ORIGINAL ARTICLE

Vol. 38 (6): 739-749, November - December, 2012

Downregulation of C3 and C4A/B complement factor fragments in plasma from patients with squamous cell carcinoma of the penis

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ABSTRACT

Purpose: To investigate the use of ClinProt technique to identify cancer markers in plasma of patients suffering from squamous cell carcinoma of the penis (SCCP).
Materials and Methods: Plasma of 36 healthy subjects and 25 patients with penile carcinoma who underwent surgical treatment between June 2010 and June 2011 was collected and analyzed by the ClinProt/MALDI/ToF technique. Then the peptides were identified from the C8 MB eluted fraction of patients' and control subjects' plasma by LIFT MS/MS.
Results: A cluster of 2 peptides (A=m/z 1897.22 ± 9 Da and B=m/z 2021.99 ± 9 Da) was able to discriminate patients from control subjects. Cross validation analysis using the whole casuistic showed 62.5% and 86.76% sensitivity and specificity, respectively. The cluster also showed very high sensitivity (100%) and specificity (97%) for SCCP patients that died due to the disease. Furthermore, patients with lymph node involvement presented sensitivity and specificity of 80% and 97%, respectively. These two peptides were identified by the proteomic approach based on a MALDI-TOF/TOF as fragments of C3 (m/z 1896.17) and C4a/b (m/z 2021.26) complement proteins.
Conclusions: The results showed that as the disease progresses, the fragments C3 and C4 A/B are less expressed in comparison with healthy subjects. These results may be useful as prognostic tools.

ARTICLE INFO

Key words:
Penis; Penis Cancer; Carcinoma, Squamous Cell; Plasma; Proteomics; Complement C3b Inactivator Proteins

Int Braz J Urol. 2012; 38: 739-49

Submitted for publication:
August 02, 2012

Accepted after revision:
October 16, 2012

INTRODUCTION

Squamous cell carcinoma of the penis (SCCP) is a rare disease in developed countries, but in emergent countries it can account for 10% of male neoplasms (1,2). Its etiology is not fully understood, but there is a strong association with poor hygienic conditions, phimosis and human papillomavirus (HPV) infection (2,3). Metastases from penile carcinoma usually spread through penile lymphatic vessels to regional nodes, especially the superficial and deep inguinal nodes, and subsequently to the iliac nodes within the pelvis. Tumor involvement of inguinal lymph nodes is the best indicator of long-term survival in patients with invasive SCCP (1). Twenty to fifty percent of

patients with penile carcinoma present inguinal involvement at diagnosis and physical examination is a not a reliable predictor or lymph nodes status (2). Therefore, reliable staging information can only be acquired through surgical procedures with subsequent histologic examination of the inguinal lymph nodes. The pathologic factors with known prognostic value, other than the presence of lymph node metastasis, include tumor thickness, grade, histologic type, lymphovascular embolization, presence of koilocytosis and stage (4-6). On the clinical nodal status there is a dilemma: a significant number of patients with palpable lymph nodes do not have metastasis, in the other hand, 20% of patients with clinically non-suspicious nodes present micrometastasis at pathological examination. Thus, prophylactic bilateral inguinal lymph node dissection is considered unnecessary in up to 80% of penile carcinoma patients with clinically negative regional lymph nodes (7).

While DNA is the information archive, proteins do the work for the cell. It has been shown that direct gene expression is only responsible for a small part of the complexity of a living organism (8). Proteomics is the large-scale study of proteins, and is associated traditionally with displaying a large number of proteins from a given cell line or organism. Curiously, there is no strict linear relationship between genes and the protein complement or 'proteome' of a cell. Proteomics is complementary to genomics, because it focuses on the gene products, which are the active agents in cells. There are two strategies for finding protein biomarkers in tissues or in biological fluids. Several possible biomarkers have been identified using a gel-based approach in bi-dimensional electrophoresis (with and without stable isotopic labeling) and mass spectrometry (9,10). However, since this approach is very laborious and time-consuming, it is not practicable in clinical chemistry laboratories. Other methods based on the biological fluid proteome prefractionation have been recently made available (11,12). The successful discovery of a proteomic profile correlated to an altered state by the ClinProt method has been reported in various human diseases, such as oral, bladder, nasopharyngeal and neck cancer (13-15). Recently, we have used this approach to find possible ccRCC biomarkers in urine and serum (12,16). In view of these results, we investigated the possibility of using the ClinProt technique to find possible plasma diagnostic markers that can better distinguish healthy subjects from patients affected by SCCP.

