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Differential expression of the components of the plasminogen activating system in human thyroid tumour derived cell lines and papillary carcinomas

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ABSTRACT

We characterised the expression of the plasminogen activators (uPA and tPA), the uPA receptor (uPAR) and the PAs inhibitors (PAI-1 and PAI-2) in human thyroid cell lines derived from normal thyroid, follicular adenoma, follicular, papillary and anaplastic carcinomas. Urokinase PA activity was detected in the supernatant of normal thyrocytes and augmented in those of all tumour cells. Quantitative RT-PCR analysis showed that uPA, uPAR and PAI-1 mRNAs increased in all carcinoma cells. Similar results were found in 13 papillary thyroid carcinoma (PTC) tissues which were mirrored in Western blot experiments. A correlation was found between tumour size and uPA mRNA increase, and higher levels of uPA and uPAR mRNAs were found in metastatic PTC. In conclusion, thyroid carcinoma cell lines and PTC overexpress uPA, uPAR and PAI-1 and the correlation of uPA and its cognate receptor with tumour size and metastasis may suggest their potential prognostic relevance in thyroid cancer.

1. Introduction

The plasminogen activating system (PAS) consists of two serine proteases, the urokinase plasminogen activator (uPA) and the tissue-type plasminogen activator (tPA), their two serpin inhibitors, the plasminogen activator inhibitor 1 (PAI-1) and 2 (PAI-2) and the glycolipid anchored cell membrane receptor for the uPA (uPAR).[1–3] It is involved in many physiological and pathological processes including clot lysis, wound healing, extracellular matrix (ECM) and basement membrane (BM) remodelling, tissue regeneration and angiogenesis.[1,2] A growing number of experimental evidences indicate that the PAS, and especially the uPA/uPAR complexes, affects tumour cell proliferation, migration, adhesion, intravasation and extrava-

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sation as well as tumour angiogenesis.[3–9] Furthermore, along with members of the matrix metalloproteinases (MMPs) family, the PAS is implicated in cancer invasion and metastatisation, in which degradation of ECM and BM allows local diffusion and spread to distant sites of malignant cells.[3–5] The role of PAS in human cancer progression is further supported by clinical evidences demonstrating that high tissue levels of PAS components correlate with a poor prognosis in several malignancies.[3,10–12] This is particularly evident in breast cancer, where uPA has been shown to be one of the most potent prognostic factors described to date, with a predictive value stronger than those of patient age, tumour size, oestrogen and progesterone receptors, HER-2/neu or p53 expression.[3,13,14] In view of their prognostic value, both uPA and PAI-1 are candidate molecular markers for routine clinical use in patients with breast cancer.[3] Moreover, the involvement of PAS at multiple steps during the neoplastic evolution represents a target for anti-cancer therapy, and a number of studies aimed either to inhibit uPA catalytic activity or to prevent uPA binding to uPAR have been performed with success on animals. The results of trials in human cancers are awaited.[15–18]

Despite that, little information regarding the expression of the PAS components during the progression of thyroid cancer is available.[19–27] For this reason, in the present study we first characterised the expression patterns of the different members of the PAS in a clonal strain of human thyrocytes derived from a normal thyroid and in different cell lines derived from a follicular adenoma and from follicular, papillary and anaplastic thyroid carcinomas. This analysis was then extended to 13 normal matched papillary thyroid carcinoma (PTC) tissues obtained following thyroidectomy.

2. Materials and methods

2.1. Cell lines and materials

The cell lines CAL-62,[28] 8305C[29] and B-CPAP[30] were purchased from German Collection of Microorganisms and Cell Cultures (DMSZ, Germany), while FTC-133[27] and MDA-MB-231[31] were bought from Interlab Cell Line Collection (ICLC, Italy).

