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Integrating radiosensitive genes improves prediction of radiosensitivity or radioresistance in patients with oesophageal cancer

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Abstract. Oesophageal cancer is a serious disease worldwide. In China, the incidence of esophageal cancer was reported to be ~478,000 in 2015. In the same year, the incidence of esophageal cancer in the United States was ~16,910. Radiotherapy serves as an important tool in the treatment of oesophageal cancer, and although radiation therapy has progressed over time, the prognosis of the majority of patients with oesophageal cancer remains poor. Additionally, the sensitivity of patients with oesophageal cancer to radiotherapy and chemotherapy is not yet clear. Although there are a number of studies on the radiosensitivity of oesophageal cancer cell lines, the vastly different results from different cell lines make them unreliable to use as a guide in clinical practice. Therefore, a common radiosensitive gene signature may provide more reliable results, and using different combinations of common gene signatures to predict the outcome of patients with oesophageal cancer may generate a unique gene signature in oesophageal cancer. In the present study, the radiosensitive index and prognostic index were calculated to predict clinical outcomes. The prognostic index of a 41-gene signature combination is the largest combination of gene signatures used for classifying oesophageal cancer patients into radiosensitive (RS) and radioresistance (RR) groups, to the best of our knowledge, and this gene signature was more effective in patients classified as having Stage III oesophageal cancer. Furthermore, four genes (*carbonyl reductase 1*, *serine/threonine kinase PAK2*, *ras-related protein Rab 13* and *twinfilin-1*) may be sufficient to classify patients into either RS or RR. Subsequent to gene enrichment analysis, the cell communication pathway was significantly different between RS and RR groups in oesophageal cancer. These results may provide useful insights in improving radiotherapy strategies in clinical decisions.

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Introduction

Oesophageal cancer remains a major national and global health problem. In the United States in 2016, oesophageal cancer accounted for >15,000 mortalities (1). In China in 2015, the incidence of oesophageal cancer was ~478,000, and the number of mortalities was estimated to be ~375,000 (2). Surgery, chemotherapy and radiotherapy are the primary strategies for patient treatment at present (3). Radiation therapy has broad applications as a vital strategy for shrinking tumours or treating regional disease in oesophageal cancer (4). Current technologies employed in radiotherapy have led to a number of advanced methods for improving treatment; however, the prognosis of oesophageal cancer remains poor, and the sensitivity of patients towards radiation is unknown (5). In the transition towards an era of personalized medicine, a powerful tool that assists clinicians in assessing which individuals are likely to be benefit from radiotherapy does not exist. In consideration of the heterogeneity between various tumour types, even for patients with the same tumour type, prognostic and therapy-predictive molecular markers are essential to improve decisions regarding cancer therapy. At the molecular level, numerous genes are responsive to radiation exposure, and a recent study proposed that identifying the gene signature may predict precise radiotherapy (6). In the past few decades, predictive radiosensitivity techniques have been developed and tested (7). In cell line experiments, the values of the surviving fraction of cells at [2] Gy(SF)2, SF5 and SF8 are defined as indicators for distinguishing radiosensitivity (RS) and radioresistance (RR), whereas patients are defined as RS and RR based on the clinical outcome (overall survival

and recurrence rate) (8). However, the majority of studies on the radiosensitivity of oesophageal cancer are primarily dependent on high-throughput microarrays to assay differential gene expression between RS and RR oesophageal cancer cell lines, and different cell lines predict markedly different RS and RR biomarkers (9-11). Although these studies may contribute to an improved understanding of the biological mechanisms underlying the development and progression of cancer to a certain extent, it is difficult to practically apply these to clinical decision-making on whether radiotherapy is an appropriate means of treatment, based on the mixed results of *in vitro* assays.

In the present study, two common radiosensitive gene signatures, which were previously validated by clinical data, were utilised (6,7). The two types of gene signatures from different sources of radiosensitive genes were used to analyse the gene expression and clinical data of patients with oesophageal cancer. Eschrich *et al* (12) and Kim *et al* (13) proposed two different gene signatures for predicting radiosensitivity. Eschrich *et al* (12) used a panel of 48 human cancer cell lines to propose a radiosensitivity index (RSI), which was modelled as a function of the combination of gene expression, tissue of origin, and *ras* and *p53* status to correlate the surviving fraction of cells at 2Gy(SF2). The model developed by Eschrich *et al* (12) predicted an RSI (10 genes), which was directly proportional to tumour radioresistance (12). A high level of RSI represents radioresistance, thus allowing for the successful prediction of a number of types of primary cancer (14-20). Although the authors previously predicted the radiosensitivity of oesophageal cancer, the sample sizes were too small (n=12), and this may have resulted in a poor prediction of the overall survival of the 12 patients with oesophageal cancer (21). Kim *et al* (13) proposed a radiosensitivity gene signature which included 31 genes based on the integrated results of four different microarray experiments. The gene signature demonstrated promising results for predicting the radiosensitivity of cancer cells; however, it has only been validated in glioblastoma. Therefore, in the present study, RSI and the 31-gene signature have been utilized to predict the outcomes of patients with oesophageal cancer using data obtained from The Cancer Genomic Atlas (TCGA).

Patients with cancer who respond to radiotherapy typically exhibit a favourable prognosis compared with those with a radioresistant cancer. Therefore, it is hypothesized that the gene expression profile of patients with oesophageal cancer may allow for the classification of individuals into RS and RR groups. In the present study, a 31-gene signature and RSI were used as predictive biomarkers for predicting the overall survival of patients with oesophageal cancer. The results obtained from the two different types of radiosensitivity gene signatures utilised did not exhibit any overlap. Thus, the signatures were combined to improve the estimation of overall survival in patients with oesophageal cancer, based on a dataset obtained from TCGA. The dataset contained information on 152 patients who received radiotherapy (https://xenabrowser.net/datapages/?cohort=GDC%20TCGA%20Esophageal%20Cancer%20(ESCA)&removeHub=https%3A%2F%2Fxena.treehouse.gi.ucsc.edu%3A443). Multivariate Cox regression analyses were used to determine the key genes for predicting RS and RR in patients with oesophageal cancer.

Materials and methods

Clinical data and gene expression data collection. Data of patients with oesophageal cancer were downloaded from TCGA data portal (https://portal.gdc.cancer.gov/). Among the cases with the gene expression profiles and clinical indexes, there were 152 cases with effective radiotherapy information, which were used for further analysis. The gene signatures associated with radiosensitivity were aggregated from two previous publications (12,13) and there were no instances of overlap in the gene signatures. Eschrich *et al* (12) indicated a linear combination of 10 genes for predicting RS and RR, whereas Kim *et al* (13) identified 31 genes integrated from four different platforms for classifying the level of sensitivity of cancer cell lines after receiving radiotherapy.

