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Original Article

Accelerated glycolysis in tumor microenvironment is associated with worse survival in triple-negative but not consistently with ER+/HER2- breast cancer

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Abstract: Metabolic reprogramming to sustain immortality is a hallmark of cancer and glycolysis is an important way to attain this. Thus, we investigate the association of glycolysis and associated pathways in the survival of breast cancer. A total of 5,176 breast cancer patients from multiple independent cohorts were analyzed. We determined the glycolytic signaling score by the degree of enrichment by Gene Set Variant Analysis and the median was used to divide each cohort into high vs low score groups. Glycolysis high breast cancer significantly enriched the hallmark cell proliferation-related gene sets (E2F targets, G2M checkpoint, and MYC targets v1 and v2) and was associated with high *MKI67* expression. In all cohorts, triple-negative breast cancer (TNBC) was associated with the highest glycolysis score. It was found that in TNBC, glycolysis high breast cancer was associated with worse survival but in ER-positive/HER2-negative breast cancer this was not observed consistently. The glycolysis high TNBC enriched multiple pro-cancerous gene sets and was infiltrated with a low level of B-cells and anti-cancerous immune cells, and significantly associated with a decreased level of cytolytic activity. It was also observed that the glycolysis was higher in the metastatic sites than in the primary breast cancer and the survival was not affected by the metastatic sites. In conclusion, accelerated glycolysis is associated with cancer cell proliferation and worse survival in TNBC.

Keywords: Breast cancer, glycolysis signaling, GSVA, prognostic biomarker, tumor microenvironment

Introduction

Metabolism is a series of life-sustaining chemical reactions in living organisms, which produces energy. Cancer cells frequently reprogram their metabolism to adapt to changes in the nutrient supply, hypoxia, and rapid growth in the tumor microenvironment (TME). Thus, metabolic reprogramming is a hallmark of cancer. Changes in the metabolism of amino acids such as glutamine and serine-glycine, fatty acids, and redox equilibrium are known, but the most famous is the "Warburg effect" proposed by Otto Warburg in the 1920s [1], which explains that cancer cells preferably generate energy not by oxidation of pyruvate in the mitochondria, but by lactic acid fermentation in the cytoplasm and subsequent glycolysis [2, 3]. Cancer cells facilitate higher glycolysis for their energy production which is required for their rapid multiplication by increased uptake of glucose. Therefore, glycolysis is one of the hallmark metabolic pathways in cancer [4, 5]. A variety of other signaling alterations and gene activation

events influence the use of this glycolysis signaling in cancer [6].

The metabolic shift from mitochondrial oxidative phosphorylation to glycolysis during hypoxia in cancer cells is well known, however, aerobic glycolysis during normoxic conditions is fascinating. The Warburg effect has several diagnostic and therapeutic implications in the field of cancer [7]. The clinical utility of the Warburg effect is apparent from its application in Positron Emission Tomography (PET) scans in which the increased glucose consumption of the cancer cells remains as the basis of tumor detection [8]. Tumor glycolysis could be used as a potential target for therapy as it is a hallmark of cancer cells. Cancer treatments targeting the transporters and enzymes in the glycolytic pathways such as glucose transporter 1 (*GLUT1*), hexokinase is under development [9]. Therefore, it is essential to understand the glycolytic pathways and changes that occur in the TMEs in tumors with high glycolysis to develop therapeutic strategies.

The most common cancer in women globally and the primary cause of cancer-related mortality in women is breast cancer [10]. In our prior studies, we reported the clinical importance of several cancer-related signaling in breast cancer using multiple transcriptomic expressions. We found that the mutation load, cancer aggressiveness, and worse patient outcomes in breast cancer are related to the abundance of reactive oxygen species [11]. In ER-positive/HER2-negative breast cancer as well as metastatic breast cancer, unfolded protein response signaling was associated with worse patient survival [12]. Recently, with the advent of immunotherapy, there has been a rapid shift in the field of breast cancer research toward understanding the immunological mechanisms and cellular metabolic pathways. As aerobic glycolysis is an essential mechanism for cancer cells to thrive, and as breast cancer shows an increased expression of glycolysis-related enzymes [13], we decide to investigate the association of tumor glycolysis in the survival of breast cancer.

Therefore, in this study, we hypothesized that the amount of glycolysis measured by our score reflects cancer cell proliferation and is associated with survival. We investigate the mechanisms associated with this using multiple independent large cohorts.

Materials and methods

Breast cancer patient cohorts

We used the Molecular Taxonomy of Breast Cancer International Consortium (METABRIC) cohort (n = 1903) and the GSE96058 cohort (the Swedish Breast Cancer Analysis Network (SCAN-B)) (n = 3273) for obtaining clinical information and transcriptomic data for our study [14, 15]. The Gene Expression Omnibus (GEO), RRID: SCR_005012, database is queried to obtain several cohorts for our study including the GSE75688 (single cell RNA sequencing data) [16], GSE110590 [17], and GSE124647 [18].

Scoring of glycolysis signaling

For scoring the enhancement level of glycolysis signaling, the "HALLMARK_GLYCOLYSIS" gene set from the Molecular Signatures Database (MSigDB) gene set collection [19], and transcriptomic data were used [20] and we calculated the glycolysis score for each breast cancer as mentioned in our prior studies [21-25].

Gene set enrichment analysis

The GSEA algorithm was used to investigate the differentiation of the enhanced signaling pathways between glycolysis low- and high-breast cancer groups with MSigDB Hallmark gene sets [26-28]. Statistical significance was referred to the value defined by GSEA, i.e., false discovery rate (FDR) < 25%.

Other scores

Each immune cell infiltrating fraction was calculated by the xCell algorithm [29-32], and cytolytic activity score (CYT) was calculated by the expression pattern of granzyme A (*GZMA*) and perforin (*PRF1*) genes to estimate the overall immune activity [33, 34].

Statistical analyses

The Kruskal-Wallis test and Mann-Whitney U test were used for the analysis of the group comparison, as described in each figure legend. The Kaplan-Meier method was used for survival analysis using log-rank test. All analy-

ses were performed and data plots were generated using R software (version 4.1.0) and Microsoft Excel (version 16). A $P < 0.05$ was considered as statistically significant in the analyses.

