

High expression of Fibroblast activation protein (FAP) predicts poor outcome in high-grade serous ovarian cancer

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Abstract

Background: High-grade serous ovarian cancer (HSOC) is a fatal form of ovarian cancer. Previous studies indicated some potential biomarkers for clinical evaluation of HSOC prognosis. However, there is a lack of systematic analysis of different expression genes (DEGs) to screen and detect significant biomarkers of HSOC.

Method and materials: TCGA database was conducted to analyze relevant genes expression in HSOC. Outcomes of candidate genes expression, including overall survival (OS) and progression-free survival (PFS), were calculated by Cox regression analysis for hazard rates (HR). Histopathological investigation of the identified genes was carried out in 151 Chinese HSOC patients to validate gene expression in different stages of HSOC.

Results: Of all 57,331 genes that were analyzed, *FAP* was identified as the only novel gene that significantly contributed to both OS and PFS of HSOC. In addition, *FAP* had a consistent expression profile between carcinoma-paracarcinoma and early-advanced stages of HSOC. Immunological tests in paraffin section also confirmed that up-regulation of *FAP* was present in advanced stage HSOC patients. Prediction of *FAP* network association suggested that *FN1* could be a potential downstream gene which further influenced HSOC survival.

Conclusions: High-level expression of *FAP* was associated with poor prognosis of HSOC via *FN1* pathway.

Introduction

Ovarian cancer is one of the major causes of death in females globally. According to 2018 global cancer statistics, 295,414 new cases and 184,799 deaths were reported (1). In gynecological oncology, ovarian cancer is less prevalent than breast cancer and cervix cancer, however the death rate of OC is the highest (1). The most recent 2020 cancer statistics in United States also confirmed that ovarian cancer is the fifth cause of deaths of females (13,940 patients, 5% of total cancer-related death), only trailing by lung & bronchus cancer, breast cancer, colon & rectum cancer, and pancreas cancer (2). According to the NIH Surveillance, Epidemiology, and End Results program (SEER) survival statistics (2009-2015), 5-year survivorship of ovarian cancer is only 47.6% (3), which remained virtually unchanged since the last decade (4).

Based on the immunohistological variation, serous ovarian cancer is the most common subtype of ovarian cancer, which could be further categorized as high-grade and low-grade neoplasm according to tumor Federation International of Gynecology and Obstetrics (FIGO) grade (5, 6). High-grade serous ovarian cancer (HSOC) is the most common, aggressive, and fatal type of ovarian cancer. Almost 30% of patients died within five years of diagnosis (7), mainly because of lack of disease-specific symptoms, prominent biomarkers, and effective therapy or targeted drugs (8-10). Despite sharing some similar histological characters and terminology, high- and low- grade SOCs are now acknowledged as two

different neoplasms (11). In 2011, the Cancer Genome Atlas (TCGA) program published the genomic and transcriptomic data of ovarian serous carcinoma, which summarized specific features of HSOC such as TP53 mutation, extensive DNA copy variation, BRCA1/BRCA2 inactive mutation, CCNE1 aberrations, and other survival-related preliminary transcriptional signatures (12-15).

Fibroblast activation protein (FAP), a cell-surface serine protease, emerges as an imperative factor in cancer-associated fibroblasts (CAFs), especially relevant to tumor occurrence and progression. Structurally, FAP consists of a cytoplasmic tail, a single transmembrane domain, and an extracellular domain (16). FAP is rarely expressed in healthy adult tissues. However, FAP is usually highly upregulated during tissue remodeling events, including cancers or cancer-associated fibroblasts (CAFs) (17-20). In addition, FAP is considered as a potential biomarker in certain tumor diagnosis and progression due to its protumorigenic specificity in both enzymatic and non-enzymatic manners (21-24).

In this study, we aim to identify potential biomarkers of HSOC survival from TCGA ovarian cancer cohort bioinformatics data. We analyzed gene expression, clinical and/or demographic information, and targeted strategies for potential HSOC biomarkers. In addition, we validated our findings by immunohistological investigation of tissues in a group of Chinese HSOC patients. Results suggest that FAP expression could be an effective biomarker for HSOC survival, which warrants further investigation as potential intervention of HSOC.

