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Advances in Brief

Up-Regulation of the Oligosaccharide Sialyl Lewis^X: A New Prognostic Parameter in Metastatic Prostate Cancer[1]

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Abstract

Metastatic prostate cancer has an unpredictable long-term prognosis. At present, there are few specific predictors to indicate the outcome for the individual patient. We have studied immunoreactivity for type-2 carbohydrate structures, known to be involved in various cell adhesion processes, in patients with metastatic prostate cancer. One group of patients (n = 26) did not progress within 3 years after orchiectomy, while another group of patients (n = 33) progressed within 1 year following castration and survived less than 2 years. Among the parameters studied, sialyl Lewis^X carbohydrate up-regulation was the only variable showing significant association with poor prognosis (P < 0.01). Sialyl Lewis^X discriminated between these two outcome groups with 71% predictability and 96% specificity. Our results indicate that up-regulation of sialyl Lewis^X is associated with hormonal-resistant, aggressive disease. This prognostic marker may, therefore, have an important role in selecting proper treatment for patients with metastatic prostate cancer.

Introduction

Adenocarcinoma of the prostate remains an important cause of cancer death and is the most common malignancy in men in Western countries (1). Approximately 50% of the tumors are discovered at an advanced stage, and the skeleton is the primary site of metastases in 85% of the patients who die of prostate cancer (1, 2). Since the first report by Huggins *et al.* (3), hormonal manipulation has been the initial treatment for metastatic prostate cancer. The aim of hormonal manipulation is to withdraw androgens or block androgen receptors at the target tissue level, since androgens stimulate normal as well as neoplastic prostate epithelial cells. The standard treatments, surgical or medical castration (orchiectomy or luteinizing hormone-releasing hormone analogues), are effective to reduce tumor growth for a variable period of time (1, 4). Unfortunately, about 20–30% of the patients do not respond to endocrine therapy. Thus far, no prognostic parameter has reliably predicted hormonal sensitivity and clinical outcome at the time of diagnosis (5, 6). In consequence, patients with hormone-resistant tumors are subjected to hormonal manipulation as a first choice of treatment, with side effects and limited effect on survival (1, 5). A future challenge is to search for treatments aimed at hormone-resistant carcinomas. A prognostic marker which reliably characterizes such tumors at the time of diagnosis might be advantageous.

All human carcinomas show changes within cell surface carbohydrates compared to their normal counterparts (7, 8). The biological significance for this aberrant glycosylation in cancer is mainly unknown, but there is increasing evidence that various cell surface carbohydrates are involved in cell-cell and cell-matrix recognition (9, 10). Type-2 cell surface carbohydrates have various adhesive functions (9–12). Especially the oligosaccharide, sialyl Lewis^X, reacts with selectins on activated endothelial cells during inflammation and is proposed to be an adhesion molecule during cancer progression and metastasizing (12–15). An immunohistochemical study of colorectal carcinomas has shown that up-regulation of sialyl-Lewis^X is an independent indicator of poor prognosis (12). The synthesis of sialyl Lewis^X was reported to be higher in the metastatic tumors than in the primary colorectal cancers.

The aim of the present study was to analyze type-2 carbohydrates in advanced prostate cancer. This study reports, for the first time, the prognostic importance of sialyl Lewis^X in metastatic prostate cancer at the time of diagnosis.

Materials and Methods

From a multicenter study analyzing the role of total androgen suppression (16), two outcome groups of patients were selected on the basis of time to progression and time to cancer-related death after surgical castration. All patients had histologically confirmed prostate carcinoma, with skeletal metastases (M1) diagnosed by bone scans. None of the patients had any previous prostate cancer therapy before tumor biopsy. The patients were followed either to disease progression or to death, with repeated clinical examinations, during a follow-up period of 3 years after the start of treatment. The first group consisted of patients who showed disease progression during the first year and death due to progression of the cancer during the subsequent year. This group was classified as therapy resistant, with poor prognosis (n = 33). The second group comprised patients who experienced disease regression with no signs of progression during a 3-year follow-up. This favorable prognostic group was classified as therapy sensitive (n = 26). The median ages in the poor and good prognostic groups were 71.4 years (56–85 years) and 73.3 years (60–85 years), respectively.

