CLINICAL STUDY

Platelet-activating factor and human thyroid cancer

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Abstract

Objective: Platelet-activating factor (PAF) is a pro-inflammatory and angiogenic lipid mediator involved in several types of cancer in humans. The levels of PAF, lyso-PAF (the PAF precursor), phospholipase A₂ activity (PLA₂, the enzymatic activity implicated in lyso-PAF formation) and acetylhydrolase activity (AHA, the PAF-degrading enzyme) were investigated in various diseased thyroid tissues.

Subjects: Control and diseased tissue of patients with a hyperplastic goitre ($n = 14$), a benign adenoma ($n = 12$) and a papillary thyroid carcinoma ($n = 15$) were investigated.

Results: PAF receptor transcripts were found in the human thyroid tissue. PAF, lyso-PAF, PLA₂ and AHA were present in control thyroid tissues, their levels being significantly correlated with each other, suggesting tiny regulations of the PAF metabolic pathways inside the thyroid gland. PAF, lyso-PAF, PLA₂ and AHA levels remained unchanged in diseased tissues of patients with a hyperplastic goitre, a benign adenoma and a papillary thyroid carcinoma. No difference was found between PAF, lyso-PAF, PLA₂ and AHA levels with respect to the TNM tumour status and the histological subtype of papillary thyroid carcinoma. No correlation was found between tissue PAF levels and those of vascular endothelial growth factor and basic fibroblast growth factor, two angiogenic growth factors involved in thyroid cancer and that mediate their effect through PAF release in breast and colorectal cancer.

Conclusion: PAF, PAF receptor transcripts and the enzymatic activities implicated in PAF production and degradation are present in the thyroid gland. While the physiological role of PAF is presently unknown in thyroid physiology, this study highlights no evidence for a potentially important role of PAF during human thyroid cancer, a result that markedly differs from breast and colorectal ones.

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Introduction

Thyroid cancer is the most common endocrine malignancy and accounts for the majority of endocrine cancer deaths each year (1). Several events have been found in association with the progression from normal thyroid tissue to thyroid cancer. They include factors that promote tumour proliferation and that affect cell differentiation, adhesion, immortalisation and death (1). Modifications in cancer cell membrane metabolism and membrane-related lipid functions are important aspects of tumour development. Thus, several lipid molecules (such as phosphatidic acid, cyclic lysophosphatidic acid, ceramide and sphingosine 1-phosphate) that act as both intracellular messengers and extracellular mediators are reported to affect tumour development and neoplasia (2–4). Alterations in phospholipid concentrations are reported in plasma and thyroid tissue of

patients with thyroid carcinoma (5, 6), and lysophosphatidic acid is a potent growth factor for human thyroid cells (7). The lipidic molecule platelet-activating factor (PAF) sparks a wide range of immunoregulatory actions (8). A phospholipase A₂ (PLA₂)-dependent process on membrane alkyl-acyl-glycerophosphocholines generates lyso-PAF (the PAF precursor), and its subsequent acetylation results in the PAF molecule. Tissue PAF concentrations are physiologically down-regulated by a specific PAF acetylhydrolase activity (AHA) (9). The PAF receptor (PAF-R) gene produces three different species of mRNA (i.e. transcript 1, transcript 2 and an elongated form of the transcript 2) which generate a unique membrane PAF-R (10, 11). During the past decade, *in vitro* studies and animal models have highlighted a potential role of PAF in cancer. Thus, PAF acts on the growth of various human tumour cell lines (12, 13), increases adhesiveness of tumour cells to vascular

endothelia (14), enhances oncogene expression (15), and can contribute to tumour development by enhancing cell motility and by stimulating the angiogenic response (16, 17). Thus, PAF is reported to mediate the effects of vascular endothelial growth factor (VEGF) (18) and basic fibroblast growth factor (bFGF) (19) that play a key role during the angiogenesis of human cancer, including thyroid ones (20). In humans, the contribution of PAF is suspected in lung, breast and colorectal cancer (17, 21–24). To improve our knowledge concerning the role of PAF in human cancer we have focused our attention on its potential involvement in thyroid carcinoma. In this study we thus investigated the levels of PAF and of the lyso-PAF precursor, and the enzymatic activities implicated in PAF production (i.e. PLA₂) and degradation (i.e. AHA) in various diseased thyroid tissues including epithelial thyroid cancer. We also searched for the presence of PAF-R transcripts in thyroid tissue and for a putative link between PAF levels and those of bFGF and VEGF.

