

Original Article

TIMP1 high expression or hypomethylation might predict favorable overall survival in colon adenocarcinoma patients

Zhenzhen Gao¹, Yaohua Fan¹, Yanhong Gu², Peng Fu¹

¹Department of Oncology, The Second Affiliated Hospital of Jiaxing University, Jiaxing, China; ²Department of Clinical Oncology, The First Affiliated Hospital of Nanjing Medical University, Nanjing, China

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Abstract: Tissue inhibitor matrix metalloproteinase 1 (TIMP1) plays a vital role in carcinogenesis, but the mechanism of its regulation remains unclear. Also, the prognostic value of TIMP1 in colon adenocarcinoma (COAD) has not been widely studied. In this report, bioinformatic analysis was conducted using R2 and the UCSC Xena browser based on data from GSE39582 in GEO datasets and in COAD cohort in TCGA database (TCGACOAD). Kaplan Meier curves of overall survival (OS) and relapse free survival (RFS) rates were generated to estimate the relationship between CXCR4 expression/methylation and OS/RFS in patients with COAD. COAD patients with high TIMP1 expression had significantly worse 5- and 10-year OS ($P < 0.05$). TIMP1 expression was higher in CpG island methylation phenotype (CIMP) group than in the non-CIMP group, whereas no significant association was identified between CIMP status and TIMP1 expression. Furthermore, TIMP1 methylation status was associated with worse OS. Thus, TIMP1 expression is regulated by DNA methylation in COAD and it might function as a potential prognostic target for colon adenocarcinoma.

Keywords: TIMP1, overall survival, COAD, DNA methylation, TCGA database

Introduction

Colon cancer (COAD) is one of the most common malignancies worldwide [1], found to be associated with a series of molecular events including somatic mutations, epigenetic alterations, and aberrant protein expression [2, 3]. Availability of better treatment options in the past 20 years have prolonged survival of colon cancer patients [4]. Furthermore, early diagnosis could have also improved the prognosis of this disease. Numerous studies have identified a large number of genetic alterations in colon carcinogenesis [5-7]. Still, recognition of the precise genetic changes is required to identify more reliable and novel biomarkers for the early detection of COAD [8].

TIMP1, pertaining to the Tissue Inhibitor of Metalloproteinases family and having included four identified members (TIMP1, TIMP2, TIMP3, and TIMP4) [3] is involved in the development and progression of COAD [9]. This protein has

been reported to inhibit the proteolytic activity of matrix metalloproteinases (MMPs) [10], which are involved in tumor invasion and development of metastatic disease [11]. TIMP1 can also induce trans-differentiation of liver fibroblasts into cancer associated fibroblasts (CAFs) that suppress apoptosis and promote the hepatocellular carcinomas via CXC-chemokine ligand 12 (CXCL12) signaling pathway [12]. Additionally, TIMP1 has been reported to decrease sensitivity of the tumor cells to chemotherapy by activating downstream pathways and exhibiting anti-apoptotic activity [3], especially by activating the PDK1 signaling pathway to protect the melanoma cells against drug induced cell death. Recent studies have explored the relationship of TIMP1 expression with the TNM stage, rate of disease free survival, risk of tumor recurrence, and the probability of liver metastasis [13]. TIMP1 is a secretory protein which can be detected in blood with enzyme linked immunosorbent assay (ELISA)

[14]. Therefore, TIMP1 has become a potential target marker of the carcinogenesis. However, more studies are necessary to elucidate the mechanism of the action of TIMP1.

Recent studies show that genome-wide methylation might have a prognostic value in COAD [15]. Tumors with relatively higher DNA methylation at CpG sites and microsatellite stable (MSS) have worse prognosis than the tumors with relatively lower level of methylation, however it is still controversial. Another study demonstrated that CpG island methylation phenotype (CIMP) positive (CIMP-positive), microsatellite instability-high (MSI-H) is usually associated with better survival outcome in stage III colon cancer [16]. However, the exact genes involved in methylation-related carcinogenesis are still not fully explored. In this study, the characteristics of the expression profile of TIMP1 in COAD patients was determined to explore association between TIMP1 expression/methylation and OS/RFS in patients with colon cancer.