Results

Glycolysis high breast cancer has accelerated cancer cell proliferation

Based upon the concept of the Warburg effect, breast cancer with elevated glycolysis is expected to be active, thus highly proliferative. To investigate whether this is the case in the bulk tumor in real-world patients, we first analyzed the relationship between glycolysis score and the measurements of cell proliferation. As expected, we found that cancer cells were the dominant type of cells with accelerated glycolysis in the TME using a single-cell sequence cohort (**Figure 1A**). By gene set enrichment analysis (GSEA), we found that in both the METABRIC and GSE96058 cohorts breast cancer with high glycolysis score significantly enhanced four cell proliferation-related gene sets in the Hallmark collection such as G2M checkpoint, MYC targets v1 and v2, and E2F targets (**Figure 1B**). The expression of *MKI67*, which is the coding gene of the widely used cell proliferation marker Ki67 is found to be significantly higher in glycolysis high breast cancer (**Figure 1C**). Nottingham histological grade was also found to be significantly correlated with glycolysis score. Triple-negative breast cancer (TNBC), the most aggressive subtype, was associated with the highest level of glycolysis score consistently in both cohorts (**Figure 1D**). Mutation count, which is reported to be associated with tumor aggressiveness, did not show a significant difference between low and high glycolysis score groups neither in the METABRIC nor in the GSE96058 cohort (**Figure 1E**). The above results showed that high glycolysis was significantly associated with accelerated cell proliferation shown by biological and clinicopathological parameters of aggressiveness in breast cancer.

In TNBC, glycolysis high breast cancer was significantly associated with inferior survival, but not consistently in ER-positive/HER2-negative subtype

Since glycolysis score was significantly associated with cancer cell proliferation, it was of interest to investigate its relationship with patient outcomes. We analyzed the survival difference between low and high glycolysis score groups by ER-positive/HER2-negative and TNBC subtypes, as each breast cancer subtype is known to have unique biological characteristics. In the entire breast cancer patients of the METABRIC cohort, we found that the high glycolysis score group was significantly associated with worse patient survival, including overall survival (OS), disease-free survival (DFS), and disease-specific survival (DSS) (**Figure 2**, whole). The result of OS was validated with the GSE96058 cohort. The high glycolysis score group was also significantly associated with inferior DFS, DSS, and OS in TNBC in the METABRIC cohort, and the OS results were validated in the GSE96058 cohort. The high glycolysis score group was significantly associated with worse OS in the ER-positive/HER2-negative subtype, in the GSE96058 cohort, however, this was not validated in the METABRIC cohort. These results revealed that in TNBC, a high glycolysis score was significantly associated with worse patient survival, but not consistently in the ER-positive/HER2-negative subtype.

Glycolysis high TNBC enriched multiple pro-cancerous gene sets, but not cell proliferation-related gene sets

As we observed that TNBC with high glycolysis was associated with worse survival, we were interested to elucidate the biological features that are associated with it. Interestingly, we found that TNBC with high glycolysis score did not enhance any of the Hallmark cell proliferation-related gene sets in either of the cohorts (**Figure 3A**). On the other hand, several other pro-cancerous Hallmark gene sets, including the gene sets for hypoxia, androgen response, peroxisome, cholesterol homeostasis, fatty acid metabolism, oxidative phosphorylation, unfolded protein response and MTORC1 signaling were significantly enriched in glycolysis high TNBC in both cohorts (**Figure 3B**). Interestingly, unlike in TNBC, in glycolysis high tumors in ER-positive/HER2-negative subtype, all these pro-cancerous Hallmark gene sets as well as all four cell proliferation-related gene sets were significantly enriched (Figure S1). These results suggest that accelerated glycolysis plays a role in the poor prognosis of TNBC patients through their interrelationship with other several hall-

