A role for m6A RNA methylation in heart failure development?

Devaux, Y.; Nossent, A.Y.; EU CardioRNA COST Action

ESC European Society of Cardiology

European Journal of Heart Failure (2020) **22**, 67–69
doi:10.1002/ejhf.1714

EDITORIAL COMMENT

A role for m6A RNA methylation in heart failure development?

Yvan Devaux[1]* and Anne Yaël Nossent[2,3], on behalf of EU-CardioRNA COST Action CA17129

[1]Cardiovascular Research Unit, Luxembourg Institute of Health, Strassen, Luxembourg; [2]Department of Laboratory Medicine and Department of Internal Medicine II, Medical University of Vienna, Vienna, Austria; and [3]Department of Surgery and Einthoven Laboratory for Experimental Medicine, Leiden University Medical Center, Leiden, The Netherlands

This article refers to 'Changes in m6A RNA methylation contribute to heart failure progression by modulating translation' by T. Berulava *et al.*, published in this issue on pages 54–66.

Socio-economical costs of heart failure

Heart failure persists as a significant cause of death and low quality of life. It is a major public health issue of which the prevalence continues to rise. In industrialized countries, heart failure affects near 2% of the adult population, a prevalence which exceeds 10% after the age of 70.[1] With an aging population, this burden is predicted to continuously rise. Healthcare costs also steadily increase and represent a significant economical burden.[2] Despite major advances in its diagnosis, treatment and prognosis, continuous efforts from the biomedical research community are warranted to discover new drugs and biomarkers to implement a personalized healthcare. Heart failure is a complex disease and its development and progression are governed by an intricate network of multiple biological pathways. The simplistic 'central dogma of molecular biology', from which proteins—building blocks of the human body—are translated from messenger RNA molecules, themselves transcribed from DNA, appears to be much more complex than previously thought. Homeostatic regulation of gene expression is paramount to the normal functioning of our body and is governed at multiple levels, including at the epigenetics level.

Epigenetics and the heart

The concept of epigenetics, defined as any phenotypic change occurring without affecting DNA sequence, has emerged as a critical aspect of cardiovascular disease and is attracting more and more attention. DNA methylation and histone modifications are major epigenetics mechanisms. Non-coding RNAs—RNA molecules lacking protein-coding potential—are also able to regulate gene expression and induce phenotypic changes without affecting the DNA sequence. They are regulated in heart failure and contribute to its development. Mostly oriented toward small non-coding RNAs, among which microRNAs have been the most widely studied, the research has recently switched to long non-coding RNAs. Both linear[3] and circular[4] RNAs are dysregulated in the failing heart and contribute to heart failure progression. Even more recently, a new strata of epigenetics mechanisms has come to light, RNA methylation. Although the importance of DNA methylation and histone modifications in epigenetics mechanisms has been recognized a long time ago, epitranscriptomics mechanisms and more precisely RNA methylation are increasingly recognized as major disease players.[5] Yet, their role in heart failure is only emerging.

RNA methylation in heart failure

In this issue of the Journal, Berulava and colleagues report the results of a study aiming to address a potential role of m6A RNA methylation in heart failure development.[6] The authors observed that changes in m6A RNA methylation affect RNA translation and protein production and are involved in heart failure progression. These results are novel and support an important role of epigenetics mechanisms in general and m6A RNA methylation in particular in the development of heart failure. This is in line with recent landmark studies revealing a role for m6A status in cardiac homeostasis and various cardiovascular disorders and repair processes.[7,8] Although both the current study, and the study by Mathiyalagan *et al.*[8] show changes in global changes in the m6A landscape and in cardiac protein synthesis during heart failure, different mechanisms for the potential functionality of m6A are reported. Where Mathiyalagan *et al.*[8] report changes in

The opinions expressed in this article are not necessarily those of the Editors of the European Journal of Heart Failure or of the European Society of Cardiology. doi: 10.1002/ejhf.1672

*Corresponding author. 1A-B rue Edison L-1445 Strassen, Luxembourg. Tel.: +352 26 970300, Fax: +352 26 970396, Email: yvan.devaux@lih.lu

messenger RNA (mRNA) stability, Berulava *et al.*[6] demonstrate that changes in protein expression are mostly the consequence of changes in polysome binding and thus in protein translation. The main difference between the two studies is the onset of heart failure; where Mathiyalagan *et al.* focus on very acute tissue damage induced by ischaemia, Berulava *et al.* choose to induce heart failure using pressure overload. Acute ischaemia affects RNA methylation in other tissues too, as a recent study demonstrates changes in the m6A landscape in the brain following acute stroke in mice.[9] Moreover, m6A may not be the only form of RNA methylation that affects heart failure. Multiple other forms of RNA methylation have been uncovered. We have recently shown that changes in a specific set of small nucleolar RNAs that guide 2'O-ribose methylation of RNA associate with heart failure in human.[10]

Strengths and limitations

Although the manuscript by Berulava and colleagues[6] has a strong bioinformatics component, which usually limits the accessibility of such reports to non-experts, the authors must be acknowledged for their efforts to render this manuscript accessible to a broad readership and especially to the clinical readership of the *European Journal of Heart Failure*.

