Physiol. Res. 40: 359–362, 1991

SHORT COMMUNICATION

Dipeptidyl Peptidase IV in the Human Lung and Spinocellular Lung Cancer

A. ŠEDO, E. KŘEPELA, E. KASAFÍREK[1], J. KRAML[2], L. KADLECOVÁ[2]

Research Institute of Tuberculosis and Respiratory Diseases,
[1]Research Institute of Pharmacy and Biochemistry and
[2]First Department of Medical Chemistry, Charles University, Faculty of Medicine, Prague

Received June 25, 1990
Accepted October 23, 1990

Summary

The isoelectric point and proportions of soluble and membrane bound dipeptidyl peptidase IV (DPP-IV) in human lung and spinocellular lung cancer tissue were tested. It was found that soluble DPP-IV is relatively less frequent in the cancer than in normal lung tissue. We demonstrated multiple molecular forms of DPP-IV in normal and cancer lung tissues, differing probably not only in the degree of sialylation. DPP-IV from lung cancer tissue consists of more basic molecular forms than that from normal lung tissue. These results suggest that the molecular properties of DPP-IV in normal and cancerous lung tissues may be different.

Key words:
Dipeptidyl peptidase IV – Lung cancer – Isoelectric focusing

Dipeptidyl peptidase IV (EC 3.4.14.5; DPP-IV) was discovered by Hopsu-Havu and Glenner (1966) in rat liver and kidney and was later purified and characterized in different tissues, cells and body fluids of many species (see reviews McDonald and Barret 1986, Křepela *et al.* 1985, Šedo and Křepela 1989).

In our previous experiments we found a higher DPP-IV activity in lung cancer than in the normal lung (Šedo 1990). The aim of this study has been to investigate molecular forms and portion of membrane-bound and soluble DPP-IV in the lung and lung cancer tissues.

The samples of lung and tumor tissues, obtained during lung surgery from patients operated for histologically diagnosed spinocellular lung carcinoma, were pulverized, homogenized and stored before DPP-IV activity determination as described elsewhere (Křepela *et al.* 1990).

DPP-IV activity in whole tissue homogenates and in supernatants after ultracentrifugation (105 000x*g*, 1 h, 2 °C) was measured by continuous rate fluorometric assay (Šedo *et al.* 1989), modified only by using 75 mmol/l Tris/HCl buffer (pH 8.0), with 7-(glycyl-L-prolylamido)-4-methylcoumarin as the substrate.

The results were expressed as a ratio of soluble and total homogenate DPP-IV activities.

Samples for isoelectric focusing (IEF) were prepared as follows. Tissue samples were pulverized in liquid nitrogen and homogenized (15 % w/v suspension) in 5 mmol/l Tris/HCl buffer, pH 7.4, with Zwittergent 3-14 detergent (10 g/l) and ethylenediaminetetraacetic acid disodium salt (2 mmol/l). After one hour solubilization at 6 °C samples were centrifuged (120 000×g, 1 h, 2 °C) and supernatants dialyzed against 5 mmol/l KCl at 6 °C. Protein concentration in particular samples (assayed according to Markwell *et al.* 1981) was 1–2 g/l. Samples treated with neuraminidase were prepared according to Kraml *et al.* (1983). Thin layer analytical IEF was performed in Agarose IEF (Pharmacia, Uppsala) in the pH range 3–10, with presence of Zwittergent 3-14 detergent (0.5 g/l), with IEF pI calibration kits (Pharmacia). DPP-IV activity on the focusograms was detected according to Lojda and Kulich (1981).

As shown in Tab. 1, the relative proportions of DPP-IV activity in the soluble fraction (supernatant after ultracentrifugation at 105 000×g) is significantly higher in control lung tissue than in lung cancer tissue.

