B3GNT3: A prognostic biomarker associated with immune cell infiltration in pancreatic adenocarcinoma

KAIWEN KONG1*, YUANYU ZHAO2*, LEILEI XIA3*, HUI JIANG1, MINGJUAN XU3 and JIANMING ZHENG1

1Department of Pathology, Changhai Hospital; 2Department of Organ Transplantation, Changzheng Hospital; 3Department of Obstetrics and Gynecology, Changhai Hospital, Navy Medical University, Shanghai 200433, P.R. China

Received March 4, 2020; Accepted September 28, 2020

DOI: 10.3892/ol.2020.12420

Abstract. Pancreatic cancer, one of the most malignant gastrointestinal tumors, is prone to liver metastasis. However, due to the lack of appropriate and comprehensive diagnostic methods, it is difficult to accurately predict the survival outcomes. Therefore, there is a need to identify effective biomarkers, such as UDP-GlcNAc:βGal β-1,3-N-acetylgalcosaminyltransferase 3 (B3GNT3), that predict the survival outcome of patients with pancreatic cancer. In the present study, based on data from 171 cases of pancreatic cancer obtained from The Cancer Genome Atlas portal, the differential expression of mRNAs was screened by comparing cancerous tissues with adjacent tissues. Univariate Cox regression analysis demonstrated that B3GNT3 had prognostic capability and could be an independent prognostic factor for pancreatic adenocarcinoma (PAAD). Using the Tumor Immune Estimation Resource tool and Tumor-Immune System Interaction Database, a potential relationship between B3GNT3 expression and immune cell infiltration was identified in pancreatic carcinoma. Furthermore, 177 samples of pancreatic carcinoma were collected and the association of CD68 expression with B3GNT3 was assessed by immunohistochemical staining. B3GNT3 expression was associated with clinical outcomes in pancreatic carcinoma and related to infiltrating levels of immune cells, which indicated that B3GNT3 could be used as an immunotherapy target for PAAD.

Introduction

Pancreatic adenocarcinoma (PAAD) is an aggressive type of malignancy, characterized by rapid progression and dismal prognosis. In most patients, PAAD is unresectable after diagnosis (1). According to National Institutes of Health statistics, regardless of tumor stage, the 5-year survival rate of patients with PAAD is only 10% based on cases of PAAD-associated mortality between 2010 and 2016 (2). PAAD exhibits a limited response to traditional therapy approaches; however, a number of novel therapies, including immunotherapy, have been tested in the clinical trial phase (3). Unfortunately, the immunosuppressive tumor microenvironment (TME) limits the efficacy of immunotherapy (4). Therefore, the discovery of novel biomarkers that characterize TMEs that would be suitable candidates for immunotherapy is urgently required for the prognostic assessment of patients with PAAD (5).

UDP-GlcNAc:βGal β-1,3-N-acetylgalcosaminyltransferase 3 (B3GNT3) is a type II transmembrane protein on the Golgi membrane that acts as the catalytic center in the synthesis of poly-N-acetyllactosamine chains and generation of the backbone components of dimeric sialyl Lewis A (6). Additionally, this gene serves vital roles in L-selectin ligand synthesis, which is involved in lymphocyte homing and trafficking. Due to its biological characteristic, B3GNT3 has been considered to be involved in the tumorigenesis of non-Hodgkin's lymphoma (7,8). B3GNT3 expression is tissue-selective. It is aberrantly expressed in the pancreas and distributed throughout the gastrointestinal tract, liver, placenta, kidney, trachea, neutrophils and lymphocytes (9,10). However, to the best of our knowledge, the role of B3GNT3 in the tumorigenesis of PAAD has not been fully revealed.

