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Generation of *Osr1* conditional mutant mice

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Summary

The *Odd-skipped related 1* (*Osr1*) gene encodes a zinc finger protein homologous to the *Drosophila* Odd-skipped transcription factor. During mouse embryogenesis, *Osr1* is expressed in multiple tissues, including the developing heart, kidney, limb, lung, and craniofacial structures. While characterization of targeted mutant mice has revealed essential roles for *Osr1* in heart and kidney development, the embryonic lethality of the *Osr1* null mice has hindered investigation of its role in many other developmental processes. We report here the generation of conditional mutant mice containing two loxP sites flanking Exon 2 of the *Osr1* gene. Mice homozygous for this targeted *Osr1^{fneo}* allele are normal and fertile. Crossing the *Osr1^{fneo/fneo}* mice with the *Ella-Cre* transgenic mice resulted in Cre-mediated deletion of the loxP-flanked Exon2 in the germ line and mice homozygous for this deletion recapitulated the *Osr1* null mutant phenotypes. The *Osr1^{fneo}* conditional mice will be valuable for tissue-specific analysis of the roles of *Osr1* in embryonic and postnatal developmental processes.

Keywords

Cre; loxP; frt; conditional inactivation; odd-skipped; *Osr1*

The Odd-skipped family of zinc finger transcription factors plays essential roles in embryonic development throughout metazoans. The *odd-skipped* gene was first identified in a large mutagenesis screen of developmental control genes in *Drosophila* as mutations in this gene caused loss of portions of the odd-numbered segments in the embryo (Nusslein-Volhard and Wieschaus, 1980; Coulter and Wieschaus, 1988). Since the characterization of the coding sequences of the *odd-skipped* gene, homologous genes have been identified in mice, human, chick, nematode, *Xenopus*, and zebrafish (Coulter et al., 1990; So and Danielian, 1999; Lan et al., 2001; Katoh, 2002; Buckley et al., 2004; Stricker et al., 2006; Tena et al., 2007; Mudumana et al., 2008). Analysis of gene knockout mice showed that *Odd-skipped related-1* (*Osr1*) is essential for heart and kidney development whereas *Osr2* plays critical roles in palate and tooth development (Lan et al., 2004; Wang et al., 2005; James et al., 2006; Mugford et al., 2008; Zhang et al., 2009). Gene knockdown studies in *Xenopus* and zebrafish showed that both *Osr1* and *Osr2* play critical roles in embryonic kidney development in these organisms (Tena et al., 2007; Mudumana et al., 2008).

During mouse embryogenesis, *Osr1* mRNA expression is first activated in the nascent intermediate mesoderm during gastrulation and subsequently in the developing heart, limb, lung, and craniofacial structures (Wang et al., 2005). While analyses of the *Osr1* null mutant mouse strains have revealed essential roles for *Osr1* in the early stages of kidney and heart development, the embryonic lethality of the mutant mice has hindered investigations of the

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roles of *Osr1* in many other developmental processes (Wang et al., 2005). To overcome this problem, we have generated mice for conditional inactivation of the *Osr1* gene using the Cre-loxP *in vivo* DNA recombination system.

The mouse *Osr1* gene contains three exons, spanning approximately 8 kb of genomic DNA sequence (FIG. 1A) (Wang et al., 2005). We isolated an *Osr1* genomic DNA fragment containing all three exons from the RPCI-22 129/SvEvTac mouse BAC library (BACPAC Resources, Children's Hospital of Oakland, Oakland, CA). To construct the targeting vector for generation of *Osr1* conditional mice, one loxP sequence was inserted into each of the two EcoRV sites in the first and second introns, respectively (FIG. 1B). An frt-flanked *neo* expression cassette was inserted immediately 3' to the loxP sequence in Intron 1 for positive selection. The resultant targeting vector contained the 3.8 kb BglII-EcoRI fragment from Intron 1 as the 5' homology arm, the 3.2 kb EcoRV-XbaI fragment containing Exon 3 as the 3' homology arm, and a diphtheria toxin-A (*DTA*) expression cassette immediately 3' to the 3' arm (FIG. 1B). Following sequence confirmation of the construct, the targeting vector was linearized and electroporated into the CJ7 mouse embryonic stem (ES) cells as previously described (Swiatek and Gridley, 1997). G418 resistant ES colonies were screened by Southern hybridization with the 3' probe to detect integration of the 3' loxP site in the second intron (FIG. 1F). Southern blots containing DNA samples of positively targeted clones were rehybridized with the 5' probe (indicated in FIG. 1A) to verify the expected integration of the loxP and *neo* sequences in the first intron (data not shown). Of 224 colonies screened, three correctly targeted ES clones were identified and two independent clones were used for generation of chimeric mice by microinjection into blastocysts of C57BL/6J mice. Chimeric male mice were bred with C57BL/6J females to test for germ line transmission of the targeted allele. Both ES clones resulted in germ line transmission. F1 mice were genotyped initially by Southern hybridization analysis using tail genomic DNA samples for the presence of the targeted *Osr1^{fneo}* allele (FIG. 1C). Heterozygous mice were intercrossed and genotyping of F2 progeny at one week after birth detected homozygous *Osr1^{fneo/fneo}* mice at expected Mendelian ratio. The homozygous *Osr1^{fneo/fneo}* mice exhibited normal life span and fertility. Some homozygous *Osr1^{fneo/fneo}* mice were bred to *FLPeR* mice (Farley et al., 2000) to remove the *neo* expression cassette in the germ line (FIG. 1D). The resultant *Osr1^{f/+}* mice were intercrossed and homozygous *Osr1^{f/f}* mice were generated and did not exhibit any detectable abnormality.

