SPANDIDOS PUBLICATIONS

Differentiation of human follicular thyroid adenomas from carcinomas by gene expression profiling

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Abstract. It is difficult to distinguish benign from malignant follicular thyroid tumors by histological or cytological examination. The goal of this study was to reveal gene expression variations between benign and malignant follicular lesions of the thyroid gland. We investigated gene expression profiles from 24 follicular thyroid tumors (12 carcinomas and 12 adenomas) and 13 normal thyroid tissues using high-density human cDNA arrays. The identification of gene expression changes was based on signal intensity ratios of tumor versus normal thyroid parenchyma. Expression patterns of a set of known genes were found to be significantly different between follicular adenomas and follicular carcinomas. Our results demonstrate a potential use of gene expression profiling for differentiating benign from malignant follicular thyroid tumors. A detailed investigation of the differentially expressed genes could give new insights into molecular pathways of malignant transformation of thyroid follicular neoplasm and may help to develop a molecular tool for the preoperative differential diagnosis.

Introduction

Thyroid carcinoma is the most common endocrine malignancy, accounting for 90% of all neuroendocrine malignancies and 1% of all malignant diseases (1,2). It is estimated that 5-10% of the population will develop a clinically significant thyroid nodule during their lifetime (3). Since the majority of thyroid nodules are benign, a major concern is to preoperatively differentiate between the benign and malignant thyroid lesions. Currently, the best diagnostic approach for such a preoperative evaluation is a fine needle aspiration (FNA) biopsy, which has greatly decreased the number of surgical intervention required for a thyroid nodule since it was introduced in 1970s (4). The diagnosis of papillary thyroid carcinoma is usually straightforward on FNA cytological examination, with a reliability of 95% (3,5). However, FNA cytology cannot discriminate between benign and malignant thyroid tumors with follicular growth features. Follicular thyroid adenoma (FTA) and follicular thyroid carcinoma (FTC) are often grouped together as indeterminate or suspicious thyroid lesions, which usually undergo hemithyroidectomy to look for invasion through the tumor capsule or the blood vessels. Accordingly, only ~20% of these indeterminate FNAs prove to be malignant (6-9). A postoperative diagnosis of malignancy leads to a second-stage complete thyroidectomy. This two-stage surgery has higher morbidity than initial total thyroidectomy. On the other hand, total thyroidectomy for all patients with suspicious thyroid lesions would result in many patients undergoing an unnecessary surgical procedure and requiring lifelong thyroid hormone replacement. Still, it is often difficult to achieve a clear cut histologic differentiation between FTA and FTC, which depends on tumor capsular penetration and vascular invasion. Thus, there is a clear need to search for molecular markers as ancillary tools for improving the preoperative diagnosis.

To date, no specific molecular markers have proved to be of practical diagnostic value for discriminating between benign and malignant follicular tumors, though many genetic alterations have been related to thyroid cancer. Among the genetic abnormalities, the *PAX8-PPARG* rearrangement and overexpression of galectin-3 (encoded by *LGALS3*) were initially reported to be promising markers for the differential diagnosis (10-12). However, other subsequent studies have shown that these molecules are also involved in benign lesions such as multinodular goiter and FTA (13-17). Gene expression profiling based on gene array technology has been used to characterize follicular thyroid tumors (18-23). Although results obtained from different research groups are not consistent, distinctive gene expression patterns have been revealed in FTC and FTA. Here, we report a filter-based cDNA array study on follicular thyroid tumors, in which differentially expressed genes were identified that can precisely distinguish between follicular adenomas and

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Abbreviations: FTA, follicular thyroid adenoma; FTC, follicular thyroid carcinoma; FNA, fine needle aspiration

Key words: gene expression profiling, cDNA array, follicular thyroid adenoma, follicular thyroid carcinoma

carcinomas. Our results support the potential use of gene expression profiling in the differential diagnosis of follicular thyroid tumor, and serve as a useful source for further investigation of molecular pathways involved in the malignant transformation of follicular thyroid neoplasm.

