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Enhanced oxidative stress and the glycolytic switch in superficial urothelial carcinoma of urinary bladder

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ABSTRACT

Objective: To examine whether oxidative stress and the glycolytic switch are correlated to tumor grading, tumor recurrence, and disease progression in urothelial carcinoma (UC) of the urinary bladder (UB).
Methods: All surgical specimens obtained from 27 patients (each containing their UC and normal tissues of UB) were subjected to a pathological examination by computerized tomography, and a portion of each specimen was used for the analysis of molecular biomarkers. The mRNA expression levels of pyruvate dehydrogenase kinase-1 (PDK1), hypoxia-inducible factor 1 alpha (HIF-1α), lactate dehydrogenase A (LDHA), pyruvate dehydrogenase, and glucose transporter protein 1 (Glut-1) were measured using TaqMan-based real-time quantitative polymerase chain reaction. In addition, 8-hydroxy-2-deoxyguanosine (8-OHdG) and the mitochondrial DNA (mtDNA) copy number were also determined.
Results: The 8-OHdG content and glycolytic genes expression were higher in UC of the UB than those in the normal tissues of UB, whereas the mtDNA copy number was depleted. According to the multivariate analysis, patients with Grade 3 tumors had higher expression levels of HIF-1α, LDHA, and Glut-1 than those with Grades 1 and 2 tumors. In addition, patients with locally recurrent tumors had a higher expression of HIF-1α and LDHA than those with nonrecurrent tumors. Furthermore, patients under disease progression had higher levels of HIF-1α and PDK1 than those not under disease progression.
Conclusions: UC of the UB manifested that the glycolytic phenotype would reflect the Warburg effect. We suggest that the molecular mechanism in the regulation of glycolytic switch in UC of the UB might provide a specific biomarker for the future development of cancer diagnosis.

1. Introduction

The occurrence of urothelial carcinoma (UC) of the urinary bladder (UB) has the fourth highest incidence of all cancers in the developed world, and patients with UC of the UB also suffer from substantial morbidity and mortality.[1] More than 90% of UB cancers present as transitional cell carcinoma, whereas approximately 5% are squamous cell carcinoma, and less than 2% are adenocarcinoma.[2] The majority of newly diagnosed cases are confined to the urothelium, which do not breach the lamina propria and lead to superficial invasion.[3] UB cancer presents most commonly as a noninvasive tumor including the frequently recurring papillary carcinomas (Ta) and the more aggressive flat carcinoma *in situ*.[4] The clinical outcomes of Ta tumors follow a pathway that is usually distinct from carcinoma *in situ* and invasive cancers (T_1–T_4). In addition, it has also been suggested that Ta tumors have a high risk of local recurrence, whereas the probabilities of disease progression and death increase with invasion.[4] This prognostic disparity highlights the importance of early detection and therapeutic intervention, and hence it is important to determine the molecular biomarkers involved in UB cancer at different tumor stages and necessary for clinical diagnosis and assessment of treatments.[5]

Hypoxia is a pervasive microenvironmental factor that affects normal development and disease progression in the human body.[6]

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Solid tumors frequently contain large regions with a low oxygen tension (hypoxia), and under such conditions can induce adaptive changes in tumor cell metabolism known as the Warburg effect.[7] Hypoxia-inducible factor 1 (HIF-1α) is a critical regulatory protein in the cellular response to hypoxia and is closely related to the angiogenic process. Several glycolytic genes are regulated by HIF-1α and result in the enhanced anaerobic glycolysis contributing to the progression of cancers.[7] Of note, HIF-1α can cause an increase in the expression of pyruvate dehydrogenase kinase-1 (PDK1), which causes the inhibition of pyruvate dehydrogenase (PDH) activity that decreases the amount of pyruvate entering the citric acid cycle, leading to decreased mitochondrial oxygen consumption rates.[8] Chai et al[9] found that an increase in HIF-1α expression was associated with tumor size, histological grade, tumor invasiveness, and recurrence in UC, and hence suggested that HIF-1α is a key regulator of the angiogenic cascade and an independent prognostic factor for disease-free survival in patients with UC. However, it remains unknown as to whether the HIF-1α-regulated expression of glycolytic genes is involved in the progression or the recurrence of UC of the UB.

