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Assessment of cell proliferation in hydatidiform mole using monoclonal antibody MIB1 to Ki-67 antigen

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Abstract

Aims—To assess the role of Ki67 immunoreactivity in predicting the clinical progress of hydatidiform mole.

Methods—Tissue from 87 hydatidiform moles, 11 normal first trimester placentas, 11 normal term placentas and 17 spontaneous abortions were examined for expression of Ki67 antigen, using the monoclonal antibody MIB1.

Results—Ki67 immunoreactivity was significantly higher in the tissue from normal first trimester placentas than in that from normal term placentas and spontaneous abortions. Among the 87 patients with hydatidiform moles studied, 20 developed persistent gestational trophoblastic disease and required subsequent treatment. There was no statistically significant difference in the Ki67 index between the 20 patients who developed persistent disease and those who did not.

Conclusion—Hydatidiform moles which give rise to persistent trophoblastic disease do not have a higher proliferative rate than those which do not. The Ki67 index is not useful for predicting the prognosis of molar pregnancies.

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About 8% to 30% of patients with hydatidiform mole require chemotherapy after primary removal due to development of persistent gestational trophoblastic disease,¹ but there seem to be few useful indicators that can predict such progression. In particular, histological grading of hydatidiform moles does not show a correlation with the clinical outcome.^{2,3}

It is now widely accepted that tumour cell kinetics directly influence the clinical course of patients who have cancer.^{4,5} The use of immunohistochemical methods has become a popular means of assessing the state of cellular proliferation in histological material, because it allows architectural and cytological features to be preserved and avoids the in vivo administration of thymidine analogues.⁶

The diagnostic and prognostic value of Ki67 immunostaining in tumours has been well documented,^{6,7} but the drawback in the use of this antibody is that its application is restricted to fresh material as the epitope does not survive routine histopathological fixation in formaldehyde or alcohol. Recently, mono-

clonal antibodies against recombinant parts of the Ki67 antigen, designated MIB1 to 3, have been raised.⁸ The immunostaining pattern as well as the morphological and cell cycle distribution of MIB1 expression is identical with that of Ki67 and so can be regarded as a true Ki67 equivalent.^{8,9} Using a modified microwave oven pretreatment of the paraffin wax sections, it was possible to detect the antigen previously defined by Ki67 in routinely formalin fixed and paraffin wax embedded tissues.^{5,6}

Studies on the status of cell proliferation in hydatidiform mole have been scanty. Our previous study on the expression of proliferating cell nuclear antigen (PCNA) in human trophoblastic disease using the monoclonal antibody PC10 showed that the PCNA index was not useful for predicting the progression of molar pregnancies to persistent trophoblastic diseases.¹⁰ As an extension of that work, the present study assesses the prognostic value of Ki67 index using the recently available monoclonal antibody MIB1.

Methods

The pathology reports of patients with a diagnosis of complete or partial hydatidiform moles treated at the Queen Mary Hospital, University of Hong Kong, were reviewed. Eighty seven cases, including 39 partial mole and 48 complete mole, treated between January 1989 and December 1990, with available blocks were retrieved. The histological features of these cases were assessed using generally agreed and accepted diagnostic criteria.^{11,12} Tissues from 11 cases of first trimester induced abortion, 11 full term placentas, and 17 cases of spontaneous abortions were also included for the study. Three of the 17 cases of spontaneous abortion show microscopic hydropic changes.

The tissues included uterine curettings and blocks from hysterectomy specimens all of which were routinely fixed in 10% formalin and embedded in paraffin wax. Sections 4 μ m thick were cut and mounted on gelatin coated glass slides and dried overnight in an oven at 37°C. The sections were dewaxed in xylene and rehydrated through graded concentrations of alcohol. Endogenous peroxidase was blocked using 3% H₂O₂ in methanol. After pretreatment with 0.5% trypsin-calcium chloride (pH = 7.8) the sections were immersed in 10 mM (pH = 6) sodium citrate buffer in a thermoresistant plastic box and were

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processed in a microwave oven for five minutes at 700W. The sections were then cooled in phosphate buffered saline before being immunostained. Immunohistochemistry was performed using the avidin-biotin complex immunoperoxidase method (Dakopatts UK). A monoclonal mouse antibody MIB1 (Immunotech SA, France) was applied at a 1 in 100 dilution and incubations were performed overnight at 4°C. Biotinylated sheep anti-mouse antibody was used as the linker molecule and diaminobenzidine-hydrogen peroxide was used as chromogen. A light haematoxylin counterstain was used. Sections were dehydrated in alcohol, cleared in xylene, and mounted.

