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ORIGINAL RESEARCH PAPER

Antibody-based biosensor to detect oncogenic splicing factor Sam68 for the diagnosis of lung cancer

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Abstract
Objective The present work aimed to investigate the potential utility of Sam68 protein as a prognostic marker in lung cancer. Then an electrochemical immunosensor is fabricated that is sufficiently sensitive to detect Sam68.
Results Analysis of stage-specific Lung cancer microarray data shows that differential expression of Sam68 is associated with cancer stage and monotonically increases from early tumor stage to advanced metastatic stage. Moreover, the higher expression of Sam68 results in reduced survival of lung cancer patients. Based on these observations, an electrochemical immunosensor was developed for the quantification of Sam68 protein. The target protein was captured by the Anti-Sam68 antibody that was immobilized on the modified Glassy carbon electrode. The stepwise assembly process was characterized by cyclic voltammetry and electrochemical impedance spectroscopy. This fabricated immunosensor displayed good analytical performance in comparison to commercial ELISA kit with good sensitivity, lower detection limit (LOD) of 10.5 pg mL^{-1}, and wide linear detection range from 1 to 5 μg mL^{-1}. This method was validated with satisfactory detection of Sam68 protein in lung adenocarcinoma cell line, NCI-H23. Besides, spike and recovery assay reconfirm that the sensor can precisely quantify Sam68 protein in a complex physiological sample.
Conclusion We conclude Sam68 as a valuable prognostic biomarker for early detection of lung cancer. Moreover, we report the first study on the development of an electrochemical immunosensor for the detection of Sam68. The fabricated immunosensor exhibit excellent analytical performance, which can accurately predict the lung cancer patient pathological state.

Keywords Splicing factor · Prognostic · Diagnostic · Biomarker · Lung cancer · Biosensors

Introduction

The Src-associated protein in mitosis (Sam68), also known as KHDRBS1, is a member of signal

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transduction and activation of RNA (STAR) family of RNA-binding proteins (Lukong and Richard 2003). Sam68 is involved in both RNA processing and signal transduction pathways due to the presence of KH-domain (K homology) and SH-domain (Src homology) (Sanchez-Jimenez and Sanchez-Margalet 2013). Therefore multimodular Sam68 links extracellular signals to RNA processing machinery in the cell (Frisone et al. 2015; Rajan et al. 2008). A series of published reports revealed overexpression of Sam68 protein/mRNA in several cancers including prostate (Busa et al. 2007), breast (Elliott and Rajan 2010), bladder (Zhang et al. 2015b), colorectal (Liao et al. 2013), ovarian (Dong et al. 2016), hepatic (Zhang et al. 2015a), tongue (Chen et al. 2016), and renal cell carcinoma (Zhang et al. 2009). Moreover, higher expression of Sam68 is associated with poor prognosis and overall survival of a various type of cancer (Chen et al. 2016; Dong et al. 2016; Elliott and Rajan 2010; Liao et al. 2013; Sumithra et al. 2019; Zhang et al. 2014, 2015b). The involvement of Sam68 protein in cancer-specific cellular processes, including cell proliferation, apoptosis, metastasis, and chemoresistance are well established (Bielli et al. 2011; Sanchez-Jimenez and Sanchez-Margalet 2013). Particularly, Sam68 mediated cancer-specific events are regulated by mis-regulation of alternative splicing in cancer, which results in the generation of oncogenic splicing isoforms (Frisone et al. 2015; Gaytan-Cervantes et al. 2017; Stockley et al. 2015). These earlier studies illustrate Sam68 as an oncogenic protein with good prognostic value in various cancers. Herein, for the first time, we show that Sam68 is a good diagnostic and prognostic biomarker for early detection of lung cancer. We observed that the expression of Sam68 is associated with pathogenic stages of lung cancer. Indeed, higher expression of Sam68 increases the lung cancer patient mortality. Besides, lung cancer remains asymptomatic in the early stage and is typically diagnosed at an advanced stage. Treatment of most late-diagnosed lung cancers remains inefficient (Gazdar et al. 2017; Khanmohammadi et al. 2020). Therefore, identification of early diagnostic and prognostic biomarkers are of urgent need. Based on the above observations, experimentally we assessed and shown the expression of Sam68 protein in NCI-H23 (human lung adenocarcinoma) cells. Next, using the recombinant Sam68, expressed in *E. coli*, we fabricated a sensitive antibody-based impedimetric sensor, which can accurately quantify the Sam68 expression in lung cancer cells. It is worth to mention that this is a first report on the analytical detection of a splicing factor for clinical diagnosis of lung cancer.

