

Human Thyroid Tumor Cell Lines Derived from Different Tumor Types Present a Common Dedifferentiated Phenotype

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Abstract

Cell lines are crucial to elucidate mechanisms of tumorigenesis and serve as tools for cancer treatment screenings. Therefore, careful validation of whether these models have conserved properties of *in vivo* tumors is highly important. Thyrocyte-derived tumors are very interesting for cancer biology studies because from one cell type, at least five histologically characterized different benign and malignant tumor types can arise. To investigate whether thyroid tumor-derived cell lines are representative *in vitro* models, characteristics of eight of those cell lines were investigated with microarrays, differentiation markers, and karyotyping. Our results indicate that these cell lines derived from differentiated and undifferentiated tumor types have evolved *in vitro* into similar phenotypes with gene expression profiles the closest to *in vivo* undifferentiated tumors. Accordingly, the absence of expression of most thyrocyte-specific genes, the nonresponsiveness to thyrotropin, as well as their large number of chromosomal abnormalities, suggest that these cell lines have acquired characteristics of fully dedifferentiated cells. They represent the outcome of an adaptation and evolution *in vitro*, which questions the reliability of these cell lines as models for differentiated tumors. However, they may represent useful models for undifferentiated cancers, and by their comparison with differentiated cells, can help to define the genes involved in the differentiation/dedifferentiation process. The use of any cell line as a model for a cancer therefore requires prior careful and thorough validation for the investigated property. [Cancer Res 2007;67(17):8113–20]

Introduction

Tumor-derived cell lines are extensively used in cancer research because they can provide insight into underlying mechanisms that play a role in tumor biology. They have a number of advantages, such as the possibility to generate sufficient quantities of research material, their use for genetic manipulation, and the opportunity to use them for both *in vitro* as well as *in vivo* (e.g., xenografts) studies. Cell lines can be useful to elucidate a number of characteristics of

tumors, such as growth kinetics and metastatic properties, and serve as first-line assays for putative treatments (1–4). Moreover, the existence of only one cell type in culture can be helpful for the interpretation of data derived from microarray studies where tumors are compared with nontumor tissues containing different cell types that can even exist in different proportions. Therefore, single cell type-derived cell lines can provide insight into the primarily deregulated genes in the transformed cells themselves. They allow analysis of tumor properties and behavior provided that they have maintained properties of *in vivo* tumors. We have tested this hypothesis using as a model tumors of the thyroid.

During the past years, a number of thyroid tumor cell lines from different pathologic origin have been developed (5–12). Thyroid tumors are interesting in this context because, compared with other tissue-derived tumors, at least five different histologically characterized types of tumors, showing differences in biological behavior and degree of differentiation, can arise from one cell type (i.e., the follicular epithelial cell or thyrocyte). These tumors include two benign tumor types (i.e., hyperfunctioning autonomous adenomas and follicular adenomas) and three malignant tumor types [i.e., differentiated follicular carcinomas (FTC), papillary carcinomas (PTC), and nondifferentiated highly aggressive anaplastic carcinomas (ATC); ref. 13]. Recent studies show that gene expression profiling by microarrays is able to distinguish *in vivo* benign from malignant thyroid tumors (14) and PTCs from FTCs (15). These findings led us to investigate the *in vitro* properties of thyroid tumors by using thyroid tumor-derived cell lines of different origin whose gene expression profiles were established and then compared with a panel of *in vivo* tumors. These commonly used cell lines were derived from one follicular adenoma (KAK-1), two FTCs (FTC-133 and WRO), three PTCs (B-CPAP, KAT-10, and TPC-1), and two ATCs (8505C and KAT-4; refs. 5–12). Because *in vivo* thyroid tumors show differences in their degree of differentiation, we investigated this aspect in the cell lines by using a number of widely used thyrocyte-specific markers, including the thyrotropin (TSH) receptor (*TSHR*), the sodium/iodide symporter (*NIS*), thyroglobulin (*Tg*), thyroperoxidase (*TPO*), the two NADPH oxidases *ThOX1* and *ThOX2*, the transcription factors *TTF1* and *TTF2*, and the paired-box containing transcription factor-8 (*PAX8*). In addition, gene expression profiles of the cell lines were compared with well-characterized differentiated cells to elucidate genes involved in differentiation. Moreover, the responsiveness of these cell lines to the physiologic stimulus TSH and to forskolin was determined, as well as their karyotypes.

Our results show that these cell lines have gene expression profiles more closely related to each other than to the *in vivo* differentiated tumors they were derived from and have characteristics of fully

Note: Supplementary data for this article are available at Cancer Research Online (<http://cancerres.aacrjournals.org/>).

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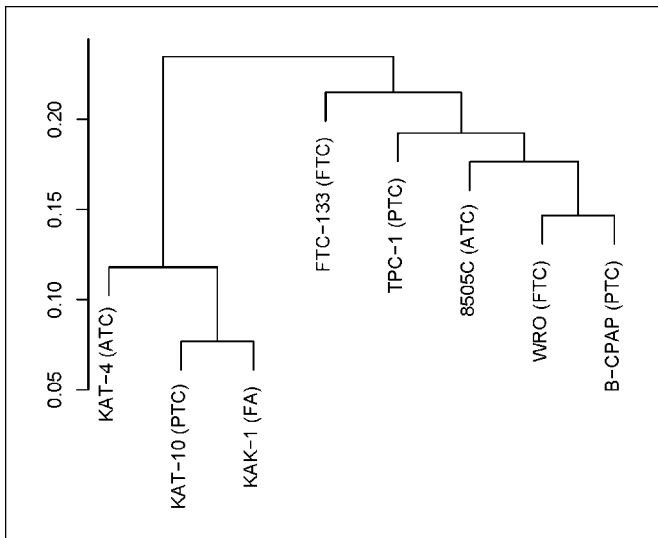


