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RESEARCH ARTICLE

Identification of Novel Molecular Network Expression in Acute Myocardial Infarction

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ARTICLE HISTORY

Received: June 15, 2019
Revised: August 05, 2019
Accepted: August 06, 2019

DOI:
10.2174/1389202920666190820142043

Abstract: ***Background*:** In the current study, we aimed to analyze the hypothesis that human myocardial-specific extracellular RNAs expression could be used for acute myocardial injury(AMI) diagnosis.

***Methodology*:** We used bioinformatics' analysis to identify RNAs linked to ubiquitin system and specific to AMI, named, (lncRNA-RP11-175K6.1), (LOC101927740), microRNA-106b-5p (miR-106b-5p) and Anaphase, promoting complex 11 (*ANapc11mRNA*). We measured the serum expression of the chosen RNAs in 69 individuals with acute coronary syndromes, 31 individuals with angina pectoris without MI and non-cardiac chest pain and 31 healthy control individuals by real-time reverse-transcription PCR.

***Results*:** Our study revealed a significant decrease in both lncRNA-RP11-175K6.*1* and *ANapc11mRNA* expression of in the sera samples of AMI patients compared to that of the two control groups alongside with significant upregulation of *miR-106b-5p*.

***Conclusion*:** Of note, the investigated serum RNAs decrease the false discovery rate of AMI to 3.2%.

Keywords: Myocardial infarction, miRNA, lncRNA, serum, diagnosis, extracellular RNAs.

1. INTRODUCTION

Myocardial Ischemia is described as decreased blood supply to the myocardium causing lack of oxygen and decreased metabolite removal. Acute Myocardial Infarction (AMI) is the first lethal cause worldwide [1, 2]. Thus we need an earlier diagnosis in order to optimize the AMI therapy that would reduce the risk of heart failure. The electrocardiogram (ECG) has limited sensitivity (50-60%) in AMI detection. In addition, many biomarkers, such as creatine kinases (CK) cardiac troponins (cTns), and lactate dehydrogenase (LDH) are widely used in the diagnosis of acute myocardial infarction patients [3]. However, the delayed release of troponins reaching peak concentrations approximately 12-24 h after infarction remains a major problem delaying the early detection of AMI. Also, cardiac troponin is elevated in cardiac conditions other than myocardial infarction such as cardiac trauma and end stage renal disease [4]. Therefore, searching for novel biomarkers of high sensitivity as well as high accuracy for detection of AMI is a great challenge.

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Several extracellular RNAs regulate the key processes linked to the pathogenesis of AMI. Many studies postulate that the expression of circulatory RNAs change over time and is affected by the duration and intensity of exposure to AMI risk factors, and may, therefore, be useful biomarkers in AMI diagnosis [5].

The ubiquitin Proteasome system (UPS) has been described as a way to remove the unwanted, or unneeded proteins [6]. It was recently found that in cardiac cells there are about nine ubiquitin ligases that act as critical players in common cardiac disease pathophysiology. Interestingly, some ubiquitin ligases, such as MDM2 and CHIP target p53 leading to proteasomal degradation and thereby protecting cardio myocytes against apoptosis in ischemia reperfusion (I.R) injury cases [7, 8]. *ANPCII* gene is an E3 ubiquitin-protein ligase complex (a member of the anaphase promoting complex) which regulated through the cell cycle being a part of The UPS (The ubiquitin proteasome system). *ANPC/C* complex functions through ubiquitination and degradation of target proteins. It also recruits the E2 ubiquitin-conjugating enzymes to the complex [9].

Many miRNAs such as miRNA-34a, miRNA-208a, miRNA-210 and miRNA-495 were studied by many researchers and were found to be important gene regulators for angiogenesis and UPS in AMI. Consequently, they are con-

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sidered to be important therapeutic tools particularly for the prevention of heart failure after AMI [10, 11]. Hence, this highlights the emergence of miRNAs as sensitive biomarkers of AMI and their possible use as new biomarkers for diagnosis and therapeutic targeting of AMI being stable in plasma, serum, and urine, as well as their cell-specific physiological functions [12, 13].

On the other hand, long noncoding RNAs (lncRNAs) were suggested by other researchers to have an association with cardiac ischemia [14, 15]; *e.g*; myocardial infarction-associated transcript, potassium voltage-gated channel, *KQT*-like subfamily HOX antisense intergenic RNA and *long noncoding RNA HOTAIR* which was found to have a protective effect in cardiac tissue and that the extracellular *HOTAIR* may be a promising biomarker for AMI detection [16-18].

In this study, we hypothesize that the chosen RNAs by *in silico* data analysis are related to UPS system and specific to AMI might be a potential biomarker panel for the detection of AMI. We first chosen AMI specific genes and linked to UPS system and their master regulators at epigenetic level through *in silico* data analysis. Then, to investigate this panel, we assessed whether *lncRNA-RP11-175K6, microRNA-106b-5p(miR-106b-5p)* and anaphase promoting complex subunit 11 (*ANAPC11*) mRNA showed significantly differential expression in sera of AMI group compared to non-cardiac chest patients and healthy volunteers.

