Circulating Anti–Matrix Metalloproteinase-7 Antibodies May Be a Potential Biomarker for Oral Squamous Cell Carcinoma

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Purpose: The present study was conducted to evaluate the diagnostic and prognostic values of serum autoantibody against matrix metalloproteinase-7 (MMP-7) in patients with oral squamous cell carcinoma (OSCC).

Materials and Methods: Anti–MMP-7 antibodies were measured in sera from 204 patients with OSCC and 212 normal controls using enzyme-linked immunosorbent assay, and clinicopathologic characteristics were correlated. Prognostic consequence was assessed with Kaplan-Meier curve and log-rank tests using Cox proportional hazard models. To check whether anti–MMP-7 antibody was related to tumor associated antigen, real-time polymerase chain reaction and western blot were used to measure MMP-7 mRNA and protein expression in tumor tissues from all 204 patients with OSCC.

Results: Serum anti–MMP-7 antibody was higher in patients with OSCC ($P < .05$), and those with poorly differentiated tumors had more anti–MMP-7 antibody than those with well to moderate tumor differentiation ($P < .01$, $P < .01$, respectively). Patients with OSCC at late TNM stages (III, IV) and lymph node metastases had relatively higher serum anti–MMP-7 antibody levels than those with earlier stages (I, II) and those who lacked lymph node metastases ($P < .05$ for the 2 comparisons). OSCC prediction sensitivity as measured by receiver operating characteristics analysis was 0.485 and specificity was 0.896 (area under the curve, 0.761; 95% confidence interval, 0.716 to 0.806). Cox analysis showed that serum anti–MMP-7 antibody positivity independently predicted poor overall survival in patients with OSCC (hazard ratio, 1.82; 95% confidence interval, 1.07 to 4.61). MMP-7 mRNA and protein expression was increased in tumor tissues from patients with OSCC and high serum anti–MMP-7 antibody.

Conclusion: Serum anti–MMP-7 antibody might be a novel diagnostic and prognostic biomarker for OSCC.

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Oral squamous cell carcinoma (OSCC) is the most frequent oral cancer, with more than 500,000 new cases diagnosed worldwide annually.[1] As such, OSCC is a substantial individual and public health burden.[2] OSCC is believed to be caused by a combination of genetic susceptibility and environmental triggers that initiate and promote this disease.[3] The survival rate of OSCC is relatively low owing to frequent metastases, poor prognosis, and high recurrence. Until recently, treatment for OSCC has been surgery, with radiotherapy or chemotherapy when indicated.[4] Unfortunately, most diagnoses are made at advanced stages, so reliable biomarkers are needed because early detection is believed to improve survival.[5]

Several antibodies against autologous cell proteins have been observed in sera of patients with various cancer types and might serve as early cancer biomarkers.[6] These antibodies are directed against autologous tumor-associated antigens (TAAs).[7] Autoantibodies are stable and easily accessed, increasing

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their value as biomarkers.[8] Studies have suggested that autoantibodies to TAAs can be detected several years before diagnosis.[9] Efforts to identify autoantibodies to TAAs as a diagnostic or prognostic biomarker are underway, and several potential autoantibody biomarkers, such as p53, Foxp3, CD25, c-myc, and heat shock protein, have been reported in sera from patients with different cancers.[10] Several autoantibodies have been identified in sera from patients with OSCC. The preoperative presence of an antibody against p53 was an independent prognostic factor that was meaningfully associated with lymph node metastases.[11,12] Also, an autoantibody to sideroflexin 3 was identified as a potential biomarker for detection of early OSCC.[13] Except for these TAAs, researchers also have investigated other candidates, such as matrix metalloproteinases (MMPs).

MMPs are zinc-dependent proteolytic enzymes that are key to the physiologic degradation of the extracellular matrix (ECM) in angiogenesis, tissue repair, and tissue morphogenesis.[14] MMP-7, the smallest molecule of the MMP family, has been found overexpressed in various tumors and increased circulating MMP-7 proteins have been correlated with metastatic disease and poor survival. As an early immune response to cancer development, increased MMP-7 autoantibodies have been identified in esophageal SCC and gastric tumors, but this was not reported for OSCC.

