ELSEVIER

CrossMark

Archives of Medical Research

Archives of Medical Research 46 (2015) 186–192

ORIGINAL ARTICLE

Matrix Metalloproteinase 14 Overexpression Is Correlated with the Progression and Poor Prognosis of Nasopharyngeal Carcinoma

Ting-hua Yan,[a,*] Zhong-hao Lin,[b,*] Jin-hua Jiang,[c,d,*] Sui-wan Lu,[a] Hua-xing Que,[a] Miao-an Chen,[b] Xiang-sheng He,[a] Gan-bo Que,[a] Ji-nan Xiao,[a] and Yuan-qing Chen[a]

[a]*Department of Internal Medicine and Hyperbaric Oxygen, Second Hospital of Longyan City, Longyan, Fujian, China*
[b]*Department of Otorhinolaryngology Head and Neck Surgery, The Affiliated Mindong Hospital of Fujian Medical University, Ningde, Fujian, China*
[c]*The First Clinical Medical College, Fujian Medical University, Fuzhou, Fujian, China*
[d]*Department of Oncology, Longyan Humanity Hospital, Longyan, Fujian, China*

Received for publication November 9, 2014; accepted March 17, 2015 (ARCMED-D-14-00647).

Background and Aims. Matrix metalloproteinase 14 (MMP14) has been identified to play a significant role in several types of cancers, but little is known about the significance of MMP14 in nasopharyngeal carcinoma (NPC) patients. The aim of this study was to explore the association of MMP14 expression with clinicopathologic features and prognosis in NPC.

Methods. MMP14 mRNA and protein expressions were examined in NPC and nasopharyngeal tissues through real-time PCR and immunohistochemistry. Meanwhile, the relationship of MMP14 expression levels with clinical features and prognosis of NPC patients was analyzed.

Results. MMP14 mRNA expression was markedly higher in NPC tissues than in nasopharyngeal epithelium tissues ($p = 0.002$). Using immunohistochemistry, staining for MMP14 protein was found in the normal nasopharyngeal epithelial cells and malignant epithelial cells, but increased expression of MMP14 was observed in NPC samples compared with normal nasopharyngeal epithelium samples ($p = 0.027$). In addition, high levels of MMP14 protein were positively correlated with the status of clinical stage ($p = 0.009$), N classification ($p = 0.006$), and distant metastasis ($p = 0.005$) of NPC patients. Patients with higher MMP14 expression had a significantly shorter overall survival time than did patients with low MMP14 expression. Multivariate analysis indicated that the level of MMP14 expression was an independent prognostic indicator ($p < 0.001$) for the survival of patients with NPC.

Conclusions. MMP14 overexpression is a potentially unfavorable prognostic factor for NPC patients. © 2015 IMSS. Published by Elsevier Inc.

Key Words: Matrix metalloproteinase-14, Nasopharyngeal carcinoma, Immunohistochemistry, Prognosis.

Introduction

Nasopharyngeal carcinoma (NPC) is a head and neck malignancy disease with a distinct racial and geographical distribution. NPC is particularly common in southern China where the annual incidence rate reaches ~20–50/100,000 persons (1). Unfortunately, the majority of NPC patients tend to present a more advanced stage of disease when first diagnosed because of its vague symptoms and internal location. Although NPC patients are sensitive to radiotherapy, treatment failure remains high due to the development of local recurrence and distant metastasis (2). Therefore, it is necessary to further investigate the precise molecular changes that are responsible for the progression and metastasis of NPC.

*These authors contributed equally to this work.

Address reprint requests to: Jin-hua Jiang, The First Clinical Medical College, Fujian Medical University, Fuzhou, 350001; Department of Oncology, Longyan Humanity Hospital, Longyan, 364000, Fujian, China; Phone/Fax: +86 0597 2180581; E-mail: freedomlongyuan@163.com

0188-4409/$ - see front matter.
http://dx.doi.org/10.1016/j.arcmed.2015.03.006

Recent studies have demonstrated that matrix metalloproteinases (MMPs) in particular are responsible for the degradation of the extracellular matrix (ECM) in tumor invasion. It has been shown that specific members of the MMP not only accelerate tumor cell invasion but also alter tumor cell behavior and promote cancer progression (3). The secreted and membrane type MMPs (MT-MMPs) are major members of the human MMP family. Matrix metalloproteinase-14 (MMP14) was the first membrane type MMP discovered and hence is also referred to as membrane type 1-matrix metalloproteinase (MT1-MMP). MMP14 has been suggested to be involved in many biological processes such as proliferation, invasion, angiogenesis, and basement membrane remodeling (4).