2. PATIENTS AND METHODS

The Ethical Committee at Ain Shams faculty of medicine granted this Pilot study an approval. The study includes 69 Egyptian patients with acute myocardial infarction at the Cardiovascular Department, Ain Shams University hospitals, 31 patients with angina pectoris, but without MI and non-cardiac chest pain according to the outcome of coronary angiography (non-cardiac causes due to gastrointestinal or lung or any other chest condition) and 31 healthy normal volunteers who have normal ECG, without any history of cardiovascular disease and were visiting the hospital for regular health checkups with matching sex and age to the patients' groups after taking an informed consent. The enrolled participants were in the period from April 2017 till October 2017. AMI diagnosis was performed through evaluating the elevated serum troponin levels, CK-MB in addition to history and clinical symptoms consistent with cardiac ischemia within 6 hours of chest pain. AMI was diagnosed on the basis of a combination of several parameters: ischemic symptoms, a pathological Q wave and elevated cTnI (cardiac troponin I) with CK-MB (creatine kinase-MB) expression. All AMI patients were diagnosed for the first time and underwent primary PCI. The patients were diagnosed according to guidelines that were adopted from American College of Cardiology/American Heart Association (2018 ESC/ACC/AHA/WHF Fourth Universal Definition) and two independent cardiologists judgment were considered. The exclusion criteria for this study were patients with a history of end-stage renal failure, hepatitis, hepatic failure, cardiomyopathy, bleeding disorders, autoimmune diseases, previous irradiation therapy to the thoracic region, arthritis or inflammatory bowel disease (IBD), chronic muscle disorders and malignancy.

Within the first 6 hours of onset of chest pain blood samples were collected for continuous assessment of cardiac markers; CK –MB and hs-cTnT. Centrifugation was done at 3,000 rpm for 15 min, separating the sera samples then it was aliquoted and eventually kept at -80°C.

2.1. Bioinformatics' Analysis

The selection of these genes firstly, focused on the literature search that ensured the ubiquitin Proteasome system (UPS) dysregulation in acute myocardial infarction. Secondly, the Gene Expression Omnibus (GEO). Gene atlas expression, Gene card database, UniProt and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment network analyses were performed to understand the potential molecular mechanisms linking UPS dysregulation to AMI pathogenesis to select anaphase promoting complex subunit 11 (*ANAPC11*) due to the following causes, linked to UPS, specific to AMI, novelty, high expression in normal cardiac tissue (Supplementary Figs. **1-5**). Thirdly, miR-106b-5p has been selected as a target to ANAPC11 mRNA due to higher number of complementarity binding site, linked to UPS dysregulation, related to cardiac injury as the pathway enrichment analysis which revealed that miR-106b-5p is linked to endocytosis, TGF-beta signaling pathway, MAPK signaling pathway, Ubiquitin mediated proteolysis (Table **1**, Supplementary Figs. **6-8**). Lastly, lncRNA-RP11-175K6.1 (LOC101927740) was identified to control the expression of the above genes by accessing a database of lncRNAs that act as ceRNAs (starbase database). We have selected lncRNA-RP11-175K6.1 based on novelty and presence of higher miRNA binding sites. We have also validated the alignment between lncRNA-RP11-175K6.1-miRNA 106b by using Claustal tool in European bioinformatics institute thus verifying the *in silico* prediction that lncRNA-RP11-175K6.1-miRNA 106b target ANapc11mRNA (Supplementary Figs. **9** and **10**).

2.2. Purification of Total RNA Including miRNA and Reverse Transcription

The extraction step of RNAs from sera sample was performed using miRNEasy RNA isolation kit (Qiagen, Hilden, Germany) as reported by manufacturer. 30µl of nuclease-free water was added to every RNA samples. NanoDrop spectrophotometer (Thermo Scientific, USA) [19] was used to get the concentration and purity of RNA. cDNA for mRNAs, lncRNA and miRNA were synthesized by miScript II RT Kit (Qiagen, Germany) in Rotor gene Thermal cycler (Thermo Electron Waltham, MA).

2.3. Differential Expression of the Selected RNAs by Real Time-Qrt PCR

ANPCII mRNA and lncRNA-RP11-175K6 expressions in sera samples were quantified by using QuantiTect SYBR Green PCR Kit and RT² SYBR Green ROX qPCR Mastermix, sequentially. RT² lncRNA qPCR Assay for Human lncRNA-RP11-175K6.1(LOC101927740 (ENST00000499583) and ANPCII QuantiTect Primer Assay (NM_001002244), Hs_ACTB_1_SG QuantiTect Primer Assay (NM_001002244) [that was used as housekeeping gene in equalization of raw data] were purchased from Qiagen, Germany.

Table 1. Gene ontology of *ANAPC11 mRNA*.

S. No.	ANapc11mRNA (retrieved from Pathways from BioSystems available at https://www.ncbi.nlm.nih.gov/gene/51529)
1.	APC-Cdc20 mediated degradation of Nek2A, organism-specific biosystem (from REACTOME)
2.	APC/C complex, organism-specific biosystem (from KEGG)
3.	APC/C complex, conserved biosystem (from KEGG)
4.	APC/C-mediated degradation of cell cycle proteins, organism-specific biosystem (from REACTOME)
5.	APC/C:Cdc20 mediated degradation of Cyclin B, organism-specific biosystem (from REACTOME)
6.	APC/C:Cdc20 mediated degradation of Securin, organism-specific biosystem (from REACTOME)
7.	APC/C:Cdc20 mediated degradation of mitotic proteins, organism-specific biosystem (from REACTOME)
8.	APC/C:Cdh1 mediated degradation of Cdc20 and other APC/C:Cdh1 targeted proteins in late mitosis/early G1, organism-specific biosystem (from REACTOME)
9.	APC:Cdc20 mediated degradation of cell cycle proteins prior to satisfation of the cell cycle checkpoint, organism-specific biosystem (from REACTOME)
10.	Activation of APC/C and APC/C:Cdc20 mediated degradation of mitotic proteins, organism-specific biosystem (from REACTOME)
11.	Adaptive Immune System, organism-specific biosystem (from REACTOME)
12.	Antigen processing: Ubiquitination & Proteasome degradation, organism-specific biosystem (from REACTOME)
13.	Autodegradation of Cdh1 by Cdh1:APC/C, organism-specific biosystem (from REACTOME)
14.	Cdc20:Phospho-APC/C mediated degradation of Cyclin A, organism-specific biosystem (from REACTOME)
15.	Cell Cycle, organism-specific biosystem (from REACTOME)
16.	Cell Cycle Checkpoints, organism-specific biosystem (from REACTOME)
17.	Cell Cycle, Mitotic, organism-specific biosystem (from REACTOME)
18.	Cell cycle, organism-specific biosystem (from KEGG)
19.	Cell cycle, conserved biosystem (from KEGG)
20.	Cellular Senescence, organism-specific biosystem (from REACTOME)
21.	Cellular responses to stress, organism-specific biosystem (from REACTOME)
22.	Class I MHC mediated antigen processing & presentation, organism-specific biosystem (from REACTOME)
23.	Conversion from APC/C:Cdc20 to APC/C:Cdh1 in late anaphase, organism-specific biosystem (from REACTOME)
24.	HTLV-I infection, organism-specific biosystem (from KEGG)
25.	HTLV-I infection, conserved biosystem (from KEGG)

