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DIAGNOSTIC IMPORTANCE OF ADENOSINE DEAMINASE ACTIVITY FOR PROGRESSION AND INVASION OF HUMAN COLON TUMORS

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Summary. *The aim of the study was to explore the usefulness of adenosine deaminase (ADA) activity as a marker of colon tumor progression and to find a quantitative expression of ADA in mucosa of colon carcinoma, polyps, adjacent to tumors, or mucosa from anastomosis after surgical resection. The highest ADA activity was obtained from mucosa adjacent to carcinoma compared to healthy tissue. The ADA activity from polyp tissue increased slightly, compared to the activity of further healthy tissue, the enzyme activity from anastomosis was also higher than the activity of corresponding healthy tissue. The highest enzyme activity of mucosa adjacent to carcinoma suggest that ADA may be included in tumor growth and progression and may be of importance as an early marker of colon carcinoma invasion. Obtained data could be useful in assessing the margins for surgical resection of colorectal carcinoma and assessing prognosis and clinical outcome of the disease after surgical resection, together with known clinicopathologic parameters.*

Key words: *Adenosine deaminase, colon carcinoma*

Introduction

Colorectal cancer is one of the most frequent cancers in humans. Considering the knowledge of molecular mechanisms of its development, the attempt is to identify a key intracellular proteins included in rapid growth and to devise more biological approaches for the diagnostic and prognostic evaluation (1,2). The epithelial tissue homeostasis is complex, it includes many opposite processes, such as proliferation, programmed cell death and differentiation. A disturbance of these processes lead to neoplastic transformation and further tumor progression (3).

Human adenosine deaminase-ADA (E.C. 3.5.4.4.) exists in various molecular forms, with different molecular weights. It plays an important role as a key enzyme involved in the salvage of purine nucleosides and recycling of purines (3). Numerous studies have revealed the highest activity and substantial molecular heterogeneity of intestinal adenosine deaminase. Beside of soluble ADA form, a particulate-membrane bound form was isolated also from normal intestine, but a cancer-specific form was isolated in some colorectal tumors (4-6).

The study was designed to evaluate the diagnostic and prognostic importance of ADA activity during human colon tumor growth and to explore its usefulnes as a possible marker of colon tumor progression and invasion.

Materials and methods

Tissue specimens used for this study were obtained from patients: with colon carcinoma (29); polyps (8); after colon carcinoma resection (6) without pathological manifestations found during colonoscopy (10). The patients had received neither chemotherapy nor radiation therapy before tumor resection. Tissue slices (obtained of mucosa adjacent to tumor tissue, tumor tissue, anastomosis, corresponding further healthy tissue and helathy tissue of patients with no pathological manifestations) were immediately homogenised by a teflon homogeniser in cold physiological saline and stored on -20^{o}C during collection. The ADA activity was measured according to the method of Pederson and Berry mofified for ammonia liberation measurement (7,8). The modified cell protein content was determined by the method of Lowry et al., (9), using bovine serum albumin as a standard. The enzyme activity was expressed in units per gram of proteins (U/g prot.) The examination of obtained results was assessed by the *t* test comparing the enzyme activity of mucosa with pathological manifestations or mucosa adjacent to tumor tissue with the activity of corresponding further healthy tissue as well as with the activity of tissue obtained from patients with no pathological manifestations.

Results

The activity of adenosine deaminase (ADA) in obatined homogenates is shown in figure 1. The highest enzyme activity was documented in mucosa adjacent to carcinoma and mucosa of carcinoma (compared with the corresponding further healthy or control mucosa). The enzyme activity from mucosa adjacent to polyp was higher compared with the activity of corresponding healthy mucosa.The enzyme activity from polyp was only slightly increased compared to corresponding healthy or control tissue. Increased ADA activity of anastomosis was registered in all patients.

