Eur J Cancer Clin Oncol, Vol. 20, No. 1, pp. 75–79, 1984.
Printed in Great Britain.

0277-5379/84$3.00+0.00

Serum Galactosyltransferase Isoenzymes as Markers for Solid Tumours in Humans*

ROSS A. DAVEY,† ROZELLE M. HARVIE, E. JANE CAHILL and JOHN A. LEVI
Bill Walsh Cancer Research Laboratory, Royal North Shore Hospital of Sydney, St. Leonards NSW 2065, Australia

Abstract—*High resolution agarose isoelectric focusing was used to compare the galactosyltransferase isoenzyme patterns of serum from 9 healthy controls with those from 38 patients with either breast, lung, ovarian, stomach or colonic cancer. At least 12 peaks of enzyme activity were found in every sample, the healthy controls having major forms with isoelectric points of 4.74, 4.87, 4.96, 5.16 and 5.23. Thirty patients (79%) had elevated levels of at least one isoenzyme and 23 (61%) had at least 3 isoenzymes elevated compared to only 10 (26%) patients who had elevated total serum galactosyltransferase activity. The isoenzymes which were most often elevated in the cancer patient group had isoelectric points of 4.93, 5.16 and 4.61. One isoenzyme with an isoelectric point of 4.43 was preferentially elevated in patients with ovarian cancer. Those isoenzymes containing little or no sialic acid were rarely elevated in cancer patients. Although no cancer-associated isoenzyme was detected the quantitative differences observed in the cancer patient group were striking.*

INTRODUCTION

GALACTOSYLTRANSFERASE is elevated in the serum of certain patients with gastrointestinal [1, 2], pancreatic [1, 3], ovarian [4, 5], lung [2, 3], liver [3] and breast cancer [6]. It has also been postulated that galactosyltransferase could be involved in neoplastic transformation [7–9] and metastatic processes [10]. This has led to a growing interest in galactosyltransferase as a possible tumour marker [9]. There have been several studies on the isoenzymes of galactosyltransferase and cancer with variable results.

Podolsky, Weiser and co-workers [1, 11, 12], using discontinuous polyacrylamide gel electrophoresis of cancer patient and control sera, reported a cancer-associated isoenzyme of galactosyltransferase which they called GTII. This is the only report of a cancer-associated isoenzyme of galactosyltransferase. Other studies using different separation techniques have reported no evidence for a cancer-associated galactosyltransferase isoenzyme. Ion-exchange chromatography was recently used to separate galactosyltransferase from pleural effusions into a GT(H) and a GT(L) form [13]. Although the GT(L) form was present in 73% of effusions from cancer patients, this form showed little specificity for neoplastic disease since 32% of patients with benign disease also had GT(L). Another study using isoelectric focusing on granular gels separated galactosyltransferase isoenzymes from various cultured normal and neoplastic cell lines [14]. Cell line-specific differences were described but the resolution was not sufficient to determine the number of isoenzymes present or whether there were any neoplastic-specific isoenzymes.

These studies relied on methods with limited resolution since it is known that purified galactosyltransferase from human milk, amniotic fluid and malignant ascites have at least 13 isoenzymes [15]. To determine whether there are cancer-associated differences in the isoenzymes of galactosyltransferase we have developed a high resolution agarose isoelectric focusing method with sufficient sensitivity to separate and detect the galactosyltransferase isoenzyme pattern contained in 0.01 ml human serum [16]. We report here the serum galactosyltransferase isoenzyme patterns of 38 patients with various cancers and 9 healthy controls.

Accepted 4 July 1983.
*This research was supported by the Bill Walsh Cancer Research Fund and in part by a grant from the New South Wales State Cancer Council.
†To whom correspondence and reprint requests should be addressed.

MATERIALS AND METHODS

Blood samples were collected from 9 healthy volunteers and 38 cancer patients with tumours at various sites (Table 1), who had not received any chemotherapy or radiotherapy in the past year. The serum was collected by centrifugation and was stored at -70°C. Total serum galactosyltransferase activity and the galactosyltransferase isoenzyme patterns were determined as previously described [16], using a 245 mm long agarose isoelectric focusing gel with a pH gradient of 4.0–6.5. These were cut into 2 mm slices and assayed for galactosyltransferase activity.

