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Proceeding Paper

Multi-Omics Analysis of *NFE2L2*-Altered TCGA-Cervical Squamous Cell Carcinoma Patients †

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Abstract: Genetic alterations in the *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 researchers and clinicians to provide personalized medicine and a quicker diagnosis. In this current study, we carried out an integrated, multi-omics, multi-database analysis of exome and transcriptomic data of *NFE2L2*-altered TCGA-Cervical squamous cell carcinoma (CSCC) patients against wild-type counterparts. Finally, we discovered the genes associated with *NFE2L2* alterations and identified the prognostic genes which could be used as potential biomarkers in the *NFE2L2*-mutated CSCC patients. Our findings might be useful in the early diagnosis of *NFE2L2*-mutated CSCC patients.

Keywords: *NFE2L2*; cervical cancer; biomarkers; therapeutic strategies; multi-omics

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1. Introduction

Cervical cancer is the fourth most common cancer 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 it has been determined that the dysregulation of NRF2 signaling due to these alterations leads to tumorigenesis, as well as drug and radiation resistance. Identifying biomarkers associated with these alterations allows researchers and clinicians to provide personalized medicine and a 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 (DEGs) 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 DEGs in these two groups. The *NFE2L2* alterations resulted in the upregulation of 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 with a web tool named DAVID (The Database for Annotation, Visualization and Integrated Discovery) [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 the TCGA-CSCC cohort. Briefly, for the TCGA-CSCC cohort, the patient samples were divided into two risk groups, the low-risk and high-risk groups, based on the prognostic index (PI).

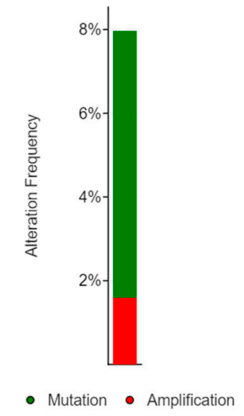
2.4. Identification of NRF2 Binding Sites by In Silico Analysis

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

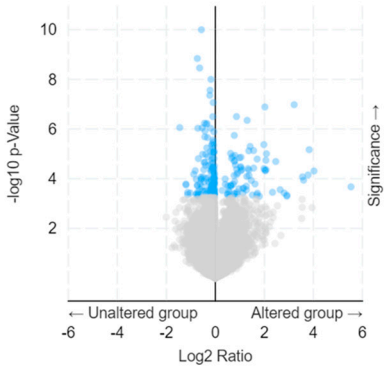
3. Results and Discussions

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

A



B



C

Gene Ontology Biological Processes		
GO BP term	Gene Count	p value
GO:0009636~response to toxic substance	4	0.00022
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

Figure 1. (**A**) Percentage of *NFE2L2* genetic alterations in TCGA-CSCC patients. (**B**) Volcano plot showing the DEGs between *NFE2L2*-altered and wild-type patients. Blue dots are significant differentially expressed genes whereas grey dots are not significant. (**C**) GO and KEGG pathway analysis of upregulated genes in *NFE2L2*-altered patients.

Next, we selected 29 upregulated genes and then performed functional annotation analysis with DAVID. Interestingly, the GO BP (biological processes) analysis revealed nine biological processes with a stringent *p*-value cut-off < 0.01, in which the majority of genes are involved in the cellular response to oxidative stress, toxic substances, phylloquinone,

farnesol, vitamin K, menaquinone, leukotriene B4 catabolic processes, and xenobiotic and retinol metabolic processes (Figure 1C and Table S2).

Next, we focused on KEGG pathway analysis (p-value < 0.05), from which we obtained six pathways. These pathways were identified to contain the genes that are involved in pentose and glucuronate interconversions, metabolic pathways, steroid hormone biosynthesis, drug metabolism, biosynthesis of cofactors, and folate biosynthesis (Table S2). Overall, functional annotation analysis showed that the genes that are upregulated in the *NFE2L2*-altered patients are important for leading to chemoresistance in CSCC patients.