Materials and methods

Identification of the association between TIMP1 expression and RFS among COAD patients

The association between TIMP1 expression and RFS (2, 5, and 10 years) in COAD patients in GSE39582 from GEO datasets and in the COAD cohort in TCGA database (TCGA-COAD) was evaluated. The R2 web-based tool (<http://r2.amc.nl>) [17] was used to generate the Kaplan-Meier survival curves for the data in GSE39582 from GEO datasets, which was composed of 585 cases including 566 COAD cases. The best cutoff was defined by the scan model. Kaplan-Meier curves of RFS were created with the auto-select best cutoff or median model.

Identification of the association between overall DNA methylation, TIMP1 methylation, and OS in COAD subtypes

Overall COAD DNA methylation status, CIMP status, TIMP1 mRNA expression, and TIMP1 methylation status in different COAD subtypes were detected using the UCSC Xena browser (<https://xenabrowser.net>) [17], which is based on TCGA-COAD cohort. The relevance of TIMP1 mRNA expression, TIMP1 methylation status,

and OS (2, 5, and 10 years) in COAD patients were further identified using the UCSC Xena browser.

Gene expression comparison of COAD cases with controls

The TIMP1 mRNA expression among COAD patients compared with normal controls was conducted by the cbiportal web-based tool (<http://www.cbiportal.org/>) [18], and verified by GEPIA (<http://gepia.cancer-pku.cn/index.html>) [19], which provides co-expression data of different genes.

Results

High TIMP1 expression indicated poor prognosis in patients with COAD

In the present study, association between TIMP1 expression and 2-, 5-, and 10-year RFS in patients with COAD was described by R2 (**Figure 1**). As shown in **Figure 1**, high TIMP1 expression was significant relative to worse 5-, and 10-year RFS ($P < 0.05$) (**Figure 1A-C**) with auto-generated cutoff value, which were verified by the UCSC Xena browser on the basis of TCGA-COAD cohort. While the correlation between high TIMP1 expression and unfavorable 2-, 5-, and 10-year RFS were not significant by setting median TIMP1 expression as cutoff ($P > 0.05$) (**Figure 1D-F**). The RFS curves showed that high TIMP1 expression was related to a worse 2-year RFS ($P = 0.046$) (**Figure 2A**), 5-year RFS ($P = 0.032$) (**Figure 2B**), and 10-year OS ($P = 0.047$) (**Figure 2C**).

Little change between TIMP1 expression and different subtypes of COAD

TIMP1 expression in different COAD subtypes in TCGA-COAD was also determined. Heatmap and the corresponding box plots showed that the MSI-H subtype had the highest TIMP1 expression, while the microsatellite instability-low (MSI-L) subtype had the lowest TIMP1 expression (**Figure S1A, S1B**). There were four subtypes (MSI-H, MSI-L, MSS, MSI-I), and the 2-year, 5-year, and 10-year overall survivals across various subtypes were not significant. The p -value of 2-year OS was 0.742 (**Figure S1C**), p -value of 5-year OS was 0.585 (**Figure S1D**), and p -value of 10-year OS was 0.485 (**Figure S1E**).

