

EXAMINING MECHANISTIC UNDERPINNINGS OF
CHEMORESISTANCE IN TRIPLE NEGATIVE
BREAST CANCER

by
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Abstract

Background: Triple negative breast cancer (TNBC) is not only the most aggressive subtype of breast cancer, but it does also not have many targeted therapeutic options due to the lack of hormone receptor expression and enrichment of HER2. TNBCs are also more prone to the development of chemoresistance and metastatic progression, which are the main obstacles to reducing TNBC-related mortality.

Question: The objective of this study is to identify targetable key node (s) that contribute to the development of chemoresistance in TNBC.

Method: Differentially expressed genes in TNBC patients with or without relapse were analyzed to select functionally important genes in chemoresistant TNBC. TRIM29 was selected based on survival analysis. Carboplatin-resistant TNBC cells were established to explore the phenotypic and molecular differences. Various growth and migration assays were used to explore the phenotype of chemoresistant TNBC cells. Expression of TRIM29 and related pathways were assessed by immunoblotting and immunofluorescence analysis. Cells with TRIM29-knockout (KO) were established by the CRISPR system.

Result: Chemoresistant TNBC cells overexpress TRIM29. Exhibiting the functional importance, overexpression of TRIM29 in MDAMB231 confers resistance to carboplatin. A stable knockout of TRIM29 in carboplatin-resistant cells results in improved response to carboplatin. Mechanistically, an enhanced expression of β -catenin and YAP1 is observed in chemoresistant as well as TRIM29-overexpressing cells. Both β -catenin and YAP1 exhibit nuclear colocalization in TRIM29-overexpressing cells. TRIM29-KO in carboplatin-resistant HCC1806 results in a drastic decrease in β -catenin level, but, paradoxically an increased level of YAP1 is observed indicating a feedback loop demanding further investigation.

Conclusion: Our findings implicate TRIM29 enrichment as an important node in chemoresistant TNBC that may concomitantly modulate YAP1 and β -catenin as downstream oncogenic effectors.

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Table of Contents

Abstract	ii
Acknowledgements	iii
Table of Contents	iv
List of Figures	v
List of Abbreviations	vi
Introduction	1
Materials and Methods	5
Results	11
Discussion	22
References	25
Curriculum Vitae	31

List of Figures

- Figure 1.** 10 genes were found to be significantly overexpressed among TNBC patients with recurrent disease. 12
- Figure 2.** TNBC patients have higher expression of TRIM29 compared with breast cancer patients with other subtypes. 13
- Figure 3.** Carboplatin-resistant cells exhibit higher migration potential compared to parental cells. 14
- Figure 4.** Carboplatin-resistant cell lines have a higher expression of TRIM29 compared with the parental cell line. 15
- Figure 5.** Overexpressing TRIM29 in MDAMB231 cells confers resistance to carboplatin. 16
- Figure 6.** Genetic knockout of TRIM29 in carboplatin-resistant HCC1806 results in decreased colony formation ability, migration potential, and they are sensitized to carboplatin in vitro. 17
- Figure 7.** Mechanistic correlation of TRIM29 with oncogenic pathways. 19
- Figure 8.** Breast tumors generated by carboplatin resistant HCC1806 with or without functional TRIM29 are similar in size and mass. 20
- Figure 9.** Ex vivo model of carboplatin resistant HCC1806 LentiV2 has better resistance to carboplatin and clonogenicity compared to TRIM29KO. 21

List of Abbreviations

ABC	ATP-binding cassette
ABCC1/MRP1	multi-drug-resistant protein-1
ABCC11/MRP8	multi-drug-resistant protein-8
ABCG2/BCRP	breast cancer resistance protein
ATDC	ataxia telangiectasia group D
ATM	ataxia-telangiectasia mutated
ATR	ATM- and Rad3-related
Chk1	checkpoint kinase 1
DDR	DNA damage response
DEGs	differentially expressed genes
DNA-PKCs	DNA-dependent protein kinases
DSBs	DNA double-stranded breaks
EGFR	epidermal growth factor receptor
FAO	fatty acid β -oxidation
HER2	human epidermal growth factor receptor 2
ICC	immunocytochemistry
IHC	immunohistochemistry
LogFC	log fold change
npmi	normalized point-mutual index
OXPPOS	oxidative phosphorylation
PAM50	Prediction Analysis of Microarray 50
ssGSEA	single sample gene set enrichment analysis
TCGA-BRCA	Cancer Genome Atlas Breast Cancer
TNBC	triple negative breast cancer
TRIM29	Tripartite Motif 29

Introduction

Introduction of Breast Cancer

Breast cancer is the most prevalent cancer among women (Sung et al., 2021). It is also the leading cause of cancer-related mortality in 110 countries. Breast cancer can be subdivided into four different subtypes based on the enrichment status of human epidermal growth factor receptor 2 (HER2) and estrogen receptors, and/or progesterone receptors (Elias, 2010). Luminal A breast cancer has a high expression of hormonal receptors but has low expression of proliferation-related genes and HER2 receptors. Luminal B is characterized by having a low expression of the hormonal receptor, varied levels of HER2 receptors, and an elevated expression of proliferation-related genes (Fan et al., 2006; Hu et al., 2006; Voduc et al., 2010). HER2 enriched breast cancer does not have overexpression of hormonal receptors but it has an enrichment of HER2. Triple negative breast cancer (TNBC) lacks the expression of HER2 and hormonal receptors (Elias, 2010).

Aggressiveness of TNBC

Compared with other breast cancer subtypes, the mean prevalence of TNBC is only 12.7% (Doval et al., 2015; Kassam et al., 2009). However, the death rate of TNBC represents 40% of all breast cancer-related mortality (Haffty et al., 2006). TNBC is akin to an increased rate of distant metastasis and relapse (Haffty et al., 2006; Rakha et al., 2007). Accordingly, TNBC is unsurprisingly associated with a diminished chance of overall and disease-free survival (Bauer, Brown, Cress, Parise, & Caggiano, 2007; Rakha et al., 2007). TNBC progresses more aggressively than other breast cancers. Upon diagnosis, the mortality rate within 5 years of diagnosis for TNBC patients is 70%, whereas other subtypes have a 44% mortality rate (Dent et al., 2007).

TNBC – Treatment Strategies

For Luminal A, Luminal B, or HER2 enriched breast tumors, endocrine therapy, and anti-HER2 therapy can be utilized to combat the malignances with or without chemotherapy. However, due to the nature of TNBC lacking the upregulation of targetable receptors, cytotoxic chemotherapy is the mainstay strategy for TNBC (Bianchini, Balko, Mayer, Sanders, & Gianni, 2016; Eastman et al., 2013; Foulkes, Smith, & Reis-Filho, 2010; Marra, Viale, & Curigliano, 2019; Pandey, Balolong-Garcia, Cruz-Ordinario, & Que,

2019). Unfortunately, among TNBC patients who underwent chemotherapy, only 20% would respond to treatment (Liedtke et al., 2008). Compared to patients with enrichment of hormonal receptors, the other 80% of TNBC patients who did not respond to treatment have a much higher chance to suffer from early recurrence and metastatic disease (Nedeljković & Damjanović, 2019), thus contributing to an elevated mortality rate.

TNBC and Chemoresistance

The big umbrella of TNBC encompasses various subtypes. There are basaloid TNBC; BRCA1 dysfunction TNBC; TNBC has EGFR (epidermal growth factor receptor) enrichment; and TNBC with overexpression of androgen-receptor pathways (Elias, 2010). In many cases, TNBC tumors may display multiple characteristics mentioned above; or they could not be differentiated explicitly (Bai, Ni, Beretov, Graham, & Li, 2021; Elias, 2010). The heterogeneity and development of chemoresistance in TNBC contribute to its malicious tendency to metastasize and relapse (Bai et al., 2021; Zhang et al., 2015). Chemoresistance in TNBC is the main obstacle contending TNBC efficiently (Bai et al., 2021). Several mechanisms have been proposed for the evolution of chemoresistance in TNBC.

ABC (ATP-binding cassette) transporters can mediate drug efflux with the investment of ATP. It has been reported to participate in the chemoresistance of many solid tumors (Sissung et al., 2010). Among the family of ABC transporters, studies indicated that TNBC is more likely to express multi-drug-resistant protein-1 (ABCC1/MRP1), multi-drug-resistant protein-8 (ABCC11/MRP8), and the breast cancer resistance protein (ABCG2/BCRP) compared to other subtypes of breast cancer (Sissung et al., 2010; L. Xu, Zhao, Wang, Zhou, & Xing, 2017; Yamada et al., 2013).

Furthermore, DNA damaging agents are a big component among the vast categories of chemotherapeutics. For these therapeutics, tumor cells can develop resistance by enhancing their DNA repair mechanisms (Wu, Siddharth, & Sharma, 2021). When DNA damaging agents induce DNA damage, DNA damage response (DDR) will be activated. In mammalian cells, major kinases that participate in DDR include DNA-PKCs (DNA-dependent protein kinases), ATM (ataxia-telangiectasia mutated), and ATR (ATM- and Rad3-related) kinases. In the case of DNA double-stranded breaks (DSBs), ATR kinase will be stimulated, thus activating ATR-Chk1 (checkpoint kinase 1), an

effector kinase (Maréchal & Zou, 2013). Subsequently, checkpoint arrest will take place, while repair mechanisms will be activated. In a subset of TNBC patients, ATR-Chk1 pathway supports the development of therapeutic resistance by preventing replication stress (Meyer et al., 2020). Moreover, it has been suggested that the combination of radiation therapy and ATR inhibitor could combat chemoresistant TNBC with a promising outcome (Tu et al., 2018).

