High expression of EMP1 predicts a poor prognosis and correlates with immune infiltrates in bladder urothelial carcinoma

BO LIN, TIANWEN ZHANG, XIN YE and HONGYU YANG

Department of Oral and Maxillofacial Surgery, Peking University Shenzhen Hospital, Shenzhen, Guangdong 518036, P.R. China

Received August 2, 2019; Accepted May 21, 2020

DOI: 10.3892/ol.2020.11841

Abstract. Epithelial membrane protein 1 (EMP1) is a key gene that regulates cell proliferation and metastatic capability in various types of cancer, and serves an important role in tumor-immune interactions. However, the association between EMP1 and clinical prognosis, as well as the presence of tumor-infiltrating lymphocytes in bladder urothelial carcinoma (BLCA) remains unclear. The present study aimed to explore the relationship between EMP1 expression and tumor immune cell infiltration in BLCA. In the present study, EMP1 expression in BLCA was analyzed using the Oncomine database, The Cancer Genome Atlas (TCGA) and the Tumor Immune Estimation Resource (TIMER). The effects of EMP1 on clinical prognosis were evaluated using the Kaplan-Meier plotter and Gene Expression Profiling Interactive Analysis. The correlations between EMP1, cancer immune infiltrates and lymphocyte abundance were determined using the TIMER and Tumor immune system interaction database. In addition, correlations between EMP1 expression and gene markers in immune infiltrates were analyzed using cBioportal. The results demonstrated that, compared with adjacent normal tissues, EMP1 was downregulated in BLCA tissues. High expression of EMP1 was significantly associated with poor overall survival (OS) in BLCA cases obtained from TCGA. Multivariate Cox analysis revealed that EMP1 was an independent predictor of OS in patients with BLCA. Gene set enrichment analysis revealed that EMP1 was associated with cancer-related pathways and was positively correlated with the levels of infiltrating CD8\(^+\) T cells, macrophages, neutrophils and dendritic cells in BLCA. Further analysis demonstrated that EMP1 was significantly associated with the enrichment of multiple types of lymphocyte. EMP1 expression exhibited a strong correlation with a range of immune markers in BLCA. In conclusion, the results of the present study demonstrated that EMP1 was associated with a poor prognosis in patients with BLCA, and that the levels of immune infiltration and multiple immunomarker groups were associated with EMP1 expression. These results suggested that EMP1 may be used as a predictive biomarker to determine the prognosis and immune infiltration in BLCA.

Introduction

The incidence of bladder urothelial carcinoma (BLCA) ranks ninth among all malignancies in the global population and fourth among all malignancies occurring in men (1). The main risk factors of BLCA are cigarette smoking, exposure to toxic industrial chemicals and gases, and genetic susceptibility (2). Although standard treatment and supportive care have improved the overall survival (OS) and quality of life, the prognosis for patients with BLCA remains poor (3).

Immune-related mechanisms serve an important role in BLCA, and immunotherapeutic strategies are considered to be a promising direction for the treatment of BLCA (4,5). Immunootherapy seeks to manipulate the patient’s own immune response to improve the clinical outcome by promoting immune cells that can kill target cancer cells (5). The receptor-ligand pairing of programmed cell death protein-1 (PD-1) has been identified to be a crucial immune checkpoint; however, current immunotherapies using anti-PD-1 have only achieved partial response in patients with advanced BLCA (6,7). In addition, an increasing number of studies have demonstrated that patients with bladder cancer with a high level of tumor-infiltrating lymphocytes exhibit improved survival (8-10). However, studies on the prognostic value of immune cell subsets in patients with BLCA have yielded completely opposite results (11). Therefore, there is an urgent need to elucidate the specific immune phenotypes of tumor-immune interactions and identify novel immune-associated therapeutic targets in BLCA.

Epithelial membrane protein 1 (EMP1) is a protein-coding gene; its expression and significance in human cancer and its biological effects have been explored in vitro, demonstrating that EMP1 significantly reduces cell migration and invasion, and increases apoptosis and caspase-9 expression in carcinoma of the nasopharynx, stomach, breast and prostate (12-16). By contrast, studies of acute lymphoblastic leukemia (ALL) have revealed that EMP1 is an indicator of poor prognosis (17). Limited information is available about the mechanism underlying the effects of EMP1. Previous studies have

Key words: epithelial membrane protein 1, immune infiltration, bladder urothelial carcinoma, expression, prognosis

Correspondence to: Dr Hongyu Yang, Department of Oral and Maxillofacial Surgery, Peking University Shenzhen Hospital, 1120 Lianhua Road, Futian, Shenzhen, Guangdong 518036, P.R. China E-mail: yanghongyu0520@163.com
suggested that EMP1 works primarily by regulating signal transduction between cells and the extracellular matrix (18). EMP1 may be associated with the proto-oncogene c-myc (19), and other studies have revealed that EMP1 is regulated by the epidermal growth factor receptor (EGFR) (20,21). In addition, EMP1 is involved in the tight connection between cells, which may cause the occurrence and development of non-small cell lung cancer by activating the PI3K/AKT pathway (22). Wang et al (23), demonstrated that EMP family members and integrins synergistically regulate cell adhesion and migration in vitro, and integrin-based cell adhesion leads to autoimmunity diseases. Therefore, previous studies have suggested that EMP1 serves an important role in tumorigenesis and tumor immunity, but the effects of this gene on the OS of patients with BLCA and the underlying function of EMP1 in tumor-immune interactions remain unclear.

The present study aimed to comprehensively analyze EMP1 expression and its association with the prognosis of patients with BLCA. In addition, the correlation between EMP1 and tumor-infiltrating immune cells in the BLCA microenvironment was determined. The correlation between EMP1 and the immune cell-specific genes reported in literature, as well as immunological checkpoint-specific genes were further studied, and the expression level of EMP1 in tumor-tissue specimens and adjacent normal tissues of patients with BLCA were compared.

Materials and methods

Patients and tissue samples. Bladder cancer and adjacent normal tissues were collected from patients with BLCA at the Peking University Shenzhen Hospital (Shenzhen, China) between September 2018 and December 2019. The specimens were all collected during bladder cancer resection, and the distance between tumor tissue and adjacent normal tissue was >2 cm. BLCA was diagnosed and classified through pathological examination based on the World Health Organization classification system (24). Specimens from patients with a history of preoperative chemotherapy were excluded. The study protocol was approved by the Ethics Committees for Human Experiments of Peking University Shenzhen Hospital. All patients signed an informed consent form before sample collection.

Image processing. The paraffin-embedded tumor sections (5 μm thick) were stained with H&E or antibodies against EMP1 (cat. no. ab230445; 1:75; Abcam) according to the routine immunohistochemical staining method (25). All images shown are wide-field light microscopy images that were acquired at sufficient resolution.

Acquisition of mRNA data. The gene expression data and corresponding clinical information were downloaded from TCGA website (https://portal.gdc.cancer.gov) for BLCA, and estimated as \( \log_2(x+1) \) transformed RSEM normalized counts (26). BLAC samples comprised samples of 404 patients with BCLA, including 28 cases with adjacent non-tumorous tissue as control group. All data were processed using R-studio software (v3.5.3) (27). The ‘ESTIMATE’ R package was used to predict the presence of infiltrating stromal/immune cells in tumor tissues using gene expression data (28).

Oncomine database analysis. The levels of EMP1 gene expression in various types of cancer were identified using the Oncomine database (https://www.oncomine.org). The threshold was determined according to the following values: P-value of 0.001 and fold-change of 2.

