Elevated Expression of A3 Adenosine Receptors in Human Colorectal Cancer Is Reflected in Peripheral Blood Cells

Stefania Gessi,1 Elena Cattabriga,1 Arianna Avitabile,1 Roberta Gafa’,2 Giovanni Lanza,2 Luigi Cavazzini,2 Nicoletta Bianchi,3 Roberto Gambari,3 Carlo Feo,4 Alberto Liboni,4 Sergio Gullini,5 Edward Leung,6 Stephen Mac-Lennan,6 and Pier Andrea Borea1

1Department of Clinical and Experimental Medicine, Pharmacology Unit and Interdisciplinary Center for the Study of Inflammation and Departments of 2Experimental and Diagnostic Medicine, 3Biochemistry and Molecular Biology, 4Surgery, Anesthesiology, and Radiology, and 5Gastroenterology, St. Anna Hospital, University of Ferrara, Ferrara, Italy; and 6King Pharmaceuticals, Cary, North Carolina

ABSTRACT

Purpose: Adenosine is a ubiquitous nucleoside that accumulates at high levels in hypoxic regions of solid tumors, and A3 adenosine receptors have been recently demonstrated to play a pivotal role in the adenosine-mediated inhibition of tumor cell proliferation. In the present work, we addressed the question of the putative relevance of A3 subtypes in colorectal adenocarcinomas.

Experimental Design: Seventy-three paired samples of tumor and surrounding peritumoral normal mucosa at a distance of 2 and 10 cm from the tumor and blood samples obtained from a cohort of 30 patients with colorectal cancer were investigated to determine the presence of A3 receptors by means of binding, immunocytochemistry, and real-time reverse transcription-polymerase chain reaction studies.

Results: As measured by receptor binding assays, the density of A3 receptor was higher in colon carcinomas as compared with normal mucosa originating from the same individuals (P < 0.05). Overexpression of A3 receptors at the protein level was confirmed by immunohistochemical studies, whereas no changes in A3 mRNA accumulation in tumors as compared with the corresponding normal tissue were revealed. The overexpression of A3 receptors in tumors was reflected in peripheral blood cells, where the density was approximately 3-fold higher compared with healthy subjects (P < 0.01). In a cohort of 10 patients studied longitudinally, expression of A3 receptors in circulating blood cells returned to normal after surgical resection for colorectal cancer.

Conclusions: This study provides the first evidence that A3 receptor plays a role in colon tumorigenesis and, more importantly, can potentially be used as a diagnostic marker or a therapeutic target for colon cancer.

INTRODUCTION

Adenosine, a ubiquitous nucleoside released from metabolically active or stressed cells, is known to act as an important regulatory molecule through its activation of cell surface receptors named A1, A2A, A2B, and A3, all of which belong to the G-protein–coupled superfamily of receptors (1). In particular, A1 and A3 inhibit adenylyl cyclase activity through G proteins, whereas A2A and A2B stimulate this enzyme via G proteins (2). Collectively, these receptors are widespread on virtually every organ and tissue and represent promising drug targets for pharmacological intervention in many pathophysiological conditions that are believed to be associated with changes of adenosine levels such as asthma, neurodegenerative disorders, chronic inflammatory diseases, and cancer (3). In solid tumors, chronic hypoxia is observed due to insufficient vascularization and limited diffusion of oxygen into the tissue (4). In this context, adenosine, derived from a decrease of cellular ATP, is released into the extracellular space and may have a significant influence on the vasculature, resistance to immune attack, and growth of tumor masses. The immunosuppressive and anti-inflammatory effects of adenosine, together with its angiogenic actions, strongly suggest that adenosine receptors could be involved in tumorigenesis (5–7). In addition, a number of studies have now reported a pivotal role of adenosine in cell cycle regulation, proliferation, and apoptosis in cells of both tumor (8–12) and nontumor origin (13). These effects depend on the extracellular concentration of adenosine, the expression of different adenosine receptor subtypes, and the signal transduction mechanisms activated after the binding of specific agonists. Several lines of evidence indicate the A3 receptor as the principle subtype responsible for adenosine-induced inhibition of tumor cell proliferation (11, 12, 14). Indeed, a dual effect in colon carcinoma-bearing mice, that is, the induction of antitumor activity concomitantly with a myeloprotective effect, has been demonstrated for A3 receptor activation. The antitumor activity was attributed to a direct antiproliferative effect, and myeloprotection was attributed to an indirect effect manifested by up-regulation of interleukin 12 and natural killer cell activity (15). In support of A3 receptor involvement in tumors, it has recently been shown that A3 receptors are highly expressed on the cell surface of tumor cells (16–19), but not in the majority of normal tissues (14). However, despite promising in vitro and animal...
studies concerning the A3-mediated reduction of tumor growth (20), convincing evidence of the presence of this adenosine subtype on solid tumors is absent. These observations constitute a rationale for studying the expression of A3 receptors in a very common and lethal malignancy such as colorectal cancer, which is the third leading cause of cancer deaths in the United States. Despite major advances in uncovering the basic biochemical and genetic alterations involved in the development and progression of colorectal cancers, treatment of this disease still relies predominantly on surgical resection. Moreover, patient prognosis is determined primarily by the stage of disease at the time of diagnosis (21). Thus, a better understanding of the molecular proteins involved in colorectal cancer cell proliferation would greatly facilitate both the discovery of new possible diagnostic and prognostic markers and the development of novel therapeutic agents. With this aim, the present study provides the first extensive analysis on the expression of A3 receptors in colon cancer tissue and normal colon mucosa, by means of binding, immunocytochemistry, and real-time reverse transcription-polymerase chain reaction (RT-PCR) experiments. Moreover, based on previous studies demonstrating that human circulating blood cells may reflect the same alterations occurring in tissues (22–24), we aimed to investigate whether changes in A3 receptor values in colon cancer tissues reflect receptor changes in neutrophils and lymphocytes of patients with colorectal cancer.

