Enzyme Patterns in Cancer

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ABSTRACT

The recent reports of the use of serum and tissue enzyme assays in primary diagnosis and then in following the course of the disease have been reviewed. These include use of bone marrow acid phosphatase, isoenzymes of both acid and alkaline phosphatase, LDH<sub>5</sub>/LDH<sub>1</sub> ratios, sialyltransferase and the combination of carcinoembryonic antigen with serum enzyme assays to help in prediction of the occurrence of hepatic metastases.

Introduction

The definition of enzyme patterns in cancer cells has been the subject of research for decades and the literature on this subject is overwhelming. For an equal period of time, clinical enzymologists have been desperately reviewing this literature and attempting to apply some of these findings to use as diagnostic or prognostic markers in patients with cancer. There have been numerous reviews of this subject and the application of enzyme tests in cancer patients. In the early years organ specific enzymes such as acid phosphatase in cancer of the prostate were studied. Then the emphasis was on the enzymes involved in glycolysis, and there was widespread interest in these "ubiquitous" enzymes.

Most recently, the theory has been proposed that cancer is a disease characterized by genetic regulation or phenotype expression of a "misprogramming of protein synthesis" manifested by ectopic polypeptide hormone synthesis, abnormal antigen expression and alteration of isoenzyme composition. The purpose of this article is to review the most recent literature on the use of enzymes in human cancer and to emphasize the misprogramming aspects and the possibility of combining the analysis of isoenzymes and tumor associated antigens or ectopically produced hormones in the evaluation of cancer patients or in predicting the success of chemotherapy.

Enzymes

ACID PHOSPHATASE

Attempts have been made to increase the utility of acid phosphatase determinations. The determination of the enzyme in serum obtained from bone marrow aspirates of the posterior superior iliac crest has been utilized by Reynolds and his associates in staging prostatic carcinomas. These authors claimed that more advanced disease could be established by such assays. Other workers
have also reported that bone marrow acid phosphatase can be helpful in prostatic cancer. In one study the utility of this determination was compared to that of bone biopsy or skeletal survey in staging prostatic cancer.\(^{11}\)

Bone biopsy was found to be more sensitive than skeletal surveys. Of 16 patients with positive bone marrow acid phosphatase, only two had positive bone biopsies at the time of the assay. Five of these 16 patients eventually developed metastases. The authors concluded that an elevation of bone marrow acid phosphatase is the initial sign of the occurrence of bone metastases in patients with prostatic cancer.

Yarrison and his associates also suggested the utility of bone marrow acid phosphatase and proposed that such an elevation is strongly suggestive of metastases to bone.\(^{32}\) They evaluated 10 patients of whom eight exhibited normal serum acid phosphatase activity. In four cases, bone marrow acid phosphatase was the only abnormal laboratory finding. In two cases, the bone scan was also abnormal and in two other patients the serum and marrow phosphatase were abnormal but the bone scan was negative. In the other patients there were no laboratory abnormalities. Marrow phosphatase elevations have also been found in other diseases. Elevations were observed in four cases of leukemia, three of myeloma, three of anemia and in one patient with metastatic oat cell carcinoma.\(^{32}\)

For many years attempts have been made to sharpen-up acid phosphatase assays and make them more useful in the initial evaluation and then in following the course of prostatic disease. The use of the tartrate-inhibited or “prostatic fraction” has not been helpful in initial diagnosis nor in following the course of treatment.\(^{19}\) There are also a significant number of false positive elevations related to rectal examination, constipation, prostatic infarcts in hyperplasia tissue or episodes of fever. Studies of acid phosphatase in prostatic fluid have not been helpful.\(^{12}\)

Acid phosphatase isoenzymes have been separated on polyacrylamide gel electrophoresis and attempts have been made to utilize this technique in evaluation of prostatic carcinoma. It has been observed that the serum of patients with prostatic carcinoma contains different isoenzymes than serum from normal persons.\(^{15,21,32}\) Chu and his associates reported studies with an immunochemical test (counter current immunoelectrophoresis) for “prostatic” acid phosphatase (PAP).\(^{6}\) They found PAP in 20 percent of serums from patients with Stage A and B diseases, 55 percent with Stage C and 80 percent of those with Stage D prostatic cancer. Abnormal values were not detected in normal persons.

Cooper and Fotti\(^{9}\) developed a radioimmunoassay for “prostatic” acid phosphatase (PAP). They found a range of 1.5 ng to 6.5 ng of PAP per 0.1 ml serum (3.99 ± 2.41 ng per 0.1 ml) in 107 normal males. The test could detect as little as 1.0 ± 0.1 ng per ml serum. These workers did not evaluate patients with cancer.

