Integrate Exploration to Identify Radiosensitive Biomarkers in Genes in PD-L1 Expression and PD-1 Check Point Pathway in Cancer

Junjie Shen
Soochow University

Jingfang Liu
The First Affiliated Hospital of Soochow University

Huijun Li
Soochow University

Lu Bai
Soochow University

Ruirui Geng
Soochow University

Jianping Cao
Soochow University

Peng Sun
(entsunpeng@126.com)
The First Affiliated Hospital of Soochow University

zaixiang tang
Soochow University  https://orcid.org/0000-0003-4981-3347

Research Article

Keywords: cancers, radiosensitivity, gene biomarkers, PD-1 check point pathway

DOI: https://doi.org/10.21203/rs.3.rs-433504/v1

License: This work is licensed under a Creative Commons Attribution 4.0 International License. Read Full License
Abstract

Purpose

Exploration to identify radiosensitive biomarkers in 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 can be thought as independent factors to identify the sensitivity of cancer patients to radiotherapy. Gene CD274 was the common 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 expanded definition to radiosensitive genes. Different types of tumors may share some common carcinogenic mechanisms and may have same RS genes.

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].

Programmed death-1 (PD-1) and its ligand PD-L1 check point pathway as a key regulatory immune checkpoint, 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 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 is 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. 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 cancer. 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 have enhanced the evidence and supplemented the previous studies. 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/PD-L1 related pathway genes as potential biomarkers to predict radiosensitive for cancer patients.

2. Materials And Methods

2.1 Data Sources
In view of the previous exploration [20–23, 25] of 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 RNAs seq 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 Table 1, 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). Figure 1 is the flow chart.
Table 1
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|
| Clinical variable          | N   | %     | HR (95%CI)          | P  |
|---------------------------|-----|-------|---------------------|----|
| 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.

### 2.2 Radiosensitive genes (RS genes)

We obtained a total of 74 genes (See Table3) 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 patients with different gene expression obtained discrepant benefit from radiotherapy. Based on the median of gene expression, the whole included participants were roughly divided into two groups as the high expression group and the low expression group. If one group (A group) had better overall survival (OS) in receiving radiotherapy (RT) than in non-RT (scenario A) while the other of the two groups (B group) had consistent OS whatever in RT group or non-RT group (scenario B) and meanwhile A group had a better OS than B group in receiving RT (scenario C) or A group had a lower OS than B group in non-RT (scenario D), we defined this gene as RS gene. That is if scenario A and scenario B happened and meantime scenario C or scenario D happened, the gene was deemed as RS gene.
2.3 Analysis methods

The relationship between genes expression levels and radiosensitivity was analyzed by the 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 included as many clinical variables as possible to screen out the best correction factors. Then the whole included participants could be divided into four groups: high-RT group, low-RT group, high-non-RT group and low-non-RT group. An example of how to identify RS gene. In high expression group, if radiotherapy had remained in the multivariate Cox regression model (HR < 1), corresponding to scenario A; and in low expression group, radiotherapy had no impact (scenario B); meanwhile in RT group, high expression group compared to low expression 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). Absolute value of r > 0.8, as strong correlation; Between 0.3 and 0.8, as a moderate correlation; Below 0.3, considered as 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. Table 1 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 the impact factors of OS. Information of HNSC and STAD see TableS1/2.

Figure2 shows the 10 RS genes after multivariate adjustment. In the one hand, the high/low expression of genes RASGRP1 and TRAF6 had no statistical difference OS in non-RT group when in RT group, high
expression 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 within high expression (RGH). High expression of Gene TIRAP had a lower OS than the low expression in non-RT group but get high OS when received RT, which was also a RGH gene. In the other hand, in non-RT group, low expression of genes CD3G, IFNG, NFKBIA, PDCD1, CD274, STAT1 and ZAP70 had much lower OS compared to the high. But these low expression genes could obtain a big promotion OS in RT group (That is so called RGL). In addition, we found that without adjustment for clinical factors, these genes were strong indicators as well (See K-M curves in Fig. 3). RS genes of HNSC and STAD see TableS4. We compared RS genes in the three tumor datasets and found some crossover genes (See Fig. 4). 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 Fig. 5A). 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 Fig. 5B).

3.3 Relationship of BRCA RS genes

We explored the correlation among these 10 RS genes expression level, the result is as shown in Fig. 6. Genes NFKBIA, RASGRP1, TIRAP and TRAF6 had a correlation coefficient of less than 0.3 with other genes (Fig. 6A). The remaining six genes were moderate correlated, r=(0.3,0.8) (Fig. 6B). Specially, there was a strong co-expression relationship between PDCD1 and ZAP70. Further analysis of PPI network (Fig. 6C) 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 (Fig. 7A) 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” (Fig. 7B).

