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Involucrin expression in urinary bladder carcinoma

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Summary. Expression of involucrin was investigated immunohistochemically in 27 cases of urinary bladder carcinoma. Although no keratinization was observed in the transitional cell carcinomas examined all displayed involucrin staining to various degrees. Involucrin expression in foci of G-I transitional cell carcinomas was classified into 3 types: type 1, a mixture of intensely stained and slightly positive cells; type 2, highly positive cells intermingled with negative tumour cells; and type 3, all tumour cells slightly positive. Undifferentiated cell carcinomas demonstrated an irregular distribution of involucrin of varying staining intensity while deposition in squamous cell carcinomas was limited to keratinized areas.

Key words: Bladder carcinoma – Involucrin – Immunoperoxidase technique

Introduction

It has been reported that involucrin is a marker of terminal keratinization in skin epidermis and other squamous cell epithelia [3, 9, 14, 18–21]. Keratin proteins are also specific markers of normal epithelial tissues and of epithelial tumours [12, 13, 15]; patterns of keratin deposition in different types of urinary bladder carcinomas have been described [1, 2]. Urothelial tumours are usually classified into papilloma, transitional cell carcinoma, squamous cell carcinoma with varying degrees of keratinization, adenocarcinomas, and undifferentiated carcinomas [4–6, 8, 10, 11, 16] and the histologic features of those carcinoma cells do not include marked keratinization except in the case of squamous cell carcinoma. The present study was concerned with immunohistochemical identification of involucrin in urinary bladder carcinomas, and comparison of its expression with that of keratins reported previously [1, 2].

Materials and methods

Materials

A total of 27 cases of urinary bladder carcinoma were investigated. The specimens were fixed in 10% formalin solution for 12 h and then processed for embedding in paraffin. Serial paraffin sections cut at 4 μm were stained with H&E for histologic orientation and immunohistochemically for involucrin. Histologically, the tumours consisted of transitional cell carcinomas (G-I, 5 cases; G-II, 7 cases; and G-III, 7 cases) and squamous cell carcinomas (G-I, 1 cases; G-II, 4 cases; and G-III, 3 cases).

Immunohistochemical method

An Involucrin Immuno Kit (Biomedical Technologies, Inc., Cambridge, USA) was used. Details of the staining method have been reported elsewhere [7, 20].

Results

Normal urothelial epithelium

Umbrella cells showed positive staining for involucrin except for the superficial zone which was devoid of antibody binding. Intermediate layer cells were positive for involucrin, while the basal cells gave very slight staining or were negative.

Hyperplastic urothelial epithelium

Basally located cells showed negative staining for involucrin, but most intermediate layer cells gave a positive reaction. Superficial cells or umbrella cells were nonreactive (Fig. 2a and b).

