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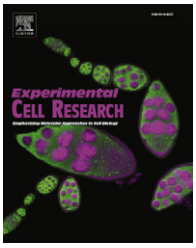

Research Article

Megakaryocyte development is normal in mice with targeted disruption of *Tescalcin*

*Supamit Ukarapong**, *Yong Bao*, *Erasmo M. Perera*, *Gary D. Berkovitz*

Division of Pediatric Endocrinology, University of Miami, Miller School of Medicine, 1601 NW 12th Avenue, Miami, FL 33136, USA

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ABSTRACT

BackgroundTescalcin is an EF-hand calcium-binding protein that interacts with the Na+/H+ exchanger 1 (NHE1). Levay and Slepak recently proposed a role for tescalcin in megakaryopoiesis that was independent of NHE1 activity. Their studies using K562 and HEL cell lines, and human CD34+ hematopoietic stem cells suggested an essential role for tescalcin in megakaryocyte differentiation.

ObjectiveTo study the role of tescalcin in megakaryocyte development using a murine model of megakaryopoiesis.

MethodsWe generated a mouse with targeted disruption of tescalcin and investigated megakaryocyte development.

ResultsTescalcin-deficient mice had a normal number of megakaryocytes and platelets. The morphology, polyploidization profile, and expression of Fli-1 in bone marrow-derived megakaryocytes were also normal.

ConclusionTescalcin does not appear to be necessary for normal megakaryocyte development.

Introduction

The tescalcin gene (*Tesc*) encodes an EF-hand calcium-binding protein and is a member of the calcineurin homologous protein (CHPs) family. Like the other members of this family, tescalcin is approximately 40% identical to the regulatory subunit of calcineurin [1,2], and all three CHPs (CHP1, CHP2, and tescalcin) inhibit calcineurin activity [2,3]. As tescalcin has both sequence and functional homology with CHP1 and CHP2, it has sometimes been referred to as CHP3 [4]. Each of the CHPs interacts with the proximal juxtamembrane C-terminal region of NHE1, and regulates activity of NHE1 as well as other NHE isoforms [4–6]. Various studies also indicate that tescalcin influences both the biosynthetic maturation and cell surface stability of NHE1 [4], consistent with the observation that tescalcin is a myristoylated protein and therefore likely to bind to the cell membrane [1,2].

Unlike the ubiquitously expressed *Chp1*, *Tesc* has a restricted pattern of expression in the embryonic and adult mouse [2,7]. NHE1 itself has been implicated in the control of cellular growth, differentiation and migration [8]. Although the precise role of tescalcin is unknown, it may influence cellular physiology through its interaction with NHE1. *Tesc* is strongly expressed in bone marrow, and a role for tescalcin in megakaryocyte development independent of NHE1 was proposed by Levay and Slepak. The authors studied megakaryopoiesis using various cell lines and concluded that tescalcin was essential for normal megakaryocyte development [9]. We developed a mouse with targeted disruption of tescalcin gene, and in this study, we report that tescalcin-deficient mice have normal megakaryopoiesis.

* *Corresponding author at:* 1601 NW 12th Ave. Suite 3044A, Miami, FL 33136, USA. Fax: +1 305 243 6309.
E-mail address: sukarapong@yahoo.com (S. Ukarapong).
Abbreviations: Tesc, Tescalcin gene.

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Materials and methods

Generation of Tesc$^{-/-}$ mice

Animal studies were performed in compliance with federal guidelines and with the University of Miami's IACUC guidelines for the use of animals. C57BL/6 inbred mice were purchased from Jackson Laboratories (Bar Harbor, Maine). Animals were housed in a temperature-controlled room with a 12-hour light/dark cycle in a pathogen free environment. Water and food were available *ad libitum*. To create *Tesc*$^{-/-}$ mutant mice, we deleted exons 3, 4, 5 and 6 by homologous recombination. Exons 4, 5 and 6 encode the single EF-hand domain of tescalcin [2]. To create the *Tesc* targeting vector, an 11.7 kb fragment containing the 1.2 kb short homology arm and the 7.5 kb long homology arm was subcloned from a BAC clone. Then, a 3.1 kb fragment containing exons 4, 5, and 6 was replaced by an inversely oriented neomycin resistance gene *(Neo)* (Fig. 1). The sequence of the targeting construct was confirmed by DNA sequencing.

The *Tesc* targeting vector was transfected into embryonic stem (ES) cells derived from a hybrid 129SvEv×C57BL/6 line by electroporation (inGenious Targeting Laboratory, Inc. Stony Brook, NY). Clones with the targeting vector integrated into their genome by homologous recombination were identified by PCR using primers A1 (5′-TCAGTCTAGTCAGGTGTCACATCC-3′) and UNI (5′-AGCGCATCGCCTTCTATCGCCTTC-3′) (Fig. 1), and by Southern blot analysis using a 5′ and a 3′ probe (Fig. 1). Cells from one ES clone were injected into C57BL/6 blastocysts and implanted into the uterus of foster mothers to generate chimeric mice. Male chimeras were mated with C57BL/6 females to produce heterozygotes (*Tesc* $^{+/-}$) mice. Heterozygous mice of mixed C57BL/6×129/SvEv background were backcrossed at least six generations into C57BL/6. Sixth generation heterozygote mice were then crossed to obtain homozygotes (*Tesc*$^{-/-}$) mice. The genotype of *Tesc*$^{-/-}$ mice was confirmed by Southern blotting as described above. Genotyping of successive generations was performed using multiplex PCR analysis of genomic DNA using *Tesc* primers C9-1Fd (5′-TGTATGATTCGGACAGTGACGG-3′) and C9-1F (5′-AGCCGACTCCTTTTCAATGTGAG-3′), and *Neo* primers as previously described [10]. We used mice between 8 and 10 weeks of age from the sixth generation for all studies.