Cancer cells must remodel their metabolism for growth (Liberti & Locasale, 2016; Shim, Chun, Lewis, & Dang, 1998). This is termed the Warburg effect (Liberti & Locasale, 2016). It is evident that cancer cells undergo oxidative phosphorylation (OXPHOS) more frequently and have elevated fatty acid β -oxidation (FAO) activities to provide energy (Bai et al., 2021). Moreover, a subset of patients with recurring TNBC tumors has co-amplification of MYC and MCL1 (Balko et al., 2014). Follow-up research found that MYC and MCL1 promote chemoresistance by expanding cancer stem cells via regulation of mitochondrial OXPHOS (Lee et al., 2017). Additionally, a novel leptin-LEPR-JAK-STAT3-dependent FAO pathway has been identified by Wang and colleagues, which stimulates cell maintenance and therapeutic resistance in TNBC (T. Wang et al., 2018).

TRIM29 is Overexpressed in TNBC Patients

There are many mechanisms underlying the chemoresistance of TNBC. We aim to identify the key node that plays many significant parts in the aggressiveness of chemoresistant TNBC. By recognizing the key node, we will be more hopeful to triumph over the aggressiveness of chemoresistant TNBC as we can act more appropriately to the specific situation.

To accomplish this goal, chemoresistant TNBC cell lines were developed; and they show enhanced migration potential in functional assays. To query possible genes that should participate in this change of phenotype, we compared differentially expressed genes between TNBC patients with and without relapse. There were 10 genes not only overexpressed in TNBC patients with recurrent disease but also enriched in TNBC patients compared to patients with other subtypes of breast cancer. After survival analysis, we locked in TRIM29 (Tripartite Motif 29) because a high expression of TRIM29 indicates poorer survivability in TNBC patients.

Introduction of TRIM29

Members of the TRIM protein family contain a B-box type 1, a B-box type 2, a RING, and three zinc-binding domains (Borden, 1998; Kuribayashi & El-Deiry, 2008; Reddy, Etkin, & Freemont, 1992; Reymond et al., 2001). They are also known to have functions in cell differentiation, development, apoptosis, and tumorigenesis (Quaderi et al., 1997). Since it has been reported that TRIM29 is involved in Ataxia telangiectasia (Kapp et al., 1992), TRIM29 is also recognized as ATDC (Ataxia telangiectasia group D). Studies in recent decays suggested that TRIM29 has roles in several biological processes, including mediation of inflammatory response, cell signaling transduction, translocation of protein, cell cycle regulation, and apoptosis (Hatakeyama, 2016; Zhao et al., 2012). Interestingly, TRIM29 is overexpressed in lung cancer (Luo, Shen, Chen, Li, & Chen, 2020), osteosarcoma (Zeng et al., 2017), pancreatic cancer, and gastric cancer (Qiu, Xiong, Deng, & Xiang, 2015). As indicated by Sun and colleagues (Sun et al., 2019), an elevated level of TRIM 29 increases the probability of metastasis in colorectal cancer patients. *In vitro* and *in vivo* results (Sun et al., 2019) also demonstrated that overexpressing TRIM29 will lead to an increased CD44 expression, which activates the Wnt- β -catenin signaling pathway and resulted in enhanced migration and invasion in colorectal cancer.

On the other hand, overexpression of TRIM29 in the Luminal A breast cancer cell line led to a reduced rate of growth (J. Liu, Welm, Boucher, Ebbert, & Bernard, 2012). Similar results were seen in TNBC and HER2 enriched breast cancer *in vitro* (Ai et al., 2014). Interestingly, even among TNBC cell lines, the expression of TRIM29 varies (Ai et al., 2014), and the role of TRIM29 remains elusive.

We found that our chemoresistant cell lines have an increased expression of TRIM29 compared to the parental cell line. In this study, our goal is to investigate if TRIM29 has a functional impact on chemoresistant cell lines, and attempt to elucidate the mechanical underpinnings behind TRIM29.

Materials and Methods

Antibodies and Reagents

A list of all primary antibodies is listed in Table 1.

Designation	Host	Source or Reference	Identifiers	Additional information
TRIM29	Mouse	Santa Cruz Biotechnology Inc	Sc-376125	1:500 dilution for western blot; 1:250 dilution for ICC
Beta-Actin	Mouse	Santa Cruz Biotechnology Inc	Sc-8432	1:3000 dilution for western blot
YAP 1	Mouse	Cell Signaling Technology	12395S	1:3000 dilution for western blot
YAP/TAZ	Rabbit	Cell Signaling Technology	8418S	1:1000 dilution for western blot; 1:300 dilution for ICC
Beta-Catenin	Mouse	Santa Cruz Biotechnology Inc	Sc-7963	1:1000 dilution for western blot; 1:300 dilution for ICC
Phospho-AKT (S473)	Rabbit	Cell Signaling Technology	193H12	1:1000 dilution for western blot
AKT	Rabbit	Cell Signaling Technology	9272S	1:1000 dilution for western blot
Notch1	Mouse	Santa Cruz Biotechnology Inc	Sc-376403	1:1000 dilution for western blot

Table 1. Table of primary antibodies used in immunoblotting and/or immunocytochemistry.

In silico Data Analysis

Differentially Expressed Gene Analysis: Normalized mRNA data from the Cancer Genome Atlas Breast Cancer (TCGA-BRCA) database was acquired from the Broad GDAC Firehose (<https://gdac.broadinstitute.org/>) and processed via Microsoft Excel. GSE43502 (Yu et al., 2013) were downloaded from Gene Expression Omnibus database (<https://www.ncbi.nlm.nih.gov/geo/>). Differentially expressed genes (DEGs) analysis was

performed on iDEP.93 for TCGA and GSE43502 data set. DEGs were shown in a volcano plot using Enhance volcano tool with default settings (Blighe, 2018). Log fold change and adjusted p-values were used to create the plots. Overexpressed genes were compared to the chemo-resistance signature driven from GSE43502. Common genes with GSE43502: ≥ 1.0 FC; TCGA: ≥ 3 FC cutoff selected for further analysis.

Survival analysis: Kaplan-Meier plots were generated by the following website: <http://kmplot.com/analysis/>. The selected cut off were based on TNBC.

Bee swarm plots and Violin plots: Targeted expression analysis on a public data set was performed on bc-GenExMiner v4.7. Expression of TRIM29 in TNBC patients categorized through IHC status was shown in a bee swarm plot. Similarly, expression through PAM50 status was revealed in violin plots. On bc-GenExMiner expression parameter followed by targeted approach was selected. Results were downloaded in the form of bee swarm and violin plots.

UALCAN analysis: Protein expression of TRIM29 in breast cancer were acquired from: <http://ualcan.path.uab.edu>.

CHAT analysis: Result of Cancer Hallmark Analysis of TRIM29 were acquired from: <https://chat.lionproject.net> (Baker et al., 2017).

Correlation analysis: Single sample gene set enrichment analysis (ssGSEA) was performed on Genepattern (broad institute web-based tool (Reich et al., 2006). The oncogenic signature “C6” chip was used to analyze TCGA raw reads of TNBC and other subtypes. Enriched score and raw reads of each patient was used to perform Pearson correlation, and highly correlated pathways were selected for further analysis and validation. Correlation plots were created through GraphPad Prism 8. A cutoff of 0.2 for 100 samples based on “ $2/\sqrt{\text{no of samples}}$ ” was used to choose positively correlated pathways and genes.

Cell Culture

Human TNBC cell lines MDAMB231 and HCC1806 were obtained from American Type Culture Collection (ATCC, Manassas, VA). Early passage of frozen cells was revived from nitrogen vapor stocks or from -80°C freezer. Short tandem repeat testing was performed for authentication. All cells were maintained at 37°C in 5% CO_2 with 95% humidity.

MDAMB231 cells were cultured in Corning DMEM supplemented with 10% fetal bovine serum and 1% antibiotic-antimycotic. HCC1806 was maintained in Corning RPMI supplemented with 10% fetal bovine serum and 1% antibiotic-antimycotic.

Carboplatin-Resistant Cell lines: For the development of resistant cell lines, 5 µg/ml carboplatin (Sigma-Aldrich, St Louis, MO) was added to culture medium every two weeks. Each treatment time is 48 hours, and then cells were maintained in normal complete culture medium until next treatment. Resistant cells used in all experiments have undergone a minimum of 15 cycles of treatment and were below 30 passages.

CRISPR Knockout: To establish carboplatin resistant HCC1806 with genetic knockout of TRIM29, we selected the guiding oligonucleotide as recommended by the CRISPR online design tool (CRISPick). The forward sequence is 5'-GAAGGAGAAGGACCGCATCA-3', whereas the reverse sequence is 5'-TGATGCGGTCCTTCTCCTTC-3', which is targeting the open reading frame of TRIM29 in exon2. After the oligonucleotides were annealed, the guide was ligated into BsmBI-digested lentiCRISPRv2 plasmid (Addgene). Subsequently, calcium phosphate was used to transfect the annealed plasmid along plasmids with viral components into HEK293. Media with viral particles were obtained from HEK293 after 48 hours of incubation. After filtration, virus-containing media is used to transduce target cells. Cells with successful infection were selected with single cell selection with 1 µg/ml puromycin. Clones successfully survived the screen were tested for fidelity of knock out via RT-PCR and immunoblotting.

