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Prognostic value of metabolic signature on 18F-FDG uptake in breast cancer patients after radiotherapy

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Radiotherapy (RT) is a major modality of postoperative treatment in breast cancer. The maximal standardized value (SUVmax) is ^{18}FDG-PET/CT derived parameter that reported to be a valuable prognostic factor in cancer patients. Herein, we aimed to identify a prognostic gene signature associated with glucose uptake for breast cancer patients after RT by leveraging the mRNA expression profiling on public datasets. The glucose uptake signature was constructed using the single sample gene set enrichment analysis (ssGSEA) algorithm and evaluated in GSE21217 where SUVmax value was measured by PET-CT directly. The prognostic value was validated in three post-RT breast cancer cohorts (GSE103744, NKI, and FUSCC databases). The patients were stratified into glucose uptake signature score-high and low groups. Patients with a higher score had worse survival than those with a lower score. Mechanistically, the glucose uptake signature was calculated in each cell type of a single-cell RNA-seq database from five breast cancer patients. Glucose uptake signature score was significantly elevated in the malignant epithelial cells compared with normal ones. The immunosuppression markers including PDCD1, TIGIT, LAG3, and HAVCR2 were significantly upregulated in the T cells bearing a high glucose uptake signature score. Collectively, our results demonstrated the potential prognostic value of a glucose uptake signature in the post-RT breast cancer patients.

INTRODUCTION

Breast cancer is the most frequently diagnosed cancer among women, which accounts for 15.5% of all cancer deaths. In the era of precision medicine, using prognostic and treatment-predictive biomarkers to assess clinical outcomes after treatment is crucial for treatment decision.

Radiotherapy (RT) is a major modality of postoperative treatment in breast cancer.[1] It has been shown that adjuvant RT after breast-conserving surgery (BCS) or mastectomy reduces local recurrence and increases survival.[2,3] In the era of molecular medicine, it is essential to identify patients who may benefit from RT. Attempts have been made to discover biomarkers to predict response to RT among patients with breast cancer. Several gene-expression-based classifiers have been presented to predict prognosis after RT,[4–7] or to classify tumors as radiosensitive or radioresistant.[6,8]

The metabolism of tumor cells is relatively different from that in normal tissue cells. Tumor cells predominantly utilize glycolysis even in the presence of ample oxygen. This phenomenon is known as the Warburg effect.[9–11] Several preclinical studies have shown that tumor glucose metabolism is highly correlated with radioresistance.[12–14] Interfering with glucose metabolism of tumor cells might reduce the amount of antioxidant metabolites and could therefore improve the therapeutic efficacy of RT.[15,16] High glucose uptake is observed during a clinical diagnosis of cancer using ^{18}F-fluorodeoxy-glucose positron emission tomography computed tomography (^{18}FDG-PET/CT).[17,18] The diagnostic and therapeutic impact of ^{18}F-FDG PET/CT is well established in many solid tumors, such as lung, and head and neck tumors.[19] In breast cancer, several studies have demonstrated the relationship between metabolic information obtained with ^{18}F-FDG PET/CT and tumor biology. Palaskas et al. has carried out genome-wide transcriptome analysis of cell lines and primary human breast tumors after determining their FDG uptake. Using 11 primary breast cancer patients as training set, they identified a "glucose uptake signature" that included genes enriched in glucose metabolic pathways, and further tested its predictive ability of radiotracer uptake in breast cancer cell lines.[20] However, whether there is a correlation between gene expression of glucose uptake related genes and the prognosis after RT remains unknown.

