245
The involvement of TWEAK and FN14 in murine autoimmune myocarditis
A. Fischer; A-M. Mueller; A. Bangert; M. Bodastahlert; R. Cetti; HA. Katus; Z. Kaya
University Hospital of Heidelberg, Cardiology Department, Heidelberg, Germany

Background: Myocarditis, defined as an inflammation of the myocardium, can be caused by autoimmune mechanisms. To group 1 we developed a mouse model of experimental autoimmune myocarditis (EAM). Little is known about the role of the innate immune system in the pathogenesis of this autoimmune disorder. However, it was already observed that the cytokine tumor necrosis factor-alpha (TNF) is present in high levels in the myocardium of EAM. Therefore, we investigated the molecular and cellular mechanisms underlying the pathogenesis of EAM in a mouse model.

Methods and Results: We investigated the involvement of TWEAK and FN14 in the pathogenesis of EAM. We found that TWEAK and FN14 expression is upregulated in the myocardium of EAM mice. Furthermore, we observed that TWEAK and FN14 expression is upregulated in the myocardium of EAM mice. These results suggest that TWEAK and FN14 may play a role in the development of EAM.

Conclusion: Our findings provide novel insights into the pathogenesis of EAM and support the hypothesis that TWEAK and FN14 contribute to the development of EAM.

246
Sympathetic neurons that innervate the heart locally modulate cardiomyocyte trophic and electrophysiological properties
N. Pianco; J. Prando; M. Franzoso; A. Di Bona; M. Campone; M. Sandri; T. Zaglia; M. Mongillo
1Venetian Institute of Molecular Medicine, Padova, Italy; 2Department of Biomedical Sciences, Padova, Italy

Purpose: The heart is an organ that is highly innervated by sympathetic neurons (SNs), and the interplay between the SNs and the cardiomyocytes (CMs) is critical for cardiac function. The purpose of this study was to investigate the molecular and cellular mechanisms underlying the trophic and electrophysiological properties of SNs in the heart.

Methods: We used a mouse model of EAM to investigate the role of SNs in the development of EAM. We found that SNs play a critical role in the development of EAM, and that the absence of SNs leads to a significant improvement in cardiac function.

Conclusion: Our findings provide novel insights into the molecular and cellular mechanisms underlying the trophic and electrophysiological properties of SNs in the heart.

247
W4R variant of CSRP3 leads to the expression of a novel alternate reading frame protein due to alternative splicing
V. Azzamati; A. Tabish; B. Buynderdż; KN. Enes; J. Hunt; R. Milner; J.V. Wisman; I. Wahlgren; M. Bohlooly; R. Kosidd
Karolinska Institute, Integrated Cardiac Metabolic Centre, Stockholm, Sweden; 2AstraZeneca R&D Innovative Medicines & Discovery Science, Cambridge, United Kingdom; 3AstraZeneca R&D, Innovative Medicines & Early Development, Cardiovascular & Metabolic Diseases, Mölndal, Sweden

Background: CSRP3 or MLP protein is expressed in striated muscle and localizes, among others, to the sarcosome Z-disc and nucleus. CSRP3 interacts with telethonin and α-actinin, acting as a cardiomyocyte mechanical stretch sensor, and plays important roles in the regulation of sarcomeric architecture. Several cardiomyopathies have been associated with variants in CSRP3. Aim: 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 212 kDa protein. RT-PCR analysis performed on Csrp3W4R/− mice revealed the presence of different CSRP3 splice variants. Notably, a Csrp3 mRNA missing exon 2 (Δ2 mRNA) is highly prevalent. Pregenic experiments conducted in various different cell lines, including neonatal rat cardiomyocytes, confirmed alternative splicing and skipping of exon 2 in the presence of 10T1/2 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 Δ2 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: Csrp3Δ:10T1/2::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.
mature the fetal heart. Here, we tested the hypotheses that (i) antenatal glucocorticoid exposure, prior to the normal increase in glucocorticoid levels, will advance fetal heart maturation and (ii) this will depend on cardiovascular glucocorticoid receptor (GR).

Male SMGRKO mice, which have Sm22α-Cre mediated deletion of GR in cardiomyocytes and vascular smooth muscle cells (VSMC), were crossed with female control (Cre-) mice to generate SMGRKO and control fetuses within the same pregnancy. Dexamethasone (Dex, 100 g/kg/d) or Vehicle (Veh) was administered in the drinking water of pregnant dams from E12.5 (n=3-6 dams/group), 2d prior to the initiation of fetal glucocorticoid synthesis. In utero high frequency ultrasound was performed at E15.5.

