Nonspecific Esterases in Normal and Neoplastic Tissues of the Syrian Hamster: A Zymogram Study

EDWARD H. KREUSSER

Department of Anatomy, Stanford University School of Medicine, Stanford, California

Summary

The normal tissues and 18 tumor types of the Syrian hamster were studied electrophoretically and stained selectively for esterases, and the 2 groups were compared. Esterase banding patterns of normal tissues appeared to be distinct from one another to a degree which roughly paralleled structural and functional differences. With very few exceptions, tumor patterns did not resemble those of parent tissues, but did exhibit a marked tendency to assume a common pattern.

Introduction

The complex interplay of genetic and extrinsic factors probably responsible for the initiation of many, if not most, neoplasias (2) must include alterations in the enzymes of cells undergoing such transformations and in the subsequent neoplastic cells themselves.

In view of this concept, it might be instructive to compare and/or contrast the occurrence of a series of enzymes in normal tissues and neoplasms derived from them. It was decided to concentrate first on an electrophoretic comparison of esterases in normal and neoplastic hamster tissues, since this has not been done heretofore and since such a study could be fitted readily into programs already under way in the laboratories of Professors R. L. Hunter and H. Kirkman at Stanford University.

Greenstein (3) has thoroughly reviewed the literature concerning enzymatic comparisons between normal and neoplastic tissues. From this survey, he has formed these generalizations: (a) Normal tissues can be recognized and distinguished from one another by means of their enzymatic compositions. (b) Tumors derived from these normal tissues conspicuously fail to inherit their parent tissues' enzymatic patterns. (c) Instead, the tumors show a tendency to converge toward a common pattern.

The studies on which these generalizations are based largely concern themselves with biochemical assays of various enzyme classes, resulting in quantitative determinations of these enzymes in various tissues and tumors. In the present study these generalizations are tested using zymograms to illustrate the multiple molecular forms of the soluble nonspecific esterases.

Within any class of enzymes, e.g., esterases, many distinct molecular types exist (4, 14). These can be distinguished by means of various physical properties. This study examines normal and neoplastic tissues in terms of the relatively narrow parameter of the variations within 1 enzyme class, the esterases. While the relative amounts of the isozymes can be estimated by observing the intensity of the bands, it should be emphasized that this is not a quantitative study, but is, instead, a qualitative one stressing presence and absence of various constituent moieties of a class, rather than units of activity of the class as a whole. It is concerned with patterns of esterases present in various tissues, trying to determine if the above statements of Greenstein can be said to hold true when examined by this newer technology.

Materials and Methods

The animal used was the Syrian hamster (Mesocricetus auratus). The tumors were provided by Dr. Hadley Kirkman, who had induced or discovered and maintained all of the tumors used in this study (7, 9). Most tumors used in the study have been maintained through many generations on previously normal host animals by s.c. transplants. Only 2 primary tumors were studied. These were an androgen/estrogen-induced scent gland epithelioma (8) and an estrogen-induced renal carcinoma (5, 6, 10, 11, 12).

All tissues were homogenized with a constant weight-to-volume ratio of 1 gm of tissue:1 ml of distilled water. These homogenates were centrifuged for 20 min at 20,000 X g in a refrigerated centrifuge. The supernatants were used in the study. It was found that these could be stored by freezing.

The zymogram technology used was modified from Markert and Hunter (14) by using 0.005 M sodium barbital in the gel and 0.05 M sodium barbital in the buffer trays (personal communication: H. O. Yokayama, 1965). Both buffers were at pH 8.6. The substrate used in all cases was a-naphthyl butyrate with Blue RR salt serving as the diazonium coupling agent. Runs were made horizontally for 2.5 hr at a potential difference of 10 volts/cm.

Several repetitions, using different animals, were made of each tissue to insure the presentation of representative zymograms for each organ. Moreover, male and female tissues were compared and found to be essentially similar, except for serum. Samples from the tumors were taken from the periphery of the growths, where vascularization was generally good and where necrosis had not occurred. Several representatives were taken of each tumor type and several samples from each tumor. All were essentially identical.

