A human tetraploid pachytene spermatocyte as the possible origin of diploid sperm: a case report

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Diploid spermatozoa represent 0.2–0.3% of all spermatozoa in the normal population and cause 8.3% of diandric triploids. Errors in meiosis I and II are the most common mechanisms by which diploid spermatozoa are produced. Endoreduplication before meiosis has been suggested as a possible origin for tetraploid meiocytes, which might, in turn, produce diploid sperm. Synaptonemal complex (SC) spreads of a fertile man were immunolabelled (SCP3, MLH1 and CENP) and hybridized with subtelomere-specific multiplex fluorescent in situ hybridization (stM-FISH) assay for SCs identification. The unexpected finding of a tetraploid pachytene cell and the identification of all of its SCs demonstrate that synapsis and crossover events can occur in human tetraploid cells. Moreover, it indicates that diploid sperm may also originate from mitotic errors (endoreduplication) occurring before meiosis.

Key words: crossing over/diploid sperm/stM-FISH/synaptonemal complex/tetraploid pachytene

Introduction

Human triploidy is one of the most frequent chromosomal abnormalities and is responsible for about 10% of all spontaneous abortions. Paternal origin of triploids is more frequent than maternal origin, and it generally occurs as a consequence of dispermy (McFadden et al., 2002). Nevertheless, diploid sperm cause 8.3% of diandric triploids. Diploid spermatozoa represent 0.2–0.3% of all spermatozoa in the normal population. Errors in meiosis I and II are the most common mechanisms by which diploid spermatozoa are produced (Zaragoza et al., 2000; Egozcue et al., 2002). It has been suggested that an endoreduplication, DNA duplication without cell division, occurring before meiosis could result in tetraploid meiocytes (Miller and Therman, 2001), which consequently might produce diploid sperm. Synapsis and crossing over between homologous chromosomes are two essential processes that must occur in meiocytes to allow meiosis to proceed. Herein is described the observation of a tetraploid pachytene spermatocyte from a fertile male in which synapsis and crossover events are observed. This could support the possible role of endoreduplication in spermatogonia as a possible origin of some diploid sperm.

Case report

A testicular biopsy was obtained from a fertile man while undergoing a vasectomy under local anaesthesia. Written consent was obtained from the patient, and the study was approved by our Institutional Ethics Committee. The biopsy was processed for synaptonemal complex (SC) spreads. These were immunolabelled with anti-SCP3, anti-MLH1 and CREST serum and subsequently hybridized with the seven-fluorochrome subtelomere-specific multiplex fluorescent in situ hybridization (stM-FISH) assay for SCs identification, as described elsewhere (Codina-Pascual et al., 2004). The identification of all SCs was performed by the projection of the stM-FISH hybridization result into the image of the immunolabelled pachytene cell.

Results and discussion

During the analysis of 105 immunolabelled pachytene cells, a nucleus with an unexpected appearance was found. The cell seemed to have twice the number of SCs as that of a normal pachytene cell (i.e. 46). Hybridization with the seven-fluorochrome stM-FISH assay allowed for the identification of all the SCs of the cell and confirmed that it was a tetraploid pachytene cell (Figure 1A).

Although in human meiotic chromosome preparations it is not unusual to find metaphase I nuclei which are apparently tetraploid, it is impossible to demonstrate that this appearance does not result from the mix-up of bivalents from two normal metaphases because, as is well known, meiosis occurs in waves on a given Sertoli cell. To the best of our knowledge, in mammals, only one tetraploid pachytene cell has been reported (Solari and Moses, 1977). This single example in mice and the nucleus herein shown, both observed among hundreds of spermatocytes analysed, indicate that tetraploidy in spermatocytes is a rare event.
Figure 1. (A) Tetraploid pachytene cell observed in a fertile man with all synaptonemal complexes (SC) identified by subtelomere-specific multiplex fluorescent in situ hybridization. SCs are in red, MLH1 foci in green and centromeres in blue. (B) Representation of the five quadrivalents of the cell. Each one of the four homologous chromosomes in the quadrivalent is drawn in a different colour. Centromeres are in black. (C) Representation of the four partially or totally unsynapsed SCs and their fully synapsed SC partner. Each one of the four homologous chromosomes is drawn in a different colour. (D) Representation of the partially synapsed Y bivalent and the fully synapsed X bivalent. (E) SC karyotype of the tetraploid pachytene cell.
This pachytene nucleus does demonstrate that human tetraploid cells can enter meiosis, and that synopsis and meiotic recombination can take place even when the genetic material is duplicated. In this cell, heterologous synopsis was not observed. Most autosomal chromosomes were present as two bivalents. Nevertheless, five quadrivalent structures were observed, which corresponded to the four chromosomes 1, 2, 9, 16 and 20 (Figure 1A and B). Unlike in a normal pachytene cell, the sex chromosomes formed two separate bivalents (Figure 1A and D). The X bivalent synapsed completely, forming an entire SC. The Y bivalent synapsed by the short arms, but the long arms remained unsynapsed. It is worth noting that each X and Y bivalent, in this tetraploid cell, contains two identical chromosomes.

The fact that the quadrivalents and some SCs were not yet fully synapsed (Figure 1A and C) indicates that this nucleus might be in an early pachytene stage. The unsynapsed regions of quadrivalents 1, 9 and 16 and the bivalent Y correspond to non-centromeric heterochromatic blocks, which have been described as the last regions to synapse (Solari et al., 1991; Codina-Pascual et al., 2006b). Moreover, heterochromatin has been proposed to act as organizing centre in the interface nucleus from animals and plants (van Driel and Fransz, 2004). Therefore, the quadrivalents of chromosomes 1, 9 and 16 and the bivalent Y largely unsynapsed in this tetraploid cell could be consequences of a structural role of non-centromeric heterochromatin blocks in the early prophase I nucleus.

In this tetraploid pachytene, meiotic recombination foci (MLH1) were present at places where synopsis had occurred. The cell had 73 MLH1 foci, i.e. more than the range described for controls (42.9–52.3) (Codina-Pascual et al., 2005) but less than twice the mean of MLH1 foci for this man (42.5, Codina-Pascual et al., 2006a). MLH1 foci were distributed similarly to a normal pachytene cell, generally, one MLH1 focus per arm in a distal location; but it is interesting to note that a different localization of MLH1 foci in some “homologous” SCs in this tetraploid cell is observed (SC7s, SC10s, SC12s, SC19s and SC21s in Figure 1E). Therefore, the variable localization of crossover events observed in a given SC among different cells (Sun et al., 2004; Codina-Pascual et al., 2006b) can also occur in a single tetraploid cell. The Y bivalent had one MLH1 focus in the short synapsed arm, possibly in the PAR1 region. The X bivalent presented one MLH1 focus in each arm with a distal distribution similar to that for the C-group chromosomes.

For years, the origin of human diploid spermatozoas has been explained by errors in meiosis I and II (Zaragoza et al., 2000; Egozcue et al., 2002). Endoreduplication was suggested to be another possible mechanism for the formation of tetraploid meiocytes (Miller and Therman, 2001), but that has never been proven. The tetraploid pachytene cell shown in this work provides evidence that diploid sperm may also originate as a consequence of mitotic errors occurring before meiosis and not only by non-disjunction at meiosis I or II.

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References

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