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FRA-1 Expression in Hyperplastic and Neoplastic Thyroid Diseases[1]

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ABSTRACT

***fra-1* gene overexpression has been shown to represent a general event in thyroid cell transformation *in vitro* and *in vivo*. Moreover, inhibition of FRA-1 protein synthesis by stable transfection with a *fra-1* antisense construct significantly reduces the malignant phenotype of the transformed thyroid cells, indicating a pivotal role of the *fra-1* gene product in the process of cellular transformation. In the attempt to define the potential use of FRA-1 protein detection in the diagnosis of thyroid diseases, we analyzed Fra-1 expression by a combination of immunohistochemistry and reverse transcription-PCR (RT-PCR) assay in 174 samples of thyroid nodules (22 nodular hyperplasias, 102 follicular adenomas, 34 papillary carcinomas, 12 follicular carcinomas, and 4 anaplastic carcinomas) representative of the spectrum of thyroid tumor pathology. FRA-1 protein was abundant in all of the carcinoma samples (50/50, 100%), with an intense staining in the nucleus and the cytoplasm. Positive staining was also found in most of the adenomas (90 of 102; 88%), but in this case, the staining was restricted to the nucleus. Similar results were obtained from the analysis of thyroid goiters; however, the number of positive cases is lower than adenomas (8 of 22; 36%); moreover, the staining was not observed in all of the cells. Conversely, no FRA-1 protein was detectable in 12 normal thyroid tissue samples used as controls. RT-PCR analysis confirmed a higher *fra-1* expression in papillary and follicular carcinomas compared with goiters and adenomas. *fra-1* expression was also analyzed on 10 fine needle aspiration biopsy (FNAB) samples by RT-PCR. *fra-1*-specific mRNA was detected in seven of the eight FNABs corresponding to thyroid nodules that were eventually diagnosed as adenomas (three of four) and carcinomas (four of four) after surgery. Conversely, no *fra-1* gene expression was observed in two FNABs derived from normal thyroid. Further studies are required before suggesting FRA-1 protein detection as a useful tool for the diagnosis of hyperplastic and neoplastic disorders of the thyroid gland.**

INTRODUCTION

The FRA-1 protein is a member of the AP-1 complex that is formed by the three Jun family members (c-Jun, JunB, and JunD) and four Fos family members (c-Fos, FosB, Fra-1, and Fra-2; Refs. 1–5). Each of these Fos-related proteins is a transcription factor that can dimerize with Jun family proteins through a leucine zipper domain and bind through a conserved basic region to very similar or identical DNA motifs. The DNA element recognized by all of these heterodimers is an AP-1 site with core sequence TGACTCA (6–8). However, the Fos proteins cannot dimerize among themselves and have no intrinsic specific DNA binding activity (9).

Modification in AP-1 activity has been often correlated with cell transformation (8). In fact, c-jun knock-out mice-derived cells are resistant to ras-mediated transformation (10). Moreover, fra-2-c-jun heterodimers play a crucial role in chicken fibroblast transformation (11). Mouse JunD antagonizes transformation by ras (12), and c-fos expression is required for malignant progression of skin tumors (13). We have demonstrated recently that thyroid neoplastic transformation induced by the v-*ras*-Ki and v-*mos* oncogenes is associated with a drastic increase of AP-1 activity, which reflects multiple compositional changes (14). The main effect is represented by a dramatic *fra-1* gene induction. Although no specific biological effect was observed after *fra-1* gene overexpression, the inhibition of the FRA-1 protein synthesis by stable transfection with a *fra-1* antisense construct significantly reduces the malignant phenotype of the transformed thyroid cells, indicating a pivotal role of the *fra-1* gene product in the process of cellular transformation (14). Our recent results showing that the induction of the *fra-1* gene is present even in cells transformed with several oncogenes (*E1A* gene of adenovirus, *RET/PTC*, v-*raf*, v-*abl*, Middle T of polyoma, v-*src*, and others) and in human thyroid carcinoma cell lines of different histotype indicate that the *fra-1* gene expression induction is a general event in the process of thyroid carcinogenesis (15). Moreover, in rat fibroblasts *fra-1* exhibits oncogenic potential because its overexpression has been shown to be unable to induce morphological transformation, but capable to stimulate anchorage-independent growth (16).

