International Journal of General Medicine

Dovepress

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ORIGINAL RESEARCH

SEMA3D Plays a Critical Role in Peptic Ulcer Disease-Related Carcinogenesis Induced by *H. pylori* Infection

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Background: Immune cell infiltration plays a critical role in regulating peptic ulcer disease (PUD) and gastrointestinal cancer (GC). However, regulators of the cell signaling hubs remain unclear.

Aim: This study characterizes genes that are differentially expressed in PUD and GC tissue samples. Bioinformatics is used to define the immune-associated hub genes associated with the malignant transfer process of PUD to GC.

Methods: Total expression data from PUD and early-stage GC tissue samples were obtained from GEO and TCGA. Differentially expressed genes were assessed and immunological enrichment analysis was performed. Protein–protein interaction (PPI) and Cytoscape analysis were used together to identify the hub genes. CIBERSORT and COX analysis were used to analyze the differentially infiltrated immune cell landscapes and determine HR scores of the hub genes.

Results: Expression data identified 437 DEGs as common to both GC and PUD tissue. Of these, 49 immune-related DEGs were grouped by function, and seven hub genes were identified by PPI analysis. The NRP2 and SEMA3D genes were then selected for survival analysis. SEMA3D had a higher hazard ratio than NRP2 and was defined as the hub for PUD carcinogenesis.

Conclusion: SEMA3D was characterized as the hub gene for PUD carcinogenesis.

Keywords: SEMA3D, peptic ulcer disease, gastric cancer, *H. pylori*, immunological micro-environment

Introduction

Helicobacter pylori (HP) is a Gram-negative gastrointestinal bacterium that infects nearly half of the world population.[1] HP infection is associated with the occurrence and progress of peptic ulcer disease (PUD) and gastrointestinal cancer (GC).[2,3] The causes of GC, and its connection to PUD, remain poorly defined. Recently, HP-induced chronic inflammation has been shown to play an important role in GC occurrence and development.[4] Prior studies defined several chemokines and cytokines involved in inflammation of the gastric epithelium.[5]

The cell microenvironment is the environment where tumor cells originate and develop. This region also consists of stromal cells, the tumor vascular system, immune cells, the extracellular matrix (ECM), and the acidic and hypoxic environment of the tumor.[6,7] Immune cells are the major cell types in the microenvironment and release many chemokines and cytokines that dictate disease outcomes in response to infection.[8] During the development of peptic ulcers caused by HP infection, NF-kB signaling is activated by inflammatory factors like IL-8.[9]

In recent years, bioinformatics tools and software have been developed to quickly explore differentially expressed target genes and identify hub genes that contribute to disease progression.[10] The CIBERSORT algorithm is a newly developed tool to assess the association between immune cell landscapes in the cell microenvironment using the existing 22 immune cell signatures.[11] This method has been successfully used to identify prognostic immune markers in lung, breast, and gastric cancer.[12,13] The present study used the CIBERSORT algorithm to calculate the proportions of 22

Received: 5 November 2021
Accepted: 11 January 2022
Published: 9 February 2022

immune cells that infiltrate the ECM during PUD and GC, based on The Cancer Genome Atlas (TCGA) (for early-stage GC) and the Gene Expression Omnibus GEO (for PUD) databases. Inflammation and differentially expressed genes (DEGs) associated with tumors were also assessed and SEMA3D was shown to correlate with the development of peptic ulcers and the immune cell signatures. SEMA3D is a member of Class-3 semaphorins (SEMA3s), which are reported to play pivotal roles in immune response, angiogenesis, apoptosis, cell migration, and local and metastatic cancer spread in pan-cancer.[14,15] SEMA3E, a gene in the same family as SEMA3D, is a lymph node metastasis-related gene expressed in gastric cancer.[16] SEMA3E deficiency dysregulates many immune cell functions both directly and indirectly.[17,18] Prior studies indicated that Semaphorin 3D and 3E have similar cellular functions, however, the exact role of SEMA3D in gastric cancer remains poorly understood.[19,20] Inappropriately regulated gastric immune responses to HP in the cell microenvironment are critical to the development of gastroduodenal disease and responses to treatment.[5] For example, CD4+T cell-derived IFN-γprovides the key stimulus for the development of gastric premalignant lesions that progress to GC.[9] SEMA3D also contributes to CD4+T cell infiltration in osteoarthritis joints.[21] Other immune cells, including macrophages, dendritic cells (DCs), B cells, and gastric epithelial cells (GECs) contribute to the mucosal response to HP infection.[18,22] DCs affect the Treg/Th17 balance induced by HP infection and indirectly activate T cells.[19,23] In the current study, SEMA3D expression was primarily found in DCs from PUD samples. Similarly, prior research indicates that SEMA3E regulates DC function.[17,20] Thus, it was hypothesized that SEMA3D contributes to gastric epithelium carcinogenesis by regulating immune cell infiltration. He findings reported here indicate that SEMA3D may play an essential role in the cell microenvironment and could serve as a promising prognostic biomarker for the malignant transformation of peptic ulcers.

Materials and Methods

Data Preparation

Gene expression data from 118 early-stage GC samples and 32 PUD gastric mucosa (uninfected or HP-infected) were downloaded from TCGA and GEO databases, respectively (Table 1. GC patients who were diagnosed with stages I or II according to the 6th and 7th editions of the AJCC Cancer Staging Manual were included in this study. Clinical information for each patient was obtained from the TCGA database following TCGA publication guidelines and data access policies. Patients were excluded if they had recurrent GC, therapies performed before admission, other observed clinical disorders, or other GC clinical stages. PUD gene expression information was obtained from the GSE60427 dataset. The microarray platform for GSE60427 was GPL1707. Eight mucosa tissue samples were included in the normal group (GSM1479654, GSM1479655, GSM1479656, GSM1479657, GSM1479670, GSM1479671, GSM1479672 and GSM1479673) and 24 samples were included in the HP+ group (GSM1479658, GSM1479659, GSM1479660, GSM1479661, GSM1479662, GSM1479663, GSM1479664, GSM1479665, GSM1479666, GSM1479667, GSM1479668, GSM1479669, GSM1479674, GSM1479675, GSM1479676, GSM1479677, GSM1479678, GSM1479679, GSM1479680, GSM1479681, GSM1479682, GSM1479683, GSM1479684 and GSM1479685). The 32 samples used for microarray analysis were selected from 293 patient subjects. All the patients provided written informed consent and the protocols were approved by the ethics committees of Oita University (Japan). Patients with PUD and GC were identified by endoscopy. Gastritis was defined as HP gastritis in the absence of peptic ulcers or gastric malignancy. Patients with a history of partial gastric resection or who had received HP eradication therapy or treatment with antibiotics, bismuth-containing compounds, H2-receptor blockers, or proton pump inhibitors within four weeks prior to the study were excluded. All the clinical information was obtained from the previous papers.[14] The number of included and excluded subjects in the study was summarized in Flow Chart (Figure S1). The protocols described above were approved by the ethics committees of Affiliated Hospital of Hebei University (AHHU20211029).

DEG Identification

Gene expression profiles were screened using the R package, and DEGs were identified in both groups. Based on PUD and GC integrated analysis, a common gene set was identified for the two groups. DEGs were determined based on an absolute value of log2 fold change (|log2FC|) >1 and a false discovery rate (FDR) <0.05. Heatmaps of DEGs were drawn

Table 1 The KEGG Enrichment Pathway List

Term Name (KEGG Pathway Data Base)	Database	ID	Input Number	Background Number	P-value	Corrected P-value	Input	Hyperlink
Cytokine-cytokine receptor interaction	KEGG PATHWAY	hsa04060	7	294	9.42E-08	1.39E-05	AMH|IL20RB|CCL14|BMP6|IFNG|TNFRSF10A|CXCL10	http://www.genome.jp/kegg-bin/show_pathway?hsa04060/hsa:53833%09red/hsa:654%09red/hsa:3627%09red/hsa:8797%09red/hsa:3458%09red/hsa:268%09red/hsa:6358%09red
Neuroactive ligand-receptor interaction	KEGG PATHWAY	hsa04080	7	338	2.37E-07	1.39E-05	PTH1R|TRH|THRB|VIPR2|PENK|TACR1|CYSLTR1	http://www.genome.jp/kegg-bin/show_pathway?hsa04080/hsa:6869%09red/hsa:7068%09red/hsa:7200%09red/hsa:5745%09red/hsa:5179%09red/hsa:7434%09red/hsa:10800%09red
Rap1 signaling pathway	KEGG PATHWAY	hsa04015	6	210	2.96E-07	1.39E-05	VAV2|AKT3|VEGFA|FGF5|FGF20|FGF21	http://www.genome.jp/kegg-bin/show_pathway?hsa04015/hsa:10000%09red/hsa:7422%09red/hsa:2250%09red/hsa:26281%09red/hsa:26291%09red/hsa:7410%09red
Pathways in cancer	KEGG PATHWAY	hsa05200	8	530	3.23E-07	1.39E-05	IFNG|BID|AKT3|VEGFA|FGF5|BIRC5|FGF20|FGF21	http://www.genome.jp/kegg-bin/show_pathway?hsa05200/hsa:637%09red/hsa:3458%09red/hsa:10000%09red/hsa:26291%09red/hsa:2250%09red/hsa:26281%09red/hsa:7422%09red/hsa:332%09red
Influenza A	KEGG PATHWAY	hsa05164	5	167	2.47E-06	7.35E-05	IFNG|CXCL10|TNFRSF10A|AKT3|BID	http://www.genome.jp/kegg-bin/show_pathway?hsa05164/hsa:637%09red/hsa:3627%09red/hsa:8797%09red/hsa:10000%09red/hsa:3458%09red
Melanoma	KEGG PATHWAY	hsa05218	4	72	2.56E-06	7.35E-05	FGF5|AKT3|FGF20|FGF21	http://www.genome.jp/kegg-bin/show_pathway?hsa05218/hsa:26281%09red/hsa:2250%09red/hsa:10000%09red/hsa:26291%09red
Axon guidance	KEGG PATHWAY	hsa04360	5	181	3.63E-06	8.91E-05	SEMA3A|PLXNB3|SEMA6D|SEMA5B|SEMA3D	http://www.genome.jp/kegg-bin/show_pathway?hsa04360/hsa:54437%09red/hsa:10371%09red/hsa:80031%09red/hsa:5365%09red/hsa:223117%09red
cAMP signaling pathway	KEGG PATHWAY	hsa04024	5	214	8.02E-06	0.000171075	AKT3|AMH|VAV2|VIPR2|NPR1	http://www.genome.jp/kegg-bin/show_pathway?hsa04024/hsa:268%09red/hsa:7410%09red/hsa:10000%09red/hsa:7434%09red/hsa:4881%09red
Viral protein interaction with cytokine and cytokine receptor	KEGG PATHWAY	hsa04061	4	100	8.95E-06	0.000171075	CXCL10|TNFRSF10A|IL20RB|CCL14	http://www.genome.jp/kegg-bin/show_pathway?hsa04061/hsa:6358%09red/hsa:53833%09red/hsa:3627%09red/hsa:8797%09red
Ras signaling pathway	KEGG PATHWAY	hsa04014	5	232	1.18E-05	0.000202148	FGF5|AKT3|VEGFA|FGF20|FGF21	http://www.genome.jp/kegg-bin/show_pathway?hsa04014/hsa:26291%09red/hsa:26281%09red/hsa:2250%09red/hsa:10000%09red/hsa:7422%09red
Natural killer cell mediated cytotoxicity	KEGG PATHWAY	hsa04650	4	131	2.50E-05	0.000391555	IFNG|TNFRSF10A|VAV2|BID	http://www.genome.jp/kegg-bin/show_pathway?hsa04650/hsa:637%09red/hsa:7410%09red/hsa:8797%09red/hsa:3458%09red
Apoptosis	KEGG PATHWAY	hsa04210	4	136	2.89E-05	0.000413901	TNFRSF10A|AKT3|BID|BIRC5	http://www.genome.jp/kegg-bin/show_pathway?hsa04210/hsa:637%09red/hsa:8797%09red/hsa:10000%09red/hsa:332%09red

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https://doi.org/10.2147/IJGM.S343635 DovePress

Table 1 (Continued).

