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Histone-lysine N-methyltransferase *SETDB1* is required for development of the bovine blastocyst

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ABSTRACT

Transcripts derived from select clades of transposable elements are among the first to appear in early mouse and human embryos, indicating transposable elements and the mechanisms that regulate their activity are fundamental to the establishment of the founding mammalian lineages. However, the mechanisms by which these parasitic sequences are involved in directing the developmental program are still poorly characterized. Transposable elements are regulated through epigenetic means, where combinatorial patterns of DNA methylation and histone 3 lysine 9 trimethylation (H3K9me3) suppress their transcription. From studies in rodents, SET domain bifurcated 1 (SETDB1) has emerged as the core methyltransferase responsible for marking transposable elements with H3K9me3 and temporally regulating their transcriptional activity. SETDB1 loss of function studies in mice reveal that although extraembryonic tissues do not require this methyltransferase, establishment of the embryo proper fails without it. As the bovine embryo initiates the processes of epigenetic programming earlier in the preimplantation phase, we sought to determine whether suppressing *SETDB1* would block the formation of the inner cell mass. We report here that bovine *SETDB1* transcripts are present throughout preimplantation development, and RNA interference–based depletion blocks embryo growth at the morula stage of development. Although we did not observe alterations in global histone methylation or transposable element transcription, we did observe increased global levels of H3K27 acetylation, an epigenetic mark associated with active enhancers. Our observations suggest that *SETDB1* might interact with the epigenetic machinery controlling enhancer function and that suppression of this methyltransferase may disrupt the bovine developmental program.

1. Introduction

Preimplantation development represents a critical window in the process of epigenetic programming as during the first few cleavage divisions, a major reorganization of chromatin structure occurs establishing the patterns of gene expression necessary to direct growth and differentiation of the mammalian embryo. This programming phase is one of the key events in the formation of the embryonic and extraembryonic cellular lineages [1,2]. Defects in the capacity of the embryo to properly establish this epigenetic program result in developmental failure or onset of disease because of inappropriate patterns of gene expression [3–5].

For the past 25 years, the preimplantation kinetics of DNA methylation have been the subject of intense study, while the dynamics of posttranslational histone methylation have remained poorly defined [6,7]. This is

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particularly true of the histone methylation machinery and processes hypothesized to demarcate the genome into regions of euchromatin and both constitutive and facultative heterochromatin. Within this realm, lysine 9 of histone 3 (H3K9) is a core target of multiple epigenetic modifiers implicated in controlling both gene transcription and supranucleosomal chromatin structure. For example, acetylated lysine 9 has been identified within the 5′ regions of transcriptionally active genes, whereas methylation at this specific residue has been linked to the nucleation of both transcriptional silencing and pericentric heterochromatin [8–11]. Interestingly, trimethylation (me3) of H3K9 is enriched within the regulatory regions and bodies of various families of transposable elements [12,13], and regulation of these parasitic nucleic acid sequences directly influences stem cell pluripotency in both primates and rodents [14–17]. Thus, understanding the mechanisms responsible for establishing and maintaining H3K9 epigenetic posttranslational marks is essential to better defining the processes of developmental programming and preimplantation lineage specification.

Six mammalian histone methyltransferases with demonstrated H3K9 catalytic activity have been identified, including EHMT1 (Glp), EHMT2 (G9a), SUV39H1, SUV39H2, SET domain bifurcated 1 (SETDB1; Eset), and SETDB2 [18]. SUV39H1 and SUV39H2 are responsible for the dynamics of pericentric heterochromatin formation [8], whereas EHMT1, EHMT2, and SETDB1 appear to regulate both protein-coding genes and transposable elements [19–21]. SETDB2 remains poorly characterized and may be involved in immune function [10]. Interestingly, although EHMT2 appears necessary to maintain DNA methylation at transposable elements, SETDB1 appears to be the core methyltransferase responsible for marking these sequences with H3K9me3 and temporally regulating their transcriptional activity [20,21]. Given the importance of transposable element sequences in regulating the pluripotency transcriptional program [14–17], better understanding the dynamics of SETDB1 during preimplantation development may be crucial to deciphering the processes directing specification of the embryonic lineage.

