Shreya *et al.*
Future Journal of Pharmaceutical Sciences (2025) 11:17
https://doi.org/10.1186/s43094-025-00768-9

Future Journal of Pharmaceutical Sciences

RESEARCH Open Access

Cancer multi-omics-based differential expression analysis and prognostic potential of identified hub targets of myco-metabolites for breast carcinoma and lung carcinoma

Singh Shreya[1,2], Debadatta Mohapatra[1,3], Gaurav Gopal Naik[1,4], Pooja Kathait[1], Soki Malang[1], Pradeep Patel[1], Shambhavi[1], Gulzar Alam[2] and Alakh N. Sahu[1*]

Abstract

Background Breast carcinoma (BC) and lung carcinoma (LC) have the highest incidence and mortality rates worldwide. In prior work, studied sample hub targets contributing to anticancer potential against BC and LC were identified through network pharmacology. In the present work, web servers UALCAN, GEPIA2, and KM plotter were used to explore the genomic and proteomic expression of these hub targets, along with their prognosis potential in BC and LC.

Results Differential hub targets SRC, MAPK3, PTPN11, JAK2, ESR1, and HAP900A1 for BC and PTPN11, JAK2, ESR1, EGFR, and MAPK3 for LC, showed good prognostic potentials. Collectively, PTPN11, JAK2, and ESR1 were overlapped differential expressed hub targets involved in the significantly good prognosis of both carcinoma.

Conclusion These differentially expressed hub targets may be taken into account for future BC and LC treatments due to their strong prognostic potential.

Keywords Breast carcinoma, GEPIA2, KM plotter, Lung carcinoma, Network pharmacology, UALCAN

Background

Breast carcinoma and lung carcinoma have the highest incidence and mortality rates, respectively, based on the estimated age-standardized incidence and mortality rates worldwide. Five-year survival rate of lung carcinoma is 22%, comparatively low to breast carcinoma [1]. With the targeted and untargeted chemo-therapeutic intervention, the acquired drug resistance develops; however, the mechanisms differ. Many factors contribute to this drug resistance: activation of compensatory molecular pathways, activation of pro-apoptotic signaling molecules, inactivation of apoptotic signaling molecules, targeted mutation, lack of DNA repair mechanism, heterogeneity of malignant tumour, epithelial-mesenchymal transition, and many more [2]. Owing to 'n' numbers of factors contributing to drug resistance, a rational combinatorial target approach (targeting compensatory molecular pathways) can be a promising alternative to tackle cancer with good prognosis potential [3].

Our previous work highlighted bio-assay guided fractionation of 1:1 v/v dichloromethane: ethanol crude extract of *Pleurotus osteratus* and myco-metabolite

*Correspondence:
Alakh N. Sahu
ansahu.phe@itbhu.ac.in
[1] Phytomedicine Research Laboratory, Department of Pharmaceutical Engineering and Technology, Indian Institute of Technology (BHU), Varanasi 221005, India
[2] SBS College of Pharmacy, Malwan, Fatehpur 212664, India
[3] Leslie Dan Faculty of Pharmacy, University of Toronto, Toronto, ON M5S 3M2, Canada
[4] Yashwantrao Bhonsale College of Pharmacy, Sawantwadi, Maharashtra 416510, India

profiling of 1:1 v/v dichloromethane: ethanol crude extract with its potent hexane and ethyl acetate fraction. Against panel of human cancer cell lines (MDA-MB-231, Hs578t, MCF, HL-60, Molt-4, A549, NCI-H 322, Panc-1, MiaPaCa-2, SCC-9, and FaDu) secreened for cell cytoxicity, their bioactivities against human breast cancer cell lines (MDA-MB-231, Hs578t, and MCF-7), and human lung cancer cell lines (A549, and NCI-H 322) were exceptionally good ($IC_{50} < 200$ µg/mL). Hexane fraction had higher cytotoxicity against the majority of the studied cancer cell lines. Through chemometric analysis, the possible myco-metabolites contributing to bioactivities of hexane fraction were betulin, solanocapsine, ophiobolin F, linoleoyl ethanolamide, (13R,14R)−7-labdene-13,14,15-triol, asterosterol, cholest-5-ene, (3b,6b,8a,12a)−8,12-epoxy-7(11)-eremophilene-6,8,12-trimethoxy-3-ol, beta-obscurine, myxalamid B, momordol, and avocadyne 4-acetate, belonging to the class of steroid, terpenoids, steroidal alkaloids, fatty alcohol, and polyketides. In continuation, we also traced the underlying mechanistic pathway involved in the cytotoxic potential of hexane fraction of 1:1 v/v dichloromethane: ethanol crude extract of *Pleurotus osteratus* via network pharmacology and experimental validation. Through network pharmacology, SRC, HSP90AA1, PIK3CA, MAPK3, AKT1, PTPN11, JAK2, ESR1, EGFR, and CDK1 were influential hub targets that contribute to the cytotoxic potential of the studied sample against breast carcinoma and lung carcinoma. Along side, in vitro and in vivo experimentation further validates results obtained from network pharmacology and signifies modulation of PI3K/AKT/mTOR signaling pathway, evidence of apoptotic bodies from fluorescence microscopy, and decrease in tumour volume and tumour weight of hexane fraction-treated Ehrlich ascites carcinoma cells bearing Swiss albino mice [4, 5]. In the present study, in order to exploit the cancer multi-omics-based differential genomic (mRNA) and proteomic expression analysis of these hub targets in cancer and normal samples, along with their prognosis potential in breast carcinoma and lung carcinoma patients, bioinformatics tools such as GEPIA2, UALCAN, and KM plotter were used. Obtained differential expressed hub targets with good prognostic potential may be considered in future therapeutic of breast carcinoma and lung carcinoma.

