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How Does TRPV6 Mediate Calcium Influx in Regulating Mitochondrial Homeostasis in Cancer Cells?

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Abstract. The present study aims to investigate the role of TRPV6 in calcium homeostasis, reactive oxygen species (ROS) levels, cell viability, and cancer growth of breast cancer MCF7 cells by knocking down the calcium channel protein TRPV6. It is anticipated that discrepancies may emerge between the experimental results and the initial predictions. These disparities may be attributed to the cell state or compensatory mechanisms. However, it is generally predicted that TRPV6 knockdown could reduce calcium concentration, upregulate ROS, decrease cell viability, and increase xenograft tumour weight. In the future, by combining advanced technologies such as mitochondrial function detection, the molecular mechanism of TRPV6 in cancer development can be further revealed.

Keywords: Breast cancer, TRPV6, Mitochondrial homeostasis

1. Introduction

Cancer is one of the leading causes of death worldwide, with around 10 million deaths occurring each year [1]. This has prompted scientists to explore the mechanisms underlying cancer growth and devise methods to eliminate it.

Breast cancer is a particularly prevalent and lethal form of the disease. Around 670,000 deaths were caused by breast cancer in 2022 [2]. Its aetiology is multifactorial, with genetic and environmental factors playing a significant role in its development [3]. Current breast cancer therapies include targeted treatment, hormonal treatment, radiation therapy, and surgery. However, therapies such as chemotherapy involve high levels of radiation and have severe side effects for patients [4].

As a result, it is essential to improve the therapies for cancers, and dig deeper to work out the mechanism of cancers, like what this paper aims to do [3].

In this work, it focuses on studying the breast cancer cells MCF7, a cell that allows long-term studying [5]. The TRPV6 is a type of ion channel that has high selectivity in MCF7 cells. The TRPV6 belongs to the transient receptor potential (TRP) channel family, comprising proteins that facilitate the flow of calcium ions across the cell membrane. And TRPV6 is highly expressed in certain types of cancer, such as breast and prostate cancer [6]. This gene, TRPV6, plays a crucial role in regulating calcium concentration in cells and thus influences cancer viability and proliferation [2]. Mitochondria are the energy center that provides energy for the cell, and they are sensitive to calcium concentration. The concentration of Ca^{2+} within the mitochondria is regulated

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by the mitochondrial calcium uniporter (MCU), which facilitates the importation of Ca^{2+} from the cell via the TRPV6, and the Ca^{2+} concentration is proportional to the ROS. Therefore, an increase in the expression of TRPV6 results in an elevated concentration of Ca^{2+} in the cytosol, which in turn leads to an increased uptake of Ca^{2+} by the mitochondria [7]. Consequently, this results in an escalation in the level of ROS, which are by-products of elevated activity within the electron transport chain (ETC) of the mitochondria [6].

Nevertheless, this study investigates how TRPV6 modulates mitochondrial homeostasis through the less well-established mechanisms by which calcium influences ROS. An increase in Ca^{2+} has been shown to increase Ca^{2}-dependent dehydrogenase activity, thereby promoting the efficiency of the tricarboxylic acid (TCA) cycle and increasing the release of nicotinamide adenine dinucleotide (NADH)to the ETC. This, in turn, leads to enhanced ETC efficiency, facilitating the transportation of more electrons within the transport chain and consequently reducing electron leaks and retentions, thus generating less ROS. The low ROS concentration is not sufficient to attack polyunsaturated fatty acids or to break DNA double strands. This prevents lipid peroxidation from occurring and increases cell viability [8]. It is therefore vital to regulate calcium and mitochondrial homeostasis to combat cancer cells [9].

Consequently, the elucidation of the mechanisms by which TRPV6 modulates the mitochondria and calcium concentration in MCF7 breast cancer cells may facilitate the development of novel therapeutic interventions and enhance existing therapeutic modalities in the treatment of cancer [6,8]. The subsequent essay will provide a comprehensive overview of the relevant literature on the subject.

2. Hypothesis

Predict that an increasing amount of knockdown of TRPV6 using siRNA decreases calcium, increases ROS, decreases the viability of MCF7 cells, and decreases the size of MCF7 xenograft tumors.

