

Squamous and glandular differentiation in urothelial bladder carcinomas. Histopathology, histochemistry and immunohistochemical expression of carcinoembryonic antigen

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Summary. This paper reports the immunochemical expression of carcinoembryonic antigen in the squamous, glandular and mixed differentiation areas observed in 190 urothelial urinary bladder carcinomas. The antigen was found to occur in 75% and 81% of all papillary and solid infiltrating carcinomas, respectively. The use of the immunohistochemical determination of CEA in improving the morphological examination of these differentiation areas and their scant presence in the prognosis of urothelial carcinomas involving squamous and/or glandular focal differentiation are discussed.

Key words: CEA - Urinary bladder - Mixed carcinoma - Metaplasia - Mucosubstances

Introduction

The determination of carcinoembryonic antigen (CEA) in urine and serum, as well as in tissues by immunohistochemical techniques (IHC) has been the subject of a great deal of work aimed at expanding the present knowledge on urothelial carcinomas (Goldenberg and Wahren, 1978; Zimmerman et al., 1980; Shevchuk et al., 1981; Jautzke and Altenaehr, 1982; Wiley et al., 1982; Fulks and Falace, 1985; López-Beltrán et al., 1986a). Notwithstanding the lack of unanimous agreement, CEA values are widely believed to be of use on account of the positive correlation between urine values and their immunohistochemical distribution, degree of cell differentiation and parietal infiltration staging (Zimmerman et al., 1980; Jautzke and Altenaehr, 1982; Jakse et al., 1983; López-Beltrán et al., 1986a).

On the other hand, there are few literature references to the expression of CEA in the squamous, glandular or mixed differentiation areas frequently observed in urothelial carcinomas with metaplastic changes, or in mixed carcinomas (Mostofi, 1954; Grace and Winter, 1968; Friedell et al., 1976; Jautzke and Altenaehr, 1982; Fulks and Falace, 1983; Webb, 1985; López-Beltrán et al., 1986b). Jautzke and Altenaehr (1982) established the immunohistochemical presence of CEA in such areas and their expression pattern; yet, no correlation was made to the degree of differentiation or tumoral infiltration staging.

This work reports the distribution of CEA in squamous, glandular and mixed areas found in a series of 190 cases of urothelial carcinoma of the bladder. Special emphasis is placed on its use for improved morphological examination and significance to the biological evolution of the tumours affected. Histogenetic considerations on the origin of these peculiar forms of urothelial differentiation are also made.

Materials and methods

A total of 190 urothelial bladder carcinomas obtained by transureteral resection were used in this study. After resection, all tumours were fixed in 10% formol and embedded in paraffin wax. Then 5µm sections were stained with hematoxyline-eosine (H&E), which allowed their classification according to histological type (papillary or solid infiltrating), degree of differentiation and parietal infiltration staging (Jewet, 1952), as well as the characterization of the metaplastic changes involved (squamous, glandular or mixed). Wherever glandular or mixed grading was found, the mucinous component was studied by the PAS technique (neutral mucines), or with the aid of Alcian Blue, pH 2.5, (acid sialomucines) and high iron diamine (HID) in the case of sulphomucines. Likewise, those tumours showing squamous, glandular or mixed differentiation were assayed for immunohistochemical CEA by Sternberger's PAP technique (1970). A control

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section in which the first antiserum was replaced with saline tris-buffer in order to detect nonspecific reactivity was used throughout. The primary antiserum used was rabbit serum in human CEA in a 1/400 dilution, which was considered to be the optimum; on the other hand, the commercial antiserum was absorbed in order to eliminate the reactivity of the non-specific cross-reacting antigen (NCA) by incubation with normal human spleen and lung. The dilutions of the secondary antiserum and the PAP complex were 1/50 and 1/100, respectively, and both, like the first antiserum, were supplied by Dako (Dakopatts, Denmark).

The statistical method used was Wolff's G-test.

Results

Out of the 190 urothelial carcinomas studied, 145 were classified histologically as papillary and 45 as solid infiltrating. Various degrees and stages of cell differentiation and parietal infiltration were found.

Squamous differentiation

The areas of squamous differentiation were found to occur in two forms, namely as wide squamous expanses consisting of wide nests and strings of polygonal cells frequently showing dyskeratosis and corneous pearls in addition to occasional intercellular bridges, or as small foci frequently unseen and made up of wide cytoplasm cells and occasional intercellular bridges, but showing no dyskeratosis or corneous pearls (Fig. 1). The immunoreactivity of CEA manifested itself in three different ways in these areas (Fig. 2): in corneous pearls and dyskeratotic cells; intracytoplasmically diffused and on the cell periphery (possibly glycocalix).

