

soluble molecules while providing significant benefits in terms of ease of administration to affected individuals. If these results can be duplicated in humans, then the resulting products will have a much greater dosing interval than that required for current products. Considering the recent explosion in the number of antibody-drug conjugates in development, this work presents an alternative strategy by demonstrating that a relatively simple carbohydrate ligand is sufficient to achieve effective, targeted delivery of multiple components. Considering nature's predominant use of carbohydrates in recognition and trafficking, advances in the field

of glycobiology should pave the way for future drug delivery research.

REFERENCES

1. Springer, A.D., and Dowdy, S.F. (2018). GalNAc-siRNA Conjugates: Leading the Way for Delivery of RNAi Therapeutics. *Nucleic Acid Ther.* 28, 109–118.
2. Holland, R.J., Lam, K., Ye, X., Martin, A.D., Wood, M.C., Palmer, L., Fraser, D., McClintock, K., Majeski, S., Jarosz, A., et al. (2021). Ligand conjugate SAR and enhanced delivery in NHP. *Mol. Ther.*, S1525-0016(21)00308-7.
3. Düzgüneş, N., Straubinger, R.M., Baldwin, P.A., Friend, D.S., and Papahadjopoulos, D. (1985). Proton-induced fusion of oleic acid-phosphatidylethanolamine liposomes. *Biochemistry* 24, 3091–3098.
4. Schlich, M., Palomba, R., Costabile, G., Mizrahy, S., Pannuzzo, M., Peer, D., and Decuzzi, P. (2021). Cytosolic delivery of nucleic acids: The case of ionizable lipid nanoparticles. *Bioeng. Transl. Med.* 6, e10213.
5. Park, K. (2017). The drug delivery field at the inflection point: Time to fight its way out of the egg. *J. Control. Release* 267, 2–14.
6. Salvati, A., Pitek, A.S., Monopoli, M.P., Prapainop, K., Bombelli, F.B., Hristov, D.R., Kelly, P.M., Åberg, C., Mahon, E., and Dawson, K.A. (2013). Transferrin-functionalized nanoparticles lose their targeting capabilities when a biomolecule corona adsorbs on the surface. *Nat. Nanotechnol.* 8, 137–143.
7. Jiskoot, W., van Schie, R.M., Carstens, M.G., and Schellekens, H. (2009). Immunological risk of injectable drug delivery systems. *Pharm. Res.* 26, 1303–1314.

Cell programming to protect the ischemic heart and limb

Mairi Brittan⁶ and Wolfram-Hubertus Zimmermann^{1,2,3,4,5}

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New strategies for myocardial protection and regeneration after myocardial infarction are urgently needed to counter the heart failure pandemic. To address this unmet medical need, Kaur and colleagues (in this issue of *Molecular Therapy*)¹ have identified in a preclinical proof-of-concept study a cocktail of modified RNA (modRNA) to endow stromal cells with angiogenic growth factor releasing activity (Figure 1). Sustained angiogenic programming was realized by lipid-nanoparticle (LNP) delivery, using formulations that have been employed safely and efficaciously in severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) vaccinations.

Reprogramming cell fate by transient expression of transcription factors has been explored for decades. A prominent, if not the most prominent example, is the overexpression of the skeletal muscle master regulator MyoD1 to reprogram fibroblasts into skeletal myoblasts.² More recently, reprogramming of somatic cells into pluripotent stem cells by the so called four Yamanaka factors (Oct3/4, Klf4, Sox 2, and *c-myc* [OKSM]) has revolutionized biomedical

research.³ To bypass the programming to an embryonic-cell-like state, direct cardiac reprogramming of myofibroblasts by overexpression of GATA4, Mef2c, Tbx5 (GMT) has been introduced⁴ with demonstrated therapeutic activity in mice with myocardial infarction.^{5,6} The original protocol employed retroviral delivery and was further refined to also convert human cells into cardiomyocyte-like cells, with, however, limited efficiency in adult human fibroblast reprogramming.⁷

