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Discriminant analysis of exfoliated cells in bladder urothelium cancer

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Abstract: Objective To explore the method of computer discriminant on exfoliated cells in bladder urothelium cancer by Pap stain. Methods The exfoliated cells in 107 urine smears included 386 uroepithelium normal exfoliated cells (UNC) , 439 urothelium dysplastic exfoliated cells (UDC) and 500 bladder urothelial cancer exfoliated cells (UCC). The cells were randomly divided into training group (n = 1077) and identifying group (n = 248) , and the chromatic and geometric shape parameters of cytoplasm and nuclear were tested. The stepwise discriminant analysis was used in cells of the training group to establish a discriminant function and analyze the rate of back substitution discriminant. The function was evaluated by cells of identifying group, and the coincidence rate was analyzed in 107 specimens. **Results** The back discriminant coincidence rate of cells in training group was 80.8%. The coincidence rate of identifying group and 107 specimens were 80.2% and 92.5% respectively. The discriminant effect was significantly better than function based on chromatics and geometric shape parameters individually ($P < 0.05$). **Conclusions** The function combined with chromatics and geometric shape parameters has good discriminant performance in bladder urothelium cancer.

Keywords: Chromatics; Geometric shape; Urothelium cancer of bladder; Exfoliated cell; Computer discriminant
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0 Introduction

In previous studies, our research group tested and analyzed the colorimetric [1] and geometric [2] parameters of exfoliated cells of bladder urothelial carcinoma. It was found that there were significant differences in the colorimetric and geometric parameters of cytoplasm and nucleus among exfoliated cells of bladder urothelial carcinoma, indicating that these parameters are valuable for distinguishing exfoliated cells; Based on these parameters, the functions for distinguishing the exfoliated cells of bladder urothelial carcinoma were established respectively, and the discriminant effects of these functions were evaluated. The results showed that colorimetry [3] or geometric parameters [4] alone could not well reflect all the characteristics of exfoliated cells of bladder urothelial carcinoma, and could not achieve satisfactory recognition results. Therefore, based on the quantitative study of colorimetry and geometric morphology, this study comprehensively applied colorimetry and geometric morphology parameters to distinguish the exfoliated cells of bladder urothelial carcinoma, so as to further improve the discrimination effect of exfoliated cells of

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bladder urothelial carcinoma.

1 Materials and methods

1.1 Materials and grouping

The urine exfoliated cell smears of 107 patients in the pathology department of Nanfang Hospital from 2006 to 2007 were collected. There were 82 males and 25 females; the average age was (59.3 ± 15.4) years; 36 patients with normal urothelium, 44 patients with dysplastic urothelium and 27 patients with bladder urothelium carcinoma (confirmed by pathological biopsy). A total of 1325 cells were collected, including 386 uroepithelium normal exfoliated cells (UNC), 439 urothelium dystrophic exfoliated cells (UDC) and 500 bladder urothelial cancer exfoliated cells (UCC). The diagnostic criteria of the cells were cited from the literature [5], and the cells between UCC and UNC were classified into UDC. There was no significant difference in age and gender among the patients in each group, which was comparable ($P > 0.05$).

1.2 Experimental method

1.2.1 Experimental instruments and software

Centrifuge (cf120, Sakura, Japan); Automatic staining instrument (varistain XY, Shandon, England); Optical microscope (bx51tf, Olympus, Japan); Micro digital camera (tk-c1481bec, JVC, Japan), image pro plus image analysis software (media cybernetics, USA).

1.2.2 Smear preparation and image acquisition

Take the first midcourse urine from the morning, centrifuge, take the sediment for smear, put it in 95% alcohol for fixation, sample the automatic staining instrument for pasteurization, pasteurization reference [6], and seal the neutral gum after dehydration. At high power mirror (400 ×) UNC, UDC and UCC images are collected under the. The imaging parameters of all cells are the same. The collected images are saved in the computer for testing. Image-proplus6.0 software was used to test the colorimetric and geometric parameters of cells. The selected colorimetric parameters are red primary color (R_p, R_n), green primary color (G_p, G_n), blue primary color (B_p, B_n), red primary color coefficient (r_p, r_n), green primary color coefficient (g_p, g_n) and blue primary color coefficient (b_p, b_n) of cytoplasm and nucleus; the geometric parameters included the area (A_c, A_n) of cell and nucleus, nucleoplasm ratio (R_{np}), long axis $(d_{\text{maj},c}, d_{\text{maj}})$ and short axis $(d_{\text{min,c}}, d_{\text{min,n}})$ of cell and nucleus, average diameter $(\bar{D}_c, \bar{D}_n)$, and perimeter (C_c, C_n); Shape factor PE (PE_c, PE_n), shape factor AR (AR_c, AR_n), normalized shape factor (RFF_c, RFF_n), roundness (Sp_c, Sp_n), Ovality (EE_c, EE_n) and shape irregularity index (FP_c, FI_n). [7]

