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Expanding the functional role of miRNAs in the establishment of permanent atrial fibrillation

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Atrial fibrillation (AF) is the most common cardiac arrhythmia and is characterized by the loss of coordinated electrical activity in the atria. The pathophysiology of AF involves initial depolarization trigger events that subsequently evolve to the establishment of a chronic condition [1]. At the cellular level, persistent AF showed a specific gene expression fingerprint characterized by a decreased expression of genes related to ion channel function and transcription factors involved in inflammation and cellular stress responses [2,3]. The role of other genetic and epigenetic players in the onset and establishment of AF is starting to be unveiled [4]. Among epigenetic factors, non-coding RNAs (ncRNAs) are key regulatory players in the control of gene expression. Some ncRNAs such as micro-RNAs (miRNAs) have been recently associated with the pathophysiology of AF [4,5]. MiRNAs are negative post-transcriptional regulators of gene expression that work as modulators of the protein levels. Despite the presence of a characteristic miRNA expression profile in AF, to our knowledge no studies reported the possible role of these negative post-transcriptional regulators in the phenotypic transition from paroxysmal to permanent FA and posterior establishment of a chronic condition. To better define this putative regulatory role, we performed an unbiased transcriptional study in left atrial tissue samples collected during surgical valvuloplasty to simultaneously characterize the miRNA and mRNA expression levels from a cohort of 14 patients: 3 showing permanent AF, 5 with paroxysmal AF and 6 controls in sinus rhythm. The study was approved by the Ethical Committee of the Santa Maria Hospital and the Faculty of Medicine of the University of Lisbon in agreement with the ethical guidelines of the 1975 Declaration of Helsinki, and all the enrolled patients provided written informed consent. Biopsies were homogenized with a bead beater and high-quality total RNA was isolated from the samples by using the Qiagen RNAeasy mini-kit, and fractionated by size prior to Illumina® library preparation for high-throughput sequencing. Libraries for small RNAs (<200 nt) and large RNAs were separately prepared and sequenced at the GeneCore facility, EMBL, Heidelberg, Germany. For mRNA transcriptome assembly, sequence reads from large RNAs were aligned with the human genome (UCSC assembly hg38) using Bowtie2 software (http://bowtie-bio.sourceforge.net/bowtie2). For miRNA characterization, small RNA reads were aligned to MiRbase 21.0 using Oasis2 software (http://oasis.dzne.de). Differentially expressed transcripts and miRNAs in paroxysmal and permanent AF were determined by comparison with the group of patients in sinus rhythm by using the Cufflinks (http://cole-trapnell-lab.github.io/cufflinks) and Oasis2 software respectively.

In order to infer functional relationships between differentially expressed miRNAs and mRNA transcripts, we constructed a regulatory network showing negative correlations between overexpressed miRNAs and their putative down-regulated mRNA transcripts (Fig. 1) using a similar strategy already described only for paroxysmal AF patients [5]. Target prediction of miRNAs was performed by the Mirwalk 2.0 algorithm (http://zmf.umm.uni-heidelberg.de/apps/zmf/mirwalk2). The number of down-regulated protein-coding mRNAs in permanent AF is considerably higher than the observed in paroxysmal AF, with an overlap of 10 transcripts between both groups. Enrichment analysis of down-regulated mRNAs in permanent AF allowed to define specific functional groups of genes related with voltage-gated ion channels, growth factors and extracellular matrix, transcription factors and acetylcholine-mediated activity (Fig. 1). Among these functional groups, the dysfunction in some of the hyperpolarization-activated cyclic nucleotide-gated (HCN) channels has been related with the onset and establishment of heart arrhythmias [6]. Some down-regulated transcription factors such as ATF3 have been associated with an angiotensin II-responsive specific spatial expression pattern in the atria, being part of the signaling pathway involved in cardiac response to neurohormonal stimulation [7]. In permanent AF, 80% of the down-regulated transcripts are putative targets of at least one the observed up-regulated miRNAs, and 74% of them are targeted simultaneously by 2 or more up-regulated miRNAs. Among the up-regulated miRNAs, miR-122-5p, miR-375 and miR-606 together were determined to be