Materials and methods

Materials

Sodium chloride (NaCl), disodium hydrogen phosphate anhydrous (Na_2HPO_4), potassium dihydrogen phosphate (KH_2PO_4), potassium chloride (KCl), glycerol, triton X-100, methanol, sodium fluoride, sodium orthovanadate, DMEM, and Bradford Reagent (Coomassie Blue G250) were procured from Himedia. Isopropyl-β-D-thiogalactopyranoside (IPTG), bovine serum albumin (BSA) phenylmethylsulfonyl fluoride (PMSF) and ELISA-TMB substrate were procured from Sigma. Glutaraldehyde was obtained from Merck. Tris–HCl and other SDS-PAGE reagents were obtained from Ameresco and Western blot reagents from Thermo scientific. Aniline, sulphuric acid, Potassium hexacyanoferrate (III) ($K_3[Fe(CN)_6]$), and Potassium hexacyanoferrate (II) ($K_4[Fe(CN)_6]$) were procured from FINAR. Fetal calf serum was purchased from GIBCO. All the reagents purchased were of laboratory grade.

Maintenance of mammalian cell lines and protein extraction

Human lung adenocarcinoma cell line, NCI-H23, was procured from National Centre for Cell Sciences (NCCS), India, and maintained in DMEM supplemented with 10% fetal calf serum at 37 °C in a humidified incubator with 5% CO_2. Total protein was isolated after discarding the media from the culture plate, and cells were washed with ice-cold PBS to remove the residual media. Ice cold RIPA buffer containing 50 mM Tris–HCl, pH 7.5, 50 mM NaCl, 1% Triton X-100, 0.1% SDS, 1 mM PMSF, 50 mM sodium fluoride, and 1 mM sodium orthovanadate was added to culture plate. The cells were scraped from the plate and sonicated. The cell lysate was centrifuged, and the supernatant was collected for further experiments.

Microarray datasets and survival analysis

Microarray dataset (GSE30219) (Rousseaux et al. 2013) with clinical information was obtained from the GEO database (www.ncbi.nlm.nih.gov/geo). The normalized mRNA expression data was taken for Sam68 expression analysis in different stages of cancer. For survival analysis, the patient samples were categorized into two groups: Sam68 high group (> 75 percentile) and Sam68 low group (< 25 percentile) based on the expression median. Kaplan and Meier (Meier 1958) method was used to perform the survival analysis, and the Log-Rank test was used to measure the statistical significance. Survival curves were generated using GraphPad Prism 7 software.

Expression and purification of recombinant Sam68 protein

Sam68 cDNA cloned in the pGEX-2T vector was gifted from David Shawalloy (Cornell University, USA). Restriction digestion and colony PCR confirmed this recombinant clone. Further pGEX-2T-Sam68 was cloned into *E. coli* BL21 strain for protein expression. GST tagged Sam68 protein (GST-Sam68) was induced with 0.5 mM IPTG for 3 h at 30 °C. Subsequently, the cells were pelleted and resuspended in lysis buffer (PBS pH 7.4 containing 0.1% Triton X-100, PMSF) and then sonicated. Recombinant GST-Sam68 protein was purified from clarified cell lysate using Sepharose 4B - Glutathione beads (Sigma) as per the manufacturer's protocol. Freshly prepared 20 mM reduced GSH in 50 mM Tris–HCl, pH 8.0 was used for elution of the recombinant protein, and the residual GSH was removed by dialysis against PBS. Recombinant protein concentration was estimated by Bradford assay [20] using BSA as a standard. The purified recombinant protein was characterized by Western blot.

