CD26 and DPP IV Expression in T Acute Lymphoblastic Leukemia Cells: Immunocytochemistry and Enzyme Cytochemistry

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Abstract. Recent studies have revealed the dipeptidyl peptidase IV (DPP IV) enzymatic activity of CD26 antigen In this paper, the possible identity of DPP IV and CD26 expression in phenotypically defined T-ALL has been examined The combination of enzyme cytochemistry and immunocytochemistry was used The correlation between the CD26 antigen expression and DPP IV positivity in the vast majority of T lymphoblasts in T-ALL patients was observed No CD26 was expressed on DPP IV negative T cells The variable CD4 and/or CD8 antigen expression, frequent CD7 positivity and absence of membrane CD3 antigen expression were the characteristic immunophenotypic features of CD26/DPP IV positive T cells CD26/DPP IV activity strongly paralleled the CD71 antigen (transferrin receptor, T cell activation/proliferation antigen) expression The phenotypic features of CD26/DPP IV positive T cells are characteristic for the relative immature cell population Noteworthy was the slight disassociation between the very high CD26 antigen expression and moderate DPP IV activity in cells of some T-ALL patients The possible existence of enzymatically inactive structures of CD26 antigen or inactive precursors of DPP IV detectable only by immunocytochemistry was discussed

Our study indicates that CD26 antigen expression is tended to identify cells with DPP IV enzymatic activity in T-ALL patients The results provide information of CD26 antigen possible involvement in the pathology of leukemic cells via its DPP IV enzyme activity

Key words: DPP IV — CD26 Antigen — T acute lymphoblastic leukemia

Introduction

Dipeptidyl peptidase IV (DPP IV, EC 3 4 14 5) is a membrane associated enzyme with an essential importance in several T cell functions Many studies suggest the specific association of DPP IV activity with cells that are functionally regarded as helper/inducer CD4+ subset DPP IV is involved in the regulation of DNA synthesis and plays an important role in the process of T cell activation and proliferation (Ansorge and Schon 1987, Schon and Ansorge 1990) In hematological pathology, DPP IV is known as a marker enzyme of diagnostic value in the lymphoproliferative disorders of T-origin (Lojda 1988)

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The significance of enzyme DPP IV in leukemic cells of T-ALL patients was assessed in our previous investigation (Klobušická and Babušíková 1998) Recently, the identity between the DPP IV and hematopoietic differentiation antigen CD26 was confirmed (Ulmer *et al* 1990, Morimoto and Schlossman 1998, Ruiz *et al* 1998)

The purpose of the present study was to evaluate the possible relationship of DPP IV to CD26 in phenotypically defined leukemic cells (peripheral blood and bone marrow) of patients suffered from T acute lymphoblastic leukemia (T-ALL) of different stage of maturation

Materials and Methods

The diagnosis of T-ALL was based on morphological and cytochemical criteria according to the standard FAB classification and on immunophenotyping Cell surface markers were detected by a standard immunofluorescence assay and FACStar flow cytometer using a panel of monoclonal antibodies to define T cell immunophenotype (CD2, membrane CD3, CD4, CD7, CD8) In addition, CD71 antigen (transferrin receptor) with its relation to proliferation was investigated

In order to demonstrate the possible identity of DPP IV and CD26 expression, the combination of enzyme cytochemistry and immunocytochemistry was used DPP IV was performed according to Lojda (Lojda 1977) with glycyl-L-proline-4-methoxy-2-naphthyl-amide as a substrate and Fast Blue B as a coupler The streptavidin-biotin-immunoperoxidase (DAKO) system was used for the demonstration of CD26 antigen The color reaction was developed using 3,3'-diaminobenzidine (DAB) as a chromogen

Results and Discussion

The strong correlation between the CD26 antigen expression and DPP IV positivity in the vast majority of T lymphoblasts in T-ALL patients was observed No CD26 antigen was expressed on DPP IV negative T cells The variable CD4 and/or CD8 antigen expression, frequent CD7 positivity and negativity for membrane CD3 antigen were the characteristic features of CD26/DPP IV active cells CD26/DPP IV paralleled the CD71 antigen expression It appears, that the CD26/DPP IV positive T cells form the population with relative immature phenotype in T-ALL patients There were no remarkable differences between the leukemic cells in peripheral blood (PB) and bone marrow (BM) in the main immunophenotypic characteristics as well as DPP IV and CD26 activity, as these cells represent in T-ALL the same leukemic clone (Table 1)

