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Adenosine deaminase and dipeptidyl peptidase- IV activities in cancerous and non cancerous human colon and gastric tissues

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Abstract : It is aimed to investigate activity changes in dipeptidyl peptidase-4 (DPP-4) and adenosine deaminase (ADA) enzymes in cancerous and noncancerous human gastric and colon tissues.

Thirty two cancerous and 32 non cancerous adjacent gastric tissues removed from patients with gastric cancer, and 18 cancerous and 18 non cancerous colon tissues from patients with colon cancer were used in the studies. In the tissues, DPP-4 and ADA activities were measured. DPP-4 activities were found to increase significantly in cancerous tissues relative to non cancerous ones (0.073±0.169 vs. 3.86±3.41, $p<0.001$ for colon and 1.25±0.95 vs. 2.52±1.05, $p<0.05$ for gastric tissue). In the ADA activities, there were however no differences between cancerous and non cancerous tissues for both cancers. In the correlation analysis performed between DPP-4 and ADA activities, weak correlations were observed.

It has been established that DPP-4 is significantly increased, but ADA is not changed in the cancerous tissues. It has been suggested that the increase in DPP-4 activity in the cancerous tissues might be of importance in the progress of cancer process, and that inhibitors of DPP-4 enzymes might give helpful results in this regard.

Keywords : Adenosine deaminase; Dipeptidyl peptidase; Cancer; Colon tissue; Gastric tissue.

INTRODUCTION

Stomach and colorectal cancers are the leading causes of death in the world. Their prognosis is poor because most patients present with advanced disease[1,2]. Pathogeneses of these cancers involve alterations in oncogenes and tumour suppressor genes, as well as other factors, such as poor hygiene, drugs, diet or smoking[3].

Dipeptidyl peptidase-4 (DPP-4 or CD26) is a

membrane-associated peptidase, also known as CD26, which exerts pleiotropic effects via peptidase activity. In addition, DPP-4 is thought to be associated with immune stimulation and resistance to anti-cancer agents. DPP-4 has also been linked to the malignant potential of T-cell lymphoma[4,5]. Treatment with anti-DPP-4 monoclonal antibody causes increased sensitivity to anti-cancer agents and greater survival. In fact, CD26 has been shown to participate in various pathways in cancer[6], which is thus thought as a novel therapeutic target. Anti-CD26 monoclonal antibody treatment results in antitumor activity against several tumor types[7,8]. In a study, it has been found that it is upregulated during enterocytic differentiation of the colon adenocarcinoma cell lines[9]. Increased DPP-4 expression is seen in various malignant tumors[10]. For example, in hepatocellular carcinoma, it is found that its activity is increased in liver specimens and serum[11-13]. Disrupted responsiveness to humoral growth regulators it the typical features of tumour progression. Dipeptidyl peptidase IV is the only enzyme capable to cleave out X-Pro dipeptides from its substrates. It is supposed that serum DPP-4 inactivates circulating bioactive peptides and protects the organism against their inappropriate systemic effects[14]. Therefore, it has been supposed that the dipeptidyl peptidase-4 and its homologues may be pro- or anti-oncogenic within the particular cancer. This contradiction has been explained by tumour type-specific local microenvironment[15,16].

Adenosine deaminase (ADA) is an enzyme (EC 3.5.4.4) involved in purine metabolism. It is needed for the breakdown of adenosine and for the turnover of nucleic acids in tissues. ADA is present in all mammalian cells, and it is thought that its primary function in is related to the immune system. However, its full physiological role is not completely understood. ADA association has also been observed with epithelial cell differentiation. It has been proposed that ADA also stimulates release of excitatory amino acids and is necessary to the coupling of A1 adenosine receptors and heterotrimeric G proteins[17].

In several studies, tissue ADA activities were found to increase, decrease or remain unchanged depending on the types of tissue and cells studied[18,19]. Additionally, in some previous studies, it has been found close association between DPP and ADA enzymes and reported that the different levels of cell surface CD26–ADA complex and relative expression of adenosine receptors on a tumor cell may lead to generation of tumor subclones as well as to participation in the well-known adenosine inhibition of cell-mediated immune responses to tumor cells[20-22]. However, no study was performed until now to elucidate possible relation between these enzymes in cancerous tissues.