Initial results show that dexamethasone increased isolomometric contraction time (indicating impaired systolic function) in control fetuses (Dex,36.3 ± 6.1ms vs Veh,25.1 ± 4.6ms; p<0.05). However, there was no significant effect in SMGRKO fetuses (Dex,31.4 ± 10.4ms vs Veh,28.0 ± 9.3ms;p=0.2). Heart rate was also decreased by Dex treatment in control (Dex,T91 ± 25pm vs Veh,227 ± 15 bpm;p=0.05), but not in SMGRKO mice (Dex,214 ± 79pm vs Veh,221 ± 79pm;p=0.5).

Comparison of Veh treated SMGRKO and control mice showed no differences in E/A wave ratio, a marker of cardiac maturity (SMGRKO+Veh,0.93 ± 0.03 vs Con+Veh,0.43 ± 0.06;p=0.49). However, in Dex treated mice, E/A wave ratio was lower in SMGRKO mice than in controls (SMGRKO+Dex,0.32 ± 0.01 vs Con+Dex,0.41 ± 0.01;p<0.01), suggesting greater cardiac immaturity in SMGRKO mice than controls following exogenous glucocorticoid treatment. This finding is consistent with previous findings that glucocorticoid effects on E/A wave ratio are independent of cardiovascular GR.

Precocious glucocorticoid exposure may impair fetal systolic heart function via activation of cardiovascular GR. It may also impair cardiac maturation via actions that are independent of cardiovascular GR. These could be mediated via GR elsewhere, via MR activation by endogenous corticosterone (in face of removal of competing GR) or via maternal or blood pressure effects. However, this requires further investigation and experiments are ongoing. Nevertheless, these data suggest that early exposure to potent synthetic glucocorticoids antenatally, prior to the normal increase in endogenous glucocorticoids, may be detrimental to cardiovascular health.

249 Uncoupling of myofilament Ca2+ sensitivty from troponin I phosphorylation by hypertrophic and dilated cardiomyopathy mutations can be reversed by EGCG and related Hsp90 inhibitors

A E. Messer, M. Papadaki, PG. Vlkhoren, A. Sheehan, SB. Marston

Imperial College London, London, United Kingdom

Introduction: Heart muscle contraction is regulated by Ca2+ and modulated via the β-adrenergic response that leads to phosphorylation of Troponin I (TnI) at Ser22/23, which changes the Ca2+ -sensitivity of the cardiac myofilament. Previously, it has been shown that mutations in sarcomeric proteins that cause Dilated Cardiomyopathy (DCM) or Hypertrophic Cardiomyopathy (HCM) abolish the relationship between TnI phosphorylation and Ca2+ -sensitivity. We have termed this “uncoupling”. 

Ca2+-desensitizers (such as Epigallocatechin-3-gallate (EGCC) and Silybin) act upon tropinin and alter the Ca2+-sensitivity of the myofilament but their relationship with TnI phosphorylation has never been studied before. Using the in vitro motility assay (IVMA) we have found that Ca2+ -desensitizers can reverse or “recouple” this relationship.

Purpose: To test the ability of EGCG and related compounds to reverse the uncoupling effect of DCM and HCM mutations.

Methods: The in vitro motility assay was mainly used in this study.

Results: EGCG decreased Ca2+ -sensitivity of phosphorylated and unphosphorylated wild-type thin filaments equally retaining the coupling. In contrast, in thin filaments with the DCM-causing TPM1 E40K mutation, Ca2+-sensitivity was uncoupled. EGCG reduced Ca2+ -sensitivity of phosphorylated but not unphosphorylated thin filaments containing DCM or HCM-causing mutations, reversing the uncoupling effect of the mutation. Thus, in the presence of EGCG, Ca2+ -sensitivity dependence on phosphorylation is restored (re-coupling). The same pattern was observed with four other DCM mutations (TPM1 E54K, TNNC1 G159D, TNNT3 K34Q, ACTC E361G).

For the HCM related mutation TNNI2 K280N mutation, tropinin from a patient with this mutation showed no difference in Ca2+ -sensitivity when compared with donor heart tropinin and the Ca2+ -sensitivity was also uncoupled. EGCG was able to restore coupling to this mutation and to other mutations in TNNI2 (114, 278-8, 9R2Q, 1E160, S179F and K273E), TPM1 (E180G) and ACTC (E99K).

However, EGCG is promiscuous and has many off target effects and so an alternative compound is needed. 30 EGCG and Silybin related drug compounds were studied using IVMA for their ability to recouple. Some of these analogues indicated potent recoupling properties and their effects were preserved across three mutations causing DCM or HCM. As both EGCG and Silybin inhibit heat shock protein 90 (Hsp90), preliminary investigations were made into 4 other Hsp90 inhibitors (Ravatresvar, Geldanamycin, Novobiocin and Pterostilbene), they were all found to be recoupers. The Ca2+-desensitising and recoupling effects of EGCG were also seen in contracting myofibrils.

Conclusion: The effect of EGCG and its analogues demonstrates that it is possible to reverse the pathological defects in troponin caused by DCM and HCM mutations pharmacologically and has the potential for treatment.