We used miScript SYBR Green PCR Kit (Qiagen /SABiosciences Corporation, Frederick, MD) for quantification of hsa-_ miR106b-5p expression in different sera samples using either Hs_ miR106_1 miScript Primer Assay targets mature miRNA: hsa- miR106 - MIMAT0000680: 5'UAAAGUGCUGACAGUGCAGAU and snord68 that was used as housekeeping gene, Each reaction was done in duplicate. Relative quantification of RNAs expression was calculated by using the method of Leviak RQ= $2^{-\Delta\Delta Ct}$ method [19].

Data analysis was performed using the Rotor Gene real time PCR (Qiagen, Hilden, Germany) which is considered negative if higher than 36 Ct value.

2.4. Statistics

The data was statistically analyzed using SPSS22. Krausakul Wallis test, chi-square test, and Spearman correlation test were used as appropriate. The receiver operating characteristic (ROC) curve was done to characterize the predictive value of the selected RNAs for AMI.

3. RESULTS

3.1. Clinical and Laboratory Data of the Study

In this study, we have not found any differences among the three investigated groups *i.e.*, age, sex and smoker: non-smoker ratio ($p>0.05$), but in serum creatinine, total Triglycerides and cardiac troponin I showed marked differences and the details are shown in (Supplementary Table **1**).

3.2. Differential Expression of Serum RNAs Among the Study Groups

We confirm our *in silico* data regarding the relationship between the three chosen RNAs by measuring the serum levels of the three RNAs in the study samples. We assessed the RNAs based on the fold change (RQ) values. We found that the AMI group had down regulated both *lncRNA-RP11-175K6.1* and RQ-*ANPCII* mRNA with concomitant up regulation of RQ-*miR-106b-5p* in comparison with the 2 control groups ($p<0.01$) (Supplementary Table **2**, Fig. **1**).

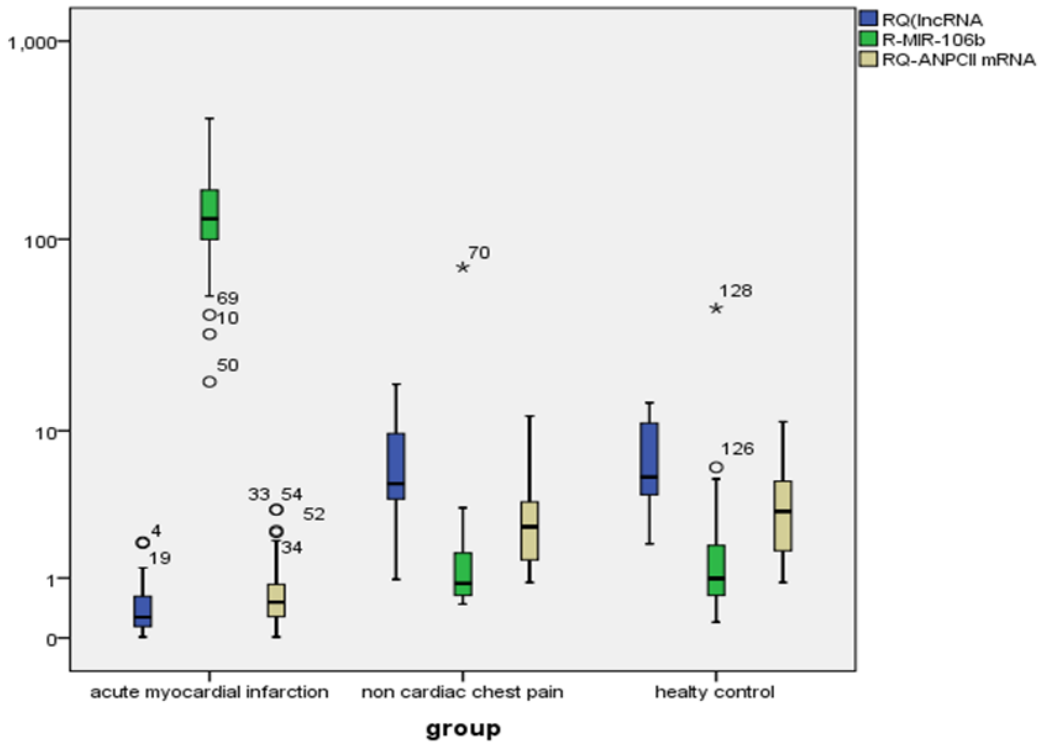

Fig. (1). Box plot shows relative expression of the serum RNAs between AMI and control groups. The data is presented as median fold changes (P<0.05). (*A higher resolution / colour version of this figure is available in the electronic copy of the article*).

Comparing AMI group to non-cardiac chest pain patients and healthy control participants by ROC curve analysis shows that the best cutoff values of *lncRNA-RP11-175K6.1*, *ANPCII* mRNA and RQ-*miR-106b-5p* were 1.36, 0.9950 and 10.94, respectively. The measured sensitivities were found to be 97.1%, 82.6% and 100%, respectively. These results indicate that these cutoff values could be used to differentiate AMI group form non cardiac chest pain patients and enrolled healthy people as illustrated in Fig. **2** (**a-c**) and Tables **2** and **3**.

