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Thamila Arianny Carlos Viana

LNCRNA AND ATRIAL FIBRILLATION: new perspectives for molecular pathophysiology

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Coorientadora: Msc. Ananília Medeiros Gomes da Silva

Orientadora: Profª. Dra. Vivian Nogueira Silbiger

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Thamila Arianny Carlos Viana

LNCRNA AND ATRIAL FIBRILLATION: new perspectives for molecular pathophysiology

BANCA EXAMINADORA:

Prof.ª Dr.ª Vivian Nogueira Silbiger

Universidade Federal do Rio Grande do Norte

Prof. Dr. André Ducati Luchessi

Universidade Federal do Rio Grande do Norte

Prof. Msc. Júlio César Vieira de Sousa

Universidade Federal do Rio Grande do Norte

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lncRNAs and atrial fibrillation: new perspectives for molecular pathophysiology

Thamila Arianny Carlos Viana[1],

[1] Laboratory of Molecular Bioanalysis and Biotechnology, Department of Clinical and Toxicology Analysis, Federal University of Rio Grande do Norte, Natal, RN, Brazil.

*Corresponding author

E-mail: thamila1@hotmail.com (TACV)

Phone number: +55 84 994268897

Abstract

Background: Many studies have demonstrated the association of atrial fibrillation (AF) with endogenous genetic regulatory mechanisms, as non-coding RNAs that could explain how the pathophysiological process occurs and suggest a possible early detection for some diseases, contributed also to the control of progression, among which are long non-coding RNAs (lncRNA). The objective of this review is to report the studies that analyzed the expression of lncRNAs in AF and the main experimental results reported and discuss the importance of these findings.

Methods and results: For the selection of studies, a search was conducted on the PubMed public database (www.ncbi.nlm.nih.gov/pubmed) using the terms "lncRNA and atrial fibrillation" or "long ncRNA and atrial fibrillation" or "non-coding long RNA and atrial fibrillation". Twenty studies were identified in this research. Of these, one is a review article, four articles in which lncRNAs were not related and fifteen represent original research. Of the 15 studies that continued in our review 10 were in tissue samples, 3 were in blood samples and 2 in vitro cell samples. No lncRNA was repeated, we attributed these variations to the sample type, method, species, and patient classification approached in the studies.

Conclusion: The role of lncRNAs and the pathophysiology of atrial fibrillation is not yet well understood, so there is a need to encourage studies to elucidate this role.

Keywords: Atrial fibrillation, lncRNAs, biomarkers, regulatory mechanisms.

1. Introduction

Atrial fibrillation is a disorder of the heart rhythm characterized by electrophysiological abnormalities, triggering the propagation of abnormal electrical impulses and consequently causing remodeling in cardiac tissue.[1] It has a significant association with high morbidity and mortality that is related to hospitalization rates, hemodynamic abnormalities and thromboembolic events such as stroke[1] considering the difficulties for rapid and effective early diagnosis and monitoring in asymptomatic patients.

Many studies have demonstrated the association of AF with endogenous genetic regulatory mechanisms, as non-coding RNAs that could explain how the pathophysiological process occurs and suggest a possible early detection for some diseases, contributed also to the control of progression.[2] These molecules have then been targets in the perspective of biomarkers for AF over a few years.[3] Studies that evaluate potential biomarkers for AF have included the small non-coding RNAs as targets for the diagnosis, monitoring and treatment of the disease. However, considering the transcriptomic advances in recent years, long non-coding RNAs (lncRNA), a new regulatory molecule has emerged as target to understand various mechanisms.

lncRNAs are distinct from short ncRNAs (miRNAs, snRNAs, tRNAs) because they has around 200 nucleotides in length. In addition, they can be expressed in biological and pathophysiological mechanisms, presenting many similarities as the messenger RNA (mRNA), can be processed, 5’ capped, and polyadenylated, which does not apply at all.[4] They are actively transcribed in the human genome and can perform important activities such as controlling pre-mRNA splicing or act as miRNA sponges.[5] This finding has

motivated further studies to assess the activity of these lncRNAs in diseases. In this review, we aimed to report the studies that analyzed the expression of these lncRNAs in AF on the experimental results of studies and discuss the importance of these findings.

