Interactome Based Identification and Validation of Prefoldin 5α for Prognosing CNS Leukemia in B-All Patients

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Research

Keywords: B-ALL, CNS Leukemia, CSF reactivity, PFDN5α, CNS leukemia prognostication

Posted Date: July 26th, 2021

DOI: <https://doi.org/10.21203/rs.3.rs-718272/v1>

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Version of Record: A version of this preprint was published at Scientific Reports on September 15th, 2022. See the published version at <https://doi.org/10.1038/s41598-022-19489-7>.

Abstract

Background

The gold standard for central nervous system (CNS) B cell acute lymphoblastic leukemia (B-ALL) diagnosis is cerebrospinal fluid (CSF) cytology with poor sensitivity. Thus, prophylactic intrathecal chemotherapy is implemented, which has severe neurological consequences necessitating discovery of CNS leukemia prognostic markers.

Methods

Two-dimensional (2D) far-western interactome analysis was performed to identify CNS+ve/-ve B-ALL CSF reactivity to lymphoblastic proteins profiled on a 2D-western blot. Those specific CNS+ve reacting spots were identified using mass spectrometry. The identified proteins were further validated by both 1D western and ELISA. An ROC curve was generated to identify the cut-off value for prognostication of CNS leukemia in B-ALL patients.

Results

Using interactome analysis with B-ALL CNS+ve/-ve CSF, we identified prefoldin 5 α (PFDN5 α), cytokine induced protein 29 (CIP29), enoyl CoA hydratase (ECH1) and peroxiredoxin 6 (PRDX6). Among these proteins, PFDN5 α showed statistically significant consistent low reactivity to CNS+ve CSF compared to CNS-ve. Further validation of PFDN5 α -CSF reactivity using 1D western and ELISA platform confirmed our interactome analysis. Receiver Operating Characteristic (ROC) curve analysis generated from B-ALL CNS +ve (n = 25) and -ve (n = 40) CSF samples identified PFDN5 α -CSF reactivity cut-off value as 0.456. In contrast, CIP29, ECH1 and PRDX6 proteins did not show any differences in CSF reactivity as seen in interactome analysis.

Conclusions

Using far-western interactome approach, we identified the prognostic significance of PFDN5 α in predicting CNS leukemia. Further validation by 1D western and ELISA confirmed the significance of differential CSF reactivity of CNS+ve/-ve B-ALL CSF to PFDN5 α towards prognostication of CNS leukemia. ROC curve analysis identified the cut-off value for CNS leukemia prediction as 0.456. Values above or below 0.456 could discriminate B-ALL patients either not requiring or requiring intrathecal chemotherapy respectively. Considering the prophylactic chemotherapy associated neurological consequences, PFDN5 α -CSF reactivity based prognosis of CNS leukemia is important in avoiding prophylactic intrathecal chemotherapy for the effective clinical management of patients.

Introduction

Central nervous system B-ALL (1) poses major challenges in the clinic with 3–5% of B-ALL patients at diagnosis and 30–40% during relapse develop CNS leukemia (2). Despite CSF cytology being the gold

standard for diagnosing CNS leukemia (3–4), the poor sensitivity results in mandatory prophylactic intrathecal chemotherapy irrespective of patients developing CNS leukemia with substantial neurological consequences (5–6). Thus, discovering prognostic markers towards predicting CNS leukemia is essential. Despite polymerase chain reaction and flow cytometric evaluation of CSF could identify 50% of CNS leukemia in B-ALL patients at presentation, lack of clinically correlative standardized data makes CNS leukemia prediction challenging (2). Similarly, T-cell immunophenotype and cytogenetic abnormalities also failed to predict CNS involvement (7–8). However, different molecular interactions seen in CNS leukemia (9–11) could be explored towards identifying prognostic markers. Therefore, we hypothesized that sub-microscopic disease specific biochemical CSF entities may interact with lymphoblastic proteins and these interactions could assist in discovering potential markers for predicting CNS leukemia. Though the daunting challenge of identifying interactions between two unknown molecules belonging to CSF and lymphoblastic proteins exists, the established far-western interactome technology was effective in deciphering molecules belonging to such interactions (12). Thus, lymphoblastic proteins and CSF components of B-ALL patients at CNS positivity (presentation/relapse) and at remission (CNS negative) were interacted to obtain an interactome profile to identify specific interactions of clinical significance. In this report, we identified and validated PFDN5 α 's prognostic significance in predicting CNS leukemia in B-ALL patients and derived a cut-off value to predict the risk of developing CNS leukemia that prophylactic intrathecal chemotherapy could be avoided.