TIMP1 expression varies among different status

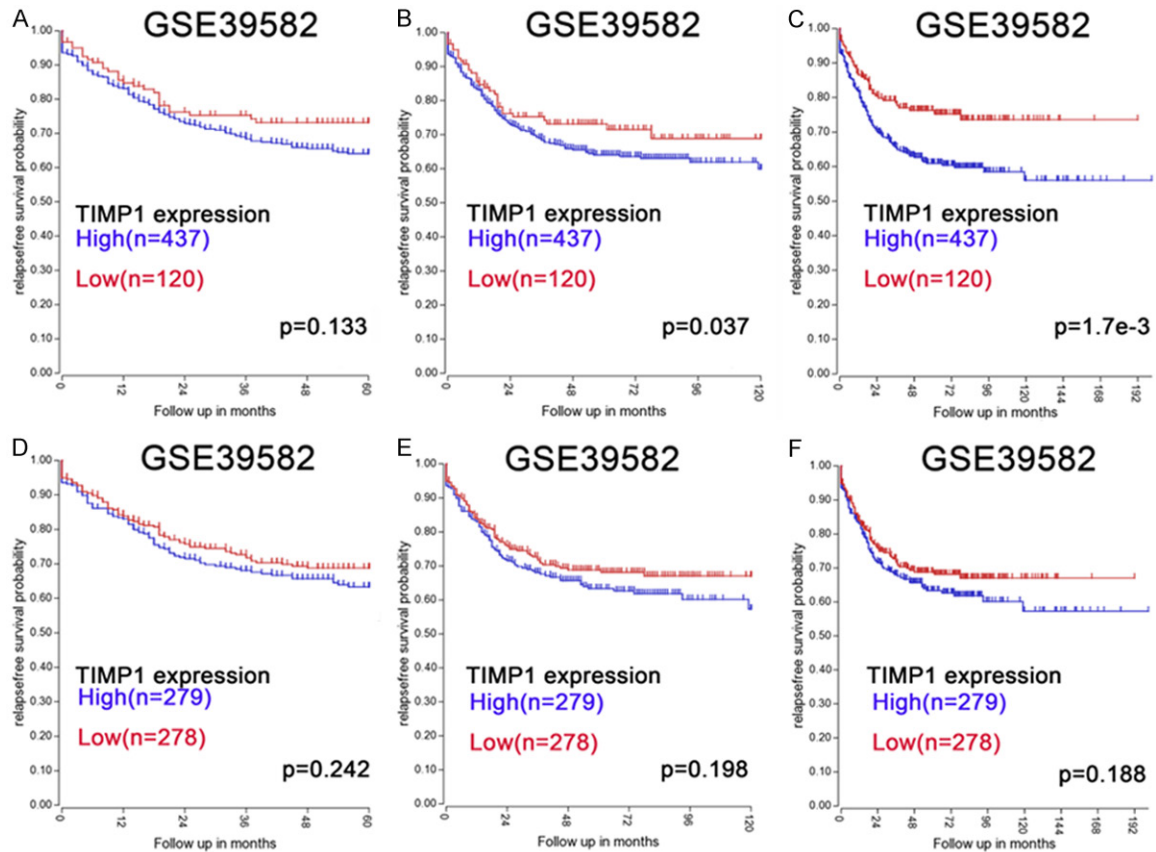


Figure 1. Kaplan-Meier curves of RFS in patients with high or low TIMP1 expression in GSE39582. RFS curves of 2 years (A), 5 years (B), and 10 years (C) were created with auto-select best cutoff. RFS curves were generated by using auto-select best cutoff. RFS curves of 2 years (D), 5 years (E), and 10 years (F) in COAD were generated with median TIMP1 expression as cutoff. Analysis was conducted using R2 tool.

