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PRINCIPAL INVESTIGATOR: Mepur H. Ravindranath, Ph.D.

CONTRACTING ORGANIZATION: John Wayne Cancer Institute
Santa Monica, California 90404

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Mepur H. Ravindranath, Ph.D.

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John Wayne Cancer Institute
Santa Monica, California 90404

E-Mail: ravi@jwci.org

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13. ABSTRACT (Maximum 200 Words)
The project objectives are [1] to identify the gangliosides [Gs] of Prostate cancer (CaP) that are immunogenic so that they can be used as targets to develop immunotherapy for prostate cancer; [2] to determine the total and specific Cap-Gs released into the blood and [3] to assess the nature of immunosuppression induced by CaP-Gs. Last year, we have found out that the neoplastic transformation of prostate epithelial cells involve Gs with Gal-GalNAc-Gal-Glucosylceramide backbone by comparing the normal prostatic epithelial with five prostate cancer cell lines, namely PC-3, DU145, LNCaP-FGC-10, LNCaP-FGC and HH870. This year, we have made novel and unique observations relevant to early diagnosis of the localized disease, which include [1] Identifying GM1b, GD1a, GalNAc-GM1b and GalNAc-GD1a as unique Gs of CaP. [2] IgM antibodies in the sera of CaP patients with localized disease (T1b/c) reacted strongly to GM1b in thin layer chromatography (TLC). [3] Patients with localized disease had high titers of GD1a. [4] A study of the total serum Gs profile was completed for all stages. [5] Using endogenous immune response, we identify that the Gs GM1b and GD1a are released into circulation. [6] The Gs GM2 and GD1b observed in CaP cell lines may be artifacts of tissue culture conditions, which are known to augment the expression of GalNAc-transferase. The endogenous immune response to GM1b and GD1a make the anti-GsIgM as potential markers of early diagnosis of localized prostate cancer. Ultimately these findings will enable formulating an allergenic CaP vaccine.

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INTRODUCTION

Project Objectives:

The project objectives are
1. to identify the gangliosides [Gs] of Prostate cancer (CaP) that are immunogenic so that they can be used as targets to develop immunotherapy for prostate cancer.
2. to determine the total and specific CaP-Gs released into the blood and
3. to assess the nature of immunosuppression induced by CaP-Gs.

Findings made in the First Year:

The major findings of the first year are as follows: Ganglioside GM1 is the major cell surface ganglioside of normal prostate epithelia. The expression of GM1 is significantly lowered in Prostate cancer cell lines (PC-3, DU145, LNCaP-FGC-10, LNCaP-FGC and HH870). On the other hand, the following gangliosides are more prominent: GM2>GD1b > GT1b (in all cell lines). GD1a is the highly expressed in PC-3 and DU145 cell lines. The immunogenicity of the CaP gangliosides was tested by comparing the serum antibody titers of healthy individuals and CaP-patients against eight different gangliosides. The gangliosides immunogenic in patients are GD1a> GT1b > GM2 > GD1b. The results enable us to identify the pathway of biosynthesis of prostate carcinoma associated gangliosides. The immunogenicity of the gangliosides suggests that they are potential targets for immunotherapy of CaP. The results lead to formulation of allogeneic CaP-vaccine with CaP-gangliosides.

BODY

Preamble: An emerging concept in tumor biology is that the tumor cells escape ceramide-mediated apoptosis by glycosylating ceramides and storing the glucosylceramides as gangliosides, the lactosylceramides with sialic acids (**1**). Gangliosides (Ggs) stored in the cytoplasm and expressed on the cell surface can be released from tumor cells into the tumor microenvironment. Ggs suppress a variety of cell-mediated immune functions (**2**). Tumor-associated Ggs also enter the circulation. While studying release of tumor gangliosides into circulation after cryosurgical ablation of colon carcinomas metastasized into the liver, we made a serendipitous observation that the gangliosides characteristic of the host tumor may induce production of IgM antibodies specific to the tumor-Ggs (**31**). Further observations on the sera of patients with early stages (AJCC stage I and/or II) sarcoma, colorectal carcinoma and melanoma (**3-5,31**) confirm the presence of antibodies specific to gangliosides characteristic of early tumors and suggest that endogenous (without involving exogenous adjuvants) immune response may be an early immunological event taking place during tumorigenesis. We strongly believe that these antibodies specific to early tumor antigens, if identified by sensitive assays, could serve as a diagnostic and prognostic marker of human cancers. While submitting evidence to show that tumor-derived Ggs in prostate cancer (CaP) may elicit IgM response in patients with subclinical and localized disease (at stage T1b/c), we seek support to immediately

establish the clinical relevance of the early endogenous IgM response to certain specific CaP-associated gangliosides.