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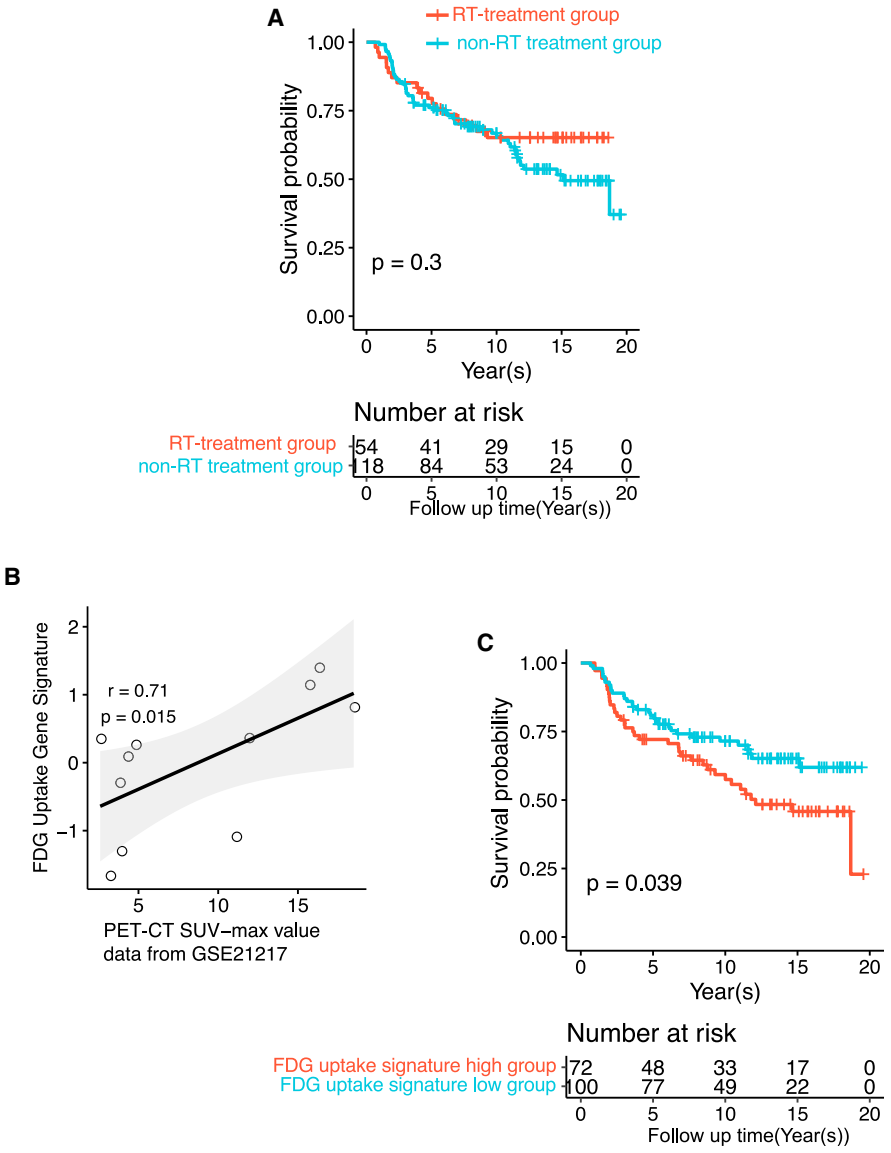

Figure 1. Development of FDG uptake gene signature

(A) Survival analysis in patients with and without RT in GSE103744. (B) Correlation analysis between glucose uptake score and SUVmax value of PET-CT in GSE21217. (C) Survival analysis in two groups of patients stratified by FDG uptake gene signature in GSE103744.

To address this question, we used clinical and genomic database to validate the prognostic value of this glucose uptake signature in post-RT breast cancer patients.

RESULTS

Correlation of glucose uptake signature score and SUVmax value

RT is considered as an effective intervention to prevent local relapse after BCS.[2] We performed survival analysis on a GSE103744 cohort that consisted of 172 patients with gene-expression data, among whom 118 patients had received RT. Unexpectedly, survival analysis showed that there was no difference of recurrence between patients with or without RT (p = 0.3, Figure 1A).

We speculate that the level of tumor glucose metabolism might be a confounding factor affecting the outcome observed. Thus, an FDG uptake gene-expression signature was derived by a single sample gene set enrichment analysis (ssGSEA) algorithm based on a total of 75 glucose metabolism related genes (the gene list is shown in Table S1). We evaluated the FDG uptake signature in an additional dataset GSE21217 in which SUVmax value was measured by PET-CT directly in breast cancer patients. For each patient, an FDG uptake gene signature score was calculated according to gene expression (Table S2). The