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fra-1 gene expression is subject to positive control by AP-1 activity (15, 17); however, AP-1-induced expression of *fra-1* depends on regulatory sequences located not only in the promoter region but also in the first intron. Because *fra-1* gene expression is prevented by the block of the HMGI-C protein synthesis, the possibility that *fra-1* gene expression is directly or indirectly regulated by the expression of the HMGI proteins should also be taken into account. The presence of five consensus binding sites for members of the HMG-box family (18) in the *fra-1* promoter is consistent with this hypothesis.

Thyroid nodules are frequently found in the general population. The large majority of them are benign, and 5–10% are eventually diagnosed as carcinomas only after surgical removal of the lesion. Evaluation of the potential of molecular markers in the diagnosis of thyroid nodules is therefore justified. In this context, we analyzed FRA-1 expression by immunohistochemistry and RT-PCR[3] in nodular lesions of the thyroid gland to assess its possible role as a diagnostic tool.

The results presented here show the expression of the FRA-1 protein in all of the thyroid carcinomas analyzed. Eighty-eight % of thyroid adenomas and 36% of goiters were positive, but in these cases the staining was restricted to the nucleus. RT-PCR analysis confirmed a lower *fra-1* gene expression in adenomas. Conversely, FRA-1 protein was not detected at all in normal thyroid tissue.

MATERIALS AND METHODS

Tissue Samples. Tissue sections for immunohistochemistry were obtained from 172 routinely processed, paraffin-embedded samples of thyroid nodules resected at Yale New Haven Hospital between 1985 and 1990 and at the Istituto dei Tumori di Napoli between 1989 and 1995. The thyroid nodular lesions were diagnosed as nodular hyperplasia (22 cases), follicular adenoma (102 cases), papillary carcinoma (34 cases), follicular carcinoma (12 cases), and anaplastic carcinoma (4 cases). Twelve samples of histologically normal thyroid were also included as controls. None of the adenomas analyzed may be considered as atypical. RNA for RT-PCR was extracted from paraffin blocks on a subset of these cases to include 4 normal thyroids, 8 nodular hyperplasias, 10 adenomas, and 15 carcinomas. Eight FNAB samples were obtained at the Istituto dei Tumori di Napoli for RNA extraction and RT-PCR. The final diagnoses were follicular adenoma (4 cases), papillary carcinoma (2 cases), and follicular carcinoma (2 cases).

Thyroid Cell Lines. The FRO cell line was derived from a human anaplastic thyroid carcinoma (19, 20). They were grown in DMEM plus 10% FCS. HTC-2 cells were established and cultured as described (21).

Immunohistochemistry. For the immunohistochemical studies of paraffin-embedded samples, 5–6-μm-thick paraffin sections were deparaffinized and then placed in a solution of absolute methanol and 0.3% hydrogen peroxide for 30 min and then washed in PBS before immunoperoxidase staining. Slides were then incubated overnight at 4°C in a humidified chamber with the primary antibodies diluted 1:100 in PBS and subsequently incubated, first with biotinylated goat antirabbit IgG for 20 min (Vectostain ABC kits; Vector Laboratories) and then with premixed reagent ABC (Vector) for 20 min. The immunostaining was performed by incubating slides in diaminobenzidine (Dako) solution containing 0.06 mM diaminobenzidine and 2 mM hydrogen peroxide in 0.05% PBS (pH 7.6) for 5 min, and after chromogen development, slides were washed, dehydrated with alcohol and xylene, and mounted with coverslips using a permanent mounting medium (Permount). Micrographs were taken on Kodak Ektachrome film with a photo Zeiss system.

The antibodies used in this study were rabbit polyclonal raised against the epitope corresponding to amino acids 3–22 mapping at the NH_2 terminus of FRA-1 of human origin. They are specific for FRA-1 and non-cross-reactive with the other members of the fos family. The immunostained samples were blindly read by two independent individuals (G. C. and G. T.).