Statistical analysis for clinical data and gene expression data. Univariate survival analysis was used to determine thedemographic and clinical factors associated with the overall survival time of patients with oesophageal cancer among 8 factors: Age, sex, histological type, radiotherapy, tumour status, smoking history, alcohol history, and Tumor-Node-Metastasis (TNM) stage. Only clinical factors with $P \leq 0.05$ (log-rank test) were analysed using a multivariate Cox regression analysis. The correlation between overall survival time and gene expression using the univariate Cox regression for each gene from the two gene signatures was used to obtain a prognostic index (PI) derived from the linear combination of gene expression and the coefficient of Cox regression.

To generate an improved model of biomarkers for predicting the RS or RR classification of patients with oesophageal cancer, the two gene signatures were combined into a novel model. Multivariate Cox regression was used to calculate the P-value of the combination of all the genes in the 41-gene signature. A combined gene-signature from two sources was used. One part of gene signature was obtained from 10 radiosensitive biomarkers and the other part was obtained from 31 radiosensitive biomarkers. Genes with $P<0.1$ were selected using multivariate Cox regression (22,23). These genes were used as a gene signature for predicting RS and RR. The PI values derived from different gene combinations were ranked according to the hazard ratio (HR) and P-value of the log-rank test. The high-risk and low-risk groups divided by the median PI value, which was estimated by the HR and the P-value of the log-rank test. Thus, a higher HR and smaller P-value represented an improved PI.

RSI. RSI is a rank-based linear regression algorithm proposed by Eschrich *et al* (12): RSI=-0.0090008 x *androgen receptor (AR)*+0.0128283 x *transcription factor AP-1 (JUN)*+0.0254552 x *signal transducer and activator of transcription 1 (STAT1)*-0.0017589 x *protein kinase C β type*-0.0038171 x transcription factor p65 + 0.1070213 x *tyrosine protein kinase ABL1 (ABL1)*-0.0002509 x small ubiquitin-related modifier 1-0.0092431 x *serine/threonine-protein kinase PAK 2 (PAK2)*-0.0204469 x *histone deacetylase 1*-0.0441683 x *interferon regulatory factor 1*.

According to Eschrich *et al* (12), the lower quartile of RSI was pre-defined as the cut-off point to divide patients into radiosensitive or radioresistant groups.

Table I. Clinical traits of oesophagus cancer with radiotherapy in The Cancer Genome Atlas database.

Factors	Death/patients	Median survival time	95% CI	Log-rank	Multivariate Cox P-value
Age					
≤60.5	30/77	1,263	557-NA	0.711	0.441
>60	30/75	764	650-NA		
Sex					
Female	5/20	NA	1,458-NA	0.144	0.790
Male	55/132	764	610-1,361		
Histological type					
Oesophagus adenocarcinoma, not otherwise specified	34/75	951	600-NA	0.84	0.243
Oesophagus squamous cell carcinoma	26/77	764	567-NA		
Radiotherapy					
Yes	8/31	855	610-1,458	0.379	0.133
No	52/121	764	567-NA		
Tumour status					
With tumour	43/66	600	484-855	0.00162	0.855
Tumour-free	16/81	NA	1,458-NA		0.660
Unknown	1/5	730	NA		
Smoking history					
≤15 years	13/29	567	283-NA	0.0156	0.557
>15 years	9/27	1,402	730-NA		0.090
Duration not specified	0/2	NA	NA		0.998
Current smoker	14/32	855	378-NA		0.356
Lifelong non-smoker	9/45	NA	NA		0.014[a]
Unknown	15/17	610	435-987		
Alcohol history					
Yes	37/107	1,361	694-NA	0.249	
No	23/48	600	480-NA		
Unknown	0/2	NA	NA		
TNM stage					
Stage 0	1/1	480	NA	0.00045	0.873
Stage I	5/19	1,781	1402		0.031[a]
Stage II	18/62	987	764-NA		0.051
Stage III	23/50	694	484-NA		0.337
Stage IV	6/6	322	136-NA		0.958
Unknown	7/14	283	161-NA		

[a]P<0.05. TNM, Tumour-Node-Metastasis; NA, not applicable; CI, confidence interval.

As an evaluation criterion and a corresponding value, the area under the curve (AUC) of the receiver-operator characteristic (ROC) curve, which is applied to assess the capacity and efficiency of a gene signature for classifying patient outcome, was utilized in the present study to verify the integrated gene signature.

Prognosis index for oesophageal cancer. As an integrated indicator of gene signature for individual patients, the PI was calculated using a linear combination of the expression value of the feature genes weighted by the Cox regression coefficient. Multivariate stepwise Cox regression was additionally used to analyse the clinical factors that were significantly associated with overall survival time by univariate survival analysis. In univariate survival analysis, log-rank test P<0.05 was considered as significance factors. The clinical variables and combination gene signature with a multivariate Cox regression significance of P≤0.1 were considered as important predictors of oesophageal cancer prognosis (23), and the PI was defined as follows: $PI=\beta_1X_1+\beta_2X_2+\ldots+\beta_iX_i$; where β_i is the Cox regression coefficient of the *i*th variable, X_i is the value of the *i*th variable and was the $\log_2$-transformed expression value of

Table II. Radiosensitivity index (10-gene signature) for predicting radiosensitivity.

Gene symbol	Uniprot accession no.	Description	Univariate Cox P-value	Coefficient	Hazard ratio	95% CI
AR	P10275	Androgen receptor	0.078	-1.331	0.264	0.06-1.16
JUN	P05412	Transcription factor AP-1	0.039	0.301	1.351	1.01-1.80
STAT1	P42224	Signal transducer and activator of transcription 1-alpha/beta	0.622	0.067	1.069	0.81-1.40
PRKCB	P05771	Protein kinase C beta type	0.836	0.031	1.03	0.76-1.40
RELA	Q04206	Transcription factor p65	0.501	-0.238	0.789	0.39-1.58
ABL1	P00519		0.745	-0.102	0.903	0.49-1.67
SUMO1	P63165	Small ubiquitin-related modifier 1	0.567	0.164	1.180	0.67-2.07
PAK2	Q13177	Serine/threonine-protein kinase PAK 2	0.995	-0.002	0.998	0.64-1.57
HDAC1	Q13547	Histone deacetylase 1	0.317	0.266	1.305	0.77-2.20
IRF1	P10914	Interferon regulatory factor 1	0.035	0.305	1.357	1.02-1.80

CI, confidence interval.

