

# **Scanning Electron Microscopy/1981**

**Part 3**

# SCANNING ELECTRON MICROSCOPY/1981/III

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of  
SCANNING ELECTRON MICROSCOPY,  
RELATED TECHNIQUES,  
and  
APPLICATIONS  
PART III

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#### DATES OF FUTURE SEM MEETINGS:

Scanning Electron Microscopy/1982 - April 25-30, Anaheim, CA, See p. xvi.  
Scanning Electron Microscopy/1983 - April 18-22, Dearborn (Detroit), MI.

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For a description of the aims and programs of the SEM Inc. (a non-profit organization), see p.xvi.

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REVIEW: A review of the chosen subject with emphasis on author's own work, placing it in context with relevant literature and putting the topic in perspective.

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Om Johari (see page ii)

# DIFFUSE NEOPLASTIC CHANGE IN UROTHELIUM FROM TUMOUR-BEARING HUMAN LOWER URINARY TRACT

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## Abstract

In nine patients, who underwent radical cystectomy for transitional cell carcinoma of the bladder, detailed maps were prepared of results of the light microscopic findings and of the surface features of the urothelium as observed by scanning electron microscopy. Scanning electron microscopy revealed a more widespread distribution of residual neoplastic disease than suspected by light microscopy alone. This observation is based on the presence of abnormal cells carrying pleomorphic microvilli in association with other cells infrequently seen in normal healthy urothelium. Such changes have been directly related to neoplastic transformation in numerous experimental studies and have also been observed in human transitional cell carcinoma. This widespread distribution of these markers for neoplastic transformation explains the multiple recurrences of transitional cell carcinoma which characterises neoplastic disease of the bladder.

## Introduction

There have been numerous previous reports of microvillous cells in specimens from human bladder cancer patients but their diagnostic value has been questioned in the light of reports of microvillous cells in normal urothelium. In a previously reported study we have examined the entire lower urinary tract from 9 normal patients and reported that microvillous cells are regularly found in the normal mature renal papilla and urethra, and a few immature microvillous cells are also seen at the surface of the bladder urothelium. Such cells are uniform in appearance and are covered with a short regular microvilli. In the present study the light microscopic and the scanning electron microscopic surface features of the bladder, ureters and urethra have been compared in 9 patients with known transitional cell neoplasia in order to determine the frequency with which microvillous cells occur in neoplastically transformed urothelium. In particular the distribution of cells with regular microvilli, comparable to those found on the normal urethra and immature bladder, was assessed as well as the presence of cells with more bizarre, pleomorphic, microvilli. This study suggests that scanning electron microscopy can indeed be used as a diagnostic tool to indicate the presence of residual neoplastic disease.

## Materials and Methods

Nine surgically removed bladders together with the attached distal ureteric stumps and the proximal or whole urethra in males and the whole urethra in females were immediately inflated and fixed in cacodylate buffered formalin pH 7.4 for 20 minutes. The bladder was then opened anteriorly and adjacent tissue blocks were taken from selected areas for light and scanning electron microscopy. In some cases adjacent areas of the ureters and urethra were also taken (table 1).

Tissues for light microscopy were further fixed in formalin, embedded in paraffin wax, sectioned and stained with haematoxylin and eosin. All tumours were graded and staged according to the U.I.C.C. system (W.H.O. monograph on bladder tumours). All previous biopsies were reviewed and similarly graded and staged. Tissues for scanning electron microscopy were further fixed in buffered formalin,

**KEY WORDS:** Bladder Cancer, Multifocal Distribution of Neoplastic Transformation, Comparison of Surface Features with Histology, Pleomorphic Microvilli.

TABLE 1 NUMBER OF SECTIONS TAKEN FROM THE CYSTECTOMY SPECIMENS

CASE	LIGHT MICROSCOPY			SCANNING ELECTRON MICROSCOPY		
	URETER	BLADDER	URETHRA	URETER	BLADDER	URETHRA
1	2	12	3	0	13	2
2	3	8	1	0	7	2
3	2	5	1	2	7	2
4	2	6	1	2	5	2
5	4	14	2	2	7	1
6	4	9	2	2	7	2
7	2	10	4	0	9	4
8	2	7	1	0	4	3
9	2	8	1 (+4) <sup>†</sup>	2	9	1

<sup>†</sup> Urethrectomy

post-osmicated, dehydrated through graded alcohols into 100% arcton† critically point dried, mounted on stubs, gold coated in a sputtering diode coating unit and examined in a JEOL scanning electron microscope JSM-35 at 15-20kV.

### Results

#### Clinical:

Five male and four female patients aged between 40 years and 64 years (mean 59 years) were examined (table 2). Eight had received prior external irradiation to the bladder at doses ranging from 4,000R to 6,000R. Only one patient had received chemotherapy. The interval between the administration of the last dose of radiotherapy and cystectomy varied considerably; from 11 years to 6 weeks. In 5 cases the interval was less than 1 year. All 9 patients underwent a radical cystectomy and urinary diversion (table 3). Case 3 died 18 months later from carcinoma of the breast. At post mortem there was no evidence of transitional cell carcinoma. Case 6 is alive 21 months later but with a probable pelvic recurrence. Case 2 is lost to follow up. The other 6 patients are alive and well for the period shown; between 1 and 4 years.

