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Transferrin Receptor Expression by Human Bladder Transitional Cell Carcinomas

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Summary. The expression of transferrin receptors (TFR) by normal and neoplastic urothelial cells was studied in "control" patients and in patients with transitional cell carcinoma of the bladder. These tumours were graded independently and consisted of 19 grade I, 30 grade II and 19 grade III lesions. TFRs were identified using a monoclonal antibody specific for TFR (OKT9) in an immunofluorescent or avidin/biotin-immunoperoxidase technique on fresh frozen sections. TFRs were not detected on normal urothelium. However, positive staining was found to increase with increasing pathological grade and stage of the tumours, ranging from 31.6% of grade I to 78.9% of grade III tumours and 51.2% of pTa (mucosa only lesions) to 87.5% of pT2/pT2+ (muscle invasion ± deeper) primary urothelial malignancies.

Key words: Transferrin receptors, Human bladder carcinoma.

Introduction

Histological grading and pathological staging have become established as most important parameters in determining treatment for patients with bladder cancer. However, the correlation between histological appearance and biological behaviour is less than perfect [5, 20]. For optimal management, the identification of aggressive and potentially aggressive tumours is desirable. Antigen expression status and flow cytometry have been examined in this context in an attempt to indicate innate biological aggressiveness [7], but, to date, acceptable predictability for individual cases has not been provided satisfactorily by these techniques.

Rapid proliferation of urothelial cells is known to occur in a variety of conditions involving the urinary tract, but is of particular importance in neoplasia. Closely associated with tumour cell proliferation is transferrin receptor expression. Transferrin receptors have been identified in a large number of cells and are considered to be essential for cell growth. This ubiquitous cell-surface glycoprotein is thought to be present on all proliferating cells [14].

The aim of this study was to record transferrin receptor status as an indicator of proliferative activity, relating this to grade and pathological stage in transitional cell bladder cancer in order to test the hypothesis that a greater proportion of invasive tumours compared to superficial tumours were positive for transferrin receptor expression.

Materials and Methods

Patients

Tissue specimens were obtained from 68 patients undergoing transurethral resection of bladder tumours. They consisted of 59 males and 9 females with a mean age of 66 ± 12 years (range 31–94 years).

Random bladder biopsies taken from patients (9 males and 13 females) with non-malignant conditions during cystoscopy were used as controls. These controls had a mean age of 62 ± 14 years with a range from 25–83 years.

Tissue Preparation

Each specimen was divided into two with one part sent for routine histopathological examination (formalin-fixed, paraffin embedded and stained with haematoxylin and eosin). The remaining part was immediately quenched in liquid nitrogen and stored at −70 °C until used. Five micron sections were cut, air dried for 2 h, fixed for 5 min in equal parts chloroform/acetone at room temperature, and wrapped in plastic film before storage at −20 °C until used. A haematoxylin and eosin slide was made from each specimen to confirm the presence of tumour. Histological grading and staging were performed on paraffin sections.

Immunohistology

Indirect immunofluorescence and avidin-biotin immunoperoxidase labelling were carried out [10].

Prior to staining, sections were rehydrated in phosphate-buffered saline (PBS), pH 7.4, for 10 min. The first layer, OKT9 (Ortho-