To the best of our knowledge, this is the first report on the potential role and the prognostic value of the mRNA expression of HIF-1α-induced glycolytic genes that are expressed in superficial UC of the UB including PDK1, lactate dehydrogenase A (LDHA), and glucose transporter protein 1 (Glut-1). By contrast, mitochondrial dysfunction can explain the metabolic shift from mitochondrial respiration to anaerobic glycolysis in cancer cells.[10] In addition, mitochondrial dysfunction-elicited reactive oxygen species (ROS) can have a severe impact on the stability of mitochondrial DNA (mtDNA) and nucleus DNA genome, which has been widely evaluated in various human cancers.[11] An oxidative DNA damage product, 8-hydroxy-2′-deoxyguanosine (8-OHdG), is considered a biomarker for various human tumors including UC of the UB.[12,13] However, the change in mtDNA copy number, which would reflect the mitochondrial function in UC of the UB, is still unknown. Therefore, the second aim of this study was to investigate whether oxidative stress-elicited mitochondrial dysfunction contributes to UC of the UB indicated by the analysis of the copy number of mtDNA and the 8-OHdG level in cancerous and normal tissues of UB.

2. Methods

2.1. Patients

A total of 27 fresh patients with UC of the UB who had first received transurethral resection of bladder tumor (TURBT) were included in this study. All patients provided the signed informed consent form prior to their surgeries according to the rules of the Institutional Review Board of Taipei City Hospital. We used continuous flow resectoscope to perform TURBT. All surgical specimens obtained from each patient including their cancerous part (including tumor and tumor base) and perilesional part (normal part) of UB tissues were sent for pathological examination and confirmation, and a portion of the tissues were immediately kept in a liquid nitrogen tank for further analysis. The cauterized tissue was eliminated using scissors carefully. Tumor grading (Grades 1, 2, and 3) was reviewed by two pathologists. All of the patients received regular follow-up including cystoscopy every 3 months for 3 years, every 6 months for 2 years, and annually after 5 years. If recurrence was noticed, repeated TURBT would be performed. Intravesical chemotherapy (mitomycin) was performed for all patients (weekly during the 1st month and monthly for 1 year).

2.2. Measurement of mRNA levels

Total RNA from UB tissues was extracted with chloroform solution after adding the TRIZol reagent (Sigma-Aldrich Chemical Co., St. Louis, MO, USA) and then precipitated with isopropanol solution followed by dissolution of toxic substance in DEPC−H_2O. An aliquot of 2 μg RNA was reverse-transcribed to cDNA with the Ready-to-Go reverse transcription-polymerase chain reaction (PCR) kit (Amersham Biosciences, Uppsala, Sweden) at 42°C for at least 16 hours. Quantification real-time PCR was performed using the LightCycler TaqMan probe-based Master kit (Roche Applied Science, Mannheim, Germany) according to the manufacturer's instructions.[14] The primer pairs and the number of probes used in this study were designed by Universal ProbeLibrary Assay Design Center (Roche), and are listed in Table 1. The threshold cycle (C_t) was applied for the quantification of gene expression in the tumor part and the normal part from TCC of UB tissues. The C_t for each gene expression was normalized with their 18S gene expression in TCC of UB tissues, respectively, and then the ratio between the tumor part and the normal part from each individual was determined for further analysis.

2.3. Determination of the 8-OHdG content

The 8-OHdG content in total DNA from UB tissues was determined by using the 8-OHdG enzyme-linked immunosorbent assay kit from Japan Institute for the Control of Aging (Fukuroi, Japan). Total DNA from tissues was isolated using phenol/chloroform extraction with the addition of butylated hydroxyl toluene (freshly prepared in ethanol). After precipitating with ice-cold 75% ethanol, we air-dried and dissolved isolated DNA in distilled water. After pretreatment of sample according to the manufacturer's instructions,[15] it was then subjected to analysis of the 8-OHdG content using the enzyme-linked immunosorbent assay kit, and the detection range of the 8-OHdG concentration in a sample is 0.125−10 ng/ml.

2.4. Measurement of mtDNA copy number

The copy number of mtDNA in UB tissues was measured with a real-time quantitative PCR (QPCR) technique using LightCycler

Table 1
Primer sequences and TaqMan probes used in this study.