3. Methods and materials

In order to test the function of TRPV6 and ascertain how it regulates calcium ion levels in the mitochondria, and how cells deal with oxidative stress after TRPV6 knockdown, four groups of experiments on MCF7 cells have been designed (see Table 1). The thapsigargin is employed as a positive control, while the scramble siRNA is utilised as a negative control. Thapsigargin is a non-competitive and irreversible SERCA inhibitor [10]. The consequence of this is the depletion of Ca^{2+} in the mitochondria and an increase in Ca^{2+} flow into the cytoplasm. This results in an increase in the Ca^{2+} concentration in the mitochondria, which in turn increases the ROS [11]. The utilisation of this positive control is twofold: firstly, it serves to validate the experiment, and secondly, it demonstrates the ability of TRPV6 to regulate the Ca^{2+} concentration within the mitochondria. The purpose of the scramble siRNAs is twofold: firstly, to ensure that the transfection process is free from contamination, and secondly, to identify and compare the functional characteristics of cells with and without TRPV6, as well as to determine the effects of TRPV6 knockdown [12].

DOI: 10.54254/2753-8818/2025.AU28312

Table 1. MCF7 cells were subjected to four distinct conditions

Experiment	Description
1	MCF7 cells+ scramble siRNA
2	MCF7 cells + scramble siRNA+ thapsigargin
3	MCF7 cells + TRPV6 siRNA
4	MCF7 cells + TRPV6 siRNA+ thapsigargin

The present study employs a drug-free approach to investigate the alterations in concentration. It utilises the single-cell cloning technique employed in Experiment 3, along with flow cytometry (FCM) and western blot analysis, to differentiate between varying concentrations of proteins and the disparate knockdown efficiencies of TRPV6. Firstly, the use of siRNAs to knock out TRPV6 genes is required, followed by the transfection of cells. Subsequently, the use of FCM is necessary for the selection of cells that have undergone successful editing. The subsequent stage of the process is the cloning stage. The expression levels of TRPV6 proteins are then tested using Western blot analysis. The TRPV6 protein expression levels are grouped into the following categories: 90% knockdown group, 70% knockdown group, 50% knockdown group and 20% knockdown group. This may facilitate a more precise understanding of the manner in which the proportion of knockdown influences mitochondrial homeostasis.

In order to respond to the aforementioned question, the present study will analyze four aspects: the Ca^{2+} concentration, the ROS activity, the viability and the cancer weight. The following essay will provide a comprehensive overview of the relevant literature on the subject.

3.1. Cell lines

3.1.1. Measurement of calcium concentration by Rhod 2 AM

The objective of this study is to ascertain whether the calcium concentration in mitochondria is influenced by the knockdown of TRPV6. Rhod 2 AM is a selective calcium fluorescence probe that is catalyzed by esterases and becomes Rhod 2, a non-fluorescent compound. It is evident that Rhod 2 is polar and accumulates in the mitochondria, thus enabling the measurement of the calcium concentration in these organelles [13]. Initially, it was necessary to prepare the working solution [14]. The Rhod-2 AM is dissolved in DMSO, and the addition of Pluronic F-127 facilitates the separation of the dye. Thereafter, the cells are to be immersed in the working solution, which has been previously prepared, and then placed in a 37°C environment for a period of between 30 and 60 minutes. Subsequently, the free dye should be removed and the cells re-cultured onto a new medium [14]. The utilisation of a MitoTracker is then recommended in order to locate the mitochondria, and then the calcium concentrations in the mitochondria can be measured.

3.1.2. Measurement of ROS activity by DCFH-DA

The ROS activity in the mitochondria can be measured by the utilisation of 2,7-dichlorofluorescein diacetate (DCFH-DA). DCFH-DA is a ROS detector that can detect substances such as H_2O_2, HOCl, and O_2 [15]. DCFH-DA has high permeability and contains a dye with high lipid solubility, allowing it to cross the membrane. When DCFH-DA reaches the mitochondria, it is reduced to DCFH. Following the interaction between DCFH and the ROS within the mitochondria, DCF exhibits green fluorescence [15]. The DCFH-DA solution must first be prepared to perform this experiment. Then,

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the cells must undergo a washing process with Phosphate Buffered Saline (PBS). The DCFH-DA solution is used to stain the cells, which are then maintained at 37 °C in a dark environment [16]. Finally, the cells must be washed twice with PBS to remove fluorescent probes that have not successfully entered the cells.