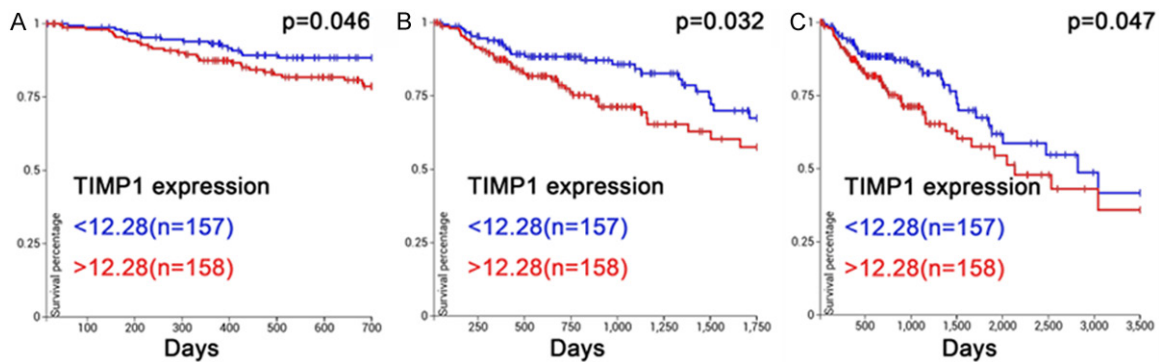


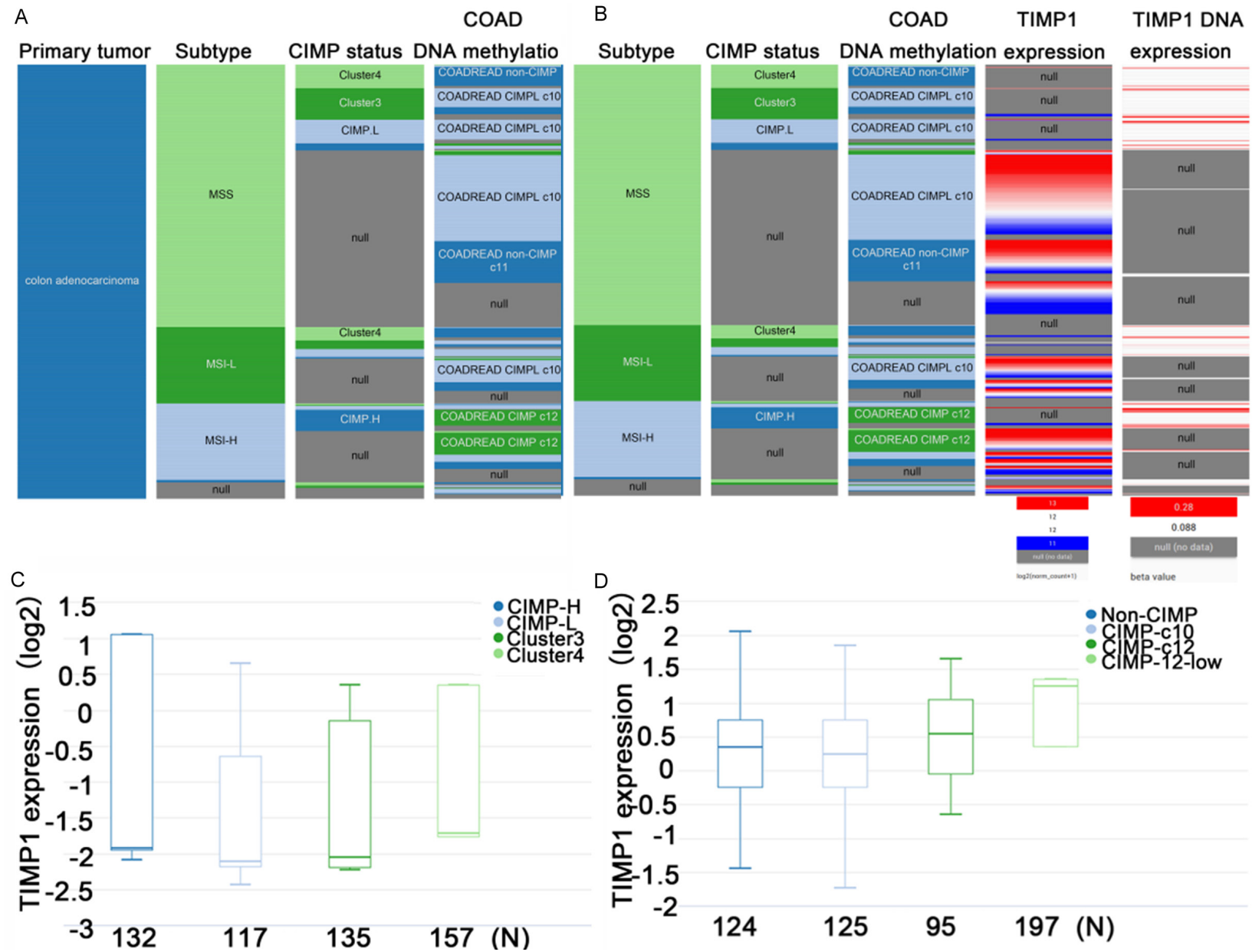
Figure 2. Kaplan-Meier curves of OS in patients with high or low TIMP1 expression in TCGA-COAD. OS curves of 2 years OS (A), 5 years OS (B) and 10 years OS (C) in COAD patients with high or low TIMP1 expression based on TCGA-COAD. OS curves were created using the UCSC Xena browser.

