Expression of ATP1AL1, a Non-Gastric Proton Pump, in Human Colorectum

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Abstract: The expression of mRNAs encoding a human nongastric proton pump (ATP1AL1) in the colorectum was investigated. The real-time PCR gave significant levels of signals not only in the distal part of human colon and rectum, but also in the proximal part of the colon. ATP1AL1 mRNA was overexpressed in 12 out of 20 human colorectal adenocarcinomas compared with the level in the accompanying normal mucosa. It is noted that astonishing levels of the mRNA overexpression were found in 4 carcinomas, which were detected even by Northern blot. The very high levels of ATP1AL1 mRNA expression in some cancer tissues may be connected to an unknown specific pathophysiological condition.

Key words: ATP1AL1, colon, cancer.

The K⁺-dependent ATPase (X⁺,K⁺-ATPase) gene family is divided into three subgroups, including the Na⁺,K⁺-ATPase, which is mainly involved in cellular homeostasis; the gastric H⁺,K⁺-ATPase, which is involved in gastric fluid acidification; and the nongastric H⁺,K⁺-ATPases, for which the identification of physiological roles is still in its infancy [1]. Complementary DNAs (cDNAs) for mammalian nongastric H⁺,K⁺-ATPases of rat [2], guinea pig [3], rabbit [4], and human [5] have been cloned. Nongastric H⁺,K⁺-ATPases of rat and guinea pig are highly localized in the distal colon, but not in the proximal colon [6–9], and these may be associated with K⁺ conservation in the colon [10, 11].

So far, the distribution of the human nongastric H⁺,K⁺-ATPase (ATP1AL1) in the colorectal tissues has not been clarified in detail. Furthermore, the physiological role of ATP1AL1 in human colon remains unclear. Here we investigated the distribution of ATP1AL1 mRNA in human tissues and the relation between the expression of ATP1AL1 mRNA and colorectal cancer.

Methods

Human colorectal carcinoma specimens were obtained from surgical resections of Japanese patients in accordance with the recommendations of the Declaration of Helsinki. Informed consents were obtained from all patients at Toyama Medical and Pharmaceutical University Hospital. In all cases, the control specimens were collected from the accompanying normal mucosa (epithelial layer), which were 5–10 cm from the carcinoma. The epithelial layer was carefully separated with scissors and pincettes. Blood vessels around the cancer tissues were carefully removed. Clinical and histological classifications according to the general rules edited by the Japanese Research Society for Cancer of the Colon and Rectum were carried out independently by three expert pathologists. Human normal gastric mucosa was obtained from sur-
gical resection of Japanese patients with stomach cancer.

Rat colonic mucosa was obtained from male Sprague-Dawley rats (weighing 200–250 g [7 weeks old] and 640–690 g [7 months old]; Japan SLC, Shizuoka, Japan) as previously described [12, 13]. The animals had free access to water and food until the day of the experiment. They were killed rapidly by stunning and cervical dislocation. All procedures were performed in accordance with the guidelines presented by the Animal Care and Use Committee of Toyama Medical and Pharmaceutical University.

Total RNAs from the human and rat tissues were prepared as described elsewhere [14, 15], and 10 μg of total RNA was separated on 1% agarose/formamide gel and transferred onto a nylon membrane (Zeta-probe GT, Bio-Rad, Hercules, CA, USA). The ready-to-use multiple tissue Northern blots (1 μg of poly A+ per each lane) were obtained from Clontech Laboratories (Palo Alto, CA, USA). PCR (polymerase chain reaction) products of ATP1AL1 (572 bp: nucleotide positions 1868–2439 and 340 bp: nucleotide positions 3033–3372), Na+,K+-ATPase (560 bp: nucleotide positions 9–568) and glyceraldehyde-3-phosphate dehydrogenase (GAPDH; 493 bp: nucleotide positions 449–941) were subcloned and sequenced. A Northern blot analysis with the 32P-labeled cDNA probe was performed as previously described [14, 15]. For the detection of rat nongastric H+,K+-ATPase, the membrane was hybridized with a 32P-labeled 572 bp ATP1AL1 fragment, which is 86% identical to that of rat nongastric H+,K+-ATPase. For the detection of ATP1AL1, the membrane was hybridized with a 340 bp ATP1AL1 fragment (3′-noncoding region), which has no cross reactivity with mRNAs of other X+,K+-ATPases. All blots were exposed to the Imaging Plate (Fuji Film, Tokyo, Japan) for 6 h (GAPDH) or 2–3 d (rat nongastric H+,K+-ATPase, ATP1AL1, and Na+,K+-ATPase). The amount of ATP1AL1 was quantified by real-time PCR (TaqMan assay) with an ABI PRISM 7700 sequence detector. In the assay, ATP1AL1-specific forward primer (nucleotide positions 1212–1233: 5′-CCATCTGTTGGTTCGACAAT-CAG-3′), the reverse primer (nucleotide positions 1324–1347: 5′-CTTGAACCTCTGCCTTGTTACA-A-3′), and the TaqMan fluorescent probe (nucleotide positions 1244–1271: 5′-CTGACACCGTGAGGAGCCATTCAACCCA-3′) were used. An amount of GAPDH was measured as control.

