Molecular Therapy
Commentary

# Keeping the Heart *Fitm2* during Chemotherapy

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https://doi.org/10.1016/j.ymthe.2018.12.002

With increasing cancer survival rates due to improved treatment options, attention to the chronic sequelae of chemotherapy is becoming increasingly more relevant. Cardiotoxicity is an important complication of several cancer therapeutic agents, but, so far, approaches to reduce cardiotoxicity have shown limited success. In this issue of *Molecular Therapy*, Gupta et al.[1] report a potential new strategy to counteract doxorubicin-induced myocardial atrophy and apoptosis: treatment with the pro-hypertrophic miR-132-212 cluster. The results unveil a new pathophysiological aspect of doxorubicin-induced cardiotoxicity and may provide a promising starting point for the development of new therapies.

Anthracycline drugs, such as doxorubicin, are highly effective, broad-spectrum anticancer agents, but cardiac side effects pose a major limitation to their use. These agents can cause severe cardiomyocyte damage, leading to degradation of the sarcomere, swelling of mitochondria, vacuolar degradation of the sarcoplasmic reticulum, and, eventually, cardiomyocyte death. Cell death may also occur in cell types other than cardiomyocytes, potentially limiting the adaptive capacity of the heart and thereby increasing the risk of future cardiovascular disease and toxicity with future administration of cardiotoxic drugs.[2] Together, these effects result in symptomatic heart failure in 2%–4% of patients treated with anthracyclines and an asymptomatic reduction of left ventricular ejection fraction (LVEF) in 9%–11% of patients.[3] While anthracycline-induced cardiotoxicity (AIC) was initially believed to comprise both an early and a late component, recent data by Cardinale et al.[4] indicated that 98% of patients with an asymptomatic decline in LVEF already showed a decrease at 12 months after chemotherapy. This suggests that most of the cardiotoxicity caused by anthracyclines is established rather soon after the onset of treatment.

Despite extensive research, the mechanisms underlying AIC have not been fully elucidated. A potential explanation for the cardiotocixity is linked to the known function of anthracyclines. Anthracyclines cause their intended cytotoxic effect in cancer cells by binding to topoisomerase 2 (Top2). Top2 is an enzyme that is important for the organization of DNA and exists in an α- and β-isoform in humans. Top2α expression is co-regulated with the cell cycle and peaks in G2/M phase. Binding of anthracyclines to Top2α inhibits DNA replication, thereby arresting the cell cycle in G1/G2 and leading to apoptosis.[3] However, Top2β is also expressed in non-proliferating cells, such as cardiomyocytes, where binding of anthracyclines leads to the suppression of peroxisome proliferator-activated receptors (PPARs), resulting in mitochondrial dysfunction and eventually cell death.[5] Additionally, it has been shown that anthracyclines can downregulate transcription factors, such as GATA4, that are essential for sarcomere maintenance.[6] Another potential underlying mechanism for AIC is through the production of reactive oxygen species (ROS). Anthracyclines interact with ferric iron to convert molecular oxygen into superoxide radicals, but can additionally interfere with enzymes in the mitochondrial respiratory chain, leading to the production of ROS. The ROS then damage cellular components that can ultimately lead to apoptosis.[2]

So far, no effective prevention or therapy has been found for AIC. A 2016 position paper by the European Society of Cardiology[7] suggested limiting cardiotoxicity by minimizing the total dose of anthracyclines, using less cardiotoxic doxorubicin analogs or formulations, or employing a non-anthracycline regimen. A cardiac protective treatment option, the use of the iron chelator dexrazoxane, has shown some therapeutic benefit.[8] Some clinical trials also suggest potential benefits of the use of ACE-inhibitors and/or beta-blockers,[9,10] but further studies are currently ongoing.

In recent years, research has focused on the function of non-coding RNAs (ncRNAs) in AIC. microRNAs (miRs) are short strands of RNA that influence the translation of mRNA into protein by causing either translational repression or degradation of mRNA.[11] miR-208a was shown to be upregulated by doxorubicin, and inhibition of this miR resulted in a reduction in apoptosis through the derepression of *Gata4* and *Bcl2*.[12] In a similar fashion, the miR-30 family was found to be downregulated in response to doxorubicin, while overexpression of miR-30e proved beneficial by reducing apoptosis and ROS production.[13]

