A novel transgenic mouse model reveals an essential role for Bcar1/p130Cas in embryonic heart development and outflow tract septation

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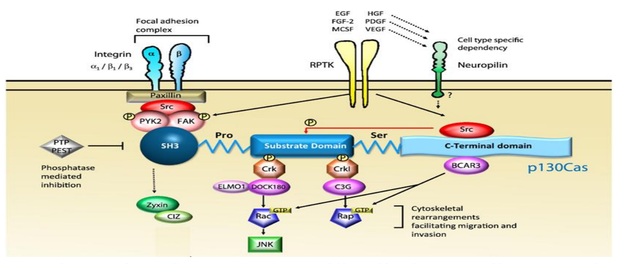
Introduction

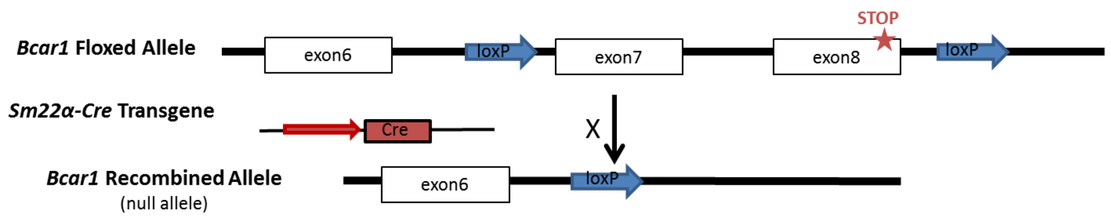
- The adapter protein p130Cas, encoded by the *Bcar1* gene, is a key regulator of cell movement, adhesion, and cell cycle control in diverse cell types [1,2].
- *Bcar1* constitutive knockout mice are embryonic lethal by embryonic days (E) 11.5-12.5, exhibiting marked systemic congestion, growth retardation and gross defects in the development of the heart, suggesting an important role for *Bcar1* in normal embryonic development [3].



» We aimed to investigate the role of *Bcar1* specifically in cardiovascular development and define the underlying cellular and molecular mechanisms disrupted following targeted *Bcar1* deletion.

Methods

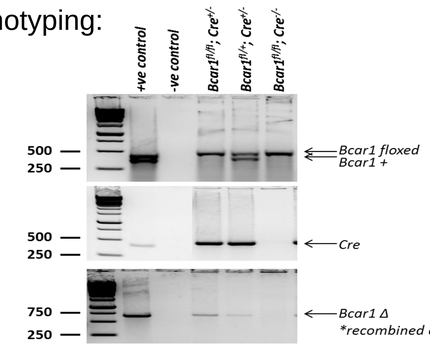
Generation of *Bcar1* conditional knockout mice



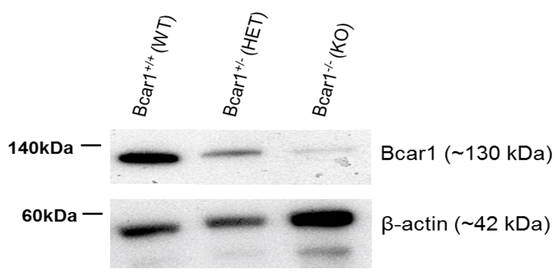
- SM22α is transiently expressed in the mouse heart during embryogenesis from ~E8.0 where it is mainly restricted to the presumptive right ventricle (bulbus cordis) and outflow tract[4].
- SM22α transcripts are first expressed in vascular smooth muscle cells at around E9.5 and continue to be expressed in all smooth muscle cells into adulthood, whereas SM22α transcripts are expressed transiently in the heart between E8.0 and E12.5[4].

Molecular Biology Techniques:

-Genotyping:



-Western Blotting:



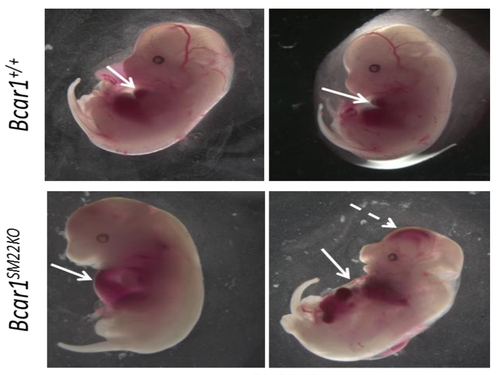
Results

➢ **Conditional knockout of *Bcar1* in SM22-expressing smooth muscle cells and heart progenitors (*Bcar1*^{SM22KO}) is embryonically lethal from E14.5.**