3.3. Correlation between Serum RNAs with the Clinicopathological Factors in AMI Group

There was no significant correlation between any of the investigated serum RNAs and clinicopathological factors in the AMI group except serum *ANPCII* mRNA expression levels with diabetes mellitus and serum *miR-106b* expression levels with smoking, sex, nitrate, blocker, ACEI and statin use; *lncRNA-RP11-175K6.1* expression levels with age, smoking, blockers, nitrate ACEI usage (Supplementary Table **3**).

Significant positive correlation was found between *lncRNA-RP11-175K6.1* and *ANPCII mRNA* among the study groups (p<0.05) (Supplementary Table **4**). Moreover, there was a significant inverse correlation between hsa-miRNA-106b-5p and *lncRNA-RP11-175K6.1* and *ANPCII mRNA* levels (r = -0.687, p<0.01 and r = -0.653, p<0.01, respectively). Remarkably, there was a significant direct correlation between hsa-miR-106b-5p and cardiac troponin I and CK-MB. There was a negative correlation between *ANPCII mRNA*, *lncRNA-RP11-175K6.1* and cardiac Troponin I level.

Regression analysis revealed that serum *miR-106b-5p* was a strong independent predictor of AMI occurrence (all p<0.01) (Supplementary Table **5**).

4. DISCUSSION

AMI can lead to congestive heart failure and malignant arrhythmia causing high morbidity and mortality [19]. Therefore, in order to provide optimized benefits to current therapies, it is necessary to identify novel, highly sensitive and specific biomarkers and their regulatory targets for the early detection of AMI. Long intergeinc non coding RNAs and microRNAs have been widely investigated as potential disease biomarkers and therapeutic targets [19]. This could be attributed to their relative higher stability as they can resist the degradation process mediated by RNase enzymes [20].

By using an integrated genetic epigenetic approach, we identified the level of expression of *lncRNA-RP11-175K6.1*(LOC101927740), *ANPCII mRNA* and *miR106 b-5p* and were found to be strongly detected in the serum of AMI patients making them potential biomarkers in diagnosing such polygenic diseases. It is supposed that the high performance requirement of AMI diagnosis does not depend upon single gene biomarker approach, so finding a network of genes that are functionally linked to each other may raise the success percentage of AMI diagnosis in comparison to conventional single-marker approach.

Ubiquitin ligases (E3s), as part of the UPS, are the master players that direct the ubiquitin addition to the target proteins and thus changing their physical properties or decreasing their activity but most importantly mark them for degradation [21, 22]. The cardiomyocyte-specific ubiquitin ligases

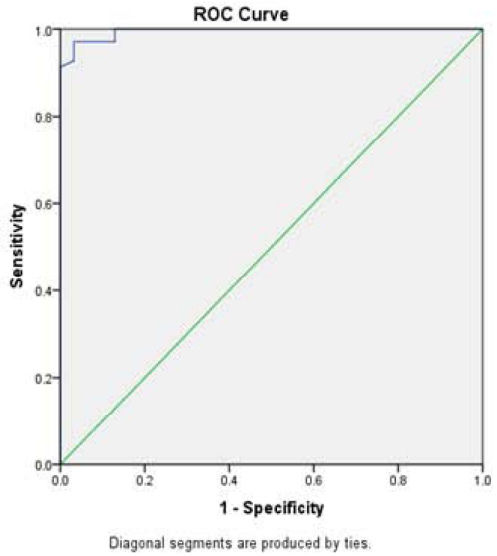

Fig. (2a). ROC curve analysis for lncRNA-RP11-175K6 used to calculate the best cut-off point to discriminate between the AMI and healthy groups. AUC [SE]=0.995 [0.024], 95% confidence interval=0.655-0.842, (*P*<0.01). (*A higher resolution / colour version of this figure is available in the electronic copy of the article*).

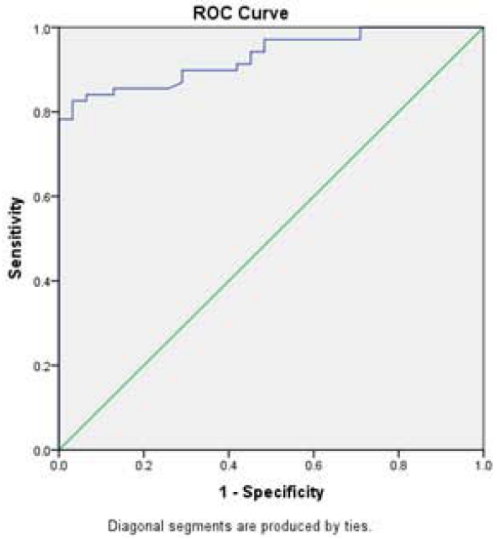

Fig. (2b). ROC curve analysis for RQ-ANPCII mRNA used to calculate the best cut-off point to discriminate between the AMI and control groups. AUC [SE]=0.930 [0.033], 95% confidence interval=0.722-0.811, (*P*<0.01). (*A higher resolution / colour version of this figure is available in the electronic copy of the article*).