3.4 external validation of BRCA RS genes

Table 2 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. Figure 8 shows the unadjusted K-M curves of ZAP70 from METABRIC.
Table 2
Associations of clinical variables with OS in METABRIC (total N = 1902).

| Variable               | Level  | 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|
|                      | N  | %    | HR (95%CI)       | P     |
|----------------------|----|------|------------------|-------|
| 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.

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, the 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 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). Such as gene IFNG, although its expression level did no effect to OS when people received RT, in non-RT group, patients with low expression of IFNG had a significantly amazing lower OS than the high (scenario D, see Fig. 2/3). And through scenario B we can see, patients with low expression of IFNG with RT had a much improved OS.

In addition, 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 the expression level of PD-L1. The expression level of CD274 has been speculated to be related to radiosensitivity of a variety of cancers [22, 23, 25].

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. Only those with high expression of these 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 (Fig. 3/5). In addition, in B genes, there were moderate co-expression relationships and interactions (Fig. 6). Functional enrichment analysis showed that most of these genes were related to T cells. Nevertheless, more experimental studies are needed to confirm the findings of this study. 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, there was also a strong co-expression relationship between PDCD1 and ZAP70 in METABRIC (r = 0.8) (See FigureS1).

This study has its merits. Firstly, we expanded the definition of radiosensitive genes and identified radiosensitivity 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 common carcinogenic mechanisms and may have same RS genes. We hope that further studies will be performed to confirm our findings.

Declarations

Data Availability

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

Conflicts of Interest

The authors declare no competing interests.

Authors’ Contributions

Study conception and design: Peng Sun and Zaixiang Tang

Data collection and clean: Huijun Li and Jianping Cao
Real data analysis and interpretation: Ruirui Geng and Lu bai

Drafting of the manuscript: Junjie Shen and Jingfang Liu

All authors reviewed the manuscript.

**Funding**

This work was supported in part by the National Natural Science Foundation of China (81773541), funded from the Priority Academic Program Development of Jiangsu Higher Education Institutions at Soochow University, the State Key Laboratory of Radiation Medicine and Protection (GZK1201919) to ZXT. Suzhou Science and Technology Project (SYS201735) to QZ, National Natural Science Foundation of China (U1967220 and 81872552) to JPC. The funding body did not play any roles in the design of the study and collection, analysis, and interpretation of data and in writing the manuscript.

**Acknowledgments**

We acknowledge the contributions of the TCGA Research Network.