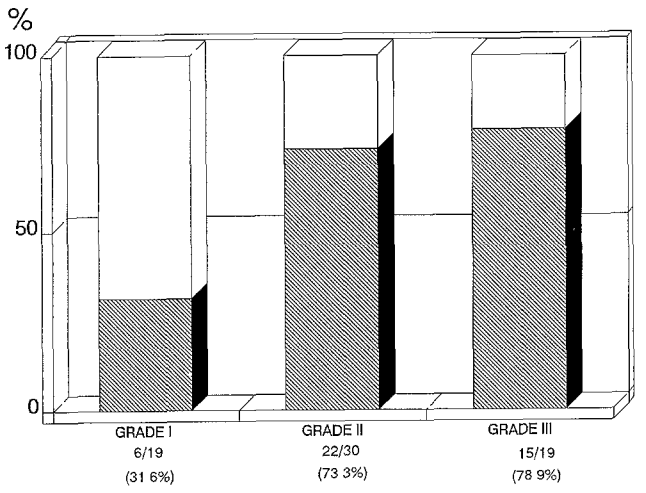


Fig. 1. TFR status related to grade

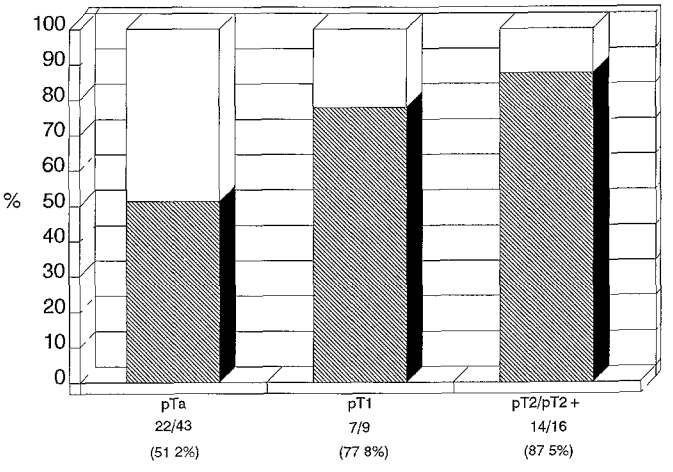


Fig. 2. TFR status related to stage

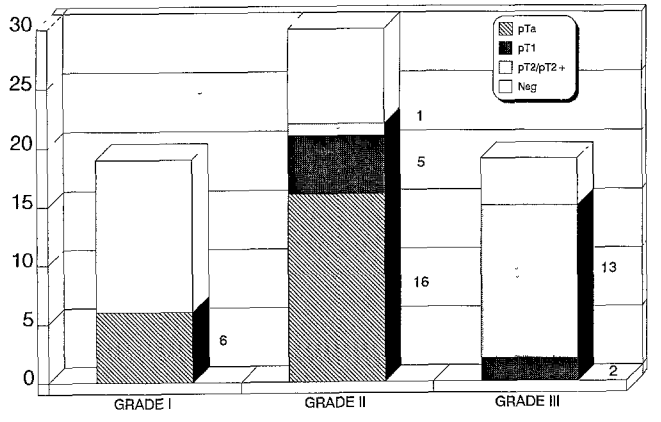


Fig. 3. TFR status related to grade and stage

mune), was applied at a 1:10 dilution in PBS for 30 min. Following incubation with the primary antibody, and after each subsequent incubation, the slides were washed thoroughly in PBS. For fluorescent microscopy, the sections were incubated with 1:20 affinity purified fluorescein isothiocyanate (FITC) conjugated goat-anti mouse immunoglobulins (Amersham) for 30 min. The sections were mounted in 70.30 glycerol/PBS and examined using a Zeiss fluorescent microscope with epi-illumination. For photographic purposes, immunoperoxidase slides were made of selected tumours. The sections were incubated with 1:20 affinity purified sheep-anti-mouse immunoglobulins conjugated with biotin (Amersham) as the second layer for 30 min, washed, then incubated with 1:50 streptavidin-horseradish peroxidase (Amersham) for thirty min. The slides were developed in 0.5% 3,3'-diaminobenzidine (Sigma Chemicals) with hydrogen peroxidase as substrate for 10 min, washed, counterstained with Harris' haematoxylin, dehydrated, cleared in xylol, and mounted with DPX. Sections were examined and scored as unseens.

Control sections included the use of PBS, normal mouse serum and an irrelevant monoclonal antibody (FN4/BA4-) which reacts with an antigen on *Fusobacterium nucleatum* [19] in place of the primary antibody.

Pathological Staging

A modification of the IUCC pathological staging system was employed in this study. pTa and pT1 indicated lesions limited to mucosa and tumour extending from mucosa into lamina propria respectively, but pT2/pT2+ was chosen to indicate those malignancies seen histologically to invade muscle, some of which clinically involved deep muscle and beyond.

Results

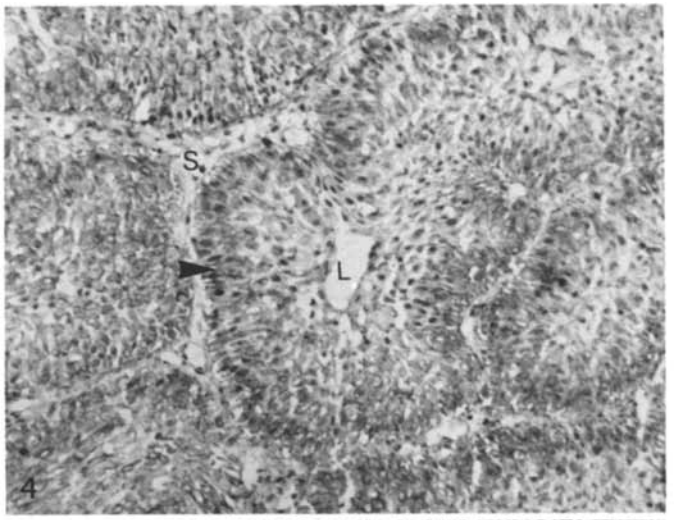
TFR positive staining was not observed in any of the random biopsies from control patients.