Dulbecco's modified Eagle's medium (DMEM), RPMI 1640 medium, HAM'S nutrient mixture F-12, phosphate buffered saline (PBS), foetal bovine serum (FBS), 0.05% trypsin, 0.02% EDTA, in PBS, L-glutamine 100× (200 mM) and penicillin/streptomycin solution 100× were purchased from EuroClone (Paignton-Devon, UK). RNAzol™ Bee was provided from Biotech Italia (Roma, Italy). Oligo(dT)$_{12-18}$ primer (0.5 μg/μl), dNTP mix (10 mM), M-MLV reverse transcriptase (200 U/μl), 5X first-strand buffer and DTT (0.1 M) were purchased from Invitrogen (Carlsbad, CA). HotMaster™ Taq DNA polymerase and Perfectprep® Gel Cleanup Kit were obtained from Eppendorf (Hamburg, Germany). All the primers were from Labtek Eurobio (Milano, Italy) and 100 bp DNA ladder was from New England BioLabs (Beverly, MA). Bovine casein, human plasminogen, sodium deoxycholate, aprotinin, leupeptin, phenylmethylsulfonyl fluoride (PMSF), sodium orthovanadate, sodium pyrophosphate and Coomassie brilliant blue R-250 were provided from Sigma Chemical Co. (St. Louis, MO). Nonidet P-40 (NP-40) was from Calbiochem (La Jolla, CA). Precision Protein Standard™, Bradford protein assay kit and electrophoresis reagents were obtained from Bio-Rad Laboratories (Hercules, CA). Microconcentrators Centricon 10 were from Millipore (Bedford, MA). Monoclonal antibodies for the uPA and its receptor (uPAR) were purchased from the American Diagnostica Inc. (Stamford, CT). The monoclonal antibody against actin was obtained from Immunological Sciences (Rome, Italy).

2.2. Cell cultures and human thyroid tissues

CAL-62 and MDA-MB-231 cell lines were cultured in DMEM containing 10% FBS v/v. 8305C and B-CPAP cells were cultured in RPMI 1640 medium with 10% FBS v/v, while the FTC-133 line in DMEM was mixed with HAM'S/F-12 at a ratio of 1:1, both supplemented with 10% FBS v/v. Two millimoles L-glutamine, 100 U/L penicillin and 100 μg/ml streptomycin were added to all the media. The clonal strain of human thyrocytes derived from a normal thyroid (HTU5) and the follicular adenoma derived cell line (HTU42) were grown as previously described.[32–34] The cells were maintained in continuous monolayer cultures at 37 °C and 5% CO_2, expanded up to 80% of confluence, then washed twice and incubated in the respective serum-free media for 24 h. At the end of this period, the conditioned media (CM) were collected and cellular residues eliminated by centrifugation at 1200 rpm for 5 min, after which the CM were concentrated 35-fold in Centricon 10 concentrators. At the same time the incubated cells were scraped in PBS, centrifuged, resuspended in RIPA buffer (50 mM Tris–HCl, pH 7.4, 1% NP-40 v/v, 0.5% sodium deoxycholate v/v, 150 mM sodium chloride, 1 mM EDTA, 1 mM sodium fluoride, 1 mM PMSF, 10 μg/ml aprotinin, 10 μg/ml leupeptin, 1 mM sodium orthovanadate and 10 mM sodium pyrophosphate in ddH_2O) and sonicated. The extracts were centrifuged at 10,000 rpm for 10 min to remove cellular fragments, and protein concentrations were determined with Bradford assay.[35]

Fragments of matched normal and tumour thyroid tissues were obtained from surgical specimens of 13 female patients (age ranging from 26 to 76 years) affected by the classical variant ($n = 7$) or the follicular variant ($n = 6$) of papillary thyroid carcinoma. Tissue samples were immediately frozen in liquid nitrogen, stored at −80 °C and then used for the preparation of total RNA or protein extracts as described below.

2.3. Substrate gel electrophoresis (zymograms)

SDS–PAGE zymograms containing 0.1% casein w/v plus 12 μg/ml plasminogen were prepared as described.[36] Conditioned media of the different cell lines were compared according to cell proteins concentrations or cell number. Aliquots of concentrated conditioned media corresponding to 2–4 μg of cell proteins were used. After the electrophoretic run, gels were rinsed once in 50 mM Tris–HCl (pH 7.4) with 2% Triton X-100 v/v and further washed in 50 mM Tris–HCl (pH 7.4). To detect the activity of the plasminogen activators, gels were incubated at 37 °C for 4 h in a buffer containing 50 mM Tris–HCl (pH 7.4) and 0.1% Triton X-100 v/v. Identical gels were incubated in the above buffers containing 10 μM aprotinin and 1 mM PMSF in order to assess the enzymes specificity. Finally, the gels were stained with a solution of 0.1% Coomassie brilliant blue in

25% methanol and 7% acetic acid, and destained in the same mixture without dye. Clear zones against the blue background indicated the presence of proteolytic activity. Samples lytic bands were compared with those formed by the MDA-MB-231 supernatant, used as a standard for the PAs activities.[31] The molecular weights of each band were evaluated in comparison with a prestained protein ladder using the UVIPRO GEL Documentation System (Eppendorf). Enzymatic bands were quantified by scanning densitometry using Molecular Analyst PC™ software for the Biorad model 670 scanning densitometer.

