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Proteomic Analysis of Candidate Prognostic Urinary Marker for Cervical Cancer

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Abstract

Urinary proteins are an important component in urine samples that can be used as biological indicators for clinical diagnosis of diseases, such as proteinuria, kidney failure, diseases in the bladder, urinary tract and related organs. In this present work, we investigated candidate protein biomarkers in urine for diagnosis of cervical cancer using proteomic approach. The urine samples were concentrated and de-salted through ultrafiltration (3 kDa), followed by Spin-50 mini-sephadex column and were then separated by two-dimensional electrophoresis (2-DE) and annotated by mass spectrometer. Comparing 2-DE results between individual healthy donors and cervical cancer, patients showed that 4 urinary proteins; Protocadherin-8, Aryl hydrocarbon receptor nuclear translocator-like protein 2, Serum albumin and Endorepellin, C-terminal domain V of perlecan were overexpressed by two-fold in the patient samples. Only the endorepellin LG3 fragment was validated by Western blot analysis. We also used 2D Western blot and nano LC-MS/MS to confirm the urinary biomarker as endorepellin LG3 fragment. Our study suggests that proteomics approach may be useful for the study of urinary proteins and provides an opportunity to discovery aberrant protein-associated diseases. We also revealed the candidate biomarkers that may be applied for cervical cancer diagnosis which benefits for medical treatment.

Introduction

According to reports from the American Cancer Society (ACS) since 2007 (National Cancer Institute, Centers of Disease Control and Prevention, North American Association of Central Cancer Registries and National Center for Health Statistic), in the USA, estimated 1,638,910 new cancer cases and 577,190 cancer deaths were diagnosed in 2012. The cancer incidence in men has been declining at the rate of 0.6% per year and was stable in women, while the cancer death rates in men and woman from 2004 to 2008 were decreased at a rate of 1.8% and 1.6% per year, respectively [1]. Cervical cancer was the third most common type of cancer and was the fourth most frequent cause of cancer death in women worldwide, with 529,512 (8.8%) new cases and 274,967 (8.2%) cancer death in 2008 [2].

Infection with human papillomavirus (HPV) is established as an initiating cause of cervical cancer, cooperating with other co-factors necessary for progression of this cancer, such as tobacco smoking, high parity, long-term hormonal contraceptive use and co-infection with HIV. Moreover, co-infection with *Chlamydia trachomatis*, herpes simplex virus type-2, immunosuppression and certain dietary deficiencies are other probable cofactors [3]. In medical case of cervical cancer, there are several methods of treatment, such as surgery [4,5], chemotherapy (hormone), radiotherapy or various combination methods [6-9]. However, the death rate of cervical cancer is still high [10]. The main problem of the cancer therapy is that the majority of patients are already in the late stage of cancer when diagnosed, where the cancer has already metastasized to other organs [11]. Therefore, many researchers tend to investigate biological indicators of cancer status and progression before clinical symptoms or enabling real-time monitoring of drug response.

Proteomic analysis is a preferred approach for identifying protein biomarkers used in diagnosis of diseases, especially cancer. In addition, proteomics has the potential to unravel basic tumor biological questions regarding the mechanisms involved in the pathogenesis of cancer. Blood and serum are sources of specimens for apply the proteomic research to clinical utilities [12-14]. However, the high concentration and the composition of serum proteins cause limitation for the study of biomarkers. Urine is an alternative body fluid that is easily obtained and non-invasively available. The protein compositions of urine are qualitatively similar to those of serum. Therefore, detection of urinary proteins may lead to the biomarkers discovery for cervical cancer, as well as seeking biomarkers from serum proteins.

In this work, 2-DE technique was used for identifying urinary protein markers, which was obtained from healthy donors and cervical cancer patients. Preparation of the human urine sample for the 2-DE analysis was discussed. The 2-DE results, protein identification and preliminary validation of particular proteins were presented. Finally, we revealed some candidate biomarkers in patient urine, which might be used for diagnosis, prognosis, staging or therapy of cervical cancer.