**References**

1. Miller KD et al (2019) Cancer treatment and survivorship statistics, 2019. CA Cancer J Clin 69(5):363–385
2. Speers C, Pierce LJ (2016) Postoperative Radiotherapy After Breast-Conserving Surgery for Early-Stage Breast Cancer: A Review. JAMA Oncol 2(8):1075–1082
3. Zhang N et al (2018) Progress of preoperative and postoperative radiotherapy in gastric cancer. World J Surg Oncol 16(1):187
4. Arvold ND et al (2011) Age, breast cancer subtype approximation, and local recurrence after breast-conserving therapy. J Clin Oncol 29(29):3885–3891
5. Meehan J et al (2020) Precision Medicine and the Role of Biomarkers of Radiotherapy Response in Breast Cancer. Front Oncol 10:628
6. Dalton WS, Friend SH (2006) Cancer biomarkers–an invitation to the table. Science 312(5777):1165–1168
7. Hirst DG, Robson T (2010) Molecular biology: the key to personalised treatment in radiation oncology? Br J Radiol 83(993):723–728
8. Eschrich SA et al (2009) A gene expression model of intrinsic tumor radiosensitivity: prediction of response and prognosis after chemoradiation. Int J Radiat Oncol Biol Phys 75(2):489–496
9. van ’t Veer LJ et al (2002) Gene expression profiling predicts clinical outcome of breast cancer. Nature 415(6871):530–536
10. Hall JS et al (2014) Investigation of radiosensitivity gene signatures in cancer cell lines. PLoS One 9(1):e86329
11. Zhang X et al (2017) Pathway-Structured Predictive Model for Cancer Survival Prediction: A Two-Stage Approach. Genetics 205(1):89–100
12. Huang S et al (2014) A novel model to combine clinical and pathway-based transcriptomic information for the prognosis prediction of breast cancer. PLoS Comput Biol 10(9):e1003851
13. Cannito S et al., SerpinB3 Differently Up-Regulates Hypoxia Inducible Factors – 1alpha and – 2alpha in Hepatocellular Carcinoma: Mechanisms Revealing Novel Potential Therapeutic Targets. Cancers (Basel), 2019. 11(12)
14. Kwon JY, Koedrith P, Seo YR (2014) Current investigations into the genotoxicity of zinc oxide and silica nanoparticles in mammalian models in vitro and in vivo: carcinogenic/genotoxic potential, relevant mechanisms and biomarkers, artifacts, and limitations. Int J Nanomedicine 9(Suppl 2):271–286
15. Li Y, Heroux P, Kyrychenko I (2012) Metabolic restriction of cancer cells in vitro causes karyotype contraction—an indicator of cancer promotion? Tumour Biol 33(1):195–205
16. Jin T et al (2020) Mitochondrial metabolic reprogramming: An important player in liver cancer progression. Cancer Lett 470:197–203
17. Keir ME et al (2008) PD-1 and its ligands in tolerance and immunity. Annu Rev Immunol 26:677–704
18. Topalian SL, Drake CG, Pardoll DM (2015) Immune checkpoint blockade: a common denominator approach to cancer therapy. Cancer Cell 27(4):450–461
19. Wang Y et al (2018) Regulation of PD-L1: Emerging Routes for Targeting Tumor Immune Evasion. Front Pharmacol 9:536
20. Jang BS, Kim IA (2018) A radiosensitivity gene signature and PD-L1 predict the clinical outcomes of patients with lower grade glioma in TCGA. Radiother Oncol 128(2):245–253
21. Jang BS, Kim IA (2017) A radiosensitivity gene signature and PD-L1 status predict clinical outcome of patients with invasive breast carcinoma in The Cancer Genome Atlas (TCGA) dataset. Radiother Oncol 124(3):403–410
22. Jang BS, Kim IA (2020) A Radiosensitivity Gene Signature and PD-L1 Status Predict Clinical Outcome of Patients with Glioblastoma Multiforme in The Cancer Genome Atlas Dataset. Cancer Res Treat 52(2):530–542
23. Lyu X et al (2019) PD-1 and PD-L1 Expression Predicts Radiosensitivity and Clinical Outcomes in Head and Neck Cancer and is Associated with HPV Infection. J Cancer 10(4):937–948
24. Shi Y (2018) Regulatory mechanisms of PD-L1 expression in cancer cells. Cancer Immunol Immunother 67(10):1481–1489
25. Du Z et al (2020) Genes Involved in the PD-L1 Pathway Might Associate with Radiosensitivity of Patients with Gastric Cancer. J Oncol 2020:7314195
26. Zhou TC et al (2017) A review of the PD-1/PD-L1 checkpoint in bladder cancer: From mediator of immune escape to target for treatment. Urol Oncol 35(1):14–20
27. Garcia-Patos P, Olmos R (2020) Multiple Imputation in Multilevel Models. A Revision of the Current Software and Usage Examples for Researchers. Span J Psychol 23:e46
28. Beesley LJ, Taylor JMG, A stacked approach for chained equations multiple imputation incorporating the substantive model. Biometrics, 2020
29. Bartlett JW et al (2015) Multiple imputation of covariates by fully conditional specification: Accommodating the substantive model. Stat Methods Med Res 24(4):462–487
30. Sakharkar MK et al., Gene Pair Correlation Coefficients in Sphingolipid Metabolic Pathway as a Potential Prognostic Biomarker for Breast Cancer. Cancers (Basel), 2020. 12(7)
31. Sun H et al (2020) Mining the proliferative diabetic retinopathy-associated genes and pathways by integrated bioinformatic analysis. Int Ophthalmol 40(2):269–279
32. Torre LA et al (2017) Global Cancer in Women: Burden and Trends. Cancer Epidemiol Biomarkers Prev 26(4):444–457
33. Miller KD et al (2016) Cancer treatment and survivorship statistics, 2016. CA Cancer J Clin 66(4):271–289
34. Hlaviland JS et al (2013) The UK Standardisation of Breast Radiotherapy (START) trials of radiotherapy hypofractionation for treatment of early breast cancer: 10-year follow-up results of two randomised controlled trials. Lancet Oncol 14(11):1086–1094
35. Albain KS et al (2010) Prognostic and predictive value of the 21-gene recurrence score assay in postmenopausal women with node-positive, oestrogen-receptor-positive breast cancer on chemotherapy: a retrospective analysis of a randomised trial. Lancet Oncol 11(1):55–65
36. Sobanski T et al (2021) Cell Metabolism and DNA Repair Pathways: Implications for Cancer Therapy. Front Cell Dev Biol 9:633305
37. Jones S et al (2008) Core signaling pathways in human pancreatic cancers revealed by global genomic analyses. Science 321(5897):1801–1806
38. Jiang X et al (2019) Role of the tumor microenvironment in PD-L1/PD-1-mediated tumor immune escape. Mol Cancer 18(1):10
39. Dong Y, Sun Q, Zhang X (2017) PD-1 and its ligands are important immune checkpoints in cancer. Oncotarget 8(2):2171–2186
40. Sun YS et al (2017) Risk Factors and Preventions of Breast Cancer. Int J Biol Sci 13(11):1387–1397
41. Fischer A et al (2010) ZAP70: a master regulator of adaptive immunity. Semin Immunopathol 32(2):107–116
42. Au-Yeung BB et al (2018) ZAP-70 in Signaling, Biology, and Disease. Annu Rev Immunol 36:127–156

