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Original Article

Galectin-8 alters immune microenvironment and promotes tumor progression

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Abstract: Galectin-8 (Gal-8), encoded by LGALS8 gene, is a unique member of the Galectin family with diverse biological functions, including tumor-modulating capabilities. Recently, evidence has accumulated supporting an essential role for Gal-8 in regulating innate and adaptive immunity, with high expression in tumors and other immune dysregulation diseases. This study reveals the role of Gal-8-induced tumor immunosuppression by analyzing animal models and clinical data of tumor-infiltrating cells. In Gal-8 expressing tumor, we found that suppressive immune cells, including Tregs and MDSCs, expanded while CD8+ cells decreased, providing direct evidence that Gal-8 regulates the tumor immune microenvironment. In addition, we not only analyzed the expression of Gal-8 in clinical samples of breast and colorectal cancer but also classified the tissue expression patterns. Further analysis revealed that Gal-8 correlates with lymph node metastasis and immunophenotyping. Consistent with animal experiments, our analysis of LGALS8 gene expression showed its negative association with infiltrated active CD8+ T cells and immune stimulatory modulators in cancers. Our study identified the potential prognostic and therapeutic value of Gal-8, and further research on developing corresponding targeted therapeutic strategies is awaited.

Keywords: Tumor microenvironment, prognosis, Galectin-8, breast cancer, colorectal cancer

Introduction

Cancer immunotherapy, a method targeting immune checkpoints to activate or block the inhibition of immune cells, has brought significant progress and challenges over the decades [1]. With resistance and an inadequate response to PD-1 and CTLA-4 targeted therapies limiting clinical outcomes, discovering novel targets has become a hot research topic [2]. In recent years, enormous attention has been drawn to target cells beyond T cells, such as myeloid-derived suppressor cells (MDSCs), which play a role in inducing and maintaining immune suppresion in the tumor microenvironment [3]. LILRB was recently recognized as a regulator of MDSCs and a potential target for tumors [4, 5] by James P. Allison and colleagues. Our previous study identified Galectin-8 (Gal-8) as a functional ligand of LILRB, which induced MDSC expansion and promoted tumor progression in vivo [6]. Gal-8, encoded by the LGALS8 gene, is a member of mammalian lectins with unique features, which emerged as an immune regulator of both innate and adaptive immunity [7-9]. It has also been shown that Gal-8 is expressed in various tumors and has some survival relevance [8]. However, we still need valid evidence on how Gal-8 affects the tumor immune microenvironment and relates to tumor immune infiltration components.

Colorectal cancer (CRC) is the third most commonly diagnosed malignancy and the second leading cause of cancer death globally [10]. There is an urgent call for alternative and effective treatment for CRC, such as immunotherapy [11]. A subset of colorectal cancer is characterized as having a significantly higher number of

somatic mutations, with a high incidence of microsatellite instability-high (MSI-H) feature [12]. Breast cancer is the most frequent malignancy in women. There is great enthusiasm to investigate immunotherapies in breast cancer, although breast cancer was previously considered to be immunologically quiescent [13].

By analyzing tissue samples, we found widespread high expression of Gal-8 in breast cancer and heterogeneous expression in colorectal cancer. We consistently found that Gal-8 regulated the suppressive tumor microenvironment in databases and *in vivo* models. In addition, we demonstrated an association with poor prognostic factors such as lymph node metastasis in clinical analyses. Thus, we aim to investigate the role of Gal-8 in microenvironment alteration to provide insight into its potential role in cancer immunotherapy.

Materials and methods

Cell culture

Mouse colorectal cancer cell line Mc38 was purchased from Kerafast. Mc38 cells were incubated in DMEM (Meilunbio) with 10% FBS (Gibco). Ectopic Flag-tagged Gal-8 plasmid was transfected into the Mc38 cells with Fugene (Promega) and a blank vector was used as control. After approximately two-week incubation supplemented with 200 μg/ml G418 (Gibco BRL) with refreshing the medium every 2-3 days, the single colonies were picked and verified by immunoblots. An optimal clone was selected and expanded.

Animal model

All animal experiments were performed strictly following the relevant ethical guidelines, approved by the Department of Laboratory Animal Science of Fudan University and the Institutional Animal Care and Use Committee of Renji Hospital, School of Medicine, Shanghai Jiaotong University. After one week of environmental adaptation, mice (female/male, six weeks old) were randomized into two groups (n=6 for each group) and injected subcutaneously with 1.5e6 Mc38 stable cells in the right flank. Since tumors became visible, the tumor sizes were recorded every 2-3 days using a vernier caliper and calculated with the formula 1/2 × A × a2 (A and a, respectively, denote the length and the width of the tumor). Per the ethical guidelines, mice would be sacrificed once the tumor volume reached 2 cm^3 or ulcers happened. Samples were collected as described.

Flow cytometry

After the acquisition, cells were washed twice with flow cytometry staining buffer (Invitrogen) three times. Then diluted fluorescent antibodies were added at the suggested concentrations and incubated per manufacturer instructions. Next, after being washed twice with staining buffer three times, samples were analyzed by MACSQuant16 (Miltenyi). FlowJo V10 was applied to analyze the data. Antibodies used in FACS, including anti-CD11b APC (Invitrogen, Cat#17-0118-42), anti-Ly-6C FITC (Biolegend, Cat#128006), and anti-Ly-6G PE-Cy5.5 (Biolegend, Cat#127616) are all commercially available.

Immunohistochemistry (IHC)

Tissue samples were deparaffinized and rehydrated, and the antigen retrieval was carried out in citrate antigen retrieval solution. After blocking endogenous peroxidase with 3% H_2O_2 for 15 min and blocking with goat serum for 1 hour, tissue samples were incubated with primary antibody overnight at 4°C, followed by incubation with biotin-conjugated secondary antibody at RT for 1 hour. DAB was used as chromogen, and nuclei were counterstained with hematoxylin. Images were scanned and captured with Olympus VS200. Primary antibodies including anti-Galectin-8 (Abcam, Cat#ab109519, RRID: AB_10861755), anti-FOXP3 (CST, CAT#12653), and anti-CD8 (Abcam, Cat#ab217344) were used per manufacturer instructions.