In the current study, Gupta et al.[1] hypothesized that overexpression of pro-hypertrophic miRs would be able to counteract AIC-induced atrophy. They show that doxorubicin causes apoptosis and a reduction in cell size in isolated neonatal rat ventricular myocytes (NRVMs). While miR-132 and miR-212 levels do not change during doxorubicin-induced cardiomyocyte toxicity, cotreatment with pre-miR-132 or pre-miR-212 protected the cells against these effects. And while *in vivo* AAV9-mediated overexpression of the cluster, as expected, resulted in cardiac hypertrophy in doxorubicin-naive animals,[14] viral overexpression of miR-132-212 during doxorubicin treatment actually ameliorated the doxorubicin-induced reduction in ejection fraction, remodeling, myofibril loss, and apoptosis.

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To elucidate the mechanism behind the cardioprotective effect of the miR-132-212 on doxorubicin-treated hearts, the authors analyzed the cardiac transcriptomes of doxorubicin-treated mice with or without viral overexpression of miR-132-212. Among the differentially regulated genes, cell-death related processes were enriched and the gene fat storage-inducing transmembrane protein 2 (Fitm2) was identified as a potential direct target of miR-132 and miR-212. Follow-up *in vitro* experiments showed that overexpression of Fitm2 was able to block the protective effects of miR-132 or miR-212 during doxorubicin-induced cardiomyocyte toxicity. Based on these data, the authors conclude that increasing the miR-132-212 cluster might be a good therapeutic means for reducing AIC and that these effects are at least partially due to a decrease in Fitm2.

Fitm2 is known to be involved in the formation of cytosolic lipid droplets and has been shown to have an important role in skeletal muscle energy metabolism.[15] Since it has not been studied for its function in cardiomyocyte biology, future studies are required to examine how its decrease would trigger hypertrophy or block AIC. As this gene was not found to be upregulated by the miR-132-212 cluster during doxorubicin treatment *in vivo*, a logical explanation might be that additional mechanisms are at play, which can explain the observed cardioprotective effects.

Another important biological question that remains relates to the long-term effect of blocking doxorubicin-induced cardiomyocyte apoptosis. Since a major cause of cardiomyocyte loss in AIC is apoptosis in response to intracellular damage, inhibition of this process by a transient early increase in miR-132/212 might be beneficial, but long-term expression might be detrimental since damaged cells are not removed. In addition, the fact that long-term overexpression of the miR-132/212 cluster under baseline conditions previously was shown to result in heart failure could underscore a level of caution regarding a long-term increase in the miRs.[14]

From a therapeutic perspective, some hurdles must be overcome before increasing levels of miR-132 and -212 can be considered a viable option for treating AIC. Increasing miR levels is possible through oligonucleotide therapy (mimicry), but targeting a specific cell type or organ is difficult. and the stability of these compounds is limited because stabilizing modifications generally preclude incorporation into RNA-induced silencing complex (RISC).[16] Viral gene therapy circumvents the stability and delivery issues, but has the risk of the patient having or developing immunity to the virus being used.[17] Additionally, even when local delivery could be achieved, sustained overexpression of miRs could have all types of unwanted off-target effects, since each miR is predicted to regulate multiple (sometimes unrelated) mRNA targets.

Currently, to avoid AIC, clinicians limit anthracycline dose, use less toxic formulations, or avoid anthracyclines altogether, even though they are very potent drugs. Therefore, there is a dire need for approaches to circumvent cardiotoxicity during anthracycline regimens. The use of a prohypertrophic miR cluster to counter the atrophy in AIC is an intriguing choice. It will be very interesting to see what future studies will reveal regarding the durability of this protective effect as well as the mechanism underlying these effects. Will it turn out that *Fitm2* fits in with the ROS paradigm because it influences ROS production by influencing cardiomyocyte energy metabolism or will a completely new mechanism of cardioprotection be revealed? Regardless of what the final outcomes will be, the work of Gupta et al.[1] presented here paves the way for a new avenue of promising research into AIC.

## CONFLICTS OF INTEREST

E.v.R. is a scientific co-founder and member of the Scientific Advisory Board of miRagen Therapeutics, Inc.

## ACKNOWLEDGMENTS

E.v.R. was supported by a consolidator grant from the European Research Council (ERC CoG 615708 MICARUS) and a network grant from Fondation Leducq and CVON REMAIN (2014-27).