RT-PCR Analysis of *fra-1* Gene Expression. RNA was extracted from paraffin-embedded blocks on 37 cases that were analyzed in parallel for FRA-1 expression by immunohistochemistry. RNA extraction was performed as described (22). Briefly, single 6–8-mm tissue sections, cut from paraffin blocks, were stirred for 20 min in 1.5-ml tubes with 1 ml of xylene. After centrifugation, the pellet was washed with 0.5 ml of ethanol and air-dried. The dried pellet was resuspended in 200 ml of 6 mg/ml proteinase K (Sigma Chemical Co., St. Louis, MO), 1 M guanidinium thiocyanate, 25 mM 2-mercaptoethanol, 0.5% Sarkosyl, and 20 mM Tris-HCl (pH 7.5) and incubated at 37°C for 18 h. RNA was then extracted with phenol and precipitated with ethanol following a standard procedure (23). Fine needle aspiration samples were washed twice with 1× PBS and then processed for RNA extraction following the same procedure. One-fifth of RNA of total RNA, digested with DNase, was reverse transcribed using random exanucleotides as primers (100 mM) and 12 units of AMV reverse transcriptase (Life Technologies, Inc.), and subsequent PCR amplification was performed as reported previously (24). Two hundred ng of cDNA were amplified in a 25-μl reaction mixture containing Taq DNA in polymerase buffer, 0.2 mM deoxynucleotide triphosphates, 1.5 mM $MgCl_2$, 0.4 mM of each primer, and 1 unit of Taq DNA polymerase (Perkin-Elmer). The PCR amplification was performed for 30 cycles (94°C for 30 s, 55°C for 2 min, and 72°C for 2 min). The specific primers for *fra-1* were: forward, 5′-GTCATTGCTAGGATACCAAAC-3′); and reverse, 5′-CACTGTCCAGCAAGGGTCTGT-3′, corresponding to the nucleotides 136–156 and 335–315, respectively (4). The amplified products were separated by 1.5% agarose electrophoresis gel and hybridized with a *fra-1* probe. Amplification of contaminating genomic DNA was excluded by control experiments in absence of reverse transcriptase. In addition, a set of primers specific for the constitutively expressed enzyme GAPDH was added to each reaction after 20 cycles of PCR to serve as internal control for the amount of cDNA tested. The GAPDH-specific primers were: forward, 5′-ACATGTTCCAATATGATTCC-3′ corresponding to the nucleotides 194–214; and reverse, 5′-TGGACTCCACGACGTACTCAG-3′ corresponding to the 336–356 nucleotides. The product of the

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[3] The abbreviations used are: RT-PCR, reverse transcription-PCR; FNAB, fine needle aspiration biopsy; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; HMGI, high-mobility group I.

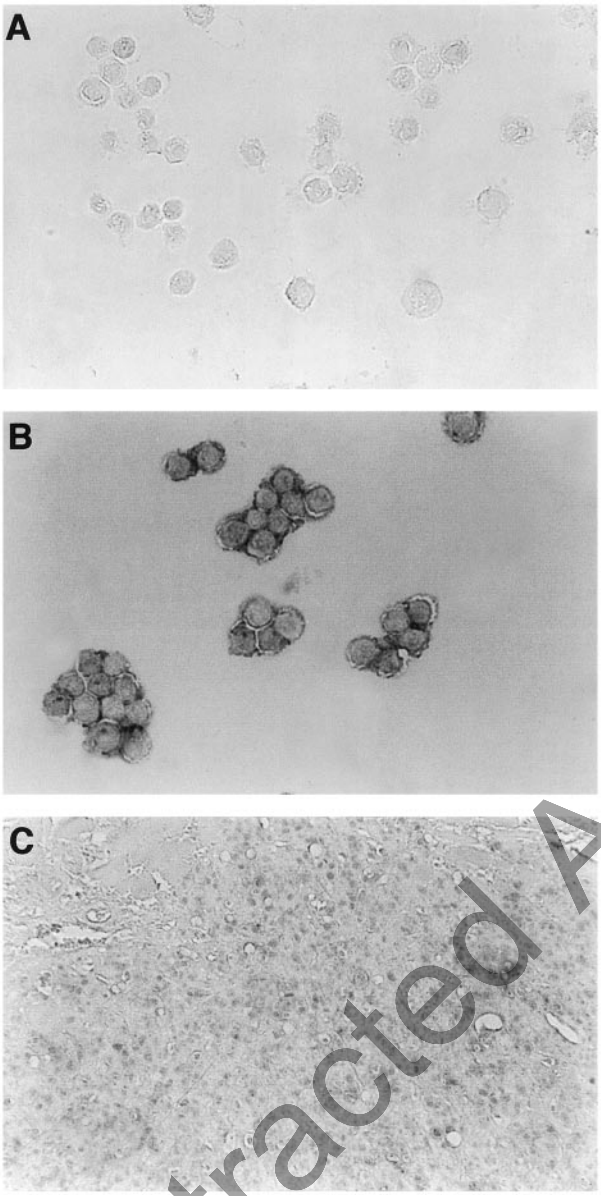

Fig. 1 Immunohistochemical analysis of FRA-1 protein in normal thyroid cells, in FRO (anaplastic carcinoma) cells, and in FRO-induced tumors. *A*, HTC 2 cells; *B*, FRO cells; *C*, tumors induced by the injection of the FRO cells into athymic mice.

