Potential Forkhead Box O 3a as Prognostic Biomarker in Prostate Cancer

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ABSTRACT

Background: Forkhead box O 3a is a transcription factor, a member of the Forkhead box O family. It has a crucial role in both oncogenesis and tumor suppression. Cellular localization and phosphorylation status are considered to be prognostic factors for cancers. Loss of Forkhead box O 3a was found in various cancers. However, its mechanism in prostate cancer remains unclear. Objective: To analyze Forkhead box O 3a as prognostic biomarker potential in prostate cancer. Methods: This analytical study was done with a cross-sectional design. The population was all cases of primary prostate cancer from the Anatomical Pathology Department in West Sumatera. The sample was 56 slides and paraffin blocks with adequate tumor tissue. Hematoxylin and eosin-stained slides were evaluated to review Gleason score. The immunohistochemistry staining of Forkhead box O 3a on paraffin blocks was carried out to analyze protein expression semi-quantitatively. Positive expression was moderate to strong interpretation. Results: The highest proportion of prostate cancer was high-grade Gleason score (8-10) 76.79% cases. The positive Forkhead box O 3a expression on tumor cells was 51.79%, predominantly in the cytoplasm. The negative expression in the high-grade Gleason score was more than positive. Statistically, these results showed a negative significant correlation of Forkhead box O 3a expression and Gleason score (p=0.038). Conclusion: Forkhead box O 3a was likely to be deactivated in prostate cancer. Abstain expression in nuclear, but overexpression in the cytoplasm of tumor cells induces loss of tumor suppression function. Thus, Forkhead box O 3a has potential as a prognostic biomarker for prostate cancer.

Keywords: *Forkhead Box O 3a, Prognostic Biomarker, Prostate Cancer.*

1. INTRODUCTION

Prostate cancer is the second most common cancer in worldwide after lung cancer in males based on GLOBOCAN 2020. It is reported 1.4 million (7.3%) new cases, increasing from 2018 (1.3 million). The incidence of prostate cancer has increased in several Asian countries in recent decades, as well as in Southeast Asia. Prostate cancer is the fifth leading cause of death by cancer in males [1][2]. New cases of prostate cancer in Indonesia were reported 13.563 (7.4%) or the fifth most common cancer in males [3].

1.1. Forkhead Box O 3a

Forkhead box O (FOXO) is a transcription factor that plays important role in the higher organisms. FOXO

the family contains four members (FOXO1, FOXO3a, FOXO4 and FOXO6) that is regulated by the phosphoinositol-3-kinase(PI3K)/Akt signalling pathway. It regulates multiple cellular processes, including cell cycle arrest, cell death, DNA damage repair, stress resistance and metabolism. Furthermore, inactivation or alteration of FOXO protein is linked to tumorigenesis in many cancers, either prostate cancer [4][5].

FOXO3a is also known as FOXO3 or forehead in rhabdomyosarcoma-like 1 (FKHRL1). The location of the FOXO3a gene is on chromosome 6q21. The FOXO3a protein consists of 5 domains, namely forkhead DNA- binding domain (DBD), 2 nuclear localization signals (NLS), nuclear export sequence (NES) dan C-terminal transactivation domain. FOXO3a has a crucial role in both oncogenesis and tumour suppression. Cellular

localization and phosphorylation status of FOXO3a is considered to be a prognostic factor for cancers. Loss of FOXO3a is found in various cancers. However, the mechanism of FOXO3a in prostate cancer remains unclear [4][6].

1.2. Gleason Score

Prostate cancer grading system according to the Gleason score currently used worldwide was introduced by Donald F. Gleason. A new grading system has been been proposed by the International Society of Urological Pathology (ISUP) in 2014 and is integrated into the 2016 edition of the WHO Classification of Tumours of the Urinary System and Male Genital Organs [7][8].

Gleason score is one of the conventional clinicopathological parameters. It is the most important prognostic factor in the progression and metastases of prostate cancer [9].