Currently, the molecular function of B3GNT3 is controversial. Ho et al (11) reported that B3GNT3 is an independent predictor for a good prognosis of neuroblastoma via the suppression of extend core (T-antigen) oligosaccharide formation. However, other researchers have revealed that B3GNT3 has a negative role in cancer. For example, Zhang et al (12) claimed that increased expression levels of B3GNT3 were associated with pelvic lymph node metastasis and poor prognosis in patients with early-stage cervical cancer. Furthermore, Gao et al (13) demonstrated that, among patients who suffer from non-small cell lung cancer, patients with high B3GNT3 expression have worse disease-free survival (DFS) time and overall survival (OS) time. According to another study, B3GNT3 is essential for...
the epidermal growth factor-induced communication of receptor
programmed cell death protein-1 (PD-1) and programmed
death-ligand 1 in triple-negative breast cancer (14). Therefore,
the downregulation of B3GNT3 may enhance cytotoxic
T cell-mediated anti-neoplastic effects. Overall, B3GNT3 may
have tumor-promoting and tumor-suppressive effects.

In the present study, The Cancer Genome Atlas (TCGA)
was used to analyze the immunohistochemical (IHC) results of
samples from a single center to evaluate the prognostic value of
B3GNT3 expression in PAAD. Gene Set Enrichment Analysis
(GSEA) was used to gain further insights into the biological
pathways involved in the pathogenesis of PAAD. Furthermore,
the association between B3GNT3 and tumor-infiltrating
immune cells in the TME was examined to identify a probable
immunotherapy target in PAAD.

Materials and methods

Data source and clinical information. The gene expression
profiles of PAAD (171 cases; workflow type, HTSeq-Counts),
and the relevant clinical information and pathologic character-
istics were downloaded from TCGA (https://portal.gdc.cancer.
gov; Project ID, TCGA-PAAD; Table I).

IHC analysis. To assess the protein expression patterns of B3GNT3
and CD68, IHC staining was performed on 177 paraffin-embedded
PAAD samples (collected from the Department of Pathology,
Changhai Hospital, Navy Medical University, Shanghai, China).
The inclusion criteria were as follows: i) Histological type of
adenocarcinoma; and ii) treatment by surgery. Among 177 cases,
115 were male and 62 were female. The age range was 32-86 years
with a mean age of 60.98±10.75 years and a median age of 62 years.
All data were collected between July 2018 and December 2019.
The IHC procedures abided by established protocols (12);
however, 16 tissue dots on the microarrays were missed during the
procedure. In brief, fresh tissues were fixed in 10% formalin for
48 h at room temperature and then dehydrated in ethanol, cleared
in xylene for transparency and embedded in molten paraffin. The
paraffin-embedded tissue block was sectioned into 4-µm thick
slices using a microtome (Leica Microsystems, Inc.). Paraffin
sections were heated, dewaxed in xylene and hydrated in different
concentrations of ethanol (100, 95, 85 and 70%) and washed in
PBS buffer (15). The sections were submerged in a high-pressure
cooker filled with EDTA antigenic retrieval buffer and heated
at 110°C for 5-10 min. Endogenous peroxidase activity
was inhibited by incubation with 3% hydrogen peroxide for 25 min,
followed by incubation with goat serum washing fluid (undiluted;
cat. no. ZLI-9056; OriGene Technologies, Inc.) to block the
non-specific protein binding for 30 min at 25°C. Subsequently,
the specimens were coated with polyclonal antibodies against
B3GNT3 (dilution, 1:100; cat. no. 18098-1-AP; ProteinTech
Group, Inc.) and CD68 (dilution, 1:800; cat. no. 76437; Cell
Signaling Technology, Inc.) (16), and incubated overnight at 4°C.
PBS replaced the primary antibodies as a negative control. After
three washes with PBS with 0.2% Tween-20, the tissue slices
were incubated with a biotinylated anti-rabbit/mouse secondary
antibody working fluid (undiluted; cat. no. PV8000-1; OriGene
Technologies, Inc.) at room temperature (25°C) for 30 min. For
visual staining, 3,3-Diaminobenzidine (cat. no. ZLI-9017; OriGene
Technologies, Inc.) was dripped on the sections. The tissue
sections were then washed with running water, counterstained
with 10% Mayer's hematoxylin at room temperature for 1-3 min,
dehydrated in anhydrous ethanol and sealed with a coverslip.

