ENZYMATIC AND CHROMOSOMAL CHARACTERIZATION OF HELA VARIANTS

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ABSTRACT

Seven strains of HeLa cells have been characterized by the number of chromosomes and the activity of the enzymes alkaline phosphatase, glucose-6-phosphate dehydrogenase, 6-phosphogluconic dehydrogenase, and lactic dehydrogenase. All seven strains were found to differ as to chromosome numbers and enzyme levels despite the fact that two strains were called HeLa and three were called HeLa S3. Three strains were found to have a stemline in which greater than 60% of the cells demonstrated a single chromosome number, and this characteristic was stable for at least 6 months. A nomenclature for these clones has been suggested by the use of the stemline chromosome number as a subscript following HeLa. These three clones were, therefore, designated HeLa65, HeLa71, and HeLa75. Karyotypes were made of the stemlines of these clones and were compared with enzyme levels. Alkaline phosphatase showed the greatest variation from cell line to cell line with a 200-fold difference in levels, whereas glucose-6-phosphate dehydrogenase showed variation in activity over a 12-fold range, lactic dehydrogenase over an 8-fold range, and 6-phosphogluconic dehydrogenase over a 2-fold range. It is suggested that human cell strains can be used for biochemical studies if they are cloned and if the clones are relatively stable at least with respect to modal chromosome number and karyotype.

HeLa cells, a human-derived cell line established by Gey, Coffman, and Kubicek (1) in 1951, have been studied extensively. Some of the molecular components of these cells which have been studied include RNA (2, 3), DNA (4, 5), protein (4, 5), and carbohydrates (6). Hsu, in 1954, reported (7) that the chromosome numbers of this cell line varied over a wide range with a hypotetraploid mode. Chu and Giles (8) studied a series of clones obtained from the parent strain and found that these clones had different modal chromosome numbers. Vogt (9) demonstrated what she felt was a causal relationship between both chromosome modal number and karyotype and the phenotypes of drug resistance or morphology in a series of HeLa variants. Recent biochemical studies dealing with regulation of enzyme activities have presented conflicting results using cell strains with identical names (10, 11). The present communication proposes a new classification for HeLa clones and presents data showing widely varying biochemical and karyological characteristics in a series of HeLa strains. The single mode chromosome number of HeLa clones, where this characteristic is evident and stable, has been used in the classification system.

MATERIALS AND METHODS

Tissue Culture Techniques

All cells were grown in Eagle's minimum essential medium1 (MEM) containing 2 µmoles of L-glutamine. HeLa cells were grown in Eagle's MEM supplemented with 10% fetal bovine serum. All other cell lines were grown in MEM containing 10% fetal bovine serum.

1 Obtained from Grand Island Biological Co., Grand Island, New York.
tamine/ml, 10% calf serum, 50 µg/ml penicillin, 50 µg/ml streptomycin, and 50 µg/ml kanamycin. Cells were grown in monolayers and were subcultured by treating the monolayer with a Versine-trypsin solution (0.7 mM EDTA plus 0.05% trypsin solution) for no longer than 7 min. Cells were obtained for enzyme studies by scraping them from the bottom of the flask into 10 ml of 0.001 M Tris-HCl, pH 7.5, in 0.154 M NaCl.

**Enzyme Assays**

Cells were obtained at a point in their growth curve when they were approaching the confluent state. After being scraped, the cells were washed twice with 0.001 M Tris-HCl, pH 7.5, in 0.154 M NaCl and then suspended in 2 ml of the same solution. A cell count was performed with a bright-line counting chamber. The cells were broken by sonification for 10 sec with a Bronwill Biosonik probe \(^2\) at 70% maximum power. The sonicate was assayed for protein content with a Bronwill Biosonik probe at 70% maximum chamber.

**Chromosome Preparation**

HeLa cells in the log growth phase were used for the preparation of chromosomes. The cultures were made 0.5 µM in Colcemid and were incubated at 37°C for 4 hr. The cells were detached with a Versine-trypsin solution (0.7 mM EDTA plus 0.05% trypsin solution). After washing the cells with Hank's buffer, the cell pellet plus 1 ml of Hanks' buffer was gently mixed with 3 ml of distilled water prewarmed to 37°C. The centrifuge tubes containing the cells were then incubated at 37°C for 10 min. Then the cells were centrifuged at 600 rpm for 5 min. The distilled water was quickly aspirated off the cells, and 4 ml of freshly prepared fixative of 3 parts ethanol and 1 part acetic acid were added carefully, without disturbing the cell pellet. After setting for 30 min at room temperature or overnight in the refrigerator, the cells were resuspended in the fixative and then centrifuged at 600 g for 5 min. The cells were resuspended in 0.5 ml of fresh fixative, and immediately applied to six or eight slides which were then passed through a flame once, burning off the fixative and spreading the chromosomes. The dried slides were stained for 40 min in Giemsa stain.

