LYSOSOMAL ENZYMES IN HELA CELLS

ICHIRO YAMAMOTO, TERUKO TOMIUCHI,
YASUAKI KAWAKUBO* AND
YASUKAZU NISHIYAMA*

*Department of Pathology, Kitasato University School
of Hygienic Sciences, Tokyo*

Received for Publication January 5, 1969

Localization and intensity of three lysosomal enzymes such as acid phosphatase, β -glucuronidase and N-aceyl- β -glucosaminidase in HeLa cells were examined during the course of cultivation. Acid phosphatase showed the most intense reaction. Localization and intensity of acid phosphatase were varied in different cell groups. Such varieties were classified as Type I to Type IV. Type I indicated young cells wherein stained granules were localized in the Golgi area. Type II indicated young cells with diffusely scattered, stained granules. Type III indicated rounded dying cells, and the whole cell was stained. Type IV indicated cells wherein the enzyme activity was almost negative. The quantitative activity of acid phosphatase was found to be high when young cells, Type I, grew in the earlier stage and when young cells, Type II, increased in the intermediate stage, and became lower as cells, Type IV, increased in the later stage. Thus, the lysosomal enzyme activity in HeLa cells seemed to consist of two factors; one is the primary lysosomes indicating cellular growth or cellular activity, the other is the secondary lysosomes indicating cellular degradation.

HeLa cells have been used for various experiments, especially for detection of antitumor effects of antibiotics (8,9). The role of lysosomal enzymes in such cells has drawn our attention, because it was thought that their autolytic function might take part in the cytotoxic effects of the antibiotics. However, in the course of our study on the cytotoxic effects of antitumor antibiotics employing HeLa cells, it was observed that even in non-treated HeLa cells the localization and activity of lysosomal enzymes altered during the course of cultivation.

The present report deals with the localization and activity of lysosomal enzymes in non-treated HeLa cells at several intervals after cultivation, and their relation to cellular degradation or cellular activity will also be discussed.

MATERIALS AND METHODS

Method of culture:

HeLa cells were cultivated at 37°C. The culture medium consisted of 10 parts of calf serum and 90 parts of Earl's balanced salt solution containing 0.1% yeast extract, 0.5% lactalbumin hydrolysate, 100 units of penicillin per ml and 100 γ of streptomycin per ml. The medium was renewed every four days. For subculture,

* Division of Pathology, The Kitasato Institute, Tokyo

a four-day-old monolayer cells were treated with trypsin and 3×10^5 cells per ml were transferred onto the surface of the coverslips placed in the floors of Leighton tubes and onto the floors of bottles, the volume of which was $55 \times 42 \times 110$ mm. Subculture medium consisted of 5 parts of calf serum and 95 parts of Earl's balanced salt solution containing 0.1% yeast extract and 0.5% lactalbumin hydrolysate, and was free of antibiotics. These cells were cultivated at 37°C and the medium was changed once 48 hours after cultivation.

Cytochemistry:

The cell sheets grown on the surface of the coverslips in Leighton tubes were fixed for 4 hours at 4°C in 4% paraformaldehyde buffered with phosphate at 48, 72, 96, 120 and 144 hours after subculture. The localization of acid phosphatase, β -glucuronidase and N-acetyl- β -glucosaminidase was examined respectively with simultaneous-azo-coupling methods employing naphthol AS-BI compounds as substrates and hexazonium pararosanilin as a capture reagent (1,5,6).

Biochemical assay:

The cell sheets grown on the floors of the bottles were scraped up with rubber policeman at the same intervals after subculture. The cells were washed three times in cold 0.25M sucrose through decantation. After freezing and thawing were repeated six times, acid phosphatase activity was determined by Bessy-Lowry's method employing disodium p-nitrophenylphosphate as substrate (2). Protein was also determined by the method of Lowry *et al.* on coloring produced by Folin-Ciocalteu's phenol reagent and by biuret reagent(7). Acid phosphatase and protein in the culture media were also measured by the same procedures.

