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# Activities of adenosine deaminase and 5'-nucleotidase in cancerous and noncancerous human colorectal tissues

A Eroğlu[1]*, O Canbolat[2], S Demirci[1], H Kocaoğlu[1], Y Eryavuz[1] and H Akgül[1]

[1]Department of Surgical Oncology, Ankara University Medical School, Ankara, Turkey; [2]Department of Biochemistry, Ankara University Medical School, Ankara, Turkey

In order to characterize human colorectal cancer, much attention has been paid to enzyme studies. However, little is known about the correlation between the levels of key enzymes of purine nucleotide pathway and some clinical and biological indicators of tumor invasiveness and aggressiveness. Adenosine deaminase (ADA) and 5'-nucleotidase (5'-NT) were measured in cancerous and cancer-free adjacent large bowel tissues from 38 patients with colorectal carcinoma. We have analyzed the relationship between the enzyme levels and some clinical and pathological parameters. The enzymes' activities were markedly higher in primary tumors than in corresponding normal mucosae. The ADA level in tumor tissue was significantly correlated with lymph node metastasis, histologic type, tumor location, and patient's age, whereas the 5'-NT level showed a significant correlation with tumor grade and tumor location. ADA activity in tumor tissues was significantly higher in patients whose clinical course remained stable than in those with recurrent diseases. The purine metabolism and salvage pathway activity of purine nucleotides are accelerated in the cancerous human colorectal tissue. Although our findings suggest that these enzymes' activities are most likely related to the same histomorphological architecture of the tumor, the authors believe that long-term follow-up studies are needed to evaluate the prognostic value of purine enzymes for colorectal cancer. *Medical Oncology* (2000) **17**, 319–324.

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## Introduction

Colorectal carcinoma is one of the most common solid tumors in humans. In order to characterize human colorectal cancer, numerous studies have focused on enzyme studies.[1-9] Alterations in the enzymology of the human colorectal tumor clearly distinguished it from that of the normal colorectal mucosa. To obtain a better understanding of purine enzymology in colorectal carcinoma, much attention has been paid to investigate the interrelations between the carcinogenic process and the activities of some enzymes of the *de novo* and salvage pathways for purine biosynthesis. However, relatively little is known about the correlation

between the levels of enzymes of the purine nucleotide pathway and clinical and biological indicators of tumor invasiveness and aggressiveness.

Adenosine deaminase (E.C.3.5.4.4.) (ADA) is an aminohydrolase that catalyzes the deamination of either adenosine or deoxyadenosine to inosine or deoxyinosine, respectively. ADA is an important enzyme in the degradation of adenine nucleotides. Because of the irreversibility of the reaction catalyzed by ADA, this enzyme reaction seems to be one of the rate-limiting steps in adenosine degradation.[10] There are several reports presenting high ADA activities in several types of cancerous tissues and cells compared to non-cancerous ones.[4,7,9,11-13] However, some investigators found low ADA activities in cancerous tissues or low lymphocyte ADA activities in cancer patients.[14-17]

5'-Nucleotidase (EC 3.1.3.5.) (5'-NT) is another enzyme functioning in nucleotide metabolism. It generates nucleosides from various types of nucleotides. Although in some studies, activity of this enzyme was found to be decreased in cancer tissues and cells,[7,15,18] some researchers found high 5'-NT activities in cancerous tissues with respect to surrounding normal tissues.[12,13]

Most of these studies have focused on purine enzymatic differences in cancerous tissues or cells as well as normal tissue, but less attention has been paid to examine any correlation between levels of the enzymes and clinicopathological parameters. Moreover, we could find no studies that have investigated the prognostic value of purine enzymes for colorectal cancer.

The aim of the study was to measure the activities of ADA and 5'-NT enzymes in cancerous and noncancerous colorectal tissues and to evaluate the relationship between the levels of the enzymes and some clinicopathological factors and clinical course of the disease.

## Materials and methods

Colorectal carcinomas were obtained at surgery from untreated patients undergoing surgical resection for therapeutic purposes between July 1998 and January 1999 at the Department of Surgical Oncology, Ankara University Medical School. 38 patients were enrolled into the study. No patient received chemotherapy or radiation therapy prior to surgery.