Glandular differentiation

The areas of glandular differentiation showed in three different ways (Fig. 3). The commonest, characterized by tube-like structures involving central secretion material, consisted of neutral mucosubstances and non-sulphurized acid sialomucines, CEA-positive on the luminal edge and lying between urothelial nests. Areas of colloid differentiation with small cell groups embedded in mucus lakes consisting of non-sulphurized acid mucines were more rarely seen; the reactivity of CEA in these was shown by its positive diffuse intracytoplasmic presence in individual cells. Finally, enteroid-like glandular structures ostensibly CEA-negative and containing non-sulphurized acid sialomucines were also detected (Fig. 4).

The mixing, in different proportions of the different squamous or glandular differentiation areas described above, resulted in the tumours with mixed differentiation areas.

Papillary tumours

All 145 papillary tumours in the series were analysed

for the presence of squamous, glandular or mixed differentiation areas in terms of their grading-staining. Thirty-two of the tumours (22.06%) showed one of these forms of differentiation, and 24 (75%) of them were immunoreactive to CEA (Table 1). No significant correlation between squamous, glandular or mixed differentiation and the tumor grading-staging was found ($p > 0.05$).

Solid infiltrating tumours

The occurrence of the various differentiation areas found in solid infiltrating tumours is shown in Table 2 as function of the tumor staging. Eleven of the 45 tumours (24.44%) showed at least one of these types of area, out of which 9 (81.81%) were CEA-positive. No statistically significant correlation was found between the occurrence of squamous, glandular or mixed differentiation and the tumor grading ($p > 0.05$).

Table 1. Distribution of the different squamous (Sq), glandular (Gl) and mixed (Mx) differentiation areas as a function of the grading-staging (G/E) and the number of CEA-positive (CEA+) cases found among the 145 papillary tumours in the series.

G/E	Sq	Gl	Mx	TOTAL
II-A	11	5	2	18
II-B	3	0	1	4
III-A	4	1	0	5
III-B	2	2	1	5
TOTAL	20	8	4	32/145 (22.06%)
CEA +	16/20 (80%)	4/8 (50%)	4/4 (100%)	24/32 (75%)

Table 2. Distribution of the different squamous (sq), glandular (Gl) and mixed (Mx) differentiation areas according to the grading/staging (G/E) in the 45 solid infiltrating tumours in the series, together with the number and distribution of CEA-positive (CEA+) cases.

G/E	Sq	Gl	Mx	TOTAL
III-B	4	2	2	8
III-C	2	0	0	2
III-D	1	0	0	1
TOTAL	7	2	2	11/45 (24.44%)
CEA +	7/7 (100)	1/2 (50%)	1/2 (50%)	9/11 (81.81%)

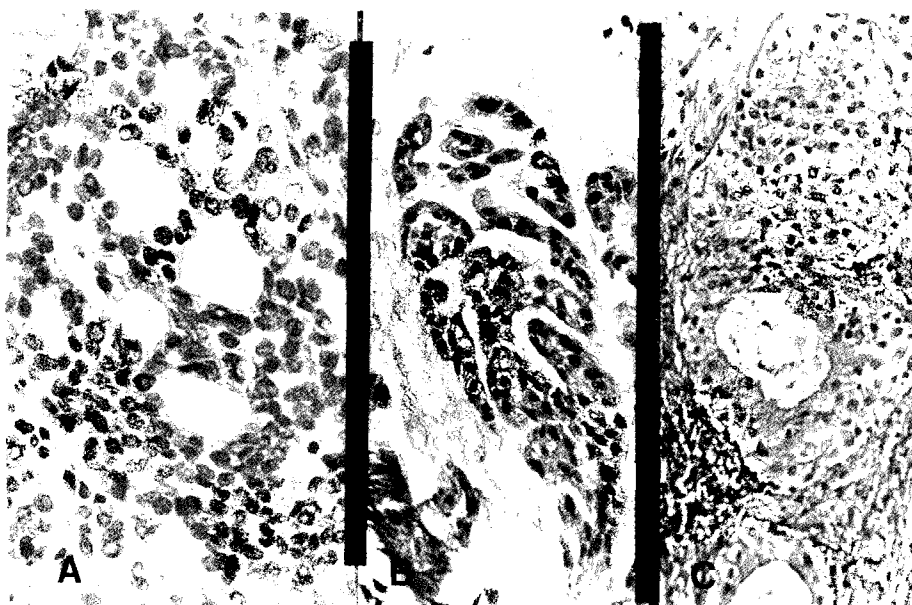


Fig. 1. Wide (A) and small (B) nest arrangement of the areas of squamous differentiation in urothelial bladder carcinomas. H.E. × 400

