Plasma proteome analysis of cervical intraepithelial neoplasia and cervical squamous cell carcinoma

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Although cervical cancer is preventable with early detection, it remains the second most common malignancy among women. An understanding of how proteins change in their expression during a particular diseased state such as cervical cancer will contribute to an understanding of how the disease develops and progresses. Potentially, it may also lead to the ability to predict the occurrence of the disease. With this in mind, we aimed to identify differentially expressed proteins in the plasma of cervical cancer patients. Plasma from control, cervical intraepithelial neoplasia (CIN) grade 3 and squamous cell carcinoma (SCC) stage IV subjects was resolved by two-dimensional gel electrophoresis and the resulting proteome profiles compared. Differentially expressed protein spots were then identified by mass spectrometry. Eighteen proteins were found to be differentially expressed in the plasma of CIN 3 and SCC stage IV samples when compared with that of controls. Competitive ELISA further validated the expression of cytokeratin 19 and tetranectin. Functional analyses of these differentially expressed proteins will provide further insight into their potential role(s) in cervical cancer-specific monitoring and therapeutics.

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1. Introduction

Cervical cancer is malignancy of the cervix uteri or cervical area. The most common histological presentation of the disease is squamous cell carcinoma (SCC), which accounts for between 80% and 85% of all cervical cancers. It is classified into four stages (I, II, III and IV) according to the Federation of International Gynecologists and Obstetricians (FIGO) staging system (Shepherd 1996). SCC is preceded by dysplasia that is also described as cervical intraepithelial neoplasia (CIN) or squamous intraepithelial lesions (SIL) (Tjalma *et al.* 2005). CIN is graded as CIN

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Abbreviations used: 2DE, two-dimensional gel electrophoresis; ACHCA, α-cyano-4-hydroxycinnamic acid;ANOVA, analysis of variance; cELISA, competitive ELISA; CIN, cervical intraepithelial neoplasia; FIGO, Federation of International Gynecologists and Obstetricians; HRP, horseradish peroxidase; IEF, isoelectric focusing; Ig, immunoglobulin; MALDI-TOF/TOF MS, matrix-assisted laser desorption/ionization time-of-flight tandem mass spectrometry; nCLU, nuclear form of clusterin; Pap, Papanicolaou; SCC, squamous cell carcinoma; sCLU, secretory form of clusterin; SIL, squamous intraepithelial lesion; TNF, tumour necrosis factor

1, 2 and 3, indicating mild, moderate and severe cervical cell changes. These pre-neoplastic changes may develop into cancer over a period of ten years or more if left unattended. It is widely accepted that the development of the disease involves continuous histopathological changes from normal epithelium through CIN to invasive cancer. However, the manner in which the cells are transformed has never been fully understood. Moreover, the effects of these transformations on the plasma protein profile are also not entirely known.

Although preventable with early detection, cervical cancer remains the second most common malignancy among women worldwide (Petignat and Roy 2007). The disease is more common in developing countries due to the lack of mass screening programmes. In Malaysia, a programme for the early detection of cervical cancer using Papanicolaou (Pap) smear was introduced three decades ago. However, women remain reluctant to undergo screening due to psychological barriers (Winarti 2008). A survey of the Malaysian population showed that the coverage of Pap smear was only 26% (MOH 1999). Following intensified health education programmes by the Malaysian government, the Ministry of Health now reports that coverage has increased to 55% of the population at risk. However, this coverage is still unsatisfactory. To date, screening programmes using Pap smear remain the most important and useful tool in the early detection of cervical cancer. However, there are limitations to the existing Pap smear screening procedures. Among them is the significant occurrence of false-positive and false-negative results (Nijhuis *et al.* 2006).

To improve the efficiency of disease screening, the current trend is to use biological and/or molecular markers in conjunction with available techniques. Current developments in molecular biology and proteomics are ushering a new era of biomarker discovery and identification of new molecular targets related to carcinogenesis. This is further assisted by the completion of the genome projects leading to high-throughput identification of disease-related proteins. These advancements may lead to an improved understanding of carcinogenesis and potentially greatly facilitate the screening, early detection and management of cancers.

