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Sialyl-lewis-X, Gleason grade and stage in non-metastatic human prostate cancer

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Early stage prostate cancers are now commonly encountered because of widespread use of screening tools. Increased cancer cell proliferation, and expression of sialyl Lewis could be important as predictors of clinical behaviour and survival in addition to histologic grade. In this study, the expression of sialyl-Lewis^x (sLe^x) was determined by immunohistochemical methods in 38 routinely processed prostate biopsies and transurethral resections preceeding radical prostatectomies for organ confined prostate cancers. Histologic grades were determined from pathologic reports and divided into two (2) groups; low grade (Gleason score 2–4) and medium grade (Gleason score 5–7). Tumour stages were based on radical prostatectomy reports and 29 were T2 and 9 were T3. SLe^x was positive in 10 of 14 (71.4%) low grade and 14 of 24 (62.5%) medium grade cancers; 22/29 (75.9%) T2 and 8/9 (88.9%) T3 were sLe^x positive; 1 of 15 (7.2%) low grade and 5 of 24 (20.8%) medium grade were strongly positive (3 +) or overexpressing sLe^x. Overexpression of sLe^x was a feature of medium grade cancer, suggesting that localized prostate cancers with increased potential for progression and metastasis exist in the clinically non-metastatic group.

***Keywords*: Human prostate cancer, sialyl-Lewis^x-grade stage**

Introduction

Cancer of the prostate, mainly adenocarcinoma, is the second most common cancer in males. This cancer accounts for 3% of all male cancer deaths. There is a component of familial inheritance of human prostate cancer and also genomic instability is demonstrable. The mainstay of treatment for early stage prostate cancer is surgery (with irradiation and anti-hormonal treatment or chemotherapy added in some cases). The 5 y death rate in early prostate cancer is 0–20%, and the rate of progression is 9–37%. Local surgical failure rates and death depend on grade, capsular involvement, and whether surgical margins are involved. Early detection of prostate cancer relies on digital rectal examination (DRG), transrectal ultrasound (TRUS) and estimation of the levels of prostate specific antigen (PSA) in serum. The possible outcome is determined by the size of the cancer, the estimated volume of cancer by transrectal ultrasound and magnetic resonance imaging (MRI), the histopathological grade, and the serum PSA level. About 30% of prostate cancers are metastatic at presentation, and of the 30% that are localized, half have reached the draining lymph nodes. Even in clinically localized prostate cancers treated by radical surgery (presumably confirmed by careful pathological examination), metastatic behaviour cannot be predicted and there may still be up to 20% mortality at 15 years. These varied behaviour patterns for prostate cancer indicate that the prognostic indicators used are not always reliable.

It is important, therefore, to search for other markers that can further predict clinical behaviour in early stage human prostate cancers. Such biological markers could then be used in conjunction with histopathological grade, PSA and stage. A possible marker for prostate cancer could be the cell surface glycoconjugate that is involved in cell/cell adhesion, sLe^x. This blood group related glycoconjugate, is a ligand for endothelial selectin (E-selectin) and participates in the binding of NK cells to target cells for cell killing. SLe^x is constitutively present in colon cancer [1] and limited distribution is described in the prostate gland [2]. Expression of sLe^x relates to metastatic behaviour [3, 4]. In colon and small cell lung cancers, increased sLe^x portends poor survival [5, 6].

The purpose of this study was to determine the relationship between the expression of sLe^x and the histological grade and the stage of human prostate cancer. It was proposed to determine the differences in expression of sLe^x between low (Gleason score 2–4) and medium grade (Gleason scores 5–7) and organ-confined stage T2 and stage T3 human prostate cancers.

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Materials and methods

Prostate cancer tissues confirmed from prostate cancers (by transrectal biopsies and transurethral resections) were used for the study. All tissues were embedded in paraffin wax. Prostate tissues were cut at 4 μm. Immunohistochemistry: the anti-sLe^x antibody was obtained from Kamiya Biomedicals (KM93) and used at a 1:40 dilution. Immunohistochemical staining were carried out as described below. Endogenous peroxidase was blocked with 0.3% H_2O_2 in methanol solution for 6 min at room temperature. The sections were washed with phosphate buffered saline (PBS pH 7.6) and treated with normal rabbit serum. Primary antibody was applied at 4 °C overnight. The biotinylated secondary antibody was used at a dilution of 1:200 at room temperature for 20 min. The reactions were developed with ABC elite (Avidin-biotin complex) (7–8 mg ml^{-1}) for 40 min at room temperature. The sections were washed and counterstained with Hematoxylin. Immunostaining intensity and distribution of the stain were used to assign values 0–3 + for the specimens; 0 = absent, 1 + mild (up to 25% of stained cells), 2 + (25–50% of stained cells), and 3 + (> 50% of stained cells). Three plus was designated as overexpression.

Results and discussion

The staining for sLe^x differed somewhat in normal and cancer cells (Figure 1). In normal cells, staining was predominantly on the cell-surface. In cancer cells, the staining was present in the cytoplasm and on the cell surface, especially in strongly-stained cells. By defining overexpression as 3 +, only 7.1% of Gleason score 2–4 and 20.8% of Gleason 5–7 showed overexpression of sLe^x (Table 1). This may be related to increased metastasis. The percentage of such cancers that lead to metastasis will probably depend on NK cell activity [6]. In contrast, 28.6% and 37.7% of both low (Gleason 2–4) and medium (Gleason 5–7) prostate cancers respectively were negative for sLe^x, indicating one-third of all prostate cancers may not be