Western blot analysis

Initially, the protein was separated on 12% SDS–PAGE and electro-transferred from gel to PVDF membrane. After blocking with 5% BSA in PBST (PBS with 0.1% tween 20), the membrane was incubated with a 1:1000 diluted rabbit anti-Sam68 antibody (Sigma). Followed by PBST wash, the membrane was incubated with 1:1000 diluted HRP-conjugated anti-rabbit immunoglobulin G (Sigma). Finally, blots were developed using Pierce^{TM} ECL Western blotting substrate (Thermo Fischer Scientific) to visualize the protein band.

ELISA

Purified GST-Sam68 protein at various concentrations (10^{-3}, 10^{-2}, 10^{-1}, 1, 5, 10 μg mL^{-1}) and different dilutions of NCI-H23 protein extract (1:100, 1:10 and crude extract) were coated in ELISA plate and above mentioned anti-Sam68 primary and secondary antibodies were used to quantify the protein. 3,3′,5,5′-Tetramethylbenzidine (TMB) liquid substrate system (Sigma) was used as a substrate, and optical density was measured at 370 nm using the Multiskan microplate reader (Thermo Fisher Scientific).

Fabrication of electrochemical immunosensor

The glassy carbon electrode (GCE) surface was polished with alumina powder and cleaned thoroughly by sonication in Milli-Q water before use. Firstly, the electropolymerization of aniline on GCE was carried out by cyclic voltammetry (0–1 V; 100 mV s^{-1} scan rate) in 0.1 M aniline and 0.5 M sulphuric acid solution to get polyaniline (PANI) modified GCE (GCE/PANI) (Jayanthi et al. 2019; Sai et al. 2006; Viswanathan et al. 2009). After rinsing with water to remove any un-polymerized aniline, 5 μl of 4% glutaraldehyde (GA) was dropped on the electrode surface and incubated for 3 h. After thorough washing with PBS, 5 μl of anti-Sam68 antibody (3 μg mL^{-1}) in PBS was added on the GCE/PANI/Glu and left for 2 h at room temperature to get immobilized. Subsequently, the GCE/PANI/Glu/Sam68-Ab electrode was rinsed, and 5 μl of 0.1% BSA was also added onto the electrode and incubated for 1 h to block any non-specific binding. The final electrode (GCE/PANI/Glu/Sam68-Ab/BSA) was stored at 4 °C until not in use.

Electrochemical experiments

All electrochemical measurements were performed with Autolab Potentiostat/Galvanostat (Metrohm, India) comprising of a conventional three-electrode system. GCE or modified GCEs were used as the working electrode, platinum electrode as a counter electrode, and Ag/AgCl (3 M KCl) as the reference

electrode. EIS and CV were performed in 0.1 M PBS (pH 7.4) with 5.0 mM redox couple,$K_3[Fe(CN)_6]$/$K_4[Fe(CN)_6]$ and 0.1 M KCl. All cyclic voltammetry (CV) experiments with the immunosensor were performed in the potential range from − 1 to + 1 V at a scan rate of 0.1 V/s. The electrochemical impedance spectroscopy (EIS) measurements were performed within 10,000 to 0.05 Hz frequency range with an amplitude of 10 mV and stepped potential 0.18 V. NOVA software (Autolab) (version 1.11.0) was used to analyze the electrochemical results. The impedance values were fitted to the Randles equivalent circuit. The charge transfer resistance (R_{ct}) at the electrode surface was calculated from the diameter of the semicircle portion at higher frequencies in the impedance spectra.