The presence of a particular protein and/or its isoforms represents the likelihood of the presence of other biologically active molecules. These in turn may correspond to cellular functions. Since cellular functions often change during different disease states, the measurement of protein expression and modification may lead to the detection and identification of a particular disease. To the best of our knowledge, there are no plasma or serum biomarkers with high sensitivity and specificity for the diagnosis and monitoring of cervical cancer. Individuals with the disease are identified following the development of symptoms, Pap smear results and/or abnormalities detected on colposcopic assessments. We therefore aimed to identify differentially expressed proteins in the plasma of cervical cancer patients as these have the potential to be developed as biomarkers for detection of the disease. These differentially expressed proteins may also play roles in disease development and manifestation.

In this study, the plasma proteome profile of healthy individuals was compared with that of patients with CIN and SCC. The plasma proteome was first resolved by two-dimensional gel electrophoresis (2DE). The resulting gels were then compared to identify proteins that were differentially expressed. The identity of these proteins was determined by matrix-assisted laser desorption/ionization time-of-flight tandem mass spectrometry (MALDI-TOF/TOF MS). The expression of three of these proteins was then confirmed by competitive ELISA (cELISA).

2. Materials and methods

2.1 *Patients and controls*

We conducted the study on 80 patients newly diagnosed with CIN and SCC of the cervix who attended the Obstetrics and Gynecology clinic, and 40 healthy volunteers at the Universiti Kebangsaan Malaysia Medical Centre between January 2004 and June 2006. All the patients were recruited with approval from the Ethical Committee of the School of Medicine, Universiti Kebangsaan Malaysia and written consent was obtained from all of them. The patients had not received any prior treatment. Cervical lesions were confirmed by Pap smear, colposcopy observation and punch biopsy.

Patients with newly diagnosed SCC of the cervix were clinically staged in accordance with the FIGO criteria. Of 40 patients with SCC of the cervix, 11 were in SCC stage I, 13 in stage II, seven in stage III and nine in stage IV. Twenty-two patients presented with CIN 1, seven had CIN 2 and 11 had CIN 3. Forty healthy volunteers with normal Pap smears and biochemical results were recruited as control subjects.

Blood samples were collected by venous arm puncture and placed in EDTA tubes. Plasma was separated by centrifugation at 800x*g* for 10 min at 4°C and kept in aliquots of 50 μl at –80°C for further analysis. Protein concentration was determined using the Bio-Rad Protein Assay kit (Bio-Rad, USA). Plasma samples of four normal controls, four CIN 3 and four SCC IV patients were subjected to 2DE. All samples were analysed in triplicate. Validation studies were then conducted by ELISA on all the 120 samples collected.

2.2 *Isoelectric focusing (IEF)*

First-dimension IEF was performed on an IPGPhor II IEF System (GE Healthcare Bio-Sciences, Uppsala, Sweden).

Plasma samples (equivalent to 100 μg protein) were diluted in buffer containing 8 M urea, 2% (w/v) CHAPS, 2% (v/v) IPG buffer pH 4–7, 2.8 M DTT and trace amounts of bromophenol blue. In-gel rehydration of Immobiline DryStrip gels (isoelectric point [p*I*] 4–7, 24 cm; GE Healthcare Bio-Sciences, Uppsala, Sweden) was then performed and the proteins separated by IEF up to 70 kVh.

A total of 100 μg protein was loaded to provide the best gel resolution as higher protein concentrations would give rise to saturated spots when the gels were silver stained and scanned using a densitometer. The presence of saturated spots would affect the linearity of the analysis, thus causing inaccurate quantitation of protein spots.

2.3 *Second-dimension SDS PAGE*

Focused IPG strips were equilibrated for 15 min in equilibration solution (6 M urea, 75 mM Tris-HCl pH 8.8, 30% [w/v] glycerol, 2% [w/v] SDS, 0.002% [w/v] bromophenol blue) containing 1% DTT and then alkylated for a further 15 min in equilibration solution containing 2.5% iodoacetamide. Electrophoresis of reduced and alkylated samples was then carried out using 24 cm 11% SDS–PAGE gels (Ettan DALT 12, GE Healthcare Bio-Sciences, Uppsala, Sweden).