Materials And Methods

Materials.

Urea and Thiourea, Bromophenol blue, Tris-base, Ammonium persulfate, Bradford protein assay reagent CHAPS, Iodoacetamide, DTT, E64, IPTG, Sodium carbonate, Sodium bicarbonate, TMB, DMSO, Aprotinin, Tryptone, SDS, Ni-agarose beads, Trypsin and Trypsin reaction buffer were from Sigma-Aldrich, USA. ASB-14-4 (tetradecanoylamidopropyl dimethyl ammonio-butane sulfonate, (Calbiochem, USA), Ampholyte (pH 3–10), IPG strips pH 3–10 and Mineral oil (biotechnology grade) (Bio-Rad), TEMED, Ethanol (Fisher Scientific, USA), Methanol (Merck, USA). Pencillin/streptomycin, Hybridoma serum free medium and Fetal bovine serum (FBS) and TOP10 from Invitrogen, USA. Protogel (National Diagnostics, USA), Amicon ultra-4 centrifugal filter unit, PVDF (Millipore, USA), Streptavidin-HRP conjugate Biotin (Thermo Scientific, USA), X-ray film and X-ray Cassette (Kodak, India), Developer and Fixer (Premier, India), Acetonitrile and Ammonium bicarbonate (Fluka, USA), ESI-Q-TOF (Bruker, Germany), Ampicillin and Kanamycin (Hi-media, India), pCMV-XL5- gene construct of PFDN5 α , CIP29, ECH1 and PRDX6 (Origene, USA). EcoRI, NotI, DNA ladder and T4 Ligase (TaKaRa, India), QIAquick kit (Qiagen, India), BL21 (Merk-Millipore, USA), pET28a vector (Merk-Millipore, USA), DH5 α (ATCC, USA), Ammonium acetate, NaCl, Tween 20, Acetone, Yeast extract, Disodium hydrogen phosphate and HCl (Nice chemicals, India), Multiplate reader (BioTek, USA).

B-ALL Patient CSF Samples.

CSF samples from B-ALL patients were collected at CNS + ve (presentation/relapse positive for blast cells)/CNS–ve remission status with written informed consent according to institutional human ethics committee guidelines with approval (IEC-AIMS-2016 Dated 25-01-2016). CSF includes sequential samples from B-ALL patients of various age groups, both genders with different cytogenetics abnormalities and treatments (Table S1).

CSF Sample processing and biotinylation.

The CSF samples obtained from the same (sequential samples) and different patients were spun down (800g for 5 minutes at 4°C) to remove any cells and were kept as aliquots at -80°C until processed. CSF biotinylation were carried out with 20mM biotin (10% v/v) to CSF for 3.5 hours at room temperature and then stored at -20°C as per Menon et al 2011 (12).

Cell culture and cell lysate preparation.

The B-lymphoblastic JM-1 cell line was obtained from National Centre for Cell Sciences, Pune, India (originally from ATCC, USA). Cells were cultured in serum-free hybridoma medium containing 10% FBS and 1% penicillin/streptomycin at 37°C in 5% CO₂ incubator as described in Xavier et al., 2010 (13). Cells were trypsin treated and spun down at 800g for 8 minutes. The pellet was PBS washed and lysed in 8M urea-2M thiourea solution with protease inhibitors E64 and Aprotinin. Further, presence of any particulate material in the lysate was dispersed by sonication and concentrated using Amicon ultra-4 centrifugal filter unit at 4500g for 45 minutes at 4°C and washed five times with 8M urea and then used for 2D PAGE profiling (13).

