Chapter

The Potential of Human Induced Pluripotent Stem Cells (hiPSCs) for the Study of Channelopathies: Advances and Future Directions

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Abstract

Human induced pluripotent stem cells (hiPSCs) have revolutionized research on ion channels and channelopathies. Channelopathies are a group of genetic disorders characterized by dysfunctional ion channels, which are responsible for the regulation of ion flow across cell membranes. These disorders can affect various organ systems, leading to a wide range of symptoms and clinical manifestations. Differentiating pluripotent stem cells into various cell types results in the possibility of creating tissue- and disease-specific cell models. These models offer the possibility to investigate the underlying mechanisms of channelopathies and develop potential therapies. Using hiPSC-derived cells has allowed crucial insights into diseases like epilepsy, long QT syndrome, and periodic paralysis. However, the full potential of hiPSCs in this field is still to be exploited. The research will most likely focus on developing more complex cell models to further investigate channel dysfunction and its pathological consequences. In addition, hiPSCs will be increasingly used in drug screening and developing personalized therapies for various diseases. This chapter outlines the past and present achievements of hiPSCs in the field of channelopathies as well as provides an outlook on future possibilities.

Keywords: neurology, cardiology, channelopathies, epilepsy, long QT syndrome, medicine, diseases, treatment

1. Introduction

1.1 Definition and classification of channelopathies

Channelopathies are a group of genetic disorders characterized by dysfunctional ion channels, which play a crucial role in the regulation of ion flow across cell membranes. These disorders can affect various organ systems, leading to a wide range of symptoms and clinical manifestations. Understanding the definition and

classification of channelopathies is essential for accurate diagnosis, appropriate management, and the development of targeted therapies [1].

Channelopathies can be classified based on several criteria, including the affected organ system, the type of ion channel involved, and the specific clinical manifestations, for example, neuronal, cardiac, and musculoskeletal channelopathies.

1.1.1 Neurological channelopathies

These channelopathies affect the nervous system, resulting in various neurological symptoms and disorders. For instance, voltage-gated sodium and potassium channel mutations can cause epilepsy, characterized by recurrent seizures and other dysfunctions of the cognitive, emotional, and neurological systems [2–4]. These seizures occur due to excessive and synchronous activity of nerve cells in the brain. Epilepsy can have various causes, including genetic predisposition, brain injury, infection, and metabolic disorders. Other neurological channelopathies include episodic ataxias, which manifest as episodes of uncoordinated movement and balance problems, and periodic paralysis, characterized by episodes of muscle weakness or paralysis [5–7].

1.1.2 Cardiac channelopathies

These channelopathies primarily affect the electrical properties of the heart, leading to arrhythmias and sudden cardiac death. Examples include Brugada syndrome, catecholaminergic polymorphic ventricular tachycardia (CPVT), familial atrial fibrillation, and long QT syndrome (LQTS) [8, 9]. The latter, long QT syndrome (LQTS), is a rare congenital disorder of the heart characterized by a delayed repolarization of the ventricles. It is characterized by prolongation of the QT interval time in the electrocardiogram (ECG). The QT interval represents the time taken to complete the depolarization and repolarization of the ventricles of the heart.

Long QT syndrome can be caused by genetic mutations that affect the function of the ion channels responsible for repolarization of the heart. However, medications such as antipsychotics can also lead to long QT syndrome. Thus, LQTS can be inherited and/or acquired [8, 10].

One of the most serious complications of long QT syndrome is torsade de pointes tachycardia, a specific form of ventricular tachycardia in which the heart beats irregularly and rapidly. It is characterized by a twisting of the QRS complex around the isoelectric line in the ECG. Torsade de pointes is a life-threatening cardiac arrhythmia, that can transform into ventricular fibrillation, which may lead to sudden cardiac death [10–12].

1.1.3 Musculoskeletal channelopathies

One example of a skeletal muscle channelopathy is myotonia congenita, which is characterized by muscle stiffness and delayed muscle relaxation after contraction. This condition is caused by mutations in chloride or sodium channels that affect the electrical properties of skeletal muscles [13, 14]. Similarly, periodic paralysis, as mentioned earlier, can also affect the muscles and lead to episodes of muscle weakness or paralysis [6, 7].

