

Overexpression of MATN3 predicts poor prognosis of gastric adenocarcinoma: based on TCGA database

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Research article

Keywords: MATN3, Gastric adenocarcinoma, The Cancer Genome Atlas, Prognosis, GSEA

Posted Date: February 13th, 2020

DOI: <https://doi.org/10.21203/rs.2.23505/v1>

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Abstract

Background Matrilin-3 (MATN3) was previously reported to be in the cartilage extracellular matrix and had a role in the development and homeostasis of cartilage and bone. We evaluated the role of MATN3 in gastric adenocarcinoma (GAC) using publicly available data from The Cancer Genome Atlas (TCGA). Methods The relationship between clinicopathologic features and MATN3 were analyzed with the Wilcoxon signed-rank test and logistic regression. Clinicopathologic variables associated with overall survival (OS) in TCGA patients using Cox regression and the Kaplan-Meier method. Gene Set Enrichment Analysis (GSEA) was performed using TCGA cohort. Results MATN3 overexpressed in GAC than that in normal tissues ($p < 0.05$), and MATN3 overexpression was significantly associated with TNM stage ($p = 0.012$), and T stage ($p < 0.01$). Kaplan-Meier survival analysis showed that GAC with MATN3 - high had a worse prognosis than that with MATN3 - low ($p < 0.01$). The univariate analysis revealed that MATN3 - high correlated significantly with a poor OS (HR: 1.86; 95% confidence interval [CI]: 0.82- 2.01; $p = 0.014$). The multivariate analysis revealed that MATN3 remained independently associated with OS, with a HR of 2.68 (CI: 0.74- 2.13; $p < 0.01$). GSEA showed that NOTCH, WNT, and MTOR signaling pathway were differentially enriched in MATN3 high expression phenotype. Conclusion Overexpression of MATN3 may be a potential prognostic biomarker of poor survival in gastric cancer, Moreover, NOTCH, WNT, and MTOR signaling pathway may be the key pathway regulated by MATN3 in GAC.

Introduction

Gastric adenocarcinoma (GAC) remains one of the most widespread tumors in the world. It was the fifth most frequently diagnosed cancer and the third leading cause of cancer death according to the data of epidemiological survey in 2018 [1]. Recurrence and metastasis is the main cause of death in patients with GAC, which seriously hinders the success of treatment [2]. GAC is a complicated multi-step disease. The pathogenesis of GAC involves genomic changes, including gene amplification, deletion, insertion or mutation, which ultimately trigger a series of epigenetic and genetic changes. Therefore, the key to the prognosis and treatment of gastric cancer is to identify key genes and understand their molecular mechanisms. The matrilin (MATNs) are a family of oligomeric extracellular matrix (ECM) proteins, which consist of at least four related proteins (matrilin-1 to 4). This family is thought to take part in the formation of filamentous networks in the extracellular matrices of various tissues. Matrilin-3 (MATN3), which is composed of one vWFA domain, four EGF-like domains, and a C-terminal coiled-coil domain, is the simplest member of the matrilin family [3,4]. Previous studies have found that MATN3 protein is present in the cartilage extracellular matrix and plays important roles in the development and homeostasis of cartilage and bone [5]. Mutations in the gene result in multiple epiphyseal dysplasia [6,7]. Until now, the expression level of MATN3 in malignant tumors, especially in GAC, remains unclear.

Thus, this study aimed to investigate the prognostic value of MATN3 expression in GAC based on the TCGA datasets. GSEA was performed to gain further insight into the biological pathways involved in GAC pathogenesis related MATN3 regulatory network.

Therefore, the purpose of this study was to evaluate the prognostic value of MATN3 expression in GAC based on TCGA, and further understand the GAC signaling pathway related to MATN3 regulation through GSEA.