**MATERIALS AND METHODS**

**Reagents.** [3H]MRE 3008F20 [5-N-(4-methoxyphenylcarbamoyl)amino-8-propyl-2(2 furyl)-pyrazolo-[4,3-e]-1,2,4-triazolo[1,5-c]pyrimidine; specific activity, 67 Ci/mmol] was synthesized at Amersham International (Buckinghamshire, United Kingdom). The A3 antibody was purchased from Alpha Diagnostic (San Antonio, TX). TaqMan MGB probe and A3 primers were obtained from Applied Biosystems (Warrington Cheshire, United Kingdom). All other reagents were of analytical grade and obtained from commercial sources.

**Patients and Tissues.** All patients included in the study had procedures performed at the St. Anna Ferrara University Hospital between September 2001 and November 2002. The protocol was approved by the local ethics committee, and informed consent was obtained from each patient. Seventy-seven subjects who underwent surgical resection for colorectal adenocarcinomas (n = 73; median age, 70 ± 10 years) or large adenomas with severe dysplasia (>2 cm in diameter; n = 4; median age, 67 ± 13 years) were included in the study. An additional four small adenomas (<1 cm in diameter) that were endoscopically removed from four patients (median age, 65 ± 11 years) have also been examined. When the surgical specimens were obtained, paired samples of primary tumor and surrounding peritumoral normal mucosa at a distance of 2 (adjacent normal mucosa) and 10 cm (remote normal mucosa) from the tumor were prepared. The specimens were examined by means of receptor binding, immunocytochemistry, and real-time RT-PCR studies. All tumors were histologically typed and graded. The characteristics of the tumors, including sex and age of patients, location, histologic type, degree of differentiation, and tumor-node-metastasis (TNM) stage, are reported in Table 1.

**Preparation of Colonic Membranes.** Colon cancer tissues and related mucosa were homogenized in PBS buffer with a Polytron (Kinematica) and centrifuged twice for 15 min at 48,000 × g. The membrane pellet was resuspended in 50 mmol/L Tris/HCl buffer, pH 7.4 (50 mmol/L Tris/HCl, 10 mmol/L MgCl2, and 1 mmol/L EDTA), and incubated with 3 IU/mL adenosine deaminase for 30 minutes at 37°C. This suspension was used for binding experiments.

**Preparation of Blood Peripheral Membrane Suspensions.** Neutrophils and lymphocytes isolated from peripheral venous blood samples (40 mL) were obtained from patients with colorectal cancer undergoing surgery and healthy volunteers. Membranes from neutrophils and lymphocytes were prepared as described previously (19, 24) and used for binding experiments.

**[3H]MRE 3008F20 Binding Assays.** [3H]MRE 3008F20 is a potent and selective A3 receptor ligand (25). In saturation experiments, membrane homogenates (80–100 μg of protein per assay) were incubated in duplicate with 10 to 12 different concentrations of [3H]MRE 3008F20. Nonspecific binding was determined in the presence of 1 μmol/L MRE 3008F20. Bound and free radioactivity were separated after an incubation time of 120 minutes at 4°C by filtering the assay mixture with a Brandel cell harvester. The protein concentration was determined according to a Bio-Rad method (26).

