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Contribution of oxidized low density lipoproteins to arrhythmogenic cardiomyopathy adipogenesis.

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Background. Arrhythmogenic Cardiomyopathy (ACM) is a genetic condition characterized by progressive fibro-fatty replacement of the ventricular myocardium and malignant arrhythmias. We recently showed that Cardiac Mesenchymal Stromal Cells (C-MSC) in ACM hearts differentiate to adipocytes, through the activation of PPAR?.

The variable penetrance and expressivity of ACM suggest the involvement of co-determinants. Physical exercise is the only accepted co-factor. Strong physical activity increases oxidative stress (OxS). 13HODE, a component of oxidized low density lipoproteins (oxLDL), is a marker of exercise-induced OxS, and has been shown in macrophages to produce fat accumulation by increasing the expression of both PPAR? and the oxLDL receptor CD36.

Purpose. To evaluate the effects of OxS and oxLDL on ACM adipogenesis and to dissect underpinning pathways.

Methods. We analyzed plasmas (n=34) and ventricular tissues (n=3) from ACM patients and matched healthy controls (HC). In vitro experiments have been carried out on ACM vs. HC human C-MSC (n=5), while in vivo experiments on the heterozygous PKP2 mouse model (PKP2+/--; n=5).

Results. Significantly higher plasmatic oxLDL were detected in ACM patients compared to HC (290.90±76.31 vs. 122.40±28.73 ng/ml; p=0.04). Moreover, oxLDL levels can discriminate between ACM patients with overt phenotype vs. their asymptomatic relatives carrying the same causative mutations (456.50±187.80 vs. 93.81±33.39 ng/ml; p=0.03). In human ventricular tissue, we observed higher OxS in ACM hearts vs. HC (malondialdehyde positivity 20.26±6.54 fold higher; p=0.004).

In basal conditions, ACM C-MSC also showed higher OxS (diclorofluorescein emission 5.64±0.80 vs. 3.60±0.36 a.u.; p=0.03) and 2.79±1.32 fold higher expression of PPAR? (p=0.04) compared to HC C-MSC. Administration of 13HODE increased lipid accumulation in ACM C-MSC (Oil Red O (ORO) staining 1.28±0.24 fold vs. untreated; p=0.02). On the contrary, treatment with the antioxidant N-Acetylcysteine (NAC) prevented lipid accumulation in ACM C-MSC (ORO staining 0.63±0.16 fold vs. untreated; p=0.03).

Lipid accumulation during adipogenic differentiation in ACM C-MSC paralleled with an increased surface expression of CD36 (R²= 0.93; p=0.03).

Despite PKP2+/- mice do not spontaneously accumulate adipocytes in the heart, C-MSC obtained from PKP2+/- mice hearts are more prone to lipid accumulation in vitro than WT cells (ORO staining 99.49±27.36 fold higher; p=0.007). The increase of plasma cholesterol and OxS by administering a high-fat diet, resulted in fibro-fatty substitution in the heart of PKP2+/- mice only (% ORO positive area 0.47±0.15% in PKP2+/- vs. 0.11±0.01% in WT; p=0.009).

Conclusions. Mutations in ACM genes are necessary but not sufficient for ACM complete penetrance. We showed that elevated OxS and oxLDL are important cofactors of adipogenesis. Further investigations could provide new approaches for pharmacological prevention of ACM adipogenic phenotype.
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