Rebuttal Letter

To

The

Editor-in-Chief

World Journal of Clinical oncology

Dear Editor,

Sub: Resubmission of original article

Ref: **Manuscript ID - Ref.: 78954**

Research Topic*: Identification of three mRNA gene signatures with prognostic value in radioresistant esophageal squamous cell carcinomas*

First let me thank you and the reviewers for assessing our manuscript and providing valuable suggestions. Now, we have addressed all the concerns raised by the reviewers and the revised version submitted using online submission portal. However, if any further modifications are needed, please feel free to contact me with any questions. I hope the revised submission is suitable for publication in World Journal of Clinical oncology. Looking forward for your response

Point-by-Point Answers to the Comments

Reviewer-1 Comments

Scientific Quality: Grade B (Very good)

Language Quality: Grade B (Minor language polishing)

Conclusion: Accept (General priority)

Specific Comments to Authors: No comments.

Response: Thank you for the comments. We have substantially performed proof reading throughout the manuscript and enhanced it by eliminating all the grammar and sentence formatting errors.

Reviewer-2 Comments

Scientific Quality: Grade C (Good)

Language Quality: Grade B (Minor language polishing)

Conclusion: Major revision

Specific Comments to Authors: In the present paper, the authors introduced “Identification of three mRNA gene signatures with prognostic value in radioresistant esophageal squamous cell

carcinomas". Overall, the work is good.

Response: Thank you for the comments. We have substantially performed proof reading throughout the manuscript and enhanced it by eliminating all the grammar and sentence formatting errors.

There are some points to improve the article. -The figures are not in high resolution.

Response: High resolution figures were provided in a spare ppt. file

The authors should enhance the quality of the figures.

Response: Quality figures specifically enhanced and included as a ppt files with all figures and attached.

-The 'keywords' should be rewritten in better words.

Response: Suitable keywords were given in the manuscript.

-The "material and methods" should be fully explained.

Response: methods and materials followed for this study were vividly explained in each and every subheading.

-The 'abstract' should be modified (it is long).

Response: A modified abstract was given in the manuscript.

Original Article

Identification of three mRNA gene signatures with prognostic value in radioresistant esophageal squamous cell carcinomas

Short Running title: Novel mRNA signatures in radiotresistant ESCC

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Abstract

Esophageal squamous cell carcinoma (ESCC) is causing a higher mortality rate due to the lack of efficient early prognosis and suitable therapeutic regimen. The prognostic role of genes responsible for the acquisition of radioresistance in ESCC patients has not been fully elucidated. The aim of this study is to establish a prognostic model by studying gene expression patterns pertinent to radioresistance in ESCC patients. Datasets were obtained from Gene expression Omnibus (GEO) and The Cancer Genome Atlas (TCGA) database. The edge R, a Bioconductor package was used to analyze different mRNA expression levels between different groups. We screened genes specifically responsible for radioresistance to estimate overall survival. Pearson correlation coefficients ($P < 0.05$) analysis was performed to confirm whether those gene expressions correlated with each other. Genes attributed to the radioresistance and overall survival were estimated by the multivariate Cox regression model through the calculation of βi, and risk score using following formulae: $\sum_{i=1}^{n} \beta i \times PSI$. We identified three prognostic mRNAs signature such as (cathepsin S mRNA), CD180 (clusters of differentiation 180 antigen) and SCIMP (SLP adapter and CSK-interacting membrane protein) indicative of radioresistance which are related to each other ($R > 0.70$ and $P < 0.05$). As to 1-year, 3-year overall survival prediction, AUC (Area Under the Curve) of the time-dependent receiver operating characteristic (ROC) curves were 0.716 and 0.841. Patients in the 'high-risk group' exhibited comparatively higher death risk and shorter survival time than the patients in low-risk group ($P < 0.0001$). Overall survival observed in the low-risk samples was effective than the high-risk samples ($P = 0.018$). Present study delineated three mRNA genes such as CTSS, CD180, and SCIMO as novel prognostic gene signatures, which may facilitate the prediction of early clinical prognosis of ESCC.