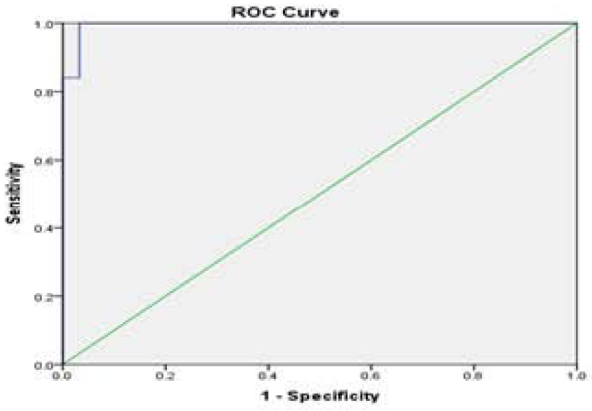

Fig. (2c). ROC curve analysis for RQ-mIR-106b used to calculate the best cut-off point to discriminate between the AMI and control groups. AUC [SE]=0.995 [0.038], 95% confidence interval=0.845-0.931, (*P*<0.01). (*A higher resolution / colour version of this figure is available in the electronic copy of the article*).

Table 2. Positivity rate of serum investigated parameter based on fold change expression (RQ) among the study groups.

-	*miR106-b-5p*	*ANPCII mRNA*	*lncRNA-RP11-175K6*
Acute myocardial infarction Positive Negative	N=69(100%) N=0(0.0%)	N=69(100%) N=0(0%)	N=2(2.8%) N=67(97.1%)
Non cardiac chest pain Positive Negative	N=1(3.22%) N=30(96.7 %)	N=1(3.2%) N=30(96.77)	N=1(3.2%) N=30(96.77%)
Control Positive Negative	N=0(0%) N=31(100 %)	N=0(0%) N=31(100 %)	N=1(3.2%) N=30(96.77%)
P $\chi^{2(a)}$	<.01 95.392	<.01 95.392	<.01 86.628

Table 3. Performance characteristics of serum investigated parameter based on fold change expression.

Biomarker	Sensitivity	Specificity	PPV (Positive Predictive Value)	NPV (Negative Predictive Value)	Accuracy
All the study groups (n=131)					
ANPCII mRNA	82.6%	96.8%	71.4%	98.3%	87%
miR106-b-5P	100%	96.8%	100%	98.6%	99%
lncRNA-RP11-175K6	97.1%	96.8%	93.8%	98.5%	97%

have been extensively studied and revealed novel functional roles for each of them [23]. ANPCII (Anaphase-promoting complex subunit 11) APC/C core complex is a cell cycle-regulated E3 ubiquitin ligase showing a high expression in heart and skeletal muscle and moreover its activators play a major role in symbiotic interactions, hormone signaling and gametogenesis [24]. Furthermore, the high expression of ANPCII mRNA is associated with chromosomal instability and residual tumor and lymphovascular invasion [25]. The overexpression of APC7 was also strongly related to primary colorectal cancer (CRC) and so it can be utilized as a potential novel biomarker of metastatic CRC [26].

Interestingly, Luo *et al.*, found an inverse correlation between ITCH E3 ubiquitin ligase and miRNA-106b and in pancreatic cancer [27]. Moreover, miR-106b was found to have a high expression level in myocardial infarction together with an angiogenesis anti-apoptotic role in cardiomyocytes through inhibition of p21 expression [28, 29]. It is reported that microRNA-106 constitutes a cluster of 25 especially miR- 106b which is significantly increased in patients with colorectal cancer [11], hepatocellular carcinoma [30], gastric cancer [31] prostate cancer [32] and renal cancer [33].

LncRNAs are stable in plasma and show disease and tissue specificity [34]. More than 1000 lncRNAs have been linked to various crucial processes, including growth, UPS, apoptosis, and cell proliferation [35]. *lncRNA-RP11-175K6* (*LOC101927740*) is located on the fifth chromosome with a strong relation with cardiomyocyte regeneration and angiogenesis [36]. *LncRNA-RP11-714G18.*1 has atheroprotective importance by suppressing vascular cell migration through RP11-714G18.1/LRP2BP/MMP1 axis expression [37].

Significantly, there was a direct correlation between *lncRNA-RP11-175K6.1* and *ANAPC11* mRNA ($P<0.05$) with inverse correlation between *lncRNA-RP11-175K6.1, ANAPC11* mRNA and *miR-106b-5p.* We hypothesized that *lncRNA-RP11-175K6.1* could act as a sponge for *miR-106b-5p* with subsequent modulation of *ANAPC11* mRNA playing a potential role in AMI pathogenesis. *miR-106b-5p* seems to inhibit *ANAPC11* mRNA expression at transcriptional or posttranscriptional level (Fig. **3**). Of note, there was inverse correlation found between *ANAPC11* mRNA and *lncRNA-RP11-175K6.1* and cardiac Troponin I level.

The study was limited by relatively small sample size from two centers in Egypt. Moreover, *in vitro* and *in vivo* mechanistic analysis is needed to clarify the deep mechanism