The IHC staining results were observed using a confocal
microscope (Olympus Corporation) and scored independently
by two pathologists blinded to the clinical characteristics in a
semi-quantitative manner. The tissue specimens were scored
based on the proportion of positive cancer cells and staining
strength. A positive reaction was defined as a cell exhibiting
red strong. For immune cells, the proportion of positive cells
was counted and the samples were classified into four groups:
0, 1, 2, 3, 4, 6, 8, 9 and 12. For statistical analysis, the scores
were multiplied by the score for the positive area (0, <5%; 1, 5‑25%;
2, 25‑75%; and 3, >75%).

Table I. Clinical information from TCGA database (project ID,
TCGA-PAAD).

| Clinical characteristics | Total, n (n=171) | Percentage |
|-------------------------|----------------|------------|
| Sex                     |                |            |
| Male                    | 93             | 54.4       |
| Female                  | 78             | 45.6       |
| Age, years              |                |            |
| ≤60                     | 57             | 33.3       |
| >60                     | 114            | 66.7       |
| T stage                 |                |            |
| T1                      | 7              | 4.1        |
| T2                      | 21             | 12.3       |
| T3,4                    | 141            | 82.5       |
| N stage                 |                |            |
| N1                      | 47             | 27.5       |
| N0                      | 119            | 69.6       |
| M stage                 |                |            |
| M1                      | 77             | 45         |
| M0                      | 4              | 2.3        |
| Grade                   |                |            |
| G1                      | 28             | 16.4       |
| G2/3/4                  | 141            | 82.5       |
| Histology               |                |            |
| PDAC                    | 140            | 81.9       |
| Others                  | 30             | 17.5       |
| MSI*                    |                |            |
| MSI-L                   | 9              | 5.3        |
| MSS                     | 137            | 80.1       |

*Some incomplete cases were excluded. PDAC, pancreatic ductal
adenocarcinoma; MSI, microsatellite instability; MSS, microsatellite
stable; MSI-L, low-frequency MSI.
GSEA. GSEA was performed to investigate the difference in survival between the high and low B3GNT3 expression groups using GSEA software (http://www.broadinstitute.org/gsea; version 4.1.0 for Windows). Gene set permutations were conducted 1,000 times for each analysis. The expression degree of B3GNT3 was used as a phenotype marker. The nominal P-value and normalized enrichment score were used to rank the enrichment pathways in each phenotype.

Tumor Immune Estimation Resource (TIMER) database analysis. The tumor immune cell infiltration characterization of PAAD was estimated using data provided by TIMER web portal (http://cistrome.dfcii.harvard.edu/TIMER/). The correlations among B3GNT3 and different immune cells and associated gene markers were explored. The related module generated scatter plots of the expression of a pair of user-defined genes in pancreatic cancer, as well as the Spearman’s correlation analysis and the estimated statistical significance.

Tumor-Immune System Interaction Database (TISIDB) analysis. The TISIDB web portal (http://cis.hku.hk/TISIDB) comprises 988 identified immune-associated oncogenes and antitumor genes, high-throughput screening techniques, exome and RNA sequencing data, and a variety of resources for immunological data collected from other public databases. It facilitates the analysis of the interaction of certain genes with immunocytes, immunomodulators and cytokines. In the present study, this database was utilized to investigate the associations between B3GNT3 expression and lymphocytes and immunomodulators (15,17).

Statistical analysis. Clinical data and B3GNT3 expression information were collected from TCGA database and analyzed using R studio (https://cran.r-project.org/; version 1.2.1335) and SPSS (version 23.0; IBM Corp). All data are presented as the mean ± SD. The association between clinicopathologic features and B3GNT3 expression was analyzed using an unpaired t-test. A univariate Cox proportional hazards model was used to evaluate risk factors associated with the survival of patients with pancreatic cancer. Subsequently, clinical parameters with P<0.05 were used in a multivariate Cox proportional hazards model to assess prognostic factors. The OS time associated with B3GNT3 expression was examined by Kaplan-Meier analysis to analyze the diversity between the high- and low-expression groups. The cut-off value of B3GNT3 expression was determined by its median value. The log-rank P-value was also computed. Clinical information, such as survival-associated IHC results, was analyzed using Cox regression analysis. The correlation analyses of B3GNT3 and immune infiltration were based on Pearson’s correlation analysis (after tests of normal distribution) and Spearman’s regression analysis. While gene set permutations were conducted 1,000 times for each analysis, other analyses were repeated only once. All statistical tests were two-sided, and P<0.05 was considered to indicate a statistically significant difference.