SM22-Cre$^{+/-}$; *Bcar1* $^{fl/+}$ X *Bcar1* $^{fl/+}$/*Bcar1* $^{fl/fl}$

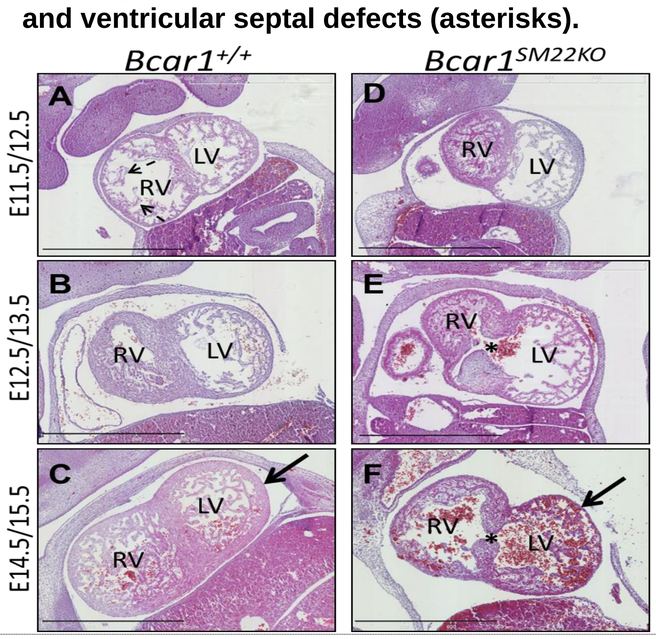
Bcar1 Genotype	Mendelian Ratio	Expected	Observed
Bcar1$^{+/+}$ (*Bcar1*$^{+/+}$, SM22-Cre$^{+/-}$; *Bcar1*$^{+/+}$, SM22-Cre$^{-/-}$; *Bcar1*$^{fl/fl}$, SM22-Cre$^{-/-}$; *Bcar1*$^{fl/+}$, SM22-Cre$^{-/-}$)	60%	73.125	85
Bcar1^{SM22HET} (*Bcar1*$^{fl/+}$, SM22-Cre$^{+/-}$)	25%	30.5	37
Bcar1^{SM22KO} (*Bcar1*$^{fl/fl}$, SM22-Cre$^{+/-}$)	15%	18.375	0
Total:	**100%**	**122**	**122**

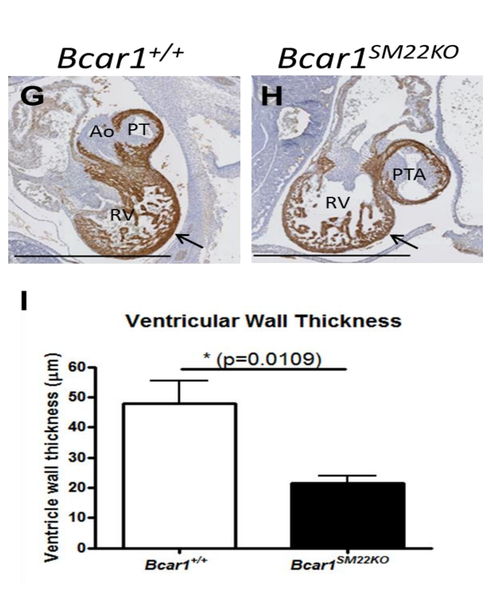
- Table of expected mendelian ratios and observed ratios, indicating embryonic lethality of *Bcar1*^{SM22KO} embryos.



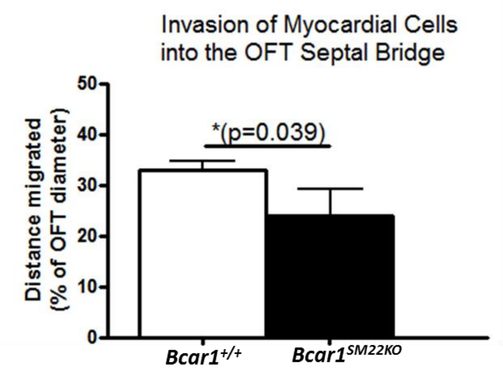
- At E14.5 *Bcar1*^{SM22KO} embryos exhibit signs of abnormal heart development (solid arrows) and haemorrhaging (dashed arrow).

