The Snail Family Gene Snai3 Is Not Essential for Embryogenesis in Mice

Cara K. Bradley¹, Christine R. Norton¹*, Ying Chen¹,², Xianghua Han¹, Carmen J. Booth³, Jeong Kyo Yoon¹, Luke T. Krebs¹, Thomas Gridley¹*

1 Center for Molecular Medicine, Maine Medical Center Research Institute, Scarborough, Maine, United States of America, 2 Graduate School of Biomedical Sciences, University of Maine, Orono, Maine, United States of America, 3 Section of Comparative Medicine, Yale University School of Medicine, New Haven, Connecticut, United States of America

Abstract

The Snail gene family encodes zinc finger-containing transcriptional repressor proteins. Three members of the Snail gene family have been described in mammals, encoded by the Snai1, Snai2, and Snai3 genes. The function of the Snai1 and Snai2 genes have been studied extensively during both vertebrate embryogenesis and tumor progression and metastasis, and play critically important roles during these processes. However, little is known about the function of the Snai3 gene and protein. We describe here generation and analysis of Snai3 conditional and null mutant mice. We also generated an EYFP-tagged Snai3 null allele that accurately reflects endogenous Snai3 gene expression, with the highest levels of expression detected in thymus and skeletal muscle. Snai3 null mutant homozgygous mice are viable and fertile, and exhibit no obvious phenotypic defects. These results demonstrate that Snai3 gene function is not essential for embryogenesis in mice.

Introduction

The Snail gene family encodes zinc finger proteins that function primarily as transcriptional repressors [1,2]. Three members of the Snail gene family have been described in mammals, encoded by the Snai1 (also called Snail), Snai2 (Slug), and Snai3 (Snun) genes. The SNAI1 and SNAI2 proteins are key regulators of the epithelial-mesenchymal transition, directly repressing transcription of genes encoding components of cell-cell adhesive complexes in epithelia. The SNAI1 and SNAI2 proteins also have demonstrated roles in other important developmental and cellular processes, such as the protection of cells from programmed cell death, the establishment of left-right asymmetry and the regulation of cell motility [3]. Snai2 gene expression is induced during muscle regeneration, and Snai2 null mice exhibit defective muscle regeneration [4]. A recent study utilized ChiP-Seq and gene expression analyses to demonstrate that a Snai1-HDAC1/2 repressive complex bound and excluded the myogenic transcription factor MyoD from its targets [5]. These authors further showed that a regulatory network involving myogenic regulatory factors, Snai1/2, and the microRNAs miR-30a and miR-206 acted as a molecular switch controlling entry into myogenic differentiation.

In contrast to the Snai1 and Snai2 genes, much less is known about the function of the Snai3 gene, which was cloned using a degenerate PCR-amplification protocol as a Snail family gene expressed in adult mouse skeletal muscle [6]. The human SNAI3 gene was subsequently identified by in silico analyses [7,8]. Originally, this gene was termed Sinuc (for Snail related gene from skeletal Muscle Cells), but it has since been renamed Snai3. The SNAI3 protein binds to the same E box sequences (CAGGTG and CACCTG) bound by the SNAI1 and SNAI2 proteins, and functions as a transcriptional repressor. Northern blot analysis revealed that the Snai3 gene was highly expressed in adult mouse skeletal muscle and thymus, was expressed at lower levels in adult heart, lung and spleen, and was also expressed during embryogenesis [6]. Analysis by in situ hybridization during mouse embryogenesis revealed that Snai3 transcripts were first observed at embryonic day (E)13.5 in skeletal muscle and diaphragm [9]. At E15.5, in addition to skeletal muscle and diaphragm expression, Snai3 transcripts also were expressed in the thymus. Skeletal muscle and thymus remained the dominant sites of Snai3 expression through the early postnatal period. We describe here generation and analysis of Snai3 null mutant mice, utilizing two different null alleles. These mice are viable and fertile, and exhibit no obvious phenotypic defects. These results demonstrate that Snai3 gene function is not essential for embryogenesis in mice.