Methods And Materials

Dynamic protein analysis of TCGA database

Gene expression data (379 cases, Workflow Type: HTSeq-Counts) and clinical information were downloaded by the *TCGAbiolink* package in R 3.6.0 (<https://www.R-project.org/>) from the official TCGA website. After screening the clinical database, 320 patients were included for G3 histologic grade indicating HSOC. Then, 2 of these patients were excluded because of lack of relevant information. Finally, a total of 318 participants were recruited in our study.

For gene expression analysis, we downloaded the entire 57,331-gene data in serous cystadenocarcinoma from the TCGA RNAseq database. After data cleaning, 19 of 57,331 genes were excluded because of their insufficient expression in total gene expression (expressions of these excluded genes were < 1 copy in all participants). Then RNA expression data were transformed to z-scores in survival analysis.

Participants recruitment

This study followed the Declaration of Helsinki and was approved by the Institution of Research Ethics Committee of the First Hospital affiliated to Soochow University. All participants were fully aware of all protocols of this study, and signed up written consent forms to authorize the utilization of their tissues and relevant information.

For the validation set of the findings in HSOC TCGA database, 151 Chinese Han patients were diagnosed and recruited by the First Hospital affiliated to Soochow University, the Nanjing Maternity and Child Health Care Hospital, and the First Hospital affiliated to the Nanjing Medical University, from January 2013 to May 2019. After surgery, patients' tumor tissues were prepared into the paraffin sections. Their demographic and clinical characteristics were collected as well.

Immunohistochemistry staining (IHC) and IHC score

IHC was performed on paraffin sections of ovarian cancer tissues to characterize target gene (FAP) expression profile. Detailed steps can be found in our previous study (25). FAP antibody (#66562) was purchased from CST company to incubate the preparing sections overnight at 4°C for further staining.

IHC of FAP was evaluated by two independent and qualified pathologists who were blinded to actual clinical outcomes. IHC scoring was then established as follows. Percentage of positive cells and intensity of staining of FAP antibody were first calculated and then divided into these three major categories: ≤ 3 , negative or weak; >3 and ≤ 6 , Moderate; >6 , strong.

Association Network prediction of targeting gene

Prediction of functional protein association networks of candidate genes was performed by STRING version 11.0 (<https://string-db.org/>) and Genecard version 4.13 (www.genecards.org). In order to increase prediction accuracy, only common predictions present in both websites were included.

GO and KEGG pathway analysis of host gene

The gene ontology (GO) functional annotation and KEGG pathway analysis of host genes of polymorphisms were carried out by using the package 'clusterProfiler' in R (version 4.0.1).

Statistical analysis

All statistical analyses were calculated in R 3.6.0. Overall survival (OS) and progression-free survival (PFS) analyses in SOC patients were conducted by Cox regression and the Kaplan-Meier method. Multivariate Cox analysis was applied to identify potential influence of FAP expression on OS and PFS at different clinical stages of HSOC. Other relevant demographic and clinical characteristics were compared by Student's *t* test or Wilcoxon test whenever appropriate between the two groups. *P* value < 0.05 was considered as statistical significance in this study.

Results

Workflow of gene identification in TCGA database

As shown in figure 1, there were 304 and 237 genes with different expressions in OS and PFS analysis, respectively. By comparing early stage (Stage I+II) with advanced stage (Stage III+IV) HSOC, we identified 544 stage-related aberrantly expressed genes in HSOC patients. After cross-checking OS, PFS and stage-

related genes, only *FAP* and *SSC5D* were still present. And, *FAP* was finally included because of its characteristics of typical enzyme-catalyzed activity in uniprot database (www.uniprot.org/) and its potential role in HSOC patient's survival (Figure 1).

Associations of FAP expression with overall survival (OS) and progression-free survival (PFS)

Cox regression analysis based on FAP expression was performed to generate survival curves in OS and PFS. As shown in figure 2A, low FAP expression group showed a significant protective effect on HSOC prognosis in OS ($P = 0.005$). Longitudinally, low FAP expression group had 91.1% survival rate in a period of 12 months, compared with 84.4% in high FAP expression group. Survival rate in 50 months decreased to 31.9% in low FAP group and 21.4% in high group, respectively (Table 1). Results of PFS also showed similar patterns to OS between high and low FAP expression groups ($P = 0.008$, figure 2B).