Patients were evaluated on the basis of age, gender, tumor stage at presentation, tumor location, depth of tumor invasion, lymph node involvement, distant metastasis, tumor grade, and histologic type of tumor. Patients' characteristics are listed in Table 1.

There were 27 male and 11 female patients. The patients ranged in age from 32–74 y (median age 53 y). The surgical samples were from the following areas of the colorectum: 2 from caecum; 3 from ascending colon; 3 from hepatic flexure; 1 from transverse colon; 1 from splenic flexure; 3 from the descending colon; 7 from the sigmoid; and 18 from the rectum.

The tumor's depth of invasion (T), lymph node status (N), and distant metastasis (M) were determined according to the criteria outlined by the TNM classification of UICC-1997.[19] Accordingly, tumor staging was as follows: there were 3 patients in stage 1

**Table 1** Clinical and pathological characteristics of patients with colorectal carcinoma

| Characteristics | No. of patients |
| --- | --- |
| Mean age (range) | 54.5 ± 12 y (32–74) |
| Sex | |
| male | 27 (71%)* |
| female | 11 (29%) |
| Tumor location | |
| rectum | 18 (47.4%) |
| colon | 20 (52.6%) |
| Depth of tumor invasion (T) | |
| T1 | 1 (2.6%) |
| T2 | 2 (5.3%) |
| T3 | 29 (76.3%) |
| T4 | 6 (15.8%) |
| Lymph node status (N) | |
| N0 | 14 (36.8%) |
| N1 | 12 (31.6%) |
| N2 | 12 (31.6%) |
| Distant metastases (M) | |
| M0 | 26 (68.4%) |
| M1 | 12 (31.6%) |
| Tumor stage | |
| stage 1 | 3 (7.9%) |
| stage 2 | 10 (26.3%) |
| stage 3 | 13 (34.2%) |
| stage 4 | 12 (31.6%) |
| Histologic type | |
| adenocancer | 32 (84.2%) |
| mucinous cancer | 6 (15.8%) |
| Tumor grade (G) | |
| G1 | 16 (42.1%) |
| G2 | 19 (50%) |
| G3 | 3 (7.9%) |

*Values in parentheses are percentages.

(T1N0M0 in 1, T2N0M0 in 2), 10 in stage 2 (T3N0M0), 13 in stage 3 (T3N1M0 in 6, T3N2M0 in 4, T4N1M0 in 2, T4N2M0 in 1), and 12 in stage 4 (T3N0M1 in 1, T3N1M1 in 3, T3N2M1 in 5, T4N1M1 in 1, T4N2M1 in 2).

12 patients were diagnosed as having synchronous hepatic metastases. Simultaneous partial hepatectomy for liver metastases was performed in 4 patients; others were treated with hepatic intraarterial chemotherapy.

The major histologic type considered in the study was adenocarcinoma (32 patients). Six tumors were considered as a mucinous carcinoma, defined as when a mucinous component was evident in more than 50% of the tumor section. Histological grading of the specimens determined that 16 were well differentiated, 19 were moderately differentiated, and 3 were poorly differentiated.

Normal looking adjacent tissues were sampled about 10 cm away from the outer edge of a tumorous region, and tumor samples were taken from the non-necrotic proliferative region after their surgical removal. The fresh surgical specimens were frozen immediately and stored at $-80°C$ until assayed.

After samples were obtained, they were immediately prepared for enzyme activity assays as given below. Tissue samples were washed out from contamined blood with cold water and were homogenized in equal amounts of cold distilled water by a Melsungen homogenizer (B. Braun, Frankfurt, Germany). The homogenate was centrifuged at 15,000 g for 60 min on a Labofuge 200 Sepatech (Heraeus Instruments Inc., South Plainfield, NJ, USA) to remove debris. Clear upper supernatant fluid was taken and the assays were carried out on this part. All the procedures mentioned above were performed at $+4°C$.

Protein was determined by the method of Lowry et al.[20] ADA and 5'-NT activities were measured as described respectively.[21,22] Results were expressed in mili international unit per mg protein (mIU/mg protein).

Data were expressed as mean ± standard deviation (s.d.). The Mann−Whitney U test was used to determine the significance of the difference between the two means. One-way analysis of variance (ANOVA) or the Kruskal−Wallis analysis as a nonparametric approximate was used for the analysis of differences among the groups. Differences between means yielding a P of less than 0.05 were considered statistically significant. The statistical analysis was performed by Statistical Package for Social Sciences (SPSS for MS Windows Release 7.0, Chicago, IL, USA).

