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Review

Role of connexin 43 in cardiovascular diseases

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ABSTRACT

Gap junctions (GJs) channels provide the basis for intercellular communication in the cardiovascular system for maintenance of the normal cardiac rhythm, regulation of vascular tone and endothelial function as well as metabolic interchange between the cells. They allow the transfer of small molecules and may enable slow calcium wave spreading, transfer of "death" or of "survival" signals. In the cardiomyocytes the most abundant isoform is Connexin 43 (Cx43). Alterations in Cx43 expression and distribution were observed in myocardium disease; i.e. in hypertrophic cardiomyopathy, heart failure and ischemia. Recent reports suggest the presence of Cx43 in the mitochondria as well, at least in the inner mitochondrial membrane, where it plays a central role in ischemic preconditioning. In this review, the current knowledge on the relationship between the remodeling of cardiac gap junctions and cardiac diseases are summarized.

Contents

1. Introduction

Gap junctions (GJs) are intracellular structures that provide connections and communication between cells, allowing the passage of ions and small molecules such as ATP, glutathione, cAMP, IP_3 and glucose (Pieperhoff and Franke, 2007). In the heart, GJs mediate electrical coupling between cardiac myocytes, forming the cell-to-cell pathways for orderly spread of the wave of electrical excitation responsible for synchronous contraction (Del Rya et al., 2015). GJ channel is composed of a hemichannel (named connexon) formed of six transmembrane proteins (connexins) embedded in the plasma membrane of one cell joined in mirror symmetry with a connexon hemichannel in the opposing cell membrane (Li et al., 2002). Twenty one genes coding for connexins have been identified, which are classified according to their

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different molecular weights that range between 26 and 60 kDa. Each connexin is constituted by four transmembrane domains, two extracellular loops and one intracellular; amino and carboxy terminal regions are both located in the cytosol (Solan and Lampe, 2005).

In the normal adult heart, there exists three main isoforms: Cx40, Cx43 and Cx45. Cx40 is expressed in the atrial myocytes, in the atrioventricular node, His-bundle and the ventricular conduction system. The expression of Cx45 is mainly localized in the sinoatrial node and the atrioventricular node (Jansen, 2010). Cx43 is the most abundant and is expressed in atrial and ventricular myocytes (Lampe and Martinez, 2004) so this review will focus on its function and role. Cx43 oligomerize in the Golgi/trans-Golgi network and, after assembly, is transported to the non-junctional plasma membrane through the cytoskeleton. Once inserted into the cell membrane, Cx43 spreads in the region where there are GJ plaques by a microtubule/dynein/β-catenin/N-cadherin-dependent pathway (Sáeza et al., 2010). Several protein kinases are implicated in Cx43 phosphorylation, such as mitogen activated protein kinase (MAPk), protein kinase C (PKC), protein kinase A (PKA), casein kinase 1 and Src (Jansen, 2010). Phosphorylation on serine 368 in the COOH-terminal regions of Cx43 provide the regulation of the turnover of GJs communication, such as trafficking, assembly/disassembly, degradation and gating of GJ channels (Popolo et al., 2013). GJ channels preferentially localize to the intercalated disks, discrete regions of cardiomyocyte-cardiomyocyte coupling in the heart, where they interact intimately with adherens junctions (Nambara et al., 2007). This system allows for intercellular communication in cardiovascular tissue and ensures the right maintenance of the cardiac rhythm, regulation of vascular tone and endothelial function, as well metabolic interchange between adjacent cells (Iwasaki et al., 2011). Abnormalities of the normal cardiac rhythm are a common, serious and often fatal complication of many forms of heart disease (Severs et al., 2008). Interest in the role of GJs in heart disease was piqued when images of diseased hearts stained for Cx43 showed that cardiac pathologies were associated with a change in the normal pattern of Cx43 in the ventricular myocardium (Popolo et al., 2014). Rather than being localized at the intercalated disk, Cx43 in human hearts after a myocardial infarction was found on the sides of the myocytes, known as the lateral membranes. Being as intercalated disk localization of Cx43 was considered important for anisotropic conduction in normal heart, this “lateralization” of Cx43 was suspected to be involved in alterations of conduction in the injured heart (Duffy, 2012). In fact, alterations in Cx43 expression and distribution were observed in myocardium disease; i.e. in hypertrophic cardiomyopathy, heart failure and ischemia (Allen, 1992).

