Original Article

Metastasis-associated colon cancer-1 is a novel prognostic marker for cervical cancer

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Received May 14, 2014; Accepted May 29, 2014; Epub June 15, 2014; Published July 1, 2014

Abstract: Aims: To investigate metastasis associated in colon cancer 1 (MACC1) expression in cervical cancer. Methods: One hundred and four paraffin-embedded cervical cancer specimens were immunohistochemically analyzed for MACC1 expression. The expression of MACC1 in 8 pairs of cervical cancer and adjacent normal cervical tissues were detected by Real-time PCR. Results: MACC1 expression was upregulated in cervical cancer tissues compared with adjacent normal cervical tissues. Patients with higher MACC1 expression had shorter overall survival time, whereas those with lower ASAP1 expression survived longer (P = 0.029). Moreover, high expression of MACC1 was correlated with FIGO stage (P = 0.039) and lymph nodes metastasis (P = 0.003) of this disease. Multivariate analysis revealed that MACC1 was an independent prognostic factor (P = 0.043) for the overall survival of cervical cancer patients. Conclusion: Our study suggests that MACC1 may contribute to tumor development and progression in cervical cancer, and that MACC1 could be a useful marker for the prognosis of cervical cancer.

Keywords: MACC1, cervical cancer, prognosis, lymph nodes metastasis

Introduction

Cervical cancer is the second common gynecologic malignancy, with more than 0.52 million new cases and 0.27 deaths globally each year [1]. More than 80% of the new cases are occurring in developing countries, where the mortality is 10 times higher than that in developed countries [2]. Cervical cancer represents a major public health concern and is associated with significant healthcare costs. HPV screening is useful for early diagnosis of cervical dysplasia and cervical cancer [3]; however, HPV screening has some shortcomings, for example, most women with a positive HPV test do not have cervical dysplasia or cancer [4]. Another limitation is that the HPV screening also includes transient viral infections that will never become cervical cancer [5]. Besides, HPV test cannot distinguish the benign from malignant lesions. Therefore, development of new biological markers for cervical cancer development would be important supplements to HPV screening test.

MACC1 (metastasis-associated in colon cancer-1) is a recently discovered gene that regulates the HGF/MET signaling pathway [6]. The MACC1 gene is located on chromosome 7 at position 7p21.1 [7]. It is primarily discovered by a genome-wide search for differently expressed genes in human colon cancer tissues [6]. As proposed by Boardman et al, MACC1 can lead to the activation of HGF/MET signaling and potentiates metastasis and recurrence of colorectal cancer [8]. Recently, it has been implicated in regulating cell migration, invasion, proliferation, colony formation, and wound healing [9]. Furthermore, MACC1 induces tumor growth and metastasis in vitro models has been demonstrated by both gain- and loss-of-function approaches [10]. Besides, it has also been proposed that MACC1 could be used as prognostic marker in cancer patients. Galimi et al. reported that overexpression of MACC1 was associated with unfavorable pathologic features in colorectal cancer [7]. Qu et al. described that overexpression of MACC1 predicts poor clinical outcome of hepatitis B virus-related
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Table 1. Distribution of MACC1 expression in cervical cancer patients according to clinico-pathologic characteristics

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>No.</th>
<th>MACC1</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (y)</td>
<td></td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>≤ 40</td>
<td>48</td>
<td>26</td>
<td>0.333</td>
</tr>
<tr>
<td>&gt; 40</td>
<td>56</td>
<td>25</td>
<td></td>
</tr>
<tr>
<td>FIGO Stage</td>
<td></td>
<td></td>
<td>0.039</td>
</tr>
<tr>
<td>IB</td>
<td>71</td>
<td>31</td>
<td></td>
</tr>
<tr>
<td>&gt; IB</td>
<td>33</td>
<td>21</td>
<td></td>
</tr>
<tr>
<td>Differentiation</td>
<td></td>
<td></td>
<td>0.973</td>
</tr>
<tr>
<td>Grade 1/2</td>
<td>43</td>
<td>21</td>
<td></td>
</tr>
<tr>
<td>Grade 3</td>
<td>61</td>
<td>30</td>
<td></td>
</tr>
<tr>
<td>LN Metastasis</td>
<td></td>
<td></td>
<td>0.003</td>
</tr>
<tr>
<td>No</td>
<td>86</td>
<td>38</td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>18</td>
<td>15</td>
<td></td>
</tr>
<tr>
<td>Recurrence</td>
<td></td>
<td></td>
<td>0.056</td>
</tr>
<tr>
<td>No</td>
<td>92</td>
<td>42</td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>12</td>
<td>9</td>
<td></td>
</tr>
<tr>
<td>Total NO. of patients</td>
<td>104</td>
<td>82</td>
<td>100</td>
</tr>
</tbody>
</table>

In the present study, we found that the expression of MACC1 was upregulated in surgical specimens of cervical cancer. Importantly, the overexpression of MACC1 is correlated with the FIGO stage and lymph node metastasis of the disease. Multivariate analysis revealed that MACC1 was an independent factor for the prediction of the prognosis of cervical cancer. Taken together, our results suggest that MACC1 plays a significant role in human cervical cancer progression and metastasis.