Results

Generation of Snai3flox, Snai3null, and Snai3-EYFP Mice

In order to assess whether the Snai3 gene plays an essential in vivo role in mice, we created three Snai3 targeted mutant alleles:
a Snai3\textsuperscript{lox} allele for conditional Snai3 gene inactivation (Fig. 1A), a Snai3\textsuperscript{null} allele, and a Snai3-EYFP knock-in allele that is also a Snai3 null allele (Fig. 1B). The Snai3\textsuperscript{null} allele was generated by Cre recombinase-mediated deletion of the Snai3\textsuperscript{lox} allele, which results in deletion of Snai3 promoter sequences and the first exon of the Snai3 gene. In the Snai3-EYFP allele, EYFP coding sequences replace Snai3 coding sequences contained in the first exon of the Snai3 gene. Mice homozygous for all three Snai3 alleles were viable and fertile. To confirm that the Snai3\textsuperscript{null} and Snai3-EYFP alleles were truly null alleles, we harvested RNA from Snai3\textsuperscript{null} /Snai3\textsuperscript{null} and Snai3-EYFP/Snai3-EYFP homozygous mutant embryos and littermate controls at E14–E15 (Fig. 1C). No Snai3 transcripts were detected in either Snai3\textsuperscript{null} /Snai3\textsuperscript{null} or Snai3-EYFP/Snai3-EYFP homozygotes, confirming that both alleles are Snai3 null alleles.

The Snai3-EYFP Allele Accurately Reflects Endogenous Snai3 Gene Expression

We assessed expression of the Snai3-EYFP allele to determine how closely it matched Snai3 RNA expression. The sites of highest Snai3 RNA expression are skeletal muscle and thymus [6,9]. By fluorescent stereomicroscopy, Snai3-EYFP expression was readily detected in both embryonic thymus and skeletal muscle at E15.5 (Fig. 2A). At E10.5, expression was also detected in both heart and the maxillary and mandibular portions of the first branchial arch (Fig. 2A). Both these sites of expression have been reported previously [6,10]. We used flow cytometry to examine in bone marrow cells and thymocytes expression of both the Snai3-EYFP allele and lineage markers of lymphoid cells. As expected [8,9], Snai3-EYFP expression was evident in both T and B cell populations (Fig. 2B, C). In the bone marrow, greater than 90% of EYFP-positive cells also expressed the pan B cell specific marker B220 (Fig. 2B). Notably, the percentage of EYFP-positive cells was greater in very early T cells (DN1), as well as cell development (Fig. 2C). However, the percentage of EYFP-positive cells were evident in very early T cells (DN1), as well as mature CD8 single positive T cells (80%). All sites of Snai3-EYFP expression were also sites of Snai3 RNA expression, as detected by quantitative RT-PCR of organs from adult mice (Fig. 2D), demonstrating that the Snai3-EYFP allele is a useful and accurate reporter of Snai3 gene expression.

Snai3 Mutant Mice do not Exhibit an Obvious Phenotype

Snai3\textsuperscript{null} and Snai3-EYFP homozygous embryos and mice did not exhibit any obvious phenotype. Both Snai3\textsuperscript{null} /Snai3\textsuperscript{null} (Table 1) and Snai3-EYFP/Snai3-EYFP homozygotes were born at expected Mendelian frequencies. Analysis of postnatal growth curves showed no differences in the rate of growth of either male or female Snai3\textsuperscript{null} homozygotes versus their heterozygous and wild type littermate controls (Fig. 3). Flow cytometry analyses of bone marrow cells and thymocytes from Snai3-EYFP homozygous mice did not reveal obvious defects in differentiation of B and T cells (Fig. 4). Since neural crest cell-specific deletion of the Snai1 gene on a Snai2 null genetic background results in craniofacial defects [11], and the Snai3 gene is expressed in the first branchial arch (Fig. 2A), we examined alcaicin-blue/alizarin-red stained skeletons from Snai3\textsuperscript{null} homozygous and littermate control mice. Analysis of these skeletal preparations did not reveal any obvious defects in the cranial, axial or limb skeletons of Snai3\textsuperscript{null} /Snai3\textsuperscript{null} mice (data not shown). We also performed extensive histopathological and clinical chemistry analyses of Snai3\textsuperscript{null} homozygous and wild type littermate control mice at eight to ten months of age. These analyses did not reveal any abnormal phenotype reproducibly present in the Snai3\textsuperscript{null} /Snai3\textsuperscript{null} mice. We conclude that the Snai3 gene is not essential for embryogenesis and normal development in mice.

Discussion

Relatively little is known about the function of the Snai3 gene and protein, compared to the numerous studies of its family members Snai1 and Snai2. The Snai3 gene was originally isolated as a Snail family gene expressed in adult mouse skeletal muscle [6].

