Brief Communication

GTD 2005

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J Med Assoc Thai 2005; 80(Suppl 2): S110-8
Full text. e-Journal: *http://www.medassocthai.org/journal*

The treatment of gestational trophoblastic disease has contrary changed the outcome of this disease since the chemotherapy era. Gestational trophoblastic tumor becomes a curable cancer since more than 80% of patients received remission from chemotherapy[1]. However, the pathogenesis of the disease is still obscured. According to the rarity of this disease, randomized clinical trial is almost impossible without the multicentre studies. Furthermore, most of the patients can be cured without histological prove of malignancy, this make the study from the tumor tissue more difficult. Nevertheless, in these recent years, the development of molecular technology facilitates progression of the knowledge in cancer medicine. There is much progress in the understanding of gestational trophoblastic disease, such as molecular genetic, oncogenes and biochemistry for the tumor marker. These new knowledge would be constructed to achieve clearer understanding of the disease in the future.

Genetic in Hydatidiform Mole

Genetic study has demonstrated that in complete hydatidiform mole are mostly diploid and composed solely from paternal DNA[2, 3]. lthough the chromosome of CHM are mostly entirely from paternal origin, mitochondria DNA is of maternal origin[4]. The role of mitochondria DNA still unclear in trophoblastic disease, but mitochondria mutation might play a role in a molecular pathogenesis of invasive gestational trophoblastic disease[5].

Approximate nearly 90% of complete moles have a 46, XX karyotype. Inspection of karyotype from moles and its parent discloses that both members of chromosome pair from CHM are traceable to one paternal chromosome. Thus, these moles result from duplication of paternal haploid set in an empty ovum[6]. The rare 46,XY can also be found[7, 8], estimate a small number range from 6-10%[9]. Heterozygous 46,XX cases can be identified in 5% of cases[10]. Theoretically, another mechanism may involve fertilization though a diploid sperm due to nondivision at meiotic division, but evidence is lacking[11]. The mechanism of loss of the maternal genome in the empty egg is unknown, but it may due to the non-disjunction during the meiotic division (Fig. 1).

Partial moles are generally triploid gestations which the extra chromosome is from maternal or paternal in origin. The possible karyotype of partial mole are69,XXY, 69,XXX or rarely 69,XYY [12-13]. The mechanism of triploid PHM may either arise through fertilization of a haploid oocyte by one sperm which double its chromosome after fertilization, or two sperm (diandric triploid), or through the fertilization of a diploid oocyte and one sperm (digynic triploid) [12, 14]. A diploid oocyte originates from failure of meiosis I or II (Fig. 2). Furthermore, it can originate from the fusion of two ova (dieggy) [15].

Biparental hydatidiform mole

The occasional diploid, biparental moles, described pathologically as CHM have been reported [16]. These biparental CHM are interesting in that they are frequently associated with women who have recurrent CHM[17-19] and more specifically with the rare families in which two or more individuals have recurrent molar pregnancies. These special categories of mole appear to represent the familial form of hydatidiform mole. The pattern of inheritance affected suggests an autosomal recessive condition. The observation showed that the women with recurrent hydatidiform mole have more than one partner suggest that the genetic defect may disturb the normal functioning of ova.

Genome imprinting

It has been clearly established that both paternal and maternal chromosomeare necessary for a balanced development of both embryonic and extra-embryonic tissues. From this hypothesis, it is likely that genomic imprinting may play a pivotal role in the development of chroriocarcinoma. A high level of H19

