Transdifferentiation of human dermal fibroblasts to smooth muscle like cells:

a novel method to study the effect of MYH11 and ACTA2 variants in the aortic aneurysm wall

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Introduction

Research on the pathogenesis of aortic aneurysms has revealed mutations in genes encoding the smooth muscle cell (SMC) contractile proteins as key underlying causes. Mutations associated with familial aortic aneurysms have been found in MYH11 (myosin heavy chain 11), ACTA2 (smooth muscle actin alpha 2) and MYLK (myosin light chain kinase) genes, which encode integral proteins of the SMC contractile apparatus. Currently, SMC can only be obtained by an invasive aortic biopsy. Therefore, the aim of this study is to transdifferentiate skin fibroblasts into SMC-like cells to provide a less invasive diagnostic test to study SMC function and mutations.

Methods

Dermal fibroblasts from 7 healthy donors and 7 patients with MYH11 or ACTA2 variants were transdifferentiated into SMC-like cells within 2 weeks by using 5ng/mL TGFβ1 and a scaffold containing collagen and elastin (matriderm). As control, cells were cultured without TGFβ1. SMC-specific markers were analyzed via qPCR, western blot and immunofluorescence. To investigate and classify the pathogenicity of the variants, cDNA sequencing was performed. Transdifferentiated SMC were additionally compared to primary SMC.

Results

The induced SMC-like cells were comparable to primary human aortic SMC in the expression of SMC specific markers on mRNA and protein level: ACTA2 (αSMA), SMTN (smoothelin) and CNN1 (calponin). Importantly, in patients with MYH11 or ACTA2 variants the effect on splicing can be demonstrated on the mRNA level in the induced SMC, allowing prediction and classification into pathogenic or non-pathogenic variants. Moreover, the variants showed different contractile protein expression, if compared to transdifferentiated SMC from the healthy donors.

Conclusions

Direct conversion of human dermal fibroblasts into SMC-like cells is a highly efficient method to investigate the pathogenic effect of variants in genes encoding the proteins of the SMC contractile apparatus. Our findings suggest the possible role of these variants in disturbed SMC contractility and aortic aneurysm formation.