Published in final edited form as:

Horm Metab Res. 2011 May ; 43(5): 364–366. doi:10.1055/s-0031-1273682.

Decrease in Dipeptidyl Peptidase IV Activity is Linked to the Efficacy of Differentiating Compounds in Follicular Thyroid Carcinoma Cell Lines

E. Fröhlich^{1,2}, E. Engel³, and R. Wahl¹

¹Internal Medicine, Department IV, University of Tübingen, Tübingen, Germany

²Center for Medical Research, Medical University of Graz, Graz, Austria

³Institute of Anatomy, University of Tübingen, Germany

Abstract

Metastasized differentiated thyroid carcinoma is treated by surgery followed by radioiodine remnant ablation. The application of differentiating agents is a possibility of increasing the efficacy of radioiodine therapy. We evaluated DPP IV and aminopeptidase N, both linked to malignancy in thyroid carcinoma, and dipeptidyl peptidase II activities in human follicular thyroid carcinoma cell lines upon treatment with retinol, apicidine, and lovastatin as differentiating agents. Decrease of dipeptidyl peptidase IV (DPP IV) activity may play a role in the differentiating action. In the human cancer cell lines FTC 138 and 238, high DPP IV and low aminopeptidase N activities were recorded. Retinol treatment induced increases in thyroid-specific protein expression [thyroglobulin and sodium-iodide symporter (NIS)], increase in iodide uptake, and decrease in thymidine uptake accompanied by decrease in DPP IV activity. Decreases in DPP IV activities were also seen upon apicidine and lovastatin treatment, which also increased differentiation of the transformed thyrocytes. Our results demonstrate a link between decrease in DPP IV activity and increase in iodide uptake upon stimulation with differentiating agents.

Introduction

Treatment of metastasized differentiated thyroid carcinoma comprises surgery and subsequent radioiodine remnant ablation. Although this treatment is very specific and often effective, recurrence occurs in 20 – 40 % of patients [1]. Several substances like retinoids and thiazolidinediones are already used in clinical trials for redifferentiation treatment of differentiated thyroid carcinoma to increase iodide uptake. Changes in transmembrane proteases such as aminopeptidase N and dipeptidylpeptidase IV have been described in the development of thyroid carcinoma [2]. Dipeptidyl peptidase IV (DPP IV, EC 3.4.14.5), a transmembrane type II glycoprotein with multifaceted function and involved in angiogenesis and invasion, is upregulated in thyroid carcinoma [3].

© Georg Thieme Verlag KG Stuttgart

Correspondence: R. Wahl, Internal Medicine Department of Endocrinology, Metabolism, Nephrology and Clinical Chemistry, University of Tübingen, Otfried-Müller-Straße 10, 72076 Tübingen, Germany, Tel.: + 49/7071/29 83136, Fax: + 49/7071/29 3136, richard.wahl@med.uni-tuebingen.de.

Most normal cells express DPP IV; human thyroid follicle cells are special because they lack activity of this enzyme. DPP IV activity, however, is expressed in differentiated thyroid carcinoma and in moderate to low levels also in anaplastic tumors. The detection of DPP IV activity was suggested as diagnostic marker especially for follicular thyroid carcinoma [4].

To identify a potential role of DPP IV in the redifferentiation of transformed thyrocytes with the perspective of using DPP IV as a new target, activity of this protease was assessed. 2 other proteinases served as controls for specificity of this effect: aminopeptidase N (APN, EC 3.4.11.2) and dipeptidyl peptidase II (DPP II, EC 3.4.14.2). APN is overexpressed in anaplastic thyroid carcinoma [2]; DPP II is expressed in normal thyrocytes [5] and appears not to be linked to malignant transformation [6].

It is known that the proteasome inhibitor bortezomib, which acts antitumorigenic in several cancer cell lines, inhibits DPP IV activity. As human thyrocytes do not express DPP IV, we screened normal porcine thyrocytes, which in contrast to normal human thyrocytes express DPP IV, for comparison to exclude a direct effect of the differentiating compounds tested on protease activities. Agents tested are retinol, the histone deacetylase inhibitor apicidine, and the hydroxymethyl-glutaryl-CoA-reductase inhibitor lovastatin.

Materials and Methods

Tissue samples and cell culture

Primary culture of porcine thyrocytes was performed as described previously [7]. Human thyroid follicular carcinoma cell lines FTC 133 and FTC 238 were obtained from ECACC (European Collection of Cell Cultures). All-*trans* retinol (Sigma), dissolved in methanol, apicidine (Sigma), dissolved in DMSO, and lovastatin (Sigma), dissolved in ethanol, were tested. Effects of the compounds were related to the respective solvents.