Results and discussion

Stage-specific expression of Sam68 is associated with poor survival of lung cancer patients

Treatment of any cancer is successful if it is detected and treated in the early stage. Effective treatment also depends on the target molecules and their association with cancer progression. The genes which are expressed in early-stage and continue the higher expression in advanced stages of cancer could be the best target for diagnosis and treatment. Therefore, we evaluated the stage-specific mRNA expression status of Sam68 using microarray dataset from GEO (GSE30219) (Rousseaux et al. 2013). The data contains 292 lung cancer samples across different stages (stage1 to stage4) and 14 healthy samples. We compared the normalized mRNA expression between cancer and healthy samples (Fig. 1a), and we observed that the expression of Sam68 in the healthy cell is not sufficiently lower compared to the cancer cell. Therefore we sub-grouped the cancer patients according to the pathological stage (Fig. 1b) and observed that the mean mRNA expression of Sam68 is monotonically and significantly increased as cancer progressed to advance stages. This indicates that increased levels of Sam68 may substantially correlate with the metastatic event in lung cancer. Next, we asked whether this aberrant expression pattern is correlated with the clinical outcomes of patients. We grouped the 292 cancer patients in a high and a low group based on the mRNA expression level of Sam68 and performed survival analysis. Kaplan Meier survival analysis and Log-rank test confirmed that the elevated mRNA expression of Sam68 is associated with poor overall survival of patients (Fig. 1c). Besides, stage-specific survival analysis reconfirms that the overall survival of the patients significantly reduced as cancer progressed to the higher stage (Fig. 1d). Our observation from Fig. 1 supports Sam68 as a good prognostic and diagnostic marker in lung cancer.

Analytical detection of recombinant Sam68 with ELISA

As Sam68 has a high prognostic value in lung cancer, it is essential to have an efficient and sensitive detection system that can be clinically applied. To detect Sam68 in protein level in cancer cells either using ELISA or antibody-based biosensor, we have cloned, expressed, and purified GST tagged Sam68 protein in *E.coli*BL21 cell. The recombinant protein of Sam68 was then characterized by Western blot (Fig. 2a). We first explored conventional ELISA. All ELISA measurements were performed in triplicate with Sam68 in the concentration range of 10^{-3}–10 μg mL^{-1} (Fig. 2b). The limit of detection (LOD) and limit of quantification (LOQ) were calculated from the expression LOD = (3 × SD)/m and LOQ = (10 × SD)/m (where SD is the estimated standard deviation from the points used to construct the calibration curve and m represents slope) (Lu et al. 2016; Shrivastava and Gupta 2011). LOD and LOQ of ELISA were determined as 1.4 μg $mL^{-1}$and 4.6 μg mL^{-1}, respectively. As ELISA was not found to be sensitive for lower concentrations of Sam68, an anti-Sam68 antibody-based immunosensor was designed for the sensitive detection of Sam68 protein.

Characterization of the immunosensor

For the fabrication of the immunosensor to detect Sam68 protein, we have used PANI as immobilization support because of its excellent conductivity and stability (Dhand et al. 2011; Fang et al. 2017). The CV (Fig. 3a) was performed to study the change in the electrical interface at each step of immunosensor fabrication. The modification of the GCE with highly conductive PANI facilitated the electron transfer kinetics at the electrode interface, evident from the

