PIONEER
ACADEMIC PUBLISHING LIMITED

Current Research in Medical Sciences
ISSN 2958-0390
www.pioneerpublisher.com/crms
Volume 4 Number 1 January 2025

BRCA Mutation Negatively Impacts TPT1/TCTP-Regulated Autophagic Response in Breast Cancer

Brian Xi[1]

[1] Milton Academy, 170 Centre St, Milton, MA 02186, United States
Correspondence: Brian Xi, Milton Academy, 170 Centre St, Milton, MA 02186, United States.

doi:10.56397/CRMS.2025.01.04

Abstract

Autophagy plays a crucial role in both cancer prevention and cancer progression. The multi-faceted process allows for the degradation of cellular components as well as their recycling for energy. TPT1 (tumor protein, translationally controlled 1) overexpression is associated with cancer progression in several tumor types, and it has been identified as a regulator of autophagic expression through its control of various upstream pathways, including mTOR and p53. Through the inhibition of p53 and the indirect promotion of mTOR, TPT1 effectively impedes the autophagic response. Commonly mutated in breast cancer tumor cells, BRCA is a tumor suppressor protein in which a mutation may lead to tumorigenesis. A search in the TCGA database revealed that TPT1 overexpression is associated with increased survivability in breast cancer patients with BRCA mutations; however, previous studies iterated the opposite, indicating TPT1 overexpression led to poor prognosis in breast cancer. In this study, we describe the influence of BRCA mutations on TPT1-mediated autophagic pathways. In breast cancer wild-type cells, TPT1 knockdown leads to increased autophagic expression and autophagosome formation while in BRCA mutant cells, the effect is negated. Furthermore, we demonstrate that BRCA mutations block TPT1-mediated upregulation of mTOR and its downstream signaling molecules. We further demonstrate that BRCA mutations do not impact AMPK phosphorylation by p53 or the repression of p53 through TPT1. Overall, these findings suggest that BRCA mutations can antagonize TPT1-mediated autophagy via the regulation of p53-independent mTOR signaling. Further understanding of the interaction between these pathways may contribute to new therapeutic approaches to treating breast cancer patients.

Keywords: TPT1/TCTP, p53, mTORC1, autophagy, BRCA, breast cancer

1. Introduction

As one of the most prominent malignancies in the world, breast cancer terrorizes humanity with its grasp on both males and females. As the leading cause of cancer deaths in females around the world, roughly 42,000 women in the US fall victim to this malignancy every year (Sancho-Garnier & Colonna 2017; Centers for Disease Control and Prevention, 2022). Though uncommon in men, breast cancer still claims nearly 20% of men diagnosed with the malignancy (Centers for Disease Control and

Prevention 2022). As a tumor suppressor, BRCA fabricates anti-oncogene proteins, helps repair DNA damage, and preserves the integrity of genetic material. As a result, lack of functioning BRCA proteins may result in the development of cancer cells (National Cancer Institute 2020). The mutation of BRCA1 and BRCA2 are two prominent oncogenes within breast cancer. Of the six commonly known molecular subgroups in breast cancer — Luminar A, Luminar B, HER2+, Triple Negative (TNBC), Claudin Low, and normal-like — BRCA mutations typically occur in TNBC, which tends to be more aggressive and difficult to treat (Mehrgou & Akouchekian, 2016). In addition to breast carcinoma, mutations in BRCA 1 or BRCA 2, located on chromosome 17q and chromosome 13q respectively, may also increase susceptibility to ovarian or prostate cancer (Mehrgou & Akouchekian, 2016). Though external factors, including contact with carcinogens and genetics, must be brought into consideration, BRCA mutations account for approximately 30% of all breast and ovarian cancer diagnostics (Mehrgou & Akouchekian, 2016).

Macroautophagy (herein referred to as "autophagy") is a homeostatic pathway that facilitates lysosome-dependent degradation and recycling of proteins, organelles, or pathogens (Li et al., 2021; Debnath et al., 2023). The process includes the fusion of autophagosomes with lysosomes to begin the degradation process of its intracellular contents (Niklaus et al., 2021). Such a function provides the cell with energy and cellular building blocks (Niklaus et al., 2021); autophagy also serves as a cancer prevention mechanism, and its dysfunction may lead to tumorigenesis. The impact of autophagy was further proven in vivo, where mouse models demonstrated that homozygous or heterozygous knockdown of essential autophagic-inducing genes resulted in a higher likelihood of developing malignancies (Galluzzi et al., 2015). Specifically, autophagy contributes to oncogene-induced cell death or senescence (Galluzzi et al., 2015). However, it has also been shown that autophagy may contribute to cancer progression; its maintenance of cancer stem cells (Galluzzi et al., 2015) and support of cell survival upon extracellular matrix detachment (Niklaus et al., 2021) among other functions establishes its role as a facilitator of tumorigenesis. Through its promotion of cell survival under nutrient stress, autophagy has also been seen as oncogenic (Glick et al., 2010). Despite this, autophagy remains an important player in carcinogenesis and is highly context-dependent, varying from a facilitator of cancer progression to functioning as a tumor suppressor.

As a regulator of autophagy, TPT1/TCTP (tumor protein, translationally controlled 1) plays an important role in the upstream signaling pathways of autophagy. Highly expressed in all eukaryotic organisms, TPT1 functions as a key regulator of cell growth, cell cycle progression, and apoptosis (Acunzo et al., 2014). It has also been shown that TPT1 maintains genomic stability following strenuous DNA-damaging circumstances (Zhang et al., 2012). In addition, it also plays a role in the inhibition of autophagy through its regulation of p53 and mammalian Target of Rapamycin (mTOR) Complex I (mTORC1). An established tumor suppressor, p53 induces cell cycle arrest, apoptosis, and senescence (Cui et al., 2021). With over 50% of tumor masses carrying a p53 mutation, it has become a commonly recognized sign of malignancies (Cui et al., 2021). mTOR, on the other hand, coordinates cellular processes including growth, survival, and autophagy (Cui et al., 2021). Split into two sections, mTORC1 and mTORC2 have distinct structures and functions, including the regulation of cell growth and proliferation (Cui et al., 2021). TPT1 was shown to promote p53 degradation through the promotion of MDM2-mediated ubiquitination (Amson et al., 2012). Such is supported by evidence in vivo, using TPT1 heterozygous mice, as well as in vitro, using homogeneous time-resolved fluorescence (HTRF) (Amson et al., 2012). Through the promotion of negative mTOR regulators, including AMPK (Feng et al., 2007), TSC2 (Feng et al., 2007), and LKB1, p53 effectively inhibits mTOR activity and cell growth and proliferation (Cui et al., 2021). Furthermore, p53 can regulate mTOR activity using miRNA at the post-transcriptional level (Cui et al., 2021). Through its inhibition of p53 activity, TPT1 indirectly promotes mTOR expression. It is well known that mTOR inhibits autophagic function. One manner in which it accomplishes this is through the phosphorylation of unc-51-like autophagy-activating kinase 1 (ULK1), one of several proteins crucial to the initiation of autophagy. mTOR phosphorylates ULK1 on serine 757 (Ser757) and serine 758 (Ser758),

interfering with the binding of AMP-activated protein kinase (AMPK) to ULK1 (Kim et al., 2011). AMPK, on the other hand, promotes autophagy through the phosphorylation of ULK1 at serine 317 (Ser317) and serine 777 (Ser777), initiating the autophagic process (Galluzzi et al., 2015).

