Biochemical and Biophysical Research Communications 425 (2012) 189–194

Contents lists available at SciVerse ScienceDirect

Biochemical and Biophysical Research Communications

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

Generation and characterization of Tmeff2 mutant mice

Tian Rui Chen [a,b], Ping Wang [a], Liberty K. Carroll [a], Ying-jiu Zhang [b], Bao-Xia Han [a], Fan Wang [a,*]

[a] Department of Cell Biology, Duke University Medical Center, Box 3709, Durham, NC 27710, USA
[b] Key Laboratory for Molecular Enzymology and Engineering of Ministry of Education, Jilin University, Changchun, China

ARTICLE INFO

Article history:
Received 13 July 2012
Available online 22 July 2012

Keywords:
Tmeff2
Mutant mouse
Central nervous system
Peripheral nervous system
Axon innervation
Growth retardation

ABSTRACT

TMEFF2 is a single-transmembrane protein containing one EGF-like and two follistatin-like domains. Some studies implicated TMEFF2 as a tumor suppressor for prostate and other cancers, whereas others reported TMEFF2 functioning as a growth factor for neurons and other cells. To gain insights into the apparently conflicting roles of TMEFF2, we generated a null allele of *Tmeff2* gene by replacing its first coding exon with human placental alkaline phosphatase cDNA (*Tmeff2*^{PLAP}). *Tmeff2*$^{PLAP/PLAP}$ homozygous mutant mice are born normal, but show growth retardation and die around weaning age. *Tmeff2* is widely expressed in the nervous system, and the *Tmeff2*^{PLAP} knock-in allele enables the visualization of neuronal innervations of skin and internal organs with a simple alkaline phosphatase staining. *Tmeff2* is also highly expressed in prostate gland and white adipose tissues (WAT). However, with the exception of reduced WAT mass, extensive anatomical and molecular analyses failed to detect any structural or molecular abnormalities in the brain, the spinal cord, the enteric nervous system, or the prostate in the *Tmeff2* mutants. No tumors were found in *Tmeff2*-mutant mice. The *Tmeff2*$^{PLAP/PLAP}$ knock-in mouse is an useful tool for studying the in vivo biological functions of TMEFF2.

1. Introduction

Tmeff2 gene encodes a protein with one epidermal growth factor (EGF) like domain, two follistatin-like domains, a single transmembrane domain, and a short cytoplasmic tail (also known as tomoregulin, TPEF and HPP1) [1–5]. Previous studies had reported conflicting functions of TMEFF2. Soluble form of TMEFF2 extracellular domain was shown to promote the survival of dopaminergic neurons [3] and cell growth in culture [6]. Consistent with the pro-survival role, elevated TMEFF2 expression has been associated with androgen-independent prostate cancers [5,7,8]. In contrast, others reported that TMEFF2 exhibited anti-proliferative effects on androgen-independent prostate cancer cell lines [9]. Furthermore, the promoter-region of TMEFF2 gene was frequently found to be hypermethylated in many cancers, suggesting a possible role of TMEFF2 as a tumor suppressor [2,9–17]. Additionally, the tumor suppressor activity of TMEFF2 was shown to depend on its cytoplasmic tail interacting with sarcosine dehydrogenase [18]. To gain insight into the in vivo physiological function of TMEFF2, we generated a null allele of *Tmeff2* gene by replacing the first coding exon of *Tmeff2* with cDNA encoding the human placental alkaline phosphatase (hPLAP). Here we report the results of anatomical and molecular characterizations of the Tmeff2-KO mice.

2. Materials and methods

2.1. Generation of Tmeff2^{PLAP} knock-in mouse

The *Tmeff2* genomic clone was subcloned using long-range PCR from genomic DNA of embryonic stem (ES) cells followed by sequencing. We constructed the targeting vector by inserting the hPLAP–ACN cassette [19] into the translation start ATG of the *Tmeff2* gene, and at the same time deleting the rest of exon 1. Targeted ES cells were generated and confirmed by Southern blotting. To detect the *Tmeff2*^{PLAP} mutant allele by PCR, PCR primers were designed as follows: TMF2–PLAP-F1, 5′-TCATGCTCTCCTTTGGTCGCAG-3′, TMF2–PLAP-B1, 5′-AAACATCTATGGTTCCCCACACC-3′, TMF2–PLAP-B2, 5′-GAGCCTCATTACCTGGGATGATG-3′. The wild-type allele produces a 537 bp fragment with TMF2–PLAP-F1 and TMF2–PLAP-B1 primers, whereas the mutant allele results in a 272 bp fragment with TMF2–PLAP-F1 and TMF2–PLAP-B2 primers. All experiments were conducted according procedures approved by The Duke University Institutional Animal Care and Use Committee.