Current approaches to early diagnosis of CaP: Adenocarcinoma of the prostate is the most common malignancy in American men, with a lifetime risk of nearly 1 in 6 (**5**). Detection of subclinical disease has the potential to decrease the rate of metastasis and increase disease-free survival (**6**). A noninvasive means for early detection would avoid unnecessary biopsy and encourage more men to seek treatment before their CaP has penetrated the capsule of the gland. Our primary objective is to develop a reliable and reproducible screening assay based on the endogenous immune response to tumor Ggs expressed by early-stage (localized) CaP. Prostate specific antigen (PSA) is the best screening tool in clinical practice since it was introduced in 1980-81 (**7,8**). The Food and Drug Administration approved PSA testing to monitor men with CaP in 1986. Serum PSA originates from prostate epithelial cells, although it is also produced by breast and salivary glands at low levels. Serum PSA together with rectal examination and transrectal ultrasonography is 79% sensitive for clinically localized CaP (**6,9**). However, serum PSA has the following limitations:

- A 5-year study by the American Cancer Society National Prostate Cancer Detection (ACS-NPCD) Project showed that only 64% of pathologically organ-confined cancers were detected through PSA-based screening (**10**).
- Serum PSA level does not increase significantly until CaP reaches a volume that exceeds 1 cc (**6,11**); only 3 to 9% of well differentiated CaP lesions <0.5 cc are detected as a result of PSA screening (**6**).
- Serum testosterone levels, drugs such as finasteride and dutesteride, and inflammation processes in the prostate affect the level of PSA (**12**).
- Benign conditions such as acute urinary retention, acute prostatitis, prostatic ischemia or infarction and BPH are also associated with elevated serum PSA levels (**6**).

Biopsy of the prostate detects CaP in 25% of men whose rectal examination results are normal but whose serum PSA levels range from 4.1to10.0 ng/ml. If the PSA level for recommending a biopsy is lowered to 2.5 ng/mL, an additional 10 to 15% of CaP cases will be identified (**13,14**) - but a substantial number of unnecessary biopsies will be performed.

To improve the power of PSA for early detection, indexes like PSA density, age-referenced PSA, volume-referenced PSA, free PSA and the ProstAsure Index have been proposed (**15**). However, PSA density (serum PSA divided by the volume of the prostate gland as measured by transrectal ultrasonography) failed to differentiate BPH from CaP when serum PSA was <4.00 ng/ml (**16**). Considerable overlap was observed in PSA density between patients with CaP and those with BPH (**15, 17, 18**). The inter-observer variation in estimating the prostate volume among different ultrasonographers makes the volume-based indices difficult to reproduce. Similarly age-referenced PSA was not significantly different from serum PSA in the ACS-NPCD Project (**19**). Comparing PSA

density, age-referenced PSA and volume-referenced PSA, Babaian et al (**20**) concluded that serum PSA is superior to these indices because of the subjectivity associated with volume estimates and the cost. A portion of the PSA molecule forms a complex with □1-antichymotrypsin (ACT) and □2-marcoglobulin (AMG) (**15,21**). The free or unbound PSA is lower in men with CaP than in those with BPH (**22,23**). Determination of the percent free PSA enhanced specificity of CaP detection without compromising sensitivity (**15,24**), particularly when the total PSA level are between 2.5 and 4.0 ng/mL (**25**).

Glycoantigens in CaP: Although oligosaccharides and sugar are important constituents of cellular and cell surface proteins and lipids, very few studies have examined their nature and antigenic properties in CaP. Glycoantigens are overexpressed in several human cancers and are recognized as tumor differentiation antigens. Our focus is on Ggs, a class of glycolipids that contain sialic acids. Ggs are formed as a result of glycosylation of ceramides accumulated in tumor cells. Ggs are membrane-bound amphophilic molecules (with 1300 to 2500 atomic mass units) with a hydrophilic head group of two or more sugars (glucose and/or galactose, neuraminic acid [sialic acid]) and a hydrophobic tail group of ceramide (sphingosine and a long chain fatty acid). The nature and distribution of Ggs differ between normal and neoplastic cells.

Based on our previous studies of human colon cancer, primary melanoma and sarcoma, we speculate that the proliferation and associated death of CaP cells *in situ* may allow tumor Ggs to leak into lymphatic and circulatory systems. Although circulating Ggs can suppress cell-mediated immune functions (**2**), there is evidence that they can also activate B lymphocytes to produce endogenous IgM antibodies. These antibodies can clear Gg molecules from the circulation (**3**).