## REFERENCES

1. Gupta, S.K., Garg, A., Avramopoulos, P., Engelhardt, S., Streckfuss-Bömeke, K., Batkai, S., and Thum, T. (2018). miR-212/132 cluster modulation prevents doxorubicin-mediated atrophy and cardiotoxicity. Mol. Ther. *27*, Published online January 2019. https://doi.org/10.1016/j.ymthe.2018.11.004.
2. Geisberg, C.A., and Sawyer, D.B. (2010). Mechanisms of anthracycline cardiotoxicity and strategies to decrease cardiac damage. Curr. Hypertens. Rep. *12*, 404–410.
3. McGowan, J.V., Chung, R., Maulik, A., Piotrowska, I., Walker, J.M., and Yellon, D.M. (2017). Anthracycline Chemotherapy and Cardiotoxicity. Cardiovasc. Drugs Ther. *31*, 63–75.
4. Cardinale, D., Colombo, A., Bacchiani, G., Tedeschi, I., Meroni, C.A., Veglia, F., Civelli, M., Lamantia, G., Colombo, N., Curigliano, G., et al. (2015). Early detection of anthracycline cardiotoxicity and improvement with heart failure therapy. Circulation *131*, 1981–1988.
5. Yang, Y., Zhang, H., Li, X., Yang, T., and Jiang, Q. (2015). Effects of PPARα/PGC-1α on the energy metabolism remodeling and apoptosis in the doxorubicin induced mice cardiomyocytes in vitro. Int. J. Clin. Exp. Pathol. *8*, 12216–12224.
6. Park, A.-M., Nagase, H., Liu, L., Vinod Kumar, S., Szwergold, N., Wong, C.-M., and Suzuki, Y.J. (2011). Mechanism of anthracycline-mediated down-regulation of GATA4 in the heart. Cardiovasc. Res. *90*, 97–104.
7. Zamorano, J.L., Lancellotti, P., Rodriguez Muñoz, D., Aboyans, V., Asteggiano, R., Galderisi, M., Habib, G., Lenihan, D.J., Lip, G.Y.H., Lyon, A.R., et al.; ESC Scientific Document Group (2016). 2016 ESC Position Paper on cancer treatments and cardiovascular toxicity developed under the auspices of the ESC Committee for Practice Guidelines: The Task Force for cancer treatments and cardiovascular toxicity of the European Society of Cardiology (ESC). Eur. Heart J. *37*, 2768–2801.
8. van Dalen, E.C., Caron, H.N., Dickinson, H.O., and Kremer, L.C.M. (2005). Cardioprotective interventions for cancer patients receiving anthracyclines. Cochrane Database Syst. Rev. *CD003917*, CD003917.
9. Bosch, X., Rovira, M., Sitges, M., Domènech, A., Ortiz-Pérez, J.T., de Caralt, T.M., et al. (2013). Enalapril and carvedilol for preventing chemotherapy-induced left ventricular systolic dysfunction in patients with malignant hemopathies: the OVERCOME trial (preventiOn of left Ventricular dysfunction with Enalapril and caRvedilol in patients submitted t. J. Am. Coll. Cardiol. *61*, 2355–2362.
10. Gulati, G., Heck, S.L., Ree, A.H., Hoffmann, P., Schulz-Menger, J., Fagerland, M.W., Gravdehaug, B., von Knobelsdorff-Brenkenhoff, F., Bratland, Å., Storås, T.H., et al. (2016). Prevention of cardiac dysfunction during adjuvant breast cancer therapy (PRADA): a 2 × 2 factorial, randomized, placebo-controlled, double-blind clinical trial of candesartan and metoprolol. Eur. Heart J. *37*, 1671–1680.

11. van Rooij, E., and Olson, E.N. (2012). MicroRNA therapeutics for cardiovascular disease: opportunities and obstacles. Nat. Rev. Drug Discov. *11*, 860–872.

12. Tony, H., Yu, K., and Qiutang, Z. (2015). MicroRNA-208a Silencing Attenuates Doxorubicin Induced Myocyte Apoptosis and Cardiac Dysfunction. Oxid. Med. Cell. Longev. *2015*, 597032.

13. Chatterjee, S., Gupta, S.K., Bär, C., and Thum, T. (2018). Non-coding RNAs: potential regulators in cardio-oncology. Am. J. Physiol. Heart Circ. Physiol., Published online November 9, 2018. https://doi.org/10.1152/ajpheart.00418.2018.