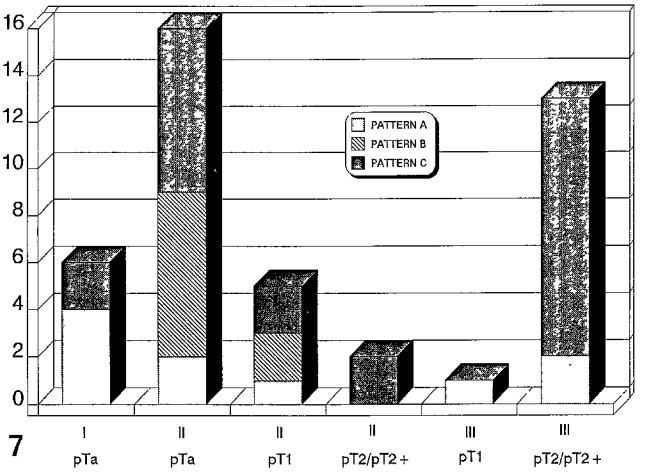
TFR positive staining was present in 6 out of 19 (31.6%) grade I, 22 out of 30 (73.3%) grade II and 15 out of 19 (78.9%) grade III tumours (Fig. 1). By pathological stage, 22 out of 43 pTa (tumour limited to mucosa), 7 out of 9 pT1 (tumour limited to lamina propria) and 14 out of 16 pT2/pT2+ (tumour involving muscle ± beyond) were TFR positive (Fig. 2). The relationship between grade and pathological stage is shown in Fig. 3. 21 out of 25 tumours not confined to mucosa (pT1 and pT2/pT2+) were TFR positive. Thus the proportion of patients whose tumours were positive for TFR was significantly greater in this group compared to those with mucosa only lesions ($p < 0.01$).

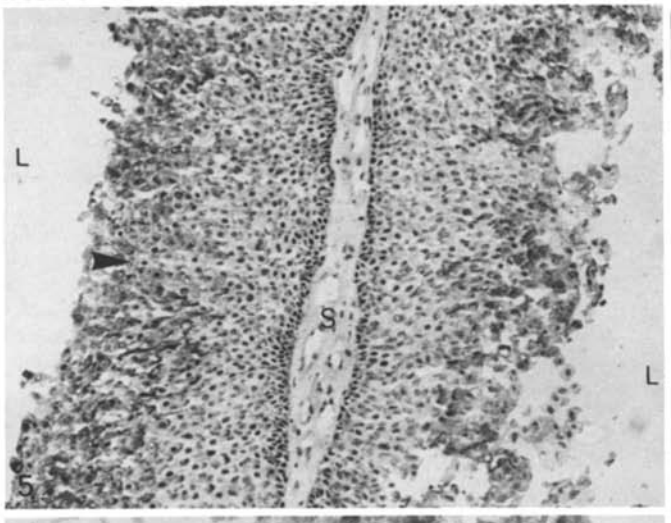
Three patterns of TFR staining were identified in sections: (a) basal epithelial cells staining most intensely (Fig. 4), (b) superficial epithelial cells staining most intensely (Fig. 5) and (c) relatively homogenous staining of the tumour cells (Fig. 6). The proportions of these subgroups are demonstrated in Fig. 7.

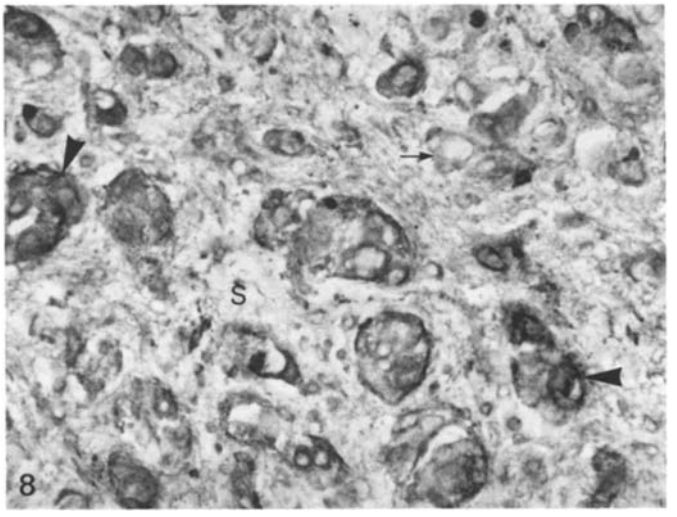
Discussion

This study reports that a proportion of primary human bladder transitional cell carcinomas display transferrin receptor positivity with light microscopy. Expression was related to histological grade and increasing pathological stage (Figs. 1, 2). A strong grade/stage relationship is recognized in transitional cell bladder cancer [5]. However, grade and stage do not necessarily indicate the state of biological dynamism of a part or of the whole of a tumour. A number of