In addition to major scientific advances in the knowledge of the role of RNA methylation in heart failure progression, the authors provide a good example of collaborative research necessary to decipher the molecular pathways implicated in complex disease processes. With a consortium of investigators with complementary expertise, researchers, clinicians, molecular biologists, bioinformaticians, and systems biologists foster the development of a new field of research. Noteworthy, collaboration of experts from both the brain and heart fields represents an invaluable resource to study the brain–heart axis, as the old concept of neurocardiology[11] may be reborn as a new field of investigation for the EU-CardioRNA COST Action (www.cardiorna.eu).[12]

Berulava *et al.*[6] provide an intriguing dataset, but also leave us with several important questions. The authors demonstrate that m6A impacts polysome binding and protein synthesis. However, effects on protein expression were confirmed on an individual basis using western blots. Given the large amount of data and bioinformatics in the paper, proteomics data obtained by mass spectrometry could have confirmed the proposed mechanism of action of m6A in this setting and would have lifted the paper to an even higher level. Other questions that the authors raise themselves, such as the potential effects of m6A on alternative splicing, as well as the regulation of m6A in other species of RNA, including microRNAs for example,[13] also remain unanswered.

Perspectives

While a better knowledge of the implication of RNA methylation in cardiovascular disease has the potential to aid in heart failure management, much remains to be done before foreseeing a translation of research findings to clinical application. The challenging issue of accurately and reproducibly measuring the extent of RNA methylation needs to be addressed. Some technological advances have been achieved but remain to be pursued.[14] It would be interesting to determine whether RNA methylation profiles differ according to heart failure aetiology. The potential of RNA methylation to be used as biomarker of cardiovascular disease is currently unknown. Considering the limitations to the use of the current gold-standard biomarker of heart failure, N-terminal pro-B-type natriuretic peptide, RNA methylation profiles may afford incremental diagnostic and prognostic value, as shown previously for circulating non-coding RNAs.[4,15,16] Sex differences shall be a central aspect of further adequately powered studies. Future studies may also look more into the regulation of m6A methylation and demethylation.

In concurrence with the study by Dorn *et al.*,[7] Berulava *et al.*[6] show that deregulation of both methylases and demethylases impacts m6A, as well as cardiac function, but although m6A is affected, levels of methylases and demethylases appear only moderately altered during heart failure development. Future studies may therefore focus on the mechanisms that determine which RNAs become hyper- vs. hypomethylated and what determines whether that will affect RNA turnover, protein production or drive, for example, alternative mRNA splicing. The differences in m6A's effects on mRNA stability in the ischaemia-induced heart failure model presented by Mathiyalagan *et al.*[8] on the one hand, and on mRNA translation in the pressure overload model presented by Berulava *et al.*[6] on the other hand, may provide a starting point for such mechanistic studies.

In conclusion, the study of Berulava and colleagues shows that regulation of m6A RNA methylation contributes to heart failure progression and supports future research in this clinically relevant and promising area.[6] An exciting additional thought is that m6A is just one of more than 150 known RNA modifications that together make up the epitranscriptome.

Funding

Dr. Devaux is Chair of the EU-CardioRNA COST ACTION CA17129 (www.cardiorna.eu), supported by COST (European Cooperation in Science and Technology). Dr. Devaux is supported by the National Research Fund of Luxembourg (grants # C14/BM/8225223 and C17/BM/11613033), the Ministry of Higher Education and Research of Luxembourg, and the Heart Foundation – Daniel Wagner. Dr Nossent is funded by a Lise Meitner grant (M2578-B30) of the Austrian Science Fund FWF.

Conflict of interest: none declared.

References

1. Ponikowski P, Voors AA, Anker SD, Bueno H, Cleland JG, Coats AJ, Falk V, González-Juanatey JR, Harjola VP, Jankowska EA, Jessup M, Linde C, Nihoyannopoulos P, Parissis JT, Pieske B, Riley JP, Rosano GM, Ruilope LM, Ruschitzka F, Rutten FH, van der Meer P. 2016 ESC Guidelines for the diagnosis and treatment of acute and chronic heart failure: The Task Force for the diagnosis and treatment of acute and chronic heart failure of the European Society of Cardiology (ESC). Developed with the special contribution of the Heart Failure Association (HFA) of the ESC. *Eur J Heart Fail* 2016;**18**: 891–975.
2. Heidenreich PA, Trogdon JG, Khavjou OA, Butler J, Dracup K, Ezekowitz MD, Finkelstein EA, Hong Y, Johnston SC, Khera A, Lloyd-Jones DM, Nelson SA,

Nichol G, Orenstein D, Wilson PW, Woo YJ. American Heart Association Advocacy Coordinating Committee; Stroke Council; Council on Cardiovascular Radiology and Intervention; Council on Clinical Cardiology; Council on Epidemiology and Prevention; Council on Arteriosclerosis, Thrombosis and Vascular Biology; Council on Cardiopulmonary, Critical Care, Perioperative and Resuscitation; Council on Cardiovascular Nursing; Council on the Kidney in Cardiovascular Disease; Council on Cardiovascular Surgery and Anesthesia; Interdisciplinary Council on Quality of Care and Outcomes ResearchForecasting the future of cardiovascular disease in the United States: a policy statement from the American Heart Association. *Circulation* 2011;**123**:933–944.

