Human Pathology (2013) **44**, 77–86

ELSEVIER

Human PATHOLOGY

www.elsevier.com/locate/humpath

Original contribution

COL4A3 expression correlates with pathogenesis, pathologic behaviors, and prognosis of gastric carcinomas ☆

Xiao-cui Nie MD[a], Jian-Ping Wang MD[a], Wan Zhu MD[a], Xiao-yan Xu PhD[a], Ya-nan Xing MD[b], Miao Yu MD[b], Yun-peng Liu MD[c], Yasuo Takano MD, PhD[d], Hua-chuan Zheng MD, PhD[a,*]

[a]*Department of Biochemistry and Molecular Biology, Institute of Pathology and Pathophysiology, College of Basic Medicine, China Medical University, Shenyang 110001, China*
[b]*Department of Surgical Oncology, The First Affiliated Hospital of China Medical University, Shenyang 110001, China*
[c]*Department of Internal Oncology, The First Affiliated Hospital of China Medical University, Shenyang 110001, China*
[d]*Clinical Research Institute, Kanagawa Cancer Center, Yokohama 241–0815, Japan*

Received 5 August 2011; revised 8 October 2011; accepted 11 October 2011

Keywords:
Carcinogenesis;
COL4A3;
Gastric carcinoma

Summary COL4A3 protein belongs to type IV collagen family and is closely linked to kidney diseases and cancer. To clarify the roles of COL4A3 in gastric carcinogenesis and subsequent progression, its expression was examined by immunohistochemistry on tissue microarrays containing gastric carcinomas, adjacent intestinal metaplasia, pure intestinal metaplasia, and gastritis. Gastric carcinoma tissue and cell lines were studied for COL4A3 expression by Western blotting and reverse transcription–polymerase chain reaction. We found that COL4A3 was differentially expressed in GES-1, AGS, BGC-823, GT-3 TKB, HGC-27, KATO-III, MGC-803, MKN28, MKN45, SCH, SGC-7901, and STKM-2 at both messenger RNA and protein levels. Carcinomas showed statistically lower COL4A3 expression than matched nonneoplastic mucosa ($P < .05$). Expression was strong in intestinal metaplasia in comparison with gastritis and carcinoma ($P < .05$). There was greater COL4A3 expression in carcinoma than gastritis ($P < .05$). Expression of COL4A3 protein was positively correlated with tumor size, lymphatic invasion, venous invasion, and TNM stage ($P < .05$). There was more COL4A3 expression in diffuse than in intestinal-type carcinomas regardless of invasion into the muscularis propria ($P < .05$). Histologically, all signet ring cell (n = 43) and mucinous (n = 12) carcinomas showed COL4A3 expression. Kaplan-Meier analysis indicated that COL4A3 expression was negatively associated with a favorable prognosis of overall, advanced, and intestinal-type gastric carcinomas ($P < .05$). Aberrant COL4A3 expression might play an important role in the pathogenesis and subsequent progression of gastric carcinoma. COL4A3 overexpression might be used as a marker of gastric intestinal metaplasia and mucinous and signet ring cell carcinoma.

☆ This study was supported by Shenyang Outstanding Talent Foundation of China; Shenyang Science and Technology Grant (1091175-1-00; F10-149-9-25; F11-264-1-10); Liaoning Science and Technology Grant; Natural Scientific Foundation of China (no. 81001093, no. 81101885, no. 81101886, no. 81172371); Project Supported by Scientific Research Fund of Hunan Provincial Education Department (L2010649); Research Fund for the Doctoral Program of Higher Education of China (no. 20102104120026) and Grant-in-aid for Scientific Research from the Ministry of Education, Culture, Sports and Technology of Japan (23659958).

* Corresponding author.
E-mail address: zheng_huachuan@hotmail.com (H. Zheng).

0046-8177/$ – see front matter.
http://dx.doi.org/10.1016/j.humpath.2011.10.028

1. Introduction

Collagen type IV is a multimeric protein composed of 3 α subunits that form a "chicken-wire" mesh network together with laminins, proteoglycans, and entactin/nidogen in extracellular matrix (ECM) or vascular basement membrane. Six α chains have been characterized, including ubiquitous α1 and α2 and tissue-specific α3 to α6. In mammals, each of these chains is composed of 3 domains: the cysteine-rich N-terminal 7S domain, the middle triple-helical domains with major collagenous Gly-Xaa-Yaa triple repeats, and the C-terminal globular noncollagenous domain (NC1). Each α chain assembles into triple helices through NC1–NC1 domain interactions [1].