Term Name (KEGG Pathway Data Base)	Database	ID	Input Number	Background Number	P-value	Corrected P-value	Input	Hyperlink
MAPK signaling pathway	KEGG PATHWAY	hsa04010	5	295	3.63E-05	0.000468473	FGF5|AKT3|VEGFA|FGF20|FGF21	http://www.genome.jp/kegg-bin/show_pathway?hsa04010/hsa:26291%09red/hsa:26281%09red/hsa:2250%09red/hsa:10000%09red/hsa:7422%09red
Breast cancer	KEGG PATHWAY	hsa05224	4	147	3.88E-05	0.000468473	FGF5|AKT3|FGF20|FGF21	http://www.genome.jp/kegg-bin/show_pathway?hsa05224/hsa:26281%09red/hsa:2250%09red/hsa:10000%09red/hsa:26291%09red
Gastric cancer	KEGG PATHWAY	hsa05226	4	149	4.09E-05	0.000468473	FGF5|AKT3|FGF20|FGF21	http://www.genome.jp/kegg-bin/show_pathway?hsa05226/hsa:26281%09red/hsa:2250%09red/hsa:10000%09red/hsa:26291%09red
Hepatitis C	KEGG PATHWAY	hsa05160	4	155	4.75E-05	0.00051021	IFNG|CXCL10|BID|AKT3	http://www.genome.jp/kegg-bin/show_pathway?hsa05160/hsa:637%09red/hsa:3627%09red/hsa:10000%09red/hsa:3458%09red
PI3K-Akt signaling pathway	KEGG PATHWAY	hsa04151	5	354	8.49E-05	0.000858733	FGF5|AKT3|VEGFA|FGF20|FGF21	http://www.genome.jp/kegg-bin/show_pathway?hsa04151/hsa:26291%09red/hsa:26281%09red/hsa:2250%09red/hsa:10000%09red/hsa:7422%09red
Chemokine signaling pathway	KEGG PATHWAY	hsa04062	4	190	0.00010258	0.000980213	AKT3|CXCL10|VAV2|CCL14	http://www.genome.jp/kegg-bin/show_pathway?hsa04062/hsa:6358%09red/hsa:7410%09red/hsa:3627%09red/hsa:10000%09red
Platinum drug resistance	KEGG PATHWAY	hsa01524	3	73	0.000120162	0.00108778	BID|AKT3|BIRC5	http://www.genome.jp/kegg-bin/show_pathway?hsa01524/hsa:637%09red/hsa:10000%09red/hsa:332%09red
Regulation of actin cytoskeleton	KEGG PATHWAY	hsa04810	4	214	0.000160568	0.001380886	FGF5|VAV2|FGF20|FGF21	http://www.genome.jp/kegg-bin/show_pathway?hsa04810/hsa:26281%09red/hsa:7410%09red/hsa:2250%09red/hsa:26291%09red
IL-17 signaling pathway	KEGG PATHWAY	hsa04657	3	93	0.000239981	0.001934399	IFNG|CXCL10|S100A8	http://www.genome.jp/kegg-bin/show_pathway?hsa04657/hsa:3458%09red/hsa:6279%09red/hsa:3627%09red
TGF-beta signaling pathway	KEGG PATHWAY	hsa04350	3	94	0.000247423	0.001934399	IFNG|AMH|BMP6	http://www.genome.jp/kegg-bin/show_pathway?hsa04350/hsa:268%09red/hsa:654%09red/hsa:3458%09red
T cell receptor signaling pathway	KEGG PATHWAY	hsa04660	3	103	0.000321205	0.002402052	AKT3|IFNG|VAV2	http://www.genome.jp/kegg-bin/show_pathway?hsa04660/hsa:3458%09red/hsa:7410%09red/hsa:10000%09red
HIF-1 signaling pathway	KEGG PATHWAY	hsa04066	3	109	0.000377492	0.002705356	IFNG|AKT3|VEGFA	http://www.genome.jp/kegg-bin/show_pathway?hsa04066/hsa:3458%09red/hsa:10000%09red/hsa:7422%09red
Fluid shear stress and atherosclerosis	KEGG PATHWAY	hsa05418	3	139	0.000753736	0.005185702	IFNG|AKT3|VEGFA	http://www.genome.jp/kegg-bin/show_pathway?hsa05418/hsa:3458%09red/hsa:10000%09red/hsa:7422%09red
Apoptosis - multiple species	KEGG PATHWAY	hsa04215	2	33	0.000882909	0.005840785	BID|BIRC5	http://www.genome.jp/kegg-bin/show_pathway?hsa04215/hsa:637%09red/hsa:332%09red
Hippo signaling pathway	KEGG PATHWAY	hsa04390	3	154	0.001007511	0.006418218	AMH|BIRC5|BMP6	http://www.genome.jp/kegg-bin/show_pathway?hsa04390/hsa:268%09red/hsa:654%09red/hsa:332%09red

https://doi.org/10.2147/IJGM.S343635 DovePress

Necroptosis	KEGG PATHWAY	hsa04217	3	162	0.001162455	0.006781484	IFNG|TNFRSF10A|BID	http://www.genome.jp/kegg-bin/show_pathway?hsa04217/hsa:637%09red/hsa:3458%09red/hsa:8797%09red
Jak-STAT signaling pathway	KEGG PATHWAY	hsa04630	3	162	0.001162455	0.006781484	IFNG|AKT3|IL20RB	http://www.genome.jp/kegg-bin/show_pathway?hsa04630/hsa:53833%09red/hsa:3458%09red/hsa:10000%09red
Hepatitis B	KEGG PATHWAY	hsa05161	3	163	0.001182817	0.006781484	BID|AKT3|BIRC5	http://www.genome.jp/kegg-bin/show_pathway?hsa05161/hsa:637%09red/hsa:10000%09red/hsa:332%09red
Bladder cancer	KEGG PATHWAY	hsa05219	2	41	0.001331445	0.007387374	TYMP|VEGFA	http://www.genome.jp/kegg-bin/show_pathway?hsa05219/hsa:1890%09red/hsa:7422%09red
Tuberculosis	KEGG PATHWAY	hsa05152	3	179	0.001539718	0.008275984	IFNG|BID|AKT3	http://www.genome.jp/kegg-bin/show_pathway?hsa05152/hsa:637%09red/hsa:3458%09red/hsa:10000%09red
Kaposi sarcoma-associated herpesvirus infection	KEGG PATHWAY	hsa05167	3	186	0.00171486	0.008938059	BID|AKT3|VEGFA	http://www.genome.jp/kegg-bin/show_pathway?hsa05167/hsa:637%09red/hsa:10000%09red/hsa:7422%09red
Focal adhesion	KEGG PATHWAY	hsa04510	3	199	0.002072159	0.010466682	AKT3|VAV2|VEGFA	http://www.genome.jp/kegg-bin/show_pathway?hsa04510/hsa:7410%09red/hsa:10000%09red/hsa:7422%09red
Epstein-Barr virus infection	KEGG PATHWAY	hsa05169	3	201	0.002130916	0.010466682	CXCL10|BID|AKT3	http://www.genome.jp/kegg-bin/show_pathway?hsa05169/hsa:637%09red/hsa:3627%09red/hsa:10000%09red
Proteoglycans in cancer	KEGG PATHWAY	hsa05205	3	203	0.002190701	0.010466682	AKT3|VAV2|VEGFA	http://www.genome.jp/kegg-bin/show_pathway?hsa05205/hsa:7410%09red/hsa:10000%09red/hsa:7422%09red
Regulation of lipolysis in adipocytes	KEGG PATHWAY	hsa04923	2	55	0.002327215	0.010818406	NPR1|AKT3	http://www.genome.jp/kegg-bin/show_pathway?hsa04923/hsa:10000%09red/hsa:4881%09red
VEGF signaling pathway	KEGG PATHWAY	hsa04370	2	59	0.002659961	0.012039822	AKT3|VEGFA	http://www.genome.jp/kegg-bin/show_pathway?hsa04370/hsa:10000%09red/hsa:7422%09red
Human cytomegalovirus infection	KEGG PATHWAY	hsa05163	3	225	0.00291796	0.012547229	BID|AKT3|VEGFA	http://www.genome.jp/kegg-bin/show_pathway?hsa05163/hsa:637%09red/hsa:10000%09red/hsa:7422%09red
Inflammatory bowel disease (IBD)	KEGG PATHWAY	hsa05321	2	65	0.003198486	0.013418038	IFNG|RORA	http://www.genome.jp/kegg-bin/show_pathway?hsa05321/hsa:3458%09red/hsa:6095%09red
Fc epsilon RI signaling pathway	KEGG PATHWAY	hsa04664	2	68	0.003485291	0.014273095	AKT3|VAV2	http://www.genome.jp/kegg-bin/show_pathway?hsa04664/hsa:7410%09red/hsa:10000%09red
Renal cell carcinoma	KEGG PATHWAY	hsa05211	2	69	0.003583469	0.014333878	AKT3|VEGFA	http://www.genome.jp/kegg-bin/show_pathway?hsa05211/hsa:10000%09red/hsa:7422%09red

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https://doi.org/10.2147/IJGM.S343635

Table 1 (Continued).

