

Upregulation of FGFR3 and HIF-1 α expression in muscle invasive bladder cancer

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ABSTRACT

BACKGROUND The major risks in patients diagnosed with non-muscle invasive bladder cancer (NMIBC) are recurrence, progression of muscle invasive bladder cancer (MIBC), and metastasis. Biological markers such as fibroblast growth factor receptor-3 (FGFR3) and hypoxia-inducible factor-1 α (HIF-1 α) are related to muscle invasiveness of bladder cancer. This study was aimed to analyze the expression of FGFR3 and HIF-1 α to predict muscle invasiveness in bladder cancer patients.

METHODS This was an observational study with a case-control design. Sixty patients with bladder cancer, who underwent histopathology examinations at the Department of Pathology, Faculty of Medicine, Universitas Sumatera Utara/H. Adam Malik Hospital from January 2012 to December 2015, were included in this study. Samples were then classified into 30 NMIBC and 30 MIBC groups. All samples were analyzed with an immunohistochemistry assay for FGFR3 and HIF-1 α . H-scores were used to determine the relationships between each group.

RESULTS FGFR3 was expressed in 29 (96.7%) patients of the NMIBC group, and 23 (76.7%) patients of the MIBC group ($p=0.026$, $OR=8.8$; 95% $CI=1.01-76.96$). HIF-1 α was expressed in only 1 (3.33%) patient of the NMIBC group, and 15 (50%) patients of the MIBC group ($p<0.001$, $OR=29$; 95% $CI=3.49-241.13$).

CONCLUSIONS There was a difference in upregulation of FGFR3 and HIF-1 α expression in both the NMIBC and MIBC groups.

KEYWORDS bladder cancer, fibroblast growth factor receptor-3, hypoxia-inducible factor-1 α , predictive factors

Bladder cancer is the 9th most prevalent malignancy worldwide and accounts for more than 380,000 cases and 150,000 deaths annually.¹ More than 90% of bladder cancers are urothelial cell carcinoma (UCC). The majority (75–80%) of all new cases of UCC are classified as a non-muscle invasive or superficial. The recurrence rates for these tumors are 50–70%, and 10–15% progress to muscle invasion over 5 years.² Bladder cancer is a type of cancer with a high incidence, and thereby warrants an attention after initial management. Based on Globocan 2012, bladder

cancer ranks 13th among the most common diseases in both sexes in Indonesia and has a 5-year prevalence of 17.794 (2.8%). The 5-year prevalence of bladder cancer in Indonesia in male patients (5.705 %) is higher than in female (1.273 %) patients. The main problems after initial therapy are higher chances of recurrence, progression, and metastasis.³ Conventional prognostic factors, including tumor staging, grading, size, and multifocality, could not predict clinical outcome in most patients with bladder cancer. Thus, several efforts to achieve markers which could predict recurrence,

progression, therapeutic response, and survival are being made.^{4,5}

As stated by Knowles and Hurst,⁶ fibroblast growth factor receptor-3 (FGFR3) is a tyrosine kinase that causes an increase in bladder cell growth and is found to change in 75% of non-muscle invasive bladder cancer (NMIBC) cases. Lerner et al⁷ also found that expression of FGFR3 was eight times higher in NMIBC cases. Deniz et al⁸ concluded that increased hypoxia-inducible factor-1 α (HIF-1 α) expression was correlated with poor prognosis. To date, studies investigating the expression of FGFR3 and HIF-1 α to predict muscle invasiveness in bladder cancer have never been conducted in Indonesia. The present study aimed to investigate the expression of FGFR3 and HIF-1 α in bladder cancer.

METHODS

A case-control study was conducted to analyze whether the expression of FGFR3 and HIF-1 α predicts muscle invasiveness in bladder cancer. Patients with muscle invasive bladder cancer (MIBC) were enrolled as cases, and patients with NMIBC were used as controls. This study was conducted at Haji Adam Malik Hospital and the Department of Pathology, Faculty of Medicine, Universitas Sumatera Utara, Medan, Indonesia, between January 2012 and December 2015. The inclusion criteria were histopathologically diagnosed bladder cancer and excellent condition of paraffin blocks. Patients with a history of previous bladder cancer, previous chemotherapy or radiotherapy, and with other malignancy were excluded from this study. This study has been approved by the hospital research ethics committee (500/TGL/KEPK FK USU-RSUP HAM/2017).