Sections were examined at high power ($\times 400$) and 20 fields per section were chosen at random. The spatial distribution of positive staining in the sections was assessed. A quantitative estimate of nuclear labelling index for MIB1 immunoreactivity (Ki67)

index was made by scoring positive nuclei/total number of counted nuclei in a minimum of 1000 trophoblastic cells in each case. Decidua, myometrium, fetal tissue and other tissues found in the sections were excluded from the analysis. The nuclei were scored as positive or negative without regard to staining intensity.

To ensure consistency of MIB1 staining between batches tissue from a colonic carcinoma was used as a known positive control in each batch. The experiment was repeated if there was any apparent fluctuation in the Ki67 index of this control. Negative controls were included by substituting TRIS-buffered saline for the primary antibody. Trypsin and microwave pre-treatments were confirmed to be necessary in the preliminary study.

All the patients with partial or complete moles in this study were followed up with a review of clinical records. Persistent gestational trophoblastic disease was diagnosed if there was a plateau in β -human chorionic gonadotrophin values for four weeks, or if there was (hCG) renewed increased in values for three consecutive weeks when pregnancy was excluded.¹³ These patients were then evaluated for further treatment with chemotherapy. Seven patients with partial mole and 13 patients with complete mole had persistently raised hCG values four weeks after giving birth and were diagnosed as having persistent gestational trophoblastic disease. Single or multiple agents were given depending on the risk scores of the patients.

Statistical analysis of the Ki67 index was performed using the Mann-Whitney U test.

Results

MIB1 staining was confined to the nuclei of cytotrophoblast cells in the normal first trimester and term placentas, spontaneous abortions, partial and complete moles; the syncytiotrophoblasts, amniotic epithelium, and endothelial cells were unreactive to MIB1 (figs 1A and B). Occasional weak nuclear staining for MIB1 was also noted in the stromal cells. The mitotic figures were particularly immunoreactive and were highlighted by MIB1 (fig 2). In a given section there was also some regional variation in staining of trophoblast cells.

The results tabulated in tables 1 and 2 show a wide range of variation in the Ki67 index in each group of patients. The differences in counts between normal first trimester and term placentas ($p = 0.0001$), as well as those between normal first trimester placentas and spontaneous abortions ($p = 0.0009$) were significant (table 1). Twenty out of the 87 cases of hydatidiform moles subsequently developed persistent gestational trophoblastic disease and required chemotherapy. The Ki67 index of those 20 patients who developed persistent disease was not significantly different ($p > 0.05$) from that of the 67 patients who had spontaneous remission of the disease (table 2).

