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The involvement of TWEAK and FN14 in murine autoimmune myocarditis
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Background: Myocarditis, defined as an inflammation of the myocardium, can be caused by autoimmune or infectious mechanisms. To investigate the role of sympathetic neurons (SNs) in the pathogenesis of this autoimmune disease, we studied the involvement of TWEAK and FN14 in the development of inflammatory cardiomyopathy.

Methods and Results: At 6 weeks of age, mice were immunized with cardiac troponin I (cTnI) to induce an EAM followed by cardiomyopathy. cTnI and reduced ejection fraction (EF). To determine the severity of myocardial damage, heart tissue levels were measured. Mouse lacking FN14 (FN14-/-) displayed significantly lower heart levels compared to wildtype (wt) littermates (FN14-/-: 625 ± 193ng/mL vs. wt: 3319 ± 1047ng/mL). Furthermore, heart tissue examination of heart sections demonstrated less inflammation and fibrosis of the myocardium in FN14-/- mice. This was accompanied by increased arrhythmia vulnerability, suggesting that in addition to controlling structural CM properties, the physiologic innervation pattern is fundamental to allow normal cardiomyocyte electrophysiology.

Conclusion: This was the first evidence, to our knowledge, of the involvement of sympathetic neurons in the pathogenesis of autoimmune myocarditis.

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Sympathetic neurons that innervate the heart locally modulate cardiomyocyte trophic and electrophysiological properties
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Purpose: The myocardium is highly innervated by Sympathetic Neurons (SNs), that distribute within the tissue with a well-defined pattern. We have previously shown that neuronal input to cardiomyocytes (CMs) represses proteolysis and activates protein synthesis, through the β2-AR dependent signaling. We here tested the hypothesis that regional differences in SN distribution reflect on heterogeneity in CM protein expression and cell size.

Results: In the mouse heart, SNs are predominantly found in the outer myocardial cell layers, and, consistently, CMs in the outer layers (EPI) are significantly larger than the similarly oriented ones in the innermost (ENDO) layers (SNs/EPI: 0.45 ± 0.06 vs. SNs/ENDO: 0.15 ± 0.02; CM volume: EPI: 8590 ± 2121 vs. ENDO: 4697 ± 1433, in μm3). Differences disappear upon SN ablation in the adult mouse, and never establish when SNs are ablated before the postnatal period in which sympathetic innervation of myocardium occurs (P3 to P21). Furthermore, systemic delivery of a β2-AR agonist, or antagonist, which diffuse throughout the myocardium resulting in hypertrophic and arrhythmia remodeling respectively, abolishes CM heterogeneity, suggesting that SNs act with a limited spatial range on neighboring CMs. In support of this we demonstrated, by ISH and RT-qPCR, that CM size heterogeneity is the result of local control on proteolysis operated by SNs. This concept holds true regardless of the innervation pattern as in the heart of other mammals, including humans, characterized by different SN distribution, CM size follows neuronal density. Moreover, using cardiac optogenetics in derenvedated hearts, we observed changes in the transmural electrophysiology and increased arrhythmia vulnerability, suggesting that in addition to controlling structural CM properties, the physiologic innervation pattern is fundamental to allow normal cardiomyocyte electrophysiology.

Conclusion: This is the first evidence, to the best of our knowledge, of either homogeneous tissue, shaped, after development, by a superimposed innervation network, through the modulation of cellular protein turnover.

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W4R variant of CSRP3 leads to the expression of a novel alternate reading frame protein due to alternative splicing
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Background: CSRP3 or MLP protein is expressed in striated muscle and localizes, among others, to the sarcomeric Z-disc and nucleus. CSRP3 interacts with telethonin and α-actinin, acting as cardiomyocyte mechanical stretch sensor, and plays important roles in the regulation of sarcomeric architecture. Several cardiomyopathies have been associated with various CSRP3 mutations. Among those, we previously demonstrated that W4R mutation (10TtoC in exon 2) in the CSRP3 gene leads to dilated and hypertrophic cardiomyopathies in patients and experimental animal models.

Aims: We investigated the molecular interplay between the W4R mutation and development of heart failure. Particularly, we focused on CSRP3 transcriptional regulation, showing that W4R mutation promoted CSRP3 alternative splicing and the translation of a novel protein: ARF-CSRP3.