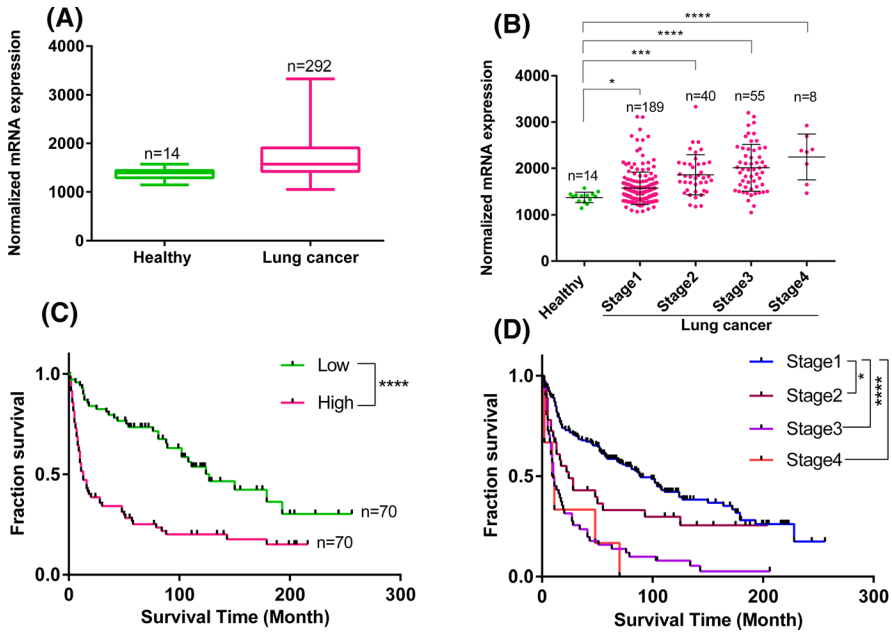

Fig. 1 Sam68 is a prognostic marker in Lung cancer. **a** The box plot shows the Sam68 mRNA expression in healthy and cancer patients. Transcriptomic data of GSE30219 was used to analyze Sam68 mRNA expression. Data were normalized using the Robust Multi-array Average (RMA) algorithm and quantile normalization techniques (Rousseaux et al. 2013). **b** Expression of Sam68 mRNA in different stages of lung cancer. The expression of Sam68 significantly increases in the higher stage. **c** Kaplan–Meier curve survival plot shows the comparison of fraction survival in higher and lower expression group of Sam68. **d** Kaplan–Meier curve shows the comparison of fraction survival in different stages of lung cancer. The significance level in each plot are represented as *P < 0.05,**P < 0.01, ***P < 0.001, ****P < 0.0001

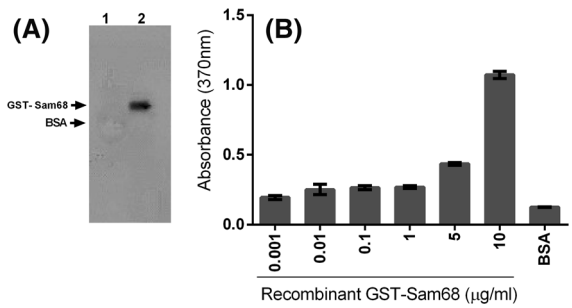

Fig.2 Characterization and detection of recombinant Sam68 protein by western blot and ELISA. **a** Western blot shows the recombinant GST tagged Sam68 protein (~ 74 KDa) (lane 2) expressed in *E.coli* BL21.Lane1: BSA (66 KDa). **b** The bar diagram shows the absorbance (370 nm) of different concentrations (0.001 to 10 μg mL^{-1}) of recombinant Sam68 protein detected by ELISA. Rabbit anti-Sam68 and Goat anti-rabbit HRP-conjugate were used as a primary and secondary antibody, respectively, and BSA was used as a negative control

increase in the peak current of the redox probe. The peak current of the redox probe was found to be decreased when PANI was cross-linked with glutaraldehyde. When the anti-Sam68 antibody was immobilized on the electrode surface (GCE/PANI/GLU/Sam68-Ab) and blocked with BSA (GCE/PANI/GLU/Sam68-Ab/BSA), the peak currents further decreased due to the insulating effect of protein molecules on the electron transfer kinetics. The change in the dielectric properties at the electrode/electrolyte interface at different stages of immunosensor fabrication was further examined with EIS. Figure 3b shows the impedance spectra obtained on different modified electrodes. The bare GCE showed a diffusional limiting electrochemical process represented by a small semicircle with a straight line in the impedance spectra. As the highly conducting PANI facilitates the electron transfer process, expectedly, a very low R_{ct} was obtained on PANI modified GCE. The cross-linking of PANI with glutaraldehyde slightly increased the R_{ct} value. When the anti-Sam68 antibody was immobilized on the electrode, there was a significant increase in the R_{ct} value, and it further increased when the antibody modified electrode surface was blocked with BSA. The increase in the

