Introduction

Colorectal cancer (CRC) is one of the leading causes of cancer mortality in the western world, and over 1 million individuals are diagnosed as CRC each year [1-3]. Surgical resection is currently highly effective for localized CRC, whereas many CRC patients still die of recurrence or tumor metastasis in the advanced stage. The overall survival (OS) rate for advanced or metastatic CRC has remained nearly unchanged over the last two decades, with the 5-year-OS of 15% while 90% for the early-stage CRCs [4]. The treatment and prognosis of CRC patients has not changed dramatically. The continued discovery and identification of novel biomarkers that are sensitive and specific for CRC is urgently needed, and will improve prevention and treatment of CRCs.

Tudor-SN protein (SND1), also known as p100, is a highly conserved protein from yeast to humans and is ubiquitously expressed. SND1 was initially identified as a factor interacting with the Epstein-Barr virus nuclear antigen 2 (EBNA2), and promotes EBNA2-dependent transcription [5,6]. Now it is mainly known as a transcriptional co-activator since its interaction with other transcription factors such as c-Myb and STAT6 [7-9]. SND1 is now recognized as a multifunctional protein modulating gene transcription, mRNA splicing, mRNA stability, mRNA translation and RNA interference (RNAi) function [10-13]. Studies have suggested critical roles of SND1 in human cancers. The up-regulation of SND1 has been reported in some types of human malignancies, including breast, prostate and hepatocellular carcinoma [14-16]. As for CRC, there are several reports that implicate the up-regulation and implication in early colon carcinogenesis [17,18]. However, much still remains unclear regarding its clinical significance.

Though SND1 could promote tumor progression through regulating calcium binding proteins, signaling pathways, non-coding RNAs or hormones in different types of other cancer [19-22], little is known about the exact mechanism of SND1 in CRC. In the present study, we first conducted the SND1 mRNA and protein expression levels...
in human CRCs and their corresponding non-cancerous colon tissues. The association of SND1 expression with the pathological characters was analyzed and its prognostic value in CRC was evaluated according to the Kaplan-Meier method. We provided evidence that SND1 might be used as a novel CRC biomarker because of its predictive significance among CRC patients.

2 Materials and Methods

2.1 Patients and Clinical Samples

Surgical samples of human colorectal cancers and paired noncancerous colon tissues were obtained from 42 patients treated at the Renmin Hospital of Wuhan University. 

Informed consent: Informed consent has been obtained from all individuals included in this study.

Ethical approval: The research related to human use has been complied with all the relevant national regulations, institutional policies and in accordance the tenets of the Helsinki Declaration, and has been approved by the authors’ institutional review board or equivalent committee.

All samples were immediately frozen in liquid nitrogen and stored at -80°C until use. The clinical and pathological characteristics of patients are shown in Table 1. Quantitative real-time PCR (qRT-PCR)

Total RNA was isolated from about 50mg of surgical samples by Trizol reagent (Life Technology, Carlsbad, CA, USA) according to the manufacturer’s instructions. Reverse transcription was performed by using oligo(dT)12-18 (Takara, Tokyo, Japan). Primer sequences for qRT-PCR amplification were as follows:

SND1:
F: 5'-GTGGACAGCGTAGTTCGGGA-3';
R: 5'-CCCACGAGACATTTCCACAC-3';
b-actin:
F: 5'-GACTTCGAGCAAGAGATGGC-3';
R: 5'-AGGAAGGAAGGCTGGAAGAG-3'.

Quantitative RT-PCR was performed in a total of 10ml of reaction mixture by ABI 7900 system (Applied Biosystems, Foster City, CA, USA) using SYBR Premix Ex Taq II reagent (Takara, Tokyo, Japan). b-actin were used as internal controls.