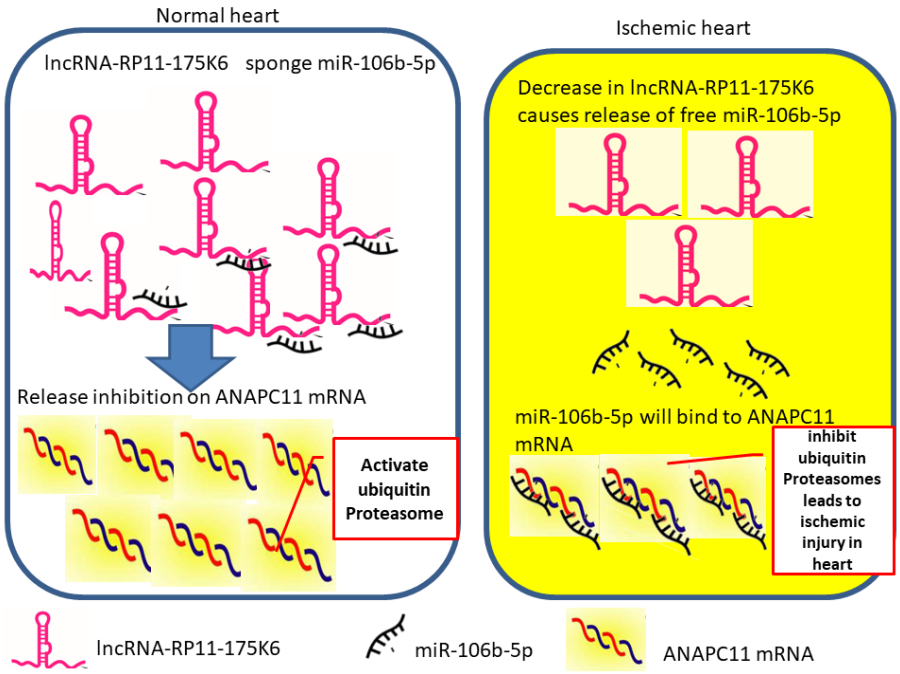

Fig. (3). A schematic diagram to map the suggested association between the chosen genes. Schematic diagram of the study hypothesis. (*A higher resolution / colour version of this figure is available in the electronic copy of the article*).

of RNA-RNA crosstalk in AMI which are strongly recommended.

CONCLUSION

We have identified dysregulation in the expression of serum *lncRNA-RP11-175K6/ miRNA -106b -5p* together with *ANAPC11* mRNA in AMI patients compared to the control groups. It may be a promising strategy for the development of an RNA based biomarker panel to discriminate AMI patients and non AMI patients. These findings may provide new tools and targets for AMI therapy. Further studies are highly recommended to detect serum (*lncRNA-RP11-175K6/ miRNA -106b -5p - ANAPC11* mRNA) in AMI patients' samples using nanoassay as a non-PCR based technique with many advantages *e.g.* direct detection of unamplified RNA network in sera samples for the early diagnosis of AMI patients, simple, rapid and cheap assay for these nucleic acids other than PCR.

AUTHOR'S CONTRIBUTIONS

Marwa Matboli has carried out *in silico* data analysis and designed *in silico* based RNA network, carried out molecular assay experiments and performed the statistical analysis. Sara H.A. Agwa has performed statistical analysis, drafting the manuscript. Ahmed K. Anwar, Amr R. Mansour, Ahmed I. Gaber, Ali E.A. Said, Paula Lwis, Marwa Hamdy participated in practical part, drafting manuscript. Sherif Sammir Elzahy has responsible for clinical samples collection with their data, and approved the final draft.

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

The study was approved by the Ethics Committee of the Faculty of Medicine, Ain Shams University, Egypt, (Approval number, FWA 000017586).

HUMAN AND ANIMAL RIGHTS

No Animals were used for studies that are the basis of this research. All the patients were diagnosed according to guidelines that were adopted from American College of Cardiology/American Heart Association (2018 ESC/ACC/AHA/ WHF Fourth Universal Definition).

CONSENT FOR PUBLICATION

Informed consent was obtained from all the patients prior to the study.

AVAILABILITY OF DATA AND MATERIALS

Not applicable.

FUNDING

This work was supported by the Egyptian Science and Technology Development Fund, RSTDG 25321.

CONFLICT OF INTEREST

The authors declare no conflict of interest, financial or otherwise.

ACKNOWLEDGEMENTS

Declared none.

SUPPLEMENTARY MATERIAL

Supplementary material is available on the publisher's website along with the published article.

REFERENCES

[1] Shi, Q.; Yang, X. Circulating microRNA and long noncoding RNA as biomarkers of cardiovascular diseases. *J. Cell. Physiol.*, **2016**,

231(4), 751-755.
[http://dx.doi.org/10.1002/jcp.25174] [PMID: 26308238]

[2] Han, Q.Y.; Wang, H.X.; Liu, X.H.; Guo, C.X.; Hua, Q.; Yu, X.H.; Li, N.; Yang, Y.Z.; Du, J.; Xia, Y.L.; Li, H.H. Circulating E3 ligases are novel and sensitive biomarkers for diagnosis of acute myocardial infarction. *Clin. Sci. (Lond.),* **2015**, *128*(11), 751-760.
[http://dx.doi.org/10.1042/CS20140663] [PMID: 25599194]

[3] Irwin, M. Faculty of 1000 evaluation for executive summary: Heart disease and stroke statistics- 2012 update: A report from the American Heart Association. F1000 - Post-publication Peer Review of the Biomedical Literature 2013.

[4] Shams-Vahdati, S.; Vand-Rajavpour, Z.; Paknezhad, S.P.; Piri, R.; Moghaddasi-Ghezeljeh, E.; Mirabolfathi, S.; Naghavi-Behzad, M. Cost-effectiveness of cardiac biomarkers as screening test in acute chest pain. *J. Cardiovasc. Thorac. Res.,* **2014**, *6*(1), 29-33.
[PMID: 24753829]

[5] Muhlestein, J.B. *Biomarkers of Infection and Risk of Coronary Heart Disease. Cardiovascular Biomarkers, 319-344,* **2006**.

[6] Calise, J.; Powell, S.R. The ubiquitin proteasome system and myocardial ischemia. *Am. J. Physiol. Heart Circ. Physiol.,* **2013**, *304*(3), H337-H349.
[http://dx.doi.org/10.1152/ajpheart.00604.2012] [PMID: 23220331]

[7] Willis, M.S.; Bevilacqua, A.; Pulinilkunnil, T.; Kienesberger, P.; Tannu, M.; Patterson, C. The role of ubiquitin ligases in cardiac disease. *J. Mol. Cell. Cardiol.,* **2014**, *71*, 43-53.
[http://dx.doi.org/10.1016/j.yjmcc.2013.11.008] [PMID: 24262338]

[8] Bader, M.; Steller, H. Regulation of cell death by the ubiquitin-proteasome system. *Curr. Opin. Cell Biol.,* **2009**, *21*(6), 878-884.
[http://dx.doi.org/10.1016/j.ceb.2009.09.005] [PMID: 19850458]