**Immunocytochemistry.** Sections (5 μm thick), obtained from formalin-fixed paraffin-embedded tissue blocks of five colorectal carcinomas and corresponding adjacent and remote normal mucosa were routinely deparaffinized, rehydrated, and rinsed in PBS. Sections were then incubated overnight at 4°C with polyclonal rabbit antibody against human A3 receptor (diluted 1:100 in PBS). An UltraVision streptavidin-biotin peroxidase detection kit (TP-060-HL; Lab Vision Corp., Fremont, CA) was used as the secondary detection system, and the peroxidase reaction was developed using diaminobenzidine tetra-chloride as chromogen. Finally, slides were lightly counterstained with Mayer hematoxylin.

**Real-Time Reverse Transcription-Polymerase Chain Reaction Experiments.** Tissue samples obtained from specimens of colorectal cancer and remote normal tissues were
removed immediately and put into TRIzol reagent for RNA extraction. Quantitative real-time RT-PCR assay (27) of A3 mRNA transcript was carried out using a gene-specific, double fluorescence-labeled TaqMan MGB probe (minor groove binder) and ABI Prism 7700 Sequence Detection System (Applied Biosystems). The following primer and probe sequences were used for real-time RT-PCR: A3 forward primer, 5'-AT-GCCTTTGGCCATTGTG-3'; A3 reverse primer, 5'-ACAATC-CACCTCTACGCTGCCT-3'; and A3 MGB probe, 5'-FAM-TCAGCCCTGGCATC-TAMRA-3' (the fluorescent reporter FAM is 6-carboxy fluorescein; the quencher TAMRA is 6-carboxy-N,N',N'-tetramethylrhodamine). For real-time RT-PCR of the reference control gene, human β-actin kits were used, and the probe was fluorescence-labeled with VIC (Applied Biosystems, Monza, Italy).

Data and Statistical Analysis. A weighted nonlinear least squares curve fitting program, LIGAND, was used for computer analysis of the data from saturation experiments (28). Significant differences between controls and patients with colorectal cancer were assessed using Student’s t test or analysis of variance and the Dunnett’s t test when required. P < 0.05 was considered significant. All data are reported as means ± SE.

RESULTS

Overexpression of A3 Adenosine Receptor Subtype in Patients with Colorectal Cancer. To address the question of the putative relevance of A3 receptor protein in human carcinogenesis and tumor progression, we analyzed the presence of A3 receptors in 73 primary colorectal carcinomas compared with adjacent normal and remote normal (RN) mucosa from the same individual. As shown in Table 2, receptor binding values of the A3 ligand [3H]MRE 3008F20 were significantly (P < 0.05) higher in tumor tissues (K_D, 8.73 ± 0.37 nmol/L; B_max, 584 ± 35 fmol/mg of protein) when compared with the corresponding values in adjacent normal mucosa (K_D, 4.56 ± 0.21 nmol/L; B_max, 295 ± 20 fmol/mg of protein) and those found in RN mucosa (K_D, 3.01 ± 0.13 nmol/L; B_max, 196 ± 10 fmol/mg of protein). To establish the basis for comparison of tumors with RN mucosa, we first examined A3 receptors in colonic mucosa derived from 14 patients who underwent surgery for nonneoplastic large bowel disease; affinity and density values were similar to those found in RN mucosa and statistically different from those found in tumor (K_D, 2.93 ± 0.19 nmol/L; B_max, 163 ± 10 fmol/mg of protein; P < 0.05). Therefore, to take into account possible interindividual variations in the expression level of A3 receptors, tumor tissue and the corresponding normal tissue obtained from the same patient were compared (Fig. 1). The interindividually variability of A3 receptor density in the tumor samples was about 2–4 fold. Moreover, the mean ratio of tumor to remote mucosa A3 receptor (T/M ratio) concentration was 3-fold, and 20 of 73 human colon carcinomas showed a 4-fold overexpression of A3 protein compared with the RN mucosa of the same donor. The T/M ratio of A3 receptor expression was examined in relation to TNM stage, which is used as the most relevant prognostic clinical variable. Although the T/M ratio of A3 receptors did not significantly correlate to TNM stage (I–IV), we observed that early-stage tumors (I and II) had a greater frequency of T/M ratio < 2 than advanced-stage (III and IV) tumors (stage I, 4 of 4 had T/M ratio < 2; stage II, 13 of 33 had T/M ratio < 2; stage III, 3 of 28 had T/M ratio < 2; stage IV, 1 of 8 had T/M ratio < 2; χ² test, P < 0.001). To further investigate whether the A3 receptor status might reflect a progression in malignancy, binding values were evaluated in four small adenomas with low-grade dysplasia and four large adenomas with high-grade dysplasia. The small adenomas had affinity and density values very similar to those of the mucosa of healthy subjects (K_D, 2.99 ± 0.50 nmol/L; B_max, 155 ± 24 fmol/mg of protein; n = 4), whereas the large adenomas showed increased binding values (K_D, 6.3 ± 0.8 nmol/L; B_max, 302 ± 15 fmol/mg of protein; n = 4; P < 0.05).