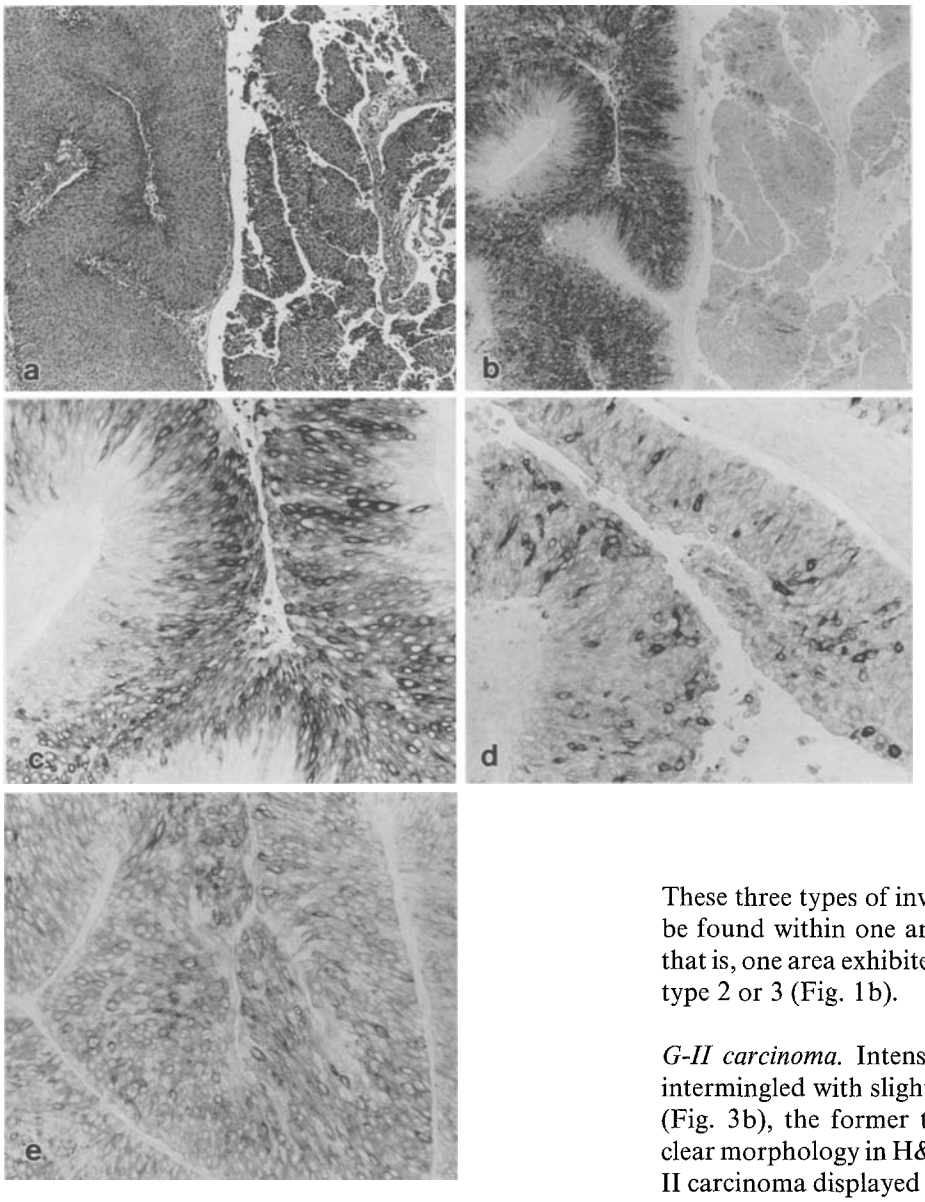

Fig. 1a–e. Transitional cell carcinoma (G-I). **a** H&E staining, ×40. **b** Involucrin staining. Serial section to **a**. Neoplastic epithelial show positive (left) or negative (right) involucrin staining reaction, ×40. **c** Higher magnification of **b**, ×100. Type I involucrin deposition: intensely staining cells and slightly stained or negative cells are mixed. **d** Type II involucrin deposition: most intensely staining cells are scattered within an involucrin negative tumour focus. **e** Type III involucrin deposition: all tumour cells show slightly positive staining

Transitional cell carcinoma

G-I carcinoma. The tumour epithelium was typically composed of numerous layers of high columnar or long-spindle shaped cells (Fig. 1a). Involucrin expression in G-I carcinoma was divided into the following 3 types: type 1, tumours in which the cells were positively stained and the basal layers of the neoplastic epithelium were usually negative, with the upper strata showing a mixture of highly and very highly stained cells (Fig. 1c); type 2, in which highly reactive tumour cells were scattered throughout neoplastic epithelium where involucrin staining was negative or very slight (Fig. 1d); and type 3, in which all of the tumour cells were uniformly, but only slightly stained (Fig. 1e). These three types of involucrin expression could often be found within one and the same tumour specimen; that is, one area exhibited type 1, while another showed type 2 or 3 (Fig. 1b).

G-II carcinoma. Intensely stained tumour cells were intermingled with slightly stained ones in tumour foci (Fig. 3b), the former type corresponding to cells of clear morphology in H&E sections. Another type of G-II carcinoma displayed foci containing strongly positive tumour cells within neoplastic areas whose involucrin staining was otherwise trace or negative (Fig. 2c and d).

G-III carcinoma. There was only irregular, slight staining for involucrin in undifferentiated tumour cells. No conspicuously reactive cells were found in G-III carcinomas (Fig. 3c and d).

Squamous cell carcinoma

Involucrin staining in the squamous cell carcinoma was dependent on the degree of keratinization, the highest deposition being detected in the hornified cells. In contrast, non-keratinized squamous tumour cells were devoid of involucrin staining (Fig. 3e and f).

