Exploration to Identify Radiosensitive Genes in PD-L1 Expression and PD-1 check Point Pathway in Cancer

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Abstract

Purpose

To identify radiosensitive genes in PD-L1 expression and PD-1 check point pathway in cancer.

Methods and Materials

Gene expression datasets and information were downloaded from TCGA. Stepwise multivariate Cox regression based on AIC was performed using stacking multiple interpolation data to identify radiosensitive (RS) genes.

Results

Among the 74 PD-1/PD-L1 pathway genes, we identified 10 RS genes in BRCA dataset, 11 RS genes in STAD dataset and 13 RS genes in HNSC dataset. These genes could be thought as independent factors and biomarkers to identify the sensitivity of cancer patients to radiotherapy. Gene CD274 was the common RS gene in the three tumor datasets. And gene ZAP70 was verified as a RS gene in the external validation. There were moderate co-expression relationships and interactions in these genes. Functional enrichment analysis showed that most of these genes were related to T cells.

Conclusions

Our study identified potential radiosensitive biomarkers of several main cancer types in an important tumor immune checkpoint pathway. New types of RS genes were identified based on the expanded definition to radiosensitive genes. Different types of tumors may share same RS genes due to the common carcinogenic mechanisms.

1. Introduction

Radiation therapy remains the primary treatment for nearly two-thirds of cancers, including the primary curative or palliative treatment for breast cancer and adjuvant therapy for radical resection of gastric cancer [1-3]. Unfortunately, because of tumor heterogeneity, tumor response rates to radiotherapy can vary conspicuously, even among patients who are diagnosed with the same tumor type [4]. Despite significant technological advances in radiation therapy for tumors in recent years, personalized radiotherapy regimens based on cancer biology have become increasingly difficult [5]. A major issue in radiation therapy is predicting cancer radiosensitivity.

Biomarkers that provide information about tumor prognosis and predict tumor's inherent radiation sensitivity or its response to treatment may be valuable in helping to personalize radiation dose, allowing clinicians to make decisions about treatment regimens for different patients, while avoiding radiation-induced toxicity in patients who are unlikely to reap the benefits from the treatment [6, 7]. Tumor molecular mapping has been used to develop radiosensitive genetic signatures and has been used to

identify prognostic or predictive biomarkers of radiation responses [8-10]. Given strong evidence of the pathway-based genetic nature of cancer, one of the main shortcomings of past studies is the failure to use prior biological information into identifying biomarkers [11]. The potential for carcinogenic mechanisms are grouped into pathways based on biological functions such as cell cycle, hypoxia, DNA damage, tumor micro-environment, immune checkpoints and others [12-16].

As a key regulatory immune checkpoint, programmed death-1 (PD-1) and its ligand PD-L1 check point pathway plays a crucial role in maintaining the balance between immune tolerance and autoimmunity [17]. Studies have shown that PD-L1 presented on the surface of the tumor cells can activate the downstream of the PD-1/PD-L1 pathway to over-inhibit T cells proliferation and differentiation [18] and thus promote immune escape and tumor growth [19]. In addition, the expression of PD-1/PD-L1 has been found associated with tumor radiosensitivity in a variety of solid tumor types also. When Bum-Sup Jang et al. [20-22] evaluated the predictive value of radiosensitive gene signatures in invasive breast carcinoma and lower grade glioma, they discovered the relationship between radiosensitive gene signatures and PD-L1. Xintong Lyu et al. [23] reported that in head and neck cancer, patients with high PD-L1 expression had better recurrence-free survival in receiving radiotherapy.

These evidences seem to indicate that PD-L1 expression and its regulation in solid tumors are affected by radiotherapy, thereby altering the outcome of patients' prognosis. In this case, it is necessary to understand the regulatory mechanism of PD-L1 in cancer. For instance, in solid tumors, up-regulation of PD-L1 is caused by activation of pro-survival pathways MAPK and PI3K/Akt as well as transcriptional factors HIF-1, STAT3 and NF-kappa B[24]. It can be supposed that genes regulating PD-1/PD-L1 check point pathway in cancer may as well associate with cancer radiosensitivity and might be useful biomarkers for predicting radiosensitive of cancers. In fact, the relationship between these genes and radiotherapy sensitivity of gastric cancer has been preliminarily investigated, and some conclusions have been obtained[25].

In this study, we explored the radiosensitivity of genes in PD-1/PD-L1 check point pathway in several cancers using reliable method and validated in an external cohort. Conclusively, for precision medicine, our work offered more evidence for using PD-1 and PD-L1 related pathway genes as potential biomarkers to predict radiosensitive for cancer patients.

2. Material And Methods

2.1 Data Sources

In view of the previous explorations [20-23, 25] to the relationship between PD-L1 and its regulatory genes to tumor radiotherapy sensitivity, we downloaded gene expression datasets for several most common cancers from The Cancer Genome Atlas (TCGA, http://cancergenome.nih.gov/) which were breast invasive carcinoma (BRCA), Glioblastoma multiforme (GBM), Head and Neck squamous cell carcinoma (HNSC), Brain Lower Grade Glioma (LGG), Liver hepatocellular carcinoma (LIHC), Lung adenocarcinoma (LUAD), Lung squamous cell carcinoma (LUSC), Stomach adenocarcinoma (STAD), respectively. The

gene expression RNAseq was generated by Illumina platform sequencing and the unit was log2(x+1) transformed RSEM normalized count. And corresponding clinical information including survival data was procured from UCSC Xena browser (https://gdc.xenahubs.net).

The corresponding expression datasets were collated to exclude normal tissues and retain tumor samples. At the same time, we examined clinical information on each type of tumor and found GBM had too few samples for no radiotherapy (n=18) while LIHC had too few samples for radiotherapy (n=14). These two datasets were abandoned. Next, we removed patients with missing survival and radiotherapy information. Patients with survival time less than 5 days were also excluded. Then multivariate Cox stepwise regression analysis (see **Table1**, **TableS1/2**) was performed on the remaining six tumor datasets, and three tumor datasets (BRCA, HNSC, STAD) whose radiotherapy was protective effect (hazard ratio, HR<1, P<0.05) were selected for subsequent analysis. In addition, we also performed external validation, using the Molecular Taxonomy of Breast Cancer International Consortium (METABRIC) cohort (https://www.cbioportal.org/datasets). **Figure1** is the flow chart.

2.2 Radiosensitive genes

We obtained a total of 74 genes (See **TableS3**) in "PD-L1 expression and PD-1 checkpoint pathway in cancer" from web of Kyoto Encyclopedia of Genes and Genomes (KEGG, https://www.kegg.jp/). These genes are involved in the upstream regulation of PD-L1 expression or play a role in downstream of the PD-1/PD-L1 pathway to inhibit T cells proliferation and differentiation [26].