Kaplan-Meier plotter database analysis. The Kaplan Meier plotter (http://kmplot.com/analysis/) is capable of assessing the effect of 54,000 genes on patient survival in 21 types of cancer (29). The association between EMP1 expression and survival in patients with BLCA was analyzed using the Kaplan-Meier plotter. The hazard ratio (HR) with 95% confidence intervals (CIs) and log-rank P-value were computed.

Tumor Immune Estimation Resource (TIMER) database analysis. TIMER (https://cistrome.shinyapps.io/timer/), which is a comprehensive resource for the systematic analysis of immune infiltrates across various types of cancer, was used in the present study to analyze the level of EMP1 expression in BLCA and the correlation between EMP1 expression and the abundance of immune infiltrates, including B cells, CD4+ T cells, CD8+ T cells, neutrophils, macrophages and dendritic cells via gene modules. The immune cell infiltration score of each patient in TCGA database was obtained using TIMER, and the patients were divided into high and low score groups based on the median value.

Immunological analysis by Tumor immune system interaction database (TISIDB). The correlations between the abundance of tumor-infiltrating lymphocytes and EMP1 expression were analyzed using TISIDB (http://cis.hku.hk/TISIDB), which is a web portal for tumor and immune system interactions integrating multiple heterogeneous data types (30). In the present study, the enrichment data of 28 immune cells provided by TISIDB, including activated CD8+ cells (Act CD8), central memory CD8 cells (Tcm CD8), effector memory CD8 cells (Tcm CD8), activated CD4+ cells (Act CD4), central memory CD4 cells (Tcm CD4), effector memory CD4 cells (Tem CD4), T follicular helper cells (Thf), gamma delta T cells (Tgd), type 1 T helper cells (Th1), type 17 T helper cells (Th17), type 2 T helper cells (Th2), regulatory T cells (Treg), activated B cells (Act B), immature B cells (Inn B), memory B cells (Mem B), natural killer (NK) cells, CD56 bright NK cells (CD56bright), CD56 dim NK cells (CD56dim), myeloid derived suppressor cells (MDSCs), NK T cells (NKT), activated dendritic cell (Act DCs), plasmacytoid DCs (pDCs), immature DCs (iDCs), macrophages, eosinophils, mast cells (Mast), monocytes and neutrophils, were used to calculate the relationship with the expression of EMP1 in BLCA.

Gene expression and survival analysis in Gene Expression Profiling Interactive Analysis (GEPIA). The online database GEPIA (http://gepia.cancer-pku.cn/index.html) was used to analyze the differential expression of EMP1 and its prognostic values.

Co-expression analysis in cBioPortal. The cBioPortal for Cancer Genomics (https://www.cbioportal.org) is an open-access, open-source resource for interactive exploration of multidimensional cancer genomics datasets (31,32) that was used in the present study to determine the correlations
between EMP1 expression and tumor-infiltrating immune cell markers. The gene markers of the tumor-infiltrating immune cells included markers of CD8⁺ T cells, T cells (general), B cells, monocytes, tumor associated macrophages (TAMs), M1 macrophages, M2 macrophages, neutrophils, NK cells, DCs, Th1 cells, Th2 cells, follicular helper T (Tfh) cells, Th17 cells, Tregs and exhausted T cells. The Spearman rank correlation analysis was used to determine the correlation coefficient. EMP1 expression was plotted on the x-axis, and the expression levels of other genes of interest were represented on the y-axis.

Gene set enrichment analysis (GSEA). To identify the potential mechanisms underlying the effects of EMP1 expression on BLCA prognosis, GSEA was performed to detect whether an a priori defined set of genes exhibited statistically significant differential expression between the high and low EMP1 expression groups. Gene sets with a P-value < 0.05 and false discovery rate (FDR) < 0.25 in the enrichment of MSigDB Collection (c2.cp.kegg.v6.2. symbols) were considered to be significantly enriched.

Statistical analysis. Survival curves were generated using the GEPIA and Kaplan-Meier databases. T test and paired t test were implemented in prism and R software, and the results were displayed in the form of pictures and tables after sorting out the results. Receiver operating characteristic (ROC) curve and area under the curve (AUC) were used to demonstrate the predictive ability of EMP1 for 3- and 5-year OS. The results generated in Oncomine are presented as P-values, fold-changes and ranks. The results of the Kaplan-Meier plots and GEPIA are displayed with HR and P or Cox P-values from a log-rank test. Univariate Cox analysis was performed to select the potential prognostic factors, and multivariate Cox analysis was performed to verify the association between EMP1 expression and survival along with other clinical features. P<0.05 was considered to indicate a statistically significant difference.

Results

Association between EMP1 expression and clinicopathologic variables in BLCA. As presented in Fig. 1A, EMP1 expression was upregulated in brain, breast, kidney and liver cancer, as well as in leukemia, lymphoma, melanoma and sarcoma compared with that in normal tissues. In addition, downregulation of EMP1 was observed in bladder, breast, cervical, colorectal, esophageal, head and neck, lung, ovarian
cancer and sarcoma in a number of data sets. Differential expression was observed between tumor and normal tissues for EMP1 in BLCA data from TCGA. The results indicated that EMP1 was upregulated in BLCA compared with adjacent normal tissues (P<0.001; Fig. 1B) and with paired adjacent healthy tissues (P<0.001; Fig. 1C). In addition, the expression of EMP1 significantly increased with clinical stage (P<0.001; Fig. 1D). The expression data of EMP1 are presented in the supplementary materials (Table S1 and Table SII).

Upregulation of EMP1 was significantly associated with advanced age (≥60 vs. <60, P=0.040), histological subtype (papillary vs. non-papillary, P=0.043), pathologic T classification (III-IV vs. I-II, P=0.008), immune score (high vs. low, P<0.001) and stromal score (high vs. low, P<0.001; Table I). However, no significant associations between EMP1 expression and sex, lymphovascular invasion, tumor recurrence, pathologic M/N classification and pathologic stage were observed.

Survival outcomes and multivariate analysis. The analysis of BLCA cases in TCGA revealed that the 5-year OS of the high EMP1 expression group was significantly lower compared with that of the low expression group (P<0.001; Fig. 2A). Receiver operating characteristic (ROC) curve analysis demonstrated the predictive ability of EMP1 for 3- and 5-year OS with the area under the curve (AUC) of 0.737 and 0.739, respectively (Fig. 2B). The association between EMP1 expression and survival outcome was further confirmed by Kaplan-Meier survival analysis (Fig. 2C). In addition, the survival analysis from TIMER dataset also showed that high levels of immune infiltration in five types of immune cells and tumor purity in the TIMER dataset. EMP1 expression was significantly correlated with tumor purity and infiltration of B cells, CD8+ T cells, macrophages, neutrophils and DCs in BLCA as shown in Fig. 3. These results suggested that EMP1 may serve a specific role in immune infiltration in BLCA. In addition, the survival analysis from TIMER dataset also showed that high levels of infiltrating CD8+ T cells were significantly associated with poor OS in patients with BLCA (P<0.006), and high expression of EMP1 predicted poor OS (P<0.01; Fig. 4).

Association between the abundance of tumor-infiltrating lymphocytes and EMP1 expression. The results obtained from TISIDB demonstrated that EMP1 expression was strongly associated with the abundance of Tcm CD8 and neutrophil cells (both correlation coefficients >0.5 and P<0.05), and moderately related with the abundance of Mem B, Act CD4, Tcm CD4, Tem CD8, Act DC, pDC, iDC, NK, NKT, eosinophil, Th1, Th2, Treg, macrophage, mast and MDSC (all correlation coefficients between 0.3-0.5 and all P<0.05) (Fig. 5).