Figure 1. Generation of Snai3 mutant alleles. (A) Construction of the Snai3 conditional null allele. Schematic representation of the wildtype Snai3 allele, the Snai3\textsuperscript{lox-neo} targeting vector, and the targeted Snai3\textsuperscript{lox-neo} allele. Exons are indicated by rectangles with coding sequences designated by gray shading, and noncoding sequences in black. LoxP sequences are marked by black triangles and FRT sites by gray triangles. An FRT-neo-FRT-loxP- cassette was inserted between exons 1 and 2, and the second loxP site was inserted 5’ to exon 1. A diphtheria toxin (DT) cassette was included for negative selection of randomly integrated clones. Hybridization probes used for Southern blot analyses are indicated. Abbreviations: B, BamHI; E, EcoRI; H, HindIII; N, NcoI; Nh, Nhel; Sa, Salt; Sp, Sphi; Spel. (B) Construction of the Snai3-EYFP\textsuperscript{neo} knock-in allele. Schematic representation of the wildtype Snai3 allele, the Snai3-EYFP\textsuperscript{neo} targeting vector, and the targeted Snai3-EYFP\textsuperscript{neo} allele. Exons are indicated by rectangles with coding sequences designated by gray shading. FRT sequences are marked by gray triangles. An EYFP-FRT-neo-FRT-loxP- cassette was inserted at the ATG site in exon1, replacing the remainder of the exon1 coding sequence. A diphtheria toxin (DT) cassette was included for negative selection. Hybridization probes used for Southern blot analyses are indicated. Restriction enzyme abbreviations are as in A. (C) Absence of Snai3 RNA expression in Snai3\textsuperscript{null}/Snai3\textsuperscript{null} and Snai3-EYFP/Snai3-EYFP embryos. Quantitative RT-PCR of relative Snai3 transcript levels in RNA isolated from Snai3\textsuperscript{null}/Snai3\textsuperscript{null}, Snai3-EYFP/Snai3-EYFP and littermate control embryos revealed the absence of Snai3 transcripts in the homozygotes, confirming that both alleles were Snai3 null alleles.

doi:10.1371/journal.pone.0065344.g001

Figure 1. Generation of Snai3 mutant alleles. (A) Construction of the Snai3 conditional null allele. Schematic representation of the wildtype Snai3 allele, the Snai3\textsuperscript{lox-neo} targeting vector, and the targeted Snai3\textsuperscript{lox-neo} allele. Exons are indicated by rectangles with coding sequences designated by gray shading, and noncoding sequences in black. LoxP sequences are marked by black triangles and FRT sites by gray triangles. An FRT-neo-FRT-loxP- cassette was inserted between exons 1 and 2, and the second loxP site was inserted 5’ to exon 1. A diphtheria toxin (DT) cassette was included for negative selection of randomly integrated clones. Hybridization probes used for Southern blot analyses are indicated. Abbreviations: B, BamHI; E, EcoRI; H, HindIII; N, NcoI; Nh, Nhel; Sa, Salt; Sp, Sphi; Spel. (B) Construction of the Snai3-EYFP\textsuperscript{neo} knock-in allele. Schematic representation of the wildtype Snai3 allele, the Snai3-EYFP\textsuperscript{neo} targeting vector, and the targeted Snai3-EYFP\textsuperscript{neo} allele. Exons are indicated by rectangles with coding sequences designated by gray shading. FRT sequences are marked by gray triangles. An EYFP-FRT-neo-FRT-loxP- cassette was inserted at the ATG site in exon1, replacing the remainder of the exon1 coding sequence. A diphtheria toxin (DT) cassette was included for negative selection. Hybridization probes used for Southern blot analyses are indicated. Restriction enzyme abbreviations are as in A. (C) Absence of Snai3 RNA expression in Snai3\textsuperscript{null}/Snai3\textsuperscript{null} and Snai3-EYFP/Snai3-EYFP embryos. Quantitative RT-PCR of relative Snai3 transcript levels in RNA isolated from Snai3\textsuperscript{null}/Snai3\textsuperscript{null}, Snai3-EYFP/Snai3-EYFP and littermate control embryos revealed the absence of Snai3 transcripts in the homozygotes, confirming that both alleles were Snai3 null alleles.

doi:10.1371/journal.pone.0065344.g001
The SNAI3 protein bound the same E2 box sequence (CAGGTG and CACCTG) recognized by the SNAI1 and SNAI2 proteins, and functioned as a transcriptional repressor. Northern blot [6] and in situ hybridization [9] analyses indicated that the Snai3 gene was expressed at highest levels in thymus and skeletal muscle. We confirmed these and other sites of expression by analyzing expression of the Snai3-EYFP allele (Fig. 2).