MATERIALD AND METHODS

Chemicals

The C8-Hydrophobic kit, α-cyano-4-hydroxycinnamic acid (HCCA) and Protmix1 were purchased from Bruker Daltonics, GmbH (Bremen, Germany). acetonitrile from Merck KGaA (Darmstadt, Germany) and methanol from Sigma-Aldrich, Inc. (St. Louis, MO).

Patients and Blood Sample Collection

Twenty-five patients with penile cancer being treated by the National Cancer Institute and Mário Kröeff Hospital were enrolled in this study after permission of the hospital's ethic committee. In addition, we used blood samples from thirty-six healthy subjects (blood donor volunteers) who underwent circumcision in Santa Veronica Hospital. Informed consent was obtained from all patients. After diagnosis of penile cancer, 5 ml of blood was collected before surgery and stored in a BD vacutainer with EDTA. About 2.5 ml of plasma was obtained after centrifugation at 1500 g for 10 min. within 4 hours of collection. The samples were frozen on dry ice and sent to Milano Bicocca University in Italy. All the samples were stored at -80 °C until being sent to Italy.

The mean age of the patients and controls was 63.56 (range, 38-90) and 60 years (range, 23-83), respectively. Follow-up was evaluated in all patients. Median follow-up was 9 months (range 1 to 36). The data collected from the patients' medical records are shown in Table-1. Due to the small number of patients, no stratification according to risk factors for penile cancer was performed.

Study Design

The entire data set, composed of 36 controls and 25 penile cancer patients, was randomly split into two groups. The first group (training data set: 28 controls and 17 penile cancer patients) was

used for the identification of signals related to peptides expressed differentially in penile cancer patients compared with controls (pattern recognition). The second group (test data set: 8 controls and 8 penile cancer patients) was used for preliminary pattern validation of the cluster.

Sample Purification

ClintProt was applied to analyze the plasma samples collected from SCCP patients. Healthy subjects and SCCP patients were randomly split into two groups for the training and the test experiments. Because of the high complexity of spectrum profiles, two algorithms were tested for biomarker discovery. After the basic statistical analysis, the two algorithms were used for the selection of a signal cluster that was able to differentiate patients from controls. Peptides were extracted from the plasma by ClintProt C8 magnetic beads according to the kit instructions. Plasma aliquots (40 µL each) were mixed with 5 µL of magnetic beads and 40 µL of the kit buffer, allowing peptides and proteins to bind to hydrophobic C8 surface of the magnetic micro particles. The supernatant was removed after 1 minute of incubation and the beads were washed twice with 45 µL and once with 15 µL of the recommend washing solution. Peptides were then eluted with 10 µL of 50% acetonitrile. The procedure was automatically carried out using the ClinProt robot (Bruker Daltonics, GmbH, Bremen, Germany), thus reducing variability.

Mass Spectrometry Analysis

Aliquots of eluted peptides (5 µL) were mixed with 10 µL of HCCA matrix solution (6.2 g/L HCCA in methanol/acetonitrile/water 50/40/10). About 1 µL of this mixture was spotted four times onto a MALDI-ToF MTP 384 target plate ground steel F (Bruker Daltonics, GmbH, Bremen, Germany). All preparation steps were performed automatically by a robot (ClinProt robot, Bruker Daltonics, GmbH, Bremen, Germany). Mass spectra were acquired by an UltrafleXtreme MALDI TOF/TOF MS instrument (Bruker Daltonics, GmbH, Bremen, Germany) operated in positive-ion linear or reflectron mode, recording m/z values from 1000-10000 Da. External calibration was performed using a set of peptide/protein standards (ProtMix1). MALDI-TOF acquisition parameters were as follows: total of 1200 shots (200 x 6), laser power about 70% (linear mode) and 40% (reflectron mode), laser movement hexagonal.