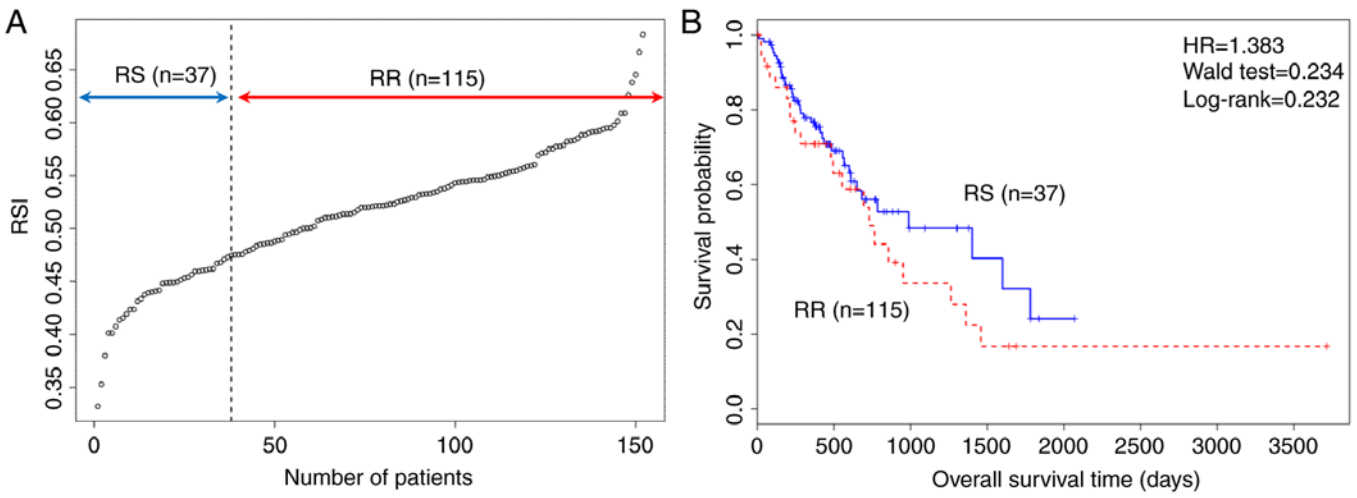

Figure 1. Standard RSI for predicting the prognosis of patients with oesophageal cancer. (A) Distribution of RSI in patients with oesophageal cancer. (B) Survival analysis comparing the RS and RR groups. P=0.232. RSI, radiosensitivity index; RS radiosensitive; RR, radioresistant; HR, hazard ratio.

each gene, and β_i was the Cox regression coefficient of the *i*th gene.

Estimating PI with different RS gene signatures. Patients with oesophageal cancer were classified into two groups (RS and RR) based on the median value of the PI (median PI value, 0.52). Kaplan-Meier curves and a two-sided log-rank test were used to compare the corresponding overall survival time and the difference in distribution of the two groups.

Gene Ontology (GO) enrichment. GO enrichment was used to analyse the functions of the genes in the 41-gene signature. Database for Annotation, Visualization and Integrated Discovery (DAVID; david.abcc.ncifcrf.gov) was used to examine the gene ontology of the selected RNAs by choosing '*Homo sapiens*' and subsequently searching the terms 'GO TERM_BP_FAT', 'GO TERM_CC_FAT', and 'GO TERM_MF_FAT' for the next step in the analysis (24,25). Abbreviations are defined as follows: BP, biological process; MF, molecular function; CC, cellular component; and FAT, function annotation chart. A Fisher's exact test was used to determine the significant categories.

Gene set enrichment analysis (GSEA). GSEA (www.broadinstitute.org/gsea) was performed using MSigDB C2 curated Kyoto Encyclopaedia of Genes and Genomes v5.2, and gene sets with a false discovery rate (FDR) value <0.1 after 1,000 permutations were considered to be significantly enriched (26). Additionally, GSEA was used to examine the differences in oesophageal cancer pathways between the RS and RR groups.

Programme implementation. The aforementioned univariate Cox regression, multivariate Cox regression and Kaplan-Meier survival curves for overall survival were analysed using R (version 3.2.4; www.R-project.org) (27) with R studio (version 1.1.463) (28) and the 'survival' package (5).

Table III. A 31-gene signature for predicting radiosensitivity.

Gene symbol	Uniprot accession no.	Description	Univariate Cox P-value	Coefficient	Hazard ratio	95% CI
ACTN1	P12814	Alpha-actinin-1	0.746	-0.054	0.947	0.68-1.31
ANXA2	P07355	Annexin A2	0.102	-0.299	0.741	0.52-1.06
ANXA5	P14668	Annexin A5	0.588	-0.097	0.907	0.64-1.29
ARHGDIB	P52566	Rho GDP-dissociation inhibitor 2	0.285	0.136	1.145	0.89-1.47
CAPNS1	P04632	Calpain small subunit 1	0.629	0.138	1.148	0.66-2.01
CBR1	P16152	Carbonyl reductase [NADPH] 1	0.791	0.031	1.032	0.82-1.30
CCND1	P24385	G1/S-specific cyclin-D1	0.900	0.012	1.012	0.84-1.22
CD63	P08962	CD63 antigen	0.687	0.075	1.077	0.75-1.55
CORO1A	P31146	Coronin-1A	0.248	0.141	1.152	0.91-1.46
CXCR4	P61073	C-X-C chemokine receptor type 4	0.756	-0.029	0.971	0.81-1.17
DAG1	Q14118	Dystroglycan	0.197	-0.200	0.818	0.60-1.11
EMP2	P54851	Epithelial membrane protein 2	0.983	0.003	1.003	0.76-1.31
HCLS1	P14317	Hematopoietic lineage cell-specific protein	0.088	0.187	1.206	0.97-1.49
HTRA1	Q92743	Serine protease HTRA1	0.210	0.163	1.177	0.91-1.52
ITGB5	P18084	Integrin beta-5	0.874	-0.032	0.969	0.65-1.43
LAPTM5	Q13571	Lysosomal-associated transmembrane 5 protein	0.121	0.152	1.164	0.96-1.41
LRMP	Q12912	Lymphoid-restricted membrane protein	0.553	0.086	1.089	0.82-1.45
MYB	P10242	Transcriptional activator Myb	0.932	-0.008	0.992	0.82-1.02
PFN2	P35080	Profilin-2	0.518	0.056	1.058	0.89-1.25
PIR	O00625	Pirin	0.043	0.237	1.268	1.01-1.59
PKM2	P14618	Pyruvate kinase PKM	0.985	0.003	1.003	0.70-1.43
PTMS	P04550	Parathymosin	0.290	0.176	1.192	0.86-1.65
PTPRC	P08575	Receptor-type tyrosine-protein phosphatase C	0.350	0.096	1.100	0.90-1.34
PTPRCAP	Q14761	Protein tyrosine phosphatase receptor	0.435	0.085	1.089	0.88-1.35
PYGB	P11216	Glycogen phosphorylase, brain form type C-associated protein	0.622	-0.063	0.939	0.73-1.21
RAB13	P51153	Ras-related protein Rab-13	0.965	0.012	1.012	0.59-1.72
RALB	P11234	Ras-related protein Ral-B	0.724	-0.077	0.926	0.60-1.42
SCRN1	Q12765	Secernin-1	0.683	0.060	1.062	0.80-1.42
SQSTM1	Q13501	Sequestosome-1	0.218	0.197	1.218	0.89-1.67
TWF1	Q12792	Twinfilin-1	0.277	0.282	1.325	0.79-2.20
WAS	P42768	Wiskott-Aldrich syndrome protein	0.246	0.137	1.147	0.91-1.45

CI, confidence interval.