Protein estimation.

Protein estimation of concentrated cell lysate was assayed using Bradford reagent and absorbance was measured at 595nm using micro plate reader.

Two-dimensional polyacrylamide gel electrophoresis (2D-PAGE).

2D-PAGE was performed essentially the same way as described in Xavier et al (13). Briefly, 200 µg total proteins from lysate sample was taken in 125 µL of rehydration buffer and rehydrated actively at 50V/20°C for 18 hours using IEF cell (Bio-Rad). After rehydration, the strip was subjected to IEF as described before (12, 13). This is followed by incubation in equilibration buffer (50mM Tris, pH 8.8, 6M urea, 30% glycerol, 2% SDS) with 0.3% DTT for 10 minutes. Subsequently, incubated with 4.5% iodoacetamide containing equilibration buffer for 10 minutes and subjected to SDS-PAGE. Proteins resolved in the gel were visualized by silver staining or used for western blotting (12).

2D-Far-western analysis using CSF.

2D-PAGE was performed using 200 µg total proteins as described above (13). For far western, biotinylated CSF sample (2.0µg concentration CSF proteins in 1ml of 5% gelatin at 4°C overnight) from

patient was probed against lymphoblastic proteins on a 2D western blot. The blots were washed 3 times 10 minutes each in TBST (Tris Buffered saline + 0.2% Tween 20) and subjected to streptavidin-HRP staining (1 in 3000 dilutions with 5% milk in TBST). The chemiluminiscence signal following CSF reactivity to protein spots was captured on X-ray film. Subsequently, the CSF reactivity pattern obtained with CNS + ve at presentation was compared with sequential CSF samples reactivity pattern from the same patient collected at different periods of remission respectively. The procedure was repeated for other patient CSF samples. The sequential samples from patients varied in time frame from 3 weeks to 1.5 years. The intra and inter patient reactivity pattern analysis between three patients resulted in identification of 4 specific spots with consistent reactivity to CNS + ve/-ve CSF.

Quantification of CSF reactive spots on 2D blot by Image J software.

The CSF reactive spots corresponding to the spots marked in Fig. 1B was measured using the Image J software by making a selection on the spot. Then the value of the background of the same blot was subtracted from the spot value and the resulting quantity was considered as the value of the spot. The internal control spot corresponding to GAPDH which showed reactivity irrespective of CNS positivity and remission was also measured in the same way as mentioned above and the value corresponding to GAPDH was used for normalizing the value of these differentially reactive spots. The normalization was done by dividing value of the spot / Value of GAPDH multiplied by average value of GAPDH.

Mass spectrometric analysis of CSF reactive lymphoblastic proteins.

Based on the comparison of the CSF reactivity profile towards proteins of lymphoblastic origin on a 2D blot, the protein spots which showed differential reactivity to CSF (spot 1–4) were cut out from the Coomassie stained gel and de-stained with 100mM ammonium bicarbonate/Acetonitrile (1:1 vol/vol) solution and spun down (12). The gel piece was dehydrated by incubation for 30 minutes at room temperature in 100% acetonitrile with occasional vortexing. Then the gel piece was dried in vacuum and ice cold trypsin reaction buffer was added to cover the gel piece. The mixture was incubated at 37°C for overnight till the gel piece becomes saturated with trypsin. The supernatant containing digested protein in the spot was collected. The proteins were identified by mass spectroscopy analysis using ESI Q-TOF mass spectrometry (12).

Cloning and expression of identified proteins using bacterial expression system.

The pCMV6-XL5-PFDN5 α /CIP29/ECH1/PRDX6 was transformed to DH5 α competent cells for plasmid amplification and subsequently purified by alkaline lysis method. The gene insert (PFDN5 α /CIP29/ECH1/PRDX6) was excised from pCMV6-XL5 vector and ligated to pET28a bacterial expression vector. Subsequently transformed into BL21 strain and optimized the conditions for expression of protein/s.