To know if the upregulated 29 genes in *NFE2L2*-altered patients have NRF2-TFBSs, we utilized LASAGNA-Search 2.0. Surprisingly, all 29 upregulated genes obtained in our study contain 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 TFBSs and upregulate 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 pan-cancer survival analysis tool, we identified five poor prognosis biomarkers with significantly higher expression (p-value < 0.05) results related to 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 indicate that the increased expression of *NFE2L2* alterations in the associated genes acts as the biomarker in CSCC patients.

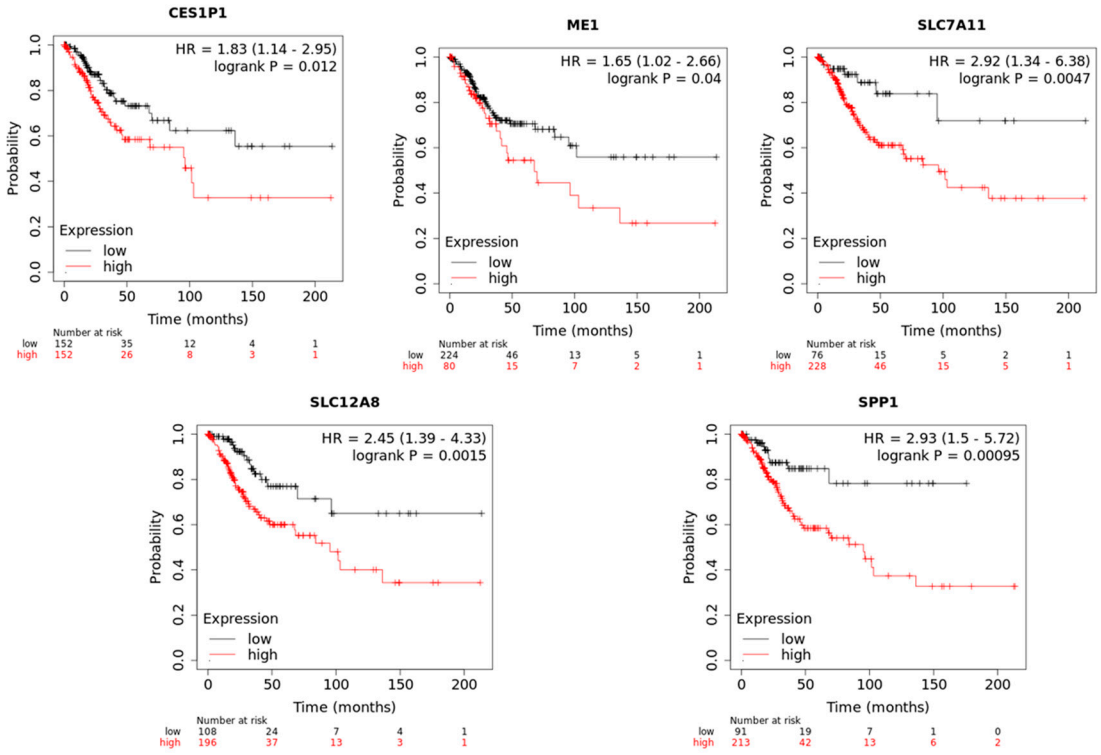


Figure 2. The KM plot showing the overall survival analysis of five genes that are highly expressed in *NFE2L2*-altered TCGA-CSCC patients.

4. Conclusions

Taken together, our results produced a list of genes that are associated with *NFE2L2* alterations in CSCC patients. The increased expression of these five *NFE2L2*-alteration-associated genes, including CES1P1, ME1, SLC7A11, SPP1, and SLC12A8, can assist in predicting poor survival in CSCC patients. These five genes may act as prognostic biomarkers and may be used to identify *NFE2L2* hyperactivity in CSCC patients. In summary, our identified five biomarkers could be possible targets in the treatment of CSCC, as the development of combined inhibitors for this five-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: https://www.mdpi.com/article/10.3390/IECC2023-14223/s1, Conference Poster: Multi-omics analysis of *NFE2L2*-mutated TCGA-cervical squamous cell carcinoma patients; Table S1: List of genes up-regulated in NFE2L2-altered patients; Table S2: Functional annotation analysis of 29 upregulated genes associated with NFE2L2 alterations; Table S3: NRF2 TFBSs in the −2 kb promoter region of 29 upregulated genes.

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