THE IMMUNOEXPRESSION PROFILE OF FGFR3 AND P53 IN PUNLMP AND UROTHELIAL BLADDER CARCINOMA AT HASAN SADIKIN HOSPITAL

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ABSTRACT

Objective: *To obtain FGFR3 and p53 immunohistochemistry of PUNLM and UBC basic data profile.* ***Material & Methods:*** *This retrospective observational analytic study used a cross-sectional design on formalin-fixed paraffin-embedded (FFPE) from patients diagnosed as PUNLMP and UBC then performed FGFR3 and p53 immunohistochemical staining. Statistical analysis of immunoexpression data using SPSS 24.0.* ***Results:*** *There are three main immunohistochemistry pattern profiles of PUNLMP and UBC in this research data, i.e. FGFR3+ p53+, FGFR+ p53- and FGFR3- p53+. There were no Cis sample obtained. FGFR+ was showed in PUNLMP, LG-PUC, HG-PUC, IUBC (pT1, pT2, pT3, pT4) with decreased percentage sequentially. As many as 51 of 54(94.44%) IUBC samples showed combination pattern with p53+ while 3 other samples were p53-.* ***Conclusion:*** *The FGFR3 and p53 immunohistochemistry profile, separately or as a pattern of combination, is in accordance with the oncogenesis molecular pathway of UBC.*

Keywords: *Urothelial bladder cancer, FGFR3, p53, immunohistochemistry.*

ABSTRAK

Tujuan: *Mendapatkan data dasar gambaran profil imunohistokimia FGFR3 dan p53 pada PUNLM dan KUKK.* ***Bahan & Cara:*** *Penelitian ini merupakan studi analitik observasional retrospektif dengan menggunakan desain potong lintang pada formalin-fixed paraffin-embedded (FFPE) dari pasien yang didiagnosis sebagai PUNLMP dan KUKK kemudian dilakukan pewarnaan imunohistokimia FGFR3 dan p53. Analisis statistik data imunoekspresi menggunakan SPSS 24.0.* ***Hasil:*** *Ada tiga pola profil imunohistokimia utama pada lesi PUNLMP dan KUKK dalam penelitian ini, yaitu FGFR3+ p53+, FGFR3+ p53- dan FGFR3- p53+. Tidak ada sampel Cis yang diperoleh. FGFR3+ ditunjukkan pada PUNLMP, LG-PUC, HG-PUC dan KUI (pT1, pT2, pT3, pT4) dengan persentase penurunan secara berurutan. Sebanyak 51 dari 54 (94,44%) sampel KUKK menunjukkan pola kombinasi dengan p53+ sementara 3 sampel lainnya adalah p53- yang merupakan pT1 UBC.* ***Simpulan:*** *Profil imunohistokimia FGFR3 dan p53, secara terpisah maupun dalam pola kombinasi sesuai dengan jalur molekuler onkogenesis UBC.*

Kata Kunci: *Kanker uretra kandung kemih, FGFR3, p53, imunohistokimia.*

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INTRODUCTION

Bladder cancer is a high-financing malignancy with regard to tight post-operative follow-up based on identification of risk grouping into low-risk and high-risk since the diagnosis of initiation is established while data on this matter at Hasan Sadikin Hospital as a government referral hospital in Indonesia is inadequate. Bladder cancer treatment continues to show a significant burden on 68.800 new diagnoses and 14.000 deaths estimated to occur in 2008.[1]

One of the main issues related to the care of bladder cancer is that treatments and testing do not equate with equivalent benefit. Providers are generally more concerned with cost effectiveness than simply with cost. An expensive and effective treatment is more acceptable than a cheap treatment that is slightly less effective. A major limitation of all cost analyses is the absence of data on the effectiveness of therapy. Because of the lifetime need for surveillance, the treatment of recurrent tumors, and the cost of complications associated with treatment, bladder cancer poses a significant economic burden.[1-2]

Bladder cancer, particularly urothelial bladder carcinoma (UBC), is grouped based on molecular pathogenesis that is divergent with

Fibroblast Growth Factor Receptor 3 (FGFR3) and p53 involvement. The population incidence also shows differences in the expression of these two tumor markers while based on the principles of management, bladder cancer is divided into 2 major groups namely Non-Muscle Invasive Bladder Cancer (NMIBC) and Muscle Invasive Bladder Cancer (MIBC).[3-4]