Because of the inherent limitations of retroviral delivery of DNA, modRNA has been investigated by many labs as an alternative means for cell type conversion. Transient expression via RNA versus stable expression of DNA after genome insertion is considered advantageous from a translational point of view. Key for transient but efficacious delivery of RNA were specific chemical modifications to prevent premature degradation and activation of the innate immune system.^{8,9} In an earlier study, Zangi and co-workers administered VEGF-A encoding modRNA by intramyocardial injection and observed vascular

regeneration by enhanced epithelial-to-mesenchymal transition of WT-1⁺ epicardium-derived cells in mice with myocardial infarction.¹⁰ In the present study, the protocol was extended by the application of 7G-modRNA, i.e., modRNA encoding for GMT + Hand2 + dominant negative (DN)-transforming growth factor β (TGF- β) + DN-Wnt8a + acid ceramidase (AC), to turn myofibroblasts into cardiac-like cells. Although direct injection of modRNA, despite its chemical stabilization, resulted in a near-complete loss of transcripts within 72 h,¹⁰ LNP delivery of 7G-modRNA enhanced angiogenic growth factor expression for the whole study duration of 4 weeks, which resulted in a clear attenuation of disease progression with

¹Institute of Pharmacology and Toxicology, University Medical Center Göttingen, Georg-August-University, Robert-Koch-Str. 40, 37075 Göttingen, Göttingen, Germany; ²German Center for Cardiovascular Research (DZHK), partner site Göttingen, Germany; ³Cluster of Excellence “Multiscale Bioimaging: from Molecular Machines to Networks of Excitable Cells” (MBExC), University of Göttingen, Göttingen, Germany; ⁴Center for Neurodegenerative Diseases (DZNE), Göttingen, Germany; ⁵Fraunhofer Institute for Translational Medicine and Pharmacology (ITMP), Göttingen, Germany; ⁶Centre for Cardiovascular Science, The Queen's Medical Research Institute, University of Edinburgh, Edinburgh EH16 4TJ, UK

Correspondence: Wolfram-Hubertus Zimmermann, MD, Institute of Pharmacology and Toxicology, University Medical Center Göttingen, Georg-August-University, Robert-Koch-Str. 40, 37075 Göttingen, Germany.

E-mail: w.zimmermann@med.uni-goettingen.de



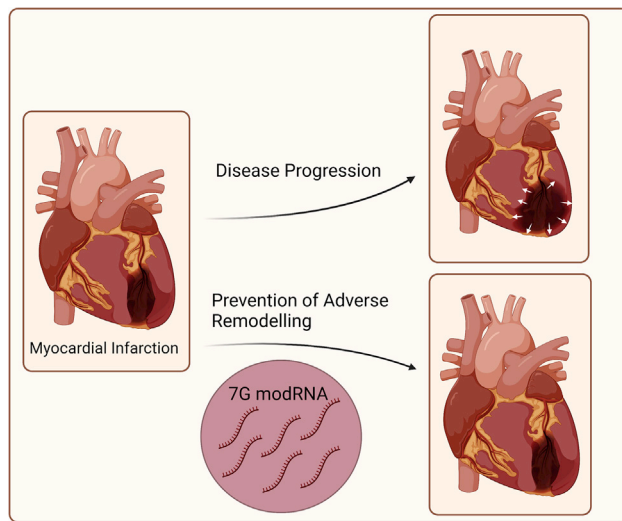


Figure 1. LNP delivery of 7G at the time of myocardial infarction attenuates disease progression

7G comprises modified RNA encoding for GATA4, Mef2c, Tbx5, Hand2, DN-TGF- β , DN-Wnt8a, and AC. After intramyocardial injection into acutely infarcted mouse hearts, expression of transcripts encoding for a broad spectrum of angiogenic growth factors (e.g., VEGF-A/B/C/D) remained enhanced for at least 4 weeks compared to controls.

markedly reduced mortality in mice with myocardial infarction. Similarly, administration of LNP-7G to the ischemic limb stabilized blood perfusion to pre-injury levels after 21 days, demonstrating a common biological activity in ischemic muscle, i.e., enhanced angiogenesis by paracrine factors.

The use of LNP for 7G delivery is particularly interesting for several reasons: (1) LNP delivery shields modRNA from degradation and thus allows for extended activity in the target tissue; (2) LNP delivery has been demonstrated to be safe and efficacious in the delivery of SARS-CoV-2 spike protein encoding RNA via the BioNTech and Moderna vaccines; and (3) further modifications of LNP are conceivable to enhance organ or cell type targeting by, for example, insertion of specific biological motifs or chemical moieties into its lipid bilayer.