Only pretreatment transurethral resections or tru-cut biopsy specimens from the primary prostate carcinomas were selected. Sections (4-μm) were cut from each formalin-fixed and paraffin-embedded tissue block. A standard ABC-peroxidase immunohistochemical technique was performed. The carbohydrates (all type-2 structures) N-acetyllactosamine, H-type 2, Lewis^Y, and sialyl Lewis^X were immunohistochemically examined by the mAbs 1B2, BE2, AH6, and SNH3, respectively (Table 1).

Histological grade (Gleason and WHO) and immunoreactivity for the four carbohydrate structures were registered. Histological grading and registration of immunoreactivity were done blindly by an experienced pathologist (A. B.). Bryne *et al.* (17) have recently suggested that cells from the most invasive parts of tumor islands give the best prognostic information. Accordingly, immunoreactivity was graded semiquantitatively within the most invasive front of tumor cell islands (Fig. 1; 0, less than 5% positive cells; +, 5–25% positive cells; ++, 25–50% positive cells; +++, 50–75% positive cells; ++++, > 75% positive cells; Ref. 17). All parameters were correlated with the two prognostic groups by means of correlation analyses. The statistical significance of correlations was examined by contingency tests, grouping the values together so as to reach a minimum number of five within each cell. The Ps were found using a χ^2 test for 2 × 2 contingencies with Yates' correction. The significance level was P < 0.05. In a few cases, when values were given on a continuous scale, one way ANOVA was used. The combined prognostic predictability of the parameters from the primary tumor were examined by stepwise backward multiple regression analysis with the outcome (cancer-specific survival) as the dependent variable.

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Table 1 *Primary monoclonal antibodies used, their isotype, dilution, and chemical structure of the respective carbohydrate antigens*

Antibody[a]	Isotype	Antigen	Chemical structure of antigen[b]	Dilution[c]
IB2	IgM	LAC	Galβ1-4GlcNAcβ1-R	1:10
BE2	IgM	H	(Fucα1-2)Galβ1-4GlcNAcβ1-R	1:10
AH6	IgM	Le^y	(Fucα1-2)Galβ1-4(Fucα1-3)GlcNAcβ1-R	1:10
SNH3	IgM	S-Le^x	(NeuAcα2-3)Galβ1-4(Fucα1-3)GlcNAcβ1-4(Fucα1-3)GlcNacβ1-R	1:10

[a] References for generation are given in Ref. 26.
[b] Further details of the chemical structures of antigens are given in Ref. 11. Gal, galactose; Glc, glucose; GlcNAc, *N*-acetylglucosamine; Fuc, fucose; Neu, neuraminic acid.
[c] Diluted from a hybridoma supernatant with an immunoglobulin content of 10–30 μg/ml.

