**Defective Channel Trafficking in A341V Mutated KCNQ1 Protein Results in Long QT Syndrome**

**Introduction**

Cardiac arrhythmias account for 200,000-300,000 sudden deaths per year in the United States alone [2][9]. While many factors contribute to irregular cardiac rhythms, one example being malfunctioning of the sinoatrial node responsible for regular electrical impulses that allow cardiomyocytes to contract, voltage-gated ion channels additionally play an essential role in electrical stability by allowing ions to flow into and out of the cell during the depolarization and repolarization stages of a cardiac action potential.

One specific inherited arrhythmic disorder, Long QT (LQT) syndrome, is characterized by a prolonged repolarization period where the time it takes for ions to return to the resting state (voltage) is longer than normal [1]. Long QT syndrome type 1 (LQT1) is a subtype of LQT syndrome which has shown to be the most common, and is associated with mutations in the KCNQ1 protein [1]. KCNQ1 and KCNE1 are two proteins that assemble on plasma membrane to form the slow delayed rectifier channel (Iks) which plays a role in repolarization of the action potential in ventricular myocytes when a shorter action potential is required [7]. Research has shown that the presence of both subunits is essential in forming Iks, for they do not properly function as voltage-gated potassium channels as individual units [7]. A point mutation in one of the proteins can result in a nonfunctional channel. While KCNQ1 is a pore-forming subunit, KCNE1 is a smaller, auxiliary subunit. A point mutation in KCNQ1, therefore, is greatly detrimental to the cell. One specific missense mutation, the A341V mutation, occurs in the S6 segment of KCNQ1 and substitutes an alanine to a valine [11].



**Figure 1**

Figure 1, retrieved from Wang et al’s article [11], illustrates the KCNQ1 protein with the N-terminus of the protein on the bottom left and the C-terminus of the protein in the bottom right. This illustration shows the location of the A341V mutation, and how it is located on the S6 segment which is exposed to the pore that is created when the Iks channel assembles.

Genotype-phenotype relationships

Researchers have concluded based off of observations from case studies that the A341V mutation in the gene coding for KCNQ1 is associated with phenotypic conditions more severe than other mutations [4]. Electrophysiological findings from experiments conducted by numerous researchers confirm this claim, as the electrophysiology of A341V mutant KCNQ1 exhibits differences from the wild-type KCNQ1. In Mikuni et al’s experiment [8], they conducted a patch clamp experiment and found that the voltage curve was different in the mutant Iks channel.

 

**Figure 2** [8]

Figures 2D (left) and 2F (right) illustrate the patch clamp results from Mikuni et al’s experiment. A patch clamp experiment creates a hole in the plasma membrane of a cell through which an electrolytic solution is run. Ions, potassium ions in this case, are passed through the channel and the electrophysiological activity can be recorded. A prolonged repolarization period is evident in figure 2F, as the ion channel containing mutant KCNQ1 takes longer to activate and is not activated to the same amplitude as the WT KCNQ1.

Such experiments demonstrate that the A341V mutation results in inefficient Iks channel functioning, yet do not explain what cellular mechanisms contribute to this occurrence. Some possible molecular mechanistic explanations, for example, include: the loss-of-function mutation perhaps causes conformational changes such as a smaller pore size in the ion channel during the gating transition, the voltage sensor domain becomes insensitive to environmental and cellular voltage changes, the ion selectivity filter is affected which would allow ions other than potassium to travel through the channel, or the protein trafficking to the plasma membrane is affected.

Defective Channel Trafficking (Cellular localization)

In Harmer et al’s study [6], researchers investigated defective channel trafficking as a possible contributing mechanism to the functional Iks channel changes. Confocal microscopy imaging was used as a methodology to analyze protein trafficking of WT and mutant A341V protein. One set of cells was transfected with WT KCNQ1-GFP and KCNE1-dsR while another set of cells was transfected with mutant A341V-GFP and KCNE1-dsR. Both proteins in either scenario were tagged with fluorescent molecules so that they could be identified under the microscope. Live cells were then fixed at the same point so that they could then be visualized under a confocal microscope. Confocal microscopy images revealed that the retention rates are relatively the same in the ER for both WT and mutant KCNQ1 proteins. These results indicated that channel trafficking remains unaffected by the A341V mutation, yet it must be considered that this confocal microscopy does not reveal cell surface expression or allow us to quantify it directly. The conclusion that channel trafficking remains unaffected is only drawn by what researchers visually interpret from the images, as shown in Figure 3.