Expression and purification of recombinant PFDN5 α /CIP29/ECH1/PRDX6 from bacteria.

200ml culture of transformed BL21 (with pET28a-PFDN5 α /CIP29/ECH1/PRDX6) was induced using 0.5mM IPTG at 37°C for protein production. After 5 hours of induction, cells were pelleted down, lysed in lysis buffer of pH 8.0 (8M Urea, 50mM Tris-Base, 500mM disodium hydrogen phosphate) containing protease inhibitors, sonicated to remove the aggregates and centrifuged at 14000rpm for 5minutes. Supernatant containing the expressed protein was collected and subjected to Ni-NTA column purification as described by the manufacturer Sigma.

Validation and Translation of B-ALL CSF reactivity to PFDN5 α for prognostication of CNS leukemia.

The purified recombinant PFDN5 α /CIP29/ECH1/PRDX6 (2 μ g) was western blotted to PVDF membrane and probed with biotinylated CSF samples from B-ALL patients as before and the reactivity was quantified using Image J software. For ELISA, 300ng of purified recombinant PFDN5 α protein coated on 96 well plates was incubated with 35ng of biotinylated CSF samples from the patients for 1hr at room temperature. Washed three times with TBST (TBS with 0.05% Tween 20) and incubated with Streptavidin HRP for 1hr at room temperature (1/7000 dilutions in 1% BSA). Washed in TBST for three times and incubated with HRP substrate TMB for 10 minutes. The reaction was stopped by adding 1N HCl and the color was measured at 450nm.

Statistical analysis

Unpaired two-tailed Students t-test assuming equal variances utilizing Graphpad Prism were carried out and statistical significance were set at $p \leq 0.05$ and represented with star (*). Receiver operating characteristic curve (ROC) analysis was performed using SPSS software version 22.0 to identify a cut off value to predict CNS positivity in B-ALL patients.

Results

Identification of specific B-ALL-CSF reacting protein spots by 2D-far-western interactome analysis. At first, CSF proteins were biotinylated (12). Since biotin forms covalent bonds with primary amines of CSF proteins, unified protein tagging was achieved demonstrated by detection of the same proteins bands resolved in SDS-PAGE by silver staining and streptavidin-HRP staining on far-western blots following SDS-PAGE (Fig. 1A). Further, far-western analysis was performed by interacting biotinylated CSF from CNS + ve/-ve samples with 2D profiled JM-1 pre-B lymphoblast cell line proteins (12, 13) to detect CSF interacting lymphoblastic proteins (Fig. 1B). Comparison of CNS + ve/-ve CSF reactivity to lymphoblastic proteins from 3 different patients with their sequential samples at each visit (comprising 10 different CSF samples) led to identification of four specific proteins spots that showed consistent CSF reactivity to CNS + ve/-ve samples (Fig: 1B, arrows) despite heterogeneity in terms of age, sex, blast percentage, difference in duration between CNS positivity and remission and cytogenetic abnormalities (Table-S1). Quantification of CSF reactivity spots following normalization against GAPDH revealed statistically significant low reactivity to PFDN5 α for CNS + ve CSF compared to CNS-ve (Figure. 1B, 1C; $p = 0.0014$). In contrast, proteins CIP29, ECH1 and PRDX6 showed significantly high reactivity with CNS + ve CSF

compared to CNS-ve (Fig. 1C; *p* values 0.0029, 0.0003, 0.0137) indicating the existence of an inverse relationship between CNS + ve and -ve CSF reactivity of these proteins to PFDN5α (Fig. 1B, 1C).

Identification and validation of B-ALL-CSF reactive spots-1-4 obtained following interactome analysis.