Preparation of the Illustration. Each sample was run side...
by side with the same liver supernatant, which acted as an arbitrary yardstick by which to compare bands of different samples. Although the runs were standardized as much as possible, the migration distances were not always the same; for example, the fastest migrating band of the standard, liver, did not always end at the same distance from the origin; the bands of the samples were similarly affected. This effect was due to slight variations in gel viscosity and voltage. Thus a flexible measuring device, such as a common tissue, run next to all samples, was needed.

In order to make the gel strips of each sample a proportionate length, relative to the rest (for Fig. 1), photographic manipulations were used. From photos taken of each sample, enlargements were made, adjusting the distance from the origin to a common band so that all were equal. In spite of this, some photos were still not in perfect proportion to the rest. This must be because the rates of migration of different molecular moieties were not altered proportionately by variations in gel and voltage. Thus, there is incomplete uniformity in the strips. The greatest variations existed in the farthest migrating bands, the same band in 2 different samples often appearing as 2 different bands. Had it been considered essential to establish complete uniformity, each sample would have been displayed next to its liver standard. Fortunately, this was not necessary, the samples comparing most easily when contiguous, as in Fig. 1.

**Observations**

There was no esterase which occurred exclusively in tumors. Neither was there any absolute difference between esterases in the tumors and in the normal tissues. There was not even a banding pattern which belonged exclusively to the tumors. One could not differentiate, for example, between the lymphoid tissues Nos. 17-19 and certain tumors Nos. 60-64. Moreover, the anterior and posterior lobes of pituitary gland Nos. 4 and 5, lacrimal gland No. 2, and seminal vesicle No. 38 were all quite similar to many of the tumor patterns. Nevertheless, certain things became clearly evident on examination of Fig. 1:

1. The *range* of staining intensity from tissue to tissue (indicative of concentration of esterases) was much greater in the normal tissues than in the tumors. No tumor stained as deeply as did liver No. 11 or kidney cortex No. 8; none so faintly as brain No. 20 or submaxillary gland No. 45. The tumor patterns displayed a range of intensities more moderate and uniform than that of normal tissues.

2. It was apparent that numerous banding patterns existed among the normal tissues, most of which were characteristic.

3. In many cases, organs of similar structure or function had similar banding patterns, e.g.: (a) The lymphoid tissues, lymph node No. 17, thymus No. 18, and spleen No. 19, all had similar bands, though the intensities were different. (b) Skin No. 15 and scent gland No. 16 (a specialized type of skin) had similarities in banding. (c) Divisions of the alimentary canal Nos. 20-30 resembled one another very closely.

However, there were some departures from the above generalizations. Many tissues expected to be similar were dissimilar; e.g., the salivary glands Nos. 44-46 were quite unlike one another, as were skeletal muscle No. 13 and tongue No. 12.

4. With the exception of 2 tumors (Nos. 47 and 48), the tumors appeared very similar to one another. Excluding these, 2 major groups could be distinguished. The renal tumors, the pancreatic islet cell tumor, and the colonic adenocarcinoma formed 1 group (Nos. 60-64), while the remaining tumors formed the other (Nos. 49-59). The renal tumor group possessed an extra band near the origin. In reality, most of the others did also, but it was too faint to be readily apparent. Thus, there was no sharp dichotomy into 2 groups.

5. A corollary to the above point is that the tumors did not tend to resemble their parent tissues. Many of the tumors arose from diffuse tissues, e.g., the melanoma No. 49, reticular cell sarcoma No. 50, and neurilemmona No. 57. Others had parent tissues of microscopic size, e.g., the cholangioma No. 51 and pancreatic islet cell tumor No. 60. It is difficult or impossible in these instances to isolate the parent tissues for purposes of comparison.