Materials and methods

Patients and tissue samples. Twenty-four patients who underwent operations at the University Hospital of Zurich or the Hospital of Zollikerberg, Zurich, Switzerland from 1998-2003 were enrolled in the study. Informed consent was obtained from each patient. An institutional review board approval for the study of human subjects was obtained from the Ethics Committees of the University Hospital of Zurich and the Hospital of Zollikerberg. The study subjects (19 women, 5 men) had a mean age of 46 years (range, 21-80 years) at the time of initial surgery and included 12 FTA and 12 FTC sufferers. All patients were euthyroid and had a normal value of the thyroid-stimulating hormone (TSH). They were not treated with any drugs before surgery. Thyroid specimens were collected intraoperatively. Each sample was snap-frozen in liquid nitrogen and stored at -80˚C. Both the tumor and surrounding health thyroid tissues were available from 13 of the 24 patients. Histologic assessment and classification were conducted according to the criteria of the WHO-classification (24). A clear cut diagnosis was achieved in all tumors. Frozen sections were taken from each tissue block and stained with hematoxylin-eosin (H&E). Representative areas containing >80% of the tumor cells or only normal thyroid tissue were selected for isolating RNA.

RNA preparation, hybridization and data generation. Extraction of total RNA, reverse transcription and hybridization were performed as previously described (25). Customer-designed cDNA filters that contain about 4,300 sequence-verified human cDNA clones, spotted in duplicate, were obtained from the Resource Center of the German Human Genome Project (RZPD; www.rzpd.de). The visualization of hybridization signals and signal normalization were conducted as described elsewhere (25). Signal intensity ratios of tumor versus the median of the 13 normal thyroid samples were calculated for each target on the array.

Data analysis. The GeneSight software (BioDiscovery, Inc., Los Angeles) was used for ratio calculation, hierarchical and k-means clustering, principal component analysis (PCA) and statistical analyses. On the basis of gene expression ratios of tumor versus normal samples, fold change analysis was conducted to detect genes with 2-fold or greater change. The fold change value was used to define the gene expression level. A gene that had a ratio value of ≥2 was considered to be up-regulated, whereas one with a ratio of ≤0.5 was regarded as down-regulated.

The gene expression profiles between the 2 tumor groups were statistically analyzed with the Student's t-test, assuming unequal variance, with a false discovery rate of 1% (P-value of <0.01) to produce a list of genes with differential expression. Hierarchical cluster analysis was performed for the differentially expressed genes to assess and illustrate a genetic similarity. The discriminative power of the selected genes was then evaluated through k-means cluster analyses in a leave-one-out procedure (LOOP), in which one sample at a time was held out. A permutation test was performed to verify the significance level for each gene by comparing its statistic to the permutation distribution of the tumor samples that were randomly divided into two groups. Three-dimensional PCA mapping was used to explore distributions of the examined samples on the basis of a set of selected genes. In PCA, the examined samples are represented by points in a three-dimensional space. The distance between any pair of points is related to the similarity between the two samples in high-dimensional space. Samples that are near each other are similar in gene expression profile and conversely, the ones that are far apart are different in expression pattern.

Results

Frequently de-regulated genes. After calculating the ratios of tumor versus the median value of normal thyroid specimens for each gene, we conducted fold change analysis to determine the most frequently de-regulated genes. The frequency of genes with at least a 2-fold alteration in thyroid tumors were assessed and summarized in Table I. No known gene was found to display a 2-fold or greater change in all follicular carcinomas or all follicular adenomas. Compared with adenomas, carcinomas showed more genes under-expressed and less genes over-expressed. The most frequently de-regulated genes, namely genes with ≥2-fold alteration detected in >8 adenomas or carcinomas, are shown in Table II.

Distinct gene expression profiles of the two tumor groups. Statistical significance in expression difference was determined by the Student's t-test for all 4,300 sequence-verified genes between the two tumor groups. Genes (304) that displayed differences at the significance level of P<0.01 underwent cluster analyses. We conducted a two-way hierarchical cluster analysis of the 24 tumors across the 304 genes. Tumors were segregated into two main histology-associated groups, one group comprising all adenomas and the other including all carcinomas (Fig. 1A). The 304 genes comprise of 131 known and 173 unknown genes. Since it is of diagnostic significance to discover molecular markers for distinguishing the benign from the malignant follicular thyroid tumors, we focused on the 131 known genes. The 24 tumors could also be divided by hierarchical clustering across the 131 known genes into two main groups, all FTAs on one side and all FTCs on the other side (Fig. 1B). The different gene expression patterns were validated by three-dimensional PCA analysis in the two tumor types (Fig. 2B). Then, we applied LOOP to perform k-means clustering. Adenomas and carcinomas were always separated on the basis of expression patterns of these genes (data not shown).

The main biological classifications of the differentially expressed genes are composed of signal transduction, transcription and post-transcription regulation, biosynthesis and metabolism, regulators of cell growth and proliferation, nucleic acid binding protein, immuno-response, transport, cell adhesion and extracellular matrix, and integral membrane proteins (Table III). The first 3 gene categories, i.e., signal

ummary of genes with ≥2-fold alteration in follicular thyroid adenoma and carcinoma.