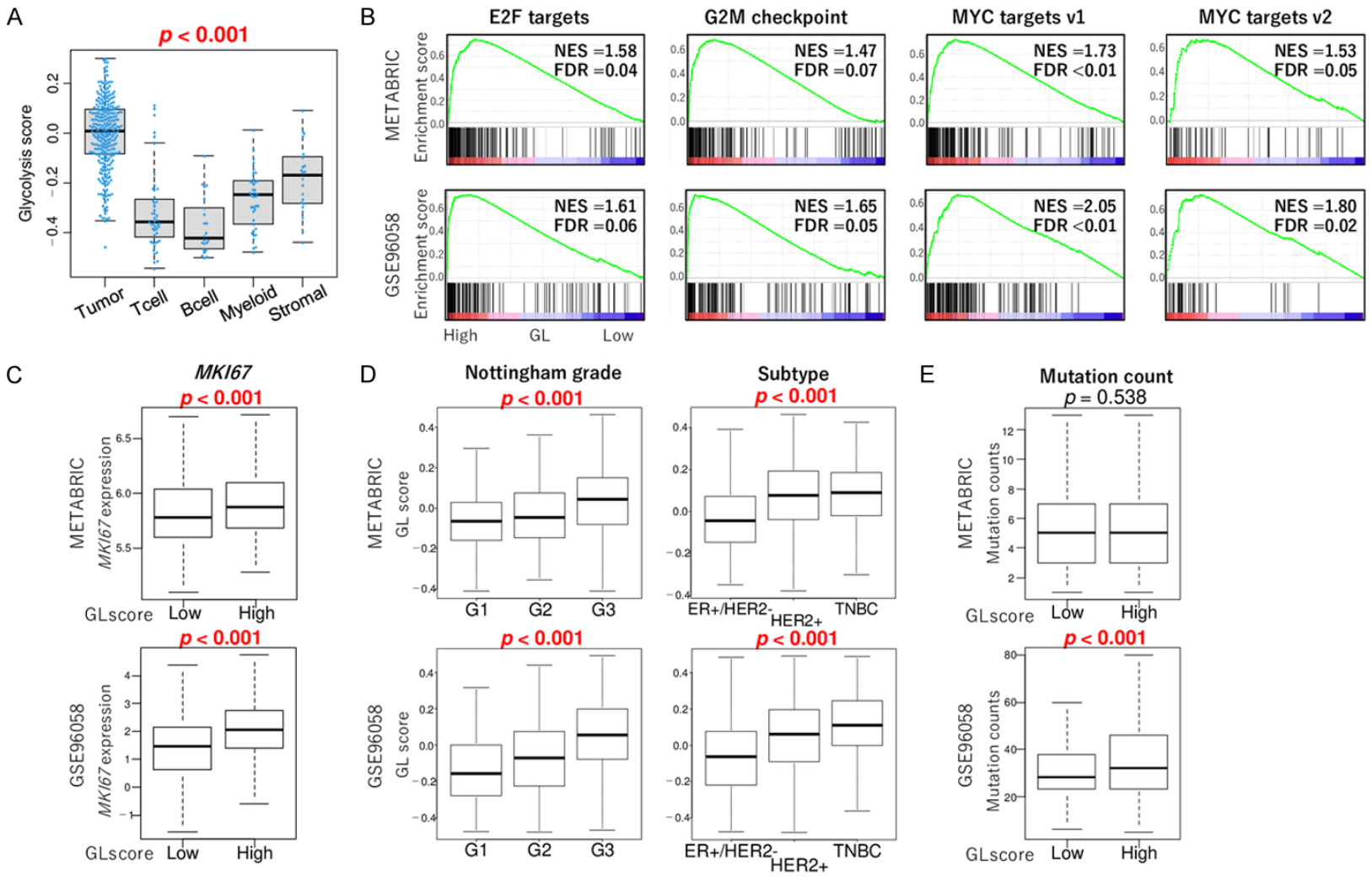

Figure 1. The biological and clinicopathological features of glycolysis score in breast cancer. A. Boxplots showing glycolysis score by cancer cells, T-cells, B-cells, myeloid cells, and stromal cells in bulk breast tumor in the GSE75688 cohort. B. Enrichment plot showing cell proliferation-related gene sets such as E2F targets, G2M checkpoint, MYC targets v1 and v2, among low and high glycolysis score groups in both the METABRIC and GSE56058 cohorts. C. Boxplots of MKI67 expression by low and high glycolysis groups. D. Boxplots of the glycolysis score by Nottingham histological grade (G1-3) and subtypes (ER-positive/HER2-negative, HER2-positive, and triple-negative breast cancer). E. Boxplots of the mutation counts by low and high glycolysis score groups. Within each cohort, the top tertile was defined as high groups. FDR, false discovery rate; NES, normalized enrichment score; TNBC, triple-negative breast cancer.

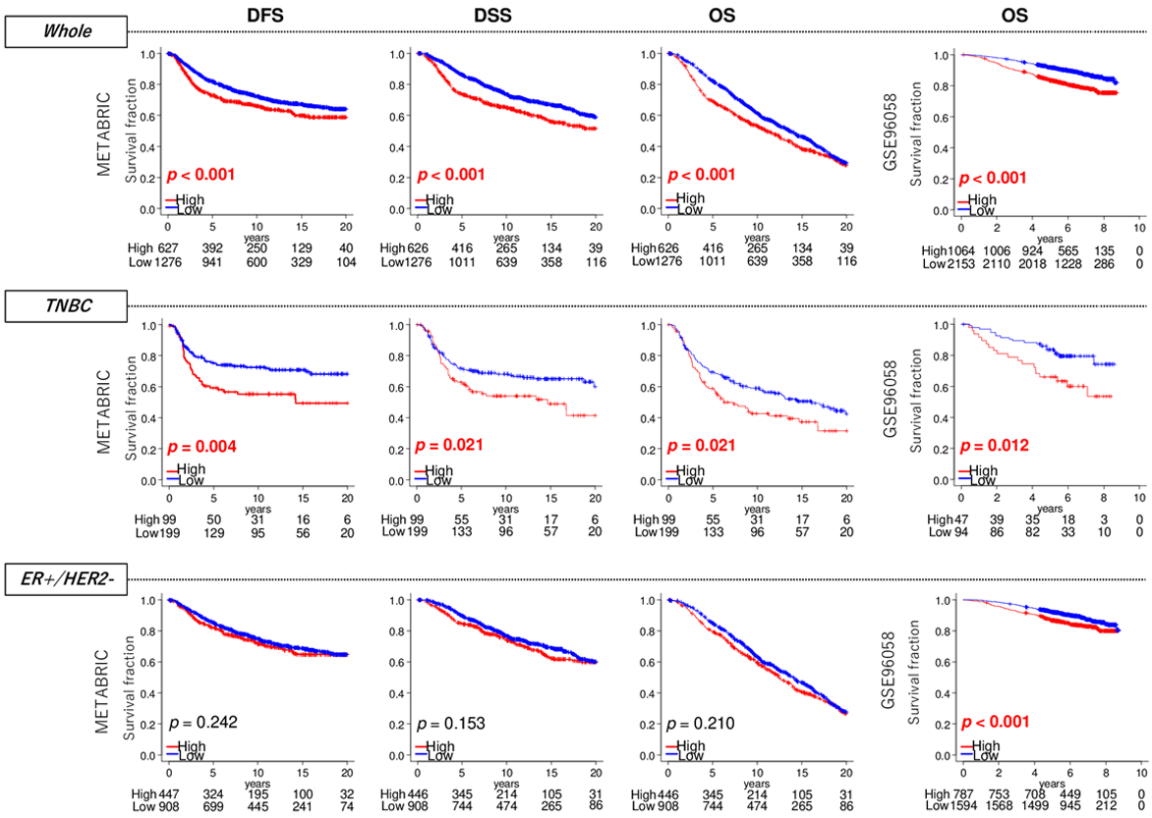

Figure 2. Patient survival fractions by low and high glycolysis score groups in the whole, TNBC, and ER-positive/HER2-negative breast cancer. Kaplan Meier curves with log-rank *P*-value of disease-free survival (DFS), disease-specific survival (DSS), and overall survival (OS) in the METABRIC and OS in the GSE96058 cohort by low and high glycolysis score groups.

mark cancers signaling, rather than cell proliferation.

