Differentiated thyroid carcinoma : treatment and clinical consequences of therapy

Hoftijzer, H.C.

2

Retinoic acid receptor and retinoid X receptor subtype expression for the differential diagnosis of thyroid neoplasms

Hendrieke C. Hoftijzer, Ying-Ying Liu, Hans Morreau, Tom van Wezel, Alberto M. Pereira, Eleonora P. Corssmit, Johannes A. Romijn, Johannes W. Smit

European Journal of Endocrinology 2009 Apr; 160 (4): 631-638

Abstract

Background

Although differential expression of retinoic acid receptor (RAR) subtypes between benign and malignant thyroid tissues has been described, their diagnostic value has not been reported.

Aim

To investigate the diagnostic accuracy of RAR and retinoid X receptor (RXR) subtype protein expression for the differential diagnosis of thyroid neoplasms.

Methods

We used a tissue array containing 93 benign thyroid tissues (normal thyroid, multinodular goiter, and follicular adenoma (FA)) and 77 thyroid carcinomas (papillary thyroid carcinoma (PTC), follicular thyroid carcinoma, and follicular variant of PTC (FVPTC)). Immunostaining was done for RAR and RXR subtypes. Staining was analyzed semi quantitatively based on receiver operating curve analyses and using hierarchical cluster analysis.

Results

We found increased expression of cytoplasmic (c) RARalpha, cRARgamma, cRXRbeta and decreased expression of nuclear (n) RARbeta, nRARgamma, and nRXRalpha in thyroid carcinomas compared with benign tissues. We found three proteins differently expressed between FA and FTC and five proteins differentially expressed between FA and FVPTC, with high diagnostic accuracies. Using cluster analysis, the combination of negative staining of membranous RXRbeta and positive staining for cRXRbeta had a high positive predictive value (98%) for malignant thyroid disease, whereas the combination of positive nRXRalpha and negative cRXRbeta staining had a high predictive value (91%) for benign thyroid lesions.

Conclusion

We conclude that differences in RAR and RXR subtype protein expression may be valuable for the differential diagnosis of thyroid neoplasms. The results of this study and especially the value of cluster analysis have to be confirmed in subsequent studies.

Introduction

The microscopical distinction between benign and malignant neoplastic thyroid nodules by conventional histology is often difficult as these lesions may share overlapping histological characteristics. Therefore, it is important to identify new markers to distinguish benign from malignant thyroid tumors. In recent years, several immunohistochemical markers have been studied to improve the differential diagnosis of thyroid lesions, using both candidate markers and unbiased approaches (1–12).

The expression of retinoid receptors may be interesting for the differentiation between benign and malignant thyroid tissues. Retinoids are important for growth, differentiation, and morphogenesis in vertebrates (13). Retinoids are derivatives of vitamin A (i.e. retinol). Retinoid receptors belong to the family of nuclear receptors and can be distinguished in retinoic acid receptors (RAR) and retinoid X receptors (RXR). According to the literature, retinoid receptors appear to be differentially expressed in benign and malignant thyroid tissues, the general picture being decreased expression of retinoid receptor subtypes in thyroid cancer (**Table 1**) (14–20), which may also have therapeutic implications (17,18,21–23). However, in these publications on retinoid receptor expression in thyroid lesions, the question whether retinoid receptor expression could be used for the differential diagnosis of thyroid neoplasms was not addressed, probably because most studies included relatively small number of patient samples or the studies included only a subset of retinoid receptors (**Table 1**).

We therefore, decided to study the diagnostic value of RAR and RXR subtype expression in benign and malignant thyroid tissues, using receiver operating curve (ROC) analyses as well as hierarchical cluster analysis (12).

Materials and methods

Thyroid tissues

We obtained one hundred and seventy histological samples from surgically removed thyroid lesions representing five different histological thyroid disorders and adjacent thyroid normal tissue from the archive of the Department of Pathology of the Leiden University Medical Center. We selected 93 benign thyroid tissues (normal n=64, multinodular goiter n=16, follicular adenoma (FA) n=13), and 77 non-medullary thyroid carcinomas (papillary thyroid carcinoma (PTC) n=53, follicular thyroid carcinoma (FTC) n=13 and follicular variant of PTC (FVPTC) n=11). All original histological diagnoses were reviewed by two independent observers. Given the variability in phenotype of follicular lesions, only micro follicular FA and widely invasive FTC's were included on which both observers agreed. Likewise, we only included encapsulated FVPTC tumors with a typical PTC nuclear pattern on which both observers agreed.

