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Macrophage migration inhibitory factor is a novel prognostic marker for human oral squamous cell carcinoma

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ABSTRACT

Macrophage migration inhibitory factor (MIF) is considered a pro-tumour factor. However, its clinical relevance in oral squamous cell carcinoma (OSCC) remains unclear. The objective of this study was to investigate the expression of MIF and its receptor CD74 in OSCC tissues, and to study the function of MIF in OSCC cells. Tissues of 90 patients with OSCC from the School of Stomatology, China Medical University were collected, and immunohistochemical staining and quantitative reverse transcription polymerase chain reaction were performed for MIF and CD74. The possible correlations between MIF and CD74 and clinical characteristics were analysed. The Kaplan-Meier analysis was used to determine the survival rates of patients. In addition, the proliferation and invasion of OSCC cells were evaluated after transfection with siRNA against MIF. MIF and CD74 levels were significantly higher in tissues of patients with OSCC than in control tissues. Moreover, MIF levels in patients with OSCC were significantly associated with cell differentiation and TNM classification. MIF expression was closely related to CD74 expression. Kaplan-Meier analysis indicated that OSCC patients with high MIF levels showed reduced overall survival and recurrenc-free survival. Furthermore, MIF expression promoted proliferation and invasion of OSCC cells. Collectively, our results reveal that MIF expression is a significant independent prognostic factor for patients with OSCC and may be a novel prognostic marker for OSCC.

1. Introduction

Oral cancer is the sixth most prevalent form of cancer, with oral squamous cell carcinomas (OSCC) exhibiting the highest morbidity of all head and neck cancers [1]. Patients diagnosed with OSCC typically have a poor prognosis because of its invasiveness and ability of migration [2]. The diagnosis of OSCC remains challenging, especially in advanced-stage cases. It is well known that the metastasis of OSCC involves interactions among multiple genes. Although significant therapeutic strategies have been developed for OSCC treatment, the 5-year survival rate is still low [3,4]. Therefore, the molecular mechanisms underlying OSCC tumorigenesis must be elucidated to identify tumour-specific biomarkers and therapeutic targets for early diagnosis and treatment of the disease.

Initial tumour growth depends on increased cell proliferation and reduced cell death, both of which are stimulated by inflammation-driven mechanisms. A pro-tumorigenic microenvironment was build up that responded to tumour cells trigger an intrinsic inflammatory. Macrophage migration inhibitory factor (MIF) is a pleiotropic cytokine associated with inflammation and tumorigenesis [5,6]. It was thus named in 1966 as it was found to inhibit the migration of macrophages [7]. Extensive studies revealed that MIF may act as a proinflammatory protein [8]. For its pleiotropic effects, there was enough evidence to confirm that MIF was capable of providing several levels of support to a developing tumour. Some reports indicate that MIF plays an important role in patients with hepatocellular carcinoma [9], breast cancer [10], nasopharyngeal carcinoma [11], pancreatic adenocarcinoma [12].

The CD74 was the first surface receptor for MIF that was described, it is normally expressed by cells associated with the immune system. CD74 also forms heterodimers with CD44 to generate receptors for MIF. CD74 has been known to be expressed on HLA Class II-positive normal cells including B cells, macrophages and dendritic cells. Also, some reports revealed that CD74 is expressed in gastric [13] and renal cell carcinomas [14].

The role of MIF and CD74 in OSCC is not very clear. Therefore, we analysed the expression of MIF and its receptor in human OSCC tissues and determined the correlation between MIF levels and clinicopathologic features of OSCC. We also investigated the correlation between MIF and CD74. Our finding revealed that MIF expression is an independent prognostic factor for shortened overall survival and

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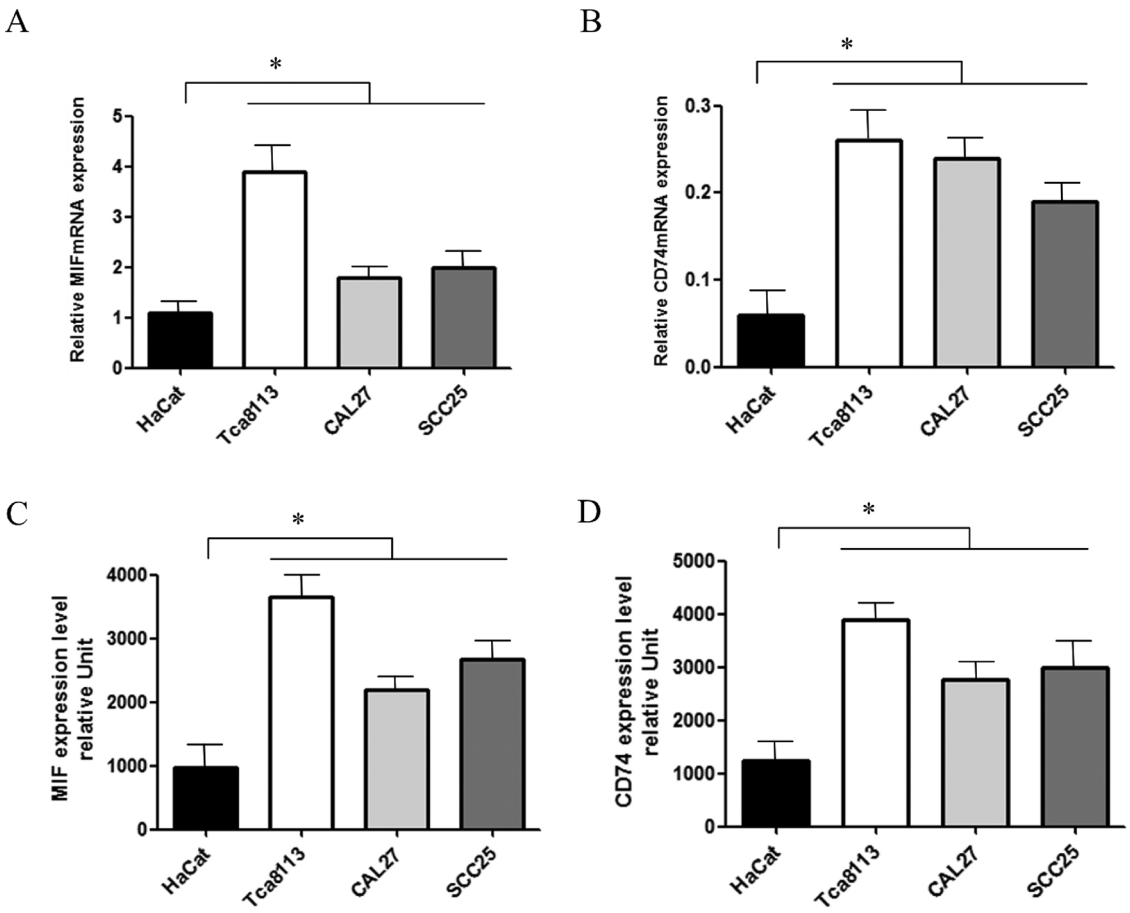

