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Studies on the Pathogenesis of Choriocarcinoma by Analysis of Restriction Fragment Length Polymorphisms[1]

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ABSTRACT

The association of complete hydatidiform mole with choriocarcinoma has long been recognized, but it is unknown whether the pathogenesis of the two are identical. We investigated the pathogenesis of these trophoblastic tumors by analyzing restriction fragment length polymorphisms using a minisatellite DNA probe to choriocarcinoma, the complete mole, and normal trophoblasts as well as the parental cells. The polymorphic fragments of the complete mole were all transmitted from the paternal DNA, but some polymorphic fragments of the paternal DNA were not recognized in the complete mole. This confirms at a molecular level the androgenetic origin of the complete mole. In some cases of choriocarcinoma, the pattern of inheritance of restriction fragment length polymorphisms was the same as that in the complete mole, whereas in others all the polymorphic fragments in tumor tissues were identical to those in the host DNA. These results suggest that the pathogenesis of choriocarcinoma varies, being completely different from that of the complete hydatidiform mole in some cases.

INTRODUCTION

Choriocarcinoma is a very malignant, frequently metastatic form of gestational trophoblastic disease. It is rather infrequent, occurring at an incidence of about 1 per 10,000 pregnancies in Japan and 1 per 20,000 pregnancies in the U.S. (1). Gestational choriocarcinoma may follow hydatidiform mole (50%), spontaneous abortion (25%), or normal pregnancy (25%). About half of cases of choriocarcinoma are preceded by hydatidiform moles, but only about 3–5% of all molar pregnancies eventuate in choriocarcinoma. A pathological diagnosis of choriocarcinoma is classified clinically as a malignant trophoblastic disease. However, a pathological diagnosis of hydatidiform mole may be classified clinically as either a benign (80%) or malignant (20%) trophoblastic disease depending on the clinical course (2). The use of both pathological and clinical classifications is often confusing and reflects complexity of diseases including their pathogenesis.

In 1977, Kajii and Ohama reported that the chromatin in hydatidiform moles is entirely of paternal origin (3). Analysis by the banding technique of the karyotypes of molar tissues and those of both parents demonstrated that paternal chromosomes were inherited in duplicate by the complete hydatidiform mole, and that no maternal chromosome was transmitted to the mole. Homologous chromosomes showed homozygous banding polymorphisms, suggesting the androgenesis of the mole; that is development of a cell under the direction of the spermatozoan nucleus, the nucleus of the oocyte being either lost or inactivated. Elucidation of the pathogenesis of choriocarcinoma by conventional cytogenetic methods is, however, difficult because the tumor cells have complex karyotypes with various chromosomal rearrangements (4).

Human genetic analysis has been revolutionalized by the use of DNA polymorphisms. The human genome contains hypervariable regions that show RFLPs[3] due to allelic variations in repeat copy number in the minisatellite. Recently, a minisatellite DNA probe, 33.15, was obtained from a human genomic library by hybridization screening with a 33-base pair tandem repeat probe constructed from the human myoglobin minisatellite (5). The band patterns detected with this probe by Southern blotting are so polymorphic that they are called "DNA fingerprints," as can be used for identification of individuals (6). The RFLP bands detected with the probe are inherited as single Mendelian codominants (5, 7). We have reported the use of this minisatellite DNA probe for determination of zygosity in multiple pregnancy (8). In the present study, we used the minisatellite DNA probe 33.15 for genetic analysis of choriocarcinoma and compared the results with those for hydatidiform mole and normal pregnancy. The results provide evidence that the pathogenesis of choriocarcinoma is heterogeneous and that the pathogenesis of some choriocarcinomas is completely different from that of complete hydatidiform moles.

MATERIALS AND METHODS

Samples. Three choriocarcinomas were obtained at the time of operation. The tumor samples were washed repeatedly with phosphate buffered saline (PBS), pH 7.2, and separated from maternal tissue with scissors under a dissecting microscope. They were classified as choriocarcinoma on the basis of their histological features. Complete hydatidiform moles and normal placentas were obtained at the time of therapeutic abortion. Parental mononuclear cells were prepared from heparinized peripheral blood by Ficoll-Hypaque sedimentation.

Extraction of High Molecular Weight DNA. High molecular weight DNA was extracted from choriocarcinomas, complete hydatidiform moles, normal placentas, and parental mononuclear cells by solubilizing the tissues or cells in 10 mM Tris, 1 mM EDTA, and 0.5% sodium dodecyl sulfate (SDS) with 50 μg/ml proteinase K. After incubation at 37°C for 16 h, the mixture was extracted twice with phenol/chloroform. DNA was precipitated with ethanol, with centrifugation in 1.5-ml tubes at 12,000 rpm in a microfuge for 5 min.

