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LOSS OF POU4F2/BRN3B IN THE STRESSED HEART COMPROMISES THE ADAPTIVE HYPERTROPHIC RESPONSE IN MALE BRN3B KO MICE, BUT NOT BRN3B KO FEMALE MICE

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Rationale A key feature of cardiovascular disease is cardiac hypertrophy, whereby terminally differentiated cardiomyocytes enlarge in response to stress. Hypertrophy can be beneficial to cardiac function (e.g., exercise or pregnancy); however pathological hypertrophy (e.g. chronic hypertension) is detrimental and leads to heart failure. The fate of cardiomyocytes during hypertrophy are dependent gene expression changes. Hence, cardiac transcription factors that control such changes by regulating target genes will be important for facilitating hypertrophy. The transcription factor POU4F2/Brn3b is highly expressed in the foetal heart and reduced in adult cardiomyocytes but is re-expressed in response to injury. Its interaction with other proteins such as the oestrogen receptor differentially regulate transcriptional activity; suggesting a difference in adaptation to physiological and pathological stress in male and female mice.

Methods and results Male and female Brn3b-KO mutant mice and wild type controls were used to assess the role of this TF in physiological (exercise on running wheels) and pathological stress (Angiotensin II infusion). Changes in cardiac function (cardiac output, etc.) were analysed using echocardiography/ultrasound, histological changes were assessed (e.g. Masson's Trichrome) and genetic changes (e.g. qPCR, Western blot) were measured at baseline and 4 weeks post treatment. Following pathological and physiological stress, cardiac function and contractility were reduced in male Brn3b-KO mice compared to baseline measurements. Exercise performance in Brn3b KO male mice was also reduced compared to WT mice. In contrast, cardiac function and contractility were not compromised in Brn3b KO female mice following physiological and pathological stress compared to baseline measurements. In addition, exercise performance in Brn3b KO female mice was better when compared to WT mice.

Conclusion Brn3b may be important for the adaptive hypertrophic response in male mice, but not necessary for female mice, suggesting complex roles for Brn3b in the stressed heart.

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THE ASSESSMENT OF THE CARDIOPROTECTIVE PROPERTIES OF METFORMIN DURING SUNITINIB-INDUCED CYTOTOXICITY

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Multi-tyrosine kinase inhibitor Sunitinib is associated with cardiotoxicity, in contrast the anti-type-2 diabetic Metformin is associated with cardioprotective properties via adenosine monophosphate-activated protein kinase (AMPK) activation.

We investigated whether treatment of rat Langendorff hearts with Metformin attenuated Sunitinib-induced cytotoxicity via

AMPK. We further investigated the cotreatment of Metformin with Sunitinib using HepG2 and HL60 cancer cell lines to reveal if potential adjunctive therapy drugs interfere with and prevent the anti-cancer activity of chemotherapy agents.

Male Sprague-Dawley rat hearts were Langendorff perfused with vehicle or, Sunitinib (1 μ M) in the absence or presence $\pm$ Metformin (50 μ M) $\pm$ S-4-Nitro-benzyl-theonine (AMPK inhibitor, NBTI) (1 μ M) for 155 min. Functional parameters were measured throughout the experiment (n=6), heart tissue was also assessed for necrosis or relevant protein phosphorylation (n=6).

HepG2 and HL60 cells treated in the presence of Sunitinib (0.1–100 μ M) $\pm$ Metformin (50/1000 μ M) $\pm$ NBTI (1 μ M) (n=6) were assessed for cell viability.

Sunitinib caused a significant increase in myocardial infarcted tissue (Vehicle=11% $\pm$ 1% vs Sunitinib=31 $\pm$ 2%, $p<0.05$). Metformin co-administration restored viable myocardial tissue (Sunitinib+Metformin=20% $\pm$ 2%, $p<0.05$), NBTI attenuated Metformin's cardioprotection (Sunitinib+Metformin+NBTI=30% $\pm$ 2%, $p<0.05$).

Sunitinib decreased cell viability in HepG2 and HL60 cells at doses 5–100 μ M (HepG2 IC₅₀=21 μ M, HL60 IC₅₀=10 μ M). Cytotoxicity of Sunitinib was maintained in presence of 0.05 mM Metformin (HepG2 IC₅₀=76 μ M, HL60 IC₅₀=22 μ M) and 1 mM Metformin (HepG2 IC₅₀=44 μ M, HL60 IC₅₀=24 μ M).

Metformin (30–1000 μ M) did not reduce HepG2 and HL60 cell viability.

This study shows for the first time that Metformin protects the hearts against Sunitinib-induced cardiotoxicity via AMPK activation which was attenuated with NBTI.

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NLRP3 INFLAMMASOME PATHWAYS IN HUMAN ENDOTHELIAL CELLS

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Endothelial cells play roles in regulating blood flow and in control of inflammation, both of which play important roles in atherogenesis. It is known that the NLRP3 inflammasome is involved in innate defence mechanisms in monocytes and macrophages and requires two steps for activation, signal 1 and signal 2, which result in the production of active IL-1 β and IL-18. The aim of this study was to investigate NLRP3 inflammasome activation in human endothelial cells. Phorbol 12-myristate 13-acetate (PMA; 5 ng/ml) differentiated THP-1 cells (positive control), human umbilical vein endothelial cells (HUVEC) and EA.hy926 cells were 'primed' for signal 1 with lipopolysaccharide (LPS) (0.1–5 μ g/ml) for 24 hours and then activated (signal 2) by exposure to ATP (300 μ M) for 1 hour. Using Western blotting, NLRP3 protein, pro-IL-1 β , pro-IL-18, active IL-1 β and active IL-18 were observed in THP-1 cells demonstrating priming and activation of the inflammasome as expected. EA.hy926 cells but not HUVEC expressed pro-IL-1 β . Inflammasome activation was confirmed by ELISA which detected any active end products in the cell culture supernatant. THP-1 cells secreted IL-1 β and IL-18 as expected. However, for endothelial cells, only EA.hy926 cells showed some low level expression of active IL-1 β . In conclusion, although