Letters to the Editor

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Molecular Cytogenetic Detection of Confined Gonadal Mosaicism in a Conceptus with Trisomy 16 Placental Mosaicism

To the Editor:

Confined placental mosaicism (CPM) is a dichotomy between the chromosomal constitution of the placental and embryonic/fetal tissues, observed in 1%–2% of all delivered pregnancies, and most commonly involves a trisomic clone confined to the placenta (Kalousek 1990). CPM has been shown to exist in three different forms (types I–III), depending on its origin and the placental cell lineages involved. In a diploid zygote, the trisomic cell line in CPM can arise from mitotic duplication of one chromosome in a specific placental cell lineage (either trophoblast or chorionic stroma), giving rise to type I or type II CPM. Rescue of a trisomic zygote, owing to chromosome loss by a postzygotic mitotic error in the embryonic progenitor cells, leads to trisomic cell-line expression in both placental lineages and is termed "type III CPM" (Kalousek et al. 1993; Robinson et al. 1997).

Embryological literature provides evidence that the chorionic stroma of the placenta and the primordial germ cells (PGCs) of the embryonic gonads share common progenitor cells (Buehr 1997), suggesting that conceptuses diagnosed with CPM involving the placental stroma may be at increased risk for gonadal mosaicism. We describe the conventional cytogenetic, molecular cytogenetic, and molecular genetic analyses of multiple fetal and placental tissues from a conceptus diagnosed with trisomy 16 placental mosaicism. Our results demonstrate the presence of trisomy 16 mosaicism in the placenta and disomy for chromosome 16 in all fetal tissues studied, except oocytes, which show mosaicism with a significant level of trisomy 16. This is the first published data documenting the existence of germ-cell mosaicism in an otherwise nonmosaic fetus, for a conceptus diagnosed with CPM.

Fresh fetal and placental tissues were obtained from a conceptus therapeutically aborted at 12 wk of gestational age and prenatally diagnosed with 100% trisomy 16 by means of cultured chorionic villus stroma. Conventional cytogenetic analysis, FISH, and microsatellite analysis were used to study the distribution of the trisomy 16 cell line in the conceptus. This study was approved by the Clinical Research Ethics Board of the University of British Columbia.

Trypsin G–banded metaphase chromosomes from cultured amnion and placental stroma were prepared in accordance with standard procedures. Trophoblast and stromal cell suspensions for FISH were obtained from chorionic villi, as described elsewhere (Henderson et al. 1996). Touch preparations of fetal lung and kidney and smears of umbilical cord blood and fetal brain were prepared by use of silanized slides. The fetal-cell preparations were fixed in 100% methanol for 5 min and then in 3:1 methanol/acetic acid for 5 min and were air dried. The fetal ovary was squashed onto silanized slides, as described by Blandau et al. (1963). By use of a chromosome 16–specific centromeric probe (D16Z2; Oncor), FISH was performed on all fresh tissues, except oocytes (see below), in accordance with the manufacturer's recommended protocol. By means of a Zeiss epifluorescence microscope, ~500 nuclei were scored, and the proportion of nuclei displaying one, two, three, or four or more hybridization signals was recorded for each sample. In addition, for each tissue type studied, FISH analysis was performed on identically processed disomic controls, to establish the cutoff values for significant levels of trisomy. From this tissue-specific control data, the lower level of trisomy detection was calculated for each tissue type, as described by Lomax et al. (1994).

For molecular cytogenetic analysis of the gonads, only meiotic prophase I oocytes were scored. Oocytes in the prophase of meiosis I can be identified by their large size and diffuse chromatin (Baker 1963) and are morphologically distinguishable from somatic cells (fig. 1). In order to select for oocytes with unpaired chromosomes and to eliminate erroneous results from paired chromosomes producing a single indiscriminate signal, two-color FISH was performed by use of both a chromosome 16–specific centromeric probe (D16Z2) and a chromosome 18–specific centromeric probe (D18Z1; Oncor). Only those oocytes in which the internal control (chromosome 18) exhibited two hybridization signals were scored for the chromosome 16 probe.

