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Immunohistochemical distribution of epithelial membrane antigen in bladder carcinomas as detected with a monoclonal antibody

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Summary. Expression of epithelial membrane antigen (EMA) was investigated immunohistochemically in 27 cases of bladder carcinoma using a monoclonal antibody. Normal urothelial epithelium showed EMA staining restricted to the upper layer of the surface epithelium. G-I transitional cell carcinomas demonstrated positive EMA staining which could be divided into the following 3 types; type 1, in which highly stained cells occurred in the upper layer of the neoplastic epithelium; type 2, in which the whole tumour focus was slightly stained; and type 3, in which cells strongly positive for EMA were scattered throughout the tumour focus. G-III (undifferentiated) transitional cell lesions exhibited irregular expression of EMA whereas squamous cell demonstrated specific intense EMA staining within keratinized tumour cells.

Key words: Bladder carcinoma – Epithelial membrane antigen – Immunoperoxidase technique

Introduction

Epithelial membrane antigen (EMA) is detectable on the luminal surfaces of glandular tissues, while normal squamous cell epithelium is negative [6, 7, 10, 12, 16, 17]. However, the antigen can be demonstrated in malignant tumours such as squamous cell carcinomas [3, 8, 10, 11, 13, 14, 17]. Although many studies have been performed on EMA distribution in a variety of tumours and normal tissues with the use of polyclonal and monoclonal antibodies [3–17], urinary bladder carcinomas have as yet escaped detailed attention. The present paper describes the immunohistochemical localization of EMA in various types of urinary bladder carcinoma, and compares the antigen distribution with that of keratin proteins and involucrin, as reported earlier in such lesions [1, 2, 4].

Materials and methods

Materials

A total of 27 cases of urinary bladder carcinoma were examined. The tumours were classified into transitional cell carcinomas (G-I, 5 cases; G-II, 7 cases; G-III, 7 cases) and squamous cell carcinomas (G-I, 1 case; G-II, 4 cases; G-III, 3 cases). All the material obtained from surgery were fixed in 10% formalin solution, and serial 4 μm paraffin sections were made for histochemical demonstration of EMA as well as for pathological examination with H&E staining.

Immunohistochemical methods

Deparaffinized sections were treated with methanol containing 0.3% H_2O_2 for 20 min to inactivate endogenous peroxidase and were rinsed thoroughly. The sections were treated as follows:

1) Reaction with normal rabbit serum (1:20, Wheaton, USA) for 20 min.

2) Reaction with mouse monoclonal anti-EMA antibody (1:40, Dakopatts, Copenhagen, Denmark) for 1 h.

3) Reaction with HRP-labelled rabbit anti-mouse immunoglobulins (1:20, Dakopatts, Copenhagen, Denmark) for 30 min.

4) Immersion for 5 min in 0.05 M Tris buffer (pH 7.6) containing 0.005% 3,3'-diaminobenzidine tetrahydrochloride (DAB) and 0.003% H_2O_2.

Results

Normal urothelial epithelium

Immunohistochemical staining for EMA in normal urothelium was confined to the surface borders and plasma membranes of cells in the upper and intermediate zones. Basally located cells did not react with the immunoreagent.

Hyperplastic epithelium and intestinal type of epithelium

EMA staining in hyperplastic epithelium was also restricted to the surfaces of the upper layers of