Keywords: Esophageal squamous cell carcinoma, CTSS, CD180, SCIMP, radioresistance, TNM stage, prognosis

Introduction

Esophageal cancer is one of the most commonly occurring gastrointestinal tumors and ranks 7th in terms of incidence and 6th leading cause of cancer death worldwide. The highest incidence rate was reported in China [1]. Esophageal cancer includes two main pathological types such as

esophageal adenocarcinoma (EA) and Esophageal squamous cell carcinoma (ESCC), and 88% of these ESCC cases originate in central and southern Asia [2]. Surgery is the conventional method of treatment for early-stage esophageal cancer patients. Neoadjuvant radiotherapy is also reported to be a crucial therapeutic modality for treating the advanced stage ESCC patients [3]. However, the differences in sensitivity of each patient to the radiation therapy could be conducive to the variable prognosis for ESCC. ESCC is an aggressive cancer accompanied by the severe malignancy and poor overall survival rate[4]. The available staging system is unclear for predicting the treatment outcome in ESCC patients and the application of cancer genomics to predict the clinical outcomes may enhance therapeutic discovery for treating ESCC [5,6].

Tumor radiotherapy can induce either a direct damage to DNA by inducing DNA double-strand breaks (DSB), or indirectly modulate cell signaling cascades to foster tumor cell death [7]. However, the clinical outcomes of radiotherapy in the most esophageal tumor patients are predominantly ascertained as the tumor cell response to radioactive rays is subject to their inherent sensitivity. Furthermore, tumor cell insensitivity is leading to the incidence of radioresistance, which is accompanied by the involvement of several cellular mechanisms such as cell cycle checkpoint regulation [8], stemness acquisition [9,10], epithelial mesenchymal transformation (EMT) [11], activation of multiple pro-survival and pro-proliferation signaling pathways [12,13]. Furthermore, the radioresistance is also mediated through tumor-associated microenvironment factors, such as hypoxia-induced HIF-1 signaling factors [14,15], tumor-associated fibroblasts [16], and tumor-associated macrophages [17].[18]. Hence, radioresistance is one of the significant reasons for the failure of radiotherapy in ESCC patients. High-throughput sequencing technology is a promising novel approach to identify the relevant genes that are related to tumor radioresistance in ESCC conditions. Maher et al identified a set of five gene microarray expression patterns including EPB41L3, RTKN, STAT5B, NMES1 and RNPC1 as the biomarkers to the neoadjuvant radiotherapy in esophageal cancer [19]. Overexpression of PTK7 can activate NF-kB to enhance raidoresistance in the radiosensitive ESCC cells [20]. Transcriptome analysis delineated that the MALAT1-ATG9B and DDIT4-MB-PLAT genes could regulate radioresistance *in vitro* models of ESCC cells

through the modulation of autophagy and hypoxia pathway [21]. The prognostic role and underlying genomic pathways pertinent to the acquisition of radioresistance in ESCC patients have not yet been fully unraveled. Therefore, it is crucial to examine in-depth analysis of biomarkers and genes pertaining to the radioresistance in ESCC for selecting novel therapeutic modalities to mitigate radioresistance during ESCC.

The current study assessed mRNA gene signatures as radioresistance markers in ESCC cells with the aid of merged mRNA data collected from the GEO and TCGA databases. The study identified three gene- signature, including CTSS, CD180 and SCIMP, that may predict development of radioresistance in ESCC cells. Furthermore, we constructed a prognostic model for radioresistant ESCC based on the risk scores associated with clinical features and the three mRNAs signature CTSS, CD180, and SCIMO.

Materials and Methods

1. GEO database search: Identifying 'radioresistance-promoting mRNAs'

Primarily the microarray profiles in GSE81812 dataset pertaining to the 'non-radiated KYSE-180 cells' and 12, 30 Gy radiated KYSE-180 cells was downloaded respectively from the GEO database (http://www.ncbi.nlm.nih.gov/geo/) to identify mRNAs contributing to the radioresistance in ESCC cells. The edgeR package (www.bioconductor.org/packages/release/bioc/html/edgeR.html) was used to analyze the differential expression of mRNA between different groups, '0 Gy group vs 12 Gy group', '0 Gy group vs 30 Gy group', using the R-statistical computing to identify mRNA genes related to radioresistance. The cutoff parameters were FDR<0.05 and |Log2FC|>2.