Figure 1. Hierarchical clustering of human thyroid tumor cell lines based on the global gene expression. For each cell line, the tumor origin, as described in the literature, is indicated in parenthesis. FA, follicular adenoma.

dedifferentiated cells. In line with these results, comparison of their overall gene expression profiles with a panel of *in vivo* thyroid tumors, containing benign and malignant tumors, showed that the cell lines group closest with undifferentiated carcinomas (i.e., ATC). Therefore, the cell lines may be useful models for undifferentiated, but not for differentiated, thyroid tumors. Their comparison with differentiated cells can elucidate the genes involved in the process of differentiation/dedifferentiation.

Materials and Methods

Cell culture. Tumor cell lines, originally described to be obtained from eight different patients, were derived from one follicular adenoma [KAK-1 (5)], two FTCs [FTC-133 (6) and WRO (UCLA-RO 82 W-1; ref. 7)], three PTCs [B-CPAP (8), KAT-10 (9), and TPC-1 (10)], and two ATCs [8505C (11) and KAT-4 (12)]. The cell lines KAK-1, KAT-10, and KAT-4 were obtained from Dr. K.B. Ain (University of Kentucky, Lexington, KY). FTC-133, WRO, B-CPAP, and 8505C were a gift from Dr. G. Brabant (Medizinische Hochschule, Hannover, Germany). TPC-1 was obtained from Dr. M. Mareel (University of Gent, Gent, Belgium). All cell lines were cultured as monolayers in a humidified atmosphere (5% CO₂) at 37°C according to the instructions of their providers (see Supplementary Materials and Methods). To investigate the effect of forskolin on differentiation expression, cell lines were deprived for 48 h from serum and thereafter incubated with forskolin (10 μmol/L) for 24 h, followed by total RNA isolation for reverse transcription-PCR (RT-PCR).

Tumor tissue samples. For the investigation of gene expression profiles of a panel of benign and malignant thyroid tumors, paired samples of nontumor and tumor thyroid tissues were obtained from patients undergoing surgery for thyroid disease. Diagnoses and preparation of a pool of hyperfunctioning autonomous adenomas (n = 5) was done as previously described (16). PTCs were obtained from the Ambroise Paré Hospital (Boulogne, France; sporadic PTCs, n = 10) and from the Institute of Oncology and Metabolism (Kiev, Ukraine; post-Chernobyl PTCs, n = 6), which also provided one FTC (n = 1) and one follicular adenoma (n = 1). ATCs were obtained from the Jules Bordet Institute (Brussels) and the Ambroise Paré Hospital (France). Final diagnoses were made by pathologists and characteristics of these tumors are described elsewhere (17).⁷ All tissues were immediately dissected, placed on ice, snap-frozen in

liquid nitrogen, and stored at -80°C until processing. The protocol was approved by the ethics committees of the institutions.

Microarrays and data analysis. Five micrograms of total RNA from the cultures and tissues were used for microarray analysis. Samples were prepared as described previously (16). Scanning of in-house cDNA microarrays containing 23,232 spots, preprocessing and normalization of raw data, and replicate averaging were done as previously described (16) with the bioconductor 1.9 bioinformatics software (18) for the R programming language 2.4.0. The samples (three ATCs and three PTCs), which were assayed according to manufacturer's instructions on oligonucleotide Affymetrix Human Genome U133 Plus 2.0 microarrays, were processed together with the bioconductor grma package. Each sample was compared to a control to obtain differential expression measurements (log₂ ratio): human thyroid tumor cell lines were compared with a pool of RNA from six primary cultures of normal human thyrocytes; PTCs, the follicular adenoma, and FTC were individually analyzed comparing directly each tumor with its corresponding nontumor adjacent tissue; ATCs were compared with a pool of normal tissues (n = 23) obtained from the same lobe as resected lesions. Unsupervised analysis was done on the basis of between-sample, correlation-based distances. Average linkage was used for hierarchical clustering (as implemented by the hclust R function), first on the 17,702 spots remaining after filtering out spots with more than two missing values across the cell lines (Fig. 1) and, second, in the two-way clustering for the display of differential expression (Fig. 5).

To assess the stability of the cell lines cluster dendrogram (Fig. 1), we used a bootstrap approach as implemented in the bioconductor pvclust package 1.2-0 (Supplementary Fig. S1). Multidimensional scaling (as implemented by the isoMDS function in the MASS 7.2-29 package for R) was done to compare the cell lines to the tissues (Fig. 2). To compare the data from two microarray platforms, we averaged the expression values for probes annotated with the same National Center for Biotechnology Information Entrez Gene identifiers within each platform and retained