TPT1 overexpression is found to be associated with higher cancer progression and lower survivability in cancer patients (Amson et al. 2012). However, TCGA data from the University of Alabama at Birmingham revealed that high TPT1 expression was associated with higher survivability in breast cancer patients with BRCA mutations (Figure 1). Therefore, we hypothesized that BRCA mutations played a crucial role in TPT1-regulated pathways, such as the autophagic response, in breast cancer. In this study, we investigate the underlying relationship between BRCA mutations and TPT1-regulated autophagic pathways in breast cancer. Our results indicate that BRCA mutations negatively regulate TPT1-mediated autophagy in breast cancer cells via mTOR signaling. However, such regulation is independent of AMPK expression or p53 activity.

2. Methods and Materials (Hypothetical Methods; Modified from Zhu et al. and Bae et al.)

2.1 Cell Culture

MDA-MB-436 and MDA-MB-231 cells were purchased from the American Type Culture Collection (ATCC, HTB-26 and HTB-130) and maintained in Dulbecco's modified essential medium (DMEM) supplemented with 10% fetal bovine serum (FBS) at 37°C and 5% CO_2. MDA-MB-436 and MDA-MB-231 cells stably expressing GFP-LC3 (MDA-MB-436 GFP-LC3 and MDA-MB-231 GFP-LC3) were also maintained in DMEM with 10% FBS.

2.2 Antibodies and Reagents

The reagents, chemicals, and antibodies used in this paper, including sources, listed below: LC3 (Novus Biologicals, NB100–2331), GFP (Santa Cruz Biotechnology, sc-9996), RFP-LC3 (EMD Millipore, 17-10143), phospho-RPS6KB (Thr389 & Ser371; Cell Signaling Technology, 9234 and 9208), RPS6KB (Cell Signaling Technology, 2708), P53 (Proteintech Group, 10442-1-AP), AMPK-α (Cell Signaling Technology, 2532), p-AMPK-α (Cell Signaling Technology, 2535), β-actin (Proteintech Group, 20536-1-AP), p-mTOR (Ser2448 & Ser2481; Cell Signaling Technology, 9234 & 2974), mTOR (Cell Signaling Technology, 2983), phospho-EIF4EBP1 (Thr37/46; Cell Signaling Technology, 2855), EIF4EBP1 (Cell Signaling Technology, 9452), bafilomycin A1 (Sigma-Aldrich, B1793), rapamycin (Sigma-Aldrich, R8781), and TPT1 (AbFrontier, YFMA10968).

2.3 Plasmids and shRNA Transfection

Monomeric RFP (mRFP)-GFP-LC3, and RFP-LC3 plasmids used for autophagosome maturation detection. TPT1 knockdown from the use of shTPT1; transfection used Attractene Transfection Reagent (Qiagen, 301005).

2.4 Stable Cell Line Generation

Stable cells were generated in accordance with standard procedure with minor modification. Transduced GFP-LC3 cells were selected with 1.4 μg/ml puromycin and infected cells were selected with 1 μg/ml of puromycin.

2.5 Autophagy Analysis

Positive controls for autophagy were obtained through rapamycin treatment. Cells were treated with rapamycin at 200 nM final concentration. Control cells were treated with DMSO.

2.6 Confocal Microscopy and Immunofluorescence Staining

Respective GFP-LC3 cells were fixed with 100% methanol then counterstained with ProLong Gold Antifade Mountant with DNA Stain DAPI (Invitrogen, P36931). For the tandem fluorescent LC3 reporter assay, respective cells were transfected with RFP-GFP LC3. After 24 hours, trials were fixed with 3.7% paraformaldehyde and counterstained with DAPI. Cell photos were taken using a fluorescence confocal microscope (Zeiss, LSM510). Quantification of autophagy was done by recording the number of LC3 puncta in 10 cells per sample. Mean values calculated from at least three separate trials.

2.7 Immunoblotting and Immunoprecipitation

Immunoblotting was performed in accordance with standard procedure with the addition of slight modifications. Samples were centrifuged at 12,000 rpm for 20 min at 4°C. Subsequently, protein samples (40 μg protein/lane) were electrophoresed on 4% and 12% SDS polyacrylamide electrophoresis (SDS-PAGE) gel. Thereafter, gel was cropped based on molecular weight for specific antibody binding, and results

were fixed with 100% methanol and transferred to polyvinylidene difluoride (PVDF) membrane (Millipore). Afterwards, the membrane was incubated overnight in 4°C. Lastly, proteins were visualized using electrochemiluminescence (ECL) (Wanlei Bio) and detected using BioImaging System (UVP Inc.). Immunoprecipitation assays were performed analogously with standard procedure with slight adjustments. In addition to centrifuging at 12,000 rpm for 15 min at 4°C, antibodies were added to lysates after spinning and rotated overnight at 4°C. 30 µl of Protein G Agarose Bead slurry (Cell Signaling Technology, 37478) were added before being washed with Phosphate Buffered Saline solution (PBS). Gel electrophoresis was performed as described previously.