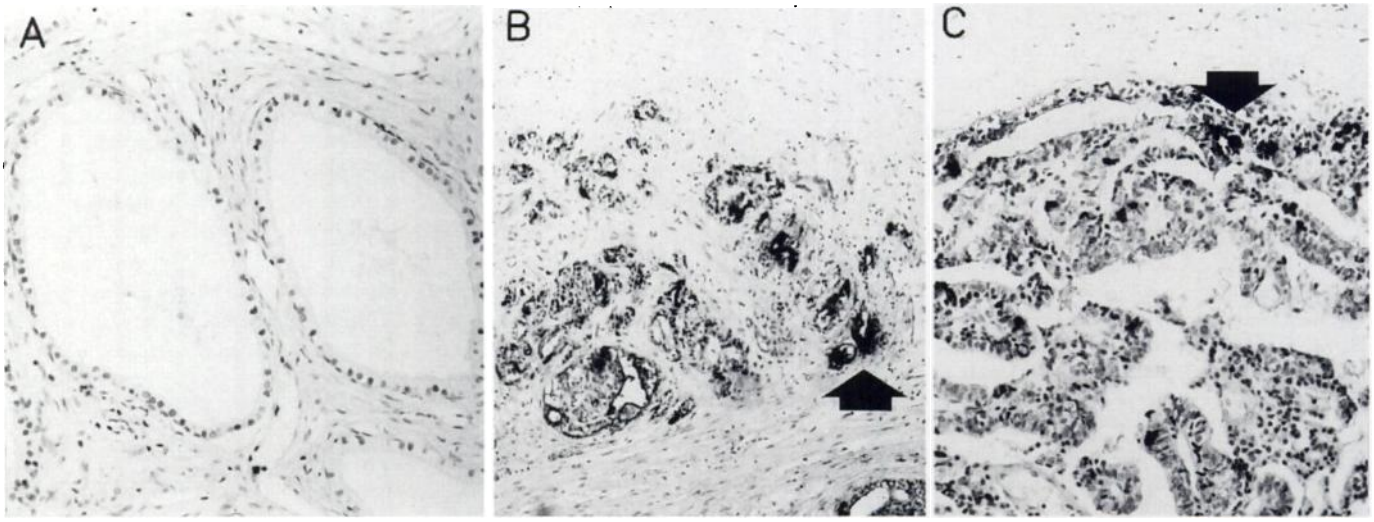

Fig. 1. Expression of sialyl Lewis^X in the primary tumor of patients with metastatic disease. *A*, no expression of sialyl Lewis^X in benign epithelium adjacent to the carcinoma. *B*, heterogeneous expression (*arrow*) of sialyl Lewis^X in the carcinoma. *C*, increased expression of sialyl Lewis^X in the most invasive front (*arrow*) of tumor cell islands compared to more central areas of the tumor island.

Results

Results from histological grading and expression of the type-2 carbohydrates are present in Table 2. The staining pattern was heterogeneous for all carbohydrates within each tumor and between tumors. Immunoreactivity for sialyl Lewis^X was strongly associated with a poor prognosis in univariate and in multivariate statistical analyses ($P < 0.01$; Table 2). Sixteen of 17 cases that expressed sialyl Lewis^X reactivity in more than 5% of the cells belonged to the poor prognostic group (Fig. 2). A further graduation of the sialyl Lewis^X-positive cases (5–25% positive cells; ++, 25–50% positive cells; +++, 50–75% positive cells) did not enhance the prognostic impact. Sialyl Lewis^X expression alone gave a 71% prognostic predictability, with a sensitivity and specificity of 52 and 96%, respectively. Immunohistochemical staining of sialyl Lewis^X was not detected in the benign epithelium adjacent to tumors, in contrast to the other carbohydrates. Neither grade as defined by WHO or Gleason nor immunoreactivity for the other sugar molecules showed significant correlations with sialyl Lewis^X or showed any prognostic importance in the univariate analysis ($P > 0.05$; Table 2). In the multivariate analysis, none of the other parameters, including a combination with sialyl Lewis^X, enhanced the prognostic value.

Discussion

The present analysis demonstrates a highly significant correlation between up-regulation of an adhesion-related sugar structure, sialyl Lewis^X, and poor prognosis for patients with metastatic prostate cancer. Among the parameters studied, sialyl Lewis^X was the only prognostic marker that could predict prognosis. To our knowledge, no other prognostic parameter has been published which can predict hormone resistance and prognosis at the time of diagnosis as reliably as this single molecule.

Histological grade and clinical stage are recognized as prognostic factors for prostate cancer patients (18–20). High grade and advanced stage have an independent adverse effect on patients' outcome (20). However, in the present analysis, histological grade (WHO and Gleason) showed no prognostic importance for patients with the same advanced clinical stage. Numerous studies on grading of prostate carcinomas, reproducibility of grading, and the predictive value of the various grading systems, especially those of Mostofi (WHO) and Gleason, have been performed (21). The conclusions are conflicting, but the lack of reproducibility and accuracy in the subjective histological assessment of tumor grading renders this method unreliable as a prognostic indicator for the individual patient (22). We have, in another publication, confirmed the conclusion that histological grading of prostate cancer has poor reproducibility and gives no prognostic information when metastases are present (23).The present finding