3.1.3. Measurement of MCF7 viability by colony formation assay

The colony formation assay is used to measure the proliferative capacity of cells [16]. Initially, the cells must be cultivated at a temperature of 37°C [17]. Subsequently, the medium should be removed and the PBS washed on two occasions. Subsequent to this, the cells should be fixed and dyed by the addition of methanol and crystal violet. Following a process of water washing and drying, the quantity of the colony can be accurately measured. An increase in proliferation is associated with an increase in cell viability [16].

3.2. Animal models

Xenograft is a method of injecting cancer genes, TRPV6, into mice, thereby inducing the growth of a cancer in the mouse. Following the conclusion of the experiment, the gene's impact on cancer growth is measured by weighting [18]. In this experiment, cancer cells must first be cultivated and then mixed with Martigel at a 1:1 proportion. Subsequently, the mixture should be injected into the posterior region of the mouse's body [19]. The duration of the experiment is typically between 21 and 30 days. Subsequent to this period, the cancers are weighed using electronic scales [18].

3.3. Statistical analysis

Following a series of four experiments, the present study sought to analyse whether the knockdown of TRPV6 resulted in a significant difference in calcium concentration, ROS activity, cell viability, and cancer weight. A statistical analysis is then performed. In this study, the unpaired t-test is utilised as the analytical tool. Firstly, the null hypothesis (H0) is to be established, namely that there is no significant change between the control group and the TRPV6 knockdown group [20]. The alternative hypothesis (H1) is predicated on the supposition that there is a significant discrepancy between the control group and the TRPV6 knockdown group [20].

Formula:

$$t = \frac{\bar{x}_1 - \bar{x}_2}{\sqrt{\frac{s_1^2}{n_1} + \frac{s_2^2}{n_2}}} \quad (1)$$

$\bar{x}$ refers to the mean values of the sample
s^2 refers to the standard deviations of the sample
n refers to the sample number

Following a rigorous process of calculation, it can be demonstrated that the result can be represented by p-values. In the event of a p-value greater than 0.05 (n = 3), the null hypothesis is substantiated by the absence of any statistically significant disparities in calcium level fluctuations. In the event of a p-value of less than 0.05 (n = 3), the null hypothesis that there have been no significant changes between the control groups and the TRPV6 knockdown groups is rejected. This outcome is the one that is preferred.

DOI: 10.54254/2753-8818/2025.AU28312

4. Results

The hypothesis advanced in this study posits that an escalation in the knockdown phenomenon results in a decline in calcium levels within the mitochondria, concomitant with an augmentation in ROS activity. This, in turn, engenders a facilitation of apoptosis among cancerous cells. It is hypothesised that four experiments will be conducted as outlined above. The results of these experiments are predicted to fall into 16 distinct categories, as illustrated in Table 2.

Table 2. The following outcomes were observed in the four assays conducted in the study

Combination results (CR#)	Increasing knockdown of TRPV6 decreases calcium by Rhod 2 AM	Increasing knockdown of TRPV6 increases ROS by DCFH-DA	Increasing knockdown of TRPV6 decreases viability by colony formation assay	Increasing knockdown of TRPV6 decreases tumors by xenograft tumor weight	Support of hypothesis
1	+	+	+	+	Full
2	+	+	+	-	Partial
3	+	+	-	+	Partial
4	+	-	+	+	Partial
5	-	+	+	+	Partial
6	+	+	-	-	Partial
7	+	-	-	+	Partial
8	-	-	+	+	Partial
9	+	-	+	-	Partial
10	-	+	-	+	Partial
11	-	+	+	-	Partial
12	+	-	-	-	Partial
13	-	+	-	-	Partial
14	-	-	+	-	Partial
15	-	-	-	+	Partial
16	-	-	-	-	Fully Contradicts

- An increase in the knockdown of TRPV6 in MCF7 cells.

The "+" symbol is used to denote alterations in measurement, analogous to the effects of thapsigargin, in contrast to the scrambled siRNA vector.

The symbol "-" is employed to indicate alterations in measurement that are analogous to the scrambled vector opposite thapsigagrin.

The results of Experiment 1 demonstrate that there is a decrease in calcium levels within the mitochondria, an increase in ROS levels, a reduction in the viability of MCF7 cells, and a decrease in the weight of cancers. These findings provide substantial evidence to support the hypothesis.