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Materials and Methods

Human urine sample collection

Fresh of clean catch first morning urine samples were collected with the presence of preservative (0.02% w/v sodium azide) from thirty-one healthy donors (aged 24-66 years) and forty-two cervical cancer patients (n=30 for squamous cell carcinoma antigen; SCCA, n=4 for adenosquamous and n=8 for adenocarcinoma cell type, stage IA1-IIIB, age range 33-69 years). The urine samples were centrifuged at 10000×g for 30 min at 4°C to remove insoluble solids. The supernatants were lyophilized and stored at -20°C until use. Cervical cancer patient urine samples were obtained through collaboration with the Maharaj Nakorn Chiang Mai Hospital. All patients were specifically informed of the use of their urine samples for these studies and signed a written consent. The Medical Ethic Committee of Chiang Mai University, Chiang Mai, Thailand reviewed and approved our research.

Lyophilized samples were resuspended in deionized water and loaded into the centrifugal filter unit with 3 kDa MWCO (Amicon, Millipore, USA) and centrifuged at 5000×g for 1 h at 4°C, followed by gel filtration column, the Sephadex gel Spin-50 mini column (BioMax Inc, MD, USA) for concentrating the proteins and remaining small interference molecules. This method was re-processed by adding deionized water, until the conductivity of the urine samples was less than 100 mS/m. Then, the urine samples were lyophilized and stored at -20°C.

Two-dimensional electrophoresis (2-DE)

For the first dimensional electrophoresis of isoelectric focusing (IEF), the lyophilized urine samples were dissolved in lysis buffer containing 7 M Urea, 4% w/v CHAPS, 4 mM Tris base, 2 M Thiourea, 1% v/v IPG buffer pH 3-10 NL and 65 mM dithiothreitol; DTT. These samples were sonicated, centrifuged at 12000×g for 15 min and then applied to IPG strips (18 cm pH 3-10 NL, pH 4-7; GE Healthcare, Sweden), with a final concentration of 200 μg protein in 350 μL determined by spectrophotometry, using the Coomassie protein assay based on the Bradford protein dye binding assay (Bio-Rad Laboratories, CA, USA) [15]. The IEF (IPGphor III, GE Healthcare Biosciences, UK) was performed under the following conditions: rehydration at 30 V for 14 h followed by 100 V for 1 h, 250 V for 1 h, 500 V for 1 h, 1000 V for 1 h, 3000 V for 1 h, 5000 V for 1 h and focusing at 8000 V for up to 64 kVhr. After IEF, the IPG strips were equilibrated with 3 mL of equilibrium buffer 1 (50 mM Tris-HCl pH 8.8, 6 M Urea, 30% v/v glycerol, 2% w/v SDS, 2% w/v DTT and bromophenol blue), and then subsequently alkylated with 3 mL of equilibrium buffer 2 (50 mM Tris-HCl pH 8.8, 6 M Urea, 30% v/v glycerol, 2% w/v SDS, 2.5% w/v iodoacetamide; IAA and bromophenol blue) for 15 min each. The IPG strip was placed on top of the 12.5% polyacylamide gel (18×18 cm, 1.5 mm) and covered with 0.5% w/v agarose. The 2-DE separation was carried out at 45 mA per gel at 4°C, until the bromophenol blue dye front reached the bottom of the gel. The 2-DE gels were stained with Sypro® Ruby (Invitrogen™ Molecular Probes™ (Eugene, OR, USA), and scanned using a Typhoon 9200 laser scanner (GE Healthcare, Sweden). In addition, the 2-DE gel images were exported to an image analysis software program, PDQuest 2-D analysis software version 7.1.3 (Bio-Rad, Hercules, CA, USA).

Protein digestion

Protein spots were manually excised from the gels and transferred to the 0.5 mL siliconized eppendorfs. The gel pieces were washed twice with 50% v/v acetonitrile (ACN) / in 25 mM ammonium bicarbonate

buffer pH 8.0 for 15 min each. The shrunk gel pieces were washed with 100% ACN and dried using a ScanVac ScanSpeed 32 concentrator (Labogene Aps, Lynge, Denmark). The dried gel pieces were swollen in 10 μL of 25 mM ammonium bicarbonate containing 0.1 μg trypsin; (sequencing grade; Promega, Madison, WI, USA). The gel pieces were crushed with a siliconized blue stick and incubated at 37°C for at least 16 h. Peptides were subsequently extracted twice with 50 μL of 50% v/v ACN/in 1% trifluoroacetic acid (TFA). The extracted solutions were then combined and dried using a ScanVac ScanSpeed 32 concentrator. The digested peptides were then resuspended in 10 μL of 0.1% TFA and the peptide solution was cleaned up using C18 ZipTip (Millipore, Bedford, MA, USA), prior to analysis by MS. Bound peptides were eluted from the ZipTip with 5 μL of 50% ACN/0.1% TFA.