Bladder cancer is a heterogeneous disease. About 75% of patients are non-invasive cases of urothelial carcinoma, which have a high recurrence rate and a low but unpredictable rate of development. Patients with infiltrating urothelial carcinoma. High increase and specific process, but again, disease behavior cannot be predicted. To date, the exact number of recurrences and tumor development is only based on clinical factors. Different results between many studies lead to there are no markers that have included in routine clinical practice.[5-6]

Clinical staging and histopathological parameters remain the "gold standards" for UBC diagnosis and prognostic prediction. However, they are not sufficient to characterize individual biological features and clinical tumor behavior. Understanding disease pathobiology could potentially add essential information to these classical criteria and contribute to more accurately predicting prognosis and refine treatment. Ideally, the clinical use of standardized prognostic and predictive biomarkers could allow the prediction of tumor recurrence through a non-invasive method, avoiding the use of invasive techniques, such as cystoscopy and biopsy, which cause significant patient discomfort and add substantial costs. Furthermore, it could allow timely prediction of UBC progression, from NMI to MI disease, particularly for high grade or carcinoma in situ lesions, guiding more vigilant surveillance and refining treatment strategies.[7]

Based on the pathogenesis of bladder cancer revealed in a number of studies it was stated that the NMIBC and MIBC lesions differ genetically and clinically. Low-grade NMIBC is characterized by a gain-of-function mutation that mainly affects classical oncogene including fibroblast growth factor receptor 3 (FGFR3) while MIBC is characterized by a loss-of-function mutation resulting of gene inactivation tumor suppressors abnormalities with the highest proportion compared to other molecules, 70%, which is p53.[8]

The FGFR3 gene gives instructions for making a protein called fibroblast growth factor receptor 3. Fibroblast growth factor receptor (FGFR) is part of a family of four fibroblast growth factor receptors that share the same structure and function which are group of tyrosine kinase (RTK) receptor signaling pathway that regulates embryogenesis, angiogenesis, tissue homeostasis, and wound repair.

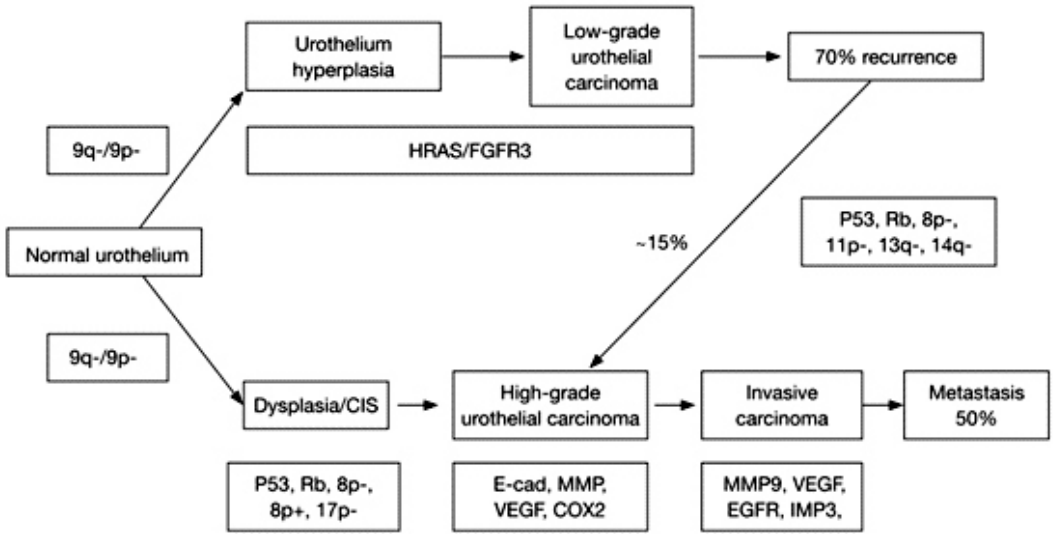

Figure 1. Divergent molecular pathways of oncogenesis in superficial and muscle-invasive urothelial carcinoma of urinary bladder.[3]

FGFR also plays an important role in various cell functions, including proliferation, differentiation, apoptosis, and migration. There are 4 transmembrane tyrosine receptors, FGFR1- 4, which are identified in the FGFR group.[3,9,10]

