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Meic1 is a putative novel member of meiosis-specific nuclear structural proteins

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ABSTRACT

Molecular mechanism underlying initiation and progression of meiosis in mammals is still largely unknown. To clarify the molecular mechanism, we isolated genes whose expression is increased at onset of meiosis by subtraction screening. Here, we identified a novel gene, named *Meic1* (meiosis-specific cohesin-like protein 1), encoding putative nuclear structural proteins. *Meic1* transcript was detectable only in embryonic day (E) 13.5 female gonads and testes. Deduced amino acid sequence encoded *Meic1* possesses a domain similar to SMC domain, which functions in chromosomal segregation during cell division. These findings indicated that *Meic1* is a novel member of molecules required for construction of meiosis-specific nuclear structure.

Key Words: *meiosis, synaptonemal complex, cohesin*

INTRODUCTION

Germ cell lineage is a sole route through which genetic information transmit into next generation. To properly transmit the genetic information, unique cellular events, such as epigenetic reprogramming, meiosis and morphological alteration, are evoked exclusively in germ cell lineage. In these events, meiosis is critical not only to reduce the ploidy of the genome, but also

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to generate genomic diversity by shuffling homologous chromosome. To accomplish the meiosis, pairs of homologous chromosomes must align during meiotic prophase, and then exchange DNA strand between homologous chromosomes. Meiosis-specific nuclear structural components, such as synaptonemal complex (SCP) and cohesin, function as glue between homologous chromosomes and ensure accurate paring of the chromosome. In mice, recent reports have shown that disruption of the meiosis- specific nuclear structural proteins causes sterility and/or aneuploidy [1-3]. Taking account into a current view that spontaneously pregnant loss in human is caused by impaired chromosomal segregation during meiosis, it seems to be important to fully clarify the molecules consisting of the meiosis-specific nuclear structure that has been remained unclear.

In mice, meiosis is induced in primordial germ cells (PGCs) at embryonic day (E) 13.5 in female or in postnatal testis in male. Although observed the sex-dependent timing of meiotic initiation in vivo, it has been thought that the initiation is cell-autonomously programmed in PGCs, based on evidence that meiosis was simultaneously induced in PGCs of both sexes according to time course of female, when PGCs were dissociated from gonadal somatic cells and then cultured in vitro [4, 5]. Additionally, it has reported that E11.5 female PGCs transferred into E11.5 male gonad could not enter into meiosis at E13.5, and, in contrary, E11.5 male PGCs transferred into E11.5 female entered into meiosis at E13.5 [4]. These findings suggest that the initiation of meiosis was intrinsically programmed in PGCs, and the exertion of program was inhibited by signals from somatic cells in male gonad.

Because meiosis was synchronously progressing in female PGCs, the cells are suitable to identify species-specific molecule underlying meiosis. In this study, we isolated a novel gene whose expression is observed exclusively in germ cells entering meiosis, and showed a possibility that the gene functioned as a nuclear structural protein maintaining meiosis-specific chromosomal segregation.

MATERIALS AND METHODS

Purification of PGCs and cDNA synthesis

Fetal gonads were collected from E11.5 or E13.5 embryos generated by mating between Oct3/4-EGFP transgenic male mice [6] and ICR female mice. Female gonads were selected, incubated with trypsin EDTA solution (Invitrogen) for 10min at 37°C, and prepared into single cell suspension. PGCs were

separated from gonadal somatic cells by cell sorting performed by FACS. mRNAs were extracted from the FACS-sorted PGCs at E13.5 and E11.5 by FastTrack™ 2.0 (Invitrogen) and then were subjected to cDNA synthesis by SMART cDNA synthesis kit (BD Biosciences).

Subtraction screening and RACE amplification

Subtraction screening by using the synthesized cDNA was performed according to Clontech PCR-Select™ cDNA subtraction kit. To isolate full length of *Meic1* gene, RACE amplification was performed by Marathon cDNA amplification kit (CLONTECH) and using specific primers as follows: TTT CTG TGT GGA CCC ATA CTG CCC for 5' RACE of *Meic1*, and TAC AGA TCC TTG CTC ATA GTA CGC for 3' RACE of *Meic1*. Sequences of isolated fragments were determined by using BigDye Terminator v1.1 Cycle Sequencing kit (Applied Biosystems).

In situ hybridization

In situ hybridization was performed as described in previously reports [7]. Briefly, fetal gonads were collected, fixed in 4% paraformaldehyde at 4°C for 12h, dehydrated in methanol, and hybridized with DIG-labbeled *meisetz/mPRDM9* RNA probe that was synthesized by using plasmids isolated by subtraction screening. After wash, the hybridized probes were visualized by incubation with anti-DIG antibody conjugated with AP (Roche), followed by reaction with BM substrate (Roche).

