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Title: The role of Takotsubo Syndrome associated miR-16 and miR-26a on cardiomyocyte contractility

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Introduction:
Takotsubo Syndrome (TTS) is an acute, but often reversible, type of severe heart failure, caused by emotional or physical stress. Initially, the symptoms resemble an acute myocardial infarction (MI). The distinction is only elucidated upon echocardiography or ventriculography, to reveal the typical pattern of TTS: apical akinesia and basal hypercontractility of the ventricle. Specific blood microRNA levels (elevated miR-16 and miR-26a) have been discovered as markers distinguishing TTS from both, MI and healthy controls. However, the aetiology of this disease is still under investigation. The effects of miR-16 and miR-26a on apical and basal cardiomyocytes (CMs) as well as on human induced pluripotent stem cell (iPSCs) CMs have never been investigated.

Methods:
Adult, male Sprague-Dawley rat CMs, separately isolated from the left ventricular apex and base were transfected with pre-miR-16, pre-miR-26a or pre-miR negative control using Lipofectamine 3000. After 48 hours, fractional shortening was measured under field stimulation (0.5 Hz, 0.5ms, 50V), using an IonOptix system. Cells were treated 1h prior to recording with 1mmol methyl-β-cyclodextrin or PBS as control. Experiments were performed and analysed blinded to all conditions.
IMR-90 iPSCs were differentiated into iPSC-CMs and enriched by metabolic selection. At day 31 following differentiation, iPSC-CMs were transfected and 48 hours afterwards, optical mapping was performed. Fluo-4 calcium transients and Fluo-Volt action potentials were recorded under perfusion and field stimulation (1&2 Hz, 5ms, 20V).

Results & Conclusions:
We observed that miR-16, previously found to be elevated in TTS patients, decreased the fractional shortening of apical rat CMs significantly. Basal CMs from the same hearts had unchanged contractility after transfection with the pre-miRs.
In iPSC-CMs none of the miRs showed any effect on calcium handling or action potentials. This could be due to the immature state of iPSC-CMs and the different spatial arrangement of signalling components.
A key difference between apical and basal cardiomyocytes seems to be the compartmentation of βARs through caveolae. Cyclodextrin is known to remove and internalize cholesterol rich parts, such as caveolae, from the cell membrane. Indeed, treatment with cyclodextrin restored contractility and abolished the effect of miR-16 in apical CMs (miR-16+PBS vs. miR-16+cyclodextrin, 2.76±0.28 % vs. 4.12±0.47 %; n= 35/7; p=0.02). Cyclodextrin had no significant effect on contractility of the apical pre-miR control and miR-26a cells, neither on basal CMs.
The observed apex-base difference in adult cells strengthens the hypothesis about the apical-specific coupling underlying TTS aetiology. Furthermore, we can demonstrate that this effect seems to be dependent on caveolae.