reaction was analyzed on a 2% agarose gel and then transferred by electroblotting to GeneScreen plus nylon membrane (DuPont, Boston, MA). DNA was fixed to the membranes by air drying and UV cross-linking, and then membranes were hybridized with a GAPDH probe. A PhosphorImager screen was briefly exposed to the membranes, and the screen was then scanned on a Molecular Dynamics PhosphorImager. The images recorded by the PhosphorImager were analyzed by volume integration with the ImageQuant software. The relative level of fra-1 expression was assessed by comparison with the level of GAPDH in the same sample.

Table 1 FRA-1 protein expression in human thyroid tumors

Histological type of thyroid specimens	No. of positive cases/no. of cases analyzed by immunohistochemistry	No. of positive cases/no. of cases analyzed by RT-PCR[a]
Normal thyroid	0/12	0/4
Goiters	8/22	3/8[b]
Follicular adenomas	90/102	8/10
Follicular carcinomas	12/12	5/5
Papillary carcinomas	34/34	10/10
Anaplastic carcinomas	4/4	

[a] RT-PCR was performed on selected cases that were also analyzed by immunohistochemistry. The cases positive by RT-PCR were also positive by immunohistochemistry.

[b] The goiter samples positive for RT-PCR were also positive by immunohistochemical analysis. Conversely, the other five cases were negative for both the assays.

RESULTS

Immunohistochemical Analysis of *fra-1* Gene Expression. Detection of FRA-1 protein by immunohistochemical analysis allows a rapid and sensitive screening of thyroid pathological tissues and is amenable to regular use as a routine diagnostic test. This technique was therefore chosen for FRA-1 protein analysis using antibodies raised, in our laboratory, against a FRA-1-specific peptide. To find the best experimental conditions, FRO cells and tumors induced by injecting the FRO cell line into athymic mice were used as positive controls (20). The FRO cell line was chosen because of its high expression of the Fra-1 protein by Western blot analysis (15). No staining was observed with human thyroid cells (HTC-2 cells; Fig. 1*A*; Ref. 21), whereas a positive nuclear staining was obtained with FRO cells (Fig. 1*B*) and FRO-induced tumors (Fig. 1*C*).

The results of the immunohistochemical study of 186 thyroid specimens are summarized in Table 1. No staining was observed in normal thyroid tissue (Fig. 2). Conversely, strong immunoreactivity was detected in all of the thyroid carcinomas analyzed (Fig. 2). The positivity appears in the nucleus, but it is also present, although slightly weaker, in the cytoplasm. This result is consistent with previous published data that showed FRA-1 protein localization in the nucleus and the cytoplasm of the COS cells (25). Eighty-eight % of follicular adenomas and 36% of the goiters were positive for FRA-1. However, in these cases, the immunoreactivity was always restricted to the nuclei. Moreover, in goiters the nuclear staining was not observed in all of the cells. In Fig. 2, we show some examples of the immunohistochemical assay. No positive signal was detected in normal thyroid (Fig. 2*A*) and in one goiter (Fig. 2*F*), whereas an intense nuclear and a weaker cytoplasmic staining was clearly observed in a papillary carcinoma (Fig. 2*C*) and in a follicular carcinoma (Fig. 2*E*). Fig. 2, *G* and *H*, shows the immunostaining of one positive goiter and one positive adenoma, respectively; the immunoreactivity is present only in the nuclei. For each case, sections were stained without the primary antibody, and in all cases, these controls were negative (Fig. 2*D*). No staining was also observed when the neoplastic tissues were analyzed after the FRA-1-specific antibodies had been preincubated with the FRA-1 control peptide (Fig. 2*B*). In Fig. 3, we show that fra-1 detection was specific for the neoplastic cells, because no

