medical sciences forum

MDPI

Proceeding Paper

Multi-omics analysis of *NFE2L2* altered TCGA-Cervical Squamous Cell Carcinoma patients

Sri Vidya Ramisetti [1], Akhileshwar Namani [2,3,*]

[1] Department of Biotechnology, GITAM School of Sciences, GITAM (Deemed to be University), Visakhapatnam, 530045, India; email: ramisetti.srividya678@gmail.com
[2] Department of Biotechnology, GITAM School of Sciences, GITAM (Deemed to be University), Visakhapatnam, 530045, India; email: anamani@gitam.edu
[3] Current address: Sri Shankara Cancer Hospital and Research Centre, Bangalore, India; email: akileshwarnamani@ssnccpr.org
*Correspondence: akileshwarnamani@ssnccpr.org.

Abstract: Genetic alterations in *NFE2L2* gene have been identified across various cancers and the dysregulation of the NRF2 pathway due to these alterations leads to drug and radioresistance in several cancers. Identification of biomarkers associated with these alterations allows the researchers and clinicians to identify the personalized medicine and quicker diagnosis. In this current study, we carried out an integrated, multi-omics, multi-database analysis of exome, transcriptomics data's of *NFE2L2* altered TCGA-Cervical squamous cell carcinoma (CSCC) patients against wild type counterparts. Finally, we discovered the genes associated with *NFE2L2* alterations, identified the prognostic genes which could be used as potential biomarkers in the *NFE2L2* mutated CSCC patients. Our finding might be useful to identify the early diagnosis of *NFE2L2* mutated CSCC patients.

Keywords: *NFE2L2*; Cervical Cancer; biomarkers; therapeutic strategies; multi-omics

Citation: Ramisetti, S.V.; Namani, A. Multi-omics analysis of *NFE2L2* altered TCGA-Cervical Squamous Cell Carcinoma patients. **2023**, *3*, x. https://doi.org/10.3390/xxxxx
Published: 16 March 2023
Publisher's Note: MDPI stays neutral with regard to jurisdictional claims in published maps and institutional affiliations.

1. Introduction

Cervical cancer is the fourth most common cancer amongst in women, accounting for approximately 6.5% of all female cancer cases worldwide [1]. The Cancer Genome Atlas (TCGA) is a publicly funded project that aims to catalog and discover major cancer-causing genome alterations to create a comprehensive "atlas" of cancer genome profiles [2]. *NFE2L2* is a gene that encodes the transcription factor NRF2 (nuclear factor erythroid 2-related factor), which is the key regulator of oxidative stress in normal cells [3]. Genetic alterations such as mutations and amplification in the *NFE2L2* gene can affect the stability, localization, and activity of the NRF2 protein. These alterations have been identified in many cancers, including cervical squamous cell carcinoma (CSCC), and dysregulation of NRF2 signaling due to these alterations leads to tumorigenesis, drug and radiation resistance. Identifying biomarkers associated with these alterations allows the researchers and clinicians to develop personalized medicine and faster diagnosis [4].

2. Methods

2.1. Identification of genetic alterations of NRF2 in TCGA-CSCC

The cBioportal for cancer genomics website was used to identify the *NFE2L2* mutational landscape and amplifications in CSCC patients from the TCGA pan-cancer study (n =251) [2,5].

2.2. Analysis of differentially expressed genes (DEG's) in NRF2 altered TCGA-CSCC

Based on the NRF2 genetic alterations of TCGA-CSCC, we stratified the total number of patients into two groups and designated them as *NFE2L2*-altered (n=20) and wild-type

(n=231) (without *NFE2L2* alterations), respectively. The mRNA expression profiles (RNA Seq-RSEM batch normalized from Illumina HiSeq_RNASeqV2) were checked to identify the DEG's in these two groups. The *NFE2L2* alterations result in upregulation of its downstream genes [6]. From the list of upregulated genes, we can conclude that they are the driving genes behind tumorigenesis and cancer progression.

2.3. Functional annotation and survival analysis

The Functional annotation of the upregulated genes from *NFE2L2* altered patients was performed by a web tool named DAVID [7]. This analysis provides the Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) Pathway information for genes.