Apoptosis, ¹²⁵I-iodide uptake, ³H-thymidine-uptake, and expression of thyroid-specific proteins (thyroglobulin and sodium-iodide symporter, NIS) were determined as described previously (e.g., [8]).

Protease activity

The cleavage of specific synthetic substrates coupled to 4-methoxy-2-naphthylamide (MNA), Gly-Pro-MNA, Ala-MNA, and Lys-Ala-MNA (Bachem) at a concentration of 0.5 mg/ml was used for histochemical localization. For quantification, peptides or amino acids coupled to 7-acetoxy-4-methylcoumarin (AMC) were used. Fluorescence was read at 360/454 nm in a fluorometer (Spectrofluor JC3C, Jobin). Protein content was determined according to Bradford.

Statistics

Differences between the means were analyzed using the student's *t*-test and p-values < 0.05 were considered significant.

Results

All of the tested proteases were strongly expressed in cultured porcine thyrocytes. DPP II activity was localized around the nucleus (Fig. 1a) and DPP IV at the apical membrane (Fig. 1b). The distribution of APN was similar to that of DPP IV.

DPP IV activity was higher in untreated FTC 238 cells (Fig. 1c) than in retinol-treated cells (Fig. 1d). DPP II activity was detected in cells located in the perinuclear region (Fig. 1e) and APN at the plasma membrane (Fig. 1f). Untreated FTC 133 and FTC 238 reacted similarly.

Correlation of changes in DPP IV with differentiation

At 13 μM retinol the effect on sodium-iodide symporter expression and on thyroglobulin expression was slightly better in FTC 238 than in FTC 133 cells, but increases in both proteins were not significant. Retinol caused no changes in protease activity in porcine thyrocytes. In FTC 238 cells DPP IV activity was significantly decreased, APN remained constant and DPP II activity was not significantly increased (Fig. 1g).

Upon addition of retinol, the iodide uptake into thyrocytes increased in a linear way from 6 to 48 h of incubation. The time course of retinol-induced iodide uptake and of DPP IV activity was similar with maximal effects after 48 h. Therefore, we recorded the response to retinol (3.25 – 26 μM) on iodide uptake and on DPP IV activity after 48 h of incubation. The increase in iodide uptake was maximal for 6.5 μM retinol. Upon treatment with 13 μM and 3.25 μM retinol, respectively, the iodide uptake was similar, but lower than for 6.5 μM retinol. No increase in relative iodide uptake compared to the (solvent) controls was recorded at 26 μM retinol. In contrast to the iodide uptake, the decrease in DPP IV activity was most pronounced at 13 and 26 μM retinol. Upon treatment with 6.5 μM retinol, DPP IV activity was similar to the solvent control.

Changes in the ratio of relative iodide uptake (iodide uptake referred to thymidine uptake), in apoptosis and in DPP IV activity of FTC 238 cells upon exposure to all differentiating agents are summarized in Table 1.

Discussion

Here, we report a link between changes in DPP IV activity and differentiating effects (increase in thyroglobulin and sodium-iodide symporter expression, decrease in thymidine uptake and increase in iodide uptake) in follicular carcinoma cell lines.

We used the determination of proteolytic activity by a classic histochemical method in this study. Thyroid carcinomas show increased DPP IV activity as differentiated tumors and increase in APN as anaplastic tumors. We observed that the follicular carcinoma cell lines FTC 133 and FTC 238 displayed higher DPP IV than APN activities. This indicates that these cells show the pattern expected for cells derived from differentiated thyroid cancer and not the one expected for cells derived from anaplastic thyroid carcinoma, which displays high APN levels and low DPP IV levels in vivo. DPP II may play a role in the release of hormone from thyroglobulin; no positive correlation of DPP II activity and malignancy has been reported [6]. It is hypothesized that DPP IV creates a microenvironment favoring the

development and progression of cancer lesions [9]. APN increases cell motility and angiogenesis in some tumors including thyroid carcinoma [2]. Upon treatment of our FTC 238 cells with retinol, DPP IV activity decreased and APN activity remained constant. As expected no changes in the activity of DPP II occurred upon treatment of FTC 238 cells with retinol.