The findings of Experiment 2 demonstrate that there is a decline in calcium levels within the mitochondria, an escalation in ROS levels, a reduction in the viability of MCF7 cells, and an augmentation in cancer weight. This outcome provides a degree of substantiation for the hypothesis.

The findings of Experiment 3 demonstrate a decline in calcium levels within the mitochondria, an escalation in ROS levels, an augmentation in the viability of MCF7 cells, and a reduction in the

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weight of cancers. This observation provides a degree of substantiation for the hypothesis.

The findings of Experiment 4 demonstrate a decline in calcium levels within the mitochondria. This decline is concomitant with a decrease in ROS levels, a reduction in the viability of MCF7 cells, and a decrease in the weight of cancers. These observations provide partial support for the hypothesis.

Experiment 5 yielded results indicating an increase in calcium levels within the mitochondria. Concurrently, this resulted in elevated levels of ROS, which in turn led to a reduction in the viability of MCF7 cells and a decrease in the weight of the cancers. This outcome provides partial support for the hypothesis.

The findings of Experiment 6 demonstrate a decline in calcium levels within the mitochondria, an escalation in ROS levels, an augmentation in MCF7 cell viability, and an escalation in cancer weight. This observation provides a degree of substantiation for the hypothesis.

The findings of Experiment 7 demonstrate a decline in calcium levels within the mitochondria, accompanied by a reduction in ROS levels, an enhancement in the viability of MCF7 cells, and a decrease in cancer weight. This observation provides partial support for the hypothesis proposed.

The findings of Experiment 8 demonstrate that an increase in calcium levels within the mitochondria is associated with a decrease in ROS levels, a reduction in the viability of MCF7 cells, and a decrease in the weight of cancers. This observation provides partial support for the hypothesis.

The results of Experiment 9 demonstrate a decline in calcium levels within the mitochondria. This decline is concomitant with a decrease in ROS levels, resulting in a reduction in the viability of MCF7 cells. Furthermore, this decline is accompanied by an increase in the weight of cancers, thereby providing partial support for the hypothesis.

The findings of Experiment 10 demonstrate that an increase in calcium levels within the mitochondria is associated with elevated levels of ROS, enhanced viability of MCF7 cells, and a reduction in cancer weight. This observation provides partial support for the hypothesis proposed.

The findings of Experiment 11 demonstrate that an increase in calcium levels within the mitochondria is associated with elevated levels of ROS, a decline in the viability of MCF7 cells, and an augmentation in the weight of cancers. This observation provides a degree of substantiation for the hypothesis.

Experiment 12 yielded findings that suggest a decrease in calcium levels within the mitochondria, alongside a decline in ROS levels, an enhancement in the viability of MCF7 cells, and an increase in cancer weight. This observation provides partial support for the hypothesis proposed.

The findings of Experiment 13 demonstrate that an increase in calcium levels within the mitochondria is associated with elevated levels of ROS, enhanced viability of MCF7 cells, and an augmentation in cancer weight. This observation provides partial support for the hypothesis proposed.

The findings of Experiment 14 demonstrate that an increase in calcium levels within the mitochondria is observed, concomitant with a decrease in ROS levels and a reduction in the viability of MCF7 cells. This is indicative of a relationship between the variables, thereby providing partial support for the hypothesis.

The results of experiment 15 demonstrate that an increase in calcium levels within the mitochondria, a decrease in ROS levels, an enhancement of MCF7 cells' viability, and a decrease in cancer weight. This provides partial support for the hypothesis.

The results of Experiment 16 demonstrate that an increase in calcium levels within the mitochondria is observed, concomitant with a decrease in ROS levels, and a corresponding increase

DOI: 10.54254/2753-8818/2025.AU28312

in the viability of MCF7 cells and the weight of cancers. This outcome serves to refute the initial hypothesis.

In this study, the expression of TRPV6 in MCF7 cells was reduced to different extents. The high-expression group (e.g., 20% knockdown) exhibited only minor reductions in calcium, viability, and tumour weight, accompanied by a modest increase in ROS. The intermediate group exhibited moderate changes. Conversely, the low-expression group (90% knockdown) demonstrated a substantial decline in calcium, viability, and tumour weight, accompanied by a pronounced increase in ROS.

