Gene 591 (2016) 320–326

Contents lists available at ScienceDirect

Gene

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

Research paper

Fibroblast growth factor receptor 1 and cytokeratin 20 expressions and their relation to prognostic variables in bladder cancer

Rehab S. Abdul-Maksoud [a], Sally M. Shalaby [a,*], Walid S.H. Elsayed [b], Saad Elkady [c]

[a] Medical Biochemistry Department, Faculty of Medicine, Zagazig University, Egypt
[b] Pathology Department, Faculty of Medicine, Zagazig University, Egypt
[c] Urology Department, Faculty of Medicine, Zagazig University, Egypt

ARTICLE INFO

Article history:
Received 6 February 2016
Received in revised form 3 May 2016
Accepted 30 May 2016
Available online 31 May 2016

Keywords:
Bladder cancer
FGFR1
Cytokeratin 20
RT-PCR
Immunohistochemical methods

ABSTRACT

Background: Tumor grade and stage are currently the most important prognostic variables in bladder cancer but establishing additional criteria is still needed for effective treatment.
Objectives: The aim of the study was to assess the expression of fibroblast growth factor receptor 1 (FGFR1) and cytokeratin 20 (CK20) in cancer bladder (CB) and to evaluate their association with the clinicopathological features of the disease.
Patients and methods: The study included 80 patients diagnosed as bladder cancer of different stages and grades and 80 patients with nonmalignant urothelial diseases of matched age and sex to the malignant group. The expressions of FGFR1 and CK20 in tissue samples were determined by RT-PCR and immunohistochemistry.
Results: The expression levels of FGFR1 and CK20 were increased in the malignant group when compared to the control group ($P < 0.001$ for each). Analysis of their expression showed that levels of FGFR1 and CK20 were significantly higher in invasive tumor stages (pT2-pT4) than in non-invasive stages (pTis, pTa, pT1) ($P < 0.001$). Interestingly, the sensitivity and specificity of combined detection with CK20 and FGFR1 for the differentiation between invasive and non-invasive stages of bladder cancer reached 97.5% and 92.5%, respectively.
Conclusion: Our results determined overexpression of both FGFR1 and CK20 in CB specimens. The alterations in the expression of FGFR1 and CK20 were associated with disease stage and grade. Lastly, combined detection of FGFR1 and CK20 had a high predictive prognostic value in differentiating invasive from non-invasive carcinoma.
© 2016 Elsevier B.V. All rights reserved.

1. Introduction

Bladder carcinoma is the most common malignancy in genitourinary tract. It accounts for 90% of urogenital malignancies (Cheung et al., 2013). Moreover, it is responsible for about 7% of all malignant diseases among Egyptians and the incidence rate of cancer bladder (CB) that ranked next to liver cancer in Egyptian males is also high (10.7%) (Ibrahim et al., 2014). The majority of bladder tumor patients (80%) present with papillary noninvasive (pTa) or superficially invasive (pT1) urothelial tumors at first presentation, whereas the remaining 20% of primary tumors are already muscle invasive (Fauconnet et al., 2009). pTa and pT1 tumors can be removed by transurethral resection (Cheung et al., 2013). However, about 70% of patients will develop disease recurrence, and up to 25% will eventually develop a more aggressive muscle-invasive disease (Jacobs et al., 2010). Histopathological examination is the only method routinely used to determine the prognosis of the patients with CB but it is subjective to individual variability and has poor sensitivity (Tosoni et al., 2000; van Rhijn et al., 2003). Therefore, identification of novel biomarkers and establishing additional criteria for monitoring tumor prognosis are still needed. Currently, oncogenes, tumor suppressor genes, growth factors, growth factor receptors, cell proliferation markers, and intermediate filament expression are of the potential predictors for tumor treatment response, recurrence, or progression (Goodison et al., 2013; Barbieri et al., 2010).

Fibroblast growth factor receptor 1(FGFR1) belongs to a family of structurally related tyrosine kinase receptors (FGFR1–4) (Cross and Reiter, 2002). It has important roles in many processes including, generation of fusion transcripts via chromosomal translocations in myeloproliferative diseases (Cross and Reiter, 2002). FGFR1 is implicated as an oncogene whose expression is increased compared with normal tissue. Activation of FGFR1 induces both mitogenic and chemotactic responses in various cell types (Welm et al., 2002). Prior studies have shown that activated FGFR1 in premalignant prostate cells exhibited accelerated progression to malignancy (Acevedo et al., 2007). Similar results were observed with sustained activation of FGFR1 in breast model, leading

Abbreviations: FGFR1, fibroblast growth factor receptor 1; CK20, cytokeratin 20; CB, cancer bladder; RT-PCR, reverse transcriptase polymerase chain reaction; HPRT, hypoxanthine phosphoribosyl transferase; TCC, transitional cell carcinoma; SCC, squamous cell carcinoma.

