Biochemical and Biophysical Research Communications 440 (2013) 8–13

Contents lists available at ScienceDirect

Biochemical and Biophysical Research Communications

journal homepage: www.elsevier.com/locate/ybbrc

Generation and characterization of a conditional deletion allele for *Lmna* in mice

Youngjo Kim, Yixian Zheng *

Department of Embryology, Carnegie Institution for Science, 3520 San Martin Dr., Baltimore, MD 21218, USA

ARTICLE INFO

Article history:
Received 22 August 2013
Available online 30 August 2013

Keywords:
Lamins
Laminopathies
Lmna
Conditional knockout
Gene targeting
Cre-loxP

ABSTRACT

Extensive efforts have been devoted to study A-type lamins because mutations in their gene, *LMNA* in humans, are associated with a number of diseases. The mouse germline mutations in the A-type lamins (encoded by *Lmna*) exhibit postnatal lethality at either 4–8 postnatal (P) weeks or P16–18 days, depending on the deletion alleles. These mice exhibit defects in several tissues including hearts and skeletal muscles. Despite numerous studies, how the germline mutation of *Lmna*, which is expressed in many postnatal tissues, affects only selected tissues remains poorly understood. Addressing the tissue specific functions of *Lmna* requires the generation and careful characterization of conditional *Lmna* null alleles. Here we report the creation of a conditional *Lmna* knockout allele in mice by introducing loxP sites flanking the second exon of *Lmna*. The $Lmna^{flox/flox}$ mice are phenotypically normal and fertile. We show that *Lmna* homozygous mutants ($Lmna^{\Delta/\Delta}$) generated by germline Cre expression display postnatal lethality at P16–18 days with defects similar to a recently reported germline *Lmna* knockout mouse that exhibits the earliest lethality compared to other germline knockout alleles. This conditional knockout mouse strain should serve as an important genetic tool to study the tissue specific roles of *Lmna*, which would contribute toward the understanding of various human diseases associated with A-type lamins.

1. Introduction

The nuclear lamina (NL) is a meshwork of proteins that lines the inner nuclear membrane and serves as a nuclear scaffold. Lamins, which belong to the type V intermediate filaments, are the major components of the NL. Lamin proteins were initially subdivided based on immunological and biochemical criteria [1,2]. A-type lamins have nearly neutral isoelectric points (pI) and are almost completely solubilized during mitosis, whereas B-type lamins have acidic pI and remain associated with nuclear envelope derived membranes during mitosis. Since the first cDNA cloning of A-type lamins in humans [3], lamins have been identified in many vertebrates. Sequence comparisons of lamin proteins have revealed that despite of overall similarity, A- and B-type lamins exhibit subclass-specific sequence variations [4–6]. It is also known that both A and B-type lamins are initially carboxymethylated and farnesylated at the C-terminal CaaX motif, but these modifications are removed from lamin-A by proteolysis (reviewed in [7]).

In the mouse, *Lmnb1* and *Lmnb2* encode lamin-B1 and lamin-B2, respectively. *Lmnb2* also expresses lamin-B3 through alternatively splicing in the testes. On the other hand, *Lmna* expresses several forms of A-type lamins, including lamin-A, -C, -AΔ10, and -C2, via alternative splicing. While B-type lamins are expressed in most cell types throughout development, the expression of A-type lamins is either lacking or very low during early development with increasing levels of expression in some cell types as development progresses [8–10]. For example, during mouse embryogenesis, the expression of A-type lamins is limited to a few tissues such as the trunk and epidermis, while in many other tissues, lamin-A/C is not detected until well after birth. In adult mice, lamin-A/C are expressed in many tissues at high levels [9].