Based on the above interactome analysis showing promising difference in reactivity between CNS + ve and CNS-ve B-ALL CSF samples, protein spots 1–4 was excised out and subjected to mass spectrometry analysis (12). The spots 1–4 was identified as PFDN5α, CIP29, ECH1 and PRDX6 respectively (Table S2). To further validate our interactome analysis results, we purified recombinant his tagged PFDN5α, CIP29, ECH1 and PRDX6 proteins following expression in bacterial expression system (Figure S1). Using purified recombinant proteins, we performed 1D western analysis with CNS+/-ve biotinylated CSF samples. Interestingly, as shown in Fig. 1B (1a vs 1d; 2a vs 2b and 3a vs 3c), PFDN5α (spot-1) showed consistently low CSF reactivity to CNS + ve and high reactivity to CNS-ve (remission) CSF samples (Fig. 2A). This observation is similar to the results of 2D-far-western blots seen in Fig. 1B (spot 1 arrows in blots 1a vs 1d; 2a vs 2b and 3a vs 3c). Notably, a statistically significant difference between CNS + ve vs -ve B-ALL CSF sample is observed ($p = 0.0018$, Fig. 2B). In contrast, purified CIP29, ECH1 and PRDX6 proteins did not show such differences in reactivity to CNS + ve/-ve CSF as shown in 2D-far-western (Figure S2 versus Fig. 1B, 1C). The differential CSF reactivity to PFDN5α between CNS + ve/-ve CSF by 1D western validates and confirms the prognostic significance of PFDN5α in CNS leukemia. Thus, PFDN5α was taken for further validation for its potential use as a prognostic marker for predicting CNS leukemia.

Consolidation of prognostic significance of PFDN5α. To further consolidate the significance of PFDN5α-CSF reactivity and to identify a cut-off value which will be useful to predict CNS leukemia, we tested the CNS + ve/-ve B-ALL CSF reactivity using another platform, namely ELISA. A total of 25 CNS + ve and 40 CNS-ve B-ALL CSF samples were analysed and the PFDN5α-CSF reactivity was plotted (Fig. 2C, Table S4). The CNS + ve and -ve B-ALL CSF samples showed a quantitative average of 0.225 ± 0.040 and 0.502 ± 0.027 respectively with statistical significance $p = 0.0001$ (Fig. 2C). ROC curve analysis revealed an area under the curve (AUC) value of 0.84 showing the utility of ROC curve in discriminating between CNS + ve/-ve patients (Fig. 2D). The cut-off value was derived as 0.456 at 88% sensitivity (22/25) and 55.0% specificity (22/40) (Table S5) towards predicting CNS leukemia.

Discussion

In the quest towards identifying a potential biomarker for predicting CNS leukemia in B-ALL patients, we used our established far-western clinical proteomics approach (12) and identified four spots that showed differential CSF reactivity to CNS + ve/-ve B-ALL CSF among the many protein spots profiled on 2D-western blot [Figure 1B (1a vs 1d; 2a vs 2b and 3a vs 3c) and 1C] The differential CSF reactivity against these four spots was significant between CNS + ve/-ve B-ALL CSF samples. These four proteins were identified as PFDN5α, CIP29, ECH1 and PRDX6 respectively and were further taken for validation to confirm their reactivity to B-ALL CSF using 1D western. Among these four proteins, only PFDN5α showed a significant difference in B-ALL CSF reactivity in 1D western against purified recombinant proteins (Fig. 2A). On the other hand, CIP29, ECH1 and PRDX6 did not show any difference in reactivity between

CNS + ve and -ve B-ALL CSF (Fig. S2). Perhaps post translational modification is essential to detect this reactivity as the recombinant proteins expressed in bacteria lack these modification compared to the JM1 lymphoblastic cell lysate used in 2D-far western blots (Fig. 1B and 1C). Notably, PFDN5 α that showed consistent pattern of reactivity in 2D far-western and 1D far western using recombinant protein indicates the potential use of PFDN5 α in predicting CNS leukemia. Interestingly, PFDN5 α not only interacts and represses c-Myc induced transcription (Table S3), but controls the expression/degradation of c-Myc (14–15). The role of c-Myc in leukemia pathogenesis is well established (14). In fact prefoldins (PFDN1-6) has been found to serve as poor prognostic markers in gastric cancer (16). Thus, PFDN5 α -CSF interactions in B-ALL CSF prone to CNS leukemia could turn out to be an important tool in the prognostication of CNS leukemia and reduce the unnecessary burden and side effects associated with prophylactic intrathecal chemotherapy. Thus, we further validated PFDN5 α -CSF reactivity using ELISA platform (Fig. 2C, D and Table S4). The results were consistent with our 2D far-western and 1D western results (Fig. 2A) thereby consolidating the finding. ROC curve analysis following ELISA identified a cut-off value of 0.456 to predict the risk of B-ALL patients developing CNS leukemia. Values below 0.456 cut-off, indicate an underlying CNS involvement requiring intrathecal chemotherapy and values above signifies better prognosis not requiring intrathecal chemotherapy. CNS leukemia prediction using PFDN5 α could bring in significant improvement in the clinical management of the disease and could avoid unnecessary prophylactic chemo burden to patients.

