394 on Snail mRNA expression in 6 and 24 hours in MDA-MB-231 cell line. (n=3, p>0.05) 65

4.35. Effect of GSK650394 on Slug mRNA expression in 6 and 24 hours in MDA-MB-231 cell line. (n=3, p>0.05) 66

4.36. Effect of GSK650394 on Twist mRNA expression in 6 and 24 hours in MDA-MB-231 cell line. (n=3, p>0.05) 66

4.37. Effect of GSK650394 on Snail mRNA expression in 6 and 24 hours in BT549 cell line. (n=3, p>0.05) 67

4.38. Effect of GSK650394 on Slug mRNA expression in 6 and 24 hours in BT549 cell line. (n=3, p>0.05) 68

4.39. Effect of GSK650394 on Twist mRNA expression in 6 and 24 hours in BT549 cell line. (n=3, p>0.05) 69

INDEX OF TABLES

Table

3.1. Primers used for real time PCR 29

3.2. Optimized real time PCR protocol 30

3.3. Lysis buffer for protein lysate preparation. 31

1. INTRODUCTION

Breast cancer is the most commonly diagnosed cancer among women, and second most common in cancer associated death. Treatment includes surgery, radiation therapy, chemotherapy, hormonal treatment and/or targeted therapies when available. (7)

Triple negative breast cancer (TNBC) is important among the breast cancer subtypes, because it lacks a targeted therapy in contrast to Luminal types (which can benefit from hormonal therapy) and HER2 positive type (which can benefit from HER2 targeted therapies like trastuzumab). Because triple negative breast cancers lack targeted therapy, they have a worse prognosis. The search for a targetable molecule for triple negative breast cancer is an ongoing important topic for researchers. (8)

Glucocorticoid receptor is shown to be overexpressed in triple negative breast cancer and it correlates with worse prognosis. (9) It has been shown that glucocorticoid receptor overexpression leads to drug resistance and increased proliferation. This effect was proven to be due to SGK1 (Serum and glucocorticoid regulated kinase 1) activation. (9)

SGK1 is a serin-threonine kinase which is a member of the AGC kinase family, and is closely related to PI3K/Akt/mTOR pathway. (10) SGK1 is activated by phosphorylation by mTORC2 and PDK1, and it protects the cell from apoptosis. (5) SGK1 has been shown to be essential for the proliferation of triple negative breast cancer cells. (11)

SGK1 upstream molecules PDK1 (Phosphoinositide-dependent Protein Kinase-1) activation and Glucocorticoid receptor overexpression have been previously shown to be linked with Epithelial-Mesenchymal Transition, which is an important step for metastasis. (12) (13) SGK1 was also proven to have a role in the phosphorylation and inhibition of GSK3 beta, a molecule which avoids the cell from going through Epithelial-Mesenchymal Transition. (14)

In this thesis study, we aimed to understand the effect of inhibition of SGK1 via GSK650394 on Epithelial-Mesenchymal Transition and thus possibly metastasis in triple negative breast cancer cells. We also aimed to examine the effect of inhibition of SGK1 on proliferation and cell cycle phase distributions, thus showing the significance of SGK1 for triple negative breast cancer cells.

2. REVIEW OF LITERATURE

2.1. Development of the Mammary Tissue

Mammary tissue is different from other tissues in that it goes through development mostly in the postnatal period. Mammary tissue begins its development in the fetal stage, but it is rudimentary at birth. In pubertal stage, epithelial growth is hormone dependent and the terminal end buds proliferate rapidly to form Terminal ductal lobulo-alveolar units. (1) (Figure 2.1.)

This tissue mostly consists of two cell types: Basal myoepithelial cells and luminal cells. Luminal cells line the apical surface and have secretory functions. Basal myoepithelial cells locate around the luminal cells and have contractile (muscle) and epithelial phenotypes. (15)

Development of the mammary tissue is controlled by various hormones and growth factors, especially steroid hormones. (1)

The signaling pathways important for the development of mammary tissue can be stated as:

- Mitogen-activated protein kinase (MAPK) pathway
- Phosphatidylinositol 3-kinase (PI3K) pathway
- Notch/CSL
- Wnt/b-catenin
- Myc

These signaling pathways and molecules have also been described as important molecules for the development of breast cancer. (16) In chapter 2.2.1. the signaling pathways critical for breast cancer will be mentioned in detail.

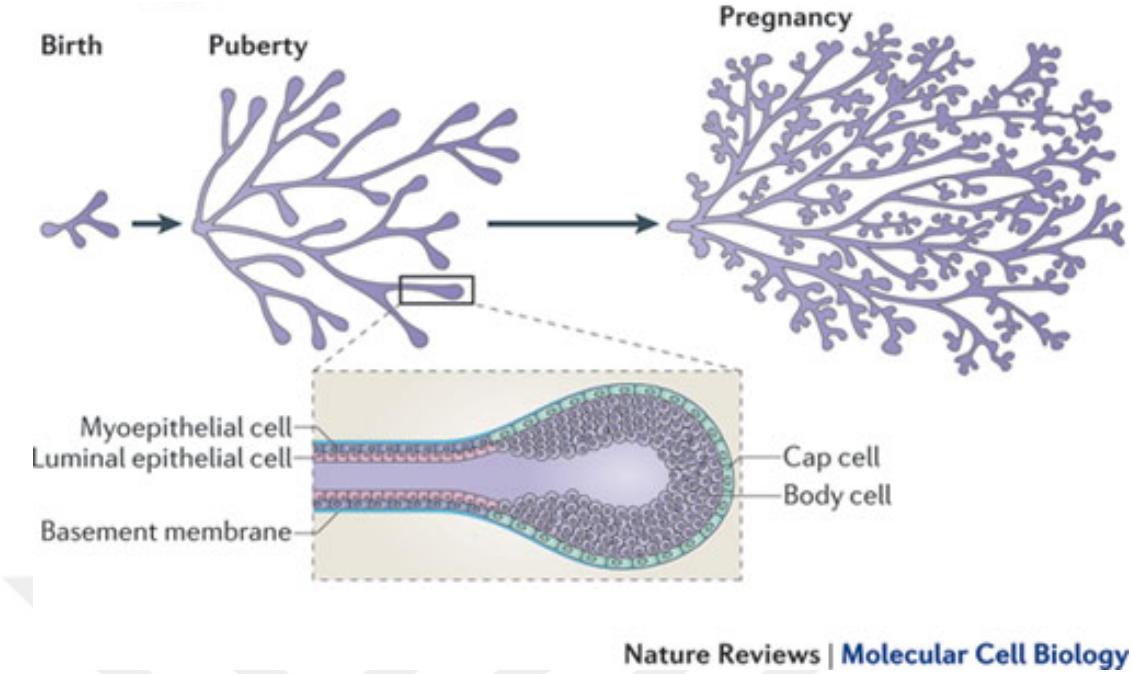


Figure 2.1. Changes in the mammary tissue in different stages of development. Adapted from (1).

2.2. Breast Cancer

Breast cancer is the most commonly diagnosed cancer among women, according to the data collected from research conducted by American Cancer Society. It is also the second most common in cancer associated death. In the United States, there is approximately 246.660 new breast cancer cases and approximately 40.450 breast cancer associated deaths expected in 2016. In the years between 1989-2012 because of the advances in early diagnosis and treatment, breast cancer associated deaths have been reduced in 36%, which means approximately 249.000 people with breast cancer avoided death. (7)

Surgical treatment is chosen considering the characteristics of the tumor, like the size or the dissemination of the tumor. The treatment comprises surgery, radiation therapy, chemotherapy (before or after surgery), hormonal treatment and/or targeted therapies. (7)

In histopathological and molecular aspects, breast cancer can be classified as; Luminal A, Luminal B, HER2-enriched, Normal mammary like, Basal-like, Claudin-low (2) (Figure 2.2.)

Hormone receptor positive breast cancer patients can benefit from hormone therapy. (17) Patients whose cancer cells overexpress HER2 growth promoting protein can benefit from targeted therapies against this protein. (18, 19)

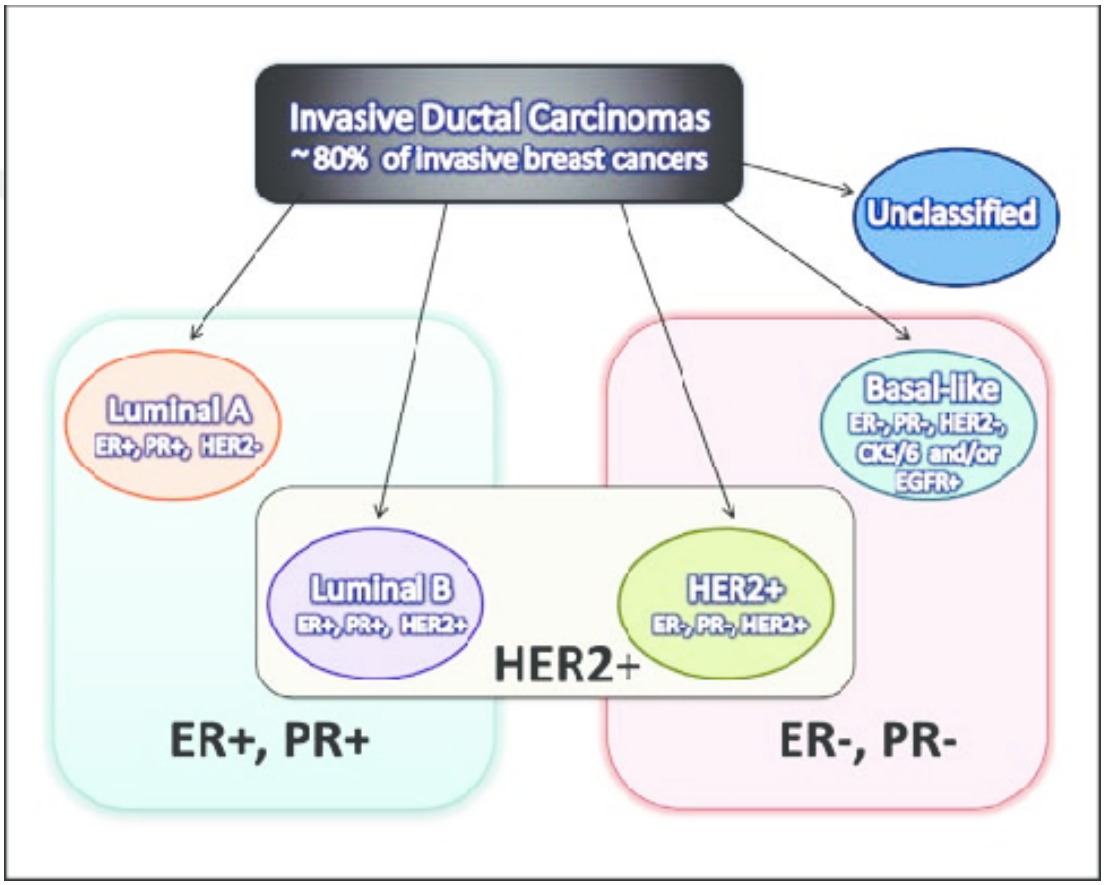


Figure 2.2. Classification of breast cancer. Adapted from (2).

2.2.1. Important Signalling Pathways in Breast Cancer

Mitogen-activated protein kinase (MAPK) pathways are induced by extracellular singals and control important cellular functions like proliferation,

differentiation, growth, apoptosis and migration. (20) MAPKKK (mitogen-activated protein kinase kinase kinase) phosphorylates and activates MAPKK (mitogen-activated protein kinase kinase) which phosphorylates and activates MAPK. In mammals six distinct groups of MAPK have been described: extracellular signal-regulated kinase (ERK)1/2, ERK3/4, ERK5, ERK7/8, Jun N-terminal kinase (JNK)1/2/3 and the p38 isoforms alpha/beta/italic gamma(ERK6)/delta. (21) Mutations in this pathway can be found in approximately 30% of breast cancers in humans. The nature of deregulation is different between individual subtypes and cancer tissues. (22)

Phosphatidylinositol 3-kinase (PI3K) pathway is a major intracellular signaling pathway that responds to hormones and growth factors, and plays a central role in the growth and proliferation of tumor cells. PI3K heterodimer, consists of a regulatory (p85) and a catalytic (p110) subunit. In response to upstream stimulation by growth factor receptor tyrosine kinases, the regulatory subunit activates the catalytic subunit. (23) The PI3Ks phosphorylate phosphatidylinositol 4,5 bisphosphate, or PIP2, to phosphatidylinositol 3,4,4-triphosphate, or PIP3, which in turn leads to the phosphorylation of Akt, a serine/threonine kinase which has a role in cancer cell cycle, survival of cells, and cell growth. mTOR is a serine/threonine protein kinase, which is found downstream of PI3K and Akt. mTOR refers to two different complexes, mTORC1 and mTORC2, which have different modes of action. mTORC1 is the target of rapamycin and rapamycin analogs, and better studied than mTORC2. (24) Alterations in PI3K pathway are very common in all types of breast cancer and associated with resistance to therapy, progression of breast cancer, EMT and metastasis. (25)

Notch pathway consists of four different receptors: NOTCH1, NOTCH2, NOTCH3, NOTCH4. Notch receptor is a hetero-oligomer that consists of a large extracellular portion which associates non-covalently with a short extracellular part, a single transmembrane portion and a small intracellular portion. Notch supports proliferation and protects cells from apoptosis, favors angiogenesis, is important for

chemoresistance and radioresistance, has a role in stem cell phenotype, and has a role in metastasis. (26) (27)

Wnt/b-catenin signaling is implicated both in mammary tissue development and mammary tumorigenesis. The activation of Wnt/b-catenin signaling, which can be defined by b-catenin nuclear expression. It has been demonstrated that Wnt/b-catenin signaling activation is especially found in a subgroup of invasive triple negative breast cancer, and it is a marker of poor clinical outcome. (28) These findings suggest that this pathway is especially critical for triple negative breast cancer development and progression. (27)

Myc protein is a basic helix-loop-helix transcription factor that needs heterodimerization with Max to regulate transcription in cancer cells. These heterodimers recruit multiple coactivators. In the context of breast cancer, Myc amplification is the most common alteration. (29) Myc target genes, such as Cyclin D2, Cdk4 (Cyclin dependent kinase 4) and Cdk (Cyclin dependent kinase) inhibitor p21Cip1, are important for cell cycle, proliferation, survival and apoptosis. (30)

AGC kinases

AGC kinase family is named after cAMP-dependent protein kinase (PKA), cGMP-dependent protein kinase (PKG) and the protein kinase C (PKC) families. (31)

- *PDK1*, is closely related to PI3K/Akt pathway. (32) PDK1 is abundantly expressed in human tissues and it was shown to be highly expressed in a lot of breast cancer cell lines. PDK phosphorylates AKT at threonine 308 (T308), and helps its activation. After being phosphorylated at T308, phosphorylation of serine 473 (S473) by mTORC2 fully activates AKT. PDK1 also has the ability to directly phosphorylate p70S6K, which is a substrate of mTOR kinase. (31) PDK1 is overexpressed in a large group of human breast cancers. In mouse

model, overexpression of PDK1 could induce mammary cell tumorigenesis. Some pharmacological inhibitors have been developed and inhibition of PDK1 shows anti-proliferative effects on cancer growth. (33) Knockdown of PDK1 is shown to weaken tumorigenesis in tumor models. Du et al showed using genetic targeting methods that inactivation of PDK1 can delay the initiation, progression and metastasis of breast cancer. Effect of PDK1 on metastasis is shown to be conducted by EMT (epithelial-mesenchymal transition) mechanism. They also showed that PDK1 deletion could suppress abnormal tumor metabolism. PDK1 is an emerging target in cancer. (12)

- *Akt/PKB family:* In response to the stimulation of growth factors, P3K is phosphorylated and activated and it forms second messenger phosphatidylinositol (3,4,5)-trisphosphate (PIP3) by phosphorylating lipids in the plasma membrane. (34) Akt (a serin threonine kinase) is recruited to the plasma membrane so that it can be fully activated. Fully activated Akt is important for cancer with its roles in key cellular functions like survival, growth, cell migration, angiogenesis. (35)
- *SGK family,* which includes the main molecule of this study, SGK1, is explained in detail in Chapter 2.3.1.
- *RSK family* (Ribosomal s6 kinase) is composed of RSK subfamily, MSK subfamily, S6K subfamily. The members of this family are highly evolutionary conserved Ser/Thr kinases that regulate a wide range of cellular processes like cell growth, motility, survival and proliferation. (36) They are downstream effectors of the Ras–extracellular signal-regulated kinase (ERK)/mitogen-activated protein kinase (MAPK) signalling cascade. (37)
- *PKA (cAMP-dependent protein kinase) family,* when cytosolic cAMP increases two cAMP molecules bind to each PKA regulatory subunit, these subunits free the active sites of the catalytic subunits and unengaged catalytic subunits interact with proteins to phosphorylate them on their Ser or Thr residues. (38)
- *PKG family (cGMP-dependent protein kinase or Protein Kinase G)* is activated by cGMP. (39)

- *PKC family* is activated by increases in diacylglycerol (DAG) or calcium ions (Ca+2) (40)
- *Aurora kinase family* functions in mitosis, there are three types of Aurora kinase: Aurora A, B and C. Aurora kinase is less known in cancer biology than the other AGC kinases. (41)
- *GRK*. GRKs are activated downstream of G protein coupled receptors (GPCR) and frequently lead to inhibition of GPCR signaling. Unlike the other members of this family, GRK isoforms aren't activated by phosphorylation. (42)
- *NDR and LATS*. Nuclear DBF2-related kinase 1 (NDR1; also known as STK38) and NDR2 (also known as STK38L) have key roles in mitosis and large tumour suppressor 1 (LATS1) and LATS2 are key members of the Hippo signaling pathway, which controls cell size. (43)
- *Rho-activated protein kinases*. Rho activated kinases consist of ROCK86, citron Rho-interacting kinase (CRIK)87, myotonin protein kinase (DMPK)88 and myotonic dystrophy kinase-related CDC42- binding kinase (MRCK). This family controls cell shape and motility by controlling cell cytoskeleton members. (44)
- *MAST*. Microtubule-associated Ser/Thr kinases (MAST) are large molecules (1309–2444 amino acids). (45)
- *SGK494*. Sugen kinase 494 (SGK494) has not been further characterized, and its function in normal development and cancer is not yet known. (10)

2.2.2. Triple Negative Breast Cancer (TNBC)

Triple negative breast cancer is named as it is because of the lack of ER (estrogen receptor), PR (progesterone receptor) and HER2 (human epidermal growth receptor 2) overexpression. Triple negative breast cancer is mostly classified under basal type of breast carcinomas, and TNBC and basal-like type of breast carcinoma are described as "distant cousins" in some articles. Basal-like breast cancer is characterized with lack of HER2 overexpression, lack or limited expression of ER and

PR, and expression of the genes in basal and myoepithelial cells in normal mammary tissue. (46)

Triple negative breast cancers tend to have higher grades than the other breast cancer subtypes. Triple negative breast cancers also have more tendency to have metastases in the brain and the lung. (47)

Triple negative breast cancer doesn't benefit from hormonal therapy or trastuzumab like the other subtypes do. (8) Therefore, chemotherapy seems to be the only option but it still hasn't been decided on a standard chemotherapy regime. (46) Triple negative breast cancer is thought to be a single clinical entity and uniformly treated with chemotherapy, but molecular profiles and other technologies have showed there is a great amount of heterogeneity between TNBC cases. (8)

BRCA1 and BRCA2 genes encode proteins that are critical for DNA integrity and genomic stability. These tumor suppressor proteins are essential for cell division, DNA replication error control, DNA repair and apoptosis. Germline mutations in these genes are characterized by basal-like tumor phenotype, ER negativity, EGFR overexpression, MYC amplification, TP53 mutations, increased genomic instability and sensitivity to DNA cross linking agents. BRCA1 mutations are rare in sporadic tumors, but in high grade breast cancers it has been shown that especially ATM, BRCA1, TP53 genes which have important roles in DNA damage response and breast tumorigenesis have loss of heterozygosity (LOH) or abnormal expression. (48)

PIK3CA mutations/amplifications (49), AKT3 amplification/mutation (50), PTEN deletion/mutation (49), INPP4B deletion (51), TSC1 changes (49) can result with the constitutive activation of PI3K/Akt pathway. FGFR1 amplification (52), EGFR amplification (53), IGF1R amplification (49), BRAF amplification/mutation (54) , KRAS amplification/mutation, HRAS mutation (54), DUSP4 deletion (55) can affect RAS/RAF/MEK pathway and result with the constitutive activation of this pathway.

RB1 deletion (56), CDK6 amplification (57), CCND1 amplification (58), CCND2 amplification (59) can reverse cell cycle processes in their favor. JAK2 amplifications (60) can constitutively activate JAK/STAT pathway. In addition to these pathways, Androgen receptor pathways (61), Notch pathway (26), JNK/AP-1 pathway (62), HIF1-alpha (63) changes can be seen in TNBC cells.

Because EGFR overexpressions are frequently seen in triple negative breast cancers, cetuximab (and other targeted therapies against EGFR), which is targeted to this mutation is clinically studied in addition to chemotherapy. (64) However, PTEN (phosphatase and tensin homologue) mutations in these cancers results with therapy resistance. (65) Mutations that result with activation in the PI3K pathway can be treated with PI3K inhibitors. (66) Triple negative breast cancer cases with activating mutations in the Ras/MAPK pathway or abberant activation of Ras/MAPK pathway because of other reasons, can be treated with MEK inhibitors. (55) Poly (adenosine diphosphate–ribose) polymerase enzyme inhibition (PARP inhibitors) which support DNA repair via base excision repair, are frequently used especially in BRCA related triple negative breast cancer. (67) (8)

Luminal androgen receptor positive TNBC cases can benefit from anti-androgenic therapy. (61) HDAC (histon deacetylase) inhibitors are important research topics in the context of epigenetic therapy. (68)

Triple negative breast cancer stands out as the richest subtype regarding cancer stem cells, especially basal-like type. (69) Cancer stem cell is also sometimes referred to as tumor initiating cell, and defines a subpopulation that has similar properties with mammary stem cells. This subpopulation has slower proliferation rate and are more resistant to chemotherapy. (70) Ras/MAPK, JAK/STAT, Wnt, TGF beta, Hedgehog, Notch pathways are especially shown important for the cancer stem cell phenotype and the inhibition of these pathways can be options for cancer stem cell targeted therapies. (71) However, lack of only one signaling pathway responsible

for this phenotype, and the heterogeneity of the progression of this phenotype eliminates the possibility of a targeted cancer stem cell therapy that can be used for all TNBC cases.

2.3 Breast Cancer and Stress Receptors

Glucocorticoid receptor emerged as a potential targetable molecule in the past years. Glucocorticoid receptor is shown to be overexpressed in triple negative breast cancer and it correlates with worse prognosis. (3) (Figure 2.3.) In contrary, in hormone receptor positive breast cancers, glucocorticoid receptor overexpression was correlated with better prognosis. This shows that glucocorticoid receptor expression is context-related and specifically important for triple negative breast cancers. (72)

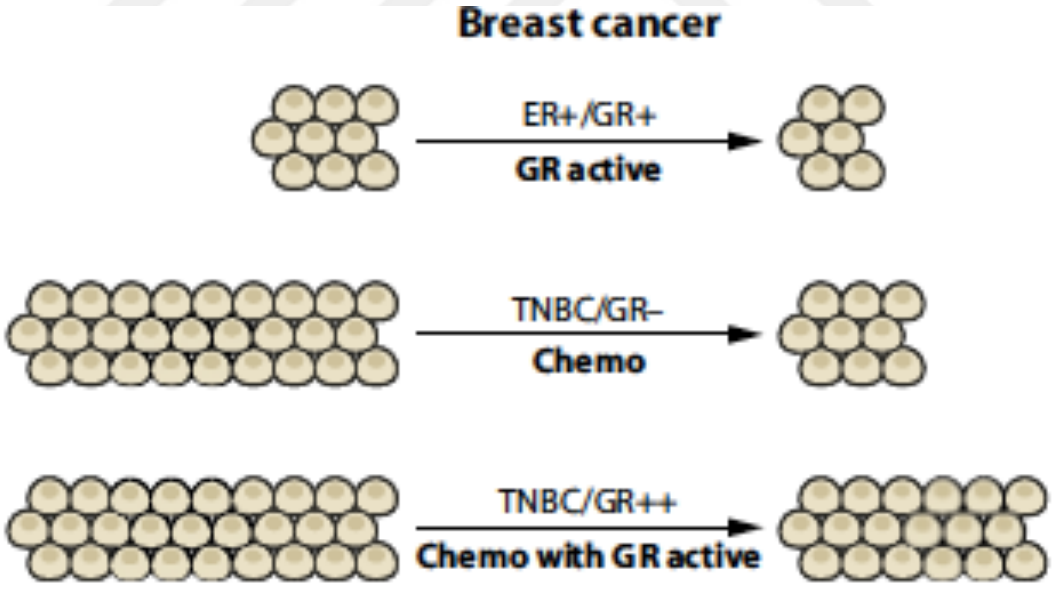


Figure 2.3. Effect of glucocorticoid receptor on hormone receptor positive and triple negative breast cancer chemotherapy sensitivity, schematically shown. Adapted from (3).

It has been shown that when glucocorticoid receptor was inhibited by mifepristone (glucocorticoid receptor antagonist), cells increased caspase associated cell death and increased sensitivity to paclitaxel. (9) Another research article showed that stress hormones (glucocorticoid, catecholamine), decrease the effect of paclitaxel with the induction of DNA damage, this study also showed that triple negative breast cancer cell lines express stress hormone receptors more abundantly than the other subtypes. (4) (Figure 2.4.) It was shown in an animal model of social isolation that stress dysregulated endocrine systems and increased the malignant burden of tumors in breast. (73)

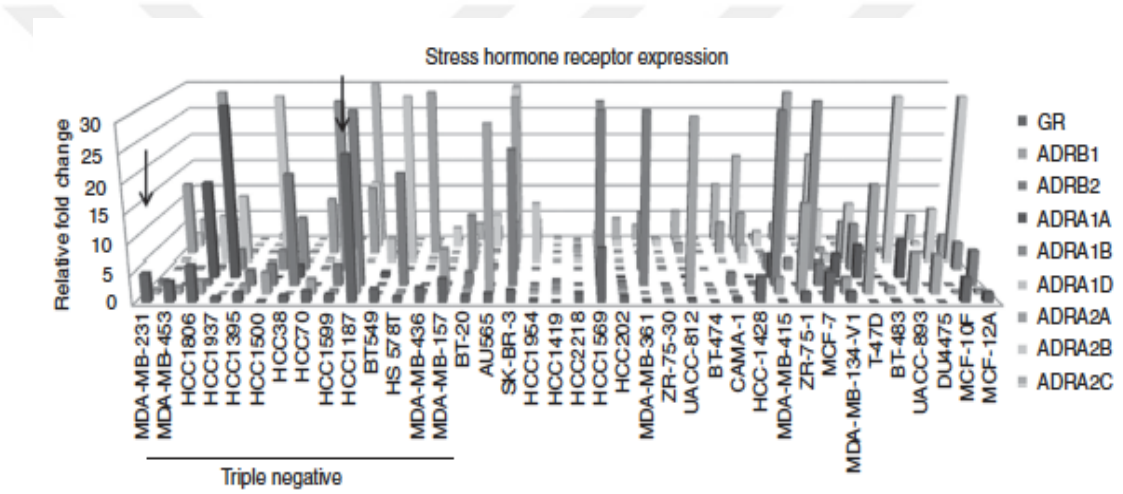


Figure 2.4. Expression of stress hormone receptors in breast cancer cell lines. Adapted from (4).

