



Correlation analysis of nuclear morphology, cytokeratin and Ki-67 expression of urothelial carcinoma cells

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Correlation analysis of nuclear morphology, cytokeratin and Ki-67 expression of urothelial carcinoma cells

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Running title: Nuclear morphology, CKs and Ki-67 in UCs

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Abstract

We aimed to delineate the morphogenesis of aberrant nuclear features of urothelial carcinoma (UC) cells in association with cytokeratin (CK) expression patterns and cell proliferation activity. Correlation analysis of the nuclear area by morphometry and the expression patterns of CK5, CK20 and Ki-67 by triple immunofluorescence analysis was applied to 1699 cells from five low-grade and seven high-grade cases of UC. The majority of UC cells showed aberrant cellular differentiation represented by abnormal CK expression patterns of CK5(+)/CK20(+) (40.5%) or CK5(-)/CK20(+) (56.0%). CK5(+)/CK20(-) cells, a phenotype of cancer stem/progenitor cells, represented a very small population (1.9%) and showed a low proliferation activity. Ki-67(+) cells showed a significantly different CK expression pattern compared with that of Ki-67(-) cells. The nuclear areas of CK5(-)/CK20(+) cells ($71.3 \pm 25.9 \mu\text{m}^2$) were significantly larger than those of CK5(+)/CK20(+) cells ($66.6 \pm 25.5 \mu\text{m}^2$). Negativity for CK5 was related to the grade of UC, and an increased number of CK5(-)/CK20(+)/Ki-67(+) cells was related to a higher malignant potential. We conclude the nuclear morphology is related to cell differentiation represented by CK expression and cell proliferative activity.

Key words: CK5, CK20, Ki-67, Nuclear morphology, Urothelial carcinoma

Text

INTRODUCTION

Normal urothelial cells usually contain small oval or round nuclei with a smooth nuclear contour, whereas urothelial carcinoma (UC) cells have an aberrant nuclear morphology (atypia) represented by large nuclei, irregular nuclear contours, and hyperchromasia. In addition, each case of UC shows variation of the nuclear morphology in which atypia becomes more prominent in high-grade cases than that in low-grade cases. The general role of distinct nuclear morphogenesis has not been elucidated in UC or malignancies of other organs. The various abnormalities responsible for nuclear atypia are thought to include genetic, chromosomal, cytoskeletal and cell cycle abnormalities. For example, nuclear atypia such as a nuclear groove and intracytoplasmic inclusion in thyroid carcinoma cells is affected by tubulin and the centrosome.¹ Capo-chichi et al. found that the loss of structural proteins of the nuclear envelope underlies aberrations in nuclear morphology as well as aneuploidy.² We have previously studied chromosomal/genetic aberrations and nuclear morphology of UC cells, and clarified a significant correlation between chromosomal/genetic changes and nuclear morphology based on individual cells.³ Thus, the nuclear features of cancer cells appear to be formed by cooperation between nuclear membrane proteins, the cytoskeleton, and chromosomes.^{1,4,8}

Abnormalities of cytoskeletal proteins are also considered as one of the factors involved in cellular atypia.^{6,9} UCs frequently show aberrant expression of some kinds of cytokeratins (CKs) including CK20 and high weight molecular (HWM)-CKs such as CK5, CK6, and CK14.⁹⁻¹³ Regardless of many past reports of aberrant expression of CKs in UC cells, few reports have studied the nuclear morphogenesis of UC cells related to cytoskeletons. Moreover, HWM-CKs and CK20 in UC cells have recently gained attention as markers of urothelial cancer stem/progenitor cells.¹⁴⁻¹⁶ Some evidence has shown that urothelial cancer stem/progenitor cells resemble basal urothelial stem cells with a low proliferation rate and long lifespan.¹⁷ Studies of normal urothelium have indicated that HWM-CKs are markers of basal urothelial cells, and CK20 is a marker of umbrella cells.^{13,17} Urothelial cancer stem/progenitor cells might include the cell population showing a HWM-CK(+)/CK20(-) phenotype and low cellular proliferation. However, the relationship is unclear between cellular morphology and cancer stem/progenitor cells or differentiated urothelial cancer cells.