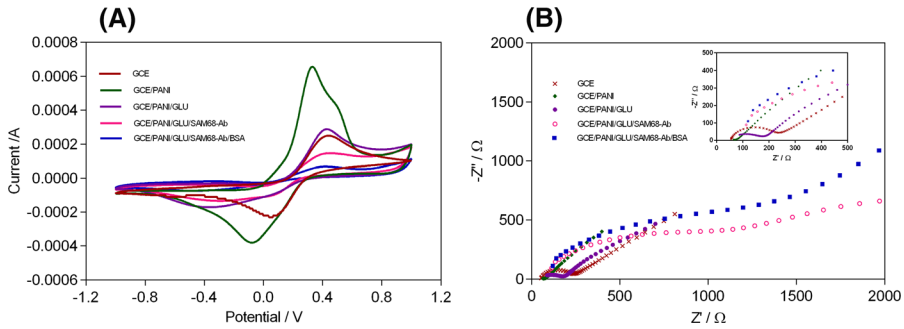

Fig. 3 **a** Cyclic voltammetry performed in 0.1 M PBS (pH 7.4) with 5.0 mM $[K_3Fe(CN)_6/K_4Fe(CN)_6]$ and 0.1 M KCl with GCE, GCE/PANI, GCE/PANI/GLU, GCE/PANI/GLU/SAM68-Ab, and GCE/PANI/GLU/SAM68-Ab/BSA electrodes (Scan rate: 100 mV s^{-1}. **b** EIS carried out in 0.1 M PBS (pH 7.4) with 5.0 mM $[K_3Fe(CN)_6/K_4Fe(CN)_6]$ and 0.1 M KCl with GCE, GCE/PANI, GCE/PANI/GLU, GCE/PANI/GLU/SAM68-Ab, and GCE/PANI/GLU/SAM68-Ab/BSA electrodes (alternating wave of 10 mV amplitude in the frequency range between 10,000 and 0.05 Hz). Figure B inset: enlarged impedance spectra

R_{ct} value may be due to the insulating effect of protein molecules on the electron transfer process.

Analytical detection of recombinant Sam68 with immunosensor

Electrochemical detection of Sam68 was carried out by incubating the fabricated immunosensor (GCE/PANI/GLU/Sam68-Ab/BSA) with various concentrations of purified Sam68 protein ranging from 0.001 to 5 µg mL^{-1} at room temperature for 1 h. At each concentration, EIS and CV were performed in triplicates (n = 3). Figure 4a, b show the resulting cyclic voltammograms and impedance spectra, respectively. Between each analysis, the immunosensor surface was regenerated by incubation in a solution containing 6 mM NaOH and 0.6% ethanol for 5 min and washing with PBS three times. As indicated in Fig. 4a, b, with increasing concentrations of Sam68 protein, there is a decrease in the peak currents and an increase in impedance. This increase in the Rct values is indicative of the recognition of Sam68 and the formation of immunocomplexes. Increasing concentration of Sam68 resulted in more bonding of target protein, leading to increased impedance. Figure 4c exhibits a linear relationship between R_{ct} and logarithmic value of Sam68 concentration (R^2 = 0.9850) (Fig. 4c). We also observed the linear relationship between R_{ct} and concentration (0.1 µg mL^{-1}–5 µg mL^{-1}) of Sam68 without using a logarithmic scale (Supplementary Fig. 1). The immunosensor gave a LOD of 10.5 pg mL^{-1} and a LOQ of 35.1 pg mL^{-1}, which