Table 1: Overview of literature on retinoic acid receptor and retinoid X receptor expression in thyroid tissue samples and carcinoma cell lines

Study	# tissue samples / cell lines		Method	Retinoid receptor investigated	Results
Rochaix *et al.* (1998) (14)	58 samples	16 PTC 2 FTC 30 CTL 6 MNG 2 FA 2 toxic goiter	Immunohistochemistry Western blot	RARβ	Reduced RARβ expression in PTC compared to normal tissue Moderate RARβ expression in one FTC, none in the other
Schmutzler *et al.* (1998) (15)	4 cell lines	FTC-133 FTC-238 HTh74 (ATC) C643 (ATC)	RT-PCR Northern blot	RARα RARβ RARγ RXRα RXRβ RXRγ	Expression RARα, β, γ, RXR α and β on all carcinoma cell lines, however lower compared to goiterous cells. Lowest in FTC cells
	18 samples	2 CTL 2 adenomas 2 unknown Ca 3 FTC 3 OTC 6 PTC			9 of 12 tumor samples decreased or absent expression of RXRβ
Takiyama *et al.* (2003) (16)	176 samples	57 PTC 40 FTC 24 ATC 28 MTC 27 FA	Immunohistochemistry RT-PCR and Western blot	RXRα RXRβ RXRγ	Decreased nuclear expression of all RXR isoforms in carcinoma PTC and FTC low nuclear expression, moderate cytoplasmic expression ATC no expression RXRγ
	3 cell lines	ARO (ATC) WRO (FTC) NPA (PTC)			FA distinct nuclear staining RXRα and RXRβ RXRγ undetectable in WRO, RXRα and RXRβ detectable in all cell lines
Schmutzler *et al.* (2004) (17)	4 cell lines	FTC-133 FTC-238 HTh74 (ATC) C643 (ATC)	RT-PCR and Northern blot	RARα RARβ	Reduced level RARβ in FTC-238 Reduced level RARα in HTh74 and C643

Table 1: Continued

Study	# tissue samples / cell lines		Method	Retinoid receptor investigated	Results
Haugen *et al.* (2004) (18)	10 samples	5 PTC 1 FTC 1 insular 1 ATC 2 FA	RT-PCR and Western blot	RARα RARβ RARγ RXRα RXRβ	RARα and γ expressed in all cell lines RARβ not expressed in FTC cells RXRα and β decreased expressed in ATC cells RXRγ expressed only in ATC
	4 cell lines	MRO-87 (FTC) WRO-82 (FTC) TAD-2 (CTL) DRO-90 (ATC)		RXRγ	RARβ expression decreased in malignant tissues RXRγ expression decreased in benign tissue and increased in malignant tissue
Elisei *et at.* (2005) (19)	24 samples	10 PTC 10 CTL 4 ATC	Immunohistochemistry	RARβ	Decreased RARβ in PTC and ATC compared to controls
Koh *et al.* (2006) (20)	3 cell lines	SNU-80 (PTC) SNU-373 (PTC) SNU-790 (ATC)		RARα RARβ RARγ	RARβ not expressed RARα detected in all three cell lines RARγ detected in SNU-80 and SNU-373

PTC= papillary thyroid carcinoma, FTC= follicular thyroid carcinoma, CTL= normal thyroid tissue, MNG= multi nodular goiter, FA= follicular adenoma, OTC= oncocytic thyroid carcinoma, ATC= anaplastic thyroid carcinoma, RT-PCR= real time peroxidase chain reaction, RA= retinoic acid, RAR= retinoic acid receptor, RXR= retinoid X receptor

Tissue microarray

Formalin-fixed, paraffin-embedded blocks routinely prepared from surgical specimens of thyroid tumors were selected for this study. Representative areas containing tumor or adjacent normal tissue were identified by a pathologist. Triplicate tissue cores with a diameter of 0.6 mm were taken from each specimen (Beecher Instruments, Silver Springs, MD, USA) and arrayed on a recipient paraffin block, using standard procedures (24).

Immunohistochemistry methods

Four micrometer consecutive tissue sections were cut from each arrayed paraffin block and prepared on pathological slides. The sections were deparaffinized in xylene followed by 0.3% hydrogen peroxide in methanol at room temperature for 20 min to block endogenous peroxidase. After rehydration, antigen retrieval was performed by microwave treatment in 0.001 M citrate buffer (pH 6.0). The sections were incubated with the following primary antibodies against RAR and RXR subtypes: anti-RARalpha monoclonal antibody 9A9A6, dilution 1:3000; anti- RARbeta monoclonal antibody 8B10B2, dilution 1:200; anti-RARgamma monoclonal antibody. 4G-7A11, dilution 1:350; anti-RXRalpha monoclonal antibody 4RX3A2, dilution 1:1000 (all gifts of Dr C Rochette-Egly, IGBMC, Illkirch, France), anti-RXRbeta polyclonal antibody sc-831, dilution 1:650 (Santa Cruz Biotechnology, Santa Cruz, CA, USA); anti-RXRgamma polyclonal antibody sc-555, dilution 1:500 (Santa Cruz). Sections were incubated overnight at room temperature with the primary antibodies, dissolved in PBS with 1% bovine serum albumin. Subsequently, the sections were incubated for 30 min with either the biotinylated rabbit-anti-mouse conjugate, dilution 1:200 or goat-anti-rabbit, dilution 1:400 (DakoCytomation, Glostrup, Denmark), followed by incubation for 30 min with the streptavidin-biotin-peroxidase conjugate. This step was performed by 10-min incubation with 3,3′- diaminobenzidine-tetrachloride substrate in a buffered 0.05-M Tris/HCl (pH 7.6) solution containing 0.002% hydrogen peroxide. Negative controls were stained with the primary antibody omitted. The sections were counterstained with hematoxylin.

