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LYSOSOMES IN MOUSE MELANOMA*

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ABSTRACT

The relation between melanoma growth and lysosomal enzyme activities was investigated using B-16 mouse melanoma. The tumor weight began to increase very rapidly after an induction period of 14 days. All the enzyme activities (unit/mg protein) investigated, except for succinic dehydrogenase, showed a rapid increase up to the ninth day after the transplant, then decreased. Tyrosinase and β-Glucuronidase activities showed, at the end of the 29 day growth period results that were equal to half of those recorded on the ninth day. Succinic dehydrogenase activity increased sharply up to the twenty-first day and then decreased. Acid phosphatase activity, on the other hand, was rather steady. An increase in lysosomal enzyme activities was not observed in the later stage of tumor growth, contrary to what had been expected.

Electron microscopic studies (1–3) revealed that mouse melanoma consists of three types of cells: a melanin producing cell, a melanin containing cell and a melanin producing and containing cell. Melanosomes were present in the melanin containing cell in the form of melanosome complexes which are now thought to be some kind of lysosomes and to be formed by autophagy. The cell containing melanosomes in various developmental stages and melanosome complexes may possibly be the old melanocyte (3). The lysosomal enzyme activities were found to be present in individual melanosomes as well as in compound melanosomes (4–6). Considering these findings, it is now necessary to study the relationship between lysosomal enzyme activities and melanoma growth in order to understand the ontogeny of the melanoma melanocyte. In an effort to elucidate the characteristics of the lysosomes in the melanoma, the subcellular distribution and latency of lysosomal enzymes were also investigated. B-16 mouse melanoma was used because it grows rapidly and usually the mice die about 30 days after the transplant.

MATERIALS AND METHODS

Isolation of subcellular fractions. B-16 mouse melanomas, 1–2 cm in diameter, were harvested from D-D strain, male mice, minced and promptly homogenized in 10 volumes of 0.25 M ice-cold sucrose solution for 30 seconds with a Potter-Elvehjem type homogenizer. All subsequent processing took place in a cold environment. The nuclear fraction was prepared by centrifuging the homogenate for 10 minutes at 700 × g. The resulting low-speed supernatant, when centrifuged at 11,000 × g for 20 minutes, yielded a sediment that was resuspended in 0.25 M sucrose and recentrifuged at 15,000 × g for 10 minutes. This sediment (the large-granule fraction) was again suspended in 0.25 M sucrose to make the large-granule preparation. The small-granule fraction was prepared by centrifuging the washes and the supernatant from the large-granule fraction in the ultracentrifuge for 60 minutes at 105,000 × g. The resulting supernatant is hereafter referred to as the soluble fraction. Enzyme assays were performed with respect to these various fractions.

Determination of various enzyme activities. Acid phosphatase was estimated by the method described by Berthet and de Duve (7). β-Glucuronidase was determined by the method of Nimmo-Smith (8) and cathepsin was done by Schamberger's modification (9) of Anson's method. Tyrosinase activity was estimated by Shimao's colorimetric method (10). Succinic dehydrogenase was measured by the method described by Schneider and Potter (11). Tyrosinase was determined as a marker of melanosomes; succinic dehydrogenase, as a marker of mitochondria and the rest, as markers of lysosomal enzymes. Protein contents were determined by Lowry's method (12) and phosphate content by Fiske and Subbarow's method (13).

RESULTS

Subcellular Distribution of Lysosomal Enzyme in B-16 Mouse Melanoma

As shown in Table I, tyrosinase and succin dehydrogenase activities were present mainly the large-granule fraction which constitut

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TABLE I

Distribution of various lysosomal enzyme activities among the subcellular fractions

	Homogenate	Nuclear fraction	Large-granule fraction	Small-granule fraction	Soluble fraction
Tyrosinase (ΔE/10 min)	8322 (178%)*	4180	7157	3041.5	418.2
Succinic dehydrogenase (μl/10 min)	1379.9 (76.4%)*	485.0	538.4	29.4	0
Cathepsin (μg-Tyrosine/30 min)	27550 (112%)*	12400	7905.5	1298.5	9265
β-Glucuronidase (μg-Nitrophenol/30 min)	4712 (71.5%)*	1080	1326	420.4	544
Acid phosphatase (μg-P/10 min)	1444 (103.7%)	500	258.6	165.4	569.5
Protein (mg)	294.5 (107.5%)*	134.3	51.5	37.8	92.5

* Recoveries are calculated in terms of the amount of homogenate.