Results and Discussion

First, we checked the distribution of nongastric H+,K+-ATPase mRNA in the rat colon by Northern blot analysis. Rat colonic mucosa (12–15 cm long) was divided into three parts (4–5 cm lengths of proximal, middle, and distal parts). Figure 1A shows that nongastric H+,K+-ATPase mRNA of 3.8 kb is highly expressed in the distal colon, but very weakly in the proximal colon. In fact the enzyme activity of H+,K+-ATPase in the rat distal colon was much higher than in the proximal colon [6], and the immunoreactivity of the anti-nongastric H+,K+-ATPase antibody was detected in apical membranes of the rat distal colon, but not in those of the proximal colon [7, 8]. In Fig. 1B, the expression pattern of nongastric H+,K+-ATPase mRNA in the colon of 7-week-old rats was essentially the same as that in A. Three 7-month-old animals and three 7-week-old animals were used.

Northern blot analysis. Rat colonic mucosa (12–15 cm long) was divided into three parts (4–5 cm lengths of proximal, middle, and distal parts). Figure 1A shows that nongastric H+,K+-ATPase mRNA of 3.8 kb is highly expressed in the distal colon, but very weakly in the proximal colon. In fact the enzyme activity of H+,K+-ATPase in the rat distal colon was much higher than in the proximal colon [6], and the immunoreactivity of the anti-nongastric H+,K+-ATPase antibody was detected in apical membranes of the rat distal colon, but not in those of the proximal colon [7, 8]. In Fig. 1B, the expression pattern of nongastric H+,K+-ATPase mRNA in the colon of 7-week-old rats was essentially the same as that in 7-month-old rats, suggesting that the aging has no effect on an expression of the nongastric H+,K+-ATPase.

Next, the distribution of ATP1AL1 mRNA in human tissues was examined by Northern blot analysis. So far, ATP1AL1 mRNA in several particular tissues and cells has been detected with RT-PCR (reverse transcription–polymerase chain reaction) [16–18], but the result of Northern blot analysis for determining the size of ATP1AL1 mRNA has not been reported. Figure 2A shows that ATP1AL1 mRNA of 4.0 kb is significantly expressed in kidney, but other tissues, in-
including several parts of the colon, give no positive signal of 4.0 kb. An expression of Na⁺,K⁺-ATPase mRNA of 3.9 kb was observed in all the tissues tested (Fig. 2A).

Using real-time PCR (TaqMan assay), we further checked the expression of ATP1AL1 mRNA in the human normal colonic mucosa by real-time PCR (TaqMan assay). The normal mucosa was obtained from the patients with colorectal cancer as described in Methods. The ages (years; mean±SE) and sex (M, male–F, female) of patients are 72±3, 5M–3F (n=8; ascending colon), 67±2, 6M–1F (n=7; transverse colon), 68±4, 3M–6F (n=9; sigmoid colon) and, 68±3, 8M–6F (n=14; rectum). In each, the arbitrary value for the amount of ATP1AL1 mRNA was divided by that of GAPDH mRNA. For the negative control experiment, normal parts of gastric mucosa from 3 patients with stomach cancer (70±3 years, 1M–2F) were used. As a positive control, human kidney mRNA (Clontech Laboratories) was used.