In this study, we defined radiosensitivity as cancer patients with different gene expression obtained discrepant benefit from radiotherapy. Based on the median of the gene expression, the whole included participants were roughly divided into two groups as the high expression group (**Hgroup**) and the low expression group (**Lgroup**). If one group (say **H group**) had better overall survival (OS) in receiving radiotherapy (RT) than in non-RT (equivalent to scenario A) while the other group (**L group**) had consistent OS whatever in RT group or non-RT group (scenario B) and meanwhile **H group** had a better OS than **L group** in receiving RT (scenario C) or **H group** had a lower OS than **L group** in non-RT (scenario D), we defined this gene as a RS gene. That is if scenario A and scenario B happened and meantime scenario C or scenario D happened, the gene was deemed as a RS gene.

2.3 Analysis methods

The identification of relationship between radiosensitivity and genes expression levels was analyzed by multivariate Cox proportional hazards models, the stepwise method based on Akaike information criterion (AIC) was used for variable selecting. The variables that remained in the model were considered to have an impact on OS. In this study, we recruited as many clinical variables as possible to screen out the best correction factors. Then the whole included participants could be divided into four groups: H-RT group, L-RT group, H-non-RT group and L-non-RT group. An example of how to identify a RS gene. In **Hgroup**, if radiotherapy had remained in the multivariate Cox regression model (HR<1), corresponding to scenario A; and in **L group**, radiotherapy had no impact (scenario B); meanwhile in RT group, **Hgroup**

compared to **L group** had a HR<1 and remained in the multivariate Cox regression model, corresponding to scenario C. This gene was considered as a RS gene.

For missing variable data, R packet *mice* (multiple imputation by chained equations) was used for multiple interpolation [27]. Next we utilized the strategy of imputation stacking, where multiple imputations of the missing data were stacked on top of each other to create a large dataset [28]. We then estimated parameters in the analysis model by fitting a weighted model for Y |X on the stacked dataset [29]. Kaplan-Meier (K-M) curves were used to show the survival curves. The log-rank test evaluated the statistically significant differences. Wilcoxon test was used to compare continuous variables that were not normal. Correlation was calculated by Pearson correlation coefficient (r). | r | >= 0.8, was considered as a strong correlation; 0.3 <= | r | < 0.8, as a moderate correlation; below 0.3, as a weak correlation [30]. The Search Tool for the Retrieval of Interacting Genes (STRING) [31] online tool was applied to analyze the protein-protein interaction (PPI) network (minimum required interaction score >= 0.4). Functions and pathways were analyzed by Gene Ontology (GO) and KEGG with p value cutoff = 0.05 and q value cutoff = 0.05. All statistical analyses were performed using the R (4.0.2). A P-value of 0.05 was considered significant. All statistical tests were two-sided.

3. Results

3.1 Identification of RS genes

We take BRCA as an example to illustrate the identification of RS genes. **Table1** shows the demographic and clinical characteristics at baseline of the included BRCA participants. A total of 979 female BRCA patients were included. The median follow-up time was 849 days (Q1:477, Q3:1678). After stepwise multivariate Cox regression analysis, radiotherapy, chemotherapy, age, surgery type, margin status, progesterone receptor (PR) status, menopause status, pathological stage, N stage, M stage were identified as impact factors of OS. Information of the results of HNSC and STAD see **TableS1/2**.

Figure2 shows the remained 10 genes in BRCA after selection by multivariate adjustment. In the one hand, high expression of genes RASGRP1 and TRAF6 had better OS in RT group than in non-RT group, and low expression of these two genes had consistent OS in both RT group and non-RT group. And high expression group receiving RT had better OS than the low. That is BRCA patients with relative high expression of RASGRP1 and TRAF6 could benefit from radiotherapy, we called them radiosensitive genes when expressed high (RGH). High expression of gene TIRAP had a lower OS than the low expression in non-RT group but got high OS when received RT, which was also considered as a RGH gene. In the other hand, genes CD3G, IFNG, NFKBIA, PDCD1, CD274, STAT1 and ZAP70 were defined as radiosensitive genes when expressed low (so called RGL). In addition, we found that without adjustment by clinical factors, these genes were strong indicators as well (See K-M curves in **Figure3**). These genes could be thought as independent factors and biomarkers to identify the sensitivity of cancer patients to radiotherapy. RS genes of HNSC and STAD see **TableS4**. We compared RS genes in the three tumor

datasets and found some crossover genes (See **Figure4**). Gene CD274 was the common gene in the three tumor datasets.

3.2 Distribution of RS genes in BRCA

We extracted BRCA patients receiving radiotherapy who survived more than 8 years (n=49) and those who survived less than 3 years (n=29). We compared the expression of the 10 RS genes in the two groups (See **Figure5A**). From the boxplot, genes RASGRP1 and TRAF6 had a significantly difference expression level ($P<0.05$) between alive group (higher) and dead group (lower). By contrast, among non-RT patients, most RGL genes had a trend that their median expression values in alive group (n=32) would be higher than those in dead group (n=29) (See **Figure5B**).

3.3 Relationship of BRCA RS genes

We explored the correlation among these 10 RS genes expression level, the result is as shown in **Figure6**. Genes NFKBIA, RASGRP1, TIRAP and TRAF6 had a correlation coefficient of less than 0.3 with other genes (**Figure6A**). The remaining six genes were moderate correlated, r=(0.3,0.8) (**Figure6B**). Specially, there was a strong co-expression relationship between PDCD1 and ZAP70. Further analysis of PPI network (**Figure6C**) shows that CD274, PDCD1, CD3G, STAT1, IFNG and ZAP70 were at the hub position.

GO and KEGG analysis of 10 RS genes to obtain the biological process (BP), molecular function (MF), cellular component (CC), and pathways. KEGG pathway analysis (**Figure7A**) showed that the 10 RS genes in BRCA mainly related to “PD-L1 expression and PD-1 checkpoint pathway in cancer” and “T cell receptor signaling pathway”. The BP of the 10 RS genes mainly related to “positive regulation of lymphocyte activation” and “T cell activation”; the CC of the 10 RS genes mainly involved in “plasma membrane signaling receptor complex” and “T cell receptor complex”; the MF of the 10 RS genes mainly associated with “tumor necrosis factor receptor binding” (**Figure7B**).

3.4 external validation of BRCA RS genes

Table2 shows the demographic and clinical characteristics at baseline of the included METABRIC participants. A total of 1902 female METABRIC patients were included. The median follow-up time was 115.6 months (Q1:61.0, Q3:184.8). After stepwise multivariate Cox regression analysis, lymph nodes, estrogen receptor (ER), HER2, age, molecular subtypes, surgery type, pathological stage, tumor size and radiotherapy were the impact factors of OS. Among the 10 RS genes of BRCA, only ZAP70 had a significant impact to OS in METABRIC cohort. **Figure8** shows the unadjusted K-M curves of ZAP70 from METABRIC.

4. Discussion

Along with some chronic diseases such as cardiovascular disease, cancer remains one of the biggest killers of human health [32]. The World Health Organization (WHO, https://www.who.int/) has recently announced on 5 March, 2021 that, breast cancer has now overtaken lung cancer as the world’s mostly

commonly-diagnosed cancer and the new global breast cancer initiative highlights renewed commitment to improve survival. At the same day, new WHO publication provides guidance on radiotherapy equipment to fight cancer like colorectal and lung cancer. Radiotherapy is remain one of the most effective tools to mitigate pain and suffering associated with advanced cancers, also, improve the quality of life and survival [33, 34]. Nevertheless, heterogeneity in terms of tumor characteristics, prognosis, and survival among cancer patients has been a persistent problem for many decades. Vast studies have shown that, the investigation of biomarkers related to radiation could provide another means by which radiotherapy could become personalized [2, 35].