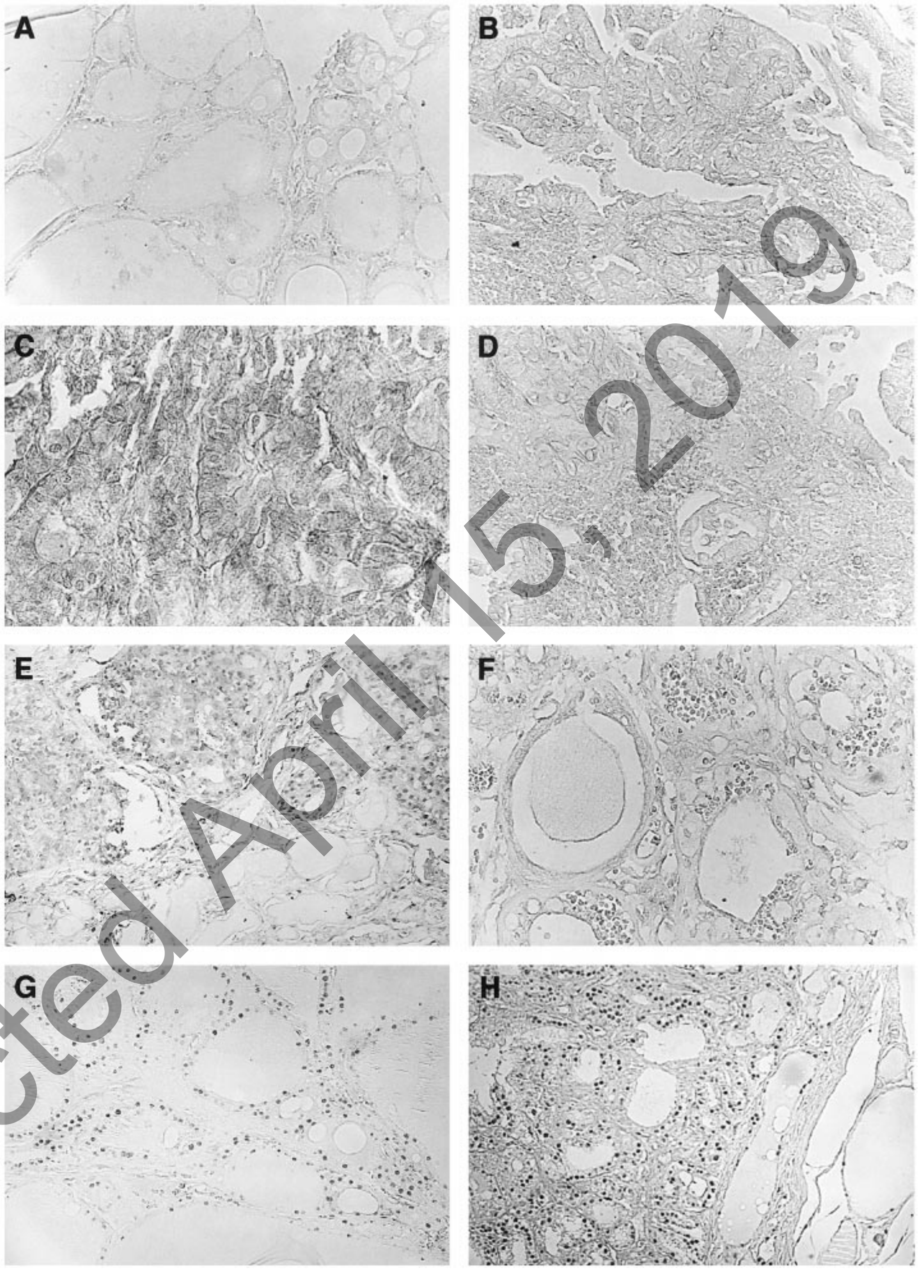

Fig. 2 Immunohistochemical analysis of FRA-1 in thyroid tissues. Paraffin sections from normal and pathological thyroid samples were analyzed by immunohistochemistry using antibodies raised against a specific FRA-1 peptide. *A*, immunostaining of a normal thyroid tissue (×200). No immunoreactivity was observed. *B*, immunostaining of a papillary thyroid carcinoma with the FRA-1 antibodies preincubated with the peptide against which antibodies were raised. No immunoreactivity was observed. *C*, immunostaining of a papillary carcinoma (×200). Strong nuclear and cytoplasmic staining was observed. *D*, immunostaining of a papillary carcinoma in the absence of the primary antibodies (×200). No immunoreactivity was observed. *E*, immunostaining of a follicular carcinoma (×200). A strong nuclear and cytoplasmic positivity was observed. *F*, immunostaining of a goiter (×200). No immunoreactivity was observed. *G*, immunostaining of a goiter (×200). A nuclear staining was observed. *H*, immunostaining of a follicular adenoma (×200). Nuclear staining was observed in the neoplastic cells.

staining was observed in the normal follicles present in the same field.