14. Ucar, A., Gupta, S.K., Fiedler, J., Erikci, E., Kardasinski, M., Batkai, S., Dangwal, S., Kumarswamy, R., Bang, C., Holzmann, A., et al. (2012). The miRNA-212/132 family regulates both cardiac hypertrophy and cardiomyocyte autophagy. Nat. Commun. *3*, 1078.

15. Miranda, D.A., Koves, T.R., Gross, D.A., Chadt, A., Al-Hasani, H., Cline, G.W., Schwartz, G.J., Muoio, D.M., and Silver, D.L. (2011). Re-patterning of skeletal muscle energy metabolism by fat storage-inducing transmembrane protein 2. J. Biol. Chem. *286*, 42188–42199.

16. van Rooij, E., Purcell, A.L., and Levin, A.A. (2012). Developing microRNA therapeutics. Circ. Res. *110*, 496–507.

17. Boutin, S., Monteilhet, V., Veron, P., Leborgne, C., Benveniste, O., Montus, M.F., and Masurier, C. (2010). Prevalence of serum IgG and neutralizing factors against adeno-associated virus (AAV) types 1, 2, 5, 6, 8, and 9 in the healthy population: implications for gene therapy using AAV vectors. Hum. Gene Ther. *21*, 704–712.

# AAV-CRISPR Persistence in the Eye of the Beholder

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https://doi.org/10.1016/j.ymthe.2018.12.007

Despite advances in genome editing technologies based on the adeno-associated virus (AAV)-CRISPR system, there are still concerns about the long-term persistence of recombinant AAV vectors in several organs (liver, muscle, eye) possibly leading to cytotoxicity or genotoxicity related to off-target effects. Indeed, there are still unanswered questions about long-lasting *in vivo* AAV persistence as a linear or circular DNA that is not targeted by epigenetic silencing in many tissues. In 2017, Kim et al.[1] reported an editing approach based on AAV-CjCas9 to downregulate *Vegfa* or the hypoxia-inducible transcription factor *Hif1a* in mice displaying age-related macular degeneration (AMD)-related pathological choroidal neovascularization (CNV) induced by laser treatment. Although partial knockdown of either *Vegfa* or *Hif1a* provided benefits and reduced the area of CNV, local opsin dysfunction near the *Vegfa*-edited cells of murine retinal pigment epithelium (RPE) was observed. Conversely, no cone dysfunction was reported upon *Hif1a* partial knockdown. Lastly, no genome-wide off-target indels, evaluated 6 weeks after intravitreal injection of AAV-CjCas9 vector, were scored, indicating that prolonged expression of AAV-CjCas9 *in vivo* did not aggravate the genotoxic risk associated with the CjCas9 nuclease. In this issue of *Molecular Therapy*, the authors now report a long-term (14 months) safety study on C57BL/6J mice intravitreally injected with AAV-CjCas9 vectors targeting *Vegfa* or *Hif1a* genes.[2] The findings continue to show that the AAV-CRISPR system in the eyes is long lasting, effective, and safe.

CRISPR/Cas9 genome editing in the retina represents a potential treatment strategy for inherited retinal dystrophies (e.g., autosomal dominant retinitis pigmentosa [adRP] and Leber congenital amaurosis [LCA]) and retinal neovascular diseases (e.g., wet AMD and proliferative diabetic retinopathy). CRISPR components have been delivered to the retina by viral and non-viral methods. Although subretinal plasmid electroporation is not suitable for therapeutic interventions in patients, it has been employed to knock down a mutant Rhodopsin gene in mouse[3] and rat[4] models of adRP. Recently, preassembled *Vegfa*-specific Cas9 ribonucleoproteins (RNPs) have been subretinally injected into a mouse model of AMD, demonstrating a significant reduction of laser-induced CNV. However, the effects were localized only to the injected area of RPE, with no transduction of the neural retina.[5]

Nonvirally-mediated transient expression of CRISPR components in the retina may reduce safety concerns, although viral delivery systems based on AAV represent the most efficient and safe tools for gene delivery to the retina. Indeed genome editing using the AAV-CRISPR system has been widely reported as efficient, safe, and precise in more than 30 published studies in mouse models[6] of diseases associated with the eyes, muscle, liver, heart, and lung. Despite the great potential of AAV vectors, their relatively small packaging capacity represents a limitation for delivering the widely used *Streptococcus pyogenes* Cas9 (SpCas9) together with guide RNAs (gRNAs) and large transgenes. Dual-vector AAV systems, smaller Cas9 orthologs, or other nucleases belonging to the type-V

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