Results: The CSRP3 gene consists of 6 exons which encode a 212a protein. RT-PCR analysis performed on C57Bl/6 W4R/+ and C57Bl/6 W4R/+ mice revealed the presence of different CSRP3 splice variants. Notably, a CSRP3 mRNA missing exon 2 at the expression of a novel alternate reading frame protein due to alternative splicing. Notably, a CSRP3 mRNA missing exon 2 (2' mRNA) is highly prevalent. Mice experiments conducted in various different cell lines, including neonatal rat cardiomyocytes, confirmed alternative splicing and skipping of exon 2 in the presence of TGFβ substitution. Interestingly, alternative splicing also impairs translation of the mature CSRP3 protein. Indeed, splicing affects wild type CSRP3, leading to its degradation through the ubiquitine proteasome system, which is rescued after treatment with MG132 (10 μM). Finally, generation of antibodies against ARF-CSRP3, which has been deduced from the 2' mRNA, led to the identification of this protein. Animals over-expressing ARF-CSRP3 develop heart failure reminiscent of what can be observed in human mutation carriers.

Conclusions and future perspectives: CSRP3 c.10ToC-W4R causes cardiomyopathy and associated heart failure via multiple molecular mechanisms, including a splicing defect leading to the expression of a novel ARF-CSRP3 mRNA and protein, loss of CSRP3 protein, defects in protein / protein interaction and mislocalization of the mature protein. Therefore, a single mutation may cause disease via multiple mechanisms and hence explain different phenotypes in different individuals.

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Glucocorticoid intervention prenatally: effects on fetal heart maturation
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Glucocorticoids are routinely administered to pregnant women at risk of pre-term delivery to mature fetal organs and improve neonatal survival. We have shown that glucocorticoid action is essential to
250 Investigating inherited HCM caused by SC02 and PRKAG2 mutations using the patients’ induced pluripotent stem cell-derived cardiomyocytes

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Mutations in PRKAG2 gene encoding the γ-subunit of AMPK cause hypertrophic cardiomyopathy (HCM) and familial Wolff-Parkinson-White syndrome (WPW). Patients with R302Q mutation in PRKAG2 suffer from sinus bradycardia, escape rhythms, atrial fibrillation and supraventricular tachycardia. This mutation affects AMPK activity and causes elevated glycogen storage in cardiomyocytes. The link between glycogen storage and WPW syndrome, HCM and arrhythmia remains unknown. A mutation in SCO2 gene encoding for mammalian cytochrome-c oxidase, a crucial part of the mitochondrial electron transport-chain, causes HCM and infants deaths. To investigate the pathological mechanisms underlying these HCM-causing mutations and search for novel pharmacological and genetic therapeutic modalities, we generated induced Pluripotent Stem Cells-derived cardiomyocytes (iPSC-CMs) from patients’ somatic cells, attempting to recapitulate the disease phenotype in vitro. The diseases we explored are: (1) HCM with familial WPW caused by R302Q mutation in PRKAG2 gene. (2) Concentric HCM caused by G1925 mutation in SCO2 gene. Successful reprogramming of respective patients’ skin-derived fibroblasts resulted in iPSC colonies expressing R302Q or G1925 mutations. Action potentials were recorded from cardiomyocytes and extracellular electrogagrams from beating cardiomyocytes clusters using patch clamp and Micro Electrode Array (MEA) techniques, respectively. (Ca2+)-transients and contractions were recorded by means of fura-2 and video edge detector, respectively. The major findings were: (1) PRKAG2 mutated iPSC-CMs exhibited spontaneous delayed afterdepolarizations (DADs), slow firing rates and irregular rhythms (the latter two at the single cell and network level). Further, these phenomena were intensified with culture age, suggesting inter-relations between glycogen storage and electrophysiological abnormalities. (2) SCO2 mutated iPSC-CMs exhibited attenuated inotropic response to isoproterenol as well as DADs and irregular rhythms. Importantly, transmission electron microscopy analysis of SCO2-mutated iPSC-CMs displayed abnormal mitochondria size and morphology. Conclusion: PRKAG2 and SCO2-mutated iPSC-CMs displayed abnormal functional features resembling the clinical phenotype expressed in patients carrying the mutations. In these cases of life threatening arrhythmias the cause is neither mutations in structural proteins nor ion channels; the cause for arrhythmias involved with hypertrophic cardiomyopathy, here, lies within mutated metabolism regulators.