Metapristone, which is a more potent molecule acquired from mifepristone, was shown to block migration and invasion thus inhibiting metastasis in the MDA-MB-231 cell line. (74) A drug combination consisting of aspirin, lysin, doxycycline and mifepristone was also shown to block metastasis. (75) Mifepristone was shown to suppress cancer stem cell phenotype using KLF5 molecule. (76)

Dexamethasone (a steroid derivate with a very powerful glucocorticoid characteristic) is used on breast cancer patients as a treatment for nausea and

vomiting which are side effects of chemotherapy. But it is thought that dexamethasone causes worse prognosis especially because of the effects of SGK1 molecule. (9)

2.3.1. Breast Cancer and SGK1 (Serum and Glucocorticoid-Regulated Kinase 1)

SGK is a serin-threonine kinase and is in the AGC kinase family and closely related to the PI3K/Akt/mTOR signaling pathway. (77) Serum and Glucocorticoid-Regulated Kinase 1 (SGK1) was at first identified as a glucocorticoid induced gene. SGKs consists of three isoforms encoded by distinct genes: SGK1, Serum and Glucocorticoid-Regulated Kinase 2 (SGK2) and Serum and Glucocorticoid-Regulated Kinase 3 (SGK3). (77)

Sgk1 expression is shown to be induced by stress situations like UV damage, H_2O_2, high temperature in addition to increase in glucocorticoids or treatment with dexomethasone. (77) (78)

SGK-1 is firstly phosphorylated by mTORC2 in the H-motif, which enables PDK1 to interact with SGK1, phosphorylate its T-loop and activate SGK1 fully. (5) (Figure 2.5.) SGK-1 protects the cell from apoptosis, especially by means of phosphorylation and inactivation of FOXO3A (Forkhead box O3). (79)

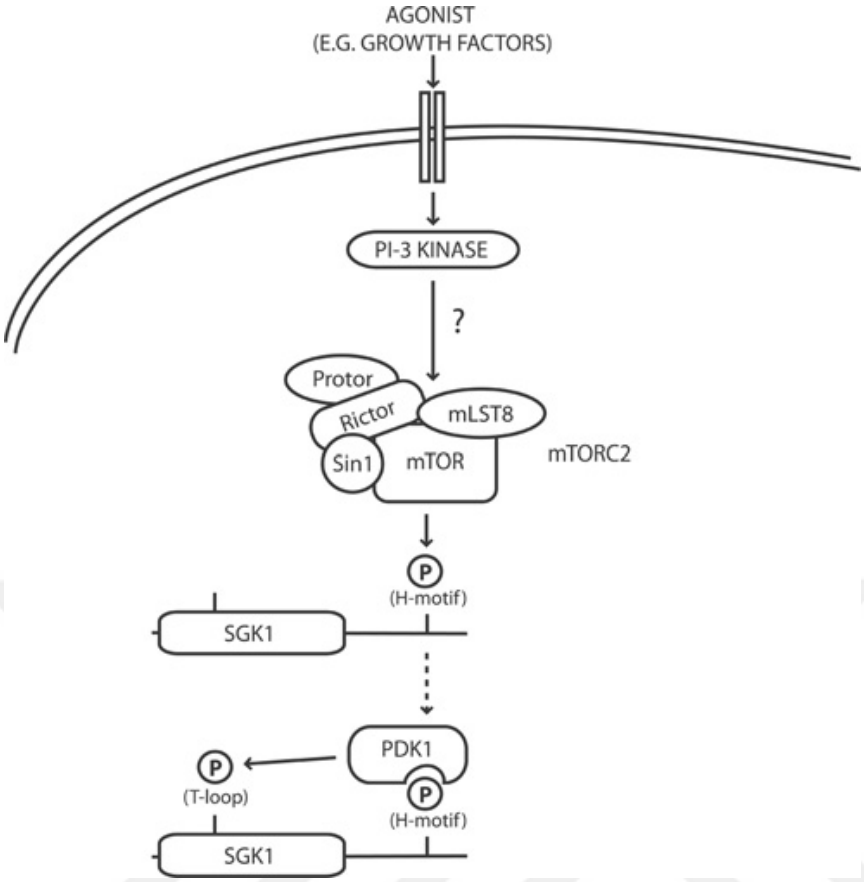


Figure 2.5. Activation of SGK1 by phosphorylation. Adapted from (5).

In a research article conducted by Sommer et al, it was shown that SGK1 provides resistance to Akt inhibitors, especially in triple negative breast cancer cell lines. This research article also showed that SGK1 mRNA and protein expressions were higher among breast cancer cell lines that were proved resistant to Akt inhibitors. (11) (Figure 2.6.)

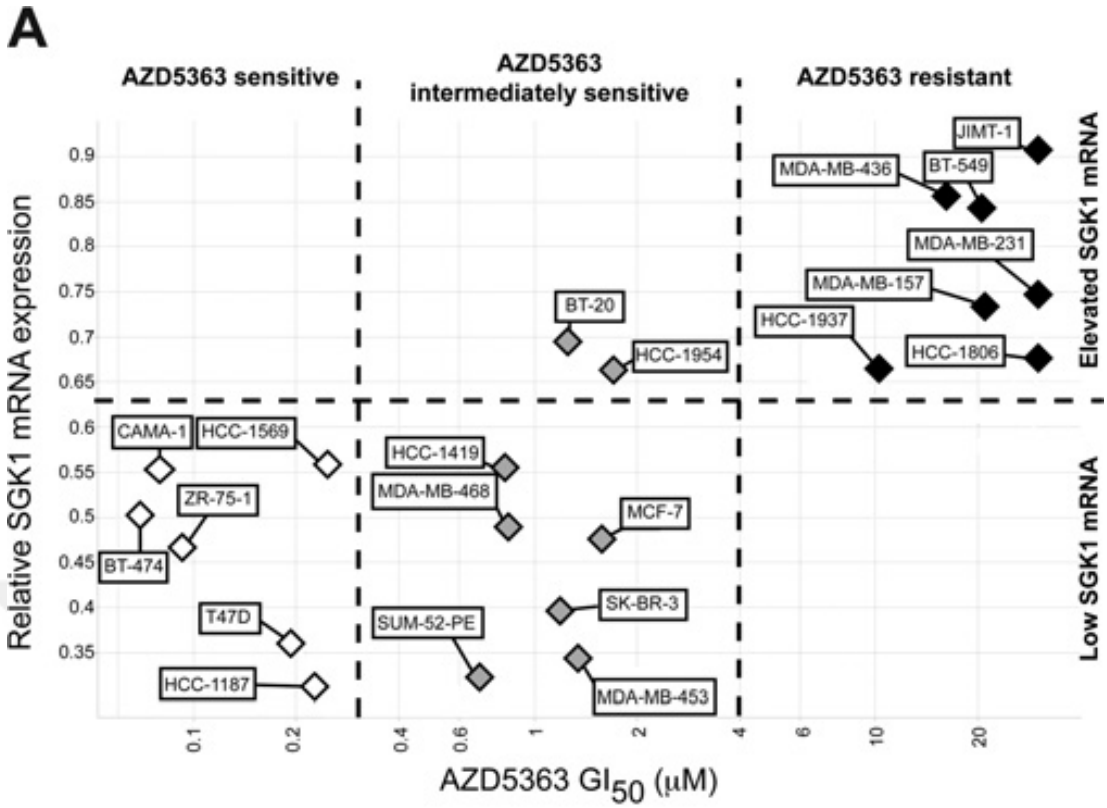


Figure 2.6. SGK1 mRNA expressions among breast cancer cell lines, compared with Akt inhibitor sensitivity. Adapted from (17).

SGK-1 and pAkt-1 were shown to be expressed comparably among breast cancer tissues and it is known that both molecules have important roles in PI3K signaling pathway. (80) SGK1 inhibitor is currently studied in vitro among different cancers, and inhibition of SGK1 blocks cell proliferation. (81) It was shown that Sgk1 activated p53 degradation and protected the cells from apoptosis, and led the cells to proliferation. (82) Also, SGK was shown to mediate survival signals by phosphorylating an important proapoptotic protein, namely FOXO3A. (83) (79) Studies considering SGK1 activity, triple negative breast cancer and importance of SGK1 inhibition in triple negative breast cancer has been proved to be becoming more significant in the recent years.

N-myc down-regulated gene (NDRG) states four genes with 50% sequence homology, which are NDRG1, NDRG2, NDRG3 and NDRG4. NDRG1 protein has three phosphorylation sites recognized by SGK (Thr346, Thr356, Thr366), each SGK1 recognition site prepares NDRG1 to phosphorylation by GSK3-beta. The exact function of these phosphorylations is to date not well known. (84)

In addition to post-translational modification, NDRG1 is also transcriptionally regulated. CpG islands in NDRG1 promotor areas show that it can be controlled with methylation. Hypoxia, iron chelators, cobalt, vitamin A, steroids and oxidative stress also affect NDRG1. (84)

NDRG1 protein is identified as a “metastasis suppressor”. In this context, it was shown that NDRG1 affects the vesicular recycling of e-cadherin and thus stabilizes it in the plasma membrane and blocks cell motility and migration. (85) This effect averts epithelial-mesenchymal transition which is an important step in metastasis, which will be explained in detail in chapter 2.4. NDRG1 also blocks TGF beta signaling and reduces TGF beta targets like Snail, Slug and Smad. NDRG1 also inhibits Wnt signaling. NDRG1 protein and mRNA expressions were shown to be downregulated in breast, prostate, colon cancer and glioma. It is estimated that NDRG1 has different impacts in various tissues and cancers. (85)

It is proposed that NDRG1 is the common substrate of GSK3 beta and SGK1, and NDRG1 phosphorylated by these molecules is inactivated. (14) In a study conducted in vascular smooth muscle cells, it was shown that SGK1 phosphorylated and inactivated GSK3 beta, which causes activation of beta catenin signaling. (86) This phenomenon was also shown in non-small cell lung cancer cell lines. This wasn’t yet proved in breast cancer. Control of GSK3 beta by SGK1 can shed light to the role of SGK1 in EMT and promotion of metastasis.

2.4.EMT (Epithelial–Mesenchymal Transition)

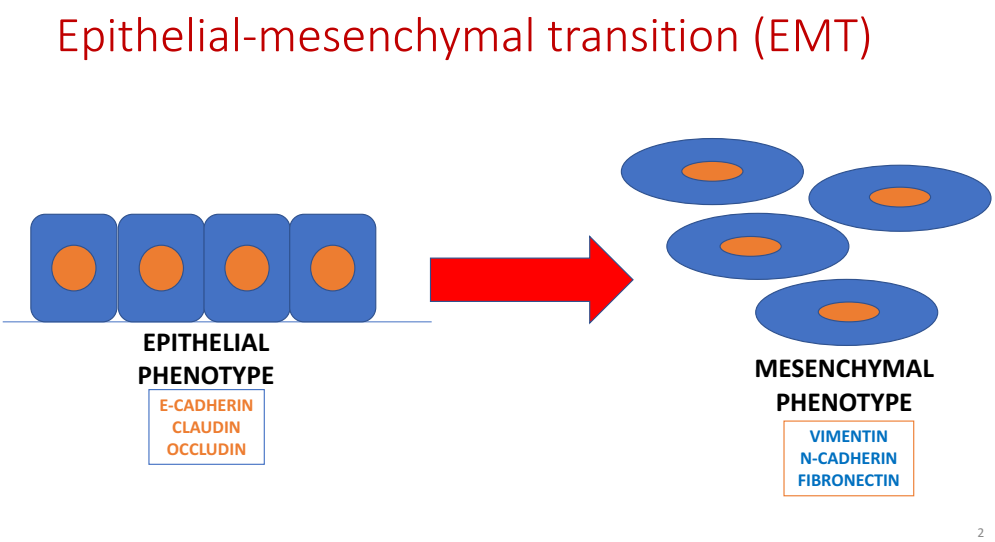


Figure 2.7. Schematic presentation of EMT.

The concept of epithelial cells downregulating their epithelial phenotypes and gaining a mesenchymal character was first set forth by Elizabeth Hay in the 1980s, in chick embryos. This process was first named epithelial-mesenchymal transformation, then changed as epithelial-mesenchymal transition to emphasize its transient nature. (87)

During EMT, cells downregulate their epithelial proteins including cell adhesion complexes. (Figure 2.7.) For example, during the phenomenon named "cadherin switch", E-cadherin downregulation is balanced by mesenchymal N-cadherin upregulation, which aids the epithelial cells to build homotypical N-cadherin interactions with mesenchymal cells rather than homotypical E-cadherin interactions with other epithelial cells. (88)

As the epithelial phenotype gives way to mesenchymal phenotype, the communication with basement membrane begins to fade and communication with

the extracellular matrix (ECM) begins to be emphasized. In accordance with this purpose, epithelial integrins disappear and gives way to integrins that are key to EMT progression. For example, as epithelial alpha 6 beta 4 integrin is epigenetically silenced, integrins that or important for the progression of EMT like alpha-5 beta 1 and alpha 1 beta 1 increase. (88)

There are various transcription factors that control the change required for EMT. Leading these changes, are SNAIL and TWIST transcription factors.

Among the SNAIL transcription factor family, the transcription factors important for EMT progression in vertebrates are SNAIL1 and SNAIL2 (also known as Slug). These factors are important in EMT during development, cancer progression and fibrosis. SNAIL1 represses epithelial genes while activating mesenchymal genes. (89) Signaling pathways that play role in EMT, for example TGF beta and WNT family proteins, Notch, growth factors signaling with Receptor Tyrosine Kinases, activate SNAIL1 in various physiological situations. (90) This activation may also happen with post-translational modifications, these post-translational modifications can change the localization of SNAIL1, changing its activation state, or lead to its degradation. (91) Glycogen synthase kinase‑3β (GSK3β) phosphorylates SNAIL1 on two serin rich motifs, leading to its degradation. First phosphorylation on serin motif, exports Serin from the nucleus, second phosphorylation results with ubiquitin mediated degradation. WNT and PI3K/Akt pathways inhibit GSK3, thus block GSK3 phosphorylation induced Snail1 degradation. Notch and NFKB pathways can destruct GSK3-Snail interaction, avoiding Snail degradation. (92)

TWIST1 and TWIST2, transcription factors among basic helix loop helix (bHLH), have important roles in EMT progression. Twist1 can reduce E-cadherin expression and increase N-cadherin expression in cancer cells independent of Snail. (93)

Signaling pathways important for EMT progression are summarized schematically in Figure 2.8.

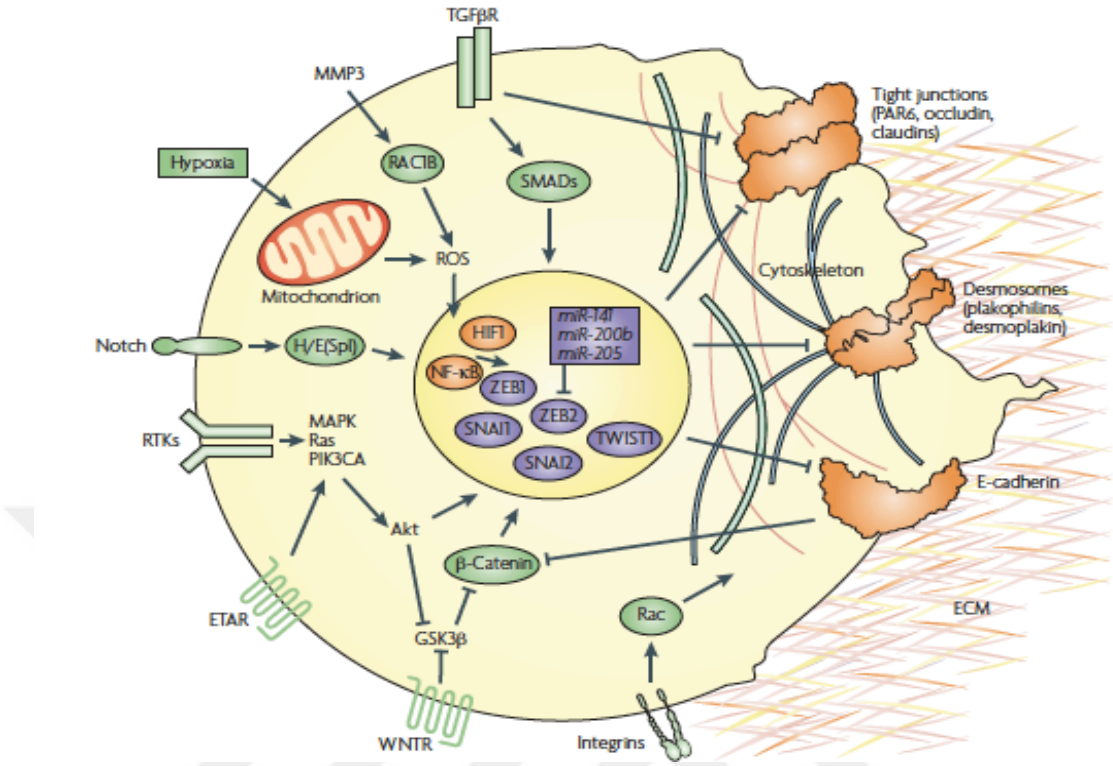


Figure 2.8. Important signaling pathways in EMT. Adapted from (6).

TGF beta plays a major role in EMT progression during development. TGF beta is also important postnatally for EMT for wound healing, fibrosis and cancer. TGF beta signaling can be examined as Smad mediated and Non-Smad mediated signaling pathways. In the SMAD mediated TGF beta downstream pathway, TGF beta binds TGF beta receptor 1 and 2, thus activates its intracellular effectors, SMAD proteins. In response to TGF beta, SMAD complexes increase expressions of various transcription factors and can also increase their activation. (88)

TGF beta can also activate the Rho-like GTPase pathway, PI3K pathway and MAPK pathway, other than orchestrating with SMAD effector proteins. These pathways are called Non-SMAD pathways downstream of TGF beta. (94)

PI3K/Akt pathway, MAPK/ERK pathway, FGF, HGF, EGF, IGF, PDGF signaling pathways take place downstream of receptor tyrosine kinases and change the activation or expression levels of important transcription factors in EMT. Inhibitors of tyrosine kinases can be used to inhibit EMT. (95) Hedgehog (95) and Notch (96) signaling pathways are also valuable for EMT development.

Other extracellular signaling pathways have also been described important in the progression of EMT, key one of these is the canonical WNT pathway. (97) Attachment of WNT ligands to the Frizzled receptor results with the inhibition of GSK3 beta, which blocks the phosphorylation, ubiquitination, degradation of beta catenin and aids the gene expression regulation of beta catenin. GSK3 beta inhibition also increases the stability of SNAIL as previously mentioned, which promotes EMT. (97)

In the context of our study, PDK1, which is an upstream molecule of SGK1, has been shown to be important for metastasis through EMT progression in a mouse model, but the molecule responsible for this EMT progression hasn’t been determined. (12) Also, glucocorticoid receptor overexpression, which induces SGK1, has been shown to be linked to EMT in previous studies, but the mechanism was not elucidated. (13) (98)

3. MATERIAL AND METHODS

This study was conducted at the Research Laboratory of Department of Basic Oncology, Hacettepe University, Oncology Institute between the years of 2015-2017.

3.1. Substances Used for This Study

The chemical and biological substances that were used in this study are listed below.

β-actin Rabbit Anti-human primary antibody (Cell Signaling Technologies-USA), GAPDH Rabbit Anti-human primary antibody (Cell Signaling Technologies-USA), SGK1 Rabbit Anti-human primary antibody (Cell Signaling Technologies-USA), pNDRG1 Rabbit Anti-human primary antibody (Cell Signaling Technologies-USA), total NDRG1 Rabbit Anti-human primary antibody (Cell Signaling Technologies-USA), pGSK3 beta Rabbit Anti-human primary antibody (Cell Signaling Technologies-USA), Vimentin Rabbit Anti-human primary antibody (Cell Signaling Technologies-USA), e-Cadherin Rabbit Anti-human primary antibody (Cell Signaling Technologies-USA), Polyclonal Goat Anti-rabbit immunoglobulins/HRP (Biolegend-USA), Anti-rabbit IgG HRP linked antibody (Cell Signaling Technologies-USA), GSK650394 SGK1 inhibitor (Adooq Biosciences-USA), Tris (Applichem-Germany), Amonium persulfate (APS) (Riedel-de Haen-Germany), Tetramethylethylenediamine (TEMED) (Amresco-USA), Crystal Violet dye (Sigma Aldrich-USA), RNAse (Thermo Fisher Scientific-USA), Propidium iodide (Abcam-UK), DMEM (Dulbecco's Modified Eagle Medium) (Gibco-USA), RPMI (Roswell Park Memorial Institute) medium (Gibco-USA), FBS (Fetal Bovine Serum) (Lonza-Switzerland), L-glutamine (Biochrom-Germany), Penicillin/Streptomycin (Biochrom-Germany), PBS (Phosphate Buffered Saline) (Sigma Aldrich-Germany), Tween 20 (Applichem-Germany), Super Signal West-femto Maximum Sensitivity Substrate (Thermo Fisher Scientific-USA), Bromphenol blue (Sigma-USA), β-mercaptoethanol (Sigma-USA), Glycerol (Gerbu-Germany), Tris-HCl (Invitrogen-USA),

NaCl (Carlo Erba-Italy), Ethylene diamine tetra acetic acid (EDTA) (Sigma-USA), Sodium dodecyl sulphate (Sigma Aldrich-Germany), Butanol (Sigma Aldrich-Germany), Glycine (Sigma-USA), NaF (Merck-Germany), Dimethyle sulphoxyde (DMSO) (WAK-Chemie Medical-Germany), Trypan blue (Sigma-USA), TGX Stain-Free™ FastCast™ Acrylamide Kit (Biorad-USA), Trans-Blot® Turbo™ RTA Mini PVDF Transfer Kit (Biorad-USA), Precision Plus Protein™ WesternC™ protein standard (Biorad-USA), Precision Protein™ StrepTactin-HRP sekonder antibody (Biorad-USA), Trans-Blot® Turbo™ Transfer Starter System (Biorad-USA), Metanol (Merck-Germany), Sodium pyrophosphate (Sigma-USA), Calyculin (Cell Signaling Technology-USA), Halt™ Protease Inhibitor Cocktail (100X) (Thermo Fisher Scientific-USA), Trypsin %0.05 (1x) (Lonza-Switzerland), Ethidium Bromide (Sigma-USA), PCR Primer (SGK1) (Iontek-Turkey), PCR Primer (β-actin) (Iontek-Turkey), PCR Primer (Snail) (Iontek-Turkey), PCR Primer (Slug) (Iontek-Turkey), PCR Primer (Twist) (Iontek-Turkey), Agarose (Amresco-USA) , Quick-RNA MiniPrep Kit (Zymo Research-USA), QuantiTect SYBR Green RT-PCR Kit (Qiagen-Germany)

3.2. Tools and Equipments

- Kodak Gel Logic 1500 Imaging System (Carestream Health Inc.-USA)
- Inverted Microscope (Olympus-England)
- Incubator (Heraeus-Germany)
- Automatic Shaker (Heidolphy-Germany)
- Microcentrifuge with Heat-changing properties (Eppendorf-Germany)
- Mini protean Tetra Cell Tank (Biorad-USA)
- PowerPac™ Power Source (Biorad-USA)
- Spectrophotometry (Spectramax-USA)
- -80 °C Deep Freezer (Thermo Electron-USA)
- Centrifuge (Heraeus-Germany)
- Ice machine (Scotsman AF200-England)
- Electrophoresis Power Source Elite 300 Plus (Wealtec-USA)

- Sensitive Scale (Denver Instrument-USA)
- Vortex (Clofton Cycline-İngiltere)
- Distillated water equipment (GFL-Germany)
- Shaker (GFL-Germany)
- Heating block (Techne Dri Bloc-England)
- Magnetic shaker (Ika RH Basic-Germany)
- Fume Hood (Ünitest-Turkey)
- Vertical Flow Hood (Thermo-Electron-USA)
- Water bath (GFL-Germany)
- +4 °C Cold Room (Alarko Carrier-Turkey)
- +4 °C Fridge (Bosch-Germany)
- -20°C Deep Freezer (Bosch-Germany)
- Thoma Microscope Slide (Fuchs Rosenthal-USA)
- -196 Liquid Nitrogen Tank

3.3. Study Plan

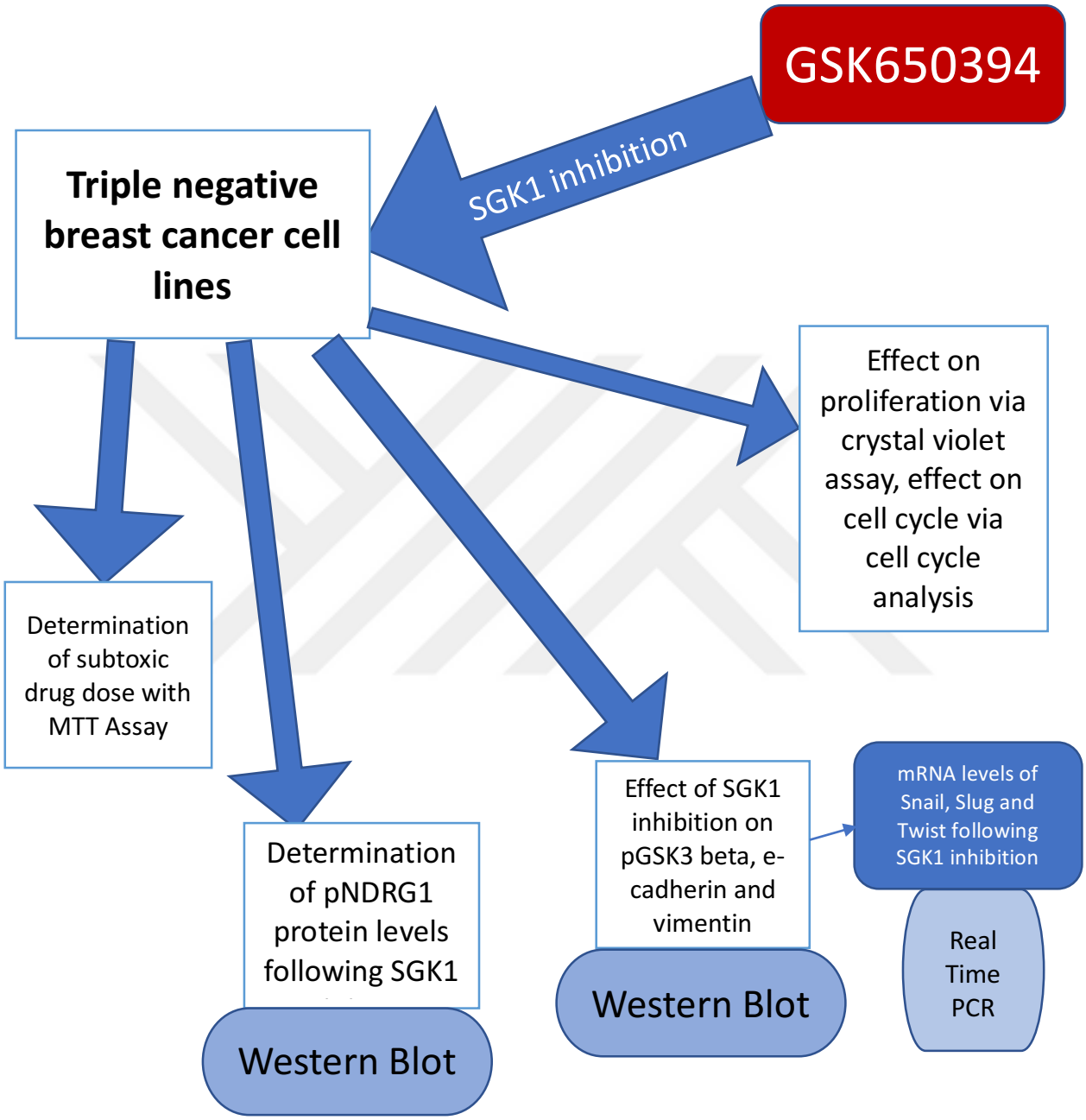


Figure 3.1: Study plan schematized.