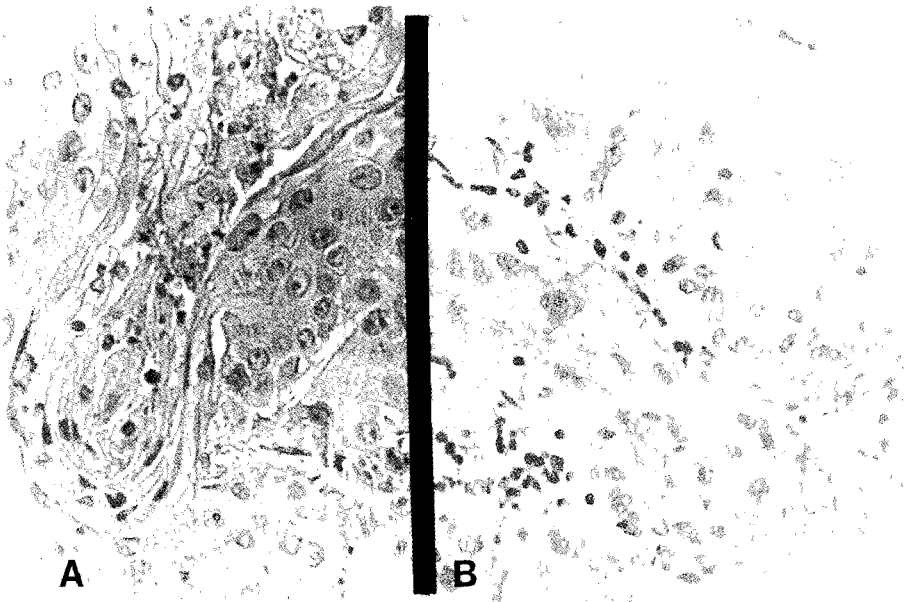


Fig. 2. Expression of CEA in corneous pearls (A), isolated cells (B) and the cell periphery (C) in areas of squamous differentiation. PAP-CEA-Methyl Green. $\times 400$

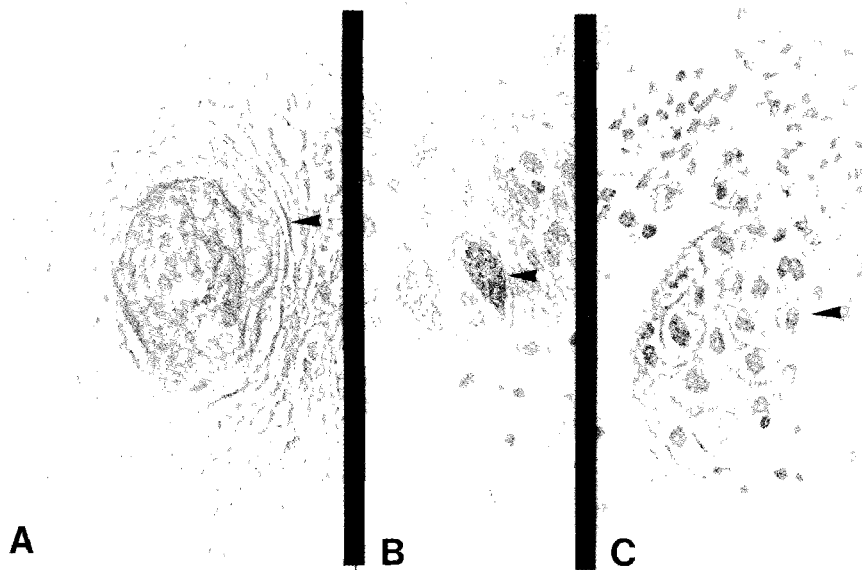


Fig. 3. Tubular (A), colloid (B) and glandular (C) arrangement as forms of glandular differentiation in urothelial bladder carcinomas. H.E. $\times 400$

