Karolinska Institutet

Department of Medicine, Solna

Bicuspid Aortic Valve-associated Aortopathy

Unraveling the Molecular Signature

AKADEMISK AVHANDLING
som för avläggande av medicine doktorsexamen vid Karolinska Institutet offentligen försvaras i CMM Lecture Hall L8:00, Karolinska Universitetssjukhuset, Solna

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ABSTRACT

Patients with bicuspid aortic valve (BAV) have an increased risk of developing ascending aortic aneurysm compared with individuals with a tricuspid aortic valve (TAV). A crucial factor involved in vascular remodeling during aneurysm development is transforming growth factor-β (TGF-β) and impaired signaling of this pathway can alter important extracellular matrix (ECM) protein such us fibronectin and collagen and thereby explaining the increased aneurysm susceptibility of BAV patients. The overall aim of this thesis was to investigate the BAV-associated aortopathy in relation to the TGF-β signaling pathway.

Alternatively spliced extra domain A (EDA) of fibronectin (FN) has an essential role in tissue repair. In paper I, the mRNA expression of FN splice forms was analyzed by Affymetrix Exon arrays in dilated and non-dilated ascending aorta of TAV (40 individuals) and BAV patients (69 individuals). EDA-FN was significantly increased only in TAV dilated aortas. Upon TGF-β treatment, vascular smooth muscle cells (vSMCs) isolated from TAV aortas were able to enhance the formation of EDA-FN whereas cells derived from BAV patients could not influence fibronectin splicing. Multivariate and univariate data analyses of mRNA expression suggested that differences in the TGF-β signaling pathway may explain the impaired EDA inclusion in BAV patients.

In paper II, multivariate techniques were applied to all exons (n = 614) of the TGF-β pathway in order to analyze alternative splicing in the TGF-β pathway. Alternative splicing mechanisms were found to be important in the development of aneurysm in both BAV and TAV patients. Furthermore, the pattern of alternative splicing is clearly different between TAV and BAV patients. Differential splicing was specific for BAV and TAV patients in 40 and 86 exons, respectively, and splicing of 61 exons were shared between the two phenotypes. This suggested that dilatation in TAV and BAV patients has different alternative splicing fingerprints in the TGF-β pathway.

In paper III, collagen homeostasis in non-dilated and dilated aorta of BAV patients was studied and compared to non-dilated and dilated aortas taken from tricuspid aortic valve patients as reference. Ascending aortas from 56 patients were used for biochemical and morphological analyses of collagen. Collagen turnover rates were similar in non-dilated and dilated aortas of BAV patients, showing that aneurysm formation in BAV is, in contrast to TAV, not associated with an increased collagen turnover. In addition, the ratio of hydroxylysyl pyridinoline (HP) to lysyl pyridinoline (LP), two distinct forms of collagen cross-linking, was lower in dilated aortas from patients with BAV, which hints at a defect in the posttranslational collagen modification associated with BAV.

In paper IV, the response to TGF-β was analyzed in primary aortic smooth muscle cells isolated from 7 BAV and 5 BAV patients and 217 genes were found differentially expressed following TGF-β1 treatment in BAV vSMCs, whereas no gene was significantly altered between treated/untreated vSMCs of TAV patients. Majority of genes were down-regulated and enriched in genes involved in angiogenesis and formation of focal adhesion. Importantly, principle component analysis based on the 217 genes demonstrated that there was a clear difference in expression of these genes in intima/media region of dilated ascending aorta of BAV and TAV patients.

In conclusion, the understanding of impairments in the TGF-β signaling pathway could be the key to unravel the molecular mechanisms underlying BAV-associated aortopathy.

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