The Kaplan–Meier Plotter [8] tool was used to evaluate the prognostic value of the 29 upregulated genes identified in the *NFE2L2* altered patients in TCGA-CSCC cohort. Briefly, for TCGA-CSCC cohort, the patient samples are divided into two risk groups such as low-risk and high-risk groups based on the prognostic index (PI).

2.4. Identification of NRF2-binding sites by in silico analysis

LASAGNA-Search 2.0 [9] is an integrated web tool for searching and visualizing transcription factor binding sites (TFBS). In this study, LASAGNA-Search 2.0 with cutoff p-values <0.001 was used to identify the NRF2 TFBS within the promoter regions of upregulated genes from *NFE2L2*-altered patients. The search was restricted to the -2 kb upstream human promoter region relative to the transcription start site.

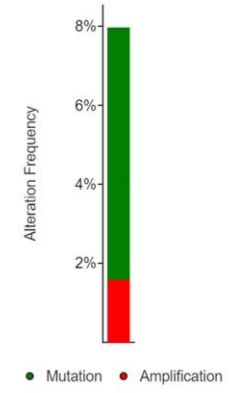
3. Results and Discussions

In TCGA-CSCC, *NFE2L2* genetic alterations occurred in 8% of patients (n=20) out of 251 patients (Figure 1 A). We then performed the DEG's analysis between *NFE2L2*-altered vs. wild-type patients by using cBioportal. As a result, we obtained 29 upregulated in *NFE2L2*-altered patients with a fold change (FC) threshold of > 1.5 and a p-value and q-value < 0.05 (Figure 1 B), Table S1. Notably, we did not find any significantly downregulated genes in the above analysis.

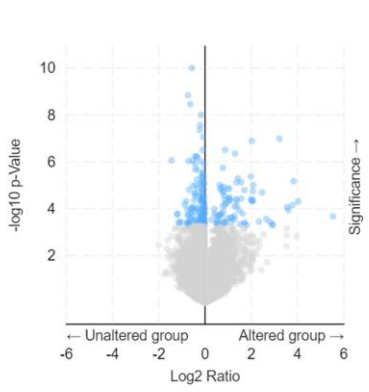
Next, we selected 29 upregulated genes and then performed functional annotation analysis with DAVID. Interestingly, the GO BP (biological processes) analysis revealed 9 biological processes with a stringent p-value cut off < 0.01, in which the majority of genes are involved in cellular response to oxidative stress, response to toxic substance, phylloquinone, farnesol, vitamin K, menaquinone, leukotriene B4 catabolic processes, Xenobiotic and retinol metabolic process (Figure 1 C), (Table S2).

Next, we focused on KEGG pathway analysis (p-value <0.05), from which we obtained 6 pathways, in which the genes that are involved in pentose and glucuronate interconversions, metabolic pathways, steroid hormone biosynthesis, drug metabolism,

A



B



C

Gene Ontology Biological Processes		
GO BP term	Gene Count	p value
GO:0009636~response to toxic substance	4	2.20E-04
GO:0042572~retinol metabolic process	3	0.002384012
GO:0016488~farnesol catabolic process	2	0.002687962
GO:0042377~vitamin K catabolic process	2	0.005368977
GO:0042361~menaquinone catabolic process	2	0.005368977
GO:0034599~cellular response to oxidative stress	3	0.006617267
GO:0036101~leukotriene B4 catabolic process	2	0.006706884
GO:0042376~phylloquinone catabolic process	2	0.006706884
GO:0006805~xenobiotic metabolic process	3	0.008248696
KEGG Pathways		
hsa00040:Pentose and glucuronate interconversions	3	0.002043251
hsa01100:Metabolic pathways	9	0.004649962
hsa00140:Steroid hormone biosynthesis	3	0.006297662
hsa00983:Drug metabolism - other enzymes	3	0.010311172
hsa01240:Biosynthesis of cofactors	3	0.034935112
hsa00790:Folate biosynthesis	2	0.049557718

biosynthesis of cofactors, folate biosynthesis were identified (Table S2). Overall, functional annotation analysis showed that the genes that are upregulated in the *NFE2L2*-altered patients are important for the chemoresistant in CSCC patients.

Figure 1. (A) Percentage of NFE2L2 genetic alterations in TCGA-CSCC patients. (B) Volcano plot showing the DEG's between NFE2L2-altered vs. wild-type patients. (C) GO and KEGG pathway analysis of upregulated genes in NFE2L2-altered patients.