To examine whether serum anti–MMP-7 antibodies also were increased in patients with OSCC, 204 patients with OSCC were compared with 212 normal controls using enzyme-linked immunosorbent assay (ELISA) with purified MMP-7 recombination protein. In addition, the possible clinical utility of anti–MMP-7 antibody as a tumor biomarker in the diagnosis and prognosis of patients with OSCC was evaluated. Further, MMP-7 mRNA and protein expression was measured in OSCC tumor tissues to check whether anti–MMP-7 antibody was related to TAA.

Materials and Methods

STUDY PATIENTS

This retrospective study included 204 consecutive patients with OSCC and 212 normal controls. All consecutive 204 patients diagnosed with OSCC were from Jinan Stomatological Hospital (Shandong, China), Chinese PLA General Hospital (Beijing, China), and Shandong Cancer Hospital and Institute (Shandong, China) from January 2008 to September 2010. OSCC diagnosis was confirmed in all cases by histologic examination. No patient underwent preoperative chemotherapy or radiotherapy. Patients with OSCC and other kinds of cancer or some autoimmune disease, such as rheumatoid arthritis, systemic lupus erythematosus, Sjögren syndrome, or diabetes, were excluded. OSCC in these patients was staged clinically based on TNM classification; for histologic grading, the Broder grading system was followed. Clinical and laboratory characteristics were obtained retrospectively from medical records. Blood (5 mL) was collected from patients with OSCC before surgery and from controls and centrifuged at 3,000*g* for 10 minutes, and sera were removed and stored at −80°C before use. Gender- and age-matched normal controls without any disease who underwent routine physical examination in the same hospital during the same period (n = 212) and were unrelated to the patients with OSCC also were recruited. All aspects of the present study were approved by the ethics committee of Jinan Stomatological Hospital and complied with the requirements of the Declaration of Helsinki. Also, written informed consent was obtained from all participants.

CLONING MMP-7 CDNA AND PURIFICATION

MMP-7 cDNA was amplified by polymerase chain reaction (PCR) from OSCC tissue using the following primers: sense, 5′-CGGGGTACCTCACTATTTCCAAATAGCCC-3′; and antisense, 5′-CCGCTCGAGTTATCCATATAGTTTCTGAATGCC-3′. Two restriction enzymes sites, including *Kpn*I and *Xho*I, were at the 5′ end of the forward and reverse primers, respectively. Then, PCR products were purified (PCR purification kit; QIAGEN, Germantown, MD). After purification, PCR products were digested with *Kpn*I and *Xba*I overnight at 37°C. The pcDNA3.1 expression vector was digested with the same restriction enzymes. Next, ligation of the insert into the expression vector using T4 DNA ligase was performed (NEB, BioLabs Inc, Lawrenceville, GA). The constructed plasmid was transformed into a BL21 competent cell and seeded in Luria-Bertani agar plates. One bacteria colony was selected and grown in Luria-Bertani buffer with carbenicillin overnight at 37°C until the optical density (600 nm) reached 0.6. Isopropyl-B-D-thiogalactopyranoside (0.75 mg/mL, 18°C, overnight) was used to induce MMP-7 expression. A purification kit was used (Takara Bio Inc, Shiga, Japan) to purify Myc-tagged MMP-7 recombinant proteins with monoclonal anti–c-Myc antibody agarose beads.

SERUM ANTI–MMP7 MEASUREMENT

EIA 96-well microtiter plates (Coster, South Elgin, IL) were coated with recombinant MMP-7 and incubated overnight at 4°C and then washed 3 times with phosphate buffered saline and Tween 20. ELISA was used to quantify the serum antibody against MMP-7. Sera samples were diluted 1:100 in sample buffer and added to wells and sample buffer was used as a negative control. Then, plates were incubated at room temperature for approximately 2 hours

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