MTT (diphenyltetrazolium bromide) Viability Assay

Cells were seeded in 96-well plates (4000 cells/well) and incubated overnight. Drugs were added for 48 hours before addition of MTT (Sigma) following manufacturer's protocol. The viability of cells was calculated in Excel.

Trypan Blue Exclusion Assay

Cells were seeded in 24-well plates (5000 cells/well) and incubated overnight. Drugs were added for 48 hours. Fuchs-Rosenthal Counting Chamber was used to count cells under phase contrast microscopy. Inviability cells were excluded with Trypan blue (Sigma-Aldrich, St Louis, MO).

Clonogenic Assay

Cells were seeded in 12-well plates (1000 cells/well) and incubated overnight. They were treated with drugs for 48 hours, and then were replaced with culture media without drug and incubated for 5-8 days. Colonies were fixed with formalin before stained with 0.1% crystal violet in 20% methanol.

Spheroid Migration Assay

Tumor spheroids were generated by resuspending cells (20000 cells/well) in an 0.5% agarose-coated 96-well plate and cultured on an orbital shaker for 48 hours at 37°C in 5% CO₂ with 95% humidity. Intact and spheric tumor spheroids were selected and plated on 12-well plate with complete culture media with or without drug for 4 days to allow proper attachment and migration. From day 4 to day 10, spheroids were observed and photographed daily. Quantification of spheroid migration was measured by Leica ImageScope software. Speed of migration was calculated and plotted using Excel.

Scratch Migration Assay

Cells were seeded in each well of the ibidi Culture-Insert 2 well (70000 cells/well) in 35 mm cell culture dishes overnight to allow formation of monolayer. After removal of the Culture-Insert, cell monolayer was washed with PBS, then it was replaced with fresh culture media. Plates were photographed immediately and were followed upon various time intervals. Quantification of wound closure was performed by Image J (Fiji) software. Speed of wound closure was calculated and plotted in Excel.

Solid Mammosphere Assay

Mammosphere media that contains 1% penicillin/streptomycin, B27 (1:50, Invitrogen-Life Technologies), 5 µg/mL, 1 µg/mL hydrocortisone (Sigma), 20 ng/mL EGF (R&D Systems), and 20 ng/mL cholera toxin (CalBiochem) in serum-free mammary epithelium basal medium (Lonza). Methylcellulose (use 5.4 mL methylcellulose per 19.6 mL mammosphere media) was added onto mammosphere media via a syringe with 16 Gauge 1 ½ inch needle. Cells were counted (20000 cells/well) and resuspended with mammosphere media before seeding onto an ultra-law attachment, 24-well plate preceding incubation for 6-9 days. Mammospheres were stained with filtered crystal violet (0.1% crystal violet in 20% methanol) for 1-2 hours before photographic collection. Quantification was performed with Image J (Fiji) software.

Transwell Migration Assay

Cells were resuspended with serum free media before seeded in the upper chambers of transwell inserts (Corning BioCoat Control Inserts, 354578). Media supplemented with serum were put in the lower chambers and incubated for 48 hours. After removal of cells in upper chamber with cotton swaps, migrated cells were fixed with formalin and stained using crystal violet (0.1% crystal violet in 20% methanol).

RNA Isolation and Quantitative RT-PCR

After cells were lysed in TRIzol Reagent (Life Technologies, Inc., Rockville, MD), chloroform-isopropanol method was used for RNA isolation, then cDNA was synthesized via iScript cDNA Synthesis Kit (Bio-Rad Hercules, CA, USA). Go Taq Green Master Mix (Promega) was used to perform RT-PCR. Actin was used as the reference gene. Sequences of reverse and forward primers and the respected parameters for incubation are stated in **Table 2**.

Target	Primer	Sequence (5' → 3')
TRIM29	Forward	CATCCTGGAGCAGAACTTCC
	Reverse	TGCTCATCAATGCACCAAAT
ACTIN	Forward	ACCATGGATGATGATATCGC
	Reverse	ACATGGCTGGGGTGTGAAG

Table 2. Table of forward and reverse primer sequences used in RT-PCR.

Protein Isolation and Western Blotting

Protein lysates were prepared with modified RIPA buffer. Protein was resolved on sodium-dodecyl sulfate polyacrylamide gel before transferred onto PVDF membrane. After immunoblotting with desired antibodies, photographic evidence of Western blot was collected using digital camera.

Transfection for Overexpression

All transfection mentioned was done by Lipofectamine 2000 (Thermo Fisher Scientific). In each well, 2.5 µg overexpression plasmid was used. Lipofectamine was added as

vehicle control for overexpression. TRIM29/ATDC overexpression plasmid was a generous gift from Dr. Simeone Diane (L. Wang et al., 2014).

Immunocytochemistry/Immunofluorescence

Cells were plated in 8-well chambers (20000 cells/well) and incubated overnight. They were fixed with 4% paraformaldehyde for 30 minutes preceding permeabilization with 0.1% Triton-X-100. Cells were blocked with 3% BSA in sterile PBS. After incubation with primary antibody in blocking buffer overnight, cells were incubated with FITC/TRITC-tagged secondary antibody (Invitrogen, 1:500 dilution with blocking buffer) for an hour at room temperature. DAPI (62248, Thermo Fisher Scientific) was used to stain nucleus before mounting. Mounted slides were visualized under Lieca F800 fluorescent microscope. With oil immersion objective, photographs were captured at 63X magnification with Lieca Elements software.

Tumor Transplantation in Mice

All animal experiments involved in this study were approved by the Johns Hopkins Institutional Animal Care and Use Committee. Animals used were SCID-MOD mice (female, 8-10 weeks) from SKCCC animal facility and were maintained in-house. HCC1806 carboplatin-resistant cell line treated with TRIM29 knocked out (TRIM29KO) and vector control (LentiV2) were suspended in PBS solution and cell count was adjusted to 1×10^6 per mL. Cell suspensions were mixed with equal parts of Matrigel. Each immunodeficient mice received 100 μ L mixture in one left and one right fat pad. Tumor sizes were recorded weekly after one week of injection.

Statistical Analysis

Experiments were carried out thrice, with triplicates of the cells each within their own well. No less than three samples were used for numerical quantification. Statistical significance was evaluated by two-tailed student *t* test using $p < 0.05$ as demonstrative for statistical significance. * Signifies a p value of < 0.05 ; ** signifies a p value of < 0.01 ; *** signifies a p value of < 0.001 .

Results

10 genes were found to be significantly overexpressed among TNBC patients with recurrent disease.

To start our inquiries, we compared differentially expressed genes (DEGs) between patients with TNBC or patients with other subtypes of breast cancer (**Fig. 1A**). Since we are specifically interested in genes that are involved in chemoresistance in TNBC, we also looked at differentially expressed genes among TNBC patients with and without recurrent disease (GSE43502, NCBI-GEO), as indicated in **Fig. 1B**. We narrowed down the DEGs with log fold change (logFC) bigger than 1. When observing the narrowed down list, there are 10 overlapping genes not only overexpressed in TNBC patients with recurrent disease but also enriched in TNBC patients compared to patients with other subtypes of breast cancer (**Fig. 1C**). The 10 overlapping genes were: ACE2, FGDBP1, K6C, KRT16, KRT81, HORMAD1, MMP12, PI3, SHC4, and TRIM29. To focus on functionally important genes that contribute to a worse prognosis in TNBC, survival analyses were performed on the 10 overlapping genes (**Fig. 1D**). According to the results of survival analyses, many of them did not show a significant impact on prognosis in TNBC, except for TRIM29. Comparing TNBC patients with high and low expression of TRIM29, those who have an enrichment of TRIM29 have significantly low survivability (Hazard Ratio = 1.86; $p = 0.00009$). Additionally, the roles of TRIM29 in cancer were investigated via Cancer Hallmark Analysis Tool (**Fig. 1E**). The visualizations in **Fig. 1E** were plotted based on normalized pointwise mutual information (npmi), which is an indicator for co-occurrence standard. As indicated in **Fig. 1E**, existing studies suggested that TRIM29 participates in invasion and metastasis, inducing angiogenesis, sustaining proliferative signaling, resisting cell death, and contributing to genome instability. Taken together, we found 10 candidate genes that are overexpressed in TNBC patients with recurrent disease. Among these 10 candidates, the high expression of TRIM29 indicates the poorest prognosis with the lowest p value. With this information in mind, we decided to explore the general expression and functionality of TRIM29 in TNBC patients.

