Thymosin β4 Is Not Required for Embryonic Viability or Vascular Development

Indroneal Banerjee, Thomas Moore-Morris, Sylvia M. Evans, Ju Chen

Rationale: Rosddeutsch et al describe a requirement for thymosin β4 (Tβ4) in vascular development. Impaired mural cell migration, differentiation, partial embryonic lethality, and hemorrhaging were observed after analysis of 2 lines of mice, one of which was germline null for Tβ4 and another in which Tβ4 was knocked down by endothelial-specific expression of Tβ4 short hairpin RNA. These data are in direct contrast to our published global and cardiac-specific Tβ4-knockout lines. Thus, the role of Tβ4 needs to be clarified to understand its importance in cardiovascular development.

Objective: To investigate and clarify the role of Tβ4 in vascular smooth muscle cell development and vessel stability.

Methods and Results: Examination of Tβ4 global knockouts did not demonstrate embryonic hemorrhaging, altered mural cell development, or lethality. Endothelial-specific knockouts also did not exhibit any embryonic lethality and were viable to adulthood.

Conclusions: Analysis of our Tβ4 global and cardiac- and endothelial-specific knockout models demonstrated that Tβ4 is dispensable for embryonic viability and vascular development. (Circ Res. 2013;112:e25-e28.)

Key Words: cardiac development ■ thymosin β4 ■ vascular biology ■ vascular smooth muscle

Understanding signaling factors that regulate the formation of the vasculature can lend significant insight into both development and disease. Thymosin β4 (Tβ4) is a 43-aa factor initially found to interact with G-actin and regulate F-actin formation.1 Recent studies have indicated that administration of Tβ4 to either postschemia reperfusion or myocardial infarction models can improve cardiovascular function and abrogate scar formation, primarily through de novo vasculature formation.2-4 Early studies using a short hairpin RNA (shRNA) knockdown approach suggested that Tβ4 acted as a key regulator of blood vessel formation (angiogenesis, vasculogenesis, and arteriogenesis).5 However, our studies using both global and cardiac-specific knockout approaches found Tβ4 to be dispensable for embryonic viability and vessel development.6

See Response, p e29

In a recent article, Rosddeutsch et al7 describe a requirement for Tβ4 in vascular development. Impaired mural cell migration and differentiation were observed after analysis of 2 lines of mice, one of which was germline null for Tβ4 and another in which Tβ4 was knocked down by endothelial-specific expression of Tβ4 shRNA. Rosddeutsch et al7 report that global knockout of Tβ4 resulted in partial lethality at the start of the study that was decreased in subsequent generations later during the study, an incompletely penetrant hemorrhagic phenotype, and decreased mural cell coverage of the aorta. Tβ4 knockdown in endothelium also resulted in partial lethality and decreased mural cell coverage.7 These data are in direct contrast to our published tβ4-knockout model.6 Thus, to clarify the role of Tβ4 in vascular formation, we further analyzed our global knockouts of Tβ4 and an endothelial-specific Tβ4 knockout not previously reported. Consistent with our previous results, we did not observe an evident phenotype, alteration in vascular development, or embryonic lethality. We conclude from these data that Tβ4 is not required for embryonic viability or vascular development.

Materials and Methods

Animal Care

All animal procedures were performed and approved by the University of California, San Diego Animal Care and Use Committee.

Generation of tβ4 Floxed, Knockout, and Endothelial-Specific Knockout Mice

tβ4-targeted mice were generated and used as previously described to a C57/B6J background.8 Endothelial-specific knockout mice were generated by crossing tβ4f/f female mice to Tie2-Cre male mice.

Immunofluorescence Analyses

Frozen sections from embryonic day (E) 14.5, E13.5, and E12.5 embryos were isolated and stained as previously described.6
Results

Mural Cell Coverage and Development Are Not Altered in Global Null Tβ4 Mutants

To clarify the role of Tβ4 in large vessel development, we examined our global Tβ4-knockout mice from E12.5 to E14.5 (Figures 1–2). Initial observation in these stages revealed no gross abnormalities or hemorrhage between wild-type and knockout samples. To examine large vessel defects, we first examined aortas in E14.5 embryos (Figure 1). At this stage, no differences were observed between examined wild-type and knockout embryos. To rule out developmental and mural cell recruitment defects, E12.5 and E13.5 aortas were stained with antibodies to α-smooth muscle actin and platelet-derived growth factor subunit-β, 2 markers of vascular smooth muscle cells (Figures 1 and 2). No differences were observed between control and Tβ4 null embryos.