2.2. Alkaline phosphatase staining for PLAP activity

AP-staining (PLAP-staining) was performed according to standard methods [19]. Briefly, the sections were inactivated at 65 °C for 6 h in PBS, and developed in staining solution (1:50 NBT/BCIP

* Corresponding author. Fax: +1 919 684 5481.
E-mail address: fan.wang@duke.edu (F. Wang).

0006-291X/$ - see front matter © 2012 Elsevier Inc. All rights reserved.
http://dx.doi.org/10.1016/j.bbrc.2012.07.064

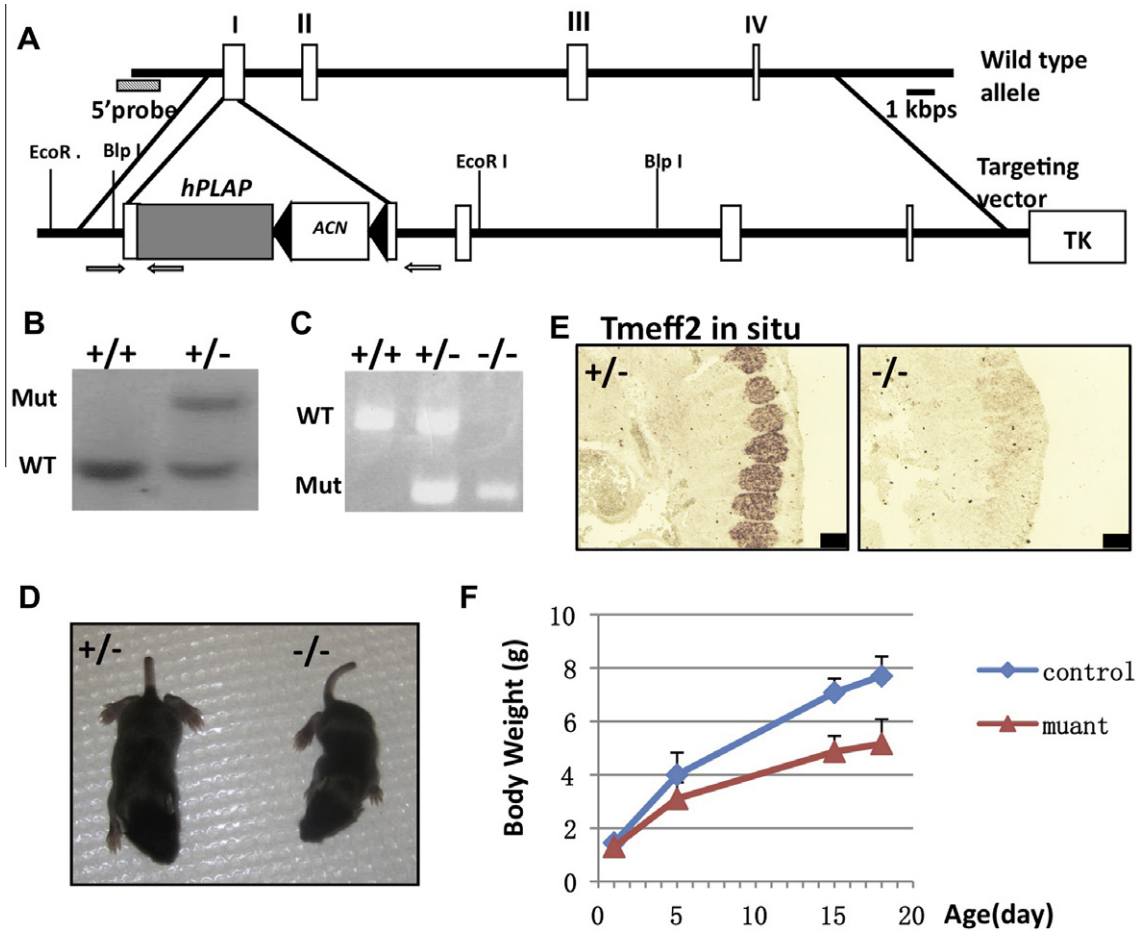

Fig. 1. Generating *Tmeff2*^{PLAP} knock-in allele and Tmeff2-KO mice. (A) Schematic representation of the targeting vector and strategy. cDNA encoding hPLAP together with the ACN (neo) cassette were used to replace start codon ATG and the rest of exon 1 of the Tmeff2 gene. Exons are represented as white boxes. The negative selection thymidine kinase cassette is designated as TK. Arrows indicate the position of primers used for PCR genotyping analysis. (B) Southern blot analysis of the genomic DNA from wildtype and targeted embryonic stem cells. (C) PCR analysis of the genotypes. (D) Compared to heterozygous littermate, Tmeff2-KO mice are smaller in size. (E) Representative images of Tmeff2 in situ hybridization results showing the expression of Tmeff2 in dorsal root ganglion (DRG) in control embryo, and the lack of in situ signal in mutant embryo. Scale bar: 100 μm. (F) The growth curve of Tmeff2$^{PLAP/+}$ (control) and Tmeff2-KO (mutant) mice (averaged from $n > 4$ for each genotype at each age).

stock solution (Roche), 0.1 M Tris–HCl, pH 9.5, 0.1 M NaCl, 5 mM $MgCl_2$).

2.3. In situ hybridization

The cDNA fragments used for in situ hybridization against choline acetyltransferase (ChAT), tyrosine hydroxylase (TH), vesicular glutamate transporter 1 (VGluT1), vesicular glutamate transporter 2 (VGluT2), glutamate decarboxylase 1 (GAD1), glutamate decarboxylase 2 (GAD2), parvalbumin (Pv), somatostatin (SST), transient receptor potential cation channel subfamily V member 1 (TrpV1), tachykinin 1 (Tac1), and neuronal nitric oxide synthase (Nos1) were individually cloned by PCR. In situ hybridization using DIG labeled probes was performed according to standard methods, and alkaline phosphatase (AP) conjugated anti-DIG antibody (Roche) was used to detect DIG.

2.4. Immunostaining

Standard immunofluorescence procedure was used. The following antibodies were used: anti-CGRP (calcitonin gene-related peptide) (1:2000; Millipore Bioscience Research Reagents/Invitrogen), anti-PGP9.5 (1:800; UltraClone), and Alexa 488-labeled anti-rabbit IgG (1:400; Invitrogen).

2.5. Oil red staining

Tissue sections were collected with a cryostat at 20 μm thickness. Sections or cultured cells were washed with PBS and fixed with PFA/PBS at room temperature for 20–30 min. Oil Red solution (60% oil red isopropanol (Electron Microscopy Sciences) and 40% dH_20) was used to stain the sections and cells for 20 min, followed by washing in PBS for three times.

2.6. In vitro differentiation of MEFs into adipocytes

Mouse embryonic fibroblasts (MEFs) were isolated from E13.5 embryos and cultured according to standard methods. In vitro induction of adipocyte differentiation was performed following a previous described method [20]. Briefly, MEFs were first cultured till complete confluence and maintained for additional 48 h. Afterward, the culture medium was replaced with induction medium (DMEM, 10% FBS, 1% NEAA, 1% Pep/Strep, 1% glutamine, 5 μg/ml insulin, 1 μM dexamethasone, 0.5 mM IBMX (3-isobutyl-1-methylxanthine), and 10 μM troglitazone) for 48 h. After induction, the cultures were placed in maintenance medium (DMEM, 10% FBS, 1% NEAA, 1% Pep/Strep, 1% glutamine, 5 μg/ml insulin, 1 μM dexamethasone, 0.5 mM IBMX (3-isobutyl-1-methylxanthine) and continued culture for 6–10 days. Oil Red staining was used to visualize the induced adipocytes.