Frequency[a]	No. genes overexpressed		No. genes underexpressed	
	FTA	FTC	FTA	FTC
≥5	947	587	350	620
≥6	632	403	184	413
≥8	269	229	42	131
≥10	17	10	2	12

[a]Number of tumors with ≥2-fold gene change in either FTA or FTC group. Each group has 12 tumors.

Table II. Genes with a 2-fold or greater alteration detected in ≥8 tumors of follicular thyroid adenoma or carcinoma group.

Accession No.	Gene	Mean		No. tumors		P-value
		FTA	FTC	FTA n=12	FTC n=12	
Overexpression						
aa036950	GENX-3414	3.48	6.15	10	11	NS
aa649213	ALOX12B	7.83	5.73	9	10	NS
aa447423	RFC3	2.63	2.84	9	10	NS
aa044065	SES2	2.16	2.88	8	10	NS
aa459286	3PAP	1.91	3.18	7	10	0.0065
r15405	PFKFB2	3.95	2.63	11	7	0.0414
ai760999	VPREB3	2.92	1.31	11	2	0.0003
ai004840	LHCGR	4.82	2.13	10	6	0.0077
ai190297	ADAMTS13	4.11	1.87	10	4	0.0021
ai200857	CST11	3.00	1.75	10	4	0.0494
n78927	MYL2	3.44	1.45	10	2	0.0012
Underexpression						
h13425	SDC2	1.13	0.27	8	11	NS
t83660	CANX	1.21	0.31	5	10	0.0036
w58337	CLPP	0.80	0.91	8	10	NS
ai701895	LENG1	1.03	0.75	8	10	NS
h01340	MAP3K10	1.10	1.47	6	10	NS
aa010529	SLC25A13	1.34	0.50	5	10	0.0400
w94954	TNNC2	1.49	0.61	4	10	0.0604
r37604	HGF	0.52	0.94	10	9	NS

NS, no significance.

transduction, transcription and post-transcription regulation, and biosynthesis and metabolism gene families, accounted for 13, 19 and 23% of the differentially expressed genes, respectively. The majority of these genes were down-regulated in FTC, compared with FTA. Only few genes were frequently up-regulated in FTC.

We then performed permutation analysis of these differentially expressed genes to find out the most significant gene signature with a small number of genes. Permutation analysis revealed 23 genes that can precisely distinguish malignant FTC from benign FTA by means of clustering and PCA mapping (Figs. 2A and C). Among the 23 genes, five genes (*BIKE*, *RAPTOR*, *PRKCD*, *PPP6C* and *PSEN2*) are associated with signal transduction, seven genes (*CDX2*, *SON*, *LSM4*, *ZDHHC2*, *DNAJA2*, *HIST1H4B* and *PHLDA1*) belong to the category of transcription and translation regulation, and five genes (*GBA3*, *PSMB6*, *URKL1*, *HARSL* and *PSMF1*) are related to protein, nucleic acid or carbohydrate biosynthesis and metabolism. The other 6 genes are members of gene families for cell growth and proliferation regulation (*ARHN*, *FTHFD* and *MLL*), immuno-reaction (*SDBCAG84* and *DBNL*) or transport (*ACCN1*).