Neurological, cardiac, and musculoskeletal channelopathies are some of the commonly classified types, but many organ systems can be affected by channelopathies,

also at the same time. The clinical manifestations can vary greatly depending on the mutation and its effects on ion channels. Cooperation between researchers, neurologists, cardiologists, and other specialists is crucial for the diagnosis, management, and treatment of channelopathies.

1.1.4 Genetic mutations

Channelopathies arise due to the dysregulation of ion channels, which can be caused by various mechanisms.

The primary cause of channelopathies are genetic mutations that can affect the structure and function of ion channel proteins. These mutations can result in the proteins' loss-of-function or gain-of-function, leading to alterations in ion channel activity. For instance, missense mutations can cause changes in the amino acid sequence of ion channels, leading to altered channel gating, conductance, or trafficking. These genetic mutations can be inherited in an autosomal dominant or recessive manner, or they may arise *de novo* [15–17].

Genetic mutations can also lead to alterations in the channels' biophysical properties. Mutations can for example affect channel gating, resulting in changes in the voltage or ligand dependence of channel gating. Mutations can also affect the conductance or ion selectivity of a channel, leading to altered ion flux. These biophysical alterations can disrupt the normal electrical signaling and ion homeostasis in cells and tissues, contributing to the pathogenesis of channelopathies [18–20].

Further, the subcellular localization of a channel can be altered by a mutation causing dysregulations in the cell biology of the respective cell. Altered cellular and/or developmental functions can cause, for example, syndactyly and bone malformation due to impaired apoptosis in Andersen-Tawil syndrome or Timothy syndrome [21, 22].

Loss-of-function mutations of ion channels can lead to reduced or absent ion channel activity, impairing the regular flow of ions across cell membranes. This can disrupt cellular excitability, neurotransmission, and other essential physiological processes [18].

Gain-of-function mutations of ion channels can lead to increased ion channel activity or altered ion selectivity. This can result in excessive ion flux, aberrant electrical signaling, and cellular dysfunction, contributing to the development of channelopathies [23].

In some channelopathies, genetic mutations can also impair the proper trafficking and localization of ion channels to the cell membrane. Mutations can disrupt the interaction of channels with chaperones, affecting their folding, assembly, and transport to the plasma membrane. As a result, the number of functional channels at the cell surface may be reduced, leading to decreased ion conductance and altered cellular excitability [24, 25].

1.1.5 Altered channel regulation by modulators

Ion channel activity can be modulated by various endogenous or exogenous factors, including ligands, second messengers, and posttranslational modifications. In channelopathies, mutations can disrupt the normal regulation of ion channels by these modulators. For example, mutations can alter the sensitivity of channels to voltage changes, impair the binding of ligands or regulatory proteins, or disrupt the phosphorylation or glycosylation sites critical for channel function. These alterations in channel regulation can lead to abnormal ion channel activity and subsequent disease manifestations [26, 27].

1.1.6 Interactions with auxiliary subunits or interacting proteins

Ion channels interact with auxiliary subunits or regulatory proteins that modulate their activity or regulate their cellular localization. Mutations in these auxiliary subunits or interacting proteins can disrupt the normal function of ion channels. For example, mutations in β subunits of voltage-gated calcium channels can affect channel trafficking, gating, or modulation by intracellular signaling molecules. Similarly, mutations in accessory proteins involved in potassium channel function, such as KCNE subunits, can impair channel activity and lead to channelopathies [28, 29].

1.1.7 Multifactorial interactions

Channelopathies can also arise from complex interactions between genetic factors, environmental triggers, and other modifying factors. These interactions can influence the severity, onset, and progression of channelopathies [1, 6].

Finally, the promotor regions of ion channels couple ion channel genesis to promotor regulations and mutations in the promotors or in the transcription factors can disrupt controlled ion channel protein generation to cause complex disease states.

1.2 Definition and properties of stem cells

Stem cells are a unique type of cells that have the potential to regenerate themselves and differentiate into different cell types in the body [30]. They play a significant role during the development of the organism, as well as in the regeneration and repair of tissues and organs in the adult body [31]. The ability to self-renew and differentiate makes stem cells an important field of research with great potential for medical research and treatment.