Expression of FAP in HSOC patients

Sections of 151 HSOC patients' tumor tissues in different stages were stained with FAP-antibody. We identified an increasing trend of FAP expression with respect to severity of cancer stages (figure 3). In addition, strong positive FAP-staining generally showed in membranous and cytosolic compartments in HSOC tissues. Figure 3b revealed the total difference scores between early stage HSOC patients and advanced patients ($P = 0.016$). Additionally, negative FAP was observed in 33 (21.85%) patients.

Prediction of network influenced by FAP

Based on STRING and Genecard, prediction of co-influence genes with FAP and their potential regulating effects was shown in figure 4. Specifically, fibronectin-1 (FN1), collagen family genes-COL1A1, COL1A2, COL3A1, COL5A2, Thy-1 cell surface antigen (THY1), and insulin (INS) were identified as co-influence genes.

FN1 was the only significant gene based on Cox regression with its hazardous influence on HSOC ($P = 0.018$, as shown in table 1). FN1 is demonstrated to over-express in ovarian cancer, which could eventually influence the formation of multicellular aggregate of ovarian cancer cells, migration and invasion of cancer cells, and aggravating platinum-resistance to deactivate chemotherapy. Additionally, epithelial-mesenchymal transition of ovarian cancer is shown to relate to aberrant expression of FN1. Therefore, FN1 and its regulatory factors, including genes, non-coding RNAs and epigenetic regulations, might be valuable candidates for ovarian cancer studies.

Considering the similar function of collagen (COL) family, we extended the search of collagen encoding genes. Additional COL genes that could influence HSOC included COL16A1 (HR = 2.50, $P = 0.001$ for Cox regression), COL5A1 (HR = 2.43, $P = 0.002$), COL8A1 (HR = 2.16, $P = 0.006$), and COL4A1 (HR = 1.83, $P = 0.035$) (Table 1).

Bioinformation of FAP in GO and KEGG analysis

For gene ontology (GO) analysis of FAP, the top three enriched GO annotations were listed as “regulation of fibrinolysis”, “negative regulation of extracellular matrix organization”, and positive regulation of execution phase of apoptosis” three in Biological Process, while as “dipeptidyl-peptidase activity”, “aminopeptidase activity”, and “metalloendopeptidase activity” in Molecular Function (figure 5). On the other hand, however, for KEGG analysis, there is no related available record for FAP in corresponding database.

Discussion

In this study we extensively searched the TCGA database for HSOC patients to identify significant biomarkers for HSOC survival. We constructed a comprehensive summary of differentially expressed genes (DEGs) of HSOC, especially with regard to patients’ survival. This is the first study, to our knowledge, to evaluate the effects of gene expression on HSOC survival through data mining.

FAP is a typical plasma membrane-bound serine protease, which is implicated in matrix digestion and invasion (26, 27). Overexpression of FAP has been investigated and believed to be associated with prognosis in many diseases, especially in cancers (20, 22, 28). Some studies have demonstrated that high FAP expression is a negative prognostic factor for epithelial ovarian cancer (29). Increased expression of FAP is believed to cause recurrence of epithelial ovarian cancer after chemotherapy (29, 30). Considering that HSOC is a common histological type of epithelial ovarian cancer, our findings in TCGA database on HSOC support this finding from a new perspective.

Based on the location of FAP in HSOC tissue, we suggest that FAP could be a strong positive cell-surface receptor. FAP has high expression in HSOC patients in both Chinese population (over 60% in our study) and other ethnics (over 50%) (30). In addition, performance of therapeutic targeting at FAP also suggests its effectiveness for cancers (31). Therefore, FAP could be a potential biomarker for drug delivery or even direct therapy for HSOC.

Cancer-associated fibroblasts (CAFs), including FAP, usually participate in extracellular matrix structure remodeling and tumor microarray reconstruction. In this study, we also retrospectively explored the TCGA database and investigated the effects of other CAF members on HSOC prognosis, such as ACTA2, PDGFR α/β , S100A4, and α SMA. These CAF members are widely adopted as biomarkers of HSOC. Nevertheless, none of these genes showed significant association with HSOC survival. This result may be partly due to negative expression of α SMA (30).