[9] Stringer, D.K.; Piper, R.C. Terminating protein ubiquitination: Hasta la vista, ubiquitin. *Cell Cycle,* **2011**, *10*(18), 3067-3071.
[http://dx.doi.org/10.4161/cc.10.18.17191] [PMID: 21926471]

[10] Yang, Y.; Cheng, H.W.; Qiu, Y.; Dupee, D.; Noonan, M.; Lin, Y.D.; Fisch, S.; Unno, K.; Sereti, K.I.; Liao, R. MicroRNA-34a plays a key role in cardiac repair and regeneration following myocardial infarction. *Circ. Res.,* **2015**, *117*(5), 450-459.
[http://dx.doi.org/10.1161/CIRCRESAHA.117.305962] [PMID: 26082557]

[11] Shyu, K.G.; Wang, B.W.; Cheng, W.P.; Lo, H.M. MicroRNA-208a increases myocardial endoglin expression and myocardial fibrosis in acute myocardial infarction. *Can. J. Cardiol.,* **2015**, *31*(5), 679-690.
[http://dx.doi.org/10.1016/j.cjca.2014.12.026] [PMID: 25936493]

[12] Fan, Z.G.; Qu, X.L.; Chu, P.; Gao, Y.L.; Gao, X.F.; Chen, S.L.; Tian, N.L. MicroRNA-210 promotes angiogenesis in acute myocardial infarction. *Mol. Med. Rep.,* **2018**, *17*(4), 5658-5665.
[http://dx.doi.org/10.3892/mmr.2018.8620] [PMID: 29484401]

[13] Juni, R.P.; Abreu, R.C.; da Costa Martins, P.A. Regulation of microvascularization in heart failure - an endothelial cell, non-coding RNAs and exosome liaison. *Noncoding RNA Res.,* **2017**, *2*(1), 45-55.
[http://dx.doi.org/10.1016/j.ncrna.2017.01.001] [PMID: 30159420]

[14] Marchese, F.P.; Huarte, M. Long non-coding RNAs and chromatin modifiers: Their place in the epigenetic code. *Epigenetics,* **2014**, *9*(1), 21-26.
[http://dx.doi.org/10.4161/epi.27472] [PMID: 24335342]

[15] Greco, S.; Gorospe, M.; Martelli, F. Noncoding RNA in age-related cardiovascular diseases. *J. Mol. Cell. Cardiol.,* **2015**, *83*, 142-155.
[http://dx.doi.org/10.1016/j.yjmcc.2015.01.011] [PMID: 25640162]

[16] Gao, L.; Liu, Y.; Guo, S.; Yao, R.; Wu, L.; Xiao, L.; Wang, Z.; Liu, Y.; Zhang, Y. Circulating long noncoding RNA HOTAIR is an essential mediator of acute myocardial infarction. *Cell. Physiol. Biochem.,* **2017**, *44*(4), 1497-1508.
[PMID: 29258067]

[17] Sano, M.; Schneider, M.D. Cyclins that don't cycle--cyclin T/cyclin-dependent kinase-9 determines cardiac muscle cell size. *Cell Cycle,* **2003**, *2*(2), 99-104.
[http://dx.doi.org/10.4161/cc.2.2.332] [PMID: 12695656]

[18] Greco, S.; Perfetti, A.; Menicanti, L.; Castelvecchio, S.; Martelli, F. Heart failure modulates long noncoding RNA expression in human left ventricles. *Eur. Heart J.,* **2013**, *34*(Suppl. 1), P3250.
[http://dx.doi.org/10.1093/eurheartj/eht309.P3250]

[19] Da Sacco, L.; Baldassarre, A.; Masotti, A. Bioinformatics tools and novel challenges in long non-coding RNAs (lncRNAs) functional analysis. *Int. J. Mol. Sci.,* **2012**, *13*(1), 97-114.
[http://dx.doi.org/10.3390/ijms13010097] [PMID: 22312241]

[20] Xuan, L.; Sun, L.; Zhang, Y.; Huang, Y.; Hou, Y.; Li, Q.; Guo, Y.; Feng, B.; Cui, L.; Wang, X.; Wang, Z.; Tian, Y.; Yu, B.; Wang, S.; Xu, C.; Zhang, M.; Du, Z.; Lu, Y.; Yang, B.F. Circulating long non-coding RNAs NRON and MHRT as novel predictive biomarkers of heart failure. *J. Cell. Mol. Med.,* **2017**, *21*(9), 1803-1814.
[http://dx.doi.org/10.1111/jcmm.13101] [PMID: 28296001]

[21] Brigant, B.; Metzinger-Le, M.V.; Rochette, J.; Metzinger, L. TRIMming down to TRIM37: Relevance to inflammation, cardiovascular disorders, and cancer in MULIBREY nanism. *Int. J. Mol. Sci*., **2018**, *20*(1), 67.
[https://doi.org/10.3390/ijms20010067]

[22] Basheer, W.A.; Harris, B.S.; Mentrup, H.L.; Abreha, M.; Thames, E.L.; Lea, J.B.; Swing, D.A.; Copeland, N.G.; Jenkins, N.A.; Price, R.L.; Matesic, L.E. Cardiomyocyte-specific overexpression of the ubiquitin ligase Wwp1 contributes to reduction in Connexin 43 and arrhythmogenesis. *J. Mol. Cell. Cardiol.,* **2015**, *88*, 1-13.
[http://dx.doi.org/10.1016/j.yjmcc.2015.09.004] [PMID: 26386426]

[23] Mota, R.; Parry, T.L.; Yates, C.C.; Qiang, Z.; Eaton, S.C.; Mwiza, J.M.; Tulasi, D.; Schisler, J.C.; Patterson, C.; Zaglia, T.; Sandri, M.; Willis, M.S. Increasing cardiomyocyte atrogin-1 reduces aging-associated fibrosis and regulates remodeling *in vivo*. *Am. J. Pathol.,* **2018**, *188*(7), 1676-1692.
[http://dx.doi.org/10.1016/j.ajpath.2018.04.007] [PMID: 29758183]

