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Original Article

Collagen 24A1, a Potential Prognostic Biomarker in Hepatocellular Carcinoma

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Abstract : The prognostic value of collagen 24A1 (COL24A1) and its association with tumor-infiltrating immune cells (TIICs) in hepatocellular carcinoma (HCC) remains unexplored. In this study, we aimed to evaluate the prognostic value of COL24A1 in HCC via comprehensive web database analysis such as various online databases, including the Tumor Immune Estimation Resource (TIMER), The University of ALabama at Birmingham CANcer data analysis, Kaplan-Meier plotter, and Gene Expression Profiling Interactive Analysis. COL24A1 mRNA expression was found to be upregulated in HCC as compared to that in normal tissues, which was associated with poor prognosis in HCC. Further, COL24A1 expression correlated positively with TIICs, including B cells, CD8+T cells, neutrophils, macrophages, and dendritic cells, which was also associated with poor prognosis in HCC. The TIMER database revealed positive correlations between COL24A1 expression and that of other collagen family members, including, COL1A1, COL5A1, COL16A1 and COL21A1 in HCC. Our findings demonstrate that COL24A1 may potentially serve as a prognostic biomarker in patients with HCC.

Keywords : Hepatocellular carcinoma, Collagen 24A1, Prognosis, Biomarker, Immune cells

INTRODUCTION

The collagen family members, comprises 28 members encoded by 43 genes, localize to cell membranes as well as the extracellular matrix and participate in cell attachment and proliferation apart from maintaining tissue shape and structure [1]. In addition, various collagen is a tumor cell matrix component and plays an important role in tumor development. Collagen 1 (COL1) is a fibrous protein found in skin, blood vessels, bones, and the heart. It plays a role in normal physiological processes, including, hemostasis and angiogenesis, as well as disease processes, such as, in cancer and atherosclerosis [2]. These proteins occupy key positions in the tumor microenvironment, participate in the processes of cancer fibrosis, and are regulated by several cancer cell transcription factors, mutant genes, and cell signaling pathways. Additionally, they influence tumor cell behavior in the proliferation and infiltration [3]. Previous studies have demonstrated the potential role of collagen 1A1 (COL1A1) as a potential biomarker in liver cancer, breast cancer, colon cancer, and gastric cancer [4,5]. High collagen 11A1 (COL11A1) expression has been shown to

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promote gastric cancer proliferation, migration, and invasion [6]. Collagen 12A1 (COL12A1) has been shown to be associated with colon cancer prognosis [7]. Notably, several collagen family members have been implicated in cancer development and progression.

Liver cancer is one of the leading causes of cancer-related deaths worldwide, with the most common histological subtype being hepatocellular carcinoma (HCC) [8]. Early diagnosis and improved treatments over the past 10 years due to technological advancements have resulted in better survival rates in patients with HCC [9,10]. Nevertheless, treatment options remain limited, and the proportion of patients treated due to HCC recurrence and metastasis, and chemotherapy-induced side effects is low. Additionally, HCC diagnosis in the early stages is hard to obtain, on account of which most patients are identified at advanced stage with poor prognosis [11]. Therefore, there is a need to develop diagnostic and prognostic biomarkers for early detection of HCC and improve survival rates.

Collagen 24A1 (COL24A1), which is known to be mainly expressed in bone tissue, is an important factor regulating osteoblast differentiation and physiological processes of bones [12]. In addition, the overexpression of COL24A1 in head and neck squamous cell carcinoma (HNSCC) has proven its potential as a prognostic biomarker [13]. Thus, studies have confirmed the possibility of biomarkers in cancer of COL24A1. We attempted to assess the potential of COL24A1, collagen family members, as an HCC biomarker, by evaluating survival rates of its expression levels in HCC, its association with tumor infiltrating immune cells (TIICs), and its association with other collagen family members via big data analysis.