In this study, we focus on the morphogenesis of aberrant nuclear features of UC cells and investigate the association with CK expression and cell proliferation activity.

MATERIALS AND METHODS

Case selection

We analyzed transurethral resected or surgically resected samples from 12 patients (10 males and two females) at the Saitama Medical University International Medical Center (Saitama, Japan). The cases consisted of 12 papillary UCs including seven non-invasive and five invasive cases. Among them, five cases were low grade and seven cases were high grade. The age of patients ranged from 46 to 79 years (average, 67.5 years old). Histological diagnosis according to the 2004 World Health Organization classification and TNM classification of Malignant Tumours seventh edition was performed based on the histological findings of hematoxylin/eosin (HE) staining of tumor sections.^{18,19} The case summaries are shown in Table 1. This study was approved by the IRB committee of the International Medical Center at Saitama Medical University (No. 08-070).

Triple immunofluorescence analysis

Triple immunofluorescence analysis was performed using the Zenon technique. Zenon Mouse IgG labeling Kits (Molecular Probes, Eugene, OR, USA) use a fluorophore-labeled Fab fragment directed against the Fc portion of an intact IgG primary antibody to form a labeling complex. Immunostaining was performed with primary antibodies against CK5 (mouse monoclonal IgG₁, clone XM26; Novocastra Laboratories Ltd, Newcastle, UK), CK20 (mouse monoclonal IgG_{2a}, clone Ks.20.8; DAKO, Glostrup, Denmark) and Ki-67 (mouse monoclonal IgG₁, clone MIB1; DAKO). These primary antibodies were labeled with Alexa Fluor 555 (Z-25005; Molecular Probes, Eugene, OR, USA), Alexa Fluor 488 (Z-25202; Molecular Probes), and Alexa Fluor 647 (Z-25108; Molecular Probes), respectively. Labeling and immunostaining was performed according to the manufacturer's instructions. Briefly, 1 µg monoclonal primary antibody was labeled with 5 µl Alexa Fluor for 1 hr, and then excessive fluorochrome was blocked with IgG blocking agent for 10 min. Sections (6 µm) were prepared from formalin-fixed paraffin-embedded blocks, and then stained with the labeled antibodies at an optimal dilution in a humidified chamber for 24 hr at 4°C. A second fixation of the tissue sections was performed in 4% formaldehyde/PBS for 15 min at room temperature. After nuclear counterstaining with 4, 6-diamino-2-phenylindole dihydrochloride (DAPI), stained tissue sections were observed and imaged under a Zeiss microscope with a CCD camera (Carl Zeiss Japan, Tokyo, Japan).

Correlation analysis of the nuclear area and expression of CKs and Ki-67

We analyzed a total of 1699 cells (an average of 142 cells per case) using an Isis Imaging system v5.1 (MetaSystems

GmbH, Altlußheim, Germany). As a morphological parameter, we measured the nuclear area. We randomly selected tumor cells in thickened epithelial structure at the surface of the tumors. The same cells were evaluated for the expression of CK5, CK20 and Ki-67. Because the values of the nuclear area were altered by histological sectioning, we analyzed the nuclear area as a relative value. Moreover, in the comparison between the nuclear area of histological sections and isolated whole cells, which were isolated from the same samples and measured in our previous report, the relative nuclear area was representative for each case by measurement of an appropriate number of cells (data not shown).³ Next, we performed a correlation analysis among the nuclear area, CK expression, and cell proliferation activity of UC cells from all cases based on the tumor grade.

Statistical analysis

The Steel-Dwass test was used to determine the significance of the association between nuclear area and four patterns of CK expression, and between CK expression patterns in Ki-67(+) and Ki-67(-) cells. We applied the Welch's t test to compare the differences of nuclear area between Ki-67(+) and Ki-67(-) cells. The chi-square test was used for comparison between low- and high-grade UC cells. A value of $P < 0.05$ was considered significant in all statistical analyses.