3. Results

3.1 TPT1 Overexpression Is Associated with High Survival in BRCA Mutated Breast Cancer Patients

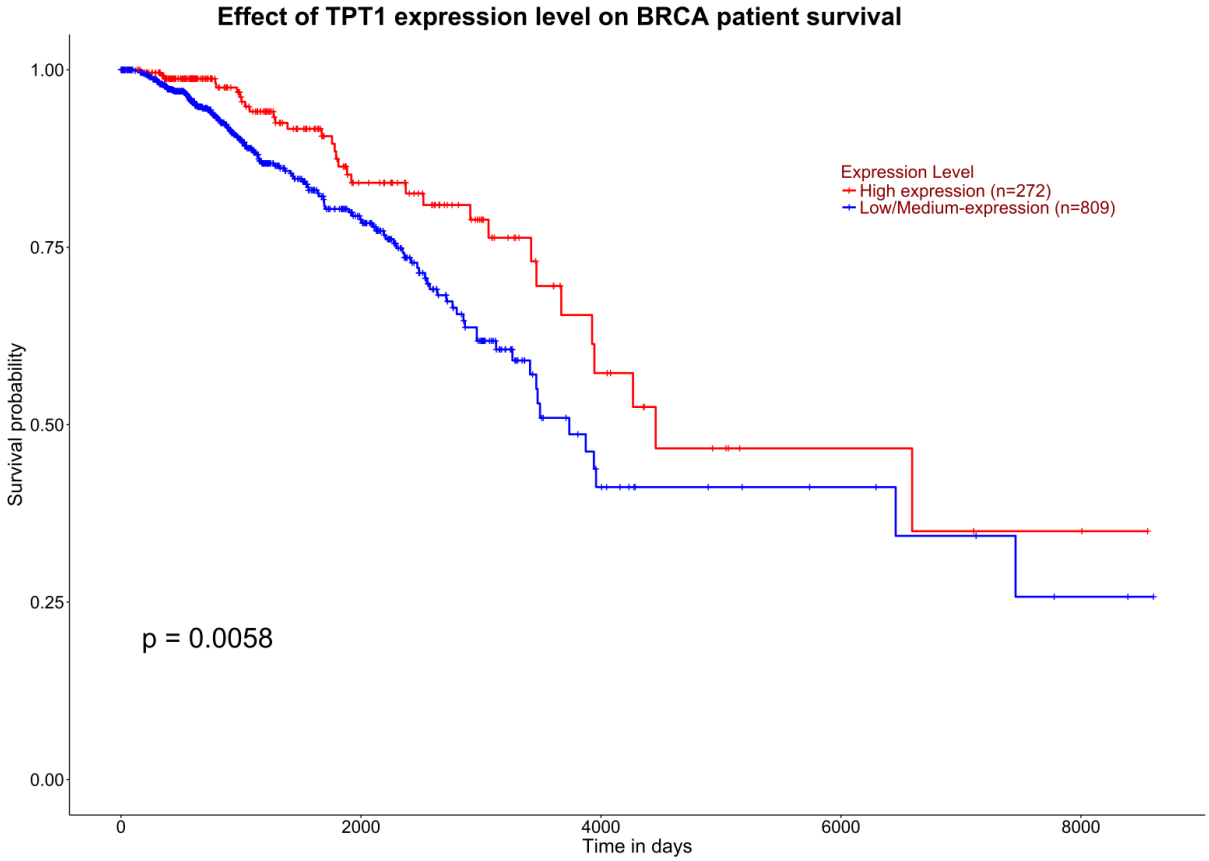

Figure 1. Obtained from UALCAN TCGA database

Data shows high expression levels of TPT1 consistently corresponds to higher survival probability in BRCA patients. On the contrary, lower expression, in 809 samples, led to poorer prognosis. $P < 0.01$.

It has been shown that in 508 breast cancer patients, high expression of TPT1 meant poor prognosis, as it is often connected with mutated p53 (Amson et al., 2012). However, TCGA data from the University of Alabama at Birmingham demonstrated that higher expression of TPT1 is associated with higher survivability in over 270 patients with a BRCA mutation, while over 800 patients with lower expression of TPT1 exhibited decreased survivability (Figure 1). The results conclusively suggest that BRCA mutations may alter TPT1-mediated pathways.

3.2 BRCA Mutations Negatively Regulates TPT1-Mediated Autophagy

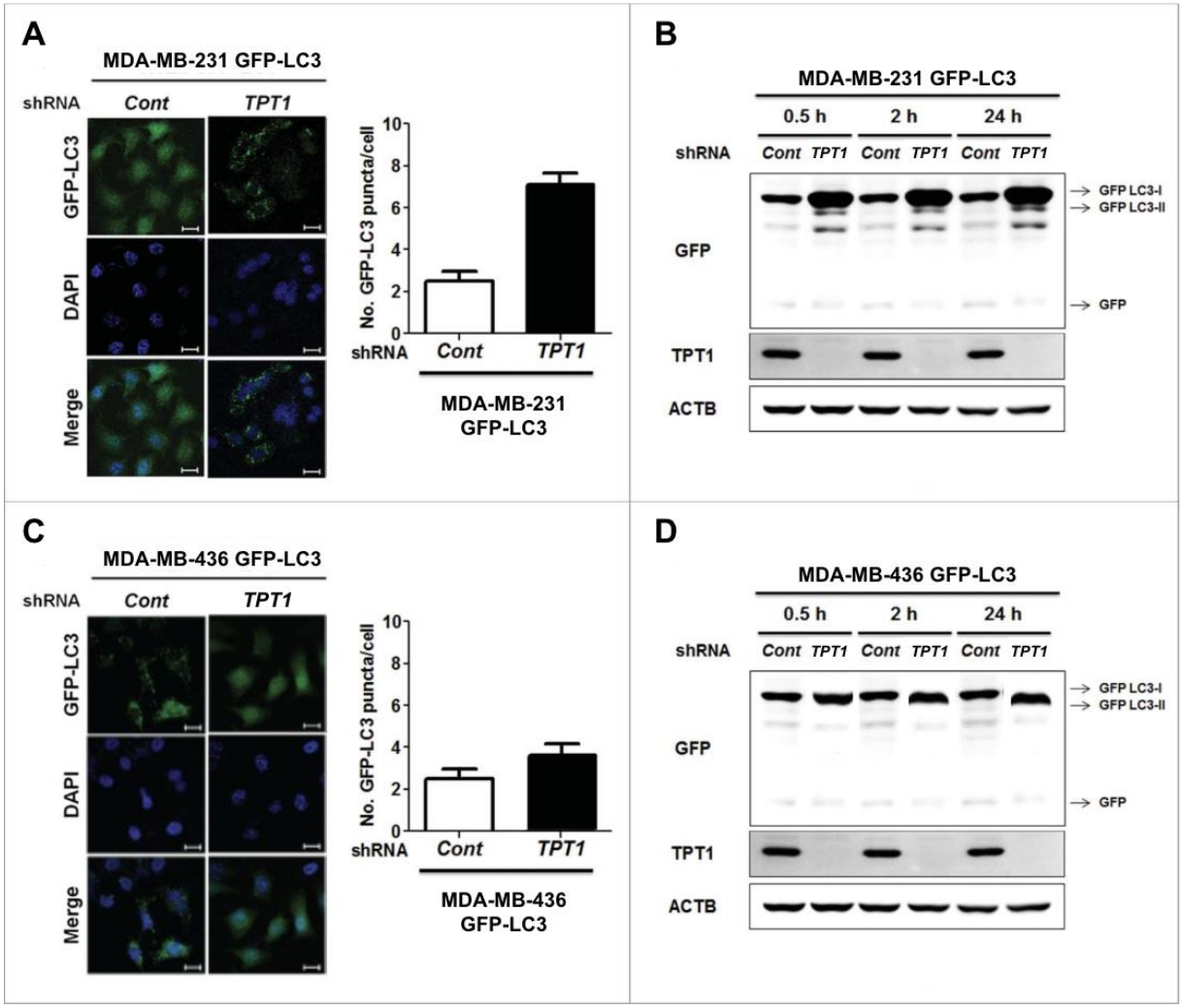

Figure 2. (Hypothetical results)

Figure modified from Bae et al (2017). BRCA negatively impacts TPT1-regulated autophagy after the knockdown of TPT1. (A and C) GFP-LC3 puncta were analyzed in respective cell lines. GFP-LC3 dots per cell quantified. Representative images were taken at x600 magnification. Cells stained with DAPI for the nucleus (blue). Scale bars: 20 μm. Presents visual contrast of autophagosome production between MDA-MB-436 and MDA-MB-231 as well as quantification. (B and D) Immunoblotted lysates from respective cells were immunoblotted with various antibodies. ACTB served as a loading control. Clearly presents a difference in autophagic response while also ruling out possible manipulation from nutrient levels.