MALDI-MS and MS/MS analysis

The samples were premixed at a 1:1 ratio of with the matrix solution (5 mg/mL α-Cyano-4-hydroxycinnamic acid; CHCA in 50% v/v ACN, 0.1% v/v TFA and 2% w/v ammonium citrate) and spotted onto the 96-wells format MALDI sample stage. Data was directly acquiesced on the Q-TOF Ultima™ MALDI instrument (MALDI™; Micromass, Manchester, UK), which was fully automated with a predefined probe motion pattern and peak intensity threshold for switching over from MS survey scanning to MS/MS, and from one MS/MS to another. Within each well, parent ions meeting the predefined criteria (any peak within the m/z 800-3000 range with intensity above 10 count ± include/exclude list) were selected from the most intense peak for CID MS/MS, using argon as the collision gas and a mass dependent ± 5 V rolling collision energy, until the end of the probe pattern was reached. The MASCOT MS/MS ion search program was used for peptide sequence searching. Search parameters allowing for oxidation of methionine, cabamidomethylation of cysteine and one missed trypsin cleavage were selected for searching the SwissProt database. The tolerance of both parent and fragment ions was set to 0.25 Da. Protein identification was repeated at least once using spots from different gels (Supporting Information Figure S1a and S1b).

1D and 2D Western blot analysis

For 1D Western blot, the 11 healthy donors and 13 cervical cancer patients were performed. The lyophilized urine samples were resuspended in sample buffer (50 mM Tris-HCl pH 6.8, β-mercaptoethanol, 10% w/v glycerol, 2% w/v SDS, and 0.1% v/v bromophenol blue) and heated at 95°C for 5 min. Ten micrograms of the sample solutions were loaded into each well of 12.5% polyacrylamide gel. The SDS-PAGE was carried out at 20 mA per gel. The prestained protein ladder marker was used as standard Mr marker proteins. For 2D Western blot, the first and second dimension separations were performed as above.

After SDS-PAGE, the proteins were transferred onto PVDF membrane (Millipore, Bedford, MA, USA), using a semi-dry apparatus (GE Healthcare, USA). The membranes were blocked with 2.5% w/v BSA in phosphate buffer saline containing 0.05% v/v Tween-20 (PBST) at room temperature for 2 h. After washing with PBST, the PVDF membranes were incubated with primary antibodies for 2 h. The excess antibodies and non-specific bindings were removed by washing with PBST, and then the HRP-conjugated secondary antibody was probed against its primary antibody. The primary and secondary antibodies used in this work are summarized in Table 1. The blot bands were visualized by enhanced chemiluminescence western blotting detection system (ECL™ kit; Perkin Elmer) and exposed to Fujifilm LAS-4000 Luminescent Image Analyzer (FUJIFILM Corporation, Japan).

Proteins	Primary antibody	Secondary antibody
PCDH8	mouse monoclonal anti-human Hp primary antibody (1:200 dilution, Santa Cruz Biotechnology) for 2 h	goat anti-mouse IgG-conjugated secondary antibody (1:3000 dilution, Abcam, Cambridge, UK) for 1 h
ARNTL2	rabbit polyclonal primary antibody (1μg/mL dilution, Abcam, Cambridge, UK) for 2 h	mouse anti-rabbit IgG-conjugated secondary antibody (1:50000 dilution, Abcam, Cambridge, UK) for 1 h
ALB	mouse monoclonal primary antibody (1:200 dilution, Santa Cruz Biotechnology) for 2 h	goat anti-mouse IgG-conjugated secondary antibody (1:3000 dilution, Abcam, Cambridge, UK) for 1 h
PGBM (Endorepellin)	mouse monoclonal heparan sulfate proteoglycan 2 antibody (domain V of perlecan) (1:100 dilution, Abcam, Cambridge, UK) for 2 h	goat anti-mouse IgM-conjugated secondary antibody (1:3000 dilution, Abcam, Cambridge, UK) for 1 h

Table 1: The primary and secondary antibody.