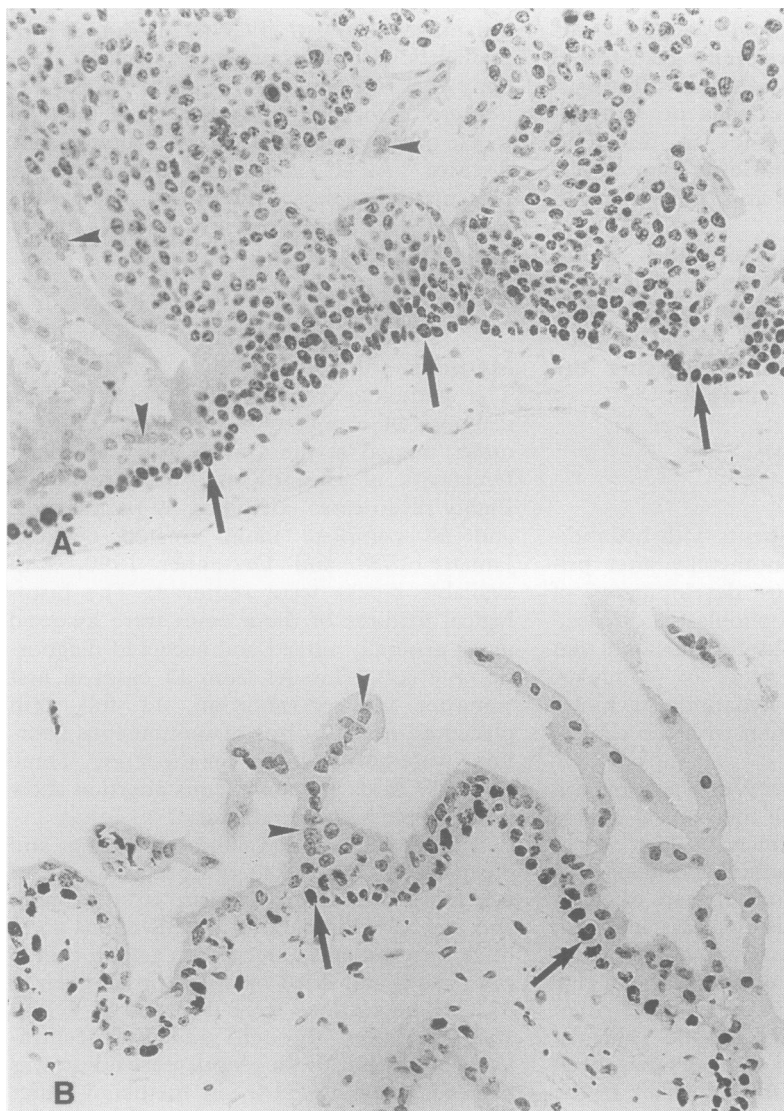


Figure 1 Immunoreactivity for Ki67 is confined to the nuclei of the cytotrophoblasts in both (A) complete and (B) partial hydatidiform moles; the syncytiotrophoblasts are negative. The positive and negative nuclei are indicated by arrows and arrowheads, respectively.

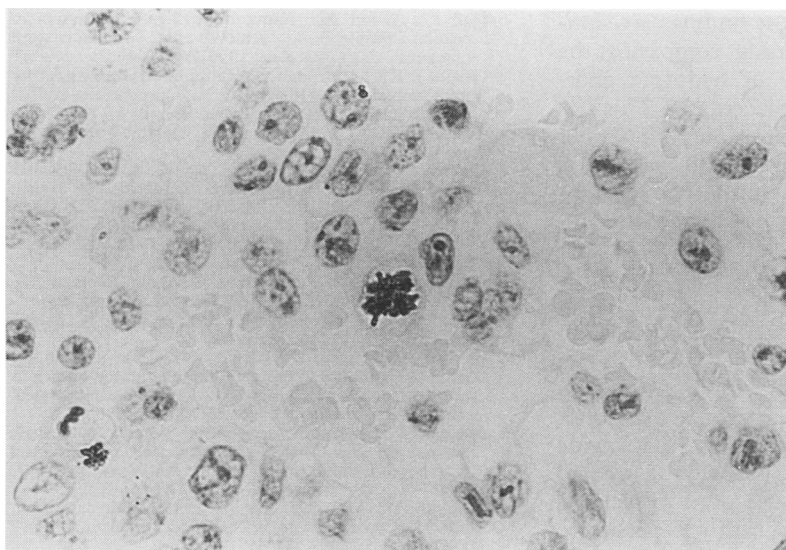


Figure 2 The mitotic figure is highlighted by the antibody MIB1.

Discussion

Many studies have searched for variables that would allow the clinical progress of a hydatidiform mole to be predicted. Historically, uterine size larger than expected for gestational dates or greater than 20 weeks' size, bilateral ovarian enlargement (theca-lutein cysts), patient's age over 40, and uterine bleeding after evacuation of the fetus have been associated with higher risk of persistent trophoblastic disease.^{14,15} However, the introduction of accurate radioimmunoassay methods for measurement of hCG has revolutionised the mode of management of hydatidiform mole.¹⁶ In effect, the hCG regression pattern remains the most specific prognostic indicator and has been adopted by most gynaecologists for management of hydatidiform mole. Nevertheless, the potential risk of patients defaulting follow up always exists and the exploration for an alternative prognostic indicator continues.

