Clinica Chimica Acta 413 (2012) 1641–1646

Contents lists available at SciVerse ScienceDirect

Clinica Chimica Acta

journal homepage: www.elsevier.com/locate/clinchim

Expression of CD147, BIGH3 and Stathmin and their potential role as diagnostic marker in patients with urothelial carcinoma of the bladder

Divya Bhagirath [a], Nitin Abrol [b], Rehan Khan [a], Manoj Sharma [c], Amlesh Seth [b], Alpana Sharma [a,*]

[a] Department of Biochemistry, All India Institute of Medical Sciences(AIIMS), New Delhi, India
[b] Department of Urology, All India Institute of Medical Sciences(AIIMS), New Delhi, India
[c] Department of Radiotherapy and Oncology, Maulana Azad Medical College and Lok Nayak Hospital, New Delhi, India

ARTICLE INFO

Article history:
Received 6 March 2012
Received in revised form 3 May 2012
Accepted 8 May 2012
Available online 14 May 2012

Keywords:
CD147
BIGH3
Stathmin
Diagnostic marker
Bladder cancer

ABSTRACT

Background: Urothelial carcinoma of the bladder is characterised by very high recurrence rate, followed up by cystoscopy which being invasive technique makes the need for non-invasive markers important for Transitional Cell Carcinoma (TCC) detection. CD147 is a transmembrane protein highly expressed in tumour cells which aids in tumour invasion and growth. BIGH3, an Extracellular matrix protein (ECM) which interacts with various ECM component in different tissue system and Stathmin(STMN1) is cytosolic microtubule destabilising protein also called as Oncoprotein18 due to its role in tumour promotion. So far the expression of BIGH3 and STMN1 remains undetermined in cancer subjects including TCC. We therefore studied the levels and molecular expression of these molecules in TCC patients, to evaluate their usefulness as diagnostic markers.

Methods: Thirty consecutive TCC patients and two sets of control- 15 Benign prostatic hyperplasia (BPH) patient and 15 healthy were taken. Serum and urine levels of these molecules were estimated by ELISA and relative mRNA expression by Q-PCR from tumour and normal urothelium. Post-Hoc analysis and ROC curve were determined to evaluate the significance and sensitivity and specificity.

Results: The mean concentrations of these molecules were found to be significantly increased ($p<0.001$) in the serum and urine of TCC patients, with varying significance in each grade for different molecules. The urinary levels of CD147 (67 pg/ml) and serum STMN1 concentration (1.38 ng/ml) showed a specific increase as compared to the controls, while BIGH3 was elevated in both serum and urine samples. Molecular (mRNA) expression was elevated in the high grade (Muscle Invasive) stage of the disease for all the molecules, with a significant 3-fold increase that correlated with disease severity being observed for STMN1. ROC analysis gave optimal combination of sensitivity and specificity for diagnosis of the disease in urine and serum sample for STMN1.

Conclusion: Of CD147, BIGH3 and STMN1, significant results were obtained for STMN1 and it could serve as the best possible diagnostic marker for TCC detection in future.

1. Introduction

Bladder cancer is the second most common urologic malignancy amongst the males after prostate cancer [1]. Transitional cell carcinoma (TCC) or urothelial carcinoma accounts for 90% of all bladder cancer, and is divided in 2 variants: Non muscle invasive or the muscle invasive form. The non-muscle invasive form has a high rate of recurrence (50%), and it progresses to muscle invasive form in almost 11% of the cases [2], hence making regular cystoscopic surveillance essential for early tumour detection. However the invasive nature of cystoscopy and low sensitivity of other diagnostic test as urine cytology emphasizes the need for more sensitive and specific markers which provide with a non-invasive means of tumor detection [3–5]. CD147 or Extracellular matrixmetalloprotienase inducer (EMMPRIN) is a transmembrane protein which is found overexpressed in various different tumours [6,7]. It induces the release of matrixmetalloproteinase (MMPs) and other angiogenic growth factors [7,8] from surrounding stromal cells upon the interactions of these cells with tumor cell, that supports the tumour growth. A soluble form of this molecule has also been identified that is formed as a result of proteolytic action of MMPs [9]. Growth factor beta inducible protein (BIGH3) is a secretory extracellular matrix (ECM) protein, which is component of various tissues serving as attachment site for different macromolecules in the ECM [10,11]. It has been found to be increased in hepatoma cells where its expression positively correlated with CD147 and it was observed to mediate the metastatic and invasive effects of