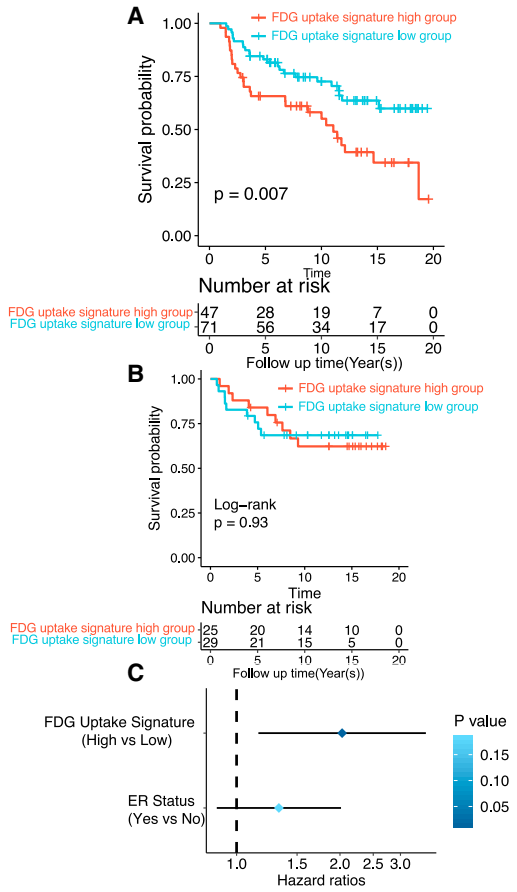

Figure 2. Validation of FDG uptake gene signature in post-RT patients in GSE103744

(A) Kaplan-Meier curves of LRFS in patients treated with RT and (B) without RT. (C) Multivariable analysis on FDG uptake gene signature and ER status.

FDG uptake score was highly correlated with SUVmax value (R = 0.71, p = 0.015, Figure 1B), suggesting that FDG uptake score was of good performance to be an alternative to predict the SUVmax value. The FDG uptake score was then calculated on 172 patients in the GSE103744 cohort. The optimum cutoff level for differentiating two groups was defined as 0.22 by the X-tile plot approach (Figure S1). Patients were divided into FDG uptake score-high (72 patients) and FDG uptake score-low (100 patients) groups according to the optimal cutoff level. Survival analysis showed that patients assigned to the FDG uptake score-low group had better local recurrence-free survival (LRFS) compared with the FDG uptake score-high group (p = 0.039, Figure 1C).

Validation of prognostic value of FDG uptake signature score for post-RT patients

To further explore the association of this signature with RT, patients in GSE103744 were classified into RT-treated group (118 patients) and non-RT-treated group (54 patients). In the RT-treated group, the survival of patients in FDG uptake score - high and FDG uptake score-low group was significantly different (p = 0.007, Figure 2A). However, in the group of non-RT-treated patients, there was no difference between FDG uptake score-low and FDG uptake score-high groups. In multivariable Cox analysis, estrogen receptor (ER) status was not associated with prognosis, while the FDG uptake gene signature score remained to be a significant factor for prognosis (Figure 2C). To confirm these results, we investigated this signature in another cohort of 319 patients from the Netherlands Cancer Institute (NKI) cohort. All the patients in this cohort had received RT. Using FDG uptake signature, patients were classified into FDG uptake score-high group (129 patients) and FDG uptake score-low group (190 patients). Similar results between the two groups were noted in this cohort. Patients in the FDG uptake score-high group had worse recurrence-free survival (RFS) (p = 0.039) than those in the FDG uptake score-low group. Five-year RFS was 62.3% in the FDG uptake score-high group and 77% in the FDG uptake score-low group, respectively (Figure 3A). Multivariate analysis showed that when adjusting for chemotherapy, endocrine therapy, ER status, and age, this FDG uptake signature remained an independent predictive factor for RFS (Figure 3B). In addition, we performed the survival analysis on Fudan University Shanghai Cancer Center (FUSCC) cohorts that enrolled 359 triple-negative breast cancer patients.[21] Survival analysis in the RT-treated patients (108 patients) revealed that the disease-free survival time of patients in the FDG uptake score-high group was significantly shorter than that in the FDG uptake score-low group (p = 0.021). However, no significant difference was observed in the non-RT-treated patients (251 patients) (Figure 3C).

