Serum ARID1A acts as a potential diagnostic biomarker for gastric cancer

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Abstract: Background: AT-rich interactive domain 1A gene (ARID1A) had been reported to act as a tumor suppressor in gastric cancer (GC), and reduced expression of ARID1A is significantly correlated with lymphatic invasion and lymph node metastasis. However, its diagnostic value in GC remains unknown. The aim of this study was to detect the expression of serum ARID1A and investigate its diagnostic value in GC. Methods: ARID1A mRNA and protein expression were assessed in 120 GC serum samples and 70 healthy control samples by quantitative real-time polymerase chain reaction (qRT-PCR) and ELISA, respectively. And the association between ARID1A expression and clinicopathologic parameters was evaluated by Chi-square test. To detect the diagnostic value of serum ARID1A, receiver operating characteristic (ROC) curve were established. Results: The expression of ARID1A was down-regulated in GC serum compared to that in healthy controls both at mRNA and protein level (P<0.001), and its low expression was closely related to tumor size, tumor grade, depth of invasion and lymph node metastasis. Besides, ARID1A expression in serum were significantly correlated with the expression of CA19-9 (P=-0.612, P<0.001) and CEA (P=-0.635, P<0.001) in GC. ROC curve showed the AUC of ARID1A for the diagnosis of GC was 0.846 which was higher than that of CA19-9 (0.626) and carcinoembryonic antigen (CEA) (0.706). Conclusions: ARID1A expression decreases in serum of GC patients, and it can be a useful marker for the diagnosis of GC to distinguish GC patients from noncancerous cohorts.

Keywords: ARID1A, gastric cancer, diagnosis, CEA, CA19-9

Introduction

Gastric cancer (GC) is the third most common type of cancers and the leading cause of cancer-associated mortality worldwide [1]. In China, there were about 300,000 deaths caused by GC each year [2]. The mechanism of GC involves a complex multi-factorial and multi-stage process that depends on interactions between gene and environment [3]. Currently, curative treatments and early diagnosis are associated with increased survival outcomes of GC patients. However, the majority of GC cases are diagnosed at later stages which lead the 5-year survival rate is only 11-40% [4]. Standard biomarkers such as carbohydrate antigen (CA) 724, CA19-9 and carcinoembryonic antigen (CEA) are useful for gastric cancer detection [5, 6], but the utility of these tumor markers are limited due to low sensitivity and specificity [7]. Therefore, new available biomarkers for the diagnosis of gastric cancer need to be detected.

AT-rich interactive domain-containing protein 1A (ARID1A), known as BAF250a, SMARCF1, or p270, belongs to a family of proteins containing a highly conserved about 100 amino acid DNA binding domain named ARID (AT-rich interacting domain) [8]. As a member of SWI/SNF complexes, ARID1A is encoded by the ARID1A gene which is located on chromosome 1p36 [9], and contribute to specific recruitment of its chromatin remodeling activity by binding transcription factors and transcriptional coactivator/corepressor complexes [10]. The down-regulation of ARID1A can result in dysfunction of the SWI/SNF complex, and inhibit transcription of various genes. In addition, ARID1A has shown to be involved in protein-protein interactions with sequence motif of LXXLL and cellular development, differentiation and proliferation [11, 12].
Diagnostic value of ARID1A in GC

Usually, ARID1A play a novel tumor suppressor role in many different cancers such as hepatocellular carcinoma and esophageal squamous cell carcinoma [13, 14]. However, its role in the diagnosis of GC remains unknown.

In this study, we detected the expression of ARID1A in 120 serum samples of GC and 70 healthy controls by qRT-PCR and ELISA, respectively. Moreover, its relationship with clinical factors of GC was analyzed. What’s more, the diagnostic value of ARID1A in GC was estimated.

Materials and methods

Patients and specimens

The study was conducted at Wuhan University and approved by the Ethics Committee of the Wuhan University. 120 patients who were diagnosed with GC were collected during 2012 to 2014. None of them had received any radio- or chemo-therapy before sampling. Besides, 70 healthy people were recruited as healthy controls. All participants signed written informed consents in advance.

5 ml blood from each sample was obtained for serum isolation. All blood samples were stranded for 1 h and centrifuged for 10 min at 3000 rpm. Then they obtained serum samples were stored at -80°C till use, respectively. In addition, the clinical parameters of each patients were recorded in a database.

RNA extraction and qRT-PCR analysis

Total RNA was isolated from the serum samples with TRizol LS reagent (Invitrogen, San Diego, CA, USA) according to the manufacturer’s instructions, respectively. Only those samples of RNA with an OD A260/A280 ratio close to a value of 2.0 were available. The first chain of cDNA was synthesized using TaqMan MicroRNA Reverse Transcription Kit (Applied Biosystems, Foster City, CA, USA). RT-PCR reaction was conducted in 7300 Real-Time PCR system (Applied Biosystems) according to the manufacturer’s protocol. GAPDH was taken as internal control. The sequences of primers for ARID1A and GAPDH were as follows: ARID1A: forward-5’-CTTCAACCTCAGCTGCCTCCA-3’ and reverse-5’-GGTCACCCACCTCATA-CTCCTTT-3’; GAPDH: forward-5’-CTCCTCCTGTTCGACAGTCGC-3’ and reverse-5’-CCCAATACGACCAAATCCGT-3’. All reactions were performed in triplicate. Relative mRNA expression of ARID1A was calculated using the comparative C_{T} (2^{-ΔΔC_{T}}) method.