50 spreads from each preparation were examined under a 100X oil immersion objective and each spread was counted twice. If repetitive counting of any particular spread did not give identical counts, the spread was recounted until consistent results were obtained. Representative spreads of the chromosome mode from each preparation were photographed with an oil immersion lens (100X) on 4- X 5-in Polaroid positive/negative film, Type 55. Enlargments with a final magnification of 4000X were made. Karyotyping was performed by cutting out the chromosomes and arranging them into groups according to the Denver-London classification (18). The three variants with a high percentage of a single modal chromosome number cells were karyotyped (HeLa61, HeLa71, and HeLa73) and at least five karyotypes were made of the predominant mode. A representative karyotype is presented.

**RESULTS**

A total of seven strains of HeLa cells have been obtained from a variety of sources and studied as to chromosome number and levels of alkaline phosphatase, lactate dehydrogenase, 6-phosphogluconic dehydrogenase, glucose-6-phosphate dehydrogenase, and inducibility of alkaline phosphatase. Two of the cell strains were obtained from a commercial source (Grand Island Biological Co.: HeLa G and HeLa S3G), two from the American Type Culture Collection (HeLa A-CCL 2 and HeLa 229-CCL 21), and three from independent research laboratories (HeLa13 from Dr. G. Melnykovich \(^3\) [10, 19], HeLa41 from Dr. M. Scharff [3, 20], and HeLa51 from Dr. L. Chessen [11]). Three of the strains (HeLa41, HeLa51, and HeLa3G) carried the designation HeLa S3,

\(^3\) This cell strain was kindly supplied by Dr. G. Melnykovich and had previously been designated S3. This strain had been obtained from the laboratory of Dr. G. Mueller, who had obtained it from Dr. Gilbert Chang.
| Name      | <56 | 56 | 57 | 58 | 59 | 60 | 61 | 62 | 63 | 64 | 65 | 66 | 67 | 68 | 69 | 70 | 71 | 72 | 73 | 74 | 75 | 76 | 77 | 78 | 79 | 80 | 81 | 82 | 83 | 84 | 85 | 86 | >86 |
|-----------|-----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|
| HeLa A    | 2   | 2  | 2  | 2  | 2  | 2  | 2  | 2  | 2  | 2  | 4  | 4  | 2  | 4  | 2  | 8  | 16 | 2  | 12 | 4  | 10 | 10 | 4  | 2  |     |    |    |    |    |
| HeLa 229  | 2   | 2  |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |
| HeLa S3G  |     |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |
| HeLa G    |     |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |
| HeLa 67   |     |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |
| HeLa H    |     |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |
| HeLa 11   |     |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |
| HeLa 12   |     |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |

* Results are expressed as per cent of cells with the indicated number of chromosomes. 50 cells were counted from each variant.
and two of the lines are called HeLa (HeLa A and HeLa G).

Table I illustrates the chromosome analyses of all seven variants in terms of percentage of the cells in the population with a given chromosome number. It is apparent that all seven strains differ from each other in this respect. HeLa A and HeLa 229 showed the most variation in chromosome number. HeLa 229 had a large number of cells with 79-81 chromosomes per cell without a definite mode. HeLa A had the majority of cells with between 71 and 84 chromosomes per cell without a definite mode. HeLa G had a bimodal chromosome distribution with 42% of the cells demonstrating 69 chromosomes and 34% having 71 chromosomes per cell. HeLa S3G had a bimodal chromosome distribution with 50% of the cells demonstrating 79 chromosomes and 38% of the cells having 75 chromosomes per cell. Three variants were studied which had single chromosome modes (Fig. 1). These variants were HeLa65 with 62% of the cells demonstrating 65 chromosomes, HeLa71 with 68% of the cells demonstrating 75 chromosomes, and HeLa71 with 62% of the cells demonstrating 71 chromosomes. We suggest that these three clones be identified by a subscript representing the major chromosome mode.

Any basis of classification should be based on a stable characteristic of the clone. For determining stability of the clones, repeat karyology was performed after 6 months of continuous subculturing. After this time period, all three clones showed almost identical percentages (Table II). Moreover, all three demonstrated the same major mode that was present in the original chromosome analysis, although there was some change in the total percentage of cells in the stemline. These changes in the percentage of cells in the stemline seemed to reflect changes in the health of the cultures themselves. In one case, HeLa65 was found to be contaminated with bacteria; chromosome analysis carried out on this contaminated culture immediately before the discovery of the contamination showed a much lower stemline percentage. A freshly thawed, uncontaminated culture gave a high percentage in the major mode.4

4 The stemline percentages indicated in Table I are probably minimal values since they were determined by counting relatively large numbers of spreads, some of which were poorly defined and, therefore, difficult to quantitate exactly. More rigorous selection of spreads suitable for counting has resulted in increased percentages of the stemline to values in excess of 75%, with HeLa65 giving, for example, 77 ± 3% the major mode.
Karyotypes were performed, on five cells, of the major mode of HeLa65, HeLa71, and HeLa75 (Figs. 2-4 and Table III). HeLa65 has four chromosomes less than the human triploid number. This karyotype deviates from the normal triploid karyotype in Group C (-4) (Fig. 2). There are six normal-appearing G group chromosomes in this variant.