2.2 Western Blotting

About 50mg of each tissue was prepared in RIPA buffer (20 mM Tris, 150mM NaCl, 1% Triton X-100, sodium pyrophosphate, b-glycerophosphate, EDTA, Na3VO4, leupeptin). The concentrations of proteins were determined by BCA methods by using BCA protein assay kit (Beyotime Institute of Biotechnology, Haimen, Jiangsu, China). Lysates were separated on SDS-PAGE by using Bio-Rad Mini-Protean Tetra Electrophoresis System and transferred to nitrocellulose membranes (GE Healthcare Bio-Science). After blocking with 5% milk in PBS for 1h at room temperature, the membranes were probed with the anti-SND1 (clone C-9, 1:1000; Santa Cruz, Dallas, TX, USA) of a-tubulin antibodies (clone B-7, 1:3000; Santa Cruz, Dallas, TX, USA) at a 1:1000 dilution in 5% BSA at 4°C overnight. After washing, the membranes were probed with HRP-conjugated anti-mouse IgG secondary antibodies (Santa Cruz, Dallas, TX, USA) for 1h. The blots were detected by enhanced chemiluminescence plus reagent (GE Healthcare Bio-Science).

2.3 Statistical Analysis

Statistical analyses were carried out by paired or grouped t-test using GraphPad Prism 5 software (GraphPad Software, La Jolla, CA, USA). Protein quantification was performed by ImageJ software. Correlation between SND1 mRNA expression and clinicopathological features was performed by χ2 test using SAS v9.2. Kaplan-Meier survival analysis was performed using a logrank test with clinical data. A P<0.05 was considered as statistically significant.

3 Results

3.1 Expression of SND1 mRNA in human CRCs and non-cancerous colon tissues

In order to investigate the expression of SND1 in CRC, real-time PCR using SND1-specific primers were performed in a total of 42 pairs of CRCs with their adjacent non-cancerous colon tissues. The results shown in Figure 1A indicate that SND1 mRNA was significantly increased in the CRC samples when compared to controls (P<0.01). Based on the features of these CRCs, subgroups including different tumor grades (I+II, III+IV) and various lymph node metastasis status were classified. The SND1 mRNA expression distribution in these subgroups was analyzed. As shown in Figure 1B, SND1 mRNA showed increased expressed in CRCs at the advanced tumor grades (III+IV). It was also more highly up-regulated in metastatic CRCs than in localized CRCs (Figure 1C). These results strongly indicate the oncogenic roles of SND1 in CRC.
3.2 SND1 protein expression in paired CRC tissues

Having confirmed the up-regulation of SND1 mRNA in CRC tissues, we next carried out western blotting experiments to further analyze the expression of SND1 protein in a series of random selected six paired CRC tissues. As shown in Figure 2A and Figure 2B, SND1 (100kDa) protein levels were significantly increased in CRCs compared to their paired noncancerous colon tissues. a-tubulin (55kDa) was used as an internal control. Together with the findings shown in Figure 1, we verified that SND1 mRNA and protein expression exhibited up-regulation in CRCs, consistent with previously published reports [17, 18].

3.3 Association of SND1 expression with various pathological characters of CRC

To determine the clinical significance of SND1 in CRCs, the associations between SND1 expression and the individual clinic pathologic feature of CRC patients were analyzed. As shown in Table 1, higher SND1 expression was positively correlated with aggressive nodal status (N1+N2, \( P=0.0261 \)), poor differentiation (\( P=0.0452 \)) and advanced tumor grades (\( P=0.0219 \)). No significant correlations between SND1 expression and other pathologic features (such as age, sex and T stage, \( P>0.05 \)) were found.
Figure 2. SND1 protein is highly up-regulated in colon cancer samples. (A) 6 pairs of human colorectal cancers surgical samples were subjected to western blotting. All 6 cases (100%) showed up-regulation compared with paired noncancerous normal colon tissues. (B) A quantitative analysis was carried out by ImageJ software.

Table 1. Correlation between clinicopathological features and SND1 expression.