2.4. *Extraction and analysis of mRNA*

Total cellular RNA was extracted from the different cell lines by the acid guanidinium thiocyanate–phenol–chloroform method of Chomczynsky and Sacchi.[37] The same protocol was used to obtain total RNA from normal and tumoural human thyroid tissues following homogenisation of the samples by ultra-turrax in guanidinium thiocyanate. The purity and integrity of the RNA preparations were checked spectroscopically and by agarose gel electrophoresis before carrying out the analytical procedures. Five micrograms of total RNA was reverse-transcribed and the obtained cDNAs were used as a template for the subsequent quantitative PCR amplifications of the different components of the plasminogen activating system with human β-actin as internal control, using specific primers described in Table 1. Controls for DNA contamination were performed omitting the reverse transcriptase or the RNA during reverse transcription. Quantitative real-time PCR assay was performed on the LightCycler instrument (Roche Diagnostics), employing the FastStart DNA Master SYBR Green I Kit (Roche Applied Sciences). The reactions were set up in a final volume of 20 μl, containing heat-activatable *Taq* polymerase, 0.5 μM of specific primers, described in Table 1, and 1.25 ng of cDNA template. Following polymerase activation (95 °C for 10 min), 40 cycles were run with 10 s denaturation at 95 °C, 10 s annealing at optimal temperatures for each primers pair and 25 s extension at 72 °C. The temperature transition rate was 20 °C/s for all steps. The double-stranded PCR product was measured once in every cycle during the extension step by detection of fluorescence emitted by the binding of SYBR Green I to the amplification products. All samples were processed in triplicate and PCR-grade water was used as a negative control. Standard run curves were generated for each gene using five serial dilutions of a cDNA mixture expressing all the genes analysed. The PCR products were analysed on 2% agarose gel and stained with ethidium bromide. To determine the specificities of amplified cDNAs, they were recovered from the gel, purified with a gel clean-up kit and subjected to sequencing reactions in presence of fluorescent-labelled nucleotides, then analysed by ABI Prism 377™ DNA sequencer (Perkin Elmer). All the obtained sequences corresponded to the expected ones (data not shown). The results were analysed at the end of the run with the LightCycler software, version 1.5 (Roche Diagnostic). The crossing points (C_p) for each reaction were determined and calculation of data was performed with the $\Delta\Delta C_p$ method, using the LightCycler relative quantification software 1.0 (Roche Diagnostics). Expression of the target genes in the tumoural thyroid tissues or tumour derived cell lines was normalised, respectively, against the expression found in the matched normal tissues or the HTU5 cells, and reported as fold of variation.

2.5. *Western blot*

Normal and tumour tissue samples were homogenised in RIPA buffer (50 mM Tris–HCl, pH 7.4, 1% NP-40 v/v, 0.5% sodium deoxycholate v/v, 150 mM sodium chloride, 1 mM EDTA, 1 mM sodium fluoride, 1 mM AEBSF, 10 μg/ml aprotinin, 10 μg/ml leupeptin, 1 mM sodium orthovanadate and 10 mM sodium pyrophosphate in ddH_2O) by ultra-turrax, sonicated and centrifuged at 10,000 rpm for 10 min. Protein concentrations in the supernatants were determined by the Bradford assay. Aliquots of 50 μg of tissue extracts were supplemented with 5X Laemmli buffer (120 mM Tris–HCl, pH 6.8, 2% SDS v/v, 10% glycerol v/v and 0.01% Bromophenol Blue w/v), heated at 95 °C for 5 min, electrophoresed on a 12.5% polyacrylamide gel v/v and transferred onto nitrocellulose membranes using the Biorad Mini Trans-Blot Cell system.

Table 1 – Primers, genomic positions, size of amplified products and annealing temperatures used in the PCR for the different components of the plasminogen activating system

Gene	Primers	Exon	Size (bp)	T_{ann} (°C)
uPA	Forward 5′-GCCATCCCGGACTATACAGA-3′ Reverse 5′-AGGCCATTCTCTTCCTTGGT-3′	8 10	417	60
tPA	Forward 5′-TCTTAGATTTCGTGTGCCAG-3′ Reverse 5′-CTCTGAGCTGTACTTCCCCG-3′	5 7	296	60
uPAR	Forward 5′-CTGGAGCTGGTGGAGAAAAG-3′ Reverse 5′-TGTTGCAGCATTTCAGGAAG-3′	3 5	406	60
PAI-1	Forward 5′-ATACTGAGTTCACCACGCCC-3′ Reverse 5′-GTGGAGAGGCTCTTGGTCTG-3′	3–4 5–6	320	62
PAI-2	Forward 5′-GGCCAAGGTGCTTCAGTTTA-3′ Reverse 5′-GGGATTTTGCCTTTGGTTTG-3′	2–3 5–6	384	62
β-Actin	Forward 5′-CAAGAGATGGCCACGGCTGCT-3′ Reverse 5′-TCCTTCTGCATCCTGTCGGCA-3′	3 4	275	62

