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Effect of transglutaminase 2 (TG2) deficiency on atherosclerotic plaque stability in the apolipoprotein E deficient mouse

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ABSTRACT

Background: Transglutaminase 2 (TG2), a cross-linking enzyme that confers supra-molecular structures with extra rigidity and resistance against proteolytic degradation, is expressed in the shoulder regions of human atherosclerotic plaques. It has been proposed that TG2 prevents tearing and promotes plaque repair at these potential weak points, and also promotes ectopic calcification of arteries. TG2 is also expressed within plaques that develop within the brachiocephalic arteries of apolipoprotein E (apoE) deficient mice.
Objectives: To determine the role that TG2 plays in plaque development and calcification, mice were bred that were doubly deficient in apoE and TG2, and were maintained on a high-fat diet for 6 months.
Results: Lesion size and composition were not significantly altered in the apoE/TG2 double-knockout mice, with the exception of a 9.7% decrease in the proportion of the plaque occupied by lipid ($p = 0.032$). The frequency of buried fibrous caps within brachiocephalic plaques was significantly higher in male than in female mice, but TG2 deficiency had no effect on either gender. The extent of lesion calcification varied markedly between individual mice, but it was not decreased in the apoE/TG2 double-knockout mice.
Conclusion: These data indicate that, in the apoE knockout mouse model of atherosclerosis, TG2 does not influence plaque composition or calcification. The data further suggest that TG2 does not influence plaque stability or repair in these mice.

1. Introduction

Plaque rupture exposes the internal constituents of the atherosclerotic plaque to the blood-stream. This promotes platelet adhesion and blood coagulation, leading to thrombosis within the affected artery. In humans, approximately 90% of these events are repaired asymptomatically [1]. Repeated cycles of rupture and repair can occur, but a strong fibrous cap covering the lesion and decreased plaque lipid content are associated with increased plaque stability. Atherosclerosis is also associated with arterial calcification, the extent of which strongly predicts the incidence of cardiovascular events [2].

Tissue repair and stabilization involve the incorporation of structural proteins into the extracellular matrix and their subsequent remodelling by proteinases and cross-linking enzymes. Amongst the various cross-linking enzymes which protect proteins from proteolysis and mechanical disruption is transglutaminase 2 (TG2) (reviewed in [3,4]). Despite lacking a conventional signal sequence, TG2 is exported from cells in response to stress, enabling it to associate with and to cross-link extracellular matrix proteins [3,4]. TG2 on the surface of macrophages also facilitates apoptotic cell engulfment [5,6]. Intracellular TG2 activity is suppressed within healthy cells, but becomes activated following injury and cross-links cellular components to minimize pro-inflammatory leakage [4]. TG2 may therefore play multiple roles in the body's response to tissue damage, and it is induced in human atherosclerotic plaques where it may stabilize against rupture [7,8]. Recently it has been suggested that TG2 enables smooth muscle cells to transform into chondrocyte-like cells [9], thus promoting ectopic calcification of atherosclerotic arteries and exerting deleterious as well as beneficial effects on lesion development [10].

Apolipoprotein E (apoE) deficient mice, maintained on a high-fat diet, develop advanced and complex plaques in the proximal brachiocephalic artery [11–13], although such plaques are rare at other anatomical sites [14]. It has been proposed that these plaques

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undergo cycles of rupture and repair [14]. The use of mice doubly deficient for apoE and for individual proteinases has identified some that promote lesion development, while indicating others that may be protective [15,16]. We are using an analogous approach to define the roles of the transglutaminases, examining, in the first instance, TG2. TG2 is a widely expressed and abundant protein, and unlike most other abundant transglutaminases, which have very restricted tissue distributions and functions, TG2 has been implicated in arterial repair. Mice with a targeted disruption of the TG2 gene develop normally for the first year of life [17,18] but subsequently develop mild abnormalities including glucose intolerance [19]. In the present study, apoE/TG2 double-knockout mice were bred and were compared (at less than 12 months of age), with matched apoE single knockout controls to determine the role of TG2 in plaque rupture and repair and in arterial calcification.