2.4 *Silver staining and image analysis*

Protein spots were visualized using the protocols described in the PlusOne™ Silver staining kit (GE Healthcare Bio-Sciences, Uppsala, Sweden). The complete protocol was followed for analytical gels. For preparative gels, the protocol was modified (Yan *et al.* 2000) so that glutaraldehyde was omitted from the sensitization step and formaldehyde omitted from the silver reaction step. Silver-stained gels were scanned (Image scanner, GE Healthcare Bio-Sciences, Uppsala, Sweden) and protein profiles compared (Image Master Platinum software, GE Healthcare Bio-Sciences, Uppsala, Sweden). Differentially expressed spots were then excised for mass spectrometric analysis.

2.5 *Mass spectrometric (MS) protein identification*

Proteins of interest were identified at the Genome Research Centre, University of Hong Kong. Briefly, excised protein spots were trypsin digested and MS analyses performed using the ABI 4800 MALDI TOF/TOF analyser (Applied Biosystems, USA) using a saturated solution of α-cyano-4-hydroxycinnamic acid (ACHCA) in 0.5% TFA/50% ACN as a matrix. MS reflector positive was set at an m/z range of 1000–4000 Da. Tamdem mass spectrometry (MS/MS) spectra were obtained with MS/MS at 2 kV (collision induced dissociation on), and the peptide fragment ion with more than five monoisotopic precursors was selected when the signal-to-noise ratio was more than 200. Combined peptide mass spectra and MS/MS data were then collected and submitted for database searching with the GPS Explorer™ 3.5 Software using the MASCOT and NCBInr search engine and databases.

2.6 *Competitive ELISA (cELISA)*

The expression dynamics of cytokeratin 19, clusterin and tetranectin were validated across all 120 plasma samples by cELISA as previously described (Xin *et al.* 2005) with some modifications. The appropriate concentrations of primary and secondary antibodies were determined with cELISA by checkerboard titration. The levels of cytokeratin 19, clusterin and tetranectin in plasma samples were reflected by their ability to inhibit the specific binding of antibody to the respective plasma proteins. Primary antibodies against cytokeratin 19, clusterin and tetranectin were obtained from Abcam (UK). The secondary antibody was horseradish peroxidase (HRP)-conjugated rabbit anti-mouse immunoglobulin (Ig)G (NEN, USA). The amount of specific proteins in the test plasma was proportional to the percentage inhibition of substrate hydrolysis. Percentage inhibition was calculated as follows (Reddington *et al.* 1991):

$$\text{Percentage inhibition} = [1 - (\text{sample OD}/\text{control OD})] \times 100$$

2.7 *Statistical analysis*

Only proteins that differed in their expression by at least two-fold were considered as being differentially expressed. Results were expressed as mean volume ratio. Statistical analysis was performed by analysis of variance (ANOVA) at $P<0.05$. Statistical analyses were performed using SPSS 11.0 (SPSS Inc., Chicago, Illinois, USA).

3. Results

3.1 *Two-dimensional gel electrophoresis*

Two-dimensional gel electrophoresis (2DE) of unfractionated whole plasma allowed the detection of more than 300 well-resolved protein spots by silver staining (figure 1). The 2DE gels were highly reproducible and image analysis performed on triplicate 2DE gels of plasma samples produced minimal inter-sample and intra-sample variation in terms of normalized volumes of plasma proteins and proteome profile. Eighteen proteins were found to be differentially expressed in the plasma of cancer patients at different stages

of the disease (table 1). In CIN 3 patients, seven proteins showed upregulated expression and four proteins showed downregulated expression when compared with controls. In SCC IV, eight proteins were upregulated and six proteins downregulated when compared with controls. Although these spots represent medium-to-high abundance proteins, previous studies on whole, unfractionated serum and plasma samples demonstrated that high-abundance proteins could be used as markers for disease states (Gianazza *et al.* 2002; Doustjalali *et al.* 2004; Yong *et al.* 2006).