Tissue microarray and analysis of Galectin-8 expression

The CRC and Breast cancer tissue microarray were purchased from BioChip (Shanghai, China) with informed patient consent. The tissue microarray matched normal tissues and primary and metastatic tumor tissue samples from 31 breast cancer patients and 47 CRC patients. IHC was performed as described and quantified based on two aspects. The area score was assigned 0 (< 5%), 1 (5~25%), 2 (25~50%), 3 (50~75%), and 4 (75~100%). The intensity

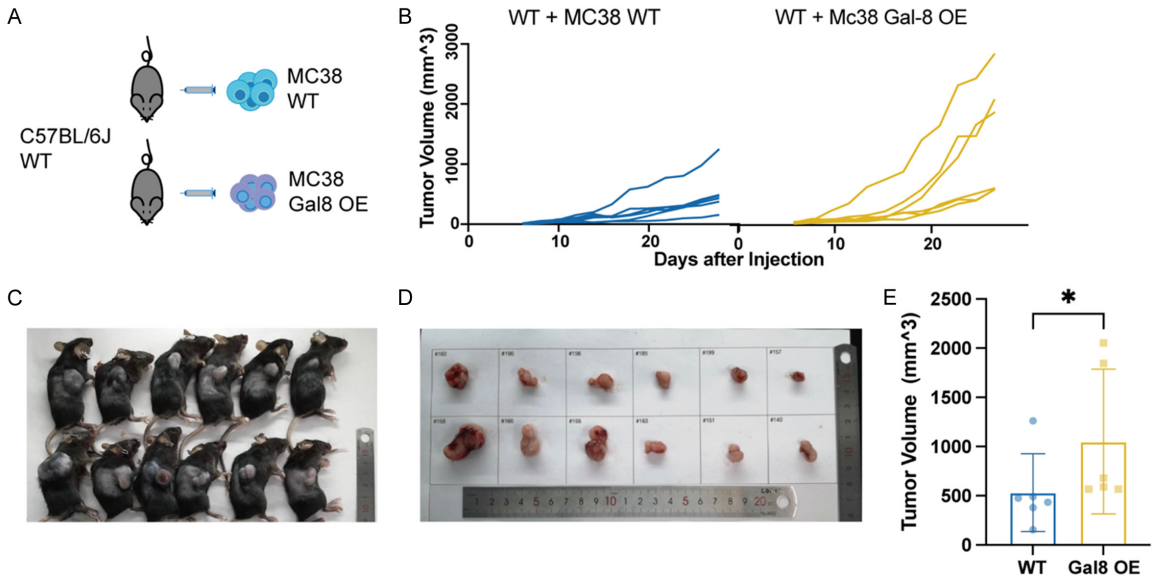


Figure 1. Gal-8 promotes tumor progression in vivo. (A) Diagram of tumor transplant mice model. Mc38 cells stably transfected with vector or Gal-8 plasmid were injected subcutaneously. (B) Tumor volume was recorded every 2 days and calculated as described. On day 28, mice were sacrificed, and the Mc38 tumors in vivo (C) and ex vivo (D) photo records were taken. (E) Mc38 tumor volume on day 28 was analyzed.

score was assigned with 0 (negative), 1 (weak), 2 (moderate), and 3 (strong). The sums of two scores (ranging from 0~7) were calculated as IHC scores. Expression upregulation was assessed by subtracting the normal tissue score from the tumor tissue score. Accordingly, upregulation was categorized as negative, weak, moderate, and strong.

Clinical data collection and processing

The mRNA expression and clinical information of breast cancer were acquired through the Xena platform (https://xena.ucsc.edu/) and the GDC data portal from TCGA [14]. The normalization of expression data was performed with the log2 (TPM+1) transformation. For breast cancer, 1097 tumor samples, 114 normal samples, and seven metastatic samples with expression and clinical data were obtained for survival and clinical feature analysis. For CRC, 380 primary tumor samples, two recurrent tumor samples, one metastatic tumor sample, and 51 normal samples with expression and clinical data were collected for survival and expression analysis grouped by age and tumor site. Due to a lack of samples with valid data for both microsatellite instability and LGALS8 expression in the TCGA COADREAD dataset, we additionally obtained 593 tumor samples with expression and clinical data from Colorectal Adenocarcinoma (TCGA, Pan-Cancer Atlas) dataset to analyze MSI status with expression and clinical features.

Statistical analysis

Data processing and visualization were performed using the ggplot2 package [15]. Patient survival was analyzed with the Kaplan-Meier method using the survival package (v3.2-7) and the survminer package. Odds ratios and corresponding 95% confidence intervals were calculated. Hypothesis testing was performed by tests appropriate for the respective data (T-test, Wilcoxon test) with a set of 5%. Pairwise analysis was applied to IHC analysis between paired normal and tumor samples. Calculations were performed with "R" statistical programming language version v4.2.1 (www.R-project.org).

Results

Galectin-8 promotes tumor progression in vivo

MC38, a C56BL/6-derived colon cancer cell line, was transfected with plasmid to construct a stable Gal-8 overexpressing cell line. Plasmid control was used for control cell line construction. Overexpression (OE) and wildtype (WT) cells were injected subcutaneously into wildtype (WT) C56BL/6J mice (**Figure 1A**).

Specifically, 1.5e6 MC38 cells were injected per individual on day 0. The subcutaneous tumors were detectable in most of the mice from day 7. Since then, tumors were measured three times a week until the tumor size was not ethically acceptable. Over time, the Gal-8 OE tumors grew faster than the WT tumor (**Figure 1B**). After mice were sacrificed on day 28, dissected tumors were photographed in vivo and ex vivo (**Figure 1C**, **1D**). The statistical comparison showed that tumors with high Gal-8 expression at 28 days overgrew that of the wildtype (**Figure 1E**).

Galectin-8 alters the tumor microenvironment and induces immune suppression in mice and human

For the mice model, peripheral blood mononuclear cells (PBMCs) were sampled for Flow cytometry in order to gate and measure the level of M-MDSC cells, defined as CD11b+, Ly-6C+, Ly-6G- cells (**Figure 2A**). As a result, the Gal-8 OE group presented the highest level of M-MDSCs (**Figure 2B**). Tumors were dissected and sliced to evaluate tumor-infiltrating immune status. IHC staining of CD8 and FoxP3 was performed on tumor samples (**Figure 2C**) to show that Gal-8 upregulated FoxP3 positive T regulator cells (Tregs) level and downregulated CD8+ T cell infiltration in MC38 tumors (**Figure 2D**, **2E**).

In order to investigate tumor-infiltrating factors in human cancer, we analyzed the correlation of LGALS8 gene expression level with immune infiltrating cells (Supplementary Figure 1A) and immune modulators (Supplementary Figure 1B) with web portal TISIDB. LGALS8 was demonstrated to negatively correlate with active CD8+ T lymphocytes (Act CD8) in multiple cancers, including BRCA, with Spearman test *p*-value of 5.71e-17 (Supplementary Figure 1C). Meanwhile, the results also showed a positive association of immature DC (iDC) and T helper 2 cell (Th2) with LGALS8 in most cancer types. The correlation of LGALS8 with iDC and Th2 in COAD was statistically significant, with *p*-values of 2.62e-11 and 2.52e-6 (Supplementary Figure 1D, 1E). LGALS8 and iDC were also significantly correlated in rectal cancer (Supplementary Figure 1F). Stimulatory receptor TNFRSF25 (also called DR3) and TNFRSF4 (also called OX40) were both negatively associated with LGALS8. The correlation coefficients of LGALS8 with DR3 and OX40 calculated by the Spearman test in breast cancer were -0.37 ($P < 2.2e-16$) and -0.379 ($P < 2.2e-16$), respectively (Supplementary Figure 1G, 1H). These two values in colorectal cancer were -0.351 ($P=1.14e-14$) and -0.285 ($P=6.27e-10$), respectively (Supplementary Figure 1I, 1J).