To understand the involvement of BRCA mutations in the TPT1 pathway, the role of TPT1 as a key regulator of autophagy was explored. TPT1-mediated autophagy was compared in MDA-MB-231 cells, which express wild-type BRCA, and MDA-MB-463 cells, which exhibit a BRCA mutation. Autophagic responses were measured with GFP puncta assay, in which the sites of autophagy can be identified by using GFP-LC3, which attaches the green fluorescent protein to autophagosomes. Through the analysis of the immunofluorescence results, the number of puncta per cell can be quantified as an indicator of the level of autophagy. As shown in Figure 2, the knockdown of TPT1 with shRNA consistently increased the autophagic response in MDA-MB-231 cells, as expected. The puncta per cell in the MDA-MB-231 stayed around 7-8, which is roughly threefold higher than that of the control cells (Figure 2A). This observation proved consistent with previous findings, which stated that TPT1 negatively regulates autophagy (Bae et al., 2017). In comparison, mutant cell line MDA-MB-436 demonstrated similar expression of autophagy within the control samples, but the shTPT1 samples exhibited decreased levels of puncta per

cell ratio, at roughly 4 (Figure 2C). This data suggests that BRCA mutations negatively regulate TPT1-pathways that lead to autophagy. Consistently, immunoblotting in these cells showed that GFP-LC3-II protein levels also increased (Figure 2B) when compared to the control in the BRCA wild-type cell line, while the opposite occurred in the mutant cell line (Figure 2D). The control samples remained consistent between the wild-type and the mutant cell lines, yet the use of shTPT1 in the mutant cells displayed a clear decrease compared to the wild-type cells. This observation further reinforces the negative role of BRCA mutations in TPT1-mediated autophagic pathways in breast cancer. To eliminate the possibility of nutrient deficiency and consumption affecting the autophagic levels, the cells were harvested at various time points and assessed. Cell lysates from the respective cell lines were harvested after 0.5 h, 2 h, and 24 h. As shown in Figure 2B, the MDA-MB-231 cell line showed consistently induced autophagy across the various time points after the knockdown of TPT1 expression. However, the shTPT1 samples of the MDA-MB-231 and MDA-MB-436 cell lines displayed a clear difference, with the mutant samples showing an evident decrease (Figure 2D). Taken together, this data suggests that BRCA mutations negatively regulate TPT1-mediated autophagic pathways in breast cancer cells.

3.3 Presence of the BRCA Mutation Negates TPT1-Mediated Autophagosome Maturation

The complete process of autophagy, known as autophagic flux, consists of autophagosome formation, autolysosome generation, and degradation (Bae et al., 2017). The increased presence of GFP-LC3 can be due to either an increase in autophagosome formation or reduced autophagic degradation. In the presence of bafilomycin A_1, an inhibitor of the degradation stage, the effect of BRCA mutations on TPT1-mediated autophagosome formation can be assessed. Consistently with previous reports, the knockdown of TPT1 with shRNA led to increased levels of autophagosome formation in the wild-type cell line, while this effect is annulled in the mutant cell line (Figure 3A and 3B). The quantitative data revealed that, after bafilomycin A_1 culturing, the control of the wild-type cells had roughly 25 puncta per cell, compared to the 30 puncta per cell of the wild shTPT1 samples. However, the mutant control samples had roughly 20 puncta per cell while the mutant shTPT1 samples exhibited only 22 puncta per cell. The results confirm that BRCA mutations inhibit the function of TPT1 pathways connecting to the formation of autophagosomes in breast cancer.

The formation of autophagosomes is followed by autolysosome generation, which involves the bonding of autophagosomes with lysosomes to further the autophagic process. Since autolysosomes have low pH values, the use of monomeric red fluorescent protein (mRFP)-GFP is used to overcome the sensitivity of the GFP fluorescence. For this experiment, the cell lines MDA-MB-436 and MDA-MB-231 were further transfected with mRFP-GFP-LC3. The wild type displayed an increase with mRFP-LC3, consistent with previous discoveries, at roughly 12 red puncta per cell (Figure 3C and 3E). Immunofluorescence results also display a clear difference between the shTPT1 and control data in the wild-type cell line (Figure 3C). However, there was no noticeable difference in the mRFP expression among the mutant cell line samples (Figure 3D). Quantitatively, the control ended up with around three red puncta per cell compared to the five red puncta per cell with shTPT1 (Figure 3F). This represented a significant difference between the mutant and wild-type cell lines under the inhibition of TPT1, with the wild type producing threefold as much red puncta as the mutant cells (Figure 3F). These results affirm that BRCA mutations prevent the TPT1-mediated autophagosome formation but also autophagosome maturation.

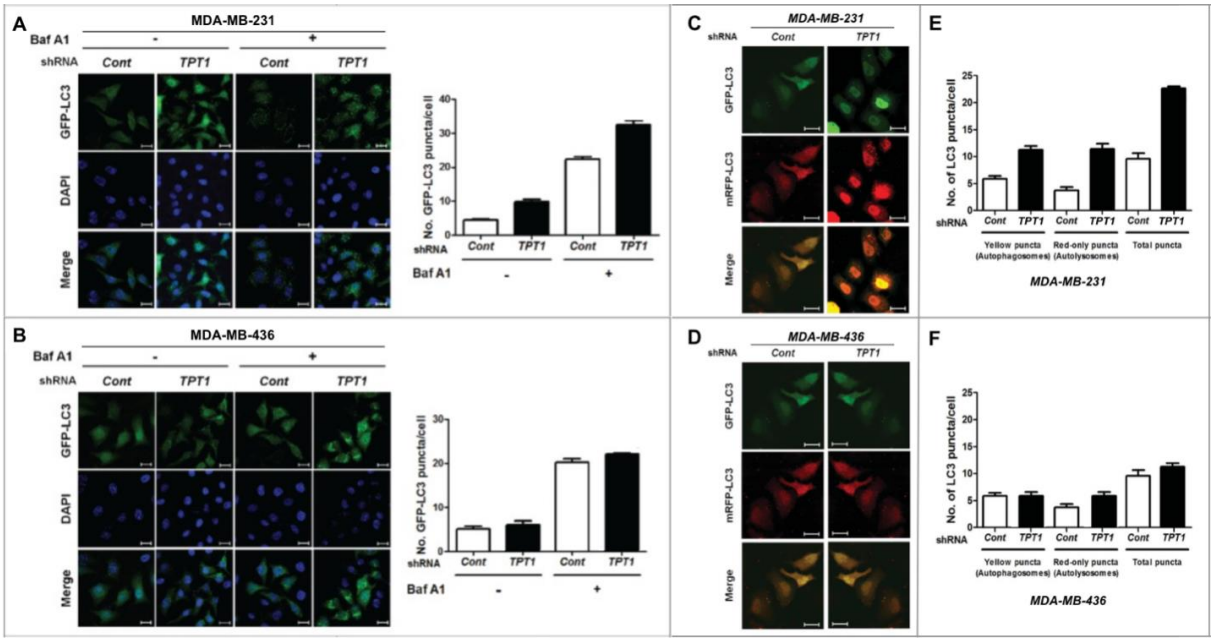

Figure 3. (Hypothetical results)

Figure modified from Bae et al (2017). BRCA mutations enhance autophagosome production and maturation under the inhibition of TPT1 in breast cancer. (A and B) Cell lines were cultured with bafilomycin A_1 before taking immunofluorescence data. Representative images were taken at x800 magnification. Scale bars: 20 μm. Clarifies the role of BRCA mutations in the autophagic flux. (C and D) Respective cell lines were transfected with mRFP-GFP-LC3 for 24 hrs. Representative images were taken at x800 magnification. Scale bars: 20 μm. Presents visual aid of autophagosome formation and autolysosome formation between contrasting cell lines. (E and F) The number of yellow puncta and red puncta was quantified for respective cell samples, and the total puncta was calculated. Quantitative representation of data presented in C and D to emphasize the contrast between the presence of BRCA and lack thereof.

