NEW INSIGHTS INTO THE MECHANISMS OF THE CARDIAC ELECTROPHYSIOLOGICAL CHANGES WITH AGING IN MICE

P. NAUD 1, A. COURBOULIN 1, J. MORISSARD 1, Y. FRELIN 1, S. DEMOLOMBE 1
1 Inserm UMR915-L’Institut du thorax, Nantes, France

Introduction — Age is the single biggest risk factor for the developing heart failure and other cardiac problems. With a large increase in the elderly population across Europe, and an associated large increase in elderly patients with chronic cardiac problems, the need to develop our understanding of the ageing process is more pressing than ever. By examining the ageing effect on the ion channel (IC) and transcriptional regulators (TR) expression of the heart, we gleaned novel information regarding the signalling potentially responsible for generating the electrophysiological remodelling with age.

Methods — By using a high-throughput quantitative approach, we investigated at a genome-scale the expression of 78 genes encoding IC, transporter subunits and Ca2+-homeostasis molecules in ventricles from embryonic days E15.5, E18.5 and postnatal day 1, day 7, day 20 and adult C57BL6 mice. We have also mapped 158 TR transcripts in the same samples, combined with bioinformatic predictions of sites recognized by the TR and their targets.

Results — Among transcripts involved in electrical signalling and Ca2+-homeostasis, 26/78 exhibited up-regulated expression, while 24 showed down-regulated expression. Using one-way hierarchical clustering analysis, we identified the TRs similarly up- and down-regulated than the ion channel genes. We hypothesized that those clustered IC and TR genes are co-regulated and that they share cis-regulatory elements. By in silico investigations, we predicted over-representation of: 1/ Esrrc on Nav1.5, Kv2.1, HCN2, RYR2, KChIP2 and Cavax201 promoters; 2/ GATA5 and GATA6 on Cav3.2, Cav1.3, Cavax262, NCX1, RIP3-2, Nedd4-2, Cx40 and Cx45 promoters, suggesting that these TRs are involved in the expression of these key ion channel for the cardiac electrical activity. These results were strengthened by inter-species conservation.

Conclusions — Our results provide novel molecular correlates in the ageing heart. The biological validations of these data will give significant potential implications for understanding the mechanisms underlying the faetal program gene re-expression in cardiac diseases.

IDENTIFICATION OF GENES INVOLVED IN AORTIC ELASTIC ANOMALIES IN THE RAT

M. OSBORNE-PELLEGRIN 1, S. FALAK 2, H. SCHULZ 2, N. HUBNER 2
1 Inserm U698, Hôpital Bichat, Paris 18, France
2 Max Delbruck Center for Molecular Medecine, Berlin 13125, Germany

The inbred Brown Norway (BN) rat presents several rare arterial phenotypes, including an aortic elastin deficit, a high incidence of PDA and the spontaneous formation of ruptures in the internal elastic lamina (RIEL) of the abdominal aorta (AA) and iliac arteries. Our previously performed genetic linkage study, using microsatellite markers, showed that these 3 arterial phenotypes do not correlate in the backcross population and so are independent, and are controlled by distinct genetic loci (Kota et al. Physiol. Genomics, 2007). The RIEL phenotype is of particular interest as it probably reflects a structural anomaly of the elastic network of the AA and the contiguous common iliac arteries. Since the human AA is highly susceptible to pathological alterations with aging, i.e. atherosclerosis and aneurysm formation, for reasons not entirely understood, the discovery of a gene influencing AA elastic network structure would be of interest. In the BN rat, RIEL is strongly linked to a locus on chromosome 5 (peak LOD score 27.4) but in this locus candidate genes are sparse and sequencing them gave negative results. We thus produced congenic rats, introgressing the chr5 segment containing the RIEL locus from BN rats on a LOU (control) genetic background. These rats express the phenotype sufficiently (30% of parental BN values) to permit further studies on recombinant offspring to try and locate the gene(s) responsible.

We have analysed genotype-phenotype correlations in a large cohort of recombinants, obtained by crossing the congenic rat LOU.BN.DSRat59-DSRat131 with parental LOU and subsequent intercrossing, in order to further define the position of the gene(s) responsible. We also performed high-density SNP mapping on chosen, informative recombinants, using >5000 SNPs discriminative between BN and LOU, and demonstrated the purity of the LOU genetic background (>99.9% outside the chr5 congenic region).

Further generations of recombinants produced from informative genitors and use of SNP genotyping in the congenic region has enabled us to locate the gene(s) responsible for a moderate RIEL phenotype in the first 6Mb region of chrom5. However, BN homozygosity down to 35 Mb causes increased severity of the phenotype.

ROLE OF SERUM RESPONSE FACTOR (SRF) ON MICRORNA EXPRESSION IN THE CARDIOVASCULAR SYSTEM

E. TRITSCH 1, W. CARPENTIER 2, Z. LI 1, M. MERICSKAY 1
1 UPMC UR4 Physiologie, Physiopathologie et Vieillissement, Paris, France
2 UPMC Plate-forme Post-Génomique (P3S), Faculté de Médecine Site Pitié-Salpêtrière, Paris, France

Serum response factor (SRF) is a transcription factor of the MADS box family that regulates essential structural and metabolic genes in many tissues. Using a mouse Cre-Lox model, we have shown previously that SRF inactivation can result in severe cardiac and intestinal failure as well as angiogenic defects.

We have performed transcriptomic analyses of gene expression alteration in the cardiac and vascular system following SRF inactivation (see other abstracts) and we found a large number of down-regulated genes but an even larger number that are up-regulated after SRF inactivation. This latter result was partly unexpected since SRF is mainly known as a positive regulator of transcription. While various hypotheses can account for this up-regulation, we chose to focus on the potential role of SRF in the control of miRNAs, which are endogenous small RNAs that can inhibit the expression of other mRNAs. Indeed, recent bioinformatic analyses revealed that more than 40 microRNAs contain SRF target sequences in their promoter region, suggesting a possible broad regulatory role of SRF for these microRNAs. It has already been shown by others that SRF regulates miR-1 and miR-133 expression during heart development, those miRs being essential for correct cardiogenesis and the control of cardiac...