Galectin-8 expression was elevated in a group of cancers especially breast cancer

Previous studies supported that Galectin-8 was elevated in various cancers [8]. By employing the TCGA pan-cancer dataset, we analyzed LGALS8 gene expression in 33 cancer types, and found that LGALS8 was significantly upregulated in 9 cancer types, including BLCA, BRCA, CESC, CHOL, ESCA, HNSC, LIHC, LUSC and STAD (Supplementary Figure 2A) [16]. Additionally, we obtained somatic mutation and CNV data from the GDC data portal. As a result, amplification is the predominant form of genetic variation in most cancer types. The prevalence of LGALS8 amplification ranges from 1~10% in different cancer types and is exceptionally high in breast cancer (Supplementary Figure 2B). The amplification prevalence is much higher than what has been reported for PD-L1 in solid tumors (0.7%) [17].

Thus, we collected paired normal and tumor samples from 31 breast cancer patients for protein-level expression evaluation. These samples included 1 ductal carcinoma in situ, 27 invasive ductal carcinomas (IDC), 1 invasive lobular carcinoma (ILC), and 1 medullary breast carcinoma (MBC). The baseline information of breast cancer patients recruited was summarized in **Table 1**. **Figure 3A** presents representative samples of three different pathological types of breast cancer. IHC scores of all tumor and normal samples are shown in **Figure 3B**. Conclusively, Gal-8 was elevated in all invasive ductal carcinoma compared to normal, while conclusions could not be drawn for other pathological due to the small sample size. Since IDC is the most common form of breast cancer, accounting for 80% of all breast cancer diagnoses, upregulation of Gal-8 in this subtype is essential. In IDC samples, the upregulation level was calculated as described, and assigned to weak (1~2), moderate (3~4), and strong (5~7) groups, with typical samples shown pairwisely (**Figure 3C**). We calculated the number and percentage of samples with different levels

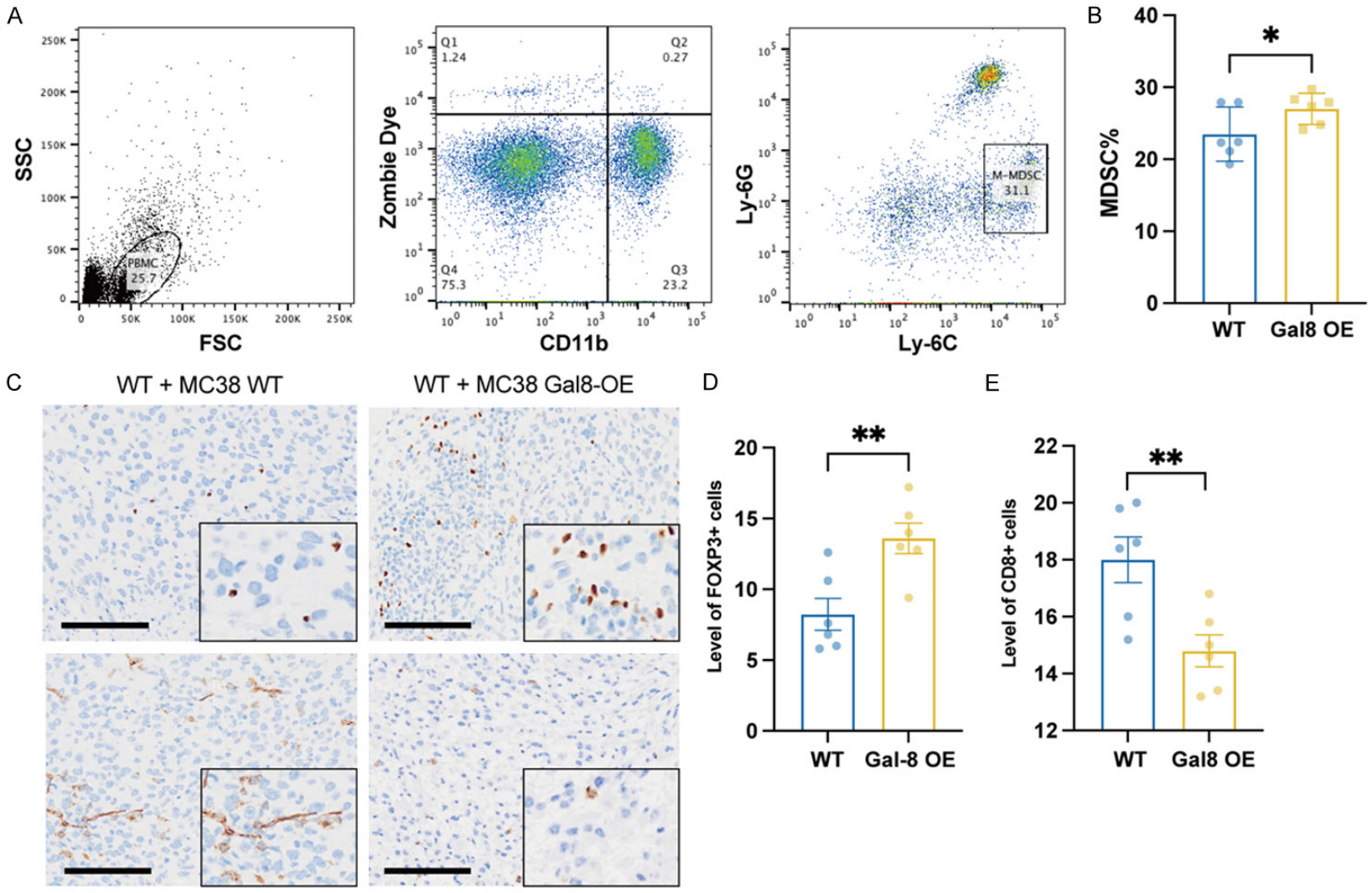


Figure 2. Gal-8 modulates the tumor microenvironment. (A) Flow cytometry gating of M-MDSC in peripheral blood of mice bearing Mc38 tumor. (B) Statistical analysis of M-MDSC in Peripheral blood of tumor-bearing mice. (C) IHC analysis was performed with dissected tumor samples, and FOXP3 (D) and CD8 (E) staining were analyzed.

Table 1. Baseline information of breast cancer patients

Characteristics	Number of cases	Percentage
Stage		
Stage 3	9	29.00%
Stage 3A	15	48.40%
Stage 3B	1	3.20%
Stage 3C	6	19.40%
Gender		
Female	31	100.00%
T		
T2	11	35.50%
T3	18	58.10%
T4	2	6.50%
N		
Nx	10	32.30%
N1	4	12.90%
N2	11	35.50%
N3	6	19.40%
M		
M0	31	100.00%
Grade		
I~II	6	19.40%
II	19	61.30%
I~III	3	9.70%
II~III	2	6.50%
III	1	3.20%
Pathological Type		
DCIS	1	3.20%
IDC	27	87.10%
ILC	1	3.20%
MBC	2	6.50%
Total	31	100.00%

of upregulation and found that Gal-8 showed moderate to strong upregulation in 89% of IDC samples (**Figure 3D**). Some of the samples were stained positive only in tumors and negative in immune and mesenchymal cells, some were positively staining in both tumors and immune cells but with a clear boundary in between, and some were stained positive in both tumors and immune cells without a clear boundary (**Figure 3E**). There is also a set of samples in which tumor cells and immune cells showed diffused expression.