2. MATERIALS AND METHODS

The population was all cases of primary prostate cancer which were collected from the Department of Anatomical Pathology archives in West Sumatera. Samples were 56 cases of prostate cancer with adequate tumour tissue. This observational study with a cross-sectional design was approved by the local research and ethical review committee. Hematoxyline and eosin (HE) stained slides and formalin-fixed paraffin-embedded (FFPE) tissue specimens were retrieved. Slides of all cases were evaluated to review Gleason score, histopathological grading, and WHO grade group based on ISUP 2014/WHO 2016. Gleason score was grouped into low- grade (Gleason score < 8) and high-grade (Gleason score 8-10). Specimens included prostatic chips and prostatectomies. Moreover, representative tissue blocks of all 56 cases were selected for immunohistochemistry (IHC) examination.

IHC staining has been carried out at the Anatomical Pathology Department of RSUP Dr. Cipto Mangunkusumo Jakarta. The antibody was primary

rabbit polyclonal anti-FOXO3a antibody (clone ab23683, Abcam, dilution of 1:200) and secondary antibody (Starr Trek Universal Link, Biocare Medical). Positive control was human normal lung. Nuclear and cytoplasmic staining for FOXO3a was semi-quantitatively evaluated. Percentage of positive cell (PP) was scored into 0,1,2,3,4 (0, 1= <10%, 2= 10-50%, 3=51-80%, 4= >80%) and staining intensity (SI) was scored 0,1,2,3 (0=negative, 1=weak, 2= intermediate, 3= strong). Percentage and intensity scores were multiplied to generate an immunoreactive score (IRS) ranging from 0 to 12. The interpretation was 0-1= negative, 2-3= mild, 4-8= moderate, 9-12= strong. A cutoff value of 4 was used to categorize FOXO3a

expression into negative and positive and scored by a pathologist with clinical data blinded. Statistical analysis for quantitative variables was mean and standard deviation. Frequency and percentage were evaluated for qualitative variables. Chi-square test was applied to determine correlation. P-value < 0,05 was taken as significant.

3. RESULTS

The result of this study is shown in Table 1. The mean age of patients was 70.68±7.99 years. The most cases was noted in 71-80 (44.64%) years old.

Table 1. The Characteristic of Subject

Characteristic	f (n = 56)	%
Age (years)		
Mean±SD	70.68±7.99	
< 51	0	0
51 – 60	7	12.50
61 – 70	19	33.93
71 – 80	25	44.64
81 – 90	5	8.93
> 90	0	0

The highest proportion of prostate cancer was high-grade Gleason score (8-10), which were Gleason score 9 (44.64%) and Gleason score 8 (28.57%). Most of prostate cancer were cases with histopathological grading poorly differentiated/ undifferentiated (76.78%) and WHO grade group 5 (48.21%) (Table 2).

Table 2. Histopathology of Prostate Cancer

Histopathology	f (n = 56)	%
Gleason Score		
6	1	1.79
7	12	21.43
8	16	28.57
9	25	44.64
10	2	3.57
Histopathological Grading		
Well (GS ≤6)	1	1.79
Moderately (GS 7)	12	21.43
Poorly/Undiff (GS 8-10)	43	76.78
WHO Grade Group		
Grade 1	1	1.79
Grade 2	8	14.29
Grade 3	4	7.14
Grade 4	16	28.57
Grade 5	27	48.21
Gleason Score Group		
Low-grade	13	23.21
High-grade	43	76.79

Histopathology based on ISUP 2014/WHO 2016 was found that Gleason score 8 with fusion gland and

cribriform of pattern 4 is the most cases, then Gleason score 9 with comedo necrosis of pattern 5 (Figure 1).
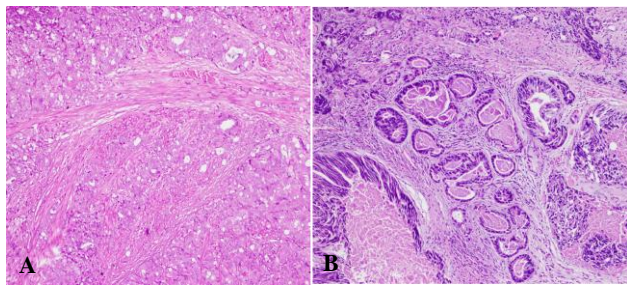

Figure 1. Histopathology of prostate cancer, A. Pattern 4 with fusion gland, B. Pattern 5 with comedo necrosis (HE, 100x).