The ROC curve was plotted using the '*survival ROC*' package (29). Log-rank test is used to test the significance of Kaplan-Meier curve (23) and Wald test is used to test Cox regression (30).

Results

Clinical characteristics of patients with oesophageal cancer. The clinical data of oesophageal cancer patients in TCGA are summarized in Table I. In total, eight clinical factors (age, sex, histological type, radiotherapy, tumour status, smoking history, alcohol history and TNM stage) were used for survival analysis.

In the present study, seven variables (age, gender, histological type, tumour status, smoking history, alcohol history and TNM stage) were tested for their association with survival. Table I demonstrates that tumour status, smoking history and TNM stage were significantly associated with overall survival in patients with oesophageal cancer in univariate survival analysis (log-rank test, $P<0.05$). Multivariate Cox regression analysis of these factors suggested TNM stage was correlated with overall survival time, and TNM stage I was closely associated with survival time (Table I). There was no significant difference in TCGA between oesophageal cancer patients treated with and without radiotherapy, and fewer patients received radiotherapy.

Table IV. Genes determined to be significant based on univariate Cox regression of the combined 41-gene signature.

Gene symbol	Uniprot accession no.	Description	Multivariate cox P-value	Coefficient	Hazard ratio	95% CI
ANXA5	P14668	Annexin A5	0.068	-0.688	0.526	0.24-1.05
TWF1	Q12792	Twinfilin-1	0.074	0.832	2.299	0.92-5.73
AR	P10275	Androgen receptor	0.009	-4.625	0.010	0.00-0.31
JUN	P05412	Transcription factor AP-1	0.093	0.387	1.472	0.94-2.31
STAT1	P42224	Signal transducer and activator of transcription 1-alpha/beta	0.041	-0.646	0.515	0.27-0.97
IRF1	P10914	Interferon regulatory factor 1	0.011	0.878	2.405	1.22-4.74

CI, confidence interval.

Table V. Cox regression analysis of prognosis index of all the different of gene signatures.

PI in Type of radiosensitivity genes	Number of genes	HR	95% CI	P-value
Standard RSI	10	1.383	0.810-2.362	0.232
PI of RSI	10	2.218	1.307-3.764	0.003
31-gene signature	31	2.402	1.410-4.093	0.001
RSI+31-gene signature	41	2.967	1.717-5.127	9.71×10^{-5}
Multivariate Cox screen	6	0.6380	0.380-1.070	0.089

PI, prognostic index; HR, hazard ratio; CI, confidence interval; RSI, radiodensity index.

Standard RSI for estimating RS and RR groups. The RSI was calculated in 152 patients with oesophageal cancer, classifying patients into two groups (RS, 25%; RR, 75%) and the cut off point for classification was 0.474. The overall survival of the two groups using a Kaplan-Meier plot is presented in Fig. 1, and the plot suggested that standard RSI was not able to satisfactorily predict overall survival of patients with oesophageal cancer.

Gene signature for predicting prognosis in TCGA oesophageal cancer cohort. Considering that the RSI did not predict overall survival, the PI of two independent gene signatures and their integration was calculated and analysed. First, the ten genes from RSI were used to perform univariate Cox regression (Table II). Subsequently, the 31-gene signature combination was analysed by univariate Cox regression in addition to the former analysis (Table III). The present study proposed that these genes may be biomarkers for predicting RR and RS in several cell lines. In the current study, Jun proto-oncogene, AP-1 transcription factor subunit (JUN), interferon regulatory factor 1 (IRF1) and pirin (PIR) were significantly associated with survival in oesophageal cancer ($P<0.05$; Tables II and III). Of the three genes, JUN is closely associated with tumour development (29) and IRF1 is a radioresistance biomarker (28). The gene PIR has rarely been reported to be associated with oesophageal cancer. PIR may act as a redox sensor for the nuclear factor κβ and is involved in stress responses (30). The present study revealed that not all genes associated with survival in oesophageal cancer ($P>0.05$). Therefore, two gene signatures for predicting RS and RR for oesophageal cancer were proposed. To identify the core genes for predicting prognosis, multivariate Cox regression was used to filter combination genes (41 genes), obtaining six genes with a $P<0.1$ as a cut-off threshold (Table IV). However, analysis of the core genes demonstrated that their combination was not significantly associated with overall survival time (HR, 0.638; 95% CI; 0.380-1.070; P=0.089; Wald test; Table V). To separate the patients into RS and RR, the median value of PI was selected (Fig. 2).

As a linear combination of the expression values of 10 genes, the PI of RSI, calculated by the aforementioned formula, was significantly relevant with overall survival time (HR, 2.218, 95% CI, 1.307-3.764; P=0.0025, Wald test; Table V). The PI of the 31-gene signature was also significantly associated with overall survival time (HR, 2.402; 95% CI, 1.410-4.093; P=0.001; Wald test; Table V). The RSI and the 31-gene signature were combined and the aforementioned process was used to calculate the PI. The results demonstrated that the PI of the combination was more significantly associated with overall survival time compared with RSI or the 31-gene signature alone (HR, 2.967; 95% CI, 1.717-5.127; $P=4.66 \times 10^{-5}$; Wald test; Table V). As demonstrated in the survival analysis and Fig. 2, the RS group had an improved prognosis compared with the RR group, particularly when considering the effect of the combination of RSI and the 31-gene signature, which had the highest HR and the most significant P-value. Therefore, the 41-gene signature may be the best biomarker for classifying patients with oesophageal cancer into RS or RR groups.

Gene signature validation in patients who had received radiotherapy. For further validation of the effectiveness and