3.2 *Identification of proteins by mass spectrometry*

From a total of 36 differentially expressed protein spots, 18 different proteins were identified. Twelve of these proteins were classified into five functional categories – acute phase proteins, transport proteins, coagulation factors, cytolysis inhibitor proteins and structural proteins (table 1). A further three were assigned to a category named 'others'. Interestingly, there were also three proteins of unknown function.

The expression of three proteins – a 21 kDa fragment of cytokeratin 19, clusterin and tetranectin – were validated by cELISA across all 120 samples. Cytokeratin 19 and tetranectin showed a similar expression profile to that observed by 2DE analyses and this was statistically significant ($P<0.05$). However, validation of the expression dynamics of clusterin by cELISA showed no significant difference in expression between the different samples (figure 2a, b and c).

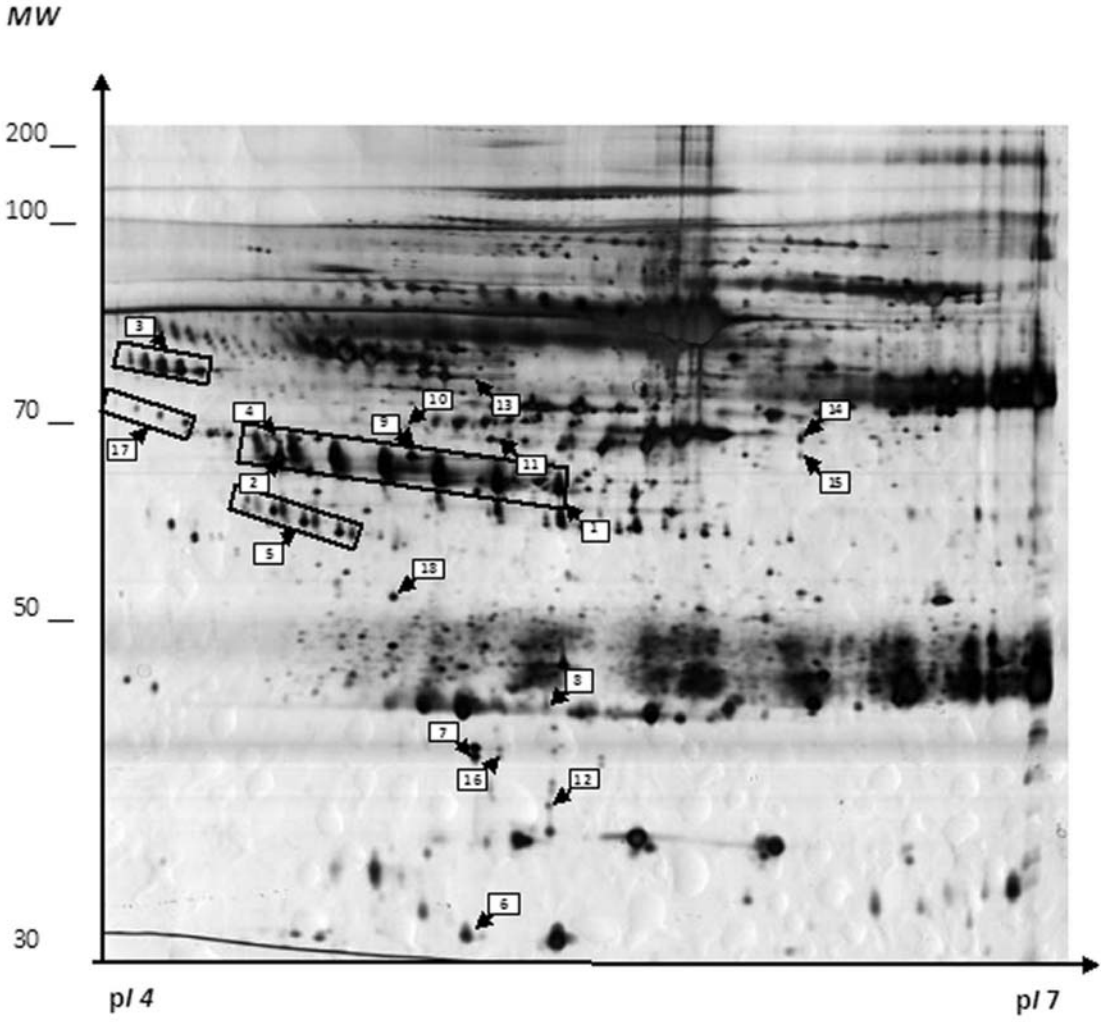

Figure 1. A representative gel of the human plasma proteome (normal control), p*I* 4–7, showing spots that are differentially expressed in cervical intraepithelial neoplasia (CIN) 3 and squamous cell carcinoma (SCC) IV. Spots identified are numbered and their details are described in table 1. MW, molecular weight; p*I*, isoelectric point.

Table 1. Differentially expressed proteins in plasma from CIN 3 and SCC IV patients identified by matrix-assisted laser desorption/ionization time-of-flight tandem mass spectrometry (MALDI-TOF MS/MS)

	Protein name and known function(s)	Accession number (NCBI)	Mw (kDa)/p*I*		Peptides matched/% coverage	*Expression change vs control/(mean volume ratio)	
			Theoretical	Experimental	(Protein score)	CIN3	SCC IV
Acute phase proteins							