The underlying biology characteristic associated with FDG uptake score

We then explored the biology characteristics of the tumor cell associated with SUVmax using single-cell RNA sequencing (RNA-seq) of five breast cancer samples.[22] Uniform Manifold Approximation and Projection (UMAP) analysis classified the cells into several clusters, including epithelial cells, immune cells, and fibroblasts, as originally defined by Wu et al[22] (Figures 4A and 4B). We calculated the FDG uptake gene signature score in each cell type of five breast cancer patients and found higher FDG uptake gene signature score cells were mainly enriched in epithelial cells (Figure 4C). The epithelial cells were further divided into malignant epithelial cells and normal epithelial cells.[22] We also observed that the cells bearing a higher FDG uptake gene signature score significantly enriched in the malignant epithelial cells compared with normal epithelial cells (Figures 4D–4F). To investigate whether the FDG uptake gene signature score is associated with the RT response on a single-cell dataset, we calculated the RT response signature from the Molecular Signatures Database (MSigDB). Correlation analysis showed that FDG uptake gene signature score is correlated with a radioresistance signature, namely "WATANABE_RECTAL_CANCER RADIOTHERAPY_RESPONSIVE_DN", which may suggest that FDG uptake gene signature score closely correlates with radioresistance of breast cancer patients as well (Figure 4G).

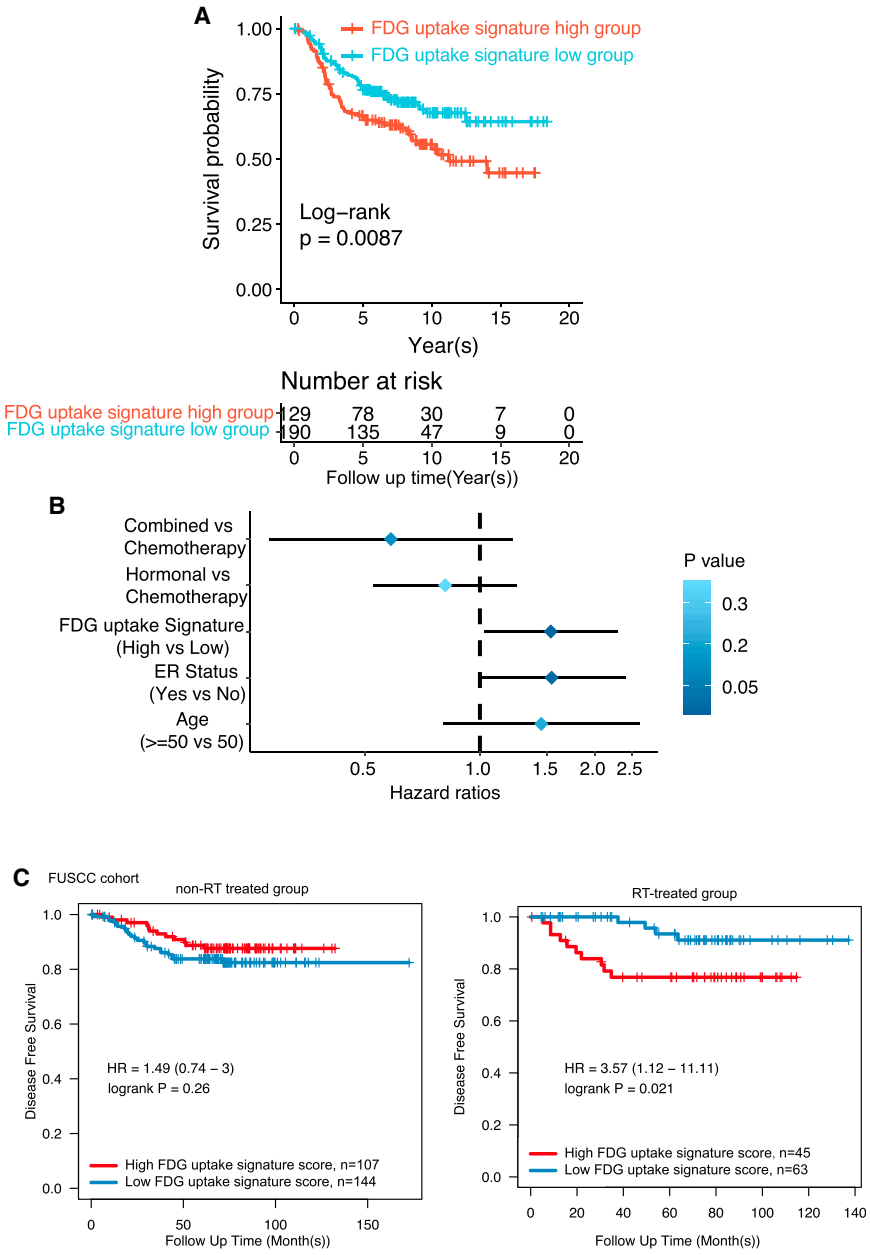

Figure 3. Validation of FDG uptake gene signature in NKI and FUSCC cohorts

(A) NKI cohort: Kaplan-Meier curves of RFS in patients treated with RT. (B) NKI cohort: Multivariable analysis on FDG uptake gene signature, chemotherapy, hormonal therapy, ER status, and age. (C) FUSCC cohort: Kaplan-Meier curves of disease-free survival in patients treated with and without RT.