Unsuccessful attempts have been made to combine carcinoembryonic antigen (CEA) and acid phosphatase assays. Chu and his associates compared carcinoembryonic antigen and phosphatase activity in prostatic fluid from patients with carcinoma of the prostate.\(^{5}\) There was no correlation between the concentration of CEA and the activity of acid phosphatase. In one case, the CEA was 81 ng per ml and the acid phosphatase 8,183 units. In another, the CEA was 0.9 ng per ml and the acid phosphatase 374 units.

**ALKALINE PHOSPHATASE**

Serum alkaline phosphatase assays have been used for many years in the
evaluation of bone and liver metastases in cancer patients. Rosen has successfully used serum serial alkaline phosphatase assays to monitor the therapy of patients with osteogenic sarcoma. The enzyme activity is sensitive to the therapeutic response and the rises and falls in alkaline phosphatase indicate the clinical status of the patients. The changes in the enzyme level occur prior to clinical evidence of response or lack of response to the drug and permit the therapist to institute changes in treatment earlier than would otherwise be possible.

The isoenzymes of alkaline phosphatase have been used to differentiate serum elevations from bone or liver disease but the main interest in alkaline phosphatase in cancer patients has been the examination of ectopically formed variants with the greatest attention to the so-called Regan isoenzyme which resembles placental phosphatase in both its physical and catalytic properties. This variant has been detected in serum of 10 to 15 percent of cancer patients, particularly in patients with ovarian or gynecologic cancer. It has been described as a carcinoplacental antigen and has been observed to reflect the clinical status of the patient.

In an extensive study of the Regan isoenzyme by the heat stability technique, Cadeau and his associates found elevations in 3 of 149 "normal" individuals (2 percent); 21 of 601 patients in a general hospital (3.5 percent); 55 of 605 patients with evidence of cancer at the time of study (9.7 percent); and 34 of 226 patients (15.0 percent) with cancer of the female breast or genitourinary tract. Elevations were found in 3 of 86 breast cancer patients without evidence of disease at the time of study (To, No, Mo) and 10 of 46 patients with active breast cancer.

Other variants of alkaline phosphatase have been described. These have been observed in some patients with a variety of cancers including cancer of the lung, pancreas, bile duct and liver and in some patients with benign diseases such as familial polyposis and ulcerative colitis. Despite reports that alkaline phosphatase isoenzymes may reflect the clinical state of the patient and the response to therapy, the precise role of their determinations has not been established. Radioimmunoassays of alkaline phosphatase in enzymes have not improved their clinical usefulness.

Lin et al observed that histaminase was related in ovarian cancer to the presence of the Regan isoenzyme. In ascitic fluid from such patients, Regan isoenzyme was found in each of 10 patients with elevated histaminase activity and in only one of nine patients with low histaminase activity.

**Lactic Dehydrogenase**

Total LDH activity has been observed elevated in the serum of patients with a variety of cancers but its elevation is not specific and elevations are observed in many cancers. In this study total lactic dehydrogenase (LDH) elevations ranged from 33 percent of patients with metastatic cervical cancer to 76 percent of patients with metastatic rectal colon cancer. Elevations were also observed in more than 50 percent of patients with primary carcinoma of the liver, uterus and lung. Clark and Srinvason concluded that LDH assays in prostatic cancer did not add to the clinical impression for diagnosis. An important area of interest is elevation of the LDH isoenzymes and in particular the LDH5 variant. Wood and his associates found that in primary carcinoma of the rectum, total LDH was elevated in 24 percent of the patients, but LDH5 was elevated in 52 percent of the patients.

The use of LDH5 to LDH1 ratios has been proposed as useful in differentiat-
ing benign from malignant tissue. The ratio of LDH$_5$ to LDH$_1$ was six times greater in cancerous breast tissue than normal breast tissue or that from patients with fibroadenoma. The LDH$_5$ proportion of the total activity rose from 4 percent in normal uterine tissue to 42 percent in cervical carcinoma. The ratio of LDH$_5$ to LDH$_1$ was greater than one in 90 of 116 specimens of prostatic carcinoma and less than one in 473 of 503 specimens of benign prostatic hypertrophy.\textsuperscript{27} In another study\textsuperscript{7} the ratio correlated with histopathology in 78.8 percent of benign prostates and only 48.4 percent of cancers. An unsuccessful attempt has been made to use LDH isoenzyme assays to define cervical dysplasia in spatula scrapings from the exocervix and endocervical canal.

Bredin, Daly and Prout studied LDH isoenzymes in bladder carcinoma.\textsuperscript{3} The mean ratios of LDH$_5$ to LDH$_1$ in tissue from patients without bladder tumors were 0.27. In patients with normal or atypical urothelium or low grade cancer (Grade I or II), the ratios were 0.47, 0.51 and 0.25; in patients with high-grade (Grade III or IV) cancer, the mean ratio was 4.74.