3.4. Cell Culture

MDA-MB-231, MDA-MB-468, BT549, MDA-MB-436 and HCC1937 cell lines were chosen with the consideration of their SGK1 levels and PTEN statuses. These cell lines were obtained from ATCC (American Type Culture Collection) and were cultured in regards with the protocols ATCC stated. The below indicated mediums were used to incubate the cell lines.

- MDA-MB-231: Low glucose DMEM, including %10 FBS, %1 Penicillin-streptomycin, 2 mM L-glutamine
- MDA-MB-468: Low glucose DMEM, including %10 FBS, %1 Penicillin-streptomycin, 2 mM L-glutamine
- BT549: RPMI, including %10 FBS, %1 Penicillin-streptomycin, 2 mM L-glutamine
- MDA-MB-436: RPMI, including %10 FBS, %1 Penicillin-streptomycin, 2 mM L-glutamine
- HCC1937: RPMI, including %10 FBS, %1 Penicillin-streptomycin, 2 mM L-glutamine

3.4.1. Passaging and Counting of Cells with Trypsin-EDTA solution

With trypsinisation procedure, the adherent breast cell lines were removed from the flask. For this procedure, firstly the medium was removed from the flask without damaging the cells. Then, PBS (Phosphate Buffered Saline) was added to flasks (2 ml for T25, 5 ml for T75 flasks), flasks were washed and then PBS was removed, cell medium remains and FBS (Fetal Bovine Serum), which reduces the enzymatic reaction of trypsin, was removed along with PBS. 500 µl Trypsin/EDTA for T25 flask, 1,5 ml Trypsin/EDTA for T75 flask, was added and flasks were slightly shaken in order to be homogenous, and incubated in the 37 ^{o}C %5 CO_2 incubator. After incubation, cells were examined via the inverted microscope in order to see if they were removed from the flask base. To stop the activity of Trypsin/EDTA, 2-5 ml

medium was added. In order to affect all the cells in the flask, media was mixed with the serological pipette. The now suspensated cells were put in a 50 ml Falcon tube and centrifuged in +4 ^{o}C and 2000 rpm for 5 minutes. After centrifugation, the supernatant which included cell residues was removed and the cell pellet was mixed with 6-10 ml full medium.

Cells were mixed thoroughly with the serological pipette, so that they could be counted on the microscopic slide. 10 µl of the suspension was taken and mixed with the trypan blue dye, so that the suspension was now diluted by 1:1. Cells were counted with the Thoma microscopic slide. Trypan blue could get through the membrane of dead cells, so the cells that were seen blue were not counted. Thoma microscopic slide has a 0.1 mm x 0.1 mm size, and the distance between the microscopic slide and coverglass is 0.1 mm. Because every space the cells were counted was 0.001 mm^3 (0.0001 ml), the mean of the counted spaces were multiplied by 10.000. Because the cell suspension was diluted with trypan blue by 1:1, the reached value was multiplied by 2 which gave us the cell number by milliliter.

After the cell counting procedure, if preferred cell number or appropriate cell passage number weren't reached yet, cell lines were passaged. With consideration of the cell numbers in the cell suspension, the number of flasks to be passaged were determined, leaving 500.000 cells for T25 flasks and 1-1,25 million cells for T75 flasks. Equal amount of cell suspensions was transferred to flasks. Flasks were filled with full medium, for T25 flasks up to 5 ml, for T75 flasks up to 15 ml. Flasks were incubated in the 37 ^{o}C %5 CO2 incubator. Flasks were examined under the inverted microscope daily, when the pH was reduced, dead cells in the flask were increased or color of the medium was changed, full medium was renewed or cells were passaged.

3.4.2. Freezing of Cells

Cell lines were put in cryovial tubes 1 x 10^6 cell/ml/tube, to be used for following experiments. DMSO (Dimethyl sulfoxide) was added to the suspension with a dilution of %10. DMSO reduces the freezing temperature of the medium and

reduces the risk of the formation of ice crystals in cells, thus the lysis of cells. Cryovial tubes were frozen in a gradually heat changing container called *Mr. Frosty*, and frozen in -86 ^{o}C for 24 hours. After this period, the cells were preserved in liquid nitrogen tanks.

3.5. Drug Incubation

After trypsinisation procedure, cell suspensions were prepared in order to include 1x10^{6} or 2x10^{6} cells. Cells were incubated in the 37 ^{o}C %5 CO2 incubator for 24 hours in order to thoroughly adhere to the surface. SGK1 inhibitor GSK650394 was prepared under the vertical flow hood in sterile conditions. The agent, which was in a crystallized form, was mixed and vortexed in DMSO, so that the final concentration was 32 mM. Stock solutions were preserved in -20 ^{o}C in volumes of 500 µl. Before removing the medium from the wells, aliquots of the stock solution were serially diluted. 24 hours later the medium was removed from the wells and GSK650394 was added in different doses (which are indicated in the Results section), and incubated for different time periods (which are indicated in the Results section). The wells that weren't incubated with GSK650394 were used as control and 2 ml medium was added to them.

3.6. MTT Assay

After cells were removed from the flask and counted, 5000 cells per well were added with 50 µl medium to a 96 well plate. We waited 24 hours for the cells to adhere to the plate surface. We did not add any drug to control wells, and we added 50 µl of twice the molarity of the necessary drug doses (1 µM, 2 µM, 4 µM and 8 µM for 0,5 µM, 1 µM, 2 µM and 4 µM), since there was already 50 µl of media in the wells, which helped us reach the preferred doses. After 48 and 72 hours of drug incubation, 25 µl of MTT (3-(4,5-Dimethylthiazol-2-yl)-2,5-Diphenyltetrazolium Bromide) was added to wells. 4 hours later, DMF (dimethylformamide) was added,

which solubilized tetrazolium crystals made by MTT in viable cells. 24 hours after DMF, plates were analyzed spectrophotometrically.

3.7.1. RNA Isolation from Cells

After treatment of the cell lines with GSK650394, RNA isolation was applied according to the protocol of Zymo QuickRNA MiniPrep RNA Isolation kit. RNA qualities and quantities were checked with the Nano-drop using 1,5 µl of sample. 260/230 value showed inorganic contamination, while 260/280 value showed organic contamination.

3.7.2. One-step Real Time PCR (Polymerase Chain Reaction)

For the mRNA expression analyses, Quantitect SYBR Green One-Step RT-PCR kit was used according to the protocol. Primers used for the reaction can be seen in Table 3.1. Mixture was prepared for the experiment with the following volumes: RT PCR Mix: 400 µl, Forward primer: 80 µl, Reverse primer: 80 µl, Quantitect RT Mix: 8 µl; and was distributed equally (14,2 µl for each) to PCR tubes. The RNA quantities acquired from the Nanodrop were used to determine the volume needed from the sample for the experiment, the targeted quantity was 1000 ng. Water was added to reach the targeted volume of 20 µl for each tube. Real time PCR conditions we have optimized can be seen in Table 3.2.

Table 3.1. Primers used for real time PCR

	FORWARD (5’-3’)	REVERSE (5’-3’)
Snail	5’-CGAGTGGTTCTTCTGCGCTA-3’	5’-CTGCTGGAAGGTAAACTCTGGA-3’
Slug	5’-CACTGCGATGCCCAGTCTA-3’	5’-TTCTCCCCCGTGTGAGTTCTAA-3’
Twist	5’-GCCGGAGACCTAGATGTCATT-3’	5’-CCCACGCCCTGTTTCTTTGA-3’
β-actin	5’-GCACTCTTCCAGCCTTCCTT-3’	5’-AATGCCAGGGTACATGGTGG-3’

Table 3.2. Optimised one step RT-PCR conditions:

Reverse transcription	50 ^{0}C	30 minutes
Activation	95 ^{o}C	15 minutes
Denaturation Annealing Extension	94 ^{o}C 57 ^{o}C 72 ^{o}C	15 seconds 30 seconds 30 seconds } 40 cycles

3.8. Western Blot and Protein Analysis

So as to show the expression of proteins, Western Blot analysis was performed. Proteins were extracted from the preferred conditions of cell lines, quantified, ran with gel electrophoresis, transferred and blotted to a PVDF membrane, incubated with primary and secondary antibodies and imaged.

3.8.1. Preparation of Protein Lysates and Protein Quantification

In order for the protein lysates to be acquired from the cells, first we prepared lysis buffer, right before we began the experiment. (Table 3.3) Because we needed the Eppendorf tubes and pipette tips to be cold, we kept them in the +4 ^{0}C refrigerator before the experiment.

Table 3.3. Lysis buffer for protein lysate preparation.

Final concentration of solutions	**Sample volumes of used solutions (for a 6 well plate, total volume: 3000 µl)**
RIPA Buffer	2850 µl
1X PIC (Protease inhibitor cocktail)	30 µl
50 mM Calyculin	30 µl
10 mM NaF	60 µl
5 mM EDTA	30 µl

After preparations were complete and the drug incubation was over, the plate was removed from the incubator and put on ice in the cold room (+4 ^{0}C) Remaining steps were all completed in the cold room. Drug and medium was removed from the wells, and wells were washed with PBS three times. 400 µl of the prepared lysis buffer was added to wells and mixed thoroughly. Plate was shaken with the shaker 100 rpm for 5 minutes. Wells were scratched with the end of pipette tip for roughly 1 minute per well. Plate was shaken with the shaker 100 rpm for 10 minutes. Wells were scratched with the end of pipette tip for roughly 1 minute per well. Plate was shaken with the shaker 100 rpm for 5 minutes. Wells were scratched with the end of pipette tip for roughly 30 seconds per well, and the acquired solution was put in an Eppendorf tube. Tubes were centrifuged in a +4^{0}C centrifuge 13000 rpm for 15 minutes. Supernatant, which included the protein lysate, was carefully removed from the tube and transferred to another tube, to be kept in -86 ^{0}C.

For the protein quantification, *BCA Assay Kit* was used. A and B solutions which included the colorimetric substances, were added to a 15 ml centrifuge tube in a ratio of 50:1. 10 µl of the protein lysates were added to wells of a 96 well plate. In order to determine the protein quantities of the lysates, BSA (*Bovine Serum Albumin*) standards, whose protein quantities were already known, were added to wells. 2 mg/ml, 1,5 mg/ml, 1 mg/ml, 0,75 mg/ml, 0,5 mg/ml, 0,25 mg/ml and 0,125

mg/ml, a total of 7 BSA protein standards were used. In order to avoid a probable colorimetric result gained from the lysis buffer, 10 µl of the lysis buffer was added to a well, which was used as a “blank” well. 200 µl of the A-B mixture was added to wells and mixed thoroughly. Plate was covered in foil in order to avoid light exposure, and shaken for roughly 30 seconds, followed by 30 minutes of incubation in the 37 ^{0}C incubator, avoiding light exposure. Plate was read in the spectrophotometer 562 nM, and the acquired values gave us the protein quantity in mg/ml.

After quantification of proteins, lysates were mixed with 4x Laemmli Sample Buffer (BioRad) and 2-mercaptoethanol. This procedure was completed under a fume hood because of the toxicity of 2-mercaptoethanol. The resulting lysate-buffer mixture was incubated for 7 minutes on a dry heat block which was heated to 95 ^{0}C beforehand, which helped the denaturation process of proteins. Denaturation of proteins is important for the binding of antibodies to them. 2-mercaptoethanol degrades the disulfide bonds of proteins. SDS provides the proteins to be charged negatively. Glycerol makes the proteins precipitate into the wells, and the bromophenol blue makes them visible while running through the gel.

3.7.2. Polyacrylamide Gel Electrophoresis

For the gel electrophoresis experiments, Mini-PROTEAN TetraCell System (Bio-Rad) was used. Gel solutions were adequately prepared in protocol with the TGX-Stain Free Fast Cast Acrylamide Solution. First, resolving gel solution was added to the TetraCell System between the glasses, then stacking gel solution was added. Immediately following the stacking gel solution, comb was put in place, in order for it to show us the wells. After the gel solutions were polymerized, which took approximately half an hour, glasses containing the gel were put in the electrophoresis tank, and the tank was filled with 1X Running Buffer (BioRad). After it was made sure that the running buffer got through the wells, lysates containing the loading buffer were loaded to wells. Tank was closed and connected to the power source, samples were run in 100 volts for approximately 2 hours.

3.8.3. Semi-dry Transfer and Blotting with *Trans-Blot Turbo system*

Semi-dry transfer and blotting was completed using Trans-Blot Turbo System according to protocol. After transfer procedure, PVDF membrane was put in a container comprising %5 nonfat milk solution. Membrane was incubated on a shaker which was set to 100 rpm in room temperature for an hour. Casein in the milk solution blocks the epitopes of proteins, augmenting the specificity of the antibody, which gives us a clearer background when imaging. After blockage with the milk solution, monoclonal primary antibodies which gave us a high affinity and specificity for the target proteins were used. Monoclonal antibodies were diluted in a ratio of 1:1000 with PBS-Tween. Membrane was incubated with the antibody solution overnight in the cold room on a 100 rpm shaker. The next day, membrane was washed with PBS-Tween three times on a 100 rpm shaker for 10 minutes each, then incubated with HRP (Horseradish Peroxidase) secondary antibody which was diluted in a ratio of 1:10000, for an hour. After incubation, membrane was washed with PBS-Tween three times on a 100 rpm shaker for 10 minutes each.

3.8.4. Chemiluminescent Imaging

For the imaging procedure, *Super Signal West-femto Maximum Sensitivity Substrate* ECL kit was used. Luminol, which is the luminescent substance in ECL, is the substrate of the peroxidase enzyme, luminol reacts with the peroxidase enzyme, oxidizes it and helps the formation of 3-aminoftalat, which creates luminescence photon energy while imaging, and shows the protein bands. Solution, prepared in protocol with the kit, was evenly distributed on the membrane and incubated avoiding light exposure for 5 minutes. After incubation, solution was removed and bands were imaged with the Kodak Gel Logic 1500 imaging System.

3.8.5. Reblotting for Loading Control Proteins

After imaging, in order to normalize and check the conditions of the Western Blot experiment, homogeneity of the beta actin and GAPDH proteins, which are housekeeping proteins and expected to be homogenous in every condition, were checked. After imaging process, membrane was incubated with PBS-Tween for 15 minutes, then blocked for an hour with %5 nonfat milk solution. Beta actin or GAPDH primary antibody was diluted in a ratio of 1:2500 with PBS-Tween. Membrane was incubated with the antibody solution overnight on a 100 rpm incubator in the cold room. The next day, membrane was washed with PBS-Tween three times on a 100 rpm shaker for 10 minutes each, then incubated with HRP (Horseradish Peroxidase) secondary antibody which was diluted in a ratio of 1:10000, for an hour. After incubation, membrane was washed with PBS-Tween three times on a 100 rpm shaker for 10 minutes each, and imaging process was completed. After imaging, the results of the beta actin bands and other protein bands were used for densitometry analysis.

3.8.6. Densitometry Analysis

Using the *Fiji* Software, density of the protein bands was detected. Protein densities of the target proteins were normalized with the densities of beta actin or GAPDH protein bands.

3.9. Crystal Violet Assay

After MDA-MB-231 cells were added to a 12-well plate, 40.000 cells per well, drug wells were incubated with GSK650394 in indicated doses, medium was added to control wells instead of GSK650394. Drug and medium was changed every other day. 5 days after the initial drug incubation, drug and medium was removed from the wells. 400 µl crystal violet mixture (%0.5 crystal violet dye, %20 methanol) was added to wells and wells were incubated with this dye for 10 minutes. After this procedure, wells were washed with PBS, macroscopically and microscopically photographed.

3.10. Cell Cycle Analysis

MDA-MB-231 cells were plated to a 6-well plate (1 million cells per well). Following 24 hours after incubation in the incubator, 1 µM GSK650394 was added to drug wells and medium was added to control wells. 24 hours later, drug and medium was removed from the wells. Cells were washed with PBS, trypsinised and transferred to a 15 ml centrifuge tube. Ethanol was added to the tube and thoroughly mixed. Tubes were incubated in +4 ^{0}C, following incubation 2 ml PBS was added and the tubes were centrifuged in 1800 rpm for 5 minutes. 1 ml PBS was added to the resulting pellet. 100 µl RNAse was added to the mixture and vortexed. PI was added and vortexed. Tubes were incubated in 37^{0}C for an hour and analyzed with flow cytometry.

3.11. Statistical Analysis

Data were analyzed with *independent groups Student t test.* Acquired values that were $p<0.05$ were considered statistically significant. Statistical analyses were conducted with the *IBM® SPSS® Statistics Version 23* program.

4. RESULTS

4.1. SGK1 mRNA and Protein Expressions in Triple Negative Breast Cancer Cells

4.1.1. SGK1 Protein Expressions in Triple Negative Breast Cancer Cells

SGK1 protein expressions were shown with Western blot method. As expected, MDA-MB-468 had a low SGK1 protein expression. MDA-MB-436 had the highest protein expression. (Figure 4.1)

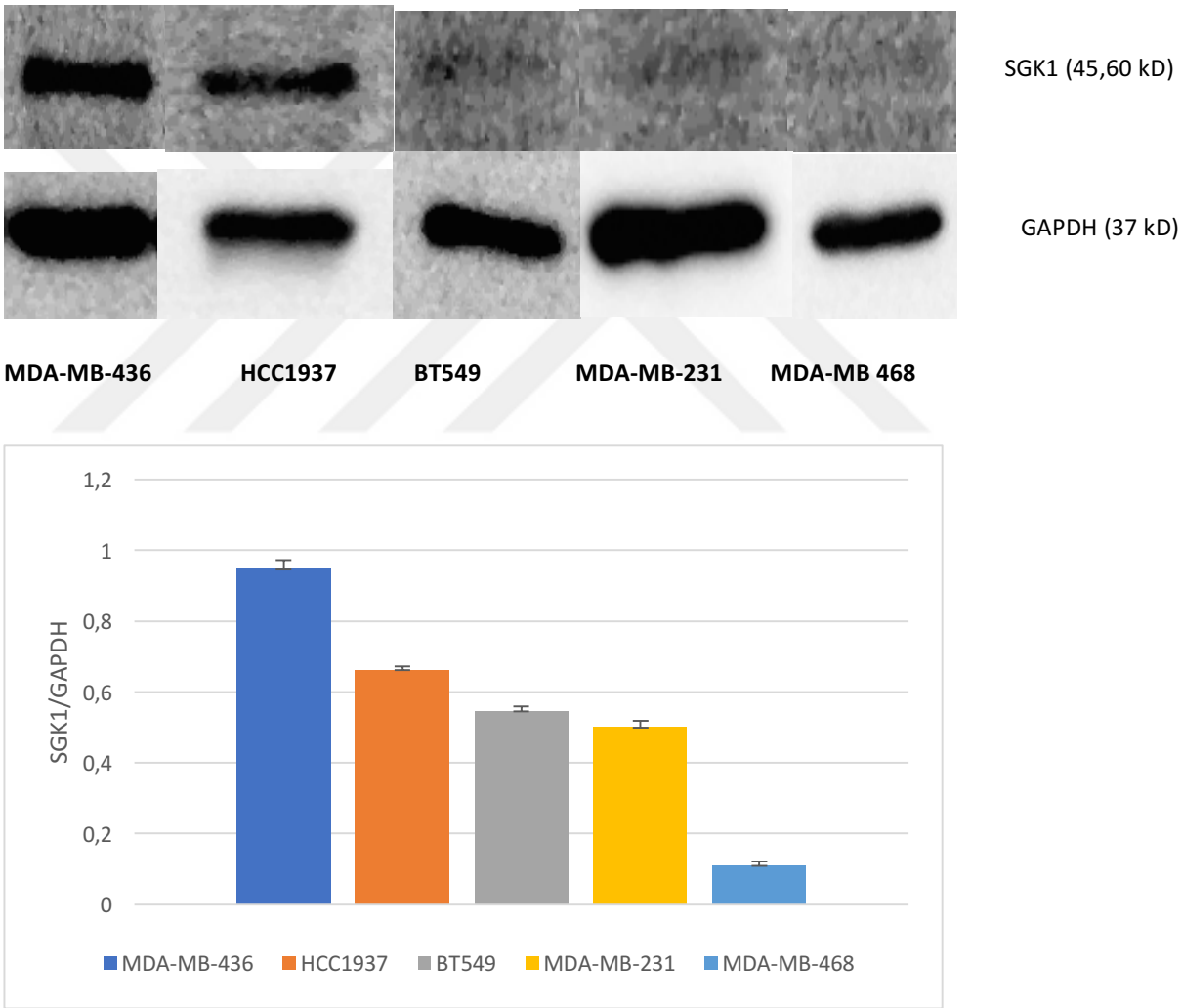


Figure 4.1. Protein levels of SGK1 in triple negative breast cancer cell lines. (n=3)

4.1.2. SGK1 mRNA Expressions in Triple Negative Breast Cancer Cells

SGK1 mRNA expressions were shown with Real Time PCR method. As expected, MDA-MB-468 had the lowest mRNA expression among the cell lines, and MDA-MB-436 had the highest mRNA expression. (Figure 4.2.)

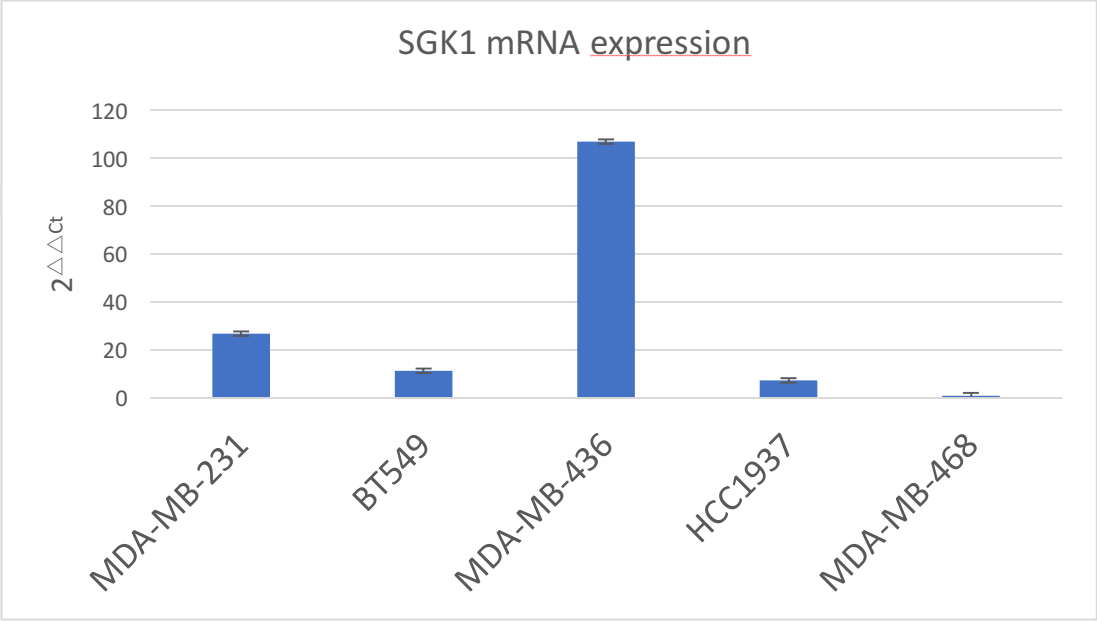


Figure 4.2. mRNA levels of SGK1 in triple negative breast cancer cell lines. (n=3)

4.2. Determination of Subtoxic Doses of GSK650394 With MTT Assay

By analyzing the literature and previous articles about the use of GSK650394 on breast cancer cell lines and other cells, we determined that the appropriate dose for our cell lines would be 1 micro molar. Considering this entity, we chose to use 0,5 micro molar, 1 micro molar, 2 micro molar and 4 micro molar doses of GSK650394 for 48 and 72 hours.

4.2.1. Determination of Subtoxic Doses of GSK650394 with MTT Assay In MDA-MB-231 Cells

We did not observe any significant cytotoxicity in MDA-MB-231 cell line with all doses tested for 48 and 72 hours. (Figures 4.3., 4.4.)