Compared with benign tumor or normal tissue, increased expression of MMP14 was observed in a variety of human cancers such as lung cancer (5,6), breast cancer (7), colon cancer (7), cervical cancer (8), prostate cancer (9), and glioblastomas (10). In addition, the correlations between MMP14 expression and clinicopathological features have been explored in a variety of human carcinomas such as breast cancer (11), lung cancer (5,6), esophageal cancer (12), gastric cancer (13), cervical cancer (8), and supraglottic cancer (14), but little is known about MMP14 in NPC patients.

In order to identify the role of MMP14 in the pathogenesis of NPC, we investigated the relationship of MMP14 protein expression with clinicopathological features including patient survival. We found that mRNA and protein expression levels of MMP14 were higher in NPC tissues than in nasopharyngeal tissues. Moreover, overexpression of MMP14 was associated with NPC progression and poor prognosis. Our results demonstrated that overexpressed MMP14 is a poor prognostic factor for NPC patient survival.

Materials and Methods

Sample Collection

A total of 20 freshly frozen NPC samples and ten normal nasopharyngeal epithelium samples were collected from the Second Hospital of Longyan City between December 2013 and June 2014. All fresh samples were immediately preserved in liquid nitrogen. One hundred and forty eight paraffin-embedded undifferentiated NPC specimens and 32 nasopharyngeal epithelium specimens were retrieved from the Second Hospital of Longyan City and Fujian Medical University between January 2005 and December 2013. No patients had received any form of tumor-specific therapy prior to diagnosis. Before the use of these clinical samples, prior consent from patients and approval from the Institutional Ethics Committee of the Second Hospital of Longyan City and Fujian Medical University were obtained. The histopathological diagnosis of all samples was diagnosed by two pathologists. Clinical staging was based on the 7th edition of the AJCC Cancer Staging Manual. In the 148 NPC cases, there were 97 males and 51 females with ages ranging from 19−72 years (median 55.98 years). The clinical follow-up time of patients ranged from 9−96 months. Overall survival (OS) was defined as the interval from the date of diagnosis to NPC-related death.

Real-Time Quantitative Reverse Transcription-Polymerase Chain Reaction (RT-qPCR)

To quantitate mRNA expression, total RNA was extracted from clinical samples with RNAiso Plus (Takara, Japan). The isolated total RNA was reverse transcribed using the PrimeScript RT Master Mix (Perfect Real Time) (Takara, Japan) for MMP14 according to the manufacturer instructions. Relative expression was calculated via the comparative cycle threshold (Ct) method and was normalized to the expression of GAPDH. The sequence-specific forward and reverse primers sequences for MMP14 mRNA were 5′-GGATACCCAATGCCCATTGGCCA-3′ and 5′-CCTC GGTGCACCATGTTTGGC-3′, respectively. Forward and reverse primers sequences for GAPDH mRNA were 5′-GCACCGTCAAGGCTGAGAAC-3′ and 5′- TGGTGA AGACGCCAGTGGA -3′, respectively. qPCR was performed using SYBR Premix Ex TaqTM II (Takara, Japan) on a LightCycler (Roche Diagnostics, USA). Relative quantification of mRNA expression was calculated using the 2-ΔΔCt method. Raw data were presented as the relative quantity of target mRNA normalized with GAPDH and relative to a calibrator sample. All qRT-PCR reactions were performed in triplicate.

Immunohistochemistry

Paraffin sections from NPC and nasopharyngeal epithelium specimens were deparaffinized in xylene and rehydrated in a descending ethanol series (100, 95, 90, 80, 70% ethanol) and double-distilled water according to standard protocols. Heat-induced antigen retrieval was performed in citrate buffer and boiled for 10 min. After antigen retrieval, sections were treated with 3% hydrogen peroxide and 1% bovine serum albumin to block endogenous peroxidase activity and nonspecific binding. The sections were incubated with MMP14 antibody (Abcam, clone EP1264Y, dilution 1:150) overnight at 4°C. After phosphate-buffered saline (PBS) washing, tissue sections were incubated with the biotinylated secondary antibody and streptavidin−horseradish peroxidase complex, each for 20 min at room temperature. Diaminobenzidine (DAB) was used as the chromogen, and tissue sections were counterstained with hematoxylin and then viewed under a bright-field microscope.