Term Name (KEGG Pathway Data Base)	Database	ID	Input Number	Background Number	P-value	Corrected P-value	Input	Hyperlink
Pancreatic cancer	KEGG PATHWAY	hsa05212	2	75	0.004199377	0.016415748	AKT3|VEGFA	http://www.genome.jp/kegg-bin/show_pathway?hsa05212/hsa:10000%09red/hsa:7422%09red
EGFR tyrosine kinase inhibitor resistance	KEGG PATHWAY	hsa01521	2	79	0.004635305	0.017717168	AKT3|VEGFA	http://www.genome.jp/kegg-bin/show_pathway?hsa01521/hsa:10000%09red/hsa:7422%09red
B cell receptor signaling pathway	KEGG PATHWAY	hsa04662	2	82	0.004975401	0.018603674	AKT3|VAV2	http://www.genome.jp/kegg-bin/show_pathway?hsa04662/hsa:7410%09red/hsa:10000%09red
Colorectal cancer	KEGG PATHWAY	hsa05210	2	86	0.005446233	0.019515668	BIRC5|AKT3	http://www.genome.jp/kegg-bin/show_pathway?hsa05210/hsa:332%09red/hsa:10000%09red
PD-L1 expression and PD-1 checkpoint pathway in cancer	KEGG PATHWAY	hsa05235	2	89	0.005812275	0.020402271	IFNG|AKT3	http://www.genome.jp/kegg-bin/show_pathway?hsa05235/hsa:3458%09red/hsa:10000%09red
Rheumatoid arthritis	KEGG PATHWAY	hsa05323	2	91	0.006062408	0.020854683	IFNG|VEGFA	http://www.genome.jp/kegg-bin/show_pathway?hsa05323/hsa:3458%09red/hsa:7422%09red
Fc gamma R-mediated phagocytosis	KEGG PATHWAY	hsa04666	2	94	0.006446702	0.021323707	AKT3|VAV2	http://www.genome.jp/kegg-bin/show_pathway?hsa04666/hsa:7410%09red/hsa:10000%09red
AGE-RAGE signaling pathway in diabetic complications	KEGG PATHWAY	hsa04933	2	100	0.007247728	0.023520929	AKT3|VEGFA	http://www.genome.jp/kegg-bin/show_pathway?hsa04933/hsa:10000%09red/hsa:7422%09red
Chagas disease (American trypanosomiasis)	KEGG PATHWAY	hsa05142	2	103	0.007664294	0.023968338	IFNG|AKT3	http://www.genome.jp/kegg-bin/show_pathway?hsa05142/hsa:3458%09red/hsa:10000%09red
Toll-like receptor signaling pathway	KEGG PATHWAY	hsa04620	2	104	0.007805507	0.023974059	CXCL10|AKT3	http://www.genome.jp/kegg-bin/show_pathway?hsa04620/hsa:3627%09red/hsa:10000%09red
Th17 cell differentiation	KEGG PATHWAY	hsa04659	2	107	0.008236179	0.02485303	IFNG|RORA	http://www.genome.jp/kegg-bin/show_pathway?hsa04659/hsa:3458%09red/hsa:6095%09red
TNF signaling pathway	KEGG PATHWAY	hsa04668	2	112	0.008977221	0.02531282	CXCL10|AKT3	http://www.genome.jp/kegg-bin/show_pathway?hsa04668/hsa:3627%09red/hsa:10000%09red
Toxoplasmosis	KEGG PATHWAY	hsa05145	2	113	0.009128891	0.02532531	IFNG|AKT3	http://www.genome.jp/kegg-bin/show_pathway?hsa05145/hsa:3458%09red/hsa:10000%09red

https://doi.org/10.2147/IJGM.S343635 DovePress

Thyroid hormone signaling pathway	KEGG PATHWAY	hsa04919	2	119	0.010062892	0.027044022	AKT3|THRB	http://www.genome.jp/kegg-bin/show_pathway?hsa04919/hsa:7068%09red/hsa:10000%09red
Sphingolipid signaling pathway	KEGG PATHWAY	hsa04071	2	119	0.010062892	0.027044022	BID|AKT3	http://www.genome.jp/kegg-bin/show_pathway?hsa04071/hsa:637%09red/hsa:10000%09red
Yersinia infection	KEGG PATHWAY	hsa05135	2	121	0.01038329	0.027475783	AKT3|VAV2	http://www.genome.jp/kegg-bin/show_pathway?hsa05135/hsa:7410%09red/hsa:10000%09red
Osteoclast differentiation	KEGG PATHWAY	hsa04380	2	128	0.011539912	0.030073711	IFNG|AKT3	http://www.genome.jp/kegg-bin/show_pathway?hsa04380/hsa:3458%09red/hsa:10000%09red
Relaxin signaling pathway	KEGG PATHWAY	hsa04926	2	130	0.011880345	0.030498796	AKT3|VEGFA	http://www.genome.jp/kegg-bin/show_pathway?hsa04926/hsa:10000%09red/hsa:7422%09red
Measles	KEGG PATHWAY	hsa05162	2	138	0.013285727	0.033605074	BID|AKT3	http://www.genome.jp/kegg-bin/show_pathway?hsa05162/hsa:637%09red/hsa:10000%09red
Non-alcoholic fatty liver disease (NAFLD)	KEGG PATHWAY	hsa04932	2	149	0.015330079	0.037668195	BID|AKT3	http://www.genome.jp/kegg-bin/show_pathway?hsa04932/hsa:637%09red/hsa:10000%09red
cGMP-PKG signaling pathway	KEGG PATHWAY	hsa04022	2	167	0.018945968	0.045259811	NPR1|AKT3	http://www.genome.jp/kegg-bin/show_pathway?hsa04022/hsa:10000%09red/hsa:4881%09red
Herpes simplex virus 1 infection	KEGG PATHWAY	hsa05168	3	492	0.023869016	0.055248506	IFNG|BID|AKT3	http://www.genome.jp/kegg-bin/show_pathway?hsa05168/hsa:637%09red/hsa:3458%09red/hsa:10000%09red
Calcium signaling pathway	KEGG PATHWAY	hsa04020	2	193	0.024733343	0.055248506	TACR1|CYSLTR1	http://www.genome.jp/kegg-bin/show_pathway?hsa04020/hsa:6869%09red/hsa:10800%09red
Human immunodeficiency virus 1 infection	KEGG PATHWAY	hsa05170	2	212	0.029360767	0.063451227	BID|AKT3	http://www.genome.jp/kegg-bin/show_pathway?hsa05170/hsa:637%09red/hsa:10000%09red
Renin-angiotensin system	KEGG PATHWAY	hsa04614	1	23	0.029512198	0.063451227	CMA1	http://www.genome.jp/kegg-bin/show_pathway?hsa04614/hsa:1215%09red
Thermogenesis	KEGG PATHWAY	hsa04714	2	231	0.034304903	0.072844979	NPR1|FGF21	http://www.genome.jp/kegg-bin/show_pathway?hsa04714/hsa:26291%09red/hsa:4881%09red
Circadian rhythm	KEGG PATHWAY	hsa04710	1	31	0.039158676	0.08018205	RORA	http://www.genome.jp/kegg-bin/show_pathway?hsa04710/hsa:6095%09red
Asthma	KEGG PATHWAY	hsa05310	1	31	0.039158676	0.08018205	EPO	http://www.genome.jp/kegg-bin/show_pathway?hsa05310/hsa:8288%09red

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https://doi.org/10.2147/IJGM.S343635

Table 1 (Continued).

Term Name (KEGG Pathway Data Base)	Database	ID	Input Number	Background Number	P-value	Corrected P-value	Input	Hyperlink
African trypanosomiasis	KEGG PATHWAY	hsa05143	1	37	0.046331832	0.093753825	IFNG	http://www.genome.jp/kegg-bin/show_pathway?hsa05143/hsa:3458%09red
Allograft rejection	KEGG PATHWAY	hsa05330	1	38	0.047522247	0.095044494	IFNG	http://www.genome.jp/kegg-bin/show_pathway?hsa05330/hsa:3458%09red
Graft-versus-host disease	KEGG PATHWAY	hsa05332	1	41	0.051084765	0.100995168	IFNG	http://www.genome.jp/kegg-bin/show_pathway?hsa05332/hsa:3458%09red
Type I diabetes mellitus	KEGG PATHWAY	hsa04940	1	43	0.053452522	0.104475384	IFNG	http://www.genome.jp/kegg-bin/show_pathway?hsa04940/hsa:3458%09red
Carbohydrate digestion and absorption	KEGG PATHWAY	hsa04973	1	44	0.054634229	0.105495522	AKT3	http://www.genome.jp/kegg-bin/show_pathway?hsa04973/hsa:10000%09red
Proteasome	KEGG PATHWAY	hsa03050	1	45	0.055814491	0.105495522	IFNG	http://www.genome.jp/kegg-bin/show_pathway?hsa03050/hsa:3458%09red
Ovarian steroidogenesis	KEGG PATHWAY	hsa04913	1	49	0.060521122	0.109395872	BMP6	http://www.genome.jp/kegg-bin/show_pathway?hsa04913/hsa:654%09red
Malaria	KEGG PATHWAY	hsa05144	1	49	0.060521122	0.109395872	IFNG	http://www.genome.jp/kegg-bin/show_pathway?hsa05144/hsa:3458%09red
Cholesterol metabolism	KEGG PATHWAY	hsa04979	1	50	0.061694183	0.109395872	ANGPTL4	http://www.genome.jp/kegg-bin/show_pathway?hsa04979/hsa:51129%09red
Endocrine and other factor-regulated calcium reabsorption	KEGG PATHWAY	hsa04961	1	50	0.061694183	0.109395872	PTH1R	http://www.genome.jp/kegg-bin/show_pathway?hsa04961/hsa:5745%09red
Amyotrophic lateral sclerosis (ALS)	KEGG PATHWAY	hsa05014	1	51	0.06286581	0.110335912	BID	http://www.genome.jp/kegg-bin/show_pathway?hsa05014/hsa:637%09red
Human papillomavirus infection	KEGG PATHWAY	hsa05165	2	330	0.064563214	0.112170433	AKT3|VEGFA	http://www.genome.jp/kegg-bin/show_pathway?hsa05165/hsa:10000%09red/hsa:7422%09red
Pyrimidine metabolism	KEGG PATHWAY	hsa00240	1	57	0.069865536	0.120168722	TYMP	http://www.genome.jp/kegg-bin/show_pathway?hsa00240/hsa:1890%09red
Endometrial cancer	KEGG PATHWAY	hsa05213	1	58	0.071027167	0.120957156	AKT3	http://www.genome.jp/kegg-bin/show_pathway?hsa05213/hsa:10000%09red
Viral myocarditis	KEGG PATHWAY	hsa05416	1	60	0.073346167	0.122480978	BID	http://www.genome.jp/kegg-bin/show_pathway?hsa05416/hsa:637%09red