3. Devaux Y, Zangrando J, Schroen B, Creemers EE, Pedrazzini T, Chang CP, Dorn GW 2nd, Thum T, Heymans S. Long noncoding RNAs in cardiac development and ageing. *Nat Rev Cardiol* 2015;**12**:415–425.
4. Devaux Y, Creemers EE, Boon RA, Werfel S, Thum T, Engelhardt S, Dimmeler S, Squire I, Cardiolinc N. Circular RNAs in heart failure. *Eur J Heart Fail* 2017;**19**:701–709.
5. Garber K. Epigenetics comes to RNA. *Science* 2019;**365**:16–17.
6. Berulava T, Buchholz E, Ellerdashvilli V, Pena T, Islam R, Lbik D, Mohamed B, Renner A, von Lewinski D, Sacherer M, Sloan KE, Bohnsack MT, Jain G, Capece V, Burkhardt S, Hasenfuss G, Fischer A, Toischer K. Changes in m6A RNA methylation contribute to heart failure progression by modulating translation. *Eur J Heart Fail* 2020;**22**:54–66.
7. Dorn LE, Lasman L, Chen J, Xu X, Hund TJ, Medvedovic M, Hanna JH, van Berlo JH, Accornero F. The N(6)-methyladenosine mRNA Methylase METTL3 controls cardiac homeostasis and hypertrophy. *Circulation* 2019;**139**:533–545.
8. Mathiyalagan P, Adamiak M, Mayourian J, Sassi Y, Liang Y, Agarwal N, Jha D, Zhang S, Kohlbrenner E, Chepurko E, Chen J, Trivieri MG, Singh R, Boucharebe R, Fish K, Ishikawa K, Lebeche D, Hajjar RJ, Sahoo S. FTO-dependent N(6)-methyladenosine regulates cardiac function during remodeling and repair. *Circulation* 2019;**139**:518–532.
9. Chokkalla AK, Mehta SL, Kim T, Chelluboina B, Kim J, Vemuganti R. Transient focal ischemia significantly alters the m(6)a epitranscriptomic tagging of RNAs in the brain. *Stroke* 2019;**50**:2912–2921.
10. Hakansson KE, Goossens EA, Trompet S, van Ingen E, de Vries MR, van der Kwast R, Ripa RS, Kastrup J, Hohensinner PJ, Kaun C, Wojta J, Bohringer S, Le Cessie S, Jukema JW, Quax PH, Nossent AY. Genetic associations and regulation of expression indicate an independent role for 14q32 snoRNAs in human cardiovascular disease. *Cardiovasc Res* 2019;**115**:1519–1532.
11. Natelson BH. Neurocardiology. An interdisciplinary area for the 80s. *Arch Neurol* 1985;**42**:178–184.
12. Gomes CPC, Agg B, Andova A, Arslan S, Baker A, Bartekova M, Beis D, Betsou F, Wettinger SB, Bugarski B, Condorelli G, Silva G, Danilin S, de Gonzalo-Calvo D, Buil A, Carmo-Fonseca M, Enguita FJ, Felekkis K, Ferdinandy P, Gyongyosi M, Hackl M, Karaduzovic-Hadziabdic K, Hellemans J, Heymans S, Hlavackova M, Hoydal MA, Jankovic A, Jusic A, Kardassis D, Kerkela R, Kuster GM, Lakkisto P, Leszek P, Lustrek M, Maegdefessel L, Martelli F, Novella S, O'Brien T, Papaneophytou C, Pedrazzini T, Pinet F, Popescu O, Potocnjak I, Robinson E, Sasson S, Scholz M, Simionescu M, Stoll M, Varga ZV, Vinciguerra M, Xuereb A, Yilmaz MB, Emanueli C, Devaux Y; EU-CardioRNA COST Action (CA17129). Catalyzing transcriptomics research in cardiovascular disease: the CardioRNA COST Action CA17129. *Noncoding RNA* 2019;**5**:E31.
13. Alarcon CR, Lee H, Goodarzi H, Halberg N, Tavazoie SF. N6-methyladenosine marks primary microRNAs for processing. *Nature* 2015;**519**:482–485.
14. Helm M, Motorin Y. Detecting RNA modifications in the epitranscriptome: predict and validate. *Nat Rev Genet* 2017;**18**:275–291.
15. Vausort M, Salgado-Somoza A, Zhang L, Leszek P, Scholz M, Teren A, Burkhardt R, Thiery J, Wagner DR, Devaux Y. Myocardial infarction-associated circular RNA predicting left ventricular dysfunction. *J Am Coll Cardiol* 2016;**68**:1247–1248.
16. Vausort M, Wagner DR, Devaux Y. Long noncoding RNAs in patients with acute myocardial infarction. *Circ Res* 2014;**115**:668–677.