Cell Surface Coat of Human and Rat Bladder Urothelium

I. Ruthenium-Red Studies in Non-Neoplastic and Neoplastic Cells

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Summary. We have studied the ultrastructure of glycocalyx at the luminal surface of normal and diseased urothelium from humans and rats with ruthenium red staining. A correlation between the thickness and staining intensity of the glycocalyx and the surface topography of the luminal surface was observed. An intensely stained thick glycocalyx was associated with prominent surface microvilli seen in the following conditions in humans: some control urothelium, inverted papilloma, well and moderately differentiated transitional cell carcinomas and mucin producing adenocarcinomas. These changes were also present in rats with FANFT-induced preneoplastic and neoplastic changes. A thin glycocalyx was associated with a scalloped luminal surface containing asymmetric unit membrane plaques and was found in some control human urothelium and in normal rat urothelium. A thin glycocalyx was also associated with the relatively smooth surface seen in poorly differentiated transitional cell carcinomas as well as in some mucin producing adenocarcinomas. We suggest that urothelial glycocalyx, as demonstrated by ruthenium red staining, correlates with the luminal surface topography rather than specific pathological condition of the bladder.

Key words: Glycocalyx – Surface topography – Urothelium – Neoplasia

Introduction

The cell surface coat (i.e., glycocalyx) of mammalian transitional cell epithelium (i.e., urothelium) contains carbohydrate moieties (Alroy et al. 1982; Candiotti et al. 1971; Ibanez et al. 1974) including glycosaminoglycans (Parsons et al. 1980). Some of the cell surface properties such as the antigenic characteristics, defensive capability, surface charge and cell surface perme-
ability are influenced by these carbohydrate moieties (Alroy et al. 1982; Parsons et al. 1980). The biological significance of the urinary bladder glyco-
ecalyx is highlighted by several observations: 1. Alterations in the urothelial glyco-
ecalyx function as morphological markers for malignant transformation (Hicks and Wakfield 1976). 2. Changes in the surface antigenic expression (i.e., ABH tissue isoantigens) of neoplastic urothelium serve to predict tumor behavior (Decenzo et al. 1975; Weinstein et al. 1981). 3. The cell surface glycans inhibit the adherence of bacteria, as well as various molecules and ions to the urothelial surface (Parsons et al. 1980). However, studies of normal and neoplastic (Alroy et al. 1982; Candidotti et al. 1971; Ibanez et al. 1974; Monis and Dorfman 1967; Parsons et al. 1980) urothelial glyco-
ecalyx have only begun to elucidate the nature and biological role of this cell element in the urinary bladder.

In the present study we examined the glyco-
ecalyx at the luminal surface of human and rat urothelial cells. We studied urothelial specimens from both patients who had non-neoplastic urologic disorders and normal rats, and compared them to: a) non-neoplastic urothelium of rats with reversible hyperplasia (Fukushima et al. 1981; Shirai et al. 1978) b) rats which were fed with the chemical carcinogen N-[4-(5-nitro-2-furyl)-2-thiazolyl] formamide (FANFT) (Cohen et al. 1976; Erturk et al. 1967) and c) rat and human neoplastic urothelium. Ruthenium red, which is a polycationic dye that binds specifically with cell surface acid polysaccharides (Luft 1971) was used to demonstrate the pattern and distribution of the acidic polysaccha-
rides of the glyco-
ecalyx. We are now reporting that the glyco-
ecalyx thickness and appearance at the luminal surface of the urinary bladder urothelium as demonstrated with ruthenium red correlates with the surface topography of the membrane.

Materials and Methods

Specimens of control and neoplastic human urinary bladder urothelium were obtained at cystectomy or by transurethral biopsies from 37 patients. They include 10 control specimens obtained from patients with various non-neoplastic urologic disorders. The patient's ages in the control group varied from 6 months to 85 years. The specimens of neoplastic urothelium include 20 specimens of transitional cell carcinoma, grades 1 to 3 and stages 0 (i.e., non-invasive) to C, 6 specimens of primary adenocarcinoma of the urinary bladder and one case of inverted papilloma. The transitional cell carcinomas were classified histologically according to the recent AFIP fascicle (Koss 1975) and staged according to the criteria of Jewett (1973). The samples were minced into 1–2 mm³ tissue blocks and were immediately fixed in cold 2% glutaraldehyde in 0.1 M cacodylate buffer pH 7.4. Specimens of control, hyperplastic and neoplastic urothe-
lium were obtained from the urinary bladders of male Fischer rats. The rat tissues were from 3 different treatment groups. Three rats had reversible regenerative urothelial hyperplasia 5 days after the application of a frozen rod to the bladder serosal surface had caused ulceration (Fukushima et al. 1981; Shirai et al. 1978). From these rats, specimens were obtained from regenerative foci and 1 cm away from such foci. Three animals were fed FANFT at a level of 0.2% of their diet by weight for 20 weeks. Specimens from these animals were obtained from bladder tumor masses and from histologically normal appearing urothelium in the blad-
ers of these rats 60 weeks after the beginning of the experiment. Control specimens were from the bladders of rats fed control diet for 60 weeks of the experiment. The animals were anesthetized with a lethal dose of 1.0 ml of nembutal i.p. (50 mg/ml) and the bladder was inflated through the urethra with cold 2% glutaraldehyde in 0.1 M cacodylate buffer. When