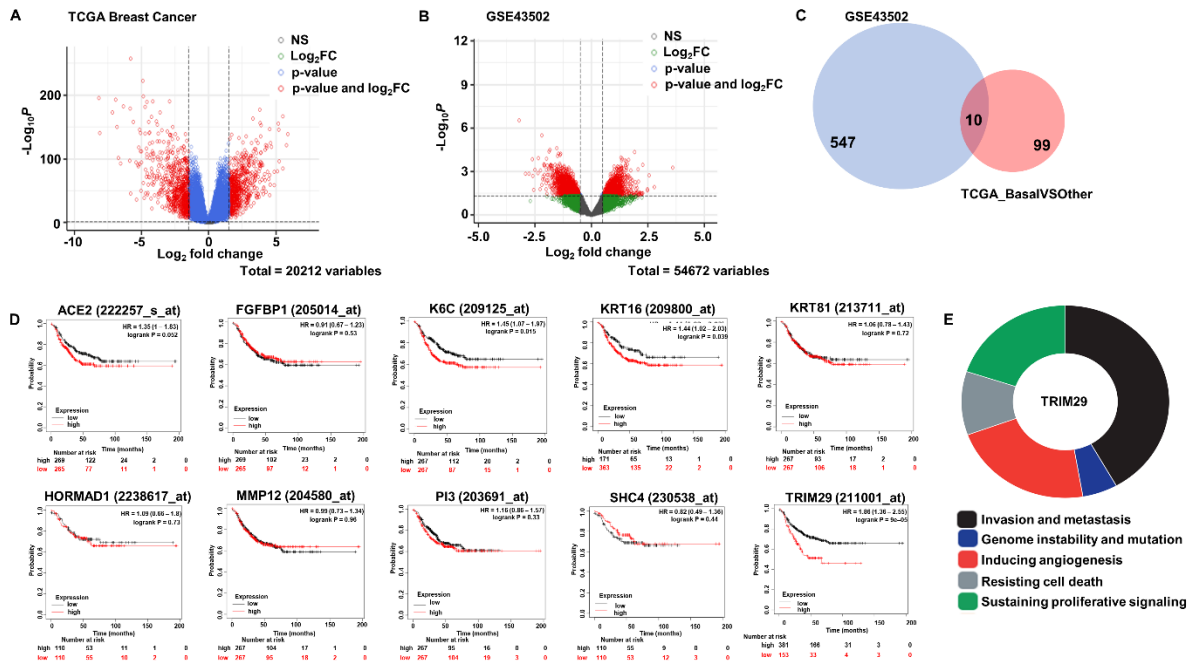


Figure 1. 10 genes were found to be significantly overexpressed among TNBC patients with recurrent disease.

(A) Volcano plot visualizing differentially expressed genes between patients with TNBC patients and patients with other subtypes of breast cancer. The gene set was acquired from TCGA database. (B) Volcano plot showing differentially expressed genes comparing TNBC patients with or without recurrent disease. Gene set used was GSE43502 from Gene Expression Omnibus. (C) Venn diagram showing numbers of overlapping genes with LogFC >1 from TCGA dataset and GSE43502 dataset. (D) Kaplan-Meier curves indicating TNBC recurrence-free survival with high- or low- expression of overlapping genes in 1C, respectively. Log-rank tests of survival patterns were used to obtain the *p* values. (E) Visualization of cancer hallmark distribution of TRIM29 in donut chart. Each color represents a cancer hallmark. The occupied area of each color is proportional to NPMI (normalized point-wise mutual information).

TRIM29 is Enriched in TNBC Patients

To ensure that TRIM29 is overexpressed in TNBC patients, we explored the expression of TRIM29 in TNBC patients compared to patients with other subtypes of breast cancers. According to RNA-seq data from the TCGA database, most TNBC patients have an elevated level of TRIM29 compared to patients with other subtypes of breast cancer. This finding is true when classified via PAM50 (Prediction Analysis of Microarray 50) status (Fig. 2A) or when classified based on IHC (immunohistochemistry) status (Fig. 2B). Additionally, data from Clinical Proteomic Tumor Analysis Consortium revealed that most subtypes of TNBC tumors have an increased protein expression of TRIM29 compared to Luminal and HER2-enriched breast cancer, except the LAR TNBC

subtype which expresses luminal androgen receptors (**Fig. 2C**). Taken together, compared with other subtypes of breast cancer, TNBC patients have a higher expression level of TRIM29 expression. We suspect that TRIM29 may have more notable functions in TNBC compared to other subtypes of breast cancer.

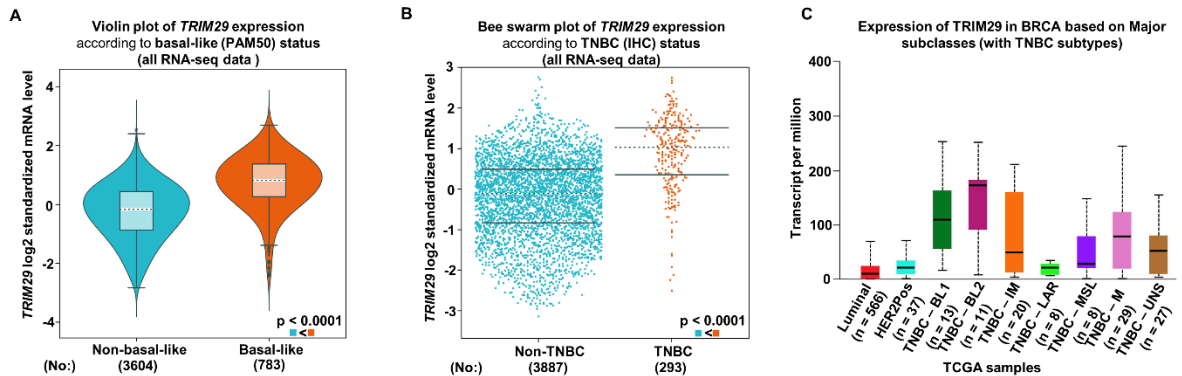


Figure 2. TNBC patients have higher expression of TRIM29 compared with breast cancer patients with other subtypes.

(A) Violin plot representing TRIM29 expression within 4387 breast cancer patients. The classification was based on PAM50 status. (B) Bee swarm plot representing TRIM29 expression in 4180 patients. Patients were categorized via IHC status. (C) UALCAN analysis indicating TRIM29 expression among patients with different subtypes of breast cancer.

Carboplatin-Resistant Cells Bear Higher Migration Potential Compared to Parental cells.

Based on the information presented above, we wish to explore the function of TRIM29 in chemoresistant TNBC. To conduct *in vivo* experiments, HCC1806 carboplatin-resistant cell line (1806_CarboR) and MDAMB231231 carboplatin-resistant cell line (231_CarboR) was developed by introducing carboplatin treatment in parental cells (1806_Ctrl and 231_Ctrl) biweekly. Acquisition of resistance of carboplatin in treated cells was confirmed via MTT assay (**Fig. 3A**). Clonogenicity of parental and CarboR cells was accessed by colony formation assay (**Fig. 3B**). We have observed that 1806_CarboR has superior clonogenicity compared to parental cells both with and without treatment of carboplatin. As of 231_CarboR, there weren't many differences in clonogenicity when treatment isn't added to the media. However, 231_CarboR is more proficient in forming colonies upon the addition of carboplatin (**Fig. 3B**).

Next, the tumor-initiating potential of carboplatin-resistant cells was accessed via mammospheres assay. As indicated in **Fig. 3C-D**, both 1806_CarboR and 231_CarboR formed more mammospheres compared to their parental cells, respectively. This suggested that the carboplatin-resistant cells exhibited a higher tumor-initiating potential compared to TNBC cells that are not resistant to carboplatin. To gauge the migration potential of the carboplatin-resistant cell lines compared to parental cells, we performed 3D (**Fig. 3E-F**) and 2D (**Fig. 3G-H**) migration assays. Both carboplatin-resistant cells move faster in both 3D and 2D settings compared to the respected parental cells. **Fig. 3I** presents the result of transwell migration comparing 1806_Ctrl and 1806_CarboR with and without carboplatin treatment. 1806_CarboR indicated superior migratory potential upon differences in chemotaxis. Moreover, the introduction of carboplatin profoundly inhibited cell migration in 1806_Ctrl, but this effect is diminished in 1806_CarboR. Based on the information presented above, the carboplatin-resistant cells not only exhibit an elevated tumor-initiating potential but also bear profound migration potential compared to the wild type cells.