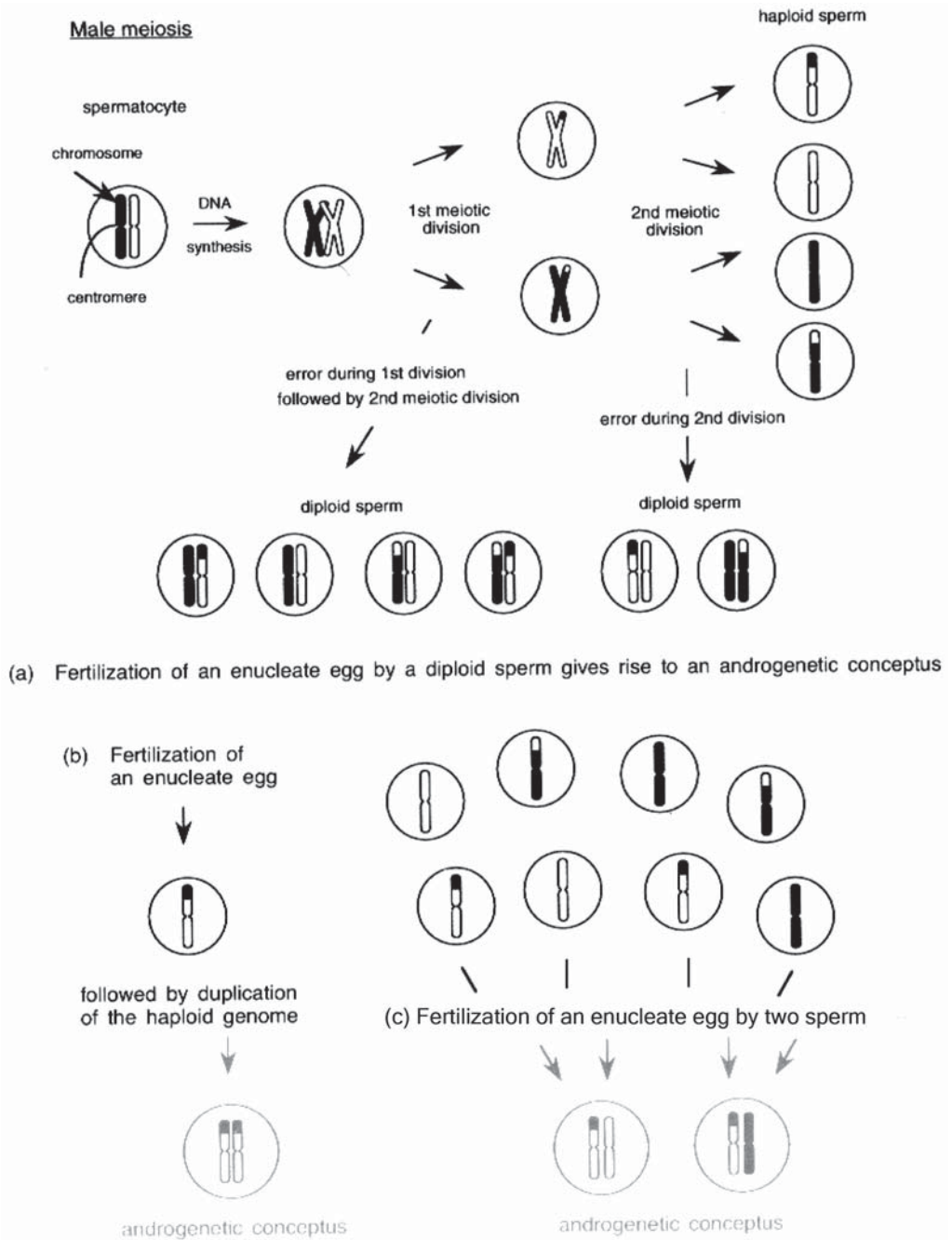

Fig. 1 The pathogenesis of complete hydatidiform mole can be demonstrated by a) Fertilization of enucleate egg by diploid sperm. b) Haploid sperm duplicate itself after fertilize to the enucleate egg. c) Fertilization of enucleate egg by two sperms.

expression, in contrast to lower level of IGF2 expression, was demonstrated in choriocarcinoma.P57 kip2, a cyclin-dependent kinase inhibitor and imprinted in maternal allele was found to be highly expressed in proliferative trophoblast of normal placenta, but expressed at low level in CHM and choriocarcinoma [20,21]. P57 kip2 play a role in regulation of trophoblastic development[21]. However, in PHM, the P57 kip2 is apparently normal but the placenta also shares the pathological feature.