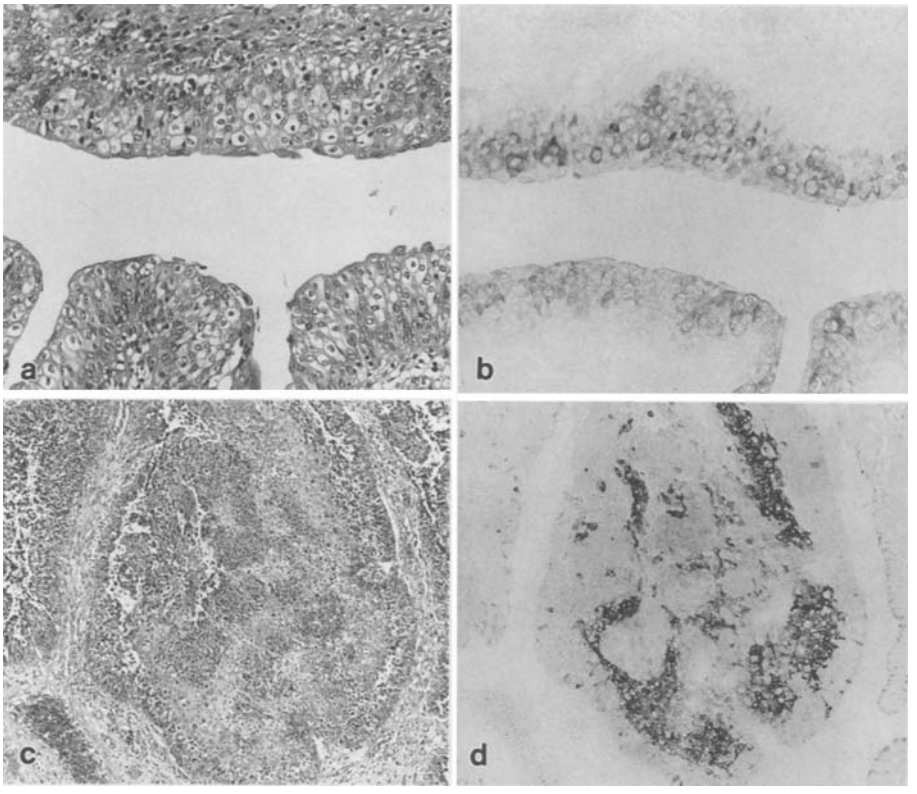

Fig. 2a–d. a and b Hyperplastic urothelial epithelium, ×100. **a** H&E staining. Hyperplastic urothelial epithelium with slight dysplasia. **b** Involucrin staining. Serial section to a. Hyperplastic urothelial epithelium demonstrates a slight reaction to involucrin. **c and d** Transitional cell carcinoma (G-II), ×100. **c** H&E staining. **d** Involucrin staining. Serial section to c, ×100. Strongly staining tumour cells are surrounded by areas showing very slight staining

Discussion

Immunohistochemical distribution of involucrin in neoplastic tissues was most striking in the keratinized cells of these tumours, and most cases with positive involucrin staining have been shown to be tumours of epidermal or squamous origin in the skin, oral or uterine cervical mucosa [9, 17–19, 21, 22]. In normal squamous cell epithelium, involucrin is expressed in the upper spinous and granular layer cells, but is absent in basal layer cells [3, 9, 14, 20, 22, 23]. The involucrin reaction in normal urothelium observed in the present study displayed a similar pattern to that found in squamous epithelium, with the exception of the negative superficial layer of umbrella cells. This finding suggested that urothelial cells may possess the potential for keratinization, although histologic features did not indicate any signs of hornification. This potential may be exhibited in a limited number of tumour cells, and so squamous-transforming cells in the transitional cell carcinoma might be expected to be involucrin-positive. Thus the three types of involucrin localization in G-I transitional cell carcinomas classified in the present study might have arisen due to variation in direction of cellular differentiation within neoplastic urothelium. The differential expression of involucrin in the tumour cells was probably directly related to latent disorganization or abnormal keratinization. A high level of involucrin staining of tumour cells is a particular sign of a high degree of keratinization, whereas no involucrin staining is an indication of little, if any, keratin in tumour cells as has been noted in squamous or epidermoid types of carcinomas [7, 9, 14, 18–20]. Histologic features of transitional cell carcinoma generally did not include hornification, as is the case with normal urothelium; however, the immunohistochemical sign of involucrin deposition in these carcinomas might also suggest a potentially active keratinizing tendency. The occurrence of positive involucrin staining in transitional cell carcinomas is one of the distinguishing markers of keratinization. Walts et al. (1985) noted that the higher grade papillary urothelial carcinomas and infiltrating urothelial and squamous cell carcinomas showed abnormal staining patterns for involucrin [21].

In the present study, the transitional cell carcinoma (G-I) displayed a rather regular distribution pattern of involucrin staining as compared with that of the undifferentiated type. This was also the case for the expression of keratin proteins, which was examined earlier. In this previous work, differentiated transitional cell carcinomas were found to have no marker