RESULTS

Correlation analysis of nuclear area and CK expression patterns

Figure 1 shows representative HE and triple immunofluorescence staining of low- and high-grade UCs. CK expression patterns were classified into four groups according to the combination of CK5 and CK20 expression, namely CK5(+)/CK20(+), CK5(+)/CK20(-), CK5(-)/CK20(+), and CK5(-)/CK20(-). Among a total of 1699 UC cells, the frequencies of CK5(+)/CK20(+), CK5(+)/CK20(-), CK5(-)/CK20(+), and CK5(-)/CK20(-) cells were 40.5%, 1.9%, 56.0%, and 3.9%, respectively (Table 2, Figure 2a). The majority of tumor cells (96.5%) showed positivity for CK20. The mean, standard deviation (SD) and median of the nuclear area of CK5(+)/CK20(+), CK5(+)/CK20(-), CK5(-)/CK20(+), and CK5(-)/CK20(-) cells were 66.6 ± 25.5 (62.5) μm^2 , 66.1 ± 21.2 (66.8) μm^2 , 71.3 ± 25.9 (66.4) μm^2 , and 68.7 ± 29.1 (62.8) μm^2 , respectively (Table 2). The nuclear areas of CK5(-)/CK20(+) cells were significantly larger than those of CK5(+)/CK20(+) cells ($P < 0.01$, Steel-Dwass test) (Table 2, Figure 3a).

Correlation analysis of nuclear area, CK expression patterns and Ki-67 positivity

Among 1699 UC cells, 239 cells were Ki-67(+) (14.1%) (Table 2). Figure 2 shows Ki-67 positivity and CK expression patterns. Among the 239 Ki-67(+) UC cells, the frequencies of CK5(+)/CK20(+), CK5(+)/CK20(-), CK5(-)/CK20(+), and CK5(-)/CK20(-) cells were 32.2%, 1.3%, 63.6%, and 2.9%, respectively (Figure 2b). On the other hand, the 1460 Ki-67(-) UC cells showed these expression patterns at 41.8%, 2.1%, 54.7%, and 1.4%, respectively (Figure 2c). There were significantly different CK expression patterns between Ki-67(+) and Ki-67(-) cells ($P < 0.01$, Steel-Dwass test). The Ki-67(+) cell population had significantly more frequent CK5(-)/CK20(+) cells and less CK5(+)/CK20(+) cells than that in the Ki-67(-) cell population ($P < 0.01$, Steel-Dwass test). The nuclear area of Ki-67(+) cells (mean = 86.0 μm^2) was significantly larger than that of Ki-67(-) cells (mean = 66.6 μm^2) ($P < 0.01$, Welch's t test). The mean, SD, and median of the nuclear area of CK5(+)/CK20(+), CK5(+)/CK20(-), CK5(-)/CK20(+), and CK5(-)/CK20(-) cells were 89.5 ± 42.3 (78.4) μm^2 , 53.1 ± 6.5 (53.1) μm^2 , 86.0 ± 32.0 (78.8) μm^2 , and 60.9 ± 35.5 (49.0) μm^2 , respectively, in the Ki-67(+) cell population, whereas, 63.7 ± 21.0 (61.5) μm^2 , 67.4 ± 21.8 (68.6) μm^2 , 68.7 ± 23.7 (64.8) μm^2 , and 71.4 ± 27.4 (65.4) μm^2 were found in the Ki-67(-) cell population, respectively (Table 2). There was no significant difference of the nuclear area between CK5(+)/CK20(+) cells and CK5(-)/CK20(+) cells among Ki-67(+) cells, whereas a significant difference of the nuclear area was found between CK5(+)/CK20(+) and CK5(-)/CK20(+) cells among Ki-67(-) cells ($P < 0.01$, Steel-Dwass test) (Figure 3b,c).

Correlation analysis of nuclear area, CK expression patterns, and the grade of UC

Figure 4 shows the difference of CK expression patterns between low- and high-grade UCs. A total of 748 and 951 cells from low- and high-grade cases were analyzed, respectively. The frequencies of CK5(+)/CK20(+), CK5(+)/CK20(-), CK5(-)/CK20(+), and CK5(-)/CK20(-) cells were 60%, 3%, 36%, and 1% in low-grade cases and 25%, 1%, 72%, and 2% in high-grade cases, respectively. In addition, CK expression patterns were significantly different between low- and high-grade UC cells ($P < 0.01$, chi-square test).



