Role of Endoglin in Maintaining Cardiac Function

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Introduction:

The focus of this project was endoglin, a co-receptor protein for the TGF-β signalling pathway. Endoglin is expressed on endothelial cells where it regulates the formation of new blood vessels from pre-existing ones, a process termed angiogenesis. It has previously been found that mice with no endoglin die early in embryogenesis from angiogenesis defects. Also, mutations in endoglin in humans are associated with the vascular disease Hereditary Haemorrhagic telangiectasia, a genetic disorder that leads to abnormal blood vessel formation in the skin, mucous membranes and major organs. It has been proposed that defects in vasomotor responses contribute to the vascular defects that occur when endoglin levels are reduced.

Aims:

•To determine the role of endoglin in cardiovascular disease by depleting endoglin in endothelial cells in adult mice.

•To assess vasomotor responses in these endoglin depleted mice using myography, and to assess cardiac function by Magnetic Resonance Imaging (MRI).

Methods:

•**Mouse Models:** 10 week old transgenic mouse line $Eng^{fl/fl}$; *Cdh5*(PAC)-Cre^{ERT2} were treated with tamoxifen (i.p. injection of 2mg/day over 5 consecutive days) to generate mice with endothelial specific depletion of endoglin (Eng-iKO^{e}). Tamoxifen treated $Eng^{fl/fl}$ mice were used as controls. Unless otherwise stated, analysis was performed 5 weeks after tamoxifen treatment.

•**Myography:** Rings of aorta were isolated and collected from 6 control and 6 endoglin mutant mice and mounted on a wire myograph filled with Krebs Solution. Concentration response curves were constructed by the cumulative application of Acetylcholine (Ach) from 1nM to 10μM to vessels already pre-contracted with the vasoconstrictor phenylephrine (Phe). Also, the contractile response to a single dose of KCl (100 mmol/L) was assessed.

•**Immunostaining:** Vessels were frozen and cut into 7 μm section. Sections were then labelled with primary antibodies to detect Endoglin or Collagen IV. Secondary antibodies conjugated with Alexa594 or Alex488 were used to detect primary antibodies. Images were taken using a Zeiss epifluorescent microscope.

•**Data analysis:** Data are presented as mean ± SEM, and n represents the number of animals. Statistical analyses were performed using Prism Software by two-way analysis of variance for repeated measurements and Mann-Whitney tests. *P< 0.05* was considered to be statistically significant (*).

Results:

Myography:

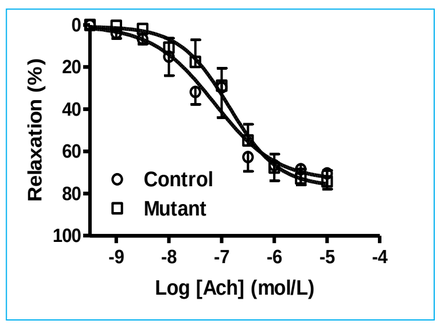

Figure 1 shows the relaxation curve in response to acetycholine. There is no significant difference between control and endoglin mutant aortas.

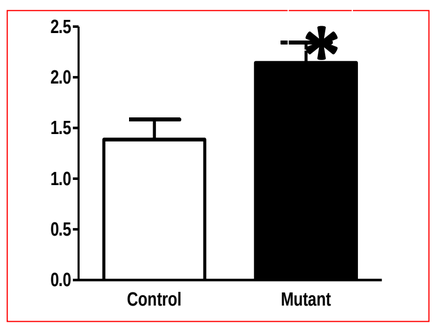

Figure 2 shows an increased contractile response to potassium chloride in the endoglin mutant aorta compared with control.