* Corresponding author at: Department of Biochemistry, All India Institute of Medical Sciences, New Delhi-110029, India. Tel.: +91 9899061974; fax: +91 11 26588641.
E-mail address: dralpanasharma@gmail.com (A. Sharma).

0009-8981/$ – see front matter © 2012 Elsevier B.V. All rights reserved.
doi:10.1016/j.cca.2012.05.005

CD147 [12]. Stathmin (STMN1), a cytosolic phosphoprotein involved in regulating the microtubule dynamics in response to the cell's need [13,14] is found overexpressed in different cancer [15–18]. It was first identified in leukemic cell lines [19], and inhibition of its expression has been shown to decrease the proliferative and invasive properties of different cancer [20,21].

Since these molecules were found overexpressed in different cancer and they are playing important role in supporting the tumor (as CD147), study of these molecules would be important to identify any change of their concentration in different stages of urothelial tumor and l useful for identification of their role as markers for the disease. Concentration of CD147 have previously been found to be increased in the urine sample from patients with invasive form of bladder tumor [22,23]. Concentration of BIGH3 and STMN1 have not been so far evaluated in patients with urothelial carcinoma. Hence evaluation of urine and serum sample for the concentration of these molecules would not only be useful in allowing non-invasive detection of bladder tumors but also in correlating these with increasing tumor grade so as to understand their role in tumor growth. We therefore accessed the concentration of these molecules in urine and serum samples as well as in tissue specimen extracted from the site of primary tumor in urothelial cancer patients. Relative mRNA expression concentration from the tumour urothelium were compared with those in the normal urothelium to observe any change in their expression with occurrence of tumour or with increasing disease severity.

2. Materials and methods

2.1. Patients and sample

Thirty consecutive urothelial carcinoma patients undergoing treatment at Department of Urology, All India Institute of Medical Sciences (AIIMS), New Delhi were included in the study. All 30 were confirmed cases of urothelial cancer as determined by the biopsy reports from the Dept. of Pathology, AIIMS and were not undergoing any chemotherapy. The patients were grouped in to following categories: High grade Muscle Invasive MI (T2) (30%), High-grade Non-Muscle Invasive NMI (T1) (26.7%) and Low grade LG (43.3%) according to the WHO/ISUP 2004 classification and T staging [24]. 80% of the included patients were males and 20% females and had an age range of 25–70 y, mean 58 y. The controls taken for the study was divided in two groups: the first group included 15 patients with Benign Prostatic Hyperplasia (BPH) undergoing treatment at Dept. of Urology, AIIMS and the second group included 15 healthy volunteers. Normal urothelium was taken from BPH patients than from adjacent normal urothelium of the carcinoma patients to make correct comparison of the expression changes in the two conditions. The age range and mean age for BPH patients and healthy group was 22–64 years, 50.3 years and 20–58 years, and 48 years, respectively. The study was approved by the Ethical Committee at AIIMS and written informed consent was taken from both patients and controls. Tumour tissue sections (2 mm each) from TCC patients were collected at the time of surgery and cold cup punch biopsies (2 mm each) of the normal urothelium were taken from BPH patients during prostate resection, that were immediately kept at −80 °C till RNA was extracted the same day. Venous blood was taken in endotoxin free vials, from either of the study groups (patients and controls) centrifuged at 3000 rpm for 10 min for separation of serum and stored at −80 °C till further use. First voided morning urine specimen were collected from patients and both the controls in sterile plastic tubes. The urine was centrifuged at 10,000 rpmm for 10 min for separation of urine supernatant which was stored at −80 °C till further use.