Since the discovery that mutations in *LMNA* cause autosomal dominant Emery–Dreifuss muscular dystrophy (EDMD) in humans [11], many additional *LMNA* mutations have been shown to cause a large group of human diseases ranging from muscular dystrophy to premature aging disease. Several germline *Lmna* mutant mouse models have been generated with the aim of understanding the mechanisms by which A-type lamins function in health and diseases [12,13]. Sullivan and colleagues showed that homozygous mice deleted of exons 8–11 of *Lmna* (referred to here as $Lmna^{\Delta 8-11/\Delta 8-11}$) in the germline develop to term with no overt abnormalities [14]. However, their postnatal growths are severely retarded, and these mice present with a subset of pathologies similar to those caused by *LMNA* mutations, including muscular dystrophy, dilated cardiomyopathy, and Charcot–Marie–Tooth syndrome in humans [15]. These mice die at around 4–8 weeks after birth. Despite the widespread use of this mouse

* Corresponding author. Fax: +1 410 243 6311.
E-mail address: zheng@ciwemb.edu (Y. Zheng).

0006-291X/$ - see front matter © 2013 Elsevier Inc. All rights reserved.
http://dx.doi.org/10.1016/j.bbrc.2013.08.082

model, recent studies show that these mice still express a truncated lamin-A protein of 54 kDa, which corresponds to the 468 amino acids encoded by exons 1–7 and 12, through alternative splicing [16]. The expression of this large lamin-A fragment might function dominant negatively because heterozygous $Lmna^{+/\Delta 8-11}$ mice develop dilated cardiomyopathies 4 weeks after birth [17], which complicates phenotypic interpretation.

Recently, another *Lmna* germline knockout mouse line has been created using gene trap (GT) technology [13]. In the $Lmna^{GT-/-}$ mouse, a gene trap cassette, consisting of a splicing acceptor and a reporter gene, is introduced into the upstream sequence of exon 2 of *Lmna*, and only the N-terminal 118 amino acids of lamin-A is expressed as a fusion with the reporter protein. $Lmna^{GT-/-}$ mice are overtly normal during embryogenesis. However, they succumb to death at 16–18 days after birth, which is much earlier than $Lmna^{\Delta 8-11/\Delta 8-11}$ mice. Therefore, $Lmna^{GT-/-}$ mice are the most severe loss-of-function mutants for A-type lamins to date.

Although mutations of *LMNA* are associated with diverse human diseases, a careful look at the diseases and animal models reveals that the defects caused by different mutations are manifested in limited tissue types including striated muscles, adipose tissues, and peripheral nerves [18]. How mutations in the broadly expressed lamin-A cause tissue-restricted defects is unclear. Several nonexclusive hypotheses have been proposed to account for the molecular basis of this selectivity [19]. For example, the mechanical stress model posits that cell types such as muscle cells that are under constant mechanical stress might be more susceptible to the alteration of the nuclear lamina [20]. The gene expression model, on the other hand, proposes that lamins influence gene expression profiles in a cell type specific manner [21,22]. Although studies using the germline *Lmna* mutant mouse models created thus far have provided support to aspects of the above hypotheses, understanding the function of *Lmna* in a given tissue requires the creation of a conditional *Lmna* knockout allele. Recently, Solovei et al. reported the use of an *Lmna* conditional knockout allele, which leads to the deletion of the last three exons (exons 10–12) of *Lmna* upon Cre expression [23]. The predicted lack of polyA signal in *Lmna* transcripts from exons 1–9 may cause their degradation. However, these transcripts could utilize a cryptic polyA signal in the absence of exons 10–12, which would lead to the generation of truncated lamin-A/C proteins. Unfortunately, the authors provided no characterization of this *Lmna* conditional knockout allele. Thus it remains unclear whether truncated lamin-A/C proteins are present upon Cre expression in these mice. To facilitate the study of the tissue autonomous function of A-type lamins, we report the generation and characterization of a different conditional *Lmna* knockout mouse model that deletes the majority of lamin-A/C proteins upon Cre-mediated recombination.

2. Materials and methods

2.1. Construction of the targeting vector

The targeting vector to generate conditional knockout alleles of *Lmna* was constructed using the recombineering protocol established by the Capecchi laboratory [24]. Briefly, an 8 kb genomic region from 2 kb upstream of exon 2 through the middle of exon 11 of *Lmna* was retrieved from a mouse BAC clone RP24-265E18, and was cloned into the pStart-K vector using Red-recombinase mediated cloning method. The construct was modified to add the 5′ and 3′ loxP sites at 100 bp upstream and downstream, respectively, from exon 2 of *Lmna* by a series of conventional and recombination-mediated cloning. The resulting plasmid was cut between exon 2 and the 3′ loxP site with the restriction enzyme MluI and ligated to an Frt-puro-Frt cassette flanked by MluI sites to obtain a positive selection marker. Finally, the modified genomic fragment of *Lmna* in the pStart-K vector was transferred by Gateway recombination to the pWS-TK6 vector containing a thymidine kinase (tk) cassette as a negative selection marker. Full DNA sequences of the *Lmna* targeting vector were verified by conventional DNA sequencing, and are available upon request. All procedures involving recombinant DNA have followed the National Institute of Health guidelines.

