



A circRNA/miR axis contributes to hypoxia-induced cardiomyocyte death

Paola Costelli

Department of Clinical and Biological Sciences, University of Torino, Istituto Interuniversitario di Miologia (IIM), Torino, Italy

Correspondence to: Paola Costelli. Department of Clinical and Biological Sciences, Corso Raffaello 30, 10125 Torino, Italy. Email: paola.costelli@unito.it..

Comment on: Wang K, Gan TY, Li N, *et al.* Circular RNA mediates cardiomyocyte death via miRNA-dependent upregulation of MTP18 expression. *Cell Death Differ* 2017;24:1111-20.

Submitted Nov 24, 2017. Accepted for publication Dec 07, 2017.

doi: 10.21037/tcr.2017.12.08

View this article at: <http://dx.doi.org/10.21037/tcr.2017.12.08>

The prevalence of cardiovascular diseases (CVD) is steadily increasing worldwide. In this regard, the 2016 report of The American Heart Association showed that the projection of global mortality due to CVD in 2030 could be about 23 million, 36% more than that reported in 2012 (1).

The vast majority of deaths due to CVD are associated with myocardial infarction, a condition characterized by cardiomyocyte death due to prolonged ischemia. About 20 min after the lack of oxygen supply infarction starts in the myocardium, leading in few hours to loss of contractility and tissue necrosis that cannot be efficiently recovered due to the poor regenerative capacity of the heart.

Myocardial cell death can occur by different mechanisms, among which necrosis, apoptosis, and autophagic cell death. Necrosis is considered an irreversible, non-regulated event characterized by the loss of cell membrane integrity and organelle (mitochondria in particular) swelling. However, also a form of 'programmed necrosis', called necroptosis has been shown to occur in myocardial infarction. As for autophagic and apoptotic death, they are tightly regulated processes. The latter is characterized by cell shrinkage with fragmentation in apoptotic bodies that maintain cell membrane integrity and is frequently dependent on the activation of caspases. By contrast, autophagic death is mainly associated with overactivation of the acidic-lysosomal proteolytic system. While apparently independent, these three types of cell death are markedly interconnected. As an example, apoptosis and autophagy are linked by the interaction among the anti-apoptotic factors Bcl2 and BclX_L and the autophagy regulator Beclin-1. Briefly, Beclin-1 contains a Bcl2 Homology-3 domain that physically interacts with Bcl2/BclX_L, resulting in Beclin-1 sequestration and autophagy inhibition. On the

other side, caspase-mediated cleavage of Beclin-1 generates a C-terminal fragment that targets mitochondria and promotes apoptosis, likely due to the release of pro-apoptotic factors (2).

The relevance of mitochondria dysfunction to the different modes of cell death occurring in heart failure is gaining a growing consensus. A significant proportion of heart mass is composed by mitochondria, since this organ has a high energy demand to sustain the contractile effort. Mitochondria turnover (e.g., biogenesis and disposal, mainly by mitophagy) is considerably high, in view of the fact that, also due to the continuous exposure to reactive oxygen species, these organelles are easily damaged. In addition, the mitochondria compartment is characterized by high plasticity, being continuously remodeled by events of fusion and/or fission. Different proteins are involved in the regulation of these latter events: mitofusins 1 and 2 (MFN1/2), localized at the outer mitochondrial membrane form homo and/or heterodimers, tethering juxtaposed mitochondria together, while optic atrophy protein 1 (OPA) is required for the fusion of inner mitochondrial membrane. On the other side, dynamin related protein (DRP) 1, a GTP-dependent protein, drives mitochondrial fission likely interacting with other proteins such as mitochondrial fission protein 1 (Fis1) and mitochondrial fission factor (Mff). Generally speaking, when the mitochondrial dynamic balance is shifted towards fusion, tubular mitochondria are formed, improving energy metabolism; by contrast, fragmented mitochondria appear when fission is enhanced, a condition that drives mitophagy and that is frequently predictive of apoptosis (3).

Evidence has been provided that short noncoding RNAs, in particular microRNAs (miRs), contribute to mitochondrial dynamics in the heart and participate to the regulation of

cardiomyocyte death. Firstly, described in *Caenorhabditis elegans* in 1993, miRs have long been considered as devoid of biological activity. Just few years ago their relevance to the regulation of mRNA expression, through accelerated degradation of mRNA and/or inhibition of translation, has become clear. The transcription of genes encoding miRs results in the production of a precursor (pri-miR) that is processed to pre-miR and subsequently to mature miR by the RNase III endonucleases Droscha and Dicer, respectively (4). In the last 10 years, miRs have been shown to participate in different biological processes such as DNA repair, oxidative stress response, cell differentiation and cell turnover.