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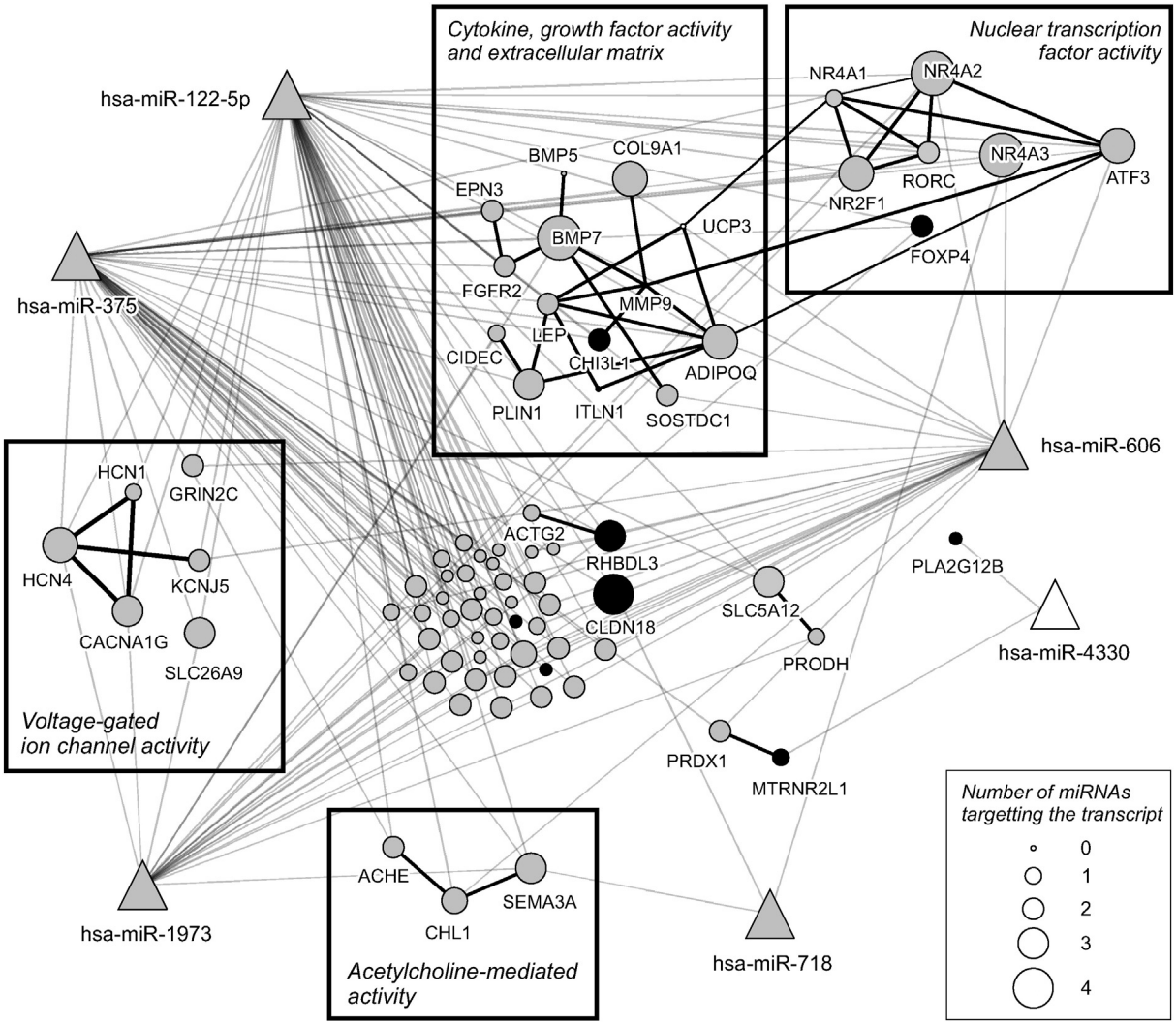

Fig. 1. Transcriptomic regulatory networks in patients with paroxysmal and permanent AF showing the predicted functional relationships between up-regulated miRNAs and their down-regulated predicted mRNA targets. Connections between symbols represent their functional associations: grey lines, miRNA-mRNA regulatory interactions determined by Mirwalk 2.0 algorithm (http://zmf.umm.uni-heidelberg.de/apps/zmf/mirwalk2); black lines, protein–protein interactions extracted from String database (http://string-db.org). Genes were grouped by functional enrichment analysis. Up-regulated miRNAs are depicted by triangles, whereas down-regulated mRNAs are represented by circles. Differentially expressed genes are represented by colours: white symbols, paroxysmal AF; grey symbols, permanent AF; and black symbols, differentially expressed mRNAs common to paroxysmal and permanent AF. Size of gene symbols is proportional to the number of predicted miRNAs targeting each transcript.

potential regulators of >65% of the down-regulated mRNAs. In contrast, in paroxysmal AF samples we only observed one up-regulated miRNAs with predicted down-regulated mRNAs showing a clear difference between initial and chronic disease stages. The presence of redundant regulatory activity mediated by miRNAs concomitant to the transcript down-regulation in permanent AF suggests that the contribution of miRNA-mediated regulatory networks is an important factor that collaborates to the establishment and stabilization of the chronic stage of the disease.

Conflict of interest

The authors report no relationships that could be construed as a conflict of interest.

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