The membranes were then washed with TBST (50 mM Tris–HCl, pH 7.5, 150 mM NaCl and 0.05% Tween-20 v/v) and saturated with 5% low fat milk w/v in TBST for 2 h at room temperature. Incubations with primary antibodies were performed for the identification of uPA and uPAR in 2.5% low fat milk w/v in TBST at 4 °C overnight. The monoclonal antibodies raised against uPA (1:1000) and uPA receptor (uPAR) (1:250) were detected with anti-mouse (1:1000) horseradish peroxidase conjugated secondary antibodies (Pierce, Rockford, IL). Samples loadings in the different Western blots were controlled with the monoclonal anti-actin antibody (1:500). The Western blots were revealed by chemiluminescence Super Signal kit from Pierce (Rockford, IL). The native human urokinase (Immunological Sciences, Rome, Italy) was used as a standard.

2.6. *Statistical analysis*

The results are expressed as means ± SEM of at least three independent experiments and values were statistically compared using the Student's *t*-test. Statistical significance of the expression at mRNA and protein levels of the different components of the plasminogen activating system in tumour tissues versus normal matched tissues were evaluated by the non-parametric Wilcoxon rank sum test using the Epistat computer program. Correlation analysis between the fold of increase of uPA mRNA and tumour size, represented by the major diameter of the lesion, was evaluated by the Pearson correlation test using the SPSS software (SPSS Inc., Chicago, IL). The results were determined to be significantly different if p values were lower than 0.05.

3. Results

The enzyme activity of the plasminogen activators (PAs) present in the 24 h serum-free conditioned media (CM) of the different cell lines, evaluated accordingly to cell protein concentrations, was analysed by zymographic analysis, in comparison to that of the CM of the breast cancer cell line MDA-MB-231, known to secrete both PAs.[31] The results revealed in the presence of the urokinase PA (uPA) activity in all cell lines analysed (Fig. 1A). Densitometric analysis of three independent experiments showed that, with respect to the HTU5 cells, uPA activity was significantly increased by 1.44 ± 0.16 fold in the CM of HTU42 cells ($p < 0.05$), by 2.97 ± 0.28 and 2.58 ± 0.14 ($p < 0.01$), respectively, in the CM of the FTC-133 and B-CPAP cell lines, by 4.41 ± 0.86 in the CM of the CAL-62 ($p < 0.05$) and by 2.30 ± 0.19 ($p < 0.01$) in that of the 8305C cells (Fig. 1B). In Fig. 1A it may also be appreciated in the CM of the CAL-62 and, to a lesser extent, in that of the FTC-133 cells, the presence of an enzymatic band of approximately 35 kDa, likely due to the low molecular weight uPA (LMW uPA).[31] In addition, a major lytic band of approximately 110 kDa, likely due to the formation of complexes between PAs and their inhibitors (PAIs), was barely detectable in the supernatant of the HTU5 cells and present at higher levels in all tumoural cell types. In normal as well as in all tumour-derived cell lines, tPA activity was never detected. Similar results were obtained when the CM from the different cell lines were compared according to the cell number. Incubation of