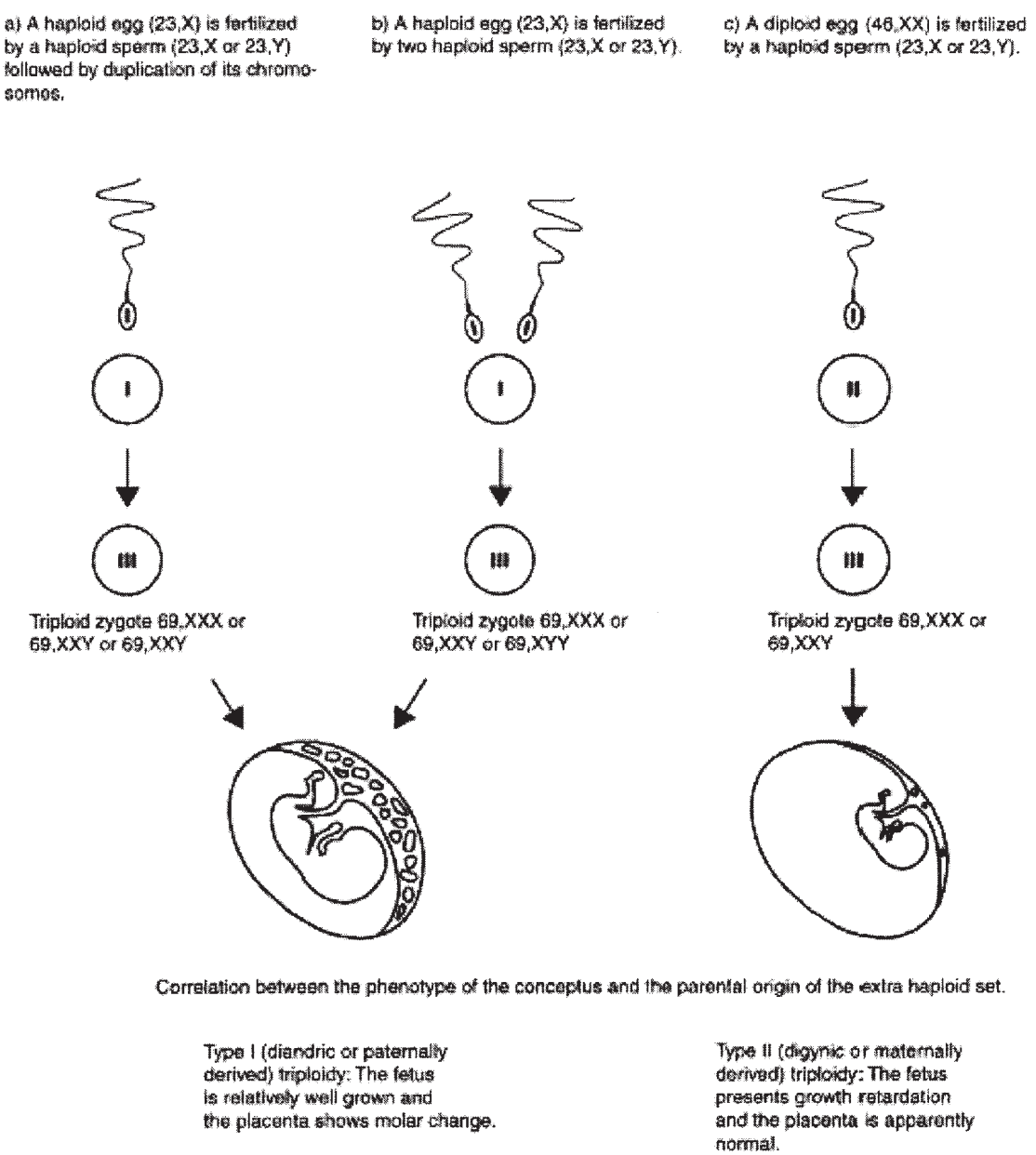

Fig. 2 The pathogenesis of partial hydatidiform mole results in two types of triploidy- diandric and digynic triploidy