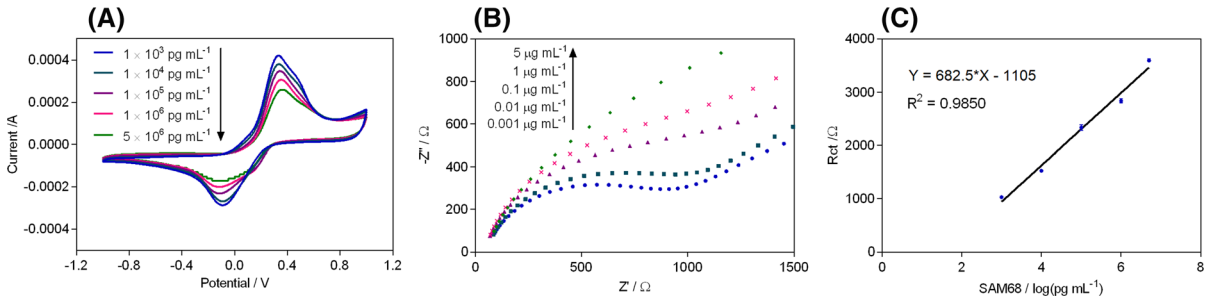

Fig. 4 **a** CV carried out in 0.1 M PBS (pH 7.4) with 5.0 mM $[K_3Fe(CN)_6/K_4Fe(CN)_6]$ and 0.1 M KCl using the fabricated immunosensor (GCE/PANI/GLU/Sam68-Ab/BSA) with increasing concentrations of Sam68 (Scan rate: 100 mV s^{-1}). **b** EIS performed in 0.1 M PBS (pH 7.4) with 5.0 mM $[K_3Fe(CN)_6/K_4Fe(CN)_6]$ and 0.1 M KCl with the immunosensor (GCE/PANI/GLU/Sam68-Ab/BSA) incubated with increasing concentrations of Sam68 (alternating wave of 10 mV amplitude in the frequency range between 10,000 and 0.05 Hz). **c** Calibration plot of the immunosensor with Sam68. Each datum point represents the average of triplicate values (n = 3). Error bars correspond to the standard deviation (SD) of three measurements (n = 3) at each concentration

are much lower compared to ELISA. Therefore the fabricated immunosensor would be efficient in detecting the low concentrations of Sam68 protein for early diagnosis of lung cancer. To date, there is no report on the development of a biosensor for the detection of Sam68. Therefore, we have compared the analytical performance of the present immunosensor with other electrochemical biosensors used for the detection of lung cancer biomarkers (Supplementary Table 1). The comparison shows the performance of the immunosensor in the present study is superior to other biosensors in terms of detection range and lower detection limit.

Storage stability of the immunosensor

The storage stability of the freshly prepared GCE based immunosensor (GCE/PANI/Glu/Sam68-Ab/BSA) was evaluated by storing the immunosensor in 0.1 M PBS (pH 7.4) at 4 °C and monitoring its electrochemical response at regular intervals. During this period, the immunosensor retained ~ 90% of the initial activity up to three weeks. The results show that the fabricated immunosensor has good analytical performance and stability that could be used up to 3 weeks when stored at 4 °C. It can be inferred that cross-linking of the antibody (biorecognition) molecules onto PANI surface through glutaraldehyde has prevented the leakage of the antibody molecules and thus stability is attributed.

Real sample analysis with the developed immunosensor

To evaluate its diagnostic application, the developed immunosensor was employed to detect Sam68 in lung cancer whole-cell extract. To check the antibody performance and protein expression level of Sam68, we performed Western blot (Fig. 5a). The results show that Sam68 protein is detected in NCI-H23 cell extract and there is no nonspecific band. Thus this antibody can be specifically used to detect Sam68 in a complex biological sample. The immunosensor was incubated at room temperature for one hour with different dilutions of NCI-H23 cell extract (1:100 dilution, 1:10 dilution, and crude extract) and the impedance spectroscopy was performed, and the concentration of Sam68 in these dilutions were identified (Table 1). Moreover, ELISA was also performed to quantify the