Results

B3GNT3 expression is associated with survival in PAAD. First, B3GNT3 expression in pancreatic cancer was investigated using TCGA data. A cohort analysis revealed that B3GNT3 expression was significantly higher in pancreatic ductal adenocarcinoma tissues than in normal tissues at the mRNA level (Fig. 1A). Kaplan Meier survival curves were generated to analyze the association between B3GNT3 expression and the prognosis of the pancreatic
KONG et al: ASSOCIATION BETWEEN B3GNT3 AND PANCREATIC CANCER

Cancer cohort using follow-up information. Patients in the high B3GNT3 expression group had a shorter OS time and DFS time [OS: Hazard ratio (HR)=2.1, P=0.00076; DFS: HR=1.6, P=0.029; Fig. 1B and C].

IHC was performed to estimate B3GNT3 protein expression in a retrospective cohort of 177 pancreatic ductal adenocarcinoma samples, among which 16 cases were censored. Immunoreactivity to the B3GNT3 antibody was detected primarily in the cytoplasm (Fig. 2). The IHC staining of B3GNT3 was positive in 137 cases, among which 41 cases (30%) were stained weakly for B3GNT3, 62 (45%) were stained moderately and 34 (25%) were stained strongly. Furthermore, Cox regression analysis indicated that the group with the highest expression levels of B3GNT3 had worst outcomes (Fig. 3).

Figure 2. Immunohistochemical analysis of B3GNT3 in pancreatic adenocarcinoma. (A) Negative, (B) weak, (C) moderate and (D) strong B3GNT3 expression in adenocarcinoma tissues. Magnification, x200. B3GNT3, βGal-1,3-N-acetylglucosaminyltransferase 3.

Figure 3. Overall survival of 161 patients with pancreatic adenocarcinoma in relation to B3GNT3 status. Patients with pancreatic cancer with higher B3GNT3 expression had poorer outcomes. The P-value shown in the figure applies to the comparison of all groups. B3GNT3, βGal-1,3-N-acetylglucosaminyltransferase 3.

Table II. Associations between overall survival and clinicopathologic characteristics in TCGA patients analyzed using Cox regression analysis.

| Clinicopathologic variable | OR   | P-value  | 95% CI      |
|----------------------------|------|----------|-------------|
| Grade (1 vs. grade 2/3/4)  | 2.160| 0.019    | 1.137-4.104|
| Stage (I vs. II/III/IV)    | 2.283| 0.038    | 1.046-4.980|
| Topography (T1/T2 vs. T3/4) | 2.021| 0.030    | 1.071-3.815|
| MSI (MSI-L vs. MSS/MSI-H)  | 0.450| 0.046    | 0.206-0.985|
| B3GNT3                     | 2.025| 0.016    | 1.325-3.093|

B3GNT3, βGal-1,3-N-acetylglucosaminyltransferase 3; MSI, microsatellite instability; MSS, microsatellite stability; MSI-H, microsatellite instability-high; MSI-L, microsatellite instability-low; OR: Odds ratio.
B3GNT3 expression is associated with clinicopathologic factors. The association between B3GNT3 expression and the clinicopathological characteristics of patients with PAAD was analyzed using data from TCGA. High B3GNT3 expression in PAAD was significantly associated with intraepithelial neoplasia, tumor/topography (18), stage and microsatellite instability (Fig. 4). The univariate analysis revealed that high expression levels of B3GNT3 were associated with poor OS time in PAAD. Other clinicopathologic variables associated with poor survival included high grade, advanced stage, high levels of tumor/topography and microsatellite instability (Table II).