DISCUSSION

UC shows various aberrant nuclear morphologies, cellular differentiation, and proliferation activities, which are the most important pathological characteristics. These pathological characteristics are strongly related to the behavior and prognosis of UCs. The aberrant nuclear morphology is generally represented by nuclear size, shape, and chromatin findings. Cellular differentiation is indicated by the histological structure and intracytoplasmic materials including the cytoskeleton. Cell proliferation activity is estimated by mitotic figures or the positive cell frequency of cell cycle related proteins such as Ki-67. CKs are intermediate filaments in the dynamic network structure of the cytoskeleton, and play an important role to maintain cellular organization and functions including differentiation, nuclear morphogenesis, and proliferation.^{5,6} Therefore, analysis of the CKs related to nuclear morphology and cellular differentiation can provide useful information for both the pathological and clinical characteristics of UCs.

In normal urothelium, CKs are useful markers for cellular differentiation. HWM-CKs such as CK5 and CK14 are restricted to the basal cell layer, whereas intermediate cells are only positive for CK18 and CK20 that are expressed by “umbrella” cells. On the other hand, our study showed that the majority of UC cells (96.5%) were positive for CK20 and more than 40% of UC cells were positive for CK5. Aberrant expression of CK5 and CK20 in UC cells is well known and applied as immunohistochemical markers for diagnosis of UCs.^{9,10,13} Analysis of the CK5/CK20 expression pattern among UCs by double immunofluorescence staining, which has been studied in few previous reports, can provide more interesting findings in terms of cellular differentiation of UCs.²⁰ In our study, CK5/CK20 expression pattern analysis revealed that the majority of UC cells were CK5(+)/CK20(+) (40.5%) or CK5(-)/CK20(+) (56.0%). Based on CK expression, CK5(+)/CK20(+) cells are thought to be basal CK5(+) cells with aberrant differentiation and expression of CK20. CK5(-)/CK20(+) cells, which were differentiating into umbrella cells, showed abnormal positioning and were found in the entire epithelial layer. UC cells with strict differentiation to intermediate cells, which are the main population in normal urothelium, are positive for CK18 and negative for both CK5 and CK20. In our study, strict differentiation to intermediate cells with negativity for CK5/CK20 was rarely detected. Furthermore, all UC cells with intermediate cell differentiation, as shown by positivity for CK18, were also positive for CK20 (data not shown). Thus, the majority of UC cells showed aberrant cellular differentiation, although some urothelial differentiation has long been recognized. Another interesting aspect of CK expression is that CK5(+)/CK20(-) cells have been recognized as tumorigenic basal cells or urothelial cancer stem/progenitor cells.¹⁵ The cancer stem cell hypothesis is now accepted and important in many kinds of carcinomas.²¹ In studies of UCs, some reports have revealed the characteristics of UC stem cells, but definite biological markers of cancer stem/progenitor cells in UCs have not been found.^{15,17,22} In our study,

CK5(+)/CK20(-) cells represented a very small population that showed a low proliferation activity, as reported previously.¹⁵ Interestingly, our study showed that CK5(+)/CK20(-) cells contained a small nucleus. From a cytopathological viewpoint, we consider that it is important to focus on the relationship between nuclear morphology and urothelial cancer stem/progenitor cells.

The cell proliferation activity of UCs is one of the most important cellular characteristics, which has been studied by many previous reports for the diagnosis of benign urothelial lesions or UC, and the prognosis of UC.²³⁻²⁵ However, there are no previous studies that focus on the differentiation of cells in the proliferative phase of the cell cycle. In our study, we detected more than 10% Ki-67(+) UC cells that showed a significantly different CK expression pattern compared with that of Ki-67(-) UC cells. Specifically, the Ki-67(+) cell population had a significantly higher frequency of CK5(-)/CK20(+) cells and a lower frequency of CK5(+)/CK20(+) cells than those in the Ki-67(-) cell population. Based on the fact that Ki-67(+) cells are usually found among the CK5(+)/CK20(-) basal cells of normal urothelium, proliferative UC cells also show aberrant cellular differentiation. We consider that CK5(-)/CK20(+) cells may have a more abnormal cellular characteristic than that of CK5(+)/CK20(+) cells in terms of cell differentiation and proliferative activity.