1.3 Statistical analysis

SPSS 13.0 was used for statistical analysis of data. 80% (1077) cells were randomly selected in SPSS and classified into the training group, including 405 uccs, 356 UDCS and 316 UNC. The remaining 20% (248) cells were cells in the recognition group, including 95 uccs, 83 UDCS and 70 UNC. The colorimetric and geometric parameters of the cells in the training group were analyzed by stepwise discriminant analysis, and the discriminant function was established to calculate the coincidence rate of the back passage; the cells in the recognition group were used to evaluate the discrimination effect of the function; at the same time, R × C table data χ^2. Test and compare the

discrimination effect established based on different parameters. The above two-sided test is adopted, and the test level is α was 0.05, with P < 0.05 as the difference was statistically significant.

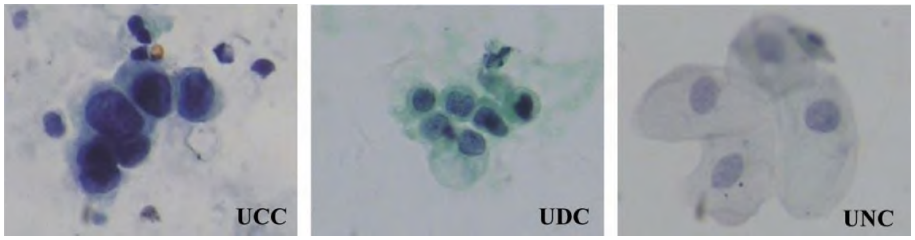

Figure 1 Micrograph of cells in each group by pasteurization (pasteurization, 400 ×)

2 Results

2.1 Cell pasteurization results

Figure 1 shows that the cell body of UNC is large, thin and oval; the nucleus is small, round or oval, in the middle, and lightly stained; More cytoplasm. UCC cells distributed in clusters with large cell bodies and were polymorphic; the nucleus is large, eccentric, and the chromatin is highly dense, deeply stained, and coarse granular; Few cytoplasm and unclear margin. UDC cells are scattered, with small cell bodies, irregular nuclei and more cytoplasm.

2.2 Function establishment

Colorimetric and geometric morphological parameters of cells in the training group were analyzed using stepwise discrimination, and the functions were established as follows:

$$\begin{cases} Y_1 = -1027.830 - 1.366R_p + 3.172R_n - 1.727B_n \\ \quad -120.212r_p + 1905.094r_n + 3149.830b_n \\ \quad -0.052A_p - 1.458A_n - 14.421R_{np} \\ \quad +20.899d_{min,n} + 15.320\overline{D}_c - 9.415\overline{D}_n \\ \quad -3.815C_c + 3.893C_n + 294.886FII_c \\ Y_2 = -1031.812 - 1.401R_p + 3.202R_n - 1.792B_n \\ \quad -97.523r_p + 1874.222r_n + 3175.587b_n \\ \quad -0.013A_p - 1.512A_n - 14.199R_{np} \\ \quad +21.550d_{min,n} + 14.022\overline{D}_c - 7.948\overline{D}_n \\ \quad -4.069C_c + 4.198C_n + 305.731FII_c \end{cases}$$

$$\begin{cases} Y_3 = -1067.262 - 1.335R_p + 3.355R_n - 2.013B_n \\ \quad -246.117r_p + 1952.221r_n + 3287.047b_n \\ \quad -0.015A_p - 1.565A_n - 11.780R_{np} \\ \quad +21.820d_{min,n} + 14.363\overline{D}_c - 7.238\overline{D}_n \\ \quad -4.134C_c + 4.266C_n + 312.083FII_c \end{cases}$$

Where Y_1, Y_2, Y_3 are the discriminant functions of UNC, UDC and UCC respectively.

2.3 Discrimination effect of function

It can be seen from table 1~3 that the discrimination rate of the function is 80.8%, that of the recognition group is 80.2%, and that of 107 smears is 92.5%. The results of statistical analysis show that the discriminant rate of the function (function 3) established by combining

colorimetry and geometric morphology parameters is significantly better than that established by applying Colorimetry (function 1) [3] and geometric morphology (function 2) [4] parameters alone ($P < 0.05$), as shown in Table 4.