RESULTS

The reproducibility and sensitivity of the galactosyltransferase isoenzyme method and the 80% recovery of activity after isoelectric focusing was similar to that previously reported [16].

A representative galactosyltransferase isoenzyme profile for each cancer site and for the healthy control group are shown in Fig. 1. The isoenzyme pattern was complex, with at least 12 isoenzyme peaks being resolved in every serum sample. The total number of isoenzymes present was probably 19 or greater. Not all isoenzymes were detected in each serum sample. The major isoenzyme forms of the healthy control group had isoelectric points of 4.33, 4.43, 4.51, 4.61, 4.74, 4.87, 4.96, 5.16 and 5.23. None of the isoenzymes detected in the 38 cancer patients were unique as all were present in the healthy control group. The qualitative similarity of all the isoenzyme profiles was striking and highlights the reproducibility of this isoenzyme assay.

In contrast, there were obvious quantitative differences between the isoenzyme profiles of the healthy control group and the cancer patient group (Fig. 1), the latter showing a general elevation of most isoenzymes. To determine the extent of these quantitative differences, each isoenzyme peak was identified by its isoelectric point and the peak height was measured. Using

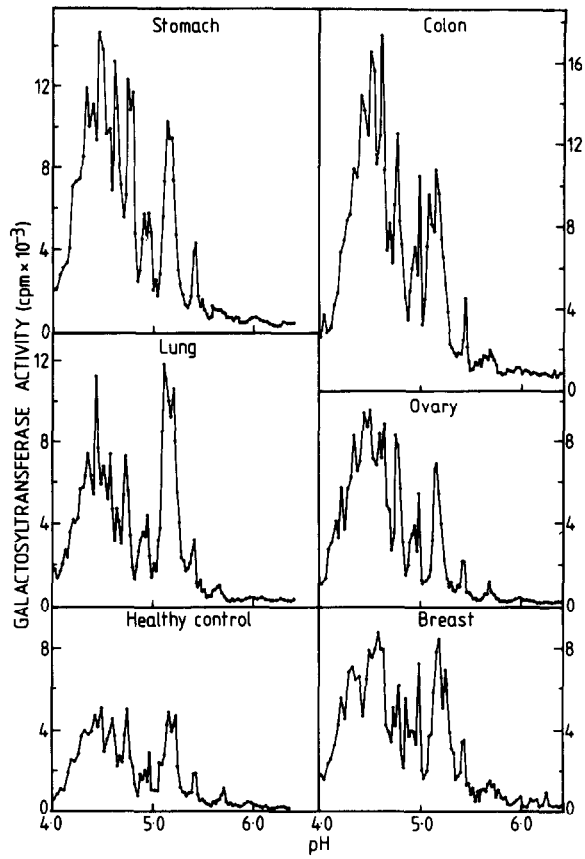

Fig. 1. Representative serum galactosyltransferase isoenzyme profiles of cancer patients with various disease sites and healthy controls.

the healthy control group the upper limit of the normal range for each isoenzyme was determined as the mean +2 S.D. Similarly, the normal upper limit of total serum galactosyltransferase was found to be 47 nmol galactose transferred/ml serum/hr. Table 1 shows that 30 (79%) of the 38 cancer patients had at least one isoenzyme elevated and 23 (61%) had at least three isoenzymes elevated. The proportion of patients with elevated isoenzymes was similar for the various sites with the exception of breast, which was lower, with only 43% having at least one

Table 1. Frequency of elevated serum galactosyltransferase activity and isoenzyme levels in cancer patients

Site	No. of patients	Percent of patients with elevated galactosyltransferase		
		Total serum activity	At least one isoenzyme elevated	At least three isoenzymes elevated
Colon	11	45	91	73
Ovary	6	16	100	67
Lung	7	29	86	57
Stomach	7	14	71	71
Breast	7	14	43	29
Total	38	26	79	61

The upper normal limit (mean +2 S.D.) for total activity was 47 nmol galactose transferred/ml serum/hr.

isoenzyme elevated and 29% having at least three elevated isoenzymes. Since only 10 (26%) of the 38 patients had elevated total serum galactosyltransferase activity, the quantitative differences in the isoenzyme profiles are superior to the level of serum galactosyltransferase activity as potential tumour markers.