Stem cells are divided into two main categories: embryonic stem cells (ES cells) and adult stem cells. ES cells originate from the inner cell mass of an embryo at a very early stage of development. They are pluripotent, which means that they have the potential to differentiate into virtually all cell types of the body [30]. Adult stem cells, on the other hand, are present in the adult body and can be found in specific tissues and organs. They are multipotent and have the potential to differentiate into various cell types within their tissue [32].

ES cells are often derived from supernumerary embryos created through assisted reproductive techniques. However, the derivation of ES cells results in the destruction of the embryo. Therefore, alternative sources of pluripotent stem cells have been explored, such as induced pluripotent stem cells. iPS cells are generated by reprogramming adult somatic cells, such as skin cells, into a pluripotent state [33]. This technique, developed by Shinya Yamanaka and his team, allows pluripotent stem cells to be derived without the need for embryo destruction [34] and has earned the Nobel Prize for Physiology and Medicine in 2012.

The properties of stem cells make them a valuable tool for regenerative medicine, disease research, and the development of new therapies. Due to their ability to self-renew, stem cells can be grown in large amounts to provide sufficient cell quantities for therapeutic applications. Their ability to differentiate enables the production of specific cell types needed for transplantation or tissue regeneration [31].

The field of stem cell research has revolutonized disease modeling. Stem cells are used to treat diseases such as blood disorders, degenerative diseases of the nervous system,

and heart disease [35, 36]. The development of iPS cells has also opened up new possibilities for personalized medicine and the study of disease mechanisms [37–39].

2. Stem cell-based models of channelopathies

As described above, channelopathies lead to serious diseases or syndromes in patients. Studying these disorders and developing effective treatments can be challenging due to the limited availability of patient samples and the complex nature of affected tissues. However, recent advancements in stem cell research have opened new avenues for modeling channelopathies in the laboratory under controlled settings. Stem cell-based models provide valuable tools to investigate the underlying mechanisms and pathophysiology, as well as potential therapeutic strategies for channelopathies.

2.1 iPS-derived cells for physiological channel research and pathophysiological research

For the characterization of basic channel activity, generic cells such as Chinese Hamster ovary (CHO) or Human embryonic kidney (HEK) cells are broadly utilized [23]. These cells can be manipulated by transfection with cDNA constructs of channels or receptors. After overexpression of these proteins, the ion channel function in the cells can be examined using the patch clamp technique to record channel activity [40].

An alternative method for examining channel activity is Two-Electrode Voltage Clamp (TEVC). This involves injecting mRNA encoding the channels of interest into the cytosol of, that is, *Xenopus laevis* frog eggs, which are then expressed and recorded [41]. In both the patch clamp technique and TEVC, the channels can be examined by applying voltages or currents. In addition, it is possible to measure the effect of activators and inhibitors on the channels and to compare wild-type and mutant channels [23].

Despite the progress made by these established methods, there are reasons to turn to new models. The main reason is the physiological relevance of the cells used. While the cell models mentioned above investigate neuronal, cardiac, and other channels, the cells used are tumor cells or oocyte cells. Moreover, two of the cell systems mentioned are not even of human origin. However, primary cells from humans are often not available for regular lab work. The advantage of using induced pluripotent stem cells (iPS) is that they can be generated from any tissue sample of the human body [33]. Thus, truly neuronal channels can be studied in neuronal cells and cardiac channels in cardiac cells. This allows the channels to be studied in a much more physiological context compared to the previously used cell systems [42].

The use of patient-derived or transgenic cell lines also allows the study of pathophysiological mechanisms of channelopathies [43]. By studying channels in cells from patients with known channelopathies, the effects of disease mutations on channel function can be investigated to gain a better understanding of the pathophysiology of these diseases.

2.2 Disease modeling and drug screening

In present and future stem cell research, a variety of established differentiation protocols are available that enable precise differentiation of stem cells in both 2D and 3D cultures [44–46]. These protocols have proven to reliably generate neuronal and cardiac cell tissues and enable the targeted generation of various electrophysiological phenotypes.