Current clinical application in genetic diagnosis

Complete or Partial hydatidiform mole or hydropic abortion

Although these three categories can be diagnosed on the basis of morphology, the distinction between complete and partial mole is less marked in the early gestation[22]. Some fetal tissue may present in early complete hydatidiform mole. Hydropic abortion may appear feature like partial mole. The study of ploidy may be useful to confirm the diagnosis[23, 24] (Fig.3).

Androgenetic or biparental complete mole

Most of complete hydatidiform moles are paternal in origin, but in rare condition, they can be originated from both paternal and maternal chromosome. This biparental complete hydatidiform mole was identified in rare families in which several sisters have repeat hydatidiform mole[18]. Linkage and homozygosity analysis suggested that in two families there is a defective gene located on chromosome 19q13.3-13.4 [25, 26]. The finding from this study provided the data to

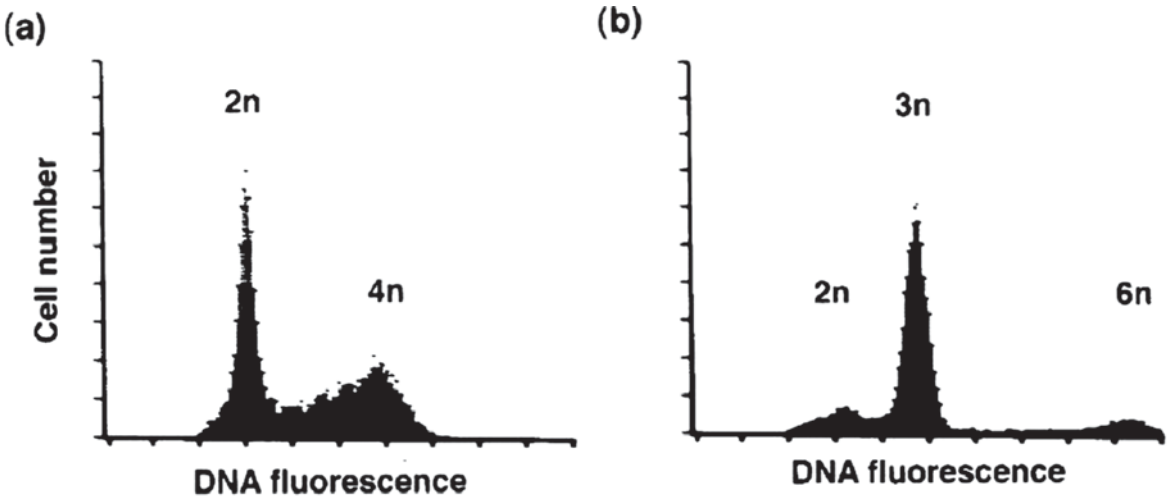

Fig. 3 (a) Flow cytometry from complete hydatidiform mole, a major diploid (2n) peak is observed with small amount of tetraploid (4n) (b) In partial hydatidiform mole, a major triploid peak is presented

support the hypothesis that complete hydatidiform moles which are biparental in origin have the higher incidence of recurrent and may have defect from the imprinted gene.

Complete hydatidiform mole with a heterozygous (dispermic or biparental) genotype has been reported increasing the risk of developing persistent trophoblastic disease from 4%for homozygous complete mole to 50% in heterozygous[3, 8, 27]. Lately, the studies using hypervariable DNA markers and polymerase chain reaction (PCR) have shown that dispermic complete mole has no greater risk to develop GTT compare to monospermic complete mole either XX or XY heterozygous[28].