Table 1 Back substitution discrimination results

Original results	Forecast results				Compliance rate (%)
	n	UCC	UDC	UNC	
UCC	405	330	75	0	81.5
UDC	356	65	283	8	79.5
UNC	316	1	58	257	81.3
Total	1077	396	416	265	80.8

Table 2 Discrimination results of cells in recognition group (n = 248)

Original results	Forecast results				Compliance rate (%)
	n	UCC	UDC	UNC	
UCC	95	77	18	0	81.1
UDC	83	19	64	0	77.1
UNC	70	3	9	58	82.9
Total	248	99	91	58	80.2

3 Discussion

Bladder urothelial carcinoma is a common tumor in the urinary system, and it is also a common malignant tumor of the urinary system [8]. Cystoscopy and urine exfoliative cytology are currently the most commonly used methods for the clinical diagnosis of bladder urothelial carcinoma [9]. At the same time, with the development of molecular biology technology, the research on the markers of bladder urothelial carcinoma has received great attention. For example, multi probe fluorescence in situ hybridization (FISH) technology [10] and the detection of bladder tumor antigen (BTA) [11] have become research hotspots, but most of them are in the research stage in China, It is not widely used in clinic.

Table 3 Discrimination results of urine smear (n = 107)

Original results	Forecast results				Compliance rate (%)
	n	Urothelial carcinoma of bladder	Dysplastic urothelium	Normal urothelium	
Urothelial carcinoma of bladder	27	26	1	0	96.3
Dysplastic urothelium	44	2	39	3	88.6
Normal urothelium	36	0	2	34	94.4
Total	107	28	42	37	92.5

Table 4 Comparison of coincidence rates of three functions [n (%)]

Function	Anaphora discrimination	Cellular discrimination	Smear discrimination
Colorimetric function (1) [3]	767(71.2)	181(73.0)	90(84.1)
Morphological structure function (2) [4]	774(71.9)	174(70.2)	87(81.3)
Colorimetry and geometric morphology function (3)	870(80.8)	199(80.2)	99(92.5)
χ^2	32.480	7.054	6.048
P	0.000	0.029	0.049

Urine exfoliative cytology has high specificity and is a non-invasive examination, but its diagnosis is highly dependent on the experience of pathologists and has strong subjectivity. With the development of computer image analysis technology, these subjective experiences can be transformed into objective parameter indicators. With the improvement of morphological quantitative diagnosis methods and standards, clinical cytology diagnosis will gradually develop from qualitative to qualitative and quantitative. Changes in cell morphology include changes in color characteristics and geometric morphology [12]. In this research group, the colorimetric and geometric parameters of exfoliated cells of bladder

urothelial carcinoma were tested, and the discriminant functions for UNC, UDC and UCC were established respectively, and the discriminant effects of the functions were evaluated. The results showed that the characteristics of exfoliated cells of bladder urothelial carcinoma could not be well reflected by colorimetric or geometric parameters alone, and there was room for improvement in the discriminant effects. Luquan et al. [13] extracted the chromaticity features and geometric morphological features of the lung cancer cell image, and used the chromaticity information and morphological information in the cell image to recognize and classify the lung cancer cells. The results show that the method combining morphology and chromaticity recognition can achieve good results. At the same time, yexiaoling et al. [14] proposed a method based on the combination of colorimetry and morphology to analyze and recognize medical cell images, and extract their feature parameters. Combined with their advantages, they can accurately identify various cell components in a relatively complex background, classify them, and are expected to achieve good recognition results.

In this study, the colorimetric and geometric parameters of urothelial exfoliated cells were systematically tested. On this basis, the discriminant effects of these parameters on UNC, UDC and UCC were discussed, and the discriminant function was established to evaluate the discriminant effect of the function. The results show that the parameter colorimetric parameters RP, RP, RN, BN, RN, BN and the geometric parameters AP, an, RNP, Dmin, N, DC, DN, CC, CN and fiic are selected into the discriminant functions of UNC, UDC and UCC, indicating that the above parameters have a certain role in identifying UNC, UDC and UCC. The coincidence rate of the function was 80.2%, among which UNC was better (82.9%), UCC was the second (81.1%), and UDC was not good (77.1%). At the same time, the results of smear discrimination showed that the discrimination effect of smear (92.5%) was significantly better than that of cell (80.2%). The diagnosis of specimen came from the comprehensive judgment of all cells on smear, and the wrong judgment of individual cells did not hinder the diagnosis of specimen; the colorimetric parameter value of UDC is between UCC and UNC, which is prone to misjudgment, resulting in poor discrimination effect. At the same time, the results also showed that the discriminant effect of the function established by combining colorimetry and geometric parameters was better than the function established by using colorimetry (73.0%) and geometric parameters (70.2%) alone, and had a high coincidence rate, indicating that the combination of colorimetry and geometric parameters can better reflect all the characteristics of bladder urothelial carcinoma, and can be used to assist clinical diagnosis and differential diagnosis.

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