Correlation between EMP1 expression and immune markers. The correlations between EMP1 expression and immune

Table I. Logistic regression of the expression of EMP1 and the clinicopathological characteristics of patients with bladder urothelial carcinoma.

| Characteristic                          | Total | Odds ratio in EMP1 expression | P-value |
|----------------------------------------|-------|--------------------------------|---------|
| Age, years (≥60 vs. <60)               | 407   | 1.64 (1.02-2.67)               | 0.04a   |
| Subtype (papillary vs. non-papillary)  | 402   | 0.65 (0.42-0.98)               | 0.04a   |
| Sex (male vs. female)                  | 407   | 0.80 (0.51-1.23)               | 0.30    |
| Lymphovascular invasion (positive vs. negative) | 273   | 1.29 (0.80-2.09)               | 0.30    |
| Recurrence (yes vs. no)                | 371   | 1.23 (0.79-1.89)               | 0.36    |
| Pathologic T classification (III-IV vs. I-II) | 374   | 1.80 (1.17-2.81)               | 8.4x10^-3a |
| Pathologic M classification (M+ vs. M0) | 200   | 2.43 (0.66-11.56)              | 0.21    |
| Pathologic N classification (N0 vs. N+) | 367   | 0.90 (0.59-1.38)               | 0.64    |
| Pathologic stage (III-IV vs. I-II)     | 405   | 1.496 (0.984-2.283)            | 0.06    |
| Stromal score (high vs. low)           | 408   | 1.65 (0.25-0.56)               | 1.91x10^-6a |
| Immune score (high vs. low)            | 408   | 1.54 (0.29-0.64)               | 2.89x10^-3a |

aP<0.05. bGrouped according to the median value. EMP1, epithelial membrane protein 1.
marker genes of the different immune cells, including CD8+ T cells, B cells, monocytes, TAMs, M1 and M2 macrophages, neutrophils, NK cells and DCs are presented in Table IV. The results revealed that the level of EMP1 expression was significantly correlated with immune markers of various immune cells. The expression levels of the majority of marker sets of monocytes, TAMs and M2 macrophages exhibited a significant correlation with EMP1 expression. In particular, chemokine (C-C motif) ligand (CCL)-2, CD68, interleukin 10 (IL10) of TAMs, prostaglandin-endoperoxide
LIN et al.: EMP1 IS ASSOCIATED WITH PROGNOSIS AND IMMUNE INFILTRATION IN BLCA

Table II. Univariate and multivariate analysis of overall survival using the Cox proportional hazard regression model (N=397).

| Parameter     | Univariate analysis |          |          | Multivariate analysis |          |          |
|---------------|---------------------|----------|----------|-----------------------|----------|----------|
|               | HR                  | 95% CI   | P-value  | HR                    | 95% CI   | P-value  |
| Age           | 1.033               | 1.017-1.049 | 4.67x10^{-5} | 1.029               | 1.012-1.046 | 5.0x10^{-4} |
| Subtype^c     | 0.650               | 0.454-0.929 | 0.018^a  | 0.814               | 0.558-1.187 | 0.286    |
| Sex           | 0.880               | 0.633-1.224 | 0.449    | 0.862               | 0.611-1.215 | 0.395    |
| Pathologic stage | 2.240             | 1.538-3.262 | 2.6x10^{-5} | 1.880               | 1.261-2.802 | 0.002^a  |
| B cell        | 0.0741              | 0.007-0.754 | 0.028    | 0.116               | 0.010-1.269 | 0.078    |
| CD4^+ T cell  | 0.286               | 0.042-1.954 | 0.202    | 1.415               | 0.047-42.416 | 0.841    |
| CD8^+ T cell  | 5.701               | 1.432-22.370 | 0.014^a  | 5.243               | 0.407-67.569 | 0.204    |
| Neutrophil    | 0.652               | 0.076-5.614 | 0.697    | 0.006               | 0.00-0.737 | 0.037^a  |
| Macrophage    | 18.825              | 4.514-78.518 | 5.62x10^{-5} | 8.867               | 1.506-52.216 | 0.016^a  |
| Dendritic     | 0.898               | 0.428-1.884 | 0.776    | 1.350               | 0.300-6.039 | 0.696    |
| Stromal score^a | 0.728            | 0.530-0.973 | 0.033^a  | 1.043               | 0.710-1.530 | 0.832    |
| Immune score^a | 1.072             | 0.795-1.446 | 0.649    | 1.015               | 0.668-1.542 | 0.944    |
| EMP1          | 8.927               | 3.713-21.466 | 1.01x10^{-6} | 6.614               | 2.390-18.301 | 2.75x10^{-4} |

^aP<0.05. ^cGrouped according to the median value. ^Papillary vs. non-papillary subtype. EMP1, epithelial membrane protein 1; HR, hazard ratio; CI, confidence interval.

Table III. Gene sets enriched analysis of upregulated EMP1 in BLCA.

| NAME                              | NES  | P-value |
|-----------------------------------|------|---------|
| KEGG ECM RECEPTOR INTERACTION      | 1.696| 0.000   |
| KEGG FOCAL ADHESION               | 1.795| 0.000   |
| KEGG PATHWAYS IN CANCER           | 1.592| 0.002   |
| KEGG NEUROTROPHIN SIGNALING PATHWAY | 1.595| 0.006   |
| KEGG AXON GUIDANCE                | 1.612| 0.008   |
| KEGG CELL ADHESION MOLECULES CAMS | 1.547| 0.008   |
| KEGG GAP JUNCTION                 | 1.527| 0.010   |
| KEGG ENDOMETRIAL CANCER           | 1.625| 0.017   |
| KEGG HEMATOPOIETIC CELL LINEAGE   | 1.484| 0.019   |
| KEGG NICOTINATE AND NICOTINAMIDE METABOLISM | 1.523| 0.023   |
| KEGG ADHERENS JUNCTION            | 1.753| 0.024   |
| KEGG GLIOMA                       | 1.559| 0.027   |
| KEGG RENAL CELL CARCINOMA         | 1.530| 0.046   |
| KEGG FC GAMMA R MEDIATED PHAGOCYTOSIS | 1.536| 0.046   |

Gene sets with P<0.05 and false discovery rate <0.25 were considered significant. NES, normalized enrichment score. EMP1, epithelial membrane protein 1; BLCA, bladder urothelial carcinoma.

synthase 2 (PTGS2), interferon regulatory factor 5 (IRF5) of the M1 phenotype, CD163, V-set and immunoglobulin domain containing 4 (VSIG4), and membrane spanning 4-domains A4A (MS4A4A) of the M2 phenotype significantly correlated with EMP1 expression in BLCA (P<0.05). Suggesting that EMP1 may regulate macrophage polarization. High EMP1 expression is associated with a high level of DC infiltration in BLCA; DC markers, such as Major Histocompatibility Complex, Class II, DP Beta 1 (HLA-DPB1) and Integrin Subunit Alpha X (ITGAX) also exhibited a significant correlation with EMP1. In addition, for Tregs, a moderate positive correlation was observed between forkhead box P3 (FOXP3), C-C Motif Chemokine Receptor 8 (CCR8) and EMP1 in BLCA. Therefore, these results further confirmed that EMP1 was associated with infiltrating immune cells in BLCA, which suggested that EMP1 may serve a crucial role in immune enhancement in the BLCA microenvironment.
immune checkpoints that have been reported in the literature were further assessed. Programmed cell death 1 ligand 1 (CD274), programmed cell death 1 ligand 2 (PDCD1LG2), hepatitis A virus cellular receptor 2 (HAVCR2), cytotoxic T-lymphocyte associated protein 4 (CTLA4), lymphocyte-activating 3 (LAG3), programmed cell death 1 (PDCD1) and T cell immunoreceptor with Ig and ITIM domains (TIGIT) were selected for analysis as they have been previously reported to be immunological checkpoint-specific genes. EMP1 was significantly associated with the expression of these genes (P<0.05; Fig. 6).