MATERIALS AND METHODS

1. Tumor Immune Estimation Resource (TIMER) database analysis

The TIMER is an online tool that enables gene expression and immune-infiltrating cell analysis across various cancer types by estimating immune infiltrate abundance using data from samples from The Cancer Genome Atlas (TCGA) [14]. We analyzed COL24A1 expression levels, clinical data and survival rates of HCC patients, and the association between COL24A1 levels and TIICs along with the prognostic value of this relationship. Further, we identified the correlation between COL24A1 expression and that of other members of the collagen family.

2. University of ALabama at Birmingham CANcer (UALCAN) database analysis

UALCAN is a database that uses TCGA data and clinical data to analyze the relative expression of genes across normal and tumor samples, including various tumor subgroups based on individual tumor grades, tumor stages, race, nodal metastasis status, and TP53 mutation status [15].

3. Kaplan-Meier (KM) plotter database analysis

The KM plotter estimates the effect of 54,000 genes on the prognosis of 21 common cancer types [16,17]. The database contains gene chips and RNA sequence data obtained from other databases. We evaluated the prognostic value of COL24A1 in HCC using this database. Hazard ratios (HR) with 95% confidence intervals (CIs) and log-rank P-values were computed.

4. Online consensus Survival for liver hepatocellular carcinoma (OSlihc) database analysis

The prognostic value of COL24A1 was confirmed using OSlihc database analysis. Survival analysis such as overall survival (OS), disease-specific survival (DSS), progression-free interval (PFI), and disease-free interval (DFI) were produced to evaluate the prognostic value of COL24A1 in OSlihc [18].

5. Statistical analysis

Gene expression data acquired from the TIMER and UALCAN databases was analyzed using online database tools. Survival analyses are shown as HR and *P*-values from a log-rank test. $P<0.05$ in the log-rank test revealed significant differences in survival rates.

RESULTS

1. Analysis of COL24A1 mRNA expression in HCC

COL24A1 expression in normal tissue and several cancers, including HCC, was analyzed using the TIMER database. The results revealed upregulation of COL24A1 expression in HCC, bladder urothelial carcinoma, breast invasive carcinoma, bladder cholangiocarcinoma, colon adenocarcinoma, esophageal carcinoma, HNSCC, lung adeno-