In the mouse, *SETDB1* transcripts (and possibly the maternal SETDB1 protein) are present in mature oocytes. Zygotic transcription of SETDB1 begins at the blastocyst stage and is essential for the survival of the mouse inner cell mass (ICM). In contrast, the extraembryonic tissues do not appear to require this methyltransferase as at Day 6.5 of gestation, null embryos display a fully developed trophoblast and ectoplacental cone, whereas the embryo proper shows a complete lack of development [22]. Given the established link between embryonic stem (ES) cell pluripotency and transposable elements, we sought to determine whether suppressing maternal SETDB1 transcripts would disrupt blastocyst formation by blocking the formation of an ICM. In mice, the transition from zygote to blastocyst takes 3.5 days with zygotic transcription initiating at the late one-cell to two-cell stage [23]. Although an RNA interference (RNAi)–based approach could be used to deplete SETDB1 messenger RNAs (mRNAs), stores of maternal SETDB1 are hypothesized to be present, and depending on the half-life of the protein, they may allow the formation of the ICM to proceed unperturbed [22]. In contrast, during bovine embryogenesis, the transition from zygote to blastocyst takes between 7 to 8 days to occur, with zygotic transcription beginning at the eight-cell stage [24]. This greater length of gestational time should allow opportunity to interrogate the consequences of *SETDB1* loss of function on both specification of the ICM and blastocyst formation. We report here that RNAi-based depletion of *SETDB1* transcripts blocks bovine preimplantation development at the morula stage and increases global levels of histone 3 lysine 27 (H3K27) acetylation, an epigenetic mark associated with active enhancers. Our results add one more piece of data to suggest that SETDB1 is essential for the earliest events in lineage specification.

2. Materials and methods

2.1. Embryo production and siRNA injection

Bovine oocytes were collected at a commercial abattoir (DeSoto Biosciences, Seymour, TN, USA) and shipped in an MOFA metal bead incubator (MOFA Global, Verona, WI, USA) at 38.5 °C overnight in sealed sterile vials containing 5% CO_2 in air-equilibrated Medium 199 with Earle's salts (Invitrogen, Life Technologies Inc., Carlsbad, CA, USA), supplemented with 10% fetal bovine serum (Hyclone, Logan, UT, USA), 1% penicillin–streptomycin (Invitrogen), 0.2-mM sodium pyruvate, 2-mM L-glutamine (Sigma Chemical Co., St. Louis, MO, USA), and 5.0 μg/mL of Folltropin (Vetoquinol, Pullman, WA, USA). The oocytes were matured in this medium for 22 to 24 hours. Matured oocytes were washed twice in warm Tyrode lactate (TL) HEPES (Vetoquinol) supplemented with 50 μg/μL of gentamicin (Invitrogen) while being handled on a 38.5 °C stage warmer (Minitube). *In vitro* fertilization was conducted using a 2-hour pre-equilibrated modified TL medium supplemented with 250-μM sodium pyruvate, 1% penicillin–streptomycin, 6 mg/mL of fatty acid–free BSA (Sigma), 20-μM penicillamine, 10-μM hypotaurine, and 10 μg/mL of heparin (Sigma) at 38.5 °C, 5% CO_2 in a humidified air incubator. Frozen semen was thawed at 35 °C for 1 minute, then separated by centrifugation at 200 × *g* for 20 minutes in a density gradient medium (Isolate; Irvine Scientific, Santa Ana, CA, USA) 50% upper and 90% lower. Supernatant was removed; sperm pellet was resuspended in 2-mL modified Tyrode's medium and centrifuged at 200 × *g* for 10 minutes to wash. The sperm pellet was removed and placed into a warm 0.65-mL microtube (VWR Scientific, Pittsburg, PA, USA) before bulk fertilizing in Nunclon four-well multidishes (VWR) containing up to 50 matured oocytes per well at a concentration of 1.0×10^6 sperm/mL. Sixteen to 18 hours after insemination, oocytes were cleaned of cumulus cells by a 2-minute vortex in 45-μL TL HEPES in a 0.65-mL microtube (VWR), washed in TL HEPES, and then randomly assigned to three different treatment groups: noninjected controls, nontargeting short interfering RNA (siRNA)–injected controls (siNULL), and injection with siRNA targeting *SETDB1* (si*SETDB1*).

Three siRNA sequences were designed to bovine *SETDB1*: *SETDB1* siRNA 545 (5′-CCGUGAAGCUAUGGCUGCC UUAAGA-3′), *SETDB1* siRNA 2799 (5′-CCUGAUGACCGAA