Materials And Methods

2.1. Downloading mRNA data and analysis

The mRNA expression data and corresponding clinicopathologic data of the stomach projects were downloaded from TCGA website. We selected patients with pathological type of adenomas or adenocarcinomas. The expression differences for discrete variables were visualized using Boxplots[8]. Finally, The mRNA expression data of 406 samples with GAC and clinicopathologic data were analyzed (Table 1).

2.2. GSEA

In this study, the association between expression of MATN3 and biological pathway was performed using Gene Set Enrichment Analysis (GSEA v2.0)[9]. An ordered list of all genes according to their correlation with MATN3 expression was firstly generated, and samples from the TCGA datasets were divided into MATN3-high and -low groups. Default settings were used and permutation analysis (1000 times) was performed to determine thresholds for significance. False Discovery Rate (FDR) was calculated. When FDR is ≤ 0.25 , the gene set is considered significantly enriched.

2.3. Statistical analysis

All the statistical analyses were performed by R (v.3.6.0). The relationships between clinicopathologic characteristics and MATN3 were analyzed with logistic regression. Cox regression and Kaplan-Meier methods were used to analyze the relationship between clinicopathologic characteristics and OS. Multivariate Cox analysis was used to determine the impact of MATN3 expression level on OS. The samples were grouped to MATN3-high group and MATN3-low group by the median value of MATN3 expression.

Results

3.1. Clinicopathologic features

We downloaded 406 GAC samples with both clinical data and gene expression data based on TCGA in June 2019 with the median age at diagnosis 65.7 years old (Table 1). Male patients accounted for the majority (63.1%).

The proportion of each pathological grade is 2.5%, 37.3% and 60.2% respectively for well, moderately and poorly differentiated. There were 56 (14.7%), 118 (31.1%), 167 (43.9%) and 39 (10.3%) patients with stage

I, II, III and IV, respectively. 267 of 389 (68.6%) cases had lymph node metastases. 27 of 388 (7%) cases had distant metastases.

3.2. Association of MATN3 expression level with clinicopathologic variables

A total of 406 GAC samples from TCGA with MATN3 expression data were analyzed. MATN3 in cancer tissues was significantly higher than that in normal tissues (Fig. 1A-B), and overexpression of MATN3 correlated significantly with the tumor clinical stage ($p = 0.012$) and T stage ($p < 0.01$) (Fig. 1C-D). Logistic regression analysis revealed that when the samples were grouped to MATN3-high group and MATN3-low group by the median value (2.34), overexpression of MATN3 was associated with poor clinicopathologic variables (Table 2). Overexpression of MATN3 in GAC was also significantly associated with pathologic TNM stage (OR = 2.66(1.33-5.48) for II vs. I, OR = 1.97 (1.01-3.94) for III vs. I, OR = 2.45 (1.02-6.12) for IV vs. I), T stage (OR = 4.97 (1.44-21.83) for T2 vs. T1, OR = 5.98 (1.9-26.45) for T3 vs. T1, OR = 6.26 (1.92-28.49) for T4 vs. T1). These results indicated that GAC with MATN3 high expression was more likely to progress to more advanced stage than GAC with MATN3 low expression.

3.3. Survival outcome

Kaplan-Meier survival analysis showed that OS of GAC with MATN3-high was significantly lower than that with MATN3-low ($p < 0.01$, Fig. 1E). Univariate analysis also showed that MATN3-high was significantly correlated with poor prognosis (hazard ratio [HR]: 1.06; 95% confidence interval [CI]: 1.02 – 1.11; $p = 0.0014$). Other clinicopathologic variables, including age, Pathologic TNM Stage, lymph nodes and distant metastasis were also associated with poor prognosis (Table 3). Furthermore, multivariate analysis showed expression level of MATN3 was an independent prognostic factor, with a HR of 1.08 (CI: 1.04- 1.13, $p < 0.01$) (Table 4).

3.4. Identification of MATN3-related signal pathways by GSEA

We further used GSEA to confirm the correlation between MATN3 expression level and signal pathways. The Fig. 2 showed that P53, NOTCH, MAPK, and MTOR [signaling pathways](#) differentially enriched in MATN3 high expression phenotype.