RESULTS

Cytochemistry:

Acid phosphatase:

Of the three enzymes examined, acid phosphatase showed the most intense reaction and was demonstrated throughout the course of cultivation. At 48 hours after cultivation, young cells predominated and there were a few mitotic cells. The young cells were varied in size and shape. Some cells were elongated with extended processes, and some were polygonal with small processes. However, the localization and intensity of acid phosphatase in young cells also showed variety, not connected with their morphology. That is, in a majority of the cells intensely stained granules were localized in the Golgi area of the cytoplasm (Fig. 1), and in the remaining cells more intensely stained granules were distributed fairly evenly in the cytoplasm (Fig. 2). Here, for the convenience to describe HeLa cells cytochemically different, young cells will be divided into two types, the former as Type I and the latter as Type II. There were also a few rounded cells which appeared to be dying. In these cells, intensely stained granules, as in the cell of Type II, were distributed throughout the cell including the nucleus. Such cells will be classified as Type III (Fig. 3). Besides, there were a few cells which were similar to young cells in shape, but somewhat swollen. In these cells, weakly

Fig. 1. A young HeLa cell, Type I. Acid phosphatase. Enzyme activity is demonstrated as intensely stained granules localized in Golgi area.

Fig. 2. A young HeLa cell, Type II. Acid phosphatase. More intensely stained granules are distributed fairly evenly in the cytoplasm.

stained granules were diffused in the cytoplasm or no stained granules were found. such cells will be classified as Type IV. In mitotic cells, intensely stained fine granules were distributed throughout the cytoplasm, and in some cells more intensely stained coarse granules were localized in the portion close to the nucleus (Fig. 4). At 72 hours after cultivation, distribution of the cellular types was not much different to that of the earlier stage, except that the cells of Type IV were observed to be increased. At 96 hours after cultivation, the cells of Type II predominated. In the later stage, at both 120 and 144 hours after cultivation, the cells of Type IV predominated. On the other hand, mitotic cells showed intense reaction throughout the course of cultivation.

β -glucuronidase and N-acetyl- β -glucosaminidase:

β -glucuronidase showed less intense reaction than acid phosphatase in the earlier stage, and its activity became almost negative at 96 hours after cultivation. N-acetyl- β -glucosaminidase showed weak reaction in the earlier stage, and became almost negative at 72 hours after cultivation. These enzymes were demonstrated as less intensely stained fine granules distributed in the variety of four types as in acid phosphatase. In mitotic cells, these enzymes were demonstrated throughout the course of cultivation.

Quantitative assay of acid phosphatase:

As shown in Fig. 5, the quantitative activity in the cells was higher in the earlier

Fig. 3. A rounded dying cell, Type III. Acid phosphatase. Stained granules are distributed throughout cell including nucleus.

Fig. 4. Mitotic cells. Acid phosphatase. One cell shows intense activity and the other contains fine, stained granules scattered in the cytoplasm. These pictures were photographed at the same magnification.

stage of cultivation, and gradually decreased as the time elapsed, except that it rose once at 96 hours after cultivation. The activity in the culture media did not show distinct alteration.

DISCUSSION

Of the three enzymes studied, acid phosphatase showed the most intense reaction and was demonstrated throughout the course of earlier stage and its activity became negative at 96 hours after cultivation. N-acetyl- β -glucosaminidase showed the weak reaction in the earlier stage and its activity became negative at 72 hours after cultivation. On the other hand, in mitotic cells these three enzymes were demonstrated as intensely stained granules in the cytoplasm throughout the course of cultivation.

Localization and intensity of acid phosphatase activity were varied in different cell groups such as young cells, rounded dying cells and swollen degenerating cells. These cell groups in the interphase were classified into four types as Type I to Type IV. The young cells could be divided into two groups, Type I and Type II. The former indicated the cell wherein intensely stained granules were localized in the Golgi area, and the latter indicated the cell wherein more intensely stained granules were fairly evenly distributed in the cytoplasm. The rounded cell was similarly

Fig. 5. Quantitative activity of acid phosphatase in HeLa cells and in the culture media. Dots indicate measured value and lines indicate mean value.

intensely stained throughout the cell including the nucleus, and classified as Type IV. Some of these results agreed with Green and Verney (4) who also described localization and intensity of acid phosphatase activity in young HeLa cells and the rounded dying cells. The cell of Type III corresponded to the rounded dying cell described by them.