2. TCGA database search: Identification of 'radioresistance-promoting mRNAs' attributed to overall survival

Gene expression profile and clinical information of ESCC patients in the TCGA database were downloaded (https://gdc-portal.nci.nih.gov/). Overall survival rates were determined to ascertain the prognostic significance of the identified radioresistance promoting mRNAs in TCGA database; the analysis of overall survival rates were carried out by using survival package in R-statistical computing through Kaplan-Meier analysis and finally compared using the Log-rank test and Cox proportional hazards regression analysis. Then, the screening of mRNAs pertaining to the radioresistance that facilitates changes in overall survival was executed.

3. Multivariate Cox regression model: Prognostic model construction of 'radioresistance-promoting mRNAs' attributed to overall survival

The mRNA related to radioresistance conducive to the mitigation in overall survival were estimated by the multivariate Cox regression model-adjusted to age, gender, grade and stage to calculate βi. The forest plot exhibited the hazards regression (HR) of the multivariate Cox regression model results. Later, risk score was estimated by using the following formulae: $\sum_{i=1}^{n} \beta i \times PSI$. By using the maximally selected rank statistics from the 'survminer' R package, all samples were divided into low-risk and high-risk groups subsequently, the survival analysis was conducted to assess prognosis differences between two groups.

4. Confirmation of the relationship between 'radioresistance-promoting mRNAs' with overall survival, stage and grade

Pearson correlation coefficients ($P < 0.05$) were calculated using r.test () in R computing in order to confirm whether the identified radioresistance-associated mRNAs were typically related to the stage and grade of ESCC. The results were shown in violin plot.

5. KEGG pathway analysis

Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment analysis of mRNAs conducive to the radioresistance in ESCC was performed using the ClusterProfile package (http://www.bioconductor.org/packages/release/bioc/html/clusterProfiler.html) for more comprehensive understanding

of biological features. P-value < 0.05 was set as the cut-off criterion in the KEGG pathway. Data pertinent to KEGG pathway analysis was attached as a supporting file.

Statistical analysis

Statistical analysis was executed with the aid of SPSS 22.0 software (IBM, Chicago, IL) and R version 3.6.0. Overall survival rate was ascertained using Kaplan-Meier Method. Multivariate Cox proportional hazards regression (HR) analysis was executed in order to ascertain the prognostic factors pertaining to the overall survival and the three gene signature, age, gender, stage and grade. $P < 0.05$ was referred to as the statistical significance. Differences between the groups were ascertained through Student t-test or paired-samples t-test.

Results

1. GEO Database Search to identify mRNA expression profiles to ascertain radioresistance-promoting mRNAs

The gene count data of 22456 mRNAs expression profiles in 41 samples of 0 Gy, 92 samples of 12 Gy and 89 samples of 30 Gy were obtained from GSE81812 dataset downloaded from GEO respectively. We identified upregulation of 1168 mRNAs in '**0 Gy group vs. 12 Gy group**' and upregulation of 497 mRNAs in '**0 Gy group vs. 30 Gy group**' by using the edgeR package. To distinguish the differentially expressed mRNAs in different X-ray levels, the top 50 mRNAs were shown in a heatmap and principal component analysis (PCA) (**Figure-1A to D**). Total 379 intersection mRNAs were screened from 0-12 Gy and 0-30 Gy as radioresistance genes.

2. Prognostic significance of radioresistance-promoting mRNAs in TCGA database

Log-rank test and Cox proportional hazards regression was adjusted for other confounding factors such as gender, age, stage and grade. These statistical analyses were used to screen for prognostic genes, and a total of 5293 mRNAs were selected due to their prognostic significance. Among them, 44 mRNAs were significantly associated with radioresistance. We selected 23

mRNAs that were negatively correlated with prognosis for further analysis. The intersection of radioresistant prognostic mRNAs was visualized in Venn diagram (**Figure-1E**).