KEY RESEARCH ACCOMPLISHMENTS and REPORTABLE OUTCOME

Gangliosides of human CaP cells differ from those of normal prostatic epithelial cells: Five CaP cell lines (PC-3, DU-145, HH-870, LNCaP-FGC and FGC-10) and normal prostate epithelial cells were grown in RPMI-1640 with 10% fetal calf serum. Gg content was analyzed by three techniques established in our laboratory: (1) Biochemical extraction of Ggs and chromatography of Gg extracts using resorcinol staining; (2) Immunostaining of the chromatograms; and (3) Direct measurement of cell-surface Ggs using an enzyme-linked immunosorbent assay (ELISA) for cell-surface antigens.

Biochemical analysis and immunostaining. When we compared the biochemical Gg profile of a normal prostatic epithelial cell line with Gg profiles of five CaP cell lines in one-dimensional **(Figure 1)** and two-dimensional **(Figure 2)** analyses, GM1a (commonly known as GM1), GM2 and GM3 were the major species in extracts of normal cells. Biochemical analysis also showed the presence of GM1b, GM2, GD1a and GT1b in PC3, DU145 and HH870.

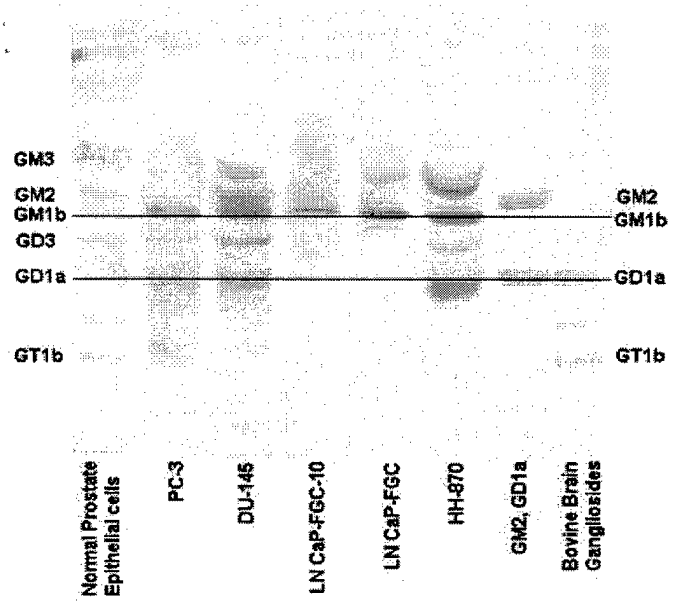

Figure 1. Unidimensional chromatogram of Ggs in normal prostate epithelial cells, five CaP cell lines, and commercially available standards (Std). Although the resolution does not permit characterization of Gg signatures, Ggs can be identified based on the mobility of the standards.

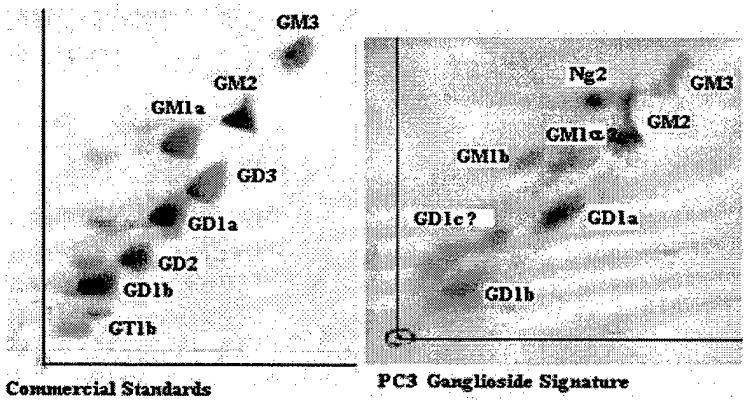

Figure 2. Resorcinol staining of a two-dimensional chromatogram shows Gg signatures. CaP cell lines and commercial standards were run on different days and therefore the positions do not strictly coincide. The labeling is based on immunostaining with GMR17.

Immunostaining of prostate cell lines with GMR17 revealed GM1b, GD1a and two spots tentatively identified as GD1c and possibly GM1 (**Figure 3**). In normal cells, immunostaining detected GM2 but not GM1a or GM1b, GT1b or GD1a. The presence of GM1b in CaP cell lines was confirmed using GM1b isolated from mouse Yac-1 cells. Because cells grown in tissue culture overexpress GalNAc-transferase (**4,42**) and GM2 is overexpressed in immunostained extracts of normal prostatic epithelial cells, GM2 and possibly GD2 and GD1b might be artifacts caused by tissue culture conditions. Our study indicates that GM1b, GD1a, GD1c and GM1 are the gangliosides most likely to be found in CaP cell lines.