Subjects and methods

Subjects

The procedure of the present study followed the rules edited by the French National Ethics. Between January 2003 and December 2003, 76 patients underwent surgery for thyroid pathology in our institution. To obtain a homogeneous group we enrolled exclusively patients clinically euthyroid and with normal concentrations of serum thyrotrophin and thyrocalcitonin. Patients operated on for autonomously solitary toxic adenoma, toxic multinodular goitre, Graves' disease, medullary or anaplastic carcinoma and less than 14 years old were excluded. Forty-one patients were investigated. They were treated by unilateral thyroidectomy, or total thyroidectomy and cervical lymph node resection if carcinoma was discovered. Fifteen patients had a papillary

thyroid carcinoma. Demographic data of these patients (including sex, age, presence or absence of an associated lymphoid thyroiditis, TNM status, and histological subtype of papillary thyroid carcinoma) are reported in Table 1. The new UICC 6th edition TNM classification system of malignant tumours was used for classifying the anatomical extent of malignant disease and patients' stage (25). Twelve patients had benign adenomas and 14 had a hyperplastic goitre. Demographic data for these patients (including sex, age and presence or absence of an associated lymphoid thyroiditis) are reported in Table 2. Specimens of the pathological thyroid tissue and the control tissue close to the pathological one were obtained during the surgical procedure. Specimens were frozen at –80°C until use.

PAF assay

Tissue samples were ethanol-extracted (80% final), purified using thin-layer chromatography (TLC), and assayed for PAF activity by aggregation of washed rabbit platelets as previously reported (21, 23, 24). The aggregating activity of samples was measured using a calibration curve obtained with 2.5–20 pg synthetic PAF (Novabiochem, Switzerland). Results were expressed as pg PAF/mg tissue. The lipid compound extracted from blood was further characterised on the basis of its aggregating activity in the presence of 0.1 mmol/l BN 52 021 (Tebu, Le Perray-en-Yvelines, France), a specific PAF-R antagonist, and its retention time during TLC.

Assay of lyso-PAF

Lyso-PAF was measured in ethanolic biopsy samples after its chemical acetylation into PAF as previously described (21, 23, 24). To summarise, ethanolic samples were dried, then mixed with 200 µl pyridine and 200 µl acetic anhydride and kept overnight, in the dark, at room temperature. After evaporation of

Table 1 Demographic data of patients with papillary thyroid carcinoma.

Patient	Sex	Age (years)	Lymphoid thyroiditis	TNM status	Stage	Histological subtype of papillary thyroid carcinoma
1	F	78	No	T4a N1b M1	IV	Tall cell variant
2	F	49	Yes	T1 N0 M0	I	Conventional
3	F	45	No	T1 N0 M0	I	Conventional
4	F	92	No	T3 N1a M0	III	Follicular variant
5	M	76	No	T2 N1b M0	IV	Follicular variant
6	F	91	No	T4a N1a M0	IV	Conventional
7	F	20	No	T2 N0 M0	II	Follicular variant
8	F	69	No	T3 N0 M0	III	Follicular variant
9	F	47	No	T3 N1b M0	IV	Conventional
10	M	63	No	T3 N1b M1	IV	Follicular variant
11	M	17	Yes	T1 N0 M0	I	Follicular variant
12	M	76	No	T4 N1a M0	IV	Conventional
13	F	64	No	T1 N0 M0	I	Conventional
14	F	56	No	T1 N0 M0	I	Conventional
15	F	41	Yes	T1 N0 M0	I	Conventional

Table 2 Demographic data of patients with benign adenomas and hyperplastic goitre.