Figure 2 shows the number of times each isoenzyme was found to be abnormally high. The isoenzymes with isoelectric points of 4.05, 4.43, 4.61, 4.93 and 5.16 were most often elevated and of these 4.43, 4.61 and 5.16 were major peaks. There were also major peaks that were seldom elevated such as 4.33, 4.87 and 5.23. The distribution of elevated isoenzymes for each cancer site was similar to that in Fig. 2, with the possible exception of isoenzyme 4.43. This isoenzyme was elevated in 5 (83%) out of 6 patients with ovarian cancer, whereas only 9 (28%) of the 32 non-ovarian cancer patients had elevated isoenzyme 4.43.

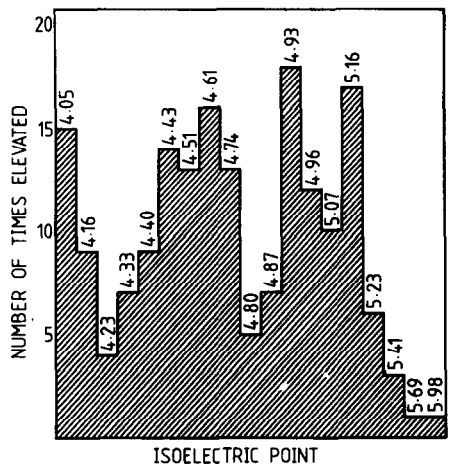

Fig. 2. The number of times each isoenzyme was elevated in the 38 cancer patients.

DISCUSSION

The high degree of resolution, sensitivity and reproducibility of the method used to determine the galactosyltransferase isoenzyme patterns have previously been discussed [16]. The degree of resolution is not sufficient to determine absolute amounts of individual isoenzymes, but it does allow peak heights to be estimated which give semi-quantitative indications of amounts. That each peak of galactosyltransferase activity is actually a true isoenzyme and not the result of a specific association of an isoenzyme with a serum component remains to be determined.

Podolsky and Weiser [1] reported that serum galactosyltransferase from patients with colonic, gastric and pancreatic cancer could be separated into two isoenzyme forms using discontinuous polyacrylamide gel electrophoresis. Subsequently they showed that the slow-migrating isoenzyme which they called GTII was present in 71% of cancer patients and was absent in all 58 healthy control sera studied [11, 12]. They concluded that GTII was a cancer-associated isoenzyme. Our analysis of the high resolution galactosyltransferase isoenzyme profiles of 38 patients with cancers at various sites and 9 healthy controls has produced no evidence of a cancer-associated isoenzyme. During our search for a suitable method to display the isoenzymes of galactosyltransferase a number of methods were evaluated and none of these resolved a cancer-associated form. The methods surveyed included ion-exchange chromatography based on the method of Podolsky and Weiser [17], several forms of electrophoresis on agarose and polyacrylamide including the method of Podolsky and Weiser [1], differential heat-inactivation, chromatofocusing and affinity chromatography on alpha-lactalbumin, asialo-agalacto-fetuin, con A, wheat-germ lectin and limulus lectin.

Gerber *et al.* [15], using polyacrylamide and granular gel isoelectric focusing of purified galactosyltransferase from cancer-patients, also reported that they found no evidence of a cancer-associated isoenzyme.

How do we explain GTII in the light of this evidence? The two most likely explanations are firstly that GTII could correspond to one or more peaks resolved by agarose isoelectric focusing and therefore GTII is present in healthy control sera. However, the resolution and sensitivity of the discontinuous electrophoresis used by Podolsky and Weiser [1] was not sufficient to detect GTII in normal sera. Secondly, GTII could be the result of an association of galactosyltransferase with some serum protein. This association survives discontinuous polyacrylamide electrophoresis, but not isoelectric focusing on agarose. The serum component responsible for the formation of GTII might be an IgG since it has recently been shown that certain IgG species co-purify with galactosyltransferase [18].