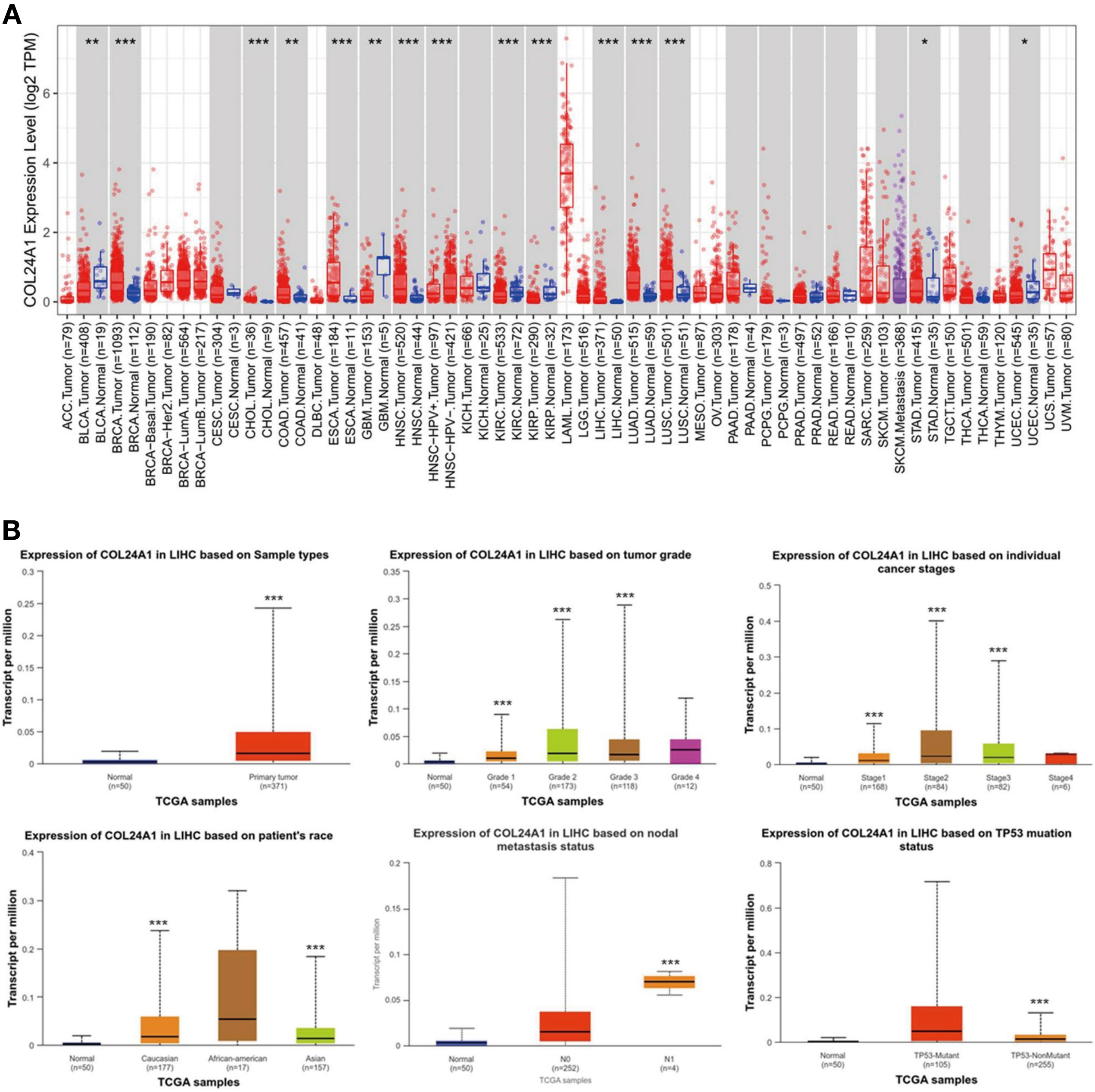

Fig. 1. Collagen 24A1 (COL24A1) mRNA expression levels in hepatocellular carcinoma (HCC). (A) High or low COL24A1 expression levels in several tumor tissues as compared to that in normal tissues as observed using the TIMER database. (B) Correlation analysis of COL24A1 expression with tumor histological subtype, tumor grade, cancer stage, age, nodal metastasis status and TP53 mutation in patients (**P* < 0.05, ***P* < 0.01, ****P* < 0.001).

carcinoma, lung squamous cell carcinoma as compared to that in normal tissues. In contrast, COL24A1 expression was found to be downregulated in glioblastoma multiforme, kidney renal clear cell carcinoma, and kidney renal papillary cell carcinoma as compared that in normal tissues (Fig. 1A). These results were further confirmed by correlation analysis using the UALCAN database, between COL24A1 expression and individual tumor grades, tumor stages, race, nodal metastasis status, and TP53 mutation status in HCC. Our results identified significant correlation between COL 24A1 and primary tumor grades (I, II, and III), stages (I, II, and III), race (Caucasian and Asian), nodal metastasis status (N0), and TP53 mutation as well as non-mutation status in HCC (Fig. 1B).