3. Determination of correlations among radioresistance-promoting mRNAs

For these 23 mRNAs, primarily we investigated whether their expressions correlated with each other in the TCGA database. Although their expressions were expressed at different levels in ESCC patients, the results showed strong correlations between three mRNAs such as CTSS, CD180, and SCIMP ($R > 0.70$ and $P < 0.05$). The correlations of 23 mRNAs were shown in the heatmap (**Figure-2A**). Hence, our research group selected these three mRNAs as our interesting radioresistance-promoting mRNAs. Correlations of these three mRNAs were shown in scattergram (**Figure-2B, 2C, 2D**).

4. Establishment of the mRNA gene signature as prognostic model for radioresistance

To explore the potential prognostic value of above three mRNA genes pertinent to radioresistance, we evaluated the overall survival rates of ESCC patients based on the expression patterns of these three mRNAs in TCGA database by using Kaplan-Meier curves. As shown in **Figure-3A**, the low expression was shown to have good overall survival [TCGA database], and the median survival time was statistically significant ($P < 0.05$), either for CTSS, CD180 or SCIMP.

Subsequently, the connection between the three mRNA gene signatures and overall survival was explored through multivariate Cox regression model adjusted to patient's age, gender, grade and stage; for which, the hazard ratio (HR) with 95% confidence interval was depicted through the forest plot (**Figure-3B**). ROC analysis for the model was shown in **Figure-3C** (AUC: 0.716 and 0.841 for 1 year, 3 years respectively). Accordingly, the risk score of each patient was conducted, and all the patients were segregated into high risk group and low risk group respectively based on the survival package; Risk score-based stratification benefits to ascertain the functional implications of mRNA signatures for their prognostic relevance. This kind of stratification can compare and analyze the differentially expressed mRNAs between the

patients groups to unravel their implications in different overall survival times. The patients of the high-risk group exhibited '**higher death risk** and **shorter survival time**' than the patients in low-risk group; the heatmap of three gene signatures such as CTSS, CD180, and SCIMP showed that the high-risk group patients typically were associated with higher expression of these gene signatures than the patients in low-risk group (**Figure-3D to 3F**). The Kaplan-Meier curves revealed that the low-risk group patients typically with low expression of these three genes exhibited a good overall survival (**Figure-3G**).

5. External validation of GEO

To further validate the prognostic value of these three mRNAs, GSE53625 was downloaded from the GEO database. As shown in **Figure-4A**, there was an association with a good survival outcome when SCIMP expression downregulated. Patients segregated into two groups based on the CTSS expression, but this was not statistically significant. Two patient groups exhibited similar survival curves for CD180 expression. In the same manner, the risk score of GEO samples was calculated, and the overall survival of patient samples in low-risk group was also higher than the patient samples in high-risk group (**Figure-4B**). The risk curve, scatter plot and heatmap results were also similar to TCGA results (**Figure-4C-E**).

6. Genes with pathological grade and TNM stage

The association between three radioresistance-promoting mRNAs such as CTSS, CD180, SCIMP and pathological grade (**Figure-5A to C**), TNM stage (**Figure-5D to F**) was ascertained. CTSS, CD180, and SCIMP genes exhibited significantly higher expression in pathological grade 2 ad 3 with well tumor difference; whereas the expression of these three radioresistance genes were comparatively higher in other tumor stages than stage I group, and early tumor period, which was exemplified by the lower expression of these three gene signatures than other grade groups.

7. Functional characteristics of the CTSS, CD180, and SCIMP mRNAs

To further explore the underlying biological features of the three mRNAs in ESCC, we performed Pearson correlation between the three mRNAs viz., CTSS, CD180, and SCIMP genes and the other mRNAs to find out coexpressed mRNAs; A total of 539 mRNAs were selected for KEGG pathway enrichment analysis ($P < 0.01$, $R > 0.4$). Our results showed that the coexpressed mRNAs were mainly enriched in 50 pathways, including NF-kB, JAK-STAT, Cell adhesion molecules (CAMs) signaling, and PD-L1 expression & PD-1 checkpoint pathways (**Figure-6**).