Conclusion

We report here for the first time the identification and validation of PFDN5 α as prognostic biomarker for prediction of CNS leukemia of B-ALL origin. The PFDN5 α -CSF reactivity analysis could avoid unnecessary prophylactic chemotherapy and chemo burden to patients which otherwise lead to severe neurological consequences. This would bring in significant improvement in the clinical management of CNS leukemia. In order to confirm our far-western finding of B-ALL PFDN5 α -CSF reactivity, we validated it using both 1D and ELISA platform using recombinant purified PFDN5 α . Further, we identified 0.456 as cut off value for predicting CNS leukemia using ROC curve analysis. Values below 0.456 indicate the patient is at risk of developing CNS leukemia and requires intrathecal chemotherapy and values above indicates that patient could be spared from intrathecal chemotherapy. Considering the current gold standard of CSF cytology analysis with poor sensitivity as the diagnosis criteria, PFDN5 α -CSF reactivity could serve as an important milestone in the clinical management of the disease to avoid the unnecessary chemo burden of prophylactic intrathecal chemotherapy to all B-ALL patients.

List Of Abbreviations

Abstract

CNS, Central Nervous System; CSF, Cerebrospinal Fluid; B-ALL, B cell acute lymphoblastic leukemia; 2D, Two-dimensional; 1D, One-dimensional; PFDN5 α , Prefoldin 5 alpha; ELISA, Enzyme Linked

Immunosorbant Assay; ROC, Receiver Operating Characteristic; AUC, Area Under the Curve; CIP29, Cytokine Inducible Protein 29; ECH1, Enoyl-CoA Hydratase 1; and PRDX6, Peroxiredoxin 6.

Methods and Materials

IPTG, Isopropyl β -D-1-thiogalactopyranoside; TBST, Tris Buffered Saline with 0.05% Tween 20; HRP, Horseradish peroxidase; SDS-PAGE, Sodium Dodecyl Sulphate–Polyacrylamide Gel Electrophoresis; DTT, Dithiothreitol; IPG, Immobilized pH gradient; IEF, Iso Electric Focusing; CHAPS, 3-[(3-cholamidopropyl)dimethylammonio]-1-propanesulfonate; TEMED, Tetramethylethylenediamine; TMB, 3,3',5,5'-Tetramethylbenzidine; ASB-14-4, tetradecanoylamidopropyl dimethyl ammonio-butane sulfonate; ESI-Q-TOF, Electrospray-ionisation quadrupole time-of-flight.

Declarations

Ethical Approval and Consent to participate: CSF samples from B-ALL patients were collected at CNS +ve (presentation/relapse positive for blast cells)/CNS–ve remission status with written informed consent according to institutional human ethics committee guidelines with approval (IEC-AIMS-2016 Dated 25-01-2016).

Consent for publication

All authors agree their consent for publication and there is no conflict of interest.

Availability of supporting data

All supporting data are provided and are available in the supplementary information.

Competing interests

The authors declare no conflict of interest. A US patent “Differential Cerebrospinal Fluid Reactivity to PFDN5 alpha for Detection of B-Cell Acute Lymphoblastic Central Nervous System Leukemia” was awarded to Krishnakumar N Menon, et al.,” (Patent Number 10261086).