UC cells show diverse abnormalities of nuclear morphology, particularly the nuclear size. A variety of factors are involved in the morphogenesis of UCs, including the cell cycle, chromosomal and genetic abnormalities, cytoskeleton, and nuclear membrane proteins. In our previous report, we studied the characteristic nuclear features of UCs by morphometry and fluorescence in situ hybridization on a cell-by-cell basis, and concluded that the number of chromosomes in each nucleus is related to the nuclear features such as nuclear swelling, irregular nuclear contour and hyperchromatism of UCs.³ We also believe that the aberrant expression of CKs³ may be related to cell morphology, because CKs have direct or indirect associations with nuclear membrane proteins and chromosomes, and may also be related to nuclear morphogenesis.^{4,7} Our study indicated that CK5(-)/CK20(+) represented the most frequent CK expression pattern, and was significantly more frequent than CK5(+)/CK20(+). Among the majority of UC cells, CK20(+) cells contained large nuclei and CK5(+) cells contained small nuclei. CK5(-)/CK20(-) cells had variously sized nuclei ranging from small to large. Moreover, the nuclear area of Ki-67(+) cells was significantly larger than that of Ki-67(-) cells. These results are compatible with our previous report showing that the nuclear size increases during G₂/M phase of the cell cycle.²⁶ Interestingly, there was no significant difference of nuclear area between CK5(+)/CK20(+) and CK5(-)/CK20(+) cells among Ki-67(+) cells, whereas the nuclear areas of CK5(-)/CK20(+) cells were significantly larger than those of CK5(+)/CK20(+) cells among Ki-67(-) cells. These results indicated that cell

proliferation activity has a greater influence on nuclear size than that of cell differentiation. Considering our past and current studies, the nuclear size appears to be related to the number of chromosomes and CK expression pattern.

Finally, CK expression patterns were significantly different between low- and high-grade UC cells. Positivity for CK20 was similar between low- and high-grade UC cells, but positivity for CK5 among low-grade UC cells was higher than that among high-grade UC cells. Specifically, CK5(+)/CK20(+) cells were more frequent in low-grade cases than that in high-grade cases, whereas CK5(-)/CK20(+) cells were more frequent in high-grade cases than that in low-grade cases. These observations suggest that the frequency of CK5(-)/CK20(+) cells is related to a larger nuclear size and a greater frequency of proliferating cells in high-grade UCs. Unexpectedly, CK5(+)/CK20(-) basal cells, which may include highly tumorigenic cancer stem/progenitor cells, were more frequent in low-grade UCs. We thought that CK5(+)/CK20(-) urothelial cells may include other population with less malignant potential in addition to cancer stem/progenitor cells. To detect true cancer stem/progenitor cell population, additional studies by other markers of UC stem cells such as aldehyde dehydrogenase activity are needed. CK5(-)/CK20(-) cells, which have no differentiation to basal cell or umbrella cell, were more frequent in high-grade UCs. Thus, we conclude that negativity for CK5 is related to the grade of UCs, and an increase of CK5(-)/CK20(+)/Ki-67(+) cells is related to a higher malignant potential.

In summary, we focused on nuclear morphology in association with the CK expression pattern and cell proliferation activity. Our results indicated that 1) the majority of UC cells show aberrant cellular differentiation represented by abnormal CK expression of CK5(+)/CK20(+) or CK5(-)/CK20(+); 2) CK5(+)/CK20(-) cells, which have a cancer stem/progenitor cell characteristic, are a very small population that show a low proliferation activity; 3) Ki-67(+) cells show a significantly different CK expression pattern compared with that of Ki-67(-) cells; 4) the nuclear areas of CK5(-)/CK20(+) cells are significantly larger than those of CK5(+)/CK20(+) cells; 5) the nuclear areas of CK5(-)/CK20(+) cells are significantly larger than those of CK5(+)/CK20(+) cells among Ki-67(-) cells; 6) negativity for CK5 is related to the grade of UC and an increase of CK5(-)/CK20(+)/Ki-67(+) cells is related to a higher malignant potential. Based on our study, we conclude that nuclear morphology is related to the CK expression pattern and proliferative activity.