Discussion

Prolonged and fractionated irradiation during radiotherapy in ESCC patients could confer to the radioresistance and distant metastasis, which may be conducive to the treatment failure [22,23]. Cancer-associated fibroblasts (CAFs) can foster the radioresistance in ESCC tumor cells through the long noncoding RNAs (lncRNA)-DNM3OS by modulating PDGFβ/PDGFRβ/FOXO1 signaling pathway. Furthermore, these findings suggested that CAFs-promoted lncRNA-DNM3OS could be a crucial target to reverse radioresistance in ESCC tumor cells. A study by Huishan Zhao et al (2019) reported the three target genes FOXL2, TCF4, and NR2F2 exhibited a significant correlation with prognosis of endometrial carcinoma; the low expression of these three genes-associated biological pathways were significantly enriched in cell cycle and fatty acid metabolism of cancer cells [24]. However, there is limited evidence to validate the gene signatures involved in conferring radioresistance in ESCC patients to delineate accurate and efficient disease prognosis [25]. Ma H et al [26] demonstrated that HMGB1 promotes radioresistance through the activation of autophagy. Furthermore, DEGs analysis of genes viz., '*CFLAR, LAMA5, ITGA6, ITGB4,* and *SDC4* ' in five signaling cascades viz., PI3K-AKT pathway, CYCS gene-based apoptosis pathway, S100AX–AKT3-related pathway, SDC4 and HSPG2 pathway, and mTOR signaling pathway were reported to be actively conducive to the radioresistance in vitro ESCC models, and tissue biopsies of ESCC patients [27]. In the present study, we, for the first time, constructed a risk

score model based on the three radioresistance-mRNAs (CTSS, CD180 and SCIMP) and clinical features of ESCC patients; this model could facilitate oncologists to predict overall survival of ESCC patients with acquired radioresistance in radiotherapy.

A research study described that the insulin-like growth factor 2 mRNA-binding protein 3 can be conducive to the development of radioresistance in ESCC [28]. miR-205 promotes radioresistance in ESCC typically through the efficient DNA repair process, impairment of apoptosis, and stimulating EMT [29]. Another factor i.e. eEF2K could foster the progression of radioresistance in ESCC [30]. In our study, the involvement of three mRNA gene signatures (CTSS, CD180, and SCIMP) in radioresistance, was analyzed by constructing the risk score model, and through the transcriptome profiling of ESCC samples between non-radiated KYSE-180 cells and 12 or 30 Gy FIR-treated KYSE-180 cells . However, the overall survival information in GSE81812 dataset is unavailable, so, we conducted univariate and multivariate Cox regression analysis of TCGA database, and found 49 radioresistance-mRNAs associated with survival, of which '23 radioresistance-mRNAs inversely correlated with survival'. After comprehensive correlation analysis, we selected three radioresistance-mRNAs (CTSS, CD180, and SCIMP) as our interesting genes that are strongly correlated with each other in TCGA database. Subramanian J et al deciphered that the well-developed genomic signatures are significantly beneficial for improving clinical outcomes in ESCC patients [31]. Results of the overall survival of patients in this study concluded that a higher risk score patients exhibited a poorer prognosis. Moreover, we downloaded the GSE53625 dataset as independent validation data to validate its prognostic role. Our result confirmed that the risk score model could also predict the survival outcome of the external validation datasets.

Among the three mRNA gene signatures, CTSS is a member of the cysteine proteases; Park, S.Y. et al. showed that the radiation-induced CTSS overexpression, which can consequently promote radioresistance; in this study, the knockdown of CTSS could induce impairment of radioresistance through the modulation of ROS-IFN-γ pathway [32]. Additionally, a plethora of research studies have found that CTSS is particularly involved in modulating autophagy

pathways [33], PI3K/Akt, Ras/Raf/MAPK signaling pathways [34], and EGFR-ERK signaling pathway [35] as these signaling cascades are more or less involved in conferring to the radioresistance. However, there are no reports available in the literature to delineate CD180, and SCIMP as other two mRNAs involved in causing radioresistance in ESCC patients. CD180 belongs to the family of Toll-like receptors. Its expression has been reported to be associated with acute or chronic leukemia [36]. SCIMP encodes a transmembrane adaptor protein that shapes host defense and inflammation via direct modulation of TLR4 [37].

