Iranian J.Publ. Hilth.
1982-83, Vol. 11, No. (1-4)

CYTOGENETIC ANALYSIS OF HeLa AND CHANG CELLS

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ABSTRACT

Based on the evaluation of two human cell lines, HeLa and Chang, aneuploidy and several marker chromosomes were found in both cells. The morphological characteristic of marker chromosomes of Chang cells were distinctly different from HeLa.

A certain submetacentric marker chromosomes was frequently present among 80% of marker chromosomes of Chang cells which distinguished this line from HeLa, which showed the various identifiable marker chromosomes. This evidence clearly established the different etiology of these two human cell lines.

INTRODUCTION

It is proposed that, chromosome changes in most human cell lines may arise from either colonal evolution or reduction - duplication cycle of stemline populations which result in chromosome changes.

Since the production of a special marker chromosome(s) is

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believed to be a casual relationship (1, 2, 3), so, a question arises:

"Do human tumour cells show a chromosome pattern specific for each etiological agent in-vivo or in-vitro".

The purpose of this study is to answer the following questions"

I. Is there any chromosome(s) abnormality common to two cases HeLa and Chang cells, as shown by the karyotype diversity and a distinct marker chromosome?

II. Is there any distinguishing characteristic karyotype between HeLa and Chang cells?

MATERIALS AND METHODS

I. Cell culture:

The cells of HeLa and Chang were cultured separately in the medium which we developed and enriched Eagle HEM supplemented with glutamine, non-essential amino acids, 10% of newborn-calf serum and antibiotics.

II. Chromosome preparation:

The cultured medium was centrifuged in a 15 ml centrifuge tube, the buffy coat was removed by adding 0.2 mcg/ml colcemid in Earl's solution to the sediment. The tube was then held on the Vortex Rotary Mixer, resulting in subjecting the cells as much as possible to the mitotic inhibitor colcemid.

The specimen was incubated at 37°C for 1 to 24 hours, then the mixture was centrifuged at 3000 RPM for 3 minutes. Prewarmed hypotonic solution (0.70% sodium citrate) was added and the mixture incubated for an additional 30 minutes.

The specimen was centrifuged again, the supernatant decanted and fresh fixative (three parts acetic acid and seven parts methanol) was added. After one hour fixation the cells were centrifuged at 3000 rpm for three minutes and fresh fixative was added. This step was repeated two or three times, depending on the nature of the fluid. The final concentration of fixative and cells was adjusted to a cloudy suspension.

The slides were prepared immediately. A drop of fluid was put on a slide. The slide preparation was made by the "air-drying method); slides were dried at a high temperature by holding the slides two seconds over a Bunsen flame.

This may improve the spreading. The slides were stained with

Giemsa stain and covered with the coverslips. The best chromosome set was found under the light microscopes, the field was photographed. The picture enlargement of the chromosome set which had a very good spread was selected. Then each individual chromosome was cut out and arranged in order of its length and centromer position to make the "Karyotype".

RESULTS

A. Similarities

I. The distribution of chromosomes showed that both of the cell lines had not been cloned. There were various cell lines in both HeLa and Chang cells. A high level of abnormal divisions of cells was present.
II. The chromosome number variation was observed from cell to cell. Up to 75% of chromosomes of Chang cells were intact and 60% of the HeLa cells were as well (Table 1.).
III. Gross abnormalities implied either cell with mode number 70 in Chang and 78 in HeLa cells.

B. Differences

I. The data (Table 1) shows that, two types of cells under investigation differ distinctly in frequency of karyotypes with marker chromosomes. Fourty one per cent of the HeLa cells contain one or more than one marker chromosome, while only 26% of the Chang cells showed multiple marker chromosomes.
II. The most important feature of Chang cells was that, 80% of the marker chromosomes of cells were a unique large submetacentric similar to group B (F. 1. 2). The individual character of their marker chromosome and HeLa marker chromosomes is given in (F. 3).
III. In general the configration of the metaphase figures in HeLa cells differs not only in number of the marker chromosomes but in the presence of various shape of marker chromosomes as well.