Cx43 expression and localization are also altered in aged mice and rat hearts and this increased heterogeneity correlates with age-associated alterations in the heart rhythm and increased atrial fibrillation in patients (Schultz, 2015a). Cx43 expression is higher in female hearts suggesting that Cx43 can be involved in sex-related differences in incidence of life-threatening arrhythmias (Knezl et al., 2008).

Even if most of the functions ascribed to Cx43 in cardiac pathophysiology are related to its function in a GJ, recent literature reports roles and functions for Cx43 outside of intercellular communication (Sakurai et al., 2013; Kalvelyte et al., 2003). The presence of Cx43 in mitochondria is well established and several studies report that mitochondrial content of Cx43 is enhanced by ischemia-reperfusion (Rodriguez-Sinovas et al., 2006) and regulates apoptosis (Goubaeva et al., 2007).

In the last decade, a new localization on Cx43 in cardiac tissue has been described. A recent report suggested that Cx43 is present in the mitochondria from mouse, rat, pig and human left ventricular myocardium, and may play a role in mediating the cardioprotective effect of ischemic preconditioning. Co-immunoprecipitation studies have shown an interaction of Cx43 with translocase of the outer membrane 20 (TOM 20), which is, with Tom5, 6, 7, 22, 40 and 70, part of the translocase of the outer membrane (TOM) protein complex and thereby of the general mitochondrial import machinery (Boengler et al., 2006). TOM 20 is the only known protein complex involved in the entering of nuclear-encoded proteins into mitochondria. The proteins bind TOM through TOM 20 and, after the recognition step, reach the internal membrane or the matrix through the TIM (Translocase of the Inner Membrane) complexes (Rodriguez-Sinovas et al., 2006). It has been demonstrated that ischemic preconditioning induces Cx43 translocation from cytosol to mitochondria with a mechanism that involves heat shock protein 90 (Hsp90) and TOM 20. ATP-dependent Hsp90 in the cytosol is implicated in the import process but mitochondrial receptors for these factors have not been established. Cytosolic Hsp90 is generally involved in the folding of newly synthesized proteins and its role in mitochondrial import may be an extension of this activity. Mitochondrial import machinery involves binding of the target protein to a chaperone (Hsp90/Hsp70), presentation to specific parts of TOM complex, and release into the inner mitochondrial membrane (Ruiz-Meana et al., 2008) (Fig. 1).

Mitochondrial Cx43 modulates the matrix potassium levels and participates in energy metabolism in the heart (Schultz et al., 2015a, 2015b). Yue et al. (2002) have demonstrated that K^+ influx, through mitochondrial ATP-dependent K^+ channels (mitoKATP), causes mitochondrial depolarization in preconditioned cardiomyocytes, an effect associated with reduced mitochondrial reactive oxygen species production and infarct size reduction. These effects are most important for the cardioprotection. Furthermore S-nitrosation of mitochondrial Cx43 increases mitochondrial permeability, especially for potassium, and leads to increased reactive oxygen species formation. The increased amount of S-nitrosation mitochondrial Cx43 by ischemic preconditioning may link nitric oxide and Cx43 in the signal transduction cascade of cardioprotective interventions (Soetkamp et al., 2014). In addition mitochondrial Cx43 play an important role in matrix calcium homeostasis; in fact, calcium overload in subsarcolemmal mitochondria was reduced by blocking Cx43-formed channels with Gap27. (Srisakuldee et al., 2014).

Furthermore, recently other studies indicate that mitochondrial Cx43 translocation is implicated in cardioprotection against doxorubicin-induced cardiotoxicity (Pecoraro et al., 2014).