For the differentiation of neuronal cell tissues, optimized protocols have been developed that allow stem cells to differentiate into specific neuronal cell types such as neurons, astrocytes, and oligodendrocytes. By combining specific growth factors and culture conditions, the desired electrophysiological properties can be studied [46–48].

Remarkable progress has also been made in the field of cardiac differentiation. The application of targeted protocols enables the efficient differentiation of stem cells into cardiac cell types such as working myocardium, atrial cells, and pacemakers. The specific combination of signals and factors can achieve the desired electrophysiological expression, which is important for the study of cardiac physiology and channelopathies [49–51].

These differentiated neuronal and cardiac cell tissues provide the opportunity to explore physiological channel activity in more detail and to study specific electrophysiological phenotypes. By using these stem cell-derived cell systems, the understanding of underlying mechanisms of channels in physiological processes is broadened. Using common and modern molecular biology methods, most notably CRISPR/Cas9, the cell lines can be modified to express channel pathologies [52]. Thus, new therapeutic approaches can be developed.

Stem cell-derived models of diseases like channelopathies offer a platform to investigate pathophysiological mechanisms at the cellular and molecular levels. By differentiating patient-specific iPSCs into disease-relevant cell types, researchers can analyze the functional consequences of the specific mutations on ion channel activity, cellular excitability, and downstream signaling pathways [53].

In the field of drug screening and safety pharmacology, stem cell-derived tissues are also playing an increasingly important role. In conventional screening, often transfected cancer cell systems or animal tissues are used, such as in patch clamps in HEK cells or mouse brain slices [54]. Stem cells also offer the possibility of drug screening in various derived cell identities to identify and explore potential candidate compounds that could alleviate disease symptoms or counteract disease progression.

However, in transfected cell systems, often only the effects on a specific channel type can be tested. In contrast, more physiologically complex cells and tissues, as derived from stem cells, offer the possibility to study the effects of drugs on an entire cell system.

In contrast to the well-established mouse brain slices, the hiPSCs offer another advantage: They are of human origin. And therefore closer to human (patho) physiology.

These advances in differentiating stem cells into specific cell types result in new possibilities for drug screening and safety pharmacology. By using stem cell-derived tissues, more comprehensive studies can be conducted on the effects of drugs on complex cell systems, leading to improved prediction of effects on the human body. This is particularly relevant for testing the efficacy and safety of potential therapeutic compounds in a disease-relevant context [48, 55].

Since all human tissues are formed from the same stem cell with the help of different differentiation protocols, the specific effects on different tissues can also be investigated, especially in the case of channel mutations. In a broader sense, the use of drugs and channel modulators on different tissue types can also be studied simultaneously.

2.3 Generation of patient-specific stem cells and personalized medicine

A significant advance in patient-specific research has been the development of techniques to generate stem cells derived from patients' tissues. Patient-derived iPS

cells are generated by reprogramming adult somatic cells from a diseased patient. This reprogramming enables the phenotypically and genotypically pathological cells to be differentiated into a wide variety of cell types [34].

The use of patient-derived iPS cells offers several advantages. Firstly, it allows the generation of stem cells that are genetically matched to the patient. This means that patient-derived iPS cells carry the patient's genetic characteristics and disease mutations, which opens up the possibility of examining the properties of the patients' cells in iPS-derived tissues of any kind [56]. As shown in the recent past, these tissues have successfully been used for modeling and studying various diseases, including neurological diseases such as Parkinson's, Alzheimer's, and Amyotrophic Lateral Sclerosis (ALS) [46, 56–58].

Cells generated from patient material are the closest pathophysiological cell state, with a 100% genotype of a patient. These patient-derived stem cells have the potential to revolutionize personalized medicine and the study of diseases.

Secondly, these cells can be used to create patient-specific cell models that can be used to study disease processes and develop tailored therapies [59].

In addition, patient-derived iPS cells can be used for personalized medicine. They enable the development of patient-specific therapies in which drugs can be tested for efficacy and safety in the patient's individual cells [60]. This approach aims to improve the efficiency of medicines and reduce potentially harmful side effects [61, 62].