Several studies have investigated the relevance of miRs to the regulation of myocardial cell death. In this regard, hypoxia-induced cardiomyocyte apoptosis has been shown to be inhibited by pathways involving miR-7a/PARP-1 or miR-138/JNK. By contrast, apoptosis can be induced by miR-320, that negatively regulates the expression of the anti-apoptotic factor IGF-1. Similarly, apoptosis induced by oxidative stress is associated with increased levels of miR-153. Not only, it seems that oxidized miRs could be involved in cardiomyocyte apoptosis. Indeed, miR oxidation appears to allow the down-regulation of genes coding for anti-apoptotic factors, such as Bcl-X_L, through mismatching (5).

Recent studies support the idea that the regulatory action exerted by miRs on cell death could also be explained by the observation that they are involved in mitochondrial bioenergetics, metabolism and dynamics. In this regard, myocardial infarction and cardiomyocyte death are prevented by overexpression of members of the miR-30 family, while miR-27 inhibits mitochondrial fission by down-regulating the expression of Mff, pushing the mitochondrial dynamic balance towards fusion, which appears to be endowed with a protective function (see above) (3).

A recent study by Wang and collaborators (6) propose that miR-652-3p directly regulates the expression of the mitochondrial protein MTP18, that pertains to the fission machinery. Indeed, the authors show that down-regulation of this protein, exerted directly by specific siRNA transfection or through miR-652-3p overexpression, results in reduced mitochondrial fission and prevents cardiomyocyte apoptosis. Similarly, miR-652-3p mimicking agents protect animals exposed to ischemia/reperfusion injury from myocardial infarction, cardiomyocyte apoptosis and mitochondrial fission. The additional and significant novelty of the present study is that the authors go further and elucidate the mechanism controlling miR-652-3p levels. Indeed, they show that the mitochondrial fission and apoptosis-related circRNA (MFACR), also known as

mm9-circ-016597, is overexpressed in myocardial infarction and directly interacts with miR-652-3p. Such interaction leads to miR-652-3p sequestration, resulting in increased MTP18 levels, that eventually contribute to mitochondrial fission and cardiomyocyte apoptosis.

CircRNAs are non-coding RNAs lacking both the 5' and 3' ends, that form a covalently closed continuous loop, a structure that confers more stability than that occurring in linear RNAs. The first circRNA was described in the early 1970s. Up to now, a lot of circRNAs, expressed by a high number of genes, have been discovered and involved in different situations such as embryonal and fetal development, carcinogenesis and infarction-associated heart failure. The mechanism of action of circRNAs is still unclear, although they have been proposed to behave as miR regulators acting as sponges, thus modulating their availability and their bioactivity. In this regard, circRNA-miR-mRNA axes have been proposed to be involved in the regulation of different biological processes. As an example, heart-related-Circ-RNA (HRCR) has been reported to directly interact and bind miR-223, contributing to heart hypertrophy; consistently, inhibition of HRCR expression in human cardiomyocytes attenuates the hypertrophic response (7).

The study by Wang and collaborators perfectly fits with this scenario. Indeed, the involvement of MTP18 in both mitochondria morphological changes and cell propensity to apoptotic death has been proposed many years ago (8). This study reports that reduced MTP18 levels are able to alter mitochondria and to stimulate apoptosis, thus suggesting a regulation that goes in the opposite way compared to that described by Wang *et al.* (6). In this regard, however, the relevance of increased MTP18 expression to the induction of mitochondrial fragmentation and apoptosis has been recently confirmed in cells different from cardiomyocytes (9). Up to now, the mechanisms underlying the expression of MTP18 have been totally unknown. Along this line, Wang and collaborators show that miR-652-3p levels are significantly reduced both in cardiomyocytes exposed to anoxia-reoxygenation and in the heart of animals in which ischemia-reperfusion injury has been induced. Overexpression of miR-652-3p results in MTP18 down-regulation as well as in protection against mitochondrial damage and apoptosis, suggesting the occurrence of a causative link between miR-652-3p and MTP18. The authors of the study elegantly demonstrate that MTP18 is a down-stream target of miR-652-3p. Also taking advantage of a recently published circRNA database, Wang and collaborators identify circRNAs that are modulated in conditions of hypoxia-reoxygenation, both *in vitro* and *in vivo*, focusing on MFACR and demonstrating that it

is able to directly interact with miR-652-3p. Finally, the authors show that modulating MFACR expression they can force changes in MTP18 levels, that are reduced when MFACR is down-regulated, and increased when MFACR is overexpressed. The observation that the levels of MFACR and miR-652-3p are inversely correlated supports the hypothesis that the former acts as a sponge able to sequester the latter, leading to increased MTP18 expression, mitochondrial fragmentation and cardiomyocyte apoptosis.