Immunohistochemistry

This study used a two-step immunohistochemistry staining method, the EnVision+Dual Link System kit, with the Santa Cruz product rabbit polyclonal human antibody FGFR3 [FGFR-3 (C-15): sc-123] for FGFR3 identification, and Santa Cruz product a mouse monoclonal human antibody HIF-1 α [HIF-1 α (H1 α 67): sc-53546] for HIF-1 α staining.

Microscopic preparations were prepared in the following manner. Paraffin blocks were cut thinly with a microtome to 4 μ m-thick sections. Each paraffin block was used for immunohistochemical FGFR3 and HIF-1 α

immunohistochemistry. The tissue was attached to a poly-L-lysine or silanized slide object glass. The FGFR3 rebound used a rabbit polyclonal human antibody FGFR3 Santa Cruz product with a 1:50 dilution and the EnVision+Dual Link System from Santa Cruz with several steps, with a primary incubation period of 30 min.

Scoring for histopathology

Staining intensity positivity values were assessed by brown color grading in epithelial cells in the cytoplasm or stroma for FGFR3 and HIF-1 α , observed by a single pathologist. This study used McCarthy's criteria to categorize the H-score.⁹ The immunohistochemical localization (HS) was scored in a semiquantitative fashion incorporating both the intensity and the distribution of specific staining. The intensity positivity value (i) plus 1, times the quantity positivity value (k); ($HS=(i + 1) \times k$). Thus, the cut-off point was determined as 3. The value was classified into four levels: a) negative=0; b) weak=+1; c) moderate=+2; and d) strong=+3.

The quantity positivity value of FGFR3 and HIF-1 α immunohistochemistry (IHC) was defined as the quantitative value in brown intensity distribution percentage per one field of view under a light microscope at a magnification of 400 times. Three levels were defined for quantity positive values: a) negative (0): IHC negative; b) focal (1): colored cells <50%; and c) diffuse (2): colored cells >50%.

Both values were combined into a single H-score value. This score was determined based on McCarty's criteria, which is the intensity positivity value (i) plus 1, times the quantity positivity value (k); ($HS=(i + 1) \times k$). Thus, the cut-off point was determined as 3. Therefore, samples were divided into two groups: one group with H-scores of 3 or greater, and the other with H-scores below 3. A negative value was acquired by determining the cut-off point involving cases with color intensity and negative control, cases with a weak, moderate, and strong positive color, and with focal quantity positivity. We used a positive control from sarcoma, placental, and liver tissue samples.

RESULTS

A total of 60 subjects were included in this study that consisted of 30 cases of MIBC and 30 NMIBC all with cases of high tumor expression. The

Table 1. Demographic characteristics of the subjects

Variables	Group		OR	95% CI	p
	MIBC, n (%) (n=30)	NMIBC, n (%) (n=30)			
Age, mean (SD)	57.07 (10.37)	55.9 (11.91)			0.687
Gender					0.647
Male	26 (86.7)	26 (86.7)			
Female	4 (13.3)	4 (13.3)			
FGFR3 expression					
Positive	23 (76.7)	29 (96.7)	8.8	1.012–76.96	0.026*
Negative	7 (23.3)	1 (3.3)			
HIF-1 α expression					
Positive	15 (50)	1 (3.3)	29	3.488–241.131	0.001*
Negative	15 (50)	29 (96.7)			

* $p < 0.05$ = statistically significant. MIBC = muscle invasive bladder cancer; NMIBC = non-muscle invasive bladder cancer; OR = odds ratio; CI = confidence interval; SD = standard deviation; FGFR3 = fibroblast growth factor receptor-3; HIF-1 α = hypoxia-inducible factor-1 α

demographic characteristics of the subjects and the immunohistochemistry results are presented in Table 1. The immunohistochemistry results of FGFR3 presented in 96.7% of the cases had positive FGFR3 (Figure 1a). The positive value of FGFR3 immunohistochemistry was significantly associated with the occurrence of muscle invasion in bladder cancer (BCa) ($p = 0.026$; OR = 8.8; 95% CI = 1.012–76.96). Positive staining of HIF-1 α also had a higher probability to be expressed in MIBC ($p < 0.001$; OR = 29; 95% CI = 3.488–241.131) (Figure 2a).