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Figure Legends

Figure 1 Representative HE and triple immunofluorescence staining of UCs.

a, c) Low-grade case (case no. 2); b, d) High-grade case (case no. 12); a, b) HE staining (original magnification, $\times 20$); c, d) Pseudocolor images of triple immunofluorescence staining (original magnification, $\times 40$). Expression of CK5 (red) and CK20 (green) was found in the cytoplasm, and Ki-67 positivity (pink) was observed in nuclei. DAPI was used for counterstaining nuclei.

Figure 2 CK expression patterns of Ki-67(+) and Ki-67(-) UC cells.

a) CK expression patterns of 1699 UC cells; b) CK expression patterns of 239 Ki-67(+) UC cells; c) CK expression patterns of 1460 Ki-67(-) UC cells.

Figure 3 Nuclear area, CK expression patterns and Ki-67 positivity.

Cell frequency of the nuclear area depended on CK expression patterns of UC cells are shown in the vertical and horizontal axes, respectively. a) All 1699 UC cells; b) 239 Ki-67(+) cells; c) 1460 Ki-67(-) cells.

Figure 4 CK expression patterns of low- and high-grade UC cells.

CK expression patterns of a) 748 low-grade UC cells and b) 951 high-grade UC cells.

tables

Table 1 Summary of cases

Case #	Age (years)	Gender	pT category	Grade	Number of analyzed cells	Nuclear area (mean \pm SD) μm^2
1	63	M	pTa	low	105	71.0 \pm 15.4
2	60	F	pTa	low	128	54.5 \pm 14.0
3	77	M	pTa	low	220	62.2 \pm 19.6
4	64	M	pTa	low	137	64.7 \pm 16.3
5	79	M	pTa	low	158	61.0 \pm 13.4
6	60	M	pTa	high	128	71.2 \pm 23.1
7	77	M	pTa	high	105	78.2 \pm 24.2
8	64	M	pT1	high	172	57.2 \pm 16.7
9	78	M	pT1	high	98	64.6 \pm 16.2
10	67	F	pT1	high	148	106.5 \pm 36.5
11	46	M	pT1	high	121	84.4 \pm 34.9
12	75	M	pT2	high	179	64.6 \pm 19.6

All cases were papillary urothelial carcinoma

Table 2 Nuclear area, cytokeratin expression pattern and Ki-67 positivity

CK expression pattern		CK5(+)/CK20(+)	CK5(+)/CK20(-)	CK5(-)/CK20(+)	CK5(-)/CK20(-)	Total
Total cells (1699)	Mean ± SD (median) μm^2	66.6 ± 25.5 (62.5)*	66.1 ± 21.2 (66.8)	71.3 ± 25.9 (66.4)*	68.7 ± 29.1 (62.8)	69.3 ± 25.9 (64.8)
Ki-67(+) cells (239)	Mean ± SD (median) μm^2	89.5 ± 42.3 (78.4)	53.1 ± 6.5 (53.1)	86.0 ± 32.0 (78.8)	60.9 ± 35.5 (49.0)	86.0 ± 35.9 (78.4)**
Ki-67(-) cells (1460)	Mean ± SD (median) μm^2	63.7 ± 21.0 (61.5)*	67.4 ± 21.8 (68.6)	68.7 ± 23.7 (64.8)*	71.4 ± 27.4 (65.4)	66.6 ± 22.7 (63.4)**