5. Discussion

A total of sixteen different prediction results were obtained, of which some supported the hypothesis and others did not. The following discussion will present a range of potential outcomes, accompanied by a detailed rationale elucidating the underlying causes of this phenomenon.

In experiment 1, the hypothesis was confirmed that all results are similar to the positive control: increasing knockdown of TRPV6 using siRNAs resulted in decreased calcium levels, increased ROS levels, decreased viability of MCF7 cells, and decreased size of MCF7 xenograft cancers. It has been demonstrated that TRPV6 plays a significant role in the regulation of calcium homeostasis within mitochondria. Disruption of this balance through TRPV6 knockdown can result in elevated levels of ROS and the subsequent apoptosis of MCF7 cells. The study hypothesizes that by reducing the expression of TRPV6 genes, it may be possible to impede cancer growth and proliferation. Further experiments can do a rescue experiment to overexpress the TRPV6 genes and rescue the functions of mitochondria, and see if the results are opposite to experiment 1, in order to prove the reliability.

In experiment 2, the remaining three indicators were found to align with the hypothesis, except cancer weight. This finding indicates that the knockdown of TRPV6 exerts an effect on cell functions, including mitochondrial homeostasis. However, the knockdown of TRPV6 did not influence the formation of cancer in living organisms. This phenomenon may be attributable to the intricate nature of the cancer microenvironment. For example, cancer can obtain nutrition from blood vessels and promote its own growth. Furthermore, the TRPV6 knockdown and the compensatory mechanisms it engenders, such as other channels (e.g., TRPV4), can lead to calcium ion influx into cells. This, in turn, can cause cancer proliferation and contribute to calcium homeostasis, thus supporting cancer growth. In the context of forthcoming experiments, an augmentation in the duration of the experimental period is likely to engender a commensurate enhancement. Moreover, a combination of experiments with calcium channel inhibitors has the potential to yield more profound experimental insights.

In Experiment 3, the viability data did not support the hypothesis that there would be no decrease. This outcome may be attributed to the relatively mild elevation of ROS levels, which was insufficient to promote the apoptosis of cells. This suggests that the threshold to trigger the subsequent apoptosis of cells was not reached. Following the conclusion of the experiment, it will be possible to ascertain whether the ROS is the key element that induces the viability change of cells by adding a ROS scavenger, NAC. Should the addition of NAC result in an increase in viability, then the presence of ROS can be confirmed and the results of the experiment can be explained.

In Experiment 4, a decline in calcium concentration, viability, and cancer weight was observed in the TRPV6 knockdown group. However, this was accompanied by a decrease in ROS levels, which contradicts the initial hypothesis. The findings of this study imply that the repression of TRPV6 expression may exert antineoplastic effects, although this effect cannot be ascribed to the ROS

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pathway. The possibility exists that a ROS-independent mechanism is implicated, or that the ROS levels were elevated before or following the detection period. Alternatively, the knockdown of TRPV6 has been demonstrated to induce antioxidant stress reactions and reduce ROS levels. Consequently, experiments can be designed that utilise the western blot technique to assess the genes NRF2 and HO-1, which encode for antioxidative stress proteins. The primary factor in question is not the generation of ROS, but rather the clearance of ROS.

The 5th experiment demonstrates that the knockdown of TRPV6 does not result in a decrease in calcium concentration within the mitochondrion; rather, it increases. It is conceivable that this phenomenon is attributable to the existence of alternative mechanisms that trigger distinct pathways for the uptake of calcium ions within the mitochondria. For instance, the mitochondrial calcium uniporter channels (MCU) on the mitochondrial membrane allow the influx of calcium ions into the mitochondria. Further experimentation should be conducted using calcium channel inhibitors to avoid calcium influx from other channels besides TRPV6. Subsequently, Rhod2AM should be used to detect the calcium concentration again to ascertain whether this is the cause of elevated calcium levels.

In Experiment 6, it was observed that the knockdown of the TRPV6 gene resulted in a stimulatory effect on increasing cell viability and cancer weight. This phenomenon may be attributed to the fact that, despite an increase in the ROS level, it does not reach the level and threshold required to induce apoptosis. Moreover, the knockdown of TRPV6 has been demonstrated to exert a number of influences on the CA-induced apoptosis pathway, including a reduction in cytochrome c release. This, in turn, has been shown to result in an increase in the viability of MFC7 cells and in cancer weights. The enhancement may also be characterised by an augmentation in the duration of the experimental period. Secondly, the mitochondrial isolation kit should be employed for the cultivation of cells. In addition, the cytochrome antibody should be utilised, along with the western blot technique, in order to assess the levels of cytochrome c.