The glycolysis high TNBC was infiltrated with a low level of anti-cancerous immune cells, and B-cells, and significantly associated with a decreased cytolytic activity

In breast cancer, immune cells in the TME are known to be an important prognostic factor. Therefore, we were interested in analyzing the association between the fraction of infiltrating immune cells and glycolysis level in the TME of TNBC. We found that in both cohorts, TNBC with higher glycolysis was significantly infiltrated with a low level of anti-cancer immune cells such as $CD4^+$ memory T cells, $CD8^+$ T cells, and dendritic cells (DC) (**Figure 4A**). On the other hand, the infiltration fraction of pro-cancerous immune cells such as T helper 2 cells (Th2), and M2 macrophages were found to have no significant association with the glycolysis score (**Figure 4B**). In the METABRIC cohort, the infiltration of Tregs was lower in glycolysis high TNBC, but that was not validated by the GSE96058 cohort (**Figure 4C**). In both cohorts, the glycolysis high TNBC significantly enhanced the cytolytic activity. These results suggested that TNBC with higher glycolysis has a lower fraction of anti-cancerous immune cells, and may be responsible for reduced patient survival.

Glycolysis high ER-positive/HER2-negative breast cancer was associated with a low level of cytolytic activity and infiltrated significantly with a higher fraction of anti-cancerous and pro-cancerous immune cells

Since the association between glycolysis and immune cells may affect patient survival in TNBC, we were then interested in studying the association between glycolysis and immunity in ER-positive/HER2-negative breast cancer. Glycolysis high ER-positive/HER2-negative breast cancer was infiltrated with a low fraction

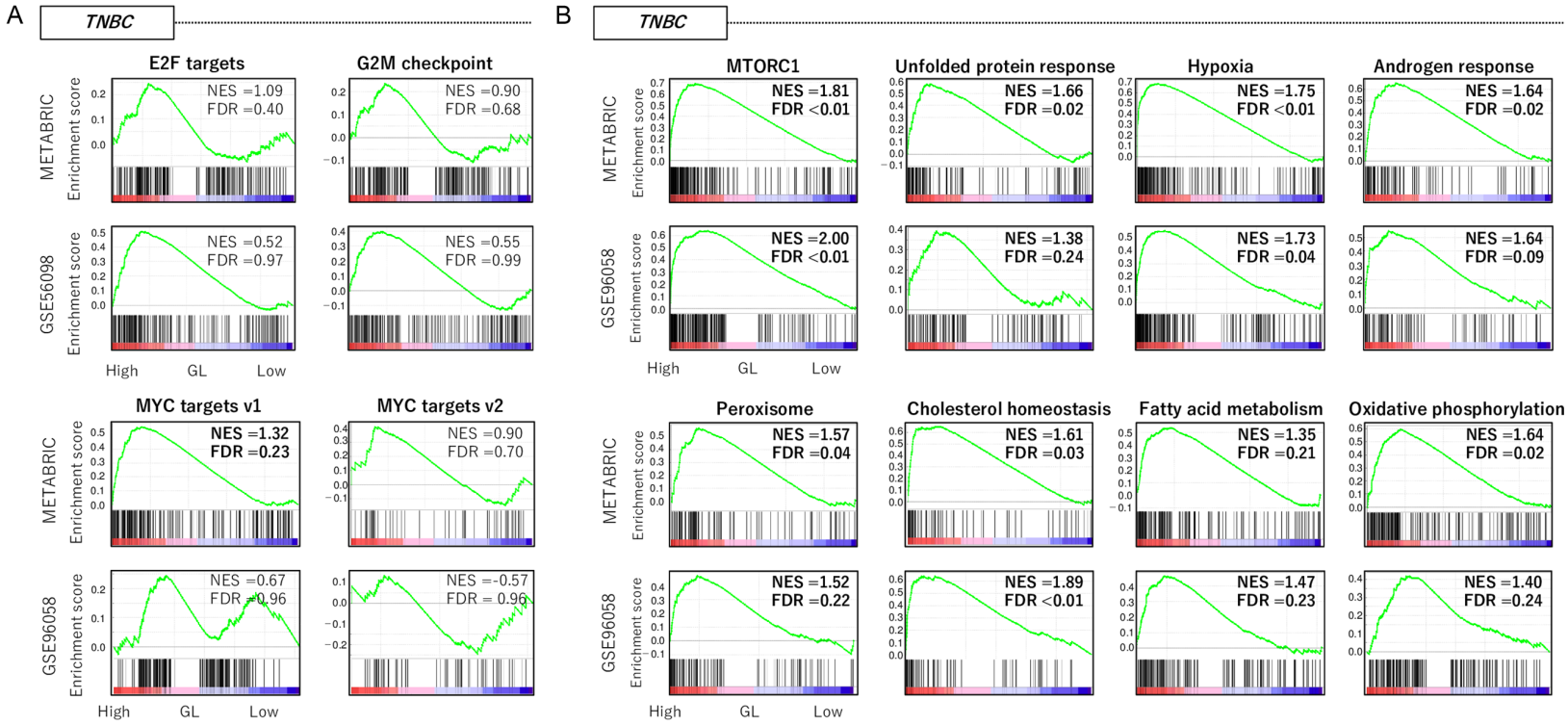

Figure 3. The biological features of the high glycolysis score TNBC in the METABRIC and GSE96058 cohorts. A. Enrichment plots showing cell proliferation-related gene sets (E2F targets, G2M checkpoint, and MYC targets v1 and v2) in high glycolysis score TNBC. B. Enrichment plots of several hallmark gene sets, which are highly enriched in TNBC with high glycolysis score, consistently in both cohorts: MTORC1, unfolded protein response, hypoxia, androgen response, peroxisome, cholesterol homeostasis, fatty acid metabolism, and oxidative phosphorylation. In each cohort, the top tertile was defined as high group. TNBC, triple-negative breast cancer; FDR, false discovery rate; NES, normalized enrichment score.