Understanding the mechanism of tumors is also a major issue in identifying effective biomarkers and potential drug targets of radiosensitivity [36, 37]. PD-1 and its ligand PD-L1 are important immune checkpoints as a potential therapeutic target in cancer[19]. PD-L1/PD-1 pathway plays a critical role in transmitting co-stimulatory molecules to activate T cells as the second signal and maintain the balance of the immune microenvironment [38]. Well, when the body is invaded by the tumors, the balance of the immune microenvironment is destroyed. PD-L1 on tumor cells may engage the PD-1 receptors resulting in suppression of T-cell mediated immune response. Studies show that therapeutic antibodies blocking the PD-1/PD-L1 pathway by targeting PD-L1 or PD-1 are highly effective in rescuing T cell anti-tumor effector functions [18, 39]. In addition, the expression level of PD-L1 seems to be related to the radiotherapy sensitivity of tumors [20, 22]. As PD-L1 expression is regulated by the upstream signaling pathway, while PD-1/PD-L1 combination is transferred to the downstream T cell regulation as the second signal, the expression level of relevant genes in regulating PD-L1 expression and in PD-1 checkpoint pathway in cancer appears to be of vital importance, which may indicate the potential sensitivity of the tumor to radiotherapy.

In this study, we identified the radiosensitivity of genes in PD-L1 expression and PD-1 checkpoint pathway in cancer using the TCGA datasets of BRCA, HNSC and STAD. Because radiotherapy had non-positive effect (HR>=1) to OS in lung cancer and LGG, we excluded these type of tumors for further exploration and perhaps they could be the subject of the next study. Then, we systematically considered clinical factors in the datasets as many as possible. We performed multiple interpolation to missing clinical variables and stacked them to perform weighted multivariate Cox regression. Therefore, the clinical variables were well controlled to ensure the reliability of the results. In the BRCA dataset, radiotherapy, chemotherapy, age, surgery type, margin status, PR status, menopause status, NM stage and pathological stage were the impact factors of OS, which were reasonable and validated [40]. In the HNSC dataset, the impact factors included radiotherapy, age, gender, TN stage, margin status, anatomic site and smoking. Notably, females OS was not as good as males (HR: 1.149(1.016, 2.066), P=0.041). And as for STAD, radiotherapy, age, gender, TN stage and residual tumor were the main influencing factors.

Totally, among genes in regulating PD-1/PD-L1 pathway in cancer, we identified 10 RS genes in BRCA dataset, 11 RS genes in STAD dataset and 13 RS genes in HNSC dataset, with overlapping genes between each other to varying degrees. CD274 was the common gene in the three tumor datasets. As known to all, CD274 is the gene that encodes PD-L1, predicting expression level of PD-L1, and has been speculated to

be related to radiosensitivity of a variety of cancers [22, 23, 25]. In addition, there were moderate co-expression relationships and interactions among most RS genes (**Figure6)**. Functional enrichment analysis showed that most of these genes were related to T cells. In the external validation, ZAP70 was verified as a RS gene. Many studies have shown that it is related to the immunity of cancers [41, 42]. Importantly, like BRCA, there was also a strong co-expression relationship between PDCD1 (correspond to PD-1) and ZAP70 in METABRIC (r=0.8) (See **FigureS1**).

Importantly, we developed a more comprehensive definition of radiosensitive genes since most studies have neglected many genes that directly affect the OS of patients without radiotherapy (scenario D). Theoretically, there are two types of radiosensitive genes. The expression level of the first type of genes (**A genes**) do not affect patients' OS, but their different expression level can influence patients' OS after radiotherapy, like RASGRP1 and TRAF6. High expression of these two genes could obtain benefit from RT. More often, however, are the second type of genes (**B genes**). Their expression could influence patients' OS, for instance, patients with low expression of CD274 had much lower OS than the high. But these patients would benefit much receiving RT. And these genes can be thought as independent factors to identify the sensitivity of cancer patients to radiotherapy (**Figure3/5)**.

This study has its merits. Firstly, we expanded the definition of radiosensitive genes and identified radiosensity of those genes in important pathway of cancer using TCGA public datasets recognized as authoritative. Secondly, we took into account as much useful clinical information as possible to control influence factors by stacking multiple interpolation data, making the results more persuasively. Thirdly, we also validated the results with a big external dataset, METABRIC, although only one gene ZAP70 was turned out to be consistent. However, this might be due to different sample sizes and large gaps in follow-up time. The limitation of this study is that we don’t have performed experimental study, also no cohort to verify the findings. In addition, because we only explored a few major cancers, more tumor types should be brought into the discussion.

In conclusion, our study identified potential radiosensitive biomarkers of several main cancer types in an important tumor immune checkpoint pathway. New types of RS genes may be identified based on expanded definition to RS genes. Different types of tumors may share same RS genes due to the common carcinogenic mechanisms. We hope that further studies will be performed to confirm our findings.

Abbreviations

RS: radiosensitive; **PD-1:** programmed death-1; **TCGA:** The Cancer Genome Atlas; **BRCA:** breast invasive carcinoma; **GBM:** Glioblastoma multiforme; **HNSC:** Head and Neck squamous cell carcinoma; **LGG:** Brain Lower Grade Glioma; **LIHC:** Liver hepatocellular carcinoma; **LUAD:** Lung adenocarcinoma; **LUSC:** Lung squamous cell carcinoma; **STAD:** Stomach adenocarcinoma; **METABRIC:** Molecular Taxonomy of Breast Cancer International Consortium; **HR:** hazard ratio; **KEGG:** Kyoto Encyclopedia of Genes and Genomes; **OS:** overall survival; **RT:** radiotherapy; **AIC:** Akaike information criterion; **K-M:** Kaplan-Meier; **STRING:**

Search Tool for the Retrieval of Interacting Genes; **PPI:** protein-protein interaction; **GO:** Gene Ontology; **PR:** progesterone receptor; **RGH:** radiosensitive genes when expressed high; **RGL:** radiosensitive genes when expressed low; **BP:** biological process; **MF:** molecular function; **CC:** cellular component; **ER:** estrogen receptor; **WHO:** World Health Organization;

Declarations

Ethics approval and consent to participate

Not applicable.

Consent to publish

Not applicable.

Availability of data and materials

We obtained the data information from TCGA. (http://cancergenome.nih.gov/)

Competing interests

The authors declare that they have no competing interests.

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Authors' Contributions

Study conception and design: P S and Z T

Data collection and clean: H L and J C

Real data analysis and interpretation: R G and L B

Drafting of the manuscript: J S and J L

All authors reviewed the manuscript.

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Tables

Table1. Associations of clinical variables with OS in BRCA (total N=979).