**Figure 3** [6]

Figures 3A and 3B above exhibit the results of the confocal microscopy experiment analyzing defective channel trafficking. With KCNQ1-GFP exhibited in green and KCNE1 exhibited in red, the yellow-colored areas in the merged figures shows where KCNQ1 and KCNE1 are colocalized. Similar patterns are exhibited in both WT and mutant proteins as KCNQ1 appears to be concentrated around the cell periphery as well as being throughout the cell, while KCNE1 is contained within the cell. Researchers determined that through ER retention levels of proteins, there is no difference in channel trafficking. Nevertheless, differences in the spread of KCNQ1 protein on the cell surface is evident in the results. A biotinylation experiment can further expand on this research to study cell surface expression of WT and mutant protein.

The phenotypic severity prompts the question of what cellular mechanisms are responsible for the electrophysiological effect of the A341V mutation. Analysis of cell surface KCNQ1 protein, both mutant and WT, can help provide insight into how channel trafficking may contribute to these changes. The purpose of this proposed experiment, therefore, is to investigate cell surface expression of WT KCNQ1 and mutant A341V KCNQ1 to see how it may be indicative of defective channel trafficking.

**Experiment**

A cell-surface biotinylation experiment can measure protein expression on the cell surface. This assay consists of a series of steps, beginning with the labelling of cell surface protein with biotin and ending with a densitometry reading from an immunoblot. The end result of this experiment is a densitometry reading which quantifies the amount of cell surface protein. This experiment can help address the proposed question as the quantification of wild-type and mutant protein can reveal differences between the two, indicative of defective channel trafficking if the amount of surface mutant KCNQ1 is less than WT surface KCNQ1.

An example of a biotinylation experiment is seen in Chen et al’s study [5]. In this experiment, researchers were aiming to investigate the differences between WT and mutant cell surface expression of KCNQ1 with the S277L mutation. The experimenters were able to conclude from the results that cell surface expression levels for mutated KCNQ1 protein were lower than the wild-type. The biotinylation experiment therefore helped answer the question of whether cell surface proteins differed in quantity in mutated vs wild-type. The results of the experiment indicate lower expression for mutated protein, and in the application of the larger question, this lower expression could be a contributing factor to inefficient Iks channels. Similarly, a biotinylation experiment can indeed be helpful in addressing my proposed question as well.

The first step to conducting the biotinylation experiment would be to perform a cell transfection. Two sets of HEK-293 (human embryonic kidney) cells, with six dishes for WT KCNQ1 and six dishes for A341V KCNQ1, will be transfected with plasmids containing the respective genes. These plasmids can be made in the laboratory using vectors or obtained from another laboratory or reliable company. For the purposes of this experiment, the plasmids containing WT and mutant genes will be obtained. The plasmids will be mixed with medium appropriate for the cells to grow and synthesize new proteins. Cell transfection will be allowed to proceed for six hours, after which the medium containing plasmids will be removed. All 12 dishes will then be incubated at physiological temperature (37oC) for 48 hours before the biotinylation experiment can proceed.

Cells in all 12 dishes will then be labelled with a biotin derivative which is impermeable to the plasma membrane, so that only proteins on the cell surface are labelled. Here, all cell surface proteins will be labelled regardless of protein type. In Chen et al’s experiment [5], a NHS-SS-biotin derivative was used, but an appropriate biotin derivative to our experiment will be necessary and the reaction will occur on ice. After labelling has been allowed to proceed for 30 minutes, the reaction will be quenched for 20 minutes using a PBS (Phosphate Buffer Saline) solution which allows excess biotin to be washed out while keeping cellular conditions stable in the buffer solution.