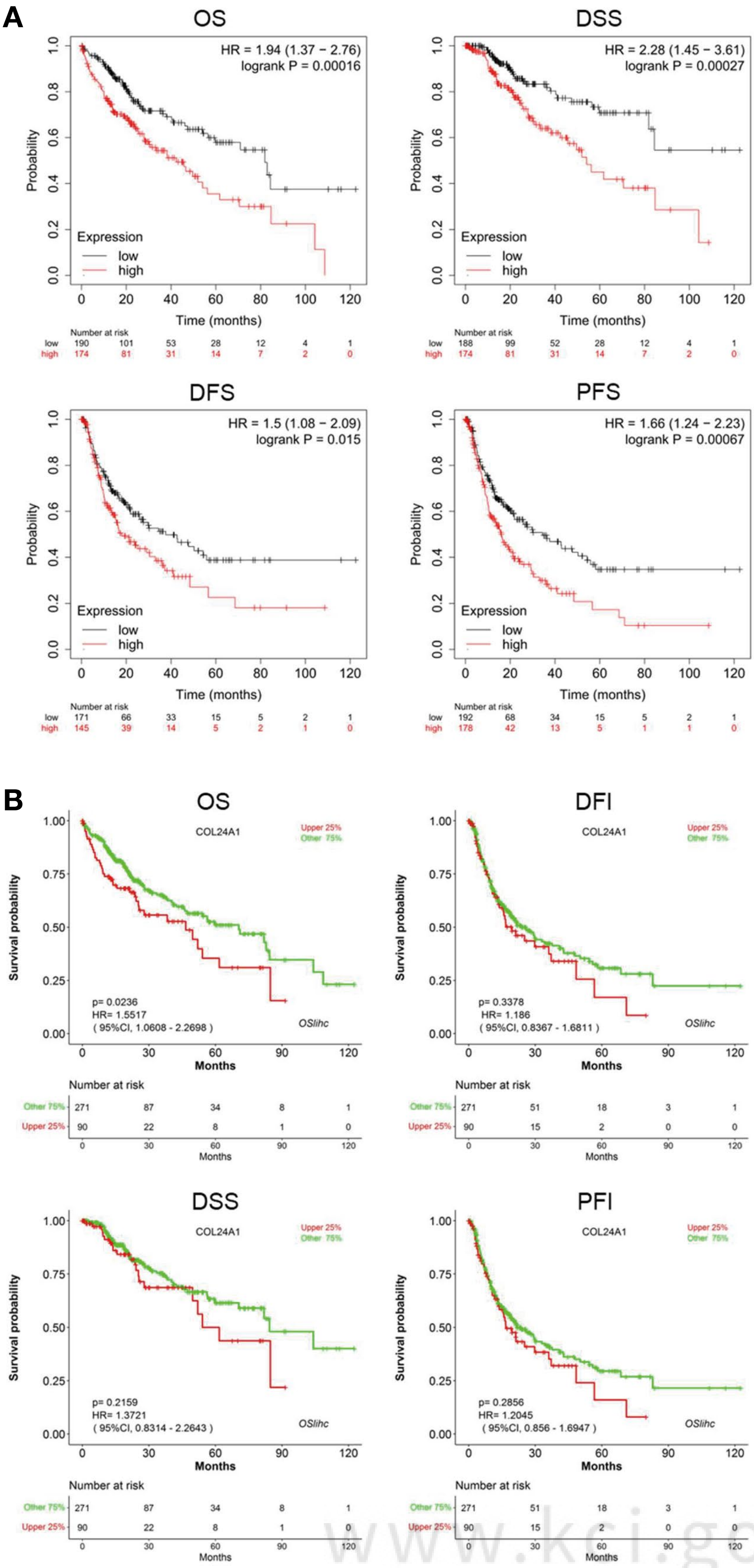

Fig. 2. The prognostic significance of Collagen 24A1 (COL24A1) expression in hepatocellular carcinoma (HCC) as observed using the Kaplan-Meier (KM) plotter (A) and OSlihc (B).