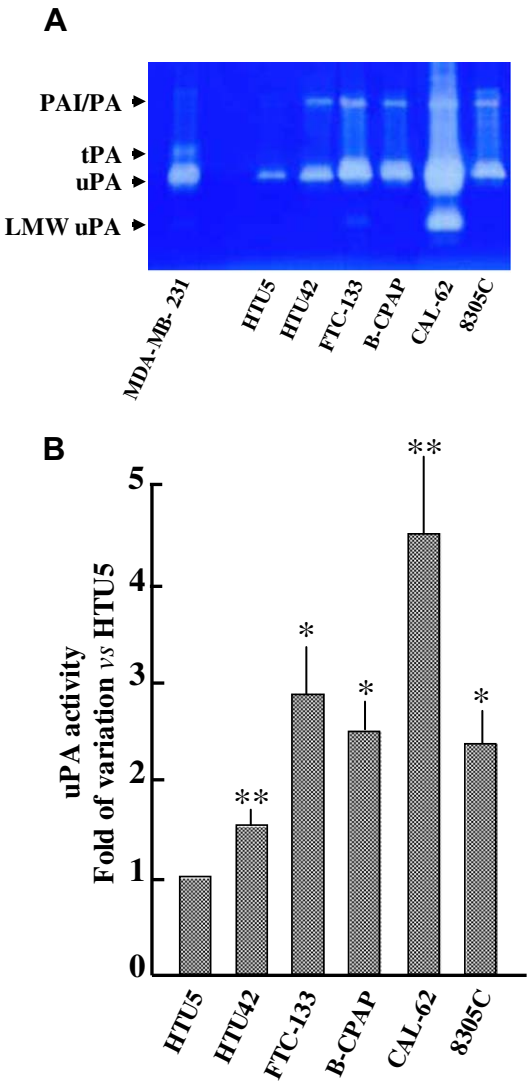

Fig. 1 – Zymographic analysis of the plasminogen activators present in the conditioned media (CM) of a clonal strain of thyrocytes derived from a normal human thyroid (HTU5 cells) and different human thyroid tumour derived cell lines. The different cell types were maintained in continuous monolayer cultures, expanded up to 80% of confluence, then washed and incubated in the respective serum-free media for 24 h. Following incubation, the CM have been collected and analysed by zymography in comparison with that of the human breast carcinoma cell line MDA-MB-231, as described in Section 2. In panel A a representative zymography out of three independent experiments is reported. In panel B the mean ± SEM of the densitometric analysis of three independent experiments is reported. Folds of variations have been calculated considering the value observed for the HTU5 cells as equal to 1. $^{*}p < 0.01$, $^{**}p < 0.05$.

gel with PMSF and aprotinin abrogated all enzyme activities (data not shown).

Quantitative RT-PCR analysis, performed on total RNA extracted from the different cell lines, demonstrated the presence of uPA mRNAs in all cell types analysed (Fig. 2). Its level, with respect to the HTU5, was not significantly changed

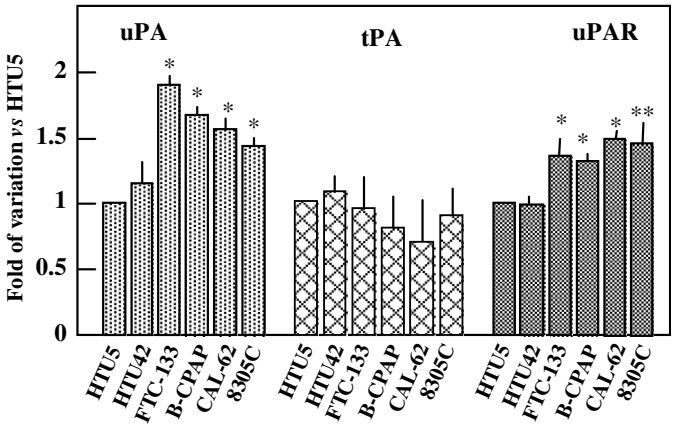

Fig. 2 – Messenger RNA levels of tPA, uPA and uPAR in a clonal strain of thyrocytes derived from a normal human thyroid (HTU5 cells) and different human thyroid tumour derived cell lines. In the figure the mean ± SEM of the results of three independent quantitative RT-PCR experiments is reported. Folds of variations have been calculated considering the ratio between tPA, uPA or uPAR and β-actin observed in HTU5 cells as equal to 1. $^{*}p < 0.01$, $^{**}p < 0.05$.

in the HTU42 cells, while a significant increase was found in all carcinoma cell lines (Fig. 2). In the same experiments we could also observe the presence of low levels of tPA mRNA, which did not considerably differ in all cell lines studied. In Fig. 2 the mRNA expression of the uPA receptor (uPAR) is also reported, which was significantly augmented in all carcinoma cell lines, but not in the HTU42 cells, with respect to the HTU5 cells. We also evaluated the expression of the two PAs inhibitors, PAI-1 and PAI-2. As evident from Fig. 3, both inhibitors were expressed by the HTU5 cells and their expression was unchanged in the HTU42 cells. However, while the level of PAI-1 mRNA was significantly increased in all malignant cell lines, that of PAI-2 was reduced in the B-CPAP cells by about 40% ($p < 0.05$), and absent in the anaplastic cells CAL-62 (Fig. 3).