1	Haptoglobin - Antioxidant, reduces pro-oxidant effect of haemoglobin/haem - Induces angiogenesis, cholesterol crystallization and inhibits prostaglandin synthesis - Anti-inflammatory effect	1212947	38.9/6.27	46.0/4.75	11/38% (478)	NS	↑ /1.33
2	Complement component 3 precursor - Component of immune system	4557385	188.6/6.02	47.5/4.64	26/15 % (496)	↑ /0.08	NS
3	Alpha-2-HS-glycoprotein - Anti-inflammatory effect, modulates phagocytosis of apoptotic cells by macrophages	29387000	40.1/5.43	60.0/4.37	7/23% (320)	NS	↓ /–0.84
4	Complement C4d variant - Component of immune system	223962	31.2/4.93	49.0/4.60	7/26% (314)	NS	↑ /0.54
5	Complement cytolysis inhibitor precursor - Component of immune system	180620	52.9/5.89	38.0/4.79	10/24% (252)	↑ /0.71	NS
Transport proteins							
6	Transthyretin - Transport protein for tyrosine	339685	12.8/5.33	15.0/5.25	3/23% (150)	NS	↑ /0.31
7	Retinol-binding protein - Transports retinol and forms complex with transthyretin	230284	21.3/5.27	23.0/5.27	7/30% (92)	↑ /0.39	NS
8	Proapolipoprotein - Involved in reverse cholesterol transport - Potent cofactor for enzyme lecithin	178775	28.9/5.45	25.5/5.45	18/60% (482)	NS	↑ /0.48
9	Apolipoprotein A-IV precursor - Glycoprotein, associates with triglyceride-rich and high-density lipoproteins	178779	43.4/5.22	47.5/5.05	29/65% (555)	↑ /0.81	NS
Coagulation factor							
10	Fibrinogen - Converted to fibrin with the presentation of prothrombin - Key role in inflammatory response	4503715	50.1/5.61	49.0/5.04	5/13% (72)	↑ /0.10	↑ /0.48
Cytolysis inhibitor protein							
11	Clusterin - Presented in nuclear (nCLU) and secretory (sCLU) form - Functional role unclear, associated with programmed cell death (apoptosis)	4102235	39.6/5.28	48.0/5.30	15/57% (579)	↑ /0.49	↑ /–0.48