Nano LC-MS/MS analysis

Protein spot 18 were excised manually from 12 gels and in-gel digestion for LC-MS/MS was performed as above. High resolution and high mass accuracy nanoflow LC-MS/MS experiments were done on a LTQ Orbitrap XL ETD mass spectrometer (Thermo Fisher Scientific, San Jose, CA) equipped with a nano electrospray ion source (New Objective, Inc.), an Agilent 1100 Series binary high-performance liquid chromatography pump (Agilent Technologies), and a Famos autosampler (LC Packings) were used. The digestion solution was injected (6 μL) at 10 μL/min flow rate onto a self packed pre column (150 μm I.D.×30 mm, 5 μm, 200 Å). Chromatographic separation was performed on a self packed reversed phase C18 nano-column (75 μm I.D.×200 mm, 3 μm, 200 Å), using 0.1% formic acid in water as mobile phase A and 0.1% formic acid in 80% acetonitrile as mobile phase B operated at 300 nL/min flow rate. Survey full-scan MS condition was at mass range (m/z) of 320-2000 and resolution of 30,000 at m/z 400. The three most intense ions were sequentially isolated for HCD (Resolution of 7500). Electrospray voltage was maintained at 1.8 kV and capillary temperature was set at 200°C.

Statistical analysis

Quantitative data was expressed as the mean ± SD. The statistical significance of differences between values was assessed using a two-tailed student's t-test. Values of $p<0.05$ were considered to indicate statistical significance.

Results

Urine samples preparation

Urine contains body filtrates such as water, salts, electrolytes and nitrogenous waste products and trace amounts of protein originating from blood plasma, the kidneys and the urogenital tract [16,17]. Normally, the protein amount found is very low in urine (the excretion protein is less than 150 mg/day). To concentrate urinary proteins and remove excess salts, various sample preparation procedures, such as ultrafiltration, centrifugation, reverse-phase separation, dialysis, lyophilization, proteins enrichment by affinity chromatography and precipitation using organic solvents have been carried out [18-25]. Unfortunately, none of these techniques were ideal for presenting urinary proteins to proteomics. Thus, the preparation of urinary proteins is a critical step of protein analysis and screening protein biomarkers. Development and optimization of techniques were required. In this work, we used a procedure optimized by our data [25] with some modification to concentrate urinary proteins, while preventing/discarding high concentrations of interfering molecules containing salts, electrolytes, and/or some nitrogenous waste products. The urine sample was conveniently concentrated by the ultrafiltration (3 kDa) of the Amicon tube. However, the conductivity meter showed that the interfering molecules in the concentrated urine were higher than the expected level (100 mS/m). The 2-DE gels of the urine sample

derived from this technique showed few spots of protein with a wide smear zone proximal to the anode (Figure 1A). Although, the Amicon tube would remove some small molecules during centrifugation, samples with as many electrolytes as urine might require more steps or more effective techniques. Thus, the concentrated urine was sequentially subjected to gel filtration column, the Sephadex gel Spin-50 mini column, to increase the effectiveness of the sample preparation procedure for proteomic work. The Spin-50 mini-column was prepacked with Sephadex G-50 hydrated in water. It is designed for desalting and purification of biological samples with a sample recovery greater than 90%.