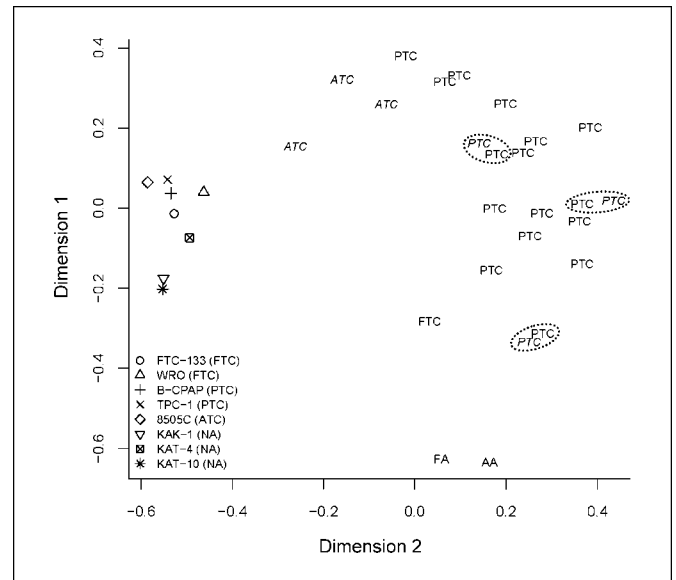


Figure 2. Global gene expression multidimensional scaling of human thyroid tumor cell lines and a panel of *in vivo* benign and malignant tumors. Benign tumors consisted of a pool of five hyperfunctioning autonomous adenomas and one follicular adenoma. Malignant carcinomas consisted of 16 PTCs, 1 FTC, and 3 undifferentiated ATCs. Cell lines, autonomous adenoma, follicular adenoma, FTC, and PTCs were hybridized on in-house-manufactured slides. Three PTC samples, already hybridized on homemade slides, and ATCs were hybridized on Affymetrix slides (*in italics*). Analysis was made based on all the genes in common between the homemade and the Affymetrix platforms. Comparison of the same PTC samples between the two platforms showed that their gene expression profiles were highly similar (*circled*). Thus, gene expression profiles from all samples can be compared regardless of the platform. Stress is 13.6%. For each cell line, the tumor origin is indicated in parenthesis. NA, not available.

⁷ L. Delys et al, in preparation.

the 3,751 genes present in both platforms. To identify genes commonly regulated across all cell lines (Supplementary Table S3), a one-class Significance Analysis of Microarrays (19) analysis was run as implemented in the bioconductor siggenes package version 1.7.1. The remaining missing values after filtering as described above were replaced by the spot-wise mean. SAM identified 6,286 significantly regulated spots (q -value < 0.01).

Reverse transcription-PCR. The expressions of *TSHR*, *NIS*, *Tg*, *TPO*, *ThOX1*, *ThOX2*, *TTF1*, *TTF2*, and *PAX8* were investigated in the eight human thyroid tumor cell lines by RT-PCR as described in Supplementary Materials and Methods. Primer sequences for the investigated genes are given in Supplementary Table S1.

Cyclic AMP measurements. Cyclic AMP (cAMP) measurements were done as described in Supplementary Materials and Methods.

Chromosome analysis. For each cell line, 15,000 to 30,000 cells were seeded in single chamber slides (Lab-Tek, Nalgen Nunc International) and cultured until they reached near confluence. Cells were harvested using standard methods. Chromosome analysis was done according to the routine GTG-banding procedure. Slides were analyzed and pictures were captured using a charge coupled device cooler camera and the software Cytovision (Applied Imaging). Karyotypes were described according to the International System for Human Cytogenetic Nomenclature (1995).

DNA profiling. Genomic DNA was extracted from the cell lines using DNeasy Tissue Kit (Qiagen) according to the manufacturer's instructions, followed by a multiplex PCR amplification of 1 to 2 ng of DNA with a AmpFLSTR Identifier PCR Amplification kit (Applied Biosystems) according to the manufacturer's protocol. PCR products were detected on 3130 ABI Prism and analyzed by Gene Mapper ID (Software v3.2, Applied Biosystems).

Results

Cell lines cluster independently of their tumor of origin. We investigated the gene expression of each cell line by hybridization onto in-house-manufactured cDNA microarray slides. The quality and reproducibility of our microarray data have been shown previously (16). Gene expression profiles from the follicular adenoma cell line KAK-1; the FTC cell lines FTC-133 and WRO; the PTC cell lines B-CPAP, TPC-1, and KAT-10; and the ATC cell lines KAT-4 and 8505C were made comparing each of the cell lines with normal human thyrocytes from primary cultures. Hierarchical clustering of these data showed a division into two groups: the cell lines KAK-1, KAT-4, and KAT-10 clustered on one side, whereas FTC-133, TPC-1, 8505C, B-CPAP, and WRO cells clustered in another group (Fig. 1). Bootstrap analysis confirmed the stability of the hierarchical clustering (Supplementary Fig. S1). Thus, the gene expression profiles of cell lines derived from the same type of thyroid tumor did not cluster together.

DNA profiling reveals a common origin for the KAT family of cell lines. The tight clustering of KAK-1, KAT-4, and KAT-10, originating from the same laboratory, indicates a high similarity between these three cell lines despite the markedly higher number of chromosomes of KAT-4 compared with KAK-1 and KAT-10 (see below). Furthermore, the KAT family (KAK-1, KAT-4, and KAT-10) shares a similar point mutation in E-cadherin, which is rare in thyroid tumors. Additionally, they contain a heterozygous mutation of B-RAF^{V600E} that is exclusively found in PTCs and in some ATCs, and they share a common genetic background (20). Our clustering results and the findings reported by Rocha et al. (20) raised the question about the exact tumor origin of these three cell lines. To further identify the origin of all cell lines, DNA profiling was done and showed that the cell lines were derived from six individuals, with KAK-1, KAT-4, and KAT-10 originating from the same individual (Supplementary Fig. S2). Additional DNA in KAT-4 indicates a contamination of this cell line by other cells. An

additional hierarchical clustering analysis, including individually each cell line of the KAT family with the five other cell lines, did not change the above results (i.e., gene expression profiles of cell lines derived from the same type of thyroid tumor do not cluster together; Supplementary Fig. S3).