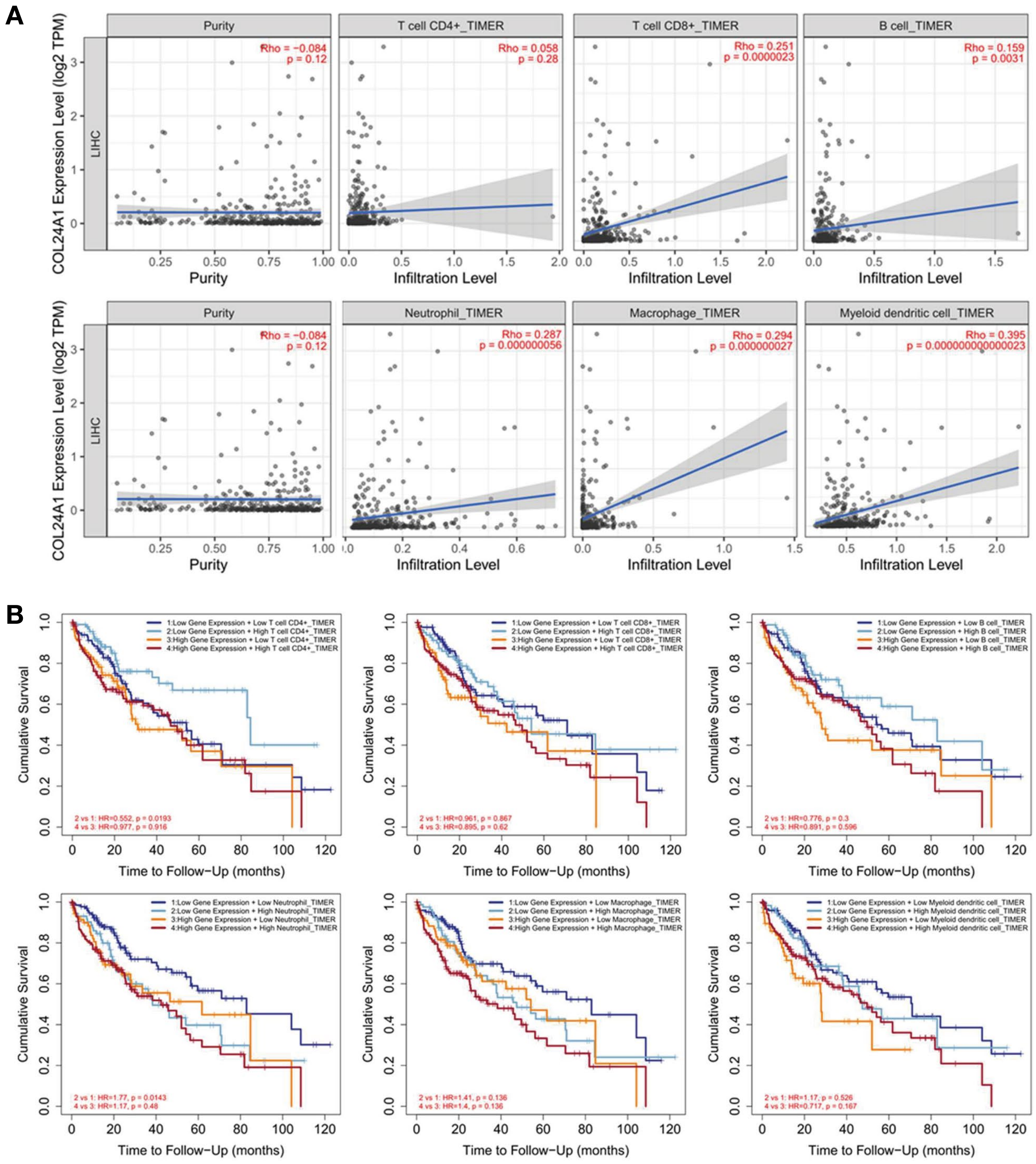

Fig. 3. Correlation analysis between Collagen 24A1 (COL24A1) expression and tumor-infiltrating immune cells (TIICs) in hepatocellular carcinoma (HCC). (A) Correlation analysis between COL24A1 levels and TIICs. (B) The prognostic value of COL24A1 expression and TIICs was analyzed using the TIMER database.