Especially for rare (channel-related) diseases, sample collection and specific therapeutic approaches are rather difficult. However, as described above, single cell samples from affected patients can be used to obtain iPSCs through reprogramming. These have the potential to be differentiated into different cell types and thus provide insights into the specific disease mechanisms of rare diseases. By modeling rare diseases with stem cells, potential target structures can be identified, and new therapeutic approaches can be developed, and researchers can target ways to restore the normal phenotype. This may involve the development of new drugs, targeted gene therapy, or the use of stem cell transplants [57].

However, in order to cope with genetic heterogeneity in patient tissues, it is advantageous to generate several cell clones from patients to be able to analyze them in parallel. As a control, clones from a close healthy family member are well suited. Additionally, these disease mutations should be corrected in the patient clones, whereas the disease-causing mutation should be introduced into the healthy clones. Via this approach, full control is given.

2.4 Electrophysiological characterization and drug testing

Stem cell-derived neurons can be studied electrophysiologically using patch-clamp techniques or multi-electrode arrays. These combined methods allow the direct measurement of action potentials, currents, and, in neurons, synaptic transmission to characterize the cells and their response to different stimuli. Thus, electrophysiological recordings are a prime tool to investigate drug effects directly on the targeted ion channel.

In this technique, a microelectrode is docked to the cell membrane to create a tight electrical contact. By applying a voltage difference and applying suction, a seal can be achieved between the electrode and the cell membrane. By applying a vacuum, a type of "patch" is created that allows the flow of ions to be measured across individual ion channels. The patch-clamp technique enables the measurement of membrane currents with high resolution and has contributed significantly to the understanding of ion channel function and signal transmission in cells [23, 63].

In cells, the membrane tightly regulates the osmolarity and maintains significant ion gradients, such as sodium (Na^+), potassium (K^+), chloride (Cl^-), and calcium (Ca^{2+}).

The approximate values for ion concentrations across human cell membranes are as follows:

1. Sodium (Na^+): The extracellular concentration of sodium ions is typically around 135–145 mM, while the intracellular concentration is lower, around 10–15 mM [64].

2. Potassium (K^+): The intracellular concentration of potassium ions is generally higher, ranging from 120 to 150 mM, while the extracellular concentration is typically around 3–5 mM [64].

3. Chloride (Cl^-): The extracellular concentration of chloride ions is around 95–110 mM, while the intracellular concentration is approximately 5–15 mM [64].

4. Calcium (Ca^{2+}): The extracellular concentration of calcium ions is around 1–2 mM, while the intracellular concentration is typically maintained at much lower levels, around 0.1–0.2 μM [64].

Overall, electrophysiological techniques such as the patch clamp technique or TEVC (two-electrode voltage clamp) are essential for investigating the functions of ion channels and the transport of ions across the membrane [65].

Extracellular derivation using microelectrode arrays or field potential amplifiers is a noninvasive method for electrophysiological characterization of cells. In this technique, electrodes are placed outside the cell to measure electric field potential emitted by the cell. Extracellular derivation allows the measurement of action potentials and synaptic activities of multiple cells simultaneously, providing a broader view of the interplay between cells [23, 65].

2.5 Understanding tissue-specific manifestations

As described above, channelopathies can affect various tissues and organs, each with its own unique cellular composition and physiology. Stem cell-based models allow researchers to generate different cell types affected by channelopathies and study their specific contributions to disease pathology. For example, patient-specific iPSCs can be differentiated into cardiomyocytes to study cardiac channelopathies or into neurons to investigate neurological channelopathies. These tissue-specific models provide a platform to elucidate the mechanisms underlying tissue-specific manifestations of channelopathies and develop targeted interventions for specific affected tissues [66, 67].

2.6 Development of cell-based therapies

In addition to drug screening, stem cell-based models hold promise for the development of cell-based therapies for channelopathies. By leveraging the differentiation potential of iPSCs, researchers can generate healthy, functional cells to replace or repair the affected tissues. For example, in cardiac channelopathies, iPSC-derived cardiomyocytes can be used as a source for cell transplantation to restore normal cardiac function – at least in mice for now [68]. Furthermore, the integration of gene

editing technologies, such as CRISPR/Cas9, with stem cell-based models opens up possibilities for correcting disease-causing mutations and generating patient-specific healthy cells for autologous transplantation [69–71].