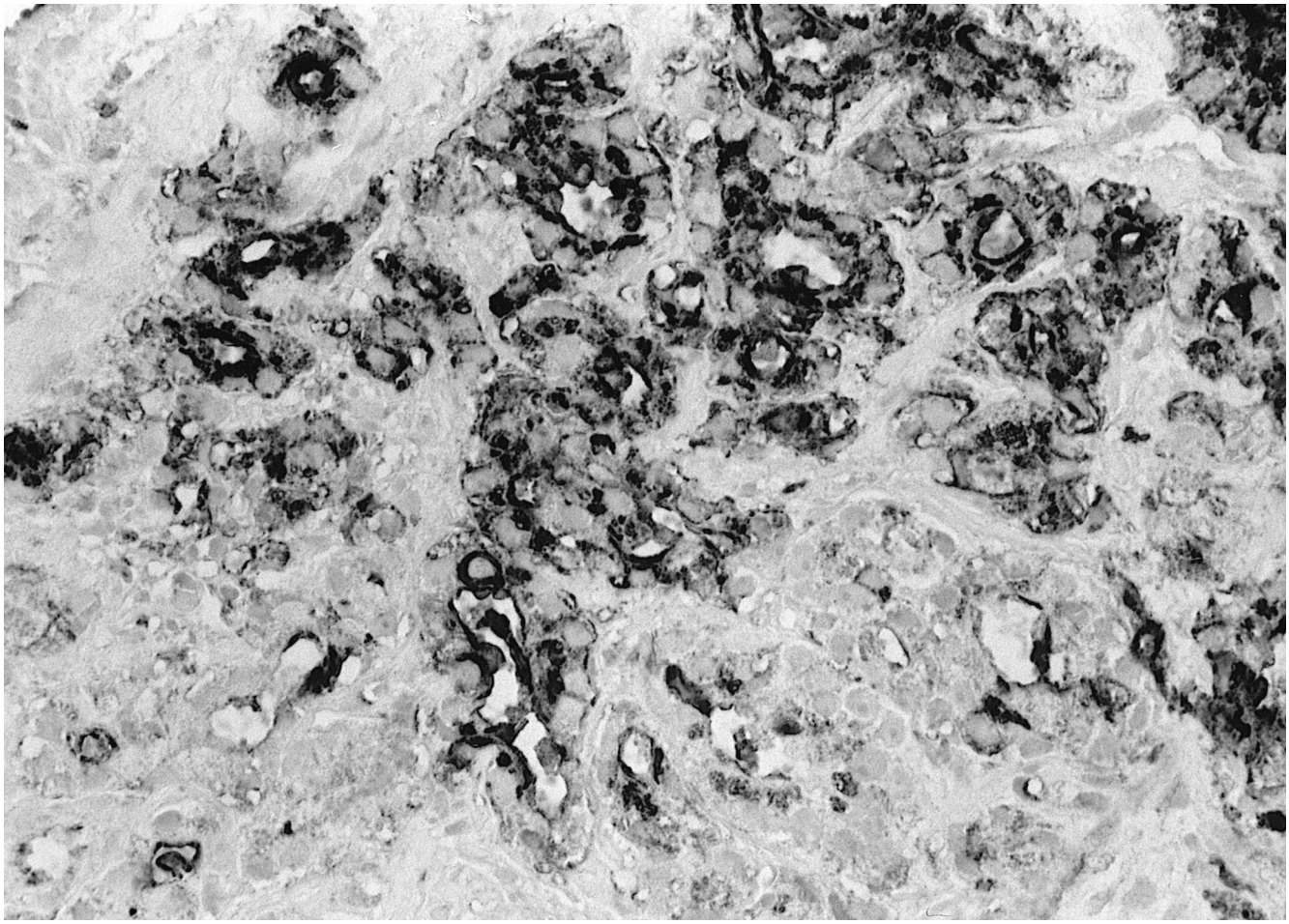

Figure 1. SLe^x immunostaining of human prostate cancer biopsy and transurethral resection (original × 250).

Table 1. Distribution of staining for sLe^x in human prostate cancer

Staining intensity	*Prostate cancer histological grade*	
	Low grade (Scores 2–4) (n = 14)	*Medium grade (Scores 5–7) (n = 24)*
0	4 (28.56%)	9 (37.7%)
1–2+	9 (64.3%)	10 (41.7%)
3 +	1 (7.1%)	5 (20.8%)

Fisher's exact test was used to calculate the significance of the differences between proportions of cancers staining for sLe^x in the low and medium groups. These differences were not significant. The Gleason scores were as reported in the biopsy report, scores 2–4 was designated as low grade cancer and scores 5–7 as intermediate grade cancer.

Table 2 SLe^x in stages T2 and T3 prostate cancers

sLe^x expression	*Stage T2 (n = 29)*	*Stage T3 (n = 9)*
Positive (1–3 +)	22 (75.9%)	8 (88.9%)
Negative (0)	7 (24.1%)	1 (11.1%)

The stage of the cancer was determined from the complete surgical report of the prostatectomy specimens. T2, palpable but limited to the prostate (involving half of the lobe, > 50% of lobe or both lobes) and T3, extracapsular involvement (*ie* seminal vesicle involved). 4/29 (13.8%) of stage T2 and 2/9 of stage T3 (22.2%) were classified as overexpressing sLe^x(3 +). There was no significant differences between sLe^x expression between T2 and T3.

susceptible to NK cell killing. These cancers may have low metastatic potential. To prevent sLe^x enhancing prostate cancer metastasis we may need sLe^x mimics, which will inhibit E-selectin [7].

Conclusions

In conclusion, therefore: (i) Biological differences between low and medium grades of human prostate cancer may be quite small based on their expression of sLe^x; (ii) sLe^x expression was similar in low and medium grade prostate cancers; (iii) no differences were noted in sLe^x expression between stages T2 and T3 (Table 2); (iv) Prostate cancers overexpressing sLe^x may possess greater propensity for metastases.

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