2. Methods

2.1. Strategy for study selection of long non-coding RNAs in atrial fibrillation search

For the selection of studies, a comprehensive literature search of original articles until October 04, 2019, of long non-coding RNAs in atrial fibrillation. The PubMed public database (www.ncbi.nlm.nih.gov/pubmed) was searched using the terms “lncRNA and atrial fibrillation” or “long ncRNA and atrial fibrillation” or “long non-coding RNA and atrial fibrillation”.

2.2. Selection of preview studies

The studies were preliminarily screened according to their title and abstract, with replicates and unrelated articles being discarded. Potentially relevant citations were then retrieved as complete manuscripts and assessed for compliance with inclusion and exclusion criteria. Articles in which the lncRNA were not shown (four research papers) or AF patients were not included were excluded. Twenty studies were identified in this search. Of them, one is review article, and fifteen represent original research. (Fig. 1)

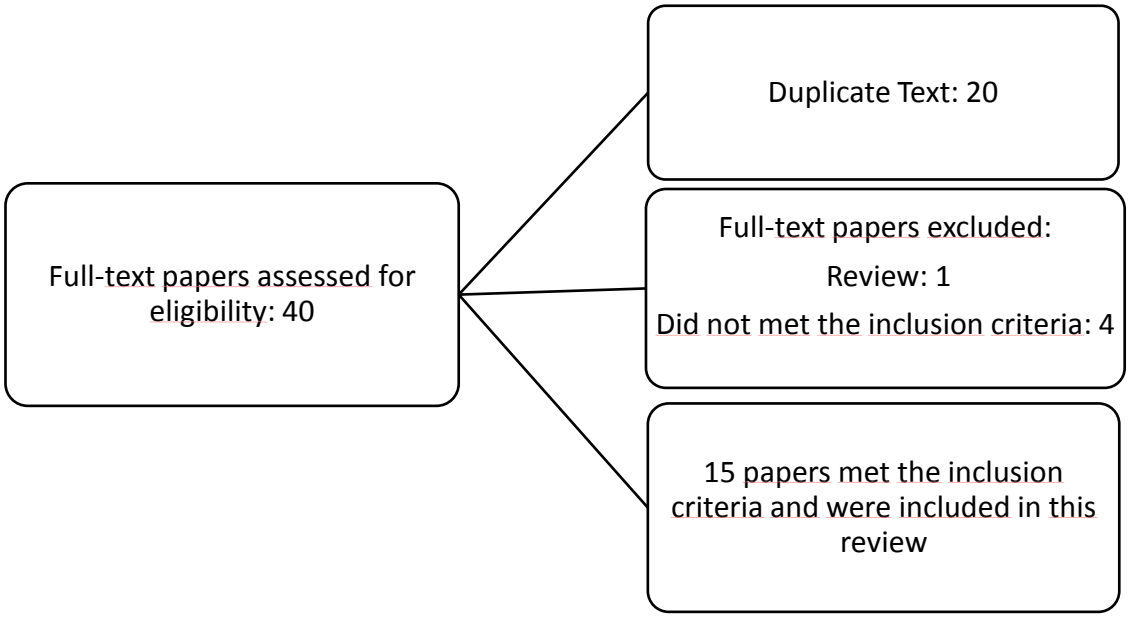

Fig.1 Flow diagram of the study selection process investigating the relationship between lncRNAs and atrial fibrillation

lncRNA expressed in atrial tissue

Ruan et al 2015[6] investigated the association of lncRNAs in AF using microarray analysis and subsequent validation by qPCR. From samples of left atrial appendage of 6 patients with rheumatic heart disease (3 with AF and 3 without AF), they identified 219 lcnRNA, in which 153 were upregulated and the other 63 were downregulated in AF compared without AF subjects. After selection of only highly conserved lncRNA and validation by qPCR, the lncRNAs ENST00000575612, uc001eqh.1, BC064139, and ENST00000425309 shown to be significantly upregulated in AF, whereas the lncRNAs TCONS_00006371, Z74666, X85157, and NR_033661 were downregulated in AF, showed a significantly different expression. In addition, lncRNAs indicated a great association with differentiation, protein tyrosine-kinase activity, calcium ion binding and renin-angiotensin system, among others.

