ELEVATIONS IN SERUM GLYCOPROTEIN:*N*-ACETYLNEURAMINIC ACID TRANSFERASES IN RATS BEARING MAMMARY TUMORS

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Received 21 May 1974

1. Introduction

Glycoproteins are increasingly being recognized as control or recognition processes in membrane-mediated events such as cell–cell agglutination, aggregation, adhesion, and 'contact inhibition' [1]. In addition, a great many studies have measured glycosyl transferase activity in 'neoplastic' cells compared with 'normal' cells, at times with conflicting outcomes [2–10]. It seems clear, however, that in most cases of neoplastic transformation the cell or tissue change is accompanied by decreased elongation of certain glycolipids and increased or altered glycoprotein production [11].

Glycoprotein synthesis proceeds, in the main, by the independent sequential addition of individual monosaccharides to the polyribosome-released polypeptide. These additions are catalyzed by glycoprotein:glycosyl transferases located predominantly in the cell's Golgi apparatus but also to a small extent in the cell's plasma membrane and mitochondria [1, 12]. Glycoprotein:glycosyl transferases – e.g., *N*-acetylgalactosaminyl [13] and a sialyl (*N*-acetylneuraminic acid; NANA) [14] transferase – have also been found in the serum of humans and animals. The tissue source and physiological function of these soluble serum transferases are not completely known. It is of interest to speculate that the serum NANA transferase may be responsible for sialation of circulating glycoproteins with terminal galactosyl residues so that these glycoproteins are retained in the circulation and not cleared by the liver [15].

This laboratory has reported [16] that human breast tumor sialyl transferase activities were greatly elevated over the control, non-involved breast tissue level. In the present study we have investigated the activity of NANA transferase in serum from rats bearing two experimental breast tumors – the R3230AC transplantable mammary tumor and the 7,12-dimethylbenz(a)anthracene (DMBA)-induced mammary tumor. We find, especially in the sera of rats bearing the R3230AC tumor, greatly elevated activity of serum NANA transferase; the magnitude of elevation is proportional to the tumor load in the R3230AC tumor-bearing animals.

2. Materials and methods

2.1. *Materials*

Fetuin [17] was purchased from Grand Island Biological Co. Sialic acid-free fetuin was prepared as described previously [6]. CMP-[^{14}C]-*N*-acetylneuraminic acid (sialic 4,5,6,7,8,9-^{14}C; specific activity 207 Ci/mole) was purchased from New England Nuclear Corporation.

2.2. *Tumor-bearing rats*

Female Fischer rats (approx. 80 g) were transplanted subcutaneously bilaterally in the axillary region with the R3230AC mammary adenocarcinoma, using a sterile trochar technique described earlier [18]. This isologous carcinoma, a slow-growing line arising from a spontaneous mammary tumor, has been classified as an autonomous, hormone-responsive tumor [19]. At various times after tumor implanta-

tion, animals were killed by cervical dislocation, and 1–2 ml of whole blood was quickly obtained by cardiac puncture and placed in tubes on ice to allow clotting. Serum was obtained by centrifugation. In a few cases, lactating female rats from our breeding colony were killed, as above, and blood was obtained for analysis. Tumor weight in these animals after 3 weeks of growth was 6–10 g (total) and after 4 weeks of growth the tumor weighed between 8 and 16 g.

Serum was also obtained from a few animals (Sprague–Dawley female rats) bearing tumors induced by gastric intubation of 7,12-dimethylbenz(a)anthracene dissolved in sesame oil (5 mg/ml × 5 doses, 1 dose per week). Animals were killed 9 weeks after the last intubation of carcinogen, at which time one animal had 5 tumors, one had 2 tumors, and one had one very small tumor.

2.3. *Complete system for assay of fetuin:sialyl transferase*

The complete system for assay of the fetuin:sialyl transferase contained 100 μl of the serum sample extract of the normal or tumor tissue (1–2 mg of protein), 50 μl of acceptor (fetuin minus sialic acid; approx. 300 μg of protein), 10 μl of 0.1 M $MnCl_2$, 20 μl of 0.1 M Tris buffer (pH 7.6), and 10 μl of CMP-[^{14}C]NANA (10^{-9} mole), to a final volume of 190 μl. For experiments in the absence of added cation the 10 μl of 0.1 M $MnCl_2$ and 10 μl of 0.1 M $MgCl_2$ were replaced with 20 μl of distilled water. This represented fetuin:sialyl or exogenous activity after subtraction of activity without acceptor present; for activity without added acceptor (i.e., activity with acceptors endogenous to the tissue), 50 μl of distilled water replaced the fetuin minus sialic acid in the assay mixture. After 30 min of incubation at 37°C the radioactivity was determined as given elsewhere [2,4,6]. The reaction was terminated with the addition of 1.0% PTA in 0.1 N HCl, washed twice with 10% trichloroacetic acid, once with ethanol: diethyl ether (2:1, v/v), and the resultant pellet (all acid insoluble materials were collected by centrifugation) dissolved in 1 N NaOH and plated on a glass fiber filter; radioactivity was determined by liquid scintillation counting. Data are cpm per mg serum protein for 30 min incubation. Optimal conditions with respect to temperature, pH, and divalent cation concentration were met in the 'plus cation' system.