Molecular profiles of cell lines are highly similar and closest to undifferentiated *in vivo* thyroid cancers. The overall gene expression profiles of the cell lines were compared with those of a panel of *in vivo* solid tumors by multidimensional scaling (Fig. 2). This algorithm reduces the high-dimension gene space into two dimensions while preserving distances between the profiles, and thereby visualizes the similarity relationships between samples. The *in vivo* tumors span the different thyroid tumor pathologies: two benign tumor types [a pool of five hyperfunctioning autonomous adenomas (16) and one follicular adenoma] and three malignant types: 1 FTC, 16 PTCs (17), and three undifferentiated ATCs.⁷ Analysis of the overall gene expression profiles showed that cell lines derived from differentiated (FTC and PTC) and undifferentiated (ATC) tumors grouped closely together and were apart from the *in vivo* tumors. *In vivo* benign tumors (autonomous adenomas and follicular adenoma) clustered closely together but were very distant from the undifferentiated ATCs, whereas PTCs and the FTC grouped in between benign tumors and ATCs. The molecular profiles of the ATCs were the closest to those of the cell lines (Fig. 2).

Cell lines have lost the expression of most of the thyroid-specific genes and do not respond to TSH. Thyrocytes are differentiated cells that are characterized by the expression of a number of genes that are involved in thyroid function. The expression of a number of these markers is decreased or lost during the process of thyroid tumorigenesis (21, 22). RT-PCR was done on the cell lines to investigate the mRNA expression of the following markers: *TSHR*, *NIS*, *Tg*, *TPO*, *ThOX1*, *ThOX2*, *TTF1*, *TTF2*, and *PAX8*. *PBGD*, a housekeeping gene, was used to normalize mRNA expression among the different samples (Fig. 3). RNA extracted from a hyperfunctioning thyroid of a patient with Graves' disease was used as a positive control. The cell lines investigated have lost the expression of most of the thyroid-specific markers, including the *TSHR*, *NIS*, *Tg*, *TPO*, and *ThOX2*. A clear *PBGD* amplification product indicated the integrity of the cDNA obtained after reverse transcription of the RNA isolated from each single sample. Although *ThOX2* could not be detected in any cell line, *ThOX1* was weakly detected in B-CPAP, KAT-4, and KAT-10. Among thyroid-transcription factors, the transcription factor *PAX8* was found in FTC-133, WRO, B-CPAP, TPC-1, and KAT-4. *TTF1* was expressed in all cell lines, and *TTF2* was faintly detected in WRO, B-CPAP, TPC-1, and KAT-10.

As the cell lines were cultured in the absence of TSH, which could be responsible for partial loss of differentiation, we investigated whether the adenylyl cyclase activator forskolin could induce the expression of thyroid-specific markers. The cell lines were incubated with 10 μ mol/L forskolin for 24 h, but in none of the cell lines did this treatment induce any of the thyroid-specific markers (Supplementary Fig. S4).

The lack of detection of the *TSHR* was further investigated by treating the cell lines for 2 h with 1 mU/mL TSH or with 1 μ mol/L forskolin in the presence of the phosphodiesterase type 4 inhibitor rolipram (25 μ mol/L) followed by measurements of cAMP levels by a RIA (Fig. 4). In none of the cell lines was an increase of the cAMP content found after treatment with TSH. TSH-treated to control ratios for all the cell lines were between 0.93 and 1.06. Incubation

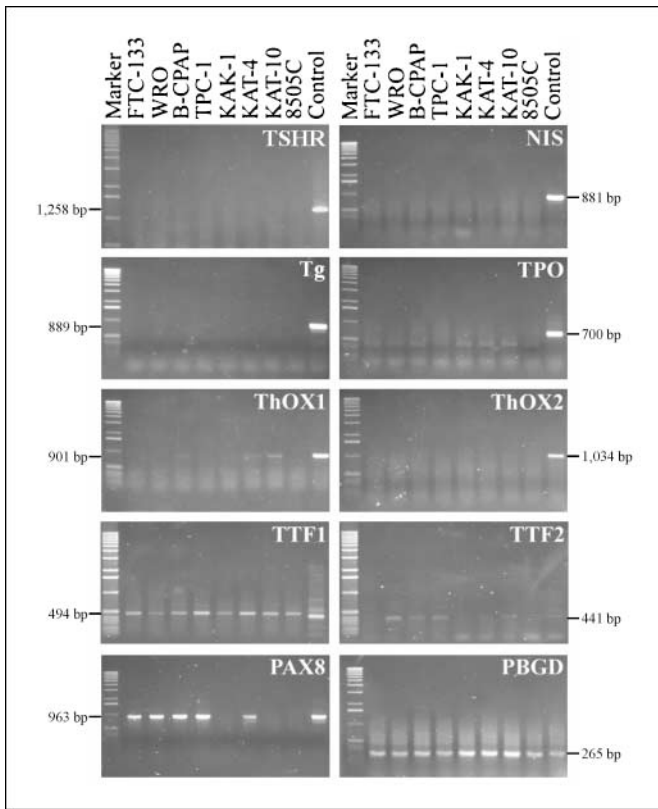


Figure 3. Semiquantitative RT-PCR analysis of the mRNA expression of thyroid-specific genes (*TSHR*, *NIS*, *Tg*, *TPO*, *ThOX1*, and *ThOX2*), thyroid-transcription factors (*TTF1*, *TTF2*, and *PAX8*), and a housekeeping gene (*PBGD*) in human thyroid tumor cell lines compared with a positive control, thyroid RNA extracted from a patient with Graves' disease.

with 10 mU/mL TSH gave similar ratios (not shown). In contrast, incubation with forskolin strongly increased cAMP levels in all cell lines, varying from a 5.2-fold (for B-CPAP) to a 2,084-fold (for KAT-10) increase versus the control (Fig. 4).