Funding.

This work was supported by a grant from Indian Council of Medical Research (ICMR), New Delhi (56/2/2016-BMS) to Dr Krishnakumar N. Menon.

Author Contributions

Conceptualization, experimental design and supervision of the project were by KNM. TX, LSV and AA performed the experiments. KNM, TX, LSV, UM, NS, PK and SKR analyzed the data and wrote the manuscript.

Acknowledgements

Dr. Tessy is research associate in the grant. We thank Mrs. Anu Vasudevan for statistics analysis and clinical oncology/heamatology department for patient samples and centre for nanosciences and molecular medicine for the infrastructure support.

References

1. Terwilliger T, Abdul-Hay M. Acute lymphoblastic leukemia: a comprehensive review and 2017 update. *Blood cancer journal*. 2017;7:e577.
2. Lenk L, Alsadeq A, Schewe DM. Involvement of the central nervous system in acute lymphoblastic leukemia: opinions on molecular mechanisms and clinical implications based on recent data. *Cancer Metastasis Rev*. 2020;39:173–87.
3. Bigner SH. (1992) Cerebrospinal fluid (CSF) Cytology: Current status and diagnostic applications. *Journal of Neuropathology and Experimental Neurology*, **51**, 235–245 Available at: <http://library1.nida.ac.th/termpaper6/sd/2554/19755.pdf>.
4. Bürger B, Zimmermann M, Mann G, Kühl J, Löning L, Riehm H, Reiter A, Schrappe M. Diagnostic cerebrospinal fluid examination in children with acute lymphoblastic leukemia: Significance of low leukocyte counts with blasts or traumatic lumbar puncture. *J Clin Oncol*. 2003;21:184–8.
5. Surapaneni UR, Cortes JE, Thomas D, O'Brien S, Giles FJ, Koller C, Faderl S, Kantarjian H. Central nervous system relapse in adults with acute lymphoblastic leukemia. *Cancer*. 2002;94:773–9.
6. Jabbour E, Thomas D, Cortes J, Kantarjian HM, O'Brien S. Central nervous system prophylaxis in adults with acute lymphoblastic leukemia. *Cancer*. 2010;116:2290–300.
7. Stefan Faderl HM, Kantarjian M, Talpaz, and Z.E. Clinical Significance of Cytogenetic Abnormalities in Adult Acute Lymphoblastic Leukemia. *Blood*. 1998;91:3995–4019.
8. Frishman-Levy L, Izraeli S. Advances in understanding the pathogenesis of CNS acute lymphoblastic leukaemia and potential for therapy. *Br J Haematol*. 2017;176:157–67.
9. Buonamici S, Trimarchi T, Ruocco MG, Reavie L, Cathelin S, Mar BG, Klinakis A, Lukyanov Y, Tseng J-C, Sen F, Gehrie E, Li M, Newcomb E, Zavadil J, Meruelo D, Lipp M, Ibrahim S, Efstratiadis A, Zagzag D, Bromberg JS, et al. CCR7 signalling as an essential regulator of CNS infiltration in T-cell leukaemia. *Nature*. 2009;459:1000–4.
10. Holland M, Castro FV, Alexander S, Smith D, Liu J, Walker M, Bitton D, Mulryan K, Ashton G, Blaylock M, Bagley S, Connolly Y, Bridgeman J, Miller C, Krishnan S, Dempsey C, Masurekar A, Stern P, Whetton A, Saha V. RAC2, AEP, and ICAM1 expression are associated with CNS disease in a mouse model of pre-B childhood acute lymphoblastic leukemia. *Blood*. 2011;118:638–49.
11. Marc Vidal ME, Cusick, and A.-L.B. Interactome Networks and Human Disease. *Cell*. 2012;144:986–98.
12. Menon KN, Steer DL, Short M, Petratos S, Smith I, Bernard CCA. (2011) A Novel Unbiased Proteomic Approach to Detect the Reactivity of Cerebrospinal Fluid in Neurological Diseases. *Molecular & Cellular Proteomics*, **10**, Available at: <http://www.mcponline.org/content/10/6/M110.000042>.