Gene name	Primer sequence	Probe number
ND1	FW: 5′-ACCATTTGCAGACGCCATAA-3′ RE: 5′-TGAAATTGTTTGGGCTA CGG-3′	[a]
18S	FW: 5′-TAGAGGGACAAG TGGCGTTC-3′ RE: 5′-CGCTGAGCCAGTCAGTGT-3′	[a]
β-Actin	FW: 5′-ATTGGCAATGAGCGGTTC-3′ RE: 5′-GGATGCCACAGGACTCCAT-3′	11
HIF-1α	FW: 5′-TTTTTCAAGCAGTAGGAATTGGA-3′ RE: 5′-TGATGTAGTAGCTGCATGATCG-3′	66
PDK1	FW: 5′-CAAGACCTCGTGTTGAGACCT-3′ RE: 5′-ACGTGATATGGGCAATCCAT-3′	20
Glut-1	FW: 5′-GGTTGTGCCATACTCATGACC-3′ RE: 5′-CAGATAGGACATCCAGGGTAGC-3′	67
LDHA	FW: 5′-TCCTGCCGGCAAACTTAG-3′ RE: 5′-GCAGATTTGGCAGAGAGTATAATG-3′	31
PDH	FW: 5′-CATCATCCTTTATTCCGTAAAGAC-3′ RE: 5′-TCCGAGAGGCAACAAGGTT-3′	74

Glut1 = glucose transporter 1; HIF-1α = hypoxia-induced factor 1 alpha; LDHA = lactate dehydrogenase A; PDH = pyruvate dehydrogenase; PDK1 = PDH kinase 1; ND1 = NADH dehydrogenase subunit 1.

[a] No probes were used for SYBR green reactions in qPCR. Probe numbers indicate the probes of the Universal Probe Set for Human in the LightCycler TaqMan Master kit (Roche Applied Science) used in this study.

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FastStart DNA Master SRBR Green I Kits (Roche Applied Sciences) according to a method described previously.[16] The relative copy number of mtDNA in UB tissues was defined by the normalization of the crossing points (C_p value) in QPCR curves between the ND1 (mtDNA-encoded) and 18S rRNA (nuclear DNA-encoded) genes by using the RelQuant software (Roche). The sequences of primers for ND1 and 18S are outlined in Table 1.

2.5. Statistical analysis

We used the Mann–Whitney test and Kruskal–Wallis test to analyze the experimental data. SPSS 8.0 for Windows was used to run the tests. A p value of <0.05 was considered statistically significant.

3. Results

The patients' ages ranged between 51 years and 80 years (mean age 61.2 years), and they were followed up for a mean duration of 18.5 months (range 12–36 months). Of the 27 patients, 21 were male and six were female; eight were Grade 1, 12 were Grade 2, and seven were Grade 3. All of the patients were diagnosed to show superficial bladder cancer (20 were stage Ta and 7 were T1) by TURBT and Computerized Tomography (CT). During the follow-up period, recurrence was noticed in 11 patients (T1, $n = 3$; Ta, $n = 8$), whereas four patients (T1, $n = 3$; Ta, $n = 1$) had disease progression (muscle invasion or distant metastasis). No mortality has been noted thus far.

The gene expression level of HIF-1α, PDK1, LDHA, PDH, and Glut-1 in the normal part and the cancerous part of UB are shown in Figure 1. We observed that the gene expression level of HIF-1α, PDK1, LDHA, and Glut-1 were more than 2-fold higher in UC of the UB than those in the normal tissues of UB (2.36-, 5.39-, 3.44-, and 2.66-fold, respectively), but the expression of PDH (1.41-fold) showed no significant difference between UC of the UB and normal UB tissues. By multivariate analysis, we further found that patients with higher tumor grading (Grade 3) had a higher level of HIF-1α, LDHA, and Glut-1 expressions than those with Grade 1 and Grade 2 tumors (Table 2). In addition, patients with local recurrent tumor were found to show a higher expression of HIF-1α and LDHA than those with nonrecurrent tumor (Table 3). Furthermore, patients with disease progression had higher HIF-1α and PDK1 expression levels (Table 4).

As shown in Figures 2 and 3, 8-OHdG in UC of the UB was significantly higher than that in each of the normal tissues of UB (9.35 ± 1.75 vs. 6.20 ± 0.82), whereas the mtDNA copy number in UC of the UB was significantly lower than that of the normal tissues of UB (0.093 ± 0.022 vs. 0.043 ± 0.026). However, after further analysis of the differences with regard to tumor grading, tumor recurrence and disease progression, no significant differences were found in each parameter (Tables 2–4).