TABLE II

Latency of the acid phosphatase activity of melanoma lysosomes

ACID PHOSPHATASE	Activity (Units/mg. Protein)
0.25 M Sucrose	10.5
0.20 M Sucrose	10.7
0.10 M Sucrose	10.3
0.05 M Sucrose	10.5

largely mitochondria, lysosomes, melanosomes and a small amount of microsomes. Cathepsin and acid phosphatase were located in not only the large-granule fraction, but in the supernatant in a considerable amount.

Latency of Lysosomal Enzymes

A series of experiments to show whether a hypotonic condition would or would not liberate the lysosomal enzymes of the melanoma cells was carried out. Homogenates of B-16 mouse melanoma and mouse liver were centrifuged at 700 × g for 10 minutes to remove cell debris and nuclei. A certain amount of distilled water was added to the supernatant to bring the final concentration of sucrose to 0.25, 0.20, 0.10 and 0.05 M, respectively. The activity of the acid phosphatase of the supernatant was not enhanced in the hypotonic solution as can be seen in Table II.

In another experiment, desoxycholate (DOC) was added to the supernatant of the melanoma tissue and mouse liver, making a final concentration of 0.1% and 0.5%. Acid phosphatase and cathepsin activities were estimated. A marked increase of the acid phosphatase activity was observed in the supernatant of the liver, but not in the tumor as shown in Figure 1. Cathepsin activity, however, increased in the presence of 0.1% DOC. Under the most hypotonic condition, when the tumor was homogenized in the distilled water, about an 80% increase was observed in cathepsin activity.

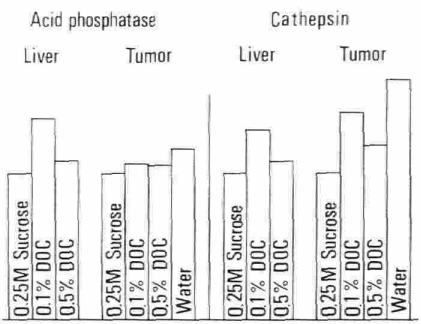

FIG. 1 Latency of various enzymes of melanoma and liver lysosomes.

Relation between Tumor Growth and the Activities of Lysosomal Enzymes

After transplanting the B-16 mouse melanoma to D-D line mice, at least 5 animals were sacrificed every fourth day for a period of 29 days.

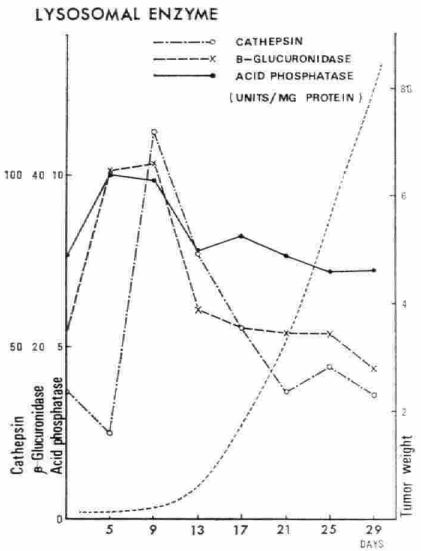

FIG. 2 Relation between lysosomal enzyme activities and melanoma growth. Cathepsin activity (μg-tyrosine/30 min/mg protein) is expressed as O-·-·-·-O. β-glucuronidase activity (μg-p-nitrophenol/30 min/mg protein) is expressed as x----x. Acid phosphatase activity (μg-P/10 min/mg protein) is expressed as •——•. Weight of melanoma (g) is expressed as ----.

Entire tumors were taken out, weighed and their sizes were measured. The supernatant fraction obtained after centrifugation at 11,000 × g for 20 minutes was used for the assay of enzymes.

The weight and size of tumors inoculated subcutaneously remained almost unchanged until 9 days after the transplant. Thereafter the tumors began to grow. The relation between weight and day is a parabolic curve, as shown in Figure 2. The growth rate became very rapid after 13 days. The tumors usually tended to be necrotic at the later stage and almost none of the animals lived longer than 29 days. The curve of acid phosphatase activity rose gradually, reaching its peak 5–9 days after the transplant and then fell gradually to the beginning level. The highest activity of β-Glucuronidase, which resembled that of acid phosphatase, was seen during the period from the fifth to the ninth day, after which its activity gradually decreased. The activity of cathepsin began at a low level, then rapidly increased, reaching its maximum on the ninth day. After that, it went down step by step until it reached the original level as seen in Figure 2. The curve of succinic dehydrogenase was approximately parallel to that of the tumor weight up to the twenty-first day and then it dropped as shown in Figure 3. Tyrosinase was rather active on the day of the transplant, as seen in Figure 4. Its activity went down to the minimal level on the fifth day, then a rapid recovery was seen. After it reached its maximum on the ninth day, it decreased again.