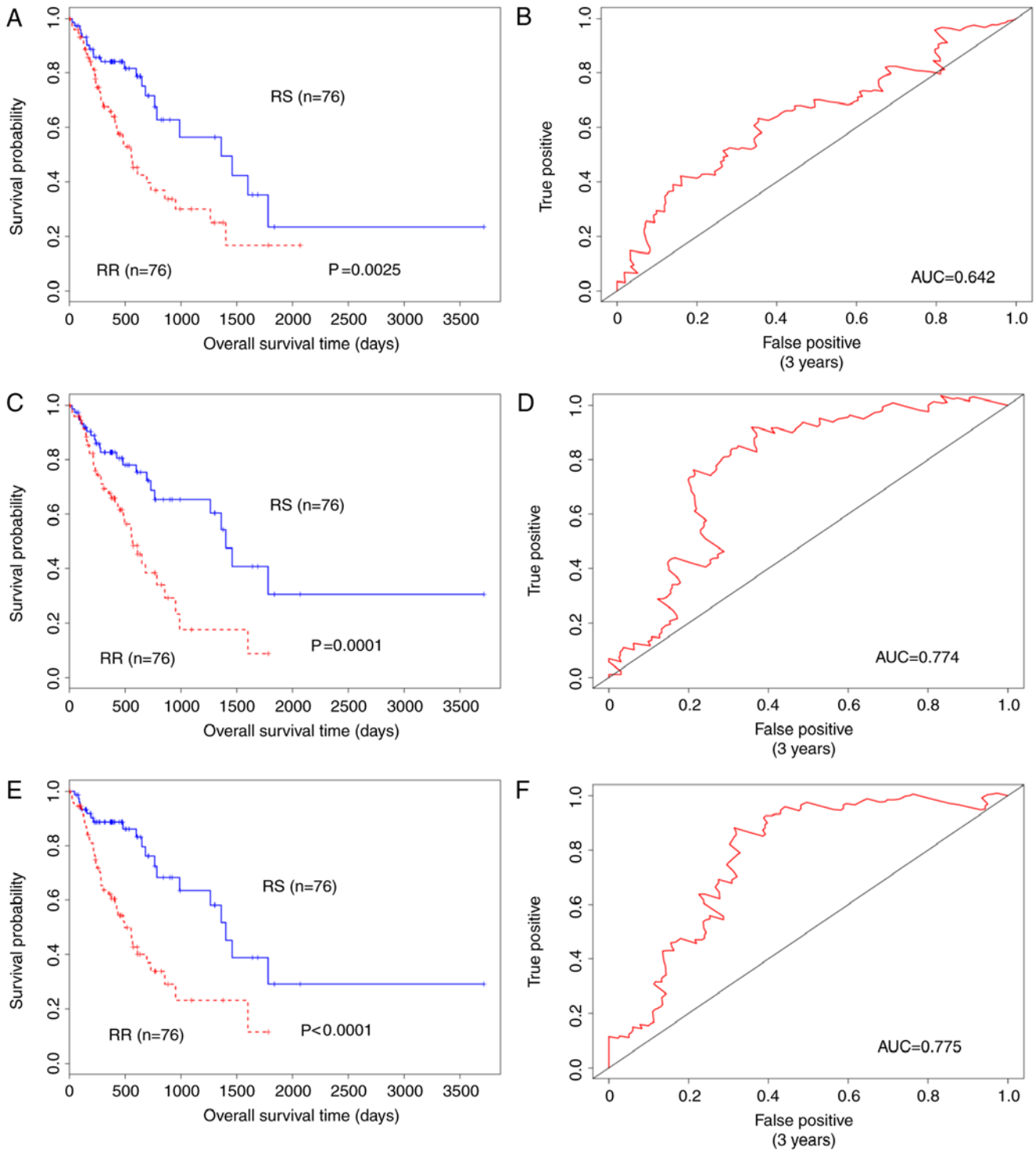

Figure 2. Survival analysis and ROC curve for estimating the radiosensitivity gene signature effect in the prognosis of oesophageal cancer patients. (A) Survival curve of the 10-gene signature PI in TCGA oesophageal cancer patients. (B) ROC curve of the 10-gene signature PI. (C) Survival curve of the 31-gene signature PI in TCGA oesophageal cancer patients. (D) ROC curve of the 31-gene signature. (E) Survival curve of the combination genes (41-gene signature) PI in TCGA oesophageal cancer patients. (F) ROC curve of the 41-gene signature PI. ROC, receiver operator characteristic; PI, prognostic index; TCGA, The Cancer Genome Atlas; RS, radiosensitive; RR, radioresistant; AUC, area under the curve.

performance of the two independent gene signature and combination models, samples from 31 patients who had received radiotherapy were selected for assessment (Fig. 3).

Additionally, with the TNM staging system being an important clinical indicator for tumours in clinical practice, in the present study, the 41-gene signature was used to predict the outcome of all stages of patients with oesophageal cancer (Fig. 4). The results demonstrated that the 41-gene signature of RS classified all stages significantly, with an improved predictive capacity for Stage II and Stage III.

Core genes for patients who have received radiotherapy. The results demonstrated that the core genes were not able to predict RS and RR groups in all patients with oesophageal

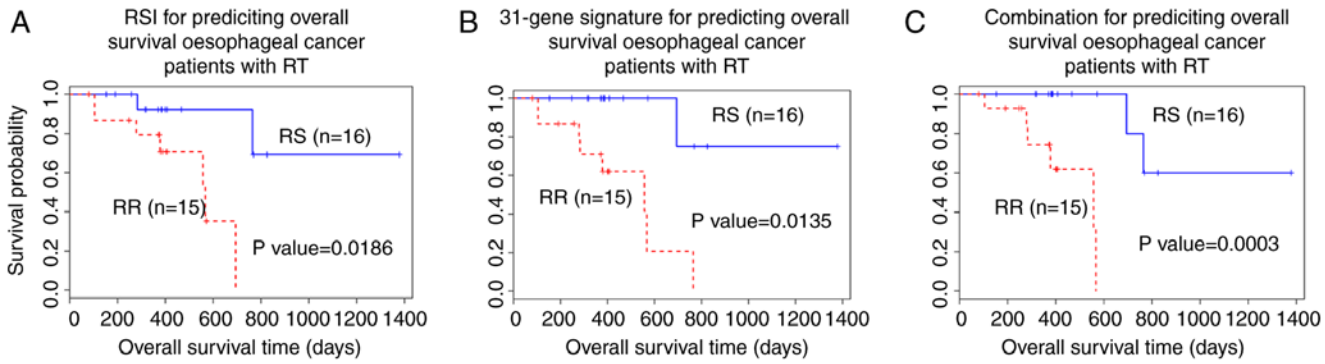

Figure 3. Comparison of the two independent gene signature models for predicting RS and RR in patients with oesophageal cancer. (A) RSI (10-gene signature combination) for predicting overall survival of patients with oesophageal cancer. Kaplan-Meier curves for the RS and RR groups separated by the RSI of the gene signature in the oesophageal cancer cohort. P=0.0186. (B) A 31-gene signature for predicting the overall survival of patients with oesophageal cancer. Kaplan-Meier curves for the RS and RR groups separated by the 31-gene signature in the oesophageal cancer cohort. P=0.0135. (C) Combination of the gene signatures for predicting overall survival of oesophageal cancer patients. Kaplan-Meier curves for the RS and RR groups separated by combination gene signature in the oesophageal cancer cohort. P=0.0003. RS, radiosensitive; RR, radioresistant; RSI, radiosensitive index; RT, radiotherapy.