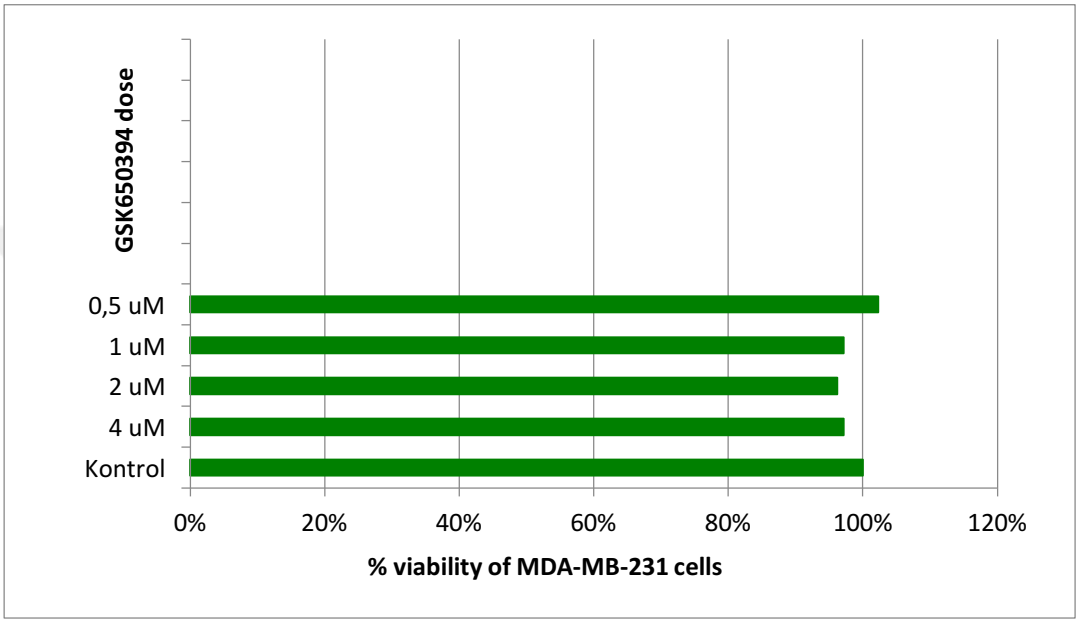


Figure 4.3. Percentage of viable MDA-MB-231 cells, after treatment with GSK650394 for 48 hours. (n=4)

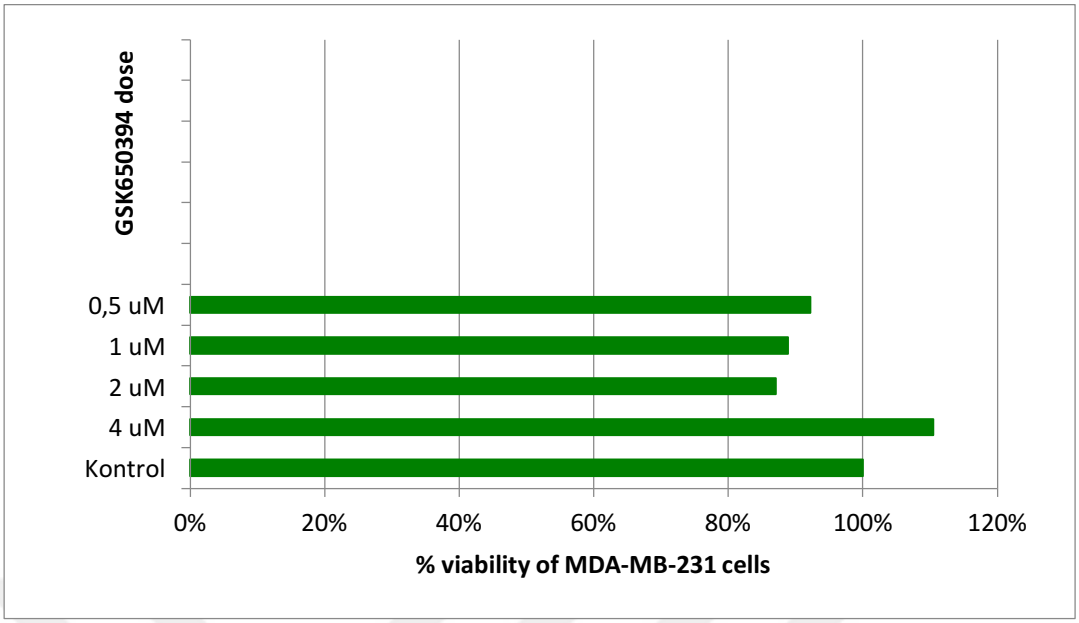


Figure 4.4. Percentage of viable MDA-MB-231 cells, after treatment with GSK650394 for 72 hours. (n=4)

4.2.2. Determination of Subtoxic Doses Of GSK650394 With MTT Assay In MDA-MB-436 Cells

We did not observe any significant cytotoxicity in MDA-MB-436 cell line with all doses tested for 48 and 72 hours. (Figures 4.5., 4.6)

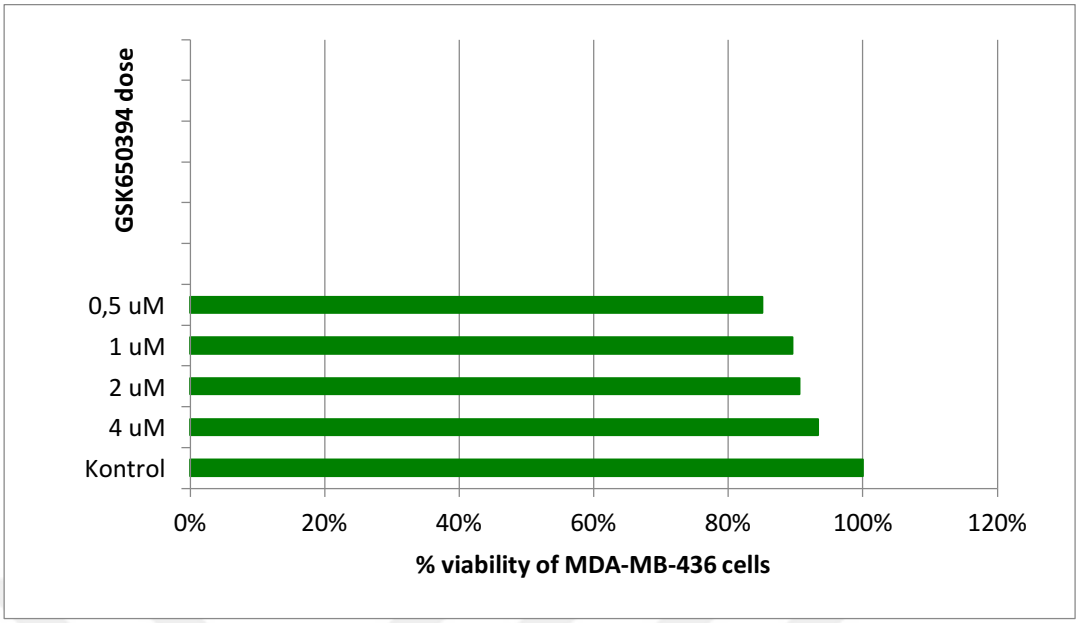


Figure 4.5. Percentage of viable MDA-MB-436 cells, after treatment with GSK650394 for 48 hours. (n=4)

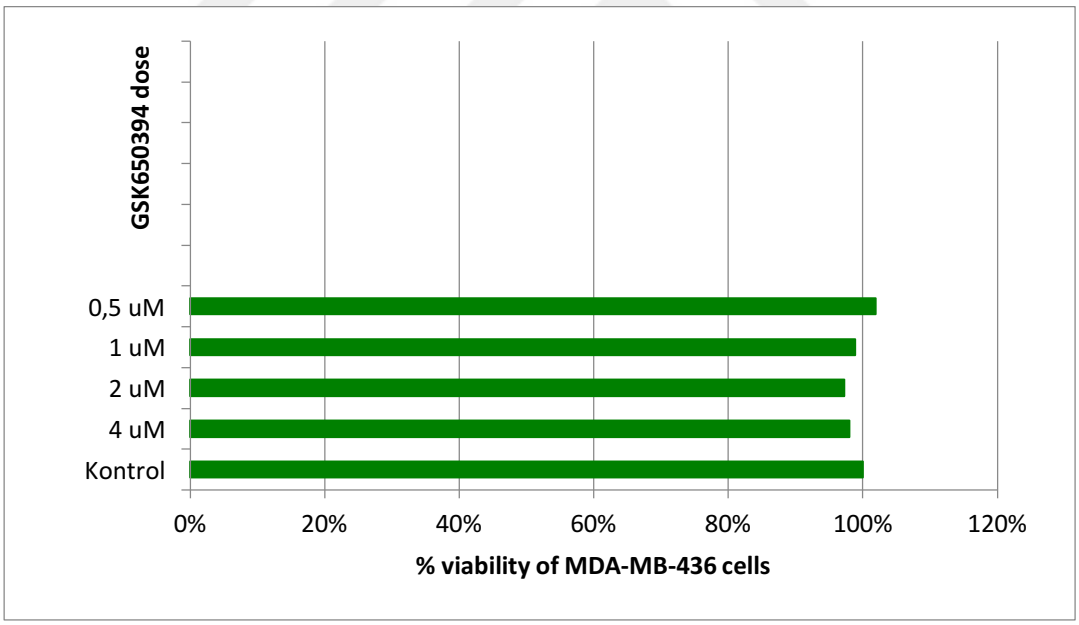


Figure 4.6. Percentage of viable MDA-MB-436 cells, after treatment with GSK650394 for 72 hours. (n=4)

4.2.3. Determination of Subtoxic Doses of GSK650394 With MTT Assay In HCC1937 Cells

We did not observe any significant cytotoxicity in HCC1937 cell line with all doses tested for 48 and 72 hours. (Figures 4.7, 4.8.)

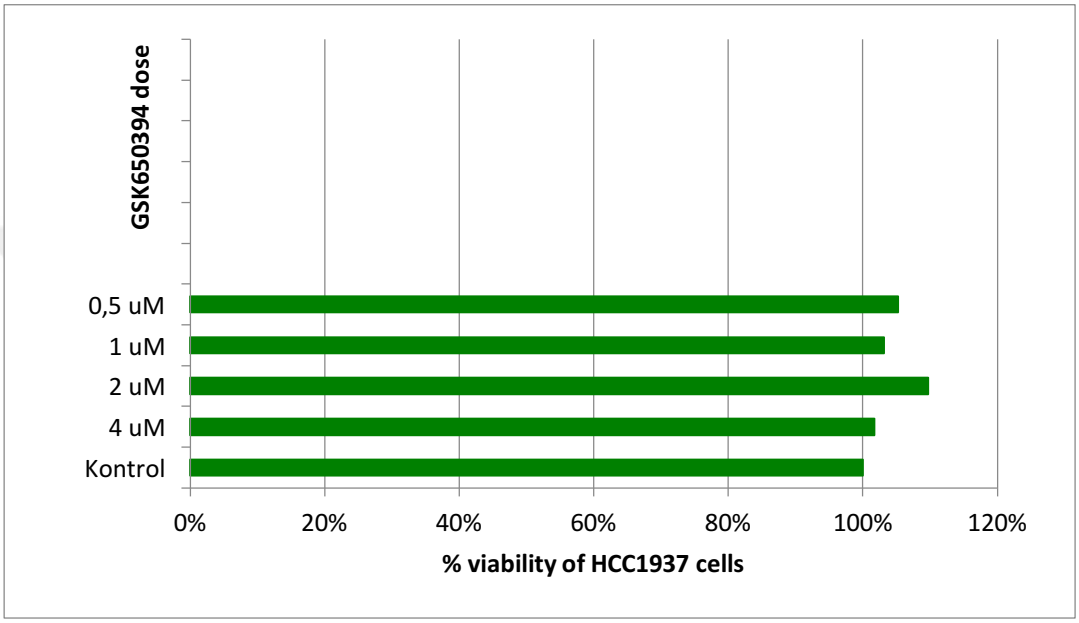


Figure 4.7. Percentage of viable HCC1937 cells, after treatment with GSK650394 for 48 hours. (n=4)

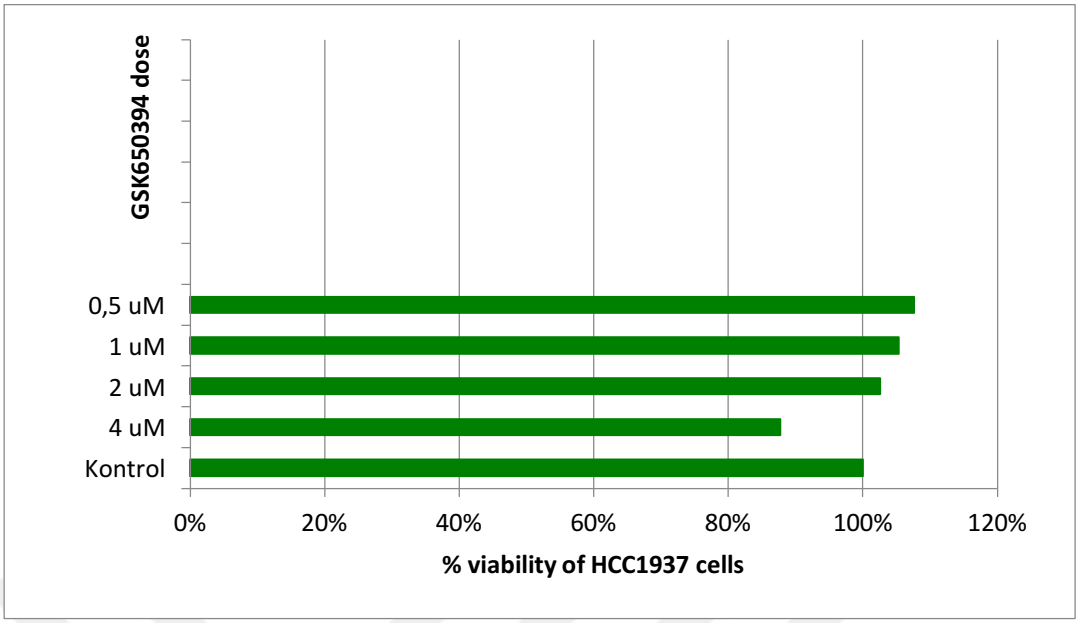


Figure 4.8. Percentage of viable HCC1937 cells, after treatment with GSK650394 for 72 hours. (n=4)

4.2.4. Determination of Subtoxic Doses of GSK650394 With MTT Assay In BT549 Cells

We did not observe any significant cytotoxicity in BT549 cell line with all doses tested for 48 and 72 hours. (Figures 4.9., 4.10.)

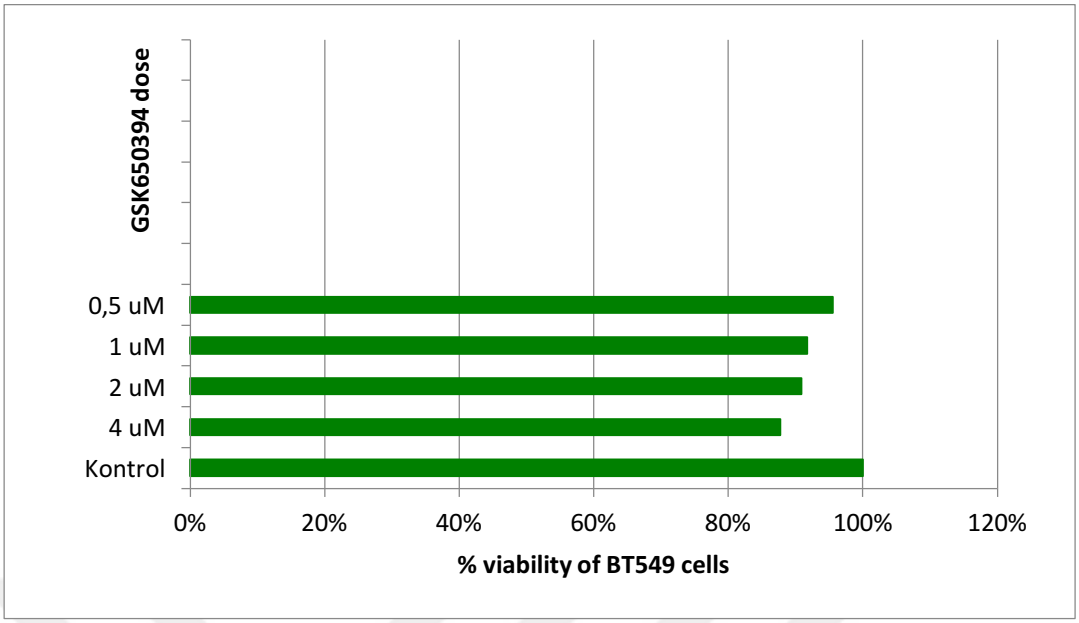


Figure 4.9. Percentage of viable BT549 cells, after treatment with GSK650394 for 48 hours. (n=4)

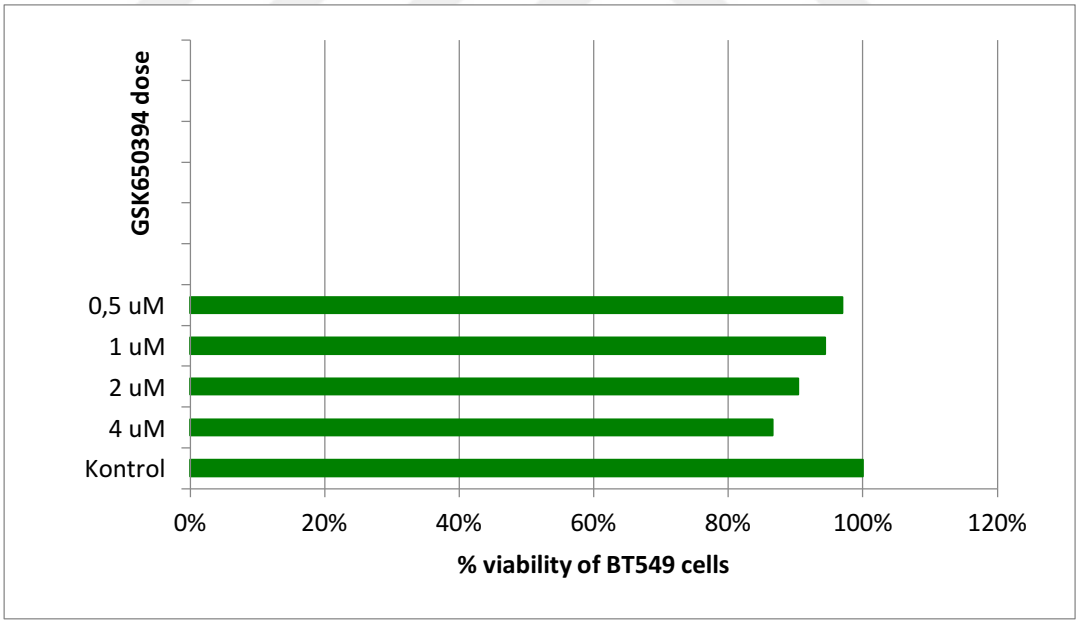


Figure 4.10. Percentage of viable BT549 cells, after treatment with GSK650394 for 72 hours. (n=4)

4.2.5. Determination of Subtoxic Doses of GSK650394 With MTT Assay In MDA-MB-468 Cells

We did not observe any significant cytotoxicity in MDA-MB-468 cell line with all doses tested for 48 and 72 hours. (Figures 4.11., 4.12.)

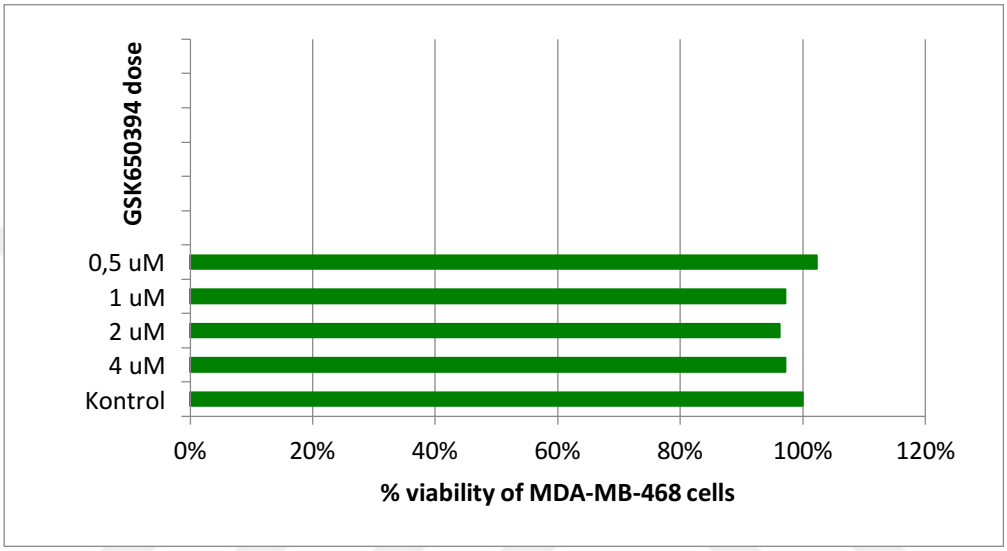


Figure 4.11. Percentage of viable MDA-MB-468 cells, after treatment with GSK650394 for 48 hours. (n=4)

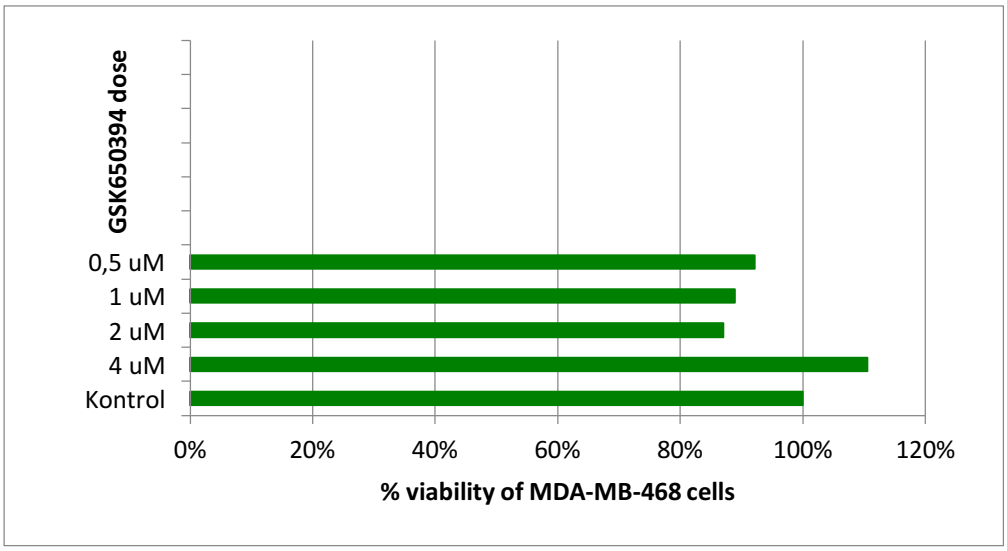


Figure 4.12. Percentage of viable MDA-MB-468 cells, after treatment with GSK650394 for 72 hours. (n=4)

4.3. Determination of SGK1 Inhibition Through Phospho-NDRG1 Protein Expression Levels in Triple Negative Breast Cancer Cell Lines

4.3.1. Determination of SGK1 Inhibition Through Phospho-NDRG1 Protein Expression Levels in MDA-MB-231 Cells

We determined 1 micro molar of GSK650394 as the dose we chose to use for our experiments, and 24 hours as the period of time needed, considering previous literature. We showed the decrease of phospho-NDRG1 protein with Western Blot method in the MDA-MB-231 cell line. (Figure 4.13.)

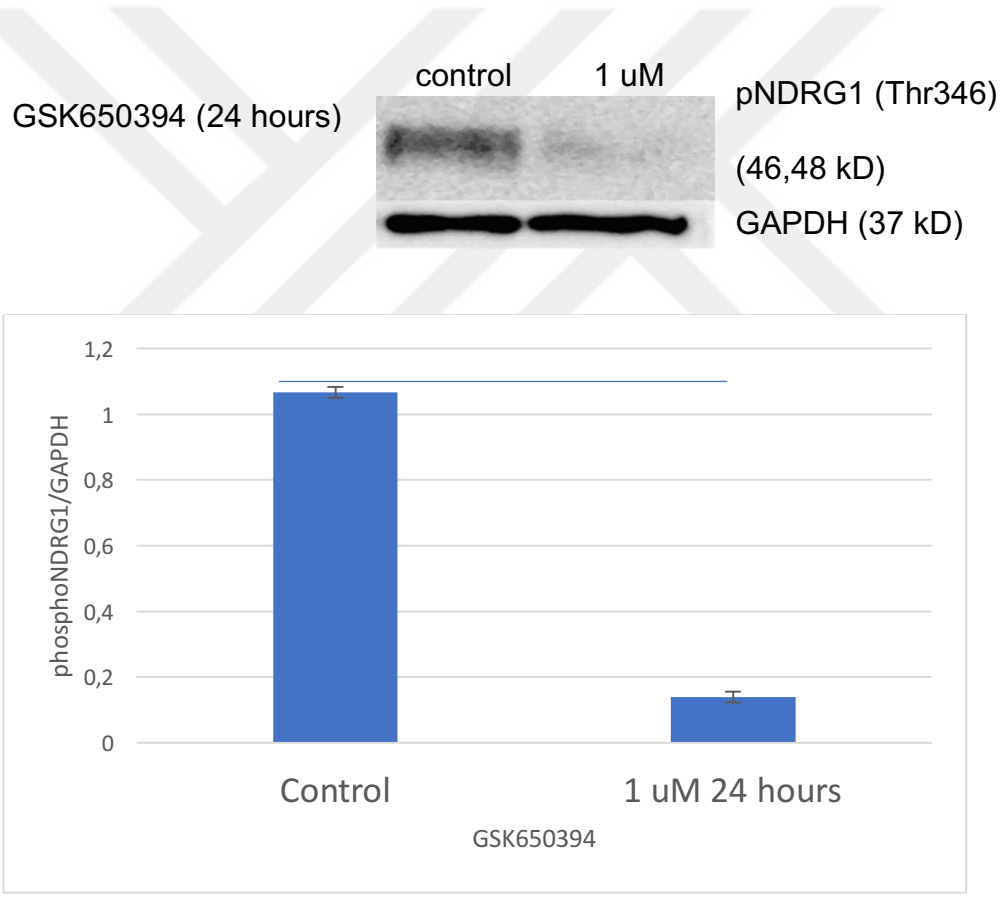


Figure 4.13. Change in the protein level of phosphorylated NDRG1 after treatment with GSK650394 in MDA-MB-231 cell line. (n=3, **p<0.05**)

4.3.2. Determination Of SGK1 Inhibition Through Phospho-NDRG1 Protein Expression Levels In BT549 Cells

We did not observe any decrease in BT549 cell line with 1 micro molar dose for 24 hours. Subsequently, we used 2 micromolar and 4 micromolar doses. We showed decrease in phospho-NDRG1 expression when we used 2 micro molar GSK650394. (Figure 4.14)

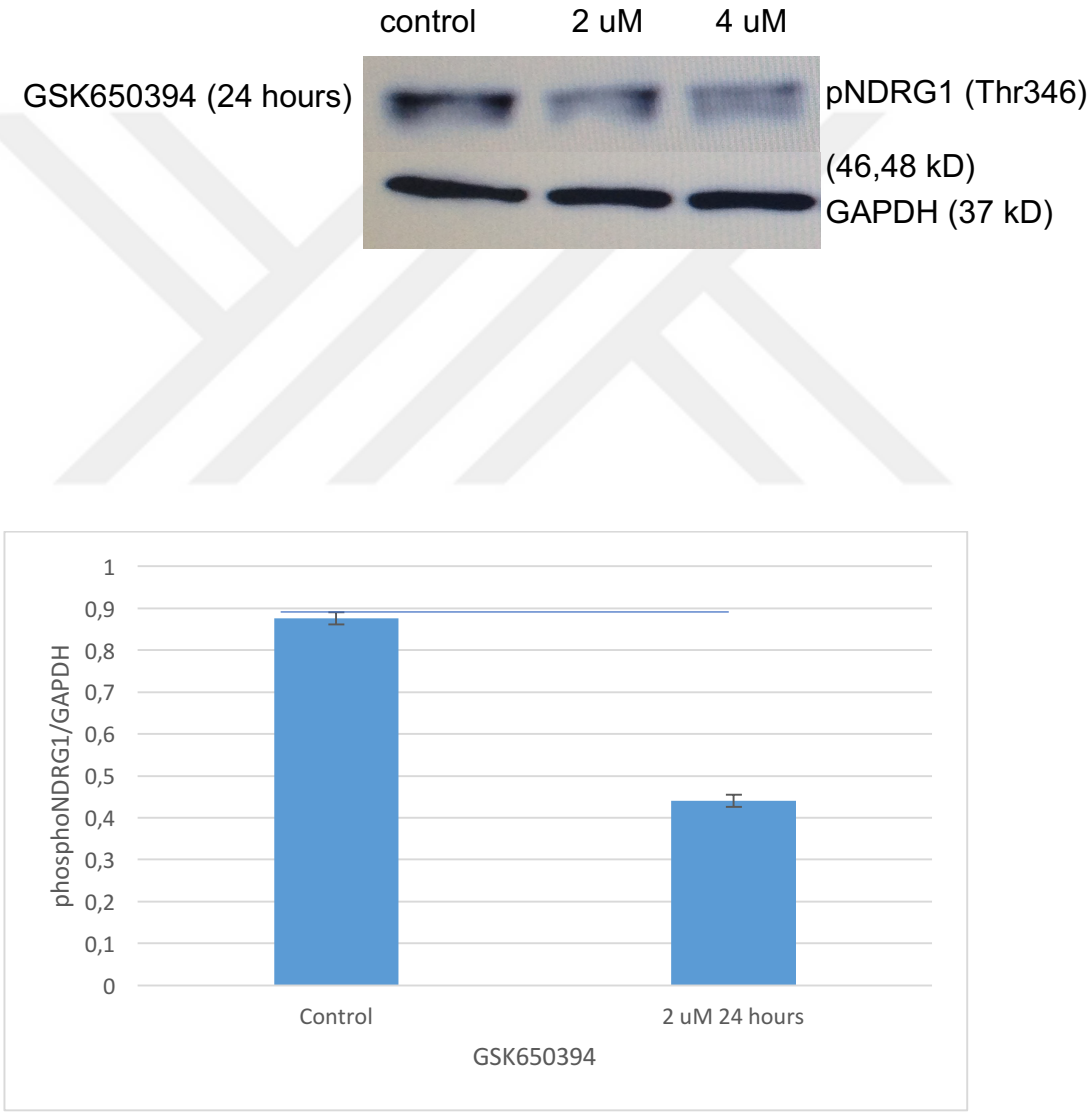


Figure 4.14. Change in the protein level of phosphorylated NDRG1 after treatment with GSK650394 in BT549 cell line. (n=3, **p<0.05**)