* Corresponding author at: Medical Biochemistry Department, Faculty of Medicine, Zagazig University, Zagazig, Egypt.
E-mail address: sallyshalaby@hotmail.com (S.M. Shalaby).

http://dx.doi.org/10.1016/j.gene.2016.05.043
0378-1119/© 2016 Elsevier B.V. All rights reserved.

to alveolar hyperplasia and invasive lesions (Reis-Filho et al., 2006). Veltman et al. (2003) found high-level amplification of 8p12 (*FGFR1*) region in 41 primary bladder tumor. Many studies have focused on the role of FGFR3 in CB, but little has been known about the expression of FGFR1 in bladder tumorigenesis (Di Martino et al., 2012).

Cytokeratins are intermediate filaments and form the cytoskeleton of the cell. Twenty different cytokeratins have been identified (Klein et al., 1998). They can be subdivided into two major types: type I, which is acidic and includes K9–K20 and type II, which is neutral/basic and includes K1–K8 (Coulombe et al., 2002). Cytokeratins can affect the availability of regulatory molecules such as heat shock proteins, apoptosis-inducing factors or signaling molecules, thus affecting the sensitivity of cells to apoptotic and proliferative stimuli (Ibrahim and Ghannam, 2012). There is a limited pattern of expression of cytokeratin 20 (CK20) in normal tissues (Moll et al., 2003). In the urothelium, its expression was restricted to superficial cells even in the presence of severe inflammation. The expression pattern is altered only in malignant urothelium (Alsheikh et al., 2001; Southgate et al., 1999). Previous studies indicated that CK20 may be a specific biomarker for detecting bladder cancer (Rajcani et al., 2013; Eissa and Matboli, 2013; Schmidt et al., 2016).

The aim of the present study was to determine the expression profiles of FGFR1 and CK20 in cancer bladder specimens and to examine whether FGFR1 or CK20 gene expression correlates with the CB clinicopathologic features as tumor stage and grade.

2. Materials and methods

2.1. Samples collection and preparation

All participants of the study recruited from the Urology Department, Zagazig University Hospitals. Informed consent was obtained from all of the participants. They were subjected to full history taking, clinical examination as well as to routine investigations as urine analysis, pelvi-abdominal ultrasonography, plain X-ray and cystoscopy. Tissue specimens were collected from 80 patients (76 men and 12 women; mean age 54.2 years) with primary carcinoma of the urinary bladder. Forty-five of these patients underwent transurethral resection. The remaining 35 were subjected to radical cystectomy. None of them had chemotherapy or radiotherapy before the surgery. Samples were taken from the patients with tumors at different clinical stages and histological grades. Complete histomorphological evaluation of biopsy specimens was performed according to the WHO classification of 1973 (Mostofi et al., 1973) and TNM classification guidelines (Sobin and Fleming, 1997) for urinary bladder tumors. Eighty histologically normal urothelial biopsies were obtained from 80 patients (63 men and 17 women; mean age 51.3 years) either from benign bladder lining of cystectomy specimens or benign prostatic urethra of transurethral prostatic resections performed for benign prostatic hyperplasia served as controls. Each specimen was divided into two parts; one was frozen at −80 °C until used for detection of cytokeratin 20 and FGFR1 by reverse transcriptase polymerase chain reaction (RT-PCR) in Medical Biochemistry Department. The second was immediately fixed in 10% formalin for histo-pathologic examination and immunohistochemical analysis in Pathology Department, Zagazig University.

2.2. Total RNA extraction

Total RNA from normal and tumor cells was extracted using RNA extraction kit (RNeasy FFPE Kit, Qiagen) following the manufacturer's instructions and quantified spectrophotometrically at 260 nm.