Longevity regulating pathway - multiple species	KEGG PATHWAY	hsa04213	1	62	0.075659496	0.125129166	AKT3	http://www.genome.jp/kegg-bin/show_pathway?hsa04213/hsa:10000%09red
Cytosolic DNA-sensing pathway	KEGG PATHWAY	hsa04623	1	63	0.076814038	0.12582871	CXCL10	http://www.genome.jp/kegg-bin/show_pathway?hsa04623/hsa:3627%09red
Non-small cell lung cancer	KEGG PATHWAY	hsa05223	1	66	0.080269195	0.128028912	AKT3	http://www.genome.jp/kegg-bin/show_pathway?hsa05223/hsa:10000%09red
Acute myeloid leukemia	KEGG PATHWAY	hsa05221	1	66	0.080269195	0.128028912	AKT3	http://www.genome.jp/kegg-bin/show_pathway?hsa05221/hsa:10000%09red
Central carbon metabolism in cancer	KEGG PATHWAY	hsa05230	1	69	0.083711683	0.128028912	AKT3	http://www.genome.jp/kegg-bin/show_pathway?hsa05230/hsa:10000%09red
Adipocytokine signaling pathway	KEGG PATHWAY	hsa04920	1	69	0.083711683	0.128028912	AKT3	http://www.genome.jp/kegg-bin/show_pathway?hsa04920/hsa:10000%09red
Renin secretion	KEGG PATHWAY	hsa04924	1	69	0.083711683	0.128028912	NPR1	http://www.genome.jp/kegg-bin/show_pathway?hsa04924/hsa:4881%09red
Prolactin signaling pathway	KEGG PATHWAY	hsa04917	1	70	0.084856372	0.128028912	AKT3	http://www.genome.jp/kegg-bin/show_pathway?hsa04917/hsa:10000%09red
RIG-I-like receptor signaling pathway	KEGG PATHWAY	hsa04622	1	70	0.084856372	0.128028912	CXCL10	http://www.genome.jp/kegg-bin/show_pathway?hsa04622/hsa:3627%09red
p53 signaling pathway	KEGG PATHWAY	hsa04115	1	72	0.087141549	0.130333446	BID	http://www.genome.jp/kegg-bin/show_pathway?hsa04115/hsa:637%09red
Leishmaniasis	KEGG PATHWAY	hsa05140	1	74	0.089421135	0.132534159	IFNG	http://www.genome.jp/kegg-bin/show_pathway?hsa05140/hsa:3458%09red
Glioma	KEGG PATHWAY	hsa05214	1	75	0.090558836	0.132534159	AKT3	http://www.genome.jp/kegg-bin/show_pathway?hsa05214/hsa:10000%09red
Chronic myeloid leukemia	KEGG PATHWAY	hsa05220	1	76	0.091695145	0.132534159	AKT3	http://www.genome.jp/kegg-bin/show_pathway?hsa05220/hsa:10000%09red
PPAR signaling pathway	KEGG PATHWAY	hsa03320	1	76	0.091695145	0.132534159	ANGPTL4	http://www.genome.jp/kegg-bin/show_pathway?hsa03320/hsa:51129%09red
Antigen processing and presentation	KEGG PATHWAY	hsa04612	1	77	0.092830063	0.133056423	IFNG	http://www.genome.jp/kegg-bin/show_pathway?hsa04612/hsa:3458%09red

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https://doi.org/10.2147/IJGM.S343635

Table 1 (Continued).

Term Name (KEGG Pathway Data Base)	Database	ID	Input Number	Background Number	P-value	Corrected P-value	Input	Hyperlink
Drug metabolism - other enzymes	KEGG PATHWAY	hsa00983	1	79	0.095095733	0.135177405	TYMP	http://www.genome.jp/kegg-bin/show_pathway?hsa00983/hsa:1890%09red
Salmonella infection	KEGG PATHWAY	hsa05132	1	83	0.099610456	0.139292669	IFNG	http://www.genome.jp/kegg-bin/show_pathway?hsa05132/hsa:3458%09red
ErbB signaling pathway	KEGG PATHWAY	hsa04012	1	85	0.101859535	0.141265897	AKT3	http://www.genome.jp/kegg-bin/show_pathway?hsa04012/hsa:10000%09red
Longevity regulating pathway	KEGG PATHWAY	hsa04211	1	89	0.106341197	0.145164173	AKT3	http://www.genome.jp/kegg-bin/show_pathway?hsa04211/hsa:10000%09red
Th1 and Th2 cell differentiation	KEGG PATHWAY	hsa04658	1	92	0.109688059	0.148553907	IFNG	http://www.genome.jp/kegg-bin/show_pathway?hsa04658/hsa:3458%09red
Small cell lung cancer	KEGG PATHWAY	hsa05222	1	93	0.110800949	0.148888776	AKT3	http://www.genome.jp/kegg-bin/show_pathway?hsa05222/hsa:10000%09red
Amoebiasis	KEGG PATHWAY	hsa05146	1	95	0.113022643	0.150696857	IFNG	http://www.genome.jp/kegg-bin/show_pathway?hsa05146/hsa:3458%09red
Prostate cancer	KEGG PATHWAY	hsa05215	1	97	0.115238898	0.150756368	AKT3	http://www.genome.jp/kegg-bin/show_pathway?hsa05215/hsa:10000%09red
Aldosterone synthesis and secretion	KEGG PATHWAY	hsa04925	1	98	0.11634499	0.150756368	NPR1	http://www.genome.jp/kegg-bin/show_pathway?hsa04925/hsa:4881%09red
Endocrine resistance	KEGG PATHWAY	hsa01522	1	98	0.11634499	0.150756368	AKT3	http://www.genome.jp/kegg-bin/show_pathway?hsa01522/hsa:10000%09red
Progesterone-mediated oocyte maturation	KEGG PATHWAY	hsa04914	1	99	0.117449728	0.150756368	AKT3	http://www.genome.jp/kegg-bin/show_pathway?hsa04914/hsa:10000%09red
Choline metabolism in cancer	KEGG PATHWAY	hsa05231	1	99	0.117449728	0.150756368	AKT3	http://www.genome.jp/kegg-bin/show_pathway?hsa05231/hsa:10000%09red
C-type lectin receptor signaling pathway	KEGG PATHWAY	hsa04625	1	104	0.122953155	0.154364545	AKT3	http://www.genome.jp/kegg-bin/show_pathway?hsa04625/hsa:10000%09red
Parathyroid hormone synthesis, secretion and action	KEGG PATHWAY	hsa04928	1	106	0.1251451	0.154855808	PTH1R	http://www.genome.jp/kegg-bin/show_pathway?hsa04928/hsa:5745%09red

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Glucagon signaling pathway	KEGG PATHWAY	hsa04922	1	106	0.1251451	0.154855808	AKT3	http://www.genome.jp/kegg-bin/show_pathway?hsa04922/hsa:10000%09red
Insulin resistance	KEGG PATHWAY	hsa04931	1	108	0.127331679	0.156436063	AKT3	http://www.genome.jp/kegg-bin/show_pathway?hsa04931/hsa:10000%09red
Cholinergic synapse	KEGG PATHWAY	hsa04725	1	112	0.131688788	0.159510362	AKT3	http://www.genome.jp/kegg-bin/show_pathway?hsa04725/hsa:10000%09red
Leukocyte transendothelial migration	KEGG PATHWAY	hsa04670	1	112	0.131688788	0.159510362	VAV2	http://www.genome.jp/kegg-bin/show_pathway?hsa04670/hsa:7410%09red
Neurotrophin signaling pathway	KEGG PATHWAY	hsa04722	1	119	0.139262506	0.167504552	AKT3	http://www.genome.jp/kegg-bin/show_pathway?hsa04722/hsa:10000%09red
AMPK signaling pathway	KEGG PATHWAY	hsa04152	1	120	0.140339169	0.167627341	AKT3	http://www.genome.jp/kegg-bin/show_pathway?hsa04152/hsa:10000%09red
Platelet activation	KEGG PATHWAY	hsa04611	1	124	0.144632646	0.171564242	AKT3	http://www.genome.jp/kegg-bin/show_pathway?hsa04611/hsa:10000%09red
Autophagy - animal	KEGG PATHWAY	hsa04140	1	128	0.148905114	0.175422464	AKT3	http://www.genome.jp/kegg-bin/show_pathway?hsa04140/hsa:10000%09red
Purine metabolism	KEGG PATHWAY	hsa00230	1	130	0.151033502	0.175619655	NPR1	http://www.genome.jp/kegg-bin/show_pathway?hsa00230/hsa:4881%09red
Dopaminergic synapse	KEGG PATHWAY	hsa04728	1	131	0.15209574	0.175619655	AKT3	http://www.genome.jp/kegg-bin/show_pathway?hsa04728/hsa:10000%09red
Vascular smooth muscle contraction	KEGG PATHWAY	hsa04270	1	132	0.153156676	0.175619655	NPR1	http://www.genome.jp/kegg-bin/show_pathway?hsa04270/hsa:4881%09red
FoxO signaling pathway	KEGG PATHWAY	hsa04068	1	132	0.153156676	0.175619655	AKT3	http://www.genome.jp/kegg-bin/show_pathway?hsa04068/hsa:10000%09red
Systemic lupus erythematosus	KEGG PATHWAY	hsa05322	1	133	0.154216311	0.175663613	IFNG	http://www.genome.jp/kegg-bin/show_pathway?hsa05322/hsa:3458%09red
Insulin signaling pathway	KEGG PATHWAY	hsa04910	1	137	0.15844188	0.178117669	AKT3	http://www.genome.jp/kegg-bin/show_pathway?hsa04910/hsa:10000%09red
Apelin signaling pathway	KEGG PATHWAY	hsa04371	1	137	0.15844188	0.178117669	AKT3	http://www.genome.jp/kegg-bin/show_pathway?hsa04371/hsa:10000%09red

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Table 1 (Continued).