GSEA identification of a B3GNT3-related signaling pathway. GS EA between the low- and high-expression B3GNT3 datasets was performed to identify differentially activated signaling pathways in pancreatic cancer (Fig. 5; Table III).
B3GNT3 expression is associated with immune infiltration levels in PAAD. The association between B3GNT3 expression and immune infiltration levels in pancreatic cancer was investigated by assessing the correlations between B3GNT3 expression and tumor immune infiltration levels using TIMER. B3GNT3 expression and immune infiltration levels, including CD4+ T cells, neutrophils, macrophages and dendritic cells, were negatively correlated in pancreatic cancer (Fig. 6), especially macrophages (|cor|=0.366). To support this result, CD68, a maker of macrophages, was assessed by immunohistochemical staining. Spearman's regression analysis was performed to evaluate the correlation of each molecule with B3GNT3 expression. The results revealed that the infiltration of macrophages exhibited a negative correlation with B3GNT3 expression (Fig. 7).

B3GNT3 expression is associated with immune signatures. The correlation between B3GNT3 expression and various immune signatures was examined by TISIDB analysis. The present study focused on tumor-infiltrating immunocytes,
immune inhibitory or stimulatory genes (including immune checkpoint gene sets) and cytokine-related genes. In Spearman correlation analysis with filtering for P<0.05 and |±rho|>0.4, B3GNT3 expression was correlated with a set of immune markers in infiltrating immune cells of pancreatic cancer, such as inducible co-stimulator (ICOS) and NECTIN2 (CD112).

Furthermore, a positive correlation was observed between B3GNT3, β Gal β-1,3-N-acetylglucosaminyltransferase 3; ICOS, inducible co-stimulator; NECTIN2, poliovirus receptor-related 2; TNFRSF, tumor necrosis factor receptor superfamily; CCL, chemokine (C-C motif) ligand; CXCL12, C-X-C motif chemokine ligand 12; CCR, C-C motif chemokine receptor.
B3GNT3 expression and poliovirus receptor-related 2 (NECTIN2) expression. Nevertheless, members of the tumor necrosis factor receptor superfamily (TNFRSF), TNFRSF8 (CD30) and TNFRSF19, exhibited negative correlations with B3GNT3 expression in pancreatic cancer. Specifically, the expression levels of chemokine (C-C motif) ligand (CCL)-2, CCL4, CCL8, CCL14, CCL11, CCL21 and CXC motif chemokine ligand 12, and associated chemokine receptors, including C-C motif chemokine receptor (CCR)1, CCR2, CCR4, CCR5 and CCR7, were significantly correlated with B3GNT3 expression (P<0.001; Fig. 8). Overall, these results demonstrated the correlation between B3GNT3 and the immune infiltrating cells in PAAD, which suggests that B3GNT3 has an important immune escape role in the TME and can be used as a target for immunotherapy.

Discussion

Studies have demonstrated that B3GNT3 is involved in non-Hodgkin lymphoma tumorigenesis (7) and the determination of malignant behaviors (12,13). In the present study, high expression levels of B3GNT3 in PAAD were positively associated with poor prognosis and advanced clinicopathological features (e.g., high grade and clinical staging) according to biomolecular informatics analysis of high-throughput RNA profiling sequencing data in TCGA. Univariate analysis revealed that high B3GNT3 expression was associated with poor OS time for PAAD. Furthermore, B3GNT3 expression was negatively associated with OS time in clinical PAAD samples from our center.