We next evaluated the mRNA levels of PAS components in 13 normal matched papillary thyroid carcinoma (PTC) tissues. Quantitative RT-PCR analysis showed that uPA, uPAR and PAI-1 mRNAs were significantly ($p < 0.01$) increased by 4.42 ± 0.95, 4.36 ± 1.73 and 3.55 ± 0.79 fold, respectively, in tumour tissues with respect to normal matched ones. On the other hand, tPA and PAI-2 mRNAs were not significantly changed (Fig. 4). The increased expression of uPA and its receptor was confirmed at

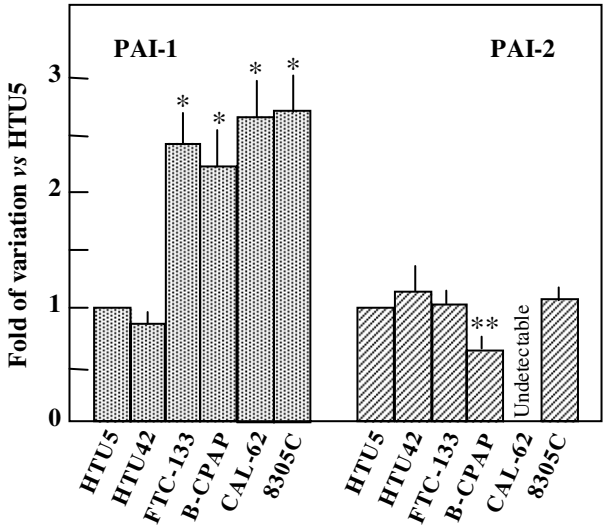

Fig. 3 – Messenger RNA levels of PAI-1 and PAI-2 in a clonal strain of thyrocytes derived from a normal human thyroid (HTU5 cells) and different human thyroid tumour derived cell lines. The levels of PAI-1 and PAI-2 mRNAs were evaluated by quantitative RT-PCR, as described in the Section 2. Data represent means ± SEM of three independent experiments. Fold of variations have been calculated considering the ratio between PAI-1 or PAI-2 and β-actin observed in HTU5 cells as equal to 1. $^{*}p < 0.01$, $^{**}p < 0.05$.

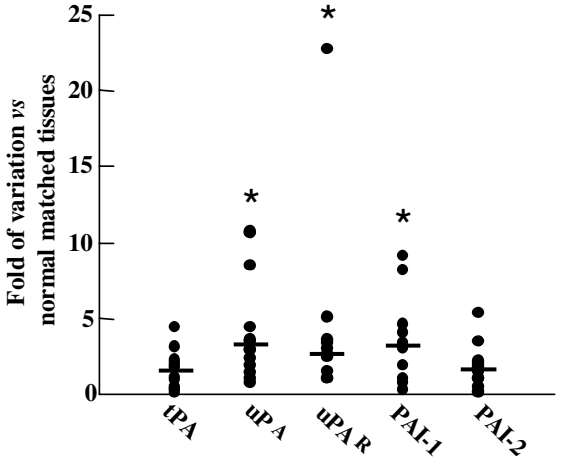

Fig. 4 – Expression of the plasminogen activating system (PAS) components in normal matched papillary thyroid carcinoma tissues (PTC). Quantitative RT-PCR analysis of PAS components in 13 normal matched PTC tissues was performed as described in Section 2. Folds of variations have been calculated considering the ratio between the different PAS components and β-actin observed in normal thyroid tissues as equal to 1. Statistical evaluation of data has been performed by the Wilcoxon test. The small bars in the graph indicate the median values. $^{*}p < 0.01$.

the protein level by Western blot experiments performed on three available normal matched PTC tissues (Fig. 5A). In particular, densitometric analysis of three independent experiments showed a significant increase of uPA and uPAR protein levels of 3.07 ± 0.73 and 4.62 ± 1.5 fold ($p < 0.01$), respectively (Fig. 5B).

We then sought to verify whether uPA, uPAR and PAI-1 mRNAs levels in PTC tissues were related with tumour size. As shown in Fig. 6, a significant ($p < 0.01$) correlation ($r = 0.709$) between uPA mRNA, expressed as fold of increase versus normal matched tissue, and tumour size was observed. On the other hand, no significant correlation was demonstrated between tumour size and uPAR or PAI-1 mRNA levels. We finally compared the fold of variation of the different PAS components mRNAs between PTC tissues from patients with (N1, $n = 4$) or without (N0, $n = 8$) lymph node metastases. As shown in Fig. 7, uPA and uPAR mRNAs were significantly higher in metastatic PTC ($p < 0.05$).