Term Name (KEGG Pathway Data Base)	Database	ID	Input Number	Background Number	P-value	Corrected P-value	Input	Hyperlink
Estrogen signaling pathway	KEGG PATHWAY	hsa04915	1	138	0.159495037	0.178137314	AKT3	http://www.genome.jp/kegg-bin/show_pathway?hsa04915/hsa:10000%09red
Signaling pathways regulating pluripotency of stem cells	KEGG PATHWAY	hsa04550	1	140	0.161597478	0.179321073	AKT3	http://www.genome.jp/kegg-bin/show_pathway?hsa04550/hsa:10000%09red
Phospholipase D signaling pathway	KEGG PATHWAY	hsa04072	1	148	0.169955848	0.187331979	AKT3	http://www.genome.jp/kegg-bin/show_pathway?hsa04072/hsa:10000%09red
Adrenergic signaling in cardiomyocytes	KEGG PATHWAY	hsa04261	1	149	0.170994887	0.187331979	AKT3	http://www.genome.jp/kegg-bin/show_pathway?hsa04261/hsa:10000%09red
Oxytocin signaling pathway	KEGG PATHWAY	hsa04921	1	153	0.175138319	0.189457805	NPR1	http://www.genome.jp/kegg-bin/show_pathway?hsa04921/hsa:4881%09red
mTOR signaling pathway	KEGG PATHWAY	hsa04150	1	153	0.175138319	0.189457805	AKT3	http://www.genome.jp/kegg-bin/show_pathway?hsa04150/hsa:10000%09red
Cellular senescence	KEGG PATHWAY	hsa04218	1	160	0.182340564	0.194798615	AKT3	http://www.genome.jp/kegg-bin/show_pathway?hsa04218/hsa:10000%09red
Hepatocellular carcinoma	KEGG PATHWAY	hsa05225	1	168	0.190496286	0.201014486	AKT3	http://www.genome.jp/kegg-bin/show_pathway?hsa05225/hsa:10000%09red
Alzheimer disease	KEGG PATHWAY	hsa05010	1	171	0.193534092	0.202974779	BID	http://www.genome.jp/kegg-bin/show_pathway?hsa05010/hsa:637%09red
Human T-cell leukemia virus 1 infection	KEGG PATHWAY	hsa05166	1	219	0.240647125	0.247852129	AKT3	http://www.genome.jp/kegg-bin/show_pathway?hsa05166/hsa:10000%09red
MicroRNAs in cancer	KEGG PATHWAY	hsa05206	1	299	0.313245477	0.320703702	VEGFA	http://www.genome.jp/kegg-bin/show_pathway?hsa05206/hsa:7422%09red
Metabolic pathways	KEGG PATHWAY	hsa01100	2	1433	0.538567906	0.541717426	NPR1|TYMP	http://www.genome.jp/kegg-bin/show_pathway?hsa01100/hsa:1890%09red/hsa:4881%09red
Term (KEGG disease data base)	**Database**	**ID**	**Input number**	**Background number**	**P-value**	**Corrected P-value**	**Input**	**Hyperlink**
Endocrine and metabolic diseases	KEGG DISEASE		3	220	0.002741264	0.012089679	SEMA3A|THRB|VEGFA	None
Immune system diseases	KEGG DISEASE		3	278	0.005229537	0.019137882	IFNG|CXCL10|EPO	None

Allergies and autoimmune diseases	KEGG DISEASE		2	93	0.006317396	0.021305726	IFNG|CXCL10	None
Avascular necrosis of femoral head	KEGG DISEASE	H01529	1	5	0.007459434	0.023759679	VEGFA	http://www.genome.jp/dbget-bin/www_bget?H01529
Potter syndrome	KEGG DISEASE	H01728	1	6	0.008697362	0.024932438	FGF20	http://www.genome.jp/dbget-bin/www_bget?H01728
Metaphyseal dysplasias	KEGG DISEASE	H00479	1	6	0.008697362	0.024932438	PTH1R	http://www.genome.jp/dbget-bin/www_bget?H00479
Glucocorticoid-induced osteonecrosis	KEGG DISEASE	H01709	1	6	0.008697362	0.024932438	VEGFA	http://www.genome.jp/dbget-bin/www_bget?H01709
Allograft rejection	KEGG DISEASE	H00083	1	11	0.014864352	0.037053167	IFNG	http://www.genome.jp/dbget-bin/www_bget?H00083
Graft-versus-host disease	KEGG DISEASE	H00084	1	12	0.016093232	0.038986422	IFNG	http://www.genome.jp/dbget-bin/www_bget?H00084
Mitochondrial DNA depletion syndrome	KEGG DISEASE	H00469	1	15	0.01977087	0.04658342	TYMP	http://www.genome.jp/dbget-bin/www_bget?H00469
Thyroid gland diseases	KEGG DISEASE		1	19	0.024653448	0.055248506	THRB	None
Allergic rhinitis	KEGG DISEASE	H01360	1	19	0.024653448	0.055248506	CXCL10	http://www.genome.jp/dbget-bin/www_bget?H01360
Hypogonadotropic hypogonadism	KEGG DISEASE	H00255	1	23	0.029512198	0.063451227	SEMA3A	http://www.genome.jp/dbget-bin/www_bget?H00255
Mouth and dental diseases	KEGG DISEASE		1	31	0.039158676	0.08018205	PTH1R	None
Other immune system diseases	KEGG DISEASE		1	45	0.055814491	0.105495522	EPO	None
Skeletal diseases	KEGG DISEASE		1	48	0.059346623	0.109395872	VEGFA	None
Congenital malformations of the urinary system	KEGG DISEASE		1	49	0.060521122	0.109395872	FGF20	None
Hypothalamus and pituitary gland diseases	KEGG DISEASE		1	60	0.073346167	0.122480978	SEMA3A	None

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https://doi.org/10.2147/IJGM.S343635

Table 1 (Continued).

Term Name (KEGG Pathway Data Base)	Database	ID	Input Number	Background Number	P-value	Corrected P-value	Input	Hyperlink
Diabetes	KEGG DISEASE		1	67	0.081418096	0.128028912	VEGFA	None
Reproductive system diseases	KEGG DISEASE		1	68	0.082565592	0.128028912	AMH	None
Digestive system diseases	KEGG DISEASE		1	81	0.097355859	0.137255801	PTH1R	None
Congenital malformations	KEGG DISEASE		3	900	0.102664169	0.141265897	PTH1R|AKT3|FGF20	None
Skin and soft tissue diseases	KEGG DISEASE		1	103	0.121855166	0.154110945	FGF5	None
Skin diseases	KEGG DISEASE		1	103	0.121855166	0.154110945	FGF5	None
Musculoskeletal diseases	KEGG DISEASE		1	156	0.178232574	0.191600017	VEGFA	None
Mitochondrial diseases	KEGG DISEASE		1	164	0.186428436	0.197936365	TYMP	None
Hematologic diseases	KEGG DISEASE		1	181	0.203579678	0.212216391	IFNG	None
Congenital malformations of the musculoskeletal system	KEGG DISEASE		1	201	0.22330449	0.231375736	PTH1R	None
Cardiovascular diseases	KEGG DISEASE		1	342	0.349410788	0.355613346	IFNG	None
Other congenital malformations	KEGG DISEASE		1	357	0.361582275	0.365836184	AKT3	None
Congenital disorders of metabolism	KEGG DISEASE		1	695	0.583662873	0.583662873	TYMP	None

using the "pheatmap" package in R and the common differentially expressed genes between the two datasets were determined using the "Venn diagrams" in R. Immune-related genes were extracted from the DEGs after KEGG enrichment analysis. Immune-related genes were downloaded from the IMMPORT database (https://www.immport.org/home).

Pathway Enrichment and Annotation

Enrichment analysis of the Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway for DEGs was performed using the KOBAS online tool (http://kobas.cbi.pku.edu.cn/index.php). KEGG analysis showed DEG enrichment in the signaling pathways.

PPI Network Analysis

The immune-associated DEGs were then used for PPI analysis. The DEG PPI network was constructed using the STRING database. Nodes with the confidence of interactive relationship > 0.7 were defined as the threshold. Subsequently, CytoHubba was utilized to identify the top ten hub genes and Venn diagrams were used to visualize common genes between the top 20 hub genes from GC and PUD patients, respectively.

Gene Set Enrichment Analysis (GSEA) and CIBERSORT Algorithm

To explore the role of the ten hub genes in regulating the cell immunological micro-environment, the CIBERSORT algorithm was applied to assess the proportion of immune cells that infiltrated the ECM using the LM22 signature. The LM22 signature consisting of 547 genes was used to identify 22 types of infiltrating immune cells. The cell fraction of the PUD and GC datasets was identified. Difference and correlation analyses were performed to explore the correlation between SMA3D expression and the types of immune cells that infiltrated the GC and PUD microenvironment ($P < 0.05$).

Clinicopathological Characteristics Analysis and Survival Analysis

GC patients were classified into a high- and low-group based on SMA3D and NRP2 expression levels. The association between clinicopathological characteristics and SMA3D and NRP2 expression was evaluated. K-M plotter was used to plot survival curves, which were compared using the Log rank test. Univariate and multivariate analyses were performed using the Cox proportional hazards model to investigate the influence of genotypes on cancer risk. $P<0.05$ was regarded as statistically significant.

Statistical Analysis

Statistical analyses were performed using R software (version 4.0.2) and GraphPad Prism. All statistical methods and appropriate R packages were described throughout the study. Hypergeometric test/Fisher's exact test was employed to perform KEGG enrichment analysis. Method proposed by Benjamini & Hochberg was used to control for the false discovery rate (FDR).[24] ANOVA (one-way) and the Tukey's test were used to compare multiple groups. Chi-square was used to analyze the correlation between SEMA3D and NRP2 expression in GC tissues and the patient clinical data. P and $q<0.05$ were considered statistically significant.