Data Analysis

Data analysis was performed using the ClinProtTools 2.1 software package (17) (Bruker Daltonics, GmbH, Bremen, Germany). ClinProTools was used for multiple spectra comparison and protein pattern identification with the following workflow: (i) spectra normalization to their total ion current; (ii) spectra recalibration using the prominent peaks; (iii) baseline subtraction and peak detection; and (iv) calculation of peak areas for each spectrum. Peak detection was done with signal-to-noise ratio of 5 and peak areas were calculated using endpoint level integration type. Spectra were also "top hat" baseline subtracted with a minimum baseline width of 10% and processed in a range of 1-10KDa. Only 1 of the 8 spectra obtained from the plasma of each subject by MALDI-TOF was used for the next statistical analysis, with the help of "Support Spectra Grouping" and "Enable Similarity Selection" options. The program, after calculation of the mean spectrum of each subject's data set, selects the spectrum that is most similar to the average for subsequent statistical analysis. Basic statistical analysis, genetic algorithm (GA) (18), and Support Vector Machine (SVM) (19) were then used for the selection of signal clusters that were able to differentiate controls from penile cancer patients. These signals were preliminarily tested for their diagnostic capability using the two different data sets separately. The receiver operating characteristic curve analysis and area under curve (AUC) calculation were performed by the ClinProTools 2.1 software to determine the diagnostic efficacy of each marker. The cut-off value ($P < 0.001$) corresponding to the highest accuracy (the lowest false negative and false positive results) was also calculated, along with specificity and sensitivity.

Peptide Identification by MALDI-TOF/TOF

The peptides were identified from the C8 MB eluted fraction of patients' and control sub-

Table 1 - Patient characteristics, histopathologic findings, pathologic staging, type of surgery and follow-up.

Pts.	Age	Race*	Grade	Vascular Invasion	Stage TNM	Surgery	Follow-up (months)	Deaths by Disease (months)
1	41	mulatto	MD***	negative	pT2N2MX	Partial amputation + bilateral inguinal limphadenectomy	12	
2	41	mulatto	MD	negative	pT3N0Mx	Partial amputation + bilateral inguinal limphadenectomy	12	
3	62	black	MD	negative	pT2N0Mx	Total amputation + bilateral inguinal limphadenectomy	36	
4	62	white	MD	negative	pT1N0Mx	Partial amputation.	2	
5	38	white	WD**	negative	pT1N2Mx	Partial amputation + bilateral inguinal limphadenectomy	1	
6	45	white	MD	negative	pT1N1Mx	Partial amputation + bilateral inguinal limphadenectomy	12	
7	51	white	MD	negative	pT2N0M0	Partial amputation + bilateral inguinal limphadenectomy	24	
8	57	black	WD	negative	pT3N2Mx	Partial amputation + bilateral inguinal limphadenectomy	18	
9	54	mulatto	MD	negative	pT2N0Mx	Partial amputation + bilateral inguinal limphadenectomy	30	
10	73	white	WD	positive	pT2N3Mx	Partial amputation + right inguinal limphadenectomy	11	11
11	76	black	MD	negative	pT2N0Mx	Partial amputation + bilateral inguinal limphadenectomy	5	5
12	57	white	MD	negative	pT3N0Mx	Partial amputation + bilateral inguinal limphadenectomy	7	

13	80	white	WD	negative	pT2N0Mx	Partial amputation + bilateral inguinal limphadenectomy	24	
14	84	white	MD	negative	pT2NxMx	Partial amputation.	3	3
15	86	white	MD	negative	pT3NxMx	Total amputation.	8	
16	64	white	MD	negative	pT3N3Mx	Partial amputation + bilateral inguinal limphadenectomy	5	5
17	80	white	MD	positive	pT4NxMx	Emasculation	8	8
18	90	white	PD****	negative	pT1NxMx	Partial amputation.	1	
19	83	white	WD	negative	pT2N3Mx	Partial amputation + bilateral inguinal limphadenectomy	8	8
20	55	mulatto	MD	negative	pT3N2Mx	Emasculation + bilateral inguinal limphadenectomy	4	
21	61	mulatto	MD	negative	pT2N0Mx	Total amputation + bilateral inguinal limphadenectomy	9	
22	71	white	MD	positive	pT2N0Mx	Partial amputation + bilateral inguinal limphadenectomy	1	
23	56	mulatto	WD	negative	pT2N0Mx	Partial amputation + bilateral inguinal limphadenectomy	4	
24	60	black	WD	negative	pT2NxMx	Total amputation.	1	
25	62	white	WD	negative	pT2N3Mx	Partial amputation + bilateral inguinal limphadenectomy	24	