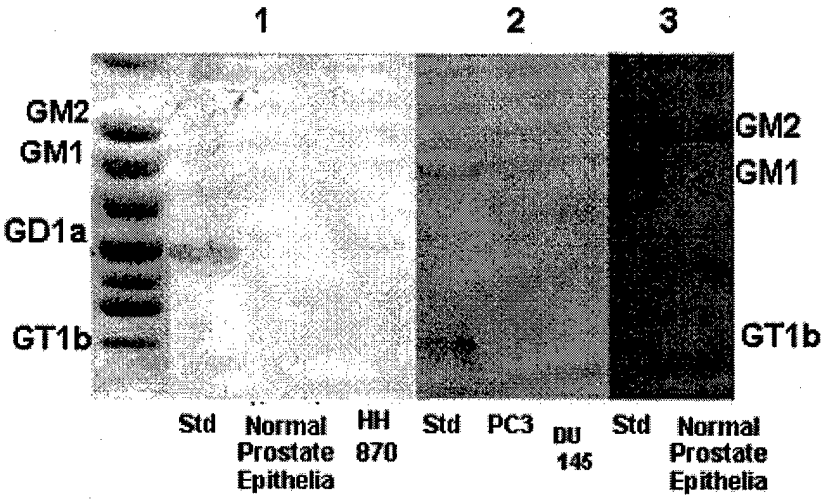

Figure 3. Immunostaining of a unidimensional chromatogram. **Panel 1**: Murine monoclonal antibody GMR17 against GD1a stained only GD1a of all the standards (Std) and HH870 but not extracts of normal prostate epithelial cells. **Panel 2**: Monoclonal antibody GMB16 against GM1a and monoclonal antibody GMR5 against GT1b stained the respective standards (Std) but not all other standards or the extracts of PC3 and DU145. **Panel 3**: Monoclonal antibody KM696 against GM2 stained extracts of normal prostate epithelial cells. In this panel we used an antibody mixture that included GMB16 (for GM1a), GMR5 (for GD1a) and KM696 (for GM2). The respective standards reacted to monoclonals but not others.

We extracted gangliosides from CaP cell lines PC3 and DU145 and then treated the Gg extracts with weak alkali to remove any O-acetyl groups. When the extracts were stained with GMR17 monoclonal antibody against GD1a, there were four distinct blue bands (**Figure 4**). GT1b was not evident. Because GMR17 does not stain GM1a, we surmised that the band corresponding to the position of GM1a was GM1b. We confirmed this by preparing Gg extracts of Yac-1 cells, which express GM1b; when we stained the extracts with GMR17, the position of GM1b from Yac-1 cells was identical to the band in PC3 and DU145 chromatograms. **Figure 5** shows two-dimensional chromatograms of PC3 and DU145 Gg extracts stained with GMR17. Sialic acid is in the terminal galactose of GM1b, in the middle galactose of GM1a, and in both positions in GD1a. Therefore the band below GD1a should have sialic acid at terminal galactose and possibly two sialic acids; it is designated as GD1c (OR GalNAc-GD1a). This identification requires further confirmation after purification with HPLC. There is another unidentified Gg above GM1b; we infer that this Gg species has a lesser atomic mass unit and we postulate that it is GM1α or GalNAc-GD1a.

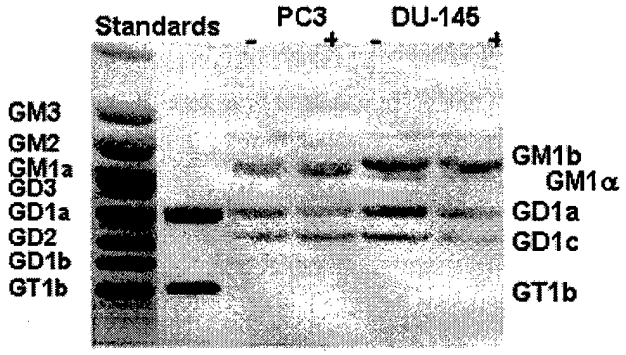

Figure 4. Immunostaining of a unidimensional chromatogram with monoclonal antibody GMR17. GMR17 is directed against GD1a but can cross-react with GT1b. GMR17 identified GD1a and GT1b on all standards; by contrast, GMR17 identified GD1a but not GT1b in extracts from the two CaP cell lines. Strikingly, GMR17 identified GM1b and possibly GM1α (GalNAc-GD1a) and GD1c(GalNAc-GD1a) in PC3 and DU145 cells. GMR17 reactivity to GM1b was confirmed by testing GM1b purified from Yac-1 cells.