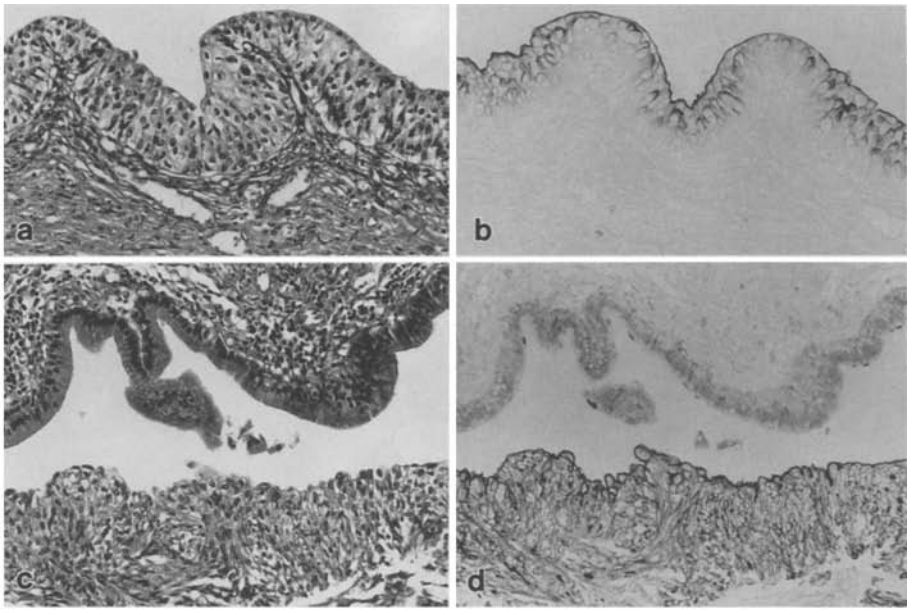

Fig. 1a–d. Hyperplastic epithelium (**a** and **b**) and intestinal type of epithelium (**c** and **d**) (× 100). **a** H&E staining. **b** EMA staining. Serial section to a. Luminal surfaces of urothelial hyperplastic cells show strong positive reaction. Staining of the plasma membrane of intermediate layer cells is slight as in normal urothelial epithelium. **c** H&E staining. High columnar cells show marked intestinal metaplasia (upper side). G-I transitional cell carcinoma (lower side). **d** EMA staining. Serial section to c. Intestinal metaplastic cells lacking a EMA positive reaction

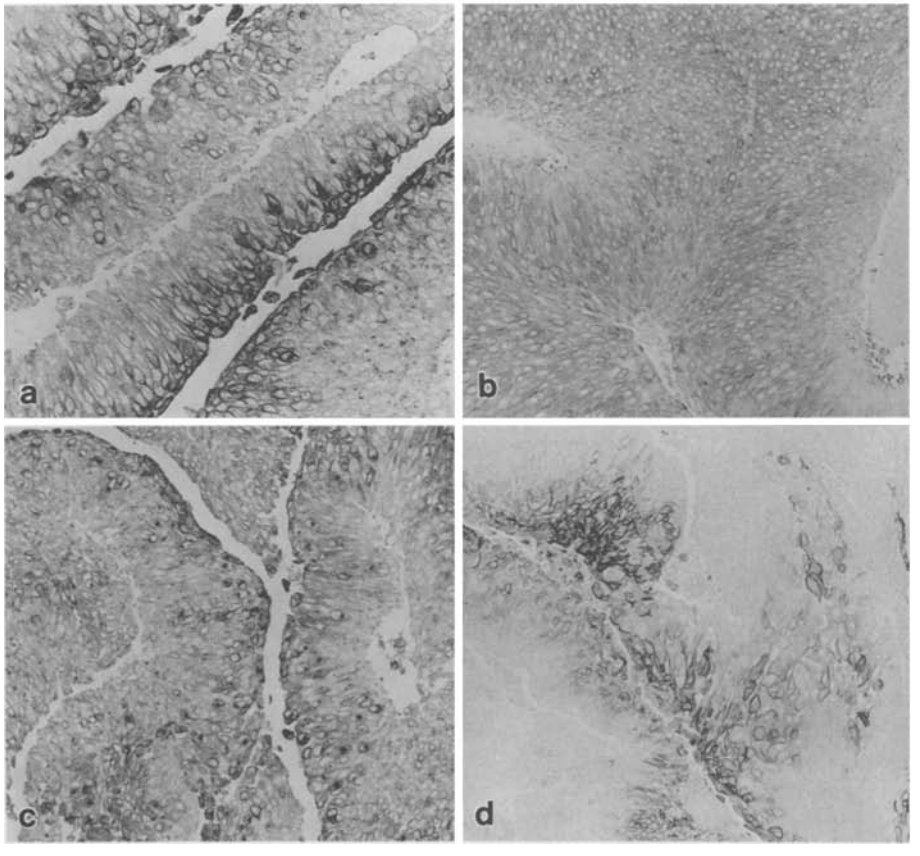

Fig. 2a–d. Transitional cell carcinoma (G-I carcinoma) (**a–c**) and squamous cell carcinoma (**d**) (× 100). **a** EMA staining (type 1). Umbrella like cells are present in the superficial layers of urothelial tumour epithelium. A strong reaction is observed from the intermediate layer to the superficial layer. **b** EMA staining (type 2). All of the tumour cells are slightly and diffusely stainable. **c** EMA staining (type 3). EMA reaction is mainly limited to plasma membranes and the positive cells are scattered within the tumour focus. **d** EMA staining reaction is limited to the plasma membranes of keratinized tumour cells