2.3. cDNA synthesis, and quantitative real-time PCR

mRNA was reverse transcribed and cDNA was synthesized with a reverse transcription kit (Reverse Transcriptase Master Mix, Roche Diagnostics) according to the manufacturer's instructions. FGFR1 and CK20 expressions were detected by RT-PCR as previously described with modifications (Tomlinson et al., 2005; Pu et al., 2008). Hypoxanthine phosphoribosyl transferase (HPRT) was used as a reference gene.

Quantitative real-time PCR analysis was performed in a mixture containing 2 μL cDNA, 10 μL qPCR Green-Master (Jena Bioscience) and 0.6 μL primers (10 μM), PCR grade water up to 20 μL. The sequence of the primers used is listed in Table 1. The following thermal cycling conditions were used for the genes: initial step at 90 °C for 2 min then incubation at 95 °C for 2 min followed by 40 cycles: 95 °C for 15 s, 60 °C for 1 min, 95 °C for 15 s.

Agarose gel electrophoretic evaluation of the RT-PCR products was routinely performed to determine the specificity of the RT-PCR reaction. The comparative threshold cycle number ($2^{-\Delta\Delta Ct}$) method was used after a validation experiment demonstrated that efficiencies of target genes and reference gene were approximately equal. Ct values define the threshold cycle of PCR, at which amplified products were detected. ΔCt was the difference in the threshold cycles for target genes and reference control. Our results were represented as fold changes in expression of genes relative to controls (−ΔΔCt) that were calculated from the arithmetic formula $2^{-\Delta\Delta Ct}$.

2.4. Immunohistochemical studies

Immunohistochemistry was performed using labeled streptavidin–biotin technique (DAKO LSAB™+/HRP kit, code K0679 and DAKO EnVision™+/HRP kits, codes K4004 and K4006, DAKO Denmark A/S, Glostrup, Denmark for CK20 and SignalStain® Boost IHC Detection Reagents (HRP, Rabbit #8114), BioNordika Denmark for FGFR1) according to the manufacturer's specifications with the DAKO AUTOSTAINER (DAKO Denmark). Formalin-fixed, paraffin-embedded tissue sections (5 μm thick) were deparaffinized and hydrated. For CK20, antigen demasking was done using heat-induced epitope retrieval with Dako Target Retrieval Solution, (10 mmol/L Tris buffer, 1 mmol/L -ethylenediamine tetra-acetic acid (EDTA), pH 9.0) for 20 min. For FGFR1, antigen demasking was performed by heating specimens in steamer in 10 mM/L sodium citrate buffer (pH 6.0); maintain at a sub-boiling temperature for 10 min. Cool slides on bench top for 30 min. Sections were stained with antibodies against CK20 (Ks 20.8, monoclonal Mouse Anti-human Cytokeratin 20, code M7019,Denmark A/S, Glostrup, Denmark) at 1:25 dilution for 30 min at room temperature, FGFR1 (FGF Receptor 1 (D8E4) XP® Rabbit mAb #9740, BioNordika Denmark) at 1:500 dilution using SignalStain® Antibody Diluent (#8112) Incubated overnight at 4 °C. Diaminobenzidine (DAB) was used as a chromogen, followed by haematoxylin counterstaining. Positive controls for CK20 and FGFR1 were colonic adenocarcinoma and beast carcinoma respectively. For negative controls, the samples were processed with diluent buffer instead of primary antibody.

CK20 is expressed in cytoplasm and FGFR1 shows membranous staining. Based on personal observations and findings derived from the previously reported literature (Tomlinson et al., 2007; Kohler et al., 2012; Jung et al., 2014), staining pattern was defined as positive or negative as follows:

For CK20, if the expression was weak/patchy (usually in umbrella cells) or no staining, it was classified as CK20 negative. Other patterns of CK 20 immunostaining, i.e., moderate to strong staining in atypical cells (usually in full thickness), were considered CK20 positive.

Table 1
Primers for FGFR1 and CK20 genes.

	Forward	Reverse
FGFR1	5′-AGGCTACAAGGTCCGTTATGC-3′	5′-TGCCGTACTCATTCTCCACAA-3′
CK20	5′-CAGACACACGGTGAACTATGG-3′	5′-GATCAGCTTCCACTGTTAGACG-3′
HPRT	5′-GACACTGGCAAAACAATGCA-3′	5′-TTCGTGGGGTCCTTTTCACC-3′

Download English Version:

https://daneshyari.com/en/article/2814779

Download Persian Version:

https://daneshyari.com/article/2814779

Daneshyari.com