Chen and colleagues[7] published a study evaluating the expression of lncRNAs in AF from pulmonary vein and left atrial area (LA-PV) and left atrial appendage (LAA) in 16 AF patients. Through the microarray methodology, 94 lncRNAs were identified and among them, the lncRNA AK055347 was one of the most expressed and therefore selected for investigation. Molecular analyzes with AK055347 revealed that the Knockdown of this lncRNA, by gene silencing, inhibited cell viability in cardiomyocytes (H9C2 cells) as well as inhibited the expression of Cyp450 and ATP synthases in these cells. Furthermore, using immunofluorescence and western blotting AK055347 shown to regulate the expression of the metabolism-associated protein MSS51 (specific mitochondrial cytochrome c oxidase (COX).

The study conducted by Wang and colleagues[8] in 2015 investigated, by transcriptome analysis, a possible association of lncRNAs in intrinsic cardiac autonomic neural

remodeling (CRA), an important factor suggested to be involved in the initiation and maintenance of PA. Using samples from previous right fat pads (ARFPs) from dult beagle dogs, sequencing identified about 61,616 putative lncRNAs. From these results, only absolute two-fold change were selected, also considering gene prediction data associated with nerve growth, nerve development, nerve migration, and neurological diseases. Then, after validating two-fold change targets, the TCONS_00032546 and TCONS_00026102 (both downregulated) were selected for analysis and functional study, and demonstrated, by in vivo silencing technique, to increase or prevent AF, taking into account neural remodeling in AF conditions.

In 2018, Zhao and colleagues[9] investigated the TCONS_00202959 lncRNA, already described in myocardial diseases, in myocardial tissues of rats with AF. For the analysis of lncRNA, they selected one group with (AF), another group with AF that received the transfection TCONS_00202959 lncRNA and a control group. From expression analysis by real-time PCR, it was interesting to note that the treatment group that received lncRNA transfection had an upregulation of TCONS_00202959 and decreased AF induction rate. On the other hand, the AF group, without lncRNA transfection, demonstrated a low expression of TCONS_00202959 and a high rate of AF induction. In addition, they observed that the expression of the TCONS_00202959 lncRNA affected certain autonomic nervous function, suggesting that this molecule can modulate expression and function of cardiac autonomic nervous function.

Another important study on lncRNA and AF evaluated lncRNAs differentially expressed in right atrium (RA) tissue of permanent atrial fibrillation patients with rheumatic heart disease. This study was conducted by Mei et al 2018[10], who performed a microarray, identifying a total of 1,909 long non-coding RNAs and 23,801 mRNAs.

Then, after analysis of lncRNAs (117 downregulated and 65 upregulated wich fold-change> 1.5) and mRNAs (47 downregulated and 85 up regulated), they observed that among the top 10 up / down regulated, lncRNAs SNORD115-22 and BC041938 had a significant up-down regulation, respectively, in AF samples compared to NSR samples, as well as 17 mRNAs were significantly dysregulated in the AF group (11 mRNAs up and 6 down regulated). Co-expression networks of lncRNAs and putative target mRNAs were performed to evaluate the relationship of these targets, demonstrating that some of these might be involved in arrhythmias by regulation of different biological processes.

In 2019, Lu and colleagues[11] investigated the functionality of GAS5 (Growth Inhibition Specificity 5) lncRNA in the progression of AF using right atrial appendage (RAA) tissue of 40 AF patients and 30 patients with sinus rhythm (SR). Data generated by qRT-PCR shows that GAS5 expression was significantly decrease in patients with AF compared to SR and the AlK5 expression, a target involved in the progression of AF, was significantly increased in AF patients than SR. To assess GAS5 activity on cell proliferation, AC16 cells were transfected with sh-GAS5 (lentiviral small hairpin RNA - sh-GAS5) and GAS5 lentiviruses (GAS5). Through of cell viability assay was observed that proliferation was inhibited after GAS5 overexpression. However, using the knockdown technique was observed that GAS5 inhibition promote cell proliferation. The authors also investigated the relationship of AlK5 and GAS5 and it was observed that in cells treated with sh-GAS5 the ALK5 expression was increased and in GAS5 lentiviruses treat was decreased.