Several points still remain to be clarified, such as the mechanisms underlying the modulations of MFACR expression as well as if mitochondrial fission and cardiomyocyte apoptosis due to stimuli different from hypoxia can also depend on the MFACR/miR-625-3p/MTP18 axis. However, the study by Wang and collaborators sheds new light on the pathogenetic mechanisms of hypoxia-induced myocardial cell death, and for the first time describes a biological function for MFACR/mm9-circ-016597. In this regard, a recent study has identified more than 1,000 circRNAs significantly modified after brain ischemia, without providing any information about their biological function (10).

As mentioned above, ischemic heart disease is the major cause of mortality worldwide. Cardiomyocyte death is the most relevant feature of myocardial infarction, a lethal condition that could be recovered by replacing these cells. In this regard, therapeutic strategies aimed to inhibit myocardial cell death and/or to activate an effective regenerative response are of great clinical interest. Along this line, future studies are warranted to investigate if the new modulator of mitochondrial fission and cardiomyocyte apoptosis MFACR could reveal a useful target to be addressed by suitable innovative therapeutic strategies.

Acknowledgments

Funding: None.

Footnote

Provenance and Peer Review: This article was commissioned and reviewed by Section Editor Dr. Chunlin Ou, MD, PhD (Cancer Research Institute of Central South University, Changsha, China).

Conflicts of Interest: The author has no conflicts of interest to declare.

Ethical Statement: The author is accountable for all aspects of the work in ensuring that questions related

to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

Open Access Statement: This is an Open Access article distributed in accordance with the Creative Commons Attribution-NonCommercial-NoDerivs 4.0 International License (CC BY-NC-ND 4.0), which permits the non-commercial replication and distribution of the article with the strict proviso that no changes or edits are made and the original work is properly cited (including links to both the formal publication through the relevant DOI and the license). See: <https://creativecommons.org/licenses/by-nc-nd/4.0/>.

References

1. Mozaffarian D, Benjamin EJ, Go AS, et al. Heart Disease and Stroke Statistics—2015 Update. *Circulation* 2015;131:e29-e322.
2. Nikolettou V, Markaki M, Palikaras K, et al. Crosstalk between apoptosis, necrosis and autophagy. *Biochim Biophys Acta* 2013;1833:3448-59.
3. Sivakumar A, Subbiah R, Balakrishnan R, et al. Cardiac mitochondrial dynamics: miR-mediated regulation during cardiac injury. *J Mol Cell Cardiol* 2017;110:26-34.
4. Ha M, Kim VN. Regulation of microRNA biogenesis. *Nat Rev Mol Cell Biol* 2014;15:509-24.
5. Sun T, Dong YH, Du W, et al. The Role of MicroRNAs in Myocardial Infarction: From Molecular Mechanism to Clinical Application. *Int J Mol Sci* 2017;18:745.
6. Wang K, Gan TY, Li N, et al. Circular RNA mediates cardiomyocyte death via miRNA-dependent upregulation of MTP18 expression. *Cell Death Differ* 2017;24:1111-20.
7. Rong D, Sun H, Li Z, et al. An emerging function of circRNA-miRNAs-mRNA axis in human diseases. *Oncotarget* 2017;8:73271-81.
8. Tondera D, Santel A, Schwarzer R, et al. Knockdown of MTP18, a Novel Phosphatidylinositol 3-Kinase-dependent Protein, Affects Mitochondrial Morphology and Induces Apoptosis. *J Biol Chem* 2004;279:31544-55.
9. Aung LH, Li R, Prabhakar BS, et al. Mitochondrial protein 18 (MTP18) plays a pro-apoptotic role in chemotherapy-induced gastric cancer cell apoptosis. *Oncotarget* 2017;8:56582-97.
10. Liu C, Zhang C, Yang J, et al. Screening circular RNA expression patterns following focal cerebral ischemia in mice. *Oncotarget* 2017;8:86535-47.

Cite this article as: Costelli P. A circRNA/miR axis contributes to hypoxia-induced cardiomyocyte death. *Transl Cancer Res* 2018;7(Suppl 1):S38-S40. doi: 10.21037/tcr.2017.12.08