Results

DEGs Identification and Enrichment Analysis

Differential analysis was performed to determine immune-related DEGs. Heatmaps showed the differential gene expression profiles of GC and PUD patients (Figure 1A and B). A total of 6032 and 2032 DEGs were identified between the stromal low-score and high-score groups in each data set, respectively. The threshold of difference was $|\log2FC| >1$ and FDR <0.05. In both the GC and PUD groups, 437 DEGs were identified as common DEGs using Venn diagrams (Figure 1C). The common DEGs were overlapped with immune-related gene sets from the IMMPORT database and 49 immune-related DEGs were grouped to identify their primary functions (Figure 1D). The top three KEGG enrichment scores indicated that these DEGs were enriched in the cytokine-cytokine receptor interaction, neuroactive ligand-receptor

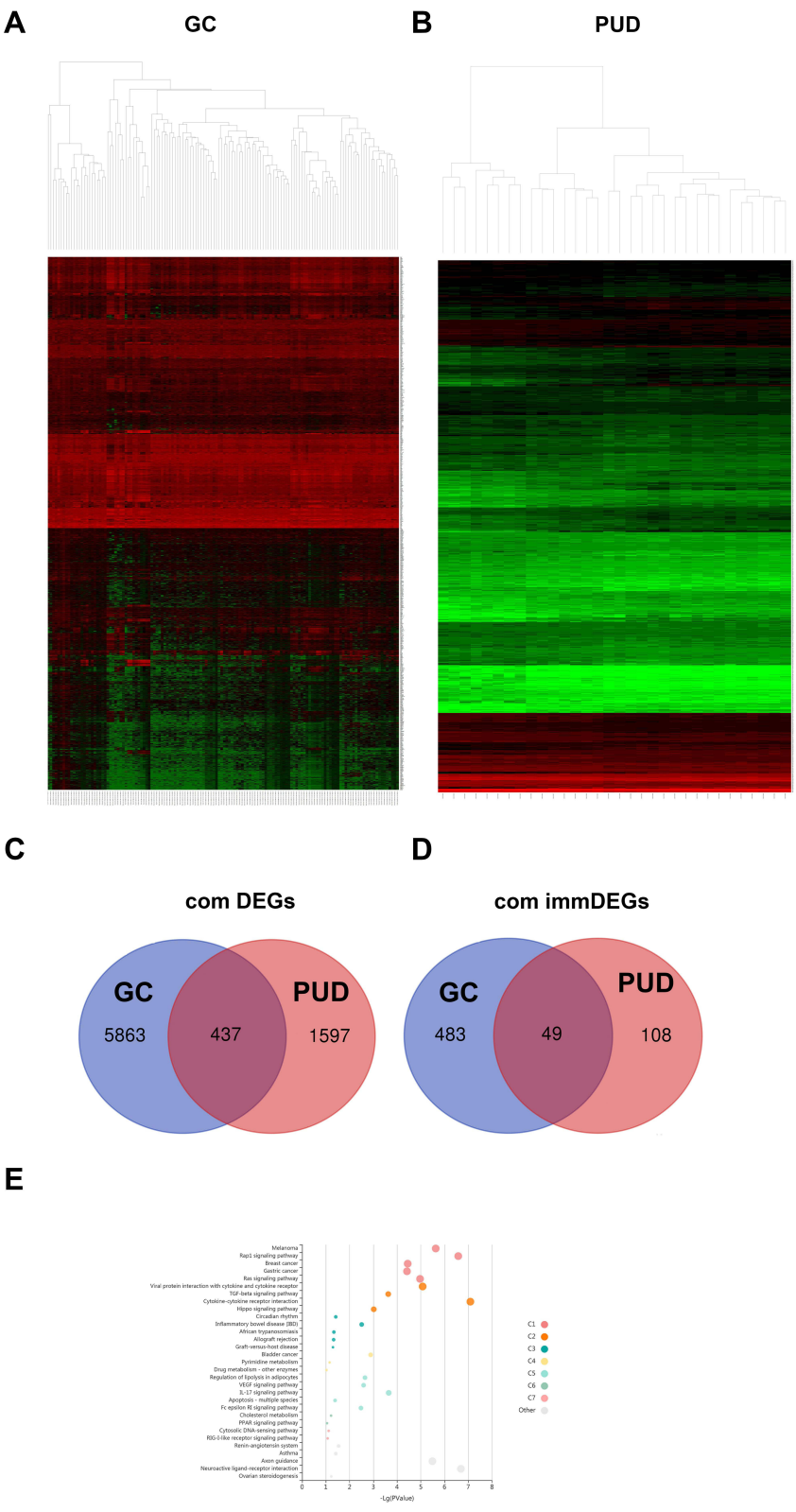

Figure 1 DEGs commonly expressed in three datasets. (**A** and **B**) Hierarchical clustering heatmap of upregulated and downregulated DEGs in the PUD or GC groups from each dataset (green points) and genes without significance (black points). The differences threshold was set as |log2FC| >1.0 and adjusted P-value <0.05. The Venn diagram shows 437 DEGs (**C**) and 49 immune-related DEG (**D**) commonly expressed across the two datasets. (**E**) KEGG enrichment analysis of immune-related DEGs.

interaction, and Rap1 signaling pathway pathways (Figure 1E). The complete pathways list of the KEGG enrichment is shown in Table 1.

PPI Network Construction

The STRINGs database was used to assess the interaction between immune-associated DEGs (Figure 2A). A PPI network was then constructed with a confidence of interactive relationship >0.7 as the threshold. CytoHubba, a plug-in of the Cytoscape software, was used to screen the hub gene through three terms of degrees, closeness, and betweenness. Seven hub genes were identified as described in the methods (Figure 2B and C).

Correlation of the Survival and Clinicopathological Characteristics with Hub Gene Expression

Of the seven hub genes, VEGFA, EPO, SPP1, IFNG, and PLXNB3 were closely related to GC progression. NRP2 and SEMA3D were selected for survival comparative analysis in the GC group. The Kaplan-Meier survival curve showed that GC patients with low expression of SEMA3Dlow had a better overall survival rate than those with high expression (Figure 3; P<0.05; adjust HR=2.446, 95% CI 1.225–4.882). In contrast, differences in NRP2 expression did not have much effect on overall survival (Figure 3B). The correlation between SEMA3D expression and clinical characteristics was assessed using COX analysis. SEMA3D expression was closely correlated with advanced disease stages but not with TNM classification, indicating that SEMASD merits a higher clinical prognostic value (Table 2, *p<0.05).

Immune Cell Infiltration Analysis and Correlation Analysis

The CIBERSORT algorithm with 22 immune cells signature was employed to perform immune cell infiltration analysis using GC and PUD tissue samples. A higher fraction of active mast cells were found in the GC group than in the PUD group (Figure 4A). As shown in Figure 4B, SEMA3D expression and active mast cells showed a significant positive correlation in the GC group, but SEMA3D expression and DCs were positively correlated in the PUD group.

Discussion

The primary goal of this study was to explore significant hub genes associated with the malignant transformation of PUD into early GC. Seven significant hub genes were identified using bioinformatics. The SEMA3D gene was found to correlate with advanced clinicopathological stages of GC and patient survival.

KEGG pathway enrichment analysis showed that DEGs regulate many types of immune response in clinical tissue. SEMA3s always require additional neuropilin (NRP) receptors to bind VEGF, and the VEGF/SEMA3s balance is a prognostic marker of disease.[25] Results from this study showed that high expression of SEMA3D and NRP2 correlated with activation of the non-canonical VEGF pathway, while VEGFA signaling was inhibited. Given the TLR4 was shown to mediate CD8+T cell activation during particular innate immune responses to disease, VEGF pathway was regarded as a critical regulator in pro-inflammatory responses.[21,26,27]

SEMA3D, which encodes a semaphorin III family secreted protein, is a critical regulator of neuron development and diverse tumorigenic processes like proliferation, invasion, and angiogenesis.[19,28,29] Abnormal SEMA3D expression is associated with a poor prognosis in many nervous system diseases and cancers.[20,25,30–32] Decreased SEMA3D expression in gastrointestinal tumors correlates significantly with colorectal cancer progression while overexpression is a favorable prognostic factor for survival.[33] SEMA3D is reported to participate in the recruitment of immune cells to the disease site.[34] Results from this study showed that SEMA3D was significantly correlated with PUD patient outcomes. These findings imply that SMA3D could serve as a potential biomarker for early diagnosis of GC.

PPI and KEGG analyses showed that SEMA3D was involved in regulating immune-related pathways and the extracellular microenvironment of GC. The proportions of immune cells that infiltrated the cellular microenvironment were estimated using the CIBERSORT algorithm and NRP2 and SEMA3D were expressed in similar cell types. SEMA3D expression was primarily correlated with three infiltrating immune cell types, CD4+T cells, DCs, and mast cells. Interestingly, SEMA3D and NER2 expression were enriched in CD4+T cells and DCs from the PUD samples but

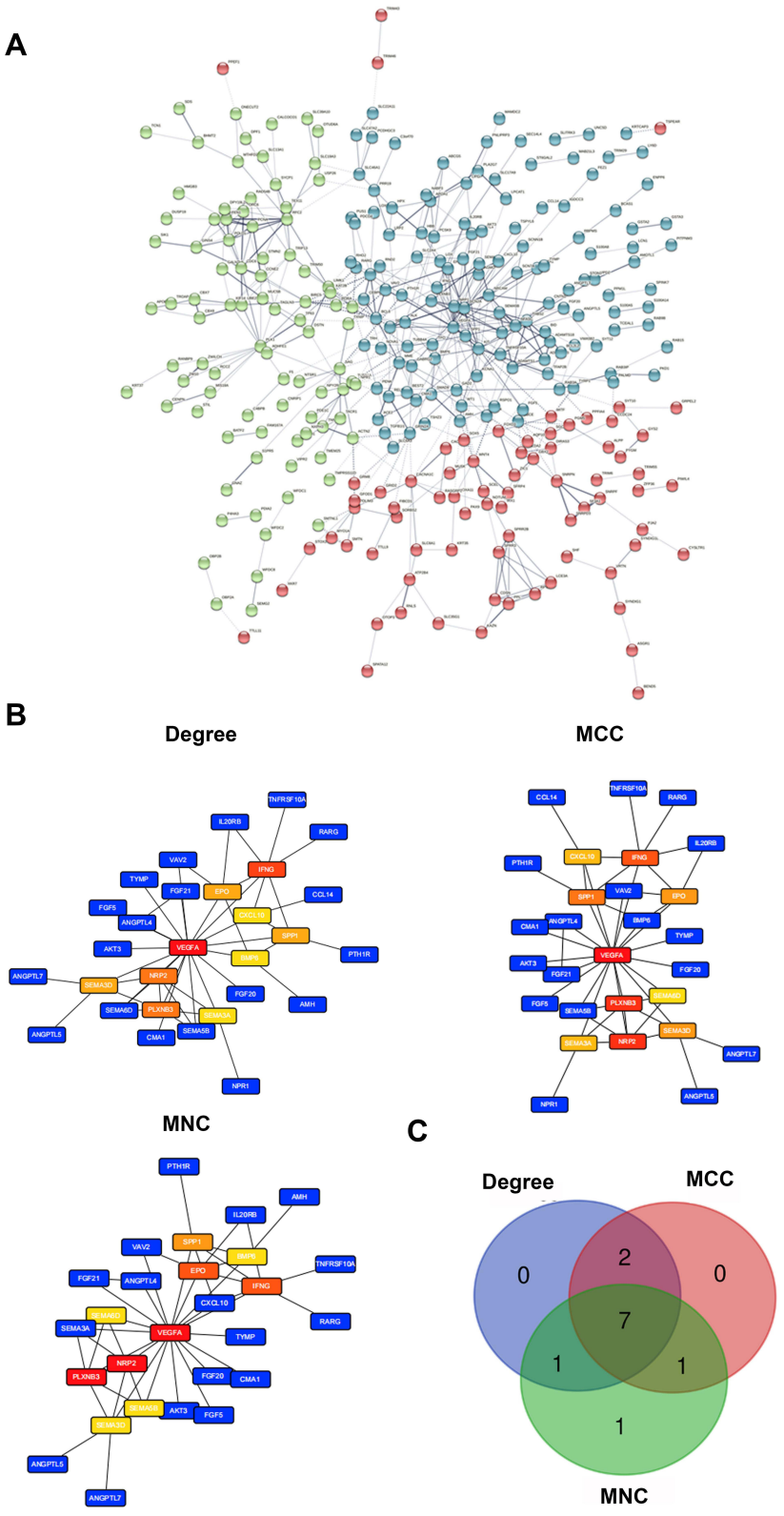

Figure 2 PPI networks and hub gene analysis of commonly expressed DEGs in the immune-related dataset. (**A**) PPI networks constructed by the STRINGs. (**B**) Major PPI network analysis of the top 10 hub genes using Cytohubba software by three methods. The node color reflects the degree of connectivity. (**C**) The Venn diagram of the three methods.