		N	%	HR (95%CI)	*P*
Radiotherapy*	yes	558	57.00	1.000	
	no	421	43.00	1.750(1.141,2.686)	0.010
Chemotherapy*	yes	831	85.58	1.000	
	no	140	14.42	2.704(1.597,4.578)	<0.001
Age*	<60	526	53.78	1.000	
	>=60	452	46.22	1.667(1.029,2.700)	0.038
Race	white	680	74.97	1.000	
	others	227	25.03	1.404(0.882,2.234)	0.153
History of cancer	no	915	93.56	1.000	
	yes	63	6.44	1.688(0.780,3.656)	0.184
Surgery type*	mastectomy	465	50.05	1.000	
	lumpectomy	235	25.30	0.824(0.477,1.421)	0.485
	other	229	24.65	0.563(0.327,0.969)	0.252
Margin status*	negative	841	89.09	1.000	
	positive/close	103	10.91	1.714(1.028,2.857)	0.039
Histology	IDC	697	71.20	1.000	
	ILC	191	19.51	0.937(0.545,1.613)	0.815
	other	91	9.30	1.653(0.916,2.985)	0.115
ER status	positive	722	77.05	1.000	
	negative	215	22.95	1.759(0.972,3.183)	0.853
PR status*	positive	626	67.02	1.000	
	negative	308	32.98	1.667(1.029,2.700)	0.062
HER2	negative	496	60.41	1.000	
	positive	142	17.30	1.018(0.594,1.743)	0.949
	indeterminate	183	22.29	0.975(0.608,1.564)	0.917
Menopausal status*	post	644	69.25	1.000	
	pre/peri	286	30.75	0.621(0.360,1.069)	0.085

T Stage	T1/T2	822	84.22	1.000	
	T3/T4	154	15.78	1.000(0.583,1.713)	0.999
N Stage*	N1/N2/N3	495	51.51	1.000	
	N0	466	48.49	0.547(0.319,0.939)	0.029
M Stage*	M0	955	98.15	1.000	
	M1	18	1.85	2.445(1.204,4.964)	0.013
Pathological stage*	I/II	717	74.84	1.000	
	III/IV	241	25.16	1.886(1.026,3.468)	0.041
Lymph nodes	0-3	593	74.40	1.000	
	>=4	204	25.60	0.693(0.405,1.186)	0.181

Abbreviations: IDC, infiltrating ductal carcinoma; ILC, infiltrating lobular carcinoma; ER, estrogen receptor; PR, progesterone receptor; TNM, tumor-node-metastasis stage.

*Clinical variables that were left after stepwise multivariate COX regression.

Table2. Associations of clinical variables with OS in METABRIC (total N=1902).

		N	%	HR (95%CI)	*P*
Radiotherapy*	yes	1137	59.78	1.000	
	no	765	40.22	1.227(1.052,1.430)	0.009
Chemotherapy	no	1506	79.18	1.000	
	yes	396	20.82	1.091(0.876,1.360)	0.463
Age*	>=60	1061	55.78	1.000	
	<60	841	44.22	0.513(0.431,0.610)	<0.001
Hormone therapy	yes	1174	61.72	1.000	
	no	728	38.28	1.064(0.912,1.241)	0.260
Surgery type*	mastectomy	1126	59.86	1.000	
	conserving	755	40.14	0.851(0.727,0.995)	0.043
Lymph nodes*	0	991	52.10	1.000	
	1-3	604	31.76	1.190(0.992,1.427)	0.061
	>=4	307	16.14	2.079(1.663,2.598)	<0.001
Cellularity	high	938	50.76	1.000	
	low	200	10.82	1.122(0.904,1.391)	0.535
	moderate	710	38.42	1.060(0.936,1.210)	0.199
Laterality	left	935	52.03	1.000	
	right	862	47.97	0.950(0.843,1.071)	0.355
Grade	G3	927	50.63	1.000	
	G1	164	8.96	0.866(0.673,1.114)	0.336
	G2	740	40.42	0.936(0.813,1.079)	0.527
ER status*	positive	1458	76.66	1.000	
	negative	444	23.34	1.272(0.992,1.629)	0.058
PR status	positive	1008	53.00	1.000	
	negative	894	47.00	1.057(0.914,1.223)	0.334
HER2*	negative	1666	87.59	1.000	
	positive	236	12.41	1.259(1.024,1.550)	0.026

Menopausal status	post	1491	78.39	1.000	
	peri	411	21.61	0.889(0.718,1.099)	0.324
Molecular subtypes*	lumA	678	35.76	1.000	
	claudin-low	198	10.44	1.047(0.778,1.411)	0.116
	her2	220	11.60	0.802(0.607,1.061)	0.250
	basal	199	10.50	1.141(0.894,1.457)	0.731
	lumB	461	24.31	1.332(1.132,1.568)	0.001
	normal	140	7.38	1.176(0.909,1.524)	0.236
Pathological stage*	II	800	57.06	1.000	
	I	478	34.09	0.734(0.613,0.880)	0.001
	III/IV	124	8.84	0.984(0.777,1.245)	0.069
Tumor size*	Median(Q1,Q3)	23	(17,30)	1.130(1.082,1.179)	<0.001

*Clinical variables that were left after stepwise multivariate COX regression.