Discussion

In this study, RNA-sequencing data based on TCGA were used for bioinformatics analysis, and it was found that the overexpression of MATN3 in GAC was associated with advanced clinical-pathological features (pathological TNM stage, T stage) and poor prognosis. We further used GSEA to study the

function of MATN3 in GAC. GSEA showed that NOTCH, WNT, and mTOR [signaling pathways](#) differentially enriched in MATN3 high expression phenotype. It suggests that MATN3 may be a potential prognostic indicator for GAC.

MATN3, as a member of the matrilin protein family, is a noncollagenous extracellular matrix [10,11]. According to previous reports [5,11,12], it is mainly distributed in cartilage around the whole body, such as articular cartilage, costal cartilage, and tracheal cartilage. In primary human chondrocytes, MATN3 can induce the expression of MMP1, MMP3, MMP13, and COX2, indicating that MATN3 can regulate extracellular matrix degradation[12]. Existing data [13,14] provide insights into the critical role of matrilin-3 in inflammation, matrix degradation, and matrix formation in cartilage development and osteoarthritis. So far, although many studies about MATN3 have been limited to epiphyseal disease, few studies have been reported on other conditions such as malignant tumors. The potential role of MATN3 in GAC was the main research content of this study.

Zhou et al. [15] found that six gene signatures, including MATN3, could be used to predict recurrence in stage III and IV patients after surgery plus chemoradiotherapy. Wu et al. [16] also found MATN3 overexpression in GAC and its prognostic significance in OS of patients. In this study, MATN3 levels were significantly increased in stage IV patients and T4 patients, and multivariate analysis suggested that MATN3 was an independent prognostic factor ($p= 0.000$). Together, all these findings suggested that high expression of MATN3 could indicate a poor prognosis for GAC patients, and MATN3 might be a pivotal target gene involved in the process of GAC cell growth and metastasis. However, the molecular mechanisms underlying differentiation remained unclear.

In our study, we observed that MATN3 high expression phenotype was associated with the following signaling pathways: NOTCH, MAPK, and MTOR [signaling pathways](#). MATN3 low expression phenotype was associated with P53 signaling which has been identified as one of the key signaling pathways that inhibit gastric cancer. Notch receptor plays different roles in the genesis and development of tumor[17]. The activation of Notch 1 and Notch 2 not only promotes the progression of gastric cancer[18,19], but also inhibits the invasion of gastric cancer[20,21]. NOTHC 3 contributes to glandular differentiation of gastric cancer[22], Notch4 promotes GC growth through the activation of Wnt1/ β -catenin signaling[23]. In addition, studies have confirmed that MTOR signaling pathway is activated in human gastric cancer and promotes cell proliferation. MTOR signaling pathway decreases after Notch inhibition, suggesting that MTOR is located downstream of Notch in gastric cancer cells[24]. In this paper, it is also proved that NOTCH, WNT and MTOR signaling pathways are active in gastric cancer cells with high expression of MATN3. However, the relationship between the expression of MATN3 and the above signaling pathways is reported for the first time, and its underlying regulatory mechanism needs to be further clarified.

However, using mRNA to predict protein expression was far from perfect [25]. This study did not evaluate the correlation between MATN3 mRNA expression and MATN3 protein expression, which was also the limitation. Further laboratory studies of gastric adenocarcinoma are needed in the future.

In conclusions, MATN3 high expression may be a potential prognostic biomarker of poor survival in gastric adenocarcinoma. Moreover, NOTCH, WNT, and MTOR **signaling pathways** may be the key pathways regulated by MATN3 in GAC. Further experimental validation is needed to demonstrate the biological impact of MATN3.

Abbreviations

GAC = Gastric adenocarcinoma, GSEA = gene set enrichment analysis, MATN3 = Matrilin-3, NES = Normalized enrichment score, NOM- p value = Nominal p value, OS = overall survival, TCGA = The Cancer Genome Atlas.