IHC staining for FOXO3a was noted that positive FOXO3a expression (51.79%) was more than negative expression (48.21%) (Table 3).

Table 3. Immunohistochemistry Staining

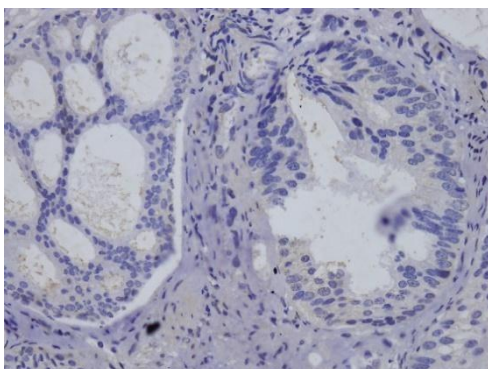| FOXO3a Expression	f (n = 56)	%
Negative	27	48.21
Positive	29	51.79

FOXO3a: Forkhead box O 3a

Figure 2. Negative FOXO3a expression of prostate cancer (400x).
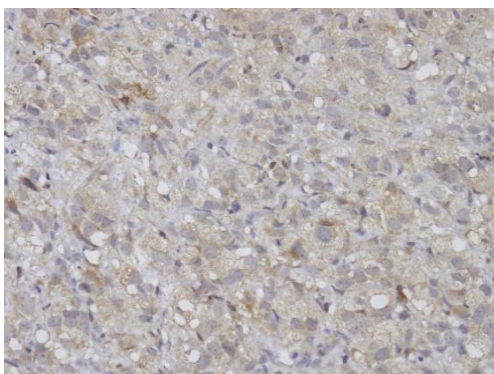

Figure 3. Mild FOXO3a expression of prostate cancer in cytoplasm (400x).

The FOXO3a IHC staining showed negative, mild, moderate, and strong expression (Figure 2-5). The positive FOXO3a expression in cytoplasm was noted predominant than nuclear.
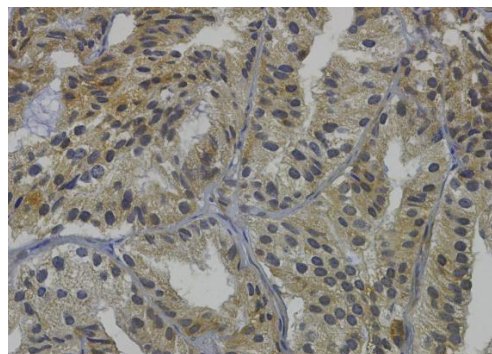

Figure 4. Moderate FOXO3a expression of prostate cancer in cytoplasm (400x).
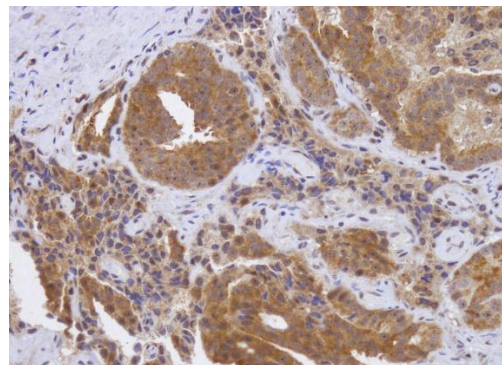

Figure 5. Strong FOXO3a expression of prostate cancer in cytoplasm (400x).

Prostate cancer with a high-grade Gleason score showed more negative FOXO3a expressions (88.89%) rather than positive, but low-grade Gleason scores showed more positive expressions (34.48%) rather than negative. Statistically, the Chi-Square test showed a negative significant correlation of FOXO3a expression and Gleason score (p = 0.038) (Table 2).