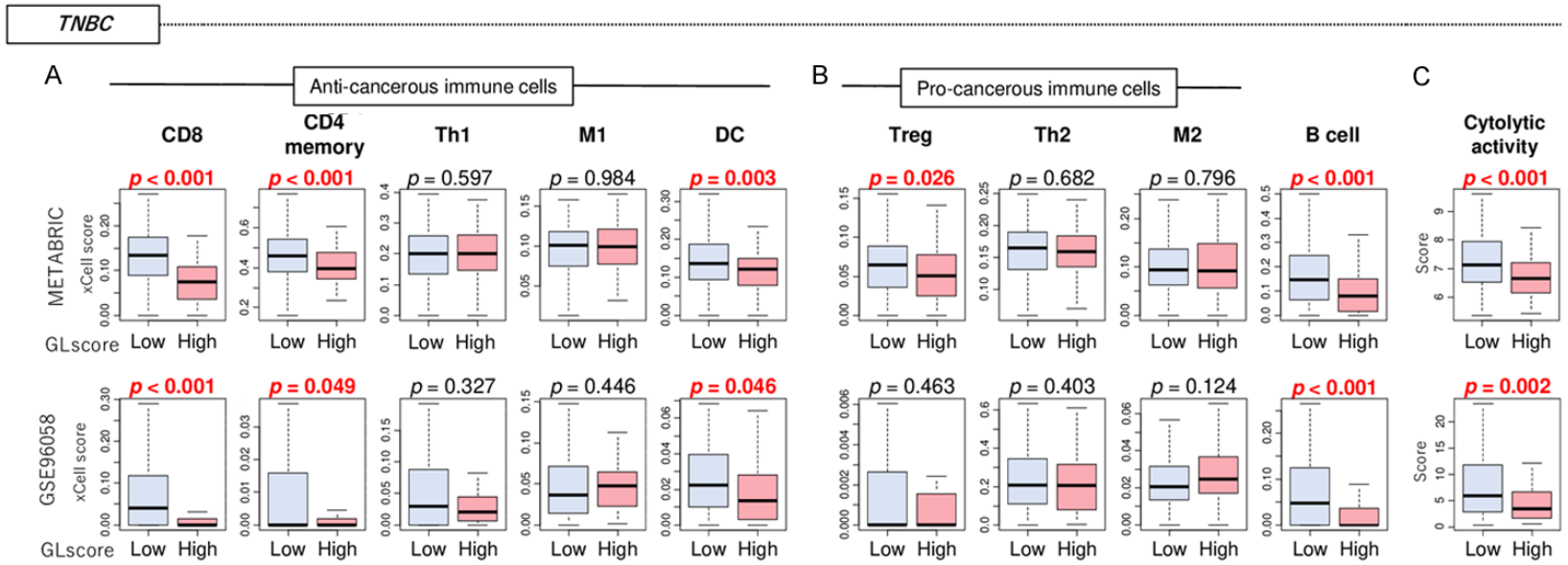

Figure 4. The cytolytic activity and infiltration fraction of immune cells in TNBC. Boxplots of (A) anti-cancerous immune cells; $CD8^+$ T cells, $CD4^+$ memory T cells, Th1 cells, M1 macrophages, and dendritic cells (DC), and (B) pro-cancerous immune cells; Tregs, Th2 cells, and M2 macrophages, and B-cells, and (C) cytolytic activity in TNBC in the METABRIC cohort. The top tertile was defined as high groups within each cohort. TNBC, triple-negative breast cancer.

of $CD8^+$ T cells and B-cells, like TNBC (**Figure 5**). However, in glycolysis high ER-positive/HER2-negative breast cancer, the infiltration of other anti-cancerous immune cells, including $CD4^+$ memory T cells, Th1 cells, and M1 macrophages were lower, and the infiltration of DC did not show significance in the METABRIC cohort (**Figure 5A**). Among pro-cancerous immune cells, the infiltration of Tregs was lower in the glycolysis high tumor, but the infiltration of Th2 and M2 macrophages were higher in the METABRIC cohort (**Figure 5B**), unlike TNBC. The results of CD8 T cells, Th1, Th2 cells, M1, M2 macrophages, and B-cells were validated with the GSE96058 cohort. Finally, similar to TNBC, glycolysis high ER-positive/HER2-negative breast cancer was significantly associated with a low level of cytolytic activity (**Figure 5C**). These results revealed that glycolysis high ER-positive/HER2-negative was infiltrated with a high fraction of anti-cancerous immune cells, unlike TNBC.

Glycolysis is accelerated in distant metastasis compared to primary breast cancer but the survival was not different based on the site of metastasis

Survival is lower in breast cancer patients with distant metastasis; thus, the utility of glycolysis score as a prognostic biomarker in patients with distant metastasis was one of our interests to learn. We found that glycolysis was elevated in distant metastatic sites such as brain, lung, bone, and liver, compared to primary breast tumor and lymph node (LN) metastasis (**Figure 6A**). Glycolysis scores were significantly elevated in liver and lung metastatic tumors, compared to the matched primary tumors from the same patients, whereas that was not the case in lymph node metastasis (**Figure 6B**). Furthermore, the glycolysis score of primary tumors with local recurrence, metastasis to liver, and soft tissue were higher than the primary tumors with bone or LN metastasis (**Figure 6C**). In the GSE124647 cohort, glycolysis score high primary breast cancer with metastasis was significantly associated with inferior overall survival (**Figure 6D** whole), which agreed with the results in METABRIC and GSE56098 cohorts (**Figure 2**). However, based on the metastasis organs, there was no significant difference was observed in survival between low and high glycolysis score groups. These results suggested that distant metastatic tumors have accelerated glycolysis compared to the primary tumor.

Discussion

In this study, we observed that a high glycolysis score in breast cancer was significantly associated with clinicopathological aggressiveness and inferior patient survival, especially in TNBC. In the bulk tumor, compared to other cells, such as T cells, B cells, myeloid, or stromal cells the glycolysis score level was higher in cancer cells. TNBC with high glycolysis enriched several pro-cancerous signaling, including MTORC1, unfolded protein response, hypoxia, androgen response, peroxisome, cholesterol homeostasis, fatty acid metabolism, and oxidative phosphorylation, but it did not enrich cell proliferation-related gene sets. On the other hand, in both METABRIC and GSE96058 cohorts, ER-positive/HER2-negative breast cancer with high glycolysis score enriched cell proliferation-related gene sets, including E2F targets, G2M checkpoint, and MYC targets v1 and v2. In both subtypes, high glycolysis score was significantly associated with low cytolytic activity and a lower infiltration fraction of $CD8^+$ T cells and B cells. Furthermore, in ER-positive/HER2-negative breast cancer, a high glycolysis score was associated with a high infiltration fraction of anti- and pro-cancerous immune cells such as Th2 cells, and M2 macrophages. Finally, the glycolysis score was significantly higher in distant metastatic tumors compared to the primary tumor, however, there was no survival difference by the level of glycolysis within the primary breast tumor based on the site of metastasis.