## Results

As shown in Figure 1, in colorectal tumors, the activity of ADA and 5'-NT was significantly increased with respect to the surrounding normal tissue ($P < 0.05$).

ADA levels in tumor tissue were higher in patients with lymph node metastases than in those without lymph node metastasis ($P < 0.05$) (Figure 2). Another significant association was observed between ADA levels and histologic type of the tumor: its expression in pure adenocarcinoma was significantly higher than in mucinous carcinoma ($P = 0.039$).

If the patient population was divided into two groups based on age, markedly increased ADA levels in tumor extracts were found in patients aged $\geq 50$ y compared to those aged $< 50$ y ($P = 0.015$). Interestingly, a similar difference was seen between the age groups in the normal tissue samples ($P = 0.032$).
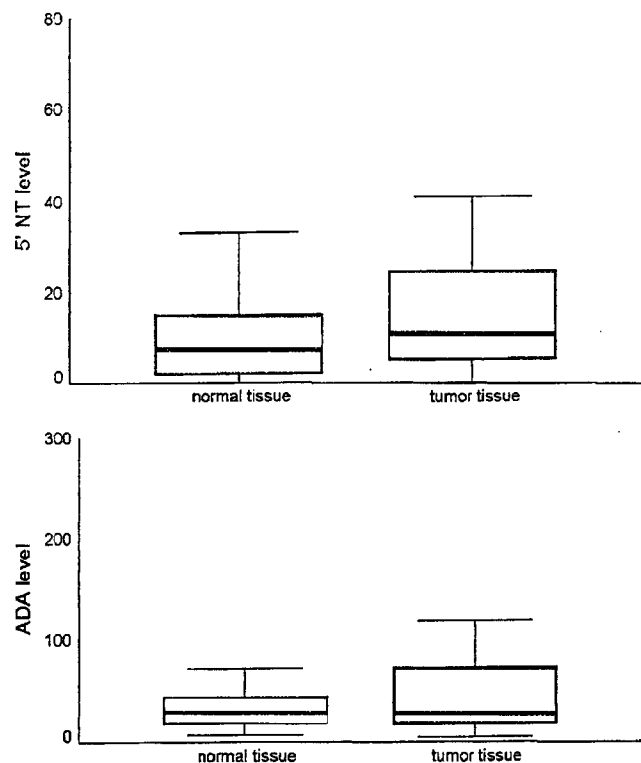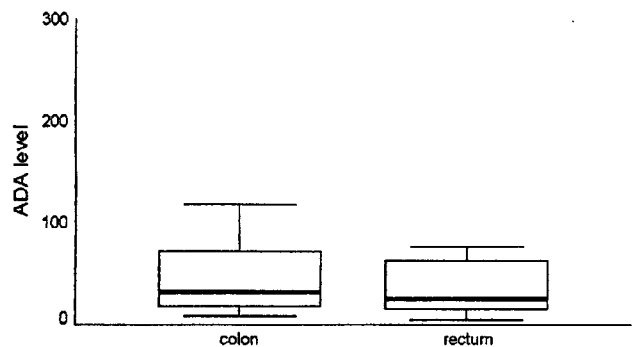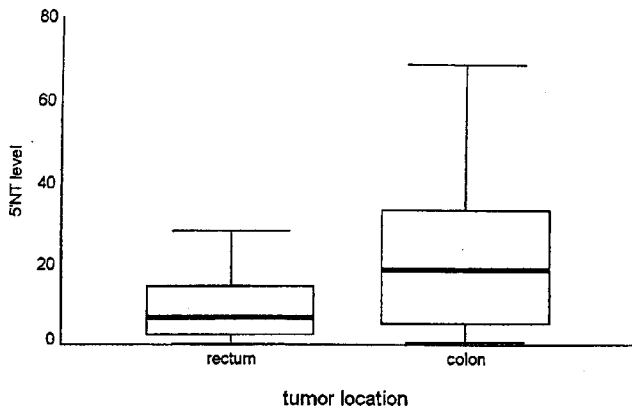