We demonstrated by qRT-PCR that the Snai3null and Snai3-EYFP alleles generated no stable Snai3 transcripts, indicating that both are Snai3 null alleles (Fig. 1C). We were surprised to find that neither the Snai3null/Snai3null nor the Snai3-EYFP/Snai3-EYFP homozygotes exhibited an obvious mutant phenotype. It should be noted that the absence of an obvious phenotype in Snai3 homozygous mutant mice in the laboratory environment does not preclude an essential role for the Snai3 gene in mice inhabiting their natural environment. However, subsequent to our initiation of these studies, a potentially relevant finding was made from a comparison of the requirements for Snail family genes in fish. While Snai3 genes are present in both the zebrafish and pufferfish (fugu) genomes, the medaka genome does not contain a Snai3 gene [12], indicating that Snai3 gene function also is not required for embryonic development in teleost fish (at least in medaka).

We have previously demonstrated genetic redundancy of the Snai1 and Snai2 genes in mice utilizing two different Cre driver lines for conditional Snai1 gene deletion on either wild type or Snai2 null genetic backgrounds, Wnt1-Cre (for neural crest cell deletion) [11] and Prrx1-Cre (for deletion in limb bud mesenchymal stem cells that give rise to chondrogenic precursors of the limb long bones) [13]. In both cases, cartilaginous precursors of endochondral bones were shorter in the Snai1/Snai2 double mutant mice, although neither of these genes was essential for delamination of neural crest cells from the dorsal neural tube through E9.5 [14]. During long bone development, our work clearly demonstrated that the Snai1 and Snai2 genes could compensate quantitatively, temporally and spatially for the other genes’ loss [13]. A similar mechanism may explain the absence of an obvious mutant phenotype in the Snai3 null mice. However, testing the functional equivalence of the SNAI1, SNAI2 and SNAI3 proteins in mice ultimately will require the generation and analysis of a series of knock-in alleles, placing the coding sequence for each Snail family member under the transcriptional control of a different family member.

A recent gain-of-function study in mice demonstrated that expression of Snai3 by retroviral transduction of hematopoietic stem cells in bone marrow chimeras resulted in a block in

Figure 2. Expression of the Snai3-EYFP allele. (A) EYFP expression is observed in thymus and in leg skeletal muscle at E15.5, and in heart, first branchial arch and somites at E10.5. EYFP expression was visualized by fluorescent stereomicroscopy. Dotted lines in the left panel outline the wild type thymus lobes. (B) Flow cytometry analysis demonstrated EYFP expression in Pre-B and B cells, but not in Pro-B cells of adult mouse bone marrow. (C) Flow cytometry analysis of thymus demonstrated EYFP expression in most T cell subsets. EYFP expression was highest in CD8 single positive T cells. (D) Quantitative RT-PCR of endogenous Snai3 RNA expression in the indicated organs of three (red bars) and seven (blue bars) week old wild type mice. Expression was normalized to beta actin RNA levels in each organ.

doi:10.1371/journal.pone.0065344.g002

Figure 3. Snai3null/Snai3null homozygous mice exhibit normal postnatal growth. Growth curves of Snai3null/Snai3null homozygous, Snai3null/+ heterozygous and wild type littermate mice. Weights (in grams) were plotted against age (in weeks). Data presented are from at least four mice in each group. Error bars indicate the standard deviations.

doi:10.1371/journal.pone.0065344.g003
lymphoid cell development [15]. However, we did not detect any obvious defects in lymphoid cell differentiation in Snai3 null mice. Two alternatives could explain the absence of a phenotype in lymphoid cells in the Snai3 null mutants. Gain-of-function studies yield phenotypes in cells that are competent to respond to the protein or signal being misregulated, but do not in and of themselves prove that this gene is required for their development in the wild type situation. Alternatively, there could be compensatory regulation (of the sort observed in long bone chondrogenesis) by another Snail family gene member, or even by an unrelated gene(s).