Following biotin labelling, cells must be lysed in order to retrieve the biotinylated proteins. In order to do so, cells in all 12 dishes are submerged in lysis buffer. Lysis buffer breaks up the contents of the cell into individual components without harming any of the individual proteins so that they can be quantified. The protein concentration of each is measured using a mass spectrometer so that the appropriate amount of biotinylated proteins can be eluted from the sample. Equal amounts of biotinylated protein will then be eluted using Neutravidin beads. This process allows for the purification of the respective WT and mutant proteins.

An appropriate amount of the purified protein sample will then be loaded into the well of an SDS-Page gel. Electrophoresis will run so that the present protein can travel throughout the gel based on its size. A size marker lane will be present in the very first lane so that the relative weights (in kilodaltons) of the distributed proteins can be determined. The gel will be transferred onto a membrane so that ECL analysis for the immunoblot can be conducted. The proteins on the membrane will be probed with specific antibodies that attach to KCNQ1 proteins and can aid their visualization. The membrane will additionally be probed with cadherin, a cell surface protein, to use as a positive control in this experiment as cadherin is always present in normal levels. In an ECL analysis using a visualization machine, the membrane will be visualized. The various bands for the respective proteins will be visible and can then be analyzed. After transferring the data to a computer, the bands with KCNQ1 protein will be quantified, the principle behind this being that every pixel is representative of a protein. The darker the bands, therefore, the more protein is present while the lighter the bands, the less protein is present.

**Discussion**

It is hypothesized that results will exhibit lighter bands for mutated A341V KCNQ1 proteins while the bands for the WT KCNQ1 proteins will be significantly darker. This would indicate that the mutation is correlated with decreased cell surface KCNQ1 protein in comparison to the WT. This specific result would point to the idea that the trafficking of mutant KCNQ1 is somehow defective. Proteins may be colocalized with other KCNE1 proteins yet they are not expressed at the same level as WT protein on the cell surface. This finding would be most consistent with Chen et al’s biotinylation experiment, as the results of their experiment revealed that the cell surface expression of mutant KCNQ1 was lower than that of the WT.

Another possibility of results is that the mutant protein shows darker bands than the WT proteins. This would indicate that somehow, an abnormally large amount of protein is trafficking to the cell surface. While this is unlikely because it would indicate that more protein is translated than in the WT, it is indeed a possibility.

One last possibility of results is that both the mutant and WT proteins are expressed equally on the cell surface. This set of results would indicate that protein trafficking remains unaffected by the A341V mutation. Rather, the molecular cause of a nonfunctional Iks channel has something to do with the ion channel itself, such as its conformation, its selectivity filter, or voltage sensor. This finding would be most consistent with Harmer et al’s experiment, as their cellular localization confocal microscopy experiment exhibited no trafficking differences.

The biotinylation experiment, while it allows for the quantification of cell surface protein, is limited in many ways. One of the biggest pitfalls of this experiment is the loss of proteins during elution. No matter how careful the researcher may be while performing the elution through Neutravidin beads, some protein will always be lost. The beads themselves may be lost, such as they might be stuck on the cap during centrifugation or perhaps the beads are non-specific for a protein, error in the results will be present. This pitfall must be accounted for. One way to combat this is to slightly modify the experimental procedure. Once the proteins have been eluted, the beads can be washed multiple times with buffer solution so that almost all protein is pulled down. This buffer solution will not harm the proteins in any way, so the proteins themselves remain unaffected but hopefully more of them are eluted to reduce error in the quantification.

While this biotinylation experiment can indeed address the proposed question, supplemental experiments are absolutely necessary to confirm the findings from these experiments. While results can never be contradictory, differing results from experiments aiming to investigate the same phenomenon indicate errors in experimental procedure. This error can be minimized by evaluating the results of multiple experiments, perhaps of different types. One way, for example, to further investigate possible defective trafficking is to conduct a live-cell imaging study under a spinning disc confocal microscope under physiological conditions. Through this imagining study, the trafficking of the proteins in a specific time course can be tracked and help provide further insight on the possibly differing trafficking phenomena of mutant and WT KCNQ1 protein.

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