Expression of EMP1 in BLCA specimens and adjacent normal tissue. Bladder cancer specimens from eight patients with pathologically confirmed BLCA were analyzed in the present study. The patients were all male, and the age range was 66-82 years. Immunohistochemical staining of BLCA tissue specimens and adjacent normal tissues revealed that EMP1 was strongly stained in adjacent normal tissues, but the staining intensity was lower in tumor tissues (Fig. 7).

Discussion

The present study examined the levels of EMP1 expression and the systematic prognostic landscape in BLCA using independent datasets in the Oncomine and TCGA databases. Differential levels of EMP1 expression between cancer and normal tissues were observed. Consistent prognostic associations of EMP1 expression in BLCA were identified, in
which high levels of EMP1 expression were associated with a poor OS rate, and further analysis using the Kaplan-Meier plotter revealed that EMP1 overexpression was associated with poor BLCA prognosis in stages III to IV. The results of the TIMER database analysis demonstrated significant positive correlations between the levels of EMP1 expression and the infiltration of CD8+ T cells, macrophages, neutrophils and DCs in BLCA; however, EMP1 expression was...
Table IV. Correlation analysis between EMP1 and the genes and markers of immune cells in cBioportal.

| Cell type       | Gene   | Spearman's correlation | P-value     |
|-----------------|--------|------------------------|-------------|
| CD8+ T cell     | CD8A   | 0.214                  | 1.30x10^{-5a} |
|                 | CD8B   | 0.092                  | 0.060       |
|                 | CD80   | 0.375                  | 4.48x10^{-15a} |
| T cell (general)| CD3D   | 0.160                  | 0.001^a     |
|                 | CD3E   | 0.240                  | 9.70x10^{-7a} |
|                 | CD2    | 0.207                  | 2.42x10^{-5a} |
| B cell          | CD19   | 0.104                  | 0.036^a     |
|                 | CD79A  | 0.177                  | 3.26x10^{-4a} |
| Monocyte        | CD86   | 0.434                  | 3.88x10^{-20a} |
|                 | CD115  | 0.417                  | 4.11x10^{-17a} |
| TAM             | CCL2   | 0.342                  | 1.24x10^{-12a} |
|                 | CD68   | 0.366                  | 2.38x10^{-14a} |
|                 | IL10   | 0.394                  | 1.25x10^{-16a} |
| M1 Macrophage   | NOS2   | 0.131                  | 0.008^a     |
|                 | IRF5   | -0.199                 | 5.37x10^{-5a} |
|                 | PTGS2  | 0.296                  | 1.10x10^{-9a} |
| M2 Macrophage   | CD163  | 0.442                  | 6.27x10^{-21a} |
|                 | VSIG4  | 0.436                  | 2.22x10^{-10a} |
|                 | MS4A4A | 0.403                  | 2.85x10^{-18a} |
| Neutrophil      | ITGAM  | 0.381                  | 1.51x10^{-15a} |
|                 | CCR7   | -0.141                 | 0.004^a     |
| Natural killer cell | KIR2DL1 | 0.200                  | 4.87x10^{-3a} |
|                 | KIR2DL3| 0.223                  | 5.35x10^{-4a} |
|                 | KIR2DL4| 0.268                  | 3.92x10^{-4a} |
|                 | KIR3DL1| 0.141                  | 0.004^a     |
|                 | KIR3DL2| 0.170                  | 5.83x10^{-4a} |
|                 | KIR3DL3| 0.067                  | 0.179       |
|                 | KIR2DS4| 0.149                  | 0.003^a     |
| Dendritic cell  | HLA-DPB1| 0.279                  | 9.81x10^{-9a} |
|                 | HLA-DQB1| 0.240                  | 9.04x10^{-7a} |
|                 | HLA-DRA | 0.270                  | 3.06x10^{-8a} |
|                 | HLA-DPA1| 0.277                  | 1.35x10^{-8a} |
|                 | CD1C   | 0.262                  | 8.34x10^{-6a} |
|                 | ITGAX  | 0.392                  | 1.99x10^{-16a} |
|                 | NRP1   | 0.455                  | 3.15x10^{-22a} |
| Th1             | TBX21  | 0.210                  | 1.98x10^{-3a} |
|                 | STAT4  | 0.364                  | 2.94x10^{-14a} |
|                 | STAT1  | 0.282                  | 6.46x10^{-9a} |
|                 | IFN-g  | 0.161                  | 0.001^a     |
|                 | TNF-a  | 0.153                  | 0.002^a     |
| Th2             | GATA3  | -0.497                 | 7.77x10^{-27a} |
|                 | STAT6  | -0.173                 | 4.48x10^{-6a} |
|                 | IL13   | 0.181                  | 2.38x10^{-4a} |
| Tfh             | BCL6   | -0.144                 | 0.004^a     |
|                 | IL21   | 0.041                  | 0.409       |
| Th17            | STAT3  | 0.431                  | 7.67x10^{-20a} |
|                 | IL17A  | -0.071                 | 0.153       |
negative correlated with the infiltration of B cells, as well as the degree of tumor purity. Further analysis revealed that the upregulation of EMP1 was significantly positively associated with an abundance of macrophages, but negatively associated with the levels of Tregs, plasma and CD4-naïve T cells. The co-expression analysis of EMP1 and the previously reported immunolabeled genes also yielded consistent results. The expression level of EMP1 in BLCA tissues was further validated using independent specimens. These results suggested that EMP1 may be a prognostic biomarker, as well as an important factor for the recruitment and regulation of infiltrating immune cells in BLCA.

EMP1 was selected as the research object mainly based on the following considerations: Through the analysis of multiple databases, a significant difference was observed in the expression of EMP1 between bladder cancer and paired normal tissues, and only one study suggested that EMP1 was expressed at low levels in bladder cancer (33). However, it was necessary to further study this gene as its function in bladder cancer was not clear. EMP1 belongs to the peripheral myelin protein 22 (PMP22) family and has high homology among the family members (34,35). PMP22 is considered to serve an important role in the immune response (36-38). Thus, it was speculated that EMP1 may exert a similar function.