Patient number	Sex	Age(years)	Disease	Lymphoid thyroiditis
1	F	48	Benign adenoma	No
2	M	68	Benign adenoma	No
3	F	35	Benign adenoma	No
4	F	40	Benign adenoma	Yes
5	F	43	Benign adenoma	No
6	F	75	Benign adenoma	Yes
7	F	39	Benign adenoma	No
8	F	50	Benign adenoma	Yes
9	F	43	Benign adenoma	No
10	F	39	Benign adenoma	Yes
11	M	55	Benign adenoma	No
12	F	43	Benign adenoma	Yes
13	F	43	Hyperplastic goitre	No
14	F	37	Hyperplastic goitre	No
15	F	35	Hyperplastic goitre	No
16	F	51	Hyperplastic goitre	No
17	F	76	Hyperplastic goitre	No
18	F	39	Hyperplastic goitre	No
19	F	73	Hyperplastic goitre	No
20	F	69	Hyperplastic goitre	No
21	M	51	Hyperplastic goiter	No
22	M	52	Hyperplastic goiter	No
23	F	26	Hyperplastic goiter	No
24	F	59	Hyperplastic goiter	No
25	F	66	Hyperplastic goiter	No
26	F	50	Hyperplastic goiter	No

the reagents and removing of the traces of pyridine with chloroform, the dried samples were retrieved with 100 µl 60% ethanol, and PAF was bioassayed as described before. The amount of lyso-PAF was established as the difference between the quantity of PAF measured before and after acetylation of the sample. Results were expressed as pg PAF/mg tissue.

AHA assay

Frozen biopsy specimens were pulverised and homogenised in 1 ml AHA buffer (NaCl, 140 mmol/l; KCl, 3 mmol/l; Hepes, 4 mmol/l; EDTA, 22 mmol/l). After centrifugation, supernatants were used for AHA, PLA₂, VEGF and bFGF determinations. AHA was assessed as previously reported (21, 23, 24). Briefly 10⁵ d.p.m. [³H]acetyl-PAF (10 Ci/mmol; NEN), 0.1 mmol/l PAF and AHA buffer (pH 8) in a final volume of 450 µl, and 50 µl of tissue extract supernatants were incubated for 30 min at 37°C. The reaction was stopped with 100 µl BSA (10%) and 400 µl trichloroacetic acid (20%). Samples were centrifuged (1500 g, 15 min) and supernatants were counted in a liquid scintillation counter. Results were expressed as fmol PAF degraded/min per mg tissue as means of duplicate assays. Variation between duplicates was less than 7%.

PLA₂ activity assay

PLA₂ levels were assessed by ELISA according to the manufacturer's recommendations (R&D Systems

Europe, Oxon, UK) and as previously described (23, 24). Results were expressed as international units (IU) per mg of tissue as means of duplicate assays. Variation between duplicates was less than 6%.

RT-PCR of PAF-R transcripts

Total RNA from tissue samples extracted with RNAwiz (Ambion, Austin, TX, USA) was reverse-transcribed and the cDNA was amplified by PCR as previously described (21, 23, 24). The human PAF-R transcript 1 sense primer was 5'-GACAGCATAGAGGCTGAGGC-3', the transcript 2 sense primer was 5'-CCTGAGCTCCCGAGAAGTCA-3' and the antisense primer was 5'-TAGCCATTAGCAATGACCCC-3. The glyceraldehyde-3-phosphate dehydrogenase (GAPDH, a positive control of PCR amplification) sense and anti-sense primers were 5'-GGCTGAGAACGGGAAGCTTG-3' and 5'-GGATG-ATGTTCTGGAGAGCC-3'. PCR products were electrophoresed on a 2% agarose gel (Gibco) and visualised by ethidium bromide staining. Expected sizes of amplified products were: PAF-R transcript 1, 225 bp; PAF-R transcript 2, 269 bp; spliced variant of PAF-R transcript 2, 351 bp; GAPDH, 439 bp.

VEGF and bFGF assays

VEGF and bFGF were detected by specific ELISA assays (DuoSet; R&D Systems Europe) according to the manufacturer's recommendation and as previously described (24, 26).

Statistical analysis

Differences between groups were assessed using the Mann–Whitney U-test. A paired Student's *t*-test was used to analyse intragroup differences. A $P < 0.05$ was considered as significant. Regression analysis was used to investigate correlations between biological values.