The 2-DE gels image of the urine samples prepared by ultrafiltration, and subsequently, Spin-50 mini column (Figure 1B) showed that the protein spots were clearer and much more numerous than results from the ultrafiltration technique alone (Figure 1A). However, the smear-zone between pH 3-5 was observed in both preparations. We suggest that some electrolytes may interact with these acidic proteins, causing the protein charge to change and become unstable. Adjusting the solution pH or adding some ions to precipitate excess electrolytes might solve this problem.
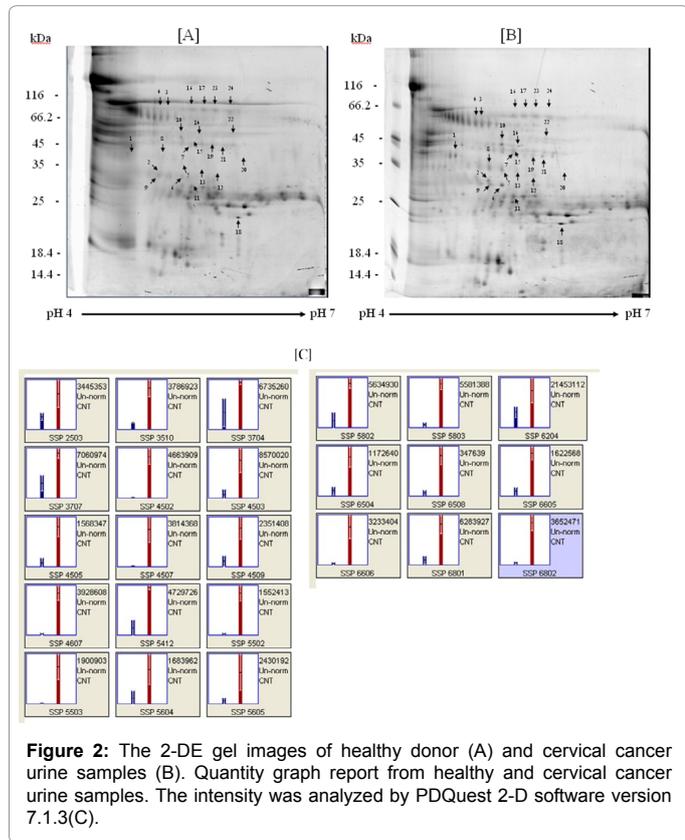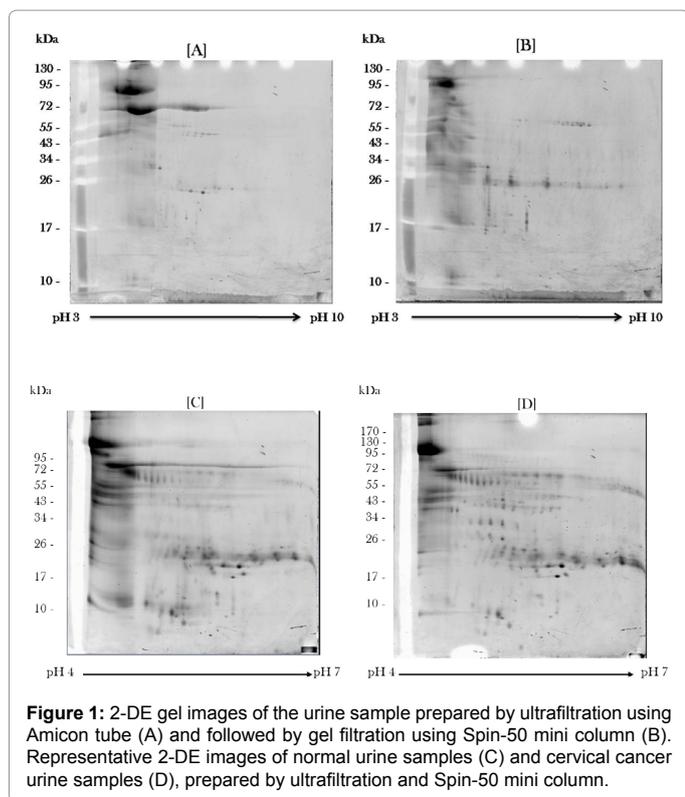
Different expression of urinary proteins in cervical cancer patients by 2-DE analysis

We applied the optimal procedure to prepare urinary protein obtained from healthy donors and cervical cancer patients. The processed protein sample from individual cases (healthy or patient) were analyzed by 2-DE. The narrow range IPG strip pH 4-7 was used instead of the pH 3-10 NL to improve spot focusing in this range. The results in Figure 1C and 1D showed a well-separated pattern of protein spots in both healthy and cervical cancer patient samples. Numerous spots migrated as a train of spots where the spot series were observed, suggesting the presence of unknown post-translational modifications (PTMs) on these proteins. Quantitative analysis of spot intensity was performed.

The quantity of protein spots of urine samples was analyzed by PDQuest 2-D analysis software version 7.1.3 in both healthy and patient gels. The software indicated that 24 protein spots were expressed differently by two-fold or more between patient and healthy sample. The synthetic gel from the gel results, provided by the image function in the PDQuest software, is shown in Figure 2A and 2B.