The efficacy of RT is closely related to the immune status of the patient. We then investigated the expression pattern of the FDG uptake gene signature score in each T cell of breast cancer. Interestingly, the cells bearing higher FDG uptake gene signature score were significantly enriched in the exhausted CD8+ T cells compared with other T cell types (Figure 5A). Previous study has shown that the upregulation of some inhibitory receptors and ligands, including programmed cell death 1 (PD-1, also known as PDCD1), T cell Immunoreceptor With Immunoglobulin and ITIM Domains (TIGIT), Lymphocyte Activating 3 (LAG3), and T cell immunoglobulin domain and mucin domain 3 (TIM-3, also known as HAVCR2), prevents RT from achieving the optimal therapeutic effect.[23] Then, the T cells were split into high and low groups according to FDG uptake gene signature score. We observed that the immunosuppression markers, including PDCD1, TIGIT, LAG3, and HAVCR2, were significantly upregulated in the T cell bearing high FDG uptake gene signature score (Figure 5B). We further investigated the relationship between FDG uptake gene signature score and four immunosuppression markers in the GSE103744 cohort. As expected, correlation test revealed that FDG uptake gene signature score was positively correlated with the mRNA expression of PDCD1, TIGIT, LAG3, and HAVCR2 (Figure 5C).

To probe the FDG uptake gene signature score associated pathways on an unbiased basis, we performed gene set enrichment analysis using microarray data of the breast cancer cohort in the GSE103744 cohort. We observed that RT resistance signature, including WATANABE_RECTAL_CANCER RADIOTHERAPY_RESPONSIVE_DN and MONNIER_POSTRADIATION_ TUMOR_ESCAPE_UP, was assigned with the high enrichment score in the samples bearing high level of FDG uptake gene signature score (Figure 6).

DISCUSSION

In this study, we identified and validated the prognostic value of a glucose uptake signature in post-RT breast cancer patients. To our knowledge, this is the first study to identify prognostic value of FDG uptake signature in post-RT breast cancer patients. In our study, the glucose uptake score-high group predicted by the FDG uptake gene signature showed worse survival compared with the glucose uptake score-low group in post-RT patients.

FDG PET/CT is widely recommended as part of the initial staging of locally advanced breast cancer, and the detection of SUVmax depends on various factors, such as their size (partial volume effect), metabolic activity, the surrounding background activity, and the serum glucose level. The decision of radiation treatment conventionally depends on

