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AUTOMATED FLOW CYTOMETRIC IDENTIFICATION OF HUMAN BLADDER CANCER CELLS BY FUNCTIONAL CELL PARAMETERS AND BLOOD-GROUP ANTIBODIES

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INTRODUCTION

Despite improvements in the recognition of DNA aneuploidies by flow cytometry, the present identification rates in bladder cancer are not sufficiently high to serve as a basis for clinical decisions. The aim of this study was to improve sensitivity and specificity of bladder tumor cell detection by a simultaneous measurement of functional cell parameters or antigenic determinants of the cellular surface together with DNA and cell volume.

MATERIAL AND METHODS

Material and preparation of samples

162 samples from patients with histologically proven transitional cell carcinoma of the bladder and 156 control samples from persons without clinical evidence of bladder tumor were investigated. The samples were obtained by bladder biopsies from the tumor site or normal mucosa (n = 69), by bladder washings (n = 131) or by spontaneously voided urine (n = 118).

DNA, intracellular pH and esterase activity. Unfixed cell samples were stained with 20 µg/ml ADB (1,4-diacetoxy-2,3-dicyano-benzene) and 40 µg/ml PI (propidium iodide) for simultaneous determination of intracellular esterase activity and cytoplasmic pH in living cells as well as of DNA in dead cells.

DNA and cell surface antigens. 70 % methanol-fixed cell samples were incubated in an indirect immunofluorescence assay first with monoclonal CEA or with Lewis blood-group antibodies and afterwards with a fluorescein-isothiocyanate (FITC) -labelled IgG-fraction of a rabbit mouse-specific antiserum as well as with 40 µg/ml PI for DNA determination.

Flow cytometry and data evaluation

Simultaneous measurement of electrical cell volume and two different fluorescence signals of the stained cells were performed with a three-parameter flow cytometer (Fluvo-Metricell/ HEKA-Elektronik / Lambrecht, FRG).

Data analysis and display were accomplished by the DIAGNOS1 program system (HEKA), which analyzes list mode data, calculates graphical display, calculates cell parameters in limited histogram areas, introduces the calculated values into a self learning database and permits automated diagnosis of abnormal cell samples. (Fig.1)

RESULTS

36 % of the tumor samples were identified by one or more aneuploid peaks in the DNA-distributions.

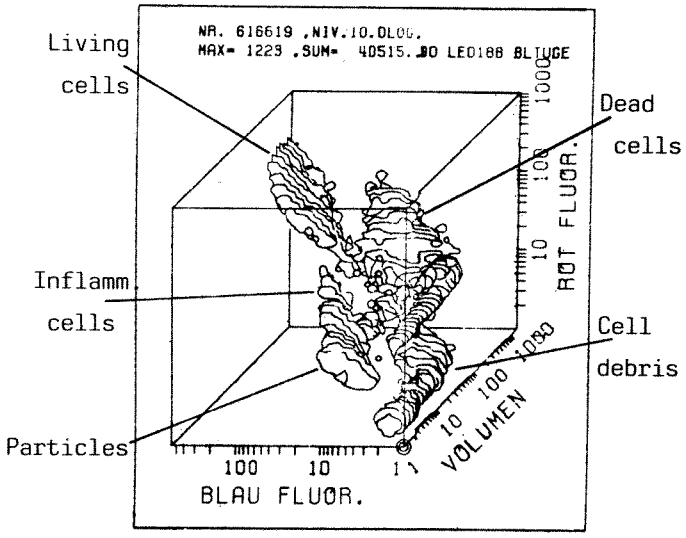

Fig. 1. Display of a simultaneous three-parameter measurement Cell volume, intracellular esterase activity (blue fluorescence) and DNA distribution (red fluorescence) of a bladder tumor sample (pT_AG_2)

The comparison of median values for the intracellular pH (Tumor: 7,22 +/-0,24, Control: 7,30 +/-0,30) showed no significant difference between tumor samples and controls. On the other hand esterase activity was clearly increased in tumor cells (Tumor: 0,24 +/-0,22, Control: 0,16 +/-0,10 arb.units; $p < 0,01$) and a good correlation ($p < 0,01$) with tumor stage and grade (according to UICC/WHO) existed. Bladder tumor samples were correctly identified in 82 % of the cases with a specificity of 88 % for normal samples, when DNA and esterase activity were jointly evaluated.

The antibody surface density for CEA and Lewis blood-group antigens was significantly lower in tumor samples than in controls (Values for the Le^a, Le^b antibody: Tumor: 0,007 +/-0,002, Control: 0,011 +/-0,002 arb.units; $p < 0,001$). Up to 91 % of the tumor samples were recognized by evaluation of DNA and monoclonal antibodies at a specificity of 86 %.

CONCLUSION

Compared to sole DNA examination the combined measurement of DNA and cell surface antigens increases the identification rate of bladder tumor cells to a maximum of 91 % with a specificity of more than 85 %. The combined determination of DNA, functional cell parameters and cell surface antigens on the same cell samples is likely to further increase tumor identification rates. Flow cytometry could then be very valuable as a screening examination for malignant cells in urine samples.

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