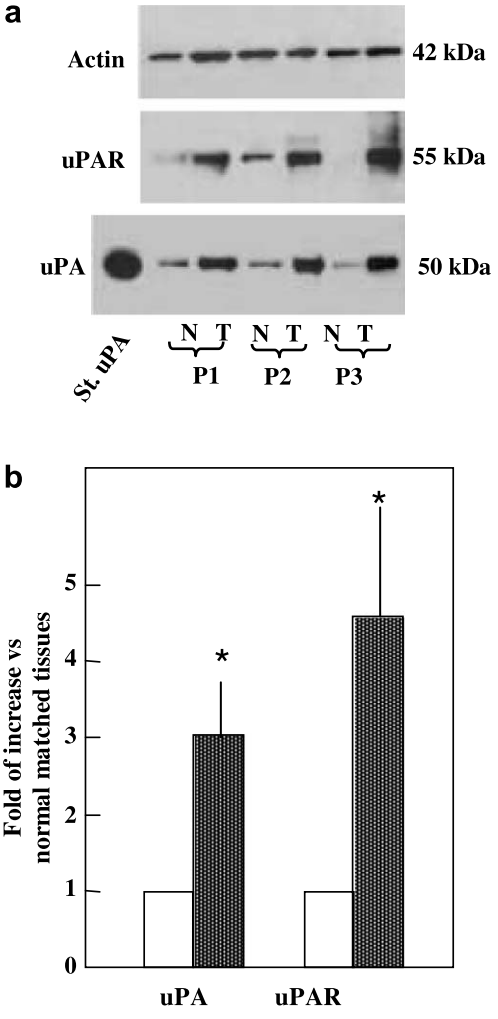

Fig. 5 – Western blot analysis of uPA and uPAR in three normal matched papillary thyroid cancer tissues. Fifty micrograms of the different tissue protein extracts was loaded in each lane and subject to Western blot as described in Section 2, using specific monoclonal antibodies against uPA, uPAR and β-actin as protein loading control. In panel A, data reported represent one out of three independent experiments. In panel B the mean ± SEM of the densitometric analysis of three independent experiments is reported. $^{*}p < 0.01$.

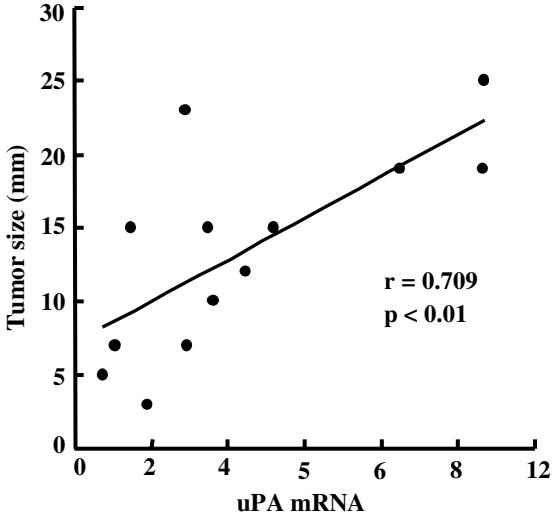

Fig. 6 – Correlation analysis between uPA mRNA and papillary thyroid cancer (PTC) sizes increases. Folds of uPA mRNA increase in PTC versus normal matched tissues were calculated as described in Section 2.

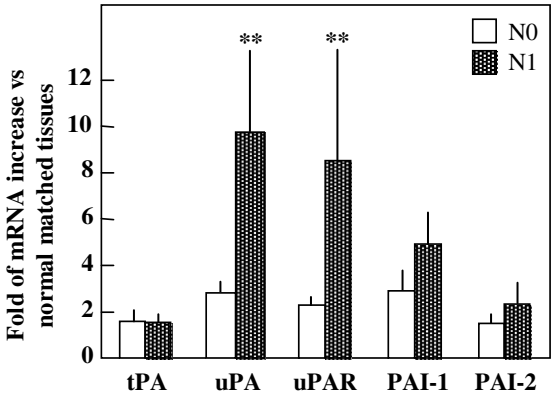

Fig. 7 – Comparison of plasminogen activating system (PAS) component mRNA levels between papillary thyroid carcinoma (PTC) tissues from patients with (N1) or without (N0) lymph node metastases. Quantitative RT-PCR analysis of the PAS components was performed, as described in Section 2, in normal matched PTC tissues obtained from eight patients without lymph node metastases and from four patients with lymph node metastases. Folds of variations have been calculated considering the ratio between the different PAS components and β-actin observed in normal thyroid tissues as equal to 1. Statistical evaluation of data has been performed by the Wilcoxon test. $^{*}p < 0.01$, $^{**}p < 0.05$.