Heterogenicity of Galectin-8 expression in CRC patient samples

It is interesting to analyze not only tumors with high Gal-8 expression such as breast cancer, because there are tumors in which Gal-8 is significantly correlated with prognosis and staging, although the expression level of Gal-8 is not much higher in tumor samples. Colorectal cancer is one of them. The expression of Gal-8 in colorectal cancer is highly heterogeneous. We used a tissue microarray with paired cancer and normal samples from 47 patients. The baseline information of recruited colorectal cancer patients was summarized in **Table 2**. Gal-8 was widely expressed in normal colorectal tissues, although it was also increased in most tumor tissues (**Figure 4A**). With the formerly mentioned method, the IHC score was counted and demonstrated in **Figure 4B**, which showed elevated Gal-8 expression in both colon and rectal tumor samples. The increase in IHC score of tumor tissues compared to normal tissues was divided into 4 groups with representative photos, which were Negative (~0), Weak (1), Moderate (2), and Strong (3~4) (**Figure 4C**). Gal-8 was detected to be moderate to strong upregulated in 44.7% of samples (**Figure 4D**). In terms of expression pattern, CRC has tumor-specific positivity, immune cell-specific positivity, tumor and immune cell positivity, and diffuse positivity, in addition to negative staining (**Figure 4E**).

A higher level of LGALS8 predicts a more advanced stage and poor prognosis

In order to analyze the clinical relevance of LGALS8, we downloaded the LGALS8 gene expression and clinical data of the TCGA BRCA and COADREAD datasets from the Xena platform. For survival analysis, patients from the upper and lower quartiles were used as the LGALS8 high- and low-expression groups. Samples with Disease specific survival (DSS) data were analyzed as described in the methods. While no statistical significance was observed for breast cancer (**Figure 5A**), there is a difference in CRC (**Figure 5B**). Then, we analyzed the clinical features of both cancer types. As shown in **Figure 5C**, LGALS8 expression was higher in patients with older age. Moreover, higher LGALS8 was detected in all stages of breast cancer, compared to normal samples (**Figure 5D**). Although there was no significant difference in LGALS8 levels between AJCC stages of breast cancer, we found significantly higher levels of LGALS8 in the group of patients with lymph node metastasis compared to N0 stage when TNM staging analysis was performed (**Figure 5E**). Interestingly, there was

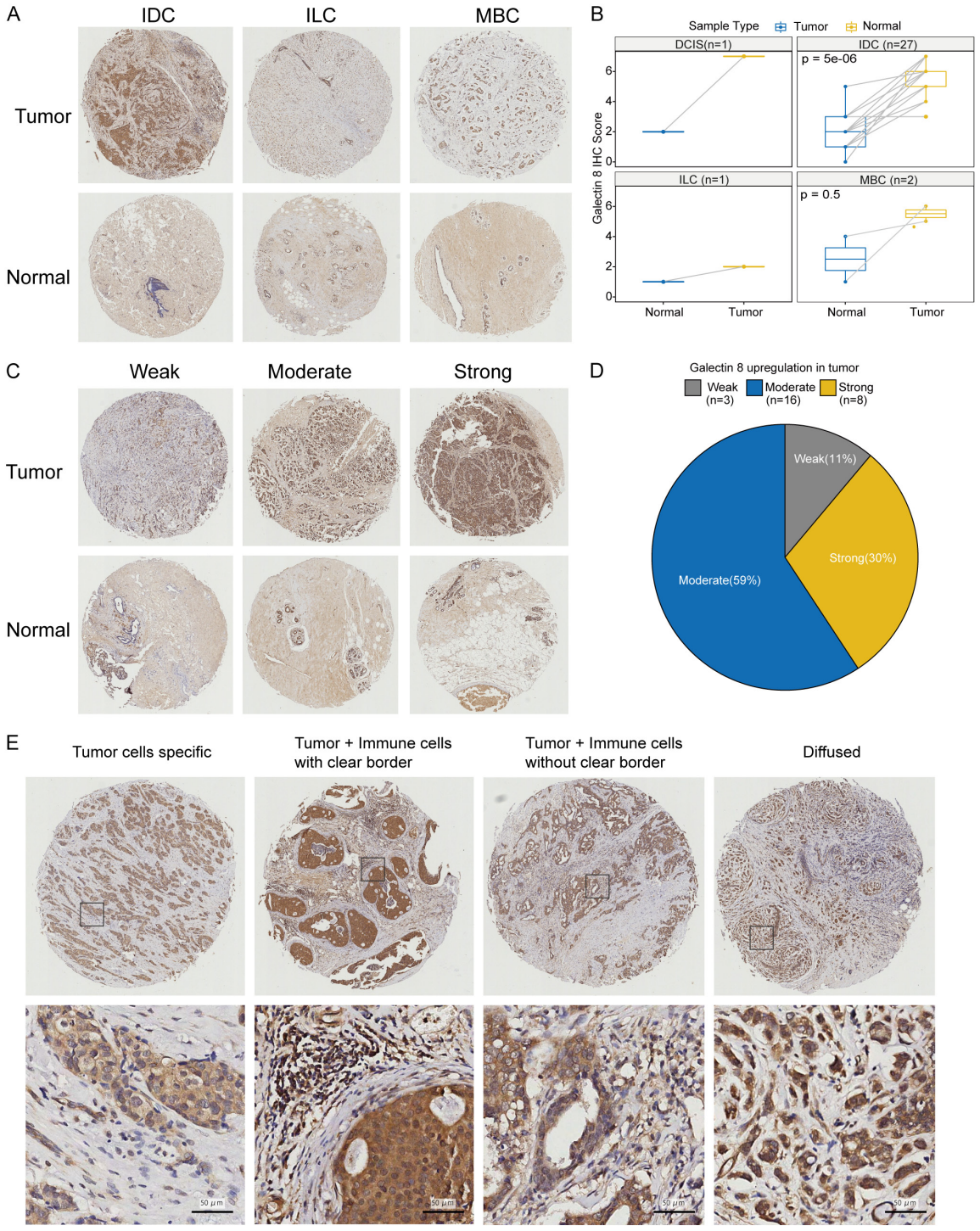


Figure 3. Gal-8 expression was elevated in breast cancer. A. Representative images of Gal-8 staining in different pathological types of breast cancer and pairwise normal tissues. B. Statistical analysis of Gal-8 staining in different pathological types of breast cancer and normal tissues pair-wisely. C. Representative images of Gal-8 grouped at different expression levels in IDC. D. Proportional statistics of the number of samples grouped at different expression levels of Gal-8 in IDC. E. Representative images of different expression patterns of Gal-8 classified in breast cancer.

also a difference of LGALS8 expression among patients who underwent different surgical procedures (**Figure 5F**). Previous study reported that Gal-8 participated in tumor progression

Table 2. Baseline information of colorectal cancer patients

Characteristics	Number of cases	Percentage
Stages		
Stage 2A	1	2.00%
Stage 3	36	73.50%
Stage 3B	1	2.00%
Gender		
Female	20	40.80%
Male	29	59.20%
Grade		
II	16	32.70%
II~III	10	20.40%
III	23	46.90%
N		
N0	12	24.50%
N1a	5	10.20%
N1b	5	10.20%
N2a	9	18.40%
N2b	18	36.70%
M		
M0	49	100.00%
Tumor site		
Ascending Colon	9	18.37%
Transverse Colon	5	10.20%
Descending Colon	4	8.16%
Sigmoid Colon	5	10.20%
Rectum	24	48.98%
Total	49	100.00%

and metastasis in mice [18]. Since clinicopathological features of different breast cancer subtypes vary greatly, we next analyzed LGALS8 expression in different immune subtypes, and found that C4 (lymphocyte depleted) type showed a higher LGALS8 level than other types (**Figure 5G**).