Results

Detection of PAF-R transcript in the thyroid gland

Since PAF acts as a local cell-to-cell mediator, its presence is only of relevance if PAF-R can be detected at the site of PAF production. As schematised in Fig. 1 (upper part), the PAF-R gene produces three different species of mRNA (i.e. transcript 1, transcript 2 and an elongated form of the transcript 2). Two 5'-non-coding exons (exons 1 and 2) are alternatively spliced to a common site on the third exon (exon 3) encoding the functional PAF-R protein. Thus, both transcripts ultimately yield the functional PAF-R. PAF-R transcripts 1 and 2 are also named 'leukocyte type' and 'tissue

Figure 1 Detection of PAF-R transcripts in the thyroid gland. RT-PCR experiments were performed on total RNA. Products were analysed on a 2% agarose gel stained with ethidium bromide. The three PAF-R mRNA transcripts are schematised in the upper panel. The dotted line is not present in the spliced mRNA. Only exon 3 encodes the functional protein. Expected sizes of amplified products were: PAF-R transcript 1, 225 bp; PAF-R transcript 2, 269 bp; spliced variant of PAF-R transcript 2, 351 bp; GAPDH, 439 bp. Sizes of PCR products and a DNA-size ladder are indicated by arrows. One representative experiment out of two is shown.

type' respectively. As shown in Fig. 1 (lower part), RT-PCR experiments indicated the presence of the three PAF-R transcripts in the thyroid gland.

PAF and lyso-PAF levels in diseased thyroid tissues

As reported in Fig. 2A, no difference was found for PAF amounts between the diseased tissue and the control tissue of patients with a hyperplastic goitre ($P = 0.18$), a benign thyroid adenoma ($P = 0.14$) and a thyroid cancer ($P = 0.41$). PAF amounts were not different ($P > 0.05$, Mann–Whitney U-test) in the control tissue of patients with a hyperplastic goitre (1.63 ± 0.45 pg/mg, $n = 14$), a benign thyroid adenoma (2.61 ± 0.53 pg/mg, $n = 12$) and a thyroid cancer (3.13 ± 0.51 pg/mg, $n = 15$). As reported in Fig. 2B, no difference was found for lyso-PAF precursor amounts between the diseased tissue and the control tissue of patients with a hyperplastic goitre ($P = 0.10$), a benign thyroid adenoma ($P = 0.19$) and a thyroid cancer ($P = 0.23$). Control tissue lyso-PAF amounts were not different ($P > 0.38$) for patients with a hyperplastic goitre (353.1 ± 35.6 pg/mg, $n = 14$), a benign thyroid adenoma (428.4 ± 54.4 pg/mg, $n = 12$) and a thyroid cancer (464.9 ± 63.8 pg/mg, $n = 15$).

PAF characterisation

The platelet-aggregating activity recovered from tissue thyroid samples was indistinguishable from synthetic PAF by the following physicochemical and biological criteria. First, it induced in a dose-dependent manner the aggregation of washed rabbit platelets that were refractory to arachidonic acid- and ADP-mediated pathways; secondly, the platelet-aggregating activity was totally inhibited by 0.1 mmol/l BN 52 021, a specific PAF-R antagonist; and thirdly, the aggregating activity exhibited on TLC a retention time similar to that of synthetic PAF (data not shown).

Enzymatic activities implicated in PAF production and degradation in diseased thyroid tissues

As reported in Fig. 3A, AHA (the PAF-degrading enzyme) levels were not different between the diseased tissue and the control tissue of patients with a hyperplastic goitre ($P = 0.26$), a benign thyroid adenoma ($P = 0.19$) and a thyroid cancer ($P = 0.31$). Control tissue AHA levels were not different ($P > 0.87$, Mann–Whitney U-test) for patients with a hyperplastic goitre (5.75 ± 0.62 fmol/min per mg, $n = 14$), a benign thyroid adenoma (6.23 ± 1.19 fmol/min per mg, $n = 12$) and a thyroid cancer (6.73 ± 1.55 fmol/min per mg, $n = 15$). As reported in Fig. 3B, no difference was found for PLA₂ (the enzymatic activity that generates the lyso-PAF precursor) levels between the diseased tissue and the control tissue of