Immunohistochemical determination of cell proliferation associated antigens has aroused the interest of histopathologists in recent years. One of the most widely used reagents in this field is the antibody Ki67. This reacts with a nuclear non-histone protein of 395 and 345 kilodaltons present in all active parts of the cell cycle—that is, G₁, S,

G₂, and mitosis—but is absent in G₀.^{17,18} This antibody has been used in a large number of studies of cell proliferation in human tissues with the aim of providing prognostic information in a range of tumour types.^{1,6,7,19} Of particular note is that expression occurs during G₁, increases during the cell cycle, and then rapidly declines after mitosis.^{18,20} Other immunohistochemical proliferation markers, such as proliferation cell nuclear antigen (PCNA), are expressed in non-cycling cells—for example in association with DNA repair, so that they tend to persist after the end of mitosis. It is easier, then, to overestimate the degree of proliferative activity in a tissue, due to retention of staining in postmitotic cells.²¹

Immunoreactivity for MIB1 in normal placentas, hydropic abortions, and hydatidiform moles was largely confined to cytotrophoblasts. This pattern confirms previous studies which have identified the cytotrophoblast as the active germinative zone, based on results obtained by autoradiography, total organ DNA analysis, flow cytometry, morphometric analysis and studies on proliferating cell nuclear antigen.^{10,22-26}

The results (table 1) show a wide range of variation in the Ki67 index obtained from each group of placental tissue. Such variation may be related to technical factors such as the time of fixation, because the tissues used in this study were archival material with no standardised fixation time. Alternatively, this range of Ki67 index may also reflect an intrinsic variation in the proliferative potential of the cytotrophoblast in different cases of each category of tissue. Moreover, variation of staining was observed in different regions of the same section suggesting a lack of synchrony in the proliferation of cytotrophoblast within the same placenta.

There was a significant difference between the Ki67 indexes of the first trimester and term placentas. This supports the previous findings that there was very active growth of the trophoblast in the first trimester placentas, and such villous trophoblast growth is much diminished in term placenta.²³⁻²⁷ Thus Ki67 expression seems to have a logical correlation with the proliferative activity in normal placentas. The Ki67 index in hydropic abortion, although lower than that for normal first trimester placentas, was much higher than

Table 1 Ki67 index (per 1000 cells) in normal first trimester and term placentas and first trimester spontaneous abortions

	Number	Mean	Median	Standard deviation	Range
Normal first trimester placentas	11	314.3	312.0	98.2	194-496
Term placentas	11	23.4	21.0	17.4	1-61
First trimester spontaneous abortions	17	150.4	139.0	128.4	11-504

Table 2 Comparison of Ki67 index (per 1000 cells) in tissue from hydatidiform moles with or without development of persistent gestational trophoblastic disease

Hydatidiform moles	Number	Mean	Median	Standard deviation	Range
With persistent trophoblastic disease	20	370.8	414.5	142.1	85-563
Spontaneous remission with no persistent trophoblastic disease	67	297.3	296	158.2	13-629

that of term placenta. These findings are similar to that found in a study comparing the PCNA immunoreactivity of hydropic abortions and first trimester placentas.²⁷ This has been explained by the possibility that after fetal death the trophoblast is still viable and continues to function at a diminished level.

The frequency with which persistent trophoblastic disease occurs after a molar gestation is dependent on the sensitivity of the follow up hCG assay, length of follow up, type of primary treatment, and terminology used in reporting sequelae.^{28,29} Studies have shown that 8%-30% of patients with hydatidiform mole will require treatment some time after primary evacuation of the fetus.¹ The wide range in the percentage of patients treated reflects the different criteria used by various investigators as to what constitutes persistent disease. In this study, using our usual criteria for persistent gestational trophoblastic disease,¹³ 17.9% (7/39) of partial moles and 27% (13/48) of complete moles subsequently required treatment. These figures, though within the range reported, were higher than the expected local figures of 11.3% for partial mole and 17.8% for complete mole as found in previous study.¹³ The discrepancy is probably due to selection of cases because only cases with available paraffin wax blocks were included in this study. More objective assessment of clinical outcome, such as documentation of subsequent development of metastasis may be used in future studies on prognostic factors in gestational trophoblastic disease.

Most studies have found that histopathological grading of hydatidiform mole, including morphometric assessment of trophoblastic hyperplasia, has little predictive value for prognosis.^{2,3} Our previous study on PCNA also showed that PCNA could not predict the progression of molar pregnancies to persistent trophoblastic disease.

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