4.3.3. Determination Of SGK1 Inhibition Through Phospho-NDRG1 Protein Expression Levels In HCC1937 Cells

We did not observe any decrease in HCC1937 cell line with 1 micro molar dose for 24 hours. Subsequently, we used 2 micro molar and 4 micro molar doses. We showed decrease in phospho-NDRG1 expression when we used 4 micro molar GSK650394. (Figure 4.15)

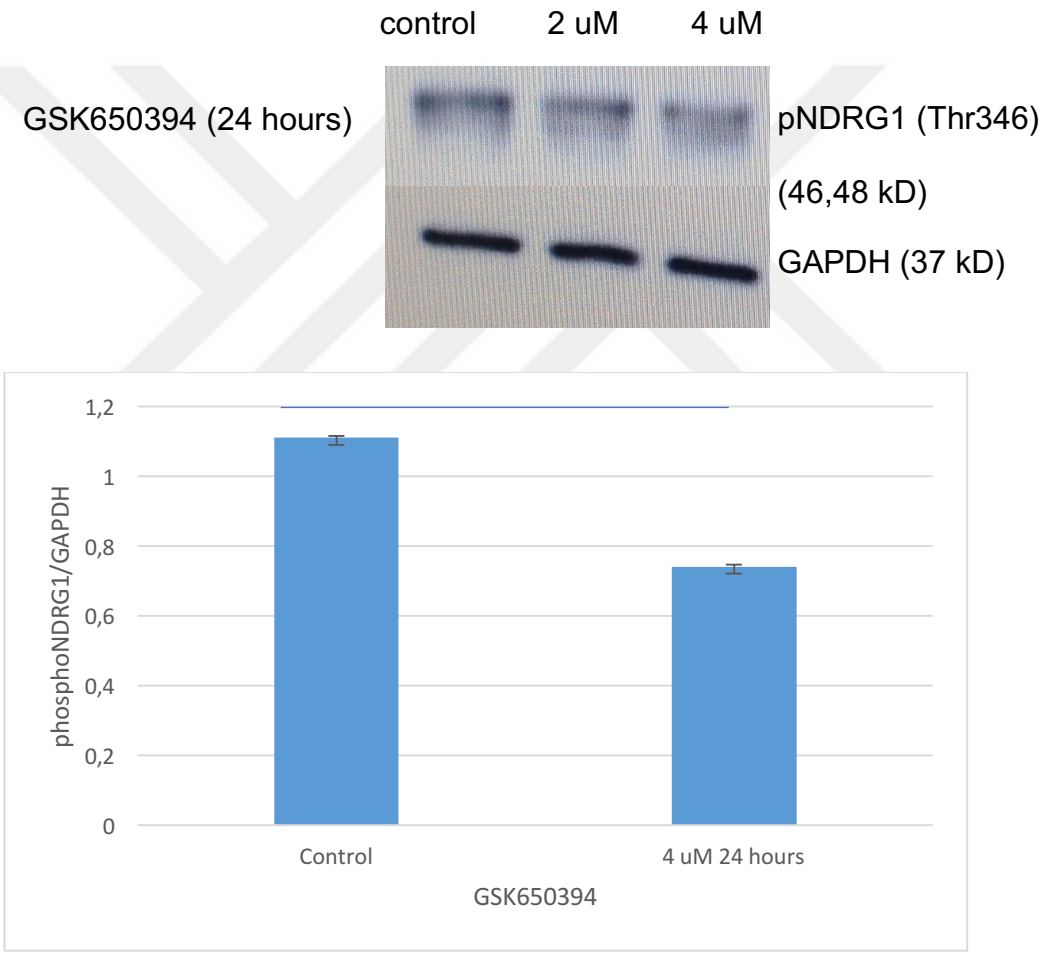
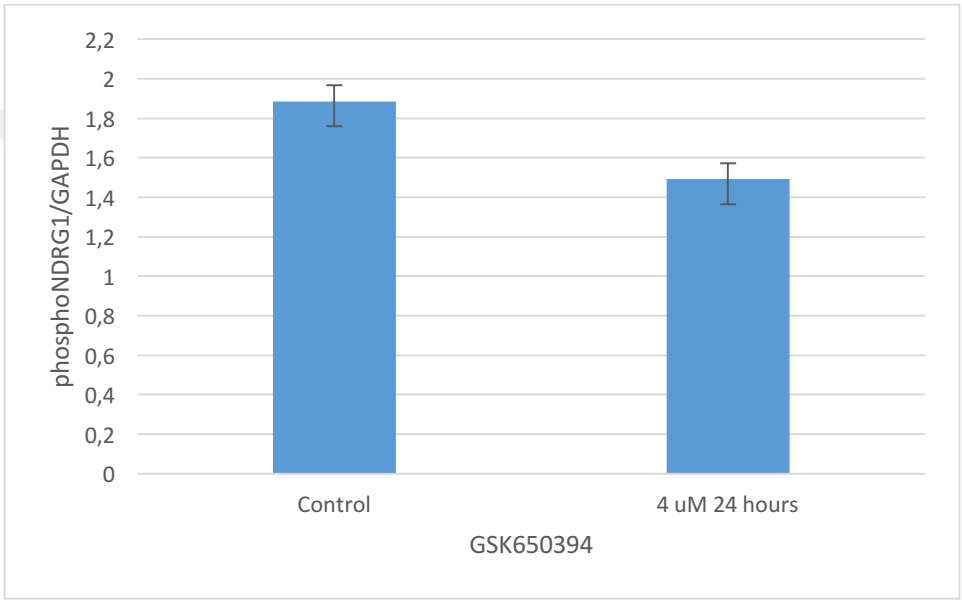


Figure 4.15. Change in the protein level of phosphorylated NDRG1 after treatment with GSK650394 in HCC1937 cell line. (n=3, **p<0.05**)

4.3.4. Determination Of SGK1 Inhibition Through Phospho-NDRG1 Protein Expression Levels In MDA-MB-436 Cells

We did not observe any decrease in phospho-NDRG1 expression when we used 1 micro molar of GSK650394 for 24 hours in MDA-MB-436 cell line. Subsequently, we used 2 micro molar and 4 micro molar doses, we did not observe inhibition with these doses either. (Figure 4.16.)



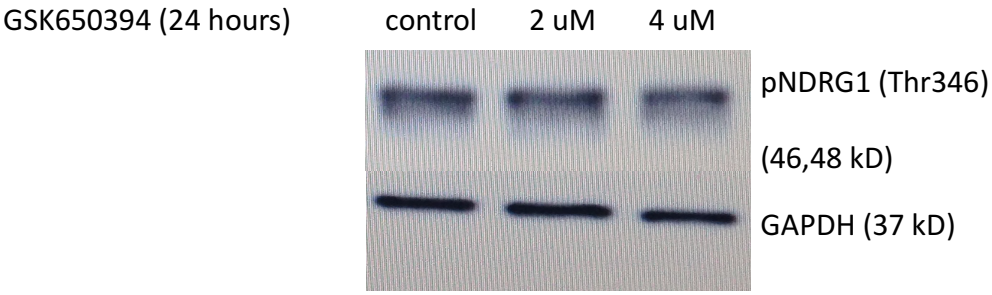


Figure 4.16. Change in the protein level of phosphorylated NDRG1 after treatment with GSK650394 in MDA-MB-436 cell line. (n=3, p>0.05)

When we did not observe any inhibition with these doses either, we planned to see a dose-response curve increasing the dose to prove that this cell line is resistant to GSK650394. (Figure 4.17)

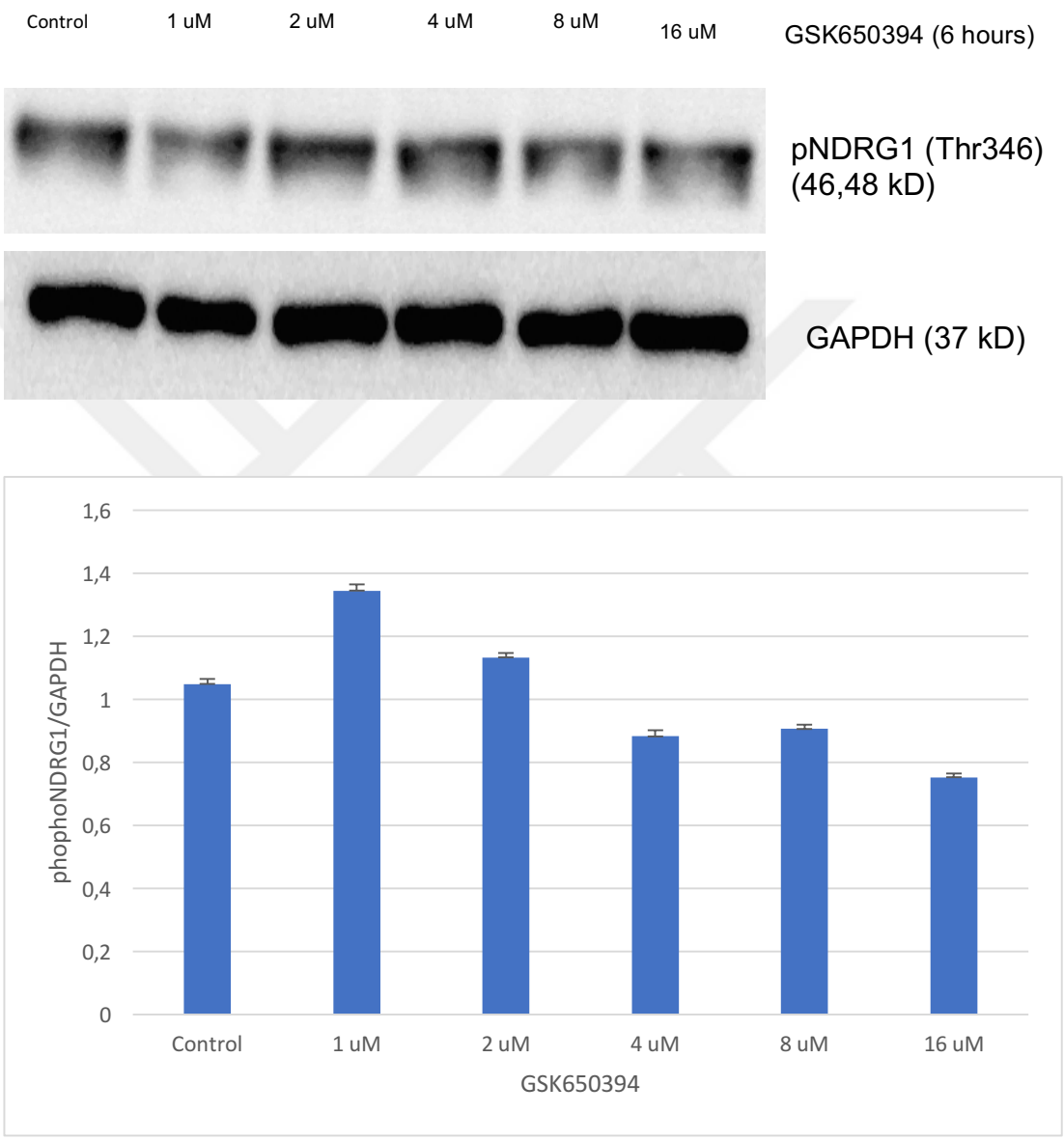


Figure 4.17. Change in the protein level of phosphorylated NDRG1 after treatment with different doses of GSK650394 for 6 hours in MDA-MB-436 cell line. (n=3)

To be sure of this resistance, we used the MDA-MB-231 cell line which we knew to be the most sensitive cell line, as control. (Figure 4.18)

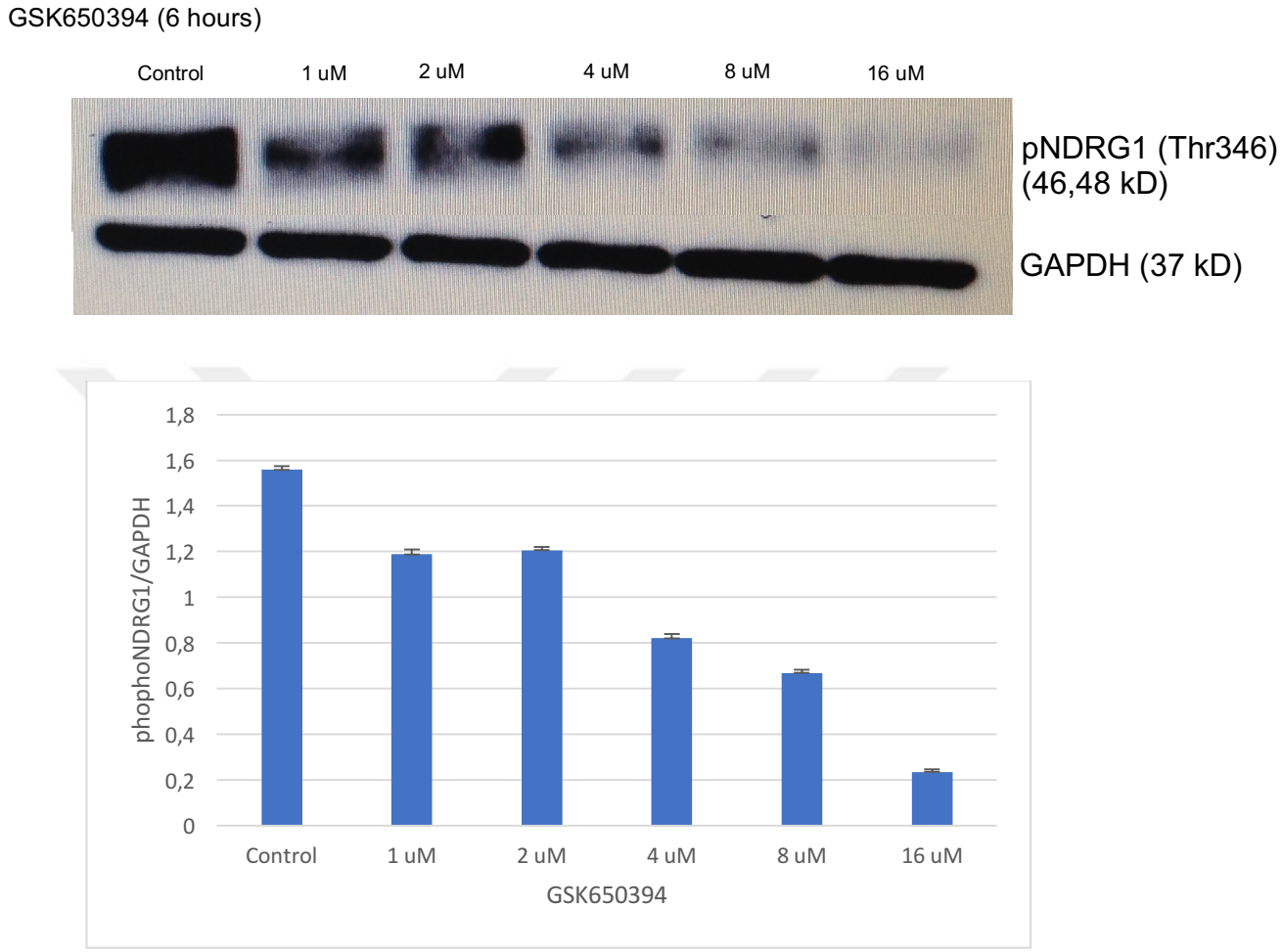


Figure 4.18. Change in the protein level of phosphorylated NDRG1 after treatment with different doses of GSK650394 for 6 hours in MDA-MB-231 cell line. (n=3)

4.3.5. Determination Of SGK1 Inhibition Through Phospho-NDRG1 Protein Expression Levels In MDA-MB-468 Cells

MDA-MB-468 was proven to have low SGK1 expression, and it was planned to be used as a negative control in our experiments. We did not observe any decrease in phospho-NDRG1 expression with 1 micro molar dose for 24 hours. (Figure 4.19.)

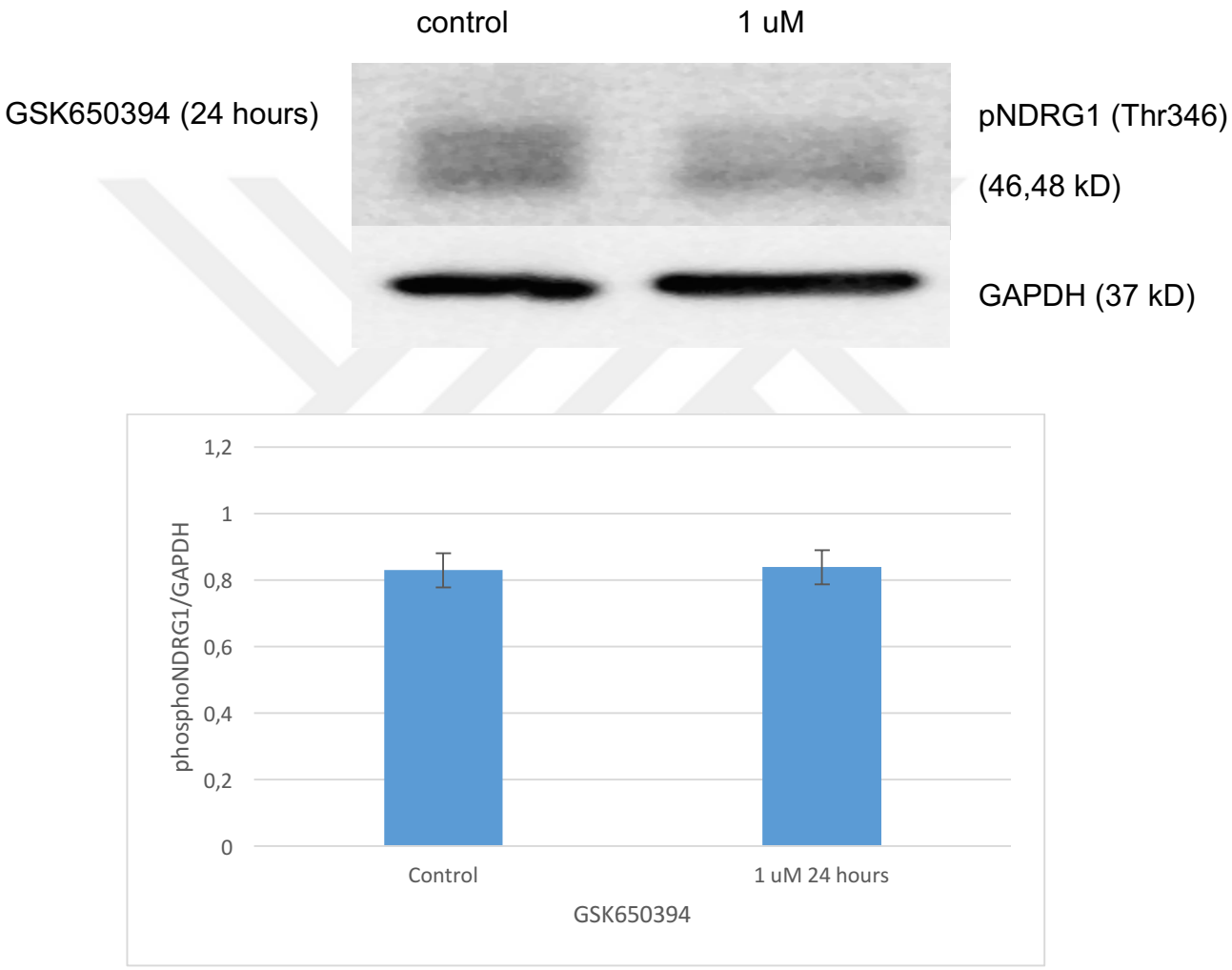


Figure 4.19. Change in the protein level of phosphorylated NDRG1 after treatment with GSK650394 in MDA-MB-468 cell line. (n=3, p>0.05)

4.4. Effect Of SGK1 Inhibition Via GSK650394 On The Proliferation Of MDA-MB-231 Cells

4.4.1. Effect Of SGK1 Inhibition Via GSK650394 On The Proliferation Of MDA-MB-231 Cells Macroscopically

MDA-MB-231 was chosen for the crystal violet (proliferation) experiments, because it was the most sensitive cell line among the chosen cell lines. Effect of GSK650394 was examined macroscopically (Figure 4.20.) with 1 micro molar and 2 micro molar GSK650394 for 5 days. It was shown that the proliferation of cells was dramatically decreased with the effect of GSK650394. (Figure 4.20.)

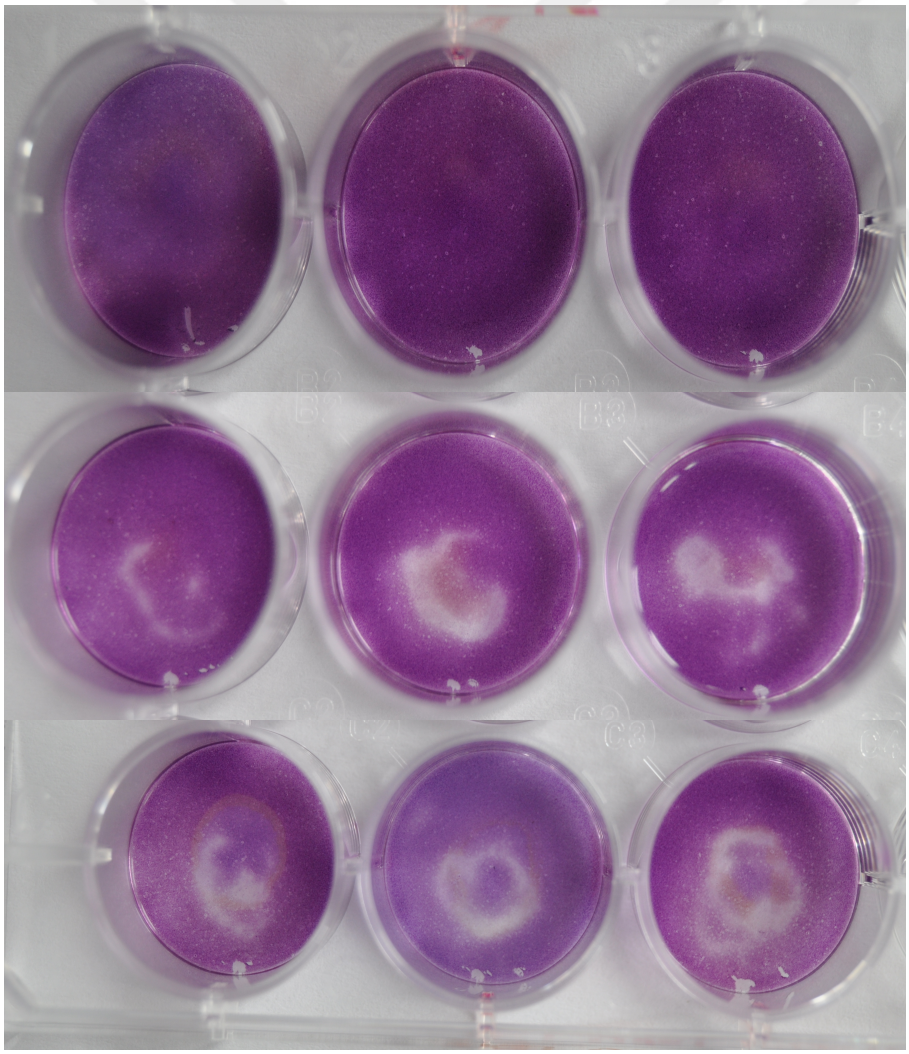


Figure 4.20. Inhibition of proliferation of MDA-MB-231 cells after treatment with GSK650394 for indicated time and doses, macroscopically imaged. (n=3)

4.4.2. Effect of SGK1 Inhibition Via GSK650394 On the Proliferation of MDA-MB-231 Cells Microscopically

Effect of GSK650394 was examined microscopically (Figure 4.21.) with 1 micro molar and 2 micro molar GSK650394 for 5 days. It was shown that the proliferation of cells was dramatically decreased with the effect of GSK650394. (Figure 4.21.)

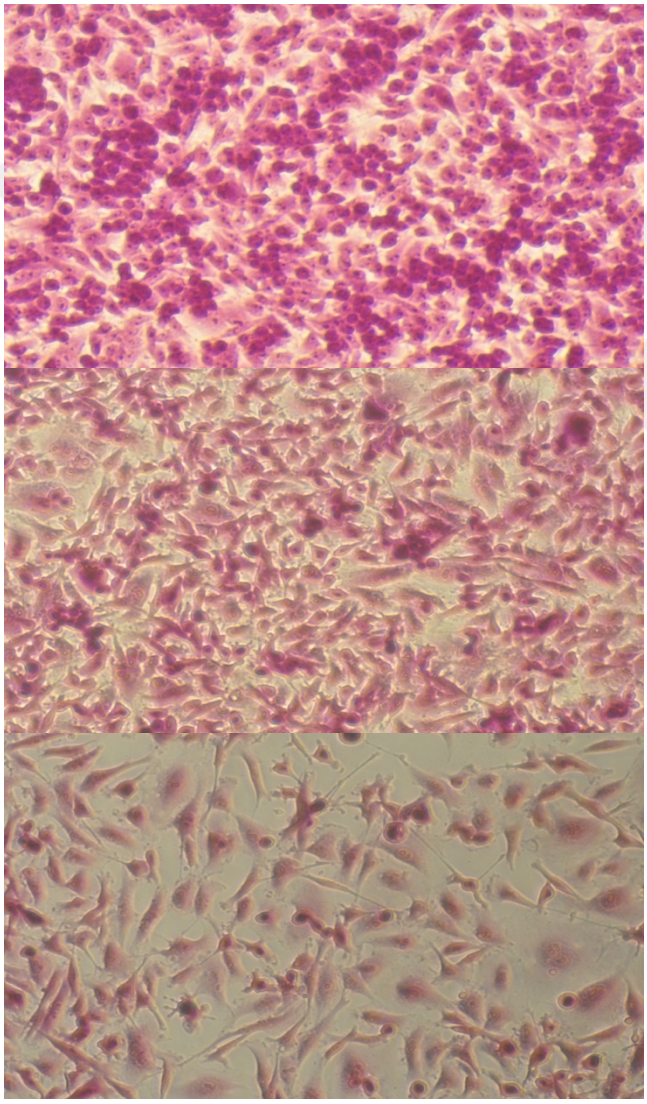


Figure 4.21. Inhibition of proliferation of MDA-MB-231 cells after treatment with GSK650394 for indicated time and doses, microscopically imaged (10x). (n=3)

4.5. Effect of SGK1 Inhibition Via GSK650394 on the Cell Cycle Phases Distribution In MDA-MB-231 Cells

Flow cytometry method was used to show the effect of SGK1 inhibition on cell cycle. It was shown that with the effect of SGK1 inhibition, the cells that were going through S phase were decreased. (Figure 4.22.)