Methods

Metabolomics and network pharmacology

In our previous work, we analyzed the cytotoxic potential of the hexane fraction *of Pleurotus osteratus* against panel of human cancer cell lines. Cytotoxic potential against a human breast cancereous cell line and human lung cancereous cell line were expectionally good, with $IC_{50} < 200$ µg/mL [5]. With the help of the network pharmacology approach, through protein–protein interaction, the identified hub targets of tentatively identified myco-metabolites were SRC, HSP90AA1, PIK3CA, MAPK3, AKT1, PTPN11, JAK2, ESR1, EGFR, and CDK1, against breast carcinoma and lung carcinoma [4].

Proteomic expression analysis

For analyzing protein expression of the identified hub targets for breast carcinoma and lung carcinoma, UALCAN webserver was used. UALCAN, a comprehensive bioinformatics tool, offers protein expression analysis options of genes using data from Clinical Proteomic Tumour Analysis Consortium (CPTAC) and the International Cancer Proteogenome Consortium (ICPC) [6].

Genomic expression analysis

Gene expression profile interactive analysis (GEPIA2) webserver was used for the genomic (mRNA) expression analysis of the identified hub targets and also for identifying the correlation of mRNA expression with different stages of breast carcinoma and lung carcinoma. GEPIA2, an updated version of GEPIA, analyses RNA sequencing expression of 8587 normal and 9736 tumour samples from The Cancer Genome Atlas (TCGA) and Genotype-Tissue Expression dataset (GTEx) [7].

Prognostic potential

The Kaplan–Meier plotter (KM plotter) uses Gene Expression Omnibus (GEO), European Genome-phenome Archive (EGA), and TCGA database for correlating the genomic expression with overall survival in more than 30,000 patients of 21 different types of tumour. For establishing the role of the identified hub targets with the overall survival in breast carcinoma and lung carcinoma, a KM plotter was used [8]. The median expression of genes served as the cutoff for classifying patients into high- and low-expressed groups.

Results

Proteomic expression analysis

Using the UCALAN web server, the protein expression of hub targets was assessed for breast carcinoma and lung carcinoma (Fig. 1). We found that SRC, HSP90AA1, MAPK3, AKT1, ESR1, EGFR, and CDK1 were significantly highly expressed in tumour sample of breast carcinoma as compared to normal sample ($p < 0.05$). Contrarily, tumour samples of breast carcinoma had significantly lower expression of the proteins PIK3CA, PTPN11, and JAK2 than normal samples ($p < 0.05$). In lung carcinoma tumour samples, significantly high expression of hub targets HSP90AA1, AKT1, EGFR, and CDK1 was observed as compared to normal samples