Materials and Methods

Ethics Statement

All experimental procedures performed on mice were in accordance with the recommendations of the Guide for the Care and Use of Laboratory Animals of the National Institutes of Health, and were approved by the Institutional Animal Care and Use Committee at Maine Medical Center (Protocol number 1023).

Construction of the Snai3^flox-neo^ Allele

To generate the Snai3^flox-neo^ targeting construct, an 8 kb EcoRI/BamH I fragment described above was cloned into pBluescript II SK with a modified multiple cloning site (SacI-EcoRI-BamH I-MluI-NotI-XhoI). The 5' distal loxP site was generated by insertion of a synthetic oligonucleotide cassette at the Sall site. A FRT-neo-FRT-loxP cassette was inserted at the SpeI site, and a diphtheria toxin-A cassette was inserted at the MluI/NotI sites to permit negative selection against random integration of the targeting vector. Cre recombinase-mediated deletion will result in deletion of Snai3 promoter sequences and the first exon of the Snai3 gene.

Construction of the Snai3-EGFPNEO^ Allele

To generate the Snai3-EGFPNEO^ targeting construct, the same 8 kb EcoRI/BamH I fragment described above was cloned into pBluescript II SK with a modified multiple cloning site (SacI-EcoRI-BamH I-MluI-NotI-XhoI). A diphtheria toxin-A cassette was inserted at the BamH I/MluI sites. Using bacterial recombinering, the coding region of exon 1 (ATG onwards) of the Snai3 gene was replaced with an “EGFP-loxP-neo-loxP” cassette. This EGFP-loxP-neo-loxP cassette was generated by PCR amplification of a vector in which the EGFP gene from pEGFP-N1 (Clontech) was inserted upstream of the loxP-neo-loxP at the SacII/XhoI sites.

Table 1. Snai3^null^/Snai3^null^ mice are born and survive at the expected Mendelian frequency.

| Genotype          | Number of litters | Number of mice | +/+     | Snai3^null^/+ | Snai3^null^/Snai3^null^ |
|-------------------|-------------------|----------------|---------|---------------|-------------------------|
|                   | 30                | 201            | 52 (25.9%) | 93 (46.3%)   | 56 (27.8%)              |

Snai3^null^/+ mice were intercrossed, and progeny genotypes were determined at weaning.

doi:10.1371/journal.pone.0065344.t001

Figure 4. Snai3-EGFP/Snai3-EGFP homozygous mice do not exhibit altered lymphoid differentiation. Flow cytometry analyses of cells from bone marrow and thymus demonstrated that Snai3-EGFP/Snai3-EGFP homozygous mice (n = 3) did not exhibit obvious defects in differentiation of B, immature T, or mature T cells.

doi:10.1371/journal.pone.0065344.g004
Electroporation of ES Cells and Generation of Mutant Mice

CJ7 ES cells [16] were electroporated with linearized Snai3loxP-neo or Snai3-EYFPneo targeting vector, placed under selection in G418, and screened for homologous recombination by Southern blot hybridization. Correctly-targeted ES cell clones were injected into C57BL/6J blastocysts to generate chimeric mice. Male chimeras were mated to female mice to obtain germline transmission of the Snai3loxP and Snai3-EYFPneo alleles. Snai3loxP+/+ heterozygous mice were mated to the Meox2-Cre deleter line [17] to excise Snai3 genomic sequences between the loxP sites and generate the Snai3null allele. Snai3-EYFP+/+ heterozygous mice also were mated to the Meox2-Cre deleter line to excise the loxP-neo-loxP cassette and generate the Snai3-EYFP allele. Snai3loxP+/+ heterozygous mice were mated to a deleter line expressing the Flpe recombinase [18] to excise the FRT-neo-FRT cassette and generate the Snai3loxP allele.

Mouse and Embryo Genotyping

For genotyping Snai3-EYFP mice and embryos, PCR primers for the Snai3-EYFP mutant allele are Snai3-F2 (CTGGTTGCGTTGGAGTGCGCATG) and EYFP-R2 (CTTGCGGGTGGTGAGCATGAA), with a product size of 306 base pairs. Genotyping primers for the wild type Snai3 allele (for use in conjunction with the Snai3-EYFP mutant allele primer set above) are Snai3-F2 and Snai3-R3 (GGTGAAGTG- GAAAATCTTAGCCCGTCT), with a product size of 354 base pairs.