* $P < 0.01$ by Steel-Dwass test** $P < 0.01$ by Welch's test

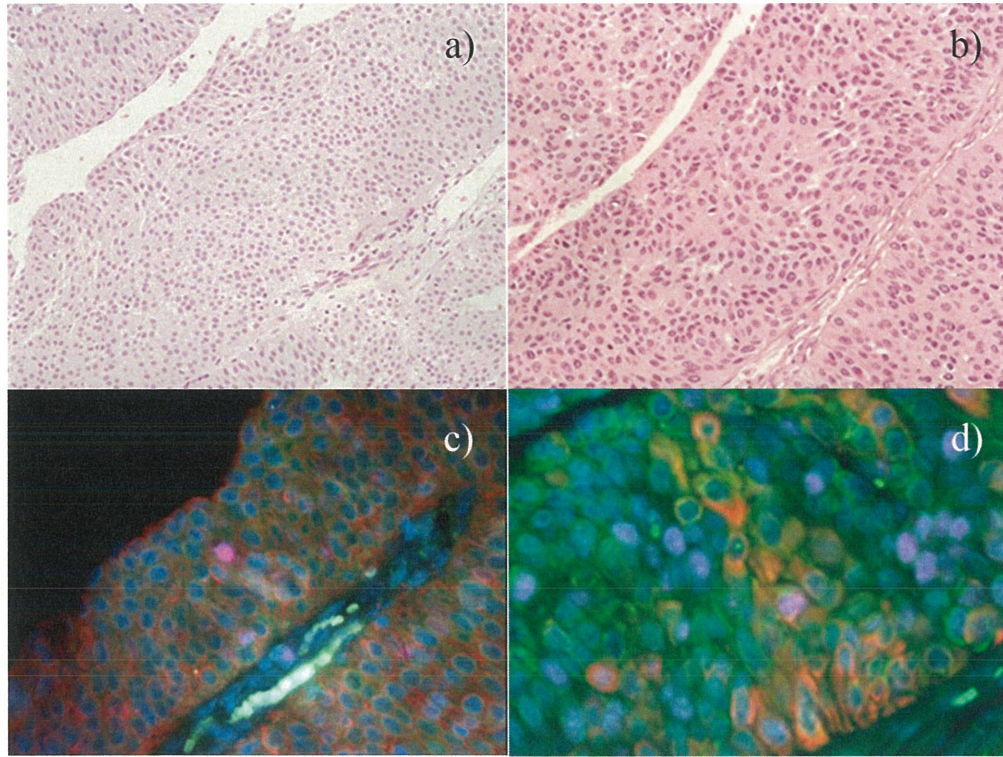


Figure 1 Representative HE and triple immunofluorescence staining of UCs.
a, c) Low-grade case (case no. 2); b, d) High-grade case (case no. 12); a, b) HE staining (original magnification, $\times 20$); c, d) Pseudocolor images of triple immunofluorescence staining (original magnification, $\times 40$). Expression of CK5 (red) and CK20 (green) was found in the cytoplasm, and Ki-67 positivity (pink) was observed in nuclei. DAPI was used for counterstaining nuclei.

160x121mm (299 x 299 DPI)