DISCUSSION

FGFR3 is a tyrosine kinase, which increases the growth of bladder cells. It is found to be mutated in 75% of cases of non-muscle invasive bladder cancer. Lerner et al⁷ reported an increase in FGFR3 expression that was eight times higher in NMIBC. Excessive expression or mutation of FGFR3 causes rapid proliferation of bladder cancer cells and inhibits the apoptotic process thus leading to the development of a hyperplastic lesion in bladder cancer.⁹ In this study, we found positive FGFR3 immunohistochemistry in 86% of subjects. In the NMIBC group, 96.7% of subjects had positive FGFR3 immunohistochemistry. The expression of FGFR3 was significantly associated with the occurrence of muscle invasion ($p = 0.026$; OR = 8.8; 95% CI = 1.012–76.96). Negative FGFR3 immunohistochemistry had an 8.8 times higher chance in the MIBC group. Bladder cancer with a positive FGFR3 had a lower recurrence rate compared with bladder cancer with a negative FGFR3 ($p = 0.004$).

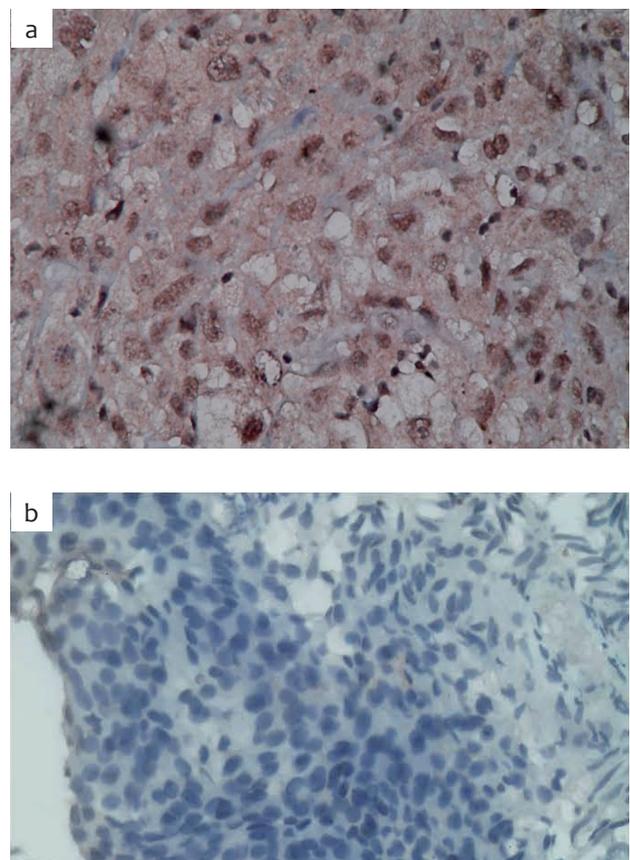


Figure 1. FGFR3 expression; (a) positive expression; (b) negative expression

Several studies have also found significant differences in FGFR3 expression in NMIBC and MIBC.^{9–12} Lindgren et al¹¹ found a significant association between FGFR3 mutation and expression. When the mutation existed, the overexpression of FGFR3 occurred, which might be because: 1) cells that had the FGFR3 mutation