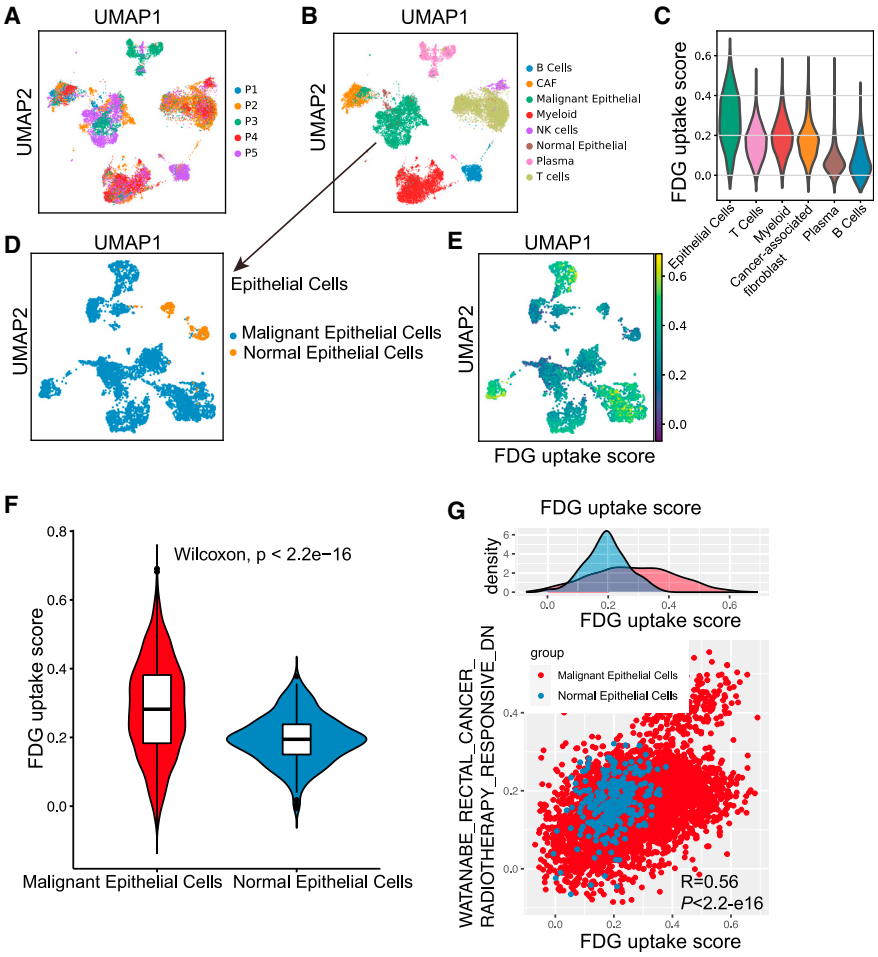

Figure 4. The FDG uptake gene signature score expression increased in malignant epithelial cells based on single-cell transcriptome

(A) UMAP plot visualized the clusters of each cell type of breast cancer. (B) Violin plot shows the expression of FDG uptake gene signature score in each cell type. (C) Visualization of UMAP colored according to malignant epithelial cells and normal epithelial cells. (D and E) UMAP plot shows the expression of FDG uptake gene signature score in epithelial cells. (F) Violin plot showed the expression of FDG uptake gene signature score in malignant epithelial cells and normal epithelial cells. (G) Scatterplot showing the correlation between FDG uptake gene signature score and RT response.

the clinical and pathological features after surgery as well. Thus, using glucose metabolism gene expression to predict the prognosis of patients after RT might be an alternative, useful tool for precise stratification of risk groups.

It has been well established that high FDG uptake might provide a unique insight into tumor cell metabolism. Numerous studies have suggested that PET parameters, such as SUVmax, depend on the biological characteristics and subtypes of breast cancer.[24–26] The prognosis of postoperative breast cancer patients with higher SUVmax is worse than that of patients with lower SUVmax.[27] Also, Wang et al. showed that PET value could predict a patient's response to chemotherapy.[28] Osborne et al. attempted to correlate ^{18}F-FDG uptake with different molecular profiles and specific genes from microarray analysis in locally advanced breast cancer; higher ^{18}F-FDG uptake was found in ER-negative tumors and multiple genes were identified to be associated with glucose use.[29] Crespo-Jara et al. has generated and validated a genomic signature for the prediction of FDG uptake in diverse metastatic tumors. Multiple biological processes were involved in this signature, including glycolysis and glucose transport.[30] Also, it has been demonstrated that the glycolytic metabolism in malignancies is highly correlated with radioresistance. Previous studies have explored the link between FDG uptake features and radiosensitivity in some cancer types. Recently, it has also been reported that SUVmax might be a good predictor of outcome after stereotactic ablative radiotherapy (SABR) for early-stage non-small cell lung cancer.[31]

Also, we investigated the biology characteristics of tumor cells associated with the FDG-uptake signature using single-cell RNA-seq data. We have shown that some immunosuppression markers were significantly upregulated in the T cell bearing a high FDG