Others, however, had accessible parent tissues. These were compared with their normal counterparts. Besides the adrenal adenocarcinoma, which will be considered shortly, 5 such comparisons were made: (a) flank organ No. 16:flank organ tumor No. 52; (b) lymphoid tissues Nos. 17-19:lymphoma No. 53; (c) liver No. 11:liver cell carcinoma No. 55; (d) ductus deferens No. 35:leiomyosarcoma from ductus deferens No. 59; and (e) kidney cortex No. 8 and kidney medulla No. 9:renal carcinomas Nos. 62-64.

On the basis of these comparisons, it was clear that the tumor tissues did not usually correspond to the tissues from which they arose. The lymphoid tissues, as mentioned before, resembled some of the tumors Nos. 60-64, though not especially the lymphoma No. 53.

There was 1 exception to this generalization, however, and a 2nd exception was possible. Adrenal adenocarcinoma No. 47 was 1. It very closely resembled adrenal gland No. 7 in its esterase pattern. Ovarian thecoma No. 48 was a possible 2nd exception. It did not resemble ovary No. 39, but it would not be expected to, for it was derived from only a small component of ovary, cells of the internal theca. It may resemble the internal theca, but this component could not be isolated in pure form for comparison. It certainly did not resemble the other tumors.

6. The degree of autonomy achieved by a formerly hormone-dependent tumor did not appear to influence its esterase pattern. The estrogen-induced primary renal carcinoma No. 62 had a pattern indistinguishable from that of the hormone-dependent transplants No. 63 and from that of a partially autonomous variant No. 64.

7. The esterase pattern of a tumor did not appear to be affected by repeated serial s.c. transplantsations. The 2 primary tumors, scent gland tumor No. 52 and renal carcinoma No. 62, were indistinguishable from other tumors, maintained by transplantation.

**Discussion**

1. It was noted in Section 3 under Observations that some tissues, expected to be similar in esterase pattern due to functional similarities, are in fact dissimilar. There are several possible explanations for these and other inconsistencies.
(a) Certain esterases may not play roles in the specific differentiated functions of tissues, e.g., in the secretion of certain substances, but may be involved in fundamental processes (1) common to many tissues, as mitosis. This may explain why anterior lobe of pituitary gland No. 4 resembled posterior lobe No. 5. This would explain also why certain tissues with complicated differentiated activities (as adrenal No. 7) displayed less complicated esterase zymograms.

b) In dissecting tissues for homogenizing, it is difficult to segregate certain tissue elements from others for accurate comparisons; e.g., certain glandular structures associated with tongue No. 12 may distort the pattern of the muscular component, rendering the homogenate unsatisfactory for comparison with that of skeletal muscle No. 13.

c) There may be a diluting-out effect, as in brain No. 20 where the myelin, which may be quite inactive enzymatically, constitutes a large proportion of the weight of the tissue. This may explain the extremely weak pattern of brain. This possibility seems likely, since the brains of embryonic or newborn animals which have less myelin produce esterase zymograms comparable in intensity to those of other organs (13).

d) Functions which superficially appear to be similar may, on closer inspection, involve quite different metabolic machinery. For example, lacrimal gland No. 2 and Harderian gland No. 1 both secrete fluid for the eye. They are quite different, however, in esterase compositions, probably a reflection of the fact that these 2 fluids are produced by quite different enzymatic pathways. The same explanation can be offered to account for the difference between brown fat No. 24 and fatty omentum No. 25.

It is clear, then, that the esterase content of a tissue, as we have exposed it, does not offer an infallible reflection of that tissue's total activity. Nevertheless, it is a fair indicator, roughly paralleling the metabolic activity of many tissues. For any 1 tissue it is an uncertain and unsatisfactory manifestation of activity; but for a whole spectrum of tissues, it shows a range of tissue activity and tends to show the uniqueness of enzymatic potential which differentiated tissues can possess.

2. All but 2 of the tumors studied resembled one another quite closely. Adrenal adenocarcinoma No. 47 did not look like the others but resembled its parent tissue, adrenal gland No. 7. Although it is not a differentiated tumor histologically, it is known to have some steroid production, though this is low. This may help to explain its similarity to the normal adrenal gland; i.e., since some of the normal activity has been preserved, some of the normal enzymatic machinery necessary for this function has therefore been preserved also.