Other groups have investigated the expression of thyroid-specific markers in some thyroid cell lines and have reported similar results, but also some discrepancies (2, 3, 7, 8, 23, 24). In particular, the detection of the *TSHR* varied considerably between groups. Different experimental conditions, such as the number of PCR cycles used or, in the case of Western blot analysis, a difference in exposure time of the membrane (24), may account for some discrepancies. However, an increase in the number of PCR cycles from 30 to 35 did not result in the detection of the *TSHR* in any of the eight cell lines (not shown).

Cell lines show a down-regulation of differentiation genes.

The differentiation status of the cell lines was further explored by comparing their gene expression profiles with those of differentiated cells: human primary cultured thyrocytes treated with TSH for 1.5 to 48 h, and with those of autonomous adenomas, highly differentiated benign thyroid tumors (Fig. 5; ref. 16). For the analysis, up-regulated genes in the differentiated cells were selected (Fig. 5); that is, up-regulated (\log_2 ratio > 1) at least at one time point after TSH treatment and which had a positive value (\log_2 ratio > 0) in autonomous adenomas (16). From the total of these 66 up-regulated genes, 14 genes had an average positive value in the cell lines (21%), whereas 52 genes had a negative value in the cell lines (78%).

From the data in Fig. 5, we found, as might be expected, that the genes that were up-regulated in the differentiated thyrocytes and in the cell lines such as *PPIF* and *EFHD2* are more likely involved in proliferation, whereas the genes up-regulated in the primary cultured thyrocytes but down-regulated in the cell lines are more likely involved in differentiation. Examples of such genes are *DIO2*, *ITPR1*, *CRABP1*, and *WARS* (Fig. 5).

Cell lines have abnormal chromosome numbers and structures. Thyroid tumors have been associated with chromosome abnormalities and gains and losses of chromosomes have been described, indicating that FTCs and ATCs are genetically less stable compared with PTCs (25). Karyotypes were determined for all the cell lines using GTG banding (Fig. 6). Numerical and structural anomalies were observed for the cell lines. The FTC cell lines FTC-133 and WRO karyograms had 62 to 71 and 68 to 77 chromosomes, respectively. The PTC cell lines B-CPAP and TPC-1 had 72 to 73 and 49 chromosomes, respectively. The ATC cell line 8505C contained 60 to 62 chromosomes. The karyograms of the KAT family showed 60 to 67 chromosomes (KAK-1), 63 to 68 chromosomes (KAT-10), and around 100 chromosomes (KAT-4; Fig. 6). The karyotype of KAT-4 is not shown because the large number of chromosome abnormalities precluded a reliable classification. Cell lines were hyperdiploid (TPC-1), hypotriploid (KAK-1, KAT-10, 8505C), hypertriploid (FTC-133, WRO, B-CPAP), or hypertetraploid (KAT-4). All karyograms were structurally abnormal and, except for TPC-1, included several unidentifiable markers (chromosomes) indicated by "A" in Fig. 6.

From the cell lines used in this study, to our knowledge, a karyotype description has been reported only for the cell lines FTC-133, B-CPAP, and WRO (7, 26, 27). Extensive cytogenetics studies on the B-CPAP cell line (27) were recently reported. The karyotype we obtained is 72,XX,-X, i(1)(p10), i(5)(p10), der(5)t(5;9)(p10;p10), der(8)t(5;8)(q13;p22-23), der(10)t(1;10)(q10;q10)del(1)(q21q32), +11, +dic(12;20)(p11.2;p11.2), -13, +14, +15, -16, der(17)inv(17)(p13q11.1)inv(q11.1q23), +19, +20, +der(20)t(7;20)(p10;p10), +der(20)add(20)(q13.2), -21, -22, +mar1, +mar2. Divergences between previously reported and present karyotypes concerned numerical and structural alterations: Particularly, dic(5;13)(q10;q10) was not recovered. For the WRO cell line, the initial chromosomal pattern (7) with 62 to 82 chromosomes and five markers was strikingly different from the pattern we obtained.

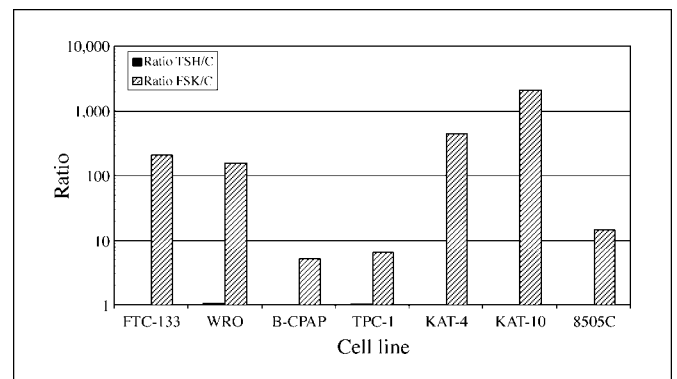


Figure 4. Representative data of cAMP measurements in the human thyroid tumor cell lines after 2 h of treatment with 1 mU/mL TSH or 1 μmol/L forskolin (FSK) compared with untreated controls. \log_{10} ratios of cAMP levels in treated and untreated cells (TSH/control; FSK/control) are plotted.

