High FABP4, associated with low-voltage areas, increases the epicardial fibrosis and its glucose metabolism


1Health Research Institute of Santiago de Compostela, Santiago de Compostela, Spain
2University Hospital of Santiago de Compostela, Cardiovascular area, Santiago de Compostela, Spain
3University Hospital of Santiago de Compostela, Heart Surgery Department, Santiago de Compostela, Spain

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Background: High plasma levels of FABP4 were associated with epicardial fat volume on persistent AF patients. FABP4 can be released by differentiated adipocytes and macrophages, which can be involved in fibrosis.

Purpose: Our main objective was to study the association between FABP4 and epicardial fibrosis, which can explain low-voltage areas (LVA) and atrial fibrillation (AF) recurrence.

Material and methods: Peripheral levels of FABP4 and Galectin-3 were analysed on AF patients undergoing pulmonary vein isolation (PVI) (n=299) and signed informed consent. During the intervention, LVA was evaluated by invasive electroanatomic voltage mapping (EAM). Epicardial (EAT) (n=6) and subcutaneous adipose tissue (SAT) (n=5) were obtained from undergoing open heart surgery patients. Stromal cells were isolated by collagenase digestion, cultured and treated with and/or FABP4 100 ng/mL and/or galectin-3 30 ng/mL for 24 hours. The proliferation and migration rate were measured by wound healing assay; mitochondrial activity was studied by MTT assay; and glucose and lactate consumption were detected by colorimetric and fluorescence enzymatic assays, respectively. Gene expression levels were assessed by real-time PCR.

Results: Regression analysis determined that the main independent predictors of LVA were age, left atrial area and FABP4 levels. Preclinical “in vitro” studies have shown that FABP4 100 ng/mL induced fibrosis in epicardial stromal cells (p=0.0039) and subcutaneous stromal cells (p=0.0009), evidenced by wound healing assay and by the increment of associated fibroblast genes (COL1A2, VIM, TGF-β1). Moreover, the high FABP4 concentration modulates the protein levels and metabolic genes expression (CPT1, FASN, ATGL, related to transport, synthesis and degradation of fatty acids, respectively) on EAT stromal cells. Glucose metabolism without lactate production was also incremented after FABP4 treatment (p<0.0001). Even, on SAT cells, released lactate was reduced after FABP4 treatment (p=0.0218).

Conclusions: Plasma FABP4 was a predictor of LVA. Our results suggest that this association might be explained by the fibrotic and metabolic role of FABP4 in epicardial cells. Future studies will shed light on the benefits on atrial remodelling with FABP4 modulators.
Figure 1. Stromal cells from epicardial adipose tissue (EAT) (n=6) and subcutaneous adipose tissue (SAT) (n=5) treated with 100 ng/mL of FABP4, 30 ng/mL of galectin-3 and both proteins together (FABGAL) for 24 hours. FABP4 and galectin-3 seem to induce fibrosis (proliferation and migration) studied by A) wound healing assay. B) Graphics showing the percentage of cell healing on wound healing assay after treatment. Scale bars: 100μm. The data was expressed as mean ± SD. The normality test was checked using Shapiro-Wilk test. When data didn't fit normality test Wilcoxon was used. The Student's t-test was used to compare the difference between control and treatments. *p<0.05, **p<0.01, ***p<0.001 vs. control (without treatment).
Figure 2. Stromal cells from epicardial adipose tissue (EAT) \((n=6)\) and subcutaneous adipose tissue (SAT) \((n=5)\) treated with 100 ng/mL of FABP4, 30 ng/mL of galectin-3 and both proteins together (FABGAL) for 24 hours. Quantification of mRNA expression levels \((\text{a.u.})\) by qPCR. The data was expressed as mean ± SD. The normality test was checked using Shapiro-Wilk test. When data didn’t fit normality test Wilcoxon was used. The Student’s \(t\)-test was used to compare the difference between control and treatments. \(^*p<0.05\), \(^{**}p<0.01\), \(^{***}p<0.001\) vs. control (without treatment).