Wu et al 2019[12] analyzed lncRNA expression in atrial samples from 7 patients RMVD (rheumatic mitral valve disease) with AF and 10 patients with sinus rhythm (SR), by microarray analysis. In the transcriptomic analysis, about 2,325 lncRNAs were found, but

only 16 were significantly expressed and among them, lncRNA-n336928 was the most upregulated. Among the mRNAs, 5 were twofold increase in AF patients compared with SR, with NPPB mRNA being the most expressed signaling to participate in small molecule metabolic process, platelet activation, respiratory electron transport chain, blood coagulation, platelet degranulation and cell adhesion (gene ontology analysis). The authors also sought to create co-expression network of the differentially expressed lncRNAs and mRNAs with a Fold change > 1.2, in which the results of the analysis showed that one mRNA or lncRNA might interact with several mRNAs or lncRNAs.

The investigation of ion channel regulation by epigenetic mechanisms brings important findings about the pathophysiology of AF. Based on this perspective, Li and colleagues[13] in 2017 evaluated the expression of lncRNAs in right atrial of rabbits with AF and controls, and by transcriptome detected 99.843 putative new lncRNAs and of these, about 1220 lncRNAs had two-fold change. Bioinformatics analyzes were performed to predict target genes with aberrant functions and interactions and by selected filters, TCONS_00075467 was used as a functional target for regulatory evaluation in electrical remodeling of AF. Still using bioinformatics analyzes the authors speculated that TCONS_00075467 could interact with miR-328, so they performed a bivariate correlation analysis that showed TCONS_00075467 expression negatively correlated with miR-328 transcript level in the AF right atrium model and also observed that TCONS_00075467 was negatively correlated with miR-328 in cardiomyocytes. They found that TCONS_00075467 knockdown positively regulates the miR-328 level, and miR328 silencing positively regulates the TCONS_00075467 level, and also that TCONS_00075467 expression was reduced following infection by miR-328 mimics. Using Western Blot data, the authors concluded that TCONS_00075467 modulates the

expression of CACNA1C (Calcium voltage gated channel subunit alpha1 C) by binding mir-128 as a ceRNA (competing endogenous RNA).

To better clarify the role of PVT1 (plasmacytoma variant translocation 1) lncRNA in the regulation of atrial fibrosis, Cao et al 2019[14] examined the expression of PVT1 by qRT-PCR in 30 patients with AF and 20 patients with sinus rhythm (SR) group, where they noted that PVT1 presented upregulation in human arterial muscle tissues of AF patients compared with SR, and was also positively correlated with collagen I and collagen III, which are two of the major ECM (extracellular matrix) proteins, that contributes to Ang-II-induced atrial fibrosis. In vitro, using human fibroblasts treated with angiotensin II (Ang-II), overexpression of PVT1 demonstrated to facilitate proliferation and production of collagen, whereas the inhibition of PVT1 had a reverse effect. The authors investigated the relationship between PVT1, mir-128-3p and Sp1 (transcription factor specificity protein 1) using bioinformatics showing a possible axis PVT1-mir-128-3p-Sp1 and that PVT1 houses binding sites putative miR-128-3p. The same was observed with the results of luciferase activity. The union of various data has suggested that PVT1 acts as a sponge for miR-128-3p to facilitate Sp1 expression. In addition, PVT1 knockdown in vivo mouse model decreased the atrial fibrosis, indicating that PVT1 may have a pro-fibrotic activity in AF.