Table 1. *(Continued)*

Structural proteins							
12	Cytokeratin 19 - Regulation of cell migration and invasion by promoting cell mobility or extracellular degradation which facilitates tumour metastasis	34783124	45.6/5.11	21.0/5.49	20/44% (199)	NS	↑ /0.93
Proteins of unknown function							
13	Unknown protein	28207863	41.6/5.14	62.4/5.31	22/50% (523)	↓ /–0.90	↓ /–1.57
14	Unknown protein (protein for MGC: 88775)	49258102	66.0/8.10	48.0/6.28	11/19% (221)	↓ /–0.50	↓ /–0.52
15	Unknown protein	33869608	65.9/8.45	46.0/6.28	12/24% (482)	↓ /–0.52	↓ /–0.52
Others							
16	Tetranectin - Involved in plasminogen activation and increases fibrinolysis - Primarily found in plasma and in the extracellular matrix of certain human carcinomas	2781225	20.6/5.80	23.4/5.35	4/65% (108)	↓ /–0.56	↓ /–0.91
17	Leucine-rich alpha 2 glycoprotein - Involved in haemostasis	15321646	38.3/6.45	52.0/4.32	9/28% (421)	↑ /0.70	↑ /0.45
18	Alpha-1-microglobulin 1/bikunin precursor - Protects against haem-induced oxidative damage	55957384	39.9/5.95	30.0/5.00	11/38% (494)	NS	↑ /1.13

*Only proteins that differed in their expression by at least two-fold at $P<0.05$ were considered as being differentially expressed. Results are expressed as mean ratio. Their relative abundance compared with the control is represented by NS (no significant change in expression), ↑ (upregulated expression) and ↓ (downregulated expression).

4. Discussion

Proteomics is opening up new avenues in cancer research, with its ability to look directly at protein expression and identify changes associated with the progression of a particular disease. Potentially, it may uncover potential biomarkers as well as provide new insights into the molecular mechanisms of disease. In this study, differential proteome analysis using 2DE gels was used to identify 18 proteins that were differentially expressed in the plasma of CIN and SCC patients. Our study design was comparable to that of Bae *et al.* (2005), who identified 35 proteins with altered expression levels in cervical cancer patients. Interestingly, although different sample types were analysed, cytokeratin 19 was upregulated in both studies.

Cytokeratins are the most abundant filament protein in epithelial cells. Their primary function is to protect epithelial cells from stress damage that may result in cell death. Modification at the amino and carboxyl terminals of cytokeratin (phosphorylation, glycosylation and transglutamination) occurs during transformation of normal cells into malignant cells (Coulombe and Omary 2002). These modification events subsequently increase filament solubility. Tumour necrosis and cell lysis in carcinoma may release cytokeratin fragments into biological fluids including blood, urine and fluid in the abdomen and lung from ascites and pleural effusion, respectively (Uenishi *et al.* 2006) by an unknown mechanism. However, the release of cytokeratin fragments is associated with the proteolytic degradation of cytokeratin during cell death, abnormal mitosis and spillover from cells undergoing proliferation and apoptosis (Oshima 2002). A previous study has shown that cytokeratin levels are relatively low in healthy subjects and elevation is associated with apoptotic resistance and malignant progression of cervical carcinoma (Yuan *et al.* 1998).

In this study, cytokeratin 19 was upregulated in the plasma of SCC patients as shown by 2DE and confirmed by cELISA (figure 2a). A cytokeratin 19 fragment, or CYFRA 21-1, has previously been identified in cervical SCC (Yuan *et al.* 1997), where elevated levels are associated with increased