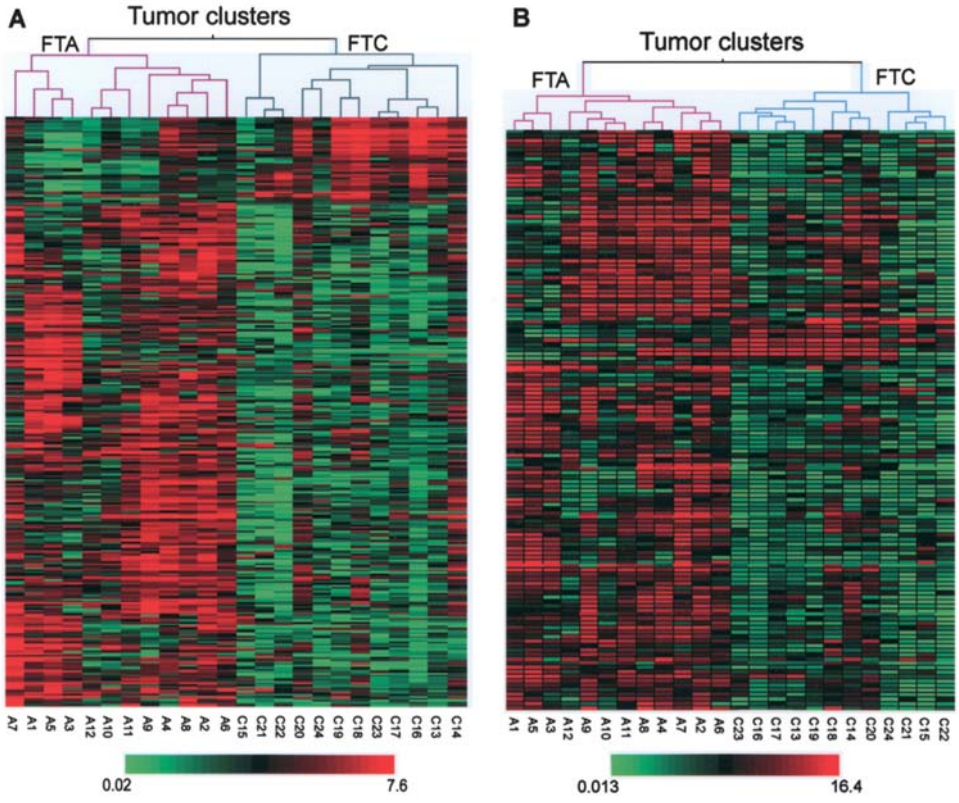

Figure 1. Hierarchical cluster analyses of 24 follicular thyroid tumors across 304 genes (A) and a set of 131 known genes (B). Expression profiles of these genes were significantly different between follicular thyroid adenomas (FTA) and carcinomas (FTC). The height of nodes of the hierarchical trees reflects the degree of similarity of gene expression. The 24 tumors examined fell into two major groups, as shown by the two branches of the dendrogram at the top (A and B). The group on the *left* includes all FTA samples, whereas the group on the *right* consists exclusively of FTC samples. The *colored bars* at the bottom indicate signal ratios of each tumor sample versus the median of 13 normal thyroid tissues. *Red*, over-expression; *green*, under-expression; *black*, unchanged expression.

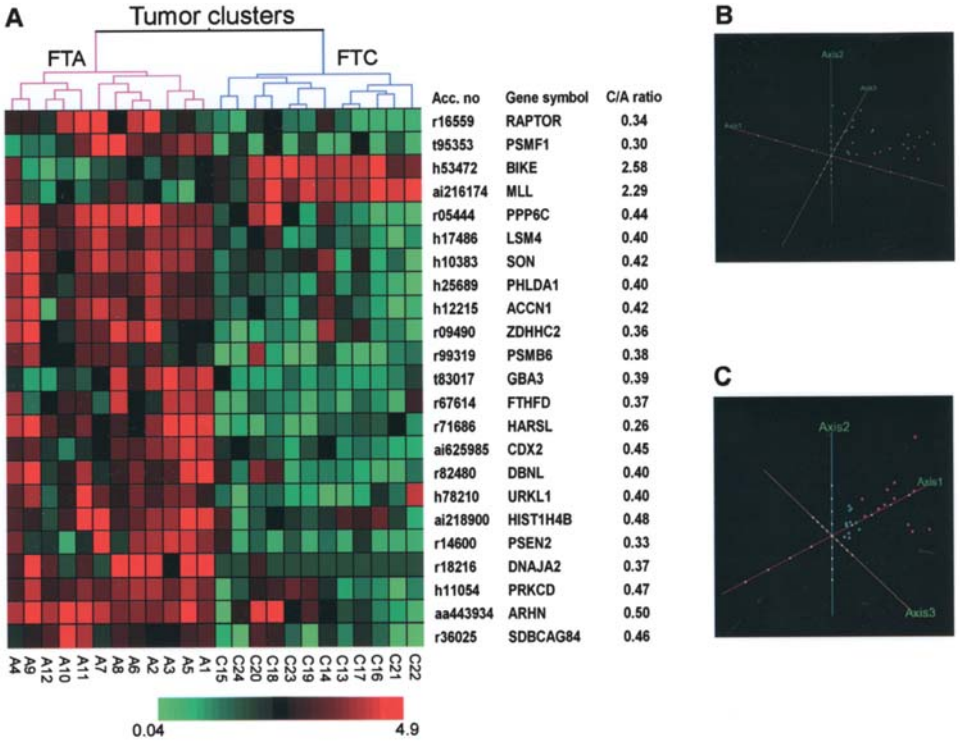

Figure 2. Hierarchical cluster analysis of 24 follicular thyroid tumors based on expression patterns of a set of 23 differentially expressed genes (A). C/A ratio indicates the mean ratio of 12 carcinomas (FTC) versus 12 adenomas (FTA). Three-dimensional principal component analysis (PCA) mappings were performed on the basis of 131 genes (B) and 23 genes (C), respectively. In PCA, red points represent FTA samples, whereas blue points represent FTC samples.