For the identification of a potential link between differentiation and DPP IV activity in transformed thyrocytes, we employed retinol, because retinoids act as differentiating agents in several cell types. If retinoids induced a reversal to normal in transformed thyrocytes an increase in iodide incorporation combined with a decreased DPP IV expression would be expected. The observations in our study are completely consistent with this hypothesis. The effect on DPP IV activity was correlated to the efficacy of retinol in FTC 238 and FTC 133 cells. FTC 133 cells showed relatively smaller increases in the expression of thyroid-specific protein such as NIS and thyroglobulin and in the ratio of iodide uptake to thymidine uptake upon retinol treatment. They also showed a smaller decrease in DPP IV activity. Apicidine also induced a decrease of DPP IV activity in conjunction with an increase in relative iodide uptake. The similar reaction pattern of the histone deacetylase inhibitor apicidine and retinol may be explained by the fact that retinoids act through HDAC-associated corepressor and through histone deacetylase-associated p160 coactivator [10]. Lovastatin, which predominantly induced apoptosis, but did not increase relative iodide uptake very much, reduced DPP IV activity to a lesser extent.

We hypothesize that a decrease of DPP IV activity may be an indicator for the efficacy of differentiating substances. Our data do not prove a causative link, and additional experiments like studies of cell lines derived from papillary and anaplastic thyroid carcinomas, the effect of DPP IV inhibitors, which are used in type 2 diabetes treatment, and experiments on RNA interference are needed.

References

1. Coelho S, Vaisman M, Carvalho D. *Curr Pharm Des.* 2005; 11:2525–2531. [PubMed: 16026305]
2. Kehlen A, Lendeckel U, Dralle H, Langner J, Hoang-Vu C. *Cancer Res.* 2003; 63:8500–8506. [PubMed: 14679016]
3. Fluge O, Bruland O, Akslen LA, Lillehaug JR, Varhaug JE. *Thyroid.* 2006; 16:161–175. [PubMed: 16676402]
4. Aratake Y, Kotani T, Tamura K, Araki Y, Kuribayashi T, Konoe K, Ohtaki S. *Am J Clin Pathol.* 1991; 96:306–310. [PubMed: 1715126]
5. Maes MB, Scharpe S, De Meester I. *Clin Chim Acta.* 2007; 380:31–49. [PubMed: 17328877]
6. Fröhlich E, Maier E, Mack A, Garbe C. *J Dermatol Sci.* 2009; 53:68–71. [PubMed: 18823758]
7. Wahl R, Brossart P, Eizenberger D, Schuch H, Kallee E. *J Endocrinol Invest.* 1992; 15:345–351. [PubMed: 1506619]
8. Fröhlich E, Czarnocka B, Brossart P, Wahl R. *Thyroid.* 2008; 18:1183–1193. [PubMed: 19014326]
9. Wilson MJ, Ruhland AR, Quast BJ, Reddy PK, Ewing SL, Sinha AA. *J Androl.* 2000; 21:220–226. [PubMed: 10714816]
10. Freemantle SJ, Spinella MJ, Dmitrovsky E. *Oncogene.* 2003; 22:7305–7315. [PubMed: 14576840]

Fig. 1.

Localization (red reaction product) and quantification of protease activity with synthetic substrates in cultured normal porcine and in the human thyroid cancer cell lines. Activity of dipeptidyl peptidase II in porcine thyrocytes is strongly expressed and localized in the perinuclear region (**a**). Activity of dipeptidyl peptidase IV in porcine thyrocytes is localized at the apical membrane facing the follicle lumen (**b**) N: nucleus, FL: follicle lumen. In FTC 238 cells, activity of dipeptidyl peptidase IV is seen in the cytoplasm of many cells (**c**). Upon treatment with 13 µM retinol (ROH), the staining decreases in all cells (**d**). FTC 238 cells show staining for dipeptidyl peptidase II activity in a granular pattern in the entire cytoplasm (**e**). Staining of FTC 238 cells for aminopeptidase N activity is low and restricted

to the plasma membrane (**f**). Changes in protease activities upon retinol treatment are minimal in porcine thyrocytes but significant for DPP IV in human FTC 238 cells ($n = 3$, **g**).

Author Manuscript

Author Manuscript

Author Manuscript

Author Manuscript

Table 1

Overview on the action of differentiating compounds on relative iodide uptake, DPP IV activity, and apoptosis in FTC 238 cells^a

Compound	N	Concentration	Relative iodide uptake	DPP IV activity	Apoptosis
Retinol	5	13 µM	2.50 ± 0.12	0.48 ± 0.16	1.1 ± 0.09
Apicidine	6	200 nM	2.25 ± 0.2	0.46 ± 0.27	1.2 ± 0.05
Lovastatin	3	37 µM	1.5 ± 0.9	0.93 ± 0.35	9.3 ± 0.16

^aThe action is indicated as changes related to solvent-related control cultures set as 1