Table 4. Correlation between FOXO3a expression with Gleason score

FOXO3a expression	Gleason score		Total f (%)	p-value
	Low-grade f (%)	High-grade f (%)		
Negative	3 (11.11)	24 (88.89)	27 (100)	0.038
Positive	10 (34.48)	19 (65.52)	29 (100)	
Total	13 (23.21)	43 (76.79)	56 (100)	

FOXO3a: Forkhead box O 3a

4. DISCUSSION

FOXO3a is a downstream effector of PI3K/Akt signaling pathway. Upon activation of growth factor receptor tyrosine kinase, PI3K/Akt becomes activated. It

subsequently phosphorylates target protein to stimulate glucose uptake, cell proliferation, and survival [10]. Activation of Akt disrupt the balance of cell survival and apoptosis by inducing pro-survival signals and inhibiting pro-apoptotic signals through its transcription factor. One of the pro-apoptotic transcription factors is FOXO3a which plays important role in tumor suppressor and oncogenesis [11].

FOXO3a is negatively regulated downstream which described as inducers of apoptosis in many different cell types. The transcriptional activity of the FOXO3a is regulated through post-translational modification (PTM) which is a fundamental process for the regulation of protein function leading to changes in subcellular localization. FOXO3a activity is regulated by various PTMs including phosphorylation, acetylation, methylation, and ubiquination. Phosphorylation of FOXO3a by Akt induces binding to protein 14-3-3, which inhibits FOXO3a binding to target DNA and induces rapid release of FOXO3a from the nucleus and simultaneously inhibits its relocation into the nucleus [4][6].

Changes in expression and modification of the transcription factor FOXO3a activity play an important role in prostate cancer progression. Akt signaling plays a key role in the regulation of FOXO3a activity, phosphorylation and deactivation of FOXO3a in prostate cancer. Increased activation of Akt causes phosphorylation of FOXO3a which has the potential to affect transcriptional activity. Phosphorylation of FOXO3a increases binding to protein 14-3-3 (a chaperone protein) to form the FOXO3a-14-3-3 complex. The complex will be removed from the nucleus and retained in the cytoplasm [12][13][14].

Protein 14-3-3 can prevent FOXO3a protein from re-entering the nucleus by masking the nuclear localization signal (NLS) domain. The decrease in FOXO3a DNA binding domain (DBD) in tumor cells nucleus of prostate cancer indicates that the low transcriptional activity of FOXO3a. Furthermore, this process inhibits pro-apoptotic and induces tumor cell pro-survival. It is associated with biologically aggressive prostate cancer. The accumulation of FOXO3a in the cytoplasm is related to the phosphorylation of FOXO3a on Ser253, so that FOXO3a exits the nucleus. FOXO3a deregulation may be due to disturbance in signal transduction. Akt activation during prostate cancer progression is thought to result from signal disturbance associated with autocrine growth factor activity [12][13][14].

This study found that positive FOXO3a expression was mainly in the cytoplasm of tumor cells, some showed both of cytoplasm and nucleus. There is statistically significant negative corelation between FOXO3a expression and Gleason score (p=0.038).

The study of Shukla et al. (2009) found the result in line with this study. The expression of FOXO3a in the cytoplasm of tumor cell prostate cancer was higher than in the nucleus or both. The expression of FOXO3a in the nucleus of the benign lesion was found to be significantly higher than low-grade (p=0.039) and high-grade (p<0.0001) prostate cancer. They reported a higher proportion of FOXO3a expression in the cytoplasm as the Gleason score increased. These results indicate a correlation between decreased FOXO3a DNA binding activity which is in line with suppression of FOXO3a transcription with an increase in Gleason score [12].

5. CONCLUSION

FOXO3a was likely to be deactivated in prostate cancer. Abstain expression of FOXO3a in nuclear, but overexpression in cytoplasm of tumor cells induced loss of tumor suppression function. Thus, FOXO3a has potential as prognostic biomarker for prostate cancer.

