



Disorders of purines and pyrimidines metabolism in human gastrointestinal tract cancer

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ABSTRACT

Patients with cancer of stomach, colon cancer and rectum showed an increase in the thymidine kinase, 5'-nucleotidase of adenylate, adenosine deaminase activities and a decrease in the thymidine phosphorylase activity, which can cause DNA biosynthesis and increase of proliferation. The lymphocyte 5'-nucleotidase of adenylate, adenosine deaminase activities are associated with cellular dysfunction and can be used as a biochemical tests of it.

Keywords: cancer, thymidine phosphorylase, thymidine kinase, 5'-nucleotidase of AMP, adenosine deaminase, blood serum, lymphocytes

INTRODUCTION

The battle against cancer is being fought on different levels from social prevention and information to basic molecular studies of cell metabolism. The age-dependent variability in nucleic acids precursors metabolism may influence both individual progression of cancer and efficacy of treatment. A comparative study of enzyme activity features of nucleotides metabolism in blood of different age patients with tumors of gastrointestinal tract T₃₋₄N_{0-x}M_{0-y} stages was carried out. Thymidilate incorporation is a limiting step for DNA replication. Thymidine kinase and thymidine phosphorylase play an important role in thymidilate synthesis by the "salvage pathway". Thymidine kinase (TK; EC 2.1.2.1) directly catalyzes the phosphorylation of deoxythymidine released from cells by DNA catabolism in the salvage pathway [1]. High tumor TK activities may be indicative of a poor response to antimetabolic chemotherapy protocols. Thymidine phosphorylase (TP; EC 2.4.2.4) plays a key role in maintaining the thymidilate balance of the nucleotide pool and controlling nucleic acid homeostasis [1]. On the other hand, the biochemical and biophysical similarities between TP and platelet-derived endothelial cell growth

factor (PD/ECGF) suggested that human TP is the same as human PD/ECGF. Therefore, this protein appears to stimulate DNA synthesis, cell growth and chemotaxis of endothelial cells in vitro and angiogenesis in vivo.

The specific signaling pathways of cancerogenesis can also be regulated by purines. While adenosine is generated as a result of hypoxia and cell necrosis in the core of tumors, there is emerging evidence that some tumors modify purine metabolism to facilitate production or retard degradation of adenosine. A high concentration of adenosine in the core of solid tumor may facilitate tumor survival by suppressing the immune system and by facilitating angiogenesis. In addition, tumors may stimulate the conversion of purine nucleotide to adenosine by increasing the expression of CD 73. CD73 or ecto-5'-nucleotidase of AMP (5'-NT; EC 3.1.3.5) is a widely expressed ecto-enzyme which catalyzes the dephosphorylation of AMP and other nucleoside monophosphates. CD73 participates in purine salvage pathway through this enzymatic activity, supplying cells with precursors for energy metabolism and nucleic acid biosynthesis. The adenosine deaminase (ADA; EC 3.5.4.4) decreases the cellular levels of adenosine. Ecto-adenosine deaminase in human cells can be bound to the cell surface and regulate the level of extracellular adenosine during tumor progression. The activities of ADA and 5'-NT are closely associated with cellular immunity [4, 2].

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MATERIALS AND METHODS

Ninety-two adult patients, 46–60 years old, with gastrointestinal cancer T₃₋₄N_{0-x}M_{0-y} stages were included in this study. Sixty-eight non-cancerous persons, 25–70 years old without gastrointestinal tract pathology formed a control group and were also examined. The study protocol was approved by Ethical Committee permission of M. Gorky Donetsk National Medical University for research involving humans. The features of enzymes activity were studied in blood serum and lymphocytes. TK activity was determined by the radioisotope method as described previously [4]. It was given in nanomoles of thymidilate formed within an hour per 1 mg of protein. TP activity was determined spectrophotometrically [4] at 300 nm according to the amount of thymine formed. 5'-NT activity was determined by the number of inorganic phosphate, ADA – at 265 nm according to the amount of inosine formed [4]. Protein concentration in biological material was determined using the method described by Lowry et al. [5]. Statistical analysis of results was performed using Medstat software package. Significance of differences was analyzed by parametric and nonparametric methods.

RESULTS AND DISCUSSION

This study demonstrated that the serum activities of TK, TP, ADA, 5'-NT strongly associated with age. There is evidence that according with the norm, the TK has the highest level of its activity in the blood at the age of 46–60 years. The relationship between parameters was assessed statistically using Spearman's rank correlation test, i.e. $\rho = 0.519$, a positive correlation between the TK activity and age was shown. Therefore, we determined the possibility of TMP synthesis increasing by the "salvage pathway" in healthy individuals aged 46–60 years. However, an age-dependent activity increase is also characteristic of TP (the index of Spearman's rank correlation, i.e. $\rho = 0.874$, a positive correlation between the TP activity and age). It is interesting to note that direct correlation between anabolism and catabolism enzymes activity (TK, TP) and age was revealed. This mechanism can regulate the velocity of their pathway of dTTP synthesis in healthy organism. The ADA and 5'-NT activities get the highest values at the age 36–45 years. A strong positive correlation between the two enzyme activities has been demonstrated ($\rho = 0.746$). So ADA and 5'-NT have cooperative effect on the adenosine levels in human organism.