To further investigate the role of B3GNT3 in pancreatic cancer, IHC analysis was performed to investigate immune cell infiltration. B3GNT3 expression was associated with macrophage infiltration. Similarly, Cerhan et al (7) reported that B3GNT3 is associated with tumor immunity and inflammation and serves an important role in lymphocyte migration and transport, leading to the survival and metastasis of non-Hodgkin's lymphoma tumor cells. Furthermore, the correlation between B3GNT3 expression and the immune-related genes suggests the potential regulatory function of B3GNT3 in tumor immunity in PAAD. Additionally, the present results revealed that B3GNT3 may inhibit regulatory T cell (Treg) migration, since the expression levels of B3GNT3 were negatively correlated with the infiltrating levels of Tregs (ICOS and TNF receptor superfamily member). ICOS is a standard T cell co-stimulating molecule that promotes T cell activation via the activation of PI3K signaling pathway, and indicated the negative correlation of malignant behaviors (12,13). In the present study, high B3GNT3 expression was associated with poor OS time for PAAD. Furthermore, B3GNT3 expression might contribute to the chemokine-mediated activity of the PI3K signaling pathway, and indicated the negative correlation between B3GNT3 expression and immunostimulator expression. CCL2 is required for tumor-associated macrophages to induce immune evasion (26), to promote cancer cell progression (27) and invasion (28). Furthermore, CCL4 is associated with a T cell-inflamed phenotype in primary and metastatic pancreatic cancer (29). Therefore, B3GNT3 as a potential unfavorable prognostic maker, might contribute to the low infiltration of immune cells and immunostimulators. For the GSEA analysis, B3GNT3 might downregulate the frequency, rate or extent of natural killer cell- and chemokine-mediated immunity. Additionally, STAT is implicated in a wide range of human cancer types, including pancreatic cancer (30-32). Therefore, B3GNT3 could increase the malignant behavior of PAAD via the regulation of the STAT cascade signaling pathway (33).

In a follow-up study, the investigation of effective immune inhibitors, such as myeloid-derived suppressor cells, tumor-associated macrophages and Tregs, particularly in the early stage of cancer, is required to develop novel immunotherapeutic agents. The present study demonstrated that B3GNT3 might be a potential target for prognostic prediction and immune therapy in patients with pancreatic carcinoma.

Acknowledgements

Not applicable.

Funding

The present study was supported by the National Natural Science Foundation of China (grant no. 81972282).

Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Authors' contributions

JZ and MX conceived and designed the experiments, and KK, LX and YZ carried out the experiments. HJ and LX analyzed the data. KK and YZ wrote the manuscript. All the authors discussed and suggested the experiments. All authors read and approved the final manuscript.

Ethics approval and consent to participate

All the human tissues used in the present study were microarrays of clinical samples from the study of national Natural Science Foundation of China (‘The Malignant Biological Behavior and Mechanism of Pancreatic Ducted Adenocarcinoma Mediated via a Novel Spliceosome MDA5’; approval no. 81972282). The present study has passed the ethics review by the Committee on Ethics of Medicine, Navy Medical University.

Patient consent for publication

Not applicable.
Competing interests

The authors declare that they have no competing interests.