**Table IV. Continued.**

| Cell type | Gene   | Spearman’s correlation | P-value  |
|-----------|--------|------------------------|----------|
| Treg      | FOXP3  | 0.254                  | 1.91x10^{-7}a |
|           | CCR8   | 0.274                  | 1.80x10^{-6}a |
|           | TGFb   | 0.314                  | 8.82x10^{-11} |

*P<0.05. TAM, tumor-associated macrophage; Th, T helper cell; Tfh, follicular helper T cell; Treg, regulatory T cell; CSF1R, colony-stimulating factor 1 receptor; CCL2, C-C motif chemokine ligand 2; IL10, interleukin 10; INOS (NOS2), nitric oxide synthase 2; IRF5, interferon regulatory factor 5; PTGS2, prostaglandin-endoperoxide synthase 2; VSIG4, V-set and immunoglobulin domain-containing 4; MS4A4A, membrane-spanning 4 domains A4A; CEACAM8, carcinoembryonic antigen-related cell adhesion molecule 8; ITGAM, integrin subunit αM; CCR7, C-C motif chemokine receptor 7; KIR2DL1, killer cell immunoglobulin-like receptor, two Ig domains and long cytoplasmic tail 1; HLA-DPB1, major histocompatibility complex class II DP β1; CD1C, CD1c molecule; ITGAX, integrin subunit αX; NRPI, neuropilin 1; TBX21, T-box 21; STAT4, signal transducer and activator of transcription 4; IFN-γ, interferon γ; TNF-α, tumor necrosis factor α; GATA3, GATA-binding protein 3; BCL6, BCL6 transcription repressor; FOXP3, forkhead box P3; CCR8, C-C motif chemokine receptor 8; STAT5B, signal transducer and activator of transcription 5B; TGFBI, transforming growth factor β1.

Figure 6. Correlations between EMP1 and immune checkpoint-related genes. Red represents a positive correlation, and blue represents a negative correlation. The size and color intensity of the circle indicates the strength of the correlation. The number in the circle indicates the correlation coefficient. BLCA, bladder urothelial carcinoma; EMP1, epithelial membrane protein 1.
Alteration of EMP1 has been implicated in different types of human cancer. Cancer cell lines transfected with EMP1 in vitro, including PC-3 prostate cancer, SW-480 colon cancer and MCF-7 breast cancer have been demonstrated to exhibit a high rate of apoptosis and a poor survival rate (14,15). The EMP1 gene expression has also been demonstrated to be downregulated in laryngeal, esophageal, head and neck, sclerosing gastric and prostate cancer, as well as in uterine fibroids compared with that in normal tissues (39-43). Sun et al (12), have demonstrated that as a tumor suppressor gene, high expression of EMP1 can improve the 5-year survival rate of patients with nasopharyngeal cancer. This result was also confirmed in gastric cancer (13), and EMP1 was reported to be associated with nodal metastasis in oral squamous cell cancer (44). EMP1 overexpression has also been established to be associated with poor OS in pediatric leukemia (17).

However, some studies have yielded different results. Lai et al (45), demonstrated that EMP1 expression levels were higher in non-small-cell lung cancer compared with those in the benign control group. When a recombinant adenovirus overexpressing EMP1 was constructed and virus-infected PC9 cells were transplanted into nude mice, the growth of the transplanted tumors could be observed. Another study by Zhang et al (46), demonstrated that EMP1 was upregulated in human gliomas, and that EMP1 expression was significantly increased in patients with World Health Organization tumor grade III-IV compared with grade I-II.

The results of the present study demonstrated that EMP1 expression was downregulated in BLCA compared with that in normal tissues, but patients with low EMP1 expression exhibited an improved OS rate compared with those in the high expression group. These conflicting results may be caused by the function of EMP1. Previous studies have demonstrated that EMP1 serves an important role in cell differentiation (35,47,48) and proliferation (34,49); thus, EMP1 promotes cell differentiation, whereas tumor cells are characterized by de-differentiation. Low degree of tumor cell differentiation leads to low expression of EMP1. In addition, EMP1 is a direct or indirect target gene of the classical proto-oncogene c-myc, which serves a role in promoting cell proliferation (34). The results of the present study also demonstrated that in patients with high EMP1 expression, the prognosis of BLCA was poor. This result was contrary to that of Peter et al (33), whose findings suggested that low expression of EMP1 was associated with an increased risk of urothelial cancer-specific mortality. These differences may be due to the histological differences among the tumors, due to the differences in the internal and external environments of the tumor cells, and even due to the differences in the methods of data collection and analysis.

There is relatively little information about the signaling pathways associated with EMP1-mediated biological processes. Silencing experiments in the T-precursor ALL and B-ALL cell lines have indicated that EMP1 may signal through the Src kinase family (17). Wang et al (50), transfected EMP1 into the esophageal cancer cell line EC9706 and reported that EMP1 inhibited the proliferation of esophageal cancer cells, arrest the tumor cells in the S phase of the cell cycle or prolong the G1 phase. However, other study suggested that the EMP1 gene serves a role in promoting cell proliferation as a target gene of the proto-oncogene c-myc (19). EMP1 is highly expressed with c-myc in active embryonic stem cells, but the expression gradually decreases as the embryo differentiates and matures (51). Currently, the mechanism of EMP1 in cell proliferation and apoptosis is not clear, but it is worth affirming that EMP1 exerts its effects mainly by regulating signal transduction between cells or between cells.
and the extracellular matrix (52). Ramnarain et al (21), have demonstrated that mutation in the EGFR gene leads to the activation of a series of downstream signals, including EMP1, as a result of which patients harboring the mutated EGFR are more likely to develop glioblastoma compared with those with wild-type EGFR. Durgan et al (53), have suggested that EMP1, as an important transcriptional target in the Ras/mitogen-activated protein kinase pathway of bronchial epithelial cells, participates in the tight junction between cells and serves an important role in tracheal morphogenesis; its deletion may be associated with lung tumors. Lai et al (45), have reported that high expression of EMP1 leads to the occurrence and development of non-small-cell lung cancer by activating the PI3K/AKT pathway.

A number of previous studies have stated that members of the EMP family affect the integrin heterodimer repertoire on the plasma membrane, and modulation of the expression or localization of EMP proteins may alter the surface repertoire of molecules (54-56). The surface molecular repertoire, including major histocompatibility complex 1 proteins, integrins and other immunoglobulin superfamily members such as CD54 and glycosylphosphatidylinositol-linked proteins may be altered as the expression of EMP2 changes (57). These results suggest that members of the EMP family may influence the development of cancer cells via the tumor immune microenvironment and ultimately affect the prognosis of patients. However, there is lack of research on the association between EMP1 and different immune cell infiltration in BLCA.

Based on previous studies, the present study further analyzed the correlation between EMP1 and the infiltration of various immune cells.

The results of the present study suggested that macrophage infiltration was an independent prognostic factor in BLCA, which was consistent with previous studies that have demonstrated significant associations between tumor-associated macrophage infiltration and shorter survival of patients with bladder cancer (10,58,59). A number of studies have reported that a high neutrophil-lymphocyte ratio is a negative predictor of bladder cancer (60-63). In addition, neutrophil infiltration is significantly associated with poor prognosis of bladder cancer (59,64). High CD4+ T-cell density has been identified to be associated with poor prognosis in patients with bladder cancer (65,66), and a high level of mature tumor-infiltrating DCs predicts progression to muscle invasion in bladder cancer (58). In the present study, EMP1 expression was positively correlated with macrophage, neutrophil, CD4+ cell and DC infiltration, and negatively correlated with B lymphocyte infiltration, indicating that EMP1 expression may be a negative regulator of tumor immunity. These results were consistent with previous studies of immune cell infiltration.

The correlation between EMP1 expression and the enrichment of neutrophils and macrophages was further confirmed in the TISIDB in the present study. EMP1 also exhibited a significant positive correlation with the enrichment of immune cells such as CD4, CD8, DCs, NK and NKT cells, which suggested that EMP1 may aggravate the prognosis of patients by affecting the level of infiltration of specific immune cells. In addition, Treg and MDSC cells are considered to be suppressors of antitumor immune responses, and their enrichment is associated with poor patient outcomes in cancer (8,67-70). However, the results of B cell enrichment in TIMER and TISIDB in the present study were not consistent, reflecting the different algorithms used for the two immune scores. Although the mechanism of EMP1 in tumor immunology is not fully understood, the correlations between EMP1 expression and immune cell infiltration implicated the role of EMP1 in regulating tumor immunology in BLCA.