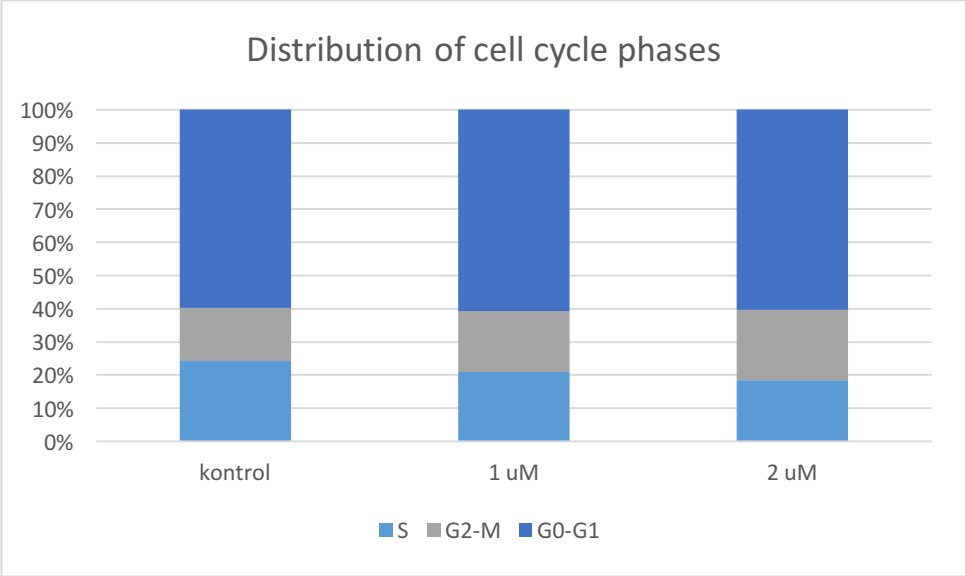


Figure 4.22. Effect of GSK650394 on the distribution of cell cycle phases. (n=3)

4.6. Effect of SGK1 Inhibition on Phospho-GSK3 Beta Expression in Triple Negative Breast Cancer Cell Lines

Effect of SGK1 inhibition on phosphorylated GSK3 beta protein expression was shown with Western Blot method, we used the GSK650394 doses that we found to be inhibitory in previous experiments. (Figures 4.23)

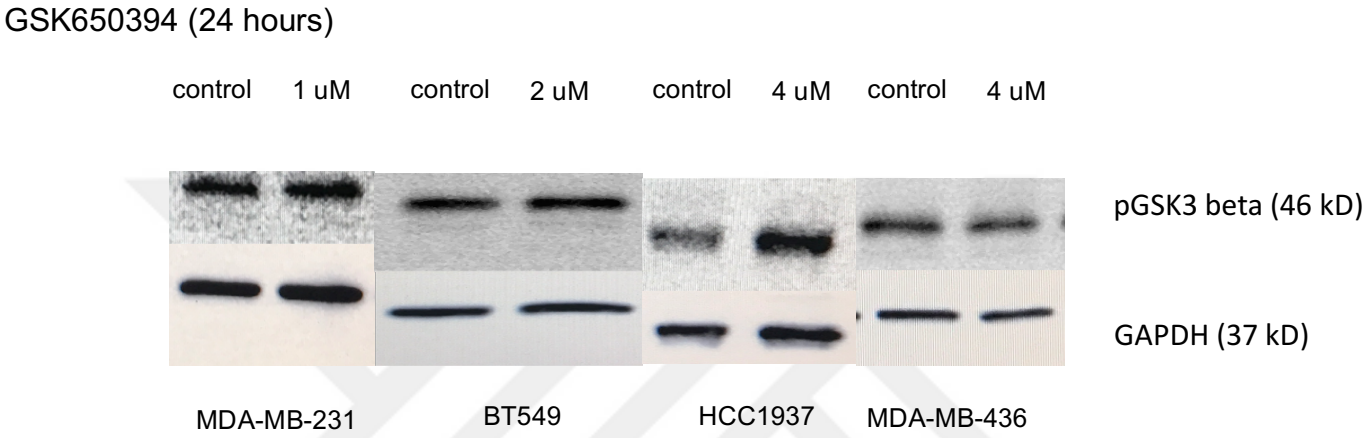


Figure 4.23. Changes in the protein level of phosphorylated GSK3ß after treatment with GSK650394 in triple negative breast cancer cell lines. (n=3)

4.6.1. Effect of SGK1 Inhibition on Phospho-GSK3 Beta Expression In MDA-MB-231 Cell Line

We did not see any change in the phosphorylated GSK3 beta expression in MDA-MB-231 cells before and after treatment with GSK650394 for 24 hours with 1 micromolar dose. (Figure 4.24.)

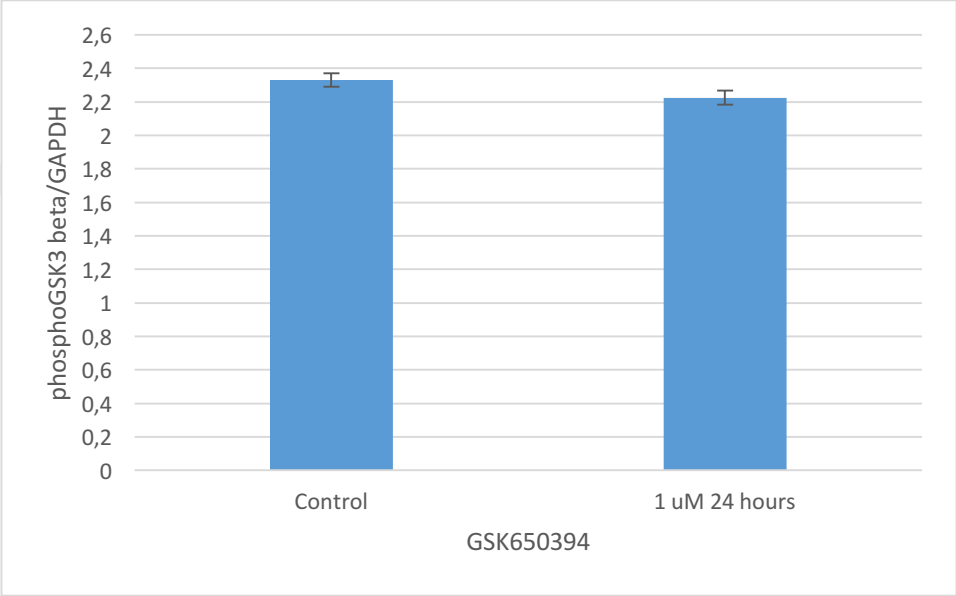


Figure 4.24. Change in the protein level of phosphorylated GSK3ß after treatment with GSK650394 in MDA-MB-231 cell line. (n=3, p>0.05)

4.6.2. Effect Of SGK1 Inhibition On Phospho-GSK3 Beta Expression In HCC1937 Cell Line

We did not see any change in the phosphorylated GSK3 beta expression in HCC1937 cells before and after treatment with GSK650394 for 24 hours with 4 micromolar dose. (Figure 4.25.)

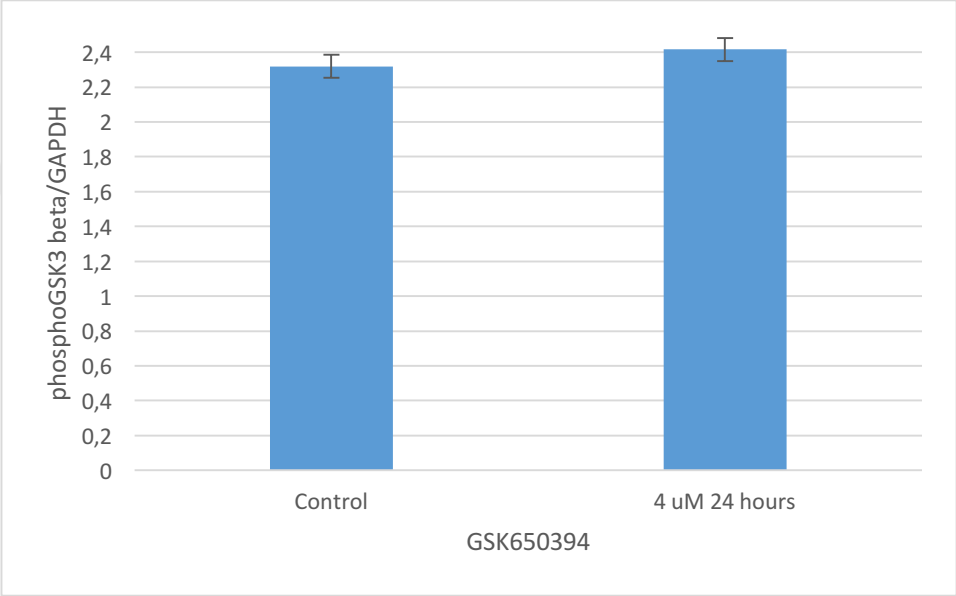


Figure 4.25. Change in the protein level of phosphorylated GSK3ß after treatment with GSK650394 in HCC1937 cell line. (n=3, p>0.05)

4.6.3. Effect of SGK1 Inhibition On Phospho-GSK3 Beta Expression In BT549 Cell Line

We did not see any change in the phosphorylated GSK3 beta expression in BT549 cells before and after treatment with GSK650394 for 24 hours with 2 micromolar dose. (Figure 4.26.)

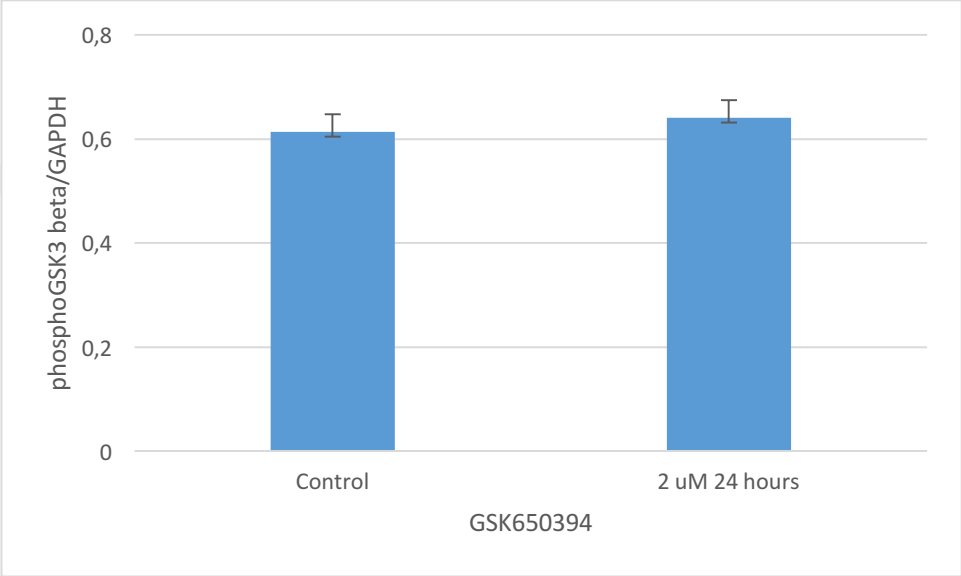


Figure 4.26. Change in the protein level of phosphorylated GSK3ß after treatment with GSK650394 in BT549 cell line. (n=3, p>0.05)

4.6.4. Effect of SGK1 Inhibition On Phospho-GSK3 Beta Expression In MDA-MB-436 Cell Line

We did not see any change in the phosphorylated GSK3 beta expression in MDA-MB-436 cells before and after treatment with GSK650394 for 24 hours with 4 micromolar dose. (Figure 4.27.)

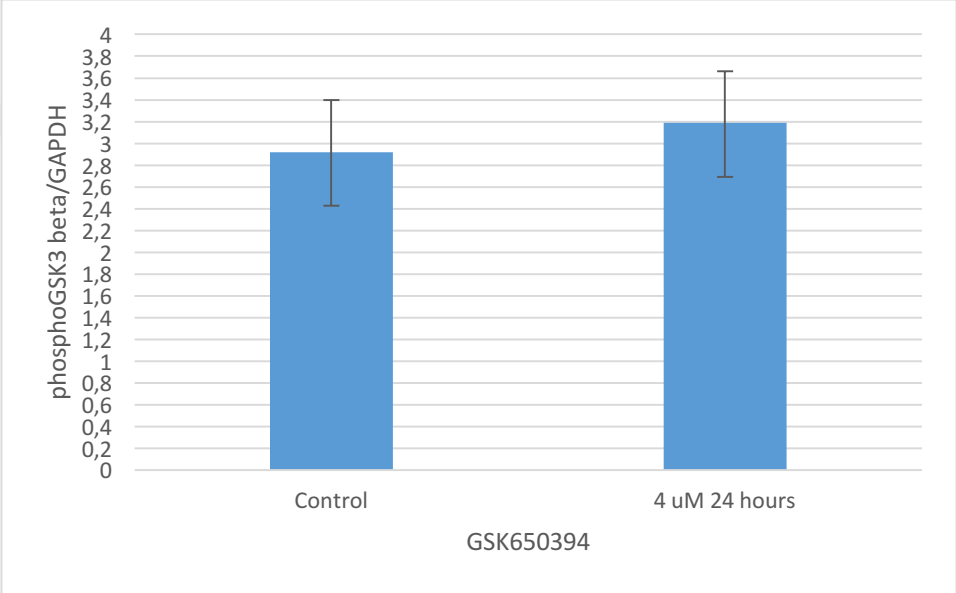


Figure 4.27. Change in the protein level of phosphorylated GSK3ß after treatment with GSK650394 in MDA-MB-436 cell line. (n=3, p>0.05)

4.7. Effect of SGK1 Inhibition Via GSK650394 On EMT Related Proteins E-Cadherin And Vimentin Protein Expressions In Triple Negative Breast Cancer Cell Lines

Effect of SGK1 inhibition on E-cadherin (which is an epithelial marker) and Vimentin (which is a mesenchymal marker), representing the effect of SGK1 inhibition on epithelial-mesenchymal transition, in the selected triple negative breast cancer cell lines were shown with Western Blot method.

4.7.1. Effect of SGK1 Inhibition Via GSK650394 on E-Cadherin Protein Expressions In Triple Negative Breast Cancer Cell Lines

E-cadherin protein expression was nearly undetectable in MDA-MB-231, BT549 and MDA-MB-436 cell lines, and we did not see any change in the protein expression of E-cadherin after GSK650394 treatment. (Figure 4.28.)

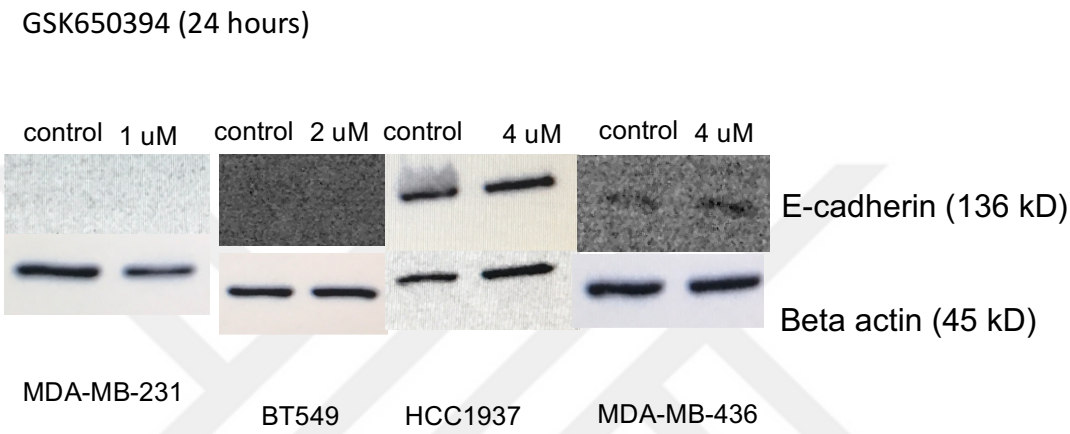


Figure 4.28. Changes in the protein levels of E-cadherin after treatment with GSK650394 in triple negative breast cancer cell lines. (n=3)

Effect Of SGK1 Inhibition Via GSK650394 On E-Cadherin Protein Expression In HCC1937 Cell Line

We did not see any change in the protein expression of E-cadherin in HCC1937 cells after treatment with GSK650394. (Figure 4.29.)

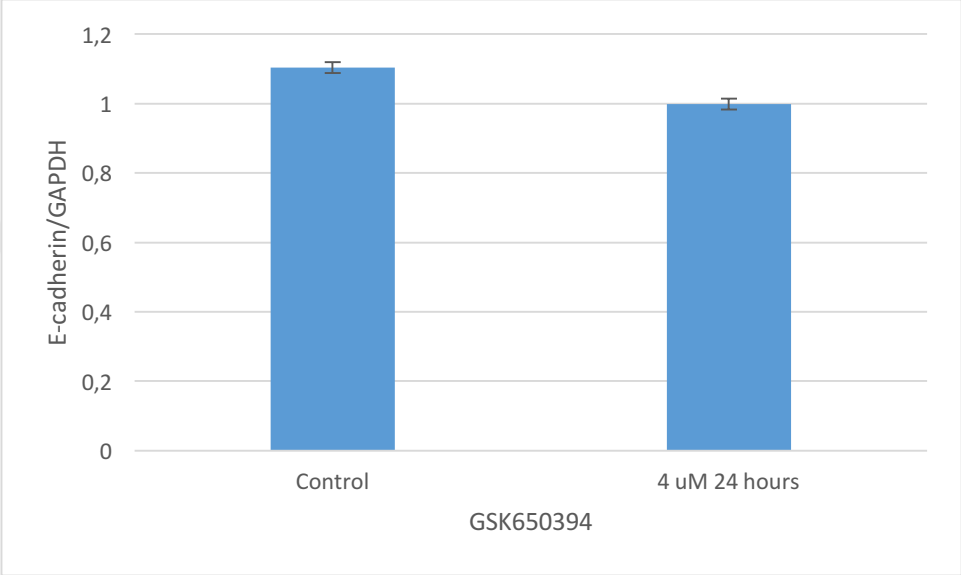


Figure 4.29. Changes in the protein levels of E-cadherin after treatment with GSK650394 in HCC1937 cell line. (n=3, p>0.05)

4.7.2. Effect of SGK1 Inhibition Via GSK650394 On Vimentin Protein Expressions in Triple Negative Breast Cancer Cell Lines

HCC1937 had a nearly undetectable vimentin expression, in contrast to the other triple negative breast cancer cell lines. (Figure 4.30.)

GSK650394 (24 hours)

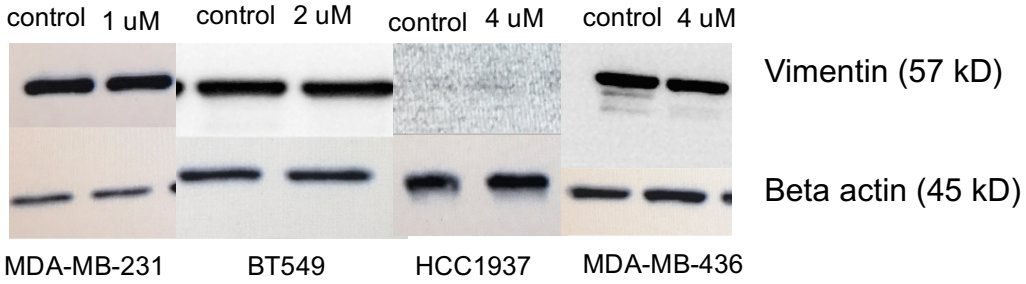


Figure 4.30. Changes in the protein levels of vimentin in response to GSK650394 in triple negative breast cancer cell lines. (n=3)

Effect Of SGK1 Inhibition Via GSK650394 On Vimentin Protein Expressions In MDA-MB-231 Cells

We did not see any change in the protein expression of vimentin in MDA-MB-231 cells after treatment with GSK650394. (Figure 4.31.)

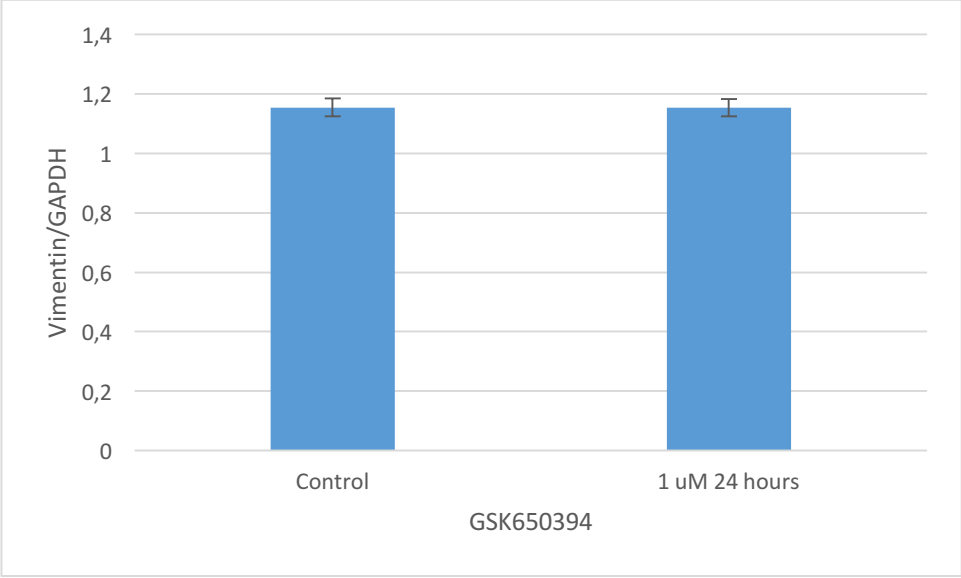


Figure 4.31. Changes in the protein levels of vimentin after treatment with GSK650394 in MDA-MB-231 cell line. (n=3, p>0.05)

Effect of SGK1 Inhibition Via GSK650394 On Vimentin Protein Expressions in BT549 Cells

We did not see any change in the protein expression of E-cadherin in BT549 cells after treatment with GSK650394. (Figure 4.32.)

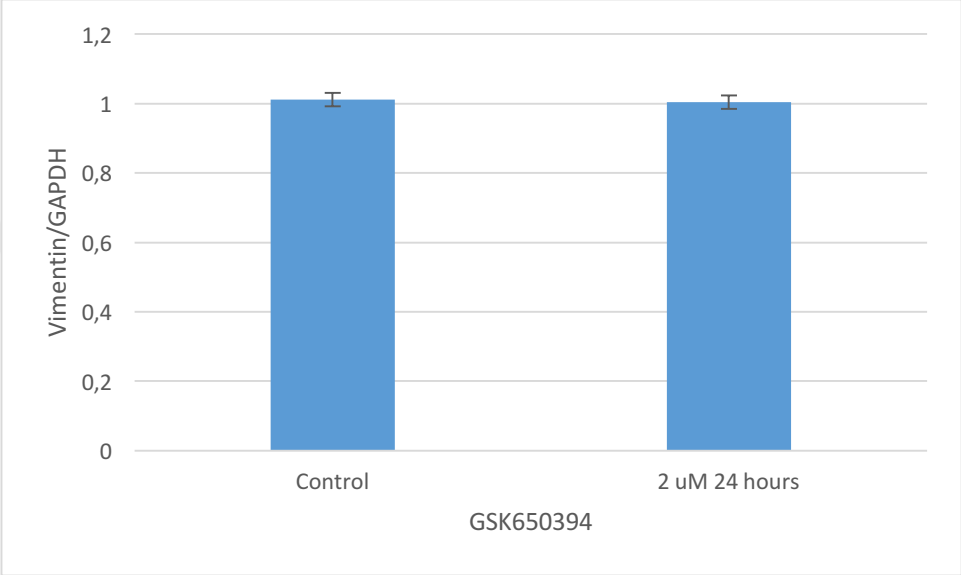


Figure 4.32. Changes in the protein levels of vimentin after treatment with GSK650394 in BT549 cell line. (n=3, p>0.05)

Effect Of SGK1 Inhibition Via GSK650394 On Vimentin Protein Expressions In MDA-MB-436 Cells

We did not see any change in the protein expression of vimentin in MDA-MB-436 cells after treatment with GSK650394. (Figure 4.33.)

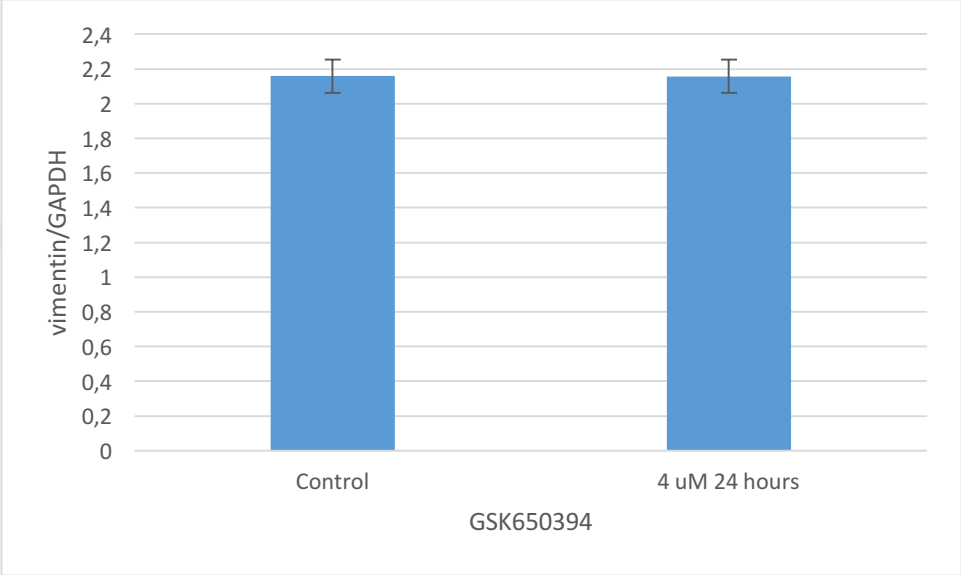


Figure 4.33. Changes in the protein levels of vimentin after treatment with GSK650394 in MDA-MB-436 cell line. (n=3, $p>0.05$)

4.8. Effect of SGK1 Inhibition Via GSK650394 On EMT Related Transcription Factors In MDA-MB-231 And BT549 Cell Lines

The effect of SGK1 inhibition on epithelial-mesenchymal transition was then further examined with Real Time PCR, showing the changes in mRNA expressions of Snail, Slug and Twist, which are EMT related transcription factors, with SGK1 inhibition. For the Real-Time PCR experiments, we used BT549 cell line, which had the most prominent expression of vimentin, thus the cell line with the most mesenchymal characteristics, and the MDA-MB-231 cell line, which was the most sensitive to GSK650394.

4.8.1. Effect of SGK1 Inhibition Via GSK650394 On EMT Related Transcription Factors In MDA-MB-231 Cells

We did not observe any effect on Snail mRNA expression after 6 and 24 hours of 1 micro molar GSK650394 incubations in MDA-MB-231 cells. (Figure 4.34.)