2.2. PCR primers and mouse strains

To generate the $Lmna^{fflox}$ allele, the *Lmna* targeting vector was linearized by AsiSI and introduced into V6.5 mouse ESCs by electroporation. After selection with 1 μg/ml puromycin (Life Technologies) and 2 μM ganciclovir (Sigma, G2536), ESC clones were screened for homologous recombination by long-range PCR of the flanking regions of the 5′ and 3′ recombination arms from the genomic DNA. The PCR primers used to screen for the homologously recombined ESC clones are as follows:

P1F: 5′-GAGAGTGTGAGAATGTCAGCTTAGAC-3′
P1R: 5′-TTTCTAGAGAATAGGAACTTCACGCG-3′
P2F: 5′-GGCTCTATGGCTTCTGAGGCGGAAAG-3′
P2R: 5′-TCCCGCTCCACCGGCAGCCTTGTCAG-3′

To generate $Lmna^{flox}$ allele in which the puromycin cassette flanked by Frt sites is deleted, $Lmna^{fflox/+}$ mice were bred with ACTB-FLPe mice (#003800, JAX lab), which express a variant of FLP1 recombinase with enhanced thermal stability in a wide variety of tissues including germ cells. $Lmna^{flox/+}$ mice were identified by PCR of the flanking region of exon 2 of *Lmna*. PCR primers used to genotype all possible *Lmna* alleles in the resulting offspring are as follows:

P3F: 5′-AACCCAGCCTCAGAAACTGGTGGATG-3′
P3R: 5′-GACAGCTCTCCTCTGAAGTGCTTGGA-3′

$Lmna^{flox/+}$ mice were intercrossed to generate $Lmna^{flox/flox}$ mice, and maintained in a mixed genetic background from CD1, 129 Sv, and C57Bl/6 J strains.

To generate the $Lmna^{\Delta}$ allele, $Lmna^{flox/+}$ mice were bred with CMV-Cre mice (#003465, JAX lab). The generation of $Lmna^{\Delta}$ allele in the F1 generation of $Lmna^{flox/+} \times$ CMV-Cre mating pairs were screened by PCR of tail DNA with the primer pair P3F/R. CMV-Cre allele was genotyped according to the PCR protocol for generic Cre from the Jackson Laboratory. To remove the CMV-Cre allele present in the F1 generation, $Lmna^{\Delta/+}$; CMV-$Cre^{+/-}$ mice were outcrossed with 129S6/SvEvTac (Taconic). $Lmna^{\Delta/+}$ mice in the F2 generation that lacked the CMV-Cre allele were used to generate the $Lmna^{\Delta/\Delta}$ mice analyzed in this study. $Lmna^{+/+}$, $Lmna^{\Delta/+}$, and $Lmna^{\Delta/\Delta}$ mice were genotyped by PCR with the primer pair P3F/R. Mice used in this study are available upon request.

2.3. Isolation of MEFs

MEFs were generated by a standard protocol. Briefly, embryos were collected from the uteri of pregnant mice at embryonic day 13.5. The heads, visceral tissues and gonads of embryos were removed. The remaining embryonic bodies were minced and trypsinized twice. Fibroblasts from each embryo were harvested by centrifugation and then plated in MEF medium [15% FBS, 100 μM β-mercaptoethanol, 2 mM L-glutamine, 0.1 mM nonessential amino acids, 1 mM sodium pyruvate, 50 μg/ml penicillin/streptomycin in Knockout DMEM (Life Technologies)]. Passage 1 MEFs were used for immunofluorescence staining and Western blotting analysis. Genomic DNA isolated from embryo heads were used for PCR genotyping.