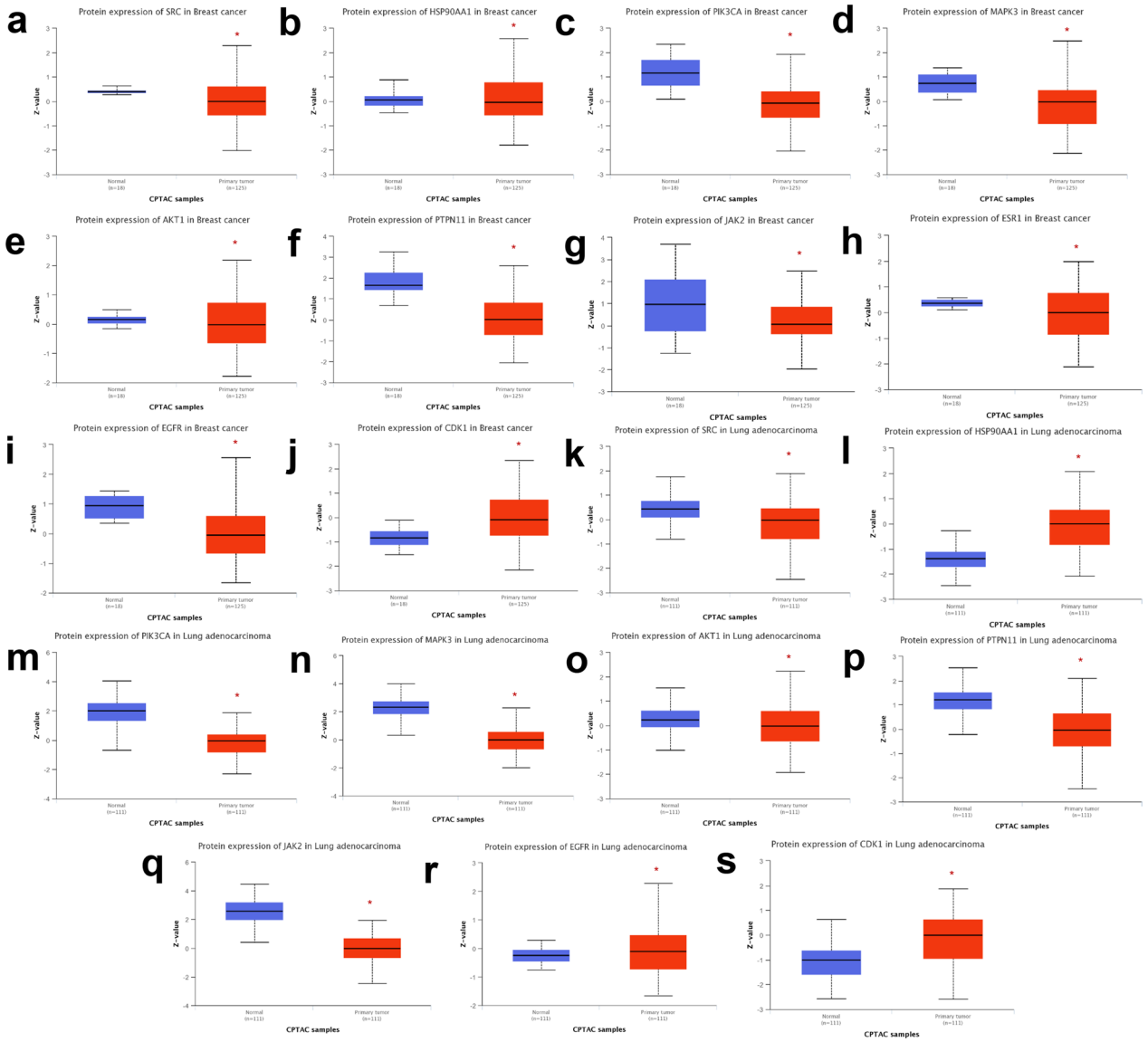

Fig. 1 Proteomic expression of hub targets in breast carcinoma (**a**–**j**) and lung carcinoma (**k**–**s**)

($p < 0.05$), whereas SRC, PIK3CA, MAPK3, PTPN11, and JAK2 hub targets were significantly lower expressed in lung carcinoma samples as compared to normal ($p < 0.05$). ESR1 protein expression in lung carcinoma was undetected.

Genomic (mRNA) expression analysis

GEPIA webseever was used for transcriptional expression study of hub targets among tumour and normal samples (Fig. 2). The genomic expression of hub targets SRC, HSP90AA1, AKT1, ESR1, and CDK1 was highly expressed in breast carcinoma and lung carcinoma as compared to normal tissue ($p < 0.05$). A significantly lower genomic expression of hub targets PI3KCA, MAPK3, JAK2, and PTPN11 was found in breast carcinoma and lung carcinoma, compared to the normal ($p < 0.05$). The mRNA expression of EGFR in lung carcinoma showed a non-significant difference with normal. In contrast to the high protein level of EGFR in breast carcinoma, the transcription level was low expressed in the breast carcinoma, followed by the normal tissue.

To study the clinical significance of hub targets in breast carcinoma and lung carcinoma patients, mRNA expression of hub targets was correlated with clinicopathological stages using GEPIA2. Transcriptional level of all hub targets (SRC, HSP90AA1, PIK3CA, MAPK3,