We identified *FN1* as the only potential gene regulated by *FAP* in HSOC. Currently, there is no direct evidence to establish the causal relationship between *FAP* and *FN1*. Nevertheless, in this study, we demonstrated similar eventual clinical outcomes induced by *FAP* and *FN1*, positive correlation between their expression, significance of their role in overall survival of HSOC, and direct regulating relationship predicted by STRING and Genecard. All these novel findings suggested *FAP* as a novel trigger for *FN1*, at least for HSOC survival. For *THY1* gene, though it was reported as a putative tumor suppressor of ovarian cancer, our study did not produce the same results as previous ones (32), despite *THY1*’s presence in our

prediction of potential *FAP* association networks. It is possible that *THY1* cooperates with *FAP* during HSOC occurrence but not in prognostic period.

Conclusion

After extensive data mining of TCGA database, we identified *FAP* as a significant biomarker for HSOC survival. *FAP* overexpression led to worse outcome of HSOC patients, especially in the advanced clinical stage. *FN1* expression is potentially down regulated by *FAP* and further influences HSOC survival.

Declarations

Conflict of Interest

The authors have no conflicts of interest to declare.

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Table

Table 1

Significant difference of Predicted gene influenced by FAP in HSOC overall survival COX analysis

Ensembl ID	Gene	β - coefficient	Hazard Rate (HR)	Standard error of coefficient	Z-value	P-value
ENSG00000078098	FAP	0.740663	2.097325	0.271298	2.7300719640	0.006332
ENSG00000115414	FN1	0.663511	1.941597	0.28008	2.3690060930	0.017836
ENSG00000084636	COL16A1	0.916471	2.500451	0.279922	3.2740280480	0.00106
ENSG00000130635	COL5A1	0.887919	2.430066	0.289441	3.0677006780	0.002157
ENSG00000144810	COL8A1	0.768551	2.15664	0.279769	2.7470888830	0.006013
ENSG00000187498	COL4A1	0.608281	1.837271	0.289627	2.1002257660	0.035709