Twin pregnancy with complete mole or partial hydatidiform mole

In partial mole, the histological appearance may show the villi with evidence of fetal tissue. Twin pregnancies consist of complete hydatidiform mole and normal fetus can also show the similar characteristics[29]. The study of ploidy can be discriminated these two condition, because the twin pregnancy with complete mole has a greater risk of developing persistent trophoblastic disease compare to partial mole.

After the diagnosis of complete hydatidiform mole, the problems with the genetic diagnosis which influences prognosis and treatment options still have 2 major questions:

1. Is that gestational or non-gestational in origin?

Because of the prognosis of gestational and non-gestational trophoblastic disease is different. Patients with non-gestational trophoblastic tumor may have poorer prognosis compare to gestational in origin. Using genetic technique is now possible to determine the origin of trophoblastic tumor (Fig. 4). The tumor with trophoblastic in origin will have both maternal and paternal DNA if it derives from previous normal pregnancy, or non-molar abortion. The DNA may show only paternal DNA if it derives from previous molar pregnancy[30].

2. What is the actual antecedent pregnancy?

The tumor arise from hydatidiform moles has a favorable prognosis compare to those which from term pregnancy or non-molar abortion. The time interval from the previous pregnancy to the diagnosis of tumor also has a significant in prognostic score. Generally, the latest previous pregnancy is perceived as the antecedent pregnancy. However, in patients with multigravida the last recognized pregnancy may not the cause of tumor (Fig. 5). Many studies have shown that the causative pregnancy may not the antecedent pregnancy[27, 30-32].

Update in tumor marker measurement

Undoubtedly, Human Chorionic Gonadrotropin (hCG) still the ideal tumor marker in this disease. However, the heterogeneity of hCG make the measurement of this marker uncertain. The problem is which tests or which techniques is the most appropriate ways to determine hCG in trophoblastic disease.

The arrival of monoclonal antibodies led the hCG immunoassay more sophisticated. There are two