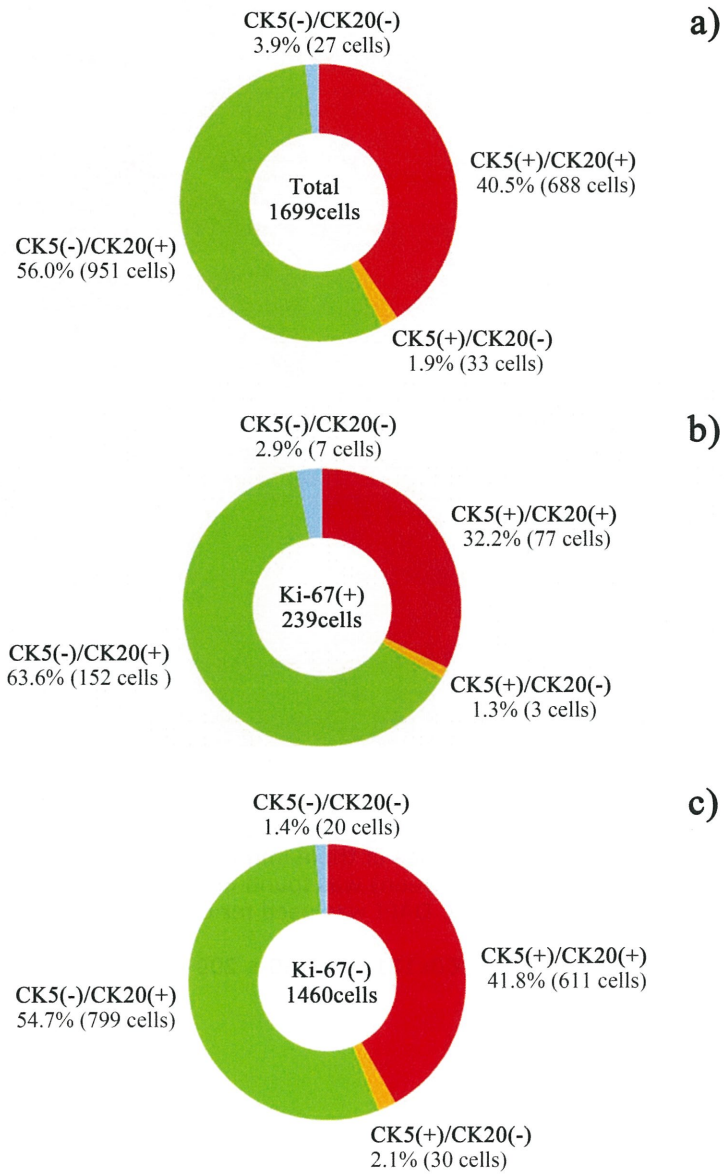


Figure 2 CK expression patterns of Ki-67(+) and Ki-67(-) UC cells.
a) CK expression patterns of 1699 UC cells; b) CK expression patterns of 239 Ki-67(+) UC cells; c) CK expression patterns of 1460 Ki-67(-) UC cells.

143x219mm (300 x 300 DPI)

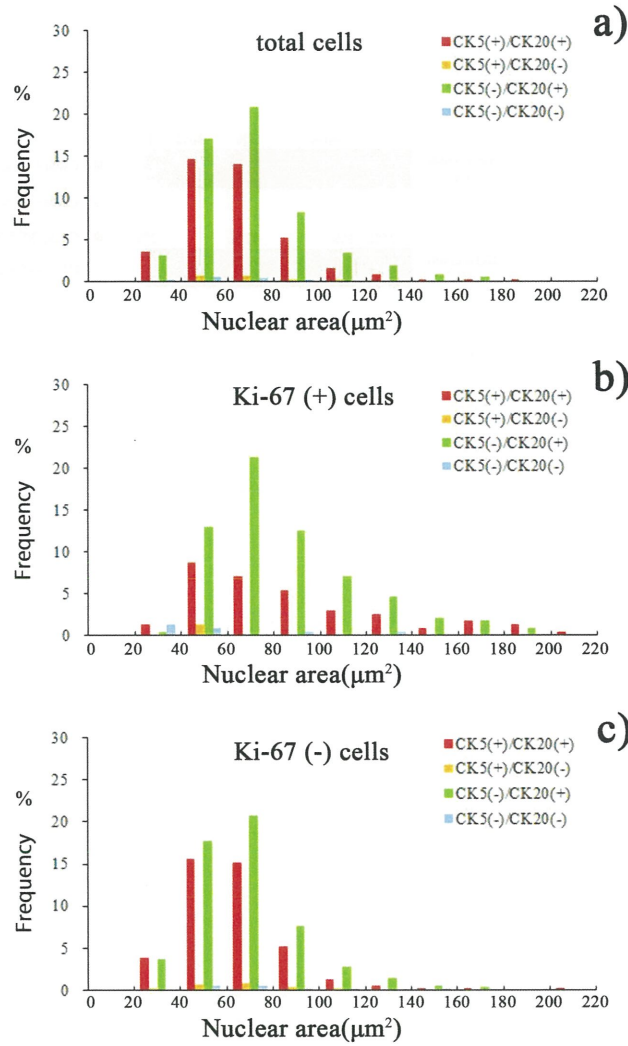


Figure 3 Nuclear area, CK expression patterns and Ki-67 positivity. Cell frequency of the nuclear area depended on CK expression patterns of UC cells are shown in the vertical and horizontal axes, respectively. a) All 1699 UC cells; b) 239 Ki-67(+) cells; c) 1460 Ki-67(-) cells.

155x219mm (300 x 300 DPI)

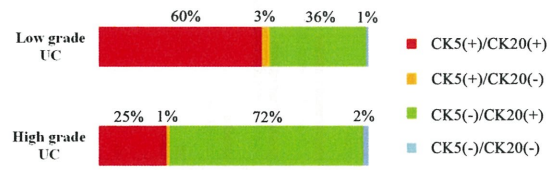


Figure 4 CK expression patterns of low- and high-grade UC cells.
CK expression patterns of a) 748 low-grade UC cells and b) 951 high-grade UC cells.

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