In cancer cells, the Warburg effect is a metabolic abnormality [35, 36]. In particular, it refers to the phenomenon in which cancer cells take up glucose at a faster rate and use less glucose for oxidative phosphorylation than normal cells. This unique metabolism has been thought to be one of the hallmarks of cancer. Proper functioning of the glycolysis and the mitochondrial oxidative phosphorylation pathways are critical for glucose energy metabolism. When mitochondrial oxidative phosphorylation is compromised by hypoxia or fatty acid oxidation overactivation, metabolic pathways are activated to compensate. However, cancer cells irreversibly acquire compensatory metabolic pathways by altering the activity of metabolic enzymes. Glutaminolysis is activated and

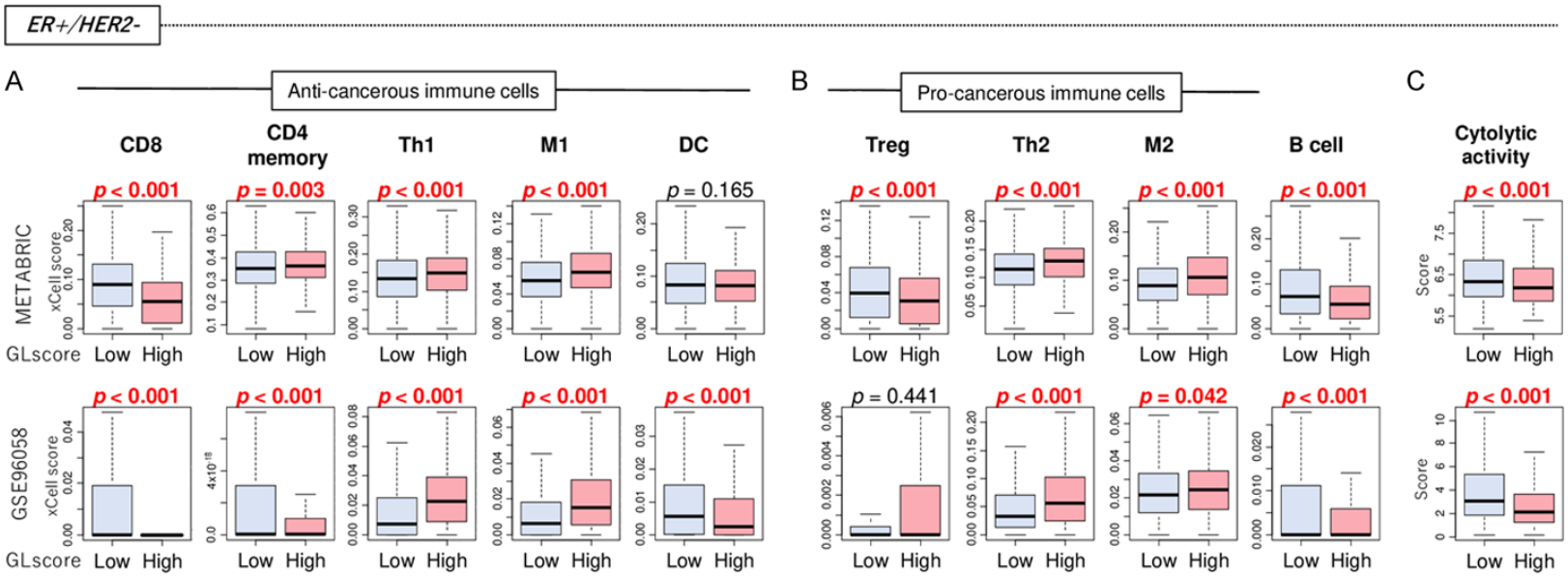

Figure 5. The cytolytic activity and infiltration fraction of immune cells in ER-positive/HER2-negative breast cancer. Boxplots of (A) anti-cancerous immune cells; $CD8^+$ T cells, $CD4^+$ memory T cells, Th1 cells, M1 macrophages, and dendritic cells (DC), and (B) pro-cancerous immune cells; Tregs, Th2 cells, and M2 macrophages, and B-cells, and (C) cytolytic activity in ER-positive/HER2-negative breast cancer in the METABRIC cohort. In each cohort, the top tertile was defined as high group. For analysis, Mann Whitney U and Kruskal-Wallis tests were used.