MATERIALS AND METHODS

Thirty two cancerous gastric tissues and 32 non cancerous adjacent gastric tissues were obtained from patients with gastric cancer by surgical operation, who were at the terminal stages of cancer. Eighteen cancer and 18 non cancer colon tissues were similarly obtained from patients with colon cancer at the terminal stages.

The study protocol was approved by local Ethics Committee. All subjects volunteered for the trial and written consent was obtained according to the Declaration of Helsinki.

Tissues were first cleaned by saline solution and the stored at - 80^{o}C until analysis. In the analysis, they were first homogenized in saline solution (20 %, w/v). After homogenization, homogenates were centrifuged at 5000 rpm for 30 min to remove debris and to obtain clear supernatant fraction. Analyses were performed in this fraction[23].

Protein concentrations of the tissues were measured by Lowry method[24] and ADA activity by the method of Guisti *et al*.[25] DPP activity was measured as described previously[26]. Statistical evaluations were made by using Wilcoxon test and P values lower than 0.05 evaluated as significant. Correlation analyses were made by using Pearson correlation test and 0.01 value level was evaluated as significant (2-tailed).

RESULTS

Results were shown in the tables. As seen from

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TABLE 1 : Mean±SD values of dipeptidyl peptidase-4 and adenosine deaminase enzyme activities (IU/mg protein) in the cancerous and non cancerous tissues

GROUPS	DPP-4	ADA
I- Non-cancerous colon tissues (n=18)	0.073±0.169	7.21±4.2
II- Cancerous colon tissues (n=18)	3.86±3.41*	7.19±5.28
III- Non-cancerous gastric tissues (n=32)	1.25±0.95	14.4±12.7
IV- Cancerous gastric tissues (n=32)	2.52±1.05**	15.2±12.8

Significantly higher than non-cancerous groups DPP-4 (*p<0.001, **p <0.05)

TABLE 2 : Correlation coefficient values between dipeptidyl peptidase-4 and adenosine deaminase enzyme activites in the the cancerous and non-cancerous tissues

Cancerous colon tissues	Non-Cancerous colon tissues	Cancerous gastric tissues	Non-cancerous gastric tissues
r:-0.302	r:-0.50	r:-0.31	r:0.49

the TABLE 1, DPP-4 activities were higher in the cancerous tissues than non cancerous ones (0.073±0.169 vs. 3.86±3.41, p<0.001 for colon, and 1.25±0.95 vs. 2.52±1.05, p<0.05 for gastric tissue). In the ADA activities, there were no differences between cancerous and non cancerous tissues for both cancers as well (14.4±12.7 vs. 15.2±12.8 for gastric tissue and 7.21±4.2 vs. 7.19±5.28 for colon tissue).

Correlation analysis results were given in the TABLE 2. As seen from the table, correlation coefficients were small between DPP-4 and ADA activities in both cancerous (r=-0.302) and non cancerous (r=-0.50) colon tissues. In the gastric tissues, there was a weak negative correlation (r=-0.31) in the cancer tissue, but a positive correlation (r= + 0.49) in normal tissue.

DISCUSSION

Elucidation of molecular mechanisms involved in carcinogenesis, and understanding the complex relation between immunity and cancer is one of the crucial steps in the cancer. Many recent studies indicate the prominent role of dipeptidyl peptidase IV in initial steps of malignant transformation, promotion and progression of tumors, acting differently as a tumor suppressor or even tumor activator. By cleaving the dipeptides from N- terminal end of peptides and polypeptides, DPP-4 controls the activity of many bioactive molecules. Besides its enzymatic activity, DPP-4 acts as receptor for adenosine deaminase (ADA), interacts with crucial receptors. Interaction with proteins, components of extracellular matrix points to its role in adhesion, invasion and metastasis of cancer cells[14,27,28].

Its involvement in regulation of apoptosis has been previously reported. Tumor-suppressing activity of DPP-4 is supported by facts that decrease and loss of DPP-4 expression and activity are found in microenvironments of specific tumors. Decrease in DPP-4 activity is shown in several carcinomas. On the contrary, elevated DPP-4 expression and/or activity are found in some other carcinomas like thyroid follicular carcinoma, astrocytic tumours, gastrointestinal stromal tumours, T and B lymphomas and leukemias etc. reveal the tumour promoting activities of this molecule[27].