Figure 2 Investigation of PAF (A) and of the lyso-PAF precursor (B) in diseased ('ill') thyroid tissues. Specimens of diseased tissues and control tissues close to the diseased were obtained during the surgical procedure from 15 patients with a thyroid cancer, 12 with a benign adenoma and 14 with a hyperplastic goitre. Results (means $\pm$ S.E.M.) are expressed as pg PAF/mg tissue and pg lyso-PAF/mg tissue. Statistical analysis was performed using Student's *t*-test for paired samples.

patients with a hyperplastic goitre ($P = 0.14$), a benign thyroid adenoma ($P = 0.34$) and a thyroid cancer ($P = 0.21$). Control tissue PLA₂ levels were not different ($P > 0.23$, Mann-Whitney U-test) for patients with a hyperplastic goitre (1.05 ± 0.11 IU/mg, $n = 14$), a benign thyroid adenoma (1.02 ± 0.18 IU/mg, $n = 12$) and a thyroid cancer (1.25 ± 0.23 IU/mg, $n = 15$).

PAF, lyso-PAF, PLA₂ and AHA values in relation to sex, associated lymphoid thyroiditis, histological subtype of papillary thyroid carcinoma and TNM status

It has been reported that PAF levels and enzymatic activities implicated in PAF production and degradation are different according to the tumour stage in colorectal carcinoma (23). As reported in Table 3, PAF and lyso-PAF amounts, PLA₂ and AHA activities were not significantly different ($P \geq 0.1$, Student's *t*-test for paired data) in tumour and non-tumour thyroid tissues according to the tumour status and patient's stage. Similarly, the sex of patients and the presence or absence of an associated lymphoid thyroiditis did not affect PAF, lyso-PAF, PLA₂ and AHA values in tumour and non-tumour tissues (Table 3). Similar results were found in patients with benign adenomas and hyperplastic goitre (data not shown).

VEGF and bFGF levels in diseased thyroid tissues

As reported in Fig. 4A, VEGF levels were significantly ($P = 0.008$) elevated in the tumour tissue of patients with a thyroid cancer (44.30 ± 10.42 pg/mg, $n = 15$) as compared with the non-tumour tissue close to the tumour (16.87 ± 2.58 pg/mg, $n = 15$). In contrast, VEGF levels were not different between the diseased tissue and the control tissue of patients with a hyperplastic goitre ($P = 0.37$) and a benign thyroid adenoma ($P = 0.35$). Control tissue VEGF levels were not different ($P > 0.26$) for patients with a hyperplastic goitre (16.23 ± 2.79 pg/mg, $n = 14$), a benign thyroid adenoma (14.48 ± 3.79 pg/mg, $n = 12$) and a thyroid cancer (16.87 ± 2.58 pg/mg, $n = 15$). As reported in Fig. 4B, bFGF levels were significantly ($P = 0.018$) elevated in the tumour tissue of patients with a thyroid cancer (76.96 ± 17.40 pg/mg, $n = 15$) as compared with the control tissue close to the tumour (42.90 ± 6.44 pg/mg, $n = 15$). In contrast bFGF levels were not different between the diseased tissue and the control tissue for patients with a hyperplastic goitre ($P = 0.12$) and a benign thyroid adenoma ($P = 0.09$). Control tissue bFGF levels were not different ($P > 0.06$) for patients with a hyperplastic goitre (31.99 ± 3.68 pg/mg, $n = 14$), a benign thyroid

Figure 3 Investigation of AHA (A) and PLA₂ activity (B) in diseased ('ill') thyroid tissues. Specimens of diseased tissues and control tissues close to the diseased were obtained during the surgical procedure from 15 patients with a thyroid cancer, 12 with a benign adenoma and 14 with a hyperplastic goitre. AHA levels (means $\pm$ S.E.M.) are expressed as fmol PAF degraded/min per mg tissue. PLA₂ levels are expressed as IU/mg tissue. Statistical analysis was performed using Student's *t*-test for paired samples.

adenoma (34.02 ± 4.23 pg/mg, $n = 12$) and an epithelial thyroid cancer (42.9 ± 6.44 pg/mg, $n = 15$).

