

Toll-Like receptor 3 mediates radiation induced calcific aortic valve disease

Background:

Thoracic radiation for the treatment of thoracic malignancies is associated with the development of calcific aortic valve disease (CAVD) including all its comorbidities. The development of CAVD after thoracic irradiation is a significant burden for affected patients, as the only treatment option is surgical aortic valve replacement. CAVD is caused by an osteoblastic phenotype switch of valvular interstitial cells (VICs), the predominant cell type in heart valves. However, the trigger for the phenotype switch remains unknown. Toll-like receptor 3 (TLR3) is part of the innate immune system, which is involved in the recognition of nucleic acids. It has been shown recently that TLR3 activation by released RNA is responsible for radiation-induced gastrointestinal syndrome. We hypothesized that TLR3 activation after radiation mediates the onset of CAVD.

Methods:

Valvular interstitial cells (VICs) were isolated from aortic valves of healthy donors undergoing heart transplantation and treated with radiation therapy (10Gy). Expression levels of TLR3, inflammatory cytokines and osteoblastic markers were compared with cells treated either with TLR3 agonist poly (I:C) or a TLR3/dsRNA complex inhibitor. Osteoblastic activity was assessed via alkaline phosphatase assay and Alizarin Red staining. Cell cycle analysis via flow cytometry was performed after radiation. Aortic valve morphology and function of aged wild-type (WT) and TLR3^{-/-} mice were analyzed via transthoracic echocardiography, microCT and histological evaluation after radiation (15Gy).

Results:

Aortic valves and VICs showed abundant TLR3 expression. Radiation of VICs resulted in significantly increased gene expression of TLR3, TNF- α , IL-6, IFN- γ , IL-10, Runx2 and BMP2 and significantly enhanced osteoblastic activity of treated cells. TLR3 inhibition resulted in prevention of osteoblastic phenotype switch after radiation in VICs. Aortic valves of radiated mice showed increased expression of TLR3 and osteoblastic markers. However, there was no osteoblastic activity after radiation in TLR3^{-/-}. Wild-type mice showed clear signs of CAVD after radiation in transthoracic echocardiographies with increased leaflet thickness, decreased valve orifice area and impaired left-ventricular function. However, there were no signs of CAVD in TLR3^{-/-} mice.

Conclusion:

Radiation leads to activation of TLR3 with concomitant initiation of calcification. Inhibition of TLR3 prevents from calcific activity after radiation. TLR3^{-/-} mice show no signs of CAVD after radiation. We show major involvement of TLR3 in the pathogenesis of CAVD after radiation. TLR3 could therefore become an effective therapeutic target for the prevention of CAVD after radiation.