Declarations

Acknowledgments

The results shown here are in whole or part based upon data generated by the TCGA Research Network: <http://cancergenome.nih.gov/>.

Funding

None.

Author contributions

Zheng GL collected the data, analyzed of data and wrote the manuscript; Zhao Y made critical revisions to the manuscript; Zheng ZC made substantial contributions to the conception, design, and coordination of the study and gave final approval of the version; all authors read and approved the final manuscript.

Ethics approval and consent to participate

Since this study only involves analysis of publically available database (TCGA) and does not contain any identifying patient information, the ethical approval of this study by Institutional review board (IRB) is not required. The TCGA data did not include the use of human subjects or personal identifying information. Thus, no informed consent was required for this part of the study.

Competing interests

The authors declare that they have no conflict of interest to this work. We declare that we do not have any commercial or associative interest that represents a conflict of interest in connection with the work submitted.

Data availability statement

No additional data are available.

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Tables

Clinical characteristics	Total (N=406)	%
Age at diagnosis	65.7[30-90]	
Gender		
male	256	63.1
female	150	36.9
Histologic Grade		
well	10	2.5
moderate	149	37.3
poor	240	60.2
Pathologic TNM Stage		
I	56	14.7
II	118	31.1
III	167	43.9
IV	39	10.3
T		
T1	23	5.8
T2	85	21.5
T3	185	46.7
T4	103	26
N		
N0	122	31.4
N1	109	28
N2	80	20.6
N3	78	20
Distant metastasis		
positive	361	93
negative	27	7

Table 1. The characteristics gastric adenocarcinoma patient based on TCGA.

Age at diagnosis (continuous)	342	0.89(0.59-1.38)	0.626
Gender[male vs. female]	344	1.02(0.66-1.58)	0.944
Histologic grade (well/moderate vs. poor)	337	1.67(0.39-8.4)	0.497
Pathologic TNM stage			
II vs. I	152	2.66(1.33-5.48)	0.006
III vs. I	185	1.97(1.01-3.94)	0.049
IV vs. I	85	2.45(1.02-6.12)	0.047
T			
T2 vs. T1	94	4.79(1.44-21.83)	0.02
T3 vs. T1	176	5.98(1.9-26.45)	0.0058
T4 vs. T1	104	6.29(1.92-28.49)	0.0055
N			
N1 vs. N0	197	0.97(0.55-1.71)	0.907
N2 vs. N0	173	1.75(0.68-2.29)	0.478
N3 vs. N0	167	1.19(0.64-2.22)	0.591
Distant metastasis (positive vs. negative)	322	0.88(0.43-2.20)	0.431

Table 2. MANT3 expression associated with clinical pathological characteristics (logistic regression).

Clinicopathologic variables	HR	HR.95L	HR.95H	p-value
Age at diagnosis	1.93	1.01	1.99	0.004
Gender	1.49	0.98	2.29	0.062
Histologic grade	1.25	0.86	1.84	0.252
Pathologic TNM Stage	1.91	1.19	1.91	0.0005
T	1.58	0.99	1.63	0.0503
N	1.85	1.05	1.49	0.0133
M	2.07	1.07	3.98	0.0302
MATN3	1.86	0.82	2.01	0.0014

Table 3. Univariate analysis of the relationship between OS and clinicopathologic variables in TCGA patients using Cox regression

Clinicopathologic variables	HR	HR.95L	HR.95H	p-value
Age at diagnosis	2.05	0.82	2.07	3.11E-05
Gender	1.52	0.97	2.36	0.0639
Histologic grade	1.36	0.89	2.05	0.1474
Pathologic TNM Stage	1.42	0.89	2.25	0.13387
T	1.14	0.81	1.61	0.45651
N	1.04	0.79	1.36	0.7846
M	2.19	0.92	5.17	0.0735
MATN3	2.68	0.74	2.13	8.77E-05