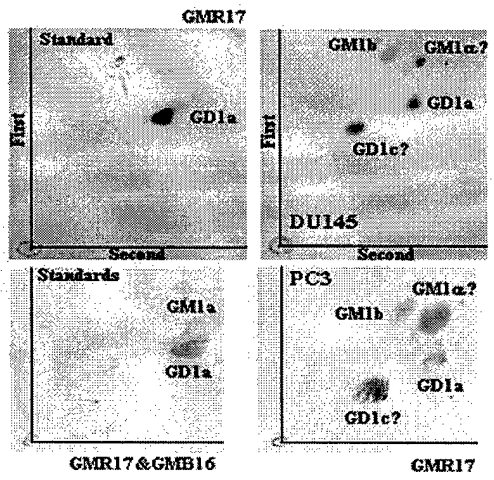

Figure 5. Immunostaining of two-dimensional chromatogram with monoclonal antibody GMR17 identifies GD1a, GM1b and possibly GD1c in PC3 and DU145 cells. Note that standards contain all the gangliosides.

Tumor gangliosides may be released into circulation. The second objective of the project is to determine whether the gangliosides from tumor cells are released into the tumor microenvironment and circulation. The results presented in **Table 1** may show that the level total gangliosides in sera of CaP patients is significantly higher than that found in BPH. However, prostatitis patients also showed high level of serum gangliosides. Specific gangliosides in the sera could not be analyzed for we have found out that the CaP gangliosides are unique, found in low levels but strongly immunogenic (*vide infra*). Therefore, we made indirect assessment of the gangliosides released from tumor by measuring endogenous immune responses of the shed gangliosides. The analyses were done with commercially available gangliosides. It should be noted that not all prostate gangliosides are commercially available. The results narrated below would point out

the possible CaP-associated gangliosides that may released into circulation.

Table 1. Serum Total Ganglioside Levels in Patients

	N	Range	Mean	Std.Dev.
CaP Stage T1	19	7.5 - 21.6	14.2	3.2
CaP Stage T2	9	6.2 - 18.9	15.3	4.0
CaP Stage T3	14	9.7 - 18.4	14.7	2.8
CaP Stage T4	10	15.5 - 21	18.3	1.7
BPH	14	8.7 - 16.2	12.8	2.4
Prostatitis	4	10.3 - 20.6	16.3	4.1

Endogenous antiganglioside IgM immune response in patients with BPH and Stage T1/T2 CaP: We have shown that necrotic tumor cells can act as a natural adjuvant to stimulate an antiganglioside response, a finding that derives support from the "Danger immune signal concept" of Metzinger (**36-38**). We analyzed anti-Gg IgM levels in sera from patients with T1c CaP and BPH (**Table 2**). Patients with BPH showed a weak IgM antibody response to GM2 and GD3, whereas the sera of patients with stage T1c CaP showed no significant response to GM1a, GM2, GM3, GD2, GD3 and GD1b. The absence of an antibody response to GM1a, GM3, GD2, and GD3 is not surprising because these gangliosides were absent or present in very low levels in CaP cell lines. However, CaP cell lines expressed GM2, GD1b and GT1b. Significantly low antibodies to these three gangliosides in vivo confirm our suspicion that they could be an artifact introduced by tissue culture conditions, as has been reported in human melanoma. CaP patients had relatively high titers of antibodies to GD1a ($p<0.05$), the major ganglioside of CaP.

Table 2. Antiganglioside IgM profile of patients with localized CaP (stage T1c) and BPH.

Disease Status		PSA (ng/ml)	Serum Ggs (mg/dL)	Anti GM1a	Anti GM2	Anti GM3	Anti GD2	Anti GD3	Anti GD1a	Anti GD1b	Anti GT1b
CaP T1c N =11	Mean	4.006	14.267	5.324	5.407	5.168	5.758	4.855	**5.923**	5.366	6.682
	STD±	2.873	4.199	1.311	0.935	1.343	1.358	1.067	**1.439**	1.325	1.519
BPH N = 10	Mean	4.372	12.100	4.280	4.703	4.352	4.561	4.256	**4.581**	4.551	5.640
	STD ±	4.852	4.271	1.043	1.602	1.079	1.263	1.120	**1.267**	1.271	1.724
		NS	NS	NS	NS	NS	NS	NS	**P<0.05**	NS	NS

The monoclonal antibody GMR17, directed against the epitope of GD1a, cross-reacted with GM1b and the other two novel gangliosides of CaP. Therefore, it is possible that the antibody reaction to GD1a observed in ELISA might be due to anti-GM1b antibody reacting to GD1a. To investigate this possibility, we carried out two-dimensional chromatography of Gg extracts from PC3 cells. One chromatogram was stained with GMR-17 (murine monoclonal antibody for GD1a) and another one was overlaid with serum obtained from a patient with T1c, grade 3/4 CaP, who showed reactivity to GD1a (**Figure 7**). The sera reacted more strongly to GM1b than to GD1a, suggesting that the presence of antibodies to the unique ganglioside of prostate cancer (GM1b).