Collagen type IV α3 (COL4A3; Goodpasture antigen) belongs to the type IV collagen family. The gene is located on human chromosome 2q 36-q37 and contains 52 exons with the encoding product being an 8114-base messenger RNA (mRNA) and 162-kDa protein. This gene is organized in a head-to-head conformation with another type IV collagen gene so that each gene pair shares a promoter [2]. The distribution of COL4A3 is limited to certain basement membranes with tissue specificity, such as glomerular basement membrane, basement membranes of the cochlea, ocular basement membrane of the anterior lens capsule, Descemet's membrane, ovarian and testicular basement membrane, and alveolar capillary basement membrane. In addition, COL4A3 is well expressed in the cytoplasm of superficial gastric mucosa; glandular cells in the cervix; trophoblasts, fractions of cells in the renal glomeruli; and parts of the intestine, plasma, and stromal cells. However, this chain is absent from epidermal basement membranes of the skin and the vascular basement membrane of the liver [3-5].

In 2000, the NC1 domain of human COL4A3 was produced as a recombinant protein (named "tumstatin"), which can suppress the proliferation of capillary endothelial cells and blood vessel formation and also induce endothelial cell-specific apoptosis [6]. Further study indicated that tumstatin could suppress the growth of human renal cell carcinoma and prostate carcinoma in mouse xenograft models associated with in vivo endothelial cell-specific apoptosis [7].

The role of COL4A3 has been studied in specific kidney pathologies. In Goodpasture syndrome, autoantibodies bind to COL4A3 in the basement membranes of the alveoli and glomeruli. The epitopes indicate that these autoantibodies are located largely in the noncollagenous C-terminal domain of COL4A3. *COL4A3* mutation within the exons that encode this C-terminal region is also linked to an autosomal recessive form of Alport syndrome [4,5]. Recent studies have shown that collagen IV has cell adhesion function and is involved in tumor invasion and metastasis, including gastric, colorectal cervical, ovarian, and breast cancers [8-18]. In addition, COL4A3 was detected around tumor clusters in well-differentiated lung carcinomas [19].

Despite a worldwide decline in incidence and mortality rate since the second half of the 20th century, gastric cancer is still ranked as the fourth most common and the second most frequent cause of death from cancer in some geographic areas. It continues to be a major health concern, although advanced diagnostic and operative techniques are widely applied in clinical practice [20,21]. Tumorigenesis and progression of gastric carcinoma are multistage processes involving a multifactorial etiology, mainly the result of gene-environmental interactions. Better understanding of the changes in gene expression during carcinogenesis, particularly identification of biomarkers for cancer diagnosis and novel targets for treatment, may improve diagnosis, treatment, and prevention. Therefore, we examined the expression of *COL4A3* mRNA and the encoded protein in gastritis, gastric intestinal metaplasia (IM), and carcinoma tissues and compared its expression with the clinicopathologic features of carcinomas.

2. Materials and methods

2.1. Cell lines and culture

Gastric carcinoma cell lines MKN28 (well-differentiated adenocarcinoma); AGS (moderately differentiated adenocarcinoma); BGC-823, MGC-803, MKN45, and SGC-7901 (poorly differentiated adenocarcinoma); KATO-III (signet ring cell carcinoma); HGC-27, GT-3 TKB, and STKM-2 (undifferentiated adenocarcinoma); and SCH (choriocarcinoma) and gastric epithelial cell line GES-1 were obtained from the Japanese Physical and Chemical Institute, Beijing Institute for Cancer Research, or the Cell Bank of the Chinese Academy of Sciences, Shanghai, China, or were kindly donated by Dr Miyaki, Kanagawa Cancer Center, Japan. They were maintained at 37°C in RPMI 1640 (BGC-823, MGC-803, MKN28, MKN45, KATO-III, SCH, and STKM-2), MEM (HGC-27), DMEM (GES-1, GT-3 TKB, and SGC-7901), or Ham F12 (AGS) medium supplemented with 10% fetal bovine serum, penicillin 100 U/mL, and streptomycin 100 μg/mL in a humidified atmosphere of 5% CO_2. All cells were harvested by centrifugation, rinsed with phosphate-buffered saline, and subjected to total protein extraction by sonication in radioimmunoprecipitation assay lysis buffer.

2.2. Subjects

Gastritis without IM or carcinoma (n = 70) and gastric IM without carcinoma (IM; n = 23) were collected from endoscopic biopsy at the Affiliated Hospital, University of Toyama, between 2006 and 2007. Gastric carcinomas (n = 411) and adjacent IM (n = 140) were collected by surgical resection at Takaoka Citizen Hospital and the Affiliated Hospital, University of Toyama, between 1993 and 2007.