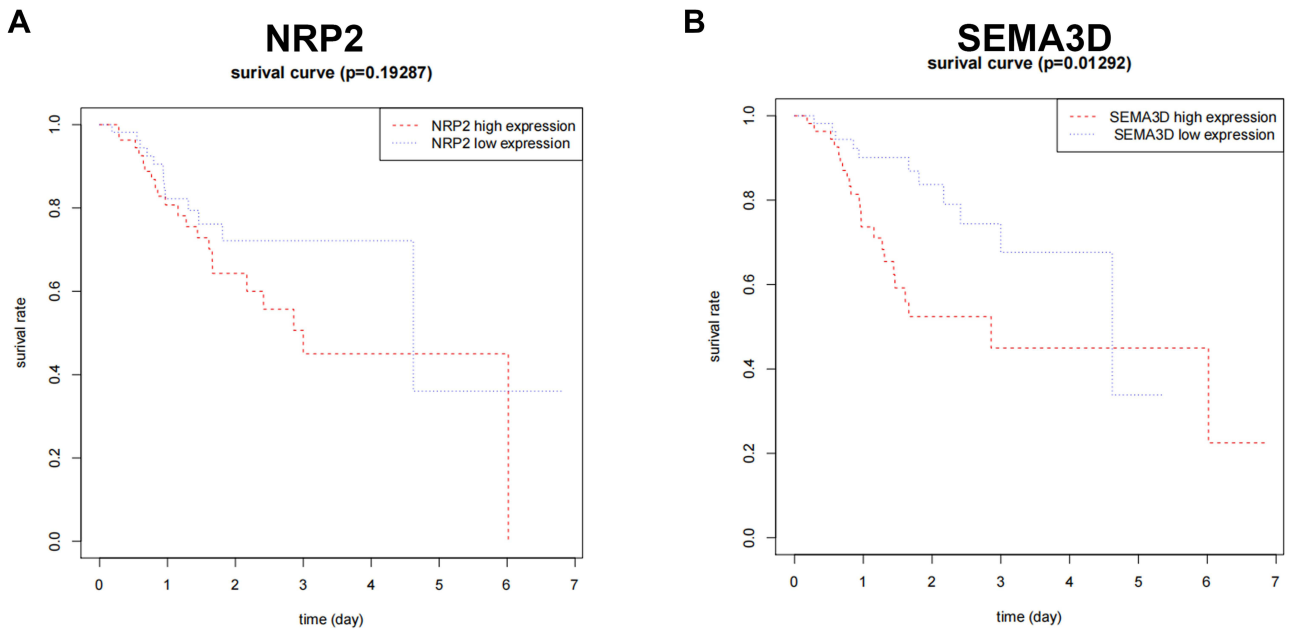

Figure 3 Correlation of NRP2 (**A**) and SEMA3D (**B**) expression with GC patient survival. COX analysis was performed to get an adjusted HR: (SEMA3D: P=0.01292; adjust HR=2.446, 95% CI 1.225–4.882), (NRP2: P=0.19287; adjust HR=1.313, 95% CI 10.6540–2.635).

primarily expressed in CD4+T cells and mast cells from the GC samples. Different DC subsets can differentially regulate T cell function. In PUD samples, DCs primarily functioned to induce T and B cell activation.[23] Mast cell function during cancer remains unclear, however. Recent studies show that mast cells promote gastric tumor cancer by releasing

Table 2 Cox Regression Analysis of Many Clinical-Pathological Characteristic in GC Dataset with SEMAD3

Variable	H R	CI (95%)	P
Univariate analysis (n =136)			
Age	2.190	1.325–3.652	0.067
Gender	0.882	0.157–2.127	0.149
T stage (T1–2/T3–4)	2.31	1.585–3.767	0.005*
N stage (N0/N1–X)	2.44	1.268–3.601	0.004*
M stage (M0/MX)	1.022	0.385–2.117	0.041*
Clinical stage (I/II)	1.688	1.512–5.786	0.003*
SEMAD3 (low/high)	2.031	1.232–2.879	0.013*
WHO histological classification	1.275	0.215–2.797	0.868
Multivariate analysis (n =136)			
Age	2.041	1.271–3.525	0.074
Clinical stage (I/II)	1.941	1.228–2.868	0.017*
SEMAD3 (low/high)	2.259	1.335–4.328	0.009*

Notes: The Chi-square and Fisher exact test were used to assess correlations between clinicopathologic features and expression of SEMA3d. The univariate and multivariate survival analysis were performed with Cox regression. All *P* values reported are from two-sided tests and the threshold for significance was set at 0.05. *p<0.05.
Abbreviations: HR, hazard ratio; CI, confident interval.

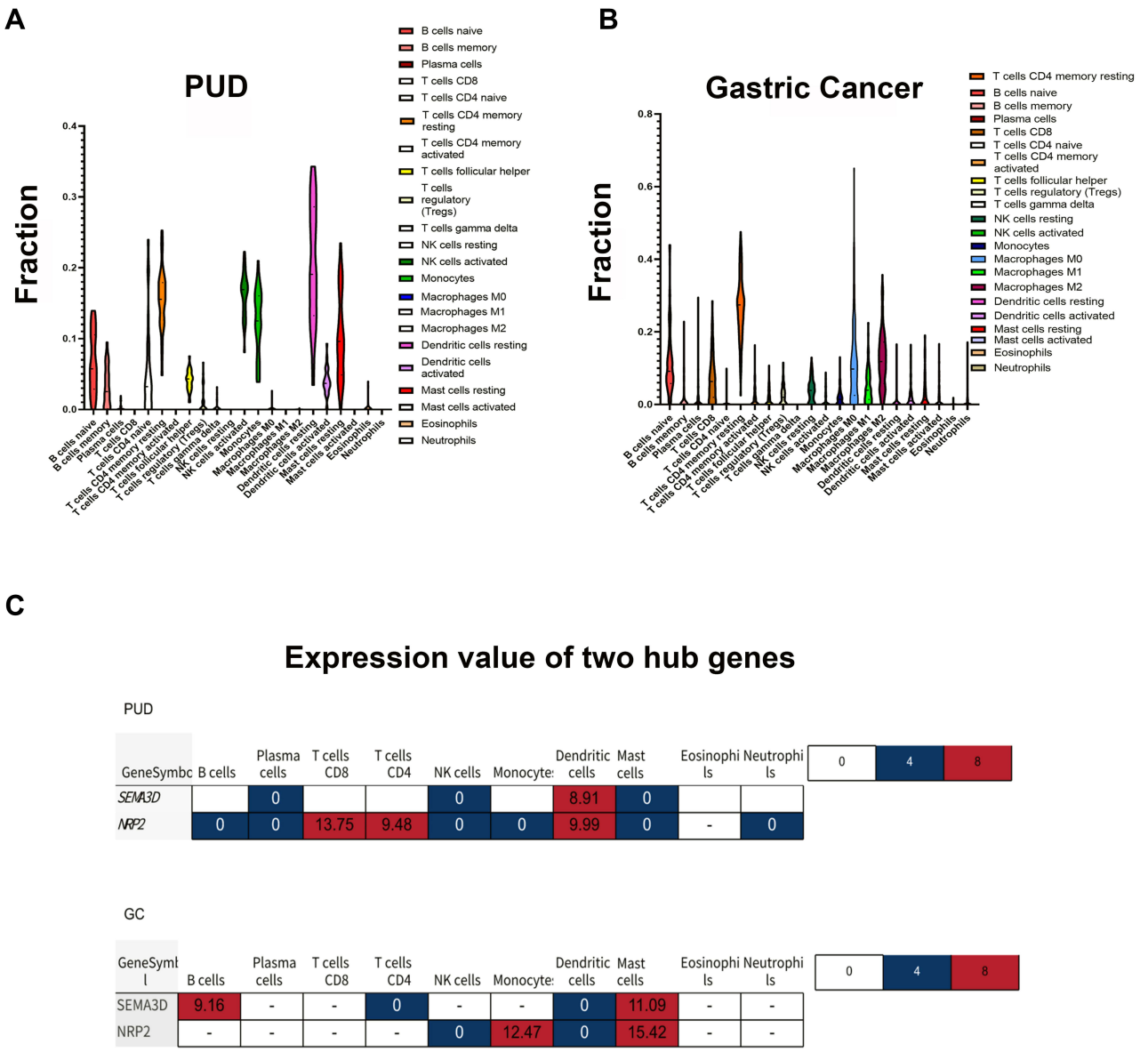

Figure 4 Immune cell infiltration analysis and correlation analysis. (**A**) Violin plot showing significant changes in immune cell infiltration in GC compared with PUD groups (P-value <0.05). (**B**) Correlation between gene expression and the relative percentages of immune cells in PUD and GC tissue.(**C**) The expression value of the two hub genes in different immune cells.

angiogenic cytokines.[35] Results from this study suggest that while SEMA3D expression in DCs from PUD tissue samples may help them to subvert the host immune response by activating T cells, SEMASD expression in mast cells may promote tumorigenesis. Similar heterogeneous functions of other SEMA3s are reported in other cancers.[36] A diagram that summarizes the findings of this study and hypothesizes how SEMA3D expression impacts DC and mast cell function is shown in Hypothetic Diagram (Figure S2). The detailed molecular mechanism of how this occurs requires additional study.

Results from this study defined seven hub genes associated with PUD-related carcinogenesis, provided strong evidence that SEMA3D correlates with tumor-related immune activation or dysfunction, and provided a new direction to study how hub gene functions during PUD and GC. However, this study does not describe the detailed mechanism by which hub genes participate in DC and mast cell function during PUD inflammation or the potential relationship between these genes and HP infection. HP-induced PUD is associated with gastric cancer, but there are few biomarkers that aid

disease prognosis in clinical practice. In this study, SEMASD was defined as a potential prognostic molecule for PUD and GC, though its mechanism of action and clinical value require further research.