TIMP1 expression is negatively regulated by DNA methylation

In the study, a comparable heatmap was obtained which was composed of COAD subtypes, CIMP status and COAD DNA methylation in

TCGA-GBM (**Figure 3A**) by the UCSC Xena browser. In the heatmap, the MSI-H subtype was identified that had the highest level of DNA methylation (**Figure 3A**). Additionally, most of the CIMP-high (CIMP-H) cases were detected in the MSI-H subgroup (**Figure 3A**). Thus, TIMP1

TIMP1 expression varies among different status



TIMP1 expression varies among different status

Figure 3. TIMP1 expression is negatively regulated by DNA methylation. Heatmap (A) of COAD DNA methylation (the lowest to the highest) in different subtypes of COAD. Heatmap (B) of CIMP status, DNA methylation, TIMP1 expression and TIMP1 DNA methylation in different subtypes of COAD. Box plots of CXCR4 expression in CIMP-H and other groups (C). TIMP1 expression in different GBM DNA methylation clusters (D). All of the analyses were performed using the UCSC Xena browser based on TCGA-COAD cohort.

expression can be regulated by its DNA methylation status. Subsequent microarray data of TIMP1 methylation demonstrated its relevance in relation to CIMP among COAD patients. As shown in **Figure 3**, TIMP1 gene was hypermethylated in the MSI-H subtype (**Figure 3B**). TIMP1 expression was higher in CIMP-H (GCIMP) group than that in other CIMP subgroups (**Figure 3B, 3C**). TIMP1 expression was identified to be decreased with the increase of total DNA methylation (**Figure 3B, 3D**).

TIMP1 methylation might be a potential prognostic indicator for colon adenocarcinoma

There was no difference between different total DNA methylation subgroups and 2-, 5-, and 10-year OS (**Figure 4A-C**). On account of high TIMP1 expression indicating poor OS in COAD patients, the methylation status was detected in association with OS. Using the Xena browser, no significant relevance between TIMP1 expression and 2-, 5-, or 10-year OS (**Figure 5A-C**) was observed in two-group analysis. In the three-group analysis, high TIMP1 expression was not associated with the worse 2-year OS ($P = 0.101$), worse 5-year OS ($P = 0.0531$), and worse 10-year OS with a trend significance (**Figure 5D-F**).

Discussion

TIMP1 is a variety of soluble protein released by various cancer cells [20], such as lung, brain, prostate, breast, colon, and several other cancer cells, whose high expression has been reported to be related to the unfavorable prognosis for a variety of cancers [21]. Inversely, higher serum level of TIMP1 in patients with metastatic breast cancer has been demonstrated to be associated with less reduced response to endocrine therapy [22], reflected by the clinical correlates including objective response (OR), time to treatment failure (TTF), and overall survival (OS). Additionally, consistent high serum levels of TIMP1 and topoisomerase II α also predict the reduced benefit from anthracycline-based chemotherapy [23]. TIMP1/CXCR4/PI3K/AKT signaling pathway has also been found to promote progres-

sion of hepatocellular carcinoma [24]. Thus, these studies show that TIMP1 and TIMP1-based signaling pathway are involved in the occurrence and progression of COAD and preserving COAD cells phenotype. The TIMP1 pathway has also been taken as an important mediator of the cancer evolution, such as increase of quantities of cancer associated fibroblasts (CAFs) residing in the outer stroma of cancer cells and resistance to radio or chemotherapy.