[24] Heyman, J.; De Veylder, L. The anaphase-promoting complex/cyclosome in control of plant development. *Mol. Plant,* **2012**, *5*(6), 1182-1194.
[http://dx.doi.org/10.1093/mp/sss094] [PMID: 23034505]

[25] Zhang, J.; Wan, L.; Dai, X.; Sun, Y.; Wei, W. Functional characterization of Anaphase Promoting Complex/Cyclosome (APC/C) E3 ubiquitin ligases in tumorigenesis. *Biochim. Biophys. Acta,* **2014**, *1845*(2), 277-293.
[PMID: 24569229]

[26] Kim, I.Y.; Kwon, H.Y.; Park, K.H.; Kim, D.S. Anaphase-Promoting Complex 7 is a prognostic factor in human colorectal cancer. *Ann. Coloproctol.,* **2017**, *33*(4), 139-145.
[http://dx.doi.org/10.3393/ac.2017.33.4.139] [PMID: 28932723]

[27] Luo, Z.L; Luo, H.J.; Fang, C.; Cheng, L. Negative correlation of ITCH E3 ubiquitin ligase and miRNA-106b dic-tates metastatic progression in pancreatic cancer. *Oncotarget,* **2016**, *7*(2), 1477-85.

[28] Liu, Z.; Yang, D.; Xie, P.; Ren, G.; Sun, G.; Zeng, X.; Sun, X. MiR-106b and MiR-15b modulate apoptosis and angiogenesis in myocardial infarction. *Cell. Physiol. Biochem.,* **2012**, *29*(5-6), 851-862.
[http://dx.doi.org/10.1159/000258197] [PMID: 22613985]

[29] Zhu, H.; Fan, G.C. Role of microRNAs in the reperfused myocardium towards post-infarct remodelling. *Cardiovasc. Res.,* **2012**, *94*(2), 284-292.
[http://dx.doi.org/10.1093/cvr/cvr291] [PMID: 22038740]

[30] Wei, R.; Huang, G.L.; Zhang, M.Y.; Li, B.K.; Zhang, H.Z.; Shi, M.; Chen, X.Q.; Huang, L.; Zhou, Q.M.; Jia, W.H.; Zheng, X.F.; Yuan, Y.F.; Wang, H.Y. Clinical significance and prognostic value of microRNA expression signatures in hepatocellular carcinoma. *Clin. Cancer Res.,* **2013**, *19*(17), 4780-4791.
[http://dx.doi.org/10.1158/1078-0432.CCR-12-2728] [PMID: 23812667]

[31] Li, F.; Liu, J.; Li, S. MicroRNA 106b ~ 25 cluster and gastric cancer. *Surg. Oncol.,* **2013**, *22*(2), e7-e10.
[http://dx.doi.org/10.1016/j.suronc.2013.01.003] [PMID: 23510949]

[32] Hudson, R.S.; Yi, M.; Esposito, D.; Glynn, S.A.; Starks, A.M.; Yang, Y.; Schetter, A.J.; Watkins, S.K.; Hurwitz, A.A.; Dorsey, T.H.; Stephens, R.M.; Croce, C.M.; Ambs, S. MicroRNA-106b-25 cluster expression is associated with early disease recurrence and targets caspase-7 and focal adhesion in human prostate cancer. *Oncogene,* **2013**, *32*(35), 4139-4147.
[http://dx.doi.org/10.1038/onc.2012.424] [PMID: 22986525]

[33] Slaby, O.; Jancovicova, J.; Lakomy, R.; Svoboda, M.; Poprach, A.; Fabian, P.; Kren, L.; Michalek, J.; Vyzula, R. Expression of miRNA-106b in conventional renal cell carcinoma is a potential marker for prediction of early metastasis after nephrectomy. *J. Exp. Clin. Cancer Res.,* **2010**, *29*(1), 90.
[http://dx.doi.org/10.1186/1756-9966-29-90] [PMID: 20609231]

[34] Yan, Y.; Zhang, B.; Liu, N.; Qi, C.; Xiao, Y.; Tian, X.; Li, T.; Liu, B. Circulating long noncoding RNA UCA1 as a novel biomarker of acute myocardial infarction. *BioMed Res. Int.,* **2016**, *2016*, 8079372.
[http://dx.doi.org/10.1155/2016/8079372] [PMID: 26949706]

[35] Yang, Y.; Cai, Y.; Wu, G.; Chen, X.; Liu, Y. Plasma long non-coding RNA, CoroMarker, a novel bi-omarker for diagnosis of coronary artery disease. *Clin. Sci. (Lond).,* **2015**, *129*(8), 675-685.

[36] Ergul, A.; Alhusban, A.; Fagan, S.C. Angiogenesis: A harmonized target for recovery after stroke. *Stroke,* **2012**, *43*(8), 2270-2274. [http://dx.doi.org/10.1161/STROKEAHA.111.642710] [PMID: 22618382]

[37] Zhang, Y.; Zheng, L.; Xu, B.M.; Tang, W.H.; Ye, Z.D.; Huang, C.; Ma, X.; Zhao, J.J.; Guo, F.X.; Kang, C.M.; Lu, J.B.; Xiu, J.C.; Li, P.; Xu, Y.J.; Xiao, L.; Wu, Q.; Hu, Y.W.; Wang, Q. LncRNA-RP11-714G18.1 suppresses vascular cell migration *via* directly targeting LRP2BP. *Immunol. Cell Biol.,* **2018**, *96*(2), 175-189. [http://dx.doi.org/10.1111/imcb.1028] [PMID: 29363163]