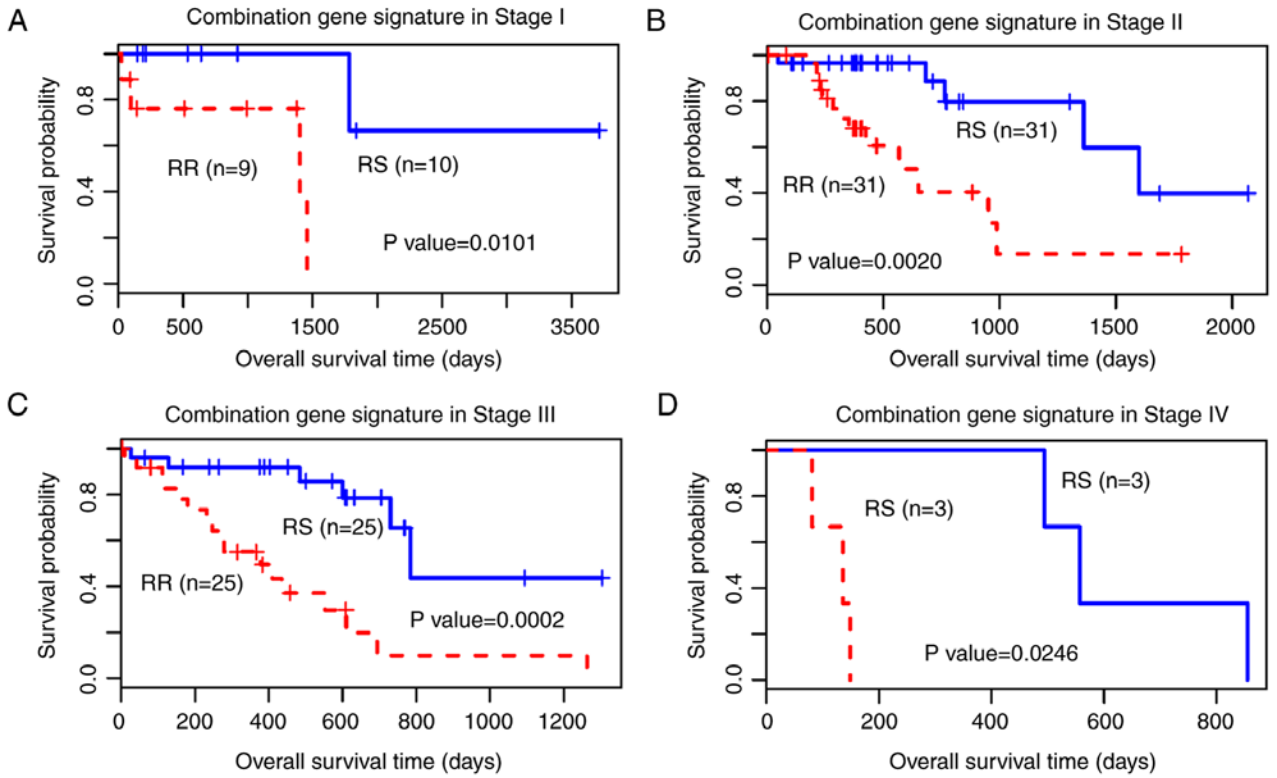

Figure 4. Combined 41-gene signature classifying the RS and RR groups by Tumor-Node-Metastasis stage of oesophageal cancer. The 41-gene signaturesignificantly classifiedoesophageal cancer patients into RS and RR groups in all stages by log-rank test. (A) Stage I. P=0.0101. (B) Stage II. P=0.0020. (C) Stage III. P=0.0002. (D) Stage IV. P=0.0246. RS, radiosensitive; RR, radioresistant.

cancer (Table V). Therefore, the core genes were tested in patients who received radiotherapy (n=31). The 41-gene signature combination performed well in predicting the prognosis in all oesophageal cancer patients and patients who had received radiotherapy. Multivariate Cox regression analysis demonstrated that the core genes [*CBR1*, *PAK2*, *ras-related protein Rab 13 (RAB13)* and *twinfilin-1(TWF1)*] may significantly predict the prognosis of patients with oesophageal cancer who had received radiotherapy (Fig. 5).

The results demonstrated that the expression of the four core genes differed between the RS and RR groups (Fig. 5A). The RS group had a significantly longer survival time compared with the RR group (P=0.0003; Fig. 5).

GO enrichment. The results indicated that the 41-gene signature combination had the highest HR and the largest significant difference between the RS and RR groups. Therefore, the GO terms associated with these 41 genes were analysed, and the results (top 10 catalogues) are presented in Fig. 6. The 41 genes were primarily associated with protein phosphorylation and protein binding (Fig. 6A and B). These genes were mainly enriched in the 'cytosol' and 'extracellular exosome' (Fig. 6C).

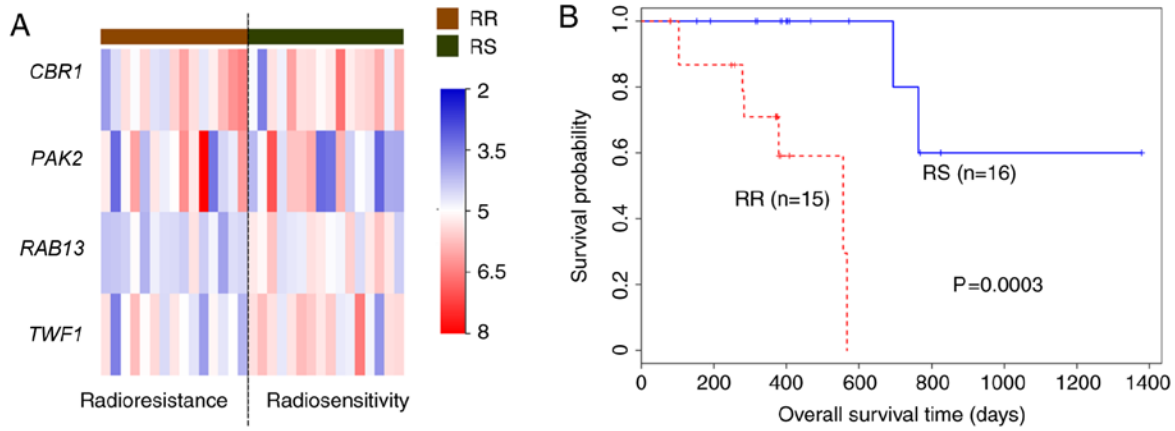

Figure 5. Core genes identified by multivariate Cox regression analysis on the 41-gene combination. (A) Heat mapdepicting the expression of the core genes in RR and RS patients. (B) Kaplan-Meier curves for the RS and RR groups separated by the core genes combination in the oesophageal cancer cohort (P=0.0003). RS, radiosensitive; RR, radioresistant; CBR1, carbonyl reductase 1; PAK2, serine/threonine-protein kinase PAK 2; RAB13, ras-related protein Rab 13; TWF1, twinfilin 1.