Table 4. Multivariate analysis of the relationship between OS and clinicopathologic variables in TCGA patients

Figures

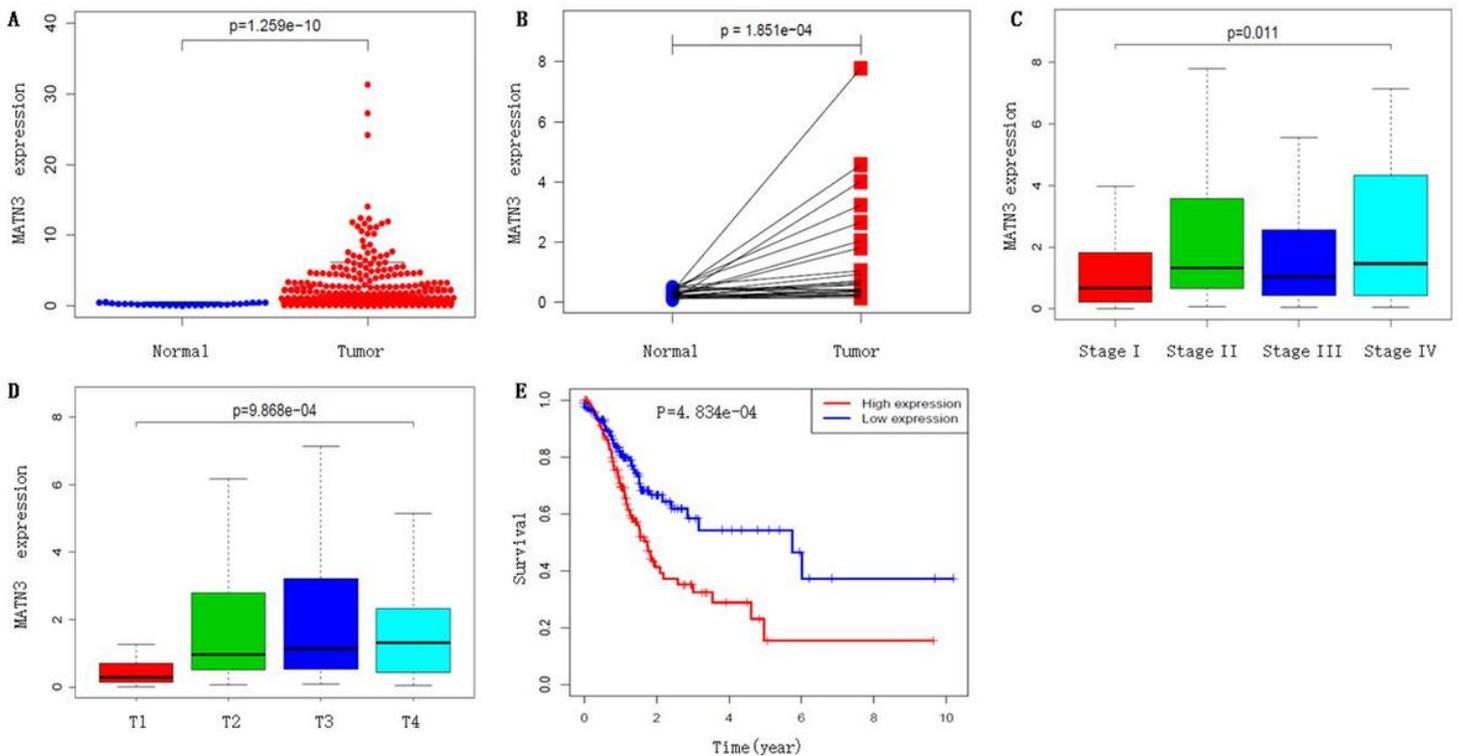


Figure 1