Cell replacement therapies and regenerative medicine are other promising aspects of stem cell applications in channelopathies. The ability of stem cells to differentiate into different cell types leads to the possibility of generating functional replacement cells that can be used in damaged tissues or organs. In the field of neuronal channelopathies, for example, stem cells could be used to generate healthy neurons to replace damaged areas in the brain or spinal cord [72]. Similarly, stem cells can be used to differentiate into cardiac cells to repair damaged heart tissue in cardiac channelopathies [73]. This approach offers promising prospects for the development of regenerative therapies that could provide long-term improvement in disease symptoms.

3. Advances in stem cell-based therapies for channelopathies

Stem cell-based therapy has made significant progress in the treatment of channelopathies in recent years. These genetic diseases are caused by mutations in the ion channels that lead to dysfunctional ion regulation. Here, we will focus on three important aspects of stem cell-based therapy for channelopathies: Gene editing and correction of disease-causing mutations, cell transplantation approaches, and challenges and future directions.

A promising approach in stem cell-based therapy for channelopathies is gene editing and the correction of disease-causing mutations. Through the development of techniques such as CRISPR/Cas9, gene editing allows the targeted modification of the genome of stem cells to correct specific mutations. Such approaches have been successfully applied to neuronal channelopathies, such as spinal muscular atrophy (SMA). Several studies showed that by correcting the disease-causing mutation in stem cells, the defective *SMN1* gene could be restored, resulting in improved motor neuron function [74, 75].

Another promising approach in stem cell-based therapy for channelopathies is the use of cell transplantation approaches. Here, differentiated stem cells or their derived cell types are transplanted into the affected tissue or organ to replace the dysfunctional cells or improve their function. In cardiac channelopathies such as long QT syndrome, it has been shown that transplantation of cardiac cells derived from stem cells can lead to an improvement in the electrical properties of the heart and a reduction in arrhythmias [76, 77].

Another example is the transplantation of encapsulated iPSC-derived β-cells competent for insulin production and glucose-level correlated release into type I diabetic pancreas of patients. This can improve the diabetic phenotype including in patients with defective insulin secretion as a consequence of defective β-cell ion channels.

In addition, stem cell-based therapy also enables a better understanding of the disease mechanisms and pathophysiology of channelopathies. By generating patient-specific induced pluripotent stem cells (iPSCs), researchers can replicate the affected cell types in the laboratory and study the effects of the disease-causing mutations on ion channel function and cell physiology. This understanding is crucial for the development of new therapeutic approaches. A study by Yazawa et al. [78] demonstrated the use of iPSCs to investigate the pathophysiological mechanisms of Timothy syndrome, a cardiac channelopathy.

However, despite the promising progress, there are also challenges and future directions in stem cell-based therapy for channelopathies. A major challenge is to generate differentiated stem cells in sufficient quantity and quality to use them for transplantation approaches. In addition, the safety and long-term efficacy of the transplanted cells must be ensured. Another future direction is to improve gene editing techniques to increase the efficiency and precision of mutation correction.

Overall, advances in stem cell-based therapy for channelopathies show promise for the treatment of these genetic diseases. Gene editing and correction of disease-causing mutations, as well as cell transplantation approaches, have the potential to alleviate the symptoms of channelopathies and improve the quality of life of affected patients. However, further research efforts and clinical trials are needed to confirm the safety, efficacy, and long-term effectiveness of these approaches.

3.1 Examples of channelopathies studied in iPSC-derived cells

Several channelopathies have been studied with the help of iPSC-derived cells. Some examples of prominent work will be briefly described hereafter.

3.1.1 Epilepsy

Research into epilepsies has benefited from the use of stem cell-derived neurons as a promising model. Stem cell-derived neurons offer the possibility to produce human neurons in the laboratory and thus to study epileptic phenomena in a controlled and reproducible system.