A report by Ling Yang et al (2019) described the activation of PI3K-Akt signaling pathway (KEGG ID: hsa05200) with upregulation of DEGs such as '*LAMA5, LAMB2, LAMB3, ITGA6,* and *ITGB4*' at 12 Gy and 30 Gy- of fractionated irradiation. Thus, PI3K-Akt is reported to be involved in protecting KYSE-180 cells from undergoing apoptosis after irradiation [27]. CYCS gene-based apoptosis pathway (KEGG ID: hsa04210) is impaired after irradiation of 12-Gy due to the induction of CYCS downregulation. KEGG pathway analysis of S100AX–AKT3 signaling depicted that the activation of this pathway could enhance the migration and metastasis of HSCC KYSE-180-12 Gy and KYE-180-30 Gy cells [27,38]. SDC4 and HSPG2 [KEGG ID: hsa05205] are the two proteoglycans that were reported to be upregulated during the irradiation of KYSE-180 cells at 12-Gy and 30-Gy. These genes are responsible for the tumor cell invasion, and metastasis [27]. In the present study, KEGG pathway analysis was executed in order to understand the underlying mechanisms of three mRNAs gene-signatures conducive to the radioresistance of ESCC. Our result showed that these mRNAs were mainly enriched in some pathways that are related to radioresistance, viz., JAK-STAT signaling pathway [39], and NF-kB signaling pathway [40]. Our results also demonstrated the radioresistance-promoting ability of these three mRNAs. Besides, these mRNAs were enriched in the immune-related pathway, such as antigen processing and presentation, cytokine-cytokine receptor interaction and Th17 cell differentiation. Hence, present research reports suggested that these three radioresistance-mRNAs gene signatures might be involved in the regulation of immune pathways conducive to ESCC radioresistance.

Conclusion

In summary, our study proved that CTSS, CD180 and SCIMP are the mRNA gene signatures which can promote the radioresistance in ESCC patients. This novel three mRNAs-based gene signature can be considered as prognostic models and have a great value for clinical prognosis of radioresistant ESCC. In conclusion, this integrated bioinformatics study reported three key genes as prognostic markers and potential targets of ESCC.

Article Highlights

- There is lack of studies to explore a significant clinical relevance for the esophageal cancer patients to choose suitable radiation dose to minimize the resistance acquired by rapidly proliferating ESCC cells during the treatment. It is possible to screen the patients with radioresistant ESCC based on the expression of these prognostic genes to choose a suitable therapeutic regimen in advance.
- It is possible to screen the patients with radioresistant ESCC based on the expression of these prognostic genes to choose a suitable therapeutic regimen in advance in the clinical practice.
- Identification of three mRNA genes such as CTSS, CD180, and SCIMO as novel prognostic gene signatures, which may facilitate the prediction of early clinical prognosis of ESCC.
- This study involves the data acquisition from the TCGA, GEO databases with patient data and analyzed the mRNA expression patterns of the prognostic genes for the acquisition of radioresistance induced through radiotherapy in ESCC patients.
- This study is a significant report which analyzed prognostic genes such as CTSS, CD180, and SCIMO pertinent to radioresistance.
- This study is useful to modulate the radiotherapy dose during continuous radiation therapy in ESCC patients.
- This study benefits to incorporate these genes for early prognosis and isolation of the clinical cases.

Author Contributions

Xiaoyan Wang (XW), Narasimha M. Beeraka (NMB), Nannan Xue (NX), Huiming Yu (HY), Ya Yang (YY), Maoxing Liu (ML), Vladimir N. Nikolenko (VNN), Junqi Liu (JL), Di Zhao (DZ) conceptualized and designed the study. NMB, XW, JL, DZ, NX, HY, VNK, and YY performed the literature analysis, and wrote the original manuscript draft. NMB, JL, DZ revised, edited, and extended the final draft. All authors have reviewed and approved the manuscript before submission.

Conflict of Interest

Authors have no competing interests.