3.4 BRCA Inhibits TPT1-Mediated mTOR Activity Through Dephosphorylation of mTOR Downstream Signals

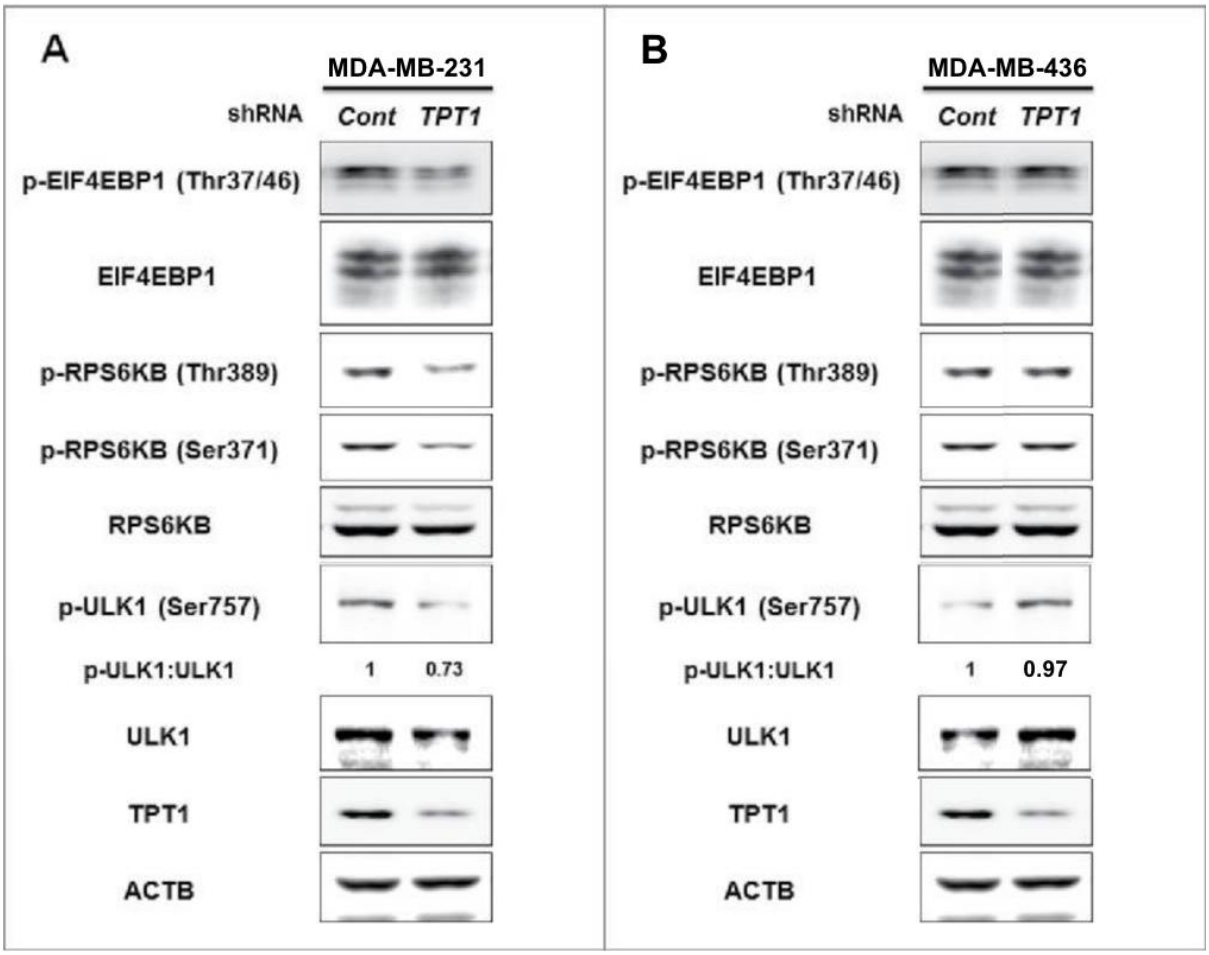

Figure 4. (Hypothetical results)

Figure modified from Bae et al (2017). Downstream signaling molecules dephosphorylated in the presence of BRCA. (A and B) Cell lysates from the respective cell lines were immunoblotted with the indicated antibodies. ACTB served as a loading control. The difference in phosphorylation rates of various mTOR downstream molecules and autophagic initiation signaling molecules between BRCA-affected and BRCA-unaffected cell samples portrayed through Western blotting.

To investigate the relationship between BRCA mutations and the mTOR component of the TPT1-mediated autophagic pathway, glucose starvation can be used to test the phosphorylation of various downstream targets of mTOR. The analysis of mTOR downstream signalers RPS6KP, EIF4EBP1, and ULK1 reveals the changes in mTOR activity. The shTPT1 data from the wild-type cell line demonstrated consistent results with previous findings as EIF4EBP1 and RPS6KB were dephosphorylated (Figure 4A). On the other hand, in the mutant cell line, the control data and the shTPT1 data revealed no noticeable difference, as shown by the immunoblotting data, suggesting that both cells produced similar quantities of mTOR downstream signalers (Figure 4B). In addition, the shTPT1 data from the wild type showed that ULK1 was dephosphorylated on the Ser757 residue (Figure 4A). This is consistent with previous findings (Bae et al., 2017) and indicates a decrease in the inhibition potential of mTOR against autophagy (Kim et al., 2011). Yet, mutant cell lysates immunoblotted with ULK1 antibodies illustrated no perceptible change as the ratio of ULK1 phosphorylation on ser757 remains almost the same at 0.97 (Figure 4B). In contrast, the wild-type cell line data saw a phosphorylation ratio of 0.73. Therefore, these results suggest that BRCA mutations negatively regulate TPT1-mediated mTOR downstream signaling pathways.