* In Brazil, race definition is not accurate due to miscegenation. The column represents the self defined skin color of the patients
** WD: well differentiated
*** MD: moderately differentiated
**** PD: poorly differentiated

jects' plasma by LIFT MS/MS. For peptide identification, the LIFT-TOF/TOF spectra were recorded in the UltrafleXtremeTM MALDI-TOF/TOF MS instrument. The fragment masses were analyzed after their ion reflector detection. Analyses were performed using the following acquisition settings: ion source 1, 7.5 kV; ion source 2, 6.7 kV; lens 3.6 kV; reflector, 29.5 kV; reflector 2, 13.95 kV; lift 1, 19 kV; lift 2, 3.15 kV; pulsed ion extraction 80 ns.

Raw MS/MS data were processed with the FlexAnalysisTM 3.3 software (Bruker Daltonics, Germany). Database searching was performed by an in-house Mascot search engine (Version: 2.3.02) using the following parameters: human Swissprot (accessed Feb. 2012- 20,317 sequences) database, no enzyme and fixed and variable modifications. MS and MS/MS tolerances were generally set at 1 Da. Only identifications with a score higher than Mascot identity thresholds were accepted.

RESULTS

The pathological findings of the SCCP are shown in Table-1. Most of the patients (n = 14) were at pT2 level, whereas the remaining were at pT1 (n = 4), pT3 (n = 6) and pT4 (n = 1) levels. Histopathology confirmed invasive SCCP in all patients. Of the 25 cases, 8 (32%) were well differentiated, 16 (64%) moderately differentiated and 1 (4%) poorly differentiated. Amputation and inguinal lymphadenectomy was performed in 20 patients. Of these patients, 11 (55%) were N0, 1 (5%) was N1, 4 (20%) were N2 and 4 (20%) were N3.

ClintProt was applied to analyze the plasma samples collected from SCCP patients and a cluster of two peaks was identified. Comparison of spectrum profiles obtained from the data set used in the training phase showed several ions differentially expressed in the two studied populations (Table-2). On the basis of the GA and SVM results, a cluster of two statistically different signals ($P < 0.05$), at m/z 2021.99 ± 9, 1897.22 ± 9, were identified as able to differentiate the populations. Preliminary statistical analysis was carried out for each marker and for the cluster of signals by the receiver operating characteristic curve analysis. The AUC of peak A at m/z 1897.22 ($P < 0.0001$) was 0.85, which corresponds to a moderately accurate test, according to the criteria suggested by Swets (20). The AUC of peak B at m/z 2021.99 ($P < 0.0001$) was 0.91, which corresponds to a highly accurate test. The combination of the two peaks indicated an improvement in the performance compared to the single signals, with specificity and sensitivity of 100% and 80%, respectively. This pattern was subsequently tested for its ability to differentiate SCCP patients from controls by external validation using data obtained from a second group of normal subjects and patients. Sensitivity and specificity were at 63.6% and 100%, respectively. Cross-validation analysis using the whole casuistic showed 62.5% and 86.76% sensitivity and specificity, respectively. The cluster of signals was also evaluated using the entire set of patient data grouped according to deaths due to disease and lymph node involvement. The results showed 100% sensitivity and 97% specificity for patients who had died of disease. Among patients with lymph node involvement, the sensitivity and specificity was 80% and 97%, respectively. Patients without lymph node involvement showed 54% sensitivity and 97% specificity (Table-3). Both peeks A and B, included in the diagnostic cluster, were under-expressed ($p < 0.05$) in patients compared with healthy subjects (Figure-1). Some of the peaks observed in the plasma protein profile could be identified by LIFT MS/MS (Table-4). In particular, signals at m/z 1897.22 (m/z 1896.17 in reflector mode) and at m/z 2021.99 (m/z 2021.26 in reflector mode) were identified by the proteomic approach based on MALDI-TOF/TOF as fragments of C3 (m/z 1896.17) and C4a/b (m/z 2021.26) complement proteins (Table-4).