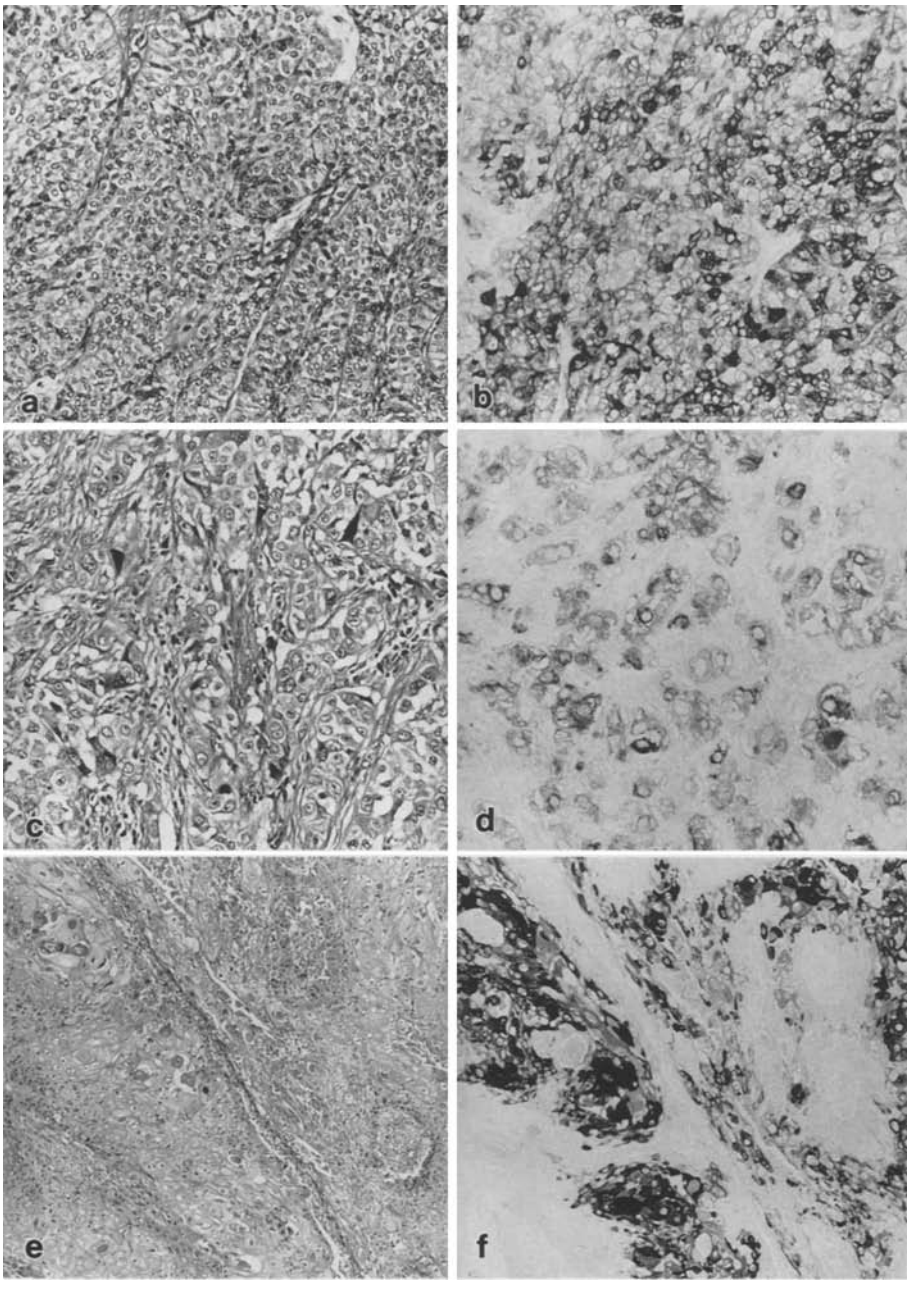

Fig. 3a–f. a and b Transitional cell carcinoma (G-II), × 100. **a** H&E staining. **b** Involucrin staining. Serial section to a. Strongly and slightly staining tumour cells are mixed in the tumour focus. **c and d** Transitional cell carcinoma (G-III), × 100, **c** H&E staining. **d** Involucrin staining. Serial section to c. Undifferentiated tumour cells are slightly positive for involucrin staining. **e and f** Squamous cell carcinoma, × 100. **e** Squamous cell carcinoma, × 100. **f** Involucrin staining. Serial section to e. Keratinized tumour cells show the strongest positive reaction. Involucrin deposition is confined to highly keratinized cells

irregular distribution of keratins. Heavy deposits of keratin proteins were observed in a only limited number of tumour cells [2], as was the case for involucrin. However, the range of staining intensity was not so great for keratin as for involucrin. Overall, transitional carcinoma cells were usually positive for keratins [2].

Squamous cell carcinoma in the bladder rarely occurs except in patients with *Schistosoma haematobium* infection [7]. In the present study the tumour cells with conspicuous involucrin staining corresponded to the highly keratinized cells within the lesions. This pattern was similar to that seen in squamous cell carcinomas of epidermis and in oral mucosa [9, 14, 17–22].

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