Stem cell-derived neurons can be used to recreate epileptic networks in the laboratory and explore the underlying mechanisms. For example, the study from Hirugashi et al. [79], in which stem cell-derived neurons were generated from patient cell samples with Dravet syndrome, a form of genetic epilepsy. The authors were able to show that these neurons were hyperexcitable and exhibited spontaneous epileptiform activity, similar to that seen in the brains of affected patients.

By using stem cell-derived neurons, researchers can more accurately investigate the specific disease mechanisms of epileptic disorders. Chen et al. [80] used stem cell-derived neurons from a cell line with a generated point mutation in *SCN1A*. This mutation leads to a misfunction in the $Na_v1.1$ α subunit, affecting neuronal function. The results provided important insights into the effects of these mutations on synaptic transmission and neuronal excitability.

KCNQ channels, also known as M channels, play a significant role in the regulation of neuronal excitability and have particular relevance in relation to epilepsy. KCNQ channels are a subset of voltage-gated potassium channels expressed in several regions of the brain, including the hippocampus, amygdala, and cortex. They are crucial for the regulation of neuronal excitability and contribute to the stabilization of resting membrane potential [18]. Mutations in the genes encoding KCNQ channels are associated with various forms of familial and sporadic epilepsy [81].

Studies have shown that mutations in the KCNQ channel genes can lead to impaired channel function, resulting in increased neuronal excitability and increased susceptibility to epileptic seizures. A particular form of epilepsy called benign familial neonatal epilepsy (BFNE) has been associated with mutations in the *KCNQ2* or *KCNQ3* gene [82].

The importance of KCNQ channels in the pathophysiology of epilepsy has sparked interest in developing therapies aimed at modulating these channels. One promising

strategy is to identify pharmacological compounds that can enhance the activity of the channels and thus reduce neuronal hyperactivity. For example, a study by Wuttke et al. [83] investigated the effect of the compound retigabine on KCNQ channels and showed that it increased channel activity, thereby reducing neuronal hyperexcitability .

Also here, stem cells have helped to deepen the understanding of the role of KCNQ channels in the development of epilepsies. By differentiating stem cells into mature neurons – either directly or via neuronal progenitor cells – researchers can study the function and expression of KCNQ channels in a neuronal context. iPSC-derived neurons from patients with *KCNQ2*-associated epileptic encephalopathies have been used to investigate the effects of *KCNQ2* mutations on neuronal excitability [84, 85]. The results showed increased neuronal excitability and provided insights into the underlying mechanisms of the disease.

Overall, studies on KCNQ channels provide deeper insight into the pathophysiology of epilepsy and result in new possibilities for the development of therapeutic approaches. By using stem cells and studying patient-derived neurons, researchers can better understand the effects of KCNQ channel mutations and develop targeted therapies to control neuronal hyperactivity in epilepsy.

When it comes to pharmacology-based therapeutical approaches, studies have shown that the use of stem cells in the investigation of neuronal channelopathies such as epilepsy can provide crucial insights into the effects of antiepileptic drugs [85–87]. This could improve the effectiveness and safety of drugs and take into account individual differences in disease response [88, 89].

3.1.2 Long QT syndrome

Long QT syndrome (LQTS) is a hereditary condition characterized by a prolonged QT interval on the electrocardiogram (ECG) that can lead to life-threatening ventricular arrhythmias. One of the main causes of LQTS is channel dysfunction, particularly of ion channels responsible for regulating cardiac repolarization.

Several ion channels are associated with LQTS. One of them is the hERG (human ether-a-go-go related gene) channel. Mutations in this potassium channel lead to impaired repolarization of the action potential in the heart and a prolonged duration of the ventricular repolarization phase, which cause LQTS2 that increases the risk of torsade de pointes tachycardia. The function of the hERG channel is to allow the rapid outflow of potassium ions during the repolarization phase of the cardiac action potential [90].

A study examined the effects of three different mutations in the hERG gene on channel function [91]. Patch-clamp techniques were used to measure potassium current in stem cell-derived cardiac cells from patients with LQTS2 and healthy controls. They found that the LQTS2 patient mutations led to reduced hERG channel activity, resulting in prolonged repolarization time and increased risk of arrhythmias. These results support the role of hERG channel dysfunction in the pathogenesis of LQTS2 [90].