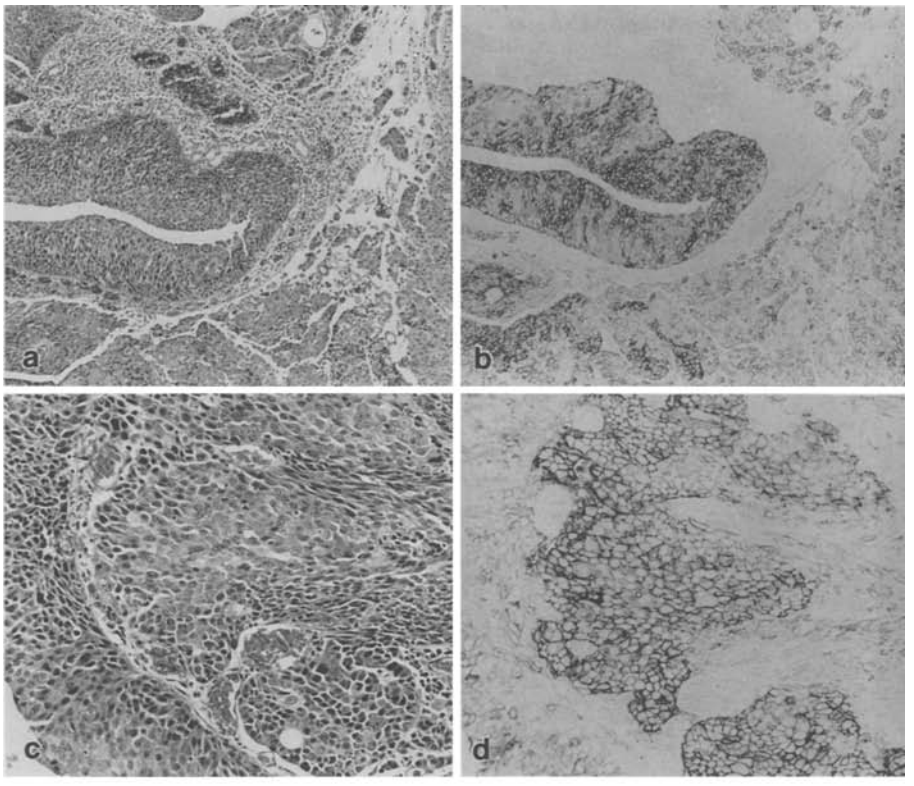

Fig. 3a–d. Transitional cell carcinoma (G-II carcinoma). **a** and **b** (×40), **c** and **d** (×100). **a** H&E staining. **b** EMA staining. Serial section to a. EMA reaction varies from strong to slight or negative. **c** H&E staining. **d** EMA staining. Serial section to c. A strong EMA reaction is observed in the plasma membranes of squamous like tumour cells