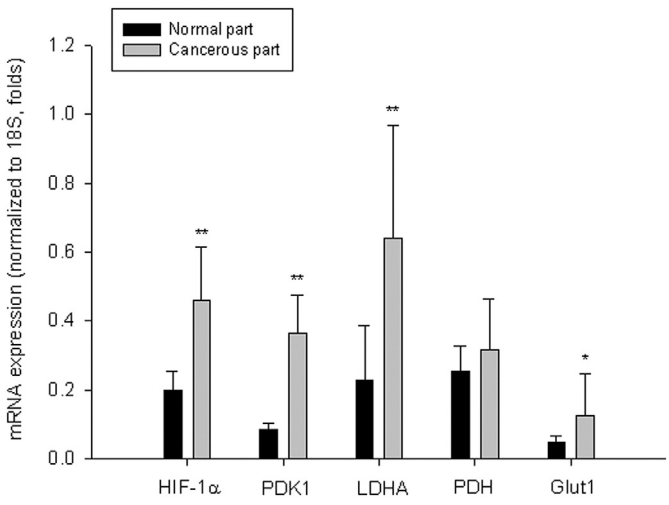

Fig. 1. The upregulated expression of HIF-1α and HIF-1α-regulated glycolytic genes in UC of UB compared with those of normal tissues. The mRNA genes expression in UB tissues (including normal part and cancerous part) was quantified using TaqMan-based real-time QPCR and the level of each gene was normalized to 18S ribosomal RNA. Data are presented as means ± SD of the results from three experiments (*$p < 0.05$, **$p < 0.01$ vs. the indicated group). HIF-1α = hypoxia-inducible factor 1 alpha; QPCR = quantitative polymerase chain reaction; SD = standard abbreviation; UB = urinary bladder; UC = urothelial carcinoma.

Table 2
Gene expression of HIF-1, PDK1, PDH, LDHA, and Glut-1, 8-OHdG and mtDNA copy number in UC of UB with different grading (1, 2, and 3).

	Grade 1 ($n = 8$)	Grade 2 ($n = 12$)	Grade 3 ($n = 7$)	p
HIF-1α*	1.82 ± 0.40	2.26 ± 0.40	3.15 ± 0.41	<0.01
PDK1	4.15 ± 2.47	5.12 ± 3.33	7.25 ± 2.69	0.78
LDHA*	2.01 ± 0.73	2.35 ± 0.87	7.13 ± 1.35	<0.01
PDH	1.57 ± 0.31	1.39 ± 0.34	1.28 ± 0.37	0.24
Glut-1*	2.05 ± 0.26	2.16 ± 0.42	4.22 ± 1.48	0.04

The ratio between cancerous and normal tissues of UB is expressed as mean ± SD. The Kruskal–Wallis test was used for statistical analysis.
*$p < 0.05$ was considered statistically significant.
Glut1 = glucose transporter 1; HIF-1α = hypoxia-induced factor 1 alpha; LDHA = lactate dehydrogenase A; mtDNA = mitochondrial DNA; 8-OHdG = 8-hydroxy-2-deoxyguanosine; PDH = pyruvate dehydrogenase; PDK1 = PDH kinase 1; ND1 = NADH dehydrogenase subunit 1; SD = standard deviation; UB = urinary bladder; UC = urothelial carcinoma.

4. Discussion

It has been suggested that enhanced glycolysis in most cancerous cells and tissues is accompanied with the tumorigenesis termed the Warburg effect.[7] The increased glycolytic metabolism of cancer cells confers a selective advantage for survival and proliferation in the unique tumor microenvironment (hypoxia condition). The role of HIF-1α is well-known factor involved in the adaptive response in most cancerous cell types to the changes in tissue oxygenation.[8] Previously, a strong relationship between HIF-1α immunoreactivity and the grade of the tumor in UB was claimed.[17] Accordingly, we observed in this study that higher mRNA expression of HIF-1α was found in UC of the UB, and more significantly related to the tumor grading, disease recurrence, and progression. Furthermore, we reported that the mRNA expressions of HIF-1α-induced glycolytic genes including LDHA, Glut-1, and PDK1, were all more than 2-fold higher in UC of the UB than those in

Table 3
Gene expression of HIF-1, PDK1, PDH, LDHA, and Glut-1, 8-OHdG, mtDNA copy number, tumor number, and diameter in UC of UB with or without recurrence.