Materials and methods

Patients and tissue specimens

This study was conducted on a total of 104 parafin-embedded cervical cancer samples, which were histopathologically and clinically diagnosed at the First People’s Hospital of Chenzhou City from 2007 to 2012. For the use of these clinical materials for research purposes, prior patient consent and approval from the Institutional Research Ethics Committee were obtained. Clinical information on the samples is summarized in supplementary Table 1. The disease stages of all the patients were classified according to the International Federation of Gynecology and Obstetrics (FIGO) guidelines for clinical staging. Eight pairs of cervical cancer tissues and the matched adjacent non-cancerous cervical tissues were frozen and stored in liquid nitrogen until further use.

RNA extraction and Real-time PCR

Total RNA from cervical cancer tissues was extracted using the Trizol Reagent (Invitrogen), according to the manufacturer’s instructions. The RNA was pretreated with RNase-free DNase (Promega), and 1 μg RNA was used for cDNA synthesis. Real-time PCR was performed using a Thermal Cycler Dice® Real-time System TP800 (Takara Bio Inc., Otsu, Japan) system. Sequences of the primers are: MACC1, forward primer 5’-TTCTTTTGATTCCTCCGGTGA-3’, reverse primer 5’-ACTCTGATGGGCATGTGCTG-3’, GAPDH forward primer 5’-GAATCTACTGGCTTTCACC-3’, reverse primer 5’-GTCATGAGCCCGTCCACGATGC-3’.

Immunohistochemistry

Immunohistochemical analysis was done to study altered protein expression in 104 cervical cancer tissues. In brief, paraffin-embedded specimens were cut into 5 μm sections and baked at 60°C for 3 h followed by deparaffinization with xylene and rehydrated. After antigenic retrieval, the sections were treated with 3% hydrogen peroxide in methanol to quench endogenous peroxidase activity, followed by incubation with 1% bovine serum albumin to block nonspecific binding. Sections were incubated with monoclonal rabbit anti-MACC1 (Sigma, USA; 1:50). After washing with PBS, the slides were incubated with prediluted secondary antibody (Abcam), followed by further incubation with diaminobenzidine (DAB). Finally, the sections were counterstained with 10% Mayer’s hematoxylin, dehydrated, and mounted.

The degree of immunostaining was scored independently by two observers based on the proportion of positively stained tumor cells and intensity of staining. Tumor cell proportion was scored as follows: 0 (no positive tumor cells), 1 (< 10% positive tumor cells), 2 (10-30% positive, 3 (31-60% positive, and 4 (61-100% positive tumor cells).
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Expression of MACC1 is up-regulated in cervical cancer tissues

To determine the expression of MACC1 mRNA in cervical cancer tissues, Real-time PCR analysis was done in paired cervical cancer tissues and adjacent normal tissues, with each pair taken from the same patient. MACC1 was found to be overexpressed in all 8 examined human primary cervical cancer samples compared with the paired normal cervical tissues (Figure 1). Interestingly, all the tumor samples displayed > 3-fold increase of MACC1 mRNA compared with adjacent normal tissues.

Relationship of MACC1 upregulation with the clinical features of cervical cancer

To investigate the potential roles of MACC1 in the progression of cervical cancer, immunohistochemistry was performed to measure MACC1 expression in 104 archived cervical cancer samples. The representative immunostaining of MACC1 in cervical cancer was shown in Figure 2A-D. Immunohistochemical staining of MACC1 levels was statistically analyzed to determine their relationship with the clinical features of cervical cancer. As shown in Table 1, MACC1 expression strongly correlated with FIGO stage ($P = 0.039$) and lymph nodes metastasis ($P = 0.003$) of patients with cervical cancer. Taken together, our data revealed a relationship between the expression of MACC1 and cervical cancer development.

Association between MACC1 expression and patient survival

Patient survival analysis indicated an inverse correlation between MACC1 expression level and the overall survival time of cervical cancer patients. As shown in Figure 3A, the length of survival time was significantly different between patients with low and high MACC1 expression ($P = 0.029$), with the low MACC1 group having a longer overall survival time. However, there was no significant relationship between MACC1 expression and patient survival.
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Figure 2. Representative images of MACC1 from immunohistochemistry assays in cervical cancer specimens (high expression for A and B; low expression for C and D) (200 x for A and C, 400 x for B and D).