The largest element (F. 4. 5), possibly a dicentric chromosome and several other markers including long metacentric, acrocentric and fragments were not observed in Chang cells.
IV. The range and mode of chromosomes were different in HeLa and Chang cells. In Chang cells the range of chromosomes was 46-160 (mode 70), but the range of chromosomes of HeLa was 46-200 and its (mode was 78). (Table 1).

DISCUSSION

The presence of the specific marker chromosome associated with the Chang cells suggests several explantions. I) The donor's cells could have given birth to these markers, 2) The tumour was a colonal derivative of a cell with these markers, 3) The cells that were established in the initial cultivation of the tumour were derivatives of a single cell in which the necessary rearrangement leading to the production of these markers had occured, finally the chromosomes evaluation in cancer or in tissue culture in vitro is not just a chance "malignancy but in a heritable state". (4.5).

We can not distinguish among these alternatives.

In 1912 Boveri (6) proposed that chromosome alterations are important in the etiology of tumour. A relationship among several types of cancer and specific chromosome abnormalities has been suspected for a long time.

Rovley in 1974 (7) has postulated that the specificities are related to the agent which induces the neoplasm. In support of this hypothesis she quotes situations where animal tumours of different types induced by the same agent have similar chromosome aberrations.

There are several well documented examples of highly specific chromosome aberration in human tumour cells.

When one considers chronic myeloid-leukemia, where about 95% of cases have the philadelphia chromosome, this hypothesis requires that all are induced by the same agent (8).

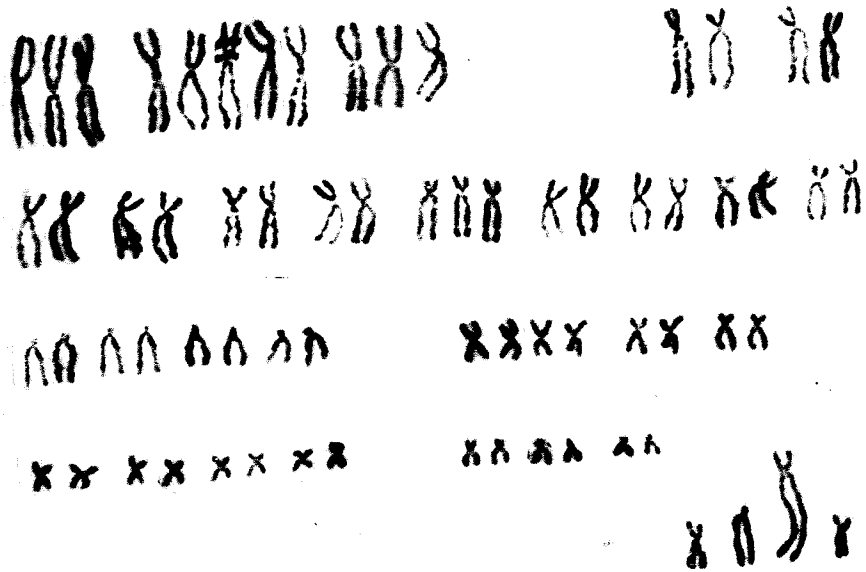
However considering the above explanations which the different etiological factor(s) induces the different marker chromosome(s), and the correlation of marker chromosome with Chang cell malignancy rather than with the presence of the HeLa genome, it can be assumed that there is a different etiology factors(s) for Chang and HeLa cell lines.

TABLE 1

Karyotype analysis of HeLa and Chang cells

Human cell lines	Total no cells	%cells with 46	Range no. Chromo	Abnormal Chromo	Making karyotype	%normal karyotype	%abnormal karyotype	%cells with marker chromo.
HeLa	100	60	46-200	.78	10	6	4	40
Chang	100	75	46-160	70	10	7	3	25

METAPHASE CHROMOSOMES OF CHANG CELL



KARYOTYPE OF CHANG CELL WITH 68 CHROMOSOMES INCLUDING LARGE SUBMETACENTRIC