Immunostaining:

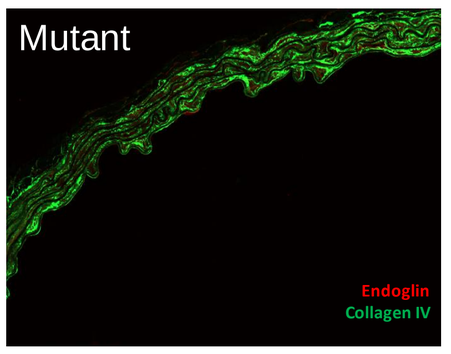

Figure 3 shows immunostaining of aortas from control. The images clearly show endoglin (red) is expressed in the endothelial cells that line the aorta in the control. In contrast note the lack of endoglin (red) in the mutant aorta. Collagen IV is a vascular basement membrane protein which appears to be present at similar levels in mutant and controls.

Heart Imaging:

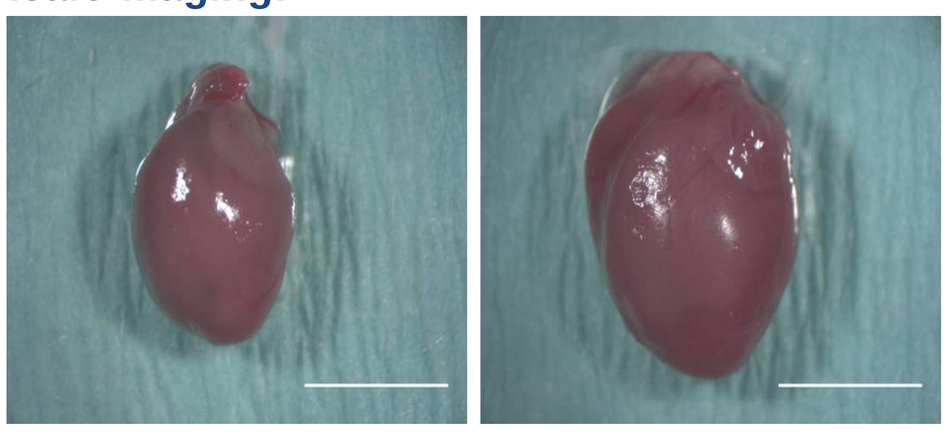

Figure 4 shows macroscopic images of hearts taken from control (left) and endoglin mutant (right) mice, 5 weeks following loss of endoglin in endothelial cells. The mutant mouse heart (right) is visibly larger than the age-matched control heart (left), indicating loss of endoglin does have an effect on cardiac function. (Image courtesy of Dr Ben Davison)

Magnetic Resonance Imaging:

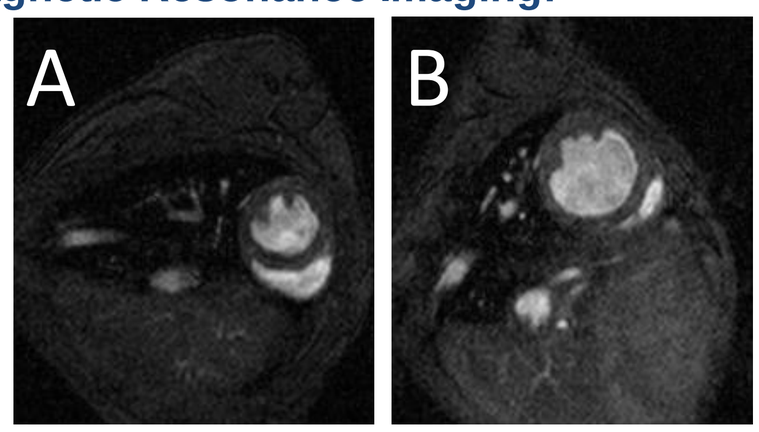

Figure 5 is a short axis view of cardiac MRI of Control (A) and endoglin mutant (B) mice demonstrating significant left ventricular remodelling in the endoglin mutant mice 6 months after initiating endoglin knockdown. Cardiac function was analysed using a horizontal bore 7 tesla Varian pre-clinical MRI. The mice were anaesthetised with isoflurane and positioned on a custom built sled which had integrated electrocardiographic, respiratory and temperature probes connected to MRI compatible monitoring equipment. In total 16 mice were analysed; recording heart dimensions during systole and diastole.