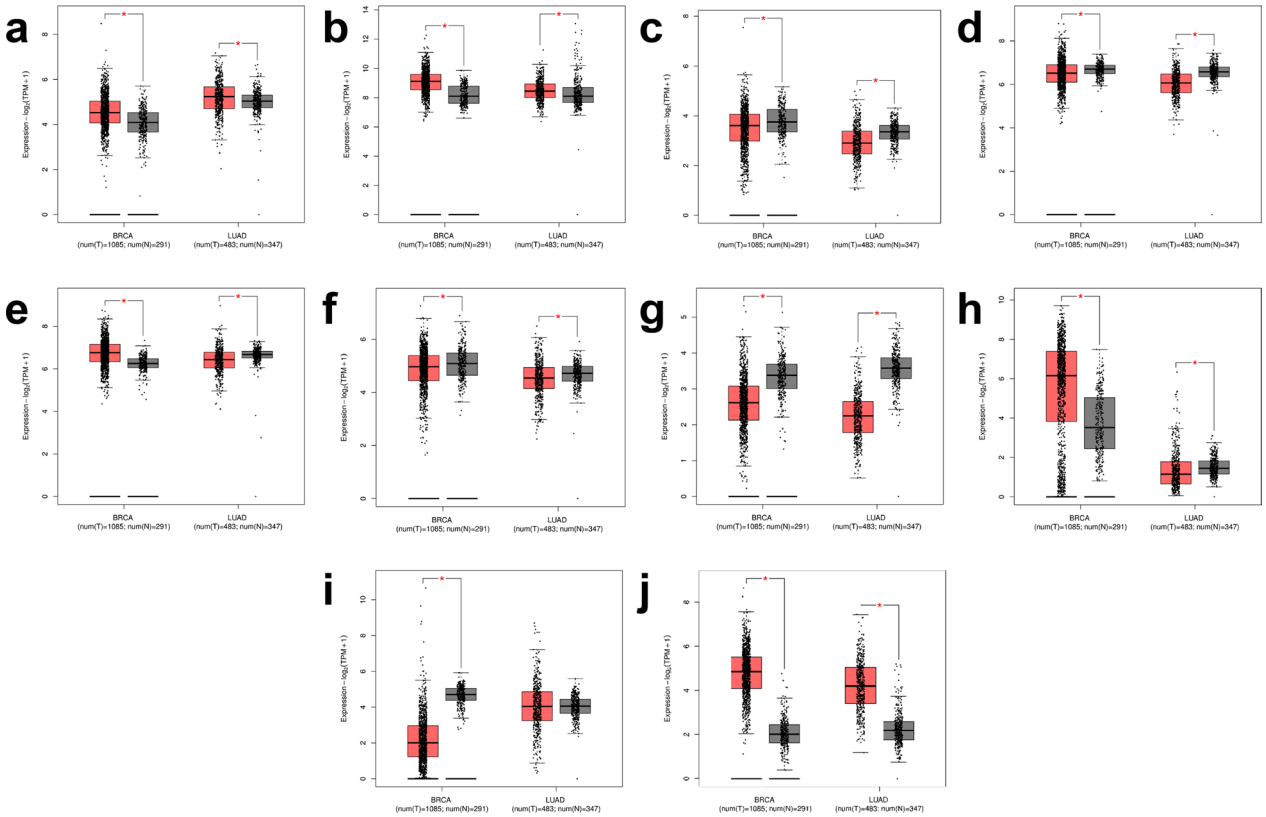

Fig. 2 Genomic (mRNA) expression of hub targets ((**a**) SRC, (**b**) HSP90AA1, (**c**) PIK3CA, (**d**) MAPK3, (**e**) AKT1, (**f**) PTPN11, (**g**) JAK2, (**h**) ESR1, (**i**) EGFR, and (**j**) CDK1) in breast carcinoma (BRCA) and lung carcinoma (LUAD)

AKT1, PTPN11, JAK2, ESR1, EGFR, and CDK1) was significantly correlated with different stages of breast carcinoma and lung carcinoma patients collectively (Fig. 3).

Prognostic potential of hub targets

For exploring the prognostic potential of hub targets for breast carcinoma and lung carcinoma, the KM plotter webserver was used, which analyzes the relationship of mRNA expression of the hub targets with the overall survival (OS). High mRNA levels of SRC, MAPK3, PTPN11, JAK2, and ESR1 and low mRNA level of HAP900A1 showed a significant good prognosis in breast carcinoma (Fig. 4), whereas PTPN11, JAK2, ESR1, and EGFR high mRNA expression and low expression of MAPK3 showed significant good prognosis in lung carcinoma. A non-significant relationship was reflected in mRNA expression of PI3KCA and AKT1 with OS for breast carcinoma and lung carcinoma. Above this hub genes, mRNA expression of HSP900A1 in lung carcinoma showed a non-significant effect on OS. The prognostic potential of CDK1 hub genes was unavailable in the KM plotter webserver.

Discussion

Hormonal therapies (related to estrogen receptors) are commonly used in therapeutic intervention of ER-positive breast cancer. Despite positive outcomes and increased survival rates to hormonal treatments, resistance to such hormonal therapies develops, owing to mutations in ESR1, such as Y537S and D538G. This estrogen receptor (ESR1 in the presented work) can cross-talk with various receptor tyrosine kinases (RTKs) like EGFR, FGFR, and HER [9] [10]. Resistance to hormonal therapies also develops due to molecular signaling through the activation of redundant or alternative RTKs, leading to the reactivation of ER-regulated transcription programs and tumour cell growth. Based on the cancer multi-omics results reported above, obtained from UALCAN and GEPIA2, the proteomic expression and genomic expression of ESR1 in breast carcinoma and lung carcinoma were significantly high as compared to normal tissue. Parallely, EGFR proteomic expression was significantly high expressed in breast carcinoma tissue than the normal tissue. The insignificant difference in proteomic and mRNA expression of EGFR in lung carcinoma was observed. In contrast to the proteomic expression of EGFR in breast