Protein identification

The 24 protein spots were found with the differential expression level higher than two-fold at p-value 0.05. These protein spots (Figure 2C) were isolated from gels, digested with trypsin and subjected to MALDI Q-TOF MS or MS/MS analysis. Seven protein spots were identified as Protocadherin-8 (spot 2), Aryl hydrocarbon receptor nuclear translocator-like protein 2 (spot 5), Serum albumin (spot 13, 16, 17, 24) and Perlecan (spot 18), as summarized in Table 2. These

Figure 1: 2-DE gel images of the urine sample prepared by ultrafiltration using Amicon tube (A) and followed by gel filtration using Spin-50 mini column (B). Representative 2-DE images of normal urine samples (C) and cervical cancer urine samples (D), prepared by ultrafiltration and Spin-50 mini column.

Figure 2: The 2-DE gel images of healthy donor (A) and cervical cancer urine samples (B). Quantity graph report from healthy and cervical cancer urine samples. The intensity was analyzed by PDQuest 2-D software version 7.1.3(C).
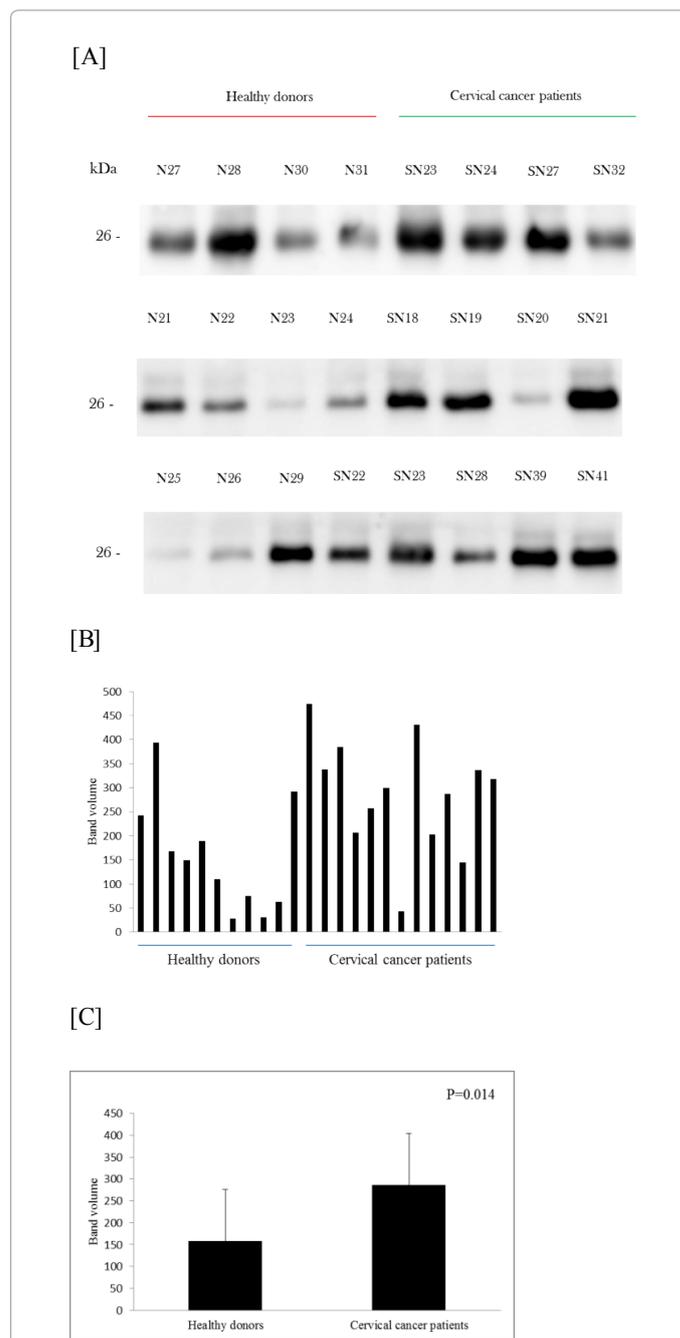
proteins were up-regulated in the cervical patient urine sample. Protein database were used to search for matching proteins and their functions

(Supporting Information Table S1). Perlecan is a large protein of 480 kDa which consist of five domains, while spot 18 had MW around 24 kDa on 2-DE gel. These finding suggest that spot 18 corresponding to the C-terminal domain V of perlecan (1% of sequence coverage), known as endorepellin LG3 fragment.