**ALDOLASE**

Other isoenzymes may play a role in the management of some cancer patients. The serum of cancer patients contains a greater proportion of aldolase A (muscle-type) than serum from normal persons. Gliomas and normal brain tissue contain aldolase C (nerve and brain variant), but in meningiomas or tissue metastatic to brain, only aldolase A (liver and fetal form) is detected.\textsuperscript{28} Banroques and his associates\textsuperscript{1} found differences in aldolase isoenzymes in a rat duodenal adenocarcinoma induced in Lewis rats by nitrosoguanidine. They found that the aldolase A and C predominated in the tumor whereas aldolase B was found in normal intestine.

In an interesting study, the aldolase isoenzymes were assayed sequentially in liver tissue following the administration of 2-acetylaminofluorene and the development of liver tumors.\textsuperscript{29} The aldolase A was 1.2 times the control after eight months, 4.9 times after nine months and 6.7 times the control value after 9.5 months of drug administration. The changes were observed long before histological changes could be seen. The conclusion was that during the carcinogenesis process in adenocarcinoma Aldolase B disappears and is replaced by Aldolase A and C.

**HEXOKINASE**

Hexokinase isoenzymes have been reported to differ in cervical tissue. Normal cervical epithelium, endometrium, myometrium and myoma have been found to have one isoenzyme which migrates on electrophoresis, just slightly beyond the origin toward the anode. Cervical carcinoma and corpus carcinoma exhibit a second isoenzyme which migrates somewhat faster.\textsuperscript{26}

**ARYL SULFATASE**

Aryl sulfatase exists in three isoenzymatic forms, A, B and C which may be differentiated by their substrate specificity. Aryl sulfatase has been found in higher concentrations in colon cancer tissue and in urine or patients with this disease. Morgan and his associates\textsuperscript{17} found that normal colon had 45 ± 68 units of activity (μM substrate converted per mg deoxyribonucleic acid) with increasing activity related to the stage and grade of the tumor. In Stage C, Grade II tissue, the activity was 77 ± 16.6 units and in Stage D, Grade III, 132 ± 36.1 units. In the urine the activity was also related to the stage of disease and 1 percent of pa-
patients with Stage A had elevations, 57 percent with Stage B and 80 percent of patients with Stage C or D disease.

**Sialyltransferase**

Bosmann and Hall observed increases in sialyltransferase activity in colon tumor tissue (1105 ± 413 units in normal colon, 2309 ± 677 units in cancerous colon). Similarly, Lamont and Isselbacher found that the levels of exogenous galactosyltransferase were higher in normal than in cancerous colon tissue. Kessel and Allen observed increased sialyltransferase activity in the serum from six of seven patients with colon cancer and elevations in serum from 56 of 65 patients with a variety of cancers. Elevations were also observed in patients with rheumatoid arthritis, but not in those with cirrhosis or in pregnant women. Further studies are needed to determine if sialyltransferase assays can be of value in measuring tumor progression, predicting metastatic spread or in evaluating therapeutic response.

**Carcinoembryonic Antigen and Serum Enzyme**

The simultaneous assay of serum enzymes and CEA has been used in colon cancer to achieve a better prediction rate of the presence of hepatic metastases than is possible by the use of the individual assays. Cooper et al, utilizing γ-glutamyltranspeptidase (γ-GTP) and CEA in patients with colorectal cancer, concluded that γ-GTP indicates that the liver is diseased and the CEA discriminates between hepatomegaly owing to metastatic cancer or benign hepatic disease. Sequential assays of both parameters may indicate the presence of hepatic metastases three to nine months before clinical confirmation.

Similar studies have been reported by Munjal et al using phosphohexose isomerase (PHI). Combined measurement of CEA and enzymes demonstrated abnormal values in over 90 percent of patients with gastrointestinal cancer metastatic to the liver.

**Tissue Enzymes and Chemotherapy**

Sartorelli and his associates have raised the interesting possibility that tissue enzyme assays might be useful in selecting patients with high probability of response to chemotherapy. Their example has been the use of 6-thioguanine in treatment of colon cancer. This drug must be converted to an active form, 6-thioguanosine 5'-phosphate. The enzyme responsible for this conversion, hypoxanthine-guanine phosphoribosyltransferase, is present in human colon tumor tissue at levels six times that in adjacent normal colon mucosa. Presumably, in those patients where the tissue enzyme concentration is low or absent, there would be no conversion of the inactive drug and the cancer would be "resistant" to its effects.

**References**

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