### Table 2

<table>
<thead>
<tr>
<th>Tissue</th>
<th>K_D (nmol/L)</th>
<th>B_max (fmol/mg of protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Healthy colon</td>
<td>2.93 ± 0.19*</td>
<td>163 ± 10*</td>
</tr>
<tr>
<td>Carcinoma</td>
<td>8.73 ± 0.37†</td>
<td>584 ± 35†</td>
</tr>
<tr>
<td>Adjacent mucosa</td>
<td>4.56 ± 0.21†</td>
<td>295 ± 20†</td>
</tr>
<tr>
<td>Remote mucosa</td>
<td>3.01 ± 0.13*</td>
<td>196 ± 10*</td>
</tr>
</tbody>
</table>

* P < 0.05 with respect to tumor; analysis was by analysis of variance, followed by Dunnett’s test.

† P < 0.05 with respect to corresponding control; analysis was by analysis of variance, followed by Dunnett’s test.
suggesting a tendency toward a greater A<sub>3</sub> receptor expression during colorectal tumor progression. Finally, no statistically significant correlation (χ² test) was observed by comparing the T/M ratio with the degree of tumor differentiation or tumor location (P ≥ 0.3).

**Overexpression of A<sub>3</sub> Adenosine Receptor Subtype in Peripheral Blood Cells of Patients with Colorectal Cancer and Normalization after Surgical Resection for Colorectal Cancer.** To evaluate the possibility of A<sub>3</sub> receptor alterations in peripheral blood cells of patients with colorectal cancer, we examined the K<sub>D</sub> and B<sub>max</sub> values of A<sub>3</sub> binding sites in neutrophils and lymphocytes of patients affected by colorectal cancer. Binding values reported in Table 3 were approximately 3-fold higher as compared with those found in healthy subjects (P < 0.01), indicating an increase in receptor number and an affinity decrease that were in agreement with the variations found in the cancer tissues (Fig. 2). Moreover, binding data obtained from three patients with small adenomas were similar to those found in healthy subjects [K<sub>D</sub>, 2.46 ± 0.52 and 2.67 ± 0.29 nmol/L; B<sub>max</sub>, 106 ± 15 and 547 ± 77 fmol/mg of protein (n = 3) in lymphocytes and neutrophils, respectively], suggesting that a progression in malignancy may be reflected by binding data obtained in blood cells. In contrast, statistical analysis of A<sub>3</sub> receptor binding values and TNM stage did not reveal a significant correlation. In a cohort of 10 patients, the peripheral A<sub>3</sub> receptor expression was studied 12 months after colorectal surgical resection. K<sub>D</sub> and B<sub>max</sub> of A<sub>3</sub> receptors on neutrophils and lymphocytes returned to normal values (Fig. 2; Table 3) and were in accord with the negative results of follow-up examinations, including human carcinoembryonic antigen (CEA), computed tomography imaging, and colonoscopy, indicating the absence of tumor recurrence.

**Immunocytochemistry.** Colon tissues consist of several types of cells including epithelial and fibroblast cells, nerve ganglia, and endothelial and smooth muscle cells of blood vessels. To assess the specificity of A<sub>3</sub> overexpression in tumor cells, the distribution of these receptors was evaluated in paired tumor and RN mucosa sections. Immunohistochemical analysis with anti-A<sub>3</sub> adenosine receptor antibody demonstrated strong cytoplasmic and cell membrane staining of the majority of neoplastic cells in the five colorectal carcinomas examined. Corresponding normal colonic mucosa taken at a distance from the tumor showed generally weak immunoreactivity in the cytoplasm and cytoplasmic membrane of epithelial cells. Fig. 3 shows the results obtained in a moderately differentiated adenocarcinoma (Fig. 3A, top panel) and in the related RN mucosa (Fig. 3B, bottom panel). Finally, positive immunostaining was observed in the nervous structures of the bowel wall, whereas a variable degree of immunoreactivity was detected in smooth muscle cells and inflammatory and stromal cells.