Discussion

The evidence of high ADA activity during rapid and stimulated growth of normal tissue is of importance in making a fully functional purine salvage pathway possible the inactivation of adenosine and 2'-deoxyadenosine, toxic metabolites for growing cells (10). The enzyme is particularly sensitive to stimulation by the growth factors and cytokines during rapid tissue proliferation (11). The results of the performed study are in agreement with a number of examinations demonstrated an increase of ADA specific activity in very rapidly growing malignances, documented as a tumor marker, while slow-growing well-differentiated tumors do not express pronounced ADA activity. (10,12). Some data suggest that ADA is not involved directly in carcinogenesis, but plays a metabolic role in supporting a rapid growth state of appropriate tissues, by the reutilisation of nucleosides, related as RNA and DNA precursors. The treatment of colon carcinoma cells with deoxycoformicine, an ADA inhibitor, resulted in inhibition of cell growth (13,14). The highest ADA activity obtained from mucosa adjacent to carcinoma may suggest its role in colon carcinoma progression and invasion. Presented results are in agreement with other results concerning the increased activity of purine salvage enzymes, including ADA, and invasiveness of colorectal adenocarcinoma (6,15). Increased ADA activity is also related to decreased or defficient ADA complexing protein (ADBP), a dimeric glycoprotein localised in normal colonic mucosa. The mouse monoclonal antibodies raised against fresh colon cancer represent also ADBP (16). The progressive transition of normal colorectal epithelium to adenoma or carcinoma is associated with the series of genetic alterations related not only to proliferation but also to apoptosis, involving the activation of oncogenes and loss of tumor suppressor genes (17). The examination of genes capable of regulating programmed cell death such as *p53* and *bcl* family suggested that high *p53* and *bcl-2* expression indicates on high dysplasia with worse outcome or that induction of *p53* may underline growth suppressive effects of novel anticancer molecules. Recent data point out the important relationship between ADA activity and regulation of programmed cell death. It was demonstrated that ADA deficiency triggers apoptotic process dependent on *p53*, as well as that introduction of p53 mutants produced a 2 to 7.5 fold increase in ADA activity. The role of *bcl-2* overexpression in preventing apoptosis concerns the interaction with dATP, product which is accumulated in the absence of ADA activity (18,19).

In conclusion, the colon cancer development requires an accumulation of numerous metabolic changes responsible for tumor proliferation, invasion and metastasis in a co-operative fashion. Presented results suggest that ADA may be included in malignant transformation of the colon epithelium, as well as in progression and invasion of human colon cancer. In this way, the estimation of ADA activity could help in assessing the margins in order to establish the extensiveness of colon resection. The difference in enzyme activity found in anastomoses could be potentially useful in the evalua-

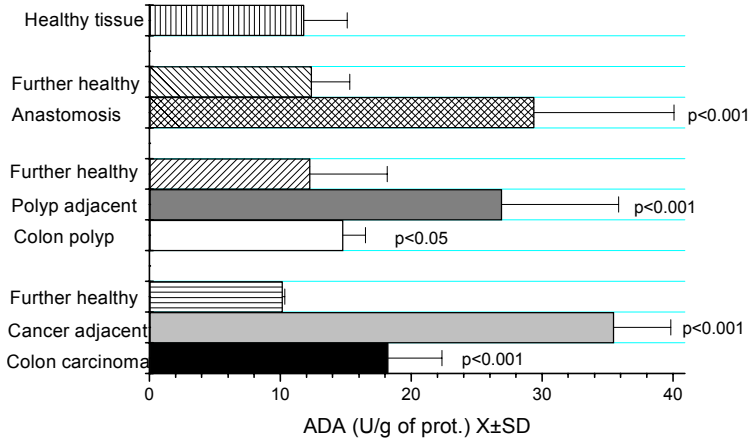

Fig. 1. Adenosine deaminase (ADA) activity in investigated colon mucosa.
Tissue specimens used for this study were obtained resected surgically or endoscopically, and ADA activity was measured according to the procedure explained in Materials and Methods. The enzyme activity was expressed as units per gram of proteins (U/g prot.) The examination of obtained results was assessed by the *t* test comparing the results with the activity of corresponding healthy tissue and control mucosa.

tion of neoplastic potential of the remaining tissue, when comparing it with known clinicopathologic prognostic factors in trying to define different clinical outcomes and to improve survival. The increase of the enzyme activity obtained from polyp-adjacent tissue point to an important proliferative potential of polyp-surrounding tissue that probably needs radical resection as well. The simplicity of measuring its activity point out its usefulness as a simple test together with known clinicopathologic examinations.

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DIJAGNOSTIČKI ZNAČAJ AKTIVNOSTI ADENOZIN DEZAMINAZE ZA PROGRESIJU I INVAZIJU HUMANOG TUMORA KOLONA

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Kratak sadržaj: *Cilj studije je bio da se ustanovi značaj praćenja aktivnosti adenozin dezaminaze (ADA) kao markera progresije tumora kolona i odredi kvantitativna ekspresija ADA u karcinomu kolona, polipu, tkivu neposredno uz tumor ili u anastomozi nakon hiruške resekcije tumora. Najveća aktivnost enzima je pokazana u tkivu neposredno uz tumor u poređenju sa zdravim tkivom. Povećanje aktivnosti ADA je bilo slabije izraženo u tkivu polipa a porast je bio i u anastomozi u poređenju sa kontrolnim zdravim delovima. Najizraženija aktivnost enzima u tkivu neposredno uz karcinom ukazuje da bi ADA mogla da bude uključena u rast i progresiju tumora i da bi mogla biti od koristi kao rani marker invazije karcinoma kolona. Dobijeni rezultati bi mogli da budu od koristi pri utvrđivanju granica hiruške resekcije kolorektalnog karcinoma, u prognozi i kliničkom ishodu oboljenja nakon hiruške resekcije, zajedno sa poznatim klinikopatološkim nalazima.*

Ključne reči: *Adenozin dezaminaza, karcinom kolona*