Conventional cytogenetic analysis of 15 metaphases

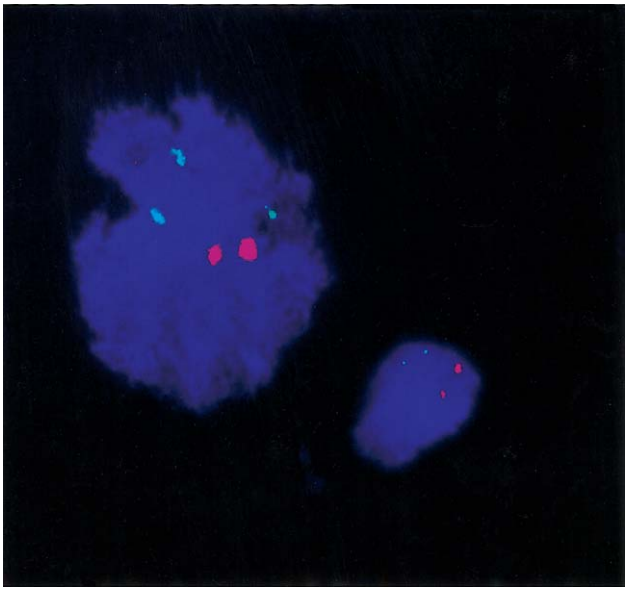

Figure 1 Two-color FISH analysis of nuclei from squash preparations of fetal ovary. The larger meiotic oocyte displays two red hybridization signals, corresponding to the centromeres of chromosome 18 (D18Z1), and three green hybridization signals, corresponding to the centromeres of chromosome 16 (D16Z2). The diffuse oocyte hybridization signals are due to the dispersed chromatin structure of an oocyte during meiosis. The smaller somatic cell shows two distinct hybridization signals, for the same probes, indicating disomy for both chromosomes 18 and 16.

from cultured placental stroma identified mosaicism, disomy, and trisomy for chromosome 16, whereas analysis of five metaphases from cultured amnion demonstrated only a diploid cell line. By use of FISH analysis, high levels of trisomy 16 were documented in trophoblast and chorionic stroma, whereas only disomy 16 was detected in fetal kidney, brain, lung, and cord blood (table 1). The finding that 26% of the oocytes displayed three hybridization signals corresponding to chromosome 16 indicates a significant level of trisomy in the germ cells of this ovary (fig. 1).

Microsatellite analysis at D16S423 demonstrated the presence of maternal uniparental disomy in fetal lung and adrenal gland (table 2). Results at D16S398 were consistent with a maternal meiotic origin of the extra chromosome 16 in placental tissues, including trophoblast, chorionic stroma, and amnion. A recombination event presumably occurred between D16S423 and D16S398, explaining reduction of maternal alleles to homozygosity for D16S423 and heterozygosity for D1S398.

This is the first published data documenting the existence of germ-cell mosaicism in an otherwise nonmosaic fetus, for a conceptus diagnosed with CPM. Our findings are consistent with trisomic zygote rescue resulting in diploid fetal somatic tissues, including blood, and placental mosaicism involving both the trophoblast and chorionic stroma. Although no mosaicism was detected in the fetal somatic tissues, the mosaicism observed in the germ cells was concordant with that found in the extraembryonic placental tissues. These results highlight the complex processes of origination and delineation of fetal and placental tissues.

In the developing human, the trophoblast is the first cell lineage to differentiate, forming the outer cells of the 16-cell morula. In the next developmental stage, blastogenesis, the trophoblast constitutes the outer layer, whereas the inner cell mass comprises multipotent cells, of which the majority become progenitors of the extraembryonic mesoderm and a smaller number give rise to the embryo/fetus proper (Markert and Petters 1978). During the 3d wk postconception and after, the progenitors of the extraembryonic mesoderm contribute to the formation of the placental stroma and the mesodermal layers of the amnion, the umbilical cord, and the secondary yolk sac (Vogler 1987). The secondary yolk sac is known to be the source of both the hematopoietic and PGC progenitors (Fujimoto et al. 1977; Vogler 1987).