**Expression of the A<sub>3</sub> Receptor Gene.** The level of expression of A<sub>3</sub> receptor protein in tumors raises the question of the underlying mechanism. One possibility is that A<sub>3</sub> receptor mRNA content is elevated in tumors. Therefore, A<sub>3</sub> receptor mRNA content was investigated on RNA from 10 paired tumor and normal tissue samples obtained from colon cancer patients by use of real-time quantitative RT-PCR. The normalized concentrations of A<sub>3</sub> receptors were determined using β-actin mRNA as endogenous internal control; the mRNA content was

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**Table 3**[^H]MRE 3008F20 binding to human lymphocytes and neutrophils of patients affected by colorectal cancer (LTC and NTC, respectively) compared with lymphocytes and neutrophils of healthy subjects (L and N, respectively)

<table>
<thead>
<tr>
<th>Cells</th>
<th>N</th>
<th>K&lt;sub&gt;D&lt;/sub&gt; (nmol/L)</th>
<th>B&lt;sub&gt;max&lt;/sub&gt; (fmol/mg of protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lymphocytes (L)</td>
<td>20</td>
<td>1.87 ± 0.20</td>
<td>122 ± 11</td>
</tr>
<tr>
<td>Lymphocytes (LTC)</td>
<td>30</td>
<td>5.50 ± 0.48*</td>
<td>371 ± 39*</td>
</tr>
<tr>
<td>Lymphocytes (SRLTC)</td>
<td>10</td>
<td>2.01 ± 0.19†</td>
<td>150 ± 12†</td>
</tr>
<tr>
<td>Neutrophils (N)</td>
<td>20</td>
<td>2.63 ± 0.15</td>
<td>588 ± 48</td>
</tr>
<tr>
<td>Neutrophils (NTC)</td>
<td>30</td>
<td>7.06 ± 0.49†</td>
<td>1687 ± 141‡</td>
</tr>
<tr>
<td>Neutrophils (SRNTC)</td>
<td>10</td>
<td>2.38 ± 0.22§</td>
<td>639 ± 54§</td>
</tr>
</tbody>
</table>

NOTE. [^H]MRE 3008F20 binding to human lymphocytes and neutrophils of 10 patients with colorectal cancer studied 12 months after surgical resection (SRLTC and SRNTC, respectively) was also included.

* P < 0.01 with respect to corresponding control (L); analysis was by Student’s t test.
† P < 0.01 with respect to corresponding TC (LTC); analysis was by Student’s t test.
‡ P < 0.01 with respect to corresponding control (N); analysis was by Student’s t test.
§ P < 0.01 with respect to corresponding TC (NTC); analysis was by Student’s t test.

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![Fig. 2](image-url) Histograms show the increase of K<sub>D</sub> (A, top panel) and B<sub>max</sub> values (B, bottom panel) of [^H]MRE 3008F20 binding to human A<sub>3</sub> adenosine receptors in lymphocytes and neutrophils obtained from patients with colorectal cancer (LTC and NTC, respectively; n = 30) with respect to control subjects (L and N, respectively; n = 20). *, P < 0.01, Student’s t test. The longitudinal analysis of A<sub>3</sub> receptor density and affinity in a cohort of patients (n = 10) studied for 12 months after surgical resection is also included (SRLTC and SRNTC in lymphocytes and neutrophils, respectively). **, P < 0.01 versus corresponding LTC and NTC, Student’s t test.
expressed as A3 receptor mRNA/β-actin mRNA. The overexpression of A3 receptor protein in tumors was not reflected by the specific A3 mRNA transcription increase. As shown in Fig. 4, we found an increased content of A3 receptor mRNA in two samples, in which a 2-fold increase was found with respect to the corresponding normal portion. In the majority of cases, the content of A3 receptor mRNA was not different from that of the corresponding nonmalignant tissue (Fig. 4). These data suggest that the elevated expression of A3 receptor protein in colon cancer was not essentially associated with an increase in A3 receptor mRNA.