2.4. *Protein*

Protein was determined by the method of Lowry et al. [20]. Bovine serum albumin was used as standard. There was no difference in protein per volume between the serum from the tumor-bearing and normal animals.

3. Results and discussion

Without added cation the normal rat serum had essentially the same activity of NANA transferase either in the presence or in the absence of added fetuin minus NANA (table 1). However, with added Mn^{2+} and Mg^{2+} the normal rat serum had about double the exogenous activity when fetuin minus NANA was added to the assay (table 2).

The data of table 1 clearly indicate that (a) serum glycoprotein:NANA transferase activity was significantly elevated in the rats bearing the R3230AC tumor, (b) the elevation in the serum glycoprotein: NANA transferase activity was essentially proportional to the load of tumor in the animal, and (c) interestingly, normal lactating animals had elevated serum glycoprotein:NANA transferase activity.

Table 2 shows data for the optimized system with both Mn^{2+} and Mg^{2+} present in the serum NANA transferase assay. Similar conclusions can be made from these data as from the data of Table 1. In the serum from animals with 4-week R3230AC tumors, the fetuin:NANA transferase activity was 3894 cpm/mg protein compared to 738 cpm/mg protein for the serum from control animals – a greater than 5-fold increase in activity.

In further experiments with the R3230AC tumor (not shown in the tables), we have in every instance been able to pick out the tumor-bearing rats (in blind studies) by levels of serum NANA transferase activity; indeed in one instance a rat bearing 4-week R3230AC tumors was found to have a value of 6811 cpm/mg protein in the 'plus cation' plus added fetuin minus NANA system. No false positives have been encountered – i.e., all values for control rat serum have been below those for R3230AC rat serum.

Preliminary work with rats bearing DMBA-induced mammary tumors shows that serum NANA transferases are also elevated in this system, but the differences from control values are not as striking or as consistent as with the transplanted R3230AC tumor (table 3).

Table 1
Serum glycoprotein:NANA transferase activity in the absence of added cation in normal rats and in rats bearing R3230AC tumors

Animals	No.	Activity (cpm/mg protein)	
		Without added fetuin minus NANA	With added fetuin minus NANA (endogenous activity subtracted)
Normal	7	410 ± 39	334 ± 23
2-week tumor	1	579	1184
3-week tumor	4	676 ± 83*	1370 ± 113**
4-week tumor	6	705 ± 68**	1612 ± 171**
Lactating normal	3	354 ± 37	699 ± 82

Experiments were performed as described in Materials and methods. Values are mean ± S.E.M.
* Significantly different from normal, $p < 0.05$ (Student's two-tailed test).
** $p < 0.01$.

Table 2
Serum glycoprotein:NANA transferase activity in the presence of added cation in normal rats and in rats bearing R3230AC tumors

Animal	No.	Activity (cpm/mg protein)	
		Without added fetuin minus NANA	With added fetuin minus NANA (endogenous activity subtracted)
Normal	7	393 ± 45	738 ± 60
2-week tumor	1	423	1184
3-week tumor	4	742 ± 51*	2850 ± 326*
4-week tumor	6	720 ± 67*	3894 ± 153*
Lactating normal	3	461 ± 147	1664 ± 165

Experiments were performed as described in Materials and methods. Values are means ± S.E.M.
* Significantly different from normal, $p < 0.01$.

Table 3
Serum glycoprotein:NANA transferase activity in serum from rats with DMBA-induced tumors

Tumor load	Activity (cpm/mg protein)			
	Without added fetuin minus NANA		With added fetuin minus NANA	
	Minus cation system	Plus cation system	Minus cation system	Plus cation system
0 (control)	411	381	421	781
10 mg	317	412	424	808
18.5 g	506	396	810	1210
23.0 g	746	789	1140	1867

Experiments were performed as described in Materials and methods.

The data presented in this paper indicate that elevations in serum NANA:glycosyl transferase activity accompany growth of mammary tumors in the rat, especially tumors of the transplantable type. This suggests that during the neoplastic growth and invasion of these tumors, these enzymes 'spill' out of the tumor cells, leave the tumor site, and enter into the host's circulation. Alternatively, the elevated sialyl transferase activity both in the tumor cell and in the serum may serve to initiate or sustain the neoplastic growth. Finally, if this phenomenon is similar to what happens in the human mammary tumor (H. B. Bosmann and T. C. Hall, work in progress), it may serve as a method of early or supportive diagnosis for human mammary malignancy.

Acknowledgements

This study was supported in part by grants CA-13220 and CA-12836 from the National Institutes of Health. H.B.B. is a Research Career Development Awardee of NIGMS.

We thank Kenneth R. Case and W. D. Rector for technical assistance.

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