Fig. 1. OSCC cell lines overexpress MIF and its receptors CD74. mRNA levels of MIF(A) and CD74 (B)、and protein levels of MIF(C) and CD74 (D) in different OSCC cell lines (Tca8113, CAL27, SCC25) and in human normal skin immortalized keratinocyte strain (HaCat) cells using RT-qPCR and western blotting. $*P < 0.05$.

recurrence-free survival of patients with OSCC. Furthermore, to clarify the potential involvement of MIF in OSCC, cancer cell functions were inveatigated after transfection with small-interfering RNA(siRNA) against MIF(siMIF). Our findings suggest that MIF could be a novel prognostic marker for OSCC.

2. Materials and methods

2.1. Patients

Ninety pairs of matched OSCC and adjacent non-cancer tissues were collected from patients who were histopathologically and clinically diagnosed with OSCC, and who underwent surgical resection at the Department of Oral and Maxillofacial Surgery at the School of Stomatology, China Medical University between 2012 and 2013. None of the patients had received chemotherapy or radiotherapy before surgery. Clinical data including age, sex, recurrence, primary location, tumour size, metastasis and clinical staging were obtained from pathological and clinical records. Tumour specimens were histologically classified based on the degree of their differentiation (well-, moderately-, and poorly differentiated), according to the International Histological Classification of Tumours [15]. Experiments using human tissues were approved by the ethics committee of the School of Stomatology, China Medical University and written informed consent was obtained from patients providing tissue specimens.

2.2. Cell culture and transfection with siRNA

Human OSCC cell lines Tca8113, CAL27, SCC25 and HaCat cells were provided by the American Type Culture Collection (ATCC, Manassas, VA, USA). Tca-8113 and SCC25 cells were maintained in the RPMI 1640 culture medium, CAL27, and HaCat cells were maintained in DMEM, 10% bovine calf serum and 1% penicillin-streptomycin solution were added to culture media. All cell lines were maintained at 37℃ in the presence of 5% CO_2.

After cultures reached 70–80% confluence, OSCC cells were trypsinized and transfected with siMIF and siControl (GenePharma Company, Shanghai, China) using Lipofectamine 2000 (Life Technologies, Carlsbad, CA, USA) in accordance with the manufacturer's instructions.Transfection efficiency was assessed using quantitative reverse transcription polymerase chain reaction (RT-qPCR) analyses.

2.3. Reverse transcription-polymerase chain reaction(RT-qPCR)

Total RNA from cells and tissues was extracted using Trizol kits (Sangon Biotech Corporation, Shanghai, China). RNA (1 μg) was reverse transcribed using PrimeScriptTM RT reagent kit an cDNA Synthesis kit (TaKaRa), according to the manufacturer's instructions. Reverse transcription reaction was set up as follows: 5× PrimeScript RT Master Mi × 4 μl, total RNA 1 μg, RNase Free dH_2O up to 20 μl, The reaction was incubated at 37 ℃ for 15 min, followed by 85℃ for 5 s. Gene expression was quantified using SYBR Premix Ex Taq II(TaKaRa, China) in 20 μl reactions set up as follows: 2 × SYBR Premix Ex TaqII (Tli RNaseH Plus) 10 μl, 10 μM forward Primer 0.4 μl, 10 μM reverse Primer 0.4 μl, ROX reference dye 0.2 μl, cDNA 2 μl, sterilized dH_2O up to 20 μl. The reaction started at 95 ℃ for 30 s, followed by 40 cycles of 95 ℃ for 5 s, 60 ℃ for 34 s, and final elongation at 60 ℃ for 1 min. Both target and reference were amplified in triplicate wells. The relative level of MIF and CD74 was calculated using the $2^{-\Delta\Delta Ct}$ method.