To know if the upregulated 29 genes in *NFE2L2*-altered patients have NRF2-TFBS, we utilised the LASAGNA Search 2.0. Surprisingly, all 29 upregulated genes obtained in our study contains NRF2-TFBSs with an upstream promoter region of -2kb relative to the transcription start site (Table S3). The results suggest that NRF2 may bind to any of these TFBS and upregulates their expression.

Our next goal was to examine whether these 29 genes play a role in the prognosis of CSCC patients. By using the KM plotter-pancancer survival analysis tool, we identified 5 poor prognosis biomarkers whose significantly higher expression (p-value < 0.05) results in poor overall survival in TCGA-CSCC patients (Figure 2). The poor prognostic genes identified in our study are CES1P1, ME1, SLC7A11, SLC12A8 and SPP1. These results clearly indicating that increased expression of *NFE2L2* alterations associated genes acts as the biomarkers in CSCC patients.

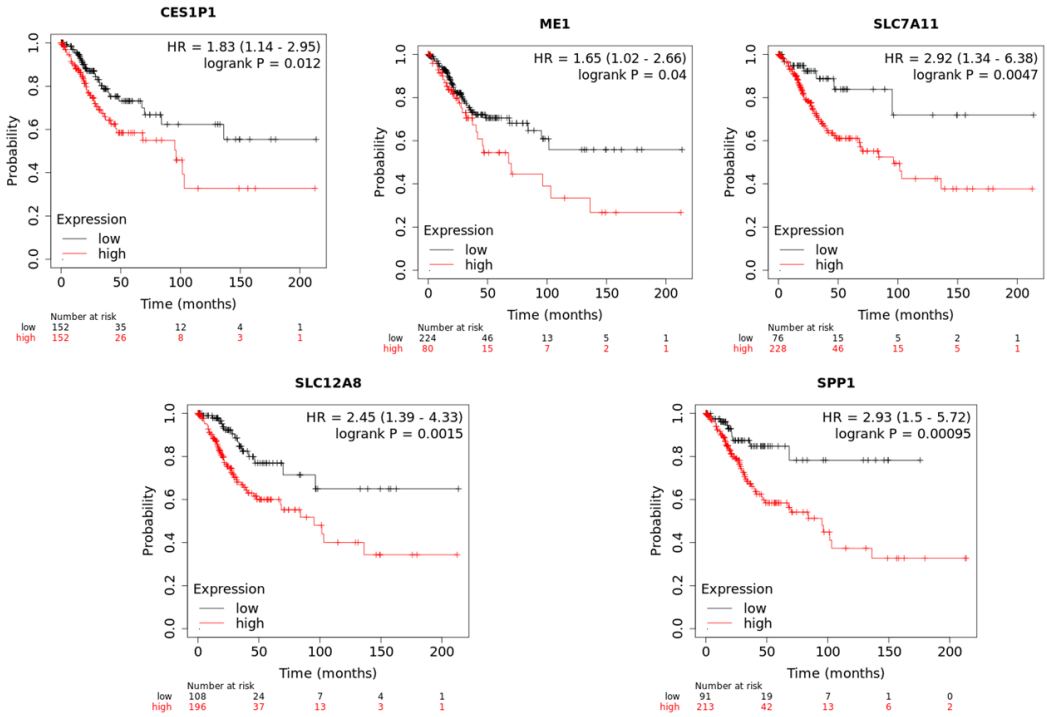


Figure 2. The KM plot showing the overall survival analysis of five genes that are highly expressed in the *NFE2L2*-altered TCGA-CSCC patients.

4. Conclusion

Taken together, our results identified a list of genes that are associated with the *NFE2L2*-alterations in CSCC patients. Increased expression of *NFE2L2*-alterations associated five genes including CES1P1, ME1, SLC7A11, SPP1 and SLC12A8 predicts poor survival in CSCC patients. These five genes may act as prognostic biomarkers and used to identify the *NFE2L2* hyperactivity in CSCC patients. In summary, our identified 5 biomarkers could be possible targets in the treatment of CSCC, in that the development of combined inhibitors for this 5 gene signature along with NRF2 could pave the way for the

development of personalized/precision medicine to suppress NRF2 -mediated tumor growth and drug resistance.