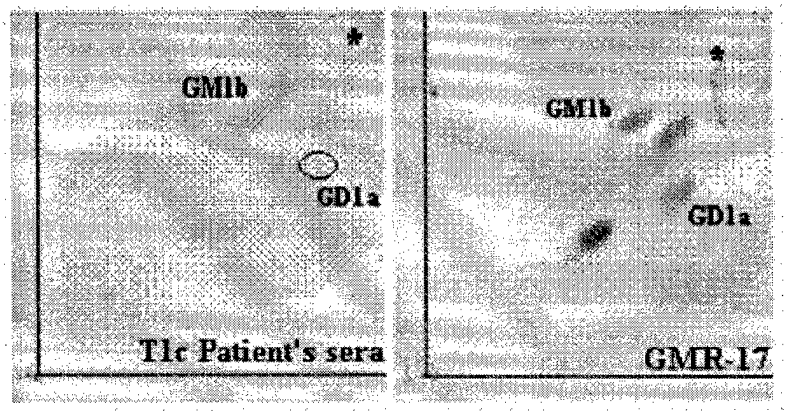

Figure 7. Serum from a patient with T1c CaP (grade 3/4) recognizes GM1b strongly and GD1a weakly, whereas monoclonal antibody GMR17 against GD1a identifies GM1b, GM1α, GD1a, and possibly GD1c. The asterisks (*) refer to the position of the yellow dye used as an indicator for the position of GM1b on the two-dimensional chromatogram.

CONCLUSIONS

The hypothesis that endogenous immune signals (antiganglioside antibodies) may predict the early evolution of CaP, alone or in combination with PSA, evolved from a series of observations. First, we compared Gg profiles of normal prostatic epithelial cells and five CaP cell lines. We found that the Gg pattern of normal prostate epithelia changed drastically upon transformation into CaP; of particular interest are four gangliosides related to GM1b. Figure 6 illustrates the biosynthetic pathway of the CaP-associated gangliosides. Second, we observed that patients with early-stage CaP (T1c) produced antibodies to CaP Ggs. Antibody to GD1a appears to be the earliest endogenous immune signal; anti-GD1a IgM antibody is prevalent in patients with CaP but not in patients with BPH or in healthy age-matched volunteers. Interestingly, antibodies to GM1, GM2, GM3, GD1b, GD2, GD3 and GT1b were negligible in these patients. We did not test GM1b or GalNAc-GD1a (GD1c?) because these gangliosides are not available commercially. However, we tested the sera from a few CaP patients for IgM antibodies to GM1b by immunostaining two-dimensional chromatograms of the Ggs (Figure 7). The markedly stronger response to GM1b than GD1a suggests that GM1b may be more immunogenic in patients.

FUTURE DIRECTIONS

We plan to purify GM1b from CaP cell lines and use this ganglioside to determine the usefulness of GM1b and GD1a as diagnostic markers for early detection of localized CaP. Although GM1b is available in murine Yac-1 cells (**40**) and murine lymphosarcoma cell lines (RAW 8.1) (**41**), we need to purify GM1b from human CaP cell line PC3, which expresses GM1b in abundance. 1nmol of Gg per well is required. For analyzing one serum sample, at least 4 nmol is needed. To analyze sera from at least 20 CaP patients and 20 BPH patients, 160 nmol is needed. Our preliminary analysis indicates that 30 million PC3 cells may provide about 10 nmol of highly purified GM1b. At least 500 million cells will

be needed for ELISA of sera from 20 CaP patients and 20 BPH patients. GM1b will be isolated by a high-performance liquid chromatography (HPLC) system (LC-10AD, Shimadzu, 11968 Challenger Court, Moor Park, CA) recently purchased for our laboratory. We have used HPLC to purify GM1b from Yac-1 cell lines (**Figure 8**), and we have immunostained thin-layer chromatograms (TLC) with GMR17 (**Figure 9**). In healthy men, IgM levels decline significantly and progressively after 50 years of age. In contrast, the level of serum PSA increases with age. Using a sensitive and validated ELISA protocol, we have observed anti-GD1a IgM antibodies in patients with localized CaP (stages T1 and T2; grades 2-5). We also tested the sera from a few CaP patients for anti-GM1b IgM antibodies by immunostaining two-dimensional chromatograms (**Figure 7**). Stronger immunoreactivity to GM1b than GD1a suggests that GM1b may be more immunogenic in patients. If so, then anti-GM1b IgM may be a better diagnostic marker than anti-GD1a IgM. If anti-GM1b proves to be highly immunogenic, this antibody will be compared with anti-GD1a IgM and serum PSA for early diagnosis of localized CaP. The antibody response will also be compared to tumor grade. We hypothesize that IgM antibodies to one or more prostate-associated Ggs such as GM1b and GD1a could be more sensitive prognostic markers in patients with localized disease.