Acknowledgements

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Availability of data and materials

All the supplementary files will be provided upon request by the journal office as the data was procured form TCGA, and GEO public databases for this study. We did not take any third party support in conducting this research, analyzing the data, or preparing the manuscript for submission. The whole process of methodology pertinent to data acquisition was given in the Methodology section.

Ethical considerations

We accessed the TCGA and GEO public databases access for the files pertinent to this study and there was no personal identification information, hence, the informed consent is not required. The first affiliated hospital of Zhengzhou university waived the informed consent due to the data acquisition particularly from the TCGA and GEO public databases as mentioned in the methodology section. All the statistical analytical procedures were executed in accordance with the relevant guidelines and regulations (Declaration of Helsinki).

Funding

None

Informed consent

Not applicable (this study does not involve human participants)

Figure Legends

Figure-1: Comparative PCA and Heatmap analysis of up-regulated mRNAs between non-radiated KYSE-180 and radiated KYSE-180 cell samples. PCAs: samples were clustered into two groups (**A**: 0 Gy group vs. 12 Gy group; **B**: 0 Gy group vs. 30 Gy group). In the heatmap, upregulated gene expressions were indicated in red whereas the downregulated gene expressions were indicated in green. The expressions of mRNAs in radiated samples were comparatively higher than non-radiated samples (**C**: 0 Gy group vs. 12 Gy group, **D**: 0 Gy group vs. 30 Gy group). **E**. Venn diagram of mRNAs attributed to the prognosis of

radioresistance in ESCC.

Figure-2: Heatmap and Scattergram depicted the correlations of mRNAs. **(A).** Heatmap exhibited correlations of 23 mRNAs attributed to the radioresistance; Scattergram exhibited correlations between two mRNA gene signatures (**B**: CTSS vs. SCIMP, **C**: CTSS vs. CD180, **D**: CD180 vs. SCIMP).

Figure-3: **(A).** Kaplan-Meier survival curves based on the expression patterns of mRNA gene signatures such as CTSS, SCIMP, and CD180 in TCGA database. **(B)**. The forest plot was established with a hazard ratio (HR) calculated through multivariate Cox regression model for adjusted age, gender, grade and stage ($P<0.05$). **(C)**. Receiver operating characteristic (ROC) analysis for 1-year, 3-year overall survival prediction. **(D)(E)(F).** the risk score distribution of the prognostic model (TCGA database) was depicted for patients segregated as high risk group and low risk group; the status of patients in the high and low risk group; A Heatmap of the three mRNA gene signatures viz., SCIMP, CD180, and CTSS, where high risk group patients significantly exhibited higher expression of these three mRNA gene signatures than low risk patients. **(G).** Kaplan–Meier survival curves for the high risk group and low risk group.

Figure-4: **(A).** Kaplan–Meier survival curves for the mRNAs signatures such as SCIMP, CD180, and CTSS of prognostic value obtained from GSE53625 dataset. **(B).** Kaplan–Meier survival curves for the high risk group and low risk group in GSE53625 dataset. **(C)(D)(E).** The risk score distribution of the prognostic model (GSE53625 dataset) was depicted for patients segregated as a high risk group and low risk group; The status of patients in the high and low risk group was indicated in the graph. A Heatmap of the three mRNA gene signatures such as SCIMP, CD180, and CTSS, where high risk group patients significantly exhibited higher expression of these three mRNA gene signatures than low risk group patients.

Figure-5: **(A)(B)(C).** Box-plots: A positive correlation between the mRNAs gene signatures such as SCIMP, CD180, and CTSS relative to the 'pathological grade' was depicted. **(D)(E)(F).**

A positive correlation between the mRNAs gene signatures viz., SCIMP, CD180, CTSS and 'TNM stage' was depicted.

Figure-6: Kyoto Encyclopedia of Genes and Genomes (KEGG) analysis of the coexpressed mRNAs. The mRNA gene signatures such as CTSS, CD180, and SCIMP genes can modulate the other coexpressed mRNAs which were mainly enriched in 50 pathways, for example, NF-kB, JAK-STAT, PD-L1 expression & PD-1 checkpoint pathway, and Cell adhesion molecules (CAMs) signaling pathways involved in cancers.

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