Supplementary Materials: The following supporting information can be downloaded at: www.mdpi.com/xxx/s1, Table S1: List of genes upregulated in *NFE2L2* altered patients; Table S2: Functional annotation analysis of 29 upregulated genes associated with *NFE2L2* alterations; Table S3: NRF2 TFBS in the -2kb promoter region of 29 upregulated genes.

Author Contributions: Conceptualization, S.V.R. and A.N.; methodology, A.N.; formal analysis, S.V.R.; investigation, S.V.R.; resources, A.N.; data curation, S.V.R.; writing—original draft preparation, AN.; writing—review and editing, A.N.; supervision, A.N.; project administration, A.N.; funding acquisition, A.N. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by Research Seed Grant (RSG) from Gandhi Institute of Technology And Management (GITAM) (Deemed to be University), grant number 2021/0093.

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: Not applicable.

Conflicts of Interest: The authors declare no conflict of interest.

References

1. Arbyn, M.; Weiderpass, E.; Bruni, L.; de Sanjose, S.; Saraiya, M.; Ferlay, J.; Bray, F. Estimates of incidence and mortality of cervical cancer in 2018: a worldwide analysis. *Lancet Glob Health* **2020**, *8*, e191-e203, doi:10.1016/S2214-109X(19)30482-6.
2. Hoadley, K.A.; Yau, C.; Hinoue, T.; Wolf, D.M.; Lazar, A.J.; Drill, E.; Shen, R.; Taylor, A.M.; Cherniack, A.D.; Thorsson, V.; et al. Cell-of-Origin Patterns Dominate the Molecular Classification of 10,000 Tumors from 33 Types of Cancer. *Cell* **2018**, *173*, 291-304 e296, doi:10.1016/j.cell.2018.03.022.
3. Cuadrado, A.; Rojo, A.I.; Wells, G.; Hayes, J.D.; Cousin, S.P.; Rumsey, W.L.; Attucks, O.C.; Franklin, S.; Levonen, A.L.; Kensler, T.W.; et al. Therapeutic targeting of the NRF2 and KEAP1 partnership in chronic diseases. *Nat Rev Drug Discov* **2019**, *18*, 295-317, doi:10.1038/s41573-018-0008-x.
4. Namani, A.; Zheng, Z.; Wang, X.J.; Tang, X. Systematic Identification of Multi Omics-based Biomarkers in KEAP1 Mutated TCGA Lung Adenocarcinoma. *J Cancer* **2019**, *10*, 6813-6821, doi:10.7150/jca.35489.
5. Cerami, E.; Gao, J.; Dogrusoz, U.; Gross, B.E.; Sumer, S.O.; Aksoy, B.A.; Jacobsen, A.; Byrne, C.J.; Heuer, M.L.; Larsson, E.; et al. The cBio cancer genomics portal: an open platform for exploring multidimensional cancer genomics data. *Cancer Discov* **2012**, *2*, 401-404, doi:10.1158/2159-8290.CD-12-0095.
6. Namani, A.; Cui, Q.Q.; Wu, Y.; Wang, H.; Wang, X.J.; Tang, X. NRF2-regulated metabolic gene signature as a prognostic biomarker in non-small cell lung cancer. *Oncotarget* **2017**, *8*, 69847-69862, doi:10.18632/oncotarget.19349.
7. Sherman, B.T.; Hao, M.; Qiu, J.; Jiao, X.; Baseler, M.W.; Lane, H.C.; Imamichi, T.; Chang, W. DAVID: a web server for functional enrichment analysis and functional annotation of gene lists (2021 update). *Nucleic Acids Res* **2022**, *50*, W216-221, doi:10.1093/nar/gkac194.
8. Nagy, A.; Munkacsy, G.; Gyorffy, B. Pancancer survival analysis of cancer hallmark genes. *Sci Rep* **2021**, *11*, 6047, doi:10.1038/s41598-021-84787-5.
9. Lee, C.; Huang, C.H. LASAGNA-Search 2.0: integrated transcription factor binding site search and visualization in a browser. *Bioinformatics* **2014**, *30*, 1923-1925, doi:10.1093/bioinformatics/btu115.