Reverse-transcriptase PCR analysis (RT-PCR)

Total RNA was isolated from adrenal gland, testis, and heart of adult *Tesc*$^{+/+}$ and *Tesc*$^{-/-}$ mice using TRIzol reagent (Invitrogen, Carlsbad, CA) and treated with RNase free DNase (Promega, Madison, WI). cDNA was prepared from 1 μg of total RNA using SuperScript II Reverse Transcriptase and an Oligo dT primer (Invitrogen, Carlsbad, CA). Multiplex PCR amplification of *Tesc* and the internal control hypoxanthine–guanine phosphoribosyltransferase (*Hprt*) gene was performed using *Tesc* specific primers C9-1Fc (5′-GCGGCGCAC-CATGGGCGCT-3′) and C9-1Rc (5′-GAGGGCGATGGTCT CCATGTTGA-3′), and *Hprt* primers previously described [1]. For analysis of *CHP1* and *CHP2* expression, we extracted total RNA from K562 cells using the RNeasy Kit (Qiagen, Valencia, CA) and synthesized first strand cDNA as described above. PCR amplification was performed using *CHP1* (CHP1-F, 5′-TGCCTTCTTTCCAGAGGGAGAGGA-3′; CHP1-R, 5′-CCGACCATCATGCGTAGCAC CTG-3′ reverse) and *CHP2* (CHP2-F, 5′-AAGCTTCTTCCCCGATGGGAGCC-3′; CHP2-R, 5′-CCTGT ACCCCAACCATCAGACGGA-3′) specific primers.

Western blot analysis

Brain and heart tissue were dissected from *Tesc*$^{+/+}$ and *Tesc*$^{-/-}$ mice, and homogenized in RIPA buffer using a Polytron (Model PT10-35). The homogenates were centrifugated at 20,000×*g* for 20 min at 4 °C to remove cell debris, and protein concentration was determined in the supernatants using the Pierce 660 nm Protein Assay (Thermo Fisher Scientific, Rockford, IL). Equal quantities of protein were separated by electrophoresis through 12% SDS-polyacrylamide gels and transferred onto nitrocellulose. The membrane was blocked for 1 h at room temperature in TBS buffer

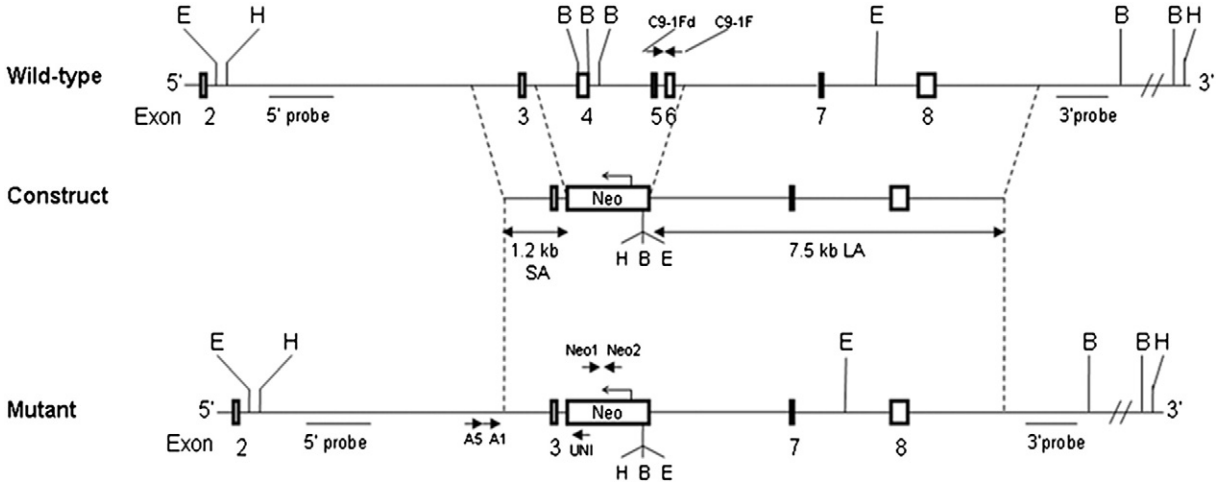

Fig. 1 – Generation of tescalcin-knockout mice. Partial genomic organization of wild type tescalcin gene with various restriction sites, the knockout construct, and the mutant allele are illustrated. The *Neo* cassette replaced exons 4, 5, and 6. Various primers used for genotyping (small single arrows), and probes for Southern blot analysis are indicated. Relevant restriction sites are BamH1, E: EcoR1, H: Hind III. The short arm (SA) and long arm (LA) of the knockout construct are indicated by the double arrows. The bent arrows on top of the *Neo* cassette indicate the orientation of *Neo* gene.