3.5 AMPK Expression Through p53 Regulation Unaffected by the Presence of BRCA Mutations

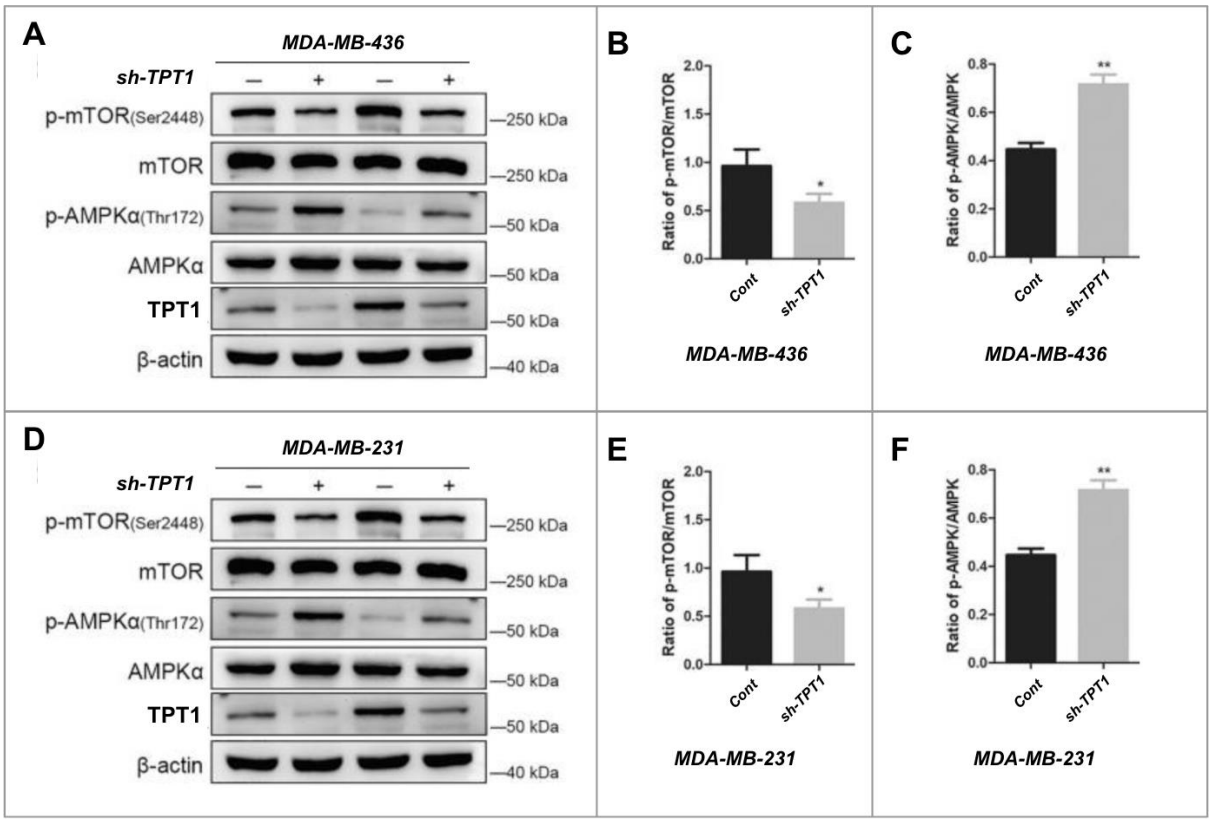

Figure 5. (Hypothetical results)

Figure modified from Zhu et al (2022). Presence of BRCA has no effect on phosphorylation of mTOR and AMPK. (A and B) MDA-MB-436 and MDA-MB-231 were transiently transfected with sh-TPT1 and immunoblotted with indicated antibodies. β-actin served as a loading control. The presence of BRCA had no effect on the phosphorylation of mTOR and AMPK. However, the inhibition of TPT1 saw an increase in AMPK phosphorylation and a decrease in mTOR phosphorylation compared to trials with increased TPT1 expression. (B and E) Phosphorylation of mTOR was quantified and the ratio between phosphorylation and dephosphorylation was calculated. Data reveals the rate of phosphorylation of respective cell lines and allows for clear comparison. (C and F) AMPK phosphorylation rate quantified between control and TPT1-inhibited, revealing the rate of phosphorylation of respective cell lines and allowing for clear comparison.

To understand whether BRCA affects other components of the TPT1-mediated autophagic pathway, levels of AMPK expression, an inhibitor of mTOR activity, through p53 can be tested to determine whether BRCA mutations regulate the upstream signaling pathways of mTOR. By inhibiting TPT1 using shTPT1 and determining the phosphorylation of mTOR and AMPK, the effect of BRCA on AMPK expression can be determined. As shown in Figure 5, the mutated cell line showed relatively similar results as that of the wild type. With TPT1 inhibited, the phosphorylation of both samples decreased compared to the control, with a ratio of roughly 0.75 between p-mTOR and mTOR (Figure 5B and 5E). Similarly, with the p-AMPK to AMPK ratio, the inhibition of TPT1 resulted in a higher rate of AMPK at roughly 0.8 compared to a rough 0.5 of the control (Figure 5C and 5F). However, further experimentation can be done in areas that involve other pathways connecting p53 and mTOR to understand whether BRCA mutations play a role in such areas. All these results indicate that BRCA does not play a role in the upstream inhibition of mTOR through p53-mediated AMPK pathways.

3.6 BRCA Mutation Does Not Play a Role in the Suppression of p53 via TPT1/TCTP

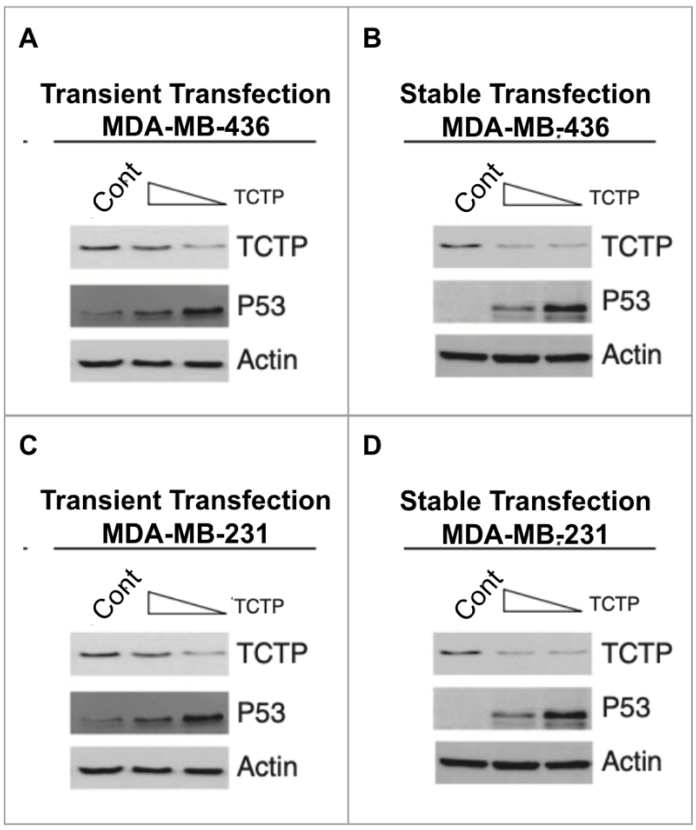

Figure 6. (Hypothetical results)

Figure modified from Amson et al (2012). TPT1/TCTP does not affect p53 expression in breast cancer cells. (A and C) MDA-MB-436 and MDA-MB-231 samples were transiently transfected with TPT1 and immunoblotted with indicated antibodies. Actin served as a loading control. As TPT1 expression decreased, p53 expression increased in respective cell lines. (B and D) MDA-MB-436 and MDA-MB-231 samples were stably transfected with TPT1 and immunoblotted with indicated antibodies. Actin served as a loading control. Samples indicated the relationship between TPT1 and p53 over the long term and solidified the lack of BRCA relevance.

