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Oxidized LDL/CD36/PPARg circuitry is a trigger of adipogenesis in arrhythmogenic cardiomyopathy.

Authors:
I Stadiotti¹, E Sommariva¹, M Casella², V Catto², A Dello Russo², L Arnaboldi³, G Milano¹, A Scopece¹, W Birchmeier⁴, E Koenig⁵, L Tumu⁶, A Corsini³, A Rossini⁵, C Tondo², G Pompilio¹, ¹Cardiology Center Monzino IRCCS, Vascular Biology and Regenerative Medicine Unit - Milan - Italy, ²Cardiology Center Monzino IRCCS, Cardiac Arrhythmia Research Center - Milan - Italy, ³University of Milan, DISFeB - Milan - Italy, ⁴Max Delbruck Center for Molecular Medicine - Berlin - Germany, ⁵Eurac Research - Bolzano - Italy, ⁶Cardiology Center Monzino IRCCS, Unit of Metabolomics and Cellular Biochemistry of Atherothrombosis - Milan - Italy,

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Background. Arrhythmogenic Cardiomyopathy (ACM) is a genetic condition hallmarked by ventricular fibro-fatty replacement and arrhythmias. Cardiac mesenchymal stromal cells (C-MSC) differentiate into adipocytes in ACM hearts, through the activation of PPAR?, caused by ACM mutations (e.g. PKP2). The clinical phenotype of ACM is variable for poorly understood reasons. The only recognized cofactor is physical exercise, which is known to increases oxidative stress. An accepted marker of exercise-induced oxidative stress is 13HODE, a component of oxLDL and direct activator of PPAR?. In macrophages, during foam cell formation, 13HODE creates a feed-forward loop increasing both PPAR? and the oxLDL receptor CD36, resulting in fat accumulation.

Purpose. To investigate oxLDL effects on ACM adipogenesis and to dissect the involved pathways.

Methods. We analyzed plasmas (n=42) and ventricular tissues (n=4) of ACM patients and matched healthy controls (HC). For in vitro experiments, ACM and HC C-MSC (n=10) have been used, while in vivo experiments have been conducted in heterozygous Pkp2 knock-out mice (Pkp2+/-; n=10).

Results. We observed higher plasma oxLDL in ACM patients compared to HC (ACM 246.70±55.89 vs HC 102.5±17.95ng/ml; p=0.019). oxLDL levels also discriminate between ACM patients with overt phenotype and their unaffected relatives carriers of the same causative mutations (p=0.03). We observed higher oxidative stress (MDA intensity 40.87±11.76 fold; p=0.015) and CD36 levels (14.72±2.10 fold; p=0.0007) in ACM ventricular tissue, compared to HC.

In basal conditions, ACM C-MSC showed greater oxidative stress (MDA intensity 8.83±2.78 fold p=0.017) and higher expression of PPAR? (1.47±0.14 fold; p=0.009) compared to HC C-MSC. The adipogenic stimulation led to a parallel increase of CD36 and lipid accumulation, mainly in ACM C-MSC (slopes statistically different p=0.016). OxLDL and 13HODE administration increased lipid accumulation in ACM C-MSC (ORO staining ACM vs ACM+oxLDL p=0.01; ACM vs ACM+13HODE p=0.014). On the contrary, the antioxidant N-Acetylcysteine (NAC) prevented lipid accumulation in ACM C-MSC (ORO staining ACM+13HODE vs ACM+13HODE+NAC p=0.0009). Through CD36 silencing of ACM C-MSC, we obtained a significantly lower lipid accumulation than non-silenced cells (ORO staining 0.35±0.10 fold; p=0.003).

Pkp2+/- mice do not spontaneously accumulate adipocytes in the heart, however Pkp2+/- C-MSC are more prone to lipid accumulation in vitro than WT cells (p=0.007). Accordingly, mice have low plasma oxLDL and cardiac oxidative stress. By increasing plasma cholesterol and oxidative stress through high fat diet, we observed fibro-fatty substitution in Pkp2+/-hearts (p=0.046).

Conclusions. These findings reveal a modulatory role of oxidized lipids in ACM adipogenesis at a cellular, tissue and clinical level, enlightening novel targets for pharmacological strategies to prevent adipogenic substitution and
consequent ACM clinical phenotypes.