<table>
<thead>
<tr>
<th>Clinicopathological Features</th>
<th>No. of Samples</th>
<th>Non-upregulated SND1</th>
<th>Upregulated SND1</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;60</td>
<td>15</td>
<td>8</td>
<td>7</td>
<td>0.7474</td>
</tr>
<tr>
<td>≥60</td>
<td>27</td>
<td>13</td>
<td>14</td>
<td></td>
</tr>
<tr>
<td>Gender</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Female</td>
<td>23</td>
<td>14</td>
<td>9</td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>19</td>
<td>7</td>
<td>12</td>
<td>0.1211</td>
</tr>
<tr>
<td>T stage</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>T1</td>
<td>2</td>
<td>2</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>T2</td>
<td>23</td>
<td>11</td>
<td>12</td>
<td></td>
</tr>
<tr>
<td>T3</td>
<td>17</td>
<td>8</td>
<td>9</td>
<td></td>
</tr>
<tr>
<td>T4</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0.5904</td>
</tr>
<tr>
<td>LN metastasis</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>N0</td>
<td>26</td>
<td>17</td>
<td>9</td>
<td></td>
</tr>
<tr>
<td>N1+N2</td>
<td>16</td>
<td>4</td>
<td>12</td>
<td>0.0261*</td>
</tr>
<tr>
<td>Tumor Grade</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>I+II</td>
<td>28</td>
<td>18</td>
<td>10</td>
<td></td>
</tr>
<tr>
<td>III+IV</td>
<td>14</td>
<td>3</td>
<td>11</td>
<td>0.0219*</td>
</tr>
<tr>
<td>Differentiation</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Well+ Moderately</td>
<td>29</td>
<td>18</td>
<td>11</td>
<td></td>
</tr>
<tr>
<td>Poorly</td>
<td>13</td>
<td>3</td>
<td>10</td>
<td>0.0452*</td>
</tr>
</tbody>
</table>

NOTE. *: P<0.05 by c2 or Continuity Adj. c2 test.
3.4 Increased SND1 expression correlates with poor CRC patient survival

Finally, to determine whether increased SND1 expression in CRC patients correlated with poorer prognosis, Kaplan-Meier survival curves were plotted using the practical 5-year overall survival (OS) resources to compare the survival rates of higher SND1 expressing CRC patients with lower SND1 expressing CRC patients. As shown in Figure 3, the data revealed that among two independent cohorts of CRC patients, the OS in the higher SND1 group was positively correlated with lower OS rate (HR=0.3428). The 5-year overall cumulative survival rate in patients with low SND1 expression was 63%, compared to 39% in those with high SND1 expression.

4 Discussion

In the present study, we confirmed that SND1 was up-regulated in CRC tissues at both the mRNA and protein levels. Notably, we found that higher SND1 mRNA expression was observed in advanced tumor grades and metastatic tumors. According to the clinical pathological characteristics, we found a positive correlation between high SND1 expression and poor differentiation, aggressive nodal status and advanced tumor grades. Recent research has demonstrated that SND1 is a poor prognostic factor in gliomas [24]. In our research focused on CRC, the results indicated that higher SND1 expression correlated with worse OS rate among CRC patients. Our study suggested the crucial clinical value of this molecule in prognosis prediction of CRC.

We speculated that overexpression of SND1 may have important roles in CRC carcinogenesis. In other types of human cancers such as non-small cell lung cancer, SND1 was found to induce chemoresistance by regulating S100A11 [19]. In hepatocellular carcinoma, it has been found that 1) SND1 could affect SMMC-7721 cells proliferation by regulating IGFBP3 expression and IGF signaling pathway [20]; 2) SND1 promotes tumor angiogenesis in human hepatocellular carcinoma through pathways that involves nuclear factor κB and miR-221 [21]; and 3) SND1 overexpression deregulates cholesterol homeostasis [22]. Of the few studies that have investigated the role of SND1 in CRC, one paper found that SND1 may contribute to the posttranscriptional regulation of key players in colon cancer development, including APC and β-catenin [18]. Accordingly, much more research is necessary to more fully understand role and mechanisms of SND1 in CRC. Future studies may begin by investigating the roles of SND1 as a transcription co-activator RNA processing factor, and protein and RNAs.

It has been demonstrated that SND1 mRNA shows remarkable up-regulation in human colon cancer tissues, even in early-stage lesions, and also in colon cancer cell lines [18]. Determining the mechanisms of its up-regulation may further help characterize its role in CRC. Currently, some reports have established that microRNA-320a (miR-320a) and miR-361-5p were the upstream regulator of SND1 by targeting to its 3'-UTR, thus leading to its
mRNA degradation in glioma and colon/gastric cancer, respectively [12,24]. Other mechanisms involving non-coding RNAs and/or transcription factors may likely be responsible for the up-regulation of SND1 in CRC.

In conclusion, we report here that the up-regulation of SND1 may be used as a novel biomarker to determine CRC prognosis.

Conflict of interest: Authors state no conflict of interest

References