Likewise, various studies have demonstrated that the inhibition of TIMP1 reduces the oncogenic behaviors of COAD cells [25]. TIMP1 silencing could attenuate the invasion and metastasis properties of COAD cells; reduce the expression of molecules regulating the survival and proliferation, such as TGF- β , IL-6, and IL-8 *in vitro*. Additionally, down-expression of TIMP1 signaling has been shown to increase the survival of animals with COAD *in vivo* [26]. On the contrary, one study showed that up-regulation of TIMP1 suppresses the hepatocellular carcinoma metastasis [14]. Therefore, currently available studies have conflicting results and further studies with large sample size are necessary to describe the role of TIMP pathways in various characteristics of the COAD cells.

In this study, the patients with high TIMP1 expression had worse 2-, 5-, and 10-year OS compared to those with low TIMP1 expression in GSE39582 in the GEO datasets by online tool R2. Data mining in the TCGA-COAD verified that high expression of TIMP1 was related to worse the 2-, 5-, and 10-year OS. Previously reported studies have not completely elucidated the mechanism of the dysregulation of TIMP1 in COAD. No significant differences were observed among the four subtypes of the TCGA-COAD cohort. Afterwards, DNA methylation and TIMP1 methylation status in different subtypes of COAD were detected by the UCSC Xena browser. MSI-H subtype had the highest level of DNA methylation, and most of the CIMP-high (CIMP-H) cases were consistent with MSI-H [14]. A gradual decrease in TIMP1 expression was observed with increasing DNA methylation. On the basis of these findings, expression of TIMP1 can be assumed to be regulated by total

TIMP1 expression varies among different status

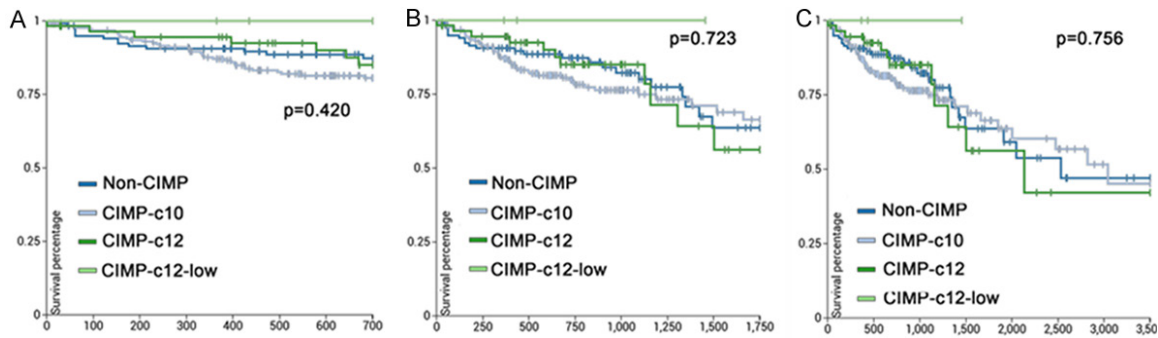


Figure 4. OS curves of 2 years, 5 years and 10 years in different COAD DNA methylation clusters. OS curves of 2 years (A), 5 years (B) and 10 year (C) in different COAD DNA methylation clusters were conducted using the UCSC Xena browser based on data in TCGA-COAD.