2. Analysis of COL24A1 prognostic value in HCC

The KM plotter and OSlihc databases were used to investigate the potential prognostic value of COL24A1 by analyzing survival rates in HCC. Upregulated COL24A1 expression was associated with poor prognosis in HCC using the KM plotter database (OS: HR = 1.94, P = 0.00016; DSS: HR = 2.28, P = 0.00027; DFS: HR = 1.5, P = 0.015; PFS:

HR = 1.66, *P* = 0.00067; Fig. 2A) and the OSlihc database (OS, HR = 1.5517, *P* = 0.0236; DFI, HR = 1.186, *P* = 0.3378; DSS, HR = 1.3721, *P* = 0.2159; DFI, HR = 1.2045, *P* = 0.2856; Fig. 2B).

3. Correlation analysis between COL24A1 and TIICs in HCC

We identified on the correlation between COL24A1 expression and TIICs in HCC using the TIMER database. The results confirmed that COL24A1 correlated positively with CD8 + T cell infiltration levels (R = 0.251, *P* = 0.0000023), B cells (R = 0.159, *P* = 0.0031), neutrophils (R = 0.287, *P* = 0.000000056), macrophages (R = 0.294, *P* = 0.000000027), and dendritic cells (R = 0.395, *P* = 0.000000000000023) in HCC (Fig. 3A). Further, an investigation into the relationship between COL24A1 and TIICs and its effect of prognosis in HCC revealed a stronger association of poor prognosis with low COL24A1 expression and CD4 + T cell infiltration levels than that with low COL24A1 expression and high CD4 + T cell infiltration levels (HR = 0.552, *P* = 0.0193). Poor prognosis was also more strongly associated with low COL24A1 expression and high neutrophil infiltration levels than that with low COL24A1 expression and low neutrophil infiltration levels (HR = 1.77, *P* = 0.0143) (Fig. 3B). Collectively, our results proved that upregulated COL24A1 expression is associated with TIICs and may thus prognosis of cancer.

4. Correlation analysis between COL24A1 and HCC gene mutations

The correlation between COL24A1 and collagen family member expression levels in HCC cells was investigated. Our results revealed that COL24A1 expression was significantly associated with that of COL1A1 (*P* = 0.0000000027), collagen 5A1 (COL5A1, *P* = 0.000000002), collagen 16A1 (COL16A1, *P* = 0.0000000055), and collagen 21A1 (COL 21A1, *P* = 0.00088) (Fig. 4).