References

1. Mizrahi J, Surana R, Valle J and Shroff R: Pancreatic cancer. Lancet 395: 2008-2020, 2020.
2. National Cancer Institute, Bethesda M: SEER cancer stat facts: Pancreatic cancer. Journal. https://seer.cancer.gov/statfacts/html/pancreas.html. Accessed December 17, 2020.
3. Ngo P, Shanshal M and Rojjan A: Immunotherapy in pancreatic cancer and the importance of tumour testing. BMJ Case Rep 13: e225574, 2020.
4. Wang S, Li Y, Xing C, Ding C, Zhang H, Chen L, You L, Dai M and Zhao Y: Tumor microenvironment in chemoresistance, metastasis and immunotherapy of pancreatic cancer. Am J Cancer Res 10: 1937-1953, 2020.
5. Christensen E, Jaffe E and Azad N: Current and emerging therapies for patients with advanced pancreatic ductal adenocarcinoma: A bright future. Lancet Oncol 21: e135-e145, 2020.
6. Hennet T, Dinter A, Kuhnert P, Mattu TS, Rudd PM and Benesi EG: Carcinoenic cloning and expression of three murine UDG-galactose: Beta-N-acetylgalosamine beta, 1,3-galactosyltransferase genes. J Biol Chem 273: 58-65, 1998.
7. Cerhan JR, Ansell SM, Fredericksen ZS, Kay NE, Liebow M, Call TG, Dogan A, Cunningham JM, Wang AH, Liu-Mares W, et al: Genetic variation in 1253 immune and inflammation genes and risk of non-Hodgkin lymphoma. Blood 110: 4455-4463, 2007.
8. Yeh JC, Hiraoka N, Petryniak B, Nakayama J, Ellisies LG, Rabuka D, Hindsagul O, Marth JD, Lowe BJ and Fukuda M: Novel sulfated lymphocyte homing receptors and their control by a Core1 extension beta 1,3-N-acetylglucosaminyltransferase. Cell 105: 957-969, 2001.
9. Shirashi N, Natsume A, Togayachi A, Endo T, Akashima T, Yamada Y, Imai N, Nakagawa S, Koizumi S, Sekine S, et al: Identification and characterization of three novel beta 1,3-N-acetylglucosaminyltransferases structurally related to the beta 1,3-galactosyltransferase family. J Biol Chem 276: 3498-3507, 2001.
10. Haider S, Wang J, Nagano A, Desai A, Arumugam P, Dumartin L, Fitzgibbon J, Hagemann T, Marshall JF, Kocher HM, et al: A multi-gene signature predicts outcome in patients with pancreatic ductal adenocarcinoma. Genome Med 6: 105, 2014.
11. Ho WL, Che MI, Chou CH, Chang HH, Jeng YM, Hsu WM, Lin KH and Huang MC: B3GNT3 expression suppresses cell migration and invasion and predicts favorable outcomes in neuroblastoma. Cancer Sci 104: 1600-1608, 2013.
12. Zhang Y, Hou T, Niu C, Song L and Zhang Y: B3GNT3 expression is a novel marker correlated with pelvic lymph node metastasis and poor clinical outcome in early-stage cervical cancer. PLoS One 10: e0144360, 2015.
13. Gao L, Zhang H, Zhang B, Zhu J, Chen C and Liu W: B3GNT3 overexpression is associated with unfavourable survival in non-small cell lung cancer. J Clin Pathol 71: 642-647, 2018.
14. Li CW, Lim SO, Chung EM, Kim YS, Park AH, Yeo J, Cha JH, Xia W, Chan LC, Kim T, et al: Eradication of triple-negative breast cancer cells by targeting glycosylated PD-L1. Cancer Cell 33: 187-201 e110, 2018.
15. Weser K, Wahlund E, Sundstrom C, Ranefall P, Bengtsson E, Wester K, Wahlund E, Sundstrom C, Ranefall P, Bengtsson E, et al: Glycosylation of CD147 affects the migratory and invasive properties of colorectal cancer cells. Oncotarget 7: 3498-3507, 2016.
16. Lucca LE, Lerner BA, Park C, DeBartolo D, Harnett B, Kumar VP, Ponath G, Raddass K, Hutterm A, Hafer DA and Pitt D: Differential expression of the T-cell inhibitor TIGIT in glioblastoma and MS. Neurol Neuroimmunol Neuroinflamm 7: 2172, 2020.
17. Ru B, Wong CN, Tong Y, Zhong JY, Zhong SSW, Wu WC, Chu KC, Wong CY, Lau CY, Chen I, et al: TISDIB: An integrated repository portal for tumor-immune system interactions. Bioinformatics 35: 4200-4202, 2019.