The FGFR3 protein extends to the cell membrane so that one end of the protein remains inside the cell and the other end is on the outer surface of the cell. This protein priority enables it to interact with specific growth factors outside the cell and receive signals that control growth and development. When this growth factor is attached to the FGFR3 protein, the protein is activated (activated), which triggers a cascade of chemical reactions in the cell that instructs the cell to undergo certain changes, such as maturity to take on special functions (differentiation).[10]

Several versions (isoforms) of the FGFR3 protein are produced from the FGFR3 gene. Different isoforms are found in various body tissues, and they interact with various growth factors. Many isoforms are found in the cells that make up bone. One special isoform of the FGFR3 protein is found specifically in cells lining the surface of the body (epithelial cells), including cells that form the outer layer of the skin, called the epidermis.[10]

FGFR3 mutations are constitutively activated and induce a number of oncogenic signaling pathways, including RAS/mitogen-activated protein kinase (MAPK), phospholipase Cc1 (PLCc1), phosphoinositide 3-kinase (PI3K) and transducer pathways and transcription signal activators (STAT).[11]

Activation mutations from FGF receptors are found in various types of malignancies with the highest prevalence of FGFR3 mutations occurring in bladder carcinoma. In other types of malignancies, FGFR receptor activation mainly occurs through gene amplification and FGF ligand-mediated signaling.[12]

In one large study, FGFR3 mutations occurred in 50% of lower and upper urinary tract tumors. During urothelial transformation, FGFR3 mutations occur earlier, as reported in preneoplastic lesions, "flat" urothelial hyperplasia. Furthermore, FGFR3 mutations occur in more than 80% of benign lesions and low-grade bladder tumors (urothelial papillomas, PUNLMP, pTa). Overexpression of FGFR3 also occurs in UBC, especially in low grade and stage lesion. Recent research shows a relationship between mutation status and protein expression, where more than 85% of mutations have a high protein level. FGFR3 dysregulation, both mutations, overexpression or both, occurs in about 80% of NMIBC and 54% of MIBC.[9]

FGFR3 mutations have been investigated as prognostic markers for recurrence, progression, and survival. In the study of Rhijn et al. it was shown that the FGFR3 mutation was predictive of recurrence in low and high-grade pTa UBC. Hernandez et al. showed that the FGFR3 mutation is a prediction of high recurrence in low-grade pTa urothelial carcinoma but does not occur in high-grade pTa UBC and pT1.[9,13]

The relationship between FGFR3 mutations and progressivity has been investigated. In the study of Burger et al, it was shown that FGFR3 status was not related to recurrence but was predictive of progression in pT1 UBC and high-grade carcinoma. Another study from Rhijn et al. showed that positive FGFR3 and Ki-67 mutations were predictive of progression in 7% of patients.[9]

The gene mutation, located on chromosome 4p16.3, has recently been identified as a molecular change that is characteristic for pTa tumors. In the largest study reported to date, 74% of pTa tumors have FGFR3 mutations as compared to 16% of T2-4 tumors. All mutations described are mutational errors located in exons 7, 10, or 15 which have previously been described as germline mutations in skeletal dysplasia syndrome. These mutations are predicted to cause constitutive activation of the receptors. In one study, mutations had been associated with a lower risk of recurrence signaling that this genetic event could identify a group of patients with a favorable disease course.[14]

In UBC, several gene mutations are obtained during a person's lifetime and only uncertain cells are present. These changes, called somatic mutations, are not inherited. Somatic mutations in the FGFR3 gene are associated with several cases of UBC. This mutation overactivated the FGFR3 protein, which may direct the bladder to grow and divide abnormally. This uncontrolled cell division leads to the formation of bladder tumors.[10]

Somatic mutations in the FGFR3 gene are associated with UBC only occurs in bladder cells.[10]

The TP53 gene gives instructions for making proteins called p53, tumor proteins. This protein acts as a tumor suppressor, which means regulating cell regulation by keeping cells from growing and dividing (proliferating) too fast or in an uncontrolled way.[15]