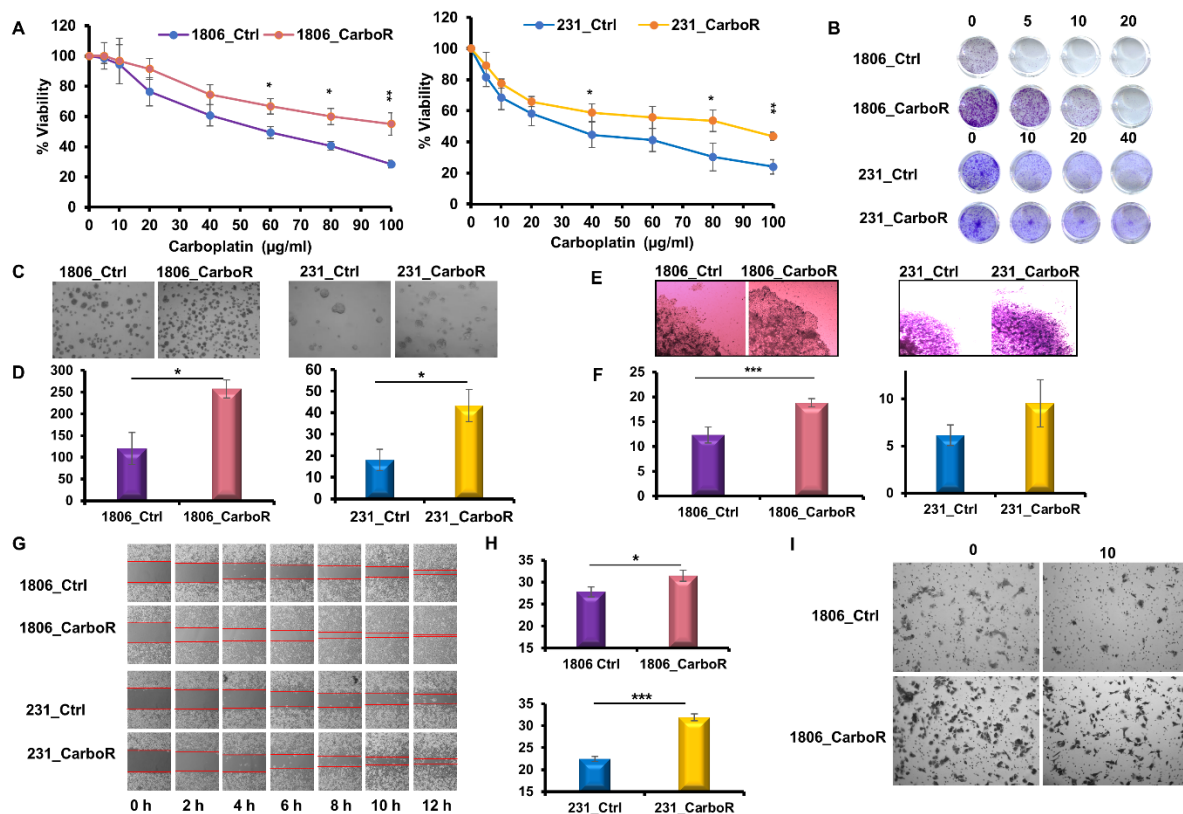


Figure 3. Carboplatin-resistant cells bear higher migration potential compared to parental cells.

(A) Result of MTT assay presented as line graph comparing % cell viability between chemoresistant cells and parental cells upon different concentrations of carboplatin. (B) Representative images of colonies formation assay of chemoresistant cells and parental cells in different treatment groups. Concentrations of carboplatin were presented in μg per mL. (C, D) Representative images of solid mammospheres. D, Bar charts below present the numbers of mammospheres. (E, F) Representative images of spheroid migration comparing chemoresistant cells and parental cells with or without treatment. The concentration of treatment was a quarter of the IC50 of parental cells. F, bar charts show the average speed of spheroid migration comparing parental and chemoresistant cells. Migration distances were recorded for 6 days to calculate the speed of migration. (G, H) Representative images showing the progression of scratch migration assay of parental and chemoresistant cells. H, bar charts indicate the average speed of 2D migration comparing the parental and chemoresistant cell lines. (I) Representative images presenting results of transwell migration comparing carboplatin-resistant cells and respected parental cells, with or without carboplatin treatment. Concentrations of carboplatin were presented in μg per mL. Data represents $n = 3$ independent experiments. * $p \leq 0.05$, ** $p \leq 0.01$, *** $p \leq 0.001$

TRIM29 is Overexpressed in TNBC Cells with Carboplatin Resistance.

Next, we investigated the protein level of TRIM29 in carboplatin-resistant cells compared to their respective parental cells (Fig. 4A). TRIM29 was overexpressed in 1806_CarboR as well as 231_CarboR compared with their parental cell lines. Similarly, as seen in immunofluorescence analysis (Fig. 4B), 1806_CarboR cells are more likely to have higher expression of TRIM29 compared to 1806_Ctrl cells. These data indicated that in TNBC, acquisition of resistance to carboplatin is correlated to overexpression of TRIM29 *in vitro*.

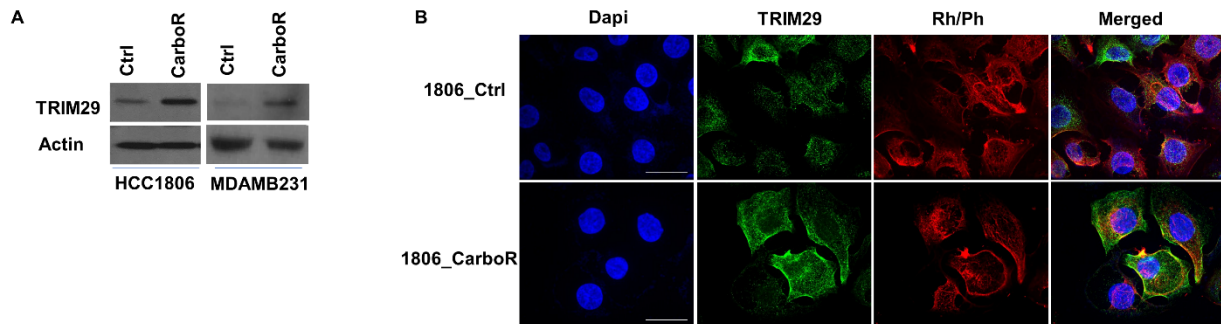


Figure 4. Carboplatin-resistant cell lines have a higher expression of TRIM29 compared with the parental cell line. (A) Result of immunoblotting of TRIM29 in parental and chemoresistant cells. The expression of Actin was used as the loading control. (B) Immunofluorescence analysis of TRIM29 in 1806_Ctrl and 1806_CarboR cells. DAPI was used to stain nuclei. Rhodamine Phalloidin was used to stain F-actin. Scale bar = 25 μ m.

Overexpressing TRIM29 in MDAMB231 Confers Resistance to Carboplatin.

To investigate the effect of TRIM29 in wild type TNBC cell line, we overexpressed TRIM29 in MDAMB231 (Fig. 5A). As indicated in Fig. 5B, when overexpressing TRIM29 in MDAMB231, they become more resistant to carboplatin compared to cells treated with the transfection vehicle. From here, we learned that overexpressing TRIM29 in wild type MDAMB231 may confer resistance to carboplatin.

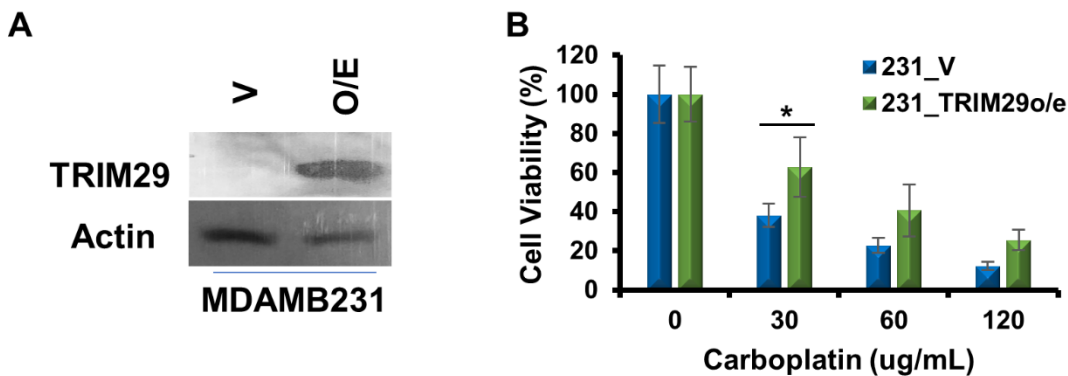


Figure 5. Overexpressing TRIM29 in MDAMB231 confers resistance to carboplatin.

(A) Immunoblotting analysis of TRIM29 in MDAMB231 treated with transfection vehicle or with TRIM29 overexpression plasmid. (B) Bar chart showing % cell viability in trypan blue exclusion assay comparing MDAMB231 treated with transfection vehicle or with TRIM29 overexpression plasmid. Data represents n = 3 independent experiments. *p \leq 0.05, **p \leq 0.01, ***p \leq 0.001.

Stable Genetic Knockout of TRIM29 in 1806_CarboR Resulted in a Decreased Clonogenicity, Migration Potential, and was Sensitized to Carboplatin Treatment.

Next, we explored the consequences of knocking out TRIM29 in carboplatin-resistant HCC1806 cells. We established 1806_CarboR with a stable genetic knockout of TRIM29 with the CRISPR system. We confirmed the stable knockout via RT-PCR and immunoblotting (Fig. 6A-B). Recalling Fig. 3B, 1806_CarboR exhibited enhanced clonogenic activity compared to 1806_Ctrl, which inspired us to investigate the baseline clonogenicity of the TRIM29KO cells. However, the impressive clonogenicity of

1806_CarboR was deprived along with the knockout of TRIM29 (**Fig 6C-D**). Since we wish to explore whether knockout of TRIM29 would deprive the chemoresistance of the 1806_CarboR, cell viability upon introduction of carboplatin treatment was assessed via trypan blue assay (**Fig 6E**). Knocking out TRIM29 in 1806_CarboR resulted in the loss of resistance to carboplatin. Similar results were seen in **Fig. 6F**, as the introduction of carboplatin profoundly decreases clonogenicity in TRIM29KO compared to 1806_CarboR treated with vector (LentiV2). Next, to examine if TRIM29 impacts the migratory potential of carboplatin-resistant 1806 cells, we performed scratch migration and spheroid migration. We observed that the speed of wound healing is slower in TRIM29KO compared to LentiV2 (**Fig. 6G-H**). Similarly, results of spheroid migration (**6I-J**) indicated that TRIM29KO has a weakened migratory potential compared to LentiV2, as TRIM29KO not only migrates less far and has a slower speed of migration compared to LentiV2. Collectively, these data suggested that TRIM29 is important in the enhanced clonogenicity and migratory potential in carboplatin-resistant HCC1806.