For genotyping Snai3loxP mice and embryos, PCR primers for the Snai3loxP allele are Snai3-loxP-F (GCAGCGACGAG- GAATGTGGCTCAGAT) and Snai3-loxP-R (AGTCGGCAGGGTAGGACGAT), with a product size of 326 base pairs. This primer set will amplify a product of 266 base pairs from the wild type Snai3 allele (this primer set flanks the region including the 5′ loxP site in the Snai3loxP allele).

Primers for the Snai3null allele (i.e., the deleted form of the Snai3loxP allele) are Snai3-loxP-F and Snai3-delR (AAGCTGG-TATGTGCTCTCCAAGTG), with a product size of 316 base pairs.

All PCR reactions were performed using ThermoPrime ReddyMix PCR Master Mix (Fisher Scientific) and the following cycling conditions: 94°C, 3 minutes; 94°C, 30 seconds; 60°C, 45 seconds; 72°C, 45 seconds × 40 cycles; 72°C, 4 minutes; 4°C hold.

Analysis of Snai3-EYFP Expression

EYFP fluorescent protein expression was analyzed in whole mount embryos through E10.5, and in partially dissected embryos at later stages. No differences were noted in expression of the Snai3-EYFPneo or Snai3-EYFP alleles. Embryos were analyzed and digital photographs taken on a Zeiss Discovery V12 fluorescence stereomicroscope. Flow cytometry for EYFP and marker protein expression was performed on suspensions of bone marrow and thymus from adult mice, as described previously [19].

Analyses of Snai3 Mutant Mice

Aclan blue-azurin red-stained skeletal preparations were generated as described previously [11]. Blood for clinical chemistry analyses, and tissues from all major organ systems of male Snai3null/Snai3null homozygous (n = 5) and wild type littermate control (n = 3) mice, as well as the reproductive tracts of an additional four homozygous and three wild type female mice, were harvested and processed for histopathological analysis. Soft tissues were fixed in neutral buffered 10% formalin (Fisher Scientific). Limbs and the skull with brain were fixed in Bouin’s solution (Rica Chemical Company). Tissues were processed, embedded in paraffin, and sectioned by routine methods (Yale Mouse Research Pathology, Section of Comparative Medicine, Yale University School of Medicine). Tissues were sectioned at 5 microns and stained with hematoxylin and eosin. Tissues were examined by routine light microscopy with an Axio Imager A1 microscope (Carl Zeiss Micro Imaging). Necropsy, histopathology, and clinical chemistry examination were performed blind to experimental genotype. Clinical chemistry assays were performed using standard methods by Antech Diagnostics (Irvine, CA). Flow cytometry to assess lymphoid differentiation was performed as described previously [19].

Quantitative RT-PCR

Organs from three- or seven-week old wild type mice, and embryos at E14–E15, were dissected and immersed in RNAlater (Ambion). Genotypes were identified from DNA samples by allele-specific PCR. Total RNA was isolated using the Qiagen Mini mRNA Extraction kit. RNA (2 μg of each sample) was reverse-transcribed with random hexamer primers (Ambion). Six nanograms of cDNA were used for real-time PCR amplification for each well, using primer sequences from Primerbank. qRT-PCR was performed using Super SYBR Green PCR Master Mix on a 7500 Real Time PCR system (Applied Biosystems) using SDS software. For each gene tested we performed three experimental replicates and four biological replicates. Snai3 primer sequences were CTGGCGCTGATCCTGAAGGT (forward primer, located in exon 2) and TGTTACCAACGTGTTCTG (reverse primer, located in exon 3). For comparison of Snai3 expression in various organs of three- or seven-week old wild type mice, gene expression levels were normalized to the βeta actin RNA level. For comparison of Snai3 expression in wild type, heterozygous and homozygous Snai3 mutant embryos, the βeta actin-normalized Snai3 RNA level in the wild type embryos was set to 1.0, to which the βeta actin-normalized Snai3 RNA levels of the heterozygous and homozygous Snai3 mutant embryos were compared.

Acknowledgments

We thank the personnel of the Molecular Phenotyping and Histopathology Core Facilities at Maine Medical Center Research Institute.