13. Xavier T, Sreepriya, Ganesan TS, Menon KN. A simple and efficient method for processing of cell lysates for two-dimensional gel electrophoresis. *Electrophoresis*. 2010;31:2429–35.
14. Fujioka Y, Taira T, Maeda Y, Tanaka S, Nishihara H, Iguchi-Aruga SM, Nagashima K, Ariga H. MM-1, a c-Myc-binding protein, is a candidate for a tumor suppressor in leukemia/lymphoma and tongue cancer. *J Biol Chem*. 2001;276:45137–44.
15. Millán-Zambrano G, Chávez S. (2014) Nuclear functions of prefoldin. *Open Biology*, 4.
16. Yesseyeva G, Aikemu B, Hong H, Yu C, Dong F, Sun J, Zang L, Zheng M, Ma J. Prefoldin subunits (PFDN1-6) serve as poor prognostic markers in gastric cancer. *Biosci Rep*. 2020;40:1–11.

Figures

Figure 1

Identification of specific CNS+ve and -ve B-ALL CSF reactivity to lymphoblastic proteins using 2D far-western interactome analysis. A) Pre B-ALL cell line (JM-1) protein lysate was prepared to obtain the proteomic profile of B-ALL proteins by two dimensional SDS-PAGE profiling and western blotting onto PVDF membrane. Far-western analysis was performed by probing profiled on PVDF membrane with biotinylated CSF samples from CNS+ve and -ve (remission) patients. The CSF reactivity pattern of sequential CSF samples (a, b, c and d) from the same patient from CNS+ve (at presentation or relapse) and CNS-ve (remission) from different time points were compared among and between patients 1-3. The protein spots that are common and unique which showed differential CSF reactivity in a consistent way in CNS +ve and -ve cases are marked with arrows (spots 1-4). Consistently CSF reacting protein spots 1-4 were selected for mass spectrometric analysis from the corresponding coomassie stained gel (Panel

A) to decipher the protein identity. The internal control GAPDH is shown by arrowheads. B) The protein identity of spots 1-4 was found to be PFDN5 α , CIP29, ECH1 and PRDX6 respectively (Table S2). Quantified CSF reactivity on the 2D blot (values of spots in 1a, 2b and 3a for CNS +ve and 1d, 2c and 3c for CNS -ve; n=3) were found to be significantly different between CNS +ve and -ve cases. C) PFDN5 α mediated transcription control of c-MYC target genes.

Figure 2

Validation analysis and quantification of CSF reactivity to purified recombinant PFDN5 α using 1D western and ELISA platforms for determination of cut-off value by ROC curve analysis for prognostication. A) Western blots showing validation of differential CSF reactivity of CNS+ve

(presentation/relapse) and CNS–ve (remission) sequential samples from patients 1, 2 and 3 towards purified recombinant PFDN5α. The pattern of CSF reactivity validates to that observed on the 2D far western blot (Figure 1A). B) Quantification of CNS+ve [51.25 ± 23.23 ; n=3; from 1a, 2b and 3a) and –ve (156.3 ± 8.27 ; n=3; from 1d, 2c and 3b) CSF reactivity to purified PFDN5α protein showing statistical significance (*p=0.0018; CNS+ve vs -ve). C) Further validation and quantification of CSF reactivity of CNS+ve (n=25) and -ve (n=40) CSF samples to purified PFDN5α by ELISA showed statistically significant difference between CNS+ve (0.225 ± 0.202) and -ve (0.502 ± 0.177) CSF samples (p=0.0001). D) Receiver operating characteristic (ROC) curve analysis of reactivity to PFDN5α towards CNS+ve and -ve CSF samples to identify cut-off value to predict the CNS positivity in patients. The ROC curve plot shows that the area under the curve is 0.84 (95% CI 0.74 to 0.95; p=0.0001) and the cut-off value at 88% sensitivity and 55.0% specificity is 0.456.

Supplementary Files

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