Figures

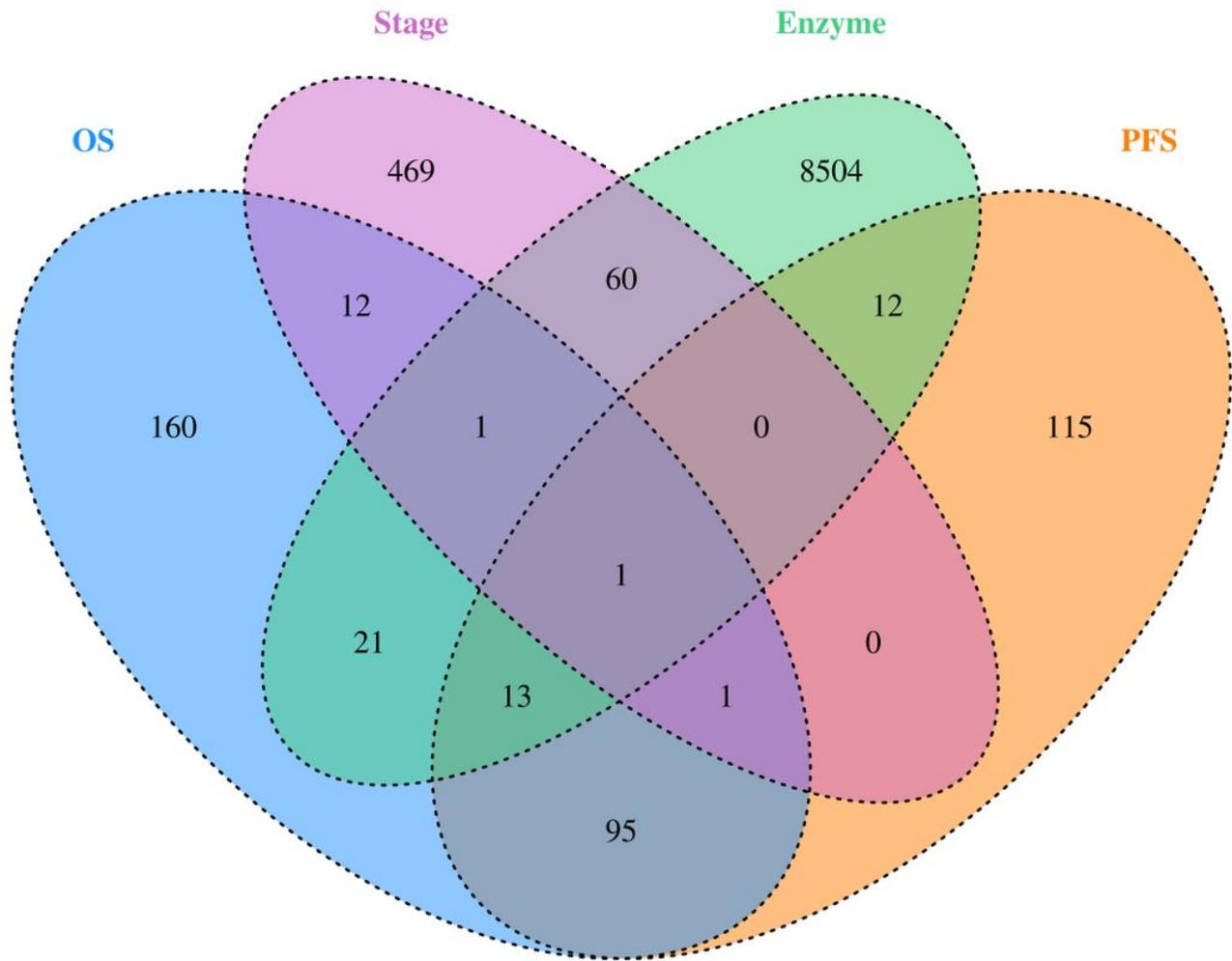


Figure 1

Venn diagram of target genes selection principle

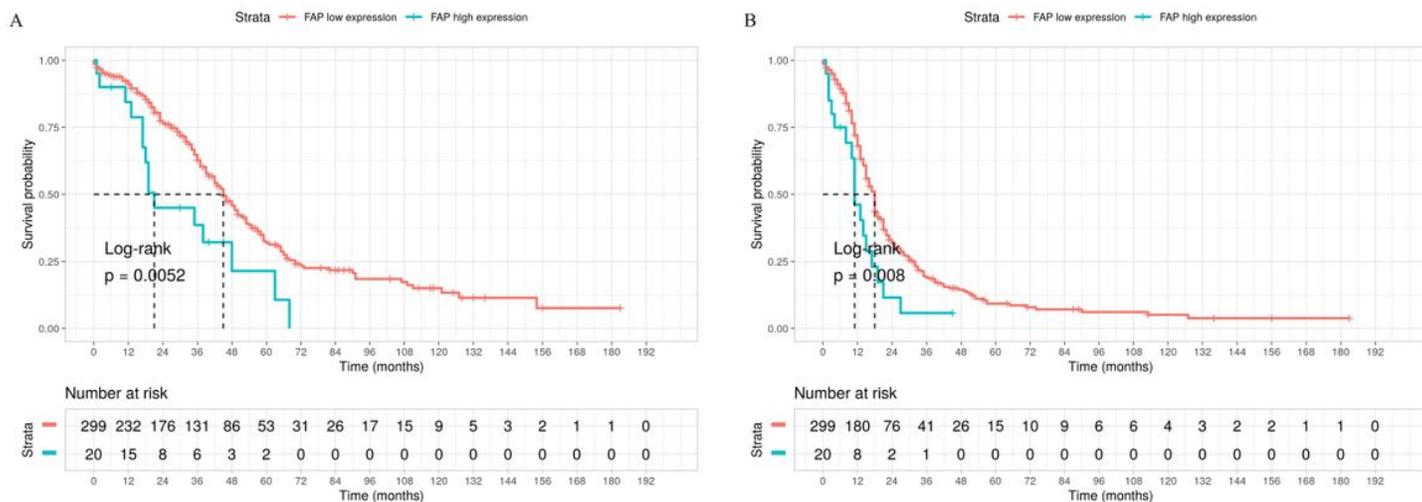


Figure 2

Impact of FAP expression on survival in HSOc patients in TCGA cohort. A. Overall Survival, OS; B. Progression Free Survival, PFS