#### Light microscopy

Table 4 shows the histology of the previous biopsies, taken within 12 months of cystectomy, related to cystectomy specimens and compares grade and stage. Diagrams indicating the location of biopsies and their morphological diagnosis were made for each patients cystectomy specimen. Representatives are shown (fig. 1a and 2a). Except for case 6 all tumours were transitional cell carcinomas. In case 6 it was of mixed type with squamous, transitional and glandular elements. At cystectomy no residual tumour was found in cases 2 and 8. In case 5 only a small focus of carcinoma-in-situ remained at the edge of an ulcer. In case 3 an ulcer was also present but it was associated with an invasive carcinoma. In cases 4, 6 and 7 large tumour masses were still present within the bladder (and urethra in case 7). In cases 1 and 9 no macroscopic tumour was seen though microscopic tumour foci were present either in the bladder wall or as in case 1 within a diverticulum in the posterior wall. In cases 6, 7, and 9 the tumour stage had increased by the time of cystectomy while in cases 1, 3, and 4 there were no change in the stage. By contrast the grade of histology did not change much. All tumours were either grade II or III. Only in one case, case 7, was the grade increased in the cystectomy specimen. Only case 6 had lymph node metastases

\*See Discussion with Reviewers

TABLE 2 CLINICAL DETAILS OF THE PATIENTS

CASE	AGE AT CYSTECTOMY	SEX	TREATMENT PRIOR TO SURGERY	
			RADIO THERAPY	INTERVAL TO CYSTECTOMY
1	62 YRS.	M	NIL	
2	64 YRS.	M	4,000R	6 WEEKS
3	40 YRS.	F	6,000R	7 WEEKS
4	63 YRS.	F	5,000R	6 WEEKS
5	64 YRS.	M	6,000R	11 YEARS
6	61 YRS.	F	4,000R	4 MONTHS
7	64 YRS.	M	R <sup>***</sup>	R <sup>***</sup>
8	58 YRS.	F	6,000R <sup>**</sup>	4 YEARS
9	55 YRS.	M	6,000R	6 WEEKS

<sup>\*\*</sup> Cyclophosphamide as well - stopped 2 years prior to cystectomy.<sup>\*\*\*</sup> Amount and interval not recorded.

TABLE 3 TYPE OF CYSTECTOMY PERFORMED AND THE SUBSEQUENT PROGRESS OF THE PATIENTS

CASE	TYPE OF CYSTECTOMY AND ANASTOMOSIS	PROGRESS
1	RC-U AND IC	ALIVE AND WELL 4 YEARS LATER
2	RC AND IC	LOST TO FOLLOW UP
3	RC-U AND US	DIED OF BREAST CARCINOMA 18 MONTHS LATER
4	RC-U AND IC	ALIVE AND WELL 18 MONTHS LATER
5	RC AND IC	ALIVE AND WELL 2 YEARS LATER
6	RC-U AND IC	ALIVE 21 MONTHS LATER WITH PROBABLE PELVIC RECURRENCE
7	RC-U AND IC	ALIVE AND WELL 15 MONTHS LATER
8	RC-U AND IC	ALIVE AND WELL 18 MONTHS LATER
9	RC <sup>**</sup> AND IC	ALIVE AND WELL 1 YEAR LATER

PC AND IC - RADICAL CYSTECTOMY AND ILEAL CONDUIT

RC-U AND IC - RADICAL CYSTO-URETHRECTOMY AND ILEAL CONDUIT

RC-U AND US - RADICAL CYSTO-URETHRECTOMY AND URETERO-SIGMOIDOSTOMY

<sup>\*\*</sup> LATER URETHRECTOMY FOR RESIDUAL TUMOUR

TABLE 4 PATHOLOGICAL GRADING AND STAGING OF SPECIMENS AND THE INTERVAL BETWEEN THE INITIAL DIAGNOSIS OF CARCINOMA AND THE CYSTECTOMY

CASE	BIOPSIES		INTERVAL BETWEEN INITIAL DIAGNOSIS AND CYSTECTOMY	CYSTECTOMY	
	GRADE	STAGE		GRADE	STAGE
1	II	P I	5 MONTHS	II	P I
2	III	P 2	3 MONTHS	NO TUMOUR	PRESENT
3	II	P I	8 MONTHS	II	P I
4	II	P I	8 YEARS	II	P 2
	II	P 2			
5		P I	12 YEARS	--	P I S
6	II	P 2 <sup>†</sup>	7 MONTHS	III	P 4 <sup>†</sup>
	III	P 2 <sup>†</sup>			
7	II	P I	1 YEAR	III	P 2
8	III	P I	10 YEARS		DYSPLASIA
9	III	P I	2 YEARS	III	P 3

<sup>†</sup> Mixed carcinoma