Association of MATN3 expression level with clinicopathologic variables

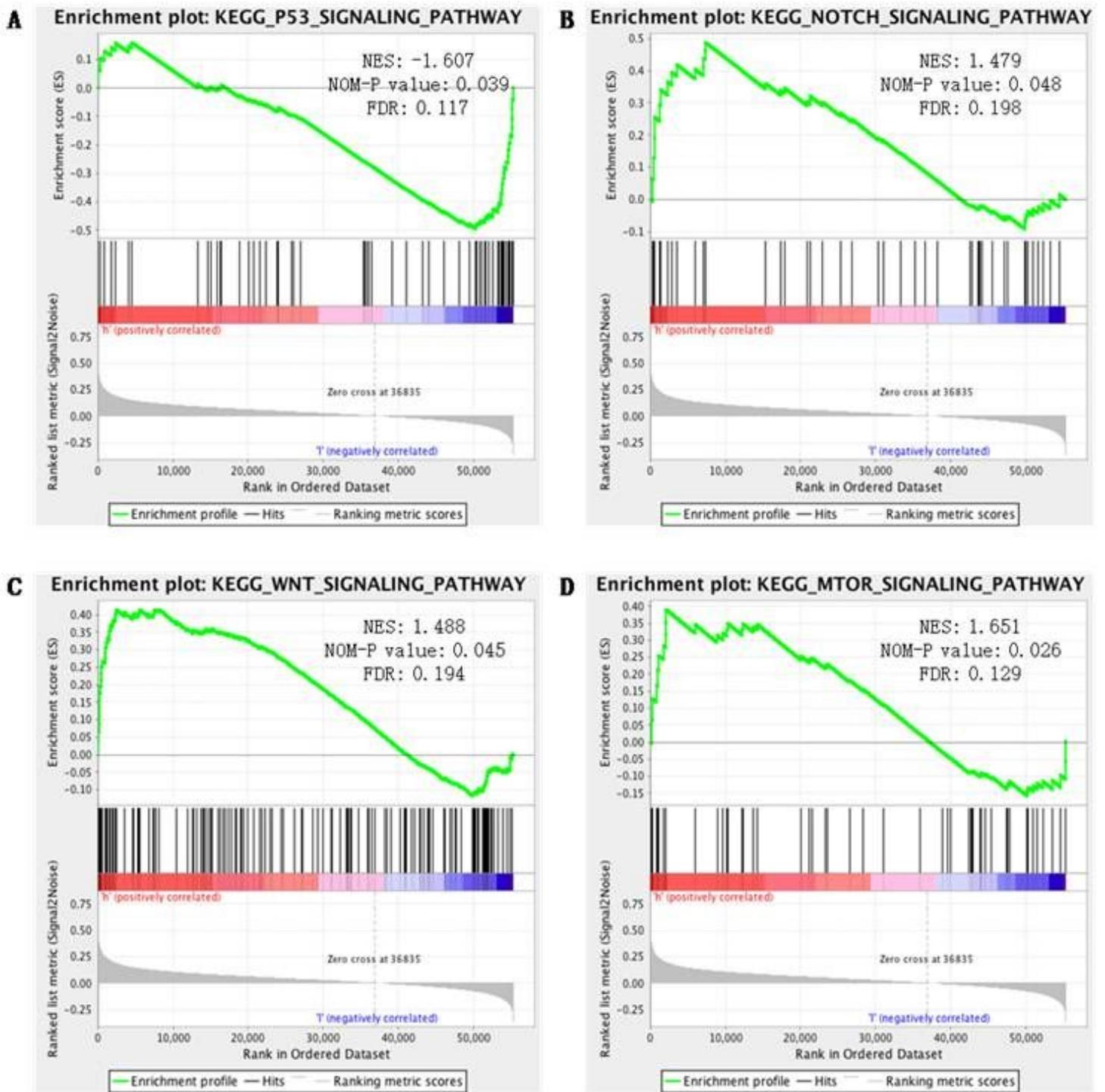


Figure 2

Identification of MATN3-related signal pathways by GSEA