Author Contributions

Conceived and designed the experiments: CKB CRN TG. Performed the experiments: CKB CRN YC XH CJ Be CY CT LK TG. Wrote the paper: CKB CRN TG. Performed the animal experiments: CKB CRN YC XH CJ Be CY CT LK TG. Analyzed the data: CKB CRN YC XH CJ Be CY CT LK TG. Contributed reagents/materials/analysis tools: CKB CRN YC XH CJ Be CY CT LK TG. Analyzed the data: CKB CRN YC XH CJ Be CY CT LK TG. Contributed reagents/materials/analysis tools: CKB CRN YC XH CJ Be CY CT LK TG. Wrote the paper: CKB CRN TG. Core Facilities at Maine Medical Center Research Institute.

References

1. Barrallo-Gimeno A, Nieto MA (2009) Evolutionary history of the Snail/Scratch superfamily. Trends Genet 25: 248–252.
2. Chiang C, Ayyanathan K (2012) Snail/Gfi-1 (SNAG) family zinc finger proteins in transcription regulation, chromatin dynamics, cell signaling, development, and disease. Cytokine Growth Factor Rev.
3. Barrallo-Gimeno A, Nieto MA (2005) The Snail genes as inducers of cell movement and survival: implications in development and cancer. Development 132: 3151–3161.
4. Zhao P, Iezzi S, Carver E, Dressman D, Gridley T, et al. (2002) Slug is a novel downstream target of Myod. Temporal profiling in muscle regeneration. J Biol Chem 277: 30091–30101.
5. Soleimani VD, Yin H, Jahani-Asl A, Ming H, Kockx CE, et al. (2012) Snail regulates MyoD binding-site occupancy to direct enhancer switching and differentiation-specific transcription in myogenesis. Mol Cell 47: 457–468.

6. Kataoka H, Murayama T, Yokode M, Mori S, Sano H, et al. (2000) A novel Snail-related transcription factor Snuc regulates basic helix-loop-helix transcription factor activities via specific E-box motifs. Nucleic Acids Res 28: 626–633.

7. Katoh M (2003) Identification and characterization of human SNAI3 (SNAI3) gene in silico. Int J Mol Med 11: 383–389.

8. Katoh M (2005) Comparative genomics on SNAI1, SNAI2, and SNAI3 orthologs. Oncol Rep 14: 1083–1086.

9. Zhuge X, Kataoka H, Tanaka M, Murayama T, Kawamoto T, et al. (2005) Expression of the novel Snai-related zinc-finger transcription factor gene Snuc during mouse development. Int J Mol Med 15: 945–948.

10. Yokoyama S, Ito Y, Ueno-Kudoh H, Shimizu H, Uchibe K, et al. (2009) A systems approach reveals that the myogenesis genome network is regulated by the transcriptional repressor RF. Dev Cell 17: 836–848.

11. Murray SA, Oram KF, Gridley T (2007) Multiple functions of Snail family genes during palate development in mice. Development 134: 1789–1797.

12. Liedtke D, Erhard I, Schartl M (2011) Snail gene expression in the medaka, Oryzias latipes. Gene Expr Patterns 11: 101–109.

13. Chen Y, Gridley T (2013) Compensatory regulation of the Snai1 and Snai2 genes during chondrogenesis. J Bone Miner Res. In press.

14. Murray SA, Gridley T (2006) Snail family genes are required for left-right asymmetry determination, but not neural crest formation, in mice. Proc Natl Acad Sci USA 103: 10300–10304.

15. Dahlem T, Cho S, Spangrude GJ, Weis JJ, Weis JH (2012) Overexpression of Sna13 suppressed lymphoid- and enhances myeloid-cell differentiation. Eur J Immunol 42: 1038–1043.

16. Swiatek PJ, Gridley T (1993) Perinatal lethality and defects in hindbrain development in mice homozygous for a targeted mutation of the zinc finger gene Krox20. Genes Dev 7: 2071–2084.

17. Tallquist MD, Soriano P (2000) Epiblast-restricted Cre expression in MORE mice: a tool to distinguish embryonic vs. extra-embryonic gene function. Genesis 26: 113–115.

18. Farley JW, Soriano P, Steffen LS, Dymecki SM (2000) Widespread recombinase expression using FLPeR (flipper) mice. Genesis 28: 106–110.

19. Krebs LT, Bradley CK, Norton CR, Xu J, Oram KF, et al. (2012) The Notch-regulated ankyrin repeat protein is required for proper anterior-posterior somite patterning in mice. Genesis 50: 366–374.