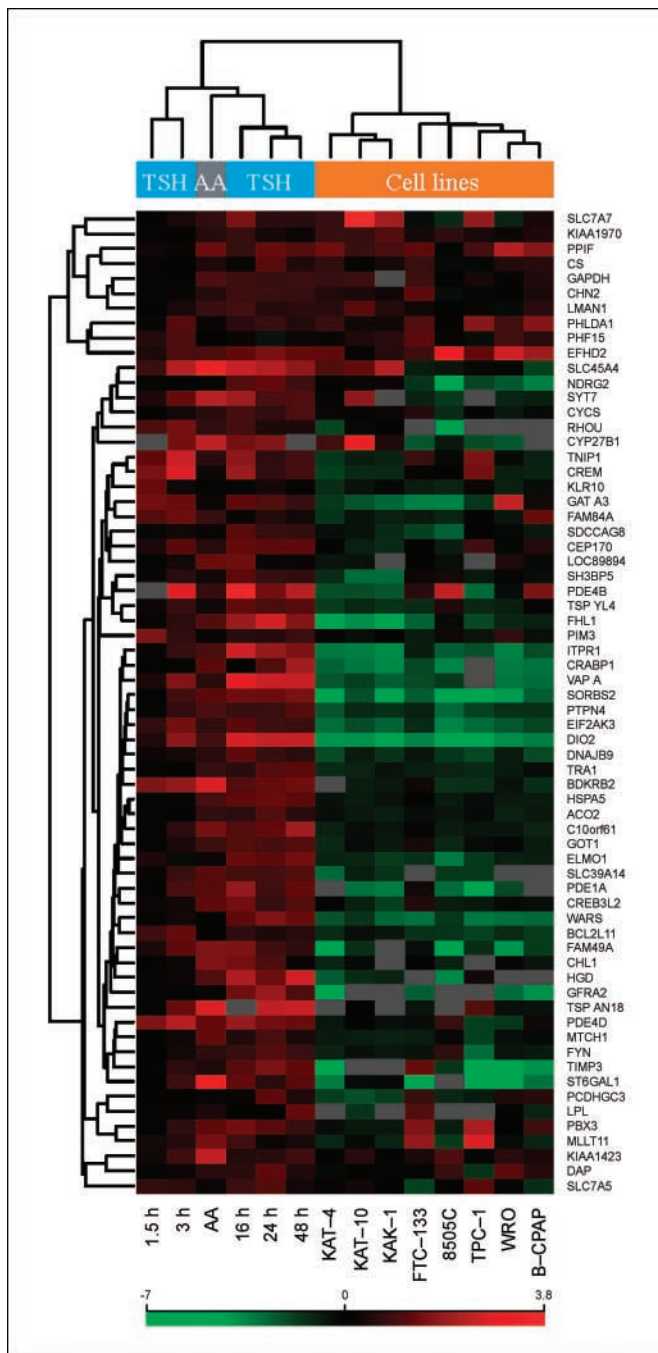


Figure 5. Hierarchical clustering displaying the modulation of thyrocyte genes in primary cultures of thyrocytes treated with TSH for different times (1.5, 3, 16, 24, and 48 h), autonomous adenomas (AA), and human thyroid tumor cell lines. Genes were defined as up-regulated (\log_2 ratio >1) in at least one time point in the TSH-treated primary cultures (16) and whose expression was larger in the autonomous adenomas compared with their corresponding normal tissue (\log_2 ratio >0). Rows, genes; columns, samples. Color scale, \log_2 ratios. Gray, missing data.

Discussion

Tumor cell lines are used as models for the process of tumorigenesis. However, before using a cell line as a model for the corresponding *in vivo* tumor, it should be verified whether the properties of the *in vivo* tumor investigated are still represented *in vitro*. Our results on thyroid tumor-derived cell lines

show that (a) gene expression profiles of thyroid cell lines originating from tumors of the same histological type or from different types are closely related to each other; (b) the expression profiles of cell lines from various origins resemble each other more than *in vivo* tumors; (c) they are closer to those of undifferentiated carcinomas; (d) the cell lines show characteristics of dedifferentiated cells because they have lost the expression of most thyroid-specific genes, have a down-regulation of most genes that are up-regulated in differentiated cells, do not respond to their physiologic stimulus (TSH), and have strongly disturbed karyotypes; (e) comparison of gene expression profiles of cell lines with differentiated thyroid cells provides insight into the genes involved in the process of differentiation/dedifferentiation.

Contrary to expectation, cell lines derived from the same type of cancer origin do not cluster together. The two FTC cell lines do not cluster together, and neither do the PTC cell lines. Indeed, correlation coefficients between gene expression profiles of cell lines derived from a similar pathology are not the highest inside the cell line group (Supplementary Table S2). Furthermore, cell lines are not closer to *in vivo* tumors of the same pathologic origin. This might be explained in part by the fact that *in vitro* conditions are compared with an *in vivo* environment, and, moreover, homogeneous cell populations (cell lines) are compared with heterogeneous populations (tissues). Solid tumors have a complex histologic organization with transformed thyrocytes, endothelial cells, fibroblasts, blood cells, and sometimes with lymphocyte infiltrates, all interacting with each other (28). Interestingly, comparison of the gene expression profiles of the cell lines with another homogeneous cell population (i.e., primary cultured autonomous adenoma cells) shows that they are not correlated (Supplementary Table S2). In contrast, the cell lines are better correlated to the *in vivo* tumors and have the highest correlation with ATCs, undifferentiated, and rapid growing tumors (Supplementary Table S2). If the separation of cell lines from the differentiated tumors would only be due to *in vitro/in vivo* differences, all the tissues should be closer to each other than to cell lines and all the cell lines should be closer to primary cultured autonomous adenoma cells than to *in vivo* tissues, which is not observed (Supplementary Table S2). On the contrary, *in vivo* benign thyroid tumors are closer to cultured cells of autonomous adenomas and more distant from *in vivo* undifferentiated cancers than the latter from cell lines (Supplementary Table S2).