Conclusions

Using comprehensive bioinformatics, this study found that the hub gene, SEMA3D, was associated with the infiltration of immune cells, in particular DCs and mast cells, into PUD and GC tissue samples. Additional research on how SEMASD impacts immune cell function in the PUD and GC dataset will help to elucidate the mechanism of malignant transformation during PUD.

Abbreviations

SEMA3s, Class-3 semaphorins; DC, dendritic cell; ECM, extracellular matrix; FDR, false discovery rate; GC, gastrointestinal cancer; GEO, Gene Expression Omnibus; HP, *Helicobacter pylori*; KEGG, Kyoto Encyclopedia of Genes and Genomes; PPI, Protein-protein interaction; PUD, Peptic ulcer disease; TCGA, The Cancer Genome Atlas.

Ethical Approval and Consent to Participate

This study was approved and conducted in compliance with the guidelines by the ethics committee of Affiliated Hospital of Hebei University (AHHU20211029). All the data used in the study was downloaded from TCGA and GEO database. Written informed consent was acquired from all enrolled patients. These original research has been carried out in accordance with the World Medical Association Declaration of Helsinki.

Author Contributions

All authors made a significant contribution to the work reported, whether that is in the conception, study design, execution, acquisition of data, analysis and interpretation, or in all these areas; took part in drafting, revising or critically reviewing the article; gave final approval of the version to be published; have agreed on the journal to which the article has been submitted; and agree to be accountable for all aspects of the work.

Funding

This research received no specific grant from any funding agency in the public, commercial, or not-for-profit sectors.

Disclosure

The authors report no conflicts of interest in this work.

References

1. Suerbaum S, Michetti P. Helicobacter pylori infection. *N Engl J Med*. 2002;347(15):1175–1186. doi:10.1056/NEJMra020542
2. Uemura N, Okamoto S, Yamamoto S, et al. Helicobacter pylori infection and the development of gastric cancer. *N Engl J Med*. 2001;345(11):784–789. doi:10.1056/NEJMoa001999
3. Camilo V, Sugiyama T, Touati E. Pathogenesis of Helicobacter pylori infection. *Helicobacter*. 2017;22(Suppl 1). PMID: 28891130. doi:10.1111/hel.12405.
4. Wessler S, Krisch LM, Elmer DP, Aberger F. From inflammation to gastric cancer - the importance of Hedgehog/GLI signaling in Helicobacter pylori-induced chronic inflammatory and neoplastic diseases. *Cell Commun Signal*. 2017;15(1):15.
5. Bagheri N, Azadegan-Dehkordi F, Rahimian G, Rafieian-Kopaei M, Shirzad H. Role of regulatory T-cells in different clinical expressions of helicobacter pylori infection. *Arch Med Res*. 2016;47(4):245–254. doi:10.1016/j.arcmed.2016.07.013
6. Valkenburg KC, de Groot AE, Pienta KJ. Targeting the tumour stroma to improve cancer therapy. *Nat Rev Clin Oncol*. 2018;15(6):366–381.
7. Chen Q, Liu G, Liu S, et al. Remodeling the tumor microenvironment with emerging nanotherapeutics. *Trends Pharmacol Sci*. 2018;39(1):59–74. doi:10.1016/j.tips.2017.10.009
8. Jiang Y, Zhang Q, Hu Y, et al. ImmunoScore signature: a prognostic and predictive tool in gastric cancer. *Ann Surg*. 2018;267(3):504–513. doi:10.1097/SLA.0000000000002116
9. Marta ŻN, Agnieszka W, Jacek P, et al. NFKB2 gene expression in patients with peptic ulcer diseases and gastric cancer. *Mol Biol Rep*. 2020;47(3):2015–2021. doi:10.1007/s11033-020-05299-5
10. Zeng D, Li M, Zhou R, et al. Tumor microenvironment characterization in gastric cancer identifies prognostic and immunotherapeutically relevant gene signatures. *Cancer Immunol Res*. 2019;7(5):737–750. doi:10.1158/2326-6066.CIR-18-0436
11. Newman AM, Liu CL, Green MR, et al. Robust enumeration of cell subsets from tissue expression profiles. *Nat Methods*. 2015;12(5):453–457. doi:10.1038/nmeth.3337

12. Bense RD, Sotiriou C, Piccart-Gebhart MJ, et al. Relevance of tumor-infiltrating immune cell composition and functionality for disease outcome in breast cancer. *J Natl Cancer Inst*. 2017;109(1):djw192. doi:10.1093/jnci/djw192
13. Chen B, Khodadoust MS, Liu CL, Newman AM, Alizadeh AA. Profiling tumor infiltrating immune cells with CIBERSORT. *Methods Mol Biol*. 2018;1711:243–259.
14. Angelopoulou E, Piperi C. Emerging role of plexins signaling in glioma progression and therapy. *Cancer Letter*. 2018;414:81–87. doi:10.1016/j.canlet.2017.11.010
15. Toledano S, Nir-Zvi I, Engelman R, Kessler O, Neufeld G. Class-3 semaphorins and their receptors: potent multifunctional modulators of tumor progression. *Int J Mol Sci*. 2019;20(3):556. doi:10.3390/ijms20030556
16. Wu J, Xiao Y, Xia C, et al. Identification of biomarkers for predicting lymph node metastasis of stomach cancer using clinical DNA methylation data. *Dis Markers*. 2017;2017:5745724. doi:10.1155/2017/5745724
17. Movassagh H, Shan L, Koussih L, et al. Semaphorin 3E deficiency dysregulates dendritic cell functions: in vitro and in vivo evidence. *PLoS One*. 2021;16(6):e0252868. doi:10.1371/journal.pone.0252868
18. Choi YI, Duke-Cohan JS, Ahmed WB, et al. PlexinD1 glycoprotein controls migration of positively selected thymocytes into the medulla. *Immunity*. 2008;29(6):888–898. doi:10.1016/j.immuni.2008.10.008
19. Aghajanian H, Choi C, Ho VC, Gupta M, Singh MK, Epstein JA. Semaphorin 3d and semaphorin 3e direct endothelial motility through distinct molecular signaling pathways. *J Biol Chem*. 2014;289(26):17971–17979.
20. Kigel B, Varshavsky A, Kessler O, Neufeld G. Successful inhibition of tumor development by specific class-3 semaphorins is associated with expression of appropriate semaphorin receptors by tumor cells. *PLoS One*. 2008;3(9):e3287.
21. Gaddis DE, Padgett LE, Wu R, Hedrick CC. Neuropilin-1 expression on CD4 T cells is atherogenic and facilitates T cell migration to the aorta in atherosclerosis. *J Immunol*. 2019;203(12):3237–3246. doi:10.4049/jimmunol.1900245
22. Reyes VE, Peniche AG. Helicobacter pylori deregulates T and B cell signaling to trigger immune evasion. *Curr Top Microbiol Immunol*. 2019;421:229.
23. Kao JY, Zhang M, Miller MJ, et al. Helicobacter pylori immune escape is mediated by dendritic cell-induced Treg skewing and Th17 suppression in mice. *Gastroenterology*. 2010;138(3):1046–1054. doi:10.1053/j.gastro.2009.11.043
24. Benjamini Y, Hochberg Y. Controlling the false discovery rate: a practical and powerful approach to multiple testing. *J R Stat Soc Series B Stat Methodol*. 1995;57(1):289–300.
25. Karayan-Tapon L, Wager M, Guilhot J, et al. Semaphorin, neuropilin and VEGF expression in glial tumours: SEMA3G, a prognostic marker? *Br J Cancer*. 2008;99(7):1153–1160.
26. Li S, Liu M, Do MH, et al. Cancer immunotherapy via targeted TGF-β signalling blockade in TH cells. *Nature*. 2020;587(7832):121–125. doi:10.1038/s41586-020-2850-3
27. Yun H, Yee MB, Lathrop KL, Kinchington PR, Hendricks RL, St Leger AJ. Production of the cytokine VEGF-A by CD4+ T and myeloid cells disrupts the corneal nerve landscape and promotes herpes stromal keratitis. *Immunity*. 2020;53(5):1050–1062.e5. doi:10.1016/j.immuni.2020.10.013
28. Berndt JD, Halloran MC. Semaphorin 3d promotes cell proliferation and neural crest cell development downstream of TCF in the zebrafish hindbrain. *Development*. 2006;133(20):3983–3992. doi:10.1242/dev.02583
29. Sabag AD, Bode J, Fink D, Kigel B, Kugler W, Neufeld G. Semaphorin-3D and semaphorin-3E inhibit the development of tumors from glioblastoma cells implanted in the cortex of the brain. *PLoS One*. 2012;7(8):e42912. doi:10.1371/journal.pone.0042912
30. Foley K, Rucki AA, Xiao Q, et al. Semaphorin 3D autocrine signaling mediates the metastatic role of annexin A2 in pancreatic cancer. *Sci Signal*. 2015;8(388):ra77. doi:10.1126/scisignal.aaa5823
31. Luzon-Toro B, Fernandez RM, Torroglosa A, et al. Mutational spectrum of semaphorin 3A and semaphorin 3D genes in Spanish Hirschsprung patients. *PLoS One*. 2013;8:e54800. doi:10.1371/journal.pone.0054800
32. Hu Z, Zhu D, Wang W, et al. Genome-wide profiling of HPV integration in cervical cancer identifies clustered genomic hot spots and a potential microhomology-mediated integration mechanism. *Nat Genet*. 2015;47(2):158–163. doi:10.1038/ng.3178
33. Wang Z, Ding M, Qian N, et al. Decreased expression of semaphorin 3D is associated with genesis and development in colorectal cancer. *World J Surg Oncol*. 2017;15(1):67. doi:10.1186/s12957-017-1128-1
34. McGeachie MJ, Wu AC, Tse SM, et al. CTNNA3 and SEMA3D: promising loci for asthma exacerbation identified through multiple genome-wide association studies. *J Allergy Clin Immunol*. 2015;136(6):1503–1510. doi:10.1016/j.jaci.2015.04.039
35. Sammarco G, Varricchi G, Ferraro V, et al. Mast cells, angiogenesis and lymphangiogenesis in human gastric cancer. *Int J Mol Sci*. 2019;20(9):2106. doi:10.3390/ijms20092106
36. Zhang X, Klamer B, Li J, Fernandez S, Li L. A pan-cancer study of class-3 semaphorins as therapeutic targets in cancer. *BMC Med Genomics*. 2020;13(Suppl 5):45. doi:10.1186/s12920-020-0682-5