Preliminary evaluation of the diagnostic efficacy was determined with an internal validation. The cluster showed a very high specificity value in an external validation test with plasma samples collected from different patients and controls (Table-3).

DISCUSSION

The area of proteomics has begun to revolutionize the study of medicine in the post genomic era, by allowing researchers to study the

Table 2 - Selection of peaks differently expressed (P < 0.05) between controls (n = 28) and penile cancer patients (n = 17) used for the training phase.

Mass	P*	P†	Ave 1‡	Ave 2§	Std D1¶	Std D2‖
6430.52	0.0195	0.00859	50.6	10.16	47.79	13.46
1897.14**	0.0283	0.00859	23.23	5.38	24.34	4.23
1865.76	0.165	0.246	8.89	1.56	13.01	1.71
6628.81	0.28	0.246	129.73	55.2	138.31	41.66
1016.3	0.422	0.546	486.1	391.81	153.1	123.32
2021.91**	0.422	0.000456	70.76	1.09	164.61	4.08
1112.17	0.422	0.0747	17.16	37.25	8.67	30.63
9420.36	0.422	0.395	23.38	14.85	13.94	10.8
1211.9	0.422	0.0423	4.53	-0.18	11.52	1.01
1229.15	0.422	0.246	6.18	10.52	5.46	5.43
3216.09	0.422	0.57	11.2	6.93	8.58	4.44
9377.51	0.422	0.5	8.02	5.25	5.81	2.94
7763.75	0.491	0.615	17.25	12.1	11.69	6.12
4100.59	0.491	0.184	2.33	-0.66	8.87	1.32
3315.24	0.518	0.71	22.33	15.47	16.59	8.47
1767.61	0.518	0.948	24.21	4.21	65.65	2.97
8764.21	0.518	0.684	6.15	2.64	7.19	5.36

* P value by t test and ANOVA; values lower than .0.05 indicate statistical relevance.
† P value calculated with the Wilcoxon/Kruskal–Wallis test; values lower than 0.05 suggest statistical relevance.
‡ Average area of peaks for control subjects.
§ Average area of peaks for SCCP patients.
¶ Standard deviation of peaks for control subjects.
‖ Standard deviation of peaks for SCCP patients.

** Marked peaks represent signals selected for the diagnostic model

Table 3 - Cluster evaluation: diagnostic efficacy of pattern based on deaths from disease and lymph node involvement.

Group	Sensitivity	Specificity
6 patients dead by disease	100%	97%
9 patients with positive lymph nodes	80%	97%
11 patients with negative lymph nodes	57%	97%

Figure 1 - Signal intensity of 2 peaks (1897.22 and 2021.99 m/z) discriminating control (class 1) and tumor (class 2).