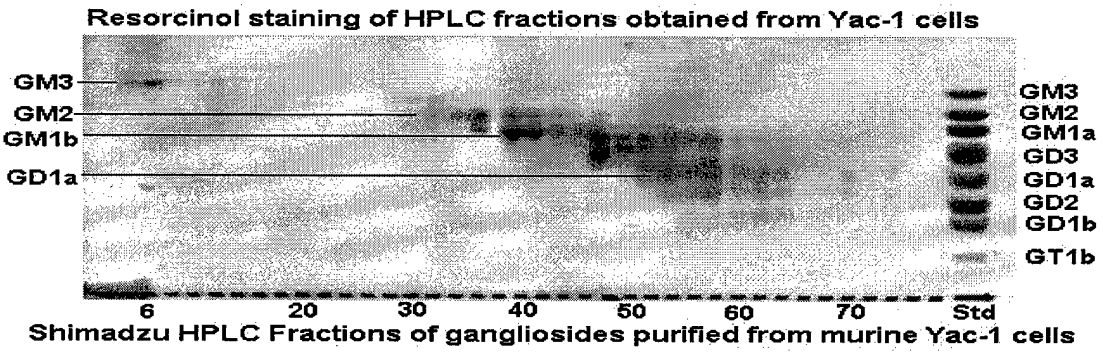

Figure 8. Purification and isolation of gangliosides from murine Yac-1 cells using Shimadzu HPLC. Contents from selected vials were resuspended in chloroform and methanol, run on thin-layer chromatography, and stained with resorcinol. Remaining vials were used for immunostaining (see Figure 9).

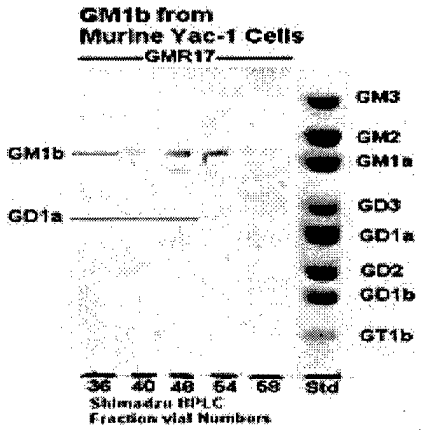

Figure 9. Immunostaining of GM1b isolated from murine Yac-1 cells using Shimadzu HPLC. Contents of selected vials were suspended in chloroform and methanol, run on thin-layer chromatography, and immunostained.

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APPENDIX

1. Mepur H. Ravindranath, Sakunthala Muthugounder, Meena Verma, Rathinam R. Selvan, Jacques Portoukalian[2], Stanley Brosman[3] and Donald L. Morton. (2003) Neoplastic transformation changes the ganglioside profile of prostatic epithelial cells. Proc Am Assoc Cancer Res 94: R2407

R2407

Neoplastic Transformation May Change the Ganglioside Profile of Prostatic Epithelial Cells

Mepur H. Ravindranath, Sakunthala Muthugounder, Meena Verma, Rathibam S. Selvan, Stanley Brosman, Jacques Portoukalian, Donald L. Morton
John Wayne Cancer Institute, Santa Monica, CA; Hoag Cancer Center, Newport Beach, CA; Pacific Clinical Research, Santa Monica, CA; Hospital Edouard-Herriot, Lyon, France

Abstract

Introduction: Neoplastic transformation changes the expression of cell-surface gangliosides in melanoma and possibly in other cancers of epithelial origin. We hypothesize that neoplastic transformation of prostate may result in alteration of the ganglioside profiles. The hypothesis is tested by comparing the ganglioside profiles of a normal prostatic epithelial cell line with six prostate cancer cell lines.

Methods: All cells were grown in RPMI with 10% fetal calf serum. The density of gangliosides on the surface of these cells was assessed by cell-suspension enzyme-linked immunosorbent assay [J.Immunol. Methods,197:51-67, 1996]. The semi-quantitative profile of the gangliosides in methanol/chloroform extracts of these cells was assessed by resorcinol staining and immunostaining of thin-layer chromatograms.

Results: In normal cells, GM3, GM2, and GM1a were the predominant gangliosides in cell extracts and on the cell surface, but their levels were negligible. In prostate cancer cell lines PC-3, DU-145 and HH-870, cell extracts contained GD1a, GM1b, GM2 and GD3, GM3, GT1b, and GD2, listed in order of decreasing concentration. The surface of these cells expressed GD1a, GM2, GD1b, and GT1b, listed in order of decreasing density. The surface of HH-870 cells also expressed GM1 and GD2. The ganglioside profile of LNCaP-FGC/FGC-10 prostate cancer cell line was more limited; GM1b was predominant in the extract, and GD1b and GM2 were predominant on the cell surface. These findings indicate that the major gangliosides of prostate cancer are GM1b, GD1a, GD1c, GM1α GD2, GM2, and GD1b.