Through the examination of TPT1/TCTP repression of p53 under the influence of BRCA mutations, it can be determined whether BRCA influences the control of TPT1 on p53. Transient transfection of decreasing amounts of TPT1 in MDA-MB-436 and MDA-MB-231 demonstrated no differences in the expression of p53 (Figure 6A and 6C). As TPT1 expression gradually decreased, the expression of p53 showed a reciprocal increase within both cell lines, as consistent with previous data (Figure 5A and 5C). Similarly, the effect of BRCA was echoed during stable transfection. The two cell lines heeded comparable results, both demonstrating increased aggregations of p53 with decreased expression of TPT1. These results signify the lack of correlation between BRCA mutations and TPT1-mediated p53 response.

4. Discussion

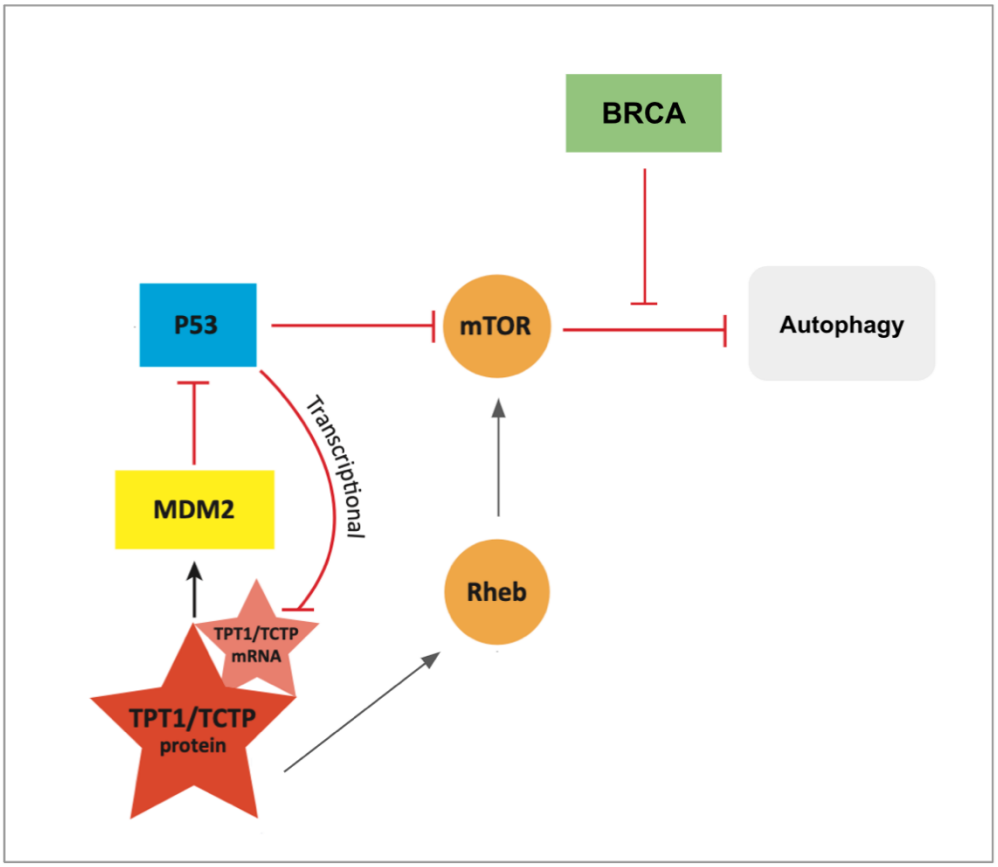

Figure 7. (Hypothetical Results)

Figure modified from Amson et al. (2013). TPT1 indirect inhibition of p53 by MDM2 results in the indirect promotion of mTOR activity, with indication of reciprocal repression (Amson et al., 2012). Furthermore, TPT1 directly promotes Rheb, which promotes mTOR activity, thereby TPT1 indirectly regulates mTOR. BRCA has been shown to inhibit mTOR-mediated autophagy, but it remains unclear whether it plays a role in the TPT1-Rheb pathway.

In this study, the role of BRCA mutations within the TPT1-mediated autophagic pathway was clarified through various in vitro experiments. A relationship between BRCA mutations and TPT1-regulated autophagy was first identified through the observation of autophagosome formation and maturation in BRCA mutant cells and BRCA wild-type cells. Similar expression levels of GFP-LC3 under the influence of bafilomycin A_1 in mutant control and shTPT1 cells emphasize the function of BRCA mutations as a negative influencer of the formation of autophagosomes. Upon further investigation into the various components of the pathway, it was found that BRCA mutations negatively regulate TPT1-mediated dephosphorylation of mTOR downstream signaling molecules. However, the lack of change between the MDA-MB-231 and MDA-MB-436 AMPK phosphorylation suggests that BRCA mutations do not play a role in the autophagic response via p53-mediated pathways. Similarly, BRCA mutations do not seem to affect TPT1-regulated p53 expression as both transient and stable transfection yielded similar results for both cell lines. This suggests that other pathways connecting to TPT1 must be considered to clearly grasp the effect of BRCA mutations on TPT1-mediated autophagic pathways.

Despite the exploration of the influence of BRCA mutations on p53-regulated AMPK expression, its connections to mTOR remain vast and unexplored. Further exploration needs to be done regarding other upstream pathways of mTOR associated with p53. Considering the extensive role of p53 in tumor suppression, it may also have other undetected regulatory functions of mTOR or autophagy. Additional experiments can be done to explore if there are other pathways connecting p53 to autophagy and the role of BRCA mutations in such pathways.

Another possibility is the direct connection between TPT1 and Rheb. Understanding whether BRCA mutations play a role in Rheb-related pathways may shed light onto the influence of BRCA on autophagy. The ubiquitination of Rheb has been shown to tightly regulate mTOR downstream signaling (Deng et al., 2019). TPT1 directly associates with Rheb through guanine nucleotide exchange activity as shown by in-vitro and in-vivo drosophila experimentation (Hsu et al., 2007). Through biochemical comparison, it has been observed that human TPT1 shows similar properties as that of drosophila, suggesting that TPT1 may regulate Rheb activity in human bodies as well and that TPT1 function is evolutionarily conserved (Hsu et al., 2007). Therefore, further experimentation could clarify the role of BRCA mutations within TPT1-mediated Rheb pathways.

TPT1 has been previously shown to be an important regulator of growth, protein synthesis, mitosis, and apoptosis (Acunzo et al., 2014). Its pivotal role in the regulation of tumor suppressor p53 highlights TPT1 as an area of interest. In addition, its key role as an antiapoptotic protein also underscores its importance in carcinomas. Furthermore, the role of autophagy as both a cancer preventer and a promoter of cancer proliferation accentuates its crucial role within tumorigenesis. A thorough understanding of the mechanism would not only allow for clinical targeting for cancer prevention, but also advance research regarding the possible hindering of cancer progression. Finally, taking into consideration the prevalence of BRCA mutations within breast cancer and others, further research may accentuate the role of BRCA in other pathways throughout the body. Nonetheless, development into the understanding of autophagy, TPT1, and BRCA would clarify such oncogenic pathways and highlight possible areas of targeted therapy.