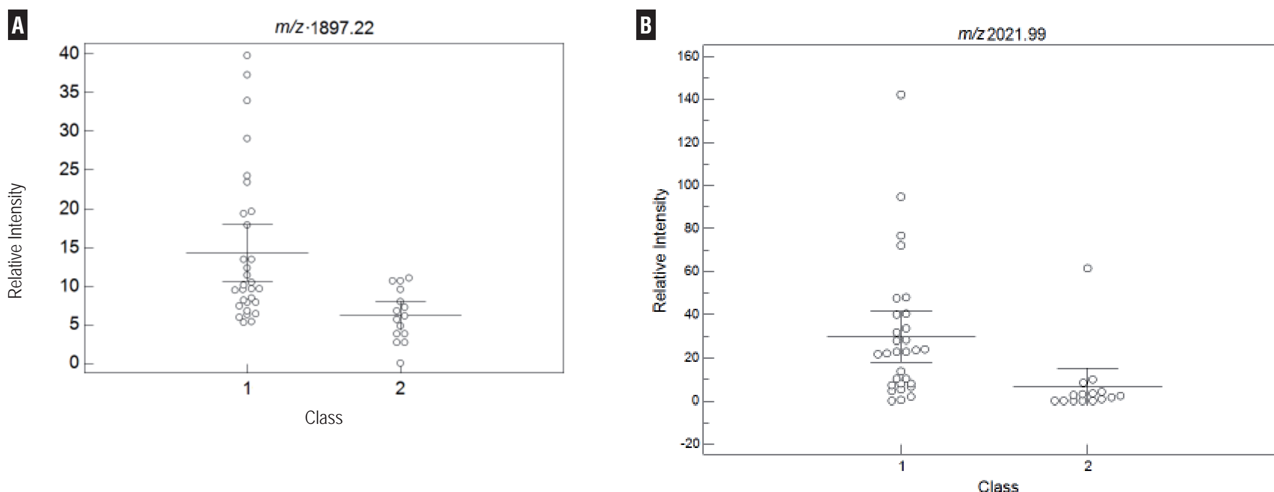

Table 4 - Identified peptides in the C8 MB eluted fraction of patients' and control subjects' plasma by LIFT MS/MS. All peptide identification scores were above Mascot identity threshold. Table reports the m/z values of the ions observed in MALDI linear and reflectron mode.

Reflectron mode m/z	Linear mode m/z	Calc. mass (Da) in all pre-fractionated plasma samples	Peptide sequence	Accession (UNIPROT)	Description	Peptide score
1896.17	1897.22	1895.024	NGFKSHALQLNNRQIR	CO4A_HUMAN CO4B_HUMAN	Complement C4-A OS=Homo sapiens GN=C4A PE=1 SV=1 Complement C4-B OS=Homo sapiens GN=C4B PE=1 SV=1	61
2021.26	2021.99	2020.097	SSKITHRIHWESASLLR	CO3_HUMAN	Complement C3 OS=Homo sapiens GN=C3 PE=1 SV=2	72

role that proteins plays in health and disease. By applying this knowledge, innovative discoveries in early cancer detection have been made, including kidney, prostate, ovarian, and bladder cancer. To our knowledge, this is the first study of penile cancer proteomics.

Although several markers have been evaluated, currently the clinical application of these markers is limited. HPV positive tumors show a variable prognostic outcome. In a previous article we reported the analysis of 80 consecutive cases of patients with penile cancers and HPV status was not significantly associated with the presence of regional metastases (21). Up to now, P53 status may correlate with survival in T1 disease but further studies are required to establish the link to lymph node spread (22) Therefore, it is still necessary to find biomarkers for early detection of metastases, prognosis and follow-up of this disease (22).

Before the use of proteomics in penile carcinoma, a critical assessment of its diagnostic and prognostic value will be required. There is a controversy about whether and when patients should undergo lymphadenectomy. The presence and the extent of metastasis to the inguinal region is the most powerful prognostic factor for survi-

val in patients with SCCP. The principal aim in the management of this disease is correct staging of patients without invasive procedures, since a presence of metastatic lymph nodes is an independent prognostic factor for survival (6). Therefore, research into potential biomarkers and biological targets is very important.

The C3, C4a and C4b complement fragments are involved in the classic and the alternative complement cascade pathway. These peptides were under-expressed ($p < 0.05$) in SCCP patients compared with healthy subjects. During disease progress, under-expression of C3, C4a/b fragments become more evident. More interestingly, we have found very high sensitivity (100%) and specificity (97%) for under-expression of these fragments in SCCP patients that have succumbed to the disease. Besides this, patients with lymph node involvement present sensitivity and specificity of 80% and 97%, respectively. In the case of patients without lymph node involvement, we obtained a sensitivity rate of 57% and specificity of 97%.

Degradation of complement system convertases was observed for patients with breast cancer (BCP). For C3, the degradation was solely on the C3d, C3g, C3α′1 and C3β. Convertases C3, which occupies a central position in the system, displays differential degradation in the BCP (5-fold more peptides than in the healthy subjects). For C4, the degradation was solely on the C4b and C4β. The degradation of the front portion of the C4b fragment was observed solely for the tested BCP (23).