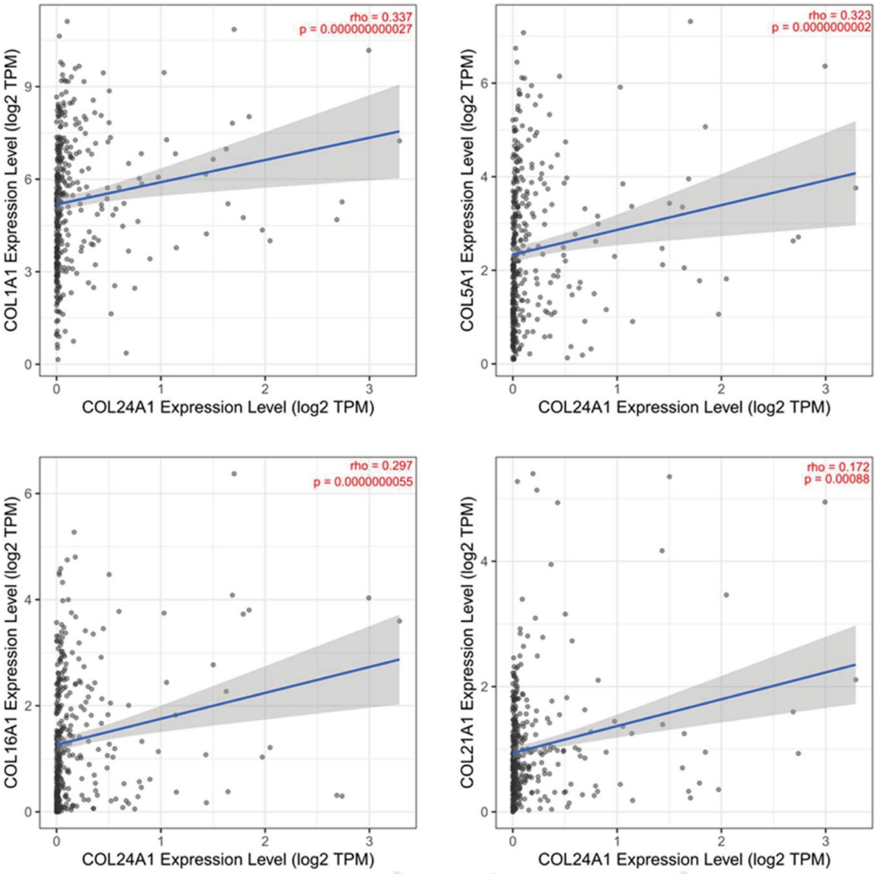

Fig. 4. Correlation analysis between Collagen 24A1 (COL24A1) expression and that of other collagen family members in hepatocellular carcinoma (HCC). Scatterplots showing the correlation between COL24A1 and other collagen family members.

DISCUSSION

Collagen is an extracellular matrix protein that cell growth and plays a structurally important role in the human body. The expression patterns of certain collagen family members have been correlated with poor prognosis in cancer. COL1 gene deletion has been found to promote cancer progression [19]. Further, COL1A1 expression is known to promote breast cancer metastasis [20]. Collagen 10A1 (COL 10A1) expression associated tumor growth and metastasis via epithelial-mesenchymal transition (EMT) in gastric cancer [21]. Additionally, COL11A1 overexpression has been confirmed in tumor cells [22]. These results imply that it may potentially serve as a prognostic cancer biomarker.

HCC has a high worldwide mortality rate and is the most common type of liver cancer caused by inflammation and fibrosis. The extremely poor prognosis is compounded by restrictions on treatment options [23,24]. Thus, several studies have focused on the identification of diagnostic and prognostic biomarkers for HCC [25-27]. The results on the analysis of survival rate in the COL24A1 expression in HCC revealed high COL24A1 expression in various cancers, including HCC, as compared to that in normal tissues. Additionally, low survival rate was found to be associated with high COL24A1 expression levels using the Kaplan-Meier database. These results were consistent with those reported by Yan et al. [28], who demonstrated higher COL24A1 expression in HCC tissues than that in normal liver tissues. Further, the results of online database analysis also confirmed a correlation between COL24A1 expression and tumor stages. Moreover, Wang et al. [29] found that overexpression of COL24A1 was significantly correlated with poor prognosis in HCC.

The immune system is considered important in caner growth. The tumor immune microenvironment regulated by TIICs influences cancer development and progression [30,31]. Our results revealed a positive correlation between several immune cells and COL24A1 expression.

We observed positive correlations between COL24A1 expression levels and that of COL1A1, COL5A1, COL16 A1, and COL21A1. COL1, a collagen family, is expressed in several fibrous tissues in the human body and is known to be involved in an abnormal EMT activation mechanism associated with tumor invasion and metastasis that results in tumor malignancy [32]. Overexpression of COL5A1 is correlation with cancer progression in ovarian cancer and glioma [33,34]. The collagen family, which has been identified as a biomarker in such a variety of cancers, needs a detailed mechanism analysis.

This study attempted to assess the potential of COL24A1 as a prognostic biomarker in HCC. We established strong associations between high COL24A1 expression levels in HCC and poor survival. Additionally, we demonstrated positive correlations between COL24A1 expression levels and TIICs, and with the expression levels of other collagen family members. Further studies are essential to validate these results.

CONFLICTS OF INTEREST

The authors have no conflicts of interest to declare.

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