Ovarian thecoma No. 28 is the 2nd tumor which doesn't fit the tumor prototype. Unfortunately, its parent tissue, theca interna, is not available for comparison; ovary No. 39, as explained above, is not an appropriate tissue for comparison. The thecoma may differ from the other tumors due to a residual functional similarity to its parent tissue and to its esterase pattern, as proposed for the adrenal adenocarcinoma.

An additional piece of information must be discussed in this connection. The pancreatic islet cell tumor No. 60 is a highly differentiated tumor histologically and is functional as well. This tumor resembled the majority of other tumors, but did not resemble pancreas No. 43. This does not preclude the explanation offered for the above 2 tumors, however, for the following reasons:

(a) It is not appropriate to compare the islet cell tumor to whole pancreas. The only meaningful comparison would be with pure islet tissue, which is not available for study. If such tissue were available it might well resemble the tumor.

(b) Esterases may not be involved in the function of all islet secretory cell types and hence would not reflect any remnant of such differentiation in the tumor.

Although these considerations are disturbing, they do not obscure the facts that 16 of the 18 tumors resembled one another very strongly and that 5 of the 6 tumors which were compared with their normal tissues of origin did not resemble them. The point to emphasize, then, is that most of the tumors changed their metabolic machineries (at least as far as esterases were concerned) from those of their parent tissues and tended to converge toward a common type.

It is logical that this phenomenon is due to the fact that, in losing their normal specific functions, the only metabolic activities left are general ones, as growth, mitosis, etc., common to many cells. In view of the few discrepancies described, however, this explanation must be a tentative one.

Conclusions

Normal tissues exhibit characteristic patterns of esterase activity which are constant for any given tissue. Moreover, the patterns tend to be unique, or are at least shared by very few other tissue types. Tumors, on the other hand, tend to reject their parent tissues' esterase compositions and adopt or retain residual compositions which are nearly identical to one another; a few exceptions have been observed.

Acknowledgments

The author wishes to express his gratitude for the invaluable encouragement and support of Drs. Hadley Kirkman and Robert L. Hunter of the Department of Anatomy, Stanford University School of Medicine, Stanford, California. The technical assistance of P. K. S. Yau and the photographic work of M. Millspa are acknowledged.

References


Edward H. Kreusser


Fig. 1. The nonspecific esterase patterns of the Syrian hamster: normal and neoplastic tissues. Abbreviations are used for the following: 9-kidney medulla; 28-glandular stomach; 47-adrenal adenocarcinoma; 50-reticular cell sarcoma; 52-flank organ tumor (scent gland hair follicle epithelioma); 54-hemangiopericytoma; 56-neurofibrosarcoma; 58-leiomyosarcoma (parent tissue thought to be vascular smooth muscle); 59-leiomyosarcoma-ductus deferens; 60-pancreatic islet cell tumor; 61-colonic adenocarcinoma; 62-spontaneous renal carcinoma; 64-partially autonomous tumor derived from hormone-dependent renal tumor. The deep band nearest the origin of 23-erythrocyte is not an esterase, but hemoglobin. The clear margin at the origin of 46-parotid gland is due to the amylase digestion of the starch at this region.
Nonspecific Esterases in Normal and Neoplastic Tissues of the Syrian Hamster: A Zymogram Study

Edward H. Kreusser

Cancer Res 1966;26:2181-2185.

Updated version
Access the most recent version of this article at:
http://cancerres.aacrjournals.org/content/26/10/2181

E-mail alerts
Sign up to receive free email-alerts related to this article or journal.

Reprints and Subscriptions
To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.

Permissions
To request permission to re-use all or part of this article, use this link http://cancerres.aacrjournals.org/content/26/10/2181.
Click on "Request Permissions" which will take you to the Copyright Clearance Center's (CCC) Rightslink site.