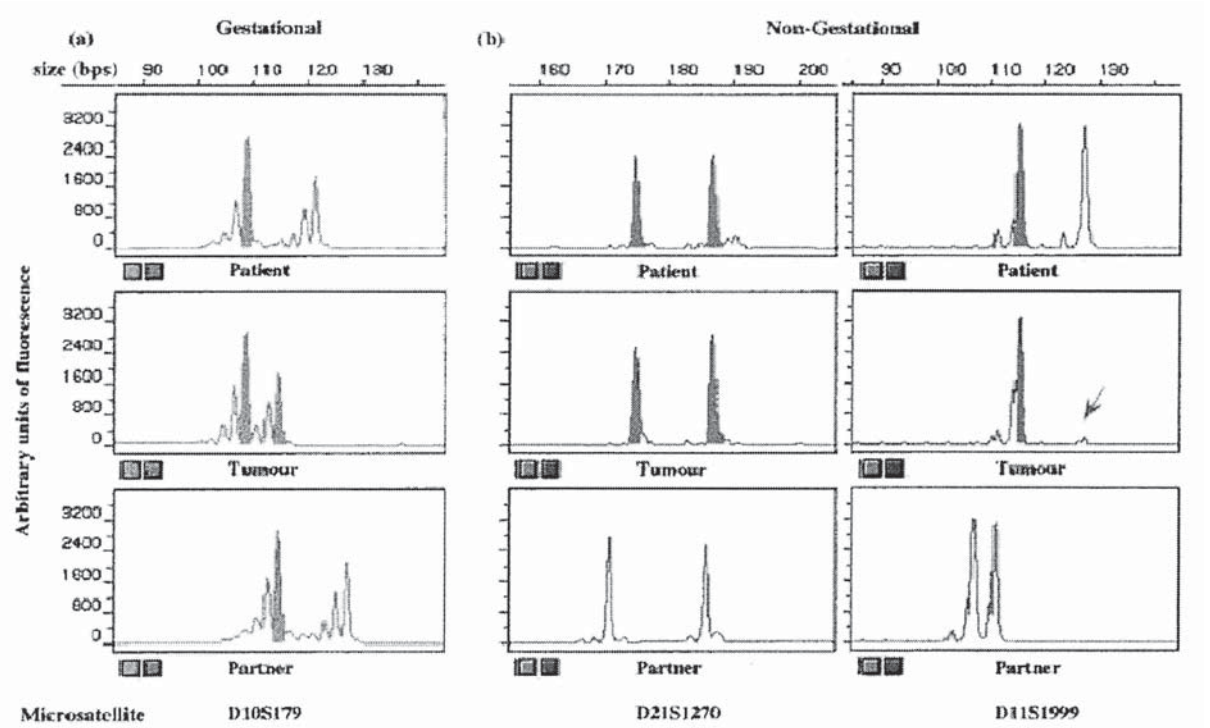

Fig. 4 (a) The informative microsatellite polymorphism identified DNA from parental blood and Trophoblastic tumor tissue following PCR amplification with fluorescent labeled primers demonstrated a tumor with 2 alleles, one derived from each parents. (b) A non-gestational tumor with 2 alleles identical to those in the patient. The right picture confirmed that the tissue is not contaminating host cell

basic types of immunoassay: the non-competitive or sandwich assay, and the competitive test. The sandwich assay is more popular because it consumes less time and most of the new sandwich assays can detect all form of hCG and its free beta subunit[33-36] (Fig. 6). All test, use at least one antibody directed against the Beta subunit to differentiate hCG and LH, and use another antibody to bind with another subunit. Thus, different commercial hCG tests may measure very different combinations of hCG-related molecules. This may not be the problems for testing and monitoring in pregnancy, in which regular hCG predominates in serum samples. It may be a problem in case of gestational trophoblastic disease, choriocarcinoma, PSTT or germ cell tumor. Because in these cases, nicked hCG, free beta-subunit or hyperglycosylated hCG are commonly found. Failure to detect these hCG variants is a common cause of failure to detect active disease [34,36,37].

At the present time, measurement of hCG is done from serum, but urine hCG also very useful in the management and diagnosis of trophoblastic disease. It is useful for the patients with long term monitor of trophoblastic disease. Furthermore, the interfering substance that causes false positive results of hCG is only present in serum. As such, the urine hCG has a clear role in management of trophoblastic disease[36, 38].

New hCG marker

Recently, hyperglycosylated hCG or invasive trophoblast antigen (ITA) is produced by invasive trophoblast cell in early pregnancy and also produced by invasive cytotrophoblast in choriocarcinoma[39]. In the near future, the measurement of hyperglycosylated hCG may potentially useful in distinguishing invasive and non-invasive gestational trophoblastic disease

Persistent low level of hCG

Persistent low concentration of hCG has been reported (usually less than 50 IU/L)[40]. The possible causes of this condition may come from the following causes:

1. False positive hCG or phantom hCG – false positive results may identified from the following criteria[41]:

- The finding of more than 5-fold differences in serum hCG results with alternative immunoassays