Although no cancer-associated isoenzyme of galactosyltransferase was detected in our study there were cancer-associated changes in the amounts of various isoenzymes. The ability of these quantitative changes to distinguish between the cancer patient group and the healthy control group is noteworthy, in that almost 80% of the cancer patient group had at least one isoenzyme level elevated and over 60% had at least three isoenzymes elevated (Table 1). These predictabilities are similar to or better than those reported for carcinoembryonic antigen [19, 20],

with the exception of that for breast cancer. The tumour marker potential of the galactosyltransferase isoenzyme profiles is also superior to total serum galactosyltransferase activity, which was elevated in only 26% of patients in this study (Table 1).

Even though 80% of patients had at least one isoenzyme elevated, it was not always the same one (Fig. 2). Rather, there were several isoenzymes which were most often elevated; these being the 4.93, 5.16, 4.61 and 4.05 forms. The 4.43 isoenzyme was preferentially elevated in patients with ovarian cancer, but not in patients with cancers at other sites. It is interesting to note that only ovarian cancer patients are reported to have consistently elevated serum levels of galactosyltransferase activity [5]. Since the sample size of six ovarian cancer patients is small, it is not possible to conclude that elevated 4.43 is related to ovarian cancer. We plan to study a larger group to determine whether the 4.43 isoenzyme has any specificity for ovarian cancer.

We reported [16] that the 5.41, 5.69 and 5.98 isoenzymes were probably not sialylated since neuraminidase treatment did not alter their isoelectric points. Figure 2 shows that these isoenzymes are rarely elevated in cancer patients. This may be the result of high levels of sialyltransferase often found in the serum of cancer patients [21, 22].

In view of the diversity of enzyme activities exhibited by the galactosyltransferases and their many sub-cellular locations [23–25], it is not surprising to find such a complex pattern of isoenzymes in serum (Fig. 1). It is against this complexity that the quantitative changes in the isoenzyme patterns of cancer patients must be assessed. Which isoenzyme changes are cancer-specific and which are secondary phenomena? It could be that some isoenzymes behave as acute-phase reactants [26]. This study set out to describe in detail the alterations in the isoenzyme pattern of galactosyltransferase of patients with various solid tumours. Although this has been achieved, the clinical significance of these changes is not yet clear.

Acknowledgement—We thank Margaret Traynor for manuscript preparation.

REFERENCES

1. PODOLSKY DK, WEISER MM. Galactosyltransferase activities in human sera: detection of a cancer-associated isoenzymne. *Biochem Biophys Res Commun* 1975, **65**, 545–551.
2. CAPEL ID, DORRELL HM, WILLIAMS DC, HANHAM IWF, LEVITT HN. Serum galactosyltransferase levels in patients with advanced cancer. *Oncology* 1982, **39**, 193–196.
3. MOTOKI T, KAWASE T, OHTA S *et al.* Galactosyltransferase activities in human sera of various diseases. *Radioisotopes* 1981, **30**, 146–149.
4. BHATTACHARYA M, CHATTERJEE SK, BARLOW JJ. Uridine 5′-diphosphate-galactose: glycoprotein galactosyltransferase activity in the ovarian cancer patient. *Cancer Res* 1976, **36**, 2096–2101.
5. CHATTERJEE SK, BHATTACHARYA M, BARLOW JJ. Glycosyltransferase and glycosidase activities in ovarian cancer patients. *Cancer Res* 1979, **39**, 1943–1951.
6. PAONE JF, WAALKES TP, BAKER RR, SHAPER JH. Serum UDP-galactosyl transferase as a potential biomarker for breast carcinoma. *J Surg Oncol* 1980, **15**, 59–66.
7. KUHNS WJ, SCHOENTAG R. Carcinoma-related alterations of glycosyltransferases in human tissues. *Cancer Res* 1981, **41**, 2767–2772.
8. KLOHS WD, MASTRANGELO R, WEISER MM. Release of glycosyltransferase and glycosidase activities from normal and transformed cell lines. *Cancer Res* 1981, **41**, 2611–2615.
9. WEISER MM, WILSON JR. Serum levels of glycosyltransferases and related glycoproteins as indicators of cancer: biological and clinical implications. *CRC Crit Rev Clin Lab Sci* 1981, **14**, 189–239.
10. CHATTERJEE SK. Glycosyltransferases in metastasizing and non-metastasizing rat mammary tumors and the release of these enzymes in the host sera. *Eur J Cancer* 1979, **15**, 1351–1356.
11. WEISER MM, PODOLSKY DK, ISSELBACHER KJ. Cancer-associated isoenzymes of serum galactosyltransferase. *Proc Natl Acad Sci USA* 1976, **73**, 1319–1322.
12. PODOLSKY DK, WEISER MM, ISSELBACHER KJ, COHEN AM. A cancer-associated galactosyltransferase isoenzyme. *N Engl J Med* 1978, **299**, 703–705.
13. KIM YD, WEBER GF, TOMITA JT, HIRATA AA. Galactosyltransferase variant in pleural effusion. *Clin Chem* 1982, **28**, 1133–1136.