Recent studies have provided possible mechanisms that may explain the association between EMP1 expression and inflammation. Wang et al (71), have demonstrated that low microRNA-31 expression in mesenchymal stem cells in patients with psoriasis causes an increase in the expression of EMP1, which in turn facilitates T lymphocyte activation. A study by Pan et al (72) has indicated that EMP1 is activated by zinc finger protein 750 and regulates signaling pathways associated with proliferation and inflammation in CAL-27 cells.

To further elucidate the possible mechanism of EMP1 expression in immunity, the present study speculated its possible function by assessing co-expression with previously reported gene markers. Co-expression of CCL-2, CD68, IL10 of TAMs, PTGS2, IRF5 of M1 phenotype, CD163, VSIG4 and MS4A4A of the M2 phenotype with EMP1 suggested that EMP1 may regulate macrophage polarization. DC markers (e.g., HLA-DBP1, HLA-DRA, BDCA-1, and ITGAX) also exhibited a significant correlation with EMP1 expression, indicating an association between DC penetration and EMP1. Together, DC and T cells can secrete IL-12 and IL-18 to activate T cell proliferation, induce CTL production, and trigger a Th1-type immune response, which are conducive to tumor clearance (73). Treg markers (FOXPI,CCR8, TGFBI) were significantly co-expressed with EMP1. FOXPI serves an essential role in maintaining homeostasis of the immune system by facilitating the acquisition of full suppressive function and stability of the Treg lineage, and by directly modulating the expansion and function of conventional T cells (74). These results indicated that EMP1 expression may serve a complex role in the immune regulation network.

Checkpoint inhibitors are monoclonal antibodies that block inhibitory checkpoint antigens and repress the stimulation of T cells, exhibiting antitumor effects (75,76). Upon chronic stimulation by tumor antigens, tumor-infiltrating T cells lose their effector functions and their ability to kill tumor cells, accompanied by a progressive increase in the diversity and number of inhibitory receptors expressed on them, including CD274, PDCDILG2, HAVCR2, CTLA4, LAG3, PDCDJ and TIGIT (75-82). Therefore, genes that were associated with immune checkpoints were selected for analysis in the present study.

The results of the present study revealed significant co-expression between EMP1 and the genes reported to be associated with immune checkpoints, suggesting that EMP1 may serve a role in BLCA by affecting these immune checkpoints. Previous results have suggested a positive correlation between EMP1 and Treg, which inhibits the immune response of other immune cells. These results suggest that EMP1 may restore the function of immune cells by inhibiting immune checkpoints. Blocking antibodies against EMP1 may be a promising treatment strategy for patients with BLCA.

The present study had certain limitations. Further research, including deep sequencing, is needed to elucidate the full spectrum of variability and any functional variants of EMP1.
in BLCA. It is also necessary to further explore the specific molecular mechanism of EMP1 in bladder cancer cells.

In conclusion, the results of the present study demonstrated that variations in the EMP1 expression levels were associated with the prognosis of patients with BLCA. High EMP1 expression was associated with a poor OS rate. In addition, these results revealed that the extent of immune cell infiltration and the diversity of immune marker expression were associated with EMP1 expression in BLCA. Therefore, the results of the present study may provide insights into the potential function of EMP1 in tumor immunology and its potential as a cancer biomarker.

Acknowledgements

No applicable.

Funding

The current study was supported by the Shenzhen Healthcare Research Project (grant no. SZLY2018022).

Availability of data and materials

All data generated or analyzed during this study are included in this published article.

Authors' contributions

HY designed the research and reviewed the manuscript. BL analyzed the data and prepared the original draft. TZ and XY performed statistical calculation and experiments. All authors read and approved the final manuscript.

Ethics approval and consent to participate

This study was approved by the Ethics Committee of Peking university Shenzhen hospital (Shenzhen, China). Signed informed consents were obtained from the patients and/or guardians.

Patient consent for publication

No applicable.

Competing interests

The authors declare that they have no competing interests.