Figures
Figure 1
Schematic of study design.
| Gene                        | P.Value | Hazard Ratio(95%CI)  | Gene                        | P.Value | Hazard Ratio(95%CI)  |
|-----------------------------|---------|---------------------|-----------------------------|---------|---------------------|
| **CD3G(RT vs non-RT)**      |         |                     | **RASGRP1(RT vs non-RT)**   |         |                     |
| high expression             | 0.216   | 0.644(0.318-1.305)  | high expression             | <0.001  | 0.306(0.158-0.595)  |
| low expression              | 0.046   | 0.543(0.312-0.945)  | low expression              | 0.706   | 0.893(0.496-1.607)  |
| **CD3G(high vs low)**       |         |                     | **RASGRP1(high vs low)**    |         |                     |
| RT                          | 0.256   | 0.683(0.361-1.219)  | RT                          | <0.001  | 0.309(0.163-0.585)  |
| non-RT                      | 0.049   | 0.545(0.288-0.995)  | non-RT                      | 0.814   | 1.064(0.613-1.847)  |
| **IFNG(RT vs non-RT)**      |         |                     | **STAT1(RT vs non-RT)**     |         |                     |
| high expression             | 0.800   | 1.136(0.559-2.308)  | high expression             | 0.551   | 1.243(0.592-2.609)  |
| low expression              | <0.001  | 0.282(0.154-0.518)  | low expression              | <0.001  | 0.363(0.210-0.630)  |
| **IFNG(high vs low)**       |         |                     | **STAT1(high vs low)**      |         |                     |
| RT                          | 0.758   | 0.932(0.506-1.720)  | RT                          | 0.23    | 1.399(0.784-2.494)  |
| non-RT                      | <0.001  | 0.310(0.165-0.582)  | non-RT                      | 0.058   | 0.612(0.335-1.120)  |
| **NFKBIA(RT vs non-RT)**    |         |                     | **TIRAP(RT vs non-RT)**     |         |                     |
| high expression             | 0.249   | 0.686(0.334-1.408)  | high expression             | <0.001  | 0.382(0.199-0.659)  |
| low expression              | 0.004   | 0.426(0.243-0.747)  | low expression              | 0.892   | 0.955(0.497-1.838)  |
| **NFKBIA(high vs low)**     |         |                     | **TIRAP(high vs low)**      |         |                     |
| RT                          | 0.512   | 0.818(0.449-1.490)  | RT                          | 0.719   | 0.900(0.505-1.605)  |
| non-RT                      | 0.004   | 0.390(0.218-0.699)  | non-RT                      | 0.018   | 1.983(1.142-3.444)  |
| **PDCD1(RT vs non-RT)**     |         |                     | **TRAF6(RT vs non-RT)**     |         |                     |
| high expression             | 0.736   | 1.038(0.509-2.115)  | high expression             | 0.017   | 0.433(0.226-0.829)  |
| low expression              | <0.001  | 0.385(0.219-0.679)  | low expression              | 0.489   | 0.800(0.425-1.506)  |
| **PDCD1(high vs low)**      |         |                     | **TRAF6(high vs low)**      |         |                     |
| RT                          | 0.843   | 1.085(0.591-1.990)  | RT                          | 0.134   | 0.648(0.358-1.175)  |
| non-RT                      | 0.006   | 0.454(0.255-0.807)  | non-RT                      | 0.306   | 1.356(0.778-2.373)  |
| **CD274(RT vs non-RT)**     |         |                     | **ZAP70(RT vs non-RT)**     |         |                     |
| high expression             | 0.160   | 0.713(0.335-1.515)  | high expression             | 0.265   | 0.775(0.380-1.580)  |
| low expression              | 0.001   | 0.432(0.249-0.751)  | low expression              | 0.003   | 0.457(0.260-0.801)  |
| **CD274(high vs low)**      |         |                     | **ZAP70(high vs low)**      |         |                     |
| RT                          | 0.617   | 0.864(0.487-1.533)  | RT                          | 0.681   | 0.895(0.495-1.617)  |
| non-RT                      | 0.103   | 0.683(0.372-1.255)  | non-RT                      | 0.016   | 0.477(0.267-0.853)  |

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
- TableS1.docx
- TableS2.docx
- TableS3.xlsx
- TableS4.xlsx