	Recurrence (−) ($n = 16$)	Recurrence (+), ($n = 11$)	p
HIF-1α*	2.12 ± 0.58	2.70 ± 0.59	0.02
PDK1	4.97 ± 4.25	5.99 ± 4.39	0.55
LDHA*	2.59 ± 0.37	4.68 ± 0.41	0.04
PDH	1.49 ± 0.32	1.31 ± 0.38	0.21
Glut-1	2.66 ± 2.53	2.67 ± 1.82	0.99

The ratio between cancerous and normal tissues of UB is expressed as mean ± SD. The Mann–Whitney test and Cox model were used for statistical analysis.
*$p < 0.05$ was considered statistically significant.
Glut1 = glucose transporter 1; HIF-1α = hypoxia-induced factor 1 alpha; LDHA = lactate dehydrogenase A; mtDNA = mitochondrial DNA; 8-OHdG = 8-hydroxy-2-deoxyguanosine; PDH = pyruvate dehydrogenase; PDK1 = PDH kinase 1; SD = standard deviation; UB = urinary bladder; UC = urothelial carcinoma.

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Table 4
Gene expression of HIF-1α, PDK1, PDH, LDHA, and Glut-1, 8-OHdG, mtDNA copy number, tumor number, and diameter in UC of UB with or without disease progression.

	Disease progression (−) ($n = 23$)	Disease progression (+) ($n = 4$)	p
HIF-1α*	2.28 ± 0.65	2.83 ± 0.33	0.03
PDK1*	5.31 ± 2.46	5.89 ± 1.49	0.04
LDHA	3.14 ± 3.13	5.19 ± 5.08	0.48
PDH	1.46 ± 0.34	1.18 ± 0.33	0.21
Glut-1	2.65 ± 2.38	2.75 ± 1.22	0.89

The ratio between cancerous and normal tissues of UB is expressed as mean ± SD. The Mann–Whitney test and Cox model was used for statistical analysis.
*$p < 0.05$ was considered statistically significant.
Glut1 = glucose transporter 1; HIF-1α = hypoxia-induced factor 1 alpha; LDHA = lactate dehydrogenase A; mtDNA = mitochondrial DNA; 8-OHdG = 8-hydroxy-2-deoxyguanosine; PDH = pyruvate dehydrogenase; PDK1 = PDH kinase 1; SD = standard deviation; UB = urinary bladder; UC = urothelial carcinoma.

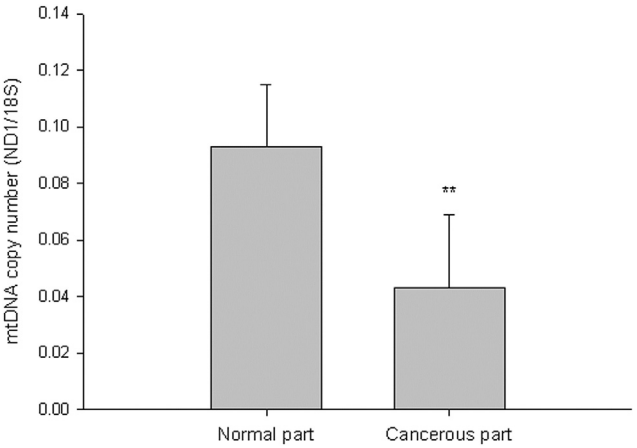

Fig. 3. Decrease in mtDNA copy number in UC of UB compared with those of normal tissues. The mtDNA copy number in UB tissues (including normal part and cancerous part) was determined using SRBR Green-based real-time QPCR. Data are presented as means ± SD of the results from three experiments (**$p < 0.01$ vs. the indicated group). mtDNA = mitochondrial DNA; QPCR = quantitative polymerase chain reaction; SD = standard abbreviation; UB = urinary bladder; UC = urothelial carcinoma.

normal tissues of UB. We suggest that the observation of glycolytic switch in UC of the UB may reflect the Warburg effect (Figure 4).[7,18]