RT-PCR Analysis of *fra-1* Gene Expression. To quantitate the levels of the *fra-1* gene expression and validate the immunohistochemical data, we analyzed 37 samples (4 normal thyroids, 8 goiters, 10 adenomas, and 15 carcinomas) for the *fra-1* mRNA levels by a semiquantitative RT/PCR assay. The results are summarized in Table 1. Fig. 4 shows a representative RT-PCR assay. Normal thyroid was negative for the *fra-1* gene expression (*Lane 1*). Three adenomas (*Lanes 3*, *4*, and *5*) of four (*Lane 2*) were positive. Conversely, all of the malignant tumors showed a significant fra-1 expression (from *Lanes 6* to *15*). In thyroid malignant neoplasias, the expression was higher (at least 4–5-fold) in comparison with benign lesions. The *fra-1* gene expression in goiters was slightly lower than that observed in adenoma samples (data not shown). Therefore, these data support the immunohistochemical findings.

Analysis of FNABs. FNAB has become an integral part of the preoperative evaluation of thyroid nodules. To evaluate the applicability of *fra-1* gene expression analysis to FNABs samples, we studied four cases of follicular adenoma and four of thyroid carcinoma (two papillary carcinomas and two follicular carcinomas). The results, shown in Table 2 and Fig. 5, are consistent with the immunohistochemical findings. The fine

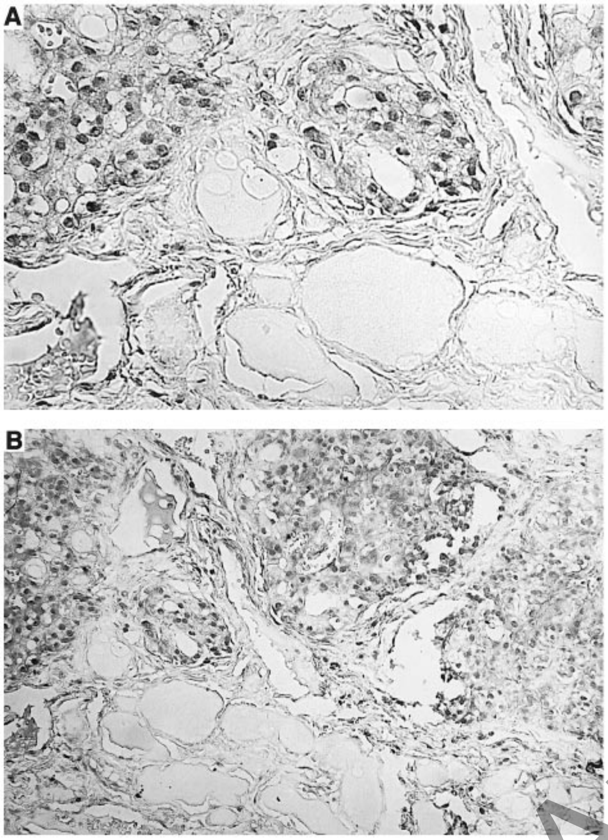

Fig. 3 FRA-1 expression is specific for thyroid neoplastic cells. *A* and *B*, immunostaining of a follicular carcinoma. A nuclear and cytoplasmatic immunostaining was specifically observed in the neoplastic cells; in fact, no staining was observed in normal thyroid follicles (on the *low side* of the figures).

Fig. 4 RT-PCR analysis of the *fra-1* gene expression in paraffin-embedded tissues. The mRNA was extracted from paraffin-embedded tissues and amplified by RT-PCR using *fra-1*-specific primers as specified in "Materials and Methods." The product of the reaction was analyzed on a 1.5% agarose gel, transferred by electroblotting to GeneScreen plus nylon membrane, and hybridized with a *fra-1* probe. The cDNA was coamplified with *GAPDH* gene as an internal control. Source of RNA: *Lane 1*, normal thyroid tissue; *Lanes 2–5*, thyroid adenomas; *Lanes 6–15*, thyroid carcinomas.