2. Role of Cx43 in ischemia-induced arrhythmias

Electrical coupling is essential for normal impulse propagation through the heart, together with proper excitability of the cardiomyocytes and normal tissue architecture. Reduced electrical coupling can increase the propensity for arrhythmias rendering the ventricle more susceptible to re-entry. This condition seems to be due to dysfunction and disorganization of Cx43. Indeed, reduction of about 90% in Cx43 expression results in about 50% decrease in the conduction velocity (Van Rijen et al., 2004). While 50% reduction in Cx43 may lead to some conduction slowing, high levels of electrical uncoupling are needed to increase arrhythmogeneity. Arrhythmias are a common complication of myocardial ischemia and infarction in humans. These pathologies are associated with progressive remodeling, loss of sarcomeres and perinuclear accumulation of glycogen. Furthermore, it was verified that the organization of GJs was markedly disordered. They are not aggregated into intercalated disks but are distributed over myocyte surfaces (Severs et al., 2004). Abnormal tissue architecture, e.g. due to increase of fibrosis, may have synergistic

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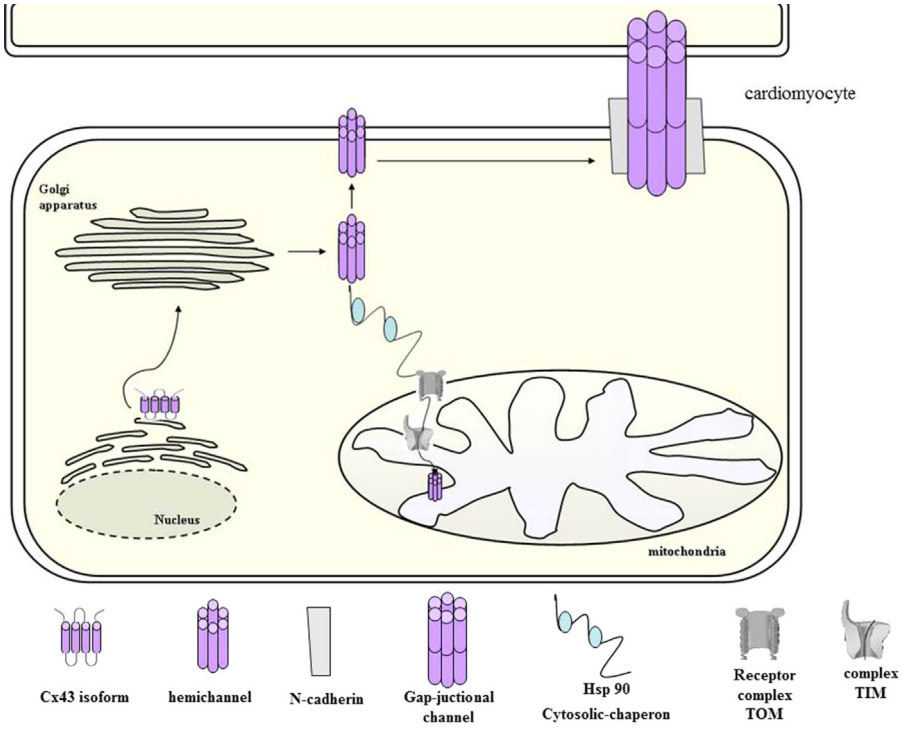

Fig. 1. Lifecycle of Cx43 During biosynthesis, Cx43 is inserted into the endoplasmatic reticulum where it correctly folds and oligomerize in the Golgi/trans-Golgi network. After assembly, Cx43 hemichannel is carried on the cell surface through the cytoskeleton. Once inserted into the cell membrane, Cx43 spreads in the region where there are GJ plaques by a microtubule/dynein/β-catenin/N-cadherin-dependent pathway. In addition, after assembly in Golgi, Cx43 may translocate from cytosol to mitochondria with a mechanism that involves the heat shock protein (Hsp90) and translocase of the outer membrane. This system involves binding of the target protein to Hsp90, presentation to specific parts of TOM complex, and release into the inner mitochondrial membrane.

effects together with reduced Cx43 expression and lead to conduction slowing (Fontes et al., 2012). Alteration of Cx43 in quantity, phosphorylation and distribution may cause cardiac electrically conductive disorder and result in arrhythmias eventually. Previous studies have demonstrated that Cx43 remodelling may account for intercellular calcium overload which is also associated with induction of ischemic arrhythmia (Gao et al., 2015).