Recently, two single nucleotide polymorphisms in the HIF-1α, P582S and A588T, were shown to cause significantly higher transcriptional activity than the wild type, and it has been reported that patients with a variant allele of HIF-1α had significantly worse poor prognosis in UC of the UB than those without a variant allele.[19] Therefore, HIF-1α-induced downstream targets, glycolytic genes, may play a vital role on the progression and recurrence in UC of the UB. Indeed, Zhou and coworkers[20] reported that by immunohistochemistry staining, the protein expressions of HIF-α and Glut-1 in UC of bladder were significantly higher than those in normal tissues near the cancerous tissues. The positive expression of Glut-1 in the UC of the UB tissue was 78%, and there also was a positive correlation between the Glut-1 expression level and the grading of cancer. In addition, Palit et al[21] indicated that the up-expression of Glut-1 in human bladder cancer was associated with a poor prognosis and a low survival rate. Likewise, we report in this study that not only HIF-1α and Glut-1, but also LDHA, were upregulated in the UC of the UB tissues compared to those of normal UB tissues, and

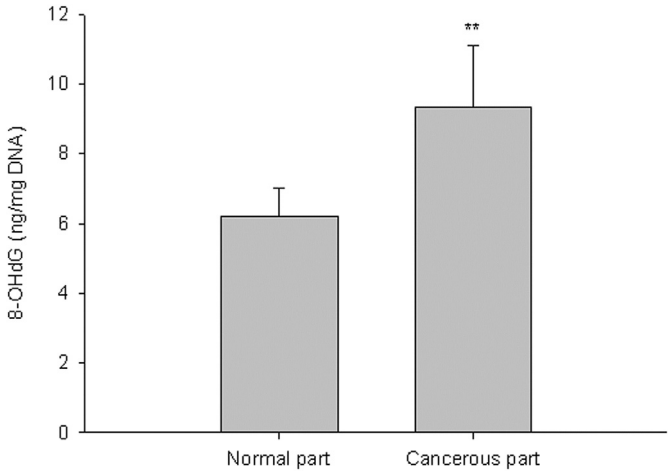

Fig. 2. Increase in 8-OHdG content in UC of UB compared with those of normal tissues. The level of oxidative DNA damage in UB tissues (including normal part and cancerous part) was evaluated by 8-OHdG content, which was quantified using the 8-OHdG ELISA kit according to previously described methods. Data are presented as means ± SD of the results from three experiments (**$p < 0.01$ vs. the indicated group). ELISA = enzyme-linked immunosorbent assay; 8-OHdG = 8-hydroxy-2-deoxyguanosine; SD = standard abbreviation; UB = urinary bladder; UC = urothelial carcinoma.

that these three genes were all correlated to the UB tumor grading. Furthermore, we also indicate that the upregulation of HIF-1α and LDHA gene expression in UC of the UB was related to the local recurrent tumor. It is also worth noting that LDHA was previously reported to be preferentially expressed in tumors including lung, breast, endometrium, and UB tissues, suggesting that those tumor cells utilize anaerobic glycolysis as a means of energy production.[22] Taken together, we suggest that there is a glycolytic switch occurring in the UC of the UB, and the expression of HIF-1α and LDHA genes would be a prognostic risk for UC of the UB with recurrence or without recurrence (Figure 4). The number of the samples assayed *ex vivo* was limited in this study, and hence, a further cell model is required for investigating the detrimental effects of HIF-1α-induced glycolytic switch in bladder cancer.

A key branch point in the glycolytic pathway is the production of pyruvate, which in anaerobic conditions is metabolized to lactate and in normoxia by PDH to acetyl-CoA. Accordingly, PDH activity is controlled by PDH kinase (PDK), which phosphorylates and inactivates its enzymatic activity.[23] Intriguingly, it has been reported that the hypoxia-inducible PDK1 is critical for the attenuation of mitochondrial ROS production, maintenance of ATP levels, and adaptation to hypoxia for the advantaged cancer survival.[8] There is a report showing that by immunochemistry staining, the upregulation of PDK1 in head and neck squamous cancer is associated with a poor prognosis.[24] In our studies, we found a higher mRNA expression of *PDK1* gene in UC of the UB compared to those of normal UB. Significantly, we also investigated if a higher expression of HIF-1α and PDK1 in patients with UC of the UB could have a higher chance of disease progression. Taken together, we contend that the striking and adverse outcome of UC of the UB with the highest PDK1 expression may be a risk for disease progression (Figure 4).