The contribution of the complement system to the control of tumor growth has been neglected for a long time, since the main emphasis has been put on cell-mediated immune response against cancer (24).The innate immune system is the first line of defense, comprised of cells and mechanisms that defend the host from infection in a non-specific manner. Taneja et al. (25) studied the plasma protein profile in patients with hepatitis E. In that study, the levels of complement proteins C3, C3f, C4, and bradykinin and kininogen were found to be lower in the plasma of hepatitis E patients compared to healthy controls. Any perturbation, such as a viral infection in the liver, should trigger a strong innate response as the first line of host defense, but the opposite was found to occur, with these proteins being in downregulation. The exact mechanism of this reduction is not understood at this time.

In our patients, the downregulation of C3 and C4 could be caused by HPV and/or EBV infection, viruses that are highly prevalent in SCCP lesions (26). Viral proteins counteract the immune response (27). This could explain the progression of the disease along with C3 and C4a/b under--expression. It is a hypothesis to be tested in the future.

The over-expression of C regulatory proteins (CRPs) in tumor cells is one way these cells protect themselves from C attack. CD46, CD55 and CD59 are thought to be the most important membrane C regulatory proteins (mCRPs), expressed both on normal and tumor cells. Tumor cells can also evade C attack by binding soluble C inhibitors from serum such as factor H (fH). It is also interesting to note that fH or a related protein is a marker for bladder cancer, suggesting a link between C resistance and escape from immune surveillance (28). CD55 has been identified as a tumor-associated antigen and a high expression level of CD55 in colorectal cancer tissue is correlated with a significant decrease in survival (29). Also, lower CD46 has been found to be inversely related with high levels of C3 deposited in renal and cervical cancer tissue (30).

In addition, in a previous study (31) we detected lower activity of natural killer cells (NK) in patients with cancer of the penis. NK cells are also part of the innate immune response and were first identified for their ability to kill tumor cells without deliberate immunization or activation. Subsequently, they were also found to be able to kill cells that are infected with certain viruses and to attack preferentially cells that lack expression of major histocompatibility complex (MHC) class I antigens. The innate immune response of patients to cancer therefore may be involved, since natural killer (NK) cell activity can be significantly decreased in SCCP patients compared to control groups (31).

Despite increasing evidence suggesting that a variety of mechanisms are responsible for tumor-related tolerance and suppression, little is

known about the timeline and reciprocal connection between these processes. The widely accepted theory of immuno-editing describes a rational, time-structured evolution of the relationship that occurs between tumor and host immune system (32,33). Tumor escape in cancer patients appears to be a cumulative process that involves tumor-derived soluble factors (TDSFs), induction of regulatory elements of various cell lineages and different anatomical environments (34). In a simplified view, tumor-induced immune subversion results from two main activities, namely the induction of tolerance toward tumor antigens and the functional suppression of the effector lymphocytes that normally counteract tumor growth (32-36).

CONCLUSIONS

Our results suggest that a proteomic approach based on magnetic beads is a useful method to discover possible clinical biomarkers. We demonstrated the capability of selected signals to differentiate SCCP patients from normal subjects. The peptides identified from the C8 MB eluted fraction of patients' and control subjects' plasma correspond to fragments of the C3 (m/z 1896.17) and C4 (m/z 2021.26) complement protein. The results showed that when the disease progresses, they are more under-expressed. These fragments are mainly downregulated in patients with metastatic involvement. The innate immune response of patients could be suppressed. Downregulation of C3 and C4A/B represents a promising prognostic tool for SCCP.

ACKNOWLEDGEMENTS

This work was supported by grants from the Italian Ministry of Universities and Research: PRIN 2006 (no. 69373), FIRB 2007 (Rete nazionale per lo studio del proteoma umano, no.RBRN07BMCT_11), FAR 2006-2011 (ex 60%), from the Italian Institute of Technology (IIT), Project SEED: "IPG-CHIP", by "FONDO PER LA PROMOZIONE DI ACCORDI ISTITUZIONALI" Regione Lombardia DGR N. 5200/2007, project no. 14546: "Network Enabled Drug Design (NEDD)" The work was also supported by grants from Programa de Oncobiologia and FAPERJ (APQ1-E26/110.812/2009), Brazil.

CONFLICT OF INTEREST

None declared.

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