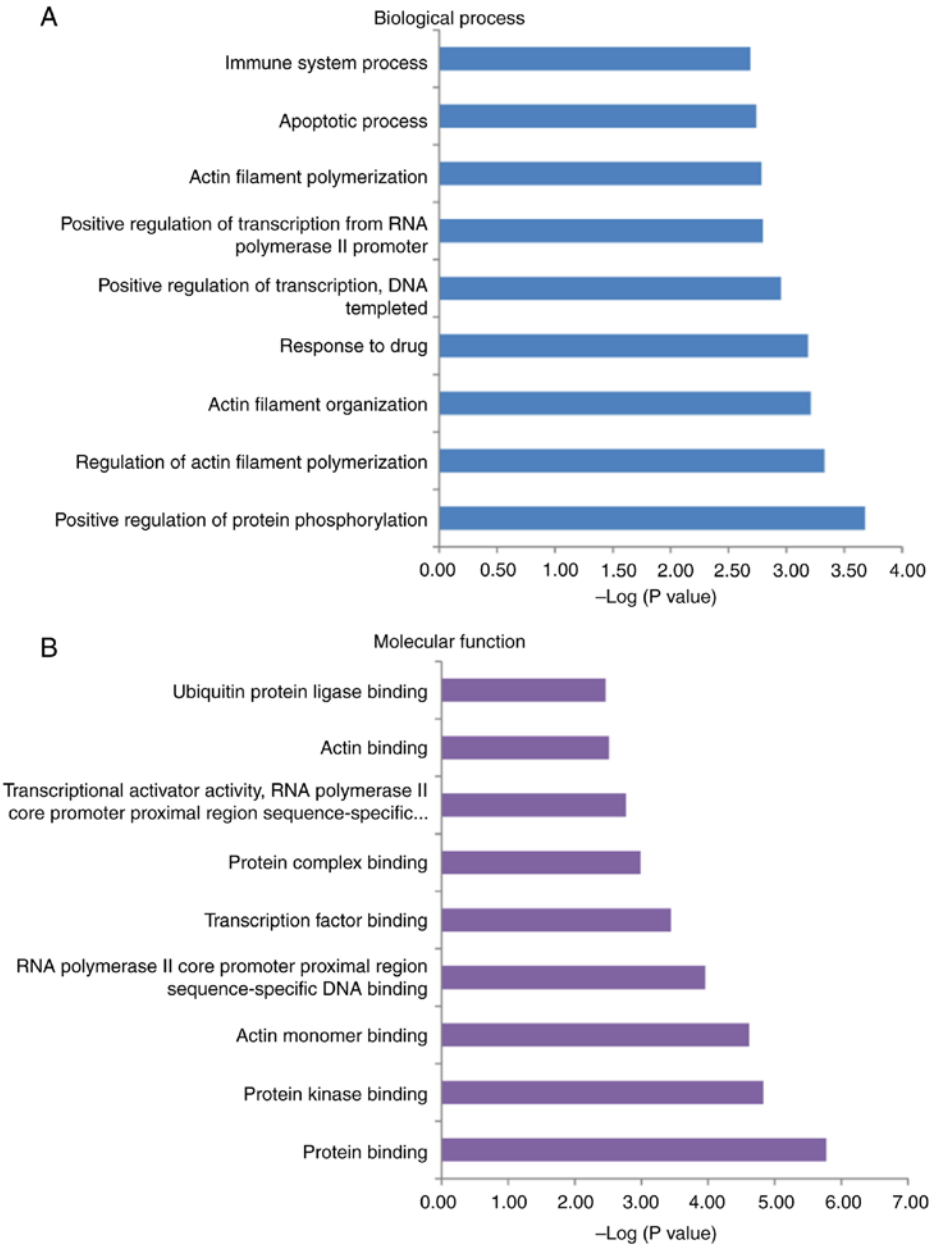

Figure 6. Gene Ontology enrichment in 41-gene signature. Gene Ontology enrichment in (A) biological process and (B) molecular function.

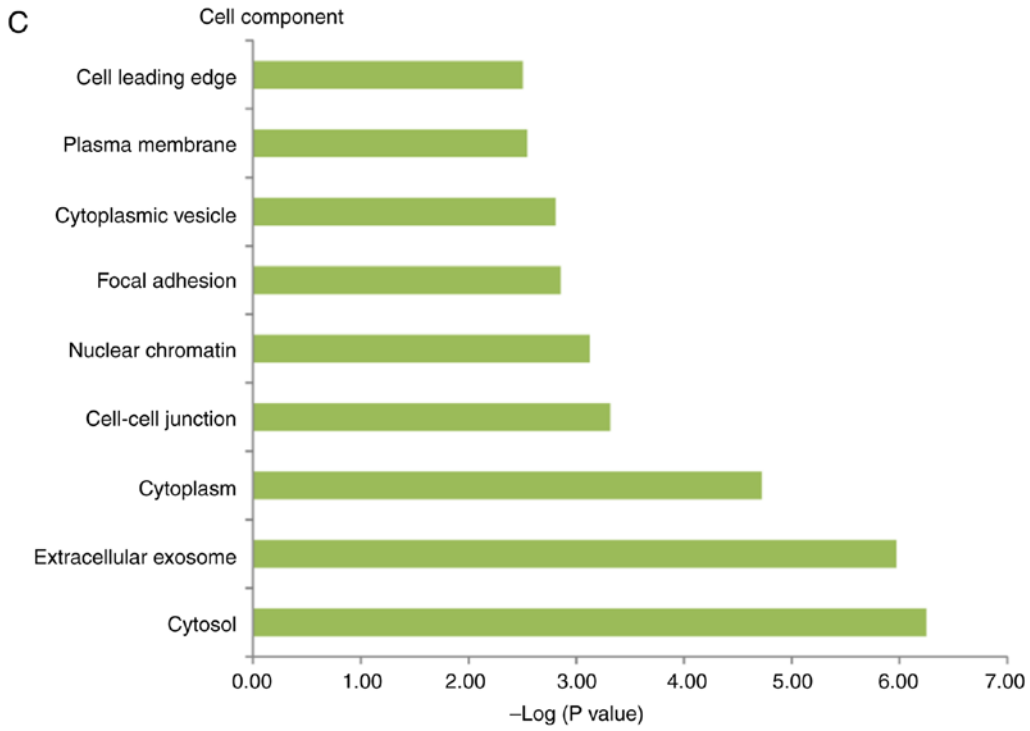

Figure 6. Continued. Gene Ontology enrichment in 41-gene signature. Gene Ontology enrichment in (C) cell component.