Genes differentially expressed between follicular thyroid adenomas and carcinomas.

Accession No.	Gene symbol	Function and description	Ca/Ad[a]	P[b]
Signal transduction				
ai089728	TJP1	Tight junction protein 1	2.4	0.0036
r51511	PTN	Pleiotrophin	0.6	0.0058
r14600	PSEN2	Presenilin 2	0.3	0.0005
h53472	BIKE	BMP-2 inducible kinase	2.6	0.0000
r16559	RAPTOR	Regulatory associated protein of mtor	0.3	0.0011
ai767059	SSH2	Slingshot 2	0.4	0.0053
r09807	CAMKK2	Calcium/calmodulin-dependent protein kinase kinase 2, ß	0.4	0.0001
r60529	NTRK3	Neurotrophic tyrosine kinase, receptor, type 3	0.4	0.0019
h12309	CARD10	Caspase recruitment domain family, member 10	0.4	0.0015
r05444	PPP6C	Protein phosphatase 6, catalytic subunit	0.4	0.0001
r62731	PRKCH	Protein kinase C, η	0.5	0.0021
h11054	PRKCD	Protein kinase C, δ	0.5	0.0000
ai033989	DUSP19	Dual specificity phosphatase 19	0.5	0.0091
r71903	PYGO2	Pygopus 2	0.5	0.0031
ai017154	NTSR2	Neurotensin receptor 2	0.6	0.0051
aa406076	ACRBP	Acrosin binding protein	0.6	0.0099
aa459286	3PAP	Phosphatidylinositol-3 phosphate 3-phosphatase adaptor subunit	1.7	0.0065
Transcription and post-transcription regulation				
h28806	SF3B2	Splicing factor 3b, subunit 2	0.4	0.0004
h17486	LSM4	LSM4 homolog, U6 small nuclear RNA associated	0.4	0.0000
n95774	LSM10	U7 snrnp-specific Sm-like protein LSM10	0.5	0.0076
r60068	DDX3	DEAD/H (Asp-Glu-Ala-Asp/His) box polypeptide 3	0.6	0.0072
n53492	ZFH4	Zinc finger homeodomain 4	0.3	0.0006
r25723	SNAPC2	Small nuclear RNA activating complex, polypeptide 2	0.3	0.0009
r09490	ZDHHC2	Zinc finger, DHHC domain containing 2	0.4	0.0001
r32478	TAF1B	TBP-associated factor, RNA polymerase I, B	0.4	0.0004
r18216	DNAJA2	Dnaj (Hsp40) homolog, subfamily A, member 2	0.4	0.0005
ai301528	HNF4A	Hepatocyte nuclear factor 4, α	0.4	0.0034
ai126286	PMFBP1	Polyamine modulated factor 1 binding protein 1	0.4	0.0017
aa621310	FOXE3	Forkhead box E3	0.4	0.0031
ai625985	CDX2	Caudal type homeo box transcription factor 2	0.5	0.0000
aa918361	PRKCBP1	Protein kinase C binding protein 1	0.5	0.0036
r64103	GTF3C3	General transcription factor IIIC, polypeptide 3, 102 kda	0.5	0.0019
aa446027	EGR2	Early growth response 2	0.5	0.0071
r14365	MEIS2	Myeloid ecotropic viral integration site 1 homolog 2	0.5	0.0091
aa400299	CEBPB	CCAAT/enhancer binding protein (C/EBP), ß	0.5	0.0020
t81077	FOXO3A	Forkhead box O3A	0.5	0.0089
aa398053	RFX2	Regulatory factor X, 2	0.6	0.0086
ai632019	ZNF259	Zinc finger protein 259	0.6	0.0074
ai189575	RING1	Ring finger protein 1	1.8	0.0007
aa035174	NR2F1	Nuclear receptor subfamily 2, group F, member 1	1.9	0.0088
r64142	EIF2B5	Eukaryotic translation initiation factor 2B, subunit 5 ε	0.5	0.0099
ai220048	RBMY1A1	RNA binding motif protein, Y chromosome, family 1, member A1	0.5	0.0025
h10383	SON	SON DNA binding protein	0.4	0.0000
aa974575	PRDM12	PR domain containing 12	0.5	0.0070
Nuclear protein				
t84575	SPP2	Secreted phosphoprotein 2	0.4	0.0029
ai218900	HIST1H4B	Histone 1, h4b	0.5	0.0010
aa868008	HIST1H4C	Histone 1, h4c	0.6	0.0073

Table III. Continued.