Remarkable was the slight disagreement between the very high presence and intensity of CD26 and moderate DPP IV positivity (100% vs 62%) in cells of some T-ALL cases The possible existence of enzymatically inactive structures of CD26 antigen (Torimoto *et al* 1992) or inactive precursors of DPP IV detectable only by immunocytochemistry (Hartel *et al* 1988) could explain this disassociation Furthermore, DPP IV could vary in T cells according to their detailed differentiation status and under certain circumstances surface membrane CD26 expression can be different from the DPP IV activity (Ruiz *et al* 1998)

Our study showed that CD26 antigen expression is tended to identify cells with DPP IV positivity in T-ALL patients The results provide some more information of CD26 antigen possible participation in the pathology of leukemic cells via its DPP IV enzyme activity

Table 1. Percentage of enzyme DPP IV and CD26 antigen positive cells in PB and BM of T-ALL patients with respect to immunophenotype, and in controls

	T-ALL			
	DPP IV+ $n = 22$	DPP IV− $n = 6$	Controls $n = 10$	p values[c]
	mean percentage ± S D			
DPP IV	78 9 ± 12 1[a]	8 4 ± 4 7	39 2 ± 2 6	0 01
	83 6 ± 14 8[b]	7 0 ± 5 5	1 3 ± 0 7	0 001
CD4	81 5 ± 12 5	10 4 ± 6 0	42 5 ± 4 3	0 01
	81 0 ± 16 2	6 4 ± 3 3	15 1 ± 5 5	0 001
CD8	82 7 ± 14 4	15 2 ± 12 9	32 9 ± 3 8	0 001
	79 7 ± 18 1	16 0 ± 12 7	13 7 ± 4 5	0 001
mCD3	9 2 ± 5 5	74 6 ± 14 9	73 7 ± 7 4	0 001
	8 5 ± 4 8	81 6 ± 13 1	10 8 ± 6 0	N S
CD7	92 4 ± 7 1	81 5 ± 14 3	67 4 ± 11 8	0 05
	91 8 ± 6 7	75 8 ± 19 0	18 0 ± 1 7	0 001
CD71	66 4 ± 17 1	15 2 ± 11 5	3 6 ± 0 9	0 001
	76 5 ± 14 2	14 7 ± 6 8	N T	N T
CD26	97 6 ± 2 3	2 5 ± 1 2	31 5 ± 3 8	0 01
	96 5 ± 3 1	2 2 ± 0 8	1 9 ± 0 7	0 001

a – percentage of positive cells in PB, b – percentage of positive cells in BM, c – significance of differences between T-ALL and control

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Mola Invasiva – Special Form of GTD

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Abstract. Invasive hydatidiform mole is a relative rare form of gestational trophoblastic disease (GTD) Most of hydatidiform moles remit after evacuation but some of them have the tendency to invade the myometrium In some rare cases the tropoblastic tissue can be found in other tissues like lungs, vulva, vagina or broad ligament The aim of the study was to demonstrate some of clinical, immunohistochemical and DNA analysis findings of a patient with a previous diagnosis of a complete hydatidiform mole

Key words: GTD — Invasive mole — DNA analysis — Immunohistochemistry

Introduction

Gestational trophoblastic disease is a heterogenous group of various lesions including hydatidiform moles and true neoplasms like choriocarcinoma and placental site trophoblastic tumor (PSTT) The main characteristics of an invasive hydatidiform mole is the penetration of hydropic degenerated villi and trophoblastic structures deep into the myometrium or invasion into the uterine vasculature The majority of moles that becomes invasive are of the complete type In immunohistochemical staining with antibodies against HCG and cytokeratins, there is an intensive possitive reaction in trophoblastic cells (Mazur and Kurman 1994, Danihel et al 1994 a,b) A partial mole can also be invasive (Gaber et al 1986) In some rare cases molar tissue is transported through the bloodstream to extrauterine sites like lungs These "metastatic foci" are ussualy detected several weeks after the evacuation of a mole from the uterus but they may occur concurrently with a mole (Hsu et al 1962, Thiele and de Alvarez 1962, Paradinas 1997)

Materials and Methods

A 47 year old woman was curretaged because of metrorhagia and increased levels of beta HCG The result of histopathological examination and DNA analysis was complete hydatidiform mole Because of USG finding of myomatous uterus, the age of the

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