Transcriptional regulation of Hexokinase-2 by BRD4 drives perivascular adipose tissue meta-inflammation in cardiometabolic disease

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Aim: To investigate BRD4-related transcriptional programmes in mouse and human models of cardiometabolic disease.
Methods: Small arteries (0.1-0.3 mm) dissected from visceral fat biopsies from healthy subjects (n=16) and patients with obesity and hypertension (n=16) were mounted on a pressurized myograph to assess the acute ex-vivo effects of BRD4 inhibition on vascular function. Vasorelaxation to acetylcholine and acetylcholine+L-NAME was evaluated, in the presence or in the absence of perivascular adipose tissue (PVAT), at baseline and after incubation with the BRD4 inhibitor RVX-208 and with selective anti-inflammatory and anti-metabolic drugs. A cardiometabolic mouse (high-fat diet+L-NAME supplementation) was orally administered RVX-208 (150 mg/kg) to test in vivo effect of chronic BRD4 inhibition. ROS and nitric oxide were assessed by confocal microscopy; protein and gene expression by Western blot and qPCR. Transcriptional changes upon BRD4 inhibition were investigated by a custom PCR array, confirmed by ChIP, and characterised by metabolomics, lipidomics and mitochondrial swelling.
Results: Endothelial-dependent vasorelaxation and vascular and perivascular TNF-alpha, IL-1beta, IL-6 were altered in cardiometabolic patients and mice. RVX-208 substantially attenuated ex-vivo vascular dysfunction, with an impact greater than anti-IL-1beta, anti-IL-6 receptor and anti-TNF-alpha. The effect was more pronounced in vessels with intact PVAT, suggesting a restoration of the PVAT anti-contractile phenotype. Gene expression profiling in PVAT unveiled hexokinase-2 (HK2) - a glycolytic enzyme implicated in mitochondrial dysfunction and inflammation - as the top downregulated gene by RVX-208 treatment. Increased binding of BRD4 to HK2 promoter in PVAT samples from cardiometabolic mice was confirmed by ChIP assays. Metabolomics assays further validated the findings by demonstrating a glycolytic shift in PVAT under disease conditions. Finally, ex vivo selective inhibition of HK2 rescued vascular dysfunction.
Conclusion: Targeting the deleterious BRD4-HK2 interplay restores cardiometabolic vascular dysfunction via reversal of the PVAT meta-inflammatory shift, highlighting a novel potential target to fight cardiometabolic pandemics.

Extracellular vesicles characterization in patients with hypertrophic cardiomyopathy

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Background: Hypertrophic cardiomyopathy (HCM) is diagnosed according to the presence of morphological and functional traits of the heart, often in the presence of genetic mutations. A specific biomarker assessing the aetiopathology of this condition is lacking. Extracellular vesicles (EVs), small particles released by all cells into biological fluids, hold promise as diagnostic and prognostic tools for cardiac diseases. We aim at characterising plasma-derived EVs isolated from 18 consecutive HCM patients and 13 healthy volunteers (CTR).
Methods: HCM underwent echocardiographic assessment and genetic testing evaluating 200 genes (NGS). EVs were isolated via ultracentrifugation from platelet-free plasma. Quantitative and qualitative assessments of EVs were performed by nanoparticle tracking analysis and transmission electron microscopy. FACS analysis was used to characterize EV subpopulations. Data are expressed as median and interquartile ranges.
Results: Most patients were male (70.8% HCM and 54% CTR) with a median age of 61 (52.5-71) years (HCM) and 47 (44-52) (CTR). Missense mutations in the *MYH7* gene were the most found in HCM. The median maximum wall thickness in HCM was 16 mm (15-19) vs 8 mm (7-9) in CTR. No differences were found in EV concentrations between HCM and CTR, respectively, $3.6*10^9$ EV/ml/cell count ($2*10^9$-$5*10^9$) and $5*10^9$ EV/ml/cell count ($4*10^9$-$6*10^9$). However, EV concentration was positively associated with the sudden cardiac death risk score in HCM (r= 0.63). Among the EVs positive for CFSE (a specific dye for EVs), those released from platelets, progenitor endothelial cells and neutrophils were increased in HCM patients vs CTR, respectively, by 1.7-, 1.5- and 1.1-fold. A strong negative association (r= -0.74) was found between progenitor endothelial cell-derived EVs and the E/E' ratio of diastolic function, a strong predictor of first cardiac events.
Conclusions: HCM patients present a peculiar phenotypic pattern of EVs that associates which diastolic function and sudden cardiac death.