References

Acunzo, J., Baylot, V., So, A., & Rocchi, P. (2014). TCTP as therapeutic target in cancers. *Cancer treatment reviews*, *40*(6), 760–769. https://doi.org/10.1016/j.ctrv.2014.02.007

Acunzo, J., Baylot, V., So, A., & Rocchi, P. (2014). The translationally controlled tumour protein (TCTP). *The International Journal of Biochemistry & Cell Biology*, *36*(3), 379-385. https://doi.org/10.1016/S1357-2725(03)00213-9

Amson, R., Pece, S., Lespagnol, A., Vyas, R., Mazzarol, G., Tosoni, D., Colaluca, I., Viale, G., Rodrigues-Ferreira, S., Wynendaele, J., Chaloin, O., Hoebeke, J., Marine, J., Di Fiore, P. P., & Telerman, A. (2012). Reciprocal repression between P53 and TCTP. *Nature Medicine*, *18*(1), 91-99. https://doi.org/10.1038/nm.2546

Amson, R., Pece, S., Marine, J. C., Di Fiore, P. P., & Telerman, A. (2013). TPT1/ TCTP-regulated pathways in phenotypic reprogramming. *Trends in cell biology*, *23*(1), 37–46. https://doi.org/10.1016/j.tcb.2012.10.002

Bae, S. Y., Byun, S., Bae, S. H., Min, D. S., Woo, H. A., & Lee, K. (2017). TPT1 (tumor protein, translationally-controlled 1) negatively regulates autophagy through the BECN1 interactome and an MTORC1-mediated pathway. *Autophagy*, *13*(5), 820–833. https://doi.org/10.1080/15548627.2017.1287650

Cui, D., Qu, R., Liu, D., Xiong, X., Liang, T., & Zhao, Y. (2021). The Cross Talk Between p53 and mTOR Pathways in Response to Physiological and Genotoxic Stresses. *Frontiers in cell and developmental biology*, *9*, 775507. https://doi.org/10.3389/fcell.2021.775507

Debnath, J., Gammoh, N., & Ryan, M. K. (2023). Autophagy and autophagy-related pathways in cancer. *Nature Reviews Molecular Cell Biology*. https://doi.org/10.1038/s41580-023-00585-z

Deng, L., Chen, L., Zhao, L., Xu, Y., Peng, X., Wang, X., Ding, L., Jin, J., Teng, H., Wang, Y., Pan, W., Yu, F., Liao, L., Li, L., Ge, X., & Wang, P. (2019). Ubiquitination of Rheb governs growth factor-induced mTORC1 activation. *Cell research*, *29*(2), 136–150. https://doi.org/10.1038/s41422-018-0120-9

Centers for Disease Control and Prevention. (n.d.). Basic Information About Breast Cancer. https://www.cdc.gov/cancer/breast/basic_info/index.htm#:~:text=About%2042%2C000%20women%20and%20500,breast%20cancer%20than%20White%20women. Accessed Jun 26, 2023.

Feng, Z., Hu, W., de Stanchina, E., Teresky, A. K.,

Jin, S., Lowe, S., & Levine, A. J. (2007). The Regulation of AMPK β1, TSC2, and PTEN Expression by p53: Stress, Cell and Tissue Specificity, and the Role of These Gene Products in Modulating the IGF-1-AKT-mTOR Pathways. *Cancer Research*, *67*(7), 3043–3053. https://doi.org/10.1158/0008-5472.CAN-06-4149

Galluzzi, L., Pietrocola, F., Bravo-San Pedro, J. M., Amaravadi, R. K., Baehrecke, E. H., Cecconi, F., Codogno, P., Debnath, J., Gewirtz, D. A., Karantza, V., Kimmelman, A., Kumar, S., Levine, B., Maiuri, M. C., Martin, S. J., Penninger, J., Piacentini, M., Rubinsztein, D. C., Simon, H. U., Simonsen, A., … Kroemer, G. (2015). Autophagy in malignant transformation and cancer progression. *The EMBO journal*, *34*(7), 856–880. https://doi.org/10.15252/embj.201490784

Glick, D., Barth, S., & Macleod, K. F. (2010). Autophagy: cellular and molecular mechanisms. *The Journal of pathology*, *221*(1), 3–12. https://doi.org/10.1002/path.2697

Hsu, Y. C., Chern, J. J., Cai, Y., Liu, M., & Choi, K. W. (2007). Drosophila TCTP is essential for growth and proliferation through regulation of dRheb GTPase. *Nature*, *445*(7129), 785–788. https://doi.org/10.1038/nature05528

Kim, J., Kundu, M., Viollet, B., & Guan, K. L. (2011). AMPK and mTOR regulate autophagy through direct phosphorylation of Ulk1. *Nature Cell Biology*, *13*(2), 132–141. https://doi.org/10.1038/ncb2152

Li, J., Chen, X., Kang, R., Zeh, H., Klionsky, D. J., & Tang, D. (2021). Regulation and function of autophagy in pancreatic cancer. *Autophagy*, *17*(11), 3275–3296. https://doi.org/10.1080/15548627.2020.1847462

Mehrgou, A., & Akouchekian, M. (2016). The importance of BRCA1 and BRCA2 genes mutations in breast cancer development. *Medical journal of the Islamic Republic of Iran*, *30*, 369.

National Cancer Institute. (n.d.). BRCA Gene Mutations: Cancer Risk and Genetic Testing. https://www.cancer.gov/about-cancer/causes-prevention/genetics/brca-fact-sheet. Accessed Jun 26, 2023.

Niklaus, N. J., Tokarchuk, I., Zbinden, M., Schläfli, A. M., Maycotte, P., & Tschan, M. P. (2021). The Multifaceted Functions of Autophagy in Breast Cancer Development and Treatment. *Cells*, *10*(6), 1447. https://doi.org/10.3390/cells10061447

Sancho-Garnier, H., & Colonna, M. (2017). Épidémiologie des cancers du sein. *La Presse Médicale*, *48*(10), 1076-1084. https://doi.org/10.1016/j.lpm.2019.09.022

Zhang, J., de Toledo, S. M., Pandey, B. N., Guo, G., Pain, D., Li, H., & Azzam, E. I. (2012). Role of the translationally controlled tumor protein in DNA damage sensing and repair. *Proceedings of the National Academy of Sciences of the United States of America*, *109*(16), E926–E933. https://doi.org/10.1073/pnas.1106300109

Zhu, Y., Jiang, X., Ding, Z., & Ming, J. (2022). Interleukin 7 inhibit autophagy via P53 regulated AMPK/mTOR signaling pathway in non-small cell lung cancer. *Scientific reports*, *12*(1), 11208. https://doi.org/10.1038/s41598-022-14742-5