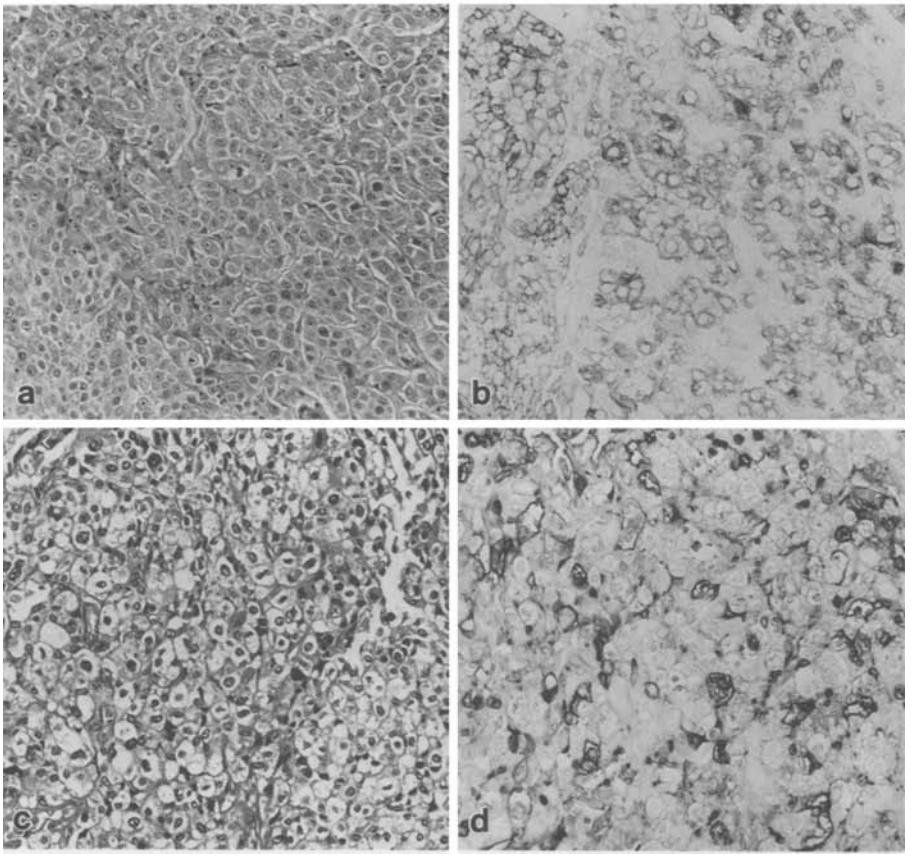

Fig. 4a–d. Transitional cell carcinoma (G-III carcinoma) (×200). **a** H&E staining. **b** EMA staining. Serial section to **a**. Some of the neoplastic cells show positive staining. **c** H&E staining. Clear cell variant. **d** EMA staining. Serial section to c. Luminal surfaces of small tubular structures show a strong positive reaction

epithelial cells (Fig. 1a and b). Intestinal type of epithelium was negative or only very slightly positive for EMA (Fig. 1c and d).

Transitional cell carcinomas

EMA staining in G-I carcinomas which showed a regular arrangement of tumour cells with papillary growth could be classified into 3 main types: type 1 showing a strongly positive EMA reaction at the tumour surface, and in surface umbrella-like cells; the basal zone being negative (Fig. 2a); type 2, in which all tumour cells displayed slight EMA staining (Fig. 2b); and type 3, EMA-positive cells being irregularly distributed throughout the tumour foci (Fig. 2c).

G-II carcinomas demonstrated an irregular distribution of EMA, with the overall staining intensity in the neoplastic cells being generally weaker than in G-I carcinomas. The localization of EMA revealed a mosaic appearance; that is, some areas displayed a strong reaction, while others were negative or only very slightly positive (Fig. 3a and b). The areas with the strongest EMA reaction contained squamous epithelium-like or squamous tumour cells, and the antigen appeared to be limited to the plasma membrane of the neoplastic cells (Fig. 3c and d).

The EMA staining in G-III carcinomas, which demonstrated features of severe malignancy, was generally reduced in individual tumour cells; however, luminal surfaces of small tubular structures displayed an abundance of EMA (Fig. 4a–d).

Squamous cell carcinoma

EMA expression in squamous cell carcinoma was confined to keratinized foci; that is, keratinized neoplastic cells were highly positive for EMA (Fig. 2d).

Cellular distribution of EMA in bladder carcinomas

EMA staining was usually limited to the plasma membrane in bladder tumour cells, and the presence of antigen was more conspicuous in differentiated tumour cells than in undifferentiated cells.

Discussion

Antibodies against EMA-isolated from human milk fat globule membranes have been used as a diagnostic tool to characterize the distribution of antigen in various tissues and neoplastic lesions [3, 8, 12]. EMA has been detected on the luminal surfaces of normal glandular tissues and their related neoplastic lesions [5, 10, 11, 13, 15]. This is the first report concerning immunohistochemical expression of EMA in urinary bladder carcinoma using a monoclonal antibody.

The surface border-positive pattern of EMA staining which was found to occur in urinary bladder umbrella cells is similarly expressed in some glandular tissues [6, 7, 9, 16]. This border-positive pattern found in normal urothelium was also characteristic for G-I (differentiated) transitional cell carcinomas and hyperplastic epithelia and suggests that surface cells in the tumour may function as protection against toxic materials in the urine as is considered to be the case for normal surface umbrella cells.

The observed cellular distribution of EMA staining in transitional cell carcinomas usually confined to the plasma membrane, is in line with findings for squamous cell carcinomas of other tissues [3, 10, 11, 13, 14]. This EMA expression may be bound to a certain type of glycoprotein due to disorganization and indicate a characteristic appearance of antigenic determinants in the respective tumour grade. It is interesting to note that EMA expression in G-I (differentiated) transitional cell carcinomas could be classified into 3 types, and that EMA distribution was often similar to that of involucrin in the tumours [1]. In squamoid tumour cells of G-II transitional carcinoma, the finding of EMA staining strongly confined to plasma membrane may indicate a sign of epithelial malignancy.

Squamous cell carcinomas in urinary bladder are rather rare in Japan, and EMA staining of this type was limited to keratinized areas as seen in squamous cell carcinomas from patients infected with *Schistosoma haematobium.* The presence of EMA in keratinized tumour cells may indicate an association between abnormal antigenic determinants and keratinization in tumour cells. EMA staining in various epidermoid carcinomas was also reported to be very strong in keratinized cells [10, 14].

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