2. Materials and methods

2.1. Animals

The maintenance of the animals, and the procedures used in these studies, were performed in accordance with the guidelines and regulations of the University of Bristol and the United Kingdom Home Office. ApoE [20] and TG2 [17] single knockout mice, both on a mixed C57BL/6, 129 strain background, were crossed and the doubly heterozygous F1 mice were interbred. Tail-tip DNA from the F2 mice and subsequent litters was genotyped by PCR. In the F2 generation, only 1 double homozygote was obtained among 98 mice ($p < 0.02$ for 1 or 0). Relative frequencies of the other genotypes were as expected. Intercrossing of apoE$^{-/-}$TG2$^{+/-}$ F2 mice yielded only 10 double homozygotes among 98 mice ($p < 0.01$ for 10 or fewer). However, the resulting double homozygotes bred successfully, generating sufficient apoE$^{-/-}$TG2$^{-/-}$ mice for study. ApoE single knockout littermates of the double-knockout mice were used to generate apoE$^{-/-}$TG2$^{+/+}$ controls.

2.2. Experimental design

Fuller experimental details are included in the online supplement. Briefly, starting at 6–8 weeks of age, 51 apoE single knockout and 66 apoE/TG2 double-knockout mice were maintained for 6 months on a high-fat rodent diet. Paraffin sections of mouse brachiocephalic arteries were used to measure the parameters listed in Table 1 [13], for immunohistochemistry and to detect the presence of $Ca_3(PO_4)_2$ deposits. Aortic sinuses from 24 randomly selected mice (six males and six females per genotype) were also processed to assess atherosclerosis. In all cases, plaque morphometry was analysed in a blinded fashion.

2.3. Immunohistochemical analysis

Sections (3 μm) of human carotid artery atheroma or mouse artery were incubated with rabbit anti-guinea pig TG2 anti-serum, or with a mouse monoclonal antibody to α-smooth muscle actin. Bound antibodies were complexed with peroxidase-linked secondary antibodies and detected with diaminobenzidine. Sections were counterstained with haematoxylin.

2.4. Determination of calcium deposits

De-waxed sections were stained with 2% Alizarin Red S in water pH 6.8, washed extensively and then counterstained with 0.005% Fast Green.

Table 1
Proximal brachiocephalic artery morphometry.

Gender	Genotype	Vessel area ($\times 10^3$ μm^2)	Media area ($\times 10^3$ μm^2)	Plaque area ($\times 10^3$ μm^2)	Lumen area ($\times 10^3$ μm^2)	Cap thickness (μm)	Plaque lipid (%)	Buried fibrous caps
Male ($n = 20$)	ApoE$^{-/-}$TG2$^{+/+}$	331.7 ± 20.3	83.5 ± 4.7	156.9 ± 19.0	141.4 ± 12.4	2.80 ± 0.26[a]	35.7 ± 1.7	2.35 ± 0.33
Female ($n = 31$)	ApoE$^{-/-}$TG2$^{+/+}$	317.8 ± 15.9	77.3 ± 4.4	165.1 ± 15.2	122.9 ± 7.5	3.62 ± 0.29	39.8 ± 1.7	1.74 ± 0.23
Both ($n = 51$)	ApoE$^{-/-}$TG2$^{+/+}$	323.3 ± 12.4	79.7 ± 3.3	161.9 ± 11.8	130.2 ± 6.7	3.30 ± 0.21	38.1 ± 1.3[b]	1.98 ± 0.20
Male ($n = 36$)	ApoE$^{-/-}$TG2$^{-/-}$	335.7 ± 8.8	82.5 ± 3.1	154.9 ± 7.6	143.7 ± 5.2	4.12 ± 0.45[a]	34.3 ± 1.5	2.14 ± 0.22
Female ($n = 30$)	ApoE$^{-/-}$TG2$^{-/-}$	298.9 ± 12.6	82.0 ± 3.7	140.8 ± 11.6	122.3 ± 7.7	3.90 ± 0.33	34.3 ± 1.7	1.51 ± 0.25
Both ($n = 66$)	ApoE$^{-/-}$TG2$^{-/-}$	318.7 ± 7.8	81.9 ± 2.4	148.9 ± 6.7	132.7 ± 4.6	4.02 ± 0.29	34.4 ± 1.1[b]	1.88 ± 0.17

Groups with the same superscript letter are significantly different from each other ($p < 0.05$).