Western blot analysis of differentially-expressed proteins

The expression level of the four candidate biomarkers, PCDH8,

Figure 3: Western blot analysis of endorepellin LG3 fragment. The focal areas of the western blot result visualized by ECL system are presented. (A) Protein band intensities of endorepellin LG3 fragment expression level in healthy and cervical cancer urine samples. (B,C) Densitometric analyses of Western blot (*t-test; p*=0.014).

ARNTL2, serum albumin and endorepellin LG3 fragment, in urine samples were validated by Western blot analysis. Specific antibodies were used to detect their corresponding protein spots on a blotted PVDF membrane. The immunoblotting result showed that only the expression level of the endorepellin LG3 fragment was significantly elevated in cervical cancer patient samples (Figure 3A). On the other hand, no significant differences between healthy and patient samples were observed for PCDH8, ARNTL2 and serum albumin (Data not shown). Endorepellin (~85 kDa) was not detected in the both healthy and cervical cancer urine samples. The expression level of endorepellin LG3 fragment (25 kDa) was quantitated by densitometer and a *t-test* analysis (Figure 3B and 3C). The result showed that the endorepellin LG3 fragment level increased at 1.8-fold in urine from the cervical cancer patients compared to healthy donors (p=0.014).

2D Western blot analysis confirmed that endorepellin LG3 fragment was over-expressed in urine of cervical cancer patients (Supporting Information Figure S2). Nano LC-MS/MS analysis was performed to confirm the representative 2D Western blot of the healthy donors and cervical cancer patients, which is shown in the Supporting Information Figure S3a and S3b). This spot was identified as endorepellin LG3 fragment (amino acids 3687-4391). The endorepellin LG3 fragment had Mascot Score of 1620, sequence coverage of 3% and 95% queries matched peptide.

Discussion

In this study, we found the protein spot 18 which was identified from the protein database search as perlecan, a core membrane protein of molecular weight 480 kDa. MW of the protein spot was estimated to be 24 kDa on 2-DE gel (Figure 2A and 2B). We found that one tryptic peptide that matched to perlecan by MS/MS, as well as PMF, resided on the C-terminal domain V of perlecan, commonly known as endorepellin (Table 2). Endorepellin blocks endothelial cell migration and capillary morphogenesis, both *in vitro* and *in vivo* [26]. Endorepellin interacts specifically with the α2β1 integrin [27], and stimulation with endorepellin induces the interaction and phosphorylation of Src homology-2 protein phosphatase-1 with integrin α2 in endothelial cells [28]. Endorepellin LG3 fragment (amino acids 3687–4391) has potent antiangiogenic properties [29].

Gonzalez et al. [30] found that perlecan has specific proteolytic cleavage by bone morphogenic protein-1 (BMP-1), and it is known that BMP-1 cleaves endorepellin at a single specific site between Asn4196 and Asp4197. Because the matched tryptic peptides were found at the C-terminal part of the known cleavage site, we suspected that the 25 kDa protein is the endorepellin LG3 fragment liberated by BMP-1 protease. These finding suggest that endorepellin LG3 fragment might be released by a concerted action of various proteases, thereby allowing the diffusion of powerful bioactive proteins within the renal parenchyma and in the urine. Endorepellin LG3 fragment is a compact globular protein with a highly negative charge (pI=5) [30]. It is known that endorepellin LG3 could bypass the anionic filter provided by the glomerular basement membrane. Besides, endorepellin, C-terminal LG3 fragment has been found in many body fluids, including blood, urine and amniotic fluid [26]. Endorepellin is secreted by pre-apoptotic endothelial cells *via* cathepsin L [31]. Endorepellin LG3 fragment has been found in patients with several cancers, including pancreatic, colon and breast cancer [26]. Indeed, endorepellin LG3 fragment is liberated *via* partial proteolysis during tissue remodeling and cancer growth, thereby representing an additional layer of control for angiogenesis [26]. Endorepellin LG3 fragment found as an effective *in vivo* antitumor vasculature agent that can be used therapeutically to treat cancer through its action of inhibiting *in vivo* tumor growth and tumor cell metabolism by specifically blocking tumor angiogenesis [32].

