

Hypo-glycosylated follistatin-like 1 for new cardiomyocyte formation

The adult human heart only has a very limited capacity to regenerate new cardiomyocytes. The final cardiomyocyte number is established shortly after birth and while there is evidence for postnatal cardiomyocyte formation, the turnover is very low being ~0.8% per year in young adults and ~0.3% per year at the age of 75.¹ The majority of studies suggest that cardiac regeneration occurs from dedifferentiation and proliferation of pre-existing cardiomyocytes, albeit not in sufficient levels to restore the cardiac contractile function after injury.² While addition of exogenously produced cardiomyocytes from human pluripotent stem cells has shown promise in re-musculating the injured myocardium, promotion of an endogenous regenerative capacity has shown promise as an attractive approach for cardiomyocyte replacement. Studies during recent years have identified several factors, including extracellular matrix proteins, soluble factors, intracellular signaling proteins, and microRNAs that can be modulated to induce the cardiomyocytes to divide.

Among these factors, glycoprotein follistatin-like protein 1 (FSTL1) has emerged as a cardioprotective and proangiogenic agent with regenerative properties.^{3–6} In 2015, data from Wei and coworkers showed that human FSTL1 protein (hFSTL1) promotes cardiomyocyte cell cycle entry and improves cardiac function following myocardial infarction (MI).⁵ hFSTL1 appeared to stimulate proliferation of immature cardiomyocytes but not mature adult ventricular cardiomyocytes. Interestingly, epicardial delivery of hFSTL1 via collagen patches loaded with recombinant hFSTL1 protein enhanced cardiac regeneration, whereas FSTL1 produced by cardiomyocytes failed to enhance cardiac regeneration. This disparity was attributed to the difference in glycosylation state of FSTL1, with bacterially produced recombinant hFSTL1 showing less extensive glycosylation. More recently, it was shown that a single replacement of asparagine with glutamine in the N-glycosylation site at position 180 of hFSTL1 is both sufficient and necessary to activate cardiomyocyte cell cycle entry and showed efficacy in limiting post-MI left ventricular remodeling.⁶

In this issue of *Molecular Therapy – Methods & Clinical Development*, Peters and coworkers investigated the significance of N-glycosylation on the regenerative activity of hFSTL1 in human induced pluripotent stem cell-derived cardiomyocytes (iPSC-CMs).⁷ To better mimic the metabolic conditions in the adult myocardium, the iPSC-CMs were matured to shift the metabolism toward oxidative phosphorylation, making the cells both susceptible to hypoxic injury and lowering their basal proliferative capacity. They find that differently from glycosylated hFSTL1, administration of non-glycosylated hFSTL1 stimulates the proliferation of iPSC-CMs. Both glycosylated and non-glycosylated hFSTL1, however, protect the iPSC-CMs from hypoxia-induced cell death. Interestingly, hypoxia stress induced FSTL1 secretion only from cardiac fibroblasts, whereas the secretion from iPSC-CMs

decreased. Further, iPSC-derived cardiac fibroblasts produced non-glycosylated form of FSTL1, whereas the iPSC-CMs only expressed high-glycosylated FSTL1. Corroborating with the current findings, a previous study found that FSTL1 produced by the cardiac fibroblasts was essential for fibroblast activation and preventing cardiac rupture following MI.⁸ In addition to promoting expression of genes regulating the cell cycle, data in the current study showed that hypo-glycosylated hFSTL1 enhanced expression of genes protecting from oxidative stress and depressed the expression of genes involved in cytolysis and immune cell activation.

The current study thus provides evidence that the glycosylation state is the key determinant of the regenerative activity of hFSTL1. These data obviously require further translational evaluation in a suitable animal model. Another burden to overcome is to provide direct evidence of actual cytokinesis in response to hypo-glycosylated hFSTL1. The authors here assessed cardiomyocyte cell cycle by analyzing for the proportion of cardiomyocytes positive for KI67, phospho-histone H3, and aurora B kinase, and by analyzing for 5-ethynyl-2'-deoxyuridine (EdU) uptake. Cardiomyocyte lineage tracing models allow defining of cardiomyocyte division in relation to cardiomyocyte DNA synthesis⁹ and could be utilized to investigate if hypo-glycosylated hFSTL1 promotes adult cardiomyocyte renewal *in vivo*. In addition, the molecular mechanisms mediating the regenerative actions of hypo-glycosylated hFSTL1 remain poorly known. As some of the actions of FSTL1 also involve fibroblasts and endothelial cells,^{4,8,10–12} a 3D iPSC cell model could be useful to decipher the signaling mechanisms involved and to investigate the effect of hypo-glycosylated hFSTL1 on interplay between the cardiac cells. Experimental models also allow investigation of the role of hypo-glycosylated hFSTL1 in modulation of myocardial fibrosis, revascularization, and regulation of inflammation that are crucial to achieve significant cardiac repair.