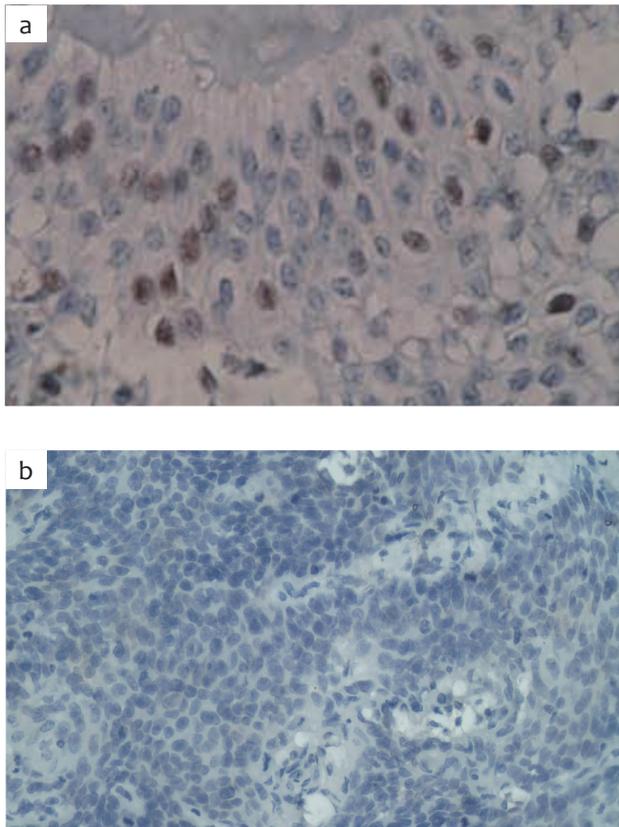


Figure 2. HIF1- α expression; (a) positive expression; (b) negative expression

would express FGFR3 which activated a signaling pathway as a response, and 2) the FGFR3 mutation caused an increased expression of the FGFR3 gene receptor autocrinally. Increased FGFR3 expression without the mutation occurred by a different mechanism. Apart from an association between the FGFR3 mutation and expression, higher FGFR3 expression found in UCC suggested the importance of the FGFR3 receptor in urothelial malignancies.¹³ These studies indicated that FGFR3 mutation analysis could be used to assess the clinical management of bladder cancer, particularly cystoscopy. The analysis of FGFR3 is based on this study and the study by Lindgren et al¹¹ that correlated the likelihood of whether NMIBC would progress to MIBC. van Rhijn et al¹⁰ inferred that in NMIBC patients with FGFR3 expression, 65% of cases would not progress to MIBC. Therefore, they proposed to decrease the number of cystoscopy procedures for evaluation. As a result, van Rhijn et al¹⁰ considered decreasing cystoscopy procedures in patients with positive FGFR3. FGFR3 is the first gene known in bladder cancer that mutated selectively in this disease and could be seen with clinical parameters.¹¹

Lindgren et al¹¹ found that FGFR3 expression correlated with the FGFR3 mutation, in which overexpression of FGFR3 was mainly due to a mutation in the FGFR3 gene, thus increasing the FGFR3 receptor.

Based on the HIF-1 α IHC, we found that 96.7% of subjects in NMIBC group showed a negative expression. Although HIF-1 α has been studied in many malignancies, only a few of them were performed in bladder cancer. The results were OR 29; 95% CI=3.488–241.131, with $p < 0.001$ as shown in Table 1. A positive HIF-1 α IHC result had a 29 times higher possibility of being produced by MIBC cancer cells. These findings are consistent with those of previous studies.^{14,15} Deniz et al⁸ found a significant association between the positive expression of HIF-1 α and both tumor grading and staging, in which higher grade and tumor stage increased the expression of HIF-1 α .

We, therefore, studied two markers at a time that expected to provide a more precise prediction for the occurrence of MIBC.¹⁶ Blick et al¹⁷ showed that FGFR3 is induced by hypoxia in bladder cancer cell lines. Thus, tumor hypoxia may represent an additional mechanism for increased levels of FGFR3 in bladder cancer. FGFR3 overexpression may provide a positive effect by preventing progression to MIBC disease.^{18,19}

The limitation of this study was the number of patients in the sample. The sample should be expanded in the future to conduct a comprehensive biomarker development study. Further prospective studies are required to assess the relative risk and appropriate cystoscopy duration for each group. This study did not include the FGFR3 mutation analysis, which is important for the expression of FGFR3 because of the limitation of the technical setting. The FGFR3 mutation was found in 75% of cases with NMIBC.⁷ van Rhijn et al¹⁰ found the FGFR3 mutation in 65% of subjects with bladder cancer (stage pTaG1-2).¹⁰ In that study, the level of FGFR3 was determined in a semiquantitative fashion, and they did not identify a significant association between FGFR3 expression levels and tumor characteristics. It is important to note is that no established cut-off points have been developed for FGFR3 IHC staining. We reported any staining as “positive” based on a previous study,²⁰ although many of our specimens showed weak staining. In conclusion, there is upregulation of both FGFR3 and HIF-1 α expression in MIBC. This result can be advantageous for using both FGFR3 and HIF-1 α as biomarkers for bladder cancer in the future.

Conflict of Interest

The authors affirm no conflict of interest in this study.

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