Accession No.	Gene symbol	Function and description	Ca/Ad[a]	P[b]
h25689	PHLDA1	Pleckstrin homology-like domain, family A, member 1	0.4	0.0000
r09545	MKI67IP	MKI67 interacting nucleolar phosphoprotein	0.4	0.0042
Biosynthesis & metabolism				
t70321	APOM	Apolipoprotein M	0.3	0.0018
ai693053	ALDH1A3	Aldehyde dehydrogenase 1 family, member A3	0.3	0.0010
h49424	GALNT9	UDP-galnac: polypeptide N-acetylgalactosaminyltransferase 9	0.4	0.0012
t83017	GBA3	Cytosolic glucosidase, ß, acid 3	0.4	0.0008
h78210	URKL1	Uridine kinase-like 1	0.4	0.0001
t96135	AGA	Aspartylglucosaminidase	0.5	0.0073
aa625737	GCAT	Glycine C-acetyltransferase	0.5	0.0054
h15299	MGLL	Monoglyceride lipase	0.5	0.0003
aa460833	PGCP	Plasma glutamate carboxypeptidase	0.6	0.0076
t66859	NDUFC1	NADH dehydrogenase (ubiquinone) 1	0.6	0.0026
r69327	SIPL	SIPL protein	0.6	0.0013
ai802786	UGT2B17	UDP glycosyltransferase 2 family, polypeptide B17	2.1	0.0051
ai831572	CYP4F11	Cytochrome P450, family 4, subfamily F, polypeptide 11	0.4	0.0027
r11209	CYP4F12	Cytochrome P450, family 4, subfamily F, polypeptide 12	0.5	0.0000
h93642	SCO1	SCO cytochrome oxidase deficient homolog 1	0.5	0.0018
r56054	B3GAT3	Beta-1,3-glucuronyltransferase 3	0.6	0.0050
r13098	DPYSL3	Dihydropyrimidinase-like 3	0.5	0.0019
w32322	ENPP4	Ectonucleotide pyrophosphatase/phosphodiesterase 4	0.6	0.0071
r69290	ENTPD1	Ectonucleoside triphosphate diphosphohydrolase 1	0.4	0.0020
r71686	HARSL	Histidyl-trna synthetase-like	0.3	0.0000
aa046808	RPS27L	Ribosomal protein S27-like	0.3	0.0015
t95353	PSMF1	Proteasome inhibitor subunit 1 (PI31)	0.3	0.0054
r99319	PSMB6	Proteasome subunit, ß type, 6	0.4	0.0001
t97170	PSMB7	Proteasome subunit, ß type, 7	0.4	0.0035
aa451675	FUT4	Fucosyltransferase 4	0.4	0.0079
r17368	PCMT1	Protein-L-isoaspartate O-methyltransferase	0.5	0.0024
aa147495	UST	Uronyl-2-sulfotransferase	0.6	0.0093
h96240	HECTD1	HECT domain containing 1	2.8	0.0054
ai190297	ADAMTS13	ADAM metalloprotease with thrombospondin type 1 motif, 13	0.5	0.0021
r88882	CPVL	Carboxypeptidase, vitellogenic-like	0.5	0.0069
Regulator of cell growth and proliferation				
h79153	INHBC	Inhibin, ß C	0.5	0.0053
n55474	MTCP1	Mature T-cell proliferation 1	0.5	0.0083
h10409	RAB40B	RAB40B, member RAS oncogene family	0.3	0.0000
aa443934	ARHN	Ras homolog gene family, member N	0.5	0.0003
ai473373	GLI	Glioma-associated oncogene homolog	0.5	0.0051
r60299	EXTL3	Exostoses (multiple)-like 3	0.5	0.0022
aa443351	ERBB2	V-erb-b2 erythroblastic leukemia viral oncogene homolog 2	2.0	0.0070
ai216174	MLL	Myeloid/lymphoid or mixed-lineage leukemia	2.3	0.0000
r67614	FTHFD	Formyltetrahydrofolate dehydrogenase	0.4	0.0000
r06442	TP53TG1	TP53 target gene 1	0.6	0.0078
Immuno-response				
r82480	DBNL	Encoding drebrin-like	0.4	0.0003
ai863022	MAGEA4	Melanoma antigen, family A, 4	0.4	0.0046
r36025	SDBCAG84	Serologically defined breast cancer antigen 84	0.5	0.0003
ai310402	LU	Lutheran blood group	0.5	0.0003
r10142	RHCE	Rhesus blood group, ccee antigens	0.5	0.0038

Continued.