ELISA analysis

Total protein was isolated from the serum of patients with GC and healthy controls. Then the serum ARID1A, CEA and CA19-9 were measured by ELISA using ELISA kit (manufactured by Panomics Inc.) according to the manufacturer’s instructions.

Statistical analysis

The statistical analyses were performed with software of SPSS version 13.0 (SPSS Inc, Chicago, IL, USA), and all data were presented as the mean ± standard deviation (SD). Stu-
Diagnostic value of ARID1A in GC

Results

Down-regulation of serum ARID1A was found in GC

Normalized to GAPDH, the serum ARID1A mRNA expression was determined by qRT-PCR assays in 120 GC serum samples and 50 healthy controls. As results showed that, the relative mRNA expression of serum ARID1A in GC was significantly decreased compared to that in normal healthy specimens (3.78±1.17 vs. 5.79±0.87, P<0.001, Figure 1). Meanwhile, the protein expression of serum ARID1A was measured by ELISA analysis. The result demonstrated that protein expression of serum ARID1A was also distinctively lower in patients with GC than that in healthy controls (Figure 2, 1.99±0.61 vs. 3.15±0.49, P<0.001).

Relationship between ARID1A expression and clinicopathological features in patients with GC

To explore whether ARID1A was involved in the development of GC, we analyzed its relationship with clinicopathological features. As shown in Table 1, low ARID1A expression in GC was significantly associated with tumor size (P=0.032), tumor grade (P=0.003), depth of invasion (P=0.009) and lymph node metastasis (P=0.008). Concretely, lower ARID1A expression was more frequently detected in GC with tumor size (≥3 cm), advanced depth of tumor invasion and positive lymph node metastasis. However, there were no association of ARID1A expression with and age (P=0.232) and gender (P=0.197).

Diagnostic value of ARID1A in GC

ROC curve was constructed to determine the ability of serum ARID1A in discriminating GC patients from normal cohorts. Additionally, the diagnostic value of CA19-9 and CEA were both evaluated respectively, too. As shown in Figure 3, the AUC of CA19-9 and CEA was severally 0.626 and 0.706 while that of ARID1A was 0.846. This indicated that the diagnostic value of ARID1A was higher than that of CA19-9 and CEA. Besides, ARID1A has a high sensitivity and specificity (80% and 77%). Furthermore, multivariable logistic regression analysis revealed that serum ARID1A could be a potential reliable diagnostic biomarker for patients with GC (OR=3.265, 95% CI=1.597-6.675, P<0.001, Table 2).

Correlation between serum ARID1A expression and CEA or CA19-9 expression

Next, we explored the relationship between ARID1A expression and the expression of CEA or CA19-9 in GC samples. Spearman’s correlation analysis revealed that ARID1A expression in serum were significantly correlated with that of CEA (P=0.635, P<0.001, Figure 4A) and CA19-9 (P=0.612, P<0.001, Figure 4B).

Table 1. Association of ARID1A expression with clinicopathological parameters in GC patients

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Cases (n=120)</th>
<th>Serum ARID1A expression (Mean ± SD)</th>
<th>P</th>
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<tbody>
<tr>
<td>Age</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>≥60</td>
<td>65</td>
<td>3.68±0.54</td>
<td>0.232</td>
</tr>
<tr>
<td>&lt;60</td>
<td>55</td>
<td>3.89±0.46</td>
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<tr>
<td>Gender</td>
<td></td>
<td></td>
<td>0.197</td>
</tr>
<tr>
<td>Female</td>
<td>52</td>
<td>3.80±0.37</td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>68</td>
<td>3.76±0.29</td>
<td></td>
</tr>
<tr>
<td>Tumor size</td>
<td></td>
<td></td>
<td>0.032</td>
</tr>
<tr>
<td>≥3 cm</td>
<td>69</td>
<td>3.47±0.44</td>
<td></td>
</tr>
<tr>
<td>&lt;3 cm</td>
<td>51</td>
<td>4.20±0.25</td>
<td></td>
</tr>
<tr>
<td>Depth of invasion</td>
<td></td>
<td></td>
<td>0.009</td>
</tr>
<tr>
<td>Early</td>
<td>56</td>
<td>3.91±0.31</td>
<td></td>
</tr>
<tr>
<td>Advanced</td>
<td>64</td>
<td>3.67±0.41</td>
<td></td>
</tr>
<tr>
<td>Tumor grade</td>
<td></td>
<td></td>
<td>0.003</td>
</tr>
<tr>
<td>1, 2</td>
<td>60</td>
<td>3.84±0.36</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>60</td>
<td>3.72±0.51</td>
<td></td>
</tr>
<tr>
<td>Lymph node metastasis</td>
<td></td>
<td></td>
<td>0.008</td>
</tr>
<tr>
<td>Positive</td>
<td>50</td>
<td>3.74±0.29</td>
<td></td>
</tr>
<tr>
<td>Negative</td>
<td>70</td>
<td>3.81±0.41</td>
<td></td>
</tr>
</tbody>
</table>