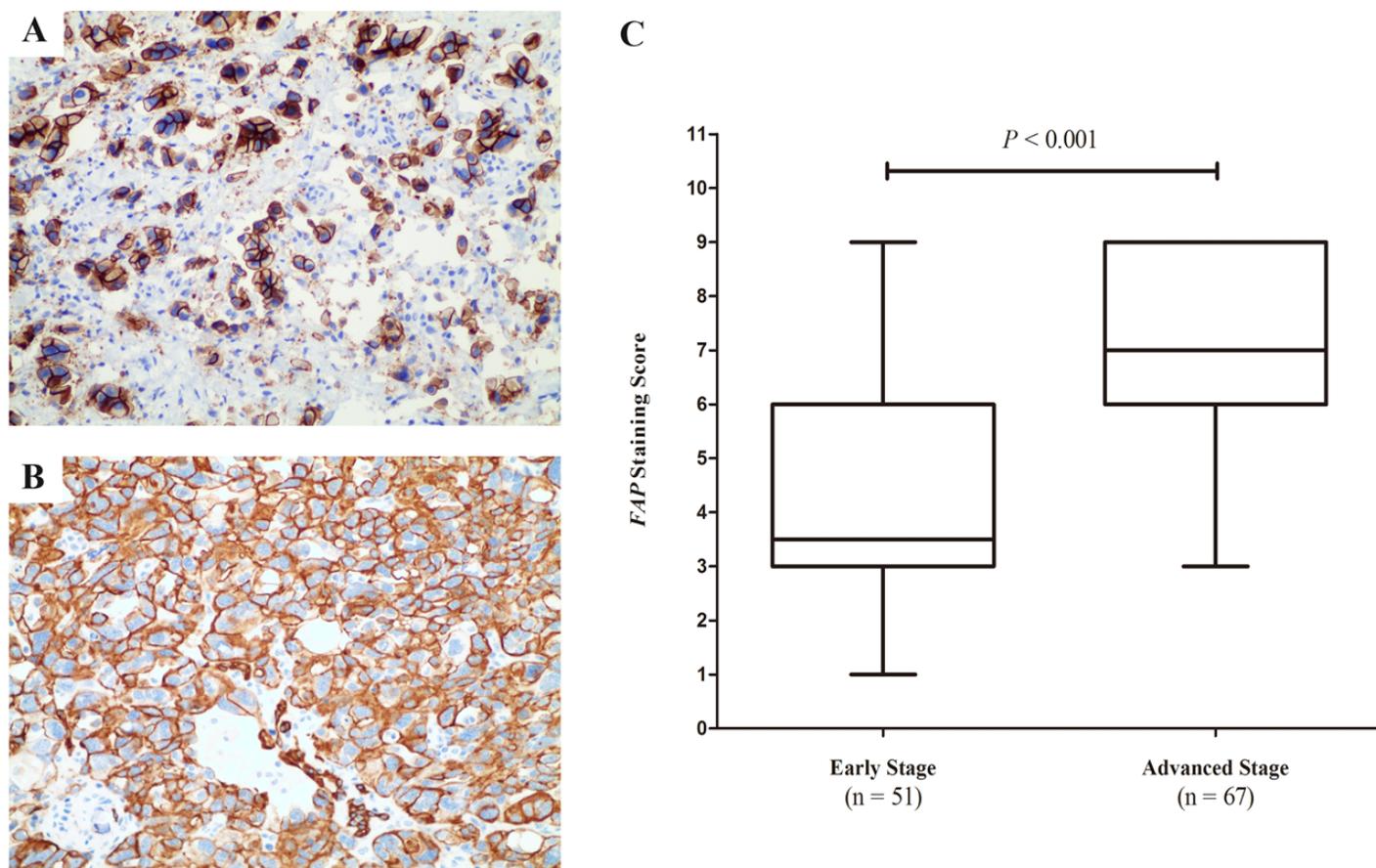


Figure 3

Expression of FAP in HSOc tissues. A. Expression of FAP in early-stage patients' tissues. B. Expression of FAP in advanced-stage patients' tissues. C. Total expression of FAP staining in patients with different

stages of HSOC.