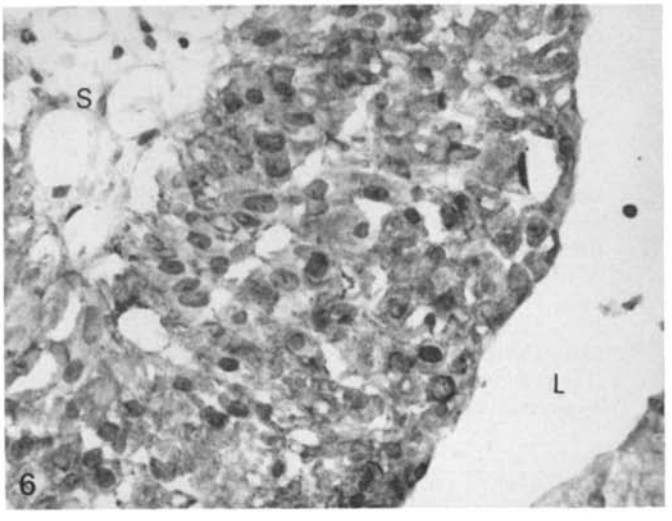


Fig. 4. Grade II tumour displaying pattern A staining, where the basal layers of tumour cells (*arrow head*) are most intensely stained. *L*, lumen; *S*, stroma. (Mag. x 100)

Fig. 5. Pattern B expression by a grade II tumour, with peripheral tumour cells most intensely expressing TFR (*arrow head*). *L*, lumen; *S*, stroma. (Mag. x 100)

Fig. 6. Relatively homogenous (pattern C) expression of TFR by a grade II tumour. *L*, lumen; *S*, stroma. (Mag. x 250)

Fig. 7. TFR patterns related to grade and stage

Fig. 8. Grade III tumour invading the lamina propria, with most tumour cells displaying TFR (*arrow head*), although some cells are apparently negative (*small arrow*). *S*, stroma. (Mag. x 250)

factors, which may be transient, have been shown to affect cell cycle activity [2, 16]. Hence, transferrin receptor status, as a marker of proliferative activity, may serve to predict tumour behaviour more effectively.

The absence of positive staining in normal urothelium and some bladder tumours does not necessarily mean that these cells are devoid of TFR. A more likely explanation is that, as these cells may not be proliferating, the number of receptors are too few to be detected by the immunofluorescence and immunoperoxidase techniques used with light microscopy. Indeed, the discrimination afforded by this apparent discrepancy may serve as a useful indicator of tumour dynamics. The significance of the three types of staining patterns seen in this study is not clear. Whether these

patterns relate to urinary transferrin levels, known to be elevated in some urothelial cancers [4], is an interesting conjecture.

It is recognized that the male to female sex ratio of 6 to 1 does not reflect the pattern of gender affliction by bladder carcinoma in this community [11] or in other Western societies [6]. The composition in this study merely reflects the manner in which patients presented to Royal Brisbane Hospital with transitional cell bladder carcinomata which were resected and collected for processing and analysis. The important point, however, is the fact that a number of primary bladder tumours, especially the higher grades, were TFR positive.

Recently, considerable activity has been addressed to the role of growth factors and growth factor receptors in tumour tissues, both being essential in cell replication [8, 9, 12, 13]. At present, these have been defined incompletely, and those identified are of small molecular size making detection difficult by presently available histochemical techniques. Oncogene product and oncofoetal antigen expression by cells have attracted recent attention [1, 18]. In any one tumour, however, a number of oncogenes may be operative, and qualitative rather than quantitative changes in oncogene products may be pertinent in cell dynamics. In contrast to these limitations with growth factor and oncogene product expression, transferrin receptor status may provide a more comprehensive appreciation of proliferative activity and, together with other phenotypic markers, prove to be an important parameter in identifying aggressive behaviour.

Antibodies to TFR have been shown to block or to inhibit cell growth [15, 17] and in this context the suggestion has been made that such antibodies may have a useful role in regulating tumour growth [3]. Therefore the findings of the present study in identifying transferrin receptors on human bladder transitional cell carcinomata may have some useful implications. Certainly, this study indicates that TFR expression deserves consideration for inclusion in phenotypical profiles as a marker of proliferative activity in primary transitional cell bladder cancer.

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