During the course of cultivation, the cells of Type I predominated in the earlier stage, the cells of Type II were relatively increased in the intermediate stage, and the cells of Type IV became predominated in the later stage. However, few cells of Type III were observed throughout the course of cultivation.

Quantitative activity of acid phosphatase in the cells was higher in the earlier stage of cultivation and gradually decreased as the time elapsed, except it rose once in the intermediate stage. Namely, the quantitative activity appeared to be high when young cells grew and became lower as swollen, degenerating cells increased. A transient increase of the activity in the intermediate stage seemed to be due to increase of the cells of Type II in this stage.

Acid phosphatase activity demonstrated in mitotic cells and young cells of Type I is apparently the primary lysosomes and this appears to indicate cellular growth or cellular activity, but not cellular degradation. The cells of Type IV, therefore, may indicate the degenerating cells which lost their lysosomes because of lowering of the cellular activity.

On the contrary, according to the concept of lysosomes(3), acid phosphatase activity demonstrated in the cells of Type II and Type III appeared to be the secondary lysosomes which perform autolytic function.

Therefore, activity of lysosomal enzymes in HeLa cells is considered to consist of two factors; one is the primary lysosomes indicating cellular growth or cellular activity and the other is the secondary lysosomes indicating cellular degradation.

ACKNOWLEDGEMENT

The authors wish to express their gratitude to Prof. H. Anzai of the Department of Public Health at Kitasato University who kindly allowed to utilize a HeLa strain in his laboratory. The authors also wish to thank Mr. M. Okamoto and Mr. T. Suzuki in the Department of Public Health at the university for their technical assistance.

REFERENCES

1. Barka, T. and Anderson, P.J.: Histochemical methods for acid phosphatase using hexazonium pararosaniline as coupler. *J. Histochem. Cytochem.* 10; 741, 1962.
2. Bessy, O.A., Lowey, O.H. and Brock, M.J.: A method for the rapid determination of alkaline phosphatase with five cubic millimeters of serum. *J. Biol. Chem.* 164; 321, 1946.
3. de Duve, C.: *The lysosome concept*. In Lysosomes, edited by A.V.S. Reuck and Margaret P. Camerson, Little, Brown and Co., Boston, 1963, p. 1.
4. Green, M.H. and Verney, E.L.: A simple method for the study of intracellular acid phosphatase in HeLa cells. *J. Histochem. Cytochem.* 4; 106, 1956.
5. Hayashi, M., Nakajima, Y. and Fishman, W.H.: The Cytologic demonstration of β -glucuronidase employing naphthol AS-BI glucuronide and hexazonium pararosaniline method; A preliminary report. *J. Histochem. Cytochem.* 12; 659, 1964.
6. Hayashi, M.: Histochemical demonstration of N-acetyl- β -glucosaminidase employing naphthol AS-BI-N-acetyl- β -glucosaminide as substrate. *J. Histochem. Cytochem.* 13; 355, 1965.
7. Lowry, O.H., Rosebrough, N.J., Farr, A.L. and Randall, R.J.; Protein measurement with the Folin phenol reagent. *J. Biol. Chem.* 193; 265, 1951.
8. Nitta, K.: Studies on the effects of actinomyces products on the culture of human carcinoma cell (strain HeLa). I. The effect of known antibiotics having no or slight tumor-inhibitory activity on HeLa cells. *Jap. Med. Sci. Biol.* 10; 277, 1957.
II. The effect of known antitumor antibiotics on HeLa cells. *Ibid.* 10; 287, 1957.
III. Comparative studies on anti-HeLa cells effects of known synthetic antitumor substances. *Ibid.* 10; 419, 1957.
IV. Studies on Sarkomycin and its derivatives. *Ibid.* 10;429, 1957.
9. Price, K.E., Buck, R.E., Schlein, A. and Siminoff, P.: A comparison of the in vitro susceptibility of HeLa and protozoan cells to antitumor antibiotics. *Cancer Res.* 22; 885, 1962.