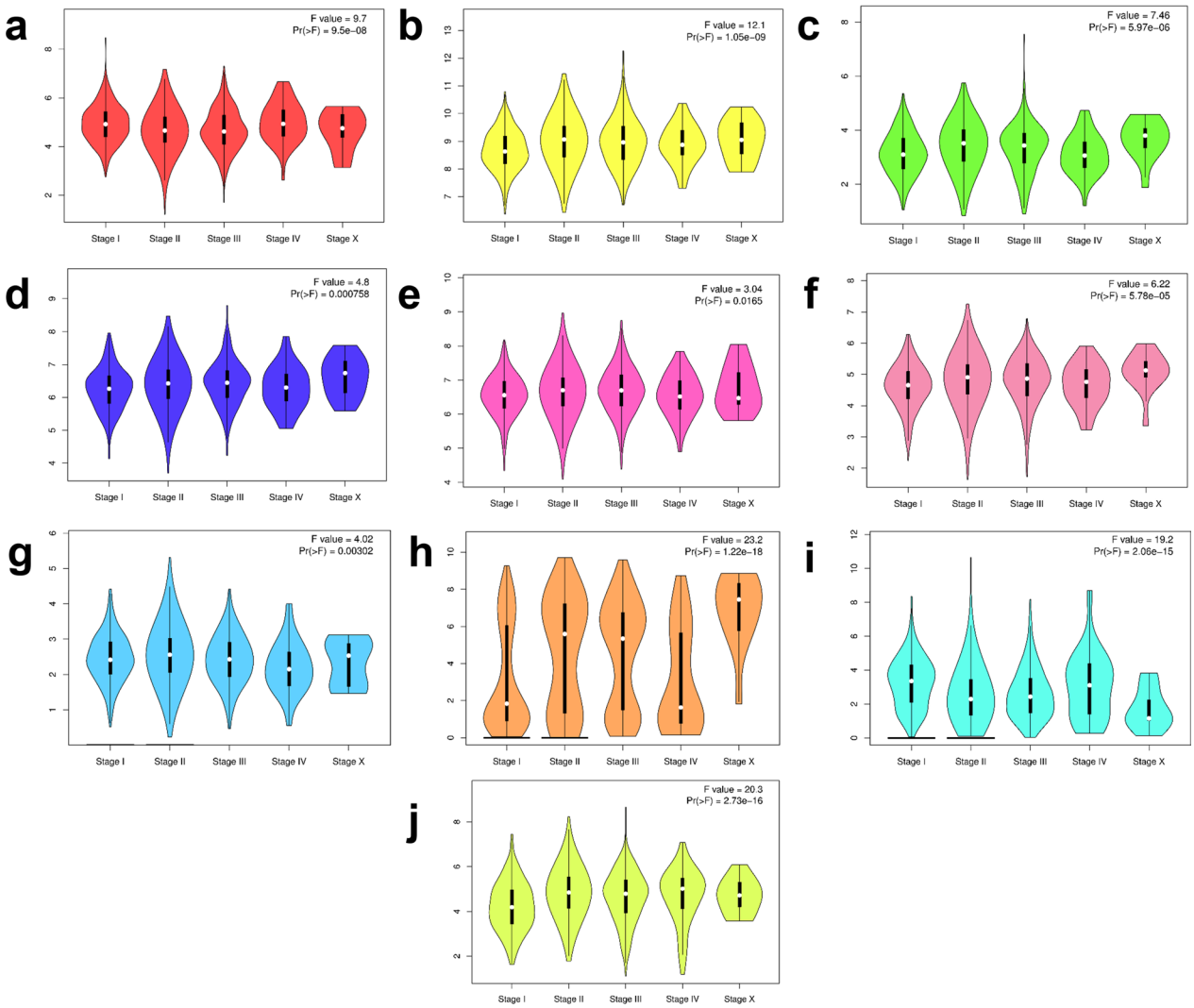

Fig. 3 Correlation of transcriptional level of hub targets ((**a**) SRC, (**b**) HSP90AA1, (**c**) PIK3CA, (**d**) MAPK3, (**e**) AKT1, (**f**) PTPN11, (**g**) JAK2, (**h**) ESR1, (**i**) EGFR, and (**j**) CDK1) with stages of breast carcinoma and lung carcinoma

carcinoma, the transcription level was significantly low expressed in breast carcinoma.

Activation of RTKs leads to activation of downstream signaling cascades (PI3K/AKT, Ras/MEK/ERK, PLC/PKC, and JAK/STAT), thereby promoting growth signal. Resistance to EGFR-targeted therapy develops due to frequent KRAS mutation and activation of other complementary RTKs members and downstream pathways [11, 12]. MAPK3, or extracellular signal-regulated kinase 1 (ERK1), is a pivotal component of the MAPK signaling pathway. Owing to mutation or overexpression of upstream signaling molecules like Ras, Raf, RTKs (HER2, EGFR), MAPK3 is overexpressed in breast and lung cancer. Besides interacting RTKs, MAPK3 can cross-talk with ER signaling and promote the transcriptional activity of ER. This overexpression contributes to progression of malignancies. Trametinib and selumetinib, MEK inhibitors, are under clinical investigation with other combinational therapies to overcome resistance (particularly HER2, EGFR) in breast and lung cancer [13–15]. PTPN11 (protein tyrosine phosphatase non-receptor type 11) also participates in downstream signal transduction of RTKs such as MAPK and PI3K/AKT signaling cascade. Dysregulation or mutation of PTPN11 drives hyperactivation of MAPK and PI3K pathways, resulting progression of malignancies in breast cancer and lung cancer [16]. As per cancer multi-omics result stated above, retrived from UALCAN and GEPIA2, the downstream signaling PI3KCA, PTPN11, and JAK2 proteomic expressions