Similar to breast cancer, stratified subgroup analysis of patient age and gender showed that LGALS8 expression was higher in tumor samples from older patients compared to younger patients. Moreover, in the younger patient group, LGALS8 expression was higher in males compared to females (**Figure 5H**). Interestingly, the analysis by clustering of microsatellite instability revealed that LGALS8 levels were significantly higher in the MSI-H samples than in the MSI-L/MSS samples (**Figure 5I**). Direct analysis of the whole sample for tumor staging and LGALS8 levels did not yield meaningful results (data not shown). However, by grouping samples for MSI status, we found a correlation between LGALS8 and tumor prognosis in the MSI-L/MSS group, in which higher levels of LGALS8 in tumor samples with higher AJCC stage (**Figure 5J**). Consistently, samples with higher T and N staging showed an increasing trend in LGALS8 (**Figure 5K**, **5L**).

Discussion

We verified in an animal model that tumors with high expression of Gal-8 grew faster. By further analyzing the immune microenvironment, we found that tumors with high Gal-8 expression had more suppressive immune cell infiltration and fewer activating immune cells, confirming the regulatory role of Gal-8 on the tumor microenvironment, which is consistent with the results of clinical analysis of immune infiltration. LGALS8 gene had a negative association with active CD8+ T cells and a positive association with immature DC cells. In the tumor microenvironment, DC cells are impaired in maturation and exert immunosuppressive effects in the immature form [19-21]. Meanwhile, immune activators DR3 and OX40 negatively correlated with LGALS8 in various cancers. Studies have shown that DR3 is associated with activation of the antitumor response of CD8 cells [22]. Thus, the negative correlation of CD8 cells and DR3 with LGALS8 reveals that LGALS8 is involved in tumor immune suppression. Another negatively associated activator, OX40, has been widely recognized as an essential target in tumor immunotherapy [23, 24], and its down-regulation in correlation with LGALS8 may imply a predictive role of LGALS8 on the effect of OX40-targeted therapy.

Using the PDC database, we found that LGALS8 gene expression was elevated in a variety of cancers compared to normal tissues, especially in breast cancer, and that the prevalence of LGALS8 amplification was higher than the reported prevalence of PD-L1 amplification in solid tumors, suggesting that LGALS8 is a potential tumor-associated gene.

LGALS8 was statistically different for breast cancer patients in choosing different surgical procedures. One of the critical factors in deciding the procedure is the lymph node metastasis. Consistently, LGALS8 expression was significantly higher in patients with advanced N

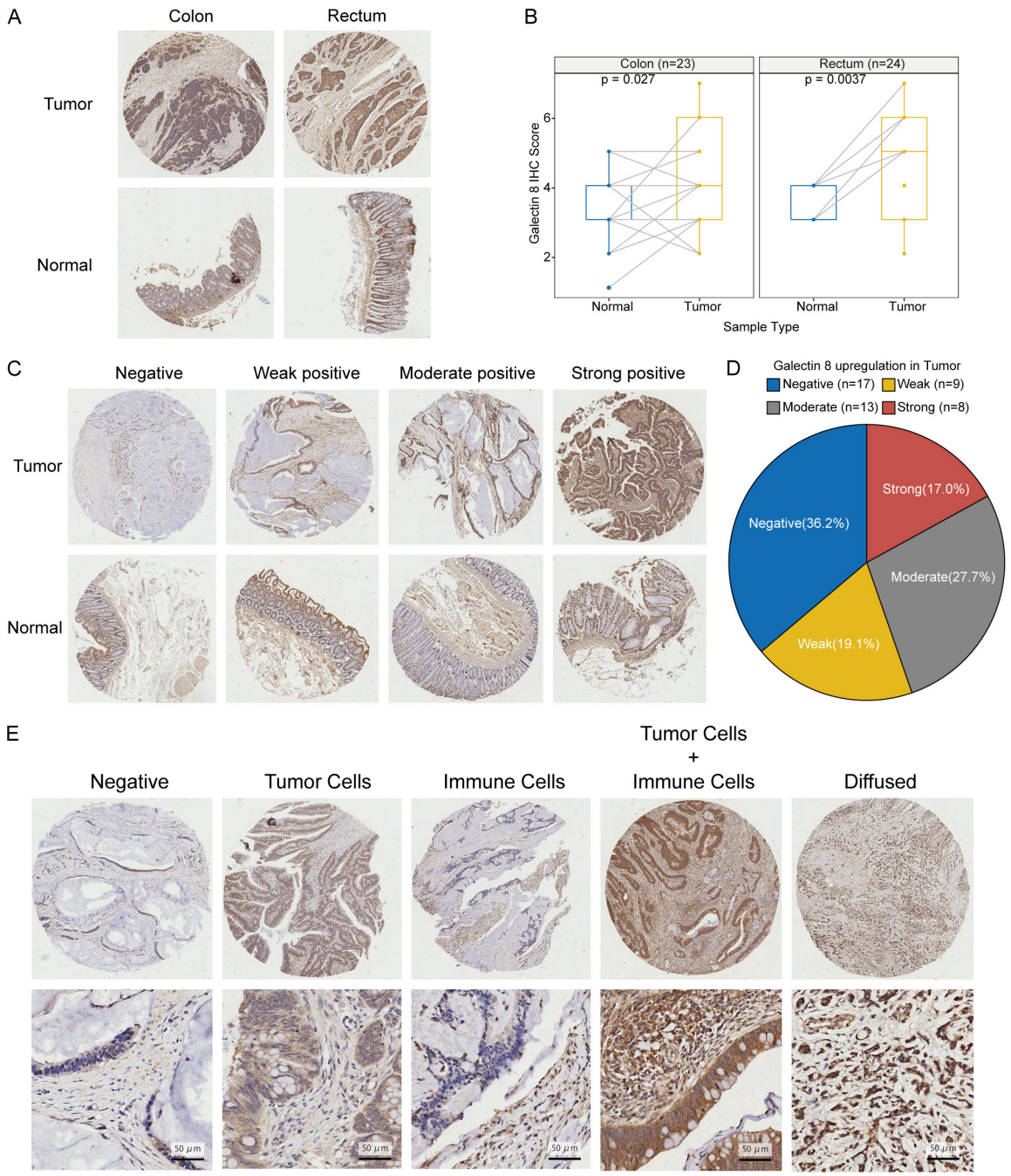
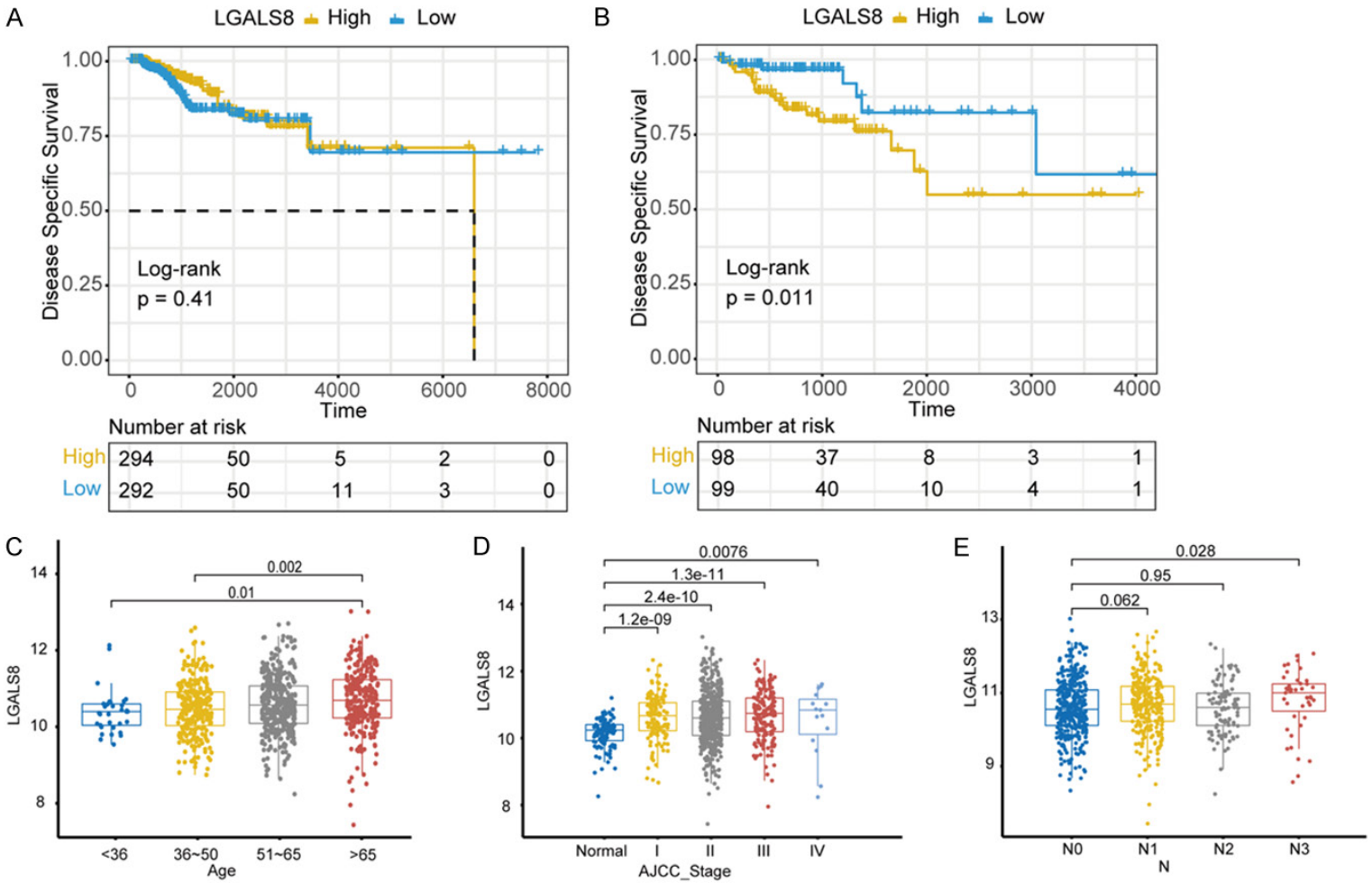