Relationships between PAF and lyso-PAF precursors with PAF catabolic enzymes and angiogenic growth factors

In control thyroid tissues, PAF levels were correlated with those of PLA₂ ($r = 0.35$, $P = 0.022$) and lyso-PAF ($r = 0.34$, $P = 0.028$). Lyso-PAF levels were correlated with those of PLA₂ ($r = 0.37$, $P = 0.016$) and AHA ($r = 0.49$, $P = 0.001$). In the thyroid cancer tissue, VEGF levels were not correlated with those of PAF ($r = 0.004$, $P = 0.98$) and lyso-PAF ($r = 0.19$, $P = 0.23$). bFGF levels were not correlated with those of PAF ($r = 0.05$, $P = 0.73$) and lyso-PAF ($r = 0.05$, $P = 0.75$). VEGF and bFGF levels were correlated in the thyroid cancer tissue ($r = 0.52$, $P = 0.0005$).

Discussion

As far as we know, this is the first clinical investigation concerning the presence of the phospholipid mediator PAF in the thyroid gland. As PAF is believed to be involved in tissue injury and cancer, it was of interest

to investigate its putative involvement in both normal and diseased thyroid tissue from patients with a hyperplastic goitre, to patients with a benign adenoma and finally patients with a thyroid cancer. Previous authors reported alterations in phospholipids levels in blood and tumour tissue of patients with thyroid carcinoma (5, 6). Furthermore, as we recently observed, alterations are found in the PAF production/degradation pathways in the tumour tissue of patients with lung and colorectal carcinoma (21, 23, 24).

The present results indicate, for the first time, that human thyroid contains PAF as well as the lyso-PAF precursor. Identification of PAF was based on the stringent functional and biophysical criteria detailed above. It is unlikely that circulating blood accounted for the amount of PAF found in the thyroid since: (i) tissue samples were extensively rinsed before being frozen; (ii) the mean amount of bioactive PAF found in blood was 10 pg/ml (27, 28), as compared with the 3 pg/mg found in thyroid tissue; (iii) similar amounts of PAF were found in tissue samples from lung and colon (21, 23); and (iv) stimulated FRTL5 cells (a normal rat thyroid cell line) produced PAF in the presence of the lyso-PAF precursor (29). The presence of the lyso-PAF precursor is consistent with the presence of a PLA₂ activity in the thyroid gland; PLA₂ mRNA

Table 3 PAF, lyso-PAF, PLA₂ and AHA levels in tumour and non-tumour tissue of patients with papillary thyroid carcinoma in relation to the sex, the presence or absence of an associated lymphoid thyroiditis, the tumour status (T), the patients' stage and the histological subtype of the papillary tumour carcinoma. Means $\pm$ S.E.M. of the indicated number (*n*) of patients. The statistical significant (*P*) between tumour and non-tumour values was analysed using student's *t*-test for paired data.