Evidence for common progenitors of the PGCs and placental stroma is provided by animal models. For the mouse, studies following the development of embryonic cell lineages show that both the extraembryonic mesoderm and the PGCs originate from common progenitors in the epiblast of the pregastrulation embryo (Lawson and Hage 1994; Buehr 1997). These studies also provide evidence that the germ-cell line is not lineage restricted at 6–6.5 d postcoitum in mice. Alkaline phosphatase, used to identify early germ cells, is first detected at ~7.2 d postcoitum, when the PGC progenitor cells move into the extraembryonic region between the endoderm and the mesoderm of the ventral part of the amniotic fold.

In the human embryo, PGC progenitors are first observed in an extraembryonic location within the sec-

Table 1

Results of FISH Analysis with Probe D16Z2

Tissue	Two Signals[a]	Three Signals[a]	n[b]	Cutoff Values (No. of Controls)[c]
Trophoblast	21	76	1,004	6.6 (6)
Stroma	17	59.3	1,648	9.7 (8)
Oocyte	64.8	26	227	7.1 (8)
Brain	85.6	6.6	501	7.7 (8)
Lung	89.4	1.2	500	8.8 (5)
Kidney	93.6	2.2	500	6.2 (4)
Cord blood	87.4	5.8	501	5.9 (9)

[a] Nuclei displaying two or three hybridization signals (zero, one, and four signals not shown).

[b] Total no. of nuclei scored.

[c] Cutoff values for significant levels of trisomy, as calculated from hybridization results from control samples.

Table 2

Results of Microsatellite Analysis of Loci on Chromosome 16

Tissue	D16S423	D16S398
Maternal blood	ab	ab
Paternal blood	cd	…
Trophoblast	…	abc
Chorionic stroma	…	abc
Amnion	…	abc
Fetal lung	aa	ab
Fetal adrenal gland	aa	…

ondary yolk sac, together with hematopoietic progenitors (Fujimoto et al. 1977). The fate of these two extraembryonically located progenitors is different. Recent evidence from studies of the mouse show that the contribution, to embryonic hematopoiesis, of the hematopoietic progenitor cells from the secondary yolk sac is transient and that definitive hematopoiesis is autonomously initiated later in the aorta-gonad-mesonephrous region of the embryo (Medvinsky and Dvierzak 1996). Thus, the PGCs represent the only permanent contribution from the secondary yolk sac, to the makeup of the embryo/fetus. The temporary sequestration of the germ line into extraembryonic regions (e.g., the secondary yolk sac in humans) has been described in many vertebrates, but the reasons for it are not understood. It has been suggested that the germ cells may be withdrawn from embryonic tissues, to escape the widespread tissue-specific methylation that occurs around the time of gastrulation (Buehr 1997).

The technical advances provided by molecular cytogenetic techniques permit accurate cytogenetic analysis of placental and fetal tissues in pregnancies with CPM and provide a unique opportunity to study the origin and interrelationship of various embryonic and extraembryonic cell lineages in mosaic conceptuses. However, since termination of a pregnancy with CPM is rare, opportunities to obtain further morphological data from human embryos or fetuses demonstrating the presence of aneuploid clones in both PGCs and chorionic stroma are infrequent. The consequences of germ-cell mosaicism likely will be specific for individual trisomic chromosomes involved in CPM. For example, some young mothers who give birth to offspring with trisomy 21 were born to mothers of advanced maternal age (Aagesen et al. 1984). It is possible that these young mothers originated as a trisomy 21 zygote that was rescued, leading to CPM 21 and trisomy 21 germ-cell mosaicism in their gonads. Further long-term prospective studies of individuals born from pregnancies with CPM are required, to document the effect of various placental aneuploidies on gonadal development and human fertility in both males and females. A diagnosis of CPM involving the chorionic stroma may represent an increased risk of chromosomal mosaicism in the germ cells and may have reproductive consequences later in life.

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