The research during past years has yielded considerable advances with respect to putative therapeutic targets to induce cardiomyocyte proliferation, but identifying the optimal treatment to replace the lost cardiomyocytes without inducing tumorigenesis poses a major challenge in the field. The findings by Peters et al.⁷ together with previous reports suggest potential for hypo-glycosylated hFSTL1 in cardiac regeneration and cardiac repair. The therapeutic approach, by using an exogenous protein such as hypo-glycosylated hFSTL1, appears as a feasible option, as the use of, for example, bioengineered epicardial patches or injectable hydrogels, allows for local delivery of the drug.

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DECLARATION OF INTERESTS

The authors declare no competing interests.

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REFERENCES

1. Bergmann, O., Zdunek, S., Felker, A., Salehpour, M., Alkass, K., Bernard, S., Sjöström, S.L., Szewczykowska, M., Jackowska, T., Dos Remedios, C., et al. (2015). Dynamics of cell generation and turnover in the human heart. *Cell* 161, 1566–1575.
2. Li, H., Hastings, M.H., Rhee, J., Trager, L.E., Roh, J.D., and Rosenzweig, A. (2020). Targeting age-related pathways in heart failure. *Circ. Res.* 126, 533–551.
3. Oshima, Y., Ouchi, N., Sato, K., Izumiya, Y., Pimentel, D.R., and Walsh, K. (2008). Follistatin-like 1 is an Akt-regulated cardioprotective factor that is secreted by the heart. *Circulation* 117, 3099–3108.
4. Ouchi, N., Oshima, Y., Ohashi, K., Higuchi, A., Ikegami, C., Izumiya, Y., and Walsh, K. (2008). Follistatin-like 1, a secreted muscle protein, promotes endothelial cell function and revascularization in ischemic tissue through a nitric-oxide synthase-dependent mechanism. *J. Biol. Chem.* 283, 32802–32811.
5. Wei, K., Serpooshan, V., Hurtado, C., Diez-Cunado, M., Zhao, M., Maruyama, S., Zhu, W., Fajardo, G., Nosedá, M., Nakamura, K., et al. (2015). Epicardial FSTL1 reconstitution regenerates the adult mammalian heart. *Nature* 525, 479–485.
6. Magadam, A., Singh, N., Kurian, A.A., Sharkar, M.T.K., Chepurko, E., and Zangi, L. (2018). Ablation of a single N-glycosylation site in human FSTL1 induces cardiomyocyte proliferation and cardiac regeneration. *Mol. Ther. Nucleic Acids* 13, 133–143.
7. Peters, M.C., Di Martino, S., Boelens, T., Qin, J., van Mil, A., Doevendans, P.A., Chamuleau, S.A.J., Sluijter, J.P.G., and Neef, K. (2022). Follistatin-like 1 promotes proliferation of matured human hypoxic iPSC-cardiomyocytes and is secreted by cardiac fibroblasts. *Mol. Ther. - Methods Clin. Develop.* 25, 3–16.
8. Maruyama, S., Nakamura, K., Papanicolaou, K.N., Sano, S., Shimizu, I., Asami, Y., van den Hoff, M.J., Ouchi, N., Recchia, F.A., and Walsh, K. (2016). Follistatin-like 1 promotes cardiac fibroblast activation and protects the heart from rupture. *EMBO Mol. Med.* 8, 949–966.
9. Johnson, J., Mohsin, S., and Houser, S.R. (2021). Cardiomyocyte proliferation as a source of new myocyte development in the adult heart. *Int. J. Mol. Sci.* 22, 1–16. 7764.
10. Ouchi, N., Asami, Y., Ohashi, K., Higuchi, A., Sono-Romanelli, S., Oshima, Y., and Walsh, K. (2010). DIP2A functions as a FSTL1 receptor. *J. Biol. Chem.* 285, 7127–7134.
11. Shimano, M., Ouchi, N., Nakamura, K., van Wijk, B., Ohashi, K., Asami, Y., Higuchi, A., Pimentel, D.R., Sam, F., Murohara, T., et al. (2011). Cardiac myocyte follistatin-like 1 functions to attenuate hypertrophy following pressure overload. *Proc. Natl. Acad. Sci. U S A* 108, E899–E906.
12. Ghim, M., Pang, K.T., Burnap, S.A., Baig, F., Yin, X., Arshad, M., Mayr, M., and Weinberg, P.D. (2021). Endothelial cells exposed to atheroprotective flow secrete follistatin-like 1 protein which reduces transcytosis and inflammation. *Atherosclerosis* 333, 56–66.