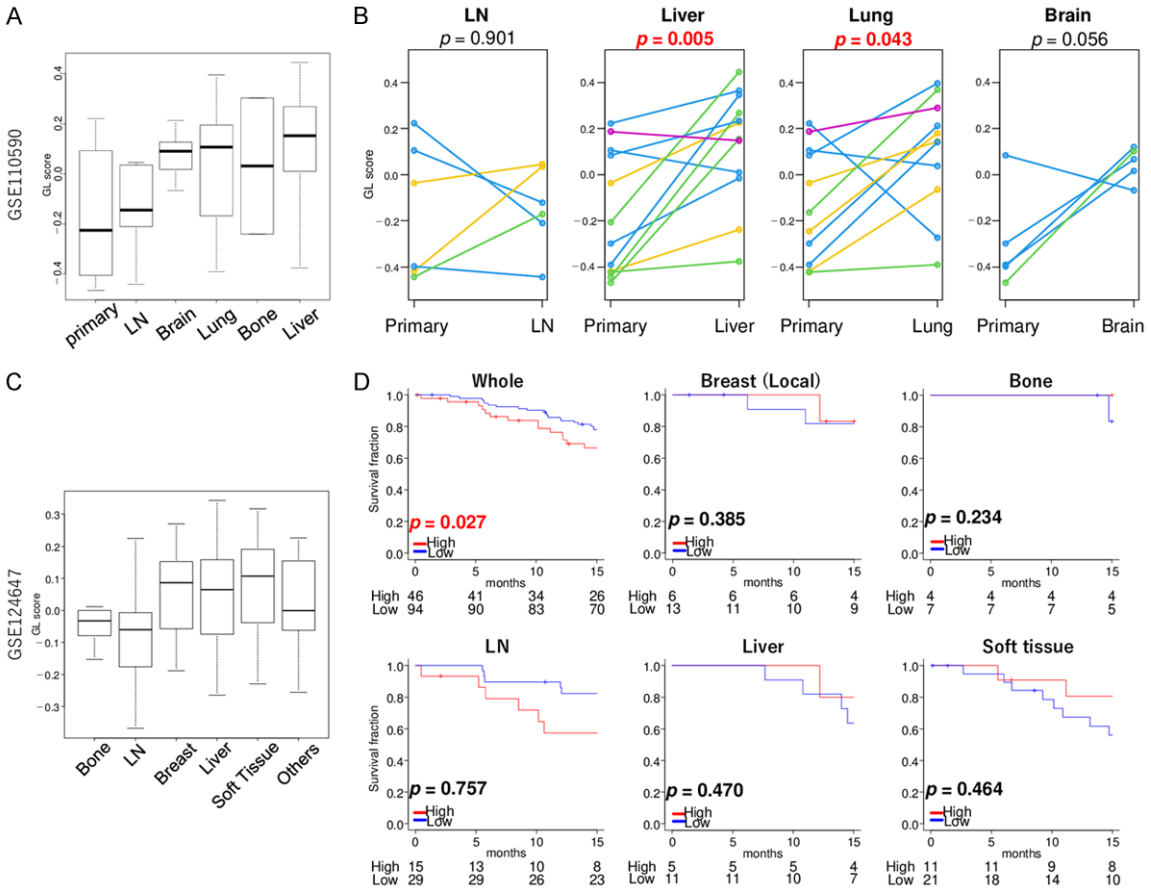

Figure 6. Association of glycolysis levels and metastasis in breast cancer. A. Boxplots of the glycolysis score by primary breast cancer (primary) and metastatic breast cancer with metastasis to the lymph node (LN), brain, lung, bone, and liver. Kruskal-Wallis test was used for the analysis. B. Matched comparison of glycolysis scores of primary breast tumors and the metastatic tumors (LN, liver, lung, and brain metastasis) from the same patients linked by the lines in the GSE110590 cohort. Paired t-test was performed for the analysis. C. Boxplots of glycolysis score by breast tumors that developed local recurrence (breast) or distant metastasis to the bone, LN, liver, soft tissue, and others in the GSE124647 cohort. For the analysis, the Kruskal-Wallis test was used. D. Kaplan-Meier survival curves of progression-free survival with log-lank *P*-value within the whole tumor, breast cancer with local recurrence, bone, LN, liver, and soft tissue.

the Warburg effect promotes fatty acid synthesis through glycolysis. Normally, glucose and fatty acid metabolism do not proceed simultaneously due to the regulation of the activity of metabolic enzymes. However, based on recent studies, it was observed that fatty acid synthesis, excessive fatty acid oxidation, and metabolic pathways such as the pentose phosphate pathway (PPP) are abnormally activated. The enhancement of the glycolytic system by the Warburg effect and fatty acid oxidation in the mitochondria results in a metabolism that simultaneously promotes fatty acid synthesis [37]. Our study results support these findings. In other words, breast cancer with a high glycolysis score enriched the pathway in peroxisome, cholesterol homeostasis, fatty acid metabolism, and oxidative phosphorylation. We also found that they may be associated with the activation of MTORC1, unfolded protein response, and androgen response. In addition, in ER-positive/HER2-negative breast cancer cell proliferation-related signaling was found to be involved in glycolysis signaling, but not in TNBC. This ability to analyze the several signaling states occurring in tumors is an advantage of in-silico translational research that is difficult to achieve with other tools.

In two large cohorts, breast cancers with high glycolysis scores were shown to have a poor prognosis in TNBC, but not in ER-positive/

HER2-negative breast cancer. Biological analysis of each subtype to determine the reason for this result revealed that high glycolysis tumor enriched MTORC1 and hypoxia signaling, which are well known to be associated with glycolysis signaling, in both subtypes. However, in the ER-positive/HER2-negative breast cancer, the cell proliferation-related gene sets were significantly enriched. We have previously shown in our studies that for the prognosis of ER-positive/HER2-negative breast cancer, the enhancement of cell proliferation signaling was important [38]. Nevertheless, given the fact that the high glycolysis group was not associated with a worse prognosis in ER-positive/HER2-negative breast cancer, it may suggest that the counterbalance of several hallmark cancer signaling may affect patient prognosis. Also, another possible explanation for this observation is that, as ER-positive/HER2-negative breast cancer has lower glycolysis levels compared to TNBC, the changes that occur in the TME of the ER-positive/HER2-negative breast cancer might not be influencing the survival.

Lactic acid produced by cancer cells in glycolysis signaling is known to induce immunosuppressive macrophages and further reduce immunity [39]. The study also showed that tumors with higher glycolysis also had higher rates of M1 and M2 macrophage infiltration, especially in ER-positive/HER2-negative breast cancer. Besides, $CD8^+$ T cell infiltration was also significantly lower, indicating significantly lower cytolytic activity. Cancer immunotherapy, which recently attracted attention due to the success of immune checkpoint inhibitors for breast cancer therapy, has been utilized to induce additional anti-tumor effects by combining immune checkpoint inhibitors with drugs that affect metabolism [40, 41]. This score, which reflects various phenomena occurring in breast cancer, especially in the TME may be expected to become a tool for future drug therapy research. Furthermore, the expression of bulk tumors is thought to reflect the metabolic activity of various cells, but this score, using single-cell sequence data, was shown to most reflect the activity of the cancer cell's glycolysis signaling. Colon cancer cell line has been demonstrated that increased glycolysis correlated with drug resistance [42]. Several studies have elucidated altered metabolism and glycolysis pathways in cancer cells [42, 43]. In light of these findings, and our study results, we can postulate that interrupting or possibly disrupting tumor glycolysis will impact tumor growth by energy depletion and may result in sensitization to therapeutics. In addition to resistance to chemotherapy, it has been reported that glycolysis signaling is also involved in resistance to radiotherapy [44]. The transketolase (*TKTL1*) which is one of the main genes in the PPP, is reported to affect the chemosensitivity of cancer cells to treatments such as cetuximab [45], and imatinib [46]. It is important to understand gene expression to comprehend cancer-related signaling, but it is known that it is difficult to understand the entire perspective of several cancer-related signal transduction which is intricately involved with a single gene [47-49]. This score, which is calculated by the expression of approximately 200 glycolysis-related genes, appears to reflect the glycolysis signaling more accurately than a single gene. Tumor metabolism, especially tumor glycolysis, which is a key feature of cancer cells, therefore represents an ideal target for therapeutic intervention. We can speculate that the score may become a new tool in the future development of cancer drugs targeting glycolysis signaling.