Discussion: Normal prostate epithelia contains GM1a, GM2, and GM3. GM1b, not found in normal prostatic epithelia, is the likely precursor of GD1a, and other uncommon gangliosides. The precursor of GM1b could be Asialo-GM1. Analysis of ganglioside profiles of prostate tumor tissue is needed to confirm these findings and identify those ganglioside antigens that are most important for diagnosis and/or immunotherapy of prostate cancer.

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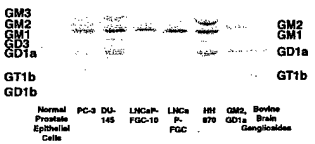
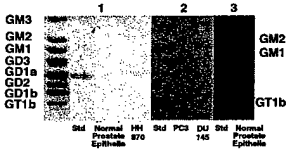
Ganglioside Signatures of CaP Cell Lines

1



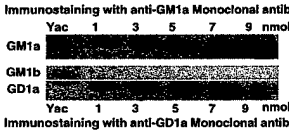
Only GM2 is Detectable in Normal Prostate Epithelia

2



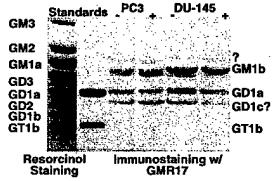
GM1b But Not GM1a Reacts to Anti-GD1a MAb

3



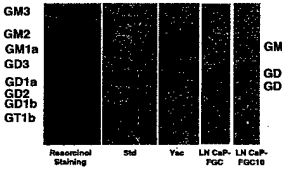
Mab GMR17 Identifies GD1a, GM1b & GD1c(?) in PC3 & DU145

4



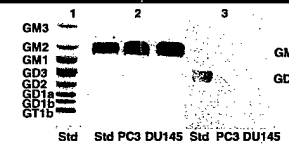
Mab GMR17 Identifies GD1a, & GD1c(?) in LN CaP-FGC/FGC10

5



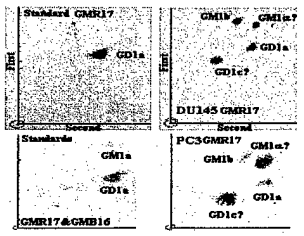
GM2 But Not GD3 Immunostained in CaP Cell Lines

6



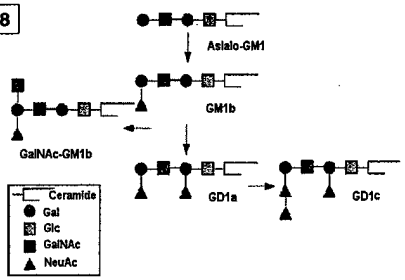
2D Immunostaining: GMR17 Reacts to Several Gangliosides of PC3 & DU145

7



Putative Biosynthetic Pathway of Prostate Cancer Associated Gangliosides

8



Results

- Resorcinol staining identified GM1, GM2 (in all cell lines), GD1a and GT1b (in PC3, DU145, and HH870) in the cell lysates. All the above gangliosides were found in traces in the extracts of normal prostatic epithelial cells.[Figure # 1].
- Except for GM2, none of the other gangliosides could be detected immunochemically in the extracts of normal prostatic epithelial cells [Figure # 2]. By immunostaining with monoclonal antibodies and standards, GM1b, GD1a, GD1c [?] [Figure # 4] and GM2 [Figure # 6] could be identified in the cell lines. GM1a, GT1b and GD3 could not be detected in any of the cancer (CaP) cell lines [Figure # 2 & 6].
- Anti-GD1a Mab GMR17, claimed to be specific for GD1a [Kotani et al., Kawashima et al., 1996], stained GM1 in all the prostate cell lines. The Mab stained GM1b fraction from murine Yac cell line [Figure # 5] but not standard GM1a [Figure # 3]. Therefore, the GM1 stained by anti-GD1a in the extracts of prostate cancer (CaP) cell lines [Figure # 4, 7] is identified as GM1b.
- Anti-GD1a Mab also stained two additional bands, one above GM1b and another below GD1a. These bands may represent GM1α and GD1c or GD1α [Figure # 4, 5, 7].
- Cell suspension ELISA with monoclonal antibodies identified GM2, GD1a and GD1b on the surface of most of the cell lines, whereas only GM1a could be detected on the surface of normal prostatic epithelial cells [Figure # 9].
- Since GM2 and its derivatives, such as GD2 and GD1b, are enhanced in the cells grown in culture due to activation of GalNAc-transferase [Tsuchida et al., 1987, Morton et al., 1994], presence of these gangliosides may be due to growth of the CaP cell lines in culture conditions.
- Putative biosynthetic pathway of CaP-Gangliosides during neoplastic transformation of normal prostate epithelial cells is indicated in the chart [Figure # 8].

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