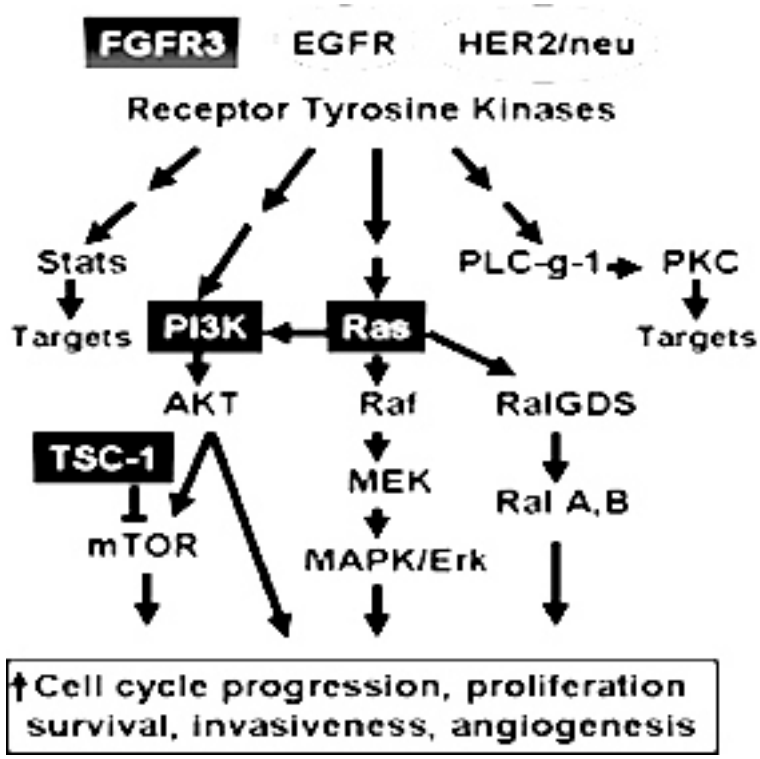

Figure 2. FGFR3 pathway.[13]

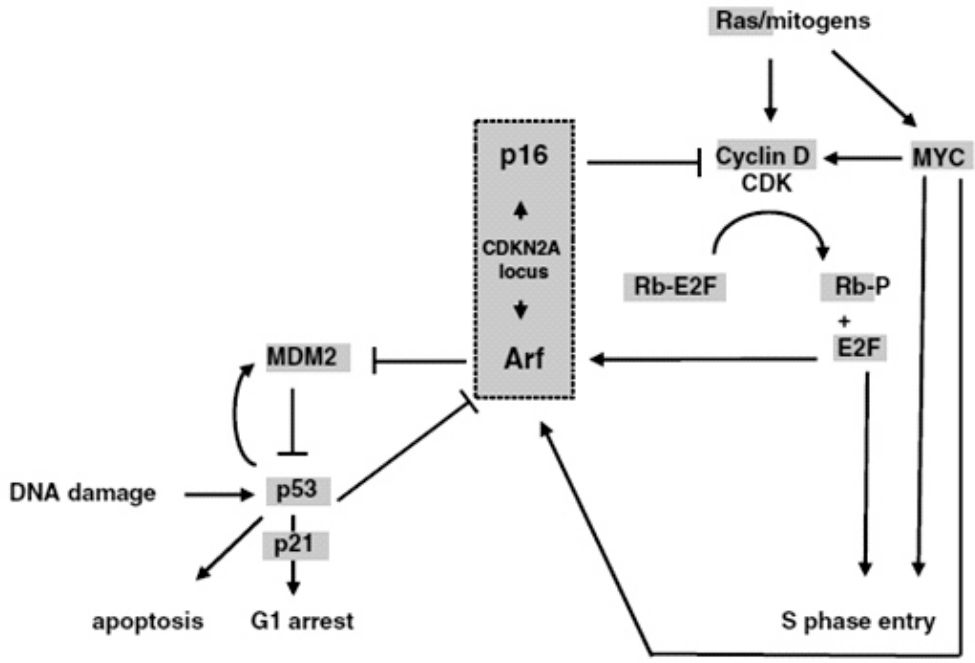

Figure 3. RB and p53.[16]

P53 protein is located in the nucleus of cells throughout the body, where it attaches (binds) directly to DNA. When DNA in cells becomes damaged by agents such as toxic chemicals, radiation, or ultraviolet (UV) rays from the sun, this protein plays an important role in determining whether DNA will be repaired or damaged cells will self-destruct (undergo apoptosis). If DNA can be repaired, p53 activates other genes to repair the damage. If DNA cannot be repaired, this protein prevents cells from dividing and signals to undergo apoptosis. By stopping cells with mutated or damaged DNA from division, p53 helps prevent tumor development.[15]