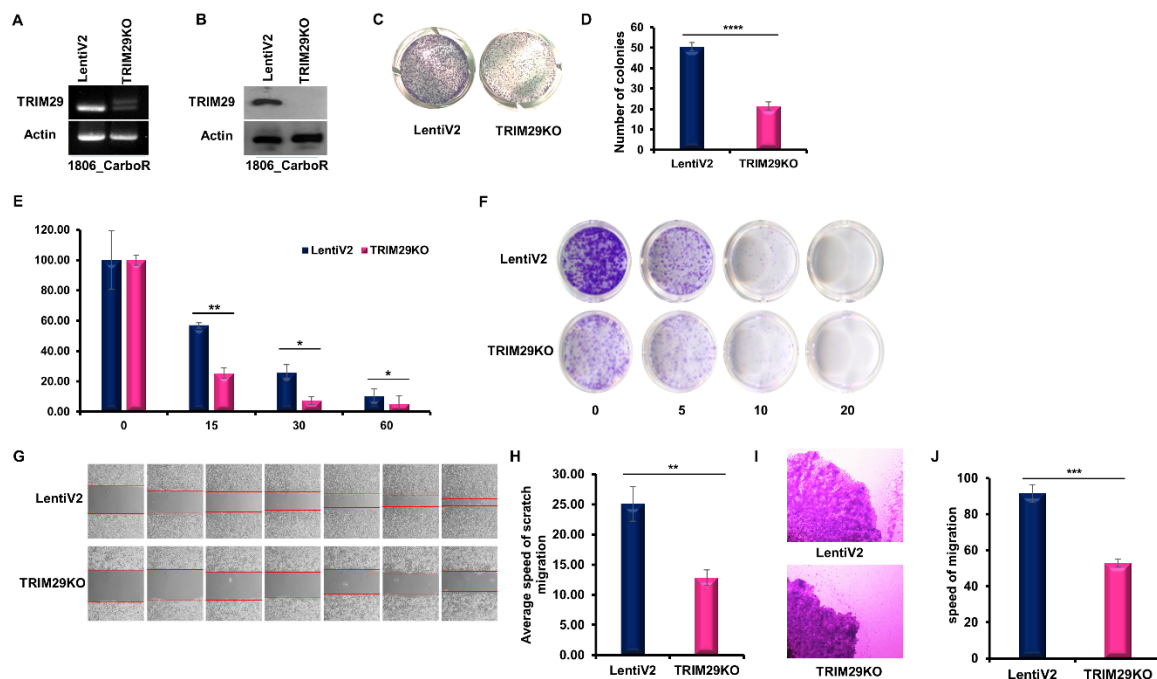


Figure 6. Genetic knockout of TRIM29 in carboplatin-resistant HCC1806 resulted in decreased colony formation ability, migration potential, and they were sensitized to carboplatin *in vitro*.

(A) Genetic Expression of TRIM29 in HCC1806 carboplatin-resistant cells with TRIM29 knocked out by CRISPR (TRIM29KO) and cells treated with vector (LentiV2) detected by RT-PCR analysis. Actin was served as the loading control. (B) Immunoblotting analysis of TRIM29, YAP1, and β -catenin in 1806 carboplatin-resistant cells with TRIM29KO or cells treated with vector. Actin was served as the loading control. (C, D) Representative images of colonies formation assay of LentiV2 and TRIM29 KO. D: Bar charts show the numbers of colonies (n > 20) as respected to C. (E) Bar chart showing % cell viability in trypan blue exclusion assay comparing LentiV2 and TRIM29KO. Concentrations of carboplatin indicated is in $\mu\text{g/mL}$. (F) Representative images of colonies formation assay of LentiV2 and TRIM29 KO. Concentrations of carboplatin indicated is in $\mu\text{g/mL}$. (G, H) Representative images showing the progression of scratch migration assay of LentiV2 and TRIM29KO cells. Bar charts indicate the average speed of 2D migration comparing LentiV2 and TRIM29KO cells. (I, J) Representative images of spheroid migration comparing LentiV2 and TRIM29KO. J, Bar charts indicate the average speed of spheroid migration comparing LentiV2 and TRIM29KO. Migration distances were recorded for 6 days to calculate the speed of migration.

Expression of TRIM29 involved in Oncogenic Pathways.

Until now, we have seen that overexpressing TRIM29 can lead to an acquisition of resistance to carboplatin while knocking out TRIM29 in carboplatin resistant HCC1806 deprives its resistance to carboplatin and resulting in a decreased clonogenicity and migration potential. Next, we wish to identify whichever oncogenic pathways are related to TRIM29 activity. After we processed raw data from TCGA with genepattern, we explored the relation of TRIM29 expression in several oncogenic pathways. We found that the expression of TRIM29 is related to the HIPPO pathway, NOTCH pathway, WNT pathway, and β -catenin (7A). Next, we examined the protein level of players involved in these pathways in 1806_Ctrl and 1806_CarboR. According to the immunoblotting results (7B), the expression of Notch1 and pJNK in 1806_Ctrl is comparable to 1806_CarboR. However, 1806_CarboR has elevated expression of β -catenin, YAP1, pAKT, and AKT, which indicated that the carboplatin-resistant 1806 has an increased activity related to WNT, HIPPO, and mTOR pathways. In Fig. 7C, immunofluorescence analysis confirmed the enrichment of β -catenin and YAP/TAZ in 1806_CarboR compared to 1806_Ctrl.

To explore whether the expression level of these players is related to the enrichment, or inhibition of TRIM29, we conducted immunoblotting analyses of YAP1 and β -catenin in HCC1806 carboplatin resistant cells with and without TRIM29KO, and in MDAMB231 with TRIM29 overexpression or treated with transfection vehicle (Fig. 7D-E). When TRIM29 is knocked out in HCC1806 carboplatin-resistant cells, it will lead to a drastic decrease in β -catenin levels. Interestingly, TRIM29KO has an enhanced YAP1

level compared to 1806_CarboR treated with an empty vector (LentiV2). On the other hand, when TRIM29 is overexpressed in MDAMB231, it will lead to an elevated level of YAP1 and β -catenin (Fig. 7E). Immunofluorescence analyses were performed to compare the localization of β -catenin and YAP/TAZ between MDAMB231 and 231 cells with TRIM29 overexpression (Fig. 7F-H). As indicated in Fig. 7F, 231 with TRIM29 overexpression has more β -catenin around the cytoplasm and nucleic membrane, and inside the nucleus. For expression of YAP/TAZ, 231 with TRIM29 overexpression has an elevated level of YAP/TAZ, and they are more likely to have YAP/TAZ localized inside the nucleus (Fig. G-H), which implied an augmentation of YAP1 activity. Taken together, in carboplatin-resistant TNBC cells, upregulation of pAKT, AKT, YAP1, and β -catenin was observed. Additionally, when overexpressed TRIM29 in MDAMB231, it will also push out the expression of YAP1 and β -catenin. However, when knocking out TRIM29 in 1806_CarboR, the expression of β -catenin will be decreased, but the YAP1 level will be increased.

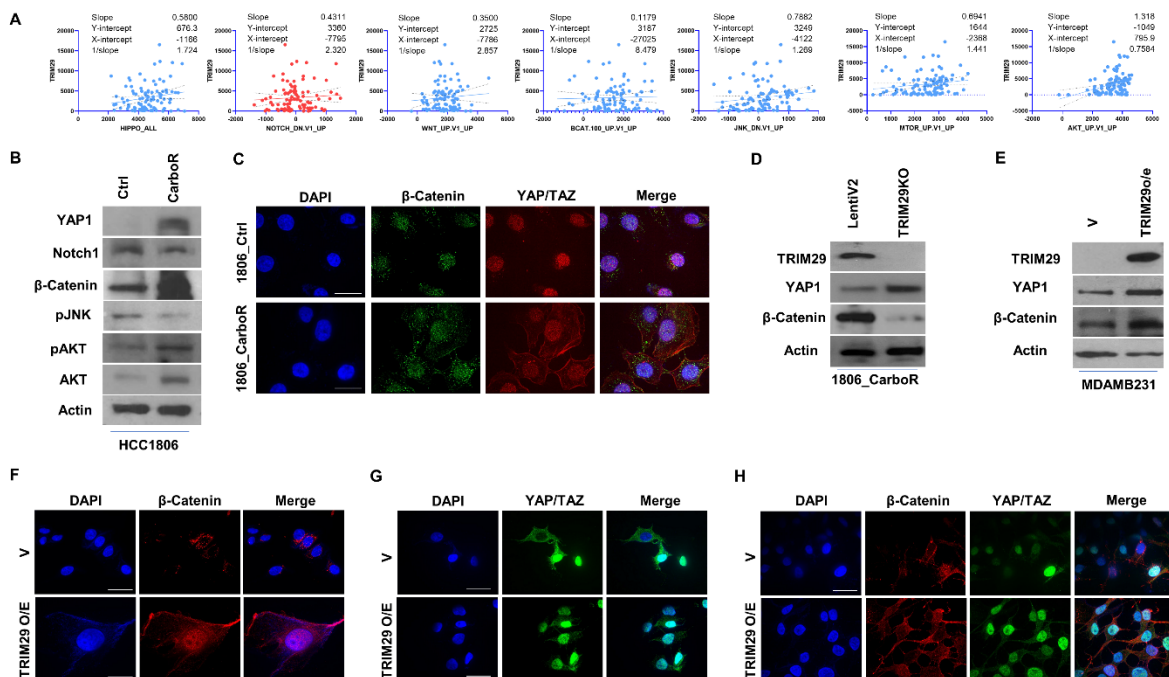


Figure 7. Mechanistic correlation of TRIM29 in oncogenic pathways.