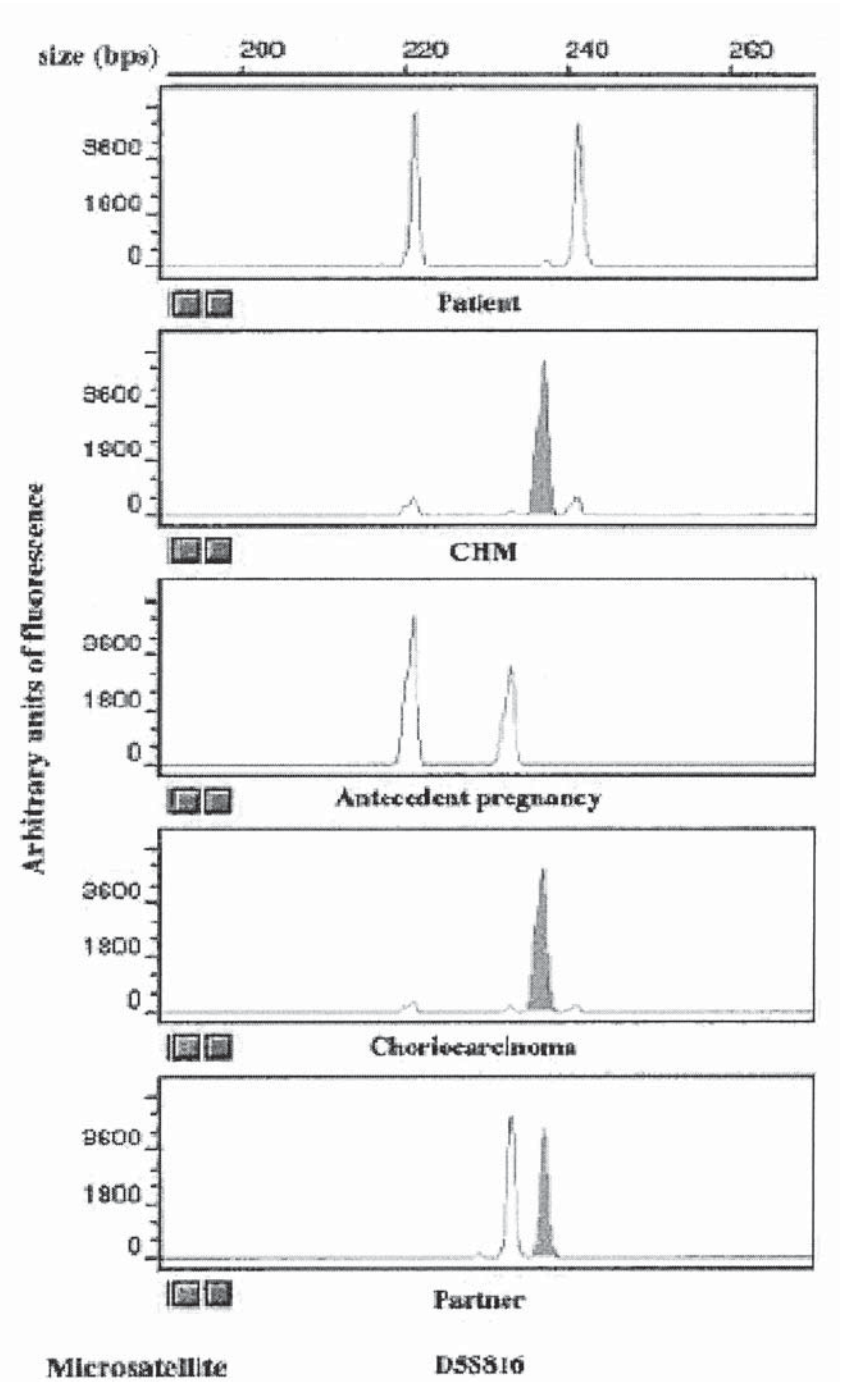

Fig. 5 (30) Identified DNA from a patient, partner, antecedent pregnancy previous mole and tumor. The tumor was genetically different from the antecedent pregnancy. The tumor was shown to be identical with the complete hydatidiform mole in the twin pregnancy with a live fetus and co-existent complete hydatidiform mole.

(critical criterion).

- The presence of hCG in serum but absence of detectable hCG in parallel urine sample (critical criterion).

- The observation of false positive results in other test for molecules not normally present in serum, such as urine beta-core fragment (confirmatory criterion).