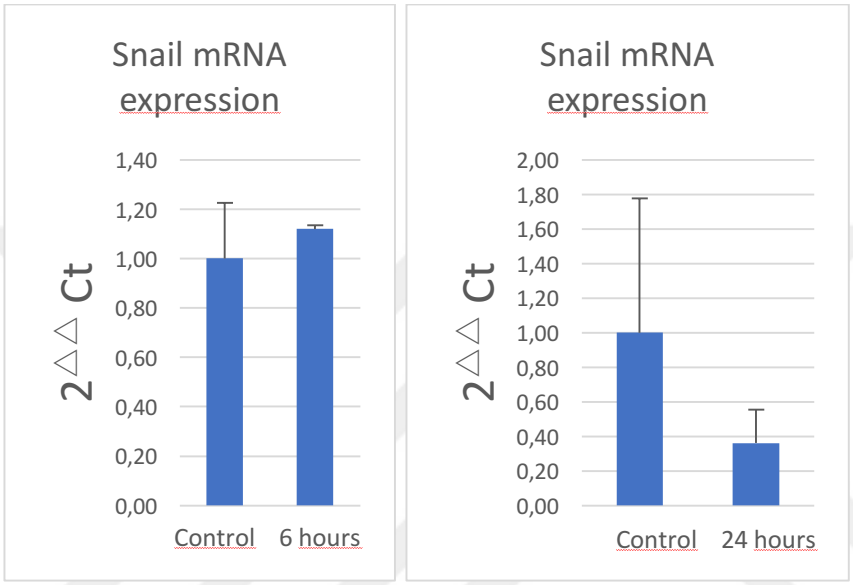


Figure 4.34. Effect of GSK650394 on Snail mRNA expression in 6 and 24 hours in MDA-MB-231 cell line. (n=3, p>0.05)

We did not observe any effect on Slug mRNA expression after 6 and 24 hours of 1 micro molar GSK650394 incubations in MDA-MB-231 cells. (Figure 4.35.)

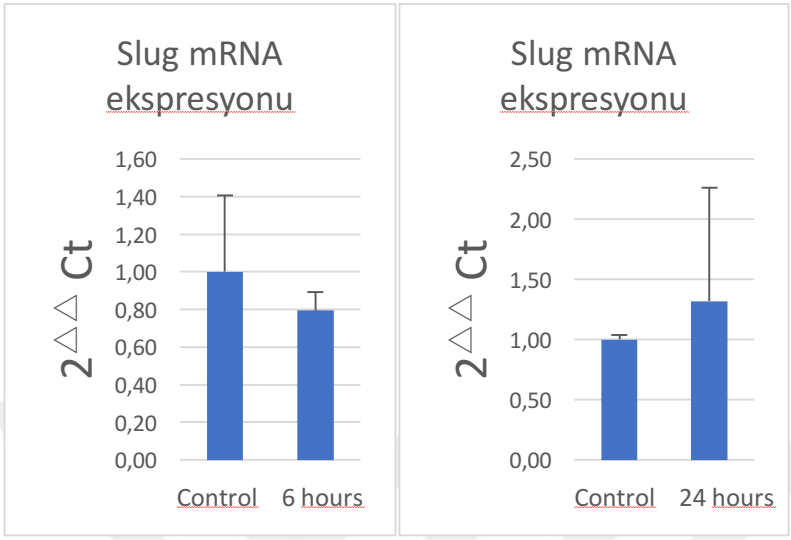


Figure 4.35. Effect of GSK650394 on Slug mRNA expression in 6 and 24 hours in MDA-MB-231 cell line. (n=3, p>0.05)

We did not observe any effect on Twist mRNA expression after 6 and 24 hours of 1 micro molar GSK650394 incubations in MDA-MB-231 cells. (Figure 4.36.)

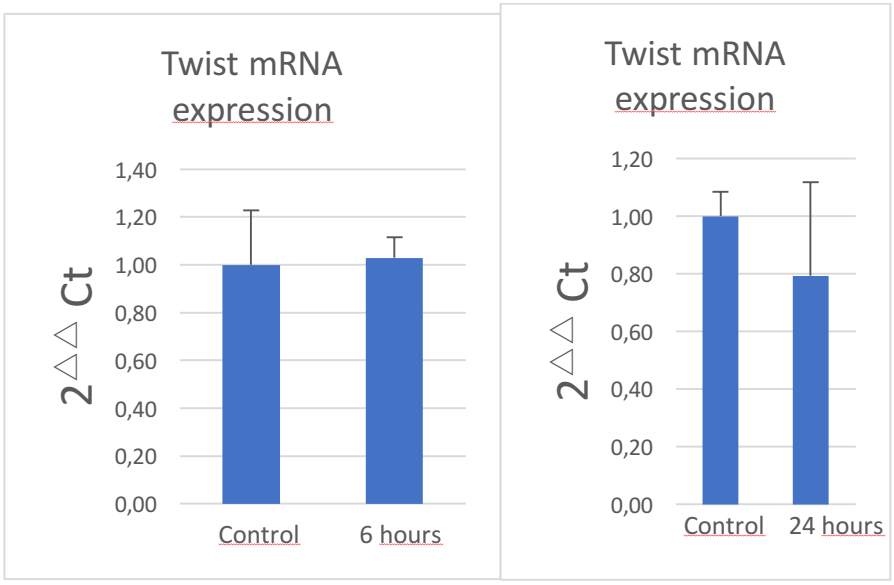


Figure 4.36. Effect of GSK650394 on Twist mRNA expression in 6 and 24 hours in MDA-MB-231 cell line. (n=3, p>0.05)

4.8.2. Effect of SGK1 Inhibition Via GSK650394 On EMT Related Transcription Factors in BT549 Cells

We did not observe any effect on Snail mRNA expression after 6 and 24 hours of 2 micro molar GSK650394 incubations in BT549 cells. (Figure 4.37)

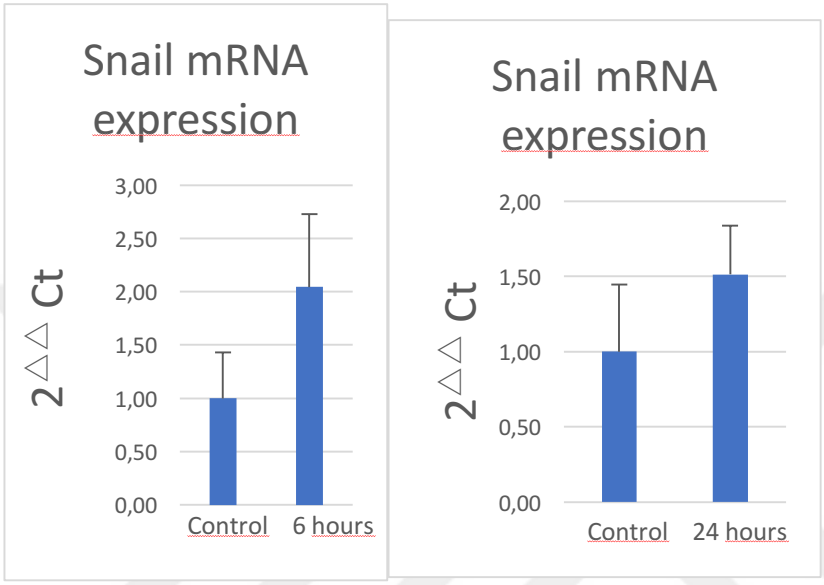


Figure 4.37. Effect of GSK650394 on Snail mRNA expression in 6 and 24 hours in BT549 cell line. (n=3, p>0.05)

We did not observe any effect on Slug mRNA expression after 6 and 24 hours of 2 micro molar GSK650394 incubations in BT549 cells. (Figure 4.38)

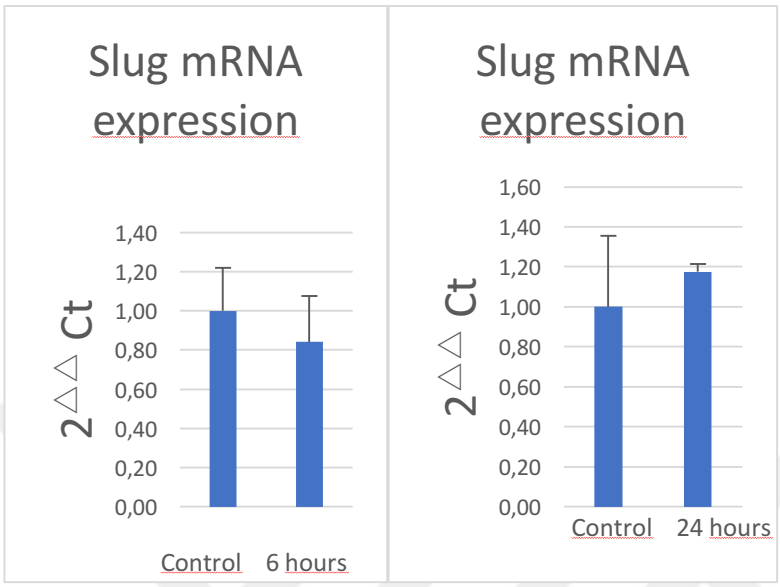


Figure 4.38. Effect of GSK650394 on Slug mRNA expression in 6 and 24 hours in BT549 cell line. (n=3, p>0.05)

We did not observe any effect on Twist mRNA expression after 6 and 24 hours of 2 micro molar GSK650394 incubations in BT549 cells. (Figure 4.39)

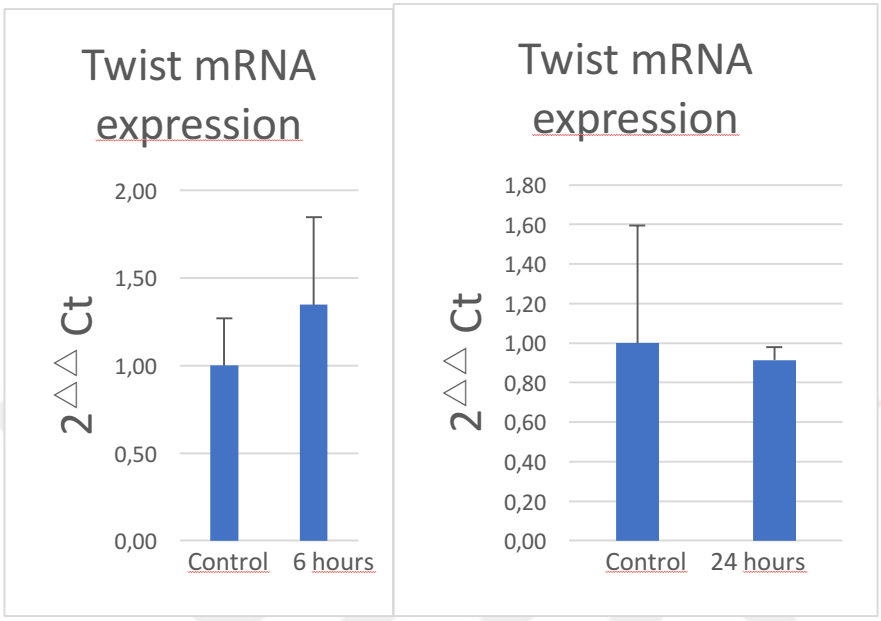


Figure 4.39. Effect of GSK650394 on Twist mRNA expression in 6 and 24 hours in BT549 cell line. (n=3, p>0.05)

5. DISCUSSION

The aim of this thesis was to determine the significance of the inhibition of SGK1 molecule in triple negative breast cancer. 5 triple negative breast cancer cell lines that differ in SGK1 expression and PTEN statuses were grown in cell culture conditions for this aim. MDA-MB-468 had a low SGK1 mRNA and protein expression according to literature and had a mutation in PTEN, we used this cell line as a negative control. MDA-MB-231 had a high SGK1 protein and mRNA expression according to literature and was PTEN wild-type. BT549, MDA-MB-436 and HCC1937 were high in SGK1 mRNA and protein expression according to literature and all of them had mutations in PTEN. In our experiments, we noticed that our cell lines also differed by their epithelial/mesenchymal phenotypes. MDA-MB-231, BT549 and MDA-MB-436 were phenotypically similar with mesenchymal cells, microscopically. However, HCC1937 was similar with epithelial cells.

MTT cytotoxic assay was used to determine the safe dose to use for further experiments. Our MTT assay results showed that we could safely use up to 4 micro molar dose of GSK650394 for our cell lines for 48 and 72 hours. This showed that GSK650394 was not cytotoxic for our cell lines up to 4 micro molar dose up to 72 hours.

SGK1 protein and mRNA expressions were consistent with literature, MDA-MB-468 had the lowest expression of SGK1 among the cell lines. The other cell lines had similar SGK1 mRNA and protein expressions.

In order to determine the doses we would use for the further experiments, we had to decide the dose that inhibited the kinase function of SGK1. So as to show the kinase function of SGK1, we used the phosphorylation of NDRG1, which was also used in previous literature to show the inhibitory function of GSK650394. Our cell lines also differed in SGK1 inhibition susceptibility. In MDA-MB-231 cell line,

consistent with the literature, we used 1 micro molar of GSK650394 for 24 hours, which was sufficient to down regulate pNDRG1 protein expression, thus inhibit SGK1 protein function. In BT549 cell line, 2 micro molar GSK650394 for 24 hours was required for the inhibition of SGK1 protein function. In HCC1937 cell line, the required dose for the inhibition of SGK1 protein function was 4 micro molar for 24 hours. For the MDA-MB-436 cell line, however, we failed to inhibit SGK1 protein function with up to 4 micro molar GSK650394 for 24 hours. In order to test this resistance to GSK650394, we compared MDA-MB-231 cell line, which was the most susceptible cell line among them, with MDA-MB-436 cell line, which we thought to be resistant to GSK650394. To test this, we prepared lysates with 1 micro molar, 2 micro molar, 4 micro molar, 8 micro molar and 16 micro molar for 6 hours. Just as expected, while we could see the gradual downregulation of pNDRG1 with the elevation of doses in MDA-MB-231, there wasn't a significant inhibition in MDA-MB-436 with up to 16 micro molar for 6 hours. We hypothesized that this resistance in MDA-MB-436, could be due to the prominent SGK1 expression of this cell line among the others. In further experiments, we used MDA-MB-436 as a resistant control.

To show the effect of GSK650394 on breast cancer cell proliferation, we used the crystal violet assay. For this experiment, we used the MDA-MB-231 cell line, which was the most susceptible cell line among the others. Consistent with the literature, we showed a dramatic decrease in proliferation when we used 1 micro molar and 2 micro molar of GSK650394 for 5 days. Dependency of the proliferation of triple negative breast cancer cells to SGK1, shows that SGK1 is an important target for inhibition in triple negative breast cancer. (11) It also shows that the negative effect of dexamethasone on the prognosis of patients can be due to SGK1.

To show the effect of GSK650394 on cell cycle, we used the flow cytometry based cell cycle assay. For this experiment, we used the MDA-MB-231 cell line, which was the most susceptible cell line among the others. Consistent with the literature, we showed that there was a decrease in the cells that were in S phase, which showed

that SGK1 had an effect on cell cycle continuation, and prevented the triple negative breast cancer cells from going through with the cell cycle, and proliferating. Effects of SGK1 on cell cycle is important for triple negative breast cancer.

It was a surprising result that GSK3 beta phosphorylation wasn't affected by inhibition of SGK1, since previous literature showed that SGK1 phosphorylated and inhibited GSK3 beta protein, we expected SGK1 inhibition would lead to the downregulation of phospho-GSK3 beta, and thus increase in its activation. (99) We can explain this effect with the significance of Wnt-beta catenin pathway in triple negative breast cancer cells. Wnt-beta catenin pathway has been shown to regulate GSK3 beta by phosphorylation and inhibition, and it is a very potent pathway for triple negative breast cancer. (97) PI3K/Akt pathway is also responsible for phosphorylating and inhibiting GSK3 beta, and it is also a prominent pathway in triple negative breast cancer. (100) Another explanation for the lack of effect can be the used low dose of GSK650394. But this dose was critical for us, considering the probable nonspecific kinase inhibitions when we increased the dose. When we specifically inhibited SGK1 with the doses that we used, we did not see any effect on phospho-GSK3.

We showed that inhibition of SGK1 in the chosen triple negative breast cancer cell lines, did not affect epithelial-mesenchymal transition markers. This could be due to the potent mesenchymal phenotype of the triple negative breast cancer cells. The cell lines that we chose (except HCC1937) had a very potent vimentin expression, and almost a nonexistent E-cadherin expression. This mesenchymal phenotype can probably be difficult to inhibit with the inhibition of one protein expression. Also, epithelial-mesenchymal transition in triple negative breast cancer cells has been proven to be affected by many different signaling pathways, among them are PI3K/Akt Pathway and Wnt-beta catenin pathway, which are important signaling pathways in triple negative breast cancer. (101)

Lack of inhibition on Snail, Slug and Twist transcription factors was not a surprising result, considering we did not see any effect on epithelial-mesenchymal transition proteins. There was also no effect with SGK1 inhibition on the mRNA levels of epithelial-mesenchymal transition related transcription factors.

In a most recent study conducted by Liu et al, inhibition of SGK1 was shown to reverse epithelial-mesenchymal transition and impair metastasis in prostate cancer cells in vitro and in vivo. (102) In this study, GSK650394 was used with a 40 micro molar dose for 24 and 48 hours. This study shows that use of GSK650394 with a higher dose, can affect the phenotype differently. Although this study shows that the results can differ if we also use GSK650394 with a higher dose, it is important not to forget that once we increase the dose, there is a risk of inhibiting different signaling pathways, most importantly Akt pathway. It has already been shown that Akt is an important regulator of epithelial-mesenchymal transition, which means that with non-specific inhibition of Akt, it is probable that epithelial-mesenchymal transition would be affected with higher doses of GSK650394. Also, in this study researchers also failed to show the downregulation of vimentin and upregulation of E-cadherin with only pharmacological inhibition, but it was shown via knockdown of SGK1 with shRNA. Taking this study into focus, we can presume that knockdown of SGK1 can also be effective in regulating epithelial-mesenchymal transition in triple negative breast cancer cells. This may be an important topic for a future research, along with the use of high dose GSK650394.

Effect of glucocorticoids on breast cancer cells is still a key novel research topic. Glucocorticoids are proven to induce chemotherapy resistance and inhibition of apoptosis, and increase the proliferation of triple negative breast cancer cells. But their effect on metastasis and epithelial-mesenchymal transition is still yet to be elucidated. A recent study on A549 cells showed that glucocorticoids inhibited EMT, which is the opposite of what we expected in triple negative breast cancer cells. (103) Effect of glucocorticoids on invasive behavior of cells, EMT and metastasis can be

different in different cell types, as the effect of glucocorticoids on proliferation and chemotherapy sensitivity of breast cancers is also context dependent. (3) It is necessary to conduct experiments with dexamethasone and mifepristone in order to find the direct effect of glucocorticoids on EMT on triple negative breast cancer cells. It can also be required to conduct in vivo experiments with glucocorticoids and SGK1 inhibitors to see the effects on metastasis.

All in all, this study showed that SGK1 can be an important target for inhibition in triple negative breast cancer.

6. CONCLUSION AND RECOMMENDATIONS

1. MDA-MB-231, MDA-MB-468, BT549, HCC1937 and MDA-MB-436 cell lines were cultured.
2. SGK1 mRNA and protein expressions of these cell lines were determined.
3. It was shown that 0,5 micro molar, 1 micro molar, 2 micro molar and 4 micro molar doses of GSK650394 was not cytotoxic for these cell lines in 48 and 72 hours.
4. Inhibitory doses of these cell lines were determined.
   MDA-MB-231: 1 micro molar, 24 hours
   BT549: 2 micro molar, 24 hours
   HCC1937: 4 micro molar, 24 hours
5. It was shown that the expression of phospho-GSK3 beta was not changed with the inhibition of SGK1.
6. It was shown that the protein expression of epithelial and mesenchymal markers, thus EMT was not changed with the inhibition of SGK1.
7. It was shown that the mRNA expression of EMT related transcription factors was not changed with the inhibition of SGK1.
8. It may be useful to conduct experiments with dexamethasone and mifepristone in order to see the direct effect of glucocorticoids on triple negative breast cancer in this context.
9. It can be beneficial to use shRNA to show the direct and specific effect of SGK1 on triple negative breast cancer.
10. The effect of GSK650394 with a higher dose on triple negative breast cancer cell lines can be investigated.

7. REFERENCES

1. Macias H, Hinck L. Mammary gland development. Wiley Interdiscip Rev Dev Biol. 2012;1(4):533-57.
2. Sandhu R, Parker JS, Jones WD, Livasy CA, Coleman WB. Microarray-Based Gene Expression Profiling for Molecular Classification of Breast Cancer and Identification of New Targets for Therapy. Laboratory Medicine. 2010;41(6):364-72.
3. Kach J, Conzen SD, Szmulewitz RZ. Targeting the glucocorticoid receptor in breast and prostate cancers. Sci Transl Med. 2015;7(305):305ps19.
4. Reeder A, Attar M, Nazario L, Bathula C, Zhang A, Hochbaum D, et al. Stress hormones reduce the efficacy of paclitaxel in triple negative breast cancer through induction of DNA damage. Br J Cancer. 2015;112(9):1461-70.
5. Garcia-Martinez JM, Alessi DR. mTOR complex 2 (mTORC2) controls hydrophobic motif phosphorylation and activation of serum- and glucocorticoid-induced protein kinase 1 (SGK1). Biochem J. 2008;416(3):375-85.
6. Polyak K, Weinberg RA. Transitions between epithelial and mesenchymal states: acquisition of malignant and stem cell traits. Nature Reviews Cancer. 2009;9:265.
7. Siegel RL, Miller KD, Jemal A. Cancer statistics, 2018. CA Cancer J Clin. 2018;68(1):7-30.
8. Bianchini G, Balko JM, Mayer IA, Sanders ME, Gianni L. Triple-negative breast cancer: challenges and opportunities of a heterogeneous disease. Nat Rev Clin Oncol. 2016;13(11):674-90.
9. Skor MN, Wonder EL, Kocherginsky M, Goyal A, Hall BA, Cai Y, et al. Glucocorticoid receptor antagonism as a novel therapy for triple-negative breast cancer. Clin Cancer Res. 2013;19(22):6163-72.
10. Pearce LR, Komander D, Alessi DR. The nuts and bolts of AGC protein kinases. Nat Rev Mol Cell Biol. 2010;11(1):9-22.
11. Sommer EM, Dry H, Cross D, Guichard S, Davies BR, Alessi DR. Elevated SGK1 predicts resistance of breast cancer cells to Akt inhibitors. Biochem J. 2013;452(3):499-508.
12. Du J, Yang M, Chen S, Li D, Chang Z, Dong Z. PDK1 promotes tumor growth and metastasis in a spontaneous breast cancer model. Oncogene. 2016;35(25):3314-23.
13. Pan D, Kocherginsky M, Conzen SD. Activation of the glucocorticoid receptor is associated with poor prognosis in estrogen receptor-negative breast cancer. Cancer Res. 2011;71(20):6360-70.
14. Murray JT, Campbell DG, Morrice N, Auld GC, Shpiro N, Marquez R, et al. Exploitation of KESTREL to identify NDRG family members as physiological substrates for SGK1 and GSK3. Biochem J. 2004;384(Pt 3):477-88.
15. Inman JL, Robertson C, Mott JD, Bissell MJ. Mammary gland development: cell fate specification, stem cells and the microenvironment. Development. 2015;142(6):1028-42.

16. Lanigan F, O'Connor D, Martin F, Gallagher WM. Molecular links between mammary gland development and breast cancer. Cell Mol Life Sci. 2007;64(24):3159-84.
17. Burstein HJ, Temin S, Anderson H, Buchholz TA, Davidson NE, Gelmon KE, et al. Adjuvant endocrine therapy for women with hormone receptor-positive breast cancer: american society of clinical oncology clinical practice guideline focused update. J Clin Oncol. 2014;32(21):2255-69.
18. Baselga J, Norton L, Albanell J, Kim YM, Mendelsohn J. Recombinant humanized anti-HER2 antibody (Herceptin) enhances the antitumor activity of paclitaxel and doxorubicin against HER2/neu overexpressing human breast cancer xenografts. Cancer Res. 1998;58(13):2825-31.
19. Goldenberg MM. Trastuzumab, a recombinant DNA-derived humanized monoclonal antibody, a novel agent for the treatment of metastatic breast cancer. Clin Ther. 1999;21(2):309-18.
20. Pearson G, Robinson F, Beers Gibson T, Xu BE, Karandikar M, Berman K, et al. Mitogen-activated protein (MAP) kinase pathways: regulation and physiological functions. Endocr Rev. 2001;22(2):153-83.
21. Krishna M, Narang H. The complexity of mitogen-activated protein kinases (MAPKs) made simple. Cell Mol Life Sci. 2008;65(22):3525-44.
22. Whyte J, Bergin O, Bianchi A, McNally S, Martin F. Key signalling nodes in mammary gland development and cancer. Mitogen-activated protein kinase signalling in experimental models of breast cancer progression and in mammary gland development. Breast Cancer Res. 2009;11(5):209.
23. Cantley LC. The phosphoinositide 3-kinase pathway. Science. 2002;296(5573):1655-7.
24. Zhao L, Vogt PK. Class I PI3K in oncogenic cellular transformation. Oncogene. 2008;27(41):5486-96.
25. Guerrero-Zotano A, Mayer IA, Arteaga CL. PI3K/AKT/mTOR: role in breast cancer progression, drug resistance, and treatment. Cancer Metastasis Rev. 2016;35(4):515-24.
26. Locatelli M, Curigliano G. Notch inhibitors and their role in the treatment of triple negative breast cancer: promises and failures. Curr Opin Oncol. 2017;29(6):411-27.
27. Rangel MC, Bertolette D, Castro NP, Klauzinska M, Cuttitta F, Salomon DS. Developmental signaling pathways regulating mammary stem cells and contributing to the etiology of triple-negative breast cancer. Breast Cancer Res Treat. 2016;156(2):211-26.
28. Dey N, Barwick BG, Moreno CS, Ordanic-Kodani M, Chen Z, Oprea-Ilies G, et al. Wnt signaling in triple negative breast cancer is associated with metastasis. BMC Cancer. 2013;13:537.
29. Chen Y, Olopade OI. MYC in breast tumor progression. Expert Rev Anticancer Ther. 2008;8(10):1689-98.
30. Hynes NE, Stoelzle T. Key signalling nodes in mammary gland development and cancer: Myc. Breast Cancer Res. 2009;11(5):210.