Figure 4. Heterogenicity of Gal-8 expression in CRC. A. Representative images of Gal-8 staining in different tumor sites of CRC and pairwise normal tissues. B. Statistical analysis of Gal-8 staining in different tumor sites of CRC and normal tissues pair-wisely. C. Representative images of Gal-8 grouped at different expression levels in CRC. D. Proportional statistics of the number of samples grouped at different expression levels of Gal-8 in CRC. E. Representative images of different expression patterns of Gal-8 classified in CRC.

stage. Differences in procedure selection suggest that LGALS8 is associated with patient prognosis. Moreover, there were significant differences in LGALS8 expression in different immune subtypes of breast cancer. LGALS8 expression was highest in the C4 group, in which lymphocytes were depleted, further suggesting the correlation between LGALS8 and immunosuppressive status. The results of IHC experiments on breast cancer samples con-


A
LGALS8 High Low
Disease Specific Survival
Log-rank
p = 0.41
Time
Number at risk
High 294 50 5 2 0
Low 292 50 11 3 0
B
LGALS8 High Low
Disease Specific Survival
Log-rank
p = 0.011
Time
Number at risk
High 98 37 8 3 1
Low 99 40 10 4 1
C
LGALS8
0.002
0.01
<36 36~50 51~65 >65
Age
D
LGALS8
0.0076
1.3e-11
2.4e-10
1.2e-09
Normal I II III IV
AJCC_Stage
E
LGALS8
0.028
0.95
0.062
N0 N1 N2 N3
N