Table 2 *fra-1* gene expression in human thyroid FNABs

Cytological diagnosis	No. of positive cases/no. of cases analyzed by RT-PCR	Histological diagnosis[a]
Normal follicular cells[b]	0/2	Normal thyroid
Follicular neoplasm	3/4	Follicular adenomas
Follicular neoplasm	2/2	Follicular carcinomas
Suspicious for papillary carcinoma	2/2	Papillary carcinomas

[a] After surgical removal of the thyroid nodule.

[b] Cytologic samples from the controlateral lobe in one patient with follicular adenoma and one patient with follicular carcinoma.

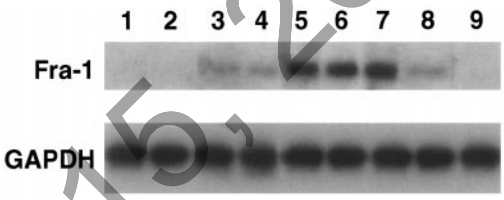

Fig. 5 RT-PCR analysis of the *fra-1* gene expression in FNAB. The mRNA was extracted from FNABs of thyroid tissue and amplified by RT-PCR using *fra-1*-specific primers as specified in "Materials and Methods." The product of the reaction was analyzed on a 2% agarose gel, transferred by electroblotting to GeneScreen plus nylon membrane, and hybridized with a *fra-1* probe. The cDNA was coamplified with *GAPDH* gene as an internal control. Sources of RNA: *Lane 1*, normal thyroid; *Lanes 2–4*, thyroid adenomas; *Lanes 5* and *6*, papillary carcinomas; *Lanes 7* and *8*, follicular carcinomas; *Lane 9*, normal thyroid.

needle biopsies from patients carrying a carcinoma showed expression of the *fra-1* gene (*Lanes 5*, *6*, *7*, and *8*), whereas the fine needle biopsy from normal thyroid (*Lanes 1* and *9*) was negative. Two adenoma samples were positive for the *fra-1* gene expression (*Lanes 3* and *4*), and one scored negative (*Lane 2*). No amplification product was achieved when a carcinoma RNA sample was analyzed in absence of reverse transcriptase (data not shown).

DISCUSSION

Thyroid tumors include a wide spectrum of lesions with different phenotypic characteristics and biological behavior: benign adenomas, differentiated carcinomas, and anaplastic carcinomas (26). We have demonstrated previously that the chromatinic proteins belonging to the group of the high mobility proteins are expressed at high levels in neoplastically transformed thyroid cells in culture and in human carcinomas, whereas they were not detected in normal thyroid and goiters and are present in 20% of adenomas (19, 20). Recently, the analysis of the AP-1 complex in thyroid cells transformed by MPSV and KiMSV revealed the induction of *fra-1* in transformed thyroid cells. This induction was abolished by blocking the HMGI protein synthesis, suggesting a regulation of the *fra-1* gene by the HMGI proteins (14). Rat and human thyroid carcinoma cell lines showed expression of the *fra-1* gene, which was absent in normal rat thyroid cells and in human thyroid tissue (15). In addition, the inhibition of FRA-1 protein synthesis by stable transfection with a *fra-1* antisense RNA vector significantly reduced the malignant phenotype of transformed thyroid cells, indicating a pivotal role for the *fra-1* gene product in the process of cellular transformation (14). Recent data showing immunohistochemical evidence of FRA-1 protein expression in a variety of breast, ovarian, and skin carcinomas[4] but not in the corresponding normal tissues suggest that fra-1 induction is a rather common event in the process of epithelial cell transformation and that FRA-1 protein detection might, therefore, represent a useful tool for the diagnosis of neoplastic and/or proliferative processes.