A lncRNA that is located only 2 kb 3 'of the PITX2 gene near the 4q25 AF chromosome susceptibility region[15] was named by Gore-Panter et al[16] in 2016 as PANCR (PITX2 Adjacent Non-Coding RNA) lncRNA which is expressed especially in the left atrium. Based on this, the authors sought to characterize this transcript of the 4q25. qRT-PCR method was used to analyze PANCR expression in 223 left atrial appendage samples subdivided into three groups based on their history of AF and their preoperative rhythm

status: no history of AF (No AF, N = 24); history of AF in sinus rhythm (SR) at time of sample collection (AF / SR, N = 78); and history of AF in AF rhythm at time of sample collection (AF / AF, N = 121). Among the results, it was observed that there is no association between PANCR levels and AF rhythm status. Using an analysis of quantitative cis-expression characteristics (cis-eQTL) the authors calculated the association of PANCR expression with SNP genotypes obtained from microarrays, and observed that AF-associated SNPs do not appear to regulate PANCR expression in left atrial appendages However, from PITX2c mRNA analysis it was observed that there is a positive and significant correlation between PANCR and PITX2c, as well as observed in H9 stem cell models that PANCR and PITX2c were co-ordinated before cardiac troponin, suggesting that these genes are expressed before that cells are fully differentiated in cardiomyocytes. Considering the important correlation between PANCR and PITX2c, functional validation was performed by knockdown in H9 differentiated cardiomyocytes and data showed that PITX2c knockdown reduced its expression (up to 40%) but did not affect PANCR expression; on the other hand, inhibition of PANCR decreased expression of both itself and PITX2c. Finally, the authors assessed whether miRNAs could be differently expressed after knockdown of these targets, so after a short sequencing it was observed that some miRNAs were significantly up- or down-regulated in this condition.

lncRNA expressed in blood

In order to discover possible lncRNAs expressed in AF, Xu et al 2016[17] evaluated the profile of these molecules in AF patients from microarray analysis and validation by quantitative PCR. They identified one hundred and seventy-seven lncRNAs of 78243 with significant differential expression levels (up and downregulation) in blood of AF patients

compared with controls. Among the hundred lncRNAs that were upregulated, the NONHSAT098586 and NONHSAG007503 were the most expressed in AF patients compared to controls, whereas that among the sevent- seven downregulated lncRNAs, the NONHSAT040387 was the lowest expression value. In addition, they constructed a co-expression network of differentially expressed correlated lncRNAs and mRNAs using bioinformatics tools and found strong associations involving lncRNAs, transcription factors and mRNAs, demonstrating, for example, that interactions between lncRNA and transcription factors such as GATA1, TAF7 and EBF1 could be very important for lncRNA expression in AF development.

Su et al 2018[18] evaluated potential lncRNAs and mRNAs differentially expressed in blood leukocytes from 34 patients paroxysmal AF (PAF) and 33 controls (no PAF) using 6 samples (three patient AF and 3 control) for microarray analysis. From a total of 2095 lncRNAs and 1584 mRNAs detected, four lncRNAs were validated by qRT-PCR. Only the ENST00000559960 and uc004aef.3 lncRNAs were significantly expressed in AF patients compared to the control (upregulated and downregulated, respectively). In order to predict potential functions of the lncRNAs in PAF, network construction was performed, and it was possible to observe that a lncRNA was associated with various protein-coding genes and an mRNA associated with several lncRNAs.

In order to investigate the role of lncRNAs in chronic AF condition, Yu et al 2017[19] examined the expression profiles of lncRNAs and inflammatory mRNAs in 6 patients with permanent AF (pmAF) and 6 healthy controls through transcriptome analysis (RNAseq) using human lymphocytes. RNAseq data showed that 148 and 102 mRNAs were up and downregulated respectively, and 45 lncRNAs were decreased and 49 lncRNAs increased in AF compared to controls. Among these, 3 lncRNAs highly

expressed (TCONS_00076385, TCONS_00194688 and TCONS_00024161) and 4 mRNAs (IFI27, IFIT2, IFI6, IDH1) were selected for validation by qRT-PCR. The results showing that they are in fact more expressed (regulated) in pmAF patients than controls. The authors also demonstrated, through a co-expression network, that 10 differentially expressed lncRNAs are co-expressed with various inflammatory mRNAs and may be related to the chronic stage of AF.