Atrial fibrillation is due to abnormal impulse initiation and/or conduction (Paul, 1986; Jansen et al., 2012) the latter is believed to be much more frequent in clinical cases. The conduction velocity is determined primarily by cardiomyocyte excitability and the GJs coupling conductance (G_j) (Jansen et al., 2012; Desplantez et al., 2012). Cardiac disease-linked GJs remodelling can decrease G_j between myocytes and increase the junctional delays between cardiomyocytes. Furthermore, it has been demonstrated that loss of Cx43 expression brings about a loss of sodium current amplitude, which is responsible for cell excitability. This means that loss of Cx43 expression involves interruption of the path between cells but also the decrease of the voltage difference that is generated by excited cell (Agullo-Pascual et al., 2014).

3. Hypertrophic cardiomyopathy

Hypertrophic cardiomyopathy is the most common genetic disease of the myocardium characterized by a thickened portion of the heart, in particular the left ventricular wall. It is associated with sudden death, especially in young adults and the incidence ratio is 1/500 in the general population (Maron, 2002; Maron et al., 2003). This cardiomyopathy involves cardiomyocyte hypertrophy, myofibrillar disarray, fibrosis and is usually associated with pressure overload due to aortic stenosis or chronic hypertension (Lips et al., 2003). Notably, ventricular alterations could be one of the strongest risk factors for hypertrophic cardiomyopathy. This damage, which causes changes in the heart conduction, prolonging the QRS interval, is used as measure of risk identification (Jacob et al., 2015). Several studies have described changes in the number, size and distribution of myocardial GJs during hypertrophic heart disease. In general, Cx43 expression appears to be unaltered or up-regulated during the initial and compensatory phase of hypertrophy, but redistributed along the cardiomyocyte surface and reduced when the hypertrophy becomes prolonged and putatively maladaptive in its progression to heart failure (Birgit et al., 2004).

In an experimental pig model of left ventricular (LV) volume-overload by creation of an aorta-vena cava fistula, it was shown that Cx43 expression initially increased during the acute hypertrophic response but decreased with the progression of hypertrophy (Formigli et al., 2003). In a rabbit model of ventricular hypertrophy by volume overload, in which an arteriovenous shunt was made between the common carotid artery and jugular vein, Cx43 mRNA expression was significantly depressed relative to sham-operated animals 12 weeks after the shunt formation (Itoh et al., 2002). In a rat model of right ventricular (RV) hypertrophy secondary to monocrotaline (MCT)-induced pulmonary hypertension and a rat model of LV hypertrophy as a result of abdominal aortic banding, a dispersion of Cx43 over the entire cell surface and a proportional decrease of Cx43 at the intercalated disc centers was observed (Uzzaman et al., 2000; Emdad et al., 2001).

Kostin et al. (2004) reported that in the left ventricles of pressure-overloaded human hearts with valvular aortic stenosis, Cx43 expression was increased in the compensated hypertrophic stage, but decreased and heterogeneously distributed throughout the ventricles in the decompensated stage. The decreased expression of Cx43 at the protein level is accompanied by a reduction

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of Cx43 mRNA, suggesting that the down-regulation of Cx43 in hypertrophic heart disease is regulated at the transcriptional level. Dupont et al. (2001) showed that the decline in Cx43 protein and mRNA was accompanied by an up-regulation of Cx40 mRNA and protein at the endocardial surface next to the Purkinje fibers in patients with idiopathic dilated cardiomyopathy. This Cx40 up-regulating response was suggested to be compensatory for the loss of Cx43. Cx45 mRNA expression, in contrast, appeared to follow the same pattern as Cx43 whereas Cx45 protein was not detectable (Yamada et al., 2003). In conjunction with a down-regulation of Cx43, Cx45 protein, but not Cx45 mRNA, was significantly increased in failing human hearts. This enhanced expression of Cx45 seems to occur in a heterogeneous pattern and is co-localized with Cx43. Heterotypic GJs of Cx43 and Cx45 have a decreased conductance leading to an increased chance for the generation of ventricular arrhythmias (Teunissen et al., 2004).

Pathologic cardiac hypertrophy is due to downregulation of the muscle-specific microRNA-1 (miR-1). It is known that Cx43 is a target of miR-1 and its dysfunction, during cardiac hypertrophy, carry out ventricular tachyarrhythmias. Curcio et al. (2013) have shown that miR-1 downregulation is associated with Cx43 increased protein levels and enhanced phosphorylation, through MAPK-ERK 1/2 activation. The increased phosphorylation of Cx43 correlated with its displacement from the GJ.