Restriction Enzyme Digestion and DNA Blotting. Samples of extracted DNA (5 μg) were digested with *Hinf*I at 37°C for 3 h, and the digests were electrophoresed in 20-cm long, 1% agarose gels in Tris-borate buffer at 90 V until all DNA fragments of less than 2 kilobases had moved off the gels. Then the DNA was transferred by blotting to a nitrocellulose filter by the method of Southern (9) and the filter was baked under vacuum at 80°C for 3 h.

Hybridization and Autoradiography. The minisatellite DNA probe 33.15, kindly provided by Alec J. Jeffreys, was used for RFLP analysis. The probe was labeled with [α-^{32}P]dCTP to a specific activity of 1.8×10^9 cpm/μg in a multiprime DNA labeling system (Amersham, England). The ^{32}P-labeled probe was hybridized to genomic blots at 65°C for 12 h in Denhardt's solution, 1 M NaCl, 50 mM Tris-HCl (pH 7.4), 10 mM EDTA, 0.1% SDS, and 0.1 mg/ml denatured sonicated salmon sperm DNA. The filter was washed once at room temperature with 2×

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[3] The abbreviation used is: RFLP, restriction fragment length polymorphism.

sodium chloride-sodium citrate (SSC) and twice at 65°C with 1 × SSC, 0.1% SDS for 30-min period.

Autoradiography was carried out using Kodak XRP-1 film (Kodak, Rochester, NY) and a Lightening Plus intensifying screen (Dupont, Boston, MA) at −80°C for 48 h.

RESULTS

The RFLP patterns of hybridization of the Jeffreys' minisatellite 33.15, to genomic DNAs were examined. Fig. 1 shows results for RFLPs from three sets of chorionic villi of normal human placenta and the parental mononuclear cells. In the 4–20-kilobase size area, the DNAs of three parents gave about 10–20 bands that were clearly resolved. Few common bands were found in any pair of individuals. Fragments of less than 4 kilobases were not strictly specific for individuals, probably due to lower genetic variability of their loci and fortuitous comigration of unrelated minisatellite fragments. All fragments in normal trophoblasts of the placenta could be traced back to one of the parents. These findings are in accordance with our previous observations and those of others that most of the large *Hinf*I fragments were present in a heterozygous state and that these heterozygous bands were transmitted to the offspring in a Mendelian fashion.

In molar tissues from complete hydatidiform moles, however, all bands were inherited only from the fathers and no mutant fragment was observed in the molar tissues (Fig. 2). The RFLP band patterns of the complete hydatidiform moles were not identical with those of the paternal ones and some polymorphic bands of the paternal DNA were not observed in molar tissues, indicating that hydatidiform moles could be due to doubling of a haploid sperm or fertilization by dispermy. These results confirm the androgenetic origin of complete hydatidiform moles.

Fig. 3 shows the hybridization patterns of DNAs extracted from choriocarcinomas of three women who underwent hysterectomy. We also obtained peripheral blood samples from all the patients and their husbands, and compared the RFLP patterns of the cells with the patterns of the choriocarcinoma tissues to determine the genetic origin of the tumor tissues. In one of three choriocarcinomas (Fig. 3), all polymorphic fragments were transmitted from the patient's husband, and some polymorphic fragments of the husband were not observed in the tumor tissue. Findings in this case were very similar to those in cases of complete hydatidiform mole, and this choriocarcinoma (case VII) clearly originated from residual molar tissues of a previous hydatidiform mole. In contrast, all the polymorphic fragments of the choriocarcinomas in Cases VIII and IX coincided with those of the respective patients, suggesting that these choriocarcinomas were not of androgenetic origin. It is interesting that the preceding pregnancies in Case VIII and Case IX were a complete hydatidiform mole and a normal pregnancy, respectively (Table 1). These results indicate that the inheritance of polymorphic band patterns in choriocarci-