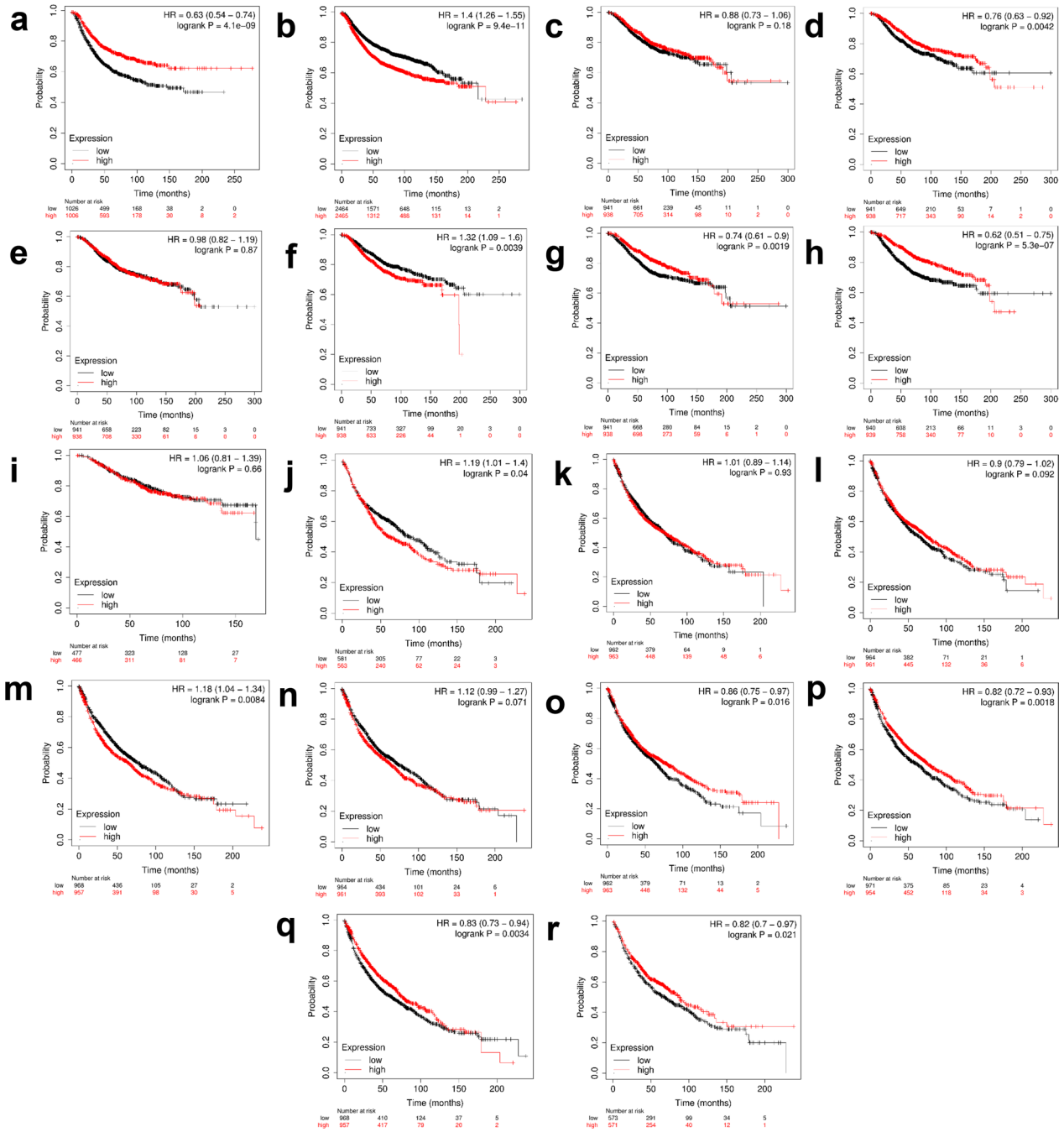

Fig. 4 Prognostic potential of hub targets ((**a**) SRC, (**b**) HSP90AA1, (**c**) PIK3CA, (**d**) MAPK3, (**e**) AKT1, (**f**) PTPN11, (**g**) JAK2, (**h**) ESR1, and (**i**) EGFR) in breast carcinoma and ((**j**) SRC, (**k**) HSP90AA1, (**l**) PIK3CA, (**m**) MAPK3, (**n**) AKT1, (**o**) PTPN11, (**p**) JAK2, (**q**) ESR1, and (**r**) EGFR) in lung carcinoma

were significantly lower expressed in breast carcinoma than in the normal tissue except AKT1 and MAPK3. On the other hand, the proteomic expression of PI3KCA, PTPN11, JAK2, and MAPK3 was significantly lower expressed in lung carcinoma as compared to normal tissue except AKT1. However, PI3KCA, PTPN11, JAK2, and MAPK3 translational expressions were significantly low expressed in both breast carcinoma and lung carcinoma, except AKT1.