Accession No.	Gene symbol	Function and description	Ca/Ad[a]	P[b]
r15360	CD14	CD14 antigen	0.5	0.0005
r14699	CDC42EP2	CDC42 effector protein 2	0.5	0.0085
h21675	HCA127	Hepatocellular carcinoma-associated antigen 127	0.6	0.0029
Ion/metal binding and transport proteins				
r01105	ABCE1	ATP-binding cassette, sub-family E, member 1	0.4	0.0016
h12215	ACCN1	Neuronal amiloride-sensitive cation channel 1	0.4	0.0000
h23053	SLC26A4	Solute carrier family 26, member 4	0.5	0.0075
r83155	TXN	Thioredoxin	0.5	0.0012
n78927	MYL2	Myosin, light polypeptide 2	0.4	0.0012
aa609449	KCNIP2	Kv channel interacting protein 2	0.5	0.0062
t83660	CANX	Calnexin	0.3	0.0036
Cell adhesion and extracellular matrix				
h68922	ITGA1	Integrin, α 1	0.5	0.0012
aa459863	ELMO2	Engulfment and cell motility 2	0.5	0.0004
h72722	MT1B	Metallothionein 1B	0.5	0.0021
Integral membrane proteins				
aa954092	RHBDL2	Rhomboid, veinlet-like 2	0.4	0.0021
r72100	SDC3	Syndecan 3	0.4	0.0085
Other				
r60010	VAMP2	Vesicle-associated membrane protein 2	0.6	0.0034
w72859	VAMP5	Vesicle-associated membrane protein 5	2.2	0.0058
aa488177	DCTN4	Dynactin 4 (p62)	0.4	0.0003
r61070	SCG3	Secretogranin III	0.4	0.0010
r68779	GMEB2	Glucocorticoid modulatory element binding protein 2	0.4	0.0071
ai004840	LHCGR	Luteinizing hormone/choriogonadotropin receptor	0.4	0.0077
r08607	ET	Hypothetical protein ET	0.4	0.0006
ai142358	NOR1	Oxidored-nitro domain-containing protein	0.4	0.0006
ai760999	VPREB3	Pre-B lymphocyte gene 3	0.4	0.0003
aa705793	SEC24D	SEC24 related gene family, member D	0.5	0.0029
ai073387	CLIP-2	Cartilage intermediate layer protein-like protein 2	0.5	0.0072
r27568	SEPP1	Selenoprotein P, plasma, 1	0.5	0.0003
r25643	VIP32	Vasopressin-induced protein, 32 kda	0.5	0.0013
aa143058	HSPC138	Hypothetical protein HSPC138	0.5	0.0073
t71617	DNM1L	Dynamin 1-like	0.5	0.0023
ai143912	K5B	Keratin 5b	0.5	0.0052
h09157	FEZ1	Fasciculation and elongation protein ζ 1	0.5	0.0053
h06531	DNAJA1	Dnaj (Hsp40) homolog, subfamily A, member 1	0.5	0.0065
r78865	BIGM103	BCG-induced gene in monocytes, clone 103	0.6	0.0038
r70257	CAPG	Capping protein (actin filament), gelsolin-like	0.6	0.0033
aa863023	RYD5	Putative ligand binding protein RYD5	0.6	0.0029
r89095	UBL5	Ubiquitin-like 5	0.6	0.0044

[a]The mean ratio of Ca (follicular thyroid carcinoma) versus Ad (follicular thyroid adenoma). [b]Student's t-test was used.

Discussion

In this study, we utilized the gene array technology to survey molecular variation of follicular thyroid lesions in an attempt to identify potential molecular markers for the differential diagnosis of FTA and FTC, and to gain new insights into the pathogenesis of the malignant transformation of thyroid lesions. We revealed a set of genes that can precisely distinguish the malignant follicular tumors from the benign ones on the basis of gene expression patterns. Our results

strengthen the view that the array technology-based gene expression profiling, when applied to FNA cytology, could certainly help the clinical management of patients with suspicious thyroid lesions. Previous studies have shown that it is possible to determine the level of transcripts by PCR using as few as 10 cells obtained from the FNA biopsy (26), and that the gene array technique is suitable for preoperative diagnosis of thyroid cancer (27).