DISCUSSION

The possibility that adenosine plays a role in the progression of cancer has aroused considerable interest in recent years (29–31). Since the observation that adenosine could be detected in the interstitial fluid surrounding a carcinoma (4), several reports have shown the effects and the possible mechanism of action of this nucleoside on tumor cell growth (11, 12, 32). However, despite the fact that several studies seem to indicate an emerging role for the A3 receptor as a good candidate for the identification of tumor cells (16–19), comparative studies performed on normal and tumor tissues are not currently available. To our knowledge, the present study provides the first extensive analysis of the presence of A3 adenosine receptors in carcinomatous tissue and paired adjacent/remote normal mucosa taken from 73 donors undergoing surgical treatment for colorectal carcinoma. By means of receptor binding studies, A3 protein was identified in all normal and malignant tissue samples. However, the amount of A3 receptors was elevated by ≥2-fold in primary colon carcinomas as compared with adjacent and remote normal mucosa, respectively (P < 0.05). To verify that the overexpression of A3 protein was specifically due to neoplastic cells, considering that colonic tumor tissues include neutrophils and other infiltrating cells, immunocytochemical studies were performed. The tumors analyzed showed a strong immunoreactivity at the level of neoplastic epithelial cells and a variable degree of positive antibody staining in smooth muscle and inflammatory cells. In contrast, the epithelial cells of corresponding normal colonic mucosa showed generally weak immunoreactivity, in agreement with previous observations obtained in human colon mucosa (33). The overexpression of A3 receptors in colorectal tumors raises the question of the underlying mechanism, in particular whether this phenomenon is due to an increase in A3 receptor gene expression. In most cases, the...
drastic differences in A3 protein levels between malignant and nonmalignant colon tissue are not due to changes in the corresponding mRNA. Although we cannot completely rule out the possibility that A3 mRNA is rapidly degraded under in vivo conditions in tumors but not in normal colon tissue, it appears more likely that posttranscriptional control mechanisms are involved in the up-regulation of the amount of A3 receptor protein in tumors. Protein expression data revealed broad interindividual variations in both normal and neoplastic tissues. By correlating the T/M ratio with various clinical variables, we found a tendency for a lower protein expression in less advanced stages (stages I and II) compared with more advanced tumor stages (III and IV), which was statistically significant using a T/M ratio of 2 to distinguish low from high expression ($P < 0.001$). Furthermore, there was a tendency toward a more pronounced expression of A3 receptors in larger adenomas as compared with smaller-sized adenomas, suggesting that the expression level of this adenosine receptor subtype may reflect the adenoma-carcinoma sequence. In light of these results, we therefore propose that A3 protein could be required during all stages of cancer development, with a major role in cancer aggressiveness. Prompted by this consideration, we intend to extend this analysis to other tumor types because the evaluation of A3 receptor protein may be useful for the prognosis and diagnosis of human malignancy. The possibility of identifying a diagnostic and prognostic cancer index is suggested by our observations in peripheral circulating blood cells. Indeed, several studies have compared receptor expression profiles in tissues and peripheral blood cells from normal and pathological conditions and found a positive association or trend (22–24). In the present study, we found that both peripheral lymphocytes and neutrophils obtained from 30 colorectal cancer patients showed a 3-fold overexpression of A3 receptors compared with blood cells from healthy donors, in line with the data found in tissues ($P < 0.01$). We did not find any association with stage, tumor site, patient age, or gender. The mechanisms of this up-regulation are not known. However, it is interesting that binding values in tissues, as in circulating blood cells, discriminate between small-sized adenomas and cancer, suggesting that this protein may be a requirement for colorectal tumor progression. According to this data, we also found in a small cohort of subjects that A3 receptor expression of circulating blood cells normalizes after surgical treatment, in accord with the negative results of follow-up exams including CEA, computed tomography scan, and colonoscopy. Hence, the good health of patients after surgical resection seems to be associated with restoration of a normal adenosinergic system, at least in terms of A3 receptor expression. We think that these findings might potentially be used for clinical applications. In particular, examination of neutrophil A3 expression (for example, in addition to CEA determination) could play a potential role in the screening of high-risk individuals or in the follow-up of patients after surgical resection.

Although our observations will have to be confirmed in a larger number of cancer patients, this study provides the first evidence that the A3 receptor plays a role in colon tumor development and, more importantly, can potentially be used as a diagnostic marker or therapeutic target for colon cancer treatment.

REFERENCES


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