Figures

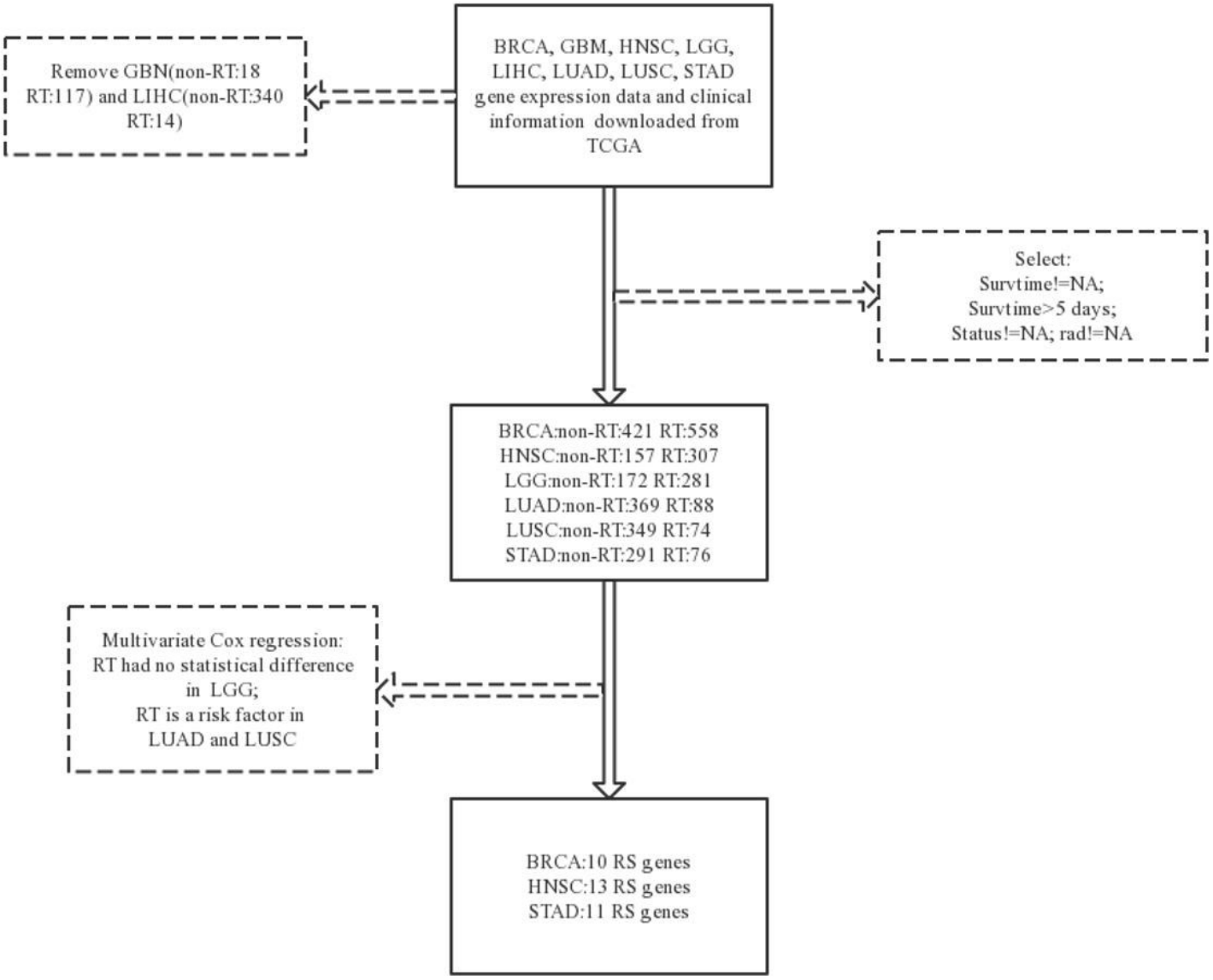

Figure 1

Schematic of study design.

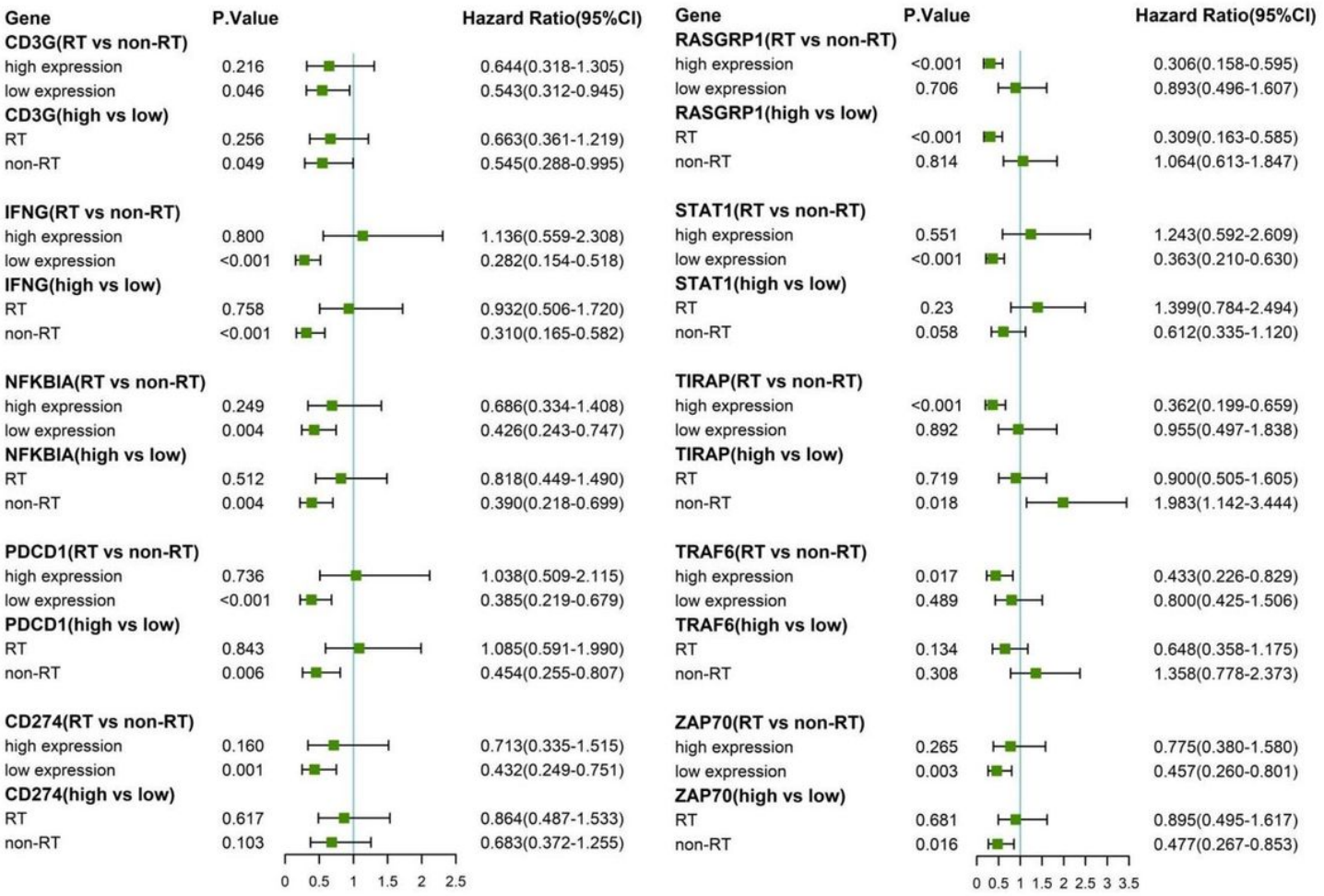

Figure 2

Forest plot for the association analysis between OS and radiotherapy under different expression levels of the 10 RS genes in BRCA. The adjusted factors include chemotherapy, age, surgery type, margin status, PR status, menopause status, N stage, M stage and pathological stage.