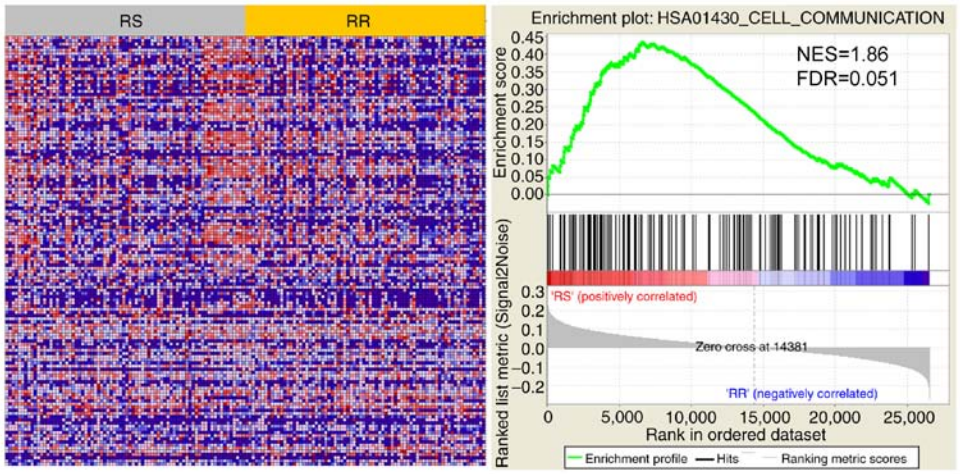

Figure 7. Gene set enrichment analysis demonstrates enrichment of the cell communication pathway classified by the 41-gene signature. RS, radiosensitive; RR, radioresistant; NES, normalized enrichment score; FDR, false discovery rate.

The results indicated that radiosensitivity and radioresistance were closely associated with these cellular components.

Identification of the 'cell communication' pathway by GSEA. The RS and RR groups were divided by the 41-gene signature to analyse the active pathway. The results demonstrated that 'cell communication' was significantly different between the RS and RR groups (Fig. 7). Using GSEA analysis, the normalized enrichment score was 1.86, and the FDR was 0.051.

Discussion

In the present study, the results suggested that integrating the two previously developed radiosensitive gene signatures (6,7) demonstrated improved performance in predicting overall survival in patients with oesophageal cancer compared with either method alone. RSI and the 31-gene signature were independently proposed, and the two signatures are related to SF2 measured from cellular radiosensitivity. The two types of gene signatures predicted clinical outcomes using univariate Cox regression analysis, and the 31-gene signature performed better compared with RSI. When the two types of gene signatures were combined, the combination (41-gene) signature demonstrated the highest HR and most significant P-value. However, when multivariate Cox regression analysis was used to screen independent genes for prognosis, the novel gene combination of 6 genes did not predict survival; demonstrating that the expression of

the 41 genes was associated with overall survival in patients with oesophageal cancer.

Compared with the previous studies on the radiosensitivity of oesophageal cancer, a common radiosensitive gene signature to predict overall survival instead of gene expression differences in cell lines was applied. For example, *cyclin-dependent kinase inhibitor 2A*, *interferon-β1*, *matrix metalloproteinase 1*, *protein S100-A4*, and*tumor necrosis factor receptor superfamily member 25* were demonstrated to be upregulated, whereas *granzyme A*, *Myc proto-oncogene*, *transforming growth factor β1* and *tumor necrosis factor-α* were downregulated (RS vs. RR cell lines) (31). In clinical practice, clinicians cannot make a distinction between whether patients are RS or RR *a priori*. In addition, different RS and RS oesophageal cancer cell lines express different biomarkers and regulation levels from 13 oesophageal cancer cell lines analysis (32). Therefore, there is no universal gene group to determine radiosensitivity. A previous study indicated that *CABPR*, *fatty acid binding protein 5*, *desmocollin-2*, *glutathione peroxidase 2*, *thioredoxin domain-containing protein*, *carbonyl reductase (CBR)3*, *dedicator of cytokinesis 8*, and *multidrug resistance-associated protein 1* were upregulated, whereas *replication protein A 70 kDa DNA-binding subunit*, *leucine zipper protein down-regulated in cancer cells*, *necdin*, and the *S-phase kinase-associated protein 1* were downregulated (32). It has been hypothesized that genes coding for proteins involved in the cell cycle and DNA repair are associated with radiosensitivity (33-35). Furthermore, a number of RS genes derived from cell lines present a significant obstacle in clinical practice as several different markers may confound clinical decision-making. Although the gene signatures used were selected from cell lines, these gene signatures were validated using a large amount of clinical data.

As radiosensitivity is difficult to study at the molecular level, RS genes are simply obtained from cellular experiments using SF2. Although a number of studies have predicted specific radiosensitive biomarkers for a limited number of cancer types (36,37), only a small number of common biomarkers for prognosis have been identified (22,38). The function of the 41-gene signature was investigated using GO. The 41 genes were primarily involved in protein phosphorylation biological processes. In particular, protein phosphorylation is closely associated with radiosensitivity (39,40). Based on the molecular function and cellular component analysis, these genes may primarily serve protein-binding functions and are located in the cytosol. Additionally, the majority of these genes (*STAT1*, *AR*, *JUN*, *PIR* and *ABL1*) serve vital roles in transcriptional regulation. The expression of transcription factors as indicators may predict radiosensitivity in cancer cells. Consequently, RS and RR groups that were classified using the 41-gene signature from GSEA were analysed, and it was demonstrated that the cell communication pathway was active in the RS group, consistent with the conclusions related to drug sensitivity in a recent study (41). However, the association between cell communication and radiosensitivity has not been studied, to the best of our knowledge.

Additionally, the four core genes (*CBR1*, *PAK2*, *RAB13* and *TWF1*) were sufficient for predicting the prognosis of patients with radiotherapy. One gene (*PAK2*) was derived from RSI and the other three genes (*CBR1*, *RAB13* and *TWF1*) were derived from the 31-gene signature. Common radiosensitivity genes were used to obtain specific special biomarkers for predicting RS and RR groups in patients with oesophageal cancer. The biomarkers from clinical data may be more useful than those from experiments with cell lines in clinical practice.

The current study had several limitations. While the relevance of specific genes for the effective prognosis prediction of oesophageal cancer was demonstrated in the current study, a limited sample size was investigated. Future clinical validation using larger sample sizes is warranted. The present study did not attempt to predict the relapse free survival (RFS) rate, as information on RFS was incomplete. However, the integrated 41-gene signature is an optimal radiosensitivity candidate for predicting the overall survival of oesophageal cancer.

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Availability of data and materials

The datasets generated and/or analyzed during the current study are available in the Cancer Genome Atlas repository (https://xenabrowser.net/datapages/?cohort=GDC%20TCGA%20Esophageal%20Cancer%20(ESCA)&removeHub=https%3A%2F%2Fxena.treehouse.gi.ucsc.edu%3A443).

Authors' contribution

QNZ and ZTB performed the analysis and wrote the manuscript. JHT was another major contributor in interpreting the biological and clinical data and writing the manuscript. XHW proposed and designed the methods for this manuscript. RFL and YL preprocessed the downloaded data. YRK and YY performed statistical analysis and validation. All authors read and approved the final manuscript.

Ethics approval and consent to participate

Not applicable.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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