Figure 3. Kaplan-Meier curves of overall survival (A) and recurrence-free survival (B) in relation to MACC1 expression in 104 cervical cancer patients.

expression and Recurrent-free survival time (Figure 3B, \( P = 0.055 \)). Multivariate analysis revealed that MACC1 expression was an independent prognostic factor of patient overall survival (Table 2). Taken together, our data suggest that MACC1 might represent a potentially useful biomarker for the prognosis of cervical cancer patients.
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Discussion

The current study has revealed that MACC1 is up-regulated in cervical cancer tissues in comparison with that in normal cervical tissues. MACC1 protein expression levels were found to significantly correlate with the progression of cervical cancer, as high level of MACC1 protein expression in cervical cancer samples is closely associated with advanced FIGO stage and lymph nodes metastasis. Moreover, statistical analysis showed that patients with higher levels of MACC1 had poorer overall survival. Our study indicates that MACC1 might represent a novel predictive marker for the clinical prognosis of the disease.

The MET tyrosine kinase is a high-affinity cell surface receptor for hepatocyte growth factor (HGF), and is involved in the control of tissue homeostasis and development [13]. The HGF/c-Met signaling pathway has been implicated as a key regulator of many biological processes including cellular growth, epithelial-mesenchymal transition, angiogenesis, cell motility, invasiveness, and metastasis. While it could be regulated by several factors, such as metastasis-associated in colon cancer-1 [8]. Stein et al. identified MACC1 as a master regulator of the HGF/Met signaling pathway. Arlt et al. demonstrated a MACC1-driven positive feedback loop that upon HGF treatment, MACC1 protein translocates from the cytoplasm to the nucleus where it binds to the promoter of the Met gene and activates HGF/Met signaling [14]. Recently, several recent reports have shown that MACC1 expression levels are highly associated with tumor development and progression [15-17]. However, the molecular mechanism underlying the biological significance of MACC1 in cervical cancer progression remains obscure.

Our study has shown that MACC1 upregulation might play a pivotal role in the development of cervical cancer. Overexpression of MACC1 in cervical cancer was identified by the assessment of MACC1 mRNA expression in cervical cancer tissues in comparison with those in adjacent normal tissues. The importance of MACC1 upregulation in cervical cancer is further demonstrated by our finding of its correlation with the poorer overall survival in patients. Multivariate analysis revealed that MACC1 expression could be used as an independent prognostic factor of overall survival in cervical cancer patients. When performing multivariate analysis to evaluate the factors affecting recurrent-free survival, the role of MACC1 is no longer significant, probably due to lower sample size. Our study is consistent with previous studies in that MACC1 plays an indispensable oncogenic role in the tumor development. These results not only suggest a promising application of MACC1 as a prognostic indicator but also warrant further studies on a possible correlation between the biological functions of MACC1 and the carcinogenesis of cervical cancer.

Interestingly, our study implicated that MACC1 overexpression is associated with advanced FIGO stage and lymph nodes metastasis, while is not associated with age or differentiation in cervical cancer patients. In previous studies, MACC1 expression was found to be correlated with vascular invasive in hepatocellular carcinoma [18]. Moreover, it was recently reported that overexpression of MACC1 may predict development of metastases in stage II colon cancer [16]. An in vivo study by Zhang et al. discovered that knockdown of MACC1 by microRNA-143 reduces cell growth and invasion in colorectal cancer cells [15]. Our study further implies the crucial role of MACC1 in tumor metastasis.

In conclusion, our findings for the first time suggest that MACC1 may be useful as a prognostic

### Table 2. Multivariate Cox regression analysis of overall survival (OS) and recurrence-free survival (RFS) in patients with cervical cancer

<table>
<thead>
<tr>
<th>Prognostic variables</th>
<th>Hazard ratio (95% CI)</th>
<th>P</th>
<th>Hazard ratio (95% CI)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (&gt; 40 vs ≤ 40)</td>
<td>2.645 (0.780-8.970)</td>
<td>0.119</td>
<td>4.529 (1.147-17.74)</td>
<td>0.031</td>
</tr>
<tr>
<td>FIGO Stage (&gt; IB vs IB)</td>
<td>1.910 (0.295-3.463)</td>
<td>0.113</td>
<td>2.013 (0.593-6.781)</td>
<td>0.018</td>
</tr>
<tr>
<td>Differentiation (Grade 3 vs 1/2)</td>
<td>1.242 (0.394-3.914)</td>
<td>0.711</td>
<td>1.181 (0.360-3.870)</td>
<td>0.784</td>
</tr>
<tr>
<td>LN Metastasis (Yes vs. No)</td>
<td>1.765 (0.501-6.224)</td>
<td>0.031</td>
<td>1.180 (0.360-3.870)</td>
<td>0.05</td>
</tr>
<tr>
<td>MACC1 expression (High vs. Low)</td>
<td>3.020 (0.770-11.844)</td>
<td>0.043</td>
<td>2.725 (0.696-10.664)</td>
<td>0.150</td>
</tr>
</tbody>
</table>
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biomarker of cervical cancer. Further studies of the underlying molecular mechanism of MACC1 involvement in the progression of cervical cancer are needed. Investigation is also required to determine whether MACC1 could be used as a therapeutic target in cervical cancer therapies.

Disclosure of conflict of interest

None.

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References