Collectively, the study by Kaur and colleagues provides important proof of concept in a small animal model for modRNA delivery to ischemic heart and limb muscle. Although the precise molecular mechanisms for angiogenic programming remain to be clarified and further optimization will be required, we anticipate an increasing body of literature reporting on LNP delivery of RNA not only to the heart but also skeletal muscle with high translational potential. It is important to note that LNP delivery of RNA encoding CRISPR-Cas9 and a single guide RNA has recently been demonstrated to be safe and efficacious in patients with transthyretin amyloidosis.¹¹ Although the goal to reprogram scar into muscle remains challenging, in particular in human models, it is exciting to see that, after many years of development, modRNA delivery is becoming increasingly attractive not only for *in vitro* manipulations but also therapeutic applications *in vivo*. Sufficiently long on-target activity will be key to clinical utility.

REFERENCES

1. Kaur, K., Hadas, Y., Kurian, A.A., Żak, M.M., Yoo, J., Mahmood, A., Girard, H., Komargodski, R., Io, T., Santini, M.P., et al. (2021). Direct reprogramming induces vascular regeneration post muscle ischemic injury. *Mol. Ther.* 29. <https://doi.org/10.1016/j.ythte.2021.07.014>.
2. Davis, R.L., Weintraub, H., and Lassar, A.B. (1987). Expression of a single transfected cDNA converts fibroblasts to myoblasts. *Cell* 51, 987–1000.
3. Takahashi, K., Tanabe, K., Ohnuki, M., Narita, M., Ichisaka, T., Tomoda, K., and Yamanaka, S. (2007). Induction of pluripotent stem cells from adult human fibroblasts by defined factors. *Cell* 131, 861–872.
4. Ieda, M., Fu, J.D., Delgado-Olguin, P., Vedantham, V., Hayashi, Y., Bruneau, B.G., and Srivastava, D. (2010). Direct reprogramming of fibroblasts into functional cardiomyocytes by defined factors. *Cell* 142, 375–386.
5. Qian, L., Huang, Y., Spencer, C.I., Foley, A., Vedantham, V., Liu, L., Conway, S.J., Fu, J.D., and Srivastava, D. (2012). In vivo reprogramming of murine cardiac fibroblasts into induced cardiomyocytes. *Nature* 485, 593–598.
6. Song, K., Nam, Y.J., Luo, X., Qi, X., Tan, W., Huang, G.N., Acharya, A., Smith, C.L., Tallquist, M.D., Neilson, E.G., et al. (2012). Heart repair by reprogramming non-myocytes with cardiac transcription factors. *Nature* 485, 599–604.
7. Garry, G.A., Bassel-Duby, R., and Olson, E.N. (2021). Direct reprogramming as a route to cardiac repair. *Semin. Cell Dev. Biol.*, Published online July 7, 2021. <https://doi.org/10.1016/j.semcdb.2021.05.019>.
8. Kormann, M.S., Hasenpusch, G., Aneja, M.K., Nica, G., Flemmer, A.W., Herber-Jonat, S., Huppmann, M., Mays, L.E., Illyeni, M., Schams, A., et al. (2011). Expression of therapeutic proteins after delivery of chemically modified mRNA in mice. *Nat. Biotechnol.* 29, 154–157.
9. Sultana, N., Magadum, A., Hadas, Y., Kondrat, J., Singh, N., Youssef, E., Calderon, D., Chepurko, E., Dubois, N., Hajjar, R.J., and Zangi, L. (2017). Optimizing cardiac delivery of modified mRNA. *Mol. Ther.* 25, 1306–1315.
10. Zangi, L., Lui, K.O., von Gise, A., Ma, Q., Ebina, W., Ptaszek, L.M., Später, D., Xu, H., Tabebordbar, M., Gorbatov, R., et al. (2013). Modified mRNA directs the fate of heart progenitor cells and induces vascular regeneration after myocardial infarction. *Nat. Biotechnol.* 31, 898–907.
11. Gillmore, J.D., Gane, E., Taubel, J., Kao, J., Fontana, M., Maitland, M.L., Seitzer, J., O'Connell, D., Walsh, K.R., Wood, K., et al. (2021). CRISPR-Cas9 *in vivo* gene editing for transthyretin amyloidosis. *N. Engl. J. Med.* 385, 493–502.