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A single nucleotide polymorphism in the matrix metalloproteinase-1 promoter enhances oral squamous cell carcinoma susceptibility in a Chinese population

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Summary We genotyped 96 oral squamous cell carcinoma (OSCC) patients for the 1G/2G polymorphism of matrix metalloproteinase-1 (MMP-1) promoter −1607 bp using PCR-RFLP. A control population of 120 frequency-matched subjects was also genotyped for the same polymorphism.The detection frequency of 2G allele was significantly higher in OSCC subjects (76%) than in the control group (56.7%). The frequency of 2G allele had a significant difference between the OSCC and controls group ($p = 0.00$, Odds Ratio, OR = 2.232, 95% CI = 1.477—3.372). The genotype 2G/2G was found in 57.3% of the OSCC, and 34.2% in the controls. The proportion of 2G homozygote (2G/2G) was significantly higher in the OSCC group when compared to controls ($p = 0.001$, OR = 2.585, 95% CI = 1.487—4.494). OSCC patients were stratified by clinicopathological parameters including gender, smoking, clinical stage and lymph node metastasis, but the only statistically significant association with MMP-1 genotype was with smoking. The results showed that a SNP in the MMP-1 promoter −1607 bp was associated with OSCC susceptibility in a Chinese population.

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Introduction

Extracellular matrix (ECM) is essential in many physiological processes, e.g., during development, growth, and repair of tissues. On the other hand, excessive proteolysis plays an important role in several pathological conditions, e.g. rheumatoid arthritis, osteoarthritis, autoimmune blistering disorders of skin, dermal photo aging, and periodontitis.[1–3] Tumor invasion, metastasis, and angiogenesis require controlled degradation of ECM, and increased expression of matrix metalloproteinases (MMPs) is associated with tumor invasion and metastasis of malignant tumors with different histogenetic origin.[4,5] At least 28 MMPs have been characterized.[6,7] MMPs are a family of metal-dependent proteolytic enzymes that mediate the degradation of ECM and basement membranes. All members of this family have a zinc- and calcium-binding catalytic domain, so that they depend on these ions for their activity. However, in addition to fostering cellular invasion by disrupting ECM barriers, MMPs can also influence the microenvironment by altering cellular signals.[8,9] Moreover; most MMPs are synthesized not only by the genetically altered cancer cells but also by adjacent and intervening stromal cells. There is also growing evidence to support an expanded role of MMPs in creating and maintaining a microenvironment that facilitates the initial stages of tumor development.[10,11]

Among the MMPs, MMP-1 is the highly expressed interstitial collagenase degrading fibrillar collagens, the most abundant protein in the human body. Overexpression of MMP-1 has been demonstrated in tumor tissues and has been suggested to be associated with tumor invasion and metastasis.[12] Overexpression of MMP-1 has been found to be associated with an overall poor prognosis in colorectal, ovarian and lung cancer.[13–15] A genetic variation in the MMP-1 promoter can influence the level of MMP-1 transcription, and hence the potential of this gene to mediate connective tissue degradation. This variation is a single nucleotide polymorphism (SNP) located at −1607 bp, where an insertion of a guanine base (G) creates the sequence, 5′-GGAT-3′, the core binding site for members of the Ets family of transcription factors.[16] The 2G allele has been shown to significantly increase the transcription activity compared to 1G allele.[16] It has demonstrated that the 2G allele displays heightened MMP-1 transcription in both tumor cells and in normal fibroblasts, and the levels of MMP-1 expression may result from the presence of the 2G allele and from elevated expression of the transcription factors that bind to this site. The presence of this allele has been associated with the development of ovarian cancer and breast cancer.[14,17]

This MMP-1 polymorphism may provide a mechanism for more aggressive matrix degradation, thereby facilitating tumor development. Association studies have been done to determine whether the MMP-1 genotype affects the risk of various types of cancers. Oral squamous cell carcinoma (OSCC) is the most prevalent malignant cancer in the oral cavity. There was no report about the association between the MMP-1 polymorphism and OSCC. So in the present study, we used DNA samples to investigate the relationship between MMP-1 polymorphism and the risk of OSCC.

Material and methods

Subjects

96 patients with pathologically proved primary OSCC from Department of Oral and Maxillofacial Surgery and Department of Oral Medicine at Hospital of Stomatology, Wuhan University in china. 120 control subjects were selected from people who for routine physical checkups, non-neoplastic minor operations, or maxillofacial trauma in the same hospital. Those with immune disorders, blood diseases, or previous neoplasms were excluded. The baseline clinical parameters for the subject population are presented in Table 1.

After informed consent was obtained, blood was drawn from the subjects and the 20 ml blood sample collected in EDTA tubes was given a code and was stored in −70 °C until further processing. An ethics review board approved this study.

DNA extraction

After defrosting, DNA was extracted by genetic DNA extracting kit (Takara, Japan). DNA was dissolved in TE buffer [10 mM Tris (PH 7.8), 1 mM EDTA]. The concentration was estimated by measurement of OD_{260}. Final preparation was stored at −20 until as template DNA for polymerase chain reaction (PCR).

PCR

The sequences of PCR primers were: forward, 5′-TCGTGAGAATGTCTTCCCATT-3′; reverse, 5′-TCTT-