“GEORGE EMIL PALADE” UNIVERSITY OF MEDICINE, PHARMACY, SCIENCE AND TECHNOLOGY OF TÂRGU MUREȘ

DOCTORAL SCHOOL

New directions in atrial fibrillation and cardiac aging: from experimental model to biomarker identification

Abstract

TÂRGU MUREȘ 2026

UNIVERSITATEA DE MEDICINĂ, FARMACIE, ȘTIINȚE ȘI TEHNOLOGIE „GEORGE EMIL PALADE” DIN TÂRGU MUREȘ

Introduction: Atrial fibrillation (AF) is the most common cardiac arrhythmia worldwide and represents a major public health concern due to its impact on morbidity, mortality, and healthcare costs. The onset and progression of AF result from a complex process of atrial remodeling involving structural, electrical, autonomic, and molecular alterations, influenced by multiple risk factors and age-related changes. Although advances over recent decades have improved the understanding of AF pathophysiology, many mechanisms involved in the development and maintenance of the arrhythmia remain incompletely understood.

In this context, experimental models of AF represent essential tools for investigating the pathophysiological mechanisms of the arrhythmia. Compared with clinical studies, these models allow controlled reproduction of conditions that promote AF, facilitating detailed analysis of the alterations associated with arrhythmia susceptibility. In parallel, the identification of molecular biomarkers capable of reflecting early changes in the atrial substrate represents an important research direction. MicroRNAs, small non-coding RNA molecules involved in the regulation of gene expression, have attracted considerable interest due to their role in cardiac remodeling processes and their detectability both in tissues and in the peripheral circulation. Thus, the analysis of microRNA expression may contribute to a better understanding of the mechanisms involved in AF and to the development of new strategies for early diagnosis, risk prediction, and identification of potential therapeutic targets.

Objectives: The objectives of this study were to investigate the mechanisms involved in susceptibility to AF and to identify diagnostic and predictive biomarkers for AF by using relevant experimental models. Specifically, the study aimed to develop and characterize an experimental model of AF, evaluate autonomic remodeling and the expression of genes associated with arrhythmia susceptibility, and identify the expression profile of atrial and circulating microRNAs involved in AF and cardiac aging. Through these approaches, the study aimed to contribute to a better understanding of the pathophysiological mechanisms of AF and to identify potential biomarkers or therapeutic targets.

Methods: To develop and characterize the experimental model of spontaneous AF induced by programmed transesophageal atrial electrical stimulation, 12 adult male Wistar rats were used. The assessment of microRNA alterations with potential diagnostic and/or predictive value for AF was performed using thirty-one male spontaneously hypertensive rats (SHR) and normotensive Wistar-Kyoto (WKY) rats. All animals included in the study were implanted with ECG radiotelemetry devices to detect arrhythmic events and analyze heart rate variability (HRV) in both the time and frequency domains. Within the experimental AF model, mRNA expression levels of hyperpolarization-activated cyclic nucleotide-gated channels (Hcn1, Hcn2, and Hcn4) and the paired-like homeodomain transcription factor 2 (Pitx2) gene were also analyzed. In parallel, to evaluate the expression profile of atrial and circulating microRNAs associated with AF and cardiac aging processes, blood and atrial tissue samples were collected from all SHR and WKY rats.

Results: Transesophageal atrial electrical stimulation induced AF in rats in the absence of facilitating factors, with arrhythmic episodes observed exclusively in stimulated animals. Furthermore, long-term atrial stimulation led to the occurrence of spontaneous AF episodes in stimulated rats compared with non-stimulated animals ($p<0.05$). Stimulated animals presented a

progressive increase in cardiac parasympathetic modulation, reflected by an increase in the SDNN parameter ($p<0.01$). Gene expression analysis revealed increased Hcn4 mRNA expression in the left atrium ($p=0.03$) and decreased Pitx2 expression ($p=0.02$).

In parallel, SHR rats presented spontaneous AF episodes, and atrial expression of miR-328 increased progressively with age ($p<0.0001$), showing a positive correlation with both circulating miR-328 levels and the number of spontaneous AF episodes (both $p<0.01$). In addition, SHR rats showed higher atrial levels of miR-203 and lower levels of miR-150, miR-99b-5p, miR-29a, and miR-30e compared with WKY animals (all $p<0.05$). Regarding cardiovascular aging, atrial miR-203 levels decreased progressively with age ($p=0.03$), whereas atrial miR-30e levels increased after adulthood ($p=0.01$), changes that were not observed in the circulation. In contrast, circulating miR-106b levels showed a progressive decline with aging ($p=0.02$).

Conclusions: The continuous increase in AF prevalence, particularly in the context of population aging, highlights the need for a better understanding of the mechanisms involved in arrhythmia initiation and maintenance, as well as the identification of reliable biomarkers. The results of the present study emphasize the importance of integrating experimental models with molecular biomarker analysis for AF investigation. The experimental AF model induced by long-term transesophageal atrial stimulation successfully reproduced spontaneous AF episodes, providing a valuable tool for studying the arrhythmia.

The findings suggest that chronic atrial stimulation induces both autonomic remodeling, characterized by increased vagal tone, and molecular atrial remodeling, reflected by increased Hcn4 expression and decreased Pitx2 expression. The persistence of AF episodes even after cessation of electrical stimulation supports the hypothesis of a stable arrhythmogenic substrate capable of maintaining the arrhythmia independently of the initial trigger.

MicroRNA expression analysis identified several molecules with potential biomarker roles in AF. Atrial levels of miR-150, miR-99b-5p, miR-29a, miR-30e, and miR-203 were associated with the presence of AF episodes, suggesting their potential utility in AF diagnosis, at least in patients from whom atrial tissue can be obtained. Notably, miR-328 showed significantly increased levels both in atrial tissue and in circulation in arrhythmic animals, indicating that this microRNA may represent a promising biomarker for both diagnosis and prediction of AF. Regarding cardiac aging, alterations in atrial miR-203 and miR-30e expression suggest their involvement in age-related remodeling processes.

Overall, the results of this study highlight both the usefulness of a new experimental model for AF investigation and the potential of specific microRNAs to serve as biomarkers of arrhythmia susceptibility. However, translation of these findings into clinical practice requires further studies in large patient cohorts to validate the identified biomarkers.