Tenascin-C deficiency improves cardiac and vascular function in diabetic cardiomyopathy in mice

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Rationale: Diabetic cardiomyopathy is known for cardiac and vascular dysfunction in lack of structural heart disease accompanied by myocardial remodeling with cardiomyocyte apoptosis, fibrosis and as endothelial dysfuntion. More recently, Tenascin-C (TN-C) upregulation in the myocardium and serum has been linked to worse outcome in diabetic and heart failure patients. However, the causative role of TN-C in the development of diabetic cardiomyopathy has not been known. **Objective:** To study the source and function of TN-C in the progression of cardiac and vascular dysfuntion in diabetes. **Methods:** AJ and TNC-KO adult male mice were repeatedly injected with streptozotocin (50mg/kg) to induce diabetes. Cardiac function was measured by echocardiography at baseline and at 18-20 weeks follow-up. Vascular endothelial function was assessed by using wire myography in isolated abdominal aorta segments. Cardiac fibrosis and coronary network geometry were assessed. In addition, the hemodynamic effects of purified human TN-C (phTN-C) on isolated working rat hearts were evaluated. At the end of the experiment LV myocardial biopsy was taken in order to measure high energy phyophates by HPLC. To clarify the potential source of TNC, a cellular model of diabetic cardiomyopathy using human induced pluripotent stem cell-derived cardiomyocytes (hiPSC-CMs) was established. Moreover, human ventricular cardiac fibroblasts (HCF) were cultured, then starvated and treated with 1) TGF-β; 2) phTN-C (10μg/ml) and TLR4 inhibitor in combination with TN-C and subsequently mRNA expression of α-SMA, TN-C, Col-1, Col-3 and ACE1 were assessed by RT-qPCR. Finally, human umbilical vein endothelial cells (HUVEC) were treated either with phTN-C (10μg/ml) or combination with TLR-4 inhibitor (TAK-242, 50nM) and analysed the expression of NADPH oxidase 1 and 4 (NOX1, NOX4), and interleukin-6 (IL-6). **Results:** Blood glucose levels of diabetic AJ and TNC-KO animals did not show difference. TN-C deficiency was accompanied by preserved ejection fraction ($p<0.05$) and preserved endothelium-dependent relaxation (at 18 weeks, $p<0.05$ and $p< 0.001$, respectively). Histology revealed less cardiac and perivascular fibrosis in TNC-KO diabetic animals than in the AJ diabetic group ($p<0.01$). Notably, larger coronary arteries showed multiple branching distally and thicker arterial walls in diabetic animals, while TNC-KO diabetic mice had richer branching systems, suggesting better left ventricular perfusion. In addition, cumulative dosage of rhTN-C (80 ng/ml) resulted in a significant reduction in cardiac output ($p<0.01$) and LV systolic pressure (($p<0.05$) in isolated rat hearts. These hemodynamic changes were accompanied by the reduction in ATP and Pcr levels in comparison with saline treatment, respectively ($p<0.01$). Mechanistically, hiPSC-CMs under diabetic conditions did not upregulate TN-C. In contrast, TGF-β treatment markedly upregulated TN-C expression in HCF ($p<0.01$). Notably, HCF exposed to rhTN-C promoted both α-SMA and ACE1 mRNA

expression, respectively ($p<0.05$). In addition, HUVEC incubated with rhTN-C showed increased expression of IL-6 and oxidative stress-related markers (NOX4) and TLR-4 inhibitor pre-treatment markedly reversed these changes. **Conclusions:** These findings highlight the underlying mechanisms of the role of TN-C in cardiovascular dysfunction in diabetes. TN-C creates an intracellular environment that facilitates fibrosis and oxidative stress, which, leads to cardiomyocyte and endothelial cell dysfunton. Thus, TN-C may be a critical mediator of the progression of cardiovascular dysfunction in diabetes as well as a potential target for therapy.