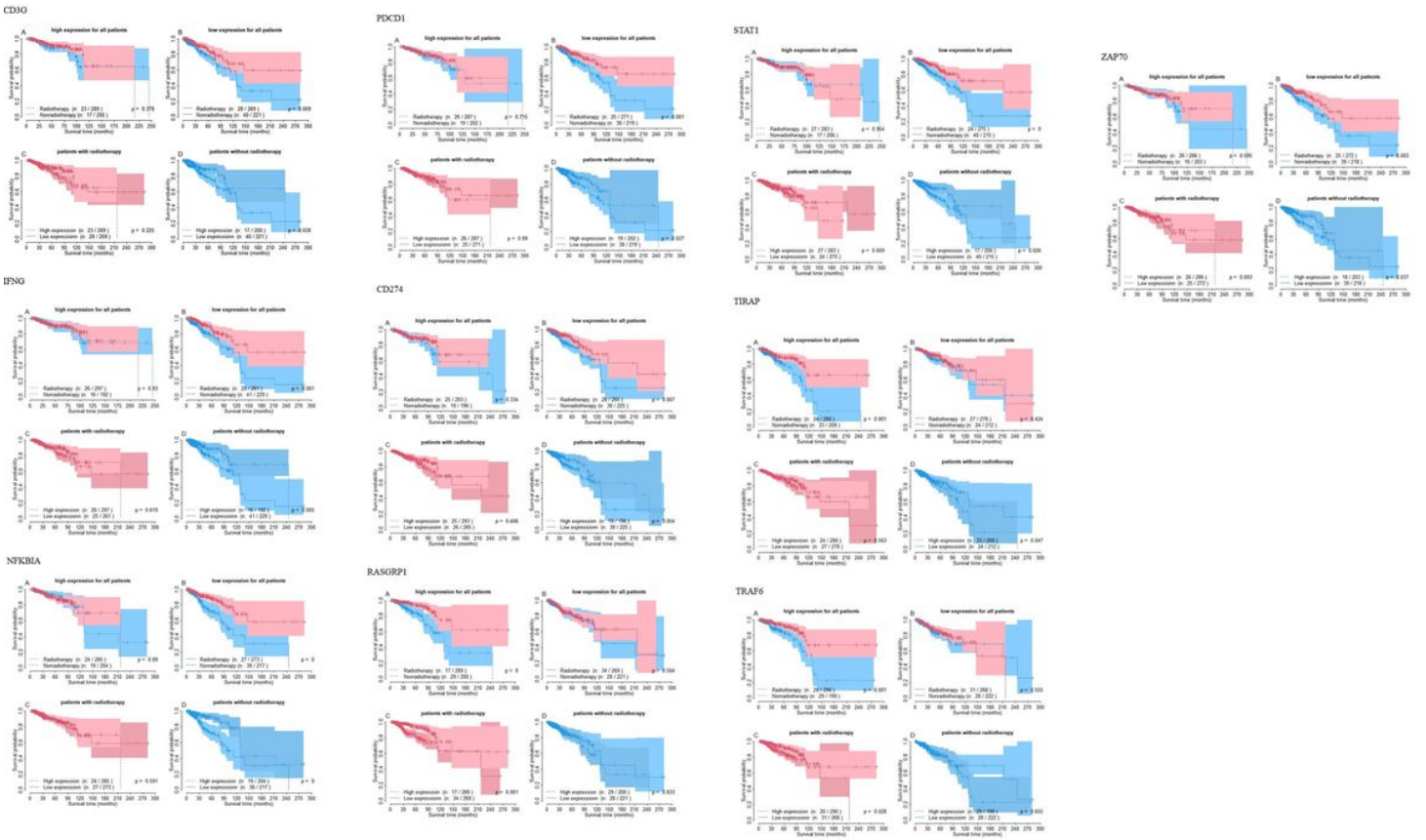

Figure 3

The unadjusted survival curves for the association analysis between OS and radiotherapy under different expression levels of the 10 RS genes in BRCA.

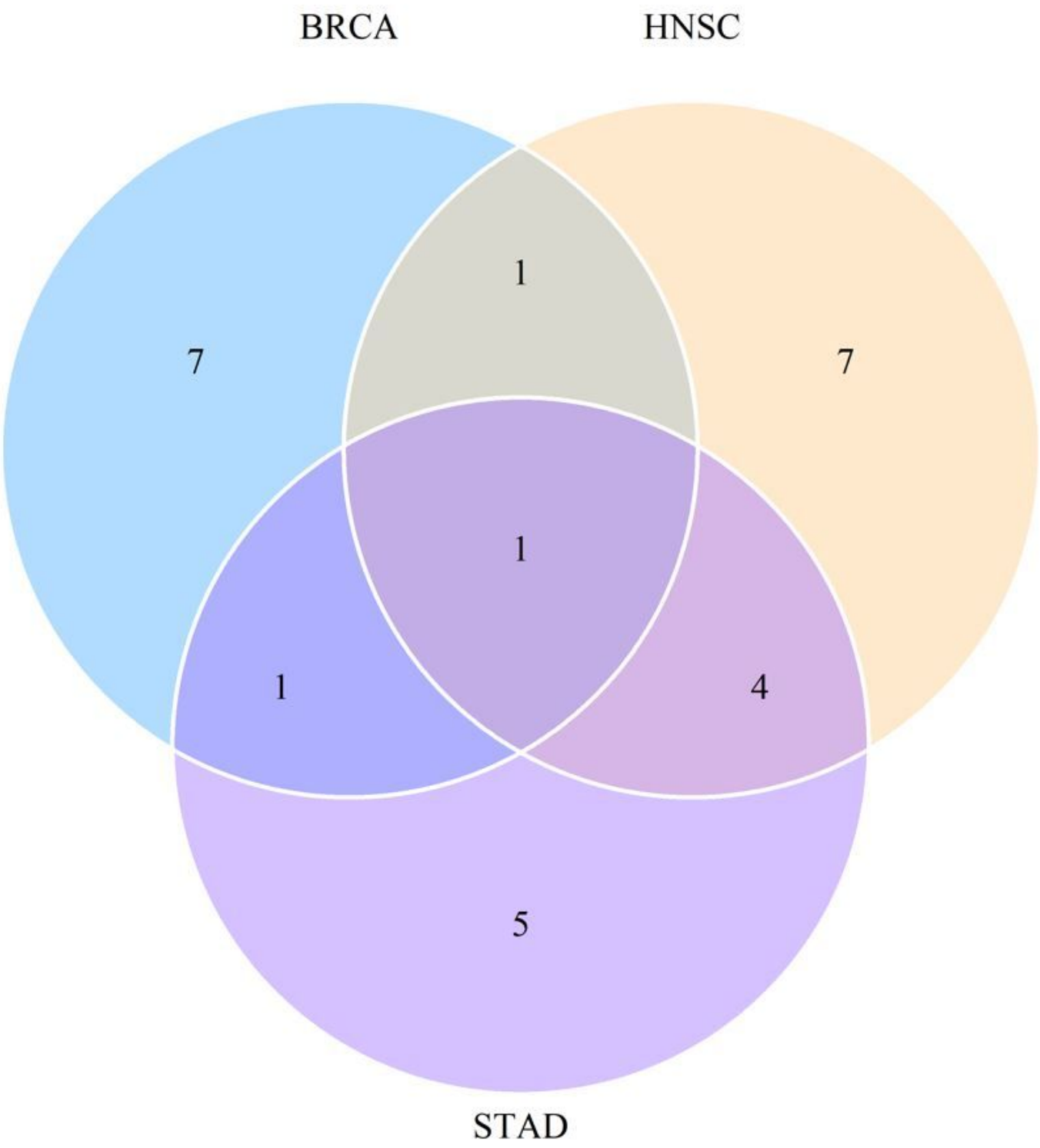

Figure 4

Venn plot for RS genes in BRCA, HNSC and STAD datasets.