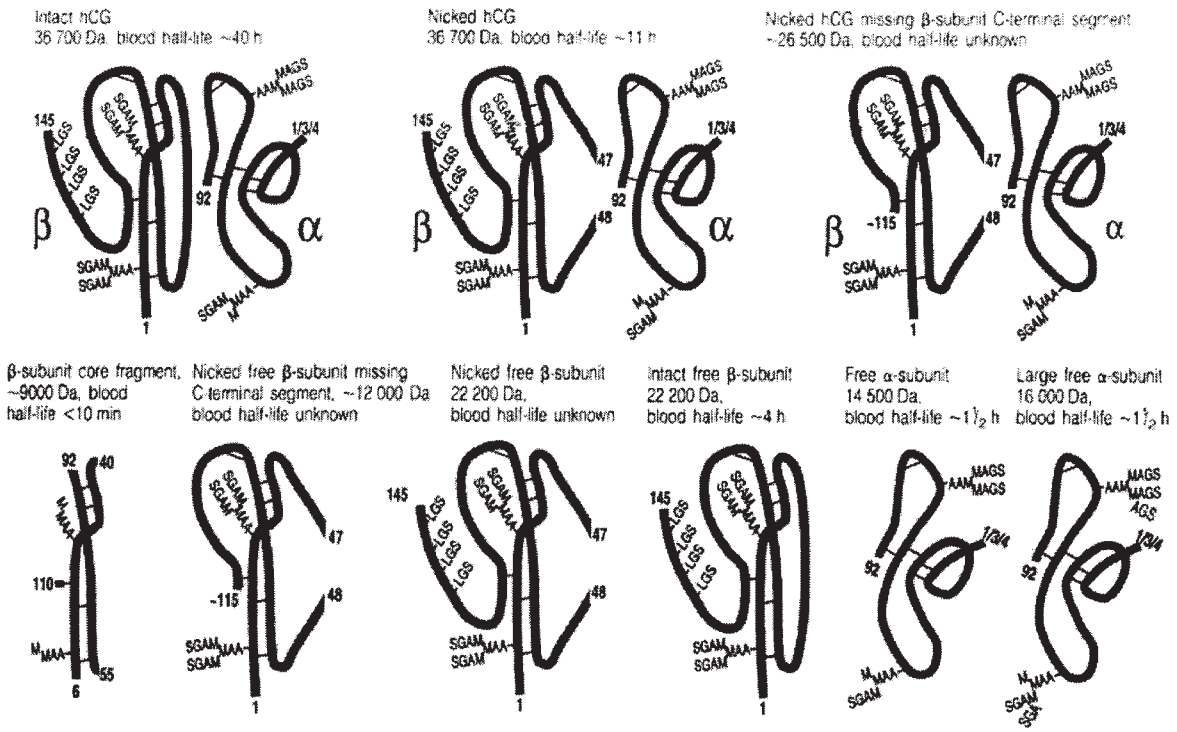

Fig. 6 The structure of hCG subunits

- The finding that a heterophilic antibody blocking agent prevented false detection (confirmatory criterion).

2. Quiescent hCG – in women with known cases of trophoblastic disease and false positive hCG have be excluded, these cases can be call "Quiescent trophoblastic disease". The study from Khanlian SA et al[40] showed that all the benign cases, hyperglycosylated hCG or invasive trophoblast antigen (ITA) accounted for less than 25% of hCG concentration. In the malignant cases, the ITA accounted more than 80% of the hCG.

3. Unexplained elevated hCG – defined as a case with persistent low level of hCG without history of gestational trophoblastic disease. It is postulated that these cases may represent sporadic normal trophoblastic cell, possibly remaining from the previous gestation.

Other tumor markers

The CA-125 has been studied in trophoblastic disease for at least 2 studies. In conclusion, this maker does not provide the information of subsequent development of persistent trophoblastic disease[42, 43]. Vascular endothelial growth factor (VEGF) has been reported increasing level in hydatidiform mole compare to normal pregnancy, while no differences were seen related to the development of persistent trophoblastic disease[44].

In summary, the knowledge in this field has much progress in this few years, but many more questions still waiting to reply. To fulfill this objective, the link between the clinical research, biochemical research in tumor marker, pathological research and molecular genetic research have to be united.

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