The somatic gene mutation TP53 has been found in several cases of UBC. Most of these mutations convert a single amino acid to p53. This modified p53 protein regulates cell proliferation and cannot trigger apoptosis in cells with mutated or damaged DNA. As a result, DNA damage can accumulate in cells. Such cells can continue to divide in an uncontrolled manner, leading to tumor growth.[15]

The pattern of p53 mutation has been widely studied in UBC. Some p53 mutations are associated with specific carcinogens while others can occur spontaneously.[15]

Based on molecular and histopathological observations, a model for the molecular pathogenesis of UBC has developed. Almost certainly this is too simple but it does provide a useful anchor for molecular studies. Mutation of FGFR3 defines the large group of superficial tumors. FGFR3 and TP53 mutation are each confined to one of the two major subgroups of UBC and currently are the best molecular markers for these groups.[16]

OBJECTIVE

This research is performed due to obtain basic data profile of FGFR3 and p53 immunohistochemistry of PUNLM and UBC cases in Hasan Sadikin Hospital as referral hospital.

MATERIAL & METHODS

The research material were paraffin block samples from patients who were diagnosed histopathologically as Papillary Urothelial Neoplasm of Low Malignant Potential (PUNLMP), Non-Invasive Low-Grade Papillary Urine Carcinoma (LGUC), High-Grade Non-Invasive Papillary Urothelial Carcinoma (HGUC)) and Infiltrating Urothelial Carcinoma bladder based on WHO 2016 classification, total sampled for the period 2015-2017.

This study was conducted using a retrospective descriptive design to look at the profiles of immunohistochemical (IHC) panels FGFR3 and p53.

Immunohistochemical staining using the primary antibodies used were FGFR3 using a monoclonal antibody FGFR3, series B-9, sc-13121, from Santa Cruiz Biotechnology with 1:100 dilution and for p53 using the p53 monoclonal antibody mouse, Pab-1801, sc-98, from Santa Cruiz Biotechnology with 1:150 dilution.

FGFR3 immunoexpression is positive if the plasma membrane is brown. The results of staining that look focal are interpreted as positive values. This FGFR3 immunoreactivity is rated as positive or negative regardless of the coloring intensity. Weak FGFR3 immunoreactivity (positive but with reduced intensity) is considered a positive result)

The evaluation of P53 immunoexpression is considered positive if the cell nucleus becomes brown. This p53 immunoreactivity is rated as positive or negative regardless of the coloring intensity.

This research has received ethical approval from the Research Ethics Committee of Padjadjaran University with Number: 381/UN6.KEP/EC/2018.

RESULTS

The number of samples in this study were 61 samples consisting of 2(3.3%) PUNLMP cases, 4(6.6%) LGUC cases, 1(1.6%) HGUC cases, 24(39.3%) IUC pT1 cases, 12 (19.7%) cases of IUC pT2, 12(19.7%) cases of IUC pT3 and 6(9.8%) cases of IUC pT4. There were no Cis sample. The characteristics of the research subject can be seen in Table 1.

Table 1 describes the characteristics of the entire study sample according to variables Gender, Age, Stage/Degree, Tumor Size, Number of Tumors, Recurrence, Smoking Habits.

Gender variables indicate that most cases of UBC occur in men. Most of the samples consisted of IUC pT1 lesions which were non-invasive muscle lesions.

UBC cases mainly occur in old age, ≥ 60 years, with an increase in the incidence of cases that are linear with an increase in age groups.

Table 1. Characteristics of Research Subjects.

Variables	n=61
Sex, frequency(%)	
Male	52(85.2%)
Female	9(14.8%)
Group of Age(years), frequency (%)	
<40	2(3.3%)
40-44	5(8.2%)
45-49	7(11.5%)
50-54	11(18.0%)
55-59	10(16.4%)
≥ 60	26(42.6%)
Tumor Stage/Grade, frequency (%)	
PUNLMP	2(3.3%)
LGUC	4(6.6%)
HGUC	1(1.6%)
pT1	24(39.3%)
pT2	12(19.7%)
pT3	12(19.7%)
pT4	6(9.8%)
Tumor Size, frequency (%)	
<3cm	4(6.6%)
≥3cm	57(93.4%)
Number of Tumor, frequency (%)	
Single	6(9.8%)
Multiple	55(90.2%)
Recurrence, frequency (%)	
Primary	24(39.3%)
Recurent	37(60.7%)
Smoking Habits, frequency (%)	
Yes	38(62.3%)
No	23(37.7%)

Table 2. Association between FGFR3 and P53 immunoexpression with histopathological stage/degree.