2.2. ELISA for determination of serum and urinary concentration of CD147, BIGH3 and Stathmin

Commercially available ELISA kits from Uscn, Life Sciences Inc. China for CD147, BIGH3 and STMN1 were used to assay concentration of these molecules in the available biological samples. The kit consisted of 96-well microtiter plates coated with antibody specific to each type of molecule, detection antibodies for identifying the antibody-protein in the plate by streptavidin-biotin labelling and TMB substrate which generated coloured product. The sample was added and assay was conducted according to the manufacturer's instruction. The absorbance of the coloured product developed at the end of the assay was quantified at wavelength 450 nm on ELISA reader. Dilutions of the standard were prepared and used for determining the standard curve for the molecules.

2.3. Quantitative mRNA expression by Real time PCR

The mRNA concentration of CD147, BIGH3 and STMN1 were analysed by relative quantitation using ABI 7500 real-time PCR (Applied Biosystems Inc., Foster City, CA). Total RNA was isolated by ethanol-chloroform precipitation, from obtained tissue sections which were minced and added to TRIZOL reagent. 1ug of the total RNA was used to prepare cDNA using random hexamers (Fermentas, Glen Bernie, MD) that was used as template in real time PCR. Twenty microliters of reaction mixture included the Maxima SYBR Green master mix (Fermentas), cDNA and the nuclease free water. The conditions used for PCR were as follows : Initial Denaturation- 95 °C for 5 min; followed by 40 cycles at 95 °C for 15 s, 60 °C for 30 s and 72 °C for 30 s. β-Actin was used as the endogenous control for quantitation. The forward and reverse primers used for each molecule were:

CD147: Forward-5′-GACGACCAGTGGGGAGAGTA-3′,
Reverse-5′-GGCCTTGTCCTCAGAGTCAG-3′,
BIGH3: Forward-5′-ATCACCAACAACATCCAGCA-3′,
Reverse-5′-CCGTTACCTTCAAGCATCGT-3′,
STMN1: Forward-5′-AAGGATCTTTCCCTGGAGGA-3′,
Reverse-5′-TGTGCCTCTCGGTTCTCTTT-3′.

Table 1
Serum and urinary levels as determined by ELISA of CD147, BIGH3 and STMN1 in urothelial carcinoma patients and controls.

Study subject(n)	CD147(pg/ml)		BIGH3 (ng/ml)		Stathmin (ng/ml)	
	Serum	Urinary	Serum	Urinary	Serum	Urinary
Total patients(TP) (30)	108.54 ± 31.8	67.08 ± 24.98	16.32 ± 10.34	10.61 ± 4.25	1.38 ± 0.61	0.48 ± 0.14
High grade (T2) muscle invasive (n = 9)	130.33 ± 31.86	85.81 ± 19.98	30.41 ± 7.06	4.42 ± 3.55	2.12 ± 0.52	0.54 ± 0.13
High grade (T1) non muscle invasive (n = 8)	99.41 ± 35.19	81.33 ± 19.53	11.99 ± 2.42	12.24 ± 3.01	1.21 ± 0.31	0.47 ± 0.16
Low grade (Ta) (n = 13)	99.07 ± 23.19	45.33 ± 10.42	9.24 ± 2.45	6.98 ± 1.74	0.98 ± 0.24	0.44 ± 0.12
BPH control (15)	83.48 ± 20.86	11.15 ± 2.75	3.08 ± 0.79	6.96 ± 1.76	0.49 ± 0.12	0.22 ± 0.05
Healthy control (15)	52.2 ± 12.29	N.D	0.36 ± 0.08	0.23 ± 0.06	0.41 ± 0.10	0.24 ± 0.06
p* (TP vs BPH controls)	0.008	0.001	0.001	0.003	0.001	0.001
p**(TP vs healthy controls)	0.001	0.001	0.001	0.001	0.001	0.001

N.D — not detectable.