lncRNA expressed in more than one sample type

Shen et al 2018[20] evaluated expression of ten AF-related lncRNAs in AF model and control group. Of these, four were more expressed in the AF model, but after treatment with Angiotensin II (involved in inducing cardiac diseases by regulating gene expression) only KCNQ1OT1 lncRNA was significantly changed. KCNQ1OT1 was used for electrophysiological and functional analysis as well as the CACNA1C gene (gene strongly involved in AF). The results demonstrated a positive effect of KCNQ1OT1 and CACNA1C in promoting atrial fibrillation, showing that knockdown of these molecules suppressed the progression of AF. In addition, by bioinformatics, the authors saw that miR-384 can simultaneously bind with KCNQ1OT1 and CACNA1C and demonstrated experimentally that KCNQ1OT1 positively regulates the expression of CACNA1C by binding miR-384. (TECIDO)

Wang et al 2019[21] evaluated the activity of the miR-24, LINC00472 lncRNA, RyR2 and JP2 in peripheral blood (N = 125 and =168) and tissue (n=18 and = 18) of patients with AF and controls as well as in cellular model (HCM and H9C2 cells). RyR2 (Ryanodine receptor type-2) and JP2 (Junctophilin-2) play an important role in the excitation-contraction process associated with AF progression. The qRT-PCR data from

plasma samples showed that the expression of LINC00472 were low and high respectively. Interestingly, in tissue samples an expression profile like plasma samples was observed, and in addition to LINC00472 mRNA, the JP2 mRNA/protein and RyR2 protein expression was decrease. The tissue samples that were also used for evaluation of methylation demonstrated that levels of CpGs were higher in AF tissues compared with normal tissues, and considering the low levels of LINC00472, there could be a clear regulatory mechanism in this process. The cellular model was used to evaluate the possible regulation of the four described targets (miR-24, LINC00472, JP2 and RyR2) and then the cells were transfected with pcDNA-LINC00472 or pcDNA-anti-miR-24 mutants showed a decreased level of miR-24 and increased levels of JP2 mRNA/protein and RyR2 protein, indicating a possible regulatory activity of miR-24 on these targets.

Upregulation	Downregulation	Sample	Authors
NONHSAT098586 NONHSAG007503	NONHSAT040387	Blood	Xu et al 2016
AK055347		Tissue	Chen et al 2016
ENST00000575612 uc001eqh.1 BC064139 ENST00000425309	TCONS_00006371 Z74666 X85157 NR_033661		Ruan et al 2015
TCONS_00202959	TCONS_00202959	Tissue	Zhao et al 2018
uc010vaf.1 DQ596229 TCONS_00023347 DQ589437 SNORD115-22 SNORD115-38 SNORD115-32 SNORD115-42 CR608741 SNORD115-6	uc001eiy.2 uc001ejh.1 DQ590126 DQ579288 BC041938 DQ576791 DQ576039 uc010yty.1 DQ595787 TCONS_00005387	Tissue	Mei et al 2018
ENST00000559960	uc004aef.3	Blood leukocytes	Su et al 2018
KCNQ1OT1		Tissue	Shen et al 2018
	TCONS_00032546 TCONS_00026102	Tissue	Wang et al 2015
	LINC00472	Tissue	Wang et al 2019

	GAS5	Tissue	Lu et al 2019
lncRNA-n336928		Tissue	Wu et al 2019
TCONS_00076385 TCONS_00194688 TCONS_00024161		Blood lymphocytes	Yu et al 2017
	TCONS_00075467	Tissue	Li et al 2017
PVT1		Tissue	Cao et al 2019
	PANCR	Tissue	Gore-Panter et al 2016

Table 1. Main lncRNAs involved with AF presented in the studies

Authors	**Model**	**Sample type**	**Method of RNA isolation**	**Method of lncRNA detection**
Xu et al 2016	Human	Blood	RNA Isolation Kit (Ambion)	Microarray/ Agilent Human LncRNA Microarray v 4.0
Chen et al 2015	Human	Pulmonary vein of the left atrial area and left atrial appendage	TRIzol reagent (Invitrogen)	Microarray/Agilent Human LncRNA Microarray V2.0
Ruan et al	Human	Left atrial	TRIzol reagent	Microarray/