31. Arencibia JM, Pastor-Flores D, Bauer AF, Schulze JO, Biondi RM. AGC protein kinases: from structural mechanism of regulation to allosteric drug development for the treatment of human diseases. Biochim Biophys Acta. 2013;1834(7):1302-21.
32. Bayascas JR. PDK1: the major transducer of PI 3-kinase actions. Curr Top Microbiol Immunol. 2010;346:9-29.
33. Yang C, Huang X, Liu H, Xiao F, Wei J, You L, et al. PDK1 inhibitor GSK2334470 exerts antitumor activity in multiple myeloma and forms a novel multitargeted combination with dual mTORC1/C2 inhibitor PP242. Oncotarget. 2017;8(24):39185-97.
34. Currie RA, Walker KS, Gray A, Deak M, Casamayor A, Downes CP, et al. Role of phosphatidylinositol 3,4,5-trisphosphate in regulating the activity and localization of 3-phosphoinositide-dependent protein kinase-1. Biochem J. 1999;337 (Pt 3):575-83.
35. Hers I, Vincent EE, Tavare JM. Akt signalling in health and disease. Cell Signal. 2011;23(10):1515-27.
36. Anjum R, Blenis J. The RSK family of kinases: emerging roles in cellular signalling. Nat Rev Mol Cell Biol. 2008;9(10):747-58.
37. Dalby KN, Morrice N, Caudwell FB, Avruch J, Cohen P. Identification of regulatory phosphorylation sites in mitogen-activated protein kinase (MAPK)-activated protein kinase-1a/p90rsk that are inducible by MAPK. J Biol Chem. 1998;273(3):1496-505.
38. Taylor SS, Buechler JA, Yonemoto W. cAMP-dependent protein kinase: framework for a diverse family of regulatory enzymes. Annu Rev Biochem. 1990;59:971-1005.
39. Lucas KA, Pitari GM, Kazerounian S, Ruiz-Stewart I, Park J, Schulz S, et al. Guanylyl cyclases and signaling by cyclic GMP. Pharmacol Rev. 2000;52(3):375-414.
40. Ikenoue T, Inoki K, Yang Q, Zhou X, Guan KL. Essential function of TORC2 in PKC and Akt turn motif phosphorylation, maturation and signalling. EMBO J. 2008;27(14):1919-31.
41. Carvajal RD, Tse A, Schwartz GK. Aurora kinases: new targets for cancer therapy. Clin Cancer Res. 2006;12(23):6869-75.
42. Penela P, Ribas C, Mayor F, Jr. Mechanisms of regulation of the expression and function of G protein-coupled receptor kinases. Cell Signal. 2003;15(11):973-81.
43. Cornils H, Kohler RS, Hergovich A, Hemmings BA. Downstream of human NDR kinases: impacting on c-myc and p21 protein stability to control cell cycle progression. Cell Cycle. 2011;10(12):1897-904.
44. Street CA, Bryan BA. Rho kinase proteins--pleiotropic modulators of cell survival and apoptosis. Anticancer Res. 2011;31(11):3645-57.
45. Garland P, Quraishe S, French P, O'Connor V. Expression of the MAST family of serine/threonine kinases. Brain Res. 2008;1195:12-9.
46. Foulkes WD, Smith IE, Reis-Filho JS. Triple-negative breast cancer. N Engl J Med. 2010;363(20):1938-48.
47. Dent R, Hanna WM, Trudeau M, Rawlinson E, Sun P, Narod SA. Pattern of metastatic spread in triple-negative breast cancer. Breast Cancer Res Treat. 2009;115(2):423-8.

48. Foulkes WD, Stefansson IM, Chappuis PO, Begin LR, Goffin JR, Wong N, et al. Germline BRCA1 mutations and a basal epithelial phenotype in breast cancer. J Natl Cancer Inst. 2003;95(19):1482-5.
49. Cossu-Rocca P, Orru S, Muroni MR, Sanges F, Sotgiu G, Ena S, et al. Analysis of PIK3CA Mutations and Activation Pathways in Triple Negative Breast Cancer. PLoS One. 2015;10(11):e0141763.
50. Chin YR, Yoshida T, Marusyk A, Beck AH, Polyak K, Toker A. Targeting Akt3 signaling in triple-negative breast cancer. Cancer Res. 2014;74(3):964-73.
51. Reed DE, Shokat KM. INPP4B and PTEN Loss Leads to PI-3,4-P2 Accumulation and Inhibition of PI3K in TNBC. Mol Cancer Res. 2017;15(6):765-75.
52. Sharpe R, Pearson A, Herrera-Abreu MT, Johnson D, Mackay A, Welti JC, et al. FGFR signaling promotes the growth of triple-negative and basal-like breast cancer cell lines both in vitro and in vivo. Clin Cancer Res. 2011;17(16):5275-86.
53. Ueno NT, Zhang D. Targeting EGFR in Triple Negative Breast Cancer. J Cancer. 2011;2:324-8.
54. Tilch E, Seidens T, Cocciardi S, Reid LE, Byrne D, Simpson PT, et al. Mutations in EGFR, BRAF and RAS are rare in triple-negative and basal-like breast cancers from Caucasian women. Breast Cancer Res Treat. 2014;143(2):385-92.
55. Giltnane JM, Balko JM. Rationale for targeting the Ras/MAPK pathway in triple-negative breast cancer. Discov Med. 2014;17(95):275-83.
56. Knudsen ES, McClendon AK, Franco J, Ertel A, Fortina P, Witkiewicz AK. RB loss contributes to aggressive tumor phenotypes in MYC-driven triple negative breast cancer. Cell Cycle. 2015;14(1):109-22.
57. Tadesse S, Yu M, Kumarasiri M, Le BT, Wang S. Targeting CDK6 in cancer: State of the art and new insights. Cell Cycle. 2015;14(20):3220-30.
58. Ortiz AB, Garcia D, Vicente Y, Palka M, Bellas C, Martin P. Prognostic significance of cyclin D1 protein expression and gene amplification in invasive breast carcinoma. PLoS One. 2017;12(11):e0188068.
59. Balko JM, Giltnane JM, Wang K, Schwarz LJ, Young CD, Cook RS, et al. Molecular profiling of the residual disease of triple-negative breast cancers after neoadjuvant chemotherapy identifies actionable therapeutic targets. Cancer Discov. 2014;4(2):232-45.
60. Balko JM, Schwarz LJ, Luo N, Estrada MV, Giltnane JM, Davila-Gonzalez D, et al. Triple-negative breast cancers with amplification of JAK2 at the 9p24 locus demonstrate JAK2-specific dependence. Sci Transl Med. 2016;8(334):334ra53.
61. Rampurwala M, Wisinski KB, O'Regan R. Role of the androgen receptor in triple-negative breast cancer. Clin Adv Hematol Oncol. 2016;14(3):186-93.
62. Ebelt ND, Kaoud TS, Edupuganti R, Van Ravenstein S, Dalby KN, Van Den Berg CL. A c-Jun N-terminal kinase inhibitor, JNK-IN-8, sensitizes triple negative breast cancer cells to lapatinib. Oncotarget. 2017;8(62):104894-912.
63. Chaturvedi P, Gilkes DM, Takano N, Semenza GL. Hypoxia-inducible factor-dependent signaling between triple-negative breast cancer cells and mesenchymal stem cells promotes macrophage recruitment. Proc Natl Acad Sci U S A. 2014;111(20):E2120-9.

64. Tomao F, Papa A, Zaccarelli E, Rossi L, Caruso D, Minozzi M, et al. Triple-negative breast cancer: new perspectives for targeted therapies. Onco Targets Ther. 2015;8:177-93.
65. Nakai K, Hung MC, Yamaguchi H. A perspective on anti-EGFR therapies targeting triple-negative breast cancer. Am J Cancer Res. 2016;6(8):1609-23.
66. Fouque A, Jean M, Weghe P, Legembre P. Review of PI3K/mTOR Inhibitors Entering Clinical Trials to Treat Triple Negative Breast Cancers. Recent Pat Anticancer Drug Discov. 2016;11(3):283-96.
67. Geenen JJJ, Linn SC, Beijnen JH, Schellens JHM. PARP Inhibitors in the Treatment of Triple-Negative Breast Cancer. Clin Pharmacokinet. 2018;57(4):427-37.
68. Fedele P, Orlando L, Cinieri S. Targeting triple negative breast cancer with histone deacetylase inhibitors. Expert Opin Investig Drugs. 2017;26(11):1199-206.
69. Atkinson RL, Yang WT, Rosen DG, Landis MD, Wong H, Lewis MT, et al. Cancer stem cell markers are enriched in normal tissue adjacent to triple negative breast cancer and inversely correlated with DNA repair deficiency. Breast Cancer Res. 2013;15(5):R77.
70. Visvader JE, Lindeman GJ. Cancer stem cells in solid tumours: accumulating evidence and unresolved questions. Nat Rev Cancer. 2008;8(10):755-68.
71. Koury J, Zhong L, Hao J. Targeting Signaling Pathways in Cancer Stem Cells for Cancer Treatment. Stem Cells Int. 2017;2017:2925869.
72. Abduljabbar R, Negm OH, Lai CF, Jerjees DA, Al-Kaabi M, Hamed MR, et al. Clinical and biological significance of glucocorticoid receptor (GR) expression in breast cancer. Breast Cancer Res Treat. 2015;150(2):335-46.
73. Hermes GL, Delgado B, Tretiakova M, Cavigelli SA, Krausz T, Conzen SD, et al. Social isolation dysregulates endocrine and behavioral stress while increasing malignant burden of spontaneous mammary tumors. Proc Natl Acad Sci U S A. 2009;106(52):22393-8.
74. Yu S, Yan C, Yang X, He S, Liu J, Qin C, et al. Pharmacoproteomic analysis reveals that metapristone (RU486 metabolite) intervenes E-cadherin and vimentin to realize cancer metastasis chemoprevention. Sci Rep. 2016;6:22388.
75. Wan L, Dong H, Xu H, Ma J, Zhu Y, Lu Y, et al. Aspirin, lysine, mifepristone and doxycycline combined can effectively and safely prevent and treat cancer metastasis: prevent seeds from gemmating on soil. Oncotarget. 2015;6(34):35157-72.
76. Liu R, Shi P, Nie Z, Liang H, Zhou Z, Chen W, et al. Mifepristone Suppresses Basal Triple-Negative Breast Cancer Stem Cells by Down-regulating KLF5 Expression. Theranostics. 2016;6(4):533-44.
77. Webster MK, Goya L, Ge Y, Maiyar AC, Firestone GL. Characterization of sgk, a novel member of the serine/threonine protein kinase gene family which is transcriptionally induced by glucocorticoids and serum. Mol Cell Biol. 1993;13(4):2031-40.
78. Leong ML, Maiyar AC, Kim B, O'Keeffe BA, Firestone GL. Expression of the serum- and glucocorticoid-inducible protein kinase, Sgk, is a cell survival response

to multiple types of environmental stress stimuli in mammary epithelial cells. J Biol Chem. 2003;278(8):5871-82.
79. Wu W, Zou M, Brickley DR, Pew T, Conzen SD. Glucocorticoid receptor activation signals through forkhead transcription factor 3a in breast cancer cells. Mol Endocrinol. 2006;20(10):2304-14.
80. Sahoo S, Brickley DR, Kocherginsky M, Conzen SD. Coordinate expression of the PI3-kinase downstream effectors serum and glucocorticoid-induced kinase (SGK-1) and Akt-1 in human breast cancer. Eur J Cancer. 2005;41(17):2754-9.
81. Talarico C, Dattilo V, D'Antona L, Menniti M, Bianco C, Ortuso F, et al. SGK1, the New Player in the Game of Resistance: Chemo-Radio Molecular Target and Strategy for Inhibition. Cell Physiol Biochem. 2016;39(5):1863-76.
82. Amato R, D'Antona L, Porciatti G, Agosti V, Menniti M, Rinaldo C, et al. Sgk1 activates MDM2-dependent p53 degradation and affects cell proliferation, survival, and differentiation. J Mol Med (Berl). 2009;87(12):1221-39.
83. Brunet A, Park J, Tran H, Hu LS, Hemmings BA, Greenberg ME. Protein kinase SGK mediates survival signals by phosphorylating the forkhead transcription factor FKHRL1 (FOXO3a). Mol Cell Biol. 2001;21(3):952-65.
84. Fang BA, Kovacevic Z, Park KC, Kalinowski DS, Jansson PJ, Lane DJ, et al. Molecular functions of the iron-regulated metastasis suppressor, NDRG1, and its potential as a molecular target for cancer therapy. Biochim Biophys Acta. 2014;1845(1):1-19.
85. Sun J, Zhang D, Bae DH, Sahni S, Jansson P, Zheng Y, et al. Metastasis suppressor, NDRG1, mediates its activity through signaling pathways and molecular motors. Carcinogenesis. 2013;34(9):1943-54.
86. Zhong W, Oguljahan B, Xiao Y, Nelson J, Hernandez L, Garcia-Barrio M, et al. Serum and glucocorticoid-regulated kinase 1 promotes vascular smooth muscle cell proliferation via regulation of beta-catenin dynamics. Cell Signal. 2014;26(12):2765-72.
87. Kalluri R, Weinberg RA. The basics of epithelial-mesenchymal transition. J Clin Invest. 2009;119(6):1420-8.
88. Lamouille S, Xu J, Derynck R. Molecular mechanisms of epithelial-mesenchymal transition. Nat Rev Mol Cell Biol. 2014;15(3):178-96.
89. Wang Y, Shi J, Chai K, Ying X, Zhou BP. The Role of Snail in EMT and Tumorigenesis. Curr Cancer Drug Targets. 2013;13(9):963-72.
90. Gonzalez DM, Medici D. Signaling mechanisms of the epithelial-mesenchymal transition. Sci Signal. 2014;7(344):re8.
91. Nieto MA, Huang RY, Jackson RA, Thiery JP. Emt: 2016. Cell. 2016;166(1):21-45.
92. Zhou BP, Deng J, Xia W, Xu J, Li YM, Gunduz M, et al. Dual regulation of Snail by GSK-3beta-mediated phosphorylation in control of epithelial-mesenchymal transition. Nat Cell Biol. 2004;6(10):931-40.
93. Yang J, Mani SA, Donaher JL, Ramaswamy S, Itzykson RA, Come C, et al. Twist, a master regulator of morphogenesis, plays an essential role in tumor metastasis. Cell. 2004;117(7):927-39.

94. Zhang YE. Non-Smad pathways in TGF-beta signaling. Cell Res. 2009;19(1):128-39.
95. Lotz-Jenne C, Luthi U, Ackerknecht S, Lehembre F, Fink T, Stritt M, et al. A high-content EMT screen identifies multiple receptor tyrosine kinase inhibitors with activity on TGFbeta receptor. Oncotarget. 2016;7(18):25983-6002.
96. Wang Z, Li Y, Kong D, Sarkar FH. The role of Notch signaling pathway in epithelial-mesenchymal transition (EMT) during development and tumor aggressiveness. Curr Drug Targets. 2010;11(6):745-51.
97. Yook JI, Li XY, Ota I, Hu C, Kim HS, Kim NH, et al. A Wnt-Axin2-GSK3beta cascade regulates Snail1 activity in breast cancer cells. Nat Cell Biol. 2006;8(12):1398-406.
98. Voutsadakis IA. Epithelial-Mesenchymal Transition (EMT) and Regulation of EMT Factors by Steroid Nuclear Receptors in Breast Cancer: A Review and in Silico Investigation. J Clin Med. 2016;5(1).
99. Xiaobo Y, Qiang L, Xiong Q, Zheng R, Jianhua Z, Zhifeng L, et al. Serum and glucocorticoid kinase 1 promoted the growth and migration of non-small cell lung cancer cells. Gene. 2016;576(1 Pt 2):339-46.
100. Wickenden JA, Watson CJ. Key signalling nodes in mammary gland development and cancer. Signalling downstream of PI3 kinase in mammary epithelium: a play in 3 Akts. Breast Cancer Res. 2010;12(2):202.
101. Xu W, Yang Z, Lu N. A new role for the PI3K/Akt signaling pathway in the epithelial-mesenchymal transition. Cell Adh Migr. 2015;9(4):317-24.
102. Liu W, Wang X, Wang Y, Dai Y, Xie Y, Ping Y, et al. SGK1 inhibition-induced autophagy impairs prostate cancer metastasis by reversing EMT. J Exp Clin Cancer Res. 2018;37(1):73.
103. Yang HW, Lee SA, Shin JM, Park IH, Lee HM. Glucocorticoids ameliorate TGF-beta1-mediated epithelial-to-mesenchymal transition of airway epithelium through MAPK and Snail/Slug signaling pathways. Sci Rep. 2017;7(1):3486.

SUPP.1. APPROVAL OF THE ETHICS COMMITTEE

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

Sayı : 16969557 – 1024
Konu :

25.10.2016

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

Sayın Prof. Dr. DOĞAN,

Kurulumuza değerlendirilmek üzere sunduğunuz GO 16/665 kayıt numaralı ve ***"Triple Negatif Meme Kanserinde Serum Ve Glukokortikoid ile İndüklenebilen Kinaz 1'in (SGK1) İnhibisyonu"*** başlıklı proje Kurulumuzun 25.10.2016 tarihli toplantısında değerlendirilmiş olup, projenin 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. Sevda F. MÜFTÜOĞLU
Başkan

Eki: Toplantı katılım tutanağı.

SUPP.2. ABSTRACTS DERIVED FROM THIS THESIS

TEOD

1 TEMEL ONKOLOJİ SEMPOZYUMU

Tümör Biyolojisi ve Genetiği

09-11 Mayıs 2018
Dokuz Eylül Üniversitesi
Kurucu Öğretim Üyeleri
Konferans Salonu, İZMİR

SÖZLÜ BİLDİRİLER

Sözlü Bildiriler 2 / Salon 2 / 11 Mayıs 2018, Cuma / 08.30-10.30 Oturum Başkanları: Prof. Dr. Kemal Ergin, Yrd. Doç. Dr. Rüksan Çehreli		
SS-10	BİREYSELLEŞTİRİLMİŞ TIP ARAŞTIRMALARINDA IN VİTRO MODEL OLARAK ORGANOİDLER	**Burçin BARAN**, Gizem ÇALIBAŞI KOÇAL, Ender ELLİDOKUZ, Yasemin BASKIN, Hülya ELLİDOKUZ
SS-11	KANSERDE IFADESİ DEĞİŞEN GENLERİN METABOLİT AĞI AÇISINDAN DEĞERLENDİRİLMESİ	**Emine Ravza ÖZTÜRK**, Alper YILMAZ
SS-12	MEME KANSERİ MODELİNDE ENDOTELYAL DİSFONKSİYONUN ETKİLERİ	**Buğra ELKAR**, Mustafa BARIŞ, Meryem ÇALIŞIR, Safiye AKTAŞ, Yasemin ÇAKIR, Mustafa SEÇİL, Osman YILMAZ, Zekiye ALTUN
SS-13	NÖROBLASTOM DENEY HAYVANI MODELİNDE MEZENKİMAL KÖK HÜCRE VE SİSPLATİN ETKİLEŞİMİNİN DEĞERLENDİRİLMESİ	Safiye AKTAŞ, **Ayşe Pınar ERÇETİN ÖZDEMİR**, Melek AYDIN, Meryem ÇALIŞIR, Ömer BEKÇİOĞLU, Osman YILMAZ, Nur OLGUN
SS-14	WİTHAFERİN-A: KÜÇÜK HÜCRELİ DIŞI AKCİĞER KANSERİ VE KÖK HÜCRELERİNDE ADAY ANTİ-TÜMÖR DOĞAL BİLEŞEN	Ayşe Pınar Erçetin ÖZDEMİR, **Melek AYDIN**, Emine GENÇKURT, Yağmur KAFALI, Sibel HIDIR, Safiye AKTAŞ
SS-15	KÜÇÜK HÜCRELİ DIŞI AKCİĞER KANSERİ TANILI HASTALARA AİT PARAFİN BLOK KESİTLERİNDE EGFR, KRAS, BRAF, PIK3CA, HER2 VE NRAS GEN MUTASYONLARININ ARAŞTIRILMASI	**Metin ÇALIŞKAN**, Gökay BOZKURT, Nezih MEYDAN, İbrahim METEOĞLU, Nur SELVİ GÜNEL
SS-16	MEME KANSERİNDE SERUM VE GLUKOKORTİKOİD İLE İNDÜKLENEBİLEN KİNAZ 1'İN (SGK1) İNHİBİSYONU	**Elif HAZNEDAROĞLU**, Hande CANPINAR, Ayşe Lale DOĞAN
SS-17	KC7F2 VE TEMOZOLOMİDE ETKEN MADDELERİNİN U87-MG GLİOMA HÜCRE MODELİ ÜZERİNDEKİ SİTOTOKSİK VE APOPTOTİK ETKİLERİ	**Zaka ABBASZADE**, Nur Selvi GÜNEL, Çağr BİRAY AVCI
SS-18	GAMMA-TOKOTRİENOL & DOXORUBİCİN KOMBİNASYON TEDAVİSİNİN OLASI TERAPATİK ETKİLERİNİN KARACİĞER KANSERİ HÜCRE HATTINDA ARAŞTIRILMASI	**Akın SEVİNÇ**, İpek ÖZYURT, Feride ZORLU SEVERCAN

www.temelonkolojisempozyumu.org

DS.1 **11:30 -11:45**
Meme Kanserinde Serum ve Glukokortikoid ile İndüklenebilen Kinaz 1'in (SGK1) İnhibisyonu
Elif Haznedaroğlu, A. Lale Doğan
Hacettepe Üniversitesi Tıp Fakültesi

DS.2 **11:45- 12:00**
Anti-Tümör İmmün Yanıta Atipik Duktal Karsinom, İnvaziv Meme Kanseri ve Normal Meme Dokusundan Elde Edilmiş Fibroblastların Etkisi
Betül Gök Yavuz, Gürcan Günaydın, Kemal Kösemehmetoğlu, Derya Karakoç, Figen Özgür, Dicle Güç
Hacettepe Üniversitesi Tıp Fakültesi

12:00-13:00 **ARA**

13:00-13:40 **9. Oturum**

Oturum Başkanları: **Yrd. Doç. Dr. Rıfat Vedat Yıldırım**
Stj. Dr. Rana Baykan

P.8 **13:00-13:05**
Değişen Yüzyılda Tıpta Profesyonelizm
Murathan Kaboğlu, Aslı Noyan, Zeynep Yalçın, İlbey Özgün, Uğur Ateş, Hatice Gökçen Taşdoğan
Danışman: **Prof. Dr. Arzu Balkan**

P.9 **13:05-13:10**
Dünyayı Sarsan Salgınlar: Sosyal, Ekonomik ve Ekolojik Koşullarla İlişkileri
Hasret Türkü Sarıkamış, Pınar Demirtaş, Sara Ferhunde, Hilal Taylan Yılmaz, Elif Hazal Külhaş, Gökhan Koç
Danışman: **Prof. Dr. Halil Necati Dedeoğlu**

9. CURRICULUM VITAE

1. PERSONAL INFORMATION

NAME, SURNAME: BIRTH DATE AND PLACE:	**Elif HAZNEDAROĞLU** 22.04.1992, ANKARA
CURRENT POSITION: MD-PhD STUDENT ADDRESS: HACETTEPE KANSER ENSTİTÜSÜ, TEMEL ONKOLOJİ ABD PHONE: +905378606796 E-MAIL: elifhaznedaroglu9@gmail.com	

2. EDUCATION

YEAR	DEGREE	UNIVERSITY	FIELD OF STUDY
2010-2018	BACHELOR'S	HACETTEPE UNIVERSITY	FACULTY OF MEDICINE (KASTAMONU)
2014-	PhD (MD-PhD Program)	HACETTEPE UNIVERSITY	TUMOR BIOLOGY AND IMMUNOLOGY

3. LANGUAGE SKILLS

English, Advanced

German, B2

4. SCHOLARSHIPS AND AWARDS

Gazi University Foundation Private School Educational Success Scholarship

Gazi University Foundation Private School Valedictorian Award

7. Medicine and Human Sciences Congress "Medicine and Art" Oral Presentation First Place Award

Vehbi Koç Foundation scholarship for MD/PhD joint program students

Travel Award from Ankara Society of Neuroimmunology for the 8th Neuroimmunology Congress

I.Basic Oncology Symposium Oral Presentation Third Place Award

5. MEMBERSHIPS

- TurkMSIC-Hacettepe (MEDISEP) (Medical Students' Exchange and Project)-Local Officer on Research Exchange (LORE) 2011-12
- HÜKÖK (Hacettepe University Stem Cell Group) membership 2011-16
- TurkMSIC (Turkish Medical Students' International Committee) membership 2011-16
- Society of Basic Oncology membership 2017-
- Society of Turkish Immunology membership 2016-
- Ankara Society of Neuroimmunology membership 2016-
- Hacettepe University Faculty of Medicine, University Promotion Committee 2016-

6. ORAL AND POSTER PRESENTATIONS

- Burcu ŞİRİN, M. Emre GEDİK, Elif HAZNEDAROĞLU, A. Lale DOĞAN, "Effect Of Rapamycin on mRNA Expression of IL-8 And CXCR1 in HER2+ Breast Cancer Cells", Poster, 3rd Molecular Immunology&Immunogenetics Congress (MIMIC 3), 27- 30 April 2016, Antalya.
- Elif HAZNEDAROĞLU, A. Lale DOĞAN, "Inhibition of Serum and Glucocorticoid Inducible Kinase 1 (SGK1) in Breast Cancer", Oral Presentation, Başkent University School of Medicine, XIX. Medical Students Symposium, 24-26 May 2017, Başkent University, Ankara.

- Serkan AKIN, Şafak AKIN, Emre GEDİK, Elif HAZNEDAROĞLU, Ayşe Lale DOĞAN, Mustafa Kadri ALTUNDAĞ, "Prognostic Value of Serum Chemerin

Level in Breast Cancer", Poster, 22. National Cancer Congress, 19-23 April 2017, Antalya.

- Elif HAZNEDAROĞLU, Hande Canpınar, A. Lale DOĞAN, "Inhibition of Serum and Glucocorticoid Inducible Kinase 1 (SGK1) in Breast Cancer", Oral Presentation, Society of Basic Oncology, I.Basic Oncology Symposium, 10-11 May 2017, Dokuz Eylül University, İzmir.

7. INTERNATIONAL CLINICAL AND RESEARCH EXPERIENCES

- Summer Exchange with IFMSA (International Federation of Medical Students' Associations), Internal Medicine, 1-30 August 2013, Ruhr Universitaet Berufsgenossenschaftliches Universitaetsklinikum Bergmannsheil, Bochum, Germany.
- Graduate research student, 28 July-22 August 2014, Experimental Therapeutics, MD Anderson Cancer Center, Houston, USA.
- Clinical clerkship with Erasmus program, Internal Medicine, Pediatrics, Dermatology Departments, 9 October-31 December 2017, Universitaetsklinikum Düsseldorf, Düsseldorf, Germany.

8. PROFESSIONAL AND SOCIAL ACTIVITIES

- Turkish Scouting Federation Scout Leader Course, Ankara, 3-5 December 2010.
- 2nd National Medical Ethics Contest, "Cancer and Ethics", 29 April 2011, Gazi University, Ankara.
- Iskender Sayek Article Group Scientific Publication Workshop, 10 December 2011, Hacettepe University, Ankara.
- TurkMSIC (Turkish Medical Students International Committee) Standing Committee on Research Exchange V. Pre-Exchange Training, 5-6 May 2012, Cerrahpaşa University, İstanbul.
- EMSA (European Medical Students' Association), European Medical Students' Congress (EMSCon'12) Organisation Committee, 3-4-5 September

2012, Hacettepe University, Ankara.

- Occupational Sign Language Certificate Course for Doctor-Patient Communication, 2014-2015, Hacettepe University, Ankara.
- "International Joint Oncology Symposium of Hacettepe University Cancer Institute & MD Anderson Cancer Center", 14-15 May 2015, Hacettepe University, Ankara.
- "XI. Immunology Symposium", 22-23 October 2015, Adana.
- "Stem Cell Day 2015", 21 November 2015, Hacettepe University, Ankara.
- "11. Hacettepe University Cancer Institute Symposium", 26-27 November 2015, Hacettepe University, Ankara.
- "Basic Oncology Methodology Course", 26 November 2015, Hacettepe University, Ankara.
- 3rd Molecular Immunology & Immunogenetics Congress (MIMIC III), 27- 30 April 2016, Antalya.
- 8th Neuroimmunology Congress, 29-31 March 2017, Cyprus.
- I. Basic Oncology Symposium, 9-11 May 2018, Dokuz Eylül University, İzmir.