4. Ischemic cardiomyopathy

The ischemic injury is a very common cause of organ dysfunction in humans, and mainly affects the heart. It is manifested by accumulation of metabolites in the extracellular compartment in combination with reduced oxygen supply. Anaerobic metabolism and lack of flow cause intra- and extra-cellular acidosis with increase in extracellular K^+. Ischemia induces closure of GJs, due to increased cytosolic Ca^{2+} concentration, reduced ATP concentration, changes in phosphorylation of Cx43 and acidification (Johansen et al., 2011). Furthermore, increased levels of intracellular Ca^{2+} and H^+ and accumulation of amphipathic lipid metabolites during ischemia promote electrical uncoupling, mediated by alterations in phosphorylation of Cx43, because acute ischemia may activate or inhibit protein kinases and phosphatases. (Beardslee et al., 2000). Additionally, Severs et al. (2004) have shown two major alterations in myocardial GJs by confocal microscopy: loss of the usual ordered distribution of GJs adjacent to infarct scars, and reduction in the quantity of GJs in myocardium distant from the infarct zone. These changes would be expected to make a significant contribution to electromechanical dysfunction in the diseased heart.

The short- and long-term prognosis after an episode of myocardial ischemia correlates well with the extent of myocardial necrosis or infarction. Rapid coronary flow restoration, or reperfusion, is the treatment of choice in patients with acute coronary syndrome. However, reperfusion is associated with additional injury that limits myocardial salvage.

It has been demonstrated that ischemic preconditioning (IP), which is a brief periods of sublethal ischemia separated by periods of reperfusion, delays the development of cell death from a subsequent prolonged episode of ischemia/reperfusion (Murry et al., 1986). A large number of stimuli able to trigger these states of increased resistance to ischemia/reperfusion have been identified. In general, IP enhances release of G-protein coupled receptor families (opioids, adenosine, bradykinin) leading to activation of cell survival pathways (downstream activation of PKC isoforms, ERK and PI3/Akt kinases) and inhibition of cell death pathway (Jeyaraman et al., 2012). Evidence for the involvement of Cx43 in IP cardioprotection comes from experiments on heterozygous Cx43-deficient mice, where IP cardioprotection is lost (Schwanke et al., 2002), as well as from ex vivo study, where the infusion of heptanol, a reversible GJ blocker, for 5 min before IP abolished the infarct size limitation induced by IP in non-treated mouse heart (Li et al., 2002).

Preconditioned hearts have been described to retain higher Cx43 levels compared to nonpreconditioned hearts (Daleau et al., 2001) and to preserve Cx43 phosphorylation during the following prolonged ischemia (Schulz et al., 2003). Electrical uncoupling, which is closely related to Cx43 dephosphorylation (Beardslee et al., 2000), is almost completely abolished by IP in rat hearts (Jain et al., 2003). Decreased channel permeability could protect cardiomyocytes against sodium and volume overload, and, indeed, cardiomyocytes become more resistant towards a hypotonic challenge once they are preconditioned (Schulz and Heusch, 2004). It is reasonable to speculate that these effects are attributable to the activation of PKC and other kinases (ERK, Akt) induced by the IP, since it has been shown that Cx43 lateralizated after ischemia is dephosphorylated (Lampe et al., 2000).

Furthermore, a key role in preconditioning seems to be played by mitochondrial Cx43. In fact, mitochondrial Cx43 hemi-channel can influence cell survival by modulating mitochondrial integrity (Boengler et al., 2005). Mitochondrial Cx43 is required for mitochondrial reactive oxygen species generation which is needed for protective signal transduction including PKCε activation downstream of the diazoxide-sensitive K_{ATP} channel (Waza et al., 2014). This was confirmed in isolated Cx43-deficient cardiomyocytes in which exposure to diazoxide resulted in markedly attenuated ROS generation and did not confer protection against subsequent ischemia-reperfusion (Ruiz-Meana et al., 2004). Furthermore, in another study it has been demonstrated that reduction of mitochondrial Cx43 by geldanamycin, an Hsp90 inhibitor, is associated with the abolition of the cardioprotective of diazoxide against cell death induced by ischemia-reperfusion (Rodriguez-Sinovas et al., 2006) (Fig. 2).