The observation that all the cell lines are closer to each other and closer to the undifferentiated than to the differentiated tumors suggests that they have evolved into a common fully dedifferentiated phenotype and have lost many of their original characteristics. Indeed, our data support this hypothesis. In all thyroid tumor cell lines, the expression of most of the thyroid-specific genes was completely lost and could not be restored after forskolin treatment. Along the accepted dedifferentiation pathway of thyroid tumors, from the benign adenomas, to the differentiated FTCs and PTCs, and the undifferentiated ATCs, *NIS* and *TPO* expression are lost first, followed by *Tg* expression, whereas *TSHR* expression is lost only in the ATCs (21, 22). *ThOX* expression is normal or slightly increased in the carcinomas (29). Thus, the cell lines correspond to the maximal dedifferentiation stage *in vivo*.

The findings on the chromosome analysis are in line with the dedifferentiated status of the cell lines. Our results did not show any similarity with chromosomal anomalies reported in

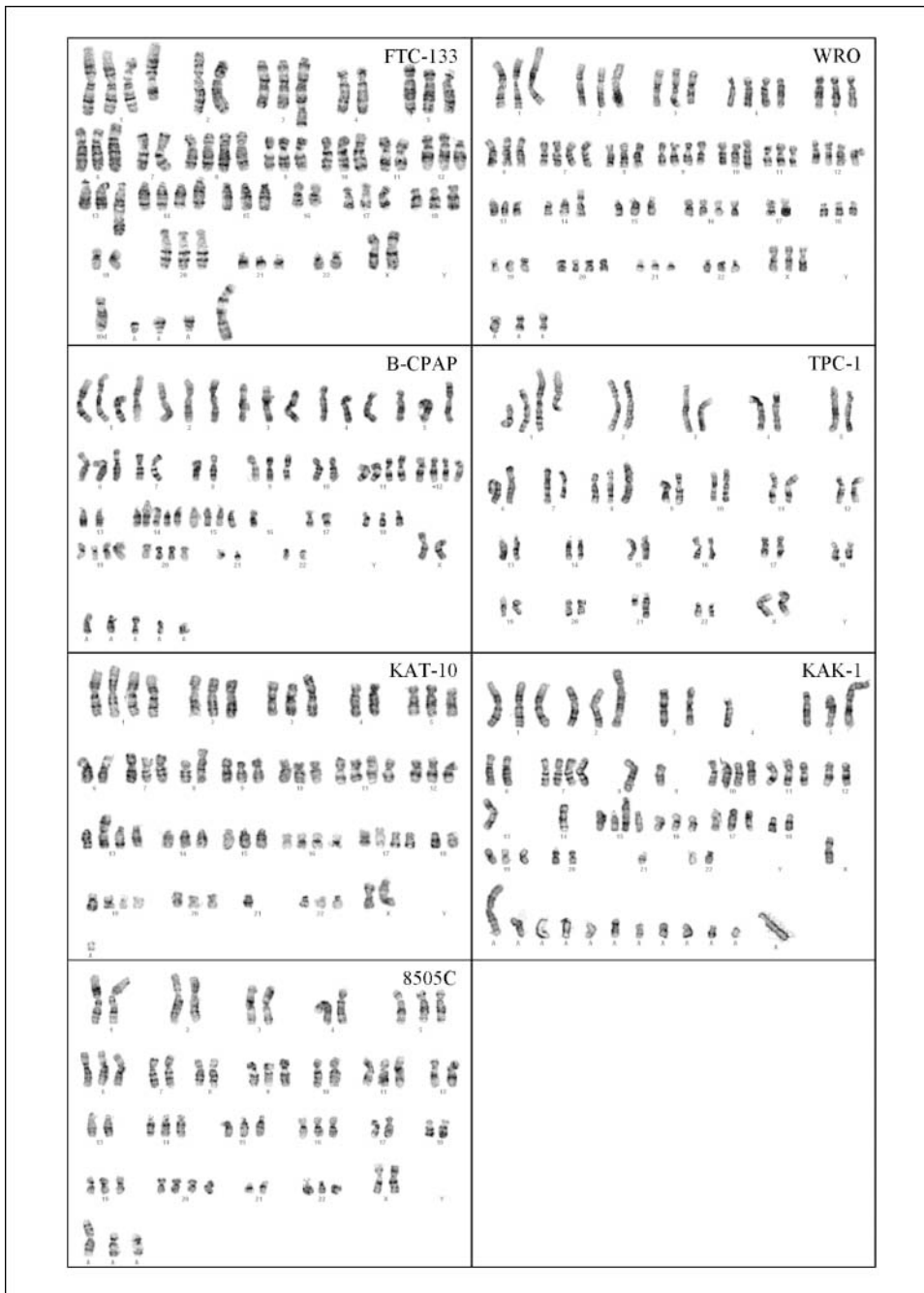


Figure 6. Karyotype analysis of human thyroid tumor cell lines.

thyroid tumors.⁸ Furthermore, the karyograms we obtained from FTC-133, B-CPAP, and WRO were not identical to the previously published ones (7, 26, 27) that suggest an *in vitro* evolution of the cell lines, as do the differences in gene expressions (i.e., *EGRI* and *FOS*) between KAK-1 and KAT-10, which have the same origin. Such irreversible differences might be explained by sub-clone selection in long-term cultures, as shown for melanoma (30) and in pituitary cell line cultures (31). They cannot be explained by the loss of *in vivo* cell heterogeneity or differences of microenvironment.