The expression levels of ATP1AL1 mRNA in the normal human colon seem to be less significant than those of rat nongastric H⁺,K⁺-ATPase mRNA, which is abundantly expressed in the distal colon. We therefore speculate that ATP1AL1 may not be a counterpart of rat nongastric H⁺,K⁺-ATPase, though the amino acid sequence of ATP1AL1 is 86% identical to that of rat nongastric H⁺,K⁺-ATPase [1].

To examine the amount of expression of ATP1AL1 mRNA in colorectal carcinomas, we obtained human tissues of colorectal well-differentiated adenocarcinomas and their accompanying normal mucosa from 20 patients. It is interesting that the real-time PCR showed that ATP1AL1 mRNA was overexpressed in...
Table 1. Clinical information of colorectal well-differentiated adenocarcinomas.

<table>
<thead>
<tr>
<th>No.</th>
<th>Age (years)</th>
<th>Sex</th>
<th>Location</th>
<th>Size (cm)</th>
<th>Stage</th>
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</thead>
<tbody>
<tr>
<td>1</td>
<td>85</td>
<td>M</td>
<td>C</td>
<td>5.0×4.5</td>
<td>II</td>
</tr>
<tr>
<td>2</td>
<td>73</td>
<td>F</td>
<td>C</td>
<td>2.5×1.5</td>
<td>0</td>
</tr>
<tr>
<td>3</td>
<td>61</td>
<td>M</td>
<td>A</td>
<td>2.8×4.8</td>
<td>0</td>
</tr>
<tr>
<td>4</td>
<td>59</td>
<td>M</td>
<td>T</td>
<td>3.0×4.0</td>
<td>IV</td>
</tr>
<tr>
<td>5</td>
<td>68</td>
<td>M</td>
<td>T</td>
<td>4.5×3.5</td>
<td>IV</td>
</tr>
<tr>
<td>6</td>
<td>67</td>
<td>F</td>
<td>T</td>
<td>5.5×4.4</td>
<td>IV</td>
</tr>
<tr>
<td>7</td>
<td>67</td>
<td>M</td>
<td>T</td>
<td>1.4×0.8</td>
<td>I</td>
</tr>
<tr>
<td>8</td>
<td>75</td>
<td>F</td>
<td>D</td>
<td>5.5×4.0</td>
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</tr>
<tr>
<td>9</td>
<td>55</td>
<td>F</td>
<td>S</td>
<td>5.0×2.0</td>
<td>I</td>
</tr>
<tr>
<td>10</td>
<td>65</td>
<td>F</td>
<td>S</td>
<td>2.5×2.0</td>
<td>II</td>
</tr>
</tbody>
</table>

Age (years) and sex (M, male; F, female) of the patient, the size (cm × cm) and location of the carcinoma (C, cecum; A, ascending colon; T, transverse colon; D, descending colon; S, sigmoid colon; R, rectum), and stage of the carcinoma according to the TNM clinical classification by the International Union Against Cancer are shown.
12 out of 20 carcinomas compared with the level in normal mucosa (Fig. 3A left and B). The significant signals of ATP1AL1 mRNA of 4.0 kb were clearly observed in several carcinomas even by Northern blot (Fig. 3C). The overexpression of ATP1AL1 mRNA was not due to a reflection of stimulated angiogenesis (increased number of blood cells) in the carcinoma, because no significant expression of ATP1AL1 was observed in peripheral leukocytes and liver (Fig. 2A). The expression of ATP1AL1 mRNA was decreased in 8 out of 20 carcinomas (Fig. 3A right and B). The change (increase or decrease) in the mRNA level of ATP1AL1 does not seem to correlate with the age and sex of the patient, the location and size of the carcinoma, and the clinical stage (Table 1 and Fig. 3).

The pathophysiological function of ATP1AL1 in various colorectal carcinomas is unknown. Apparently, further study to clarify the mechanism of increase and decrease in the level of ATP1AL1 mRNA in the carcinoma is necessary. In summary, we found that ATP1AL1 mRNA is weakly but widely expressed in all the parts of normal colonic mucosa. The very high levels of ATP1AL1 mRNA expression in some cancer tissues may be connected to an unknown specific pathophysiological condition.

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REFERENCES