5. Pharmacological agents

There are currently under study compounds able to inhibit Cx43 hemichannel, thereby limiting adenosine triphosphate loss and volume overload (De Vuyst et al., 2011). Among all synthesized compounds, those that are most promising for their effects on the hearts are the Cx mimetic peptides Gap-26 and Gap-27 and the antiarrythmic peptides AAP10, ZP123, GAP-134 and RXP-E, and. Gap-26 and Gap-27 decrease infarct size and area at risk and closes Cx43 hemichannels (Hawat et al., 2010)

AAP10 improves gap junctional conductance, reduces ischemia-induced internalization of Cx43 (De Vuyst et al., 2011). Interestingly, AAP10 acts only on Cx43 present at the intercalated discs, but not at the lateral sides. This suggests that AAP10 somehow interferes with the mechanisms responsible for the directed incorporation and localization of Cx43 in the plasma membrane. Furthermore, AAP10 maintaines Cx43 at the polar membrane in the ischemic zone, which might be due to reduced internalization from the cell pole or enhanced incorporation at that site (Jozwiak and Dhein, 2008). ZP123, increases Cx43 protein levels in a concentration-dependent manner in cultured neonatal ventricular cardiomyocytes after 24 h (Stahlhut et al., 2006). This effect is partly due to an increased Cx43 synthesis, and partly a consequence of a decreased degradation and phosphorylation. GAP-134, a small dipeptide analogue of ZP123, enhances conduction velocity and gap junctional coupling probably by an indirect route, as there was no change in Cx43 and Cx40 mRNA levels, nor in the spatial distribution of Cx43 in the atria after 14 days of oral GAP-134 administration (Laurent et al., 2009).

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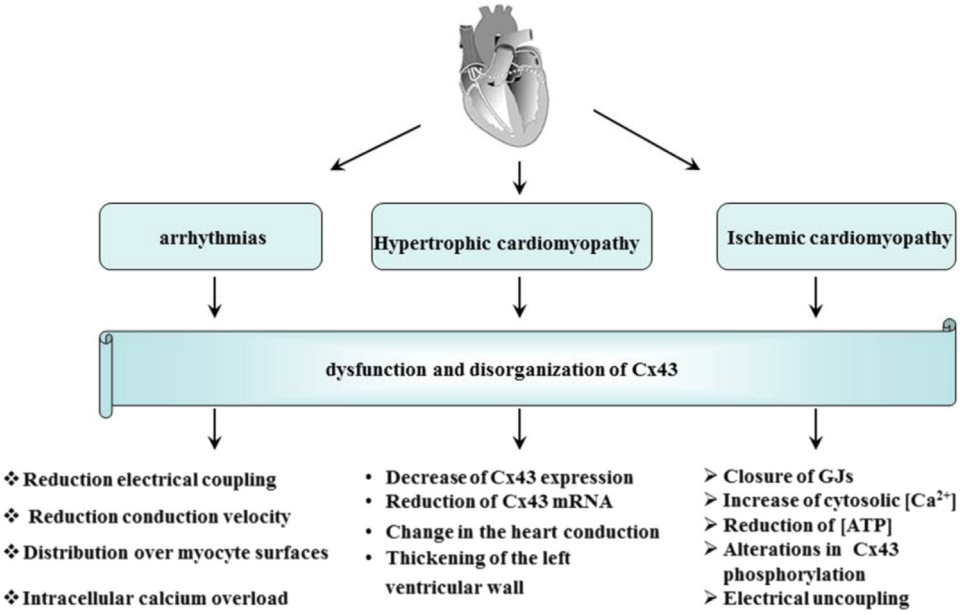

Fig. 2. Schematic diagram that shows the effects of Cx43 dysfunction and disorganization in the main heart disease.

GAP-134 is in Phase 2 clinical trials for myocardial infarction and in the Phase 1 for arrhythmia.

6. Conclusion and future perspective

In this review we analyzed the functional and structural abnormalities of Cx43 in heart diseases. Cx43 lateralization is an event common to all cardiovascular diseases which, in fact, have abnormal rhythms and conduction. In recent years, reports have highlighted the important role of connexin in mitochondria.This opens up a fascinating field of research, not only in the regulation and transmission of electric impulse but also in vitality of the cardiomyocytes.

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