	Sample	PAF (pg/mg)	Lyso-PAF (pg/mg)	PLA ₂ (IU/mg)	AHA (fmol/min per mg)
Female (<i>n</i> = 11)	Non-tumour	3.35 $\pm$ 0.67	395.9 $\pm$ 51.4	0.93 $\pm$ 0.14	6.41 $\pm$ 1.94
	Tumour	3.26 $\pm$ 0.93 (<i>P</i> = 0.47)	503.8 $\pm$ 77.1 (<i>P</i> = 0.12)	1.37 $\pm$ 0.38 (<i>P</i> = 0.16)	6.18 $\pm$ 1.15 (<i>P</i> = 0.44)
Male (<i>n</i> = 4)	Non-tumour	2.53 $\pm$ 0.47	485.9 $\pm$ 143.2	1.22 $\pm$ 0.17	7.26 $\pm$ 1.29
	Tumour	2.01 $\pm$ 0.83 (<i>P</i> = 0.25)	489.2 $\pm$ 89.6 (<i>P</i> = 0.49)	0.77 $\pm$ 0.23 (<i>P</i> = 0.10)	4.56 $\pm$ 1.22 (<i>P</i> = 0.15)
Associated lymphoid thyroiditis (<i>n</i> = 12)	Non-tumour	3.23 $\pm$ 0.63	400.4 $\pm$ 57.2	1.13 $\pm$ 0.11	6.57 $\pm$ 1.85
	Tumour	3.05 $\pm$ 0.89 (<i>P</i> = 0.43)	471.7 $\pm$ 71.9 (<i>P</i> = 0.19)	1.32 $\pm$ 0.35 (<i>P</i> = 0.32)	5.73 $\pm$ 1.09 (<i>P</i> = 0.30)
No associated lymphoid thyroiditis (<i>n</i> = 3)	Non-tumour	2.70 $\pm$ 0.40	491.9 $\pm$ 132.7	0.58 $\pm$ 0.23	5.58 $\pm$ 1.15
	Tumour	2.44 $\pm$ 0.82 (<i>P</i> = 0.30)	608.7 $\pm$ 62.3 (<i>P</i> = 0.23)	0.80 $\pm$ 0.41 (<i>P</i> = 0.35)	5.17 $\pm$ 1.93 (<i>P</i> = 0.44)
T1 + T2 (<i>n</i> = 8)	Non-tumour	2.46 $\pm$ 0.39	476.2 $\pm$ 74.9	1.07 $\pm$ 0.16	6.09 $\pm$ 0.93
	Tumour	2.41 $\pm$ 0.54 (<i>P</i> = 0.49)	478.2 $\pm$ 87.8 (<i>P</i> = 0.49)	1.13 $\pm$ 0.37 (<i>P</i> = 0.44)	4.41 $\pm$ 0.82 (<i>P</i> = 0.11)
T3 + T4 (<i>n</i> = 7)	Non-tumour	3.92 $\pm$ 0.93	355.5 $\pm$ 67.7	0.97 $\pm$ 0.16	7.41 $\pm$ 3.02
	Tumour	3.52 $\pm$ 1.45 (<i>P</i> = 0.41)	524.3 $\pm$ 86.3 (<i>P</i> = 0.10)	1.31 $\pm$ 0.48 (<i>P</i> = 0.27)	7.28 $\pm$ 1.57 (<i>P</i> = 0.49)
Stage I + II (<i>n</i> = 7)	Non-tumour	2.33 $\pm$ 0.44	423.3 $\pm$ 65.5	1.02 $\pm$ 0.17	5.43 $\pm$ 0.76
	Tumour	2.51 $\pm$ 0.61 (<i>P</i> = 0.41)	466.3 $\pm$ 100.3 (<i>P</i> = 0.37)	1.24 $\pm$ 0.41 (<i>P</i> = 0.31)	4.58 $\pm$ 0.93 (<i>P</i> = 0.20)
Stage III + IV (<i>n</i> = 8)	Non-tumour	3.82 $\pm$ 0.82	413.3 $\pm$ 82.3	1.03 $\pm$ 0.15	7.70 $\pm$ 2.65
	Tumour	3.30 $\pm$ 1.28 (<i>P</i> = 0.37)	527.9 $\pm$ 74.9 (<i>P</i> = 0.10)	1.19 $\pm$ 0.44 (<i>P</i> = 0.38)	6.77 $\pm$ 1.45 (<i>P</i> = 0.18)
Conventional papillary thyroid carcinoma (<i>n</i> = 8)	Non-tumour	2.42 $\pm$ 0.46	414.7 $\pm$ 54.4	1.08 $\pm$ 0.17	8.09 $\pm$ 2.47
	Tumour	3.58 $\pm$ 1.29 (<i>P</i> = 0.19)	462.7 $\pm$ 88.3 (<i>P</i> = 0.33)	1.40 $\pm$ 0.45 (<i>P</i> = 0.28)	6.51 $\pm$ 1.16 (<i>P</i> = 0.20)
Follicular variant (<i>n</i> = 6)	Non-tumour	3.26 $\pm$ 0.88	404.5 $\pm$ 111.9	0.97 $\pm$ 0.17	5.30 $\pm$ 1.37
	Tumour	2.12 $\pm$ 0.55 (<i>P</i> = 0.15)	506.6 $\pm$ 89.9 (<i>P</i> = 0.19)	1.01 $\pm$ 0.42 (<i>P</i> = 0.46)	4.88 $\pm$ 1.70 (<i>P</i> = 0.43)