DPP-4 has been associated with cancer[29]. The non-enzymatic role of this enzyme as an extracellular anchorage for ADA may be important in tumourigenesis. Although the presence of extracellular ADA is independent of DPP-4 expression[21,30], proliferating cells accumulate high extracellular concentrations of adenosine, which may be toxic to the cells[31]. Therefore, the different levels of cell surface DPP-4–ADA complex and relative expression of adenosine receptors on a tumor cell may lead to generation of tumor subclones as well as to participation in the adenosine inhibition of cell-mediated immune responses. In addition, it has recently been reported that DPP-4–ADA may form a ternary complex with plasminogen. Binding of plasminogen to cell surface receptors promotes its conversion to

plasmin, which is required for proteolysis in several physiological and pathological processes including tumor cell invasion and metastasis[22].

In early studies[32,33], a loss of DPP-4 expression was reported in some tumor tissues. Even, DPP-4 was found to be absent in metastatic tumors[34,35]. An inverse correlation was also observed in endometrial cancers. Interestingly, DPP-4 expression is found to be lower than in early stages, or may even be absent[15,36,37]. The protective role was first contrasted in 1999[38]. Resarchers found tumor suppressor functions for DPP-4 in cell lines from non-small cell lung and prostatic carcinomas[16,38] and concluded that DPP-4 regulates the activities of locally produced mitogenic peptides involved in cancer development. In fact, deregulation of the DASH expression pattern was observed in a number of human tumors. In a study, decline in expression of DPP-4 was observed in tumors of the gastrointestinal tract. Moreover, increased levels of adenosine were measured in the hypoxic tumor focus of colon carcinoma. A high concentration of adenosine was related to decreased levels of DPP-4 and its enzymatic activity as well as to reduced binding of ADA and fibronectin. It has been speculated that this process could alter anti-tumor immune response and facilitate invasion and metastasis of carcinoma cells[39].

A seeming contradiction in some tumors could not be fully explained so far. Hypothetically, due to its multifunctional, cell-specific repertoire, DPP-4 might in parallel facilitate some prooncogenic processes[40], whilst at the level of the transformed cell itself it could play an anti-proliferative and thus anti-oncogenic role[16,41]. The resulting pro- or anti-oncogenic effect probably depends on tumor type. Although the functional studies of DASH molecules are not clear, DASH seems suitable candidate for further evaluations[20].

As to the results of the present study, it has been observed that DPP-4 activities were found to increase significantly in cancerous tissues relative to non cancerous ones (0.073±0.169 vs. 3.86±3.41, $p<0.001$ for colon and 1.25±0.95 vs. 2.52±1.05, $p<0.05$ for gastric tissue). In the ADA activities, there were however no differences between cancerous and non cancerous tissues (14.4±12.7 vs. 15.2±12.8 for gastric tissue, and 7.21±4.2 vs. 7.19±5.28 for colon tissue, $p>0.05$). In the correlation analysis between DPP-4 and ADA activities, weak correlations were found in the cancerous (r=-0.302) and non cancerous (r=-0.50) colon tissues. In the gastric tissues, weak negative correlation (r=-0.31) was found in cancer tissue, but a positive correlation (r=+0.49) in normal tissue. As to the relation between DPP-4 and ADA, no meaningful relations are observed. However, DPP-4 activities are clearly higher in the cancerous tissues relative to non cancerous ones (TABLE 1).

The importance of this finding is not clear for us for the time being. However, it is quite possible that increased DPP-4 activity play part in some steps of carcinogenic process in the gastric and colon tissues including metastatic events. Although further efforts are obviously needed before reaching final decision on the subject, it seems that substances having inhibitory potential for DPP-4 may be of importance in controlling the initial steps of malignant transformation, promotion and progression of tumors including gastric and colon tumors.

CONCLUSIONS

Our results show that DPP-4 is significantly increased in the cancerous tissues, and that substances having potential to inhibit DPP-4 might give helpful results in this regard. Subject however needs further studies before final evaluation.

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