Endothelial-Specific Deletion of Tβ4 Does Not Result in Embryonic Lethality

Rossdeutsch et al.7 observed 12% lethality in embryos with endothelial-specific shRNA-mediated knockdown of Tβ4. As stated in previous publications, shRNA knockdown can have off-target effects. To further investigate this possibility, we used the same Tie2-Cre used by Rossdeutsch et al.7,10 Our Tβ4f/f females were bred to Tie2-Cre male mice. Male Tie2-Cre mice were also used by Rossdeutsch et al in their knockdown studies. Of the 34 postnatal male mice, 18 were Tβ4f/y and 16 were Tβ4f/y;Tie2-Cre+, demonstrating lack of embryonic lethality in endothelial-specific knockouts of Tβ4. These data are similar to our global and cardiac-specific knockouts of Tβ4, where we did not observe any postnatal lethality or vascular phenotype.

Discussion

Rossdeutsch et al.7 report that global Tβ4-knockout mice present with hemorrhage and defective mural cell recruitment, resulting in partial lethality between E10.5 and E14.5. These data are in direct contrast to our published global Tβ4-knockout model. In the Discussion section of their article, Rossdeutsch et al refer to our article7 and state that, from our data, “Tβ4 knockout aortas had an apparent reduction in α-SMA+cell coverage within the vessel wall relative to wild-type controls at E14.5.”7 We believe this to be a misinterpretation of our data. In our publication6 we did not report, nor observe, differences in mural cell coverage in any of our samples at E14.5. Data showing α-smooth muscle actin staining of aortic vascular smooth muscle were meant to illustrate maintenance of vascular smooth muscle within the aorta and were not meant to be a quantitative assessment. However, given the observations by Rossdeutsch et al, we have specifically examined aortas in multiple sections from E12.5 to E14.5 Tβ4 null and control embryos and found no differences between control and Tβ4 null embryos (Figures 1 and 2). We also did not observe any hemorrhaging in any of the embryos in our studies. Thus, our data do not support an essential role for Tβ4 in vessel development.

Rossdeutsch et al.7 also suggest that differences in observed phenotypes between our Tβ4 null mutants and theirs can be attributed to either strain background–dependent effects or distinct gene targeting strategies. Initially, Rossdeutsch et al.7 observed 40% lethality of their global null Tβ4 mice in a mixed C57/B6J and 129Sv background, with reduction to 20% lethality when bred into a pure C57/B6J background.
However, our global null mice were bred into a C57/B6J background and yet did not display any phenotype. It is possible that differences in observed phenotypes between the 2 Tβ4 null mutant lines may result from variables, such as environment, diet, and infectious state, which may differ between the 2 mouse facilities.

An alternative explanation to strain- or facility-dependent phenotype is that the targeted embryonic stem cells used to establish the Rossdeutsch mutant mouse line contained Tβ4-independent lethal mutations that may have been bred out over successive generations. Furthermore, the possibility raised by Rossdeutsch et al2 that differences in targeting strategies, in particular the retention of the Neo cassette in the Rossdeutsch et al targeted allele, could also account for discrepant phenotypes is a possibility. However, if this is the case, it is unlikely to reflect differences in targeting Tβ4 function, as exon 2, encompassing 75% of the coding sequence, is deleted in both knockouts and similarly Tβ4 protein is absent, as demonstrated by protein analysis, in both knockouts. Therefore, if the different phenotypes are a result of retention of the Neo gene in the Rossdeutsch Tβ4 null allele, observed phenotypes may result from non-Tβ4-related functions.

Rossdeutsch et al2 argue that there are no off-target effects with their shRNA gene silencing approach and that “the differences between our global knockout and knockdown mice suggest that the shRNA knockdown approach used by Rossdeutsch et al may have off-target effects that result in observed phenotypes. The phenotype observed in their global Tβ4-knockout mice may result from perturbation of Tβ4-independent events. Together, these data do not support an essential role for Tβ4 in vessel development or embryonic viability.”

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Disclosures

None.

References


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