SRC, a non-receptor protein kinase, is activated by diverse classes of cellular components such as integrins, G protein-coupled receptors, receptor tyrosine kinases, and steroid receptors. Upon activation, SRC triggers multitude downstream signaling pathways PI3K/AKT,

Ras/MEK/ERK, PLC/PKC, and JAK/STAT, thereby controlling diverse spectrum of biological activities including gene transcription, cell adhesion, cell cycle progression, and migration. Dosatinib, sarcotinib, and bosutinib are SRC inhibitors that are under clinical trials for breast cancer and lung cancer as monotherapy and as combination therapies. Transcriptional overexpression of SRC was observed in breast and lung cancer tissue as compared to normal tissue. This overexpression significantly contributes to the progression of malignancies [17]. While HSP90A1 (heat shock protein 90 alpha family), a molecular chaperone, plays crucial roles in folding, stability and activity of several oncogenic signaling proteins (HER2, EGFR, and ER). It also contributes to activation of downstream MAPK and PI3K signaling pathway contributing to proliferation, differentiation, and cell survival. Inhibitors such as geldanamycin bind to the ATP-binding pocket of HSP90, inhibit its chaperone function, and lead to the degradation of oncogenic proteins [18]. Besides this, dysregulation of CDK1s also results in uncontrolled cancer cell proliferation. In accordance with cancer multi-omics result described above, analyzed by UALCAN and GEPIA2, the proteomic and genomic expression of HSP90AA1 and CDK1s were significantly higher expressed in breast carcinoma and lung carcinoma. While SRC proteomic and genomic expressions were highly expressed in breast carcinoma. In contrast, in lung carcinoma, proteomic expression of SRC was significantly low expressed, and genomic expression was highly expressed.

Moreover, transcriptional levels of all hub targets (SRC, HSP90AA1, PIK3CA, MAPK3, AKT1, PTPN11, JAK2, ESR1, EGFR, and CDK1) were significantly correlated with different stages of breast carcinoma and lung carcinoma patients collectively.

Conclusion

Comparatively, for breast carcinoma, differentially expressed hub targets such as SRC, MAPK3, PTPN11, JAK2, ESR1, and HAP900A1 and differential expressed hub targets PTPN11, JAK2, ESR1, EGFR, and MAPK3 for lung carcinoma showed significant good prognostic potential. Collectively, PTPN11, JAK2, and ESR1 were overlapped differential expressed hub targets involved in the significantly good prognosis of both carcinoma (breast carcinoma and lung carcinoma). These differentially expressed hub targets may be taken into account for future breast carcinoma and lung carcinoma treatments due to their strong prognostic potential.

Abbreviations

CDK1	Cyclin-dependent kinase 1
CPTAC	Clinical proteomic tumour analysis consortium
ICPC	International cancer proteogenome consortium
EGA	European genome-phenome archive
EGFR	Epidermal growth factor receptor
ESR1	Estrogen receptor 1
GEO	Gene expression omnibus
GEPIA2	Gene expression profile interactive analysis 2
GTEx	Genotype-tissue expression dataset
JAK2	Janus kinase 2
KM plotter	Kaplan meier plotter
MAPK3	Mitogen-activated protein kinase 3
PIK3CA	Phosphatidylinositol-4,5-bisphosphate 3-kinase catalytic subunit alpha
PTPN11	Protein tyrosine phosphatase non-receptor type 11
TCGA	The cancer genome atlas

Acknowledgements

Ministry of Human Resource Development (MHRD), Government of India financial assistance provided to Singh Shreya, as a teaching assistantship is greatly acknowledged.

Author contributions

SS performed most of the experimental work, which included the conception and design of the work along with the acquisition, analysis, and interpretation of data. DM, GN, PK, SM, PP, and S also contributed to the acquisition, analysis, and interpretation of data. GA and AS contributed to the supervision, conception, and design of the work. The first draft of the manuscript was written by SS, and further revisions from other co-authors were included. All authors have read and approved the final manuscript.

Funding

Ministry of Human Resource Development (MHRD), Government of India financial assistance provided to Singh Shreya, as a teaching assistantship is greatly acknowledged.

Availability of data and materials

Most of the data are available in the main manuscript.

Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Competing interests

The authors declare no conflicts of interest.