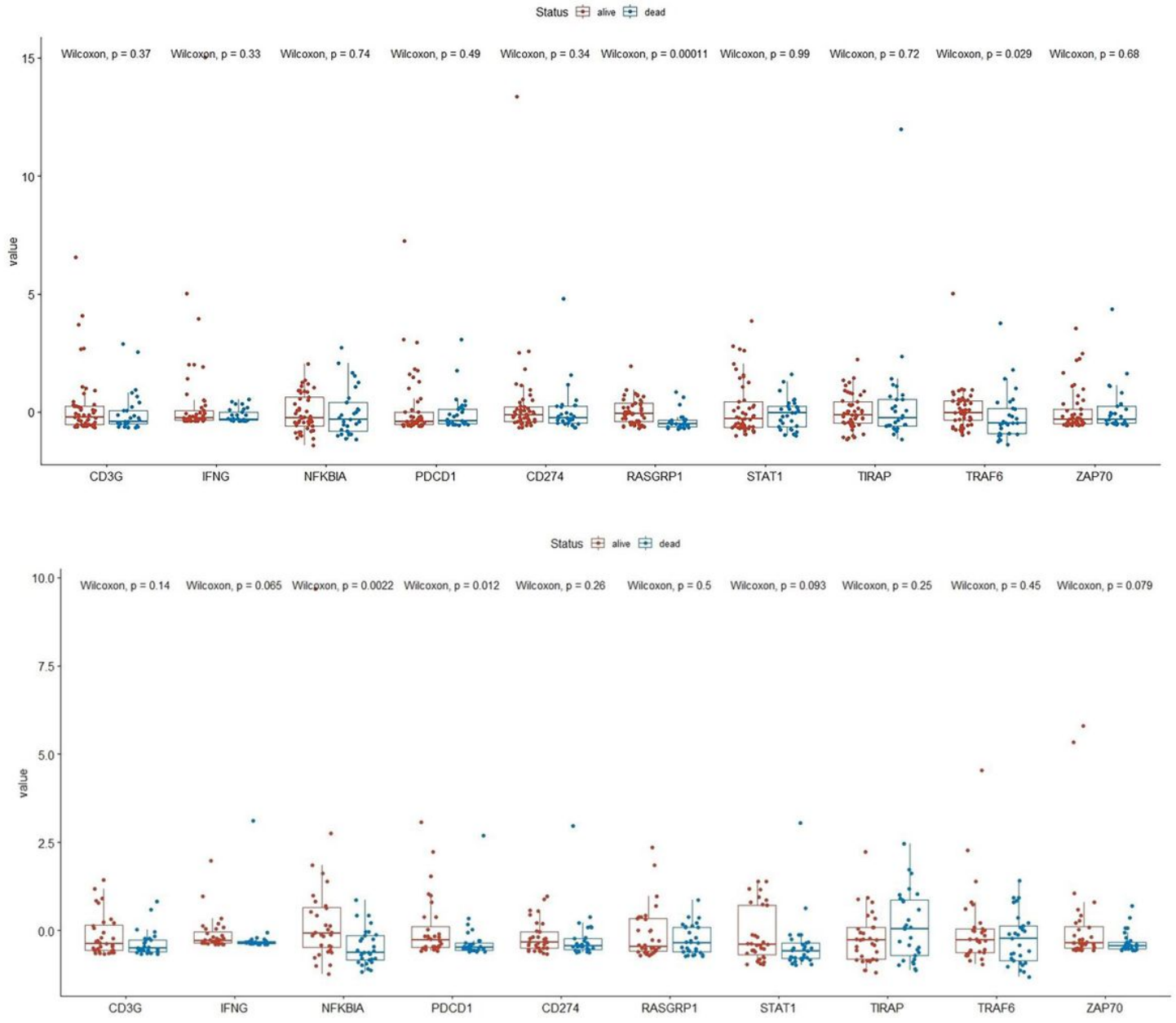

Figure 5

Box plots for the expression distribution of 10 RS genes in BRCA patients. (A) Patients received radiotherapy. (B) Patients did not receive radiotherapy.

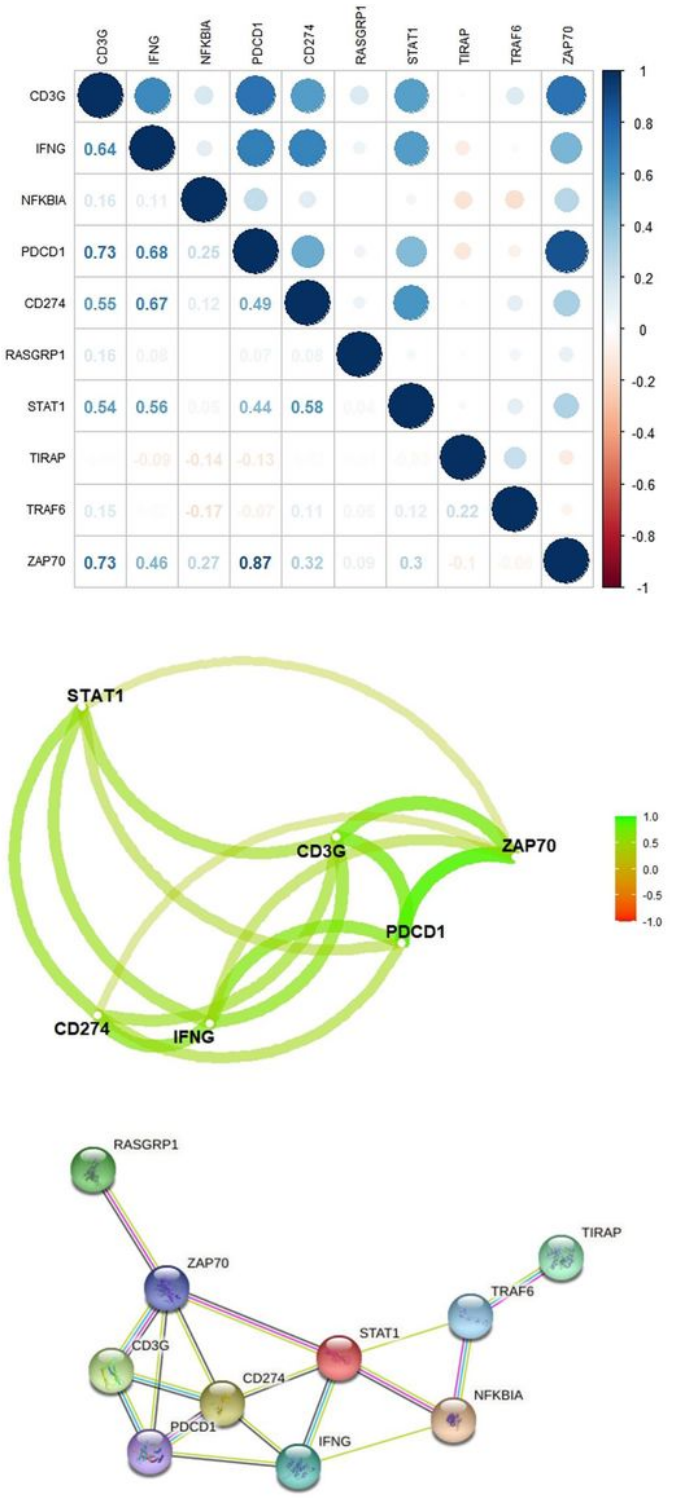

Figure 6

Correlation among the 10 RS genes in BRCA. (A) The plot for correlation of expression levels of the 10 RS genes. (B) The plot for relationship among the six genes. (C) PPI network for the 10 RS genes.