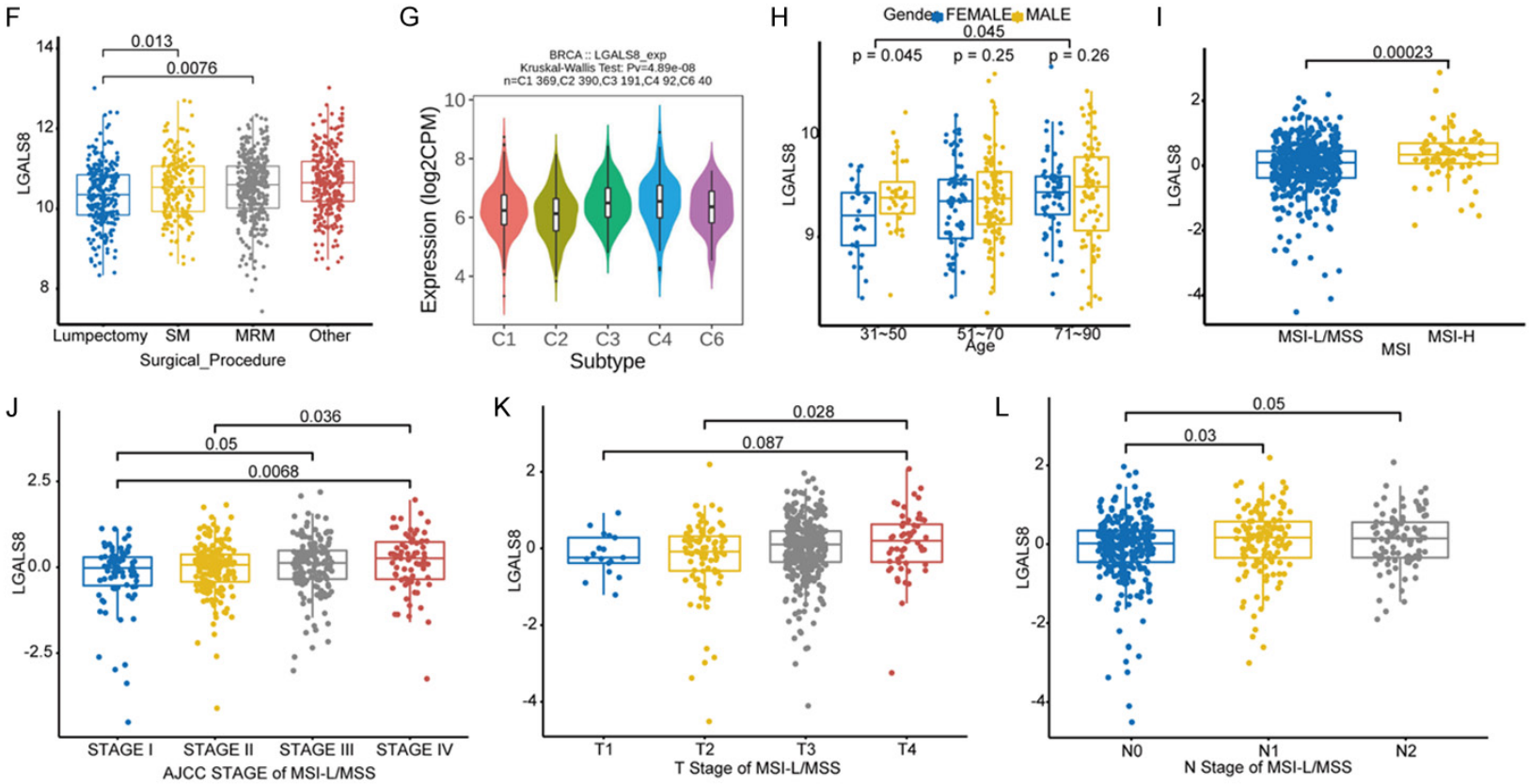


Figure 5. A higher level of LGALS8 predicts more advanced stage and poor prognosis. A. Disease specific survival analysis of LGALS8 high and low expression groups in breast cancer. B. Disease specific survival analysis of LGALS8 high and low expression groups in CRC. C. Age-stratified LGALS8 expression analysis of breast cancer. D. Stage-stratified LGALS8 expression analysis of breast cancer. E. N Stage-stratified LGALS8 expression analysis of breast cancer. F. Analysis of LGALS8 expression in breast cancer from patients who underwent different surgical procedures. G. Analysis of LGALS8 expression in different immune subtypes of breast cancer, including C1 (wound healing), C2 (IFN-gamma dominant), C3 (inflammatory), C4 (lymphocyte depleted), and C6 (TGF-b dominant). H. Age and gender-stratified LGALS8 expression analysis of CRC. I. Analysis of LGALS8 expression in CRC with different MSI status. J. Stage-stratified LGALS8 expression analysis of MSI-L/MSS CRC. K. T Stage-stratified LGALS8 expression analysis of MSI-L/MSS CRC. L. N Stage-stratified LGALS8 expression analysis of MSI-L/MSS CRC.

firmed Gal-8 elevation at the protein level in IDC samples. Despite inter-individual variability, Gal-8 had moderate to intense high expression in more than half of the tumor samples. Differently, LGALS8 was not significantly upregulated in CRC but was associated with disease-specific survival, and protein-level elevation was detected by tissue analysis. LGALS8 was higher in the MSI-H group, which had a high mutation burden. High levels of somatic mutation rates, which can lead to neoantigen generation, may stimulate antitumor immune responses [25] and bring preferable outcomes [26, 27]. The negative role of Gal-8 in the prognosis of colorectal cancer can be well explained by the suppression of tumor immunity by Gal-8. In addition, in the MSI-L/MSS group, LGALS8 increased with tumor progression.

In summary, the LGALS8 gene has potential value in the prognosis of both breast and colorectal cancers, with increased expression at the protein level compared to normal tissue. LGALS8 tends to have higher expression in subtypes with higher tumor mutational load, further suggesting its relevance to immune cell infiltration and regulation of immune factors. Conclusively, Gal-8 is a potential tumor therapeutic target. The current analysis is not sufficient to explain the mechanisms behind Gal-8 immune regulation, but it provided the direction of the target cells and pathways. Further studies are needed to support the therapeutic effect of this target on tumor immunity.

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Disclosure of conflict of interest

None.