DISCUSSION

According to a series of reports by De Duve *et al.* (14), lysosomes are mainly found in the light mitochondrial fraction. Lysosomal enzymes, cathepsin, β-Glucuronidase and acid phosphatase in the melanoma, were found to exist, not only in the mitochondrial fraction, but also in the supernatant in a considerable amount. The different distribution pattern obtained might be due to tissue specificity (15, 16). The following possibility is also present. In melanoma tissue, the microfocal degradation of melanosomes as

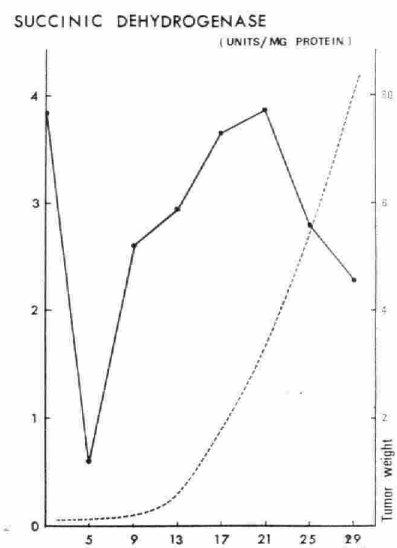

FIG. 3 Relation between succinic dehydrogenase activity and tumor growth. Succinic dehydrogenase activity (μl/10 min/mg protein) is expressed as •——•. Weight of melanoma is expressed as ----.

well as other organelles takes place continuously in the melanin containing cells (17). Therefore, when melanoma tissue is homogenized, the contents of such large autophagosomes, the melanosome complexes, are distributed among all subfractions of the melanoma tissue. It is well supported by the electron-microscopic observations on various cell fractions isolated from B-16 mouse melanoma (18).

Lysosomal enzymes are usually activated by hypotonicity, detergents, freezing and thawing or mechanical procedures (19). The nature of lysosomal enzymes is such that latency is not uniform (20–25). The latency of lysosomal enzymes of melanoma was investigated. It was shown, as indicated in Table I and Figure 2, that the activity of cathepsin increased by DOC treatment and hypotonicity in the same way it did in the liver. On the other hand, the activity of acid phosphatase was not increased by either the DOC treatment or the hypotonicity, since there was no latency present.

Assuming that melanoma melanocytes may be changed into the other two types of cells in which microfocal degradation of cell components takes place, it can be hypothesized that the lysosomal activity of the whole tumor will increase as a tumor grows. The results obtained in the comparison between tumor growth and lysosomal enzyme activities were rather contrary to what was anticipated. The cathepsin activity per tissue wet weight decreased at the late stage and the activity of the other two enzymes also decreased. Acid phosphatase activity, on the other hand, remained unchanged during tumor growth. Paris *et al.* (25) reported that in rapidly growing mammary gland carcinomas, the lysosomal enzyme activities are lower than those of the original tissue, and also it is known that lysosomes tend to disappear in dividing cells (26). The concentration of the lysosomal enzymes increased in regressing mammary tumors and, in contrast, the concentration of several non-lysosomal enzymes failed to increase (9).

Although the exact reasons for these phenomena obtained in this experiment are not known at the present time, the necrosis of a tumor at the late stage is probably due, not only to the activity of lysosomes in the melanin containing cells, but also to the general deterioration in the tumor tissue caused by insufficient blood supply, nutritional shortage and so on.

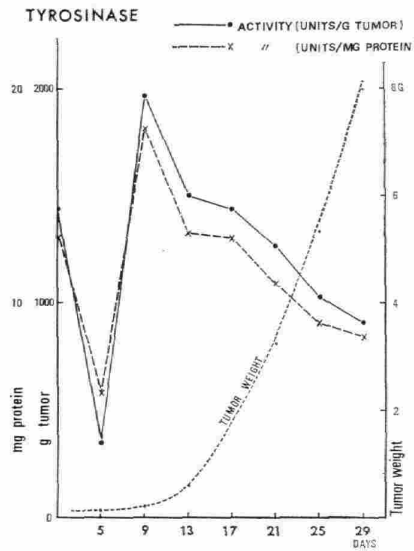

FIG. 4 Relation between tyrosinase activity and tumor growth. Tyrosinase activity (ΔE/10 min/mg protein) is expressed as x----x. Tyrosinase activity (ΔE/10 min/g tumor) is expressed as •——•. Weight of melanoma (g) is expressed as ----.

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