18. van RoesSEL S, Kasumova G, Verheij J, Najarian RM, Maggino L, de Pastena M, Malleo G, Marchegiani G, Salvia R, Ng SC, et al: International validation of the eighth edition of the American joint committee on cancer (AJCC) TNM staging system in patients with resected pancreatic cancer. JAMA Surg 153: e183617, 2018.
19. Chen H, Fu T, Suh WK, Tsavachidou D, Wen S, Gao J, Tang DN, He Q, Sun J and Sharma P: CD4 T cells require ICOS-mediated PI3K signaling to increase T-Bet expression in the setting of anti-CTLA-4 therapy. Cancer Immunol Res 2: 167-170, 2014.
20. Liang MH, Shin SJ, Shin SK, Kim DJ, Zo JJ, Shim YM, Lee SE, Cho BC, Park SY, Choi YL and Kim HR: High CD3 and ICOS and low TIM-3 expression predict favourable survival in resected oesophageal squamous cell carcinoma. Sci Rep 9: 20197, 2019.
21. Wang J, He M, Shi W, Sha H, Feng J, Wang S and Wang Y: Inducible costimulator (ICOS) enhances the cytotoxic activity of cytokine-induced killer cells against gallbladder cancer in vitro and in vivo. Cancer Invest 27: 244-250, 2009.
22. Yang J, Liu J, Chen Y, Tang W, Bo K, Sun Y and Chen J: Investigation of ICOS, CD28 and CD80 polymorphisms with the risk of hepatocellular carcinoma: A case-control study in eastern Chinese population. Biosci Rep 39: BS20181824, 2019.
23. Whelan S, Ophir E, Kotturi MF, Levy O, Ganguy S, Leung L, Vaknin I, Kumar S, Dassa L, Hansen K, et al: PVRIG and PVRL2 are induced in cancer and inhibit CD8+ T-cell function. Cancer Immunol Res 7: 261-269, 2019.
24. Liang S, Yang Z, Li D, Miao X, Yang L, Zou Q and Yuan Y: The clinical and pathological significance of nectin-2 and DDX3 expression in pancreatic ductal adenocarcinomas. Dis Markers 2015: 379586, 2015.
25. Oshima T, Sato S, Kato J, Ito Y, Watanabe T, Tsuji I, Hori A, Kurokawa T and Kubo T: Nectin-2 is a potential target for antibody therapy of breast and ovarian cancers. Molecular Cancer 12: 60, 2013.
26. Yang H, Zhang Q, Xu M, Wang L, Chen B, Feng Y, Li Y, Zhang X, Cui W and Jia X: CCL2-CCR2 axis recruits tumor associated macrophages to induce immune evasion through PD-1 signaling in esophageal carcinogenesis. Mol Cancer 19: 41, 2020.
27. Liu Q, Song J, Pan Y, Shi D, Yang C, Wang S and Xiong B: Wnt5a/CaMKII/ERK/CCL2 axis is required for tumor-associated macrophages to promote colorectal cancer progression. Int J Biol Sci 16: 1023-1034, 2020.
28. He M, Wu W, Chang C, Miyamoto H, Liu X, Jiang K and Yeh S: Estrogen receptor α promotes lung cancer cell invasion via increase of and cross-talk with infiltrated macrophages through the CCL2/CCL22/MMP9 and CXC12/L2/CXCR4 signaling pathways. Mol Oncol 14: 1779-1799, 2020.
29. Romero JM, Gruning B, Yang H, Bavi PP, Jhaveri A, Masoomian M, Fischer SE, Zhang A, Denroche RE, Lungu IM, et al: A four-chemokine signature is associated with a T-cell-inflamed phenotype in primary and metastatic pancreatic cancer. Clin Cancer Res 26: 1997-2010, 2020.
30. Ko HJ and Kim YJ: Signal transducer and activator of transcription proteins: Regulators of myeloid-derived suppressor cell-mediated immunosuppression in cancer. Arch Pharm Res 39: 1597-1608, 2016.
31. Kim BH, Yi EH and Ye SK: Signal transducer and activator of transcription 3 as a therapeutic target for cancer and the tumor microenvironment. Arch Pharm Res 39: 1085-1099, 2016.
32. Wei D, Le X, Zheng L, Wang L, Frey JA, Gao AC, Peng Z, Huang S, Xiong HQ, Abbruzzese JL and Xie K: Stat3 activation regulates the expression of vascular endothelial growth factor and human pancreatic cancer angiogenesis and metastasis. Oncogene 22: 319-329, 2003.
33. Scholz A, Heinze S, Detjen KM, Peters M, Welzel M, Hafler P, Schirner M, Wiedenmann B and Rosewicz S: Activated signal transducer and activator of transcription 3 (STAT3) supports the malignant phenotype of human pancreatic cancer. Gastroenterology 125: 891-905, 2003.