47 kDa Protein Sequence : a Candidate for Bladder Cancer Biomarkers

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Full Title	**47 kDa Protein Sequence : a Candidate for Bladder Cancer Biomarkers**
Article Type	Research Article
Keywords	Bladder Cancer, Diagnosis, 47 kDa Protein, cytokeratin 14
Abstract	**Introduction:**In the globe, bladder cancer (BC) is the ninth most prevalent malignancy. The issue is that there are currently no biomarkers with high diagnostic accuracies for BC. Previous studies revealed that BC patients selectively express the 47 kDa protein. This study will identify the sequence from 47kDa protein as the basis for making antibodies to future bladder cancer biomarkers. **Method:** 47 kDaPeptides were analysed byelectrospray ionisation mass spectrometry using the Shimadzu Prominence nanoHPLC system [Shimadzu] coupled to a 5600 TripleTOF mass spectrometer [Sciex].Tryptic peptides were loaded onto an Agilent Zorbax 300SB-C18, 3.5 µm [AgilentTechnologies] and separated with a linear gradient of water/acetonitrile/0.1% formicacid (v/v). Spectra were analysed to identify proteins of interest using Mascotsequence matching software [Matrix Science] with UniProtdatabase **Results:** In the 47 kDa protein sequencing performed by the sequencing method, the following sequences were obtained:42’ APSTY

	GGGLS VSSSR 56'; 117' VTMQN LNDRL ASYLD KVRAL EEANA DLEVK IRDWY QR 153'; 161' DYSPY FK 167'; 176' ILTAT VDNAN VLLQI DNARL AADDF RTKYE TELNL RMSVE ADING LRRVL DELTL AR 232'; 252' NHEEE MNALR GQVGG DVNVE MDAAP GVDLS RILNE MRDQY EK 293'; 301' DAEEW FFTKT EELNR EVATN SELVQ SGKSE ISELR RTMQN LEIEL QSQLS MKASL ENSLE ETKGR 265; 406' TRLEQ EIATY RRLLE GEDAH LSSSQ FSSGS QSSR 469'; 460' VVSTH EQVLR 469'. The matching process was carried out with the mascot from the results of the sequenced pieces obtained from the extraction. Obtained a series of sequences that match Keratin, type I cytoskeletal 14—obtained index score of 908 and sequence coverage of 56%. **Conclussion :**Sequenced protein, showed that 47kDa protein is closest to type1cytoskeletal keratin 14, can be a candidate for bladder cancer biomarkers.
Novelty	The novelty of this research is sequencing 47kDa protein, that specifically expressed in the bladder cancer patients, a candidate for promising bladder cancer biomarkers
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Institutional Review Board Statement	The study was conducted according to the guidelines of the Declaration of Helsinki, and approved by Ethics Committee of Saiful Anwar Hospital Number 400/099/K.3/302/2021
Informed Consent	Informed consent was obtained from the subjects involved in the study.

Statement	Written informed consent has been obtained from the patient(s) to publish this paper
Data Availability Statement:	-
Conflicts of Interest:	The authors declare no conflict of interest

Introduction

One of the cancers that can develop in the urinary tract is bladder cancer (BC). Transitional cell carcinoma (TCC), the most common kind of bladder cancer, develops from the cells that line the bladder's epithelium.(1) BC is the ninth-leading cause of cancer worldwide. There have been 430,000 cases of BC this day.(2) The problem is the lack of accessible basic diagnostic kits for use by healthcare professionals in peripheral health facilities. The high recurrence rate of BC, 50–70% of newly diagnosed tumors return within five years, and 10–20% of individuals develop to an invasive disease, is another risk factor. Therefore, those who have BC should be under constant observation.(3)

One of the molecular biology disciplines with the highest rate of expansion is proteomics, which is concerned with a methodical approach to the evaluation of protein expression in cells or organisms. In a preliminary investigation, researchers from the Faculty of Medicine at Universitas Brawijaya discovered that both healthy bladder epithelial cells and cancer cells in the bladder had a protein with a molecular weight of 122 kDa. A protein with a molecular weight of 69 kDa, was exclusively discovered in healthy bladder epithelial cells. Additionally, cells from bladder cancer were the only ones to contain a protein with a molecular weight of 47 kDa.(4)