In addition to the lack of expression of various differentiation and determination markers in the cell lines, and to their disturbed karyotypes, several of them have inactivated p53 (32–34) and activated nuclear factor-κB (1) contrary to differentiated tumors; that is, they have progressed *in vitro* toward a genotype closer to ATCs (35). It is interesting in this respect that Castedo et al. (36) have shown that in the presence of active p53, tetraploid cells, precursors of aneuploid cells, die. Thus, it is probable that the loss of p53 has been a necessary step in the *in vitro* evolution to aneuploidy in cell lines derived from PTCs.

The expression of transcription factors (*TTF1*, *TTF2*, *PAX8*) is altered or reduced in thyroid tumors, in particular in poorly differentiated tumors (22). It is interesting to note that some of the

⁸ <http://cgap.nci.nih.gov/Chromosomes/CytList>

cell lines still express the three transcription factors but do not express *Tg* or *TPO*, the expressions of which are regulated by these factors. This might be explained by the fact that the expression of these transcription factors is decreased, because their overexpression induces expression of thyroglobulin as previously shown for WRO (37) and also, as shown by the sequence of gene expression in embryos, that other factor(s) are probably necessary (38). Thus, the cell lines retain the expression of thyroid-transcription factors, suggesting that they have lost the expression of their differentiation but not completely their thyroid determination. As discussed later, they retain some of their original characteristics.

Dedifferentiation is characterized by a loss of specific markers and a subsequent gain of proliferative capacity. Tumor-derived immortal cell lines have a much higher proliferation rate than the *in vivo* differentiated thyroid tumors. Among the genes overexpressed in all cell lines, we found genes encoding proteins involved in general metabolism, cell cycle, and proliferation (e.g., *PRIMI*, *RPA1*, *HMGB2*, *CNAP1*, *RRM1*, *HMG2*, *PCNT1*, *SLC1A5*, *RPS23*, *TOP2A*). Genes involved in immune recognition (e.g., *CD200*, *HLA-DRA*, *HLA-DQB1*, *CDA08*) were down-regulated (Supplementary Table S3). In addition to the well-defined thyrocyte markers as described above, we further investigated the dedifferentiated status of the cell lines by comparing gene expression profiles of differentiated thyrocytes both from normal thyrocytes treated with TSH *in vitro* and from autonomous adenomas *in vivo* (16). We reasoned that genes regulated in common in both differentiated thyrocytes and cell lines might be involved in cell proliferation, whereas inversely regulated genes would be involved in differentiation/dedifferentiation. Genes that are related to specific thyroid function, such as *CRABP1*, *DIO2*, *VAPA*, *WARS*, and *MACF1* (16), are underexpressed in all the cell lines as they are in thyroid carcinogenesis *in vivo* (14, 39, 40). This approach of comparing differentiated cells with cell lines might have useful applications in tumor types from other origin, because the process of dedifferentiation is common in most tumor types and a major determinant of their evolution into highly aggressive tumors. It may lead to the identification of new diagnostic markers and/or therapeutic targets.

Although part of the difference with *in vivo* tumors could be explained by loss of tissue heterogeneity and microenvironment differences, our results thus suggest that the cell lines have evolved into dedifferentiated cells, which might explain that their gene expression profiles are very distinct from *in vivo* differentiated tumors but are closer to undifferentiated carcinomas and are closer to each other. This evolution is confirmed by several other facts: the aneuploidy, the generality of p53-inactivating mutations, both irreversible, and the complete loss of expression of differentiation genes in all the cell lines but not in FTCs or PTCs *in vivo*. Moreover, CpG island methylation of promoters is higher in cell lines than in differentiated carcinomas, and such profiles are different between different types of carcinomas and between these and their derived cell lines (41). This does not imply that the cell lines have lost all the characteristics of the original cancers; for

example, they retain the causal oncogenic event BRAF activation for the B-CPAP cell line (42) and RET/PTC1 rearrangement for the TPC-1 cells (43), as well as the expression of thyroid-specific transcription factors. They are, however, largely different and thus not valid models of the original differentiated tumor but when validated for a given property could be used as models for undifferentiated cancer.

The literature also shows that gene expression profiles from cell lines derived from cancers of other solid tissues, such as breast, lung, and brain, are separated from the tumor of origin, which is, however, little commented upon (28, 44–47). Breast cancers cluster separately from breast cancer-derived cell lines and from cultured human mammary epithelial cells (47). In another study, breast cancer- and leukemia-derived cell lines cluster in one group, again apart from breast cancer and normal breast tissues, which cluster together in a second group (28). Thus, the congruence of gene expression patterns from cell lines originating from different tumor types of the same tissue is far greater than the differences of expression that still allow distinguishing cell lines of different origins. Interestingly, although breast cancer cell lines are separated from *in vivo* tumors, it has been shown that they do cluster according to the cell type from which they originate (i.e., basal, luminal, and stromal-like groups; ref. 48); however, in contrast, this is not shown for lung cancer cell lines (44). A meta-analysis on the NCI60 panel of cell lines and a large number of *in vivo* tumors derived from various origins showed that the cell lines group together despite their different originating tissues (49).

The evolution of the cell lines from the *in vivo* tumors could have several, not necessarily exclusive, explanations: Cell lines could result from an *in vitro* selection of a fraction of cancer stem cells, from an adaptation to the *in vitro* environment or from an *in vitro* Darwinian selection by this new environment. The latter explanation is supported by the very different evolution of glioma cells depending on the presence of serum in the medium (50) by the long-term history of the cells, the changed karyotypes, and the expression of genes related to proliferation instead of differentiation. A role of an adaptation to another microenvironment is supported by the striking change in gene expression taking place when a cell line generates a xenograft (50).

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