Sex Chromatin of Hydatidiform Moles

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The placenta is a homograft which is tolerated by the host for an unusually long time. The hydatidiform mole may be said to be even more successful in this respect for it can survive longer and penetrate further in the host even in the absence of a viable foetus. Recent observations that a large number of hydatidiform moles are chromatin positive are of much interest, but so far the reported series have been small. In the Far East molar pregnancies occur with a greater frequency. This study is based on 185 cases of hydatidiform moles collected from Malaya, and it is hoped that this large material may furnish additional data on the nuclear sex of these tumours.

Materials and Methods

The material was collected from the Pathology Division of the Institute for Medical Research, Kuala Lumpur, Malaya, during 1965 and 1966. There were 185 cases of hydatidiform moles. Two sections were taken from each. One was stained by haematoxylin and eosin for routine examination, and the other was stained by the Feulgen method counterstained with Fast Green for chromatin study.

The 1/12 oil immersion objective was used throughout. Observations were made on both cytotrophoblast cells and stromal cells with well-preserved interphase nuclei. Only those cases where at least 100 cells of either type could be counted were included in the final results. Using this criterion, it was found that there were 86 moles with both stromal and trophoblast cells suitable for assessment, a further 14 where only the trophoblast cells could be analysed, and another 8 where only the stromal cells could be analysed.

Results

The percentage incidence of sex chromatin in cytotrophoblast cells and stromal cells is represented by the histogram in Fig. 1. It can be seen that for stromal cells the incidence shows a distinct bimodal distribution, with one peak between 0-4 and the other around 25-29. There are 78 chromatin positive cases and 14 chromatin negative ones.

FIG. 1. Percentage incidence of sex chromatin in cytotrophoblast cells and stromal cells.

The cytotrophoblast cells, on the other hand, show a continuous distribution, and it is not possible to separate the cases into low and high incidence groups. They have a much lower chromatin count throughout the range. The largest difference recorded is a case where the incidence of sex chromatin is 48% in the stroma and only 8% in the trophoblast.

Cells with more than one chromatin body are not seen either in the stroma or the trophoblast.

Fig. 2 shows that the percentage frequency of sex chromatin in both stromal and trophoblast cells is independent of maternal age.

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FIG. 2. Relation between maternal age and percentage incidence of sex chromatin in cytotrophoblast and stromal cells. ○, stromal cells, ●, cytotrophoblast cells.

The approximate age of the mole can be deduced from the length of the period of amenorrhoea. Fig. 3 shows that the chromatin count of stromal and trophoblast cells does not vary with the stage of development of the mole.

Discussion

When Park (1957) applied the technique of nuclear sexing to trophoblast tumours, he found that the chromatin counts could not be separated into low or high incidence groups. Later investigations, however, revealed a preponderance of chromatin positive cases among these tumours. Tominaga and Page (1966) collected the scattered observations from the literature and calculated that 88% of the reported hydatidiform moles were chromatin positive. Of their own 19 cases, 18 were chromatin positive, with the percentages falling into two distinct groups: those of 18% or more and those of 5% or less. More recently, Wei and Chiang (1967) in a tissue culture study of molar tissue found that 10 of their 14 cases (71.4%) were chromatin positive.

In the present series, 83% of the moles are chromatin positive. The chromatin counts are in a bi-

modal distribution, with a group of 4% or less and another of 15% or more in the stromal cells. The cytotrophoblast cells, on the other hand, cannot be separated into high or low incidence groups, and have a lower chromatin count throughout the range. One explanation for this may be that the trophoblast cells are rapidly dividing. Miles (1960) and Therkelsen and Petersen (1962) have demonstrated that rapidly dividing female cells may show a reduced incidence in the percentage of chromatin positive cells. A disparity between the chromatin status of stromal and trophoblast cells was also observed by Atkin and Klinger (1962). In their case, sex chromatin was absent in the trophoblast but present in the stroma. Chromosome counts and DNA measurements of the trophoblast revealed a near-triploid mode which suggested that the sex chromatin might be lost with the increase in chromosome number of the trophoblast.

It is notable that, in the present series, cells with more than one chromatin body have not been observed in either stromal cells or trophoblast. Márquez-Monter (1962) found that 1 out of his 55 moles showed double sex chromatin in both stromal and trophoblast cells. Atkin and Klinger reported

FIG. 3. Relation between period of amenorrhoea and percentage incidence of sex chromatin in cytotrophoblast and stromal cells
○, stromal cells, ●, cytotrophoblast cells.

another mole with similar findings. Chromosome analysis of this case revealed a triploid (3A3X) complement. It is interesting to note that male polyploid cells, on the other hand, rarely exhibit the sex chromatin body (Klinger and Schwarzacher, 1960).

Another 3 cases of moles with aneuploid chromosome constitutions were reported by Stolte *et al.* (1960). Makino, Sasaki, and Fukuschima (1963) showed that there was an increasing tendency of a shift towards tetraploidy and heteroploidy in the more malignant varieties of trophoblastic tumours, but this feature was not so marked in benign hydatidiform moles. In these, most of the cells were diploid and had a female karyotype. Atkin (1965) also found that 4 of his 6 cases of moles had a normal female complement. Rather than being a basic feature of all moles, aneuploidy may therefore be associated with its malignant transformation.

A molar placenta may occasionally be associated with an abnormal foetus. Beischer, Fortune, and Fitzgerald (1967) found that of the 52 cases of co-existent foetus associated with a single molar placenta abstracted from the literature, 8 showed physical abnormalities. In their own case, chromo-

some analysis of both foetal and molar tissue revealed a triploid complement. Szulman (1965) reported triploidy in a mole which was attached to a macerated foetus. Atkin and Klinger analysed the sex chromatin of various foetal tissues in their case and found a mosaic condition.

Park found that the percentage incidence of sex chromatin in trophoblast tumours decreased linearly with increasing maternal age. The present study does not confirm these findings (Fig. 2). Moreover, the sex chromatin frequency is not affected by the stage of development of the mole (Fig. 3).

Several points of interest emerge from the preceding review. The chromosome complement of molar tissue is frequently normal. In these cases, female karyotypes predominate. This enhances the significance of the observation that the majority of moles are chromatin positive. On the other hand, moles may have an abnormal chromosome constitution. Aneuploid changes may be reflected in the cells by the presence of double sex chromatin bodies or the disappearance of this structure. The disparity observed in the chromatin count between stromal and trophoblast cells seems to indicate that

aneuploid changes, when they do occur, are more prevalent among the cells of the trophoblast. Finally, a molar placenta may be associated with a foetus with both physical and chromosomal abnormalities. A triploid constitution appears to be the type of abnormality most frequently encountered. In these cases, double sex chromatin bodies can be demonstrated in various foetal tissues as well as in stromal and trophoblast cells.

Summary

Of 185 cases of hydatidiform moles available, just over half of these are found to be sufficiently well preserved for sex chromatin to be analysed in the cytotrophoblast and stromal cells.

The percentage incidence of sex chromatin in stromal cells can be separated into low and high incidence groups; here 83% of the cases are chromatin positive.

The cytotrophoblast cells, on the other hand, cannot be separated into low and high incidence groups and have a much lower chromatin count throughout the range.

The chromatin count in cytotrophoblast and stromal cells is not affected by maternal age or the stage of development of the mole.

The reliability of the chromatin body as an indicator of genetic sex in hydatidiform moles is discussed.

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