Utilizing a 47 kDaprotein based polyclonal antibody from BC epithelial cells, the immunocytochemical test had a 100% sensitivity value and a 30% specificity. The sensitivity value of polyclonal antibody of 47 kDAprotein were higher than Bladder Tumor Antigen STAT (BTA-STAT), Urinary Bladder Cancer Rapid (UBC Rapid), Bladder Tumor Antigen - TRAK assay (BTA - TRAK), Urinary Bladder Cancer Rapid - immunoradioassay (UBC IRMA) nuclear matrix protease-22 (NMP-22) and telomeric repeat amplification protocol (TRAP) were thought to be based on the characteristics of the targeted antigen. (5–7) This study will try to explore the sequence of 47 kDa protein, a promising candidate for BC biomarkers.

Method

The procedure for preparing the 47-kDa protein was carried out as follows before the sequencing process started: 10 to 100 picomoles of the 47 kDa protein are required for sequencing. In order to dissolve the liquid sample, use acetonitrile or propanol. Samples were provided on a PVDF membrane measuring 40 mm2. Cysteine was located by alkylation. Additionally, the tris or glycine-containing primary amines were removed from the 47-kDa protein. Dialysis was done with the goal of keeping the salt content under 2 mM. To get rid of the glycine from the SDS-PAGE, the protein has to be electroblotted onto the PVDF membrane. The membrane was then thoroughly washed 4 or 5 times to get rid of any remaining tris or glycine.

By hydrolyzing a 47 kDa protein in 6 M hydrochloric acid for 24 hours at 100°C, the amino acid was determined. Chromatography is used to separate hydrolyzed amino acids, and the ninhydrin technique is used to determine how much of each amino acid is present. The number of amino acids was counted using the absorbance measurement. Using the Sanger, fluoro 2,4-dinitrobenzene (FDBN), or dansyl chloride techniques, the N-terminal amino acids were sequenced. Using carboxypeptidase enzymes, the C-terminal sequence was determined. Beta-mercaptoethanol and iodoacetic acid were added to eliminate disulfide linkages that can obstruct sequencing. To convert big polypeptides into smaller polypeptides containing 15–25 amino acids, specific protease enzymes were introduced.

SDS-PAGE gels were used to separate proteins. Put the protein up for electroblotting on a sturdy surface, such a PVDF membrane. An automated protein sequencer received protein feeds that were connected to the PVDF membrane. when the protein on the solid support is exposed to Edman's reagent, it modifies the N-terminal amino acid. Under acidic circumstances, the changed N-terminal amino acids were hydrolyzed and identified by chromatography. Based on this approach, automated amino acid sequencers are produced. Utilizing Mascott sequence matching software built on the UniProt database, the amino acid sequence will be examined.

Result

Sequencing Determination and Equalizing sequencing

The following sequences were found in the 47 kDa protein sequences produced by the sequencing technique:

1. 42’ APSTY GGGLS VSSSR 56’
2. 117’ VTMQN LNDRL ASYLD KVRAL EEANA DLEVK IRDWY QR 153’

3. 161’ DYSPY FK 167’
4. 176’ ILTAT VDNAN VLLQI DNARL AADDF RTKYE TELNL RMSVE ADING LRRVL DELTL AR 232’
5. 252’ NHEEE MNALR GQVGG DVNVE MDAAP GVDLS RILNE MRDQY EK 293’
6. 301’ DAEEW FFTKT EELNR EVATN SELVQ SGKSE ISELR RTMQN LEIEL QSQLS MKASL ENSLE ETKGR 265
7. 406’ TRLEQ EIATY RRLLE GEDAH LSSSQ FSSGS QSSR 469’
8. 460’ VVSTH EQVLR 469’

The sequenced fragments acquired from the extraction were compared to the Mascot during the matching procedure. The sequences match with Keratin, type I cytoskeletal 14 (Cytokeratin 14/CK14); they received an index score of 908 and 56% of the sequences were covered.