**Figure 1** 5'-nucleotidase (5'NT) (above) and adenosine deaminase (ADA) levels in colorectal tumor tissues.
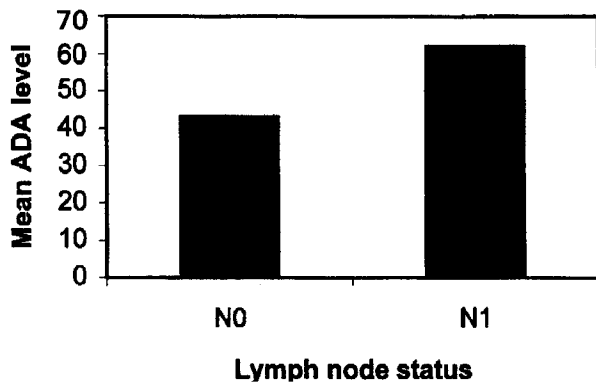
**Figure 2** *ADA levels in tumor tissue according to lymph node metastases.*

There was no correlation between the ADA levels in tumor tissues and tumor stage, depth of tumor invasion, distant metastasis, and gender.

ADA and 5'-NT levels in colon tumor tissues were significantly elevated compared to rectum cancer tissues ($P < 0.05$) (Figure 3). Elevated 5'-NT values in tumor tissue were more common in well-differentiated tumors than in moderately and poorly differentiated

tumors ($P = 0.019$) (Figure 4). No obvious relationship was noticed between this enzyme activity and patient's age, gender, tumor stage, lymph node metastasis, distant metastasis, depth of tumor invasion, or histologic type of the tumor ($P > 0.05$).

Patients had been followed for a mean of 12 months (range: 9–15 months). During this period recurrent disease or progression of liver metastases developed in 8 patients. When the ADA and 5'-NT levels in individual tumors were compared according to the clinical course of the disease, it was seen that ADA enzyme activity in tumor tissue was significantly lower in patients with recurrent diseases than in those with stable disease ($P = 0.018$) (Figure 5). No similar association was seen for 5'-NT activity. In addition, patients who remained disease-free showed an increase ADA activity in tumor tissue compared to tumor-free tissue ($P = 0.0132$). In contrast, patients in whom the disease



**Figure 3** *5'NT (above) and ADA (below) activities in tumor tissues according to the tumor location.*
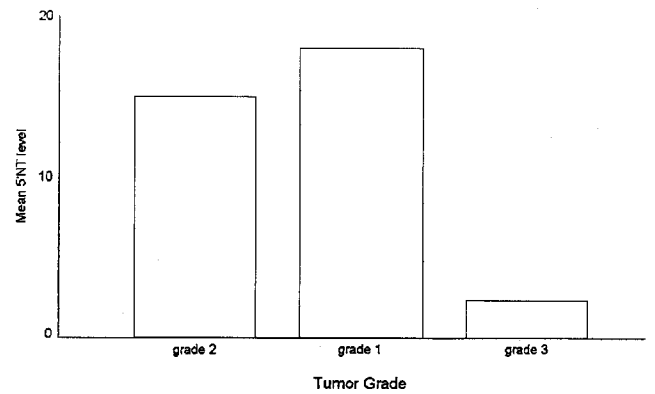


**Figure 4** *5'NT level in tumor tissues according to tumor grade.*
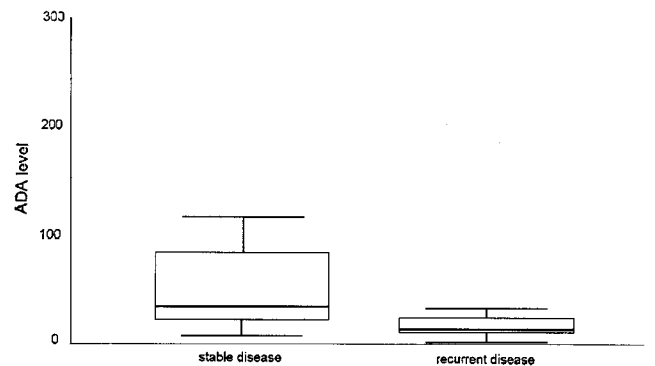


**Figure 5** *ADA activity in tumor tissue according to the clinical course of the colorectal cancer.*

progressed or recurred had a decrease in ADA levels of tumor tissues compared to normal tissues ($P = 0.0357$).