Magnetic Resonance Imaging:

Figures 6-9 indicate results obtained from cardiac MRI in both control mice (Blue Bars) and Endoglin knockout mice (Red Bars).The week 5 time point data is courtesy of Dr Ben Davison

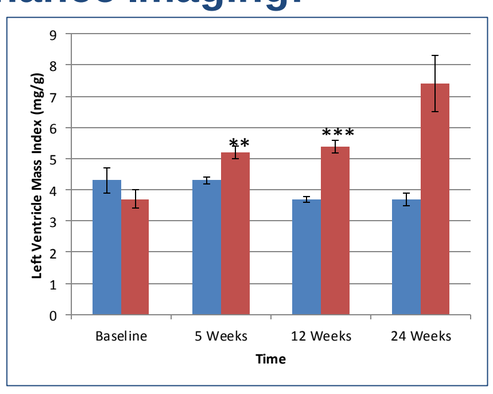

Figure 6 shows a significant increase in the left ventricle mass index in the Endoglin knockout mice compared to a slight decrease in that of the control mice.

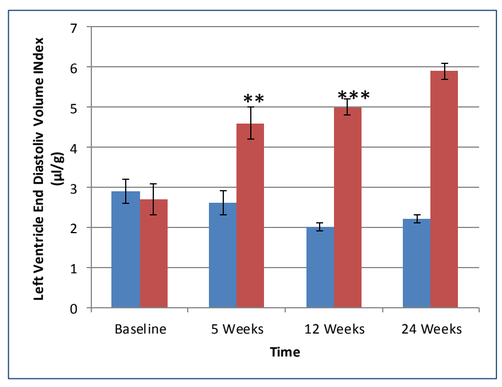

Figure 7 shows a large increase in the left ventricular end diastolic volume index in the Endoglin knockout mice compared to that of the control mice

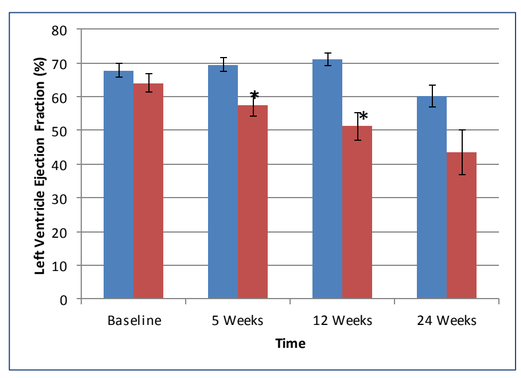

Figure 8 shows a decrease in the left ventricular ejection fraction of the Endoglin knockout mice compared with the control mice.

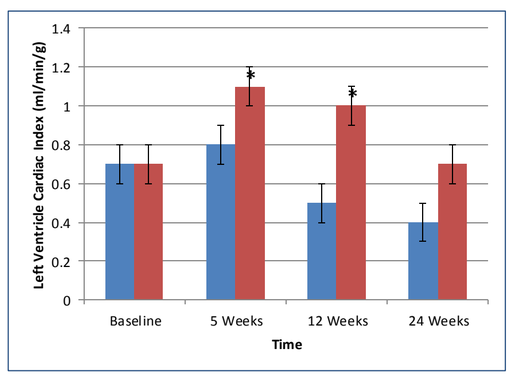

Figure 9 indicates left ventricular cardiac index, which decreased in the control mice, whereas it increased in the endoglin knockout mice at weeks 5 and 12 before falling at 24 weeks.

Conclusions:

- Depletion of endoglin in the aortic endothelium of the mutant mice was confirmed by immunohistochemistry. Aortas from the endoglin knockout mice show increased contractile responses in phenylephrine and potassium chloride compared to control aortas. This suggests endoglin is important in regulating the aortic contractile response.
- Endoglin mutant mice showed significant left ventricular remodelling. The cardiac MRI results show increased ventricle volumes, stroke volumes and cardiac index in the endoglin mutant mice. This suggests that endoglin is important for maintaining the structure and function of the heart.