The blood serum is the most available material for patients' monitoring. According to the obtained findings as well as the results of our previous studies [4, 6], the activity of serum enzymes correlates with their activity in the tissues. Our findings are confirmed by the results of other authors [7]. In this study, the key enzymes of thymidine

and adenosine metabolism were determined in the blood serum of patients with stomach cancer, colon cancer, tumors of rectum (table 2).

Table 1. The activity of thymidine and adenosine metabolism enzymes in the blood serum in control group at the different age

Age, years	TK nmol/mg•h	TP nmol/mg•min	ADA nmol/mg•min	5'-NT AMP nmol/mg•min
25-35	2.67±0.34	36.16±2.15	0.79±0.19	6.10±0.65
36-45	2.96±0.51	41.61±3.12	1.83±0.14	5.48±0.52
46-60	3.87±0.71	47.88±2.62	1.74±0.22	5.76±0.26
61-70	3.05±0.32	52.01±2.89	2.18±0.45	5.63±0.46

Table 2. The serum enzymes activity of patients with cancers of gastrointestinal tract T₃₋₄N_{0-x}M_{0-y} stages at the age of 46–60 years

Cancer localizations	TK nmol/mg•h	TP nmol/mg•min	ADA nmol/mg•min	5'-NT AMP nmol/mg•min
Control	3.87±0.71	47.88±2.62	1.74±0.22	5.76±0.26
Stomach	6.78±0.98**	14.65±2.38***	7.58±0.84**	9.99±0.83*
Colon	4.98±0.34*	18.06±1.13**	8.21±0.86**	9.71±1.35**
Rectum	4.78±0.14*	19.32±2.67**	5.69±0.49**	12.11±1.07**

Authentic differences versus the norm: * p<0.05, ** p<0.01, *** p<0.001

Serum TK activity increases with the cancers of different localizations. The TP activity in blood serum of patients with cancer sharply decreases. This disorder may be one of the reasons of dTTP synthesis elevation and higher rate of proliferation. Blood serum changes of TK and TP activity of stomach cancer patients correlates with their activity peculiarities in the tissues. ADA and 5'-NT activity is increased in all patients with both cancer of stomach and intestinal cancer. It is correlated with TK and TP activity changes, because in this case, the inhibitory action of adenosine on the pyrimidine synthesis will be removed. There are specific nucleotide metabolism changes which tumors have to increase the biosynthesis of DNA and proliferative activity [8].

Tumor immunosurveillance contributes to the control of cancer growth. Many mechanisms can be used by cancer cells to avoid the antitumor immune response. One such mechanism relies on the capacity of cancer cells and of the tumor microenvironment to generate adenosine, a major molecule involved in antitumor T-cell response suppression. So another aspect of the given study was to evaluate the white blood cells dysfunction according to the analysis of purines metabolism disorders in groups of patients with gastrointestinal tract tumors. To reveal prognostic possibilities of biochemical indexes, the research of blast transformation reaction in lymphocytes of patients aged 46–60 was conducted parallel with ADA and 5'-NT activity determining. A decrease of blast transformation reaction index was observed in cancer patients (from 65.44±2.40 in the control group to 16.38±2.33 in case of cancer development). It has been found that the lymphocytes activities of ADA, 5'-NT were decreased in all patients groups, but not proportionally. It has been found that lymphocytes of ADA activity decrease in case of cancer of stomach up to 89.63±3.65 nmol/10⁶c•h in comparison with control (178.38±12.07 nmol/10⁶c•h, p<0.01). This is

accompanied by 5'-NT activity decreasing up to 36.76 ± 0.92 nmol/10⁶c·h (48.03 ± 5.70 nmol/10⁶c·h, $p < 0.05$). We demonstrated that ADA activity in lymphocytes of colon cancer patients is lower by 7.3 times as compared to norm (24.17 ± 2.31 nmol/10⁶c·h, $p < 0.001$), but the 5'-NT activity is lower by 1.7 times according to the norm only (29.02 ± 0.74 nmol/10⁶c·h, $p < 0.01$). In case of tumor localization in rectum, ADA activity is lower by 5.5 times (32.33 ± 0.58 nmol/10⁶c·h, $p < 0.001$), but the 5'-NT activity is lower by 2,3 times ($20,98 \pm 0.59$ nmol/10⁶c·h, $p < 0.01$). So, it is very likely that disordinated activity of 5'-NT and ADA contribute to the elevated levels of extracellular adenosine during tumor progression and its regulatory effects realization [8]. Individual analysis of lymphocytes functional power showed that ADA, 5'-NT activity changes was accompanied by the decline of cellular immune function (the blast transformation reactions of lymphocytes were decreased in all cases of cancer development).

CONCLUSIONS

In patients with cancers of gastrointestinal tract the nucleotide enzyme activity changes can be responsible for some increase in DNA biosynthesis and proliferation. A practical application of the present study is that the investigation of 5'-NT, ADA lymphocyte activity can be used as a biochemical test of lymphocyte dysfunction.

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