## Discussion

Cancer is characterized by a significant change in enzymatic programs. The activities of the enzymes of purine salvage and *de novo* biosynthesis are higher than degradative enzyme activities.[2,6,7,9] There are marked purine enzymatic differences between normal and neoplastic human colon cells.

ADA levels have been studied in patients with various tumors. Some authors suggest that high ADA and 5'-NT activities play an important part in the salvage pathway activity of the cancerous tissues and cells[7,18] while others propose that increased ADA activity may be a compensatory mechanism against toxic accumulation of its substrates due to accelerated purine and pyrimidine metabolism in the cancerous tissue and cells.[23,24]

Although in several studies ADA activities were found to be increased in cancerous tissue, some authors measured low activity values in tumor tissues or lymphocytes from cancer patients.[14–17,25] In order to explain the discrepancy, further investigation is required.

In this study, we have assayed ADA and 5'-NT levels in a series of colorectal tumors and in the normal tissue adjacent to them. For ADA, the observed increase in activity in tumors as compared to normal tissues is in agreement with previously published findings.[4,7,9,11–13] However, we could not have detailed information of the physiological significance of increased ADA activities in cancerous tissue. It seems to be a secondary phenomenon, which reflects rapid accelerated purine turnover and salvage pathway activity of nucleic acid metabolism associated with tissue proliferation.

Natsumeda *et al* reported that elevated activity of the *de novo* enzymes of purine biosynthesis and high activities of the salvage enzymes should provide an increased capacity for purine biosynthesis in the colorectal tumors.[6] They also emphasized that complete blocking of the *de novo* pathway cannot be successful for chemotherapy because of the high salvage enzymatic capacity of human colon carcinomas. Similar observations indicating increased activities of *de novo* and salvage enzymes of pyrimidine biosynthesis were reported in human colorectal carcinoma.[3,5,8]

Ten Kate *et al* demonstrated that there was an increase of the ADA activity in the tumors compared to normal tissue.[4] However, in their study, there was no correlation between any of the clinicopathological parameters and the ADA levels. In our study the ADA level was significantly related to lymph node metastasis, tumor location, histologic type, and patient's age.

Data concerning cancer progression suggest that ADA levels in tumor tissues did not decrease with the tumor stage. Our results seemed to be contrary to that of Sanfilippo *et al*, who found that ADA levels were related to tumor stage, ie low ADA levels were found in patients with advanced tumors.[9] In addition, they could not find any enzyme activities among the histologic grading. In their preliminary report, it was found that ADA activity was increased in breast cancer with respect to normal tissue but not in colon cancer with respect to normal tissue.[7]

Sufrin *et al* studied ADA activity in patients with renal cell carcinoma.[16] They demonstrated that no statistically significant correlation between tumor stage, its grade, its histologic cell pattern and lymphocyte ADA levels was found. They also reported that their patients with progressive diseases had a decline in lymphocyte ADA levels. Our results are basically in agreement with those of Sufrin *et al*. In our series the patients whose clinical course remained stable demonstrated a significant difference in ADA activity between tumor and tumor-free tissues. Patients in whom disease progressed or recurred had a decrease in ADA levels of tumor tissues compared to normal tissues.

In our study the ADA activity was found to be significantly related to tumor location. Site-specific differences were detected within the colorectal region (colon *vs* rectum). The type of the cell of origin of the tumor is probably important in determining the expression of ADA. It may partly account for the observed tumor-to-tumor variation in expression of the enzyme.

5'-NT was investigated in order to evaluate the program of enzymes degrading mononucleotides to nucleosides. Although Sanfilippo *et al* reported that no significant difference was observed in 5'-NT activity between cancerous and noncancerous colorectal tissues,[7] in our study 5'-NT activity in tumor tissue was significantly higher than in tumor-free tissue. Moreover, it was seen that its level was correlated to the tumor grade.

In conclusion, since ADA and 5'-NT levels were correlated with some clinical and pathological characteristics, the increased enzymes' activities in colorectal cancer patients might be due to the cancer bearing status. Although our results demonstrated that aggressive colorectal cancer was associated with decreased ADA level, the prognostic significance of the enzyme activity requires further evaluation. It is emphasized that decreased ADA activity in cancerous tissue might be a useful indicator for predicting the subsequent development of recurrent disease.

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