4. Discussion

Several reports demonstrate that the components of the PAS, along with matrix metalloproteinases (MMPs) family members, are involved in multiple aspects of the neoplastic

progression.[3,5–9] Despite that, little information regarding the expression of PAS in normal thyroid follicular cells and its role in thyroid tumours is available. In the present study, we have first characterised the expression of the PAS components in a clonal strain of human thyrocytes derived from a normal thyroid (HTU5), and cell lines derived from the different histological types of thyroid tumours, including a follicular adenoma. Our results demonstrate that thyrocyte transformation is associated with increased expression of uPA, uPAR and PAI-1, while PAI-2 was either unchanged or decreased. Importantly, the same changes were observed in papillary thyroid carcinoma, with respect to matched normal tissues. These findings corroborate our previous study demonstrating that different MMPs, including MMP-1, -2, -9, -11, -13 and -14, were either increased or induced *de novo* in different carcinoma cell types, compared to normal human thyrocytes.[38] Since plasmin as well as uPA can directly activate pro-MMPs, all together these observations suggest the increased competence of malignant thyroid tumour cells to degrade and invade ECM and BM, required for tumour progression and metastatisation. In this context, it has been demonstrated that the capacity to degrade ECM by one of the carcinoma cell lines here analysed, the FTC-133, was strongly reduced by the inhibition of either uPA or MMPs.[27] Furthermore, we here demonstrate in tumour tissues a significant correlation between uPA mRNA increase and PTC size. In addition, the expression of both uPA and its cognate receptor was higher in metastatic PTC. Considering that tumour size and lymph node metastases are major prognostic factors for differentiated thyroid cancers,[39] these observations, if confirmed in larger case studies, suggest that uPA could represent a valuable prognostic factor in thyroid cancer, as reported for other malignancies.[3,13,14]

The expression of PAs in thyroid carcinomas has been documented in very few studies[23,24,27] and, to the best of our knowledge, the experiments here reported represent the first comparison of PAs expression among cell lines derived from normal thyroid and different histotypes of human thyroid tumours. In the former and in normal thyroid tissues we demonstrated the presence of uPA and, at a lower level, of tPA mRNAs; however, we did not detect tPA proteolytic activity in cell conditioned media, while uPA activity was clearly found. This would suggest that tPA mRNA in normal human thyrocytes is translated at levels which fall below the sensitivity of our zymographic analysis. In all tumour cell lines and PTC tested, tPA mRNA was unchanged, while a significant increase of uPA mRNA and activity was found in all carcinoma cell lines, and to a minor extent, in the follicular adenoma derived cell line HTU42. The same expression profile was found in PTC with respect to normal matched tissue.

We also observed a significant induction of uPAR gene expression in all carcinoma cell lines and PTC, with respect to HTU5 cells and normal matched tissue. These findings corroborate a previous immunohistochemical study in which it was suggested that the analysis of uPAR expression might be useful for the differential diagnosis of follicular adenoma from carcinoma, and that uPAR may have a prognostic value for PTC.[25] In this context, it is relevant that uPAR mRNA level is higher in metastatic PTC with respect to the non-metastatic ones. Urokinase PAR, whose action was originally thought to be limited to the activation of the zymogen uPA at the leading edge of the cells, is now considered an important initiator of signal-transduction pathways able to elicit a plethora of cellular responses, including adhesion, differentiation and proliferation.[6] Moreover, it also has to be considered that the upregulation of the uPA/uPAR system may hold a role in the activation of latent growth factors able to drive thyrocyte proliferation, such as the cleavage of the hepatocyte growth factor (HGF) allowing its dimerisation and binding to its receptor Met.[26]

The role of the two PAIs in tumour progression still remains controversial. PAI-1 is considered the primary inhibitor of uPA, and its interaction with the uPA bound to uPAR causes the rapid internalisation of the complexes. Consequently, an increased expression of PAIs in cancer tissues would be expected to correlate with a low probability of metastasis and with a favourable outcome. On the contrary, high levels of PAI-1 expression are associated with aggressive disease and poor prognosis in several types of cancers.[2,3,12] Several hypotheses have been put forward to explain these observations, including an essential role of PAI-1 in tumour neoangiogenesis, modulation of cells adhesion, migration and inhibition of apoptosis.[3] Our results demonstrated that PAI-1 mRNA is significantly increased in all malignant cell lines and PTC, with respect to HTU5 cells and matched normal tissues. However, in our study no correlation between PAI-1 mRNA increases and PTC tumour size could be observed. In addition, similar PAI-1 mRNA levels were found in metastatic and non-metastatic PTC. Regarding PAI-2, its expression was found either unchanged or suppressed in the carcinoma cell lines and not significantly changed in PTC with respect to matched normal thyroid tissues.

In conclusion, we demonstrated that both human thyroid carcinoma derived cell lines and PTC overexpress uPA, uPAR and PAI-1, and that uPA overexpression correlates with PTC size. Furthermore, the expression of uPA and its cognate receptor was higher in metastatic PTC, thus suggesting their potential prognostic relevance in thyroid cancers, as proved for other malignancies.

Conflict of interest statement

None declared.

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