Notably, many of the differentially expressed genes identified in this study are related to signal transduction, transcription and post-transcription regulation, and the biosynthesis and metabolism of protein, fat, carbohydrate and nuclear acid. This finding is generally consistent with previous reports (28,29), implying that abnormal changes in signaling pathways and/or transcription and post-transcription regulation result in disturbances in the biosynthesis and metabolism of proteins, which in turn impact the metabolisms of other substances such as fat, carbohydrate and nuclear acid. Most of the identified genes have not been reported previously in the context of thyroid malignancies. A further detailed evaluation of these genes would provide important information about the relationship between FTA and FTC and the underlying mechanisms of thyroid malignant transformation.

In our previous cDNA array study on follicular and papillary thyroid cancer (25), it was shown that the majority of genes with altered expression were involved in both follicular (FTC) and papillary thyroid carcinomas (PTC). Similarly, the results presented here also demonstrates that both FTC and FTA share a majority of genetic alterations. Only a small number of genes were found to be differentially expressed between FTC and PTC or between FTC and FTA. The shared molecular characteristics reflect the close histological and biological relationship of the three types of thyroid tumors, while the differentially expressed genes may contribute to their different cellular appearance and biological behavior.

It is noteworthy that none of these differentially expressed genes were consistently up- or down-regulated in all FTC or all FTA samples and that these genes, only when they are used as a whole, could correctly partition the samples into the histopathology-associated groups. This type of assignment is based on the distinctive expression pattern of a gene signature containing a set of genes, rather than the degree of up or down-regulation of any particular gene. This phenomenon is consistent with the knowledge that multiple genetic events are involved in the thyroid tumorigenesis. In theory, an initial slight abnormality of a specific gene could greatly impact other genes in its pathway and result in disturbances in biosynthesis and metabolism of many related proteins, which in turn affect cell regulation, differentiation and growth. The degree of multiple molecular alterations may be much more evident than that of the causative specific gene, making it difficult to identify the causative gene. Therefore, a specific gene, which is found in a univariate study to be associated with thyroid cancer, may not turn out to be the best multivariate factor for predictive and diagnostic purposes. Our results demonstrate that, for the purpose of differential diagnosis of follicular thyroid lesions, individual genes might be less important.

The present study provides a clear example of how gene expression profiling could be highly useful for the diagnosis of thyroid cancer. Several similar studies have been published and different gene signatures described as being useful for distinguishing between the benign and malignant thyroid tumors (18-23,29). Some research groups have even shown that a combination of several genes can be used for this differential diagnostic purpose. For example, Weber *et al* proposed a three-gene combination (*CCND2*, *PCSK2* and *PLAB*) as a classifier, since they evaluated the expression level of the 3 genes by real-time PCR from a set of 80 differentially expressed genes they had identified (20), and Mazzanti *et al* described a predictor model using 6 or 10 genes selected from 47 genes differentially expressed between FTA and FTC (19). However, gene signatures reported to date in regard to the differentiation of FTA from FTC, like in other human tumors, differ among different research groups. Although some labs could partly confirm their gene array results by real-time PCR or by using an independent group of test samples, consistent findings have not been obtained by others. This apparent lack of reproducibility, which has been construed as a weakness of genome-wide gene expression data, may arise from the intrinsic properties of different array platforms themselves (30,31). In addition, other possible causes of the discordance in gene expression profiling include: (i) different experimental conditions, e.g., variations in labeling and hybridization, (ii) different procedures in the gene selection, which may lead to the identification of different sets of targets, and (iii) different statistical models for data analysis. The disharmony is a challenge for developing diagnostic and prognostic tools. To eliminate this discordance, international co-operative efforts are required to develop a set of standards and refine these array technologies, which are now in progress.

Nonetheless, our study and other similar ones suggest that gene expression profiling can be used to differentiate a benign from a malignant tumor and therefore is a promising tool in the future for clinical decision making. A combination of the genes identified in this study and the ones detected in other studies might increase the reliability of the differential diagnosis of a follicular thyroid tumor. Clearly, further work is needed to evaluate the different sets of genes identified by different research groups, in order to develop a clinically useful testing system. It is important that such a testing system must be rigorously evaluated using larger data sets to obtain reliable performance estimates. We hope that the results presented here will provide a useful source for developing such a testing system and therefore facilitate differential diagnoses of thyroid nodules to eliminate unnecessary operations for patients with suspicious thyroid lesions.

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