We have previously reported that cell proliferation signaling is prognostically important in breast cancer [38, 50]. In this study, we showed that the glycolysis pathway plays a pivotal role in predicting patient outcomes. Furthermore, metastatic tumors, especially those metastasizing to prognostically relevant organs, showed even higher glycolysis activity than primary tumors. Although bioinformatics can investigate interrelationships regarding various signaling pathways, and changes in the TME, in vivo/vitro studies, are essential to observe the underlying mechanisms.

Multiple independent cohorts from completely different backgrounds were utilized in this study. The strength of this approach is that the results were validated by multiple large independent cohorts regardless of epidemiological, national, regional, racial, ethnical, and clinical differences, that minimizes the sampling biases. The weakness of this approach is that because weutilized the already reported cohorts, some of the critical parameters such as details on treatments were not available, thus additional research is needed to understand

the impact of different therapeutics in glycolysis high tumors. We found a significant relationship between glycolysis and tumor immunity, but additional experiments are needed to elucidate the underlying mechanisms. Finally, as our study is a retrospective study, it has several limitations, and prospective studies analyzing the relationship of glycolysis signaling with patient outcomes are needed in the future.

Conclusions

Breast cancer with higher glycolysis was associated with clinicopathological aggressiveness, and worse patient survival, especially TNBC. The relationship between glycolysis signaling and tumor immunity might be playing a pertinent role in the patient's outcome.

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Disclosure of conflict of interest

None.

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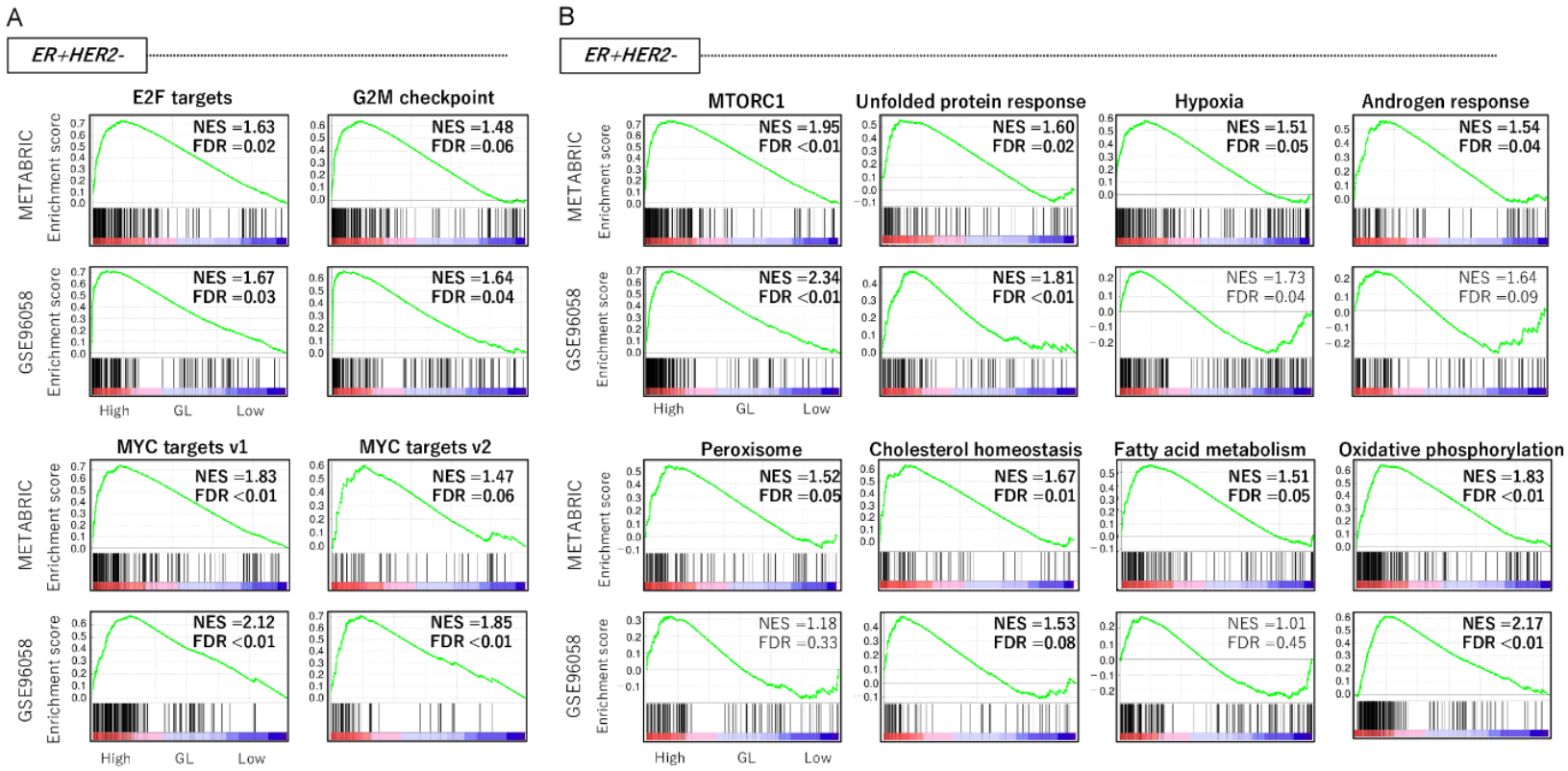

Figure S1. The biological features of the high glycolysis score ER+/HER2- breast cancer in the METABRIC and GSE96058 cohorts. A. Enrichment plots showing cell proliferation-related gene sets (E2F targets, G2M checkpoint, and MYC targets v1 and v2) in high glycolysis score ER+/HER2- breast cancer. B. Enrichment plots of several hallmark gene sets, which are highly enriched in ER+/HER2- breast cancer with high glycolysis score, consistently in both cohorts: MTORC1, unfolded protein response, hypoxia, androgen response, peroxisome, cholesterol homeostasis, fatty acid metabolism, and oxidative phosphorylation. In each cohort, the top tertile was defined as high group. FDR, false discovery rate; NES, normalized enrichment score.