Northern hybridization

Total RNAs were extracted from each tissue by Trizol reagent (Invitrogen). The total RNAs (10μg) were separated by electrophoresis in 1.2% denaturing agarose gels, transferred onto nylon membrane, and hybridized with ^{32}P-labbeled *Meic1* cDNA fragment isolated by subtraction screening. The hybridized membrane was washed, and then ^{32}P activity on the membrane was determined by BAS2000 imaging analyzer (Fuji film)

RESULTS AND DISCUSSION

Genes whose expression is exclusively increased at onset of meiosis, was enriched by subtracting cDNAs of mitotic female primordial germ cells (PGCs) at embryonic day (E) 11.5 from those of meiotic female PGCs at E13.5.

A number of genes (400 clones) identified by the screening were sequenced and compared by NCBI BLAST search. By the comparison, functionally unknown genes were selected. Next, the nucleotide

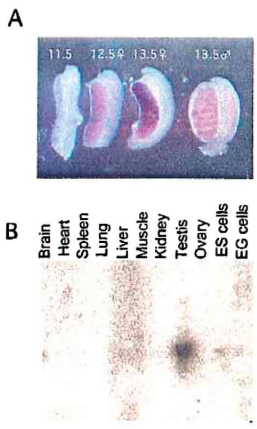

Fig. 1 Expression analysis of *Meic1* transcript. (**A**) *In situ* hybridization analysis of *Meic1* expression in fetal gonads. Developmental stage and sex of the gonads were indicated in the figure. Sex of the gonads was determined by seminiferous tubules formation. (**B**) Northern blot analysis of *Meic1* expression in adult tissues indicated. *Gapdh* is shown as a control.

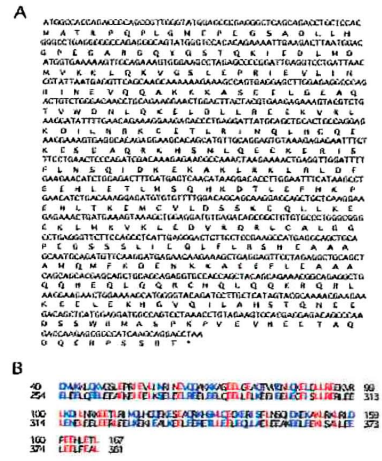

Fig. 2. Nucleotide and amino acid sequences of *Meic1* gene
(A) Shown are nucleotide (upper) and amino acid (lower) sequences of *Meic1*. (B) Comparison to CDD by BLAST search. Shown are alignment of amino acid sequences of Meic1 (upper) and typical SMC domain (lower). Conserved and similar amino acid residues are highlighted in red and blue, respectively.

sequences of the genes were compared with EST database, and the genes whose transcripts were detectable preferentially in EST database from germ cell lineages were selected. Finally, deduced amino acid sequences encoded the selected genes were compared to the Conserved Domain Database (CDD, http://www.ncbi.nlm.nih.gov/Structure/cdd/cdd.shtml) by BLAST search to predict the functions of the genes.

By the criteria described above, we focused on a gene, named *Meic1* (meiosis-specific cohesin-like protein 1). First, we assessed whether the transcript was expressed exclusively in germ cells entering meiosis. *In situ* hybridization analyses revealed, as expected, that *Meic1* transcript was detectable only in E13.5 female gonads but not in male gonads (Figure 1A). Consistent with a fact that maturation of germ cells in fetal female gonad occurs in an anterior-to-posterior wave [8], *Meic1* transcript was more strongly expressed in the anterior part than in the posterior part of the gonad at E13.5 (Figure 1A). In adult tissues, *Meic1* transcript was detectable only in testis (Figure 1B). These results confirmed that *Meic1* transcript was exclusively expressed at onset of meiosis, and suggested that *Meic1* play important role in meiosis.

Full length of *Meic1* transcript was

isolated by RACE amplification. As the results, *Meic1* gene encodes 987 bp of open reading frame (Figure 2A) and is located on distal region of chromosome 7 in which a cluster of imprinting genes are located. Comparison to CDD by BLAST search revealed that deduced amino acid sequence encoded by *Meic1* gene possess a domain similar to SMC domain (Figure 2B). It has been characterized that proteins possessing SMC domain play important roles on maintenance of chromosomal structure during meiosis, as well as mitosis [9-13]. During meiotic prophase, characteristic nuclear structural proteins were emerged in nuclei, resulting in synaptonemal complex formation. Until now, it has been clarified that a number of molecules, such as cohesin, components of synaptonemal complex, and DNA recombination molecules and cyclin-related molecule are essential to properly form synaptonemal complex. In addition to the previously known molecules, *Meic1* may be a novel member of meiosis-specific nuclear structural proteins, based on criteria of the expression pattern and the possession of SMC domain. In yeast, it has intensively investigated that meiosis-specific cohesin is critical for segregation of each homologous chromosome and sister chromosome during meiosis MI and MII phase, respectively [11, 12, 14]. Recently, also in mammals, it has suggested that meiosis-specific cohesin is dispensable for proper chromosomal segregation during meiosis [3, 13, 15-17]. We speculate that *Meic1* also play an important role on chromosomal segregation during meiosis by interacting with the cohesions. The possibility will be verified by further experiment by biochemical method and analysis of *Meic1* deficient mice.

