ABSTRACT

Background: Abdominal aortic aneurysm (AAA) is an age-related disease characterized by progressive degradation of elastic lamellae, defective collagen architecture and medial smooth muscle cell loss. We previously demonstrated that knocking out the serine protease granzyme B (GZMB) reduces incidence and severity of AAA in mice; however, while GZMB is known for its role in apoptosis, it also accumulates extracellularly during inflammation and can cleave extracellular matrix (ECM) components such as decorin and fibrillin-1. We hypothesized that GZMB contributes to AAA development through the degradation of vascular ECM and that the inhibition of extracellular GZMB would reduce the incidence and severity of AAA progression.

Methods: Human aneurysmal samples were obtained and apolipoprotein E(apoE)-knockout (KO), GZMB/apoE-double knockout (GDKO) and perforin/apoE-DKO (PDKO) mice were implanted with osmotic minipumps releasing angiotensin II for 28 days to induce AAA formation. Additional apoE-KO mice were injected with the GZMB inhibitor, serpin A3N (SA3N, 4-120 μg/kg) or anti-GZMB neutralizing antibody (1 mg/kg) prior to pump implantation. Tissues were assessed for aneurysm pathology, inflammation and ECM composition. Collagen content was analysed by second harmonic generation and transmission electron microscopy.

Results: Human aneurysmal tissues showed elevated levels of GZMB immunopositivity compared to controls. A significant reduction in AAA incidence and severity was observed in GDKO mice compared to apoE-KO, whereas perforin deficiency was not protective against AAA. A dose-dependent reduction in the frequency of aortic rupture was observed in mice that received serpin or anti-GZMB antibody treatment. Pre-incubation with serpin prevented decorin cleavage by GZMB *in vitro.* Reduced GZMB and a corresponding reduction in loss of adventitial decorin were observed in serpin and anti-GZMB-treated mice while collagen density was increased. Adventitial collagen from serpin-treated mice exhibited significantly higher fibre density and reduced fibril size irregularity.

Conclusions: GZMB promotes destruction of the elastic lamellae via degradation of fibrillin-1 and destabilization of elastic microfibrils while GZMB-mediated degradation of decorin contributes to loss of adventitial collagen organization and density. The extracellular inhibition of GZMB prevented decorin loss and enabled a beneficial remodelling of adventitial collagen in response to medial injury, leading to higher vessel tensile strength and increased resistance to aortic rupture.