(A) Results of correlation of TRIM29 expression to oncogenic pathways. Cut off = 0.2. (B) Immunoblotting analysis of YAP1, AKT, pAKT, and β -catenin in 1806 parental cells and chemoresistant cells. (C) Immunofluorescence analysis of YAP/TAZ, and β -catenin in 1806

parental cells and chemoresistant cells. DAPI was used to stain nuclei. Scale bar = 25 μm . (D) Immunoblotting analysis of TRIM29, YAP1, and β -catenin in 1806 carboplatin-resistant cells with TRIM29KO or cells treated with vector. Actin was served as the loading control. (E) Immunoblotting analysis of TRIM29, YAP1, and β -catenin in MDAMB231 treated with transfection vehicle or with TRIM29 overexpression plasmid. Actin was served as the loading control. (F) Immunofluorescence analysis of β -catenin in MDAMB231 treated with transfection vehicle or with TRIM29 overexpression plasmid. DAPI was used to stain nuclei. Scale bar = 25 μm . (G) Immunofluorescence analysis of YAP/TAZ in MDAMB231 treated with transfection vehicle or with TRIM29 overexpression plasmid. DAPI was used to stain nuclei. Scale bar = 25 μm . (H) Immunofluorescence analysis of β -catenin and YAP/TAZ in MDAMB231 treated with transfection vehicle or with TRIM29 overexpression plasmid. DAPI was used to stain nuclei. Scale bar = 25 μm .

Carboplatin resistant HCC1806 with TRIM29KO forms larger tumors in immunodeficient mice.

Encouraged by our *in vitro* results, we explored the behavior of carboplatin resistant HCC1806 with and without knock out of TRIM29 in immunodeficient mice. Breast tumors from mice were collected 3 months after injection. We observed that the tumors generated from TRIM29KO cells were not smaller than tumors generated from cells treated with LentiV2 (**Fig 8**).

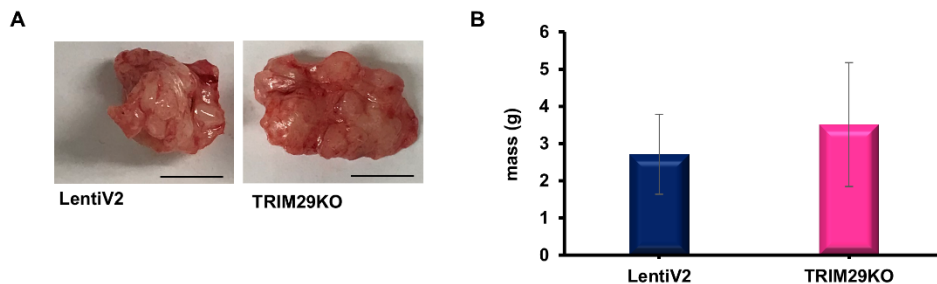


Figure 8. Breast Tumors Generated by Carboplatin Resistant HCC1806 with or without Functional TRIM29 are Similar in Size and Mass.

(A) Representative image of left breast tumors excised upon sacrifice. Scale bar = 1 cm. (B) Bar charts indicate the average total mass of left, and right tumors excised from mice injected with HCC1806_CarboR LentiV2 or HCC1806_CarboR TRIM29KO.

An *Ex vivo* model of carboplatin resistant HCC1806 with TRIM29KO has poor clonogenicity and does not show therapeutic resistance to carboplatin

Using breast tumors excised from mice, an *ex vivo* model of LentiV2 and TRIM29KO was established. Results of immunoblotting suggested that TRIM29 knocked out was sustained throughout the development of cancer in mice (**Fig. 9A**). We are

curious to see if *ex vivo* LentiV2 and TRIM29KO cells have similar or opposite phenotypes compared to *in vitro* LentiV2 and TRIM29KO cells. As presented in **Fig. 9B**, the clonogenicity of the *ex vivo* model is similar to the *in vitro* results (**Fig 6F**). Additionally, *ex vivo* cells of TRIM29KO are less viable upon carboplatin treatment compared to *ex vivo* LentiV2 (**Fig. 9E**), the pattern of this result is akin to what we saw in the *in vitro* LentiV2 and TRIM29 model (**Fig. 6E**). Taken together, our experimental results indicated that the *ex vivo* model of TRIM29KO has a similar phenotype compared to the *in vivo* model.

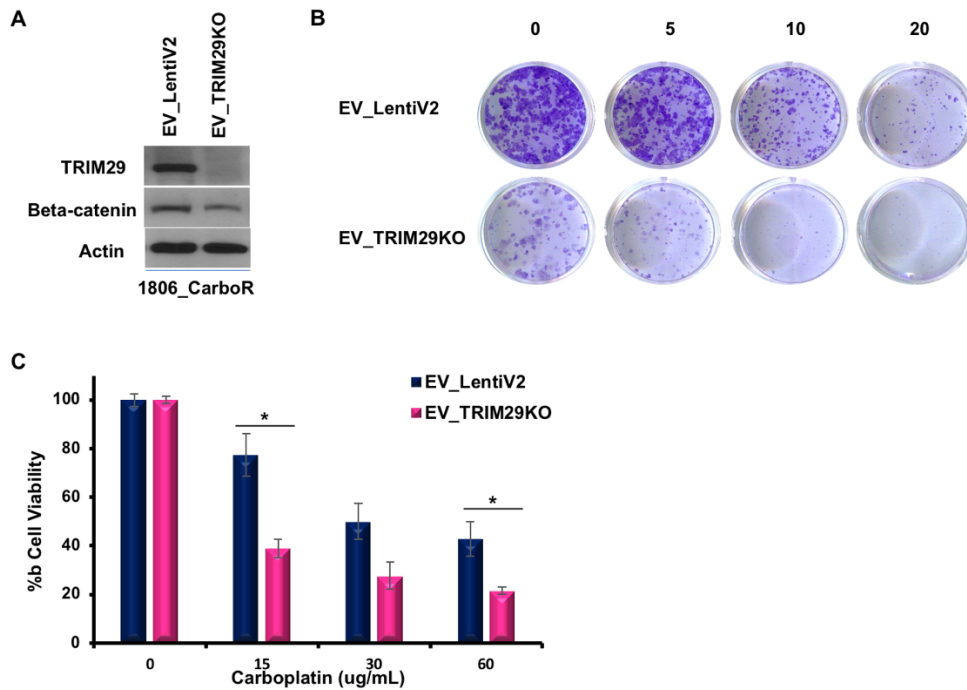


Figure 9. Ex Vivo Model of Carboplatin Resistant HCC1806 LentiV2 has better resistance to carboplatin and clonogenicity compared to TRIM29KO.

(A) Immunoblotting analysis of TRIM29 and β -catenin comparing *ex vivo* (EV) LentiV2 and TRIM29KO. Actin was served as the loading control. (B) Representative images of colonies formation assay of *ex vivo* LentiV2 and TRIM29KO with or without carboplatin treatment. Concentrations of carboplatin were presented in μg per mL. (C) Bar chart showing % cell viability in trypan blue exclusion assay comparing LentiV2 and TRIM29KO.

Discussion

The goal of our study is to identify the key node that underlines the TNBC therapeutic resistance. After several rounds of analysis, we decided to explore the function of TRIM29 in chemoresistant TNBC. Our *in vitro* results indicated that overexpressing TRIM29 in wild type TNBC cells confers resistance to carboplatin. On the other hand, knocking out TRIM29 in carboplatin resistant TNBC cells sensitizes them to carboplatin. It also led to diminished clonogenicity and migration potential. Inspired by the phenotypic changes in TRIM29 overexpression or inhibition, we performed correlation analyses of TRIM29 in oncogenic pathways. These findings along with immunoblotting results indicated that TRIM29 is related to an increased YAP1 and β -catenin.

TRIM29 is upregulated in several different types of cancers. In non-small cell lung cancer, TRIM29 contributes to oncogenesis by modulation of cell cycle-related proteins (W. Zhan et al., 2015) and suppressing TRIM29 in lung cancer *in vitro* sensitizes them to chemotherapy (C. Liu, Huang, Hou, Hu, & Li, 2015). In bladder cancer, Bcl family protein and cyclin D1/E levels are enhanced by TRIM29 through the PKC-NF- κ B signaling pathway, thus resulting in less apoptosis while an increase in proliferation signal (Tan, Liu, & Wu, 2016). Moreover, by activating methyltransferase 3A, TRIM29 indirectly suppresses PTEN via epigenetic regulation and contributes to poor prognosis in bladder cancer (Frstrup et al., 2013; Huang et al., 2013; Palmboos et al., 2015). TRIM29 is also a prognosis factor in cervical cancer. As indicated by Xu and colleagues (R. Xu, Hu, Zhang, Jiang, & Wang, 2016), TRIM29 has the leverage to activate the Wnt/ β -Catenin pathway thus contributing to epithelial-mesenchymal transition (EMT), cell proliferation, colony formation, and migration. In colorectal cancer, TRIM29 enhances the Warburg effect by altering the PKM1/PKM2 ratio, which strengthens the malignant behavior of cancer (Han et al., 2021). Moreover, TRIM29 has also been shown to contribute to chemoresistance in lung cancer (C. Liu et al., 2015) and ovarian cancer (Hao et al., 2021).

