2020

The NEMP family supports metazoan fertility and nuclear envelope stiffness

Yonit Tsatskis

Ariz Mohammad

Karen Krchma

Jun Wu

Didier Hodzic

See next page for additional authors

Authors

Yonit Tsatskis, Ariz Mohammad, Karen Krchma, Jun Wu, Didier Hodzic, Daniel Potter, Tim Schedl, Kyunghee Choi, Helen McNeill, and et al

GENETICS

The NEMP family supports metazoan fertility and nuclear envelope stiffness

Yonit Tsatskis[1]*, Robyn Rosenfeld[1,2]*, Joel D. Pearson[1]*, Curtis Boswell[2,3]*, Yi Qu[1], Kyunga Kim[1,4], Lacramioara Fabian[5], Ariz Mohammad[6], Xian Wang[7,8], Michael I. Robson[9], Karen Krchma[10], Jun Wu[10], João Gonçalves[1], Didier Hodzic[11], Shu Wu[1], Daniel Potter[11], Laurence Pelletier[1,2], Wade H. Dunham[1], Anne-Claude Gingras[1,2], Yu Sun[7,8], Jin Meng[12], Dorothea Godt[12], Tim Schedl[6], Brian Ciruna[2,3], Kyunghee Choi[10,13], John R. B. Perry[14], Rod Bremner[1,15], Eric C. Schirmer[9], Julie A. Brill[2,16], Andrea Jurisicova[1,4,17]†, Helen McNeill[1,2,11]†

Human genome-wide association studies have linked single-nucleotide polymorphisms (SNPs) in NEMP1 (nuclear envelope membrane protein 1) with early menopause; however, it is unclear whether NEMP1 has any role in fertility. We show that whole-animal loss of NEMP1 homologs in Drosophila, Caenorhabditis elegans, zebrafish, and mice leads to sterility or early loss of fertility. Loss of Nemp leads to nuclear shaping defects, most prominently in the germ line. Biochemical, biophysical, and genetic studies reveal that NEMP proteins support the mechanical stiffness of the germline nuclear envelope via formation of a NEMP-EMERIN complex. These data indicate that the germline nuclear envelope has specialized mechanical properties and that NEMP proteins play essential and conserved roles in fertility.

INTRODUCTION

Reproductive life span in women is dictated by the finite number of germ cells created during fetal life and loss during aging (1). Premature ovarian insufficiency (i.e., menopause before age of 40) affects ~1 to 2% of women and is a leading cause of infertility in the Western world. Examination of published human genome-wide association studies data revealed that single-nucleotide polymorphisms (SNPs) near the NEMP1 (nuclear envelope membrane protein 1) locus show an association with reduced age at menopause (2, 3). We analyzed an additional cohort of 106,353 women from the UK Biobank study (4) and confirmed an association between NEMP1 and age of menopause, with the most significant association in a region ~300 kb away from NEMP1 (rs2277339, $P = 7 \times 10^{-51}$) (fig. S1A). Published Hi-C data from human ovaries indicate that rs2277339 physically interacts with the NEMP1 locus (fig. S1B), with chromatin changes consistent with the possibility that this is a distant enhancer element for NEMP1. In addition, we found an association ($P = 7 \times 10^{-3}$) with a previously unknown variant identified in the Genotype-Tissue Expression (GTEx) study (rs114352356) (5). This SNP encodes a rare nonsynonymous disruptive allele, which lies in the NEMP1 coding region, and is associated with 3.64-year (standard error 1.23) earlier onset of menopause (fig. S1A).

RESULTS

NEMP1 is a poorly understood, multi-transmembrane nuclear envelope (NE) protein with homologs found in all metazoans (Fig. 1A). No genetic models exist for Nemp function in any system. We first examined Drosophila, which has a single Nemp gene (CG9723). We found that dNemp is ubiquitously expressed in all examined larval and pupal tissues (fig. S2). Super-resolution microscopy revealed that dNemp localizes to inner nuclear membranes (INMs) (Fig. 1, B to D), as does Xenopus and human NEMP1 (6, 7).

We generated two independent dNemp mutants and found that $dNemp^{-/-}$ males and females are sterile (Fig. 1, E, F, and G, and fig. S3A). Loss of dNemp also resulted in ~40% pupal lethality (Fig. 1H). Bacterial artificial chromosome (BAC) rescue constructs containing the genomic region of dNemp (Fig. 1I) or tubulin-Gal4–driven expression of dNemp complementary DNA (cDNA) (Fig. 1, E and H) fully restored fertility and viability, confirming that these phenotypes are caused by the loss of dNemp. Human NEMP1 could rescue viability and partially rescue $dNemp^{-/-}$ sterility, suggesting that the role of NEMP in fertility may be conserved (Fig. 1, E and H, and fig. S3B).

$dNemp^{-/-}$ mutants had extremely small testes and ovaries (Fig. 1, F and G). Germline dNemp knockdown led to fewer germ cells in testes (Fig. 1J) and ovaries (Fig. 1K). Somatic knockdown also caused defects in gonad structure and infertility (fig. S3, C and D). Thus, dNemp is required by both somatic and germline cells for fertility.

In $dNemp^{-/-}$ testes, the stem cell niche and surrounding self-renewing germline and somatic stem cells appeared normal (figs. S3, E to H, and S4, A and B). However, subsequent development was disrupted, with a markedly reduced number of $Vasa^{+}$ germ cells (fig. S4, C and D), reduced proliferation (fig. S4, E to G), and disorganization

[1]Lunenfeld-Tanenbaum Research Institute, Sinai Health System, Toronto, ON M5G 1X5, Canada. [2]Department of Molecular Genetics, University of Toronto, Toronto, ON M5S 1A8, Canada. [3]Program in Developmental and Stem Cell Biology, The Hospital for Sick Children, Toronto, ON, Canada. [4]Department of Physiology, University of Toronto, 1 King's College Circle, Toronto, ON M5S 1A8, Canada. [5]Genome and Genome Biology Program, The Hospital for Sick Children, Toronto, ON M5G 0A4, Canada. [6]Department of Genetics, School of Medicine, Washington University School of Medicine, St. Louis, MO 63110, USA. [7]Department of Mechanical and Industrial Engineering, University of Toronto, Toronto, ON M5S 3G8, Canada. [8]Institute of Biomaterials and Biomedical, University of Toronto, Toronto, ON M5S 3G9, Canada. [9]Wellcome Centre for Cell Biology and Institute of Cell Biology, University of Edinburgh, Edinburgh EH9 3BF, UK. [10]Department of Pathology and Immunology, Washington University School of Medicine, St. Louis, MO 63110, USA. [11]Department of Developmental Biology, Washington University School of Medicine, St. Louis, MO 63110, USA. [12]Department of Cell and Systems Biology, University of Toronto, Toronto, ON, Canada. [13]Graduate School of Biotechnology, Kyung Hee University, Yong In, South Korea. [14]MRC Epidemiology Unit, Cambridge Biomedical Campus, University of Cambridge School of Clinical Medicine, Cambridge, UK. [15]Departments of Ophthalmology and Visual Science and Laboratory Medicine and Pathobiology, University of Toronto, Toronto, ON M5S 1A8, Canada. [16]Cell Biology Program, The Hospital for Sick Children, Toronto, ON M5G 0A4, Canada. [17]Department of Obstetrics and Gynecology, University of Toronto, Toronto, ON M5G 1E2, Canada.
*These authors contributed equally to this work.
†Corresponding author. Email: mcneillh@wustl.edu (H.M.); jurisicova@lunenfeld.ca (A.J.)