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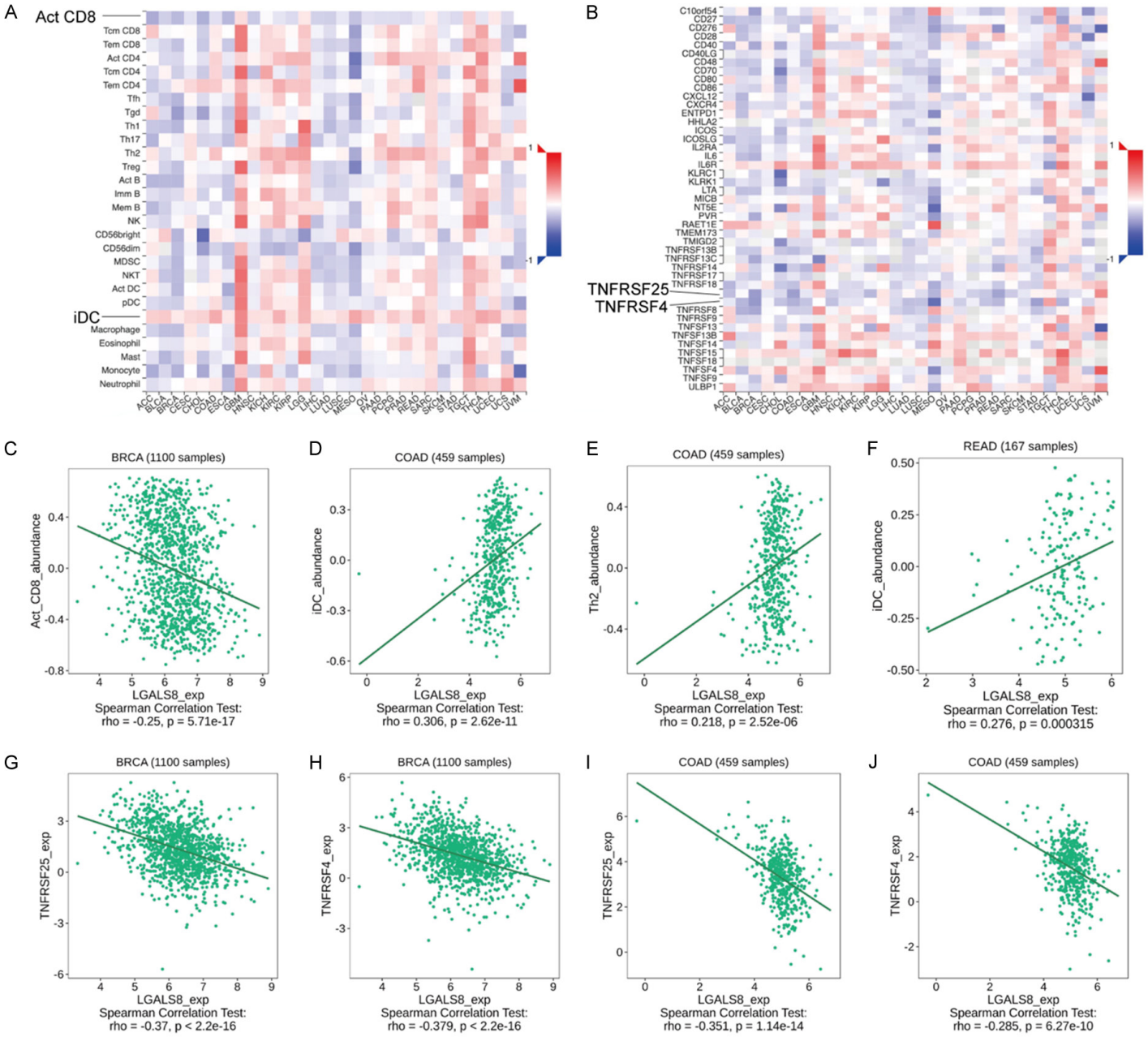
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Tgd
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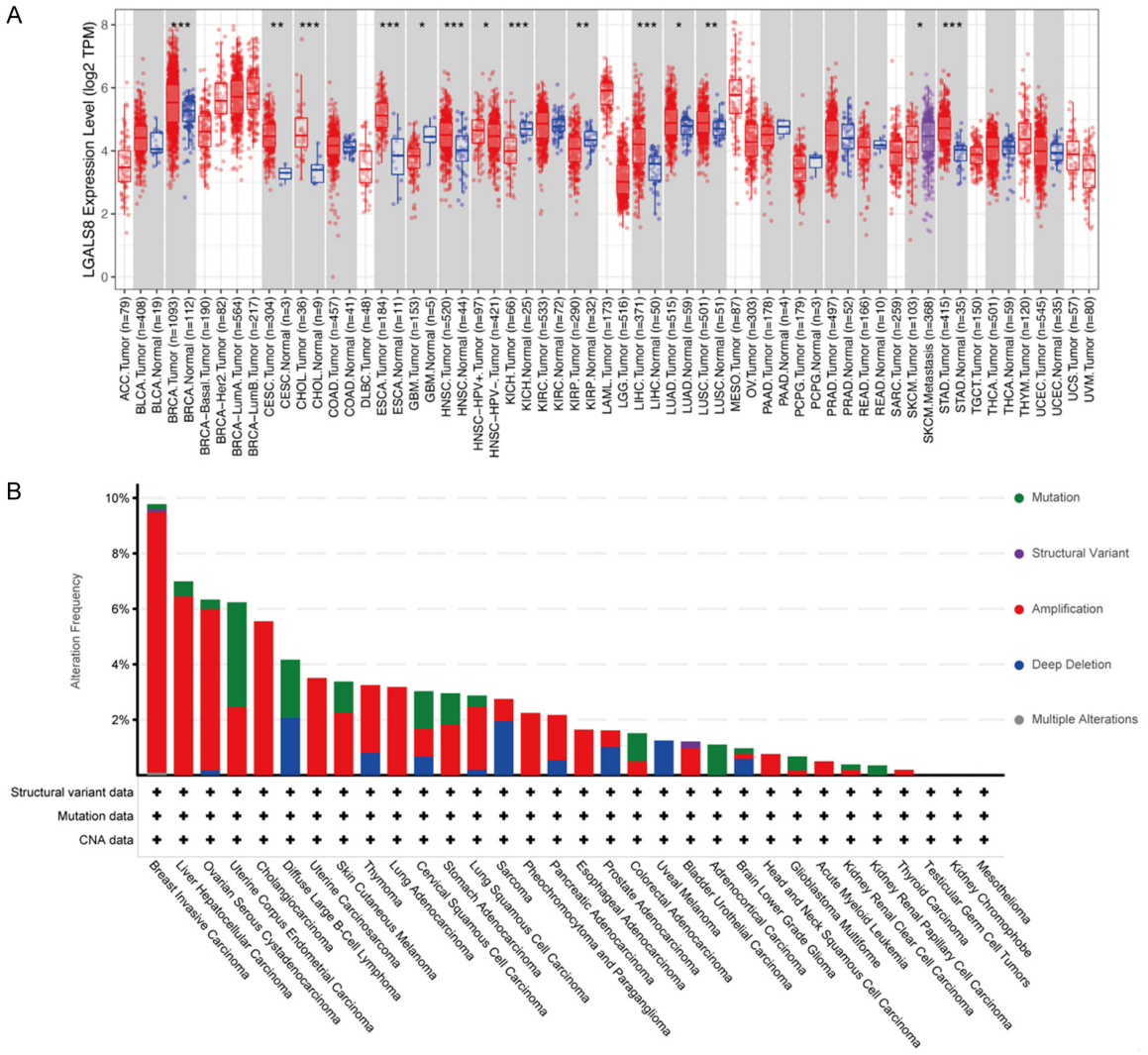
Th17
Th2
Treg
Act B
Imm B
Mem B
NK
CD56bright
CD56dim
MDSC
NKT
Act DC
pDC
iDC
Macrophage
Eosinophil
Mast
Monocyte
Neutrophil
B
TNFRSF25
TNFRSF4
C
BRCA (1100 samples)
Act_CD8_abundance
LGALS8_exp
Spearman Correlation Test:
rho = -0.25, p = 5.71e-17
D
COAD (459 samples)
iDC_abundance
LGALS8_exp
Spearman Correlation Test:
rho = 0.306, p = 2.62e-11
E
COAD (459 samples)
Th2_abundance
LGALS8_exp
Spearman Correlation Test:
rho = 0.218, p = 2.52e-06
F
READ (167 samples)
iDC_abundance
LGALS8_exp
Spearman Correlation Test:
rho = 0.276, p = 0.000315
G
BRCA (1100 samples)
TNFRSF25_exp
LGALS8_exp
Spearman Correlation Test:
rho = -0.37, p < 2.2e-16
H
BRCA (1100 samples)
TNFRSF4_exp
LGALS8_exp
Spearman Correlation Test:
rho = -0.379, p < 2.2e-16
I
COAD (459 samples)
TNFRSF25_exp
LGALS8_exp
Spearman Correlation Test:
rho = -0.351, p = 1.14e-14
J
COAD (459 samples)
TNFRSF4_exp
LGALS8_exp
Spearman Correlation Test:
rho = -0.285, p = 6.27e-10

Supplementary Figure 1. Correlation analysis of LGALS8 gene expression and immune infiltration in human cancers. (A) Heat map of LGALS8 correlation analysis with tumor-infiltrating immune cells. (B) Heat map of LGALS8 correlation analysis with immune modulators. (C) Spearman correlation test of LGALS8 and active CD8+ T lymphocytes in BRCA data set. (D) Spearman correlation test of LGALS8 and immature DC in COAD data set. (E) Spearman correlation test of LGALS8 and T helper 2 cell in COAD dataset. (F) Spearman correlation test of LGALS8 and immature DC in READ dataset. (G) Spearman correlation test of LGALS8 and TNFRSF25 in BRCA data set. (H) Spearman correlation test of LGALS8 and TNFRSF4 in BRCA data set. (I) Spearman correlation test of LGALS8 and TNFRSF25 in COAD data set. (J) Spearman correlation test of LGALS8 and TNFRSF4 in COAD data set.



Supplementary Figure 2. LGALS8 expression and gene alteration in pan-cancer. (A) Comparison of LGALS8 expression in pan-cancerous tumors and normal samples. (B) Statistical analysis of the types and frequency of genetic variants in pan-cancerous tumors.