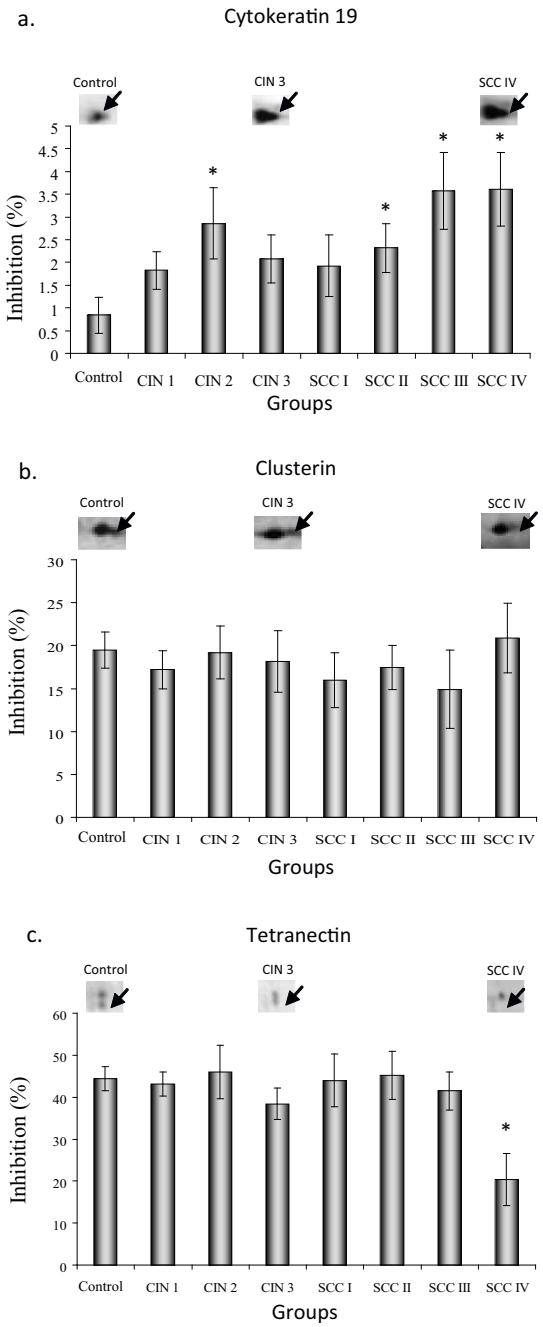

Figure 2. Analysis of plasma cytokeratin 19 **(a)**, clusterin **(b)** and tetranectin **(c)** expression by competitive ELISA. Competitive ELISA was performed using primary antibodies specific for these proteins in the presence of plasma from control subjects, and those with cervical intraepithelial neoplasia (CIN) 1, CIN 2 and CIN 3, and squamous cell carcinoma (SCC) I, SCC II, SCC III and SCC IV. Analyses were performed in triplicate. The amount of antigen in the test plasma is proportional to the percentage inhibition of substrate hydrolysis. 2DE resolution of the three proteins is depicted above the control, CIN 3 and SCC IV sample groups. Asterisks denote statistically significant differences (P<0.05) when compared with the control.

tumour number and size (Uenishi *et al.* 2006). Cytokeratins play a dual role in apoptosis (Marceau *et al.* 2007), and type I cytokeratins (cytokeratins 14, 15, 17, 18 and 19) are substrates for caspases (Oshima 2002). *In vitro* experiments have demonstrated that fragments of cytokeratins are released from cells into the extracellular space as soon as they are cleaved by caspases (Barak *et al.* 2004). However, recent studies have also revealed an unexpected protective role for cytokeratin during tumour necrosis factor (TNF)- and Fas-mediated apoptosis. Cytokeratin cleavage by caspases subsequently limits the availability of active caspases, which are required for apoptosis, and hence facilitates the process of carcinogenesis. The upregulation of cytokeratin 19 in SCC patients observed in this study may be attributed to cleavage of cytokeratins by caspase, of which the fragment (~21 kDa) is then released into the bloodstream of CIN and SCC patients.

We also identified a ~48 kDa clusterin that was upregulated in CIN patients and downregulated in SCC patients. Clusterin, also known as apolipoprotein J, is a glycoprotein that is widely distributed in various tissues and its expression correlates with cell response to stress, cell damage recovery, senescence, tumorigenesis and apoptosis (Shannan *et al.* 2006). Clusterin expression is complex, as the protein appears as an 80 kDa secretory form (sCLU), composed of 40 kDa alpha and beta subunits, and a ~55 kDa nuclear form (nCLU). A previous study has proposed that sCLU has a cytoprotective effect, whereas elevated levels of nCLU may trigger cell death (Criswell *et al.* 2003). Overexpression of sCLU has been demonstrated in various malignancies, such as colorectal (Chen *et al.* 2003), prostate (Pins *et al.* 2004), breast (Itahara *et al.* 2007), renal (Kurahashi *et al.* 2005) and liver cancers (Kang *et al.* 2004).