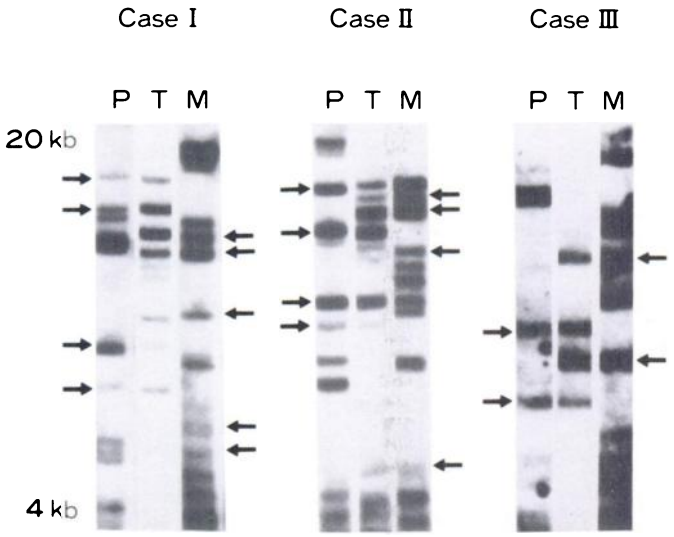

Fig. 1. Band patterns of the RFLPs of DNA of three cases (*Cases I–III*) in normal trophoblasts (*T*) and the paternal (*P*) and maternal (*M*) mononuclear cells with the 33.15 minisatellite probe. The polymorphic band in trophoblastic tissues could be identified as of either paternal (*arrows on the left*) or maternal (*arrows on the right*) origin.

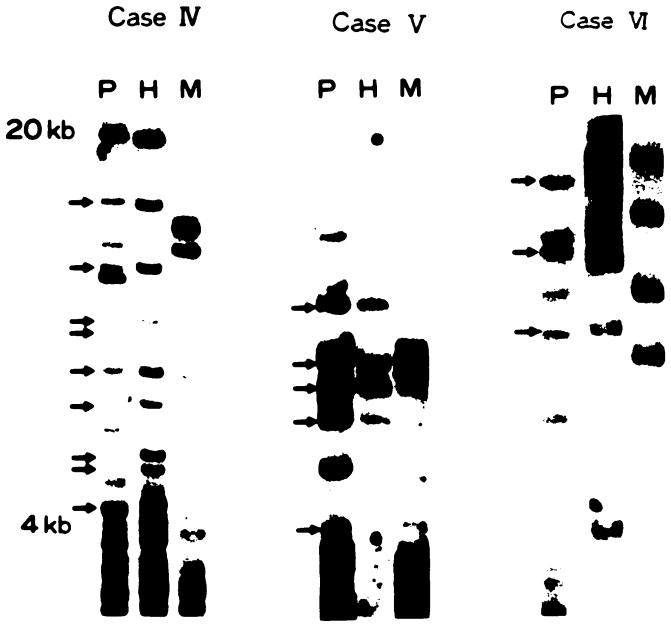

Fig. 2. RFLP analysis of DNAs of three complete hydatidiform moles (*H*) (*Cases IV–VI*) and of their paternal (*P*) and maternal (*M*) DNAs with the 33.15 minisatellite probe. All polymorphic bands in molar tissues could be identified as of paternal origin (*arrows*), but some of the paternal bands were not detected in molar tissues.

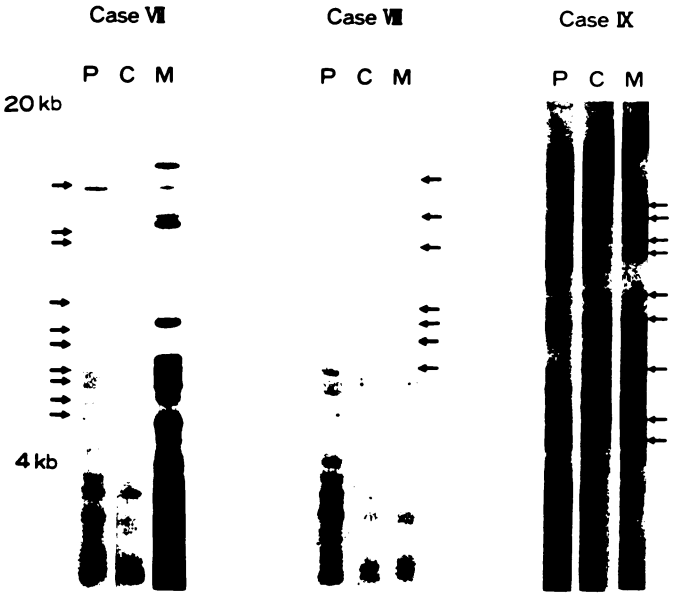

Fig. 3. RFLP analysis of DNAs of three cases of choriocarcinoma (*C*) (*Cases VII–IX*) and the patient's (*M*) and her husband's (*P*) mononuclear cells with the 33.15 minisatellite probe. *Arrows*, polymorphic bands shared with choriocarcinoma tissues.