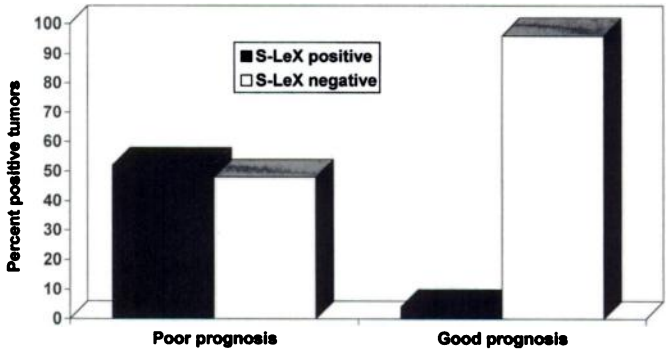

Fig. 2. Sialyl Lewis^X expression in the hormone-resistant, *i.e.*, poor, prognostic group compared to the hormone-sensitive, *i.e.*, good, prognostic group. Expression of Sialyl Lewis^X at time of diagnosis strongly indicates a limited response to endocrine treatment and poor prognosis.

Table 2 *Prognostic impact of histological grading and immunostaining of some type 2 carbohydrate structures in two outcome groups of patients with metastatic prostate cancer*

Variables[a]	No. of patients and percentage				P
	Poor prognosis		Good prognosis		
WHO grade					NS[b]
1	5	15%	2	8%	
2	11	33%	8	31%	
3	17	52%	16	61%	
Total	33	100%	26	100%	
Gleason score					NS
2–4	6	18%	3	12%	
5–7	22	67%	19	73%	
8–10	5	15%	4	15%	
Total	33	100%	26	100%	
N-Acetyllactosamine					NS
0	11	35%	8	31%	
1	3	9%	4	15%	
2	7	22%	6	23%	
3	9	28%	6	23%	
4	2	6%	2	8%	
Total	32	100%	26	100%	
H-antigen					NS
0	11	33%	12	46%	
1	6	18%	2	8%	
2	6	18%	8	31%	
3	10	33%	4	15%	
4	0	0	0	0	
Total	33	100%	26	100%	
Lewis^Y					NS
0	7	22%	7	29%	
1	2	6%	1	4%	
2	9	28%	4	17%	
3	10	31%	10	42%	
4	4	13%	2	8%	
Total	32	100%	24	100%	
Sialyl Lewis^X					0.0039
0	15	47%	24	96%	
1	7	22%	0	0	
2	4	13%	1	4%	
3	4	13%	0	0	
4	2	6%	0	0	
Total	32	100%	25	100%	

[a] 0, <5% cells positive; 1, 5–25% cells positive; 2, 25–50% cells positive; 3, 50–75% cells positive; and 4, >75% cells positive.
[b] NS, not significant.

that sialyl Lewis^X is able to distinguish between hormone-sensitive and hormone-resistant carcinomas may, therefore, be of clinical importance.

The biological functions of the presently studied type-2 carbohydrates are mostly unknown, but accumulating evidence indicates that some of these are involved in cell adhesion processes during embryonal development, cell migration in wound healing, and cancer progression and metastasis (9, 10, 12, 13, 24, 25). A recent interesting *in vitro* study by Sawada *et al.* (15) showed that colon cancer cell adhesion to E-selectin on activated endothelial cells could be inhibited by soluble glycoproteins containing sialyl Lewis^X epitopes but not by the same glycoprotein without sialyl Lewis^X. Furthermore, these authors found that highly metastatic tumor cells expressing a larger amount of sialyl Lewis^X adhere more strongly to endothelial cells than the corresponding low metastatic carcinoma cells expressing less sialyl Lewis^X. Their work further supports the hypothesis that inhibition of selectin-carbohydrate interactions can be a future treatment in metastasis prevention (13, 15). Our present results also support the hypothesis that sialyl-Lewis^X is involved in tumor dissemination, since advanced prostatic tumors expressing sialyl Lewis^X strongly indicate an aggressive disease with poor prognosis. The results obtained clearly indicate that patients with metastatic prostate cancer expressing sialyl Lewis^X have hormone-resistant carcinomas with poor prognosis. This finding may be of value for future treatment planning and has increased the understanding of this common disease.

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