References

1. Bray F, Ferlay J, Soerjomataram I, Siegel RL, Torre LA and Jemal A: Global Cancer Statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA Cancer J Clin 68: 394-424, 2018.
2. Burger M, Catto JW, Dalbagni G, Groshen HB, Herr H, Karakiewicz P, Kassouf W, Kiemeney LA, La Vecchia C, Shariat S, et al: Epidemiology and risk factors of urothelial bladder cancer. Eur Urol 63: 234-241, 2013.
3. Cumberbatch MKG, Jubber J, Black PC, Esperto F, Figueroa JD, Kamat AM, Kiemeney L, Lotan Y, Pang K, Silverman DT, et al: Epidemiology of bladder cancer: A systematic review and contemporary update of risk factors in 2018. Eur Urol 74: 784-795, 2018.
4. Obara W, Eto M, Mimata H, Kohri K, Mitsuhashi N, Miura I, Shuin T, Miki T, Koie T, Fujimoto H, et al: A phase I/II study of cancer peptide vaccine S-288310 in patients with advanced urothelial carcinoma of the bladder. Ann Oncol 28: 798-803, 2017.
5. Lavoie JM, Bidnur S, Black PC and Eglit BJ: Expanding immunotherapy options for bladder cancer: commentary on: Pembrolizumab as second-line therapy for advanced urothelial carcinoma. Urology 106: I-2, 2017.
6. Hamilou Z, Lavaudi P and Loriot Y: Atezolizumab in urothelial bladder carcinoma. Future Oncol 14: 331-341, 2018.
7. Katam AM, Bellmunt J, Galsky MD, Konety BR, Lamm DL, Langham D, Lee CT, Milowsky MI, O'Donnell MA, O'Donnell PH, et al: Society for Immunotherapy of Cancer consensus statement on immunotherapy for the treatment of bladder cancer. J Immunother Cancer 5: 68, 2017.
8. Liu YN, Zhang H, Zhang L, Cai TT, Huang DJ, He J, Ni HH, Zhou FJ, Zhang XS and Li J: Sphingosine 1 phosphate receptor-1 (S1P1) promotes tumor-associated regulatory T cell expansion: Leading to poor survival in bladder cancer. Cell Death Dis 10: 50, 2019.
9. Mukherjee N, Ji N, Hurez V, Curiel TJ, Montgomery MO, Braun AJ, Nicolas M, Aguileria M, Kaushik D, Liu Q, et al: Intratumoral CD56bright natural killer cells are associated with improved survival in bladder cancer. Oncotarget 9: 36492-36502, 2018.
10. Sjödahl G, Lövgren K, Lauss M, Chebil G, Patschan O, Gudjonsson S, Månsson W, Fernö M, Leandersson K, Lindgren D, et al: Infiltration of CD3+ and CD68+ cells in bladder cancer is subtype specific and affects the outcome of patients with muscle-invasive tumors. Urol Oncol 32: 791-797, 2014.
11. Wang B, Xie S, Bi J, Liu Z, Zeng H, Huang H, Xue M, He Z, Yang M, Yu H, et al: Elevated pre-existing lymphocytic infiltration in tumour stroma predict poor prognosis in resectable urothelial carcinoma of the bladder. Histopathology 75: 354-364, 2019.
12. Sun GG, Lu YF, Fu ZZ, Cheng YJ and Hu WN: EMP1 inhibits nasopharyngeal cancer cell growth and metastasis through induction apoptosis and angiogenesis. Tumour Biol 35: 335-351, 2014.
13. Sun G, Zhao G, Lu Y, Wang Y and Yang C: Association of EMP1 with gastric carcinoma invasion, survival and prognosis. Int J Oncol 45: 1091-1098, 2014.
14. Sun GG, Wang YD, Cui DW, Cheng YJ and Hu WN: Epithelial membrane protein 1 negatively regulates cell growth and metastasis in colorectal carcinoma. World J Gastroenterol 20: 4001-4010, 2014.
15. Sun GG, Wang YD, Lu YF and Hu WN: EMP1, a member of a new family of antiproliferative genes in breast carcinoma. Tumour Biol 35: 3347-3354, 2014.
16. Sun GG, Wang YD, Cui DW, Cheng YJ and Hu WN: EMP1 regulates caspase-9 and VEGF expression and suppresses prostate cancer cell proliferation and invasion. Tumour Biol 35: 3455-3462, 2014.
17. Ariës IM, Jerchel IS, van den Dungen RE, van der Berk LC, Boer JM, Horstmann MA, Escherich G, Pieters R, Cramer TD and den Boer ML: EMP1, a novel poor prognostic factor in pediatric leukemia regulates prednisolone resistance, cell proliferation, migration and adhesion. Leukemia 28: 1828-1837, 2014.
18. Jain A, Tindell CA, Laux I, Hunter JB, Curran J, Galkin A, Afar DE, Aronson N, Shak S, Natale RB, et al: Epithelial membrane protein-1 is a biomarker of geltinin resistance. Proc Natl Acad Sci USA 102: 11858-11863, 2005.
19. Ben-Porath I and Benvenisty N: Characterization of a tumor-associated gene, a member of a novel family of genes encoding membrane glycoproteins. Gene 183: 69-75, 1996.
20. Li YQ, Xue T, Wang L, Xu ZC, Xi ZQ, Yuan J, Wang XF, Chen YM, Zhang M and Yao L: Up-regulation of epithelial membrane protein-1 in the temporal neocortex of patients with intractable epilepsy. Neurochem Res 34: 1594-1602, 2009.
21. Ramnarain DB, Park S, Lee DY, Hatamapa KJ, Scoggin SO, Ota H, Libermann TA, Raisanen JM, Ashby R, Wang ET, et al: Differential gene expression analysis reveals generation of an autocrine loop by a mutant epidermal growth factor receptor in glioma cells. Cancer Res 66: 867-876, 2006.
22. De Marco C, Laudanna C, Rinaldo N, Oliveira DM, Ravo M, Weisz A, Cecarelli M, Caia E, Rizzuto A, Zoppoli P, et al: Specific gene expression signatures induced by the multiple oncogenic alterations that occur within the PTEN/PI3K/ABL pathway in lung cancer. PLoS One 12: e0178865, 2017.
23. Wang YW, Li WM, Wu WJ, Chay CY, Chang TY, Sun Y, Cheng CJ, Shiee YL, Su SJ, Cheng HL, et al: Epithelial membrane protein 2 is a prognostic indicator for patients with urothelial carcinoma of the upper urinary tract. Am J Pathol 183: 706-719, 2013.
24. Humphrey PA, Moch H, Cubilla AL, Ulbright TM and Reuter VE: The 2016 WHO Classification of Tumours of the Urinary System and Male Genital Organs-Part B: Prostate and bladder tumours. Eur Urol 70: 106-119, 2016.
25. Demirag GG, Kelleli M, Kemal Y, and Yacel I: Epithelial membrane protein 1 expression in ovarian serous tumors. Oncol Lett 11: 2140-2144, 2016.
26. Li B and Dewey CN: RSEM: Accurate transcript quantification from RNA-Seq data with or without a reference genome. BMC Bioinformatics 14: 278, 2013.
27. Team R: Core: A language and environment for statistical computing. R Foundation for Statistical Computing, 2014.
28. Yoshikara K, Shahmoradgoli M, Martinez E, Vegrasa R, Kim H, Torres-Garcia W, Treviño V, Shen H, Laird PW, Levine WD, et al: Inferring tumour purity and stromal and immune cell admixture from expression data. Nat Commun 4: 2312, 2013.
29. Nagy A, Lánzczky A, Menyhárt O and Győrffy B: Validation of immune cell admixture from expression data. Nat Commun 4: 2612, 2013.
30. Arslan AA, Gold LI, Mittal K, Belitskaya-Levy I, Suen TC, Belmondo V, Tang MS and Toniolo P: Gene expression studies provide clues for the development of bladder cancer. Prostate Cancer Prostatic Dis 10: 167-174, 2007.
31. Wei Q, Li M, Fu X, Tang K, Na Y, Jiang M and Li Y: Global expression analysis of the hormonally androgen-independent prostate cancer. Prostate Cancer Prostatic Dis 10: 167-174, 2007.
32. Aslan AA, Gold LI, Mittal K, Suen TC, Belmondo V, Tang MS and Toniole P: Gene expression studies provide clues for the pathogenesis of uterine leiomyoma: New evidence and a novel hypothesis. Hum Reprod 20: 852-863, 2005.
33. Zhang J, Cao W, Xu Q and Chen WT: The expression of EMP1 is downregulated in oral squamous cell carcinoma and possibly associated with tumour metastasis. J Clin Pathol 64: 25-29, 2011.
34. Lai S, Wang G, Cao X, Li Z, Hu J and Wang J: EMP-1 promotes tumorigenesis of NSCLC through PI3K/AKT pathway. J Huazhong Univ Sci Technol Med Sci 32: 834-838, 2012.
35. Zhang H, Lu Y and He J: Expression of epithelial membrane protein 1 in human esophageal cancer and its clinical implications. Zhongguo Zhongliu Shengwu Zhiliao Zazhi 14: 466-470, 2007.
36. Tsuravishili G, Bouchal J, Baumforth K, Wei W, Dziechciarkova M, Ehrmann J, Klein J, Fridman E, Skarda J, Srovnal J, et al: Novel markers for differentiation of lobular and ductal invasive breast carcinomas by laser microdissection and microarray analysis. BMC Cancer 7: 55, 2007.
37. Mackay A, Jones C, Dexter T, Silva RL, Bulmer K, Jones A, Simpson P, Harris RA, Patel KS, Neville AM, et al: cDNA microarray analysis of genes associated with ERBB2 (HER2/neu) overexpression in luminal mammary luminal epithelial cells. Oncogene 22: 2680-2688, 2003.
38. Lobiger CS, Magyar JP, Taylor V, Wulf P, Welcher AA, Program AE and Suter U: Identification and characterization of a cDNA and the structural gene encoding the mouse epithelial membrane protein-1. Genomics 36: 379-387, 1996.
39. Wang HT, Kong JP, Ding F, Wang QX, Wang MR, Liu LX, Wu M and Liu ZH: Analysis of gene expression profile induced by EMP-1 in esophageal cancer cells using cDNA Microarray. World J Gastroenterol 9: 392-398, 2003.
40. Ruegg CL, Wu HY, Fagnoni FF, Engleman EG and Lau R: EMP-1, a novel growth factor, is overexpressed by a subset of progenitor/pre-B lymphocytes negative for cytoplasmic mu-chain. J Immunol 157: 72-80, 1996.
41. Wang YW, Cheng YL, Ding YR, Chou LH and Chow NH: EMP1, EMP 2, and EMP3 as novel therapeutic targets in human esophageal cancer. Biochim Biophys Acta 1686: 108-117, 2004.
42. Duran J, Tao G, Walters MS, Florey O, Schmidt A, Arbelaez V, Rosen N, Crystal RG and Hall A: SOS1 and Ras regulate epithelial tight junction formation in the human airway using EMP1. EMBO Rep 16: 87-96, 2015.
43. Morales SA, Telander DG, Mareninov S, Nagy A, Wadehra M, Braun J and Gordon LK: Anti-EMP2 diabody blocks epithelial membrane protein 2 (EMP2) and FAK mediated collagen gel contraction in ARPE-19 cells. Exp Eye Res 102: 10-16, 2012.
44. Wadehra M, Forbes A, Pushkarna N, Goodllick L, Gordon LK, Williams CJ and Braun J: Epithelial membrane protein-2 regulates surface expression of alphavbeta3 integrin in the endometrium. Dev Biol 287: 336-345, 2005.
45. Morales SA, Mareninov S, Wadehra M, Zhang L, Goodllick L, Braun J and Gordon LK: FAK activation and the role of epithelial membrane protein 2 (EMP2) in collagen gel contraction. Invest Ophthalmol Vis Sci 50: 462-469, 2009.
46. Wadehra M, Goodllick L and Braun J: The tetraspans protein EMP2 modulates the surface expression of cavelons and glycosylphosphatidylinositol-linked proteins. Mol Biol Cell 15: 4083-4093, 2004.
47. Ayari C, LaRue H, Hovington H, Caron A, Bergeron A, Tébû T, Fradet V and Fradet Y: High level of mature tumor-infiltrating dendritic cells predicts progression to muscle invasion in bladder cancer. Hum Pathol 44: 1630-1637, 2013.
48. Pichler R, Fritz J, Zavadi, Schäfer G, Kulig Z and Brunner A: Tumor-infiltrating immune cell subpopulations influence the oncologic outcome after intravesical Bacillus Calmette-Guérin therapy in bladder cancer. Oncotarget 7: 39916-39930, 2016.
49. Kuriakose MA, Chen WT, He ZM, Sikora AG, Zhang P, Zhang ZY, Qiu WL, Hsu DF, McMunn-Coffran C, Brown SM, et al: Analysis of gene expression profile induced by cDNA from RNA-Seq data with or without a reference genome. BMC Bioinformatics 11: 285, 2010.
50. Ruegg CL, Wu HY, Fagnoni FF, Engleman EG and Lau R: EMP-1, a novel growth factor, is overexpressed by a subset of progenitor/pre-B lymphocytes negative for cytoplasmic mu-chain. J Immunol 157: 72-80, 1996.
51. Wang YW, Cheng YL, Ding YR, Chou LH and Chow NH: EMP1, EMP 2, and EMP3 as novel therapeutic targets in human esophageal cancer. Biochim Biophys Acta 1686: 108-117, 2004.
52. Duran J, Tao G, Walters MS, Florey O, Schmidt A, Arbelaez V, Rosen N, Crystal RG and Hall A: SOS1 and Ras regulate epithelial tight junction formation in the human airway using EMP1. EMBO Rep 16: 87-96, 2015.
53. Morales SA, Telander DG, Mareninov S, Nagy A, Wadehra M, Braun J and Gordon LK: Anti-EMP2 diabody blocks epithelial membrane protein 2 (EMP2) and FAK mediated collagen gel contraction in ARPE-19 cells. Exp Eye Res 102: 10-16, 2012.
54. Pichler R, Fritz J, Zavadi, Schäfer G, Kulig Z and Brunner A: Tumor-infiltrating immune cell subpopulations influence the oncologic outcome after intravesical Bacillus Calmette-Guérin therapy in bladder cancer. Oncotarget 7: 39916-39930, 2016.
55. Kuriakose MA, Chen WT, He ZM, Sikora AG, Zhang P, Zhang ZY, Qiu WL, Hsu DF, McMunn-Coffran C, Brown SM, et al: Analysis of gene expression profile induced by cDNA from RNA-Seq data with or without a reference genome. BMC Bioinformatics 11: 285, 2010.
56. Ruegg CL, Wu HY, Fagnoni FF, Engleman EG and Lau R: EMP-1, a novel growth factor, is overexpressed by a subset of progenitor/pre-B lymphocytes negative for cytoplasmic mu-chain. J Immunol 157: 72-80, 1996.
65. Zhang Q, Hao C, Cheng G, Wang L, Wang X, Li C, Qiu J and Ding K: High CD4+ T cell density is associated with poor prognosis in patients with non-muscle-invasive bladder cancer. Int J Clin Exp Pathol 8: 11510‑11516, 2015.