Table 1 *Summary of results obtained by analysis of RFLPs in normal trophoblast, complete hydatidiform moles, and choriocarcinomas*

Histological diagnosis Case no.	Patient's age (years)	Gestational week	Antecedent pregnancy	RFLPs
Normal trophoblast				
I	25	8		Parental
II	30	7		Parental
III	22	7		Parental
Complete hydatidiform mole				
IV	21	9		Androgenic
V	25	11		Androgenic
VI	41	9		Androgenic
Choriocarcinoma				
VII	50		Mole	Androgeneic
VIII	31		Mole	Gynecogeneic
IX	27		Normal pregnancy	Gynecogeneic

nomas is not always similar to that in hydatidiform moles. Thus we conclude that the pathogenesis of some choriocarcinomas is different from that of complete moles.

DISCUSSION

Complete hydatidiform moles have been shown by karyotype analysis to be of androgenetic origin (3, 10–12). However, exact karyotype analysis of choriocarcinomas is very difficult because of chromosomal changes in tumor cells, and so the pathogenesis of these tumors is still unknown. In the present study, we use a newly developed method, RFLP analysis with the minisatellite DNA probe 33.15, and clearly demonstrate that not all choriocarcinomas are androgenetic in origin. Chromosomal changes of choriocarcinoma cells that interfere with karyotype analysis of the tumor cells did not affect RFLP analysis with the DNA probe 33.15. This could be due to the low frequent appearance of alleles of new length by chromosomal changes, resulting in no mutant fragments in the DNA fingerprints of choriocarcinomas.

The DNA probe 33.15 detects highly variable regions, that are widely dispersed in the human genome, and gives specific individual DNA fingerprints consisting of 10–20 polymorphic fragments. As estimated from the longest polymorphic fragment, the mean frequency was very low (<0.04) and the mean heterogeneity rose to 96% (5, 6). As these polymorphic fragments are inherited in a Mendelian fashion, RFLP analysis is useful in many genetic investigations, such as antenatal diagnosis, zygosity determination of multiple pregnancy (8, 13, 14), mapping of human linkage groups (15), indirect localization of genetic disease loci by linkage (16), and analysis of the role of mitotic nondisjunction and recombination in inherited cancer (17). Using this line of approach, we previously demonstrated a new application of Jeffrey's minisatellite DNA probe for genetic analysis of hydatidiform moles and showed its value for verification of androgenesis as a cause of the complete mole (18).

The difference between the patterns of inheritance of RFLPs in choriocarcinomas and complete hydatidiform moles indicates that the pathogenesis of choriocarcinoma is heterogeneous. In some cases of choriocarcinoma, residual tissues of antecedent complete moles could show malignant transformation (Case VII). In other cases (Case VIII and Case IX), the choriocarcinomas were of host origin, and were probably derived from a diploid oocyte with inability to undergo a first meiotic division.

Chromosomal abnormality in fetuses seems to increase with maternal age, and induces spontaneous abortion, molar pregnancy, or birth of neonates with various abnormal chromosomes. There is a report that 10–12% of the neonates born to parturients of more than 45 years old have chromosomal abnormalities, whereas less than 1% of the neonates of parturients of under 37 years old have abnormal chromosomes (19). The risk of complete hydatidiform mole is also 10 times greater in women of more than 40 years old than in those of less than 35 years old (20–22). These phenomena might be due to the aging of oocyte, which induces chromosomal disjunction, anucleation, or the absence of meiosis. The higher incidence of choriocarcinoma in older women (23, 24) supports our hypothesis that some choriocarcinomas are derived from diploid oocytes that do not undergo a first meiotic division.

It should be noted that in one of the present patients with choriocarcinoma the preceding pregnancy was a complete hydatidiform mole, and the RFLP pattern of the choriocarcinoma was derived completely from the patient. In this case, it is unlikely that residual hydatidiform mole transformed into a choriocarcinoma. Thus by RFLP analysis of gestational choriocarcinomas, the involvement of an antecedent pregnancy can be distinguished by excluding a paternal contribution to the genome in a choriocarcinoma.

Contamination of choriocarcinoma samples used for DNA fingerprinting with the patient's tissues could affect the results. However, histological examination showed that the specimens used in this work for DNA preparation were not appreciably contaminated with host tissues. Furthermore, Northern blot analysis of the choriocarcinoma samples used for DNA fingerprinting demonstrated high expression of the mRNA of human chorionic gonadotropin β-subunit, indicating that the materials employed were trophoblastic tumors (data not shown).

We conclude that the pathogenesis of choriocarcinoma varies and that this tumor is the same disease entity as complete hydatidiform mole in only some cases.

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