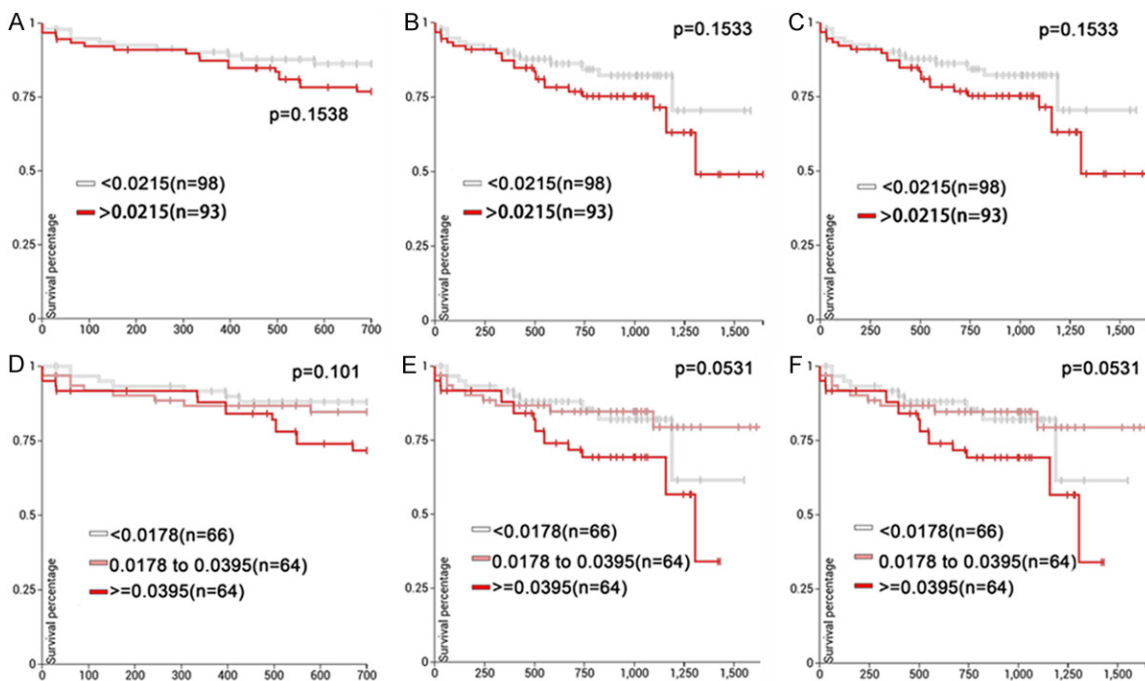


Figure 5. TIMP1 methylation might be a prognostic target of favorable OS in patients with COAD. Two groups (A-C) and three groups (D-F) OS curves of 2 years (A and D), 5 years (B and E) and 10 years (C and F) in the COAD patients with different level of TIMP1 DNA methylation were conducted with the UCSC Xena browser.

DNA methylation in COAD. Patients with high TIMP1 methylation had marginally better 5-year OS ($P = 0.0531$), suggesting that the prognostic value of total genome-wide methylation and TIMP1 methylation in COAD.

Due to experimental limitations, the potential correlation between TIMP1 expression/methylation and other recognized prognostic factors in COAD was not examined. Therefore, to study the prognostic value of TIMP1 methylation, future studies with a large patient cohort using multivariate analysis will be more informative.

Conclusion

In summary, this study shows that TIMP1 expression is regulated by DNA methylation in COAD. Low TIMP1 expression and high TIMP1 DNA methylation might be potential indicators of favorable OS.

Disclosure of conflict of interest

None.

Address correspondence to: Peng Fu, Department of Oncology, The Second Affiliated Hospital of Jia-

xing University, 1518 Huanchen Rd, Jiaxing 3140-00, China. Tel: (+86)13736845410; E-mail: fupeng24@163.com