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  1 MTTCSRQFTS SSSMKGSCGI GGGIGGGSSR ISSVLAGGSC RAPSTYGGGL
 51 SVSSSRFSSG GACGLGGGYG GGFSSSSSSF GSGFGGGYGG GLGAGLGGGF
101 GGGFAGGDGL LVGSEKVTMQ NLNDRLASYL DKVRALEEAN ADLEVKIRDW
151 YQRQRPAEIK DYSPYFKTIE DLRNKILTAT VDNANVLLQI DNARLAADDF
201 RTKYETELNL RMSVEADING LRRVLDELTL ARADLEMQIE SLKEELAYLK
251 KNHEEEMNAL RGQVGGDVNV EMDAAPGVDL SRILNEMRDQ YEKMAEKNRK
301 DAEEWFFTKT EELNREVATN SELVQSGKSE ISELRRTMQN LEIELQSQLS
351 MKASLENSLE ETKGRYCMQL AQIQEMIGSV EEQLAQLRCE MEQQNQEYKI
401 LLDVKTRLEQ EIATYRRLLE GEDAHLSSSQ FSSGSQSSRD VTSSSRQIRT
451 KVMDVHDGKV VSTHEQVLRT KN
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Figure 1 Keratin sequence, type I cytoskeletal 14(Cytokeratin 14/CK14), red color shows the 47kDA protein sequence obtained from the urine of bladder cancer patients.

Discussion

47 kDa protein sequence match with Keratin, type I cytoskeletal 14 (Cytokeratin 14/CK14); they received an index score of 908 and 56% of the sequences were covered. We will explore the role of CK 14 in the BC screening and grading of BC and reccurencies.

CK 14 in Bladder Cancer Screening

Considered to be a sign of epithelial cell differentiation is CK14 expression. According to one study, CK14 was expressed in urothelial BC with a squamous cell componentor bladder squamous cell carcinomas (SCC).(8) Another study found that the bladder cancer model mice produced by N-butyl-N-(4-hydroxybutyl) nitrosamine (BBN) had strong CK14 expression. CK14 expression is low in healthy mice, however it greatly elevated in animals treated with BBN. (9) These investigations demonstrated that CK14 expression has role in the detection and prediction of BC caused by bladder epithelial metaplasia. and it is potentially beneficial for bladder malignancies screening.

CK 14 in Bladder Cancer Grading

Some characteristics of a malignant tumor include rapid progression to an undifferentiated histological structure and invasiveness. To develop an effective treatment strategy for each patient, it is crucial to determine the aggressiveness of the malignancy. 10% to 20% of instances of bladder cancer result in an invasive tumor. Due to its high propensity for distant metastases and hence increased mortality, muscle invasive bladder cancer. (10). Invasive urothelial carcinomas showed low expression of CK14, according to Gruver (2012). (11) In contrast to these findings, Nakayama (2011) showed increasing expression of CK14 with the highest expression seen in CIS as squamous intraepithelial neoplasia advanced. (12) A recent study also revealed that because of its invasive characteristics, urothelial carcinoma with increased CK14 expression was linked to extremely bad outcomes. (13-14)CK14 expression in leading cells in the case of collective invasion has been noted in a variety of literatures. (15-16) In general, higher levels of malignancy are linked to increased CK14 expression. (17) The potential role of CK14 may help to explain this occurrence. Alam (2011) found that cell proliferation and cell cycle progression were inhibited in CK14 knockdown cells. This was made feasible by the removal of CK14, which decreased PI3K/Akt signaling, which is involved in cell proliferation, and enhanced Notch-1 signaling, which is involved in cell differentiation. (18) The expression of CK14 may allow for the stratification of bladder cancer patients according to the degree of metaplasia and the detection of bladder cancer's invasiveness. But further research is still required.

CK14 in Predicting Bladder Cancer Recurrence

The cancer is frequently involves of recurrence. When cancer cells are not completely eliminated throughout the treatments, this event may happen more frequently. In 50% to 70% of instances, bladder cancer will return within 5 years from the initial diagnosis. Greater CK14 expression may indicate a greater grade of BC, as was previously described. Increased CK14 expression also suggested a higher bladder cancer recurrence risk. (13) High CK20 and low CK5 expression have also been shown to be indicators of poor recurrence-free survival. (19)

Conclusion

47kDa protein sequenceis closest to type1 cytoskeletal keratin 14, can be a candidate for bladder cancer biomarkers.

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