REFERENCES

[1]   H. Sung *et al.*, "Global Cancer Statistics 2020: GLOBOCAN Estimates of Incidence and Mortality Worldwide for 36 Cancers in 185 Countries," *CA. Cancer J. Clin.*, vol. 71, no. 3, pp. 209–249, 2021, doi: 10.3322/caac.21660.

[2]   F. Bray, J. Ferlay, I. Soerjomataram, R. L. Siegel, L. A. Torre, and A. Jemal, "Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries," *CA. Cancer J. Clin.*, vol. 68, no. 6, pp. 394–424, 2018, doi: 10.3322/caac.21492.

[3]   The Global Cancer Observatory, "Cancer Incident in Indonesia," *Int. Agency Res. Cancer*, vol. 858, pp. 1–2, 2020.

[4]   Y. Liu *et al.*, "Critical role of FOXO3a in carcinogenesis," *Mol. Cancer*, vol. 17, no. 1, pp. 1–12, 2018, doi: 10.1186/s12943-018-0856-3.

[5]   A. E. Webb and A. Brunet, "FOXO transcription factors: Key regulators of cellular quality control," *Trends Biochem. Sci.*, vol. 39, no. 4, pp. 159–169, 2014, doi: 10.1016/j.tibs.2014.02.003.

[6]   M. Hornsveld, T. B. Dansen, P. W. Derksen, and B. M. T. Burgering, "Re-evaluating the role of FOXOs in cancer," *Semin. Cancer Biol.*, vol. 50, no. May 2017, pp. 90–100, 2018, doi: 10.1016/j.semcancer.2017.11.017.

[7]   J. I. Epstein, L. Egevad, M. B. Amin, B. Delahunt, J. R. Srigley, and P. A. Humphrey, "The 2014 International Society of Urological

Pathology (ISUP) Consensus Conference on Gleason Grading of Prostatic Carcinoma," *Am. J. Surg. Pathol.*, vol. 40, no. 2, pp. 244–252, 2016, doi: 10.1097/pas.0000000000000530.

[8]   P. A. Humphrey, J. I. Epstein, and G. J. Netto, "Tumours of the prostate," in *WHO classification of tumours of the urinary system and male genital organs*, 4th ed., H. Moch, P. A. Humphrey, and T. M. Ulbright, Eds. Lyon: IARC Press, 2016, pp. 138–61.

[9]   R. B. Shah and M. Zhou, "Recent advances in prostate cancer pathology: Gleason grading and beyond," *Pathol. Int.*, vol. 66, no. 5, pp. 260–272, 2016, doi: 10.1111/pin.12398.

[10]   B. D. Manning and A. Toker, "AKT/PKB Signaling: Navigating the Network," *Cell*, vol. 169, no. 3. Elsevier Inc., pp. 381–405, 2017, doi: 10.1016/j.cell.2017.04.001.

[11]   T. P. Das, S. Suman, H. Alatassi, M. K. Ankem, and C. Damodaran, "Inhibition of AKT promotes FOXO3a-dependent apoptosis in prostate cancer," *Cell Death Dis.*, vol. 7, no. 2, 2016, doi: 10.1038/cddis.2015.403.

[12]   S. Shukla, M. Shukla, G. T. MacLennan, P. Fu, and S. Gupta, "Deregulation of FOXO3A during prostate cancer progression," *Int. J. Oncol.*, vol. 34, no. 6, pp. 1613–1620, 2009, doi: 10.3892/ijo_00000291.

[13]   S. Shukla, "FOXO3a: A Potential Target in Prostate Cancer NIH Public Access," *Austin J Urol*, vol. 1, no. 1, pp. 1–4, 2014.

[14]   T. Obsil and V. Obsilova, "Structural basis for DNA recognition by FOXO proteins," *Biochim. Biophys. Acta - Mol. Cell Res.*, vol. 1813, no. 11, pp. 1946–1953, 2011, doi: 10.1016/j.bbamcr.2010.11.025.