By contrast, mitochondrial dysfunction has been thought to play a key role in tumorigenesis, and a wide spectrum of mutations and depletion in mtDNA have been identified in several human cancers.[10] At least in part, mitochondrial dysfunction can explain the metabolic shift in cancer cells from mitochondrial respiration to anaerobic glycolysis owing to the energy demand for cancer cells' survival. Consequently, mitochondrial dysfunction-elicited ROS could severely damage DNA molecules, resulting in the genomic instability that contribute to tumorigenesis.[25] 8-OHdG, one of the

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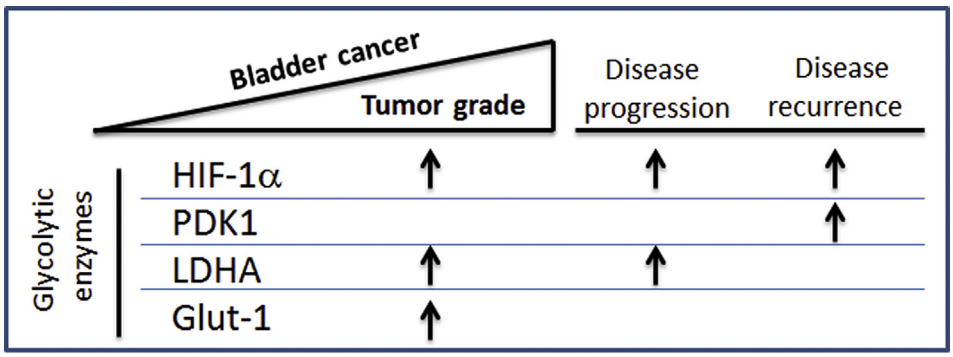

Fig. 4. Overview of glycolytic shift in bladder cancer. Upregulated expression of glycolytic genes including HIF-1α, PDK1, LDHA, and Glut-1 are observed in UC of UB compared with those of normal tissues, but not for PDH, a mitochondrial protein. The gene expression of HIF-1α is more significantly related to tumor grading, disease recurrence, and progression. Glut-1 = glucose transporter protein 1; HIF-1α = hypoxia-inducible factor 1 alpha; LDHA = lactate dehydrogenase A; PDH = pyruvate dehydrogenase; PDK1, pyruvate dehydrogenase kinase-1; UB = urinary bladder; UC = urothelial carcinoma.

major DNA base-modified products, is induced by hydroxyl radical, singlet oxygen, or photodynamic action. Previously, Wada and colleagues[26] reported that the levels of 8-OHdG in bladder and renal cancers tissues were significantly higher than in the neighboring noncancerous tissues. Our data are consistent with their findings, but the increase in 8-OHdG in UC of the UB has no significant correlation with the tumor grading, disease recurrence, or progression. However, it was reported by Soini et al[27] that higher levels of serum and urine 8-OHdG in patients with the UC of the UB were associated with poor prognosis.

Mitochondrial dysfunction-elicited ROS can induce the alteration of mtDNA copy number in different cancerous tissues, and this effect has also been widely studied.[28] It has been reported that higher mtDNA copy number in whole blood was found to be associated with increased risk of breast cancer,[29] but a low mtDNA copy number in whole blood was also reported to be associated with renal cell carcinoma.[30] The contradictory results suggest that the role of mtDNA copy number in human cancer is complicated and might be cancer site-specific. Of note, the decrease in mtDNA copy number has been observed in lung cancer, hepatocellular carcinoma, and gastric cancers, especially in lesions of advanced-stage cancers.[10] The decreased mtDNA copy number might be an index of disease aggressiveness or progression in these human cancers.[10] Nevertheless, the observations of the present study for the first time reported that the mtDNA copy number in UC of the UB was lower than that in the normal tissues of UB, even though no significant difference was observed between tumor grading, progression, and recurrence of tumors.

In conclusion, the glycolytic shift that we demonstrated in UC of the UB might provide a specific biomarker for the future development of cancer diagnosis. We suggest that higher expression levels of HIF-1α and PDK1 in patients with superficial UC of the UB might imply that they have a higher risk of disease progression and poor prognosis.

Conflicts of interest

None.

Acknowledgments

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