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Developmental Biology 312 (2007) 419–426
DEVELOPMENTAL BIOLOGY
www.elsevier.com/developmentalbiology

Centromeric DNA hypomethylation as an epigenetic signature discriminates between germ and somatic cell lineages

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Received for publication 14 June 2007; revised 20 September 2007; accepted 21 September 2007
Available online 29 September 2007

Abstract

Germ cells have unique features strikingly distinguishable from somatic cells. The functional divergence between these two cell lineages has been postulated to result from epigenetic mechanisms. Here we show that the chromosomal centric and pericentric (C/P) regions in male and female germline cells are specifically DNA-hypomethylated, despite the hypermethylation status in somatic cells. In multipotent germline stem cells, the C/P region was initially hypomethylated and then shifted to the hypermethylation status during differentiation into somatic lineage *in vitro*. Moreover, the somatic-type hypermethylation pattern was maintained in the somatic cell-derived nuclear transfer embryos throughout preimplantation development. These results imply that the identity of germ cell lineage may be warranted by the hypomethylation status of the C/P region as an epigenetic signature.

Keywords: Mouse; DNA methylation; Germ cell lineage; Somatic cell cloning; Centric/pericentric region; Epigenetics

Introduction

Germ cells capable of transmitting genetic information to next generation have the totipotency acquired by germ-cell-specific nuclear events, including meiotic division and global reprogramming processes. In mouse embryogenesis, primordial germ cells (PGCs), which are present in a posterior region of the primitive streak in the extra-embryonic mesoderm, migrate into the embryonic mesoderm, enter into the genital ridge at embryonic day 10.5 (E10.5), and continue to proliferate mitotically until E13.5. Female PGCs enter the prophase of first meiotic division around E13.5, where they are arrested until the later stages during the postnatal life (McLaren, 1994). Male PGCs, on the other hand, enter the mitotic arrest around E13.5 and resume proliferation postnatally. Germ cells are distinguished from somatic cells by histological characteristics and specific gene expression during embryogenesis (Extavour and Akam, 2003). It is also known that the epigenetic features of germ and somatic cells discriminate between these two cell lineages (Shiota et al., 2002). However, the definitive conclusion on the epigenetic regulation has not yet been drawn.

Cytosine methylation of CpG dinucleotides is a major epigenetic modification of DNA and plays a crucial role(s) in gene regulation such as X chromosome inactivation, genome imprinting, and inactivation of transposable elements (Li, 2002). *De novo* methylation of particular genes is also thought to control the cell differentiation process during embryogenesis (Reik et al., 2001). In this study, to elucidate the epigenetic features involved in the functional divergences between germ and somatic cells, we have focused on the DNA methylation status of chromosomal centric and pericentric (C/P) regions, owing to germ cell-dominant expression of the pericentric transcripts (She et al., 2004).

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doi:10.1016/j.ydbio.2007.09.041

Materials and methods

Southern blot analysis

Frozen mouse tissues (0.1 g) were crushed, placed in a lysis buffer (1 ml) consisting of 50 mM Tris/HCl, pH 8.0, 0.1 M NaCl, 20 mM EDTA, 1% SDS, proteinase K (0.1 mg/ml), and RNase A (0.08 mg/ml) and incubated at 50 °C with gentle rocking overnight. In epididymal sperm, 2-mercaptoethanol was added to the lysis buffer at a final concentration of 0.5% to reduce the disulfide bonds of the nuclear proteins. Genomic DNA was extracted with phenol-chloroform, precipitated with ethanol, and dissolved in 10 mM Tris/HCl, pH 8.0, containing 1 mM EDTA (TE). The DNA samples were digested at 37 °C with *Msp*I or *Hpa*II, separated by electrophoresis on agarose gels (50 ng DNA/lane), transferred to Hybond-N^+ membranes (GE Healthcare Bio-Sciences, Piscataway, NJ), and hybridized to ^{32}P-labeled probes at 55 °C overnight, as described previously (Kashiwabara et al., 2002). The DNA probes were amplified by polymerase chain reaction (PCR) using the following sets of oligonucleotide primers: 5′-GACGACTTGAAAAATGACGAAATC-3′ and 5′-CATATTCCAGGTCCTTCAGTGTGC-3′ for major satellite; 5′-CATGGAAAATGATAAAAACC-3′ and 5′-CATCTAATATGTTCTACAGTGTGG-3′ for minor satellite; 5′-ACATTCGCCGTTACAAGATGGCGCTGA-3′ and 5′-AATTGTTATTAGACGCGTTCTCACGCC-3′ for intracisternal A particle (IAP); 5′-ATCTTGGTCCCGGGACTCCAAGGAACTTA-3′ and 5′-GTTAGTAGTTATAGTTGACTCTGTTTAGAG-3′ for long interspersed nuclear elements 1 (LINE1 or L1 sequences).