Table 1. Association of ARID1A expression with clinicopathological parameters in GC patients

P<0.05 was considered to be statistically significant.
Diagnostic value of ARID1A in GC

The high incidence and mortality rates of GC were influenced by many factors such as ineffective screening, diagnosis, and treatment approaches [15, 16]. Despite molecular many markers of early diagnosis such as special genes, IncRNAs and miRNAs have been confirmed for GC detection in previous studies, the low sensitivity and specificity still made the diagnosis of GC inaccurate and not in time [17]. Thus, more effective biomarkers for GC early diagnosis are urgently needed.

ARID1A usually plays an important role in many cancers development with alterations in ARID1A gene or dysregulation of ARID1A expression. During the past researches, ARID1A gene has been classified as a novel tumor suppressor with loss expression in endometrial carcinomas, lung cancers and renal cancers [18-20]. Specifically, ARID1A could modulate cell-cycle-related genes, such as CDKN1A (p21), SMAD3, C-MYC and E2F to suppress tumor cellular proliferation [21-23]. Moreover, reduced ARID1A expression was considered to be caused by ARID1A frame shift and nonsense mutations. Currently, mutations of ARID1A were also found in various of cancers such as colon and rectal cancer, bladder cancer, pancreatic cancer, renal carcinoma, Barrett’s esophagus, large B-cell lymphoma, endometrial tumors [24-30]. Concretely, these mutations could lead to mRNA decay, protein folding incorrectly or functional defects. In GC, down-regulation expression of ARID1A was verified to be significantly associated with lymphatic invasion and lymph node metastasis [31, 32], and this expression pattern could enhance gastric cancer cell migration and invasion via downregulation of E-cadherin transcription [33]. Functional studies revealed that the reduced expression of ARID1A was associated with poor prognosis in primary GC or in GC [34, 35]. However, whether ARID1A could be a diagnostic biomarker for GC has not been reported so far.

In the present study, our results indicated that ARID1A expression was significantly reduced in GC serum samples compared to that in normal healthy controls, which was consistent with previously published reports [35]. This might reveal that ARID1A could be a tumor suppressor in GC. And the reduced ARID1A expression was associated with larger tumor, advanced depth of tumor invasion and positive lymph node

![Figure 3. Diagnostic significance of ARID1A, CEA and CA19-9 in GC. A. ROC curve showed the performance of serum ARID1A expression in discriminating GC patients from normal healthy controls. B, C. ROC curve of CEA, CA-199 in the serum of the gastric cancer patients and healthy controls.]

Table 2. Multivariable logistic regression analyses for estimating the diagnostic value of serum ARID1A in GC

<table>
<thead>
<tr>
<th>Parameters</th>
<th>OR (95% CI)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, ≥60 vs. &lt;60</td>
<td>1.634 (0.799-3.341)</td>
<td>0.178</td>
</tr>
<tr>
<td>Gender, female vs. male</td>
<td>1.015 (0.495-3.341)</td>
<td>2.084</td>
</tr>
<tr>
<td>Serum ARID1A, ≥3.78 vs. &lt;3.78</td>
<td>3.265 (1.597-6.675)</td>
<td>0.001</td>
</tr>
</tbody>
</table>
Diagnostic value of ARID1A in GC

Figure 4. Correlation of the ARID1A with CEA (A) and CA19-9 (B) in GC patients.

Then we further explored the diagnostic value of ARID1A. ROC curves and multivariable logistic regression analyses manifested that serum ARID1A could distinguish GC patients from normal cohorts and could be used as a potential reliable biomarker for the clinical diagnosis of GC, which has not been reported so far. On the basis of previous studies, we further observed the predictive values of CEA and CA19-9, and found ARID1A had a more accurately diagnostic value. As classical biomarkers, CEA and CA19-9 have previously been the commonly used markers in GC, however, they could be detected in different types of carcinoma with exhibiting low specificity and sensitivity [17]. Additionally, we determined the first study to examine the correlation of expression between ARID1A and CA19-9 or CEA. The results showed that with upregulation of CA19-9 or CEA expression, ARID1A expression was significantly decreased. But whether CA19-9 or CEA attenuated ARID1A expression in GC serum, our studies were no involved.

In summary, our findings confirm that ARID1A expression is decreased in GC, and it can be a potential diagnostic biomarker for GC. However, it is still necessary to detect new evidence for the diagnostic value of ARID1A in GC.

Acknowledgements

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Disclosure of conflict of interest

None.

References

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Diagnostic value of ARID1A in GC