2015		appendage tissue.	(Invitrogen)	Agilent One-Color Microarray-Based Gene Expression Analysis Low for Input Quick Amp Labeling kit (v6.0)
Zhao et al 2018	Rat	Myocardial tissue	The RNA extraction kit was purchased from Axygen (Tewksbury).	qPCR/ reverse transcription kit was purchased from Axygen (Tewksbury).
Mei et al 2018	Human	Right atrial tissue	TRIzol reagent (Invitrogen)	Microarray/ GeneChip Human Transcriptome Array 2.0 (HTA2.0) (Affymetrix,)
Su et al 2018	Human	Blood leukocytes	TRIzol reagent (Invitrogen)	Microarray/ Human Arraystar LncRNA Microarray V3.0
Shen et al 2018	Rat	Myocardial tissue and cellular model	TRIzol reagent (Invitrogen)	qRT-PCR/ Applied Biosystems Step One Plus (Applied Biosystems)

Wang et al 2015	Canine	Right fat pads	TRIzol reagent (Invitrogen)	RNA-seq/ illumina Hiseq 2500 with a 50 bp single end protocol (Illumina).
Wang et al 2019	Human	Blood/plasma, cardiac tissue and cellular model	TRIzol reagent (Invitrogen)	Kit PyroMark Gold Q96 (Qiagen,) for plasma and kit SYBR Premix EX Taq (Takara) for cardiac tissue.
Lu et al 2019	Human	Right atrial appendage tissue	TRIzol reagent (Invitrogen)	qRT-PCR/ Transcription Kit (TaKaRa)
Wu et al 2019	Human	Atrial tissue	TRIzol reagent (Invitrogen)	Microarray/ Affymetrix Human Transcriptome Array 2.0
Yu et al 2017	Human	Blood lymphocytes	TRIzol reagent (Invitrogen)	RNA-seq/ The Illumina 4000 platform was applied to RNAseq with a 2 * 150 bp model. (Illumina).
Li et al 2017	Rabbit	Right atrial tissue	TRIzol reagent (Invitrogen)	RNA-seq/ Illumina Hiseq 2500 with a 50 bp single end protocol (Illumina).

Cao et al 2019	Human	Atrial muscle tissues and atrial fibroblasts	TRIzol reagent (Invitrogen)	qRT-PCR/ Reverse Transcription System Kit (Takara).
Gore-Panter et al 2016	Human	Left atrial appendage tissue	TRIzol reagent (Invitrogen)	A similar qRT-PCR assay, but normalized to cyclophilin A/ PPIA, Applied Biosystems Assay No. Hs04194521_s1.

Table 2. A review of sample preparation protocols and methods of lncRNA detection

3. Discussion

In the search PubMed public database (www.ncbi.nlm.nih.gov/pubmed) we found 20 articles, after applying our filters left 15. We note that the all studies are recent (less than 5 years) (table 1); of the 15 studies that continued in our review 10 were in tissue samples, 3 were in blood samples and 2 in vitro cell samples.

No lncRNA was repeated in the work done, we can attribute this to the variability of samples (atrial tissue, blood, cell models) and also of chosen species (mouse, rabbit, canine, human, mice), since some authors chose to use animal models, the variation of technique (RNAseq, qPCR, microarray) may also be a factor, since in the analyzed works there is no standard method to identify these lncRNAs. Another factor may be the types of AF patients (AF paroxysmal, AF permanent), and controls used (healthy patients, sinus rhythm, rheumatic mitral valve disease) (table 2).

Therefore, we suggest that international consortia be made with groups of researchers with a common objective, in which the inclusion and exclusion criteria, the type of sample, the processing and the analysis of the data are well defined. In order to better understand the role of lncRNAs in the pathophysiology of atrial fibrillation, or even to find a lncRNA as a serum biomarker for AF.

4. Conclusion

There are still few studies that support lncRNA for atrial fibrillation, so it is important to encourage further studies that attempt to elucidate the role of lncRNAs in the pathophysiology of atrial fibrillation and to validate these lncRNAs with larger cohorts or their targets in AF.

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