Cell collection

Preimplantation embryos were prepared by fertilizing epididymal sperm with metaphase II-arrested eggs from 8-week-old ICR mice (Japan SLC, Shizuoka, Japan) *in vitro* and incubated in KSOM medium (Lawitts and Biggers, 1993), as described previously (Yamagata et al., 2005). Embryos at E6.5, E7.5, and E10.5 were obtained from the uterus of naturally mated ICR mice. Spermatogenic cells were dispersed from seminiferous tubules of ICR mice in Hepes/CZB medium (Kimura and Yanagimachi, 1995) by gentle pipetting, separated into various differentiation stages, and collected one by one

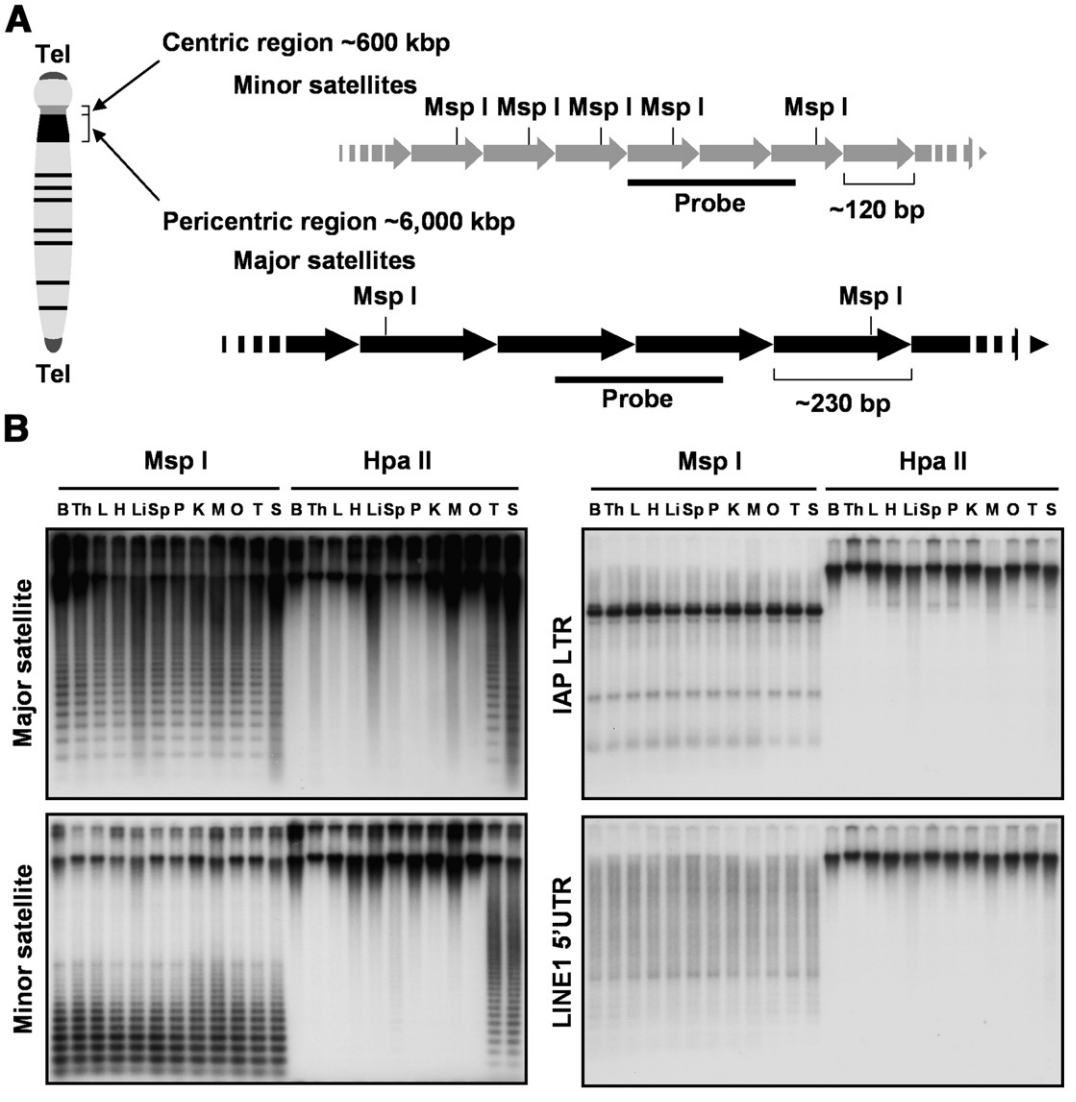

Fig. 1. DNA methylation status of major and minor satellites in mouse tissues. (A) The C/P region containing the major and minor satellites on mouse chromosome. The minor satellites consisting of 120-bp monomers (arrows) are tandemly repeated over approximately 600 kbp on the chromosome. The major satellite region (approximately 6000 kbp) is formed by 230-bp monomer repeats. Bars represent the DNA probes for Southern blot analysis. Note that the *Msp*I site (CCGG) is frequently present in the minor satellite. Tel, telomere. (B) Southern blot analysis. Mouse genomic DNAs from various tissues and epididymal sperm were digested with methylation-sensitive (*Hpa*II) or insensitive (*Msp*I) restriction enzyme and hybridized to DNA fragments of the major satellite, minor satellite, IAP LTR, and 5′-untranslated region of LINE1 (5′UTR). B, brain; Th, thymus; L, lung; H, heart; Li, liver; Sp, spleen; P, pancreas; K, kidney; M, muscle; O, ovary; T, testis: S, sperm.