The aim of the present study was to investigate whether the detection of the FRA-1 protein in the thyroid neoplastic samples could have been of some help in the diagnosis. In the present study, we analyzed 50 thyroid carcinomas (34 papillary, 12 follicular, and 4 anaplastic), 102 adenomas, 22 goiters, and 12 thyroid normal tissue samples for the presence of the FRA-1 protein by immunohistochemical analysis and RT-PCR. The presence of the FRA-1 protein was not detected in normal thyroid; it did not, however, appear restricted to thyroid malignant neoplasias. In fact, 36% of the hyperplastic nodules analyzed were positive immunohistochemically for the presence of *fra-1* gene product. However, the modality of staining was different from goiters and adenomas in comparison with the malignant neoplasias, because nuclear and cytoplasmic staining was observed in papillary and follicular carcinomas, whereas the immunoreactivity was restricted to nuclei in positive adenomas and goiters. These results were confirmed by RT-PCR analysis. In fact, they show a higher *fra-1* gene expression in carcinomas, compared with adenomas and goiters. Analysis of the FNAB confirms the expression in the case of benign and malignant tumors but not in normal thyroid samples. Therefore, the presence of the FRA-1 protein, which is not detectable at all in normal human thyroid, surely indicates a thyroid cell proliferation, and then it is nevertheless a marker for proliferative processes in thyroid (both hyperplastic and neoplastic). Moreover, the kind of immunoreactivity may allow a discrimination between malignant and benign neoplasias, because the positivity is restricted to the nuclei in adenomas. Finally, FRA-1 protein detection may represent, in association with expression analysis of other genes, such as *HMGI(Y)* and/or β-*10 thymosin* (27), a useful adjuvant for the diagnosis of thyroid lesions. In fact, HMGI(Y) and FRA-1 proteins are absent in normal thyroid and are induced in all carcinomas. However, a differential expression was observed in thyroid adenomas; FRA-1 protein is present in most adenomas (88% of cases), whereas HMGI(Y) protein is detectable in only ~20% of follicular adenomas (28). Therefore, the detection in thyroid adenomas of FRA-1, but not HMGI (Y), might exclude the diagnosis of follicular carcinomas. Conversely, this diagnosis might be taken in consideration when both these proteins are expressed, especially if FRA-1 protein is detected in the cytoplasm as well. At the same time, the negativity of a thyroid sample for both the genes might exclude the diagnosis of a hyperproliferative process. However, to assess the potential use of FRA-1 detection in the diagnosis of thyroid neoplasias, a higher number of normal thyroid, follicular, and anaplastic carcinoma samples must be analyzed.

The expression of the *fra-1* gene in human adenomas is consistent with the results regarding *in vitro* thyroid cell transformation. In fact, we showed that *fra-1* was expressed in all of the rat thyroid transformed cell lines, even in those that did not show a fully malignant phenotype, such as PC E1A, PC PTC, and PC Harvey (15).

In conclusion, *fra-1* gene activation appears to be an early event in the process of thyroid carcinogenesis, and its detection may represent, together with the analysis of other markers, a useful tool in the diagnosis of human thyroid neoplastic diseases.

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Retraction: FRA-1 Expression in Hyperplastic and Neoplastic Thyroid Diseases

Gennaro Chiappetta, Giovanni Tallini, Maria Cristina De Biasio, Francesca Pentimalli, Filomena de Nigris, Simona Losito, Monica Fedele, Sabrina Battista, Pasquale Verde, Massimo Santoro, and Alfredo Fusco

This article (1) has been retracted at the request of the editors. In Fig. 4, the GADPH lanes appear to be repeated as triplets, and Fra-1 lanes 6 through 15 appear to be duplicated and rotated in the same blots. The authors provided RT-PCR images that exhibit results similar to the claims in the article, but they have not been able to locate the original blots to address the concerns regarding potential image modification.

A copy of this Retraction Notice was sent to the last known e-mail addresses for 10 of 11 authors. One author (Giovanni Tallini) agreed to the retraction; four authors (Gennaro Chiappetta, Filomena de Nigris, Sabrina Battista, and Alfredo Fusco) did not agree to the retraction; five authors (Francesca Pentimalli, Simona Losito, Monica Fedele, Pasquale Verde, and Massimo Santoro) did not respond; the one remaining author (Maria Cristina De Biasio) could not be located.

Reference

1. Chiappetta G, Tallini G, De Biasio MC, Pentimalli F, de Nigris F, Losito S, et al. FRA-1 expression in hyperplastic and neoplastic thyroid diseases. Clin Cancer Res 2000;6:4300–6.

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FRA-1 Expression in Hyperplastic and Neoplastic Thyroid Diseases

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