Endorepellin LG3 fragment has been found at elevated level in the urine of patients with end stage renal disease and in the amniotic fluid of pregnant women with symptoms of premature rupture of fetal membranes [33,34]. Circulating LG3 levels are reduced in breast cancer patients, suggesting that reduced titers might be a useful biomarker for cancer progression and invasion [35]. Parker et al. [36] reported that endorepellin LG3 peptide is present in the urine of physically active mine workers. It is also possible that the activity mediated the release of endorepellin LG3 fragment into the circulation. In addition, endorepellin LG3 fragment has been found to increase in the urine of patients with IgA nephropathy (IgAN) and may be served as a urinary biomarker for IgAN [37].

Spot no.	Protein name	Acc. No.[a]	Theor. MW/pI[b]	Observed MW/pI	Queries Matched	Mowse score	Matching peptide sequences [c]
2	PCDH8_HUMAN, Protocadherin-8	O95206	112.9/5.35	32.38/5.01	3	40	(R) LVHVEVEVRDVNDHAPR (F) (R) IAEYNLTLVAEDRGAPPLR (T) (K) KDLINHMQSGLWACTAECK (I)
5	BMAL2_HUMAN, Aryl hydrocarbon receptor nuclear translocator-like protein 2	Q8WYA1	70.8/7.01	35.46/5.19	3	45	(R) SWPPNIVGMEEER (N) (R) LQSSSYLDDSSPTGLMK (D) (K) ELFPPSPSEMGELEATR (Q)
13	ALBU_HUMAN, Serum albumin	P02768	69.3/5.92	34.79/5.38	1	32	(K) KVPQVSTPTLVEVSR (N)
16	ALBU_HUMAN, Serum albumin	P02768	69.3/5.92	64.3/5.46	1	36	(R) RHPDYSVVLLLR (L)
17	ALBU_HUMAN, Serum albumin	P02768	69.3/5.92	64.29/5.60	4	79	(R) RHPDYSVVLLLR (L) (K) KVPQVSTPTLVEVSR (N) (R) RHPYFYAPELLFFAK (R) (K) VFDEFKPLVEEPQNLIK (Q)
18	PGBM_HUMAN, Basement membrane- specific heparan sulfate proteoglycan core protein (Endorepellin; the C-terminal domain V of perlecan)	P98160	468.5 / 6.06	23.96/5.93	1	60	(R) SLPEVPETIELEVR (T)
24	ALBU_HUMAN, Serum albumin	P02768	69.3/5.92	62.78/5.92	3	70	(R) RHPDYSVVLLLR (L) (K) KVPQVSTPTLVEVSR (N) (R) RHPYFYAPELLFFAK (R)

a) Accession number according to SwissProt database
b) pI and MW values are calculated by the MASCOT software
c) Matching peptide sequences and percentage of amino acid sequence coverage are given by MASCOT for the identified protein

Table 2: Protein annotation of candidate protein markers in cervical cancer urine based on the SwissProt database.

Conclusion

In this work, an alternative and convenient technique to reduce any interference in urine prior to presenting it to the gel-based approaches is shown. With this technique, we presented a high-resolution view of urinary protein derived from cervical cancer patients. Through proteomic tools, the four candidate biomarkers, PCDH8, ARNTL2, serum albumin and endorepellin, the C-terminal domain V of perlecan, were shown to be significantly up-regulated in cervical cancer urine patients. The validation of protein expression by Western blot analysis showed the significantly altered expression of endorepellin LG3 fragment, which may be applied as a potential parameter for cervical cancer diagnosis, prognosis or therapeutic application. Further investigations of the endorepellin LG3 fragment protein will lead to verification of protein markers in large-scale study for various human material. The relationship of endorepellin LG3 fragment in cervical cancer is necessary for understanding and clarifying the view of biological mechanisms of cervical cancer.

Conflict of Interest

The authors have declared no conflict of interest.

Acknowledgements

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