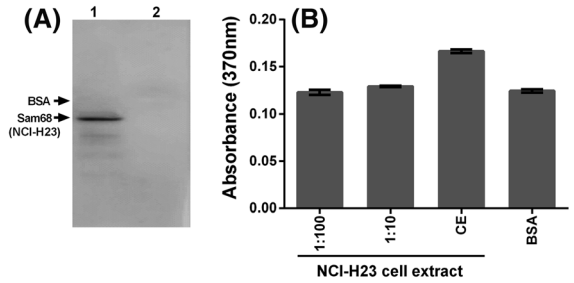

Fig. 5 Detection of Sam68 in NCI-H23 cell line. **a** Western blot shows the expression of Sam68 (~ 60 KDa) in the human lung cancer cell line, NCI-H23 (lane1). Lane2: BSA (66 KDa). **b** Different dilution of NCI-H23 cell extract was used to detect Sam68 in ELISA [1:100, 1:10 indicate the dilution of crude extract (CE)]. Rabbit anti-Sam68 and Goat anti-rabbit HRP-conjugate were used as the primary and secondary antibody, respectively, and BSA was used as a negative control

Sam68 protein in these dilutions (Fig. 5b). As the concentration of Sam68 in different dilutions of NCI-H23 cell extract is very low, the ELISA method was unable to differentiate between the amounts of protein as noted in previous results (Fig. 2b). The immunosensor response was further recorded with 0.01, 0.1, and 1 μg mL^{-1} recombinant Sam68 spiked in 1:100 dilution of NCI-H23 cell extract. The results in Table 2 indicate that the immunosensor is showing a good recovery of Sam68 protein in NCI-H23 cell extract, which is in the range of 92.37–109.37. The superior recovery indicates that the fabricated immunosensor can selectively detect Sam68 in a complex biological sample without the matrix effect of the cell lysate.

Conclusion

Lung cancer remains latent in an early stage and is typically diagnosed at an advanced stage. This makes both early diagnosis and treatment challenging. Therefore identification of early diagnostic biomarkers is of urgent need, which is a critical determinant for the initiation of appropriate treatment. In the present study, we demonstrated Sam68 as a stage-specific diagnostic and prognostic biomarker in lung cancer through microarray dataset analysis. Since low levels of the biomarker is expressed in early pathological stages, a simple and efficient detection system is required to quantify Sam68 protein**.** Thus, we designed an immunosensor that can correctly measure the Sam68 expression level in lung cancer patients. We

Table 1 Determination of Sam68 protein concentration using the immunosensor in different dilutions of NCI-H23 cell extract

NCI-H23 cell extract dilutions	Sam68 found by immunosensor (μg mL^{-1})
1:100	0.001017081
1:10	0.006959364
Crude extract	0.513819864

Table 2 Determination of Sam68 protein spiked in NCI-H23 cell extract using the immunosensor

S. no	Sam68 spiked (μg mL^{-1})	Sam68 in 1:100 dilution of NCI-H23 cell extract detected by immunosensor (μg mL^{-1})	Expected Sam68 concentration (μg mL^{-1})	Total Sam68 concentration detected with immunosensor (μg mL^{-1})	Recovery (%)	RSD (%)
1	0.01	0.00101708	0.011017	0.01205	109.37	2.33946
2	0.1	0.00101708	0.101017081	0.102329	101.29	2.333013
3	1	0.00101708	1.001017081	0.924698	92.37	0.852126

employed glutaraldehyde cross-linked PANI as immobilization support for developing the immunosensor. In comparison with ELISA, the analytical performance of immunosensor was found to be superior in terms of a wide detection range (1 ng mL^{-1} to 5 μg mL^{-1}), very low LOD (10.5 pg mL^{-1}) and LOQ (35.1 pg mL^{-1}). We validated sensor performance with a lung cancer cell line (NCI-H23). The immobilization strategy applied here shows the promising result to determine the Sam68 protein concentration in a complex biological sample. The development of immunosensor to detect Sam68 and its diagnostic and prognostic importance shows a promise for better lung cancer patient care. Moreover, this report represents the first demonstration of a splicing factor as an early cancer biomarker and development of a label-free electrochemical immunosensor for diagnosis.

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Author contributions BS performed the experiments with ABD.VSPKSAJ and US optimized the sensor protocol. HCM and RG helped BS for protein expression and purification. ABD and US designed the study. ABD, US, and BS wrote the manuscript.

Compliance with ethical standards

Conflict of interest All the authors declare that there is no competing interest.

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