IHC	Histopathological stage / degree									
					Infiltrating urothelial carcinoma					
	PUNLMP (n=2)	LGUC (n=4)	HGUC (n=1)	CIS (n=0)	pT1 (n=24)	pT2 (n=12)	pT3 (n=12)	pT4 (n=6)	n=61	p
FGFR3										**0.730**
Positive	2(100.0%)	4(100.0%)	1(100.0%)	0(0.0%)	19(79.2%)	10(83.3%)	8(66.7%)	4(66.7%)	48(78.7%)	
Negative	0(0.0%)	0(0.0%)	0(0.0%)	0(0.0%)	5(20.8%)	2(16.7%)	4(33.3%)	2(33.3%)	13(21.3%)	
P53										**0.432**
Positive	2(100.0%)	3(75.0%)	1(100.0%)	0(0.0%)	21(87.5%)	12(100.0%)	12(100.0%)	6(100.0%)	57(93.4%)	
Negative	0(0.0%)	1(25.0%)	0(0.0%)	0(0.0%)	3(12.5%)	0(0.0%)	0(0.0%)	0(0.0%)	4(6.6%)	

Description: Categorical data p-value is calculated based on the Chi-Square test with an alternative Kolmogorov Smirnov and Exact Fisher test if the terms of Chi-Square are not met. The value of significance based on the value of p <0.05. * Sign indicates p-value <0.05 means significant or statistically significant.

Table 3. Association between FGFR3 and P53 imunoexpression patterns with histopathological stage/degree.

IHC	Histopathological stage / degree								n=61	p
	PUNLMP (n=2)	LGUC (n=4)	HGUC (n=1)	CIS (n0)	Infiltrating urothelial carcinoma					
					pT1 (n=24)	pT2 (n=12)	pT3 (n=12)	pT4 (n=6)		
Immunoexpression Pattern										**0.696**
FGFR3+ P53+	2(100.0%)	3(75.0%)	1(100.0%)	0(0.0%)	16(66.7%)	10(83.3%)	8(66.7%)	4(66.7%)	44(72.1%)	
FGFR3+ P53-	0(0.0%)	1(25.0%)	0(0.0%)	0(0.0%)	3(12.5%)	0(0.0%)	0(0.0%)	0(0.0%)	4(6.6%)	
FGFR3- P53+	0(0.0%)	0(0.0%)	0(0.0%)	0(0.0%)	5(20.8%)	2(16.7%)	4(33.3%)	2(33.3%)	13(21.3%)	
FGFR3- P53-	0(0.0%)	0(0.0%)	0(0.0%)	0(0.0%)	0(0.0%)	0(0.0%)	0(0.0%)	0(0.0%)	0(0.0%)	

Most UBC lesion samples showed tumor size ≥3cm and the number of multiple lesions with a total of 37 (60.7%) samples in the study were UBC cases that experienced recurrence.The presence of smoking habits was stated in 38(62.3%) research samples.

Immunohistochemical (IHC) staining FGFR3 and p53 in the study sample gave results of the interpretation shown in Table 2 while the combination pattern of FGFR3 and p53 immunoexpression can be seen in Table 3.

For analysis on categorical data of FGFR3 and p53 Imunoexpression in Table 2 and the FGFR3 and p53 Imunoexpression Patterns in Table 3 above were tested using the Chi-Square statistical test.

The Chi-Square statistical test results in the above research group obtained information that the P-value in the FGFR3 and p53 Imunoexpression variables was greater than 0.05 (P value> 0.05) which means that there was no statistically significant percentage difference between the FGFR3 and p53 Imunoexpression variables in the staging group/histopathological degree. There was no statistically significant difference in percentage between the patterns of Imunoexpression in the histopathological stage/degree group.