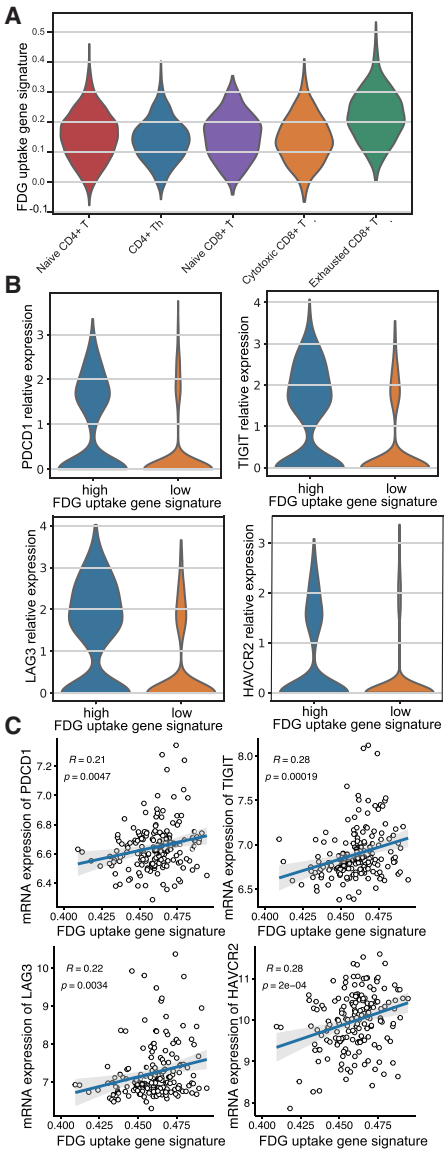

Figure 5. The FDG uptake gene signature score expression increased in exhausted T cells based on single-cell transcriptome

(A) Violin plot shows the expression of FDG uptake gene signature score in each T cell type. (B) Violin plot shows the expression of PDCD1, TIGIT, LAG3, and HAVCR2 in the T cells bearing high and low FDG uptake gene signature score. (C) FDG uptake gene signature score was significantly correlated with the expression of PDCD1, LAG3, TIGIT, and HAVCR2 in the GSE103744 cohort.

uptake gene signature score. Although glucose in T cell activation and effector functions have been demonstrated to be important, their roles in T cell exhaustion remain undetermined.[32] Here we hypothesize that elevated tumor oxygen consumption contributes to T cell exhaustion and immune evasion. The reason is that elevated glucose consumption resulting in cancer cell glucose deprivation in the tumor environment has been found to dampen the tumoricidal activity of tumor-infiltrating lymphocytes in a mouse melanoma and sarcoma model.[33,34] More and more evidence suggests that tumor glycolysis plays a key role in instigating immunosuppressive networks that are critical for immune evasion. Several recent studies have begun to establish the relationship of tumor-intrinsic metabolism to successful immunotherapy. For instance, it has been reported that increased glycolytic metabolism in melanoma cells is associated with resistance to adoptive T cell therapy and checkpoint blockade.[35]

There are several limitations in our study. First, tumor tissues are necessary to get gene profiling information, and their clinical utility is to be further validated and has not yet been introduced into clinical routine. In clinical practice, gene signature might be less convenient than immunohistochemical markers. Second, the NKI dataset lacks some clinical information, such as TNM stage, HER2, and histological type, so multivariable Cox regression has been adjusted for the available information. Third, in this study, only retrospective cohorts were used for the validation, prospective studies are warranted to validate these results.

In summary, we demonstrated the potential prognostic value of a glucose uptake signature in post-RT breast cancer patients. More prospective data are warranted for the use of signature in the clinical setting.

MATERIALS AND METHODS

Study design and patients

By mining RNA expression profiling of samples, we developed a gene-expression-based signature using a gene set variation analysis (GSVA) algorithm from gene-expression data of GSE21217.[20] The association of the signature and SUVmax was tested. To validate this signature, we performed survival analysis on the Sjöström (GSE103744), NKI, and FUSCC datasets. In the Sjöström (GSE103744) dataset, 172 patients undergoing BCS with or without RT were collected.[36] The median follow-up time was 9.2 years (range, 0.6–19.6). LRFS was the clinical endpoint for training purposes. Patients were divided into SUVmax-high and SUVmax-low groups according to the signature. To further test these results among patients treated with RT only, we investigated the gene signature in another cohort of 319 patients (NKI dataset).[37–39] In this cohort, all the patients with breast cancer had undergone breast conserving therapy, including surgery and RT. The median follow-up time was 7.1 years (range, 0.05–18.4 years). For this validation cohort, RFS was used as the endpoint since information about LRFS was not available. As per the FUSCC cohort, 360 patients with mRNA sequencing data were retrieved from the NCBI Sequence Read Archive (SRA ID:SRP157974). One sample with obscure RT status was removed for downstream analysis. Follow-up time was updated until May 2021.