Additionally, *KCNQ1*, a gene encoding the KCNQ1 ion channel, can lead to impaired function of the channel and is associated with long QT syndrome 1, when mutated [92]. The KCNQ1 channel is responsible for regulating potassium ion flow during the repolarization phase of the action potential in the heart [93–96].

Another ion channel associated with LQTS is the *SCN5A* sodium channel. Mutations in this channel can lead to reduced sodium influx during the depolarization phase and impair repolarization of the action potential. This also leads to a prolonged

QT interval (LQTS3) and an increased risk of arrhythmias [97]. Recent studies have investigated the function of *SCN5A* mutations in stem cell-derived cardiac cells and found that these mutations resulted in decreased sodium channel activity, which explains the impaired repolarization and prolonged QT time [98, 99].

iPS-derived cardiomyocytes have been used to study the electrophysiological behavior of single LQTS phenotypic cells [100–102]. For this purpose, the stem cells were first mutated to known LQTS genotypes, and then, the cells were differentiated and examined. In doing so, they examined not only LQTS but also other forms of arrhythmia. These cells could then be used in further steps to measure potential pharmaceuticals directly on the physiological cell systems and their immediate effect.

In summary, the hERG and KCNQ1 potassium channels as well as the SCN5A sodium channel play a central role in inherited LQTS 1-3. These channels are critical for the normal repolarization of the heart, and mutations in their genes lead to impaired ionic currents and prolonged repolarization, increasing the risk of life-threatening arrhythmias, and have been modeled in iPSC-derived cardiomyocytes.

Studies in stem cell-derived cardiac cells allow us to have a closer look at channel dysfunction and can be used as a screening platform to test direct therapeutic options at the physiological cellular level.

3.1.3 Periodic paralysis

Periodic paralysis, also known as paroxysmal paralysis, is a rare neurological disorder characterized by episodic weakness or paralysis of the muscles [13, 103]. These syndromes pose a challenge to the medical community because they are often difficult to diagnose and treat. Genetic mutations, particularly in the *CACNA1S*, *Kir2.1,* or *SCN4A* genes, have been linked to hypokalemic periodic paralysis and Andersen-Tawil syndrome [21, 103, 104].

The impaired function of ion channels in muscles leads to altered electrical excitability and impaired contractility of muscle fibers [19]. The underlying mechanisms of periodic paralysis are complex and can range from channel hyperpolarization to excessive depolarization [19]. This leads to impaired action potential formation and conduction, which in turn leads to characteristic paralysis attacks.

The use of stem cells, particularly induced pluripotent stem cells (iPSCs), has ushered in a new era also in the study of periodic paralysis [105, 106]. iPSCs can be produced from patient cells and allow the generation of specific cell types affected by the disease, such as muscle cells or neurons. These *in vitro* models allow researchers to study the effects of genetic mutations on the function of ion channels and the physiological properties of the affected cells.

The use of stem cells has not only contributed to a better understanding of the pathophysiology of periodic paralysis but also opened up new approaches for therapy development [106].

The study of periodic paralysis has already made and will make further significant progress thanks to advances in stem cell research and molecular genetic techniques. Further investigation of these disorders, particularly regarding their genetic diversity and the development of personalized therapies, will help to improve the quality of life of those affected.

4. Conclusion and future directions

Stem cell research has provided valuable insights into the understanding of channelopathies and offers promising avenues for the development of novel therapies. By generating disease-specific stem cell models, researchers have been able to study the underlying mechanisms of channelopathies and screen potential therapeutic agents. Additionally, stem cell-based approaches, such as gene editing and cell transplantation, hold great potential for the treatment of channelopathies. However, ethical considerations and regulatory frameworks need to be carefully addressed to ensure the responsible and ethical use of stem cells in research and clinical applications.

By specifically correcting genetic mutations in iPSCs or modulating ion channel activity in affected cells, potential treatment strategies can be explored. In addition, transplantation of healthy stem cells or differentiated cells into animal models provides new opportunities to study the efficacy of therapies.

Conflict of interest

The authors declare no conflict of interest.

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