To test for Cre mediated inactivation of the *Osr1* conditional allele *in vivo*, we crossed the *Osr1^{fneo/fneo}* mice with *E11a-Cre* transgenic mice, which express Cre in the early embryo and causes deletion of loxP-flanked sequences in most tissues including the germ line (Lakso et al., 1996). Whereas the resultant *Osr1^{ΔE2/+}* mice were normal, analysis of embryos from *Osr1^{ΔE2/+}* heterozygous intercrosses revealed that the *Osr1^{ΔE2/ΔE2}* mutant embryos died before birth (FIG. 2, FIG. 3). The *Osr1^{ΔE2/ΔE2}* embryos phenocopied the phenotypes of the *Osr1^{-/-}* mutant embryos as previously reported (Wang et al., 2005), with most of them dying at around E12.0 (FIG. 3, B and D). Similar to the *Osr1^{-/-}* embryos, a small percentage of *Osr1^{ΔE2/ΔE2}* embryos survived to late gestation and had generalized edema (FIG. 3E). Histological analysis showed that all *Osr1^{ΔE2/ΔE2}* embryos lacked metanephric kidney and had heart defects (data not shown), similar to the *Osr1^{-/-}* embryos as previously reported (Wang et al., 2005). These data indicate that Cre-mediated deletion of the loxP-flanked Exon-2 abolished *Osr1* gene function. Thus, the *Osr1^{fneo}* conditional mice will be valuable for tissue-specific genetic analysis of the molecular pathways involving *Osr1* in many developmental processes.

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FIG. 1.

Generation of *Osr1* conditional mutant mice. (A) Schematic representation of the *Osr1* gene. Boxes represent exons and are labeled E1, E2, and E3, respectively. The protein-coding regions of Exons 2 and 3 are shaded, with the positions of the translation start (ATG) and stop (TAA) codons indicated. Positions corresponding to the Southern hybridization probes used for screening for correct targeting of the *Osr1* locus are also indicated. Restriction sites are: B, BamHI; Bg, BglII; R, EcoRV; X, XbaI. (B) The targeting vector consists the BglII/EcoRV fragment from Intron 1 of the *Osr1* gene as the 5' homology arm, followed by an loxP sequences, an frt-flanked *neo* expression cassette, the EcoRV fragment containing Exon 2 of the *Osr1* gene, another loxP sequence, the EcoRV/XbaI fragment containing Exon 3 as the 3' homology arm, and an DTA expression cassette. (C) Schematic representation of the targeted *Osr1^{neo}* allele. (D) Schematic representation of the *Osr1^f* allele, following FLP-mediated deletion of the *neo* expression cassette from the *Osr1^{neo}* allele. (E) Schematic representation of the *Osr1^{ΔE2}* allele, following Cre-mediated deletion of the loxP-flanked Exon 2. (F) Southern hybridization band patterns of BamHI-digested DNA samples from two non-targeted clones (Numbers 56 and 57) and one correctly targeted clone (Number 58) using the 3' Southern probe indicated in A, which hybridizes to the wildtype fragment of 5.3 Kb and a fragment of 4.4 Kb from the correctly targeted *Osr1^{neo}* allele.

FIG. 2.

PCR genotyping of a litter of E12 embryos from intercross of a pair of *Osr1*^{ΔE2/+} mice. The PCR primers used are: ODDCKO1 (5'-GAGTGTAGCGTCTTGTGGACAG-3', from the antisense strand in Exon 3), ODDCKO2 (5'-GCACTTTGCTCGATCTTGCTTG-3', from sense strand upstream of the EcoRV site in Intron II), ODDCKO3 (5'-GGTTCTGATATTGTTGTAGGACAG-3', from sense strand upstream of the EcoRV site in Intron I). The ODDCKO1 and ODDCKO2 primers produce a PCR product of 220 bp from the wildtype allele, whereas ODDCKO1 and ODDCKO3 amplify a PCR product of 320 bp from the *Osr1*^{ΔE2} allele. +/+, wildtype; +/Δ, *Osr1*^{ΔE2/+} heterozygous; Δ/Δ, *Osr1*^{ΔE2/ΔE2} homozygous. M, DNA molecular size markers, with the corresponding fragment sizes marked to the right.

FIG. 3.

The *Osr1*^{ΔE2/ΔE2} embryos die prenatally. (A, B) A pair of *Osr1*^{ΔE2/+} heterozygous (A) and *Osr1*^{ΔE2/ΔE2} homozygous (B) embryos harvested at E12.0. Note the *Osr1*^{ΔE2/ΔE2} homozygous embryo had dilated epicardium (arrow in B) and showed hemorrhaging in the trunk region. (C–E) *Osr1*^{ΔE2/+} heterozygous (C) and *Osr1*^{ΔE2/ΔE2} homozygous (D and E) embryos harvested at E14.5. The mutant embryo shown in D was dead and arrested development by E12.5 (arrowhead points to the forelimb bud, which shows a characteristic E12 limb morphology, in contrast to the well formed digits in the heterozygous littermate in C). The mutant embryo shown in E was alive but had generalized edema (arrow). These phenotypes are identical to those seen in the *Osr1*^{−/−} mutant embryos as previously reported (Wang et al., 2005).