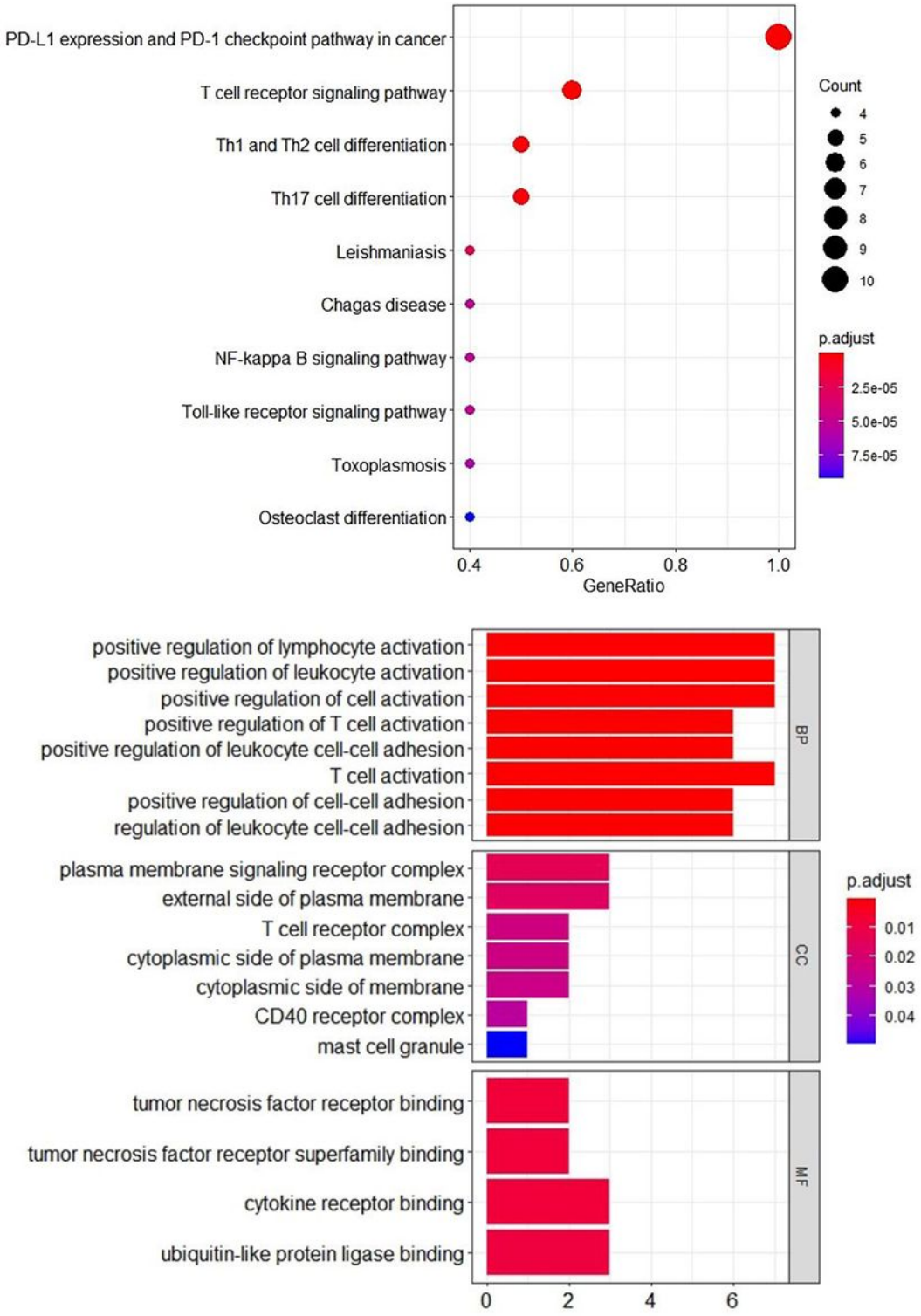

Figure 7

GO and KEGG analysis plot for the 10 RS genes in BRCA. (A) Bubble plot for KEGG analysis. (B) Bar plot for GO analysis.

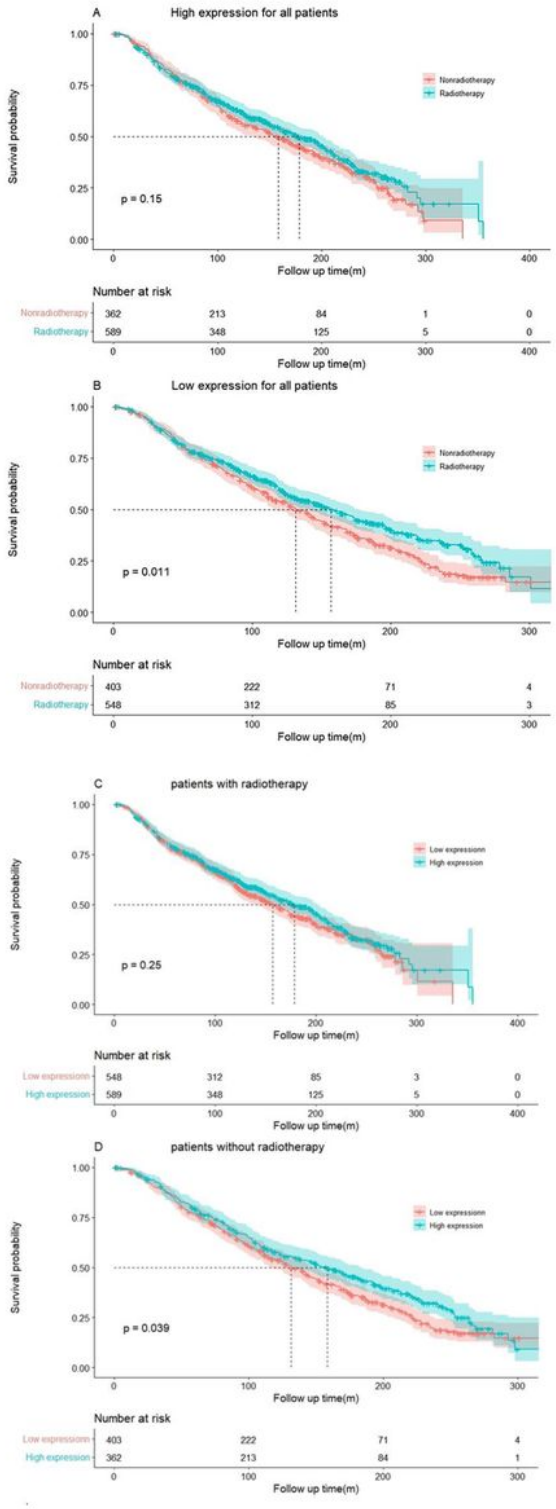

Figure 8

The unadjusted survival curves for the association analysis between OS and radiotherapy under different expression levels of ZAP70 in METABRIC.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- FigureS1.docx
- Stable1.docx
- Stable2.docx
- Stable3.xlsx
- Stable4.xlsx