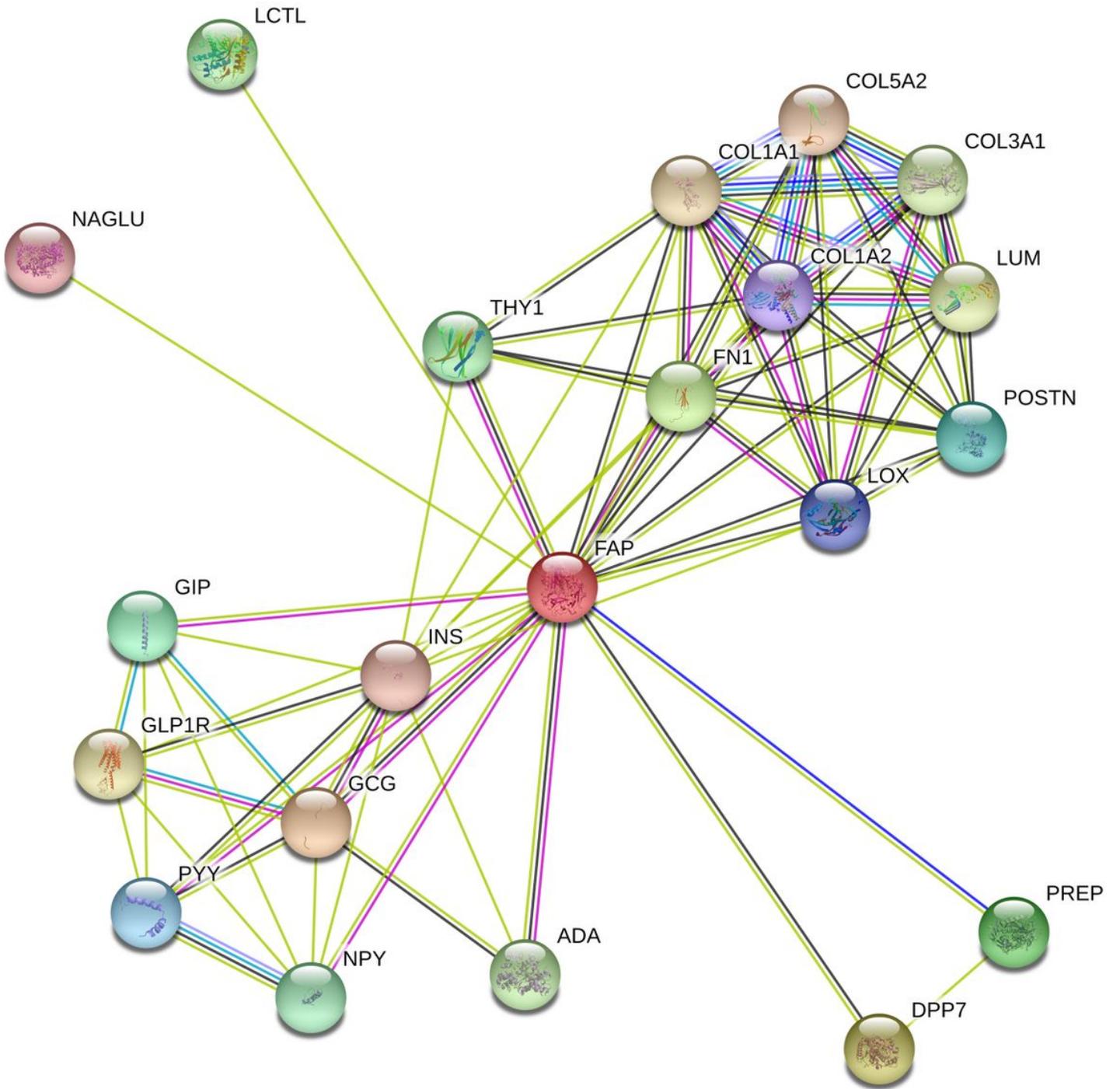


Figure 4

Prediction of FAP influence in gene regulation.