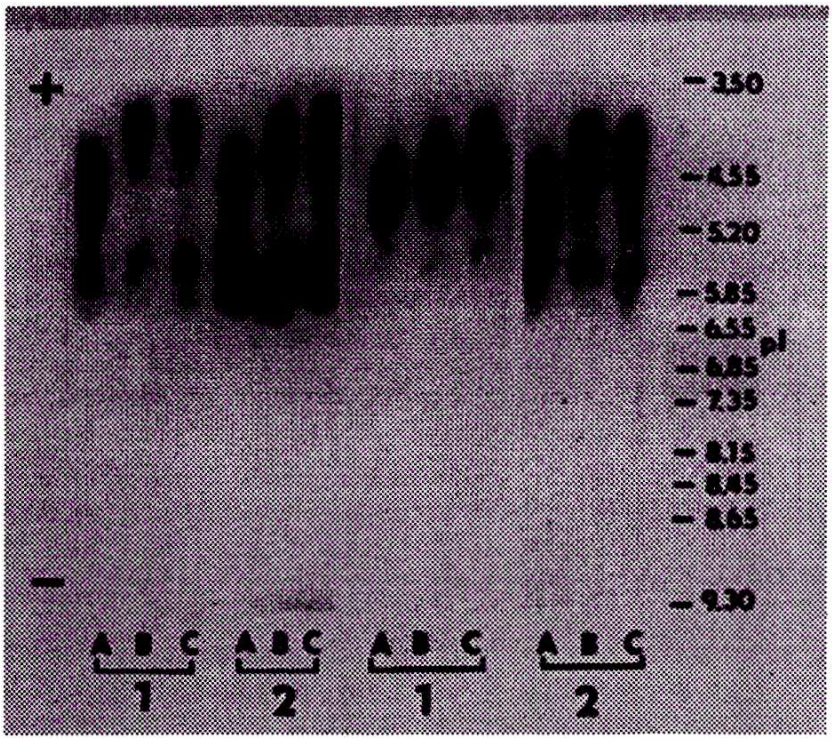

Fig. 1
Thin layer analytical isoelectric focusing of dipeptidyl peptidase IV from two matched pairs of lung (1) and lung spinocellular cancer (2) tissue. A – samples after neuraminidase treatment, in the presence of Pharmalyte 3-10 and 0.4 mol/l $CaCl_2$; B – samples prepared as A, but without neuraminidase; C – control samples, Pharmalyte 3-10 and $CaCl_2$-free.

The zymograms (Fig. 1) displayed the presence of multiple molecular forms of DPP-IV obtained after solubilization from both normal and cancer tissues. However, DPP-IV from lung cancer seems to contain a higher number of basic

molecular forms than from normal lung tissue. Only some of the acidic forms of DPP-IV were shifted to more basic ones after treatment with neuraminidase.

Table 1

Part of soluble (S) dipeptidyl peptidase IV in the whole homogenate (H) activity (nkat/ml) in lung and lung spinocellular cancer tissues

Case	S/H ratio	
	Lung tissue	Cancer tissue
1	0.070	0.070
2	0.145	0.380
3	0.171	0.045
4	0.125	0.051
5	0.554	0.100
6	0.300	0.041
Mean	0.228	0.057
SD	0.177	0.023
p* =	0.041	

* *Statistical significance of difference between S/H ratios in lung and lung cancer tissues (t-test)*

DPP-IV in lung cancer tissue has not been studied so far. Our observations suggest that soluble DPP-IV is relatively less frequent in spinocellular lung cancer tissue than in normal lung tissue. The heterogeneity of DPP-IV molecular forms, revealed by IEF, is probably not only due to a different degree of sialylation, but also to some other kind of posttranslational modifications.

The observed alterations of DPP-IV in spinocellular lung carcinoma in comparison to normal lung tissue might be complex in nature. They may involve differences in cellular composition of studied tissues as well as in the biosynthesis, processing, trafficking and delivery of the enzyme in DPP-IV expressing cells.

At present we are searching for other differences between DPP-IV from cancer and normal lung tissues and for a source of various forms of DPP-IV both in the normal and cancer lung tissues at the cellular and subcellular level.

References

HOPSU-HAVU V.K., GLENNER G.G.: A new dipeptide naphthylamidase hydrolyzing glycyl-prolyl-β-naphthylamide. *Histochemie* 7: 197–201, 1966.

KRAML J., KOLÍNSKÁ J., KADLECOVÁ L., ZÁKOSTELECKÁ M., LOJDA Z.: Analytical isoelectric focusing of a rat intestinal brush-border enzymes: postnatal changes and effect of neuraminidase in vitro. *FEBS Lett* 151: 193–196, 1983.

KŘEPELA E., KASAFÍREK E., NOVÁK K., VIKLICKÝ J.: Increased cathepsin B activity in human lung tumors. *Neoplasma* 37: 61–70, 1990.

KŘEPELA E., VIČAR J., ŽIŽKOVÁ L., KASAFÍREK E., KOLÁŘ Z., LICHNOVSKÝ V.: Dipeptidyl peptidase IV in mammalian lungs. *Lung* **163**: 33–54, 1985.

LOJDA Z., KULICH J.: The usefulness of the analytical electrofocusing in a thin-layer gel (PAG) in the histochemistry of enzymes cleaving peptide bonds. *Histochemistry* **73**: 311–319, 1981.

MARKWELL M.A.K., HAAS S.M., TOLBERT N.E., BIEBER L.L.: Protein determination in membrane and lipoprotein samples: manual and automated procedures. *Methods Enzymol.* **72**: 296–303, 1981.

McDONALD J.K., BARRETT A.J.: *Mammalian proteases. A Glossary and Bibliography*. Vol. 2, *Exopeptidases*, Academic Press, London, 1986.

ŠEDO A.: *Dipeptidyl Peptidase IV in Some Neoplastic Diseases* (in Czech). Ph.D. thesis, Faculty of General Medicine, Charles University, Prague, 1990.

ŠEDO A., KŘEPELA E.: Dipeptidyl peptidase IV in human lymphocytes and lymphatic organs (in Czech) *Čs. fysiol.* **34**: 69–84, 1989.

ŠEDO A., KŘEPELA E., KASAFÍREK E.: A kinetic fluorometric assay of dipeptidyl peptidase IV in viable human blood mononuclear cells. *Biochimie (Paris)* **71**: 757–761, 1989.

Reprint Requests:
Dr. A. Šedo, Research Institute of Tuberculosis and Respiratory Diseases, Department of Biochemistry, CS–180 71 Prague 8, Budínova 67.