HeLa71 is characterized by having two chromosomes more than the normal human triploid number. The karyotype, illustrated in Fig. 3, demonstrates two additional chromosomes in

### Table II

<table>
<thead>
<tr>
<th>Name</th>
<th>Chromosome No.*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>02</td>
</tr>
<tr>
<td>HeLa65</td>
<td>2</td>
</tr>
<tr>
<td>HeLa71</td>
<td>2</td>
</tr>
<tr>
<td>HeLa75</td>
<td>2</td>
</tr>
</tbody>
</table>

*Results are expressed as per cent of cells with the indicated number of chromosomes. 50 cells were counted from each variant.

**Figure 2** Karyotype of the stemline of HeLa65 arranged according to the Denver-London classification.
Group G, one of which is abnormally small. There are two abnormal chromosomes in Group D, one of which has much longer arms than the other Group D chromosomes, and another marker chromosome which has one long arm and shows a secondary constriction toward the end of the longer arm. Although the shorter arm may represent a weak point in the chromosome at the region of constriction with a resultant loss of a part of the chromosome during preparation of the slide, four of the five karyotypes of the major mode demonstrated this marker chromosome.

HeLa71 (Fig. 4) has six chromosomes more than the human triploid number. Deviations from triploid are as follows: Group C (+1), Group D (+2), Group E (+1), and Group G (+2). Group G, however, contains only five normal-appearing acrocentric chromosomes and three minute chromosomes.

Table III illustrates the reproducibility of karyotypes of the stemlines from HeLa65, HeLa71, and HeLa75. As would be expected, the greatest amount of variation occurred in Groups C, D, and E which are the most difficult to classify.

Table IV illustrates the biochemical characteristics of these HeLa variants. Alkaline phosphatase showed the greatest variation from cell line to cell line with a 200-fold difference in level from HeLa A (low) to HeLa S3 (high). Three of the lines have been found to increase in alkaline phosphatase activity when grown in the presence of hydrocortisone (HeLa A, HeLa 229, HeLa65—which is indicated by a + in the last column of Table IV), and all of these lines are relatively low in alkaline phosphatase activity. This observation would tend to confirm the observation of Maio and De Carli (21) that tissue culture strains low in alkaline phosphatase have increased levels of this enzyme after cultivation with hydrocortisone.

**Figure 3** Karyotype of the stemline of HeLa71 arranged according to the Denver-London classification.
Although alkaline phosphatase showed the greatest variation in level of activity for all the strains considered, glucose-6-phosphate dehydrogenase showed variation in activity over a 12-fold range, lactic dehydrogenase over an 8-fold range, and 6-phosphogluconic dehydrogenase over a 2-fold range. The enzymes did not always vary in the same direction: HeLa A demonstrated a low alkaline phosphatase and a high glucose-6-phosphate dehydrogenase, whereas HeLa 7 demonstrated a relatively high alkaline phosphatase and a low glucose-6-phosphate dehydrogenase. On the
TABLE IV

Enzyme Activities in HeLa Variants

<table>
<thead>
<tr>
<th>Name</th>
<th>Alkaline phosphatase*</th>
<th>Lactic dehydrogenase†</th>
<th>6-Phosphogluconic dehydrogenase§</th>
<th>Glucose-6-phosphate dehydrogenase¶</th>
<th>Effect of hydrocortisone on alkaline phosphatase levels¶</th>
</tr>
</thead>
<tbody>
<tr>
<td>HeLa A</td>
<td>0.003</td>
<td>1.7</td>
<td>0.081</td>
<td>0.44</td>
<td>+</td>
</tr>
<tr>
<td>HeLa 229</td>
<td>0.010</td>
<td>0.94</td>
<td>0.086</td>
<td>0.30</td>
<td>+</td>
</tr>
<tr>
<td>HeLa S3G</td>
<td>0.74</td>
<td>0.32</td>
<td>0.11</td>
<td>0.68</td>
<td>-</td>
</tr>
<tr>
<td>HeLa G</td>
<td>0.12</td>
<td>2.6</td>
<td>0.046</td>
<td>0.15</td>
<td>-</td>
</tr>
<tr>
<td>HeLa145</td>
<td>0.006</td>
<td>1.6</td>
<td>0.052</td>
<td>0.12</td>
<td>+</td>
</tr>
<tr>
<td>HeLa171</td>
<td>0.56</td>
<td>2.5</td>
<td>0.066</td>
<td>0.099</td>
<td>-</td>
</tr>
<tr>
<td>HeLa175</td>
<td>0.41</td>
<td>1.9</td>
<td>0.069</td>
<td>0.055</td>
<td>-</td>
</tr>
</tbody>
</table>