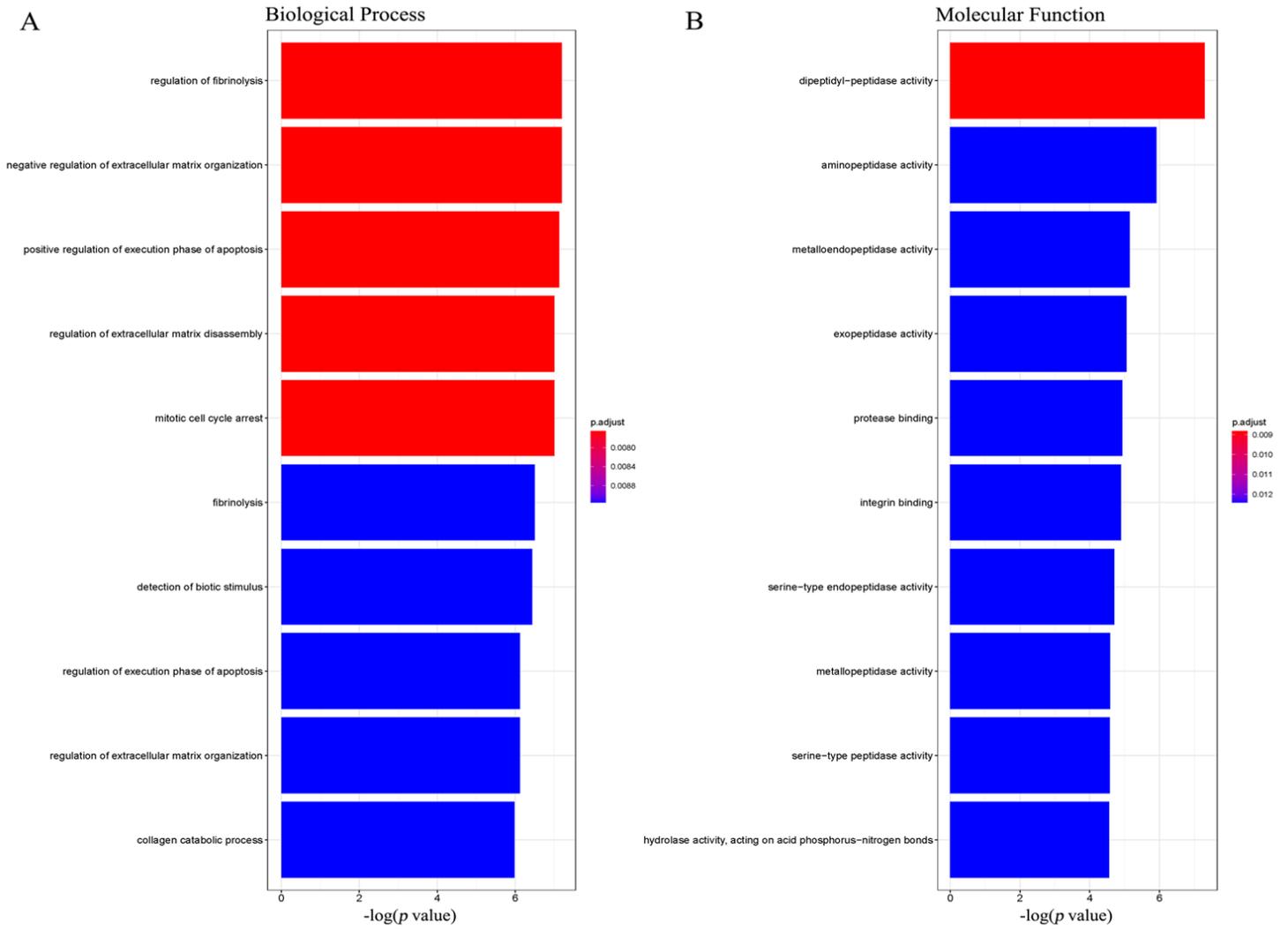


Figure 5

GO annotations of FAP. A. Biological Process. B. Molecular Function.