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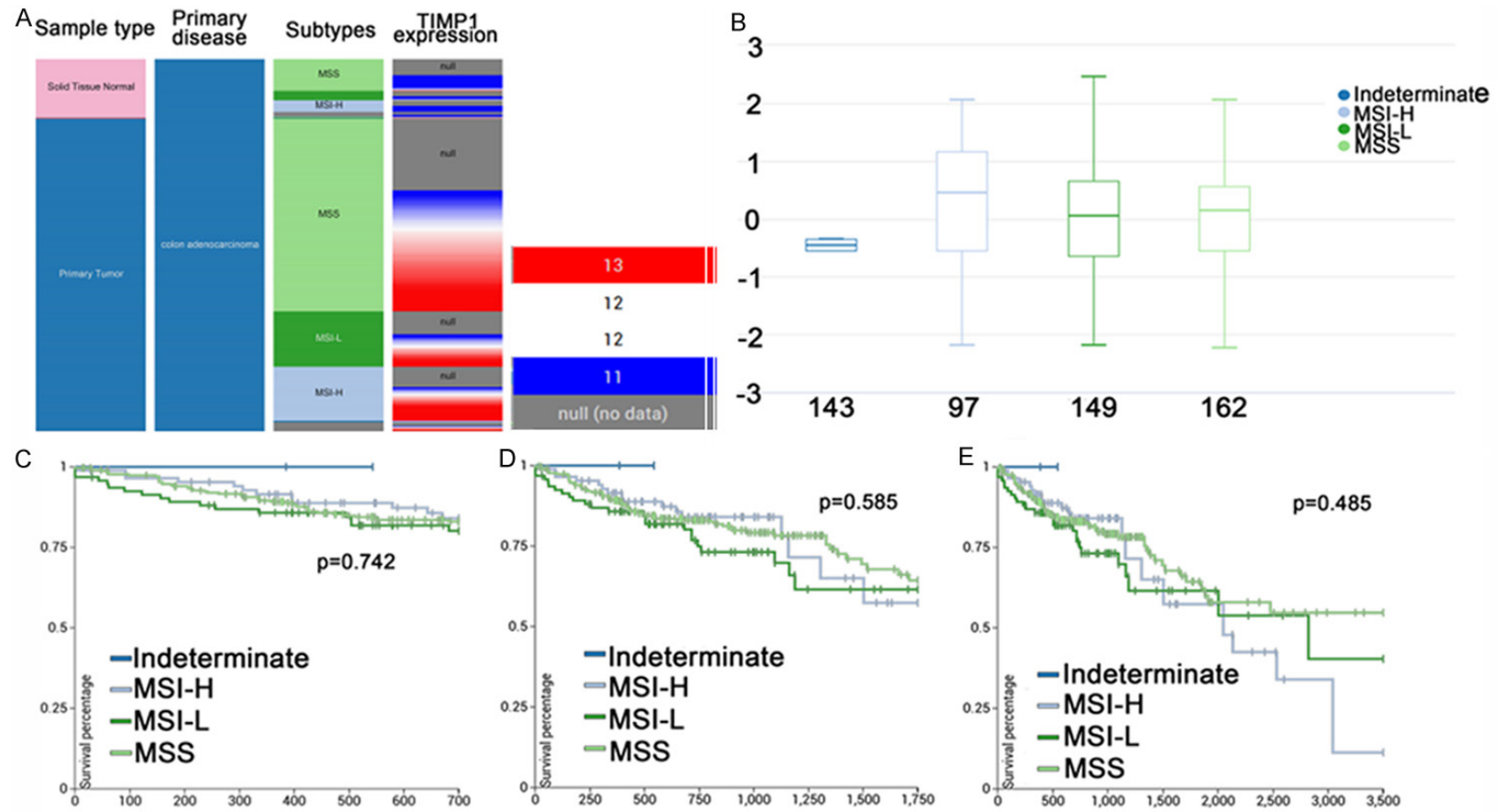


Figure S1. No significant expression changes of TIMP1 were observed among different subtypes of COAD. Heatmap (A) and box plots (B) of TIMP1 expression in different subtypes of COAD were created, and Kaplan-Meier curves of 2-year OS (C), 5-year OS (D) and 10-year OS (E) were performed by the UCSC Xena browser.