It has been proposed that, during carcinogenesis, clusterin may act in a biphasic manner as a tumour attenuator and an enhancer of the malignant phenotype (Thomas-Tikhonenko *et al.* 2004). High levels of nCLU may be protective against the early stages of neoplastic growth. However, the lack of nCLU and an elevated level of sCLU during the advanced stages of cancer increase susceptibility to tumorigenesis. We propose that the ~48 kDa clusterin identified in our study is the nCLU form, which increased in CIN and decreased in SCC in accordance with its biphasic mode of action. However, although a similar trend was observed on 2DE, the expression dynamics of clusterin could not be statistically confirmed by cELISA (figure 2b).

We also verified the expression dynamics of tetranectin by cELISA and found it to be downregulated in SCC patients (figure 2c) in a statistically significant manner. Tetranectin is a C-type lectin plasminogen-binding protein. Its binding enhances plasminogen activation, and increases fibrinolysis (Holtet *et al.* 1997). Fibrinolysis degrades the stroma and

facilitates invasion by tumour cells. Tetranectin has also been implicated in cancer progression, where it affects the activity of angiostatin. Angiostatin is an endogenous angiogenesis inhibitor that is formed by proteolytic degradation of plasminogen in cancer tissues. Tetranectin is hypothesized to bind to angiostatin, thereby modifying its functional activity and promoting angiogenesis (Mogues *et al.* 2004). Fibrinolysis and angiogenesis are important processes related to cancer growth, invasiveness and metastasis (Mogues *et al.* 2004). Increased expression of tetranectin in malignant tumour tissue results in decreased levels of tetranectin in the blood (Hogdall *et al.* 1995). Based on these observations, one possible explanation for the lower tetranectin levels in the plasma of cancer patients is that tetranectin is removed from the blood by proteolytically active and malignant tumours.

Several acute-phase proteins – haptoglobin, alpha-2-HS-glycoprotein, complement C4d variant, complement component 3 precursor and complement cytolysis inhibitor precursor – were also identified as being differentially expressed. An increase in the expression of acute-phase proteins in the blood of cancer patients has been previously reported (Ferrero *et al.* 2005; Zhao *et al.* 2007). However, these proteins may not be specific enough for cancer diagnosis as they are also seen in other diseases involving inflammation. It is likely that changes in the level of acute-phase proteins in cancer may be due to the host response to tumour necrosis and bleeding.

Several proteins that were differentially expressed in our 2DE analysis of the plasma from CIN and SCC patients have been previously shown to be similarly expressed in other cancers of the female reproductive organs. Alpha-2-HS glycoprotein is downregulated (Pawlik *et al.* 2006) and fibrinogen is upregulated (Somiari *et al.* 2005) in breast cancer patients. Increased expression of haptoglobin and transthyretin has been observed in ovarian cancer patients (Ye *et al.* 2003; Alaoui-Jamali and Xu 2006). The similar expression profile of these proteins may be due to a common biological function in the female reproductive organs.

We also identified three proteins of unknown function that were downregulated in the plasma of CIN and SCC patients. These proteins may be interesting candidates for further studies. Two other proteins, leucine-rich α2 glycoprotein and α1 microglobulin, were upregulated in the serum of cervical cancer patients. Further functional analysis of these proteins may provide insights into their possible physiological roles in tumour aetiology.

This study demonstrates the feasibility of using 2DE in identifying differentially expressed proteins that may be involved in the progression of cervical cancer from CIN to SCC. However, it is clear from the analysis that higher resolution maps displaying a larger proportion of the plasma proteome need to be constructed to fully examine the changes that may be related to cancer. Furthermore, changes in the proteome observed in this analysis require validation over a much larger sample size for sensitivity, and different cancer types for specificity. While it is unlikely that the change in expression of a single protein will be a specific indicator for a particular disease such as cervical cancer, an understanding of how a number of different proteins behave in combination may provide an indication of the occurrence of a disease. Ongoing investigation into the roles of these differentially expressed proteins will determine their possible use and specificity in the diagnosis and monitoring of cervical cancer.

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