66. Pfannstiel C, Strissel PL, Chiappinelli KB, Sicie D, Wach S, Wirtz RM, Wullweber A, Taubert H, Breyer J, Otto W, et al; BRIDGE Consortium, Germany; BRIDGE Consortium, Germany; BRIDGE Consortium, Germany: The Tumor Immune Microenvironment Drives a Prognostic Relevance That Correlates with Bladder Cancer Subtypes. Cancer Immunol Res 7: 923‑938, 2019.

67. Wu K, Tan MY, Jiang JT, Mu XY, Wang JR, Zhou WJ, Wang X, Li MQ, He YY and Liu ZH: Cisplatin inhibits the progression of bladder cancer by selectively depleting G‑MDSCs: A novel chemoinmunomodulating strategy. Clin Immunol 193: 60‑69, 2018.

68. Smith SG, Baltz JL, Koppolu BP, Ravindranathan S, Nguyen K and Zaharoff DA: Immunological mechanisms of intravesical chitosan/interleukin‑12 immunotherapy against murine bladder cancer. OncolImmunology 6: e1259050, 2016.

69. Horn T, Laus J, Setz AK, Maurer T, Schmid SC, Wolf P, Haller B, Winkler M, Retz M, Nawroth R, et al: Urinary bladder cancer tregs suppress MMP2 and potentially regulate invasiveness. Cancer Immunol Res 6: 528-538, 2018.

70. Chan AW, Zhang Z, Chong CC, Tin EK, Chow C and Wong N: Genomic landscape of lymphoepithelioma-like hepatocellular carcinoma. J Pathol 249: 166‑172, 2019.

71. Yoshikawa T, Nakatsugawa M, Suzuki S, Shirakawa H, Nobuoka D, Sakemura N, Motomura Y, Tanaka Y, Hayashi S and Nakatsura T: HLA-A2‑restricted glycan‑3‑peptide‑specific CTL clones induced by peptide vaccine show high avidity and antigen‑specific killing activity against tumor cells. Cancer Sci 102: 918‑925, 2011.

72. Germeau C, Mu W, Schiavetti F, Lurquin C, Henry E, Vigneron N, Brasseur F, Lethé B, De Plaen E, Velu T, et al; High frequency of antitumor T cells in the blood of melanoma patients before and after vaccination with tumor antigens. J Exp Med 201: 241‑248, 2005.

73. Takashima Y, Kawaguchi A, Sato R, Yoshida K, Hayano A, Homma J, Fukai J, Iwadate Y, Kajiwara K, Ishizawa S, et al: Differential expression of individual transcript variants of PD-1 and PD-L2 genes on Th-1/Th-2 status is guaranteed for prognosis prediction in PCNSL. Sci Rep 9: 10004, 2019.