* Alkaline phosphatase activity is expressed as µmoles of p-nitrophenol formed per min per mg protein.
† Lactic dehydrogenase activity is expressed as µmoles of NAD converted to NADH per min per mg protein.
§ 6-Phosphogluconic dehydrogenase activity is expressed as µmoles NADP converted to NADPH per min per mg protein.
¶ Glucose-6-phosphate dehydrogenase activity is expressed as µmoles NADP converted to NADPH per min per mg protein.
¶ Plus (+) indicates increased activity when cells are cultured with hydrocortisone; Minus (−) indicates the opposite or no effect.

other hand, this variation was not always an inverse relationship, as shown by HeLa S3G which had the highest alkaline phosphatase and glucose-6-phosphate dehydrogenase.

DISCUSSION

This study presents data in support of a system of classifying HeLa variants that would avoid the confusing situation inherent in the present nomenclature of HeLa cells. The HeLa cells from three different sources, all called S3, demonstrate markedly different chromosomal characteristics and levels of enzymes. Although the suggested nomenclature based on stemline chromosome number has the obvious defect of not being able to characterize completely the HeLa variant, at least it will prevent the comparison of results obtained from different laboratories with obviously different variants, such as those described.

Cultures of HeLa cells have been thought to be rather unstable as to chromosomal characteristics (22) despite the work of Chu and Giles (8) and Vogt (9) who showed that specific clones of HeLa cells had quite sharp modal chromosome numbers. The evidence we have accumulated to date also suggests that certain clones are stable with repeated subculturing for at least 6 months and that the chromosome mode of the clone is a characteristic of that clone.

Both Vogt (9) and Hsu (22) showed that culture conditions could be used to alter the cell populations with the selection of cells genetically and phenotypically different from the original stemline. Our results do not necessarily indicate that all methods of culturing will result in instability of the genetic and phenotypic characteristics of all cell strains. Vogt (9) was able to increase the percentage of cells in the stemline to 100% of a single chromosome number, and we would suggest that under stable conditions of environment this cell strain could be maintained as a sufficiently "pure" strain to allow it to be characterized.

Spurna and Hill (23) have presented evidence which they say demonstrates the stability of HeLa and L cells for 7 months under stable conditions of culture. Although they show a stable stemline for HeLa and L cells, their HeLa S3 cells did change modal chromosome number from 68 on the 12th passage to 70 on the 43rd passage. One finding which they do not discuss, but which may be pertinent, is the skew of their ideograms to the low side of the modal number. One reason for this could be the loss of chromosomes from cells of the modal number with a resultant decrease of
the percentage of cells in the stemline. Their findings would suggest that the HeLa (wild strain) and L cells were fairly stable and were adapted to the growth conditions whereas the HeLa S3 cells which they studied were undergoing some adaptation to their growth conditions and thus had not stabilized. Our results along with those of the other investigators mentioned above would suggest that this drift in characteristics of the culture can occur from alterations in methods of culture, richness of the medium, materials added to the medium, and type and amount of serum used in the cultures.

Hsu (22) has discussed the evolution of tissue culture strains under the influence of the multiple-selecting factors operative in the tissue culture situation. Although evolution undoubtedly operates on an accelerated scale under the artificial environment of the tissue culture conditions, it is possible that, by frequent cloning and examination of the karyotype of the strains and their biochemical parameters, phenotypically stable clones of HeLa cells can be established and maintained to allow studies of biochemical genetics in human cell strains. Until this objective is achieved for human strains, it will not be possible to carry out studies with human cells comparable to those carried out with bacteria. With respect to modal chromosome number, it is interesting to note that variation in enzyme level. HeLa tumors is associated with increasing aneuploidy (22) suggests that chromosomal imbalance alone may result in alterations in enzyme level. HeLa and similar human cell strains offer a unique opportunity for studying the effect of chromosomal imbalance on enzyme levels and control mechanisms within human cells.

Necessary extensions of this study should be directed at cloning the various HeLa strains so as to determine the stability of clones so obtained, the variations of modal number with culture conditions, the karyology and biochemistry of the clones, and the relationship of variation in number of specific chromosomes with variation in enzyme levels.

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