Figure 4 Investigation of VEGF (A) and bFGF (B) in diseased ('ill') thyroid tissues. Specimens of diseased tissues and control tissues close to the diseased were obtained during the surgical procedure from 15 patients with a thyroid cancer, 12 with a benign adenoma and 14 with a hyperplastic goitre. VEGF and bFGF levels (means $\pm$ S.E.M.) are expressed in pg/mg tissue. Statistical analysis was performed using Student's *t*-test for paired samples.

transcripts being previously detected in the human thyroid gland (7, 30). PAF can generate biological responses detectable at levels as low as 10 fmol/l. Thus regulating PAF levels is evidently important, since elevated or decreased levels of PAF might result in pathological effects. The present results reveal, for the first time, the presence of the PAF AHA in the human thyroid gland; such enzymatic activity being previously found in rat FRTL5 cells (29). Finally, since PAF acts as a local cell-to-cell mediator, its presence is only of relevance if PAF-R can be detected at the site of PAF production. Functional PAF-R is found on cultured porcine thyroid cells (31). In the present study we detected the presence of the three PAF-R mRNA transcripts in the human thyroid gland. At this time the significance of these different PAF-R mRNA transcripts in PAF physiology is still an open question. However, it may be suggested that transcription of a single gene from multiple promoters provides additional flexibility in the control of gene expression. Thus, PAF-R transcripts, PAF and the lyso-PAF precursor are detected in the thyroid tissue. A significant link is found between PAF and lyso-PAF values and with those of PLA₂ and AHA (two enzymatic activities implicated in PAF formation and degradation respectively),

suggesting subtle regulations of PAF metabolic pathways inside the thyroid gland. At this time the role of PAF in the thyroid physiology remains an open question. Only a few results are available concerning the effect of PAF on thyroid cell functions. Thus, PAF is reported to reduce cAMP production from porcine thyroid cells in response to thyreostimulin (31), and to increase the growth of GEJ cells (human thyroid hybrid cells) and their thyreostimulin receptor expression (32).

Several molecular abnormalities are found during the progression from normal thyroid tissue to thyroid cancer (1). They include elevated expression of oncogenes and angiogenic growth factors, alteration of normal cell-to-cell contact, and reduction of apoptosis. Numerous data report that PAF can participate to all these events (12–19). Since elevated levels of PAF are reported in breast (17, 22) and colorectal carcinomas (23) and PAF AHA is dramatically increased in lung ones (21), it was thus tempting to speculate on an alteration of PAF metabolic pathways in thyroid cancer. In fact, results clearly indicate that PAF and lyso-PAF amounts, PLA₂ and AHA are not different in the tumour and the non-tumour thyroid tissue and that their levels are similar to those found in other

diseased thyroid tissues. Moreover no difference was found between PAF, lyso-PAF, PLA₂ and AHA levels with respect to the TNM status of patients and the histological subtype of papillary thyroid carcinoma. It is unlikely that technical problems prevented us correctly assessing PAF, lyso-PAF, PLA₂ and AHA tissue levels since: (i) similar experimental protocols have been used with success to investigate alterations of these biological parameters in lung, colorectal and liver tissues (21, 23, 24), and (ii) VEGF and bFGF amounts were markedly elevated in the tumour tissue of thyroid cancer as compared with non-tumour tissue. These latter results were in agreement with previous studies highlighting elevated levels of these two angiogenic growth factors in thyroid cancer (20). Finally, no link was found in tumour tissue between PAF levels and those of VEGF and bFGF, suggesting that their angiogenic effects in thyroid cancer were not mediated by PAF.

In conclusion, PAF and the lyso-PAF precursor are present in the thyroid gland. PAF-R transcripts and the enzymatic activities implicated in lyso-PAF formation (i.e. PLA₂) and PAF degradation (i.e. AHA) are also detected. Altogether these results argue for a tiny regulation of PAF levels inside the thyroid gland. However, at the present time, the role of PAF in its physiology remains an open question. Finally, tissue PAF, lyso-PAF, PLA₂ and AHA levels are not altered in various diseased thyroid tissues including papillary thyroid cancer, a result that markedly differs from previous data obtained with other types of human cancer.

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