DISCUSSION

Characteristic data in this study indicate that the majority of UBC cases that occur at Hasan Sadikin Hospital in the period 2015-2017 which was as much as 85.2% occurred in men. This is consistent with epidemiological data on the incidence of UBC in various countries including Asia, which states that the incidence of UBC for men is 3–4 times higher than for women.[17,18]

The characteristics of this study sample based on age groups showed a tendency to increase incidence according to an increase in age groups. The age group with the largest number of samples is the age group of ≥ 60 years of 42.6%. The same finding was revealed by Cheng et al. that most cases of UBC occur in old age, 6th and 7th decades.[1]

Most of the cases in this study sample, 24(39.3%) were IUC pT1 cases. This is in accordance with the statement from Kang et al., Ferlay et al., and Kamat et al. which states that most, 70-80% of cases of UBC at the initial diagnosis are cases of non- muscle invasive. However, this is not entirely in line if the observations are emphasized in 2 large groups of UBC based on the principles of management, namely non-muscle invasive UBC and muscle-invasive KKK.[4,8,11,18]

Muscle invasive KKK lesions consisting of IUC pT2 to pT4 included 30 (49.18%) study samples while non-invasive muscle KKK lesions consisting of PUNLMP cases, LGUC, HGUC, and IUC pT1 included 31 (50.82%) cases of research samples. So it appears that the difference between the two groups is not too large.

This may be explained by the condition of this research conducted at advanced health facilities. Hasan Sadikin Hospital is the highest referral (Top Referral Hospital) in West Java Province, also a National Referral Hospital so that the cases obtained here are advanced cases or cases of recurrent.

This situation also provided an explanation for the data on the characteristics of the sample, which were mostly, 90.2%, were cases of multiple lesions and 93.4% of cases showed tumor size ≥3cm.

The characteristic data of the sample showed that most of the cases came from patients with a smoking habit history, 62.3%. This is in accordance with the statement from Otsuka et al. and Netto et al.[20-21]

The results of the FGFR3 immuno-histochemical (IHC) examination in this study

showed that all low-grade lesion samples, PUNLMP and LGUC, gave positive FGFR3 results. This is in accordance with the statement of Poussel et al. and Otsuka et al. which states that the FGFR3 mutation was found in 77% of low-grade non-invasive lesions. In addition, there were also positive FGFR3 results in muscle-invasive lesions that were in accordance with the statement from Cheng et al. which states that invasive tumors can also show overexpression of FGFR3 in almost 40% of cases. [20-21]

The p53 IHC examination showed positive results in 100% of IUC pT2, pT3, and pT4 cases, respectively. This is consistent with the pathogenesis of UBC revealed by van Rhijn et al., Netto et al. and Zhao et al. [2,5,22]

The pattern of expression of FGFR3 and p53 in this study included 3 combination patterns, namely FGFR3+ p53+, FGFR3+ p53- and FGFR3- p53+. All three patterns are seen in the IUC pT1, pT2, pT3, and pT4 cases with varying percentages. However, the results of FGFR3+ p53- were not found in IUC cases which are included in muscle-invasive lesions (IUC pT2, pT3, and pT4) so this suggests the possibility that the patterns of FGFR3+ p53+ and FGFR3- p53+ are markers of muscle-invasive lesions.

Immunohistochemistry results which show a combination pattern of FGFR3+ p53+ and FGFR3- p53+ in muscle-invasive lesions can describe the history of the lesion based on divergent pathogenesis of UBC. Muscle invasive lesions with FGFR3+ p53+ IHC results imply the possibility that these invasive muscle lesions are low-grade lesions that undergo progression while the FGFR3- p53+ pattern implies that these muscle-invasive lesions are most likely primary invasive muscle lesions.

Another pattern shown by IUC pT1 lesions, namely FGFR3+ p53- shows the possibility of these lesions being low-grade lesions that experience progression without involving changes in p53.

Other results are shown by PUNLMP and LGUC lesions which are obtained by a combination pattern of FGFR3+ p53+ and FGFR3+ p53-. This situation implies the possibility that the lower grade lesions are likely to experience progression into muscle-invasive lesions.

CONCLUSION

The FGFR3 and p53 immunohistochemistry profile, separately or as a pattern of combination, is in accordance with the oncogenesis molecular pathway of UBC.

ACKNOWLEDGMENT

The authors gratefully acknowledge the Padjadjaran University for contributing to the financing of this research through an UNPAD internal grant. The authors also particularly thank the Oncology and Stem Cell study center of UNPAD in facilitating matters regarding the conduct of research until the publication process.

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