Received: 8 March 2024 Accepted: 13 January 2025
Published online: 05 February 2025

References

1. GLOBACAN 2020. (n.d.). Retrieved from https://gco.iarc.fr/today/fact-sheets-cancers
2. Holohan C, Van Schaeybroeck S, Longley DB, Johnston PG (2013) Cancer drug resistance: an evolving paradigm. Nat Rev Cancer 13(10):714–726. https://doi.org/10.1038/nrc3599
3. Hopkins AL (2008) Network pharmacology: the next paradigm in drug discovery. Nat Chem Biol 4(11):682–690. https://doi.org/10.1038/nchembio.118
4. Shreya S, Kumar DN, Mohapatra D, Jaiswal S, Naik GG, Guru SK, Sahu AN (2023) Tracing the anti-cancer mechanism of pleurotus osteratus by the integrative approach of network pharmacology and experimental studies. Appl Biochem Biotechnol 195(1):152–171. https://doi.org/10.1007/s12010-022-04111-3
5. Shreya S, Kasote D, Mohapatra D, Naik GG, Guru SK, Sreenivasulu N, Sahu AN (2023) Chemometric-based analysis of metabolomics studies

of bioactive fractions of pleurotus osteratus and their correlation with in vitro anti-cancer activity. Appl Biochem Biotechnol 195(7):4602–4616. https://doi.org/10.1007/s12010-023-04325-z
6. Chandrashekar DS, Karthikeyan SK, Korla PK, Patel H, Shovon AR, Athar M, Netto GJ, Qin ZS, Kumar S, Manne U, Creighton CJ, Varambally S (2022) UALCAN: an update to the integrated cancer data analysis platform. Neoplasia 25:18–27. https://doi.org/10.1016/j.neo.2022.01.001
7. Tang Z, Kang B, Li C, Chen T, Zhang Z (2019) GEPIA2: an enhanced web server for large-scale expression profiling and interactive analysis. Nucleic Acids Res 47(W1):W556–W560. https://doi.org/10.1093/nar/gkz430
8. Lánczky A, Győrffy B (2021) Web-based survival analysis tool tailored for medical research (KMplot): development and implementation. J Med Internet Res 23(7):e27633. https://doi.org/10.2196/27633
9. Borg Å, Baldetorp B, Fernö M, Killander D, Olsson H, Ryden S, Sigurdsson H (1994) ERBB2 amplification is associated with tamoxifen resistance in steroid-receptor positive breast cancer. Cancer Lett 81(2):137–144. https://doi.org/10.1016/0304-3835(94)90194-5
10. Newby JC, Johnston SR, Smith IE, Dowsett M (1997) Expression of epidermal growth factor receptor and c-erbB2 during the development of tamoxifen resistance in human breast cancer. Clin Cancer Res: Off J Am Assoc Cancer Res 3(9):1643–1651
11. Luo M, Fu L-W (2014) Redundant kinase activation and resistance of EGFR-tyrosine kinase inhibitors. Am J Cancer Res 4(6):608–628
12. Ludovini V, Bianconi F, Pistola L, Chiari R, Minotti V, Colella R, Crinò L (2011) Phosphoinositide-3-kinase catalytic alpha and KRAS mutations are important predictors of resistance to therapy with epidermal growth factor receptor tyrosine kinase inhibitors in patients with advanced non-small cell lung cancer. J Thorac Oncol 6(4):707–715. https://doi.org/10.1097/JTO.0b013e31820a3a6b
13. Lugowska I, Koseła-Paterczyk H, Kozak K, Rutkowski P (2015) Trametinib: a MEK inhibitor for management of metastatic melanoma. Onco Targets Ther 8:2251–2259. https://doi.org/10.2147/OTT.S72951
14 Lee S, Rauch J, Kolch W (2020) Targeting MAPK signaling in cancer: mechanisms of drug resistance and sensitivity. Int J Mol Sci 21(3):1102. https://doi.org/10.3390/ijms21031102
15. Mas C, Boda B, CaulFuty M, Huang S, Wiszniewski L, Constant S (2015) Antitumour efficacy of the selumetinib and trametinib MEK inhibitors in a combined human airway-tumour-stroma lung cancer model. J Biotechnol 205:111–119. https://doi.org/10.1016/j.jbiotec.2015.01.012
16 Cao Y, Duan H, Su A, Xu L, Lai B (2022) A pan-cancer analysis confirms PTPN11's potential as a prognostic and immunological biomarker. Aging 14(13):5590–5610. https://doi.org/10.18632/aging.204171
17. Luo J, Zou H, Guo Y, Tong T, Ye L, Zhu C, Li P (2022) SRC kinase-mediated signaling pathways and targeted therapies in breast cancer. Breast Cancer Res 24(1):99. https://doi.org/10.1186/s13058-022-01596-y
18 Abdullah O, Omran Z (2024) Geldanamycins: potent Hsp90 inhibitors with significant potential in cancer therapy. Int J Mol Sci 25(20):11293. https://doi.org/10.3390/ijms252011293

Publisher's Note