14. WHITEHEAD JS, FEARNEY FJ, KIM YS. Glycosyltransferase and glycosidase activities in cultured human fetal and colonic adenocarcinoma cell lines. *Cancer Res* 1979, **39**, 1259–1263.
15. GERBER AC, KOZDROWSKI I, WYSS SR, BERGER EG. The charge heterogeneity of soluble human galactosyltransferases isolated from milk, amniotic fluid and malignant ascites. *Eur J Biochem* 1979, **93**, 453–460.
16. DAVEY R, BOWEN R, CAHILL J. Analysis of soluble galactosyltransferase isoenzyme patterns using high resolution agarose isoelectricfocusing. *Biochem Int* 1983, **6**, 643–651.
17. PODOLSKY DK, WEISER MM. Purification of galactosyltransferase "isoenzymes" I and II. *J Biol Chem* 1979, **254**, 3983–3990.
18. WILSON JR, WEISER MM, ALBINI B *et al.* Co-purification of soluble human galactosyltransferase and immunoglobulins. *Biochem Biophys Res Commun* 1982, **105**, 737–744.
19. HANSEN HJ, SYNDER JJ, MILLER E *et al.* Carcinoembryonic antigen (CEA) assay. *Hum Pathol* 1974, **5**, 139–147.
20. MARTIN EW JR, KIBBEY WE, DIVECCHIA L, ANDERSON G, CATALANO P, MINTON JP. Carcinoembryonic antigen. Clinical and historical aspects. *Cancer* 1976, **37**, 62–81.
21. KESSEL D, ALLEN J. Elevated plasma sialyltransferase in the cancer patient. *Cancer Res* 1975, **35**, 670–672.
22. BERGE P-G, WILHELM A, SCHRIEWER H, WUST G. Serum-sialyltransferase activity in cancer patients. *Klin Wochenschr* 1982, **60**, 445–449.
23. PATT LM, GRIMES WJ. Cell surface glycolipid and glycoprotein glycosyltransferases of normal and transformed cells. *J Biol Chem* 1974, **249**, 4157–4165.
24. SHUR BD, ROTH S. Cell surface glycosyltransferases. *Biochim Biophys Acta* 1975, **415**, 473–512.
25. ROTH J, BERGER EG. Immunocytochemical localization of galactosyltransferase in HeLa cells: codistribution with thiamine pyrophosphatase in trans-Golgi cisternae. *J Cell Biol* 1982, **92**, 223–229.
26. CANONICO PG, LITTLE JS, POWANDA MC, BOSTIAN KA, BEISEL WR. Elevated glycosyltransferase activities in infected or traumatized hosts: nonspecific response to inflammation. *Infect Immun* 1980, **29**, 114–118.