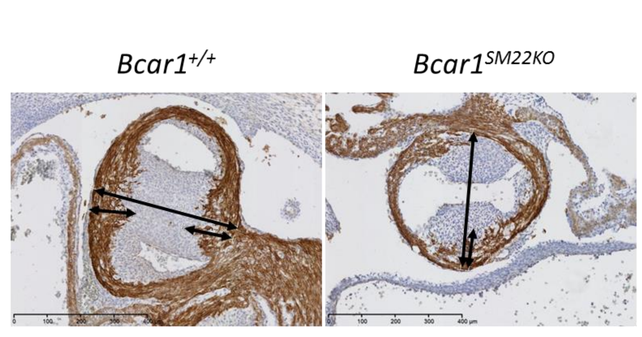
➢ **Bcar1^{SM22KO} embryos display defects in ventricular development, which include a thinning myocardial wall (solid arrows), reduced trabeculations (dashed arrows) and ventricular septal defects (asterisks).**



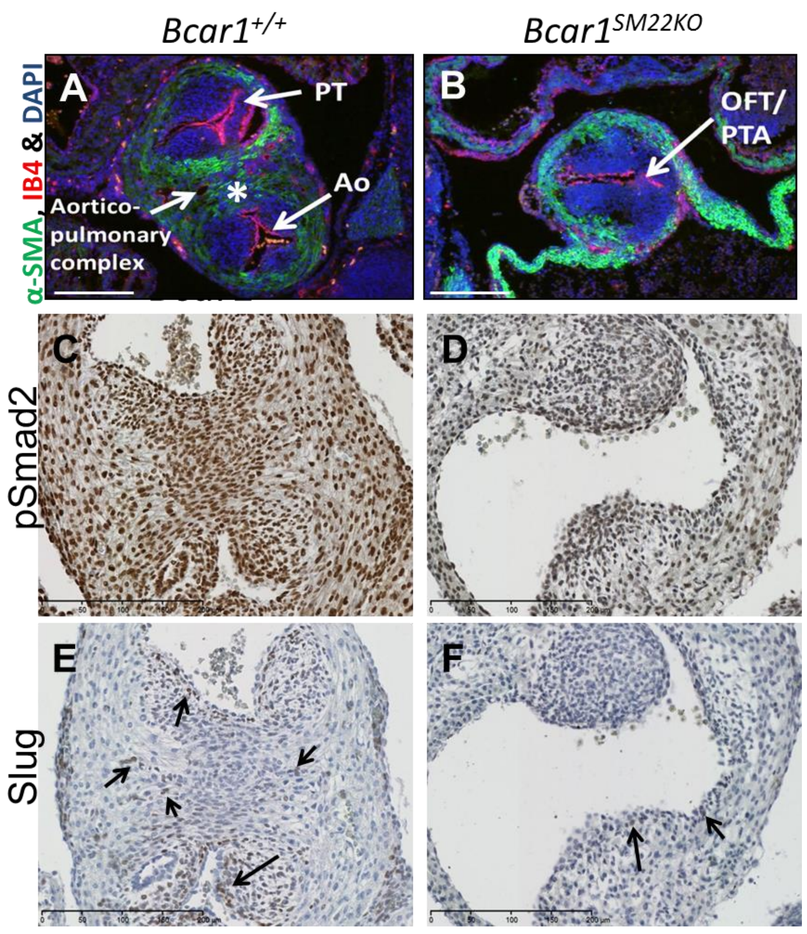


➢ **Bcar1^{SM22KO} embryos exhibit signs of defective myocardialisation of the outflow tract (OFT), as well as disrupted expression of proteins involved in epithelial-to-mesenchymal transformation (EMT).**





- At E14.5 a significant reduction in the invasion of MF20 positive myocardial cells into the developing outflow tract septum was observed in the *Bcar1*^{SM22KO} embryos compared to littermate controls.



- At E14.5 a muscularised septum expressing α-smooth muscle actin (α-SMA), which separates the aorta (Ao) and pulmonary trunk (PT), has formed in the controls (A). This is absent in the *Bcar1*^{SM22KO} embryos leading to persistent truncus arteriosus (PTA) (B).
- A clear reduction in pSmad2 expression in the outflow tract is observed in the *Bcar1*^{SM22KO} embryos (D) compared to littermate controls at E14.5 (C).
- Reduced expression of the transcription factor Slug is observed in the *Bcar1*^{SM22KO} embryos (F), indicated by the reduced number of Slug positive cells (arrows), compared to littermate controls at E14.5 (E).

Conclusions

• Conditional knockout of Bcar1 in early smooth muscle cell and cardiomyocyte progenitors, using the SM22-Cre line, (*Bcar1*^{SM22KO}) is lethal from embryonic day (E)14.5.

• *Bcar1*^{SM22KO} embryos exhibit defective myocardialisation and failure of outflow tract (OFT) septation, and disrupted expression of proteins involved in epithelial-to-mesenchymal transformation (EMT).

• Our work reveals a cell-specific requirement for Bcar1 in early myogenic lineages and cardiac progenitors and indicates an important role for Bcar1 in OFT myocardialisation and EMT.

References:
[1] Barrett A, et al. P130cas: A key signalling node in health and disease. Cellular signalling. 2013;25:766-777
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[3] Honda H, et al. Cardiovascular anomaly, impaired actin bundling and resistance to src-induced transformation in mice lacking p130cas. Nature genetics. 1998;19:361-365.
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Declaration of interest: None.