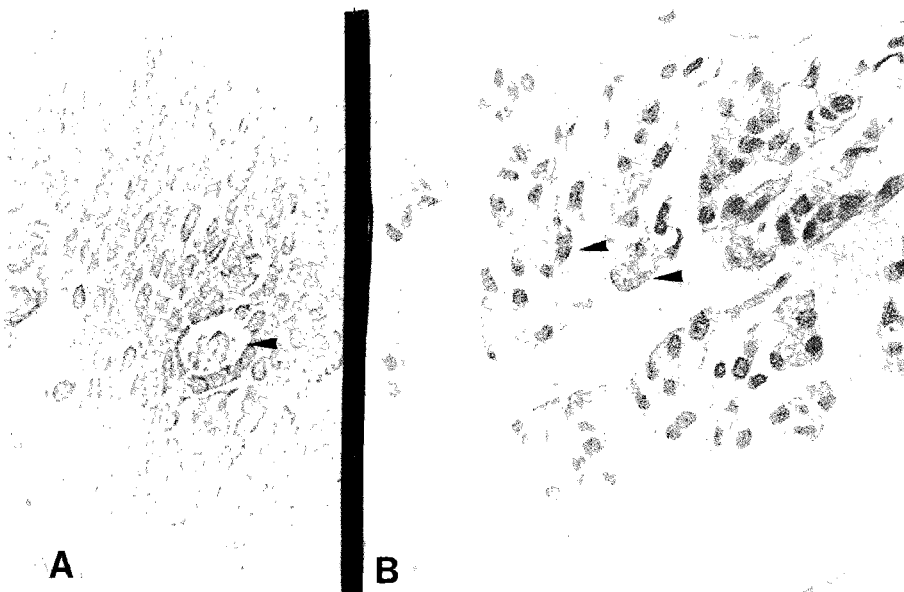


Fig. 4. Expression of CEA in the luminal edge of tubular structures (A) or diffused intracytoplasmically throughout colloid differentiation areas (B). A: PAP-CEA-Methyl Green. $\times 400$. B: PAP-CEA-haematoxylin. $\times 400$

Discussion

The occurrence of squamous or glandular differentiation areas in certain urothelial carcinomas poses several questions of interest, such as their histogenesis and incorporation into the diagnosis of the tumours showing them and whether or not they are CEA-positive.

As far as the histogenesis of these peculiar differentiation foci is concerned, there is still much controversy and a lack of agreement between different authors. The urothelium is known to react to irritating mechanical or infectious phenomena, frequently undergoing squamous and -less often- glandular metaplastic changes, which emphasizes its metaplastic potential. This can be accounted for on the basis of the preservation of the capacity of change into both types of epithelium, which is indeed a significant urothelial singularity (Dimmette et al., 1956; Gordon, 1963; O'flynn and Mullaney, 1964). This metaplastic potential of the urothelium is also shown in urothelial tumours, where it is found to occur with more or less the same frequency both in papillary and solid infiltrating tumours, irrespective of their grading and staging (Ward, 1970), in zones for which some authors propose the denomination «differentiation areas» instead of «metaplastic changes» and including the tumours bearing them in a group of mixed urinary bladder carcinomas (Mostofi et al., 1974; Friedell et al., 1976; Webb, 1985).

As with earlier findings, squamous differentiation areas were found to occur more frequently than glandular ones, though no satisfactory explanation can yet be given for this cell prevalence or even for the appearance of cell differentiation foci. With regard to this, vesical chronic irritation has been prompted as a major factor of, among others, glandular differentiation and the occurrence of mixed carcinomas (Grace and Winter, 1968; Melicow, 1974); other authors believe this association to be purely coincidental, which, accordingly, emphasizes the metaplastic potential of the urothelium that is preserved in neoplastic transformations (Ward, 1970). These findings, alongside with the observation of differentiation foci in both high and low degree tumours, does in fact suggest that this potential is so strongly associated with the urothelial cell that, once shown, it is preserved throughout the long evolution of urothelial carcinomas, even in cases of advanced cell anaplasia.

From this point of view, the prognosis of urothelial tumours involving squamous and/or glandular differentiation areas would be related to the grading-staging of the urothelial portion, which is generally the prevailing element (Ward, 1970). Thus, from a histoprognostic viewpoint, these cases involving a prevailing urothelial component should be distinguished from those in which the squamous and/or glandular element is prevalent and which are given an ominous prognosis (Webb, 1985).

The immunohistochemical determination of CEA would be of relative use as, on the one hand, it would be of aid in examining small squamous and/or glandular differentiation foci, while on the other, it would allow the appropriate reclassification of urothelial carcinomas where small differentiation areas frequently go unseen

(Jautzke and Altenaehr, 1982; Fulks and Falace, 1985; López-Beltrán et al., 1980b). As regards its prognostic relevance, the significance of the expression of CEA to these mixed carcinomas would be related to the greater biological aggressiveness of urothelial carcinomas containing it (Jautzke and Altenaehr, 1982; López-Beltrán et al., 1986a) and, particularly, to mixed carcinomas showing prevailing urothelial elements.

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