In experiment 7, the hypothesis was refuted, as evidenced by a decline in ROS levels and an increase in viability, which are opposite from the positive control. In circumstances where the ROS levels are elevated, the antioxidant defence mechanism may be initiated. This process is governed by a regulatory factor known as nuclear factor erythroid 2-related factor 2 (NRF2). NRF2 has the capacity to stimulate the expression of antioxidant genes, including HO-1. This process can result in the clearance of ROS. In order to facilitate future experimentation, it is imperative to verify the expression of NRF2 in the experiment using Western blot analysis. This will assist in determining whether the decrease in ROS levels and viability is attributable to other factors.

As demonstrated in Experiment 8, there is a concomitant increase in calcium levels and ROS activity. This phenomenon may be ascribed to the suboptimal knockdown of TRPV6 genes, which exerts a negligible effect on ion concentration and ROS activity. In order to achieve further enhancement, it would be advisable to undertake the repetition of experiments on multiple occasions in order to ascertain the calcium level. In addition, the use of qPCR to test the mRNA level of TRPV6 would ensure the TRPV6 knockdown efficiency. In addition, the employment of a more sensitive ROS probe would facilitate enhanced detection.

Experiment 9 demonstrates an incongruity between the hypothesised and observed results, with a decline in ROS level and an increase in cancer weight. The low ROS activity indicates that the antioxidant defense occurs as mentioned above. It is also hypothesised that Ca^{2+} depletion may have a detrimental effect on cell viability. Nevertheless, the data demonstrate that this may also be accompanied by a process of ROS-independent cell death, indicating that the increase in cancer weight may be influenced by the cell cycle. In order to verify the results obtained, it is

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recommended that future experimentation be conducted on the subject of cyclic protein expression, such as cyclin D, cyclin E, and cyclin A. The use of the Western blot to measure cyclin protein level is advised, with a view to elucidating the mechanisms involved in cell viability.

Experiment 10 provides evidence that there is an increase in both calcium concentration and cell viability. This phenomenon may be attributable to an increase in Ca^{2+} concentration. Since the calcium ions become prevalent, they induce the pathways that regulate cell cycles. For instance, an increase in the transcription of MKPII2 results in elevated levels of cyclin C and D. Additionally, an increase in calcium ion concentration also activates the NFAT/CREB pathway, leading to the proliferation of cells in growth. In preparation for the forthcoming experiment, the utilisation of Western blot analysis will be employed to measure cyclin protein levels.

Experiment 11 showed that the calcium concentration and cancer weight exhibited an inverse relationship, as predicted by the hypothesis, with both variables having an increasing trend. This may be attributable to the suboptimal efficacy of the TRPV6 knockdown, which results in elevated calcium levels. Alternatively, the observed increase in cancer weight may be attributable to mechanisms independent of ROS activity that the increasing calcium ion level might activate the NFAT / CREB pathway, a pathway that leads to cell growth by regulating cyclins. Consequently, it is imperative to quantify the knockdown efficiency by using a Western blot

In experiment 12, it was observed that, in addition to alterations in calcium concentration, modifications were also evident in all three assays. This phenomenon may be attributable to a decline in mitochondrial calcium concentration, concomitant with elevated ROS production within the ETC. Consequently, the low level of ROS prevents DNA strand breaks and increases cell viability, thereby counteracting the development of cancerous growths. Subsequently, the 5-ethynyl-2'-deoxyuridine (EdU) experiment should be employed to ascertain whether DNA synthesis is occurring at a high frequency. This indicates a high probability of cell viability and proliferation, which can serve as evidence to support the initial hypothesis.

In experiment 13, it is evident that all four assay results demonstrate an increase in trend. It has been hypothesised that the inefficacy of the knockdown may be the cause of the increase in Ca^{2+} and ROS activity. However, it should be noted that the increase in ROS does not reach the threshold required to trigger apoptosis in cells. Consequently, this results in an increase in cell viability and cancer weight. It is recommended that future experimentation utilise the western blot technique to assess the knockdown efficiency of the TRPV6 gene. Subsequent analysis of the level of ROS can be conducted using a fluorescent probe such as MitoSOX to ascertain whether it can trigger cell apoptosis.