Here, we isolated a novel member of meiosis-specific structural proteins. By BLAST search, homologue of this protein was also detectable in other organisms including human (data not shown). These facts raise a possibility that loss of these genes might cause abnormal chromosomal segregation resulting in spontaneous pregnant loss in human. This possibility will be also evaluated by functional analysis of this gene.

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REFERENCES

1) Yuan, L., Liu, J. G., Zhao, J., Brundell, E., Daneholt, B. & Hoog, C. The murine SCP3 gene is required for synaptonemal complex assembly, chromosome synapsis, and male fertility. *Mol. Cell* 5, 73-83, 2000.
2) Yuan, L., Liu, J. G., Hoja, M. R., Wilbertz, J., Nordqvist, K. & Hoog, C. Female germ cell aneuploidy and embryo death in mice lacking the meiosis-specific protein SCP3. *Science* 296, 1115-1118, 2002.
3) Bannister, L. A., Reinholdt, L. G., Munroe, R. J. & Schimenti, J. C. Positional cloning and characterization of mouse mei8, a disrupted allelle of the meiotic cohesin Rec8. *Genesis* 40, 184-194, 2004.
4) McLaren, A. & Southee, D. Entry of mouse embryonic germ cells into meiosis. *Dev. Biol.* 187, 107-113, 1997.
5) Chuma, S. & Nakatsuji, N. Autonomous transition into meiosis of mouse fetal germ cells in vitro and its inhibition by gp130-mediated signaling. *Dev. Biol.* 229, 468-479, 2001.
6) Yoshimizu, T., Sugiyama, N., De Felice, M., Yeom, Y. I., Ohbo, K., Masuko, K., Obinata, M., Abe, K., Scholer, H. R. & Matsui, Y. Germline-specific expression of the Oct-4/green fluorescent protein (GFP) transgene in mice. *Dev. Growth. Differ.* 41, 675-684, 1999.
7) Yamamoto, M. & Matsui, Y. Testis-specific expression of a novel mouse defensin-like gene, Tdl. *Mech. Dev.* 116, 217-221, 2002.
8) Menke, D. B., Koubova, J. & Page, D. C. Sexual differentiation of germ cells in XX mouse gonads occurs in an anterior-to-posterior wave. *Dev. Biol.* 262, 303-312, 2003.
9) Michaelis, C., Ciosk, R. & Nasmyth, K. Cohesins: chromosomal proteins that prevent premature separation of sister chromatids. *Cell* 91, 35-45, 1997.
10) Toth, A., Ciosk, R., Uhlmann, F., Galova, M., Schleiffer, A. & Nasmyth, K. Yeast cohesin complex requires a conserved protein, Eco1p(Ctf7), to establish cohesion between sister chromatids during DNA replication. *Genes Dev.* 13, 320-333, 1999.
11) Klein, F., Mahr, P., Galova, M., Buonomo, S. B., Michaelis, C., Nairz, K. & Nasmyth, K. A central role for cohesins in sister chromatid cohesion, formation of axial elements, and recombination during yeast meiosis. *Cell* 98, 91-103, 1999.
12) Watanabe, Y. & Nurse, P. Cohesin Rec8 is required for reductional chromosome segregation at meiosis. *Nature* 400, 461-464, 1999.

13) Revenkova, E., Eijpe, M., Heyting, C., Gross, B. & Jessberger, R. Novel meiosis-specific isoform of mammalian SMC1. *Mol. Cell Biol.* 21, 6984-6998, 2001.

14) Buonomo, S. B., Clyne, R. K., Fuchs, J., Loidl, J., Uhlmann, F. & Nasmyth, K. Disjunction of homologous chromosomes in meiosis I depends on proteolytic cleavage of the meiotic cohesin Rec8 by separin. *Cell* 103, 387-398, 2000.

15) Prieto, I., Suja, J. A., Pezzi, N., Kremer, L., Martinez, A. C., Rufas, J. S. & Barbero, J. L. Mammalian STAG3 is a cohesin specific to sister chromatid arms in meiosis I. *Nat. Cell Biol.* 3, 761-766, 2001.

16) Parisi, S., McKay, M. J., Molnar, M., Thompson, M. A., van der Spek, P. J., van Drunen-Schoenmaker, E., Kanaar, R., Lehmann, E., Hoeijmakers, J. H. & Kohli, J. Rec8p, a meiotic recombination and sister chromatid cohesion phosphoprotein of the Rad21p family conserved from fission yeast to humans. *Mol. Cell Biol.* 19, 3515-3528, 1999.

17) Lee, J., Yokota, T. & Yamashita, M. Analyses of mRNA expression patterns of cohesin subunits Rad21 and Rec8 in mice: germ cell-specific expression of rec8 mRNA in both male and female mice. *Zool. Sci.* 19, 53, 2002.