Development of FDG uptake signature

To construct the FDG uptake gene signature, we used ssGSEA that defined an enrichment score to represent the degree of absolute enrichment of a gene set in each sample within a given gene list.[20] ssGSEA is a methodology to calculate separate enrichment scores for each pairing of

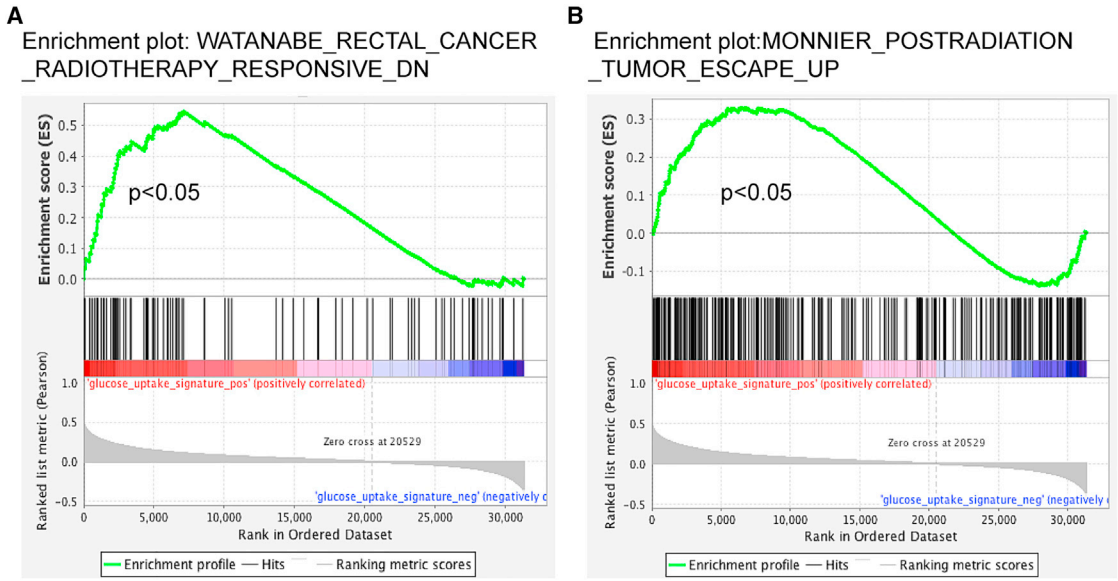

Figure 6. FDG uptake gene signature score associated pathways
(A and B) Gene set enrichment analysis on the GSE103744 cohort.

a sample and gene set. Each ssGSEA enrichment score represents the degree to which the genes in a particular gene set are coordinately up- or downregulated within a sample. The ssGSEA analysis were performed in R package GSVA. The association of the signature and SUVmax was tested.

Determination of FDG uptake gene signature score cutoff value

The prognostic ability of the FDG uptake gene signature score was analyzed by Kaplan-Meier survival analysis. The X-tile was used to implement the optimal cutoff point of FDG uptake score. X-TILE software 3.6.1 (Yale University School of Medicine, New Haven, CT) was used to assess the X-tile analysis.

Survival analysis and multivariate analyses

We used the log rank test to assess the survival data between different risk groups stratified by gene signature at the optimal cutoff. Multivariable Cox regression analyses were applied to analyze the independent prognostic effect of the signature. Statistical analysis was performed with R software (version 3.6.1) and statistical levels were two-sided; statistical significance was set at 0.05.

Single-cell RNA-seq analysis

External single-cell mRNA-seq data have been described by Wu et al.[22] The Python package Scanpy (version 1.4.6) was used to analyze these dataset. Raw data consisting of gene-expression values in count value were used for downstream analysis. Before clustering, the full dataset or a subset thereof was filtered for highly variable genes (min_mean = 0.0125, max_mean = 3, min_disp = 0.5) and scaled. Clustering was performed on the top 50 principal components of the data using the UMAP algorithm with resolution = 0.3.

SUPPLEMENTAL INFORMATION

Supplemental information can be found online at https://doi.org/10.1016/j.omto.2021.10.008.

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AUTHOR CONTRIBUTIONS

J.M., E.D., L.Z., Z.-z.Y, and X.-m.G. conceived and designed the manuscript. J.M., E.D., L.Z., and W.S. performed the data collection. J.M., E.D., X.M, J.-l.M., X.-m.Z., and X.-x.C. performed the analysis. J.M., E.D., and L.Z. drafted the manuscript. X.-l.Y., Y.-z.J., J.W., Z.-m.S., Z.-z.Y., and X.-m.G. reviewed the data and finalized the manuscript.

DECLARATION OF INTERESTS

The authors declare that they have no competing interests.

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