In breast cancer, it has been reported that TRIM29 functions as a tumor suppressor (Dükel et al., 2016; Guo, Wang, Han, Zhang, & Li, 2017; J. Liu et al., 2012). Yet, the role of TRIM29 in chemoresistant TNBC remains unclear. As encouraged by our *in silico* result, which indicated that TRIM29 is enriched in TNBC patients with recurrent disease; and that a higher expression of TRIM29 indicates a diminished rate of

recurrence-free prognosis, we hypothesized that TRIM29 could be functionally important in chemoresistant TNBC.

We observed that when overexpressing TRIM29 in MDAMB231, β -catenin will be overexpressed. β -catenin is the key effector of the Wnt pathway (Valenta, Hausmann, & Basler, 2012). The Wnt pathway is a highly conserved signaling cascade in mammalian cells. It also has profound implications for oncogenesis, cancer stemness, immune surveillance, and metastasis (Blick et al., 2008; DiMeo et al., 2009; Gupta et al., 2005; Kulka, Fukuishi, Rottem, Mekori, & Metcalfe, 2006; T. Zhan, Rindtorff, & Boutros, 2017). When the Wnt pathway is inactive, a destruction complex would bound to β -catenin, which will lead to proteasomal degradation of β -catenin. Inversely, when the Wnt pathway is active, β -catenin will dislodge from the destruction complex and thus won't be degraded. Accumulation of β -catenin will increase the probability of β -catenin translocating into the nucleus and act as a transcription factor that enhances cell growth, proliferation, and migration. In TNBC, an elevated expression of β -catenin is related to a decreased overall survival. Other than that, TNBC cells with β -catenin knocked down resulted in severe deficiencies in migration and anchorage-independent clonogenicity (J. Xu, Prosperi, Choudhury, Olopade, & Goss, 2015). Additionally, inhibition of β -catenin in TNBC cells resulted in sensitization to doxorubicin and cisplatin (J. Xu et al., 2015), which implied the importance of β -catenin in chemoresistant TNBC.

HIPPO is another well-known pathway that is involved in cell proliferation and apoptosis. When the HIPPO pathway is on, the protein cascade will result in the phosphorylation of YAP/TAZ. YAP and TAZ are transcriptional co-activators. They commonly interact with TEAD transcriptional factors to stimulate the transcription of corresponding genes (Plouffe et al., 2018). YAP/TAZ are phosphorylated and sequestered in the cytoplasm when HIPPO is on. In contrast, when HIPPO is off, YAP/TAZ can translocate into the nucleus and interact with TEAD, resulting in the transcription of genes that promote proliferation (Moroishi et al., 2015). Activation of YAP/TAZ contributes to the aggressiveness in many different cancers (Zanconato, Cordenosi, & Piccolo, 2016). In breast carcinoma, activation of YAP/TAZ has a significant impact on metastasis, tumorigenesis, stemness, and therapeutic resistance (Zanconato et al., 2016). Linking TRIM29 to YAP1, a recent study reported that TRIM29

increases proliferation in pancreatic cancer by directly binding to YAP1, thus preventing ubiquitination of YAP1 (Deng et al., 2021). In our study, overexpressing TRIM29 in MDAMB231 resulted in an enrichment of YAP1. Intriguingly, we also observed an elevated level of YAP1 in TRIM29KO cells compared to vector control. It is unclear at this point why YAP1 is elevated with TRIM29KO but this interesting observation needs further enquiry into plausible feedback loops. Consistent with our *in vitro* findings, the breast tumors generated from carboplatin-resistant HCC1806 cells with TRIM29KO were not smaller than those generated from carboplatin-resistant HCC1806 with competent TRIM29. We suspect that some compensatory actions have taken place.

Our silico analysis pointed out that TRIM29 is enriched in chemoresistance TNBC. In the present study, we explored the impact of TRIM29 in the TNBC cell lines. According to our results, overexpressing TRIM29 in MDAMB231 will result in elevated levels of YAP1 and β -catenin. TRIM29 overexpression in MDAMB231 also confers resistance to carboplatin *in vitro*. We also found that genetic knockout of TRIM29 in carboplatin resistant HCC1806 would result in a decreased level of β -catenin, but not YAP1. This could be a result of a compensatory mechanism that needs to be explored. The upregulation of YAP1 in the TRIM29KO carboplatin resistant cells may explain the larger tumor formed in mice.

Moreover, we noted that knocking out TRIM29 in carboplatin resistant HCC1806 significantly hindered its clonogenicity and migratory potential. It also resulted in deprivation of resistance to carboplatin. In closing, our result indicated that TRIM29 is functionally important for chemoresistance and migration in TNBC. Inhibitors for TRIM29 have not yet been found. Yet, since our findings also suggested that YAP1 and β -catenin are the downstream effectors of TRIM29, it could be a good idea to incorporate YAP1 inhibitors (Verteporfin, CA3) or β -catenin inhibitors (RXC004) with the combination of chemotherapy for TNBC patients.

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Curriculum Vitae

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RESEARCH INTEREST

The area of study in which I am most enthusiastic about is cancer-related research. I wish to improve medical treatment and/or contribute to disease prevention.

CURRENT RESEARCH WORK

Triple-negative breast cancer (TNBC) is a highly aggressive subtype of breast cancer. Although its prevalence is rather humble (12.7%), it is responsible of 40% of breast cancer-related mortality. Since chemotherapy is the mainstay of TNBC treatment, development of chemoresistance greatly contribute to the poor survival rate of TNBC. In my current research, I am trying to look for functionally important molecular factor that contribute to the maliciousness of TNBC.

EDUCATION

Master of Science, Biochemistry and Molecular Biology – Johns Hopkins University – Expected in June 2022

- 1st year thesis: Genetic Screening in cancer: in vivo intervention with CRISPR – a key that opens many doors
 - Advisor: Dr. Jiou Wang
- 2nd year thesis: To Study the Chemoresistance Mystery of TNBC
 - Advisor: Dr. Dipali Sharma

Bachelor of Science, Biomedical Sciences – University of Washington, Tacoma – June 2020

- Capstone Title: Examining the Role of Phosphorylation in the SK-rich Region of the Kinetochore-Associated Stu2 Protein in Budding Yeast
 - Advisor: Dr. Jack Vincent

Associate of Science, Biochemistry – Seattle Central College, Seattle – Aug 2016

OTHER EXPERIENCE

- Reviewed a manuscript from *International Journal of Cancer Research and Molecular Mechanisms*
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PUBLICATION

- **Wu Q, Siddharth S, Sharma D.** 2021. Triple Negative Breast Cancer: A Mountain Yet to Be Scaled Despite the Triumphs. *Cancers*. 13(15), 3697.

RESEARCH EXPERIENCE

Research Associate

Winter 2020 – Spring 2022

Johns Hopkins School of Medicine, Baltimore, MD

Supervisor: Dr. Dipali Sharma

- Skillful in maintaining human cell culture and performing functionality assays.
- Ability to perform quantitative assay such as RT-qPCR, conventional PCR, and immunoblotting.
- Presented updated research data to supervisor regularly.
- Identified candidate genes that are upregulated in triple negative cancer patients with chemoresistant tumor with GSEA.

Undergraduate Research Associate

Summer 2019 – Spring 2020

University of Washington, Tacoma

Supervisor: Dr. Jack Vincent

Collaborator: The Biggins Lab at the Fred Hutchinson Cancer Research Center

- Designed PCR primers for site-directed mutagenesis in budding yeast.
- Performed spot tests to compare the growth between mutant and parental strains.
- Confirmed the induced mutation via DNA sequencing and PCR reaction.
- Presented the research outcome to the Biggins Lab in Fred Hutchinson Cancer Research Center.

LABORATORY SKILLS

Molecular biology:

Analytical DNA gel electrophoresis, RT-qPCR, recombinant DNA cloning techniques, restriction digestion, primer design, DNA-RNA isolation, cDNA synthesis

Microbiology

Basic microbiology laboratory techniques, transformation of bacteria and yeast, culture bacteria and yeast

Biochemistry:

Immunoblotting, immunoprecipitation, purification, and extraction of DNA by gel electrophoresis

Tissue/cell culture:

Cell transformation, cell transfection, cell line maintenance, viability assays and functionality assays

In silico data analysis:

Gene set enrichment analysis (GSEA), data curation and analysis from publicly available databases (Gene Expression Omnibus, Expression Atlas-EMBL), UALCAN, survival analysis using KM plotter, CHAT (cancer hallmark analysis tool) analysis

Animal experiment (mice):

Tumor measurement, necropsy, establishment of cell line-derived xenograft

HONORS & AWARDS

2020 *Magna cum laude*, UW Tacoma

2020 SAMURS Biomedical Sciences Poster 2nd Place, UW Tacoma