In experiment 14, the decline in the viability of MCF7 cells aligns with the positive control, while other variables demonstrate non-conformity. Specifically, there is an increase in calcium levels, a decrease in ROS, and an increase in cancer weight. This phenomenon may show calcium overload and impaired mitochondrial function. The subsequent result of this impairment is metabolic depression and reduced ROS, which reduces cell viability. However, it is important to note that cancer may continue to grow in response to compensatory mechanisms, such as metabolic reprogramming. In future experiments, the functionality of the mitochondrion can be assessed by the mitochondrial membrane potential measurement ($\Delta\psi m$). The mitochondrial integrity was evaluated by fluorescence microscopy.

In Experiment 15, the results obtained for the cancer weight were consistent with those of the positive control. These results did not support the hypothesis, and all are consistent with the negative control. Consequently, this may be attributable to the low knockdown efficiency, which could result in suboptimal outcomes in the knockdown of the TRPV6 gene. In order to verify the efficacy of the

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knockdown, it is imperative that rigorous analytical techniques are employed. Such techniques include, but are not limited to, quantitative PCR (qPCR) and western blot analysis. Following this verification, the experiments involving Rhod 2 AM, DCFH-DA, colony formation assay, and xenograft should be repeated.

In the final experiment, the results obtained did not support the hypothesis. Evidence indicates that TRPV6 exerts minimal influence on mitochondrial homeostasis and does not function directly in the promotion of cancerous growth. However, this finding does not indicate a failure of the experiment; it merely suggests that TRPV6 may not be the factor responsible for regulating MCF7 breast cells. Consequently, it is possible to modify the experiment's models. For instance, the cancer model can be altered from breast cancer to prostate cancer to analyse the role of TRPV6. Alternatively, experiments can be conducted that also test other channels, such as TRPV5, to ascertain whether there are compensatory mechanisms.

A decline in calcium ion concentration, viability, and cancer weight is indicative of a heightened knockdown efficiency of TRPV6 genes. Concurrently, a substantial augmentation in ROS levels is observed, signifying the pivotal function of TRPV6 in mitochondrial homeostasis. To substantiate these findings, a rescue experiment was conducted by overexpressing the TRPV6 gene, which served to reinforce the conclusions drawn.

With regard to the intermediate efficiency, the results demonstrate a slight alteration in the concentrations of Ca^{2+}, the activity of ROS, cell viability, and the weight of the cancerous tissue. Evidence suggests that TRPV6 may affect mitochondrial homeostasis and cancer growth. However, results demonstrate that TRPV6 is active, but the threshold is not met, so there are minimal changes. In the groups exhibiting low knockdown efficiency, no statistically significant alterations were observed in calcium concentration, ROS activity, viability, and cancer weight. These findings suggest that TRPV6 may not function as a regulator of mitochondrial homeostasis and may not impact cancer growth. It is recommended that future experiments, such as qPCR, should be carried out.

In conclusion, the increase of TRPV6 knockdown will result in changes in calcium concentration, ROS activity, cell viability, and cancer weight.

6. Conclusion

The present study investigated the role of calcium channel protein TRPV6 in cellular calcium homeostasis, oxidative stress, cell viability, and cancer weights. In this study, Rhod 2 AM was utilised to assess the calcium concentration in mitochondria, while DCFH-DA was employed to ascertain the levels of ROS. Furthermore, clone formation experiments were used to measure cell viability, and xenograft mouse models were used to assess the cancer weights. A total of 16 potential outcomes were deduced. The study's results indicated that TRPV6 may influence the cellular oxidative state by modulating the concentration of calcium ions within the mitochondria.

In conclusion, the results obtained have enhanced our comprehension of TRPV6's function in cancer-related regulation. This has been achieved by emphasising the diversity and intricacy of TRPV6’s functions. Furthermore, it has been demonstrated that a drug targeting strategy that is unidirectional may encounter limitations. It is recommended that future research integrate clustered regularly interspaced short palindromic repeats (CRISPR) gene editing and mitochondrial function detection. This integrated approach will facilitate the analysis of how TRPV6 contributes to cancer development. As a result, this paper aims to promote the identification of more effective treatment methods for breast cancer.

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