Immunohistochemical scoring

A semi quantitative assessment of immunohistochemical scoring was performed including both the intensity of staining and the percentage of positive cells. The percentage of cells with positive staining was scored as follows: >0–20%: ‘1’, >20–50%: ‘2’, >50–70%: ‘3’, and >70–100% ‘4’. The staining intensity was scored as faint: ‘1’, intermediate: ‘2’, and intense: ‘3’. Scores for proportion of positive cells and intensity were multiplied. Nuclear, cytoplasmic, and membranous staining was scored inde-

pendently. The total score per sample therefore ranged from 0 to 12. Score results for triplicate samples were averaged.

Statistical analyses

Statistical analyses were performed using SPSS 14.0 (SPSS Inc., Chicago, IL, USA). Initially, staining scores for every individual antibody were expressed as mean ± SD per histological category (**Table 2**). The next step was the analysis of differences in staining scores for each antibody between malignant *versus* benign tissues, malignant *versus* normal tissues, FA *versus* FTC and FA *versus* FVPTC using the Mann-Whitney test. For each differentially expressed antibody between two histological categories, the optimal cut-off value for the distinction between the two categories was determined by receiver operating characteristic (ROC) analysis. In theory, this could give different cut-off values for one antibody for different comparisons. Only antibodies with sensitivities and specificities above 70% were included in further analyses. In addition to the individual protein markers, the analysis of the diagnostic accuracy of panels of antibodies was performed using hierarchical clustering analysis of tissue microarray data using Cluster and TreeView (Cluster and TreeView 2.11; Eisen Lab, University of California at Berkeley, CA, USA) (12,25). Input for these analyses was the individual staining score per sample for each antibody. A P-value of <0.05 was considered significant.

Table 2: Results of retinoid receptor staining

	Normal n=64	**Multi nodular goiter** n=16	**Follicular adenoma** n=13	**FTC** n=13	**FVPTC** n=11	**PTC** n=53
Nuclear RARα	4.47 ± 1.98	4.06 ± 1.46	4.88 ± 1.67	6.02 ± 3.71	1.12 ± 2.39	2.56 ± 2.43
Cytoplasmic RARα	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	2.43 ± 4.47	1.67 ± 2.42	1.21 ± 2.47
Nuclear RARβ	8.53 ± 2.20	8.62 ± 1.42	8.63 ± 2.21	5.62 ± 3.37	5.78 ± 3.12	6.21 ± 3.18
Nuclear RARγ	4.56 ± 2.59	3.17 ± 1.40	3.17 ± 2.26	1.61 ± 1.58	0.85 ± 1.92	2.00 ± 2.63
Cytoplasmic RARγ	0.00 ± 0.00	0.00 ± 0.00	0.55 ± 1.81	1.78 ± 3.54	0.54 ± 1.80	0.93 ± 2.58
Nuclear RXRα	2.29 ± 2.00	2.27 ± 2.03	2.64 ± 2.60	0.44 ± 0.12	0.00 ± 0.00	0.37 ± 1.10
Nuclear RXRβ	0.71 ± 1.62	0.36 ± 1.21	0.00 ± 0.00	0.00 ± 0.00	0.30 ± 0.95	0.32 ± 1.33
Cytoplasmic RXRβ	0.07 ± 0.53	0.00 ± 0.00	2.18 ± 4.85	5.99 ± 4.89	2.68 ± 4.54	6.80 ± 4.55
Membranous RXRβ	1.66 ± 2.06	3.34 ± 2.05	4.53 ± 2.69	1.88 ± 1.88	1.41 ± 2.84	0.92 ± 2.20

Scoring method: The percentage of cells with positive staining was scored: >0–20%: '1', >20–50%: '2', >50–70%: '3', and >70–100% '4'. The intensity was scored as faint: '1', intermediate: '2', and intense: '3'. These scores were multiplied by each other for a combination score. Score results for triplicate samples were averaged. Distinctive scores were categorized according to nuclear, cytoplasm and membranous staining patterns. Data are mean ± SD. RAR= Retinoic Acid Receptor RXR= Retinoid X Receptor

Results

RAR and RXR expression in thyroid lesions: benign versus malignant

The scores for expression of RAR and RXR receptor subclasses are shown in **Table 2**. Benign tissue samples had an overall lower expression of cytoplasmic RARalpha (cRARalpha), cytoplasmic RARgamma (cRARgamma), and cytoplasmic RXRbeta (cRXRbeta) and a higher expression of nuclear RARalpha (nRARalpha), nuclear RARbeta (nRARbeta), nuclear RARgamma (nRARgamma) and nuclear RXRalpha (nRXRalpha) compared with malignant tissues. FA scored particularly high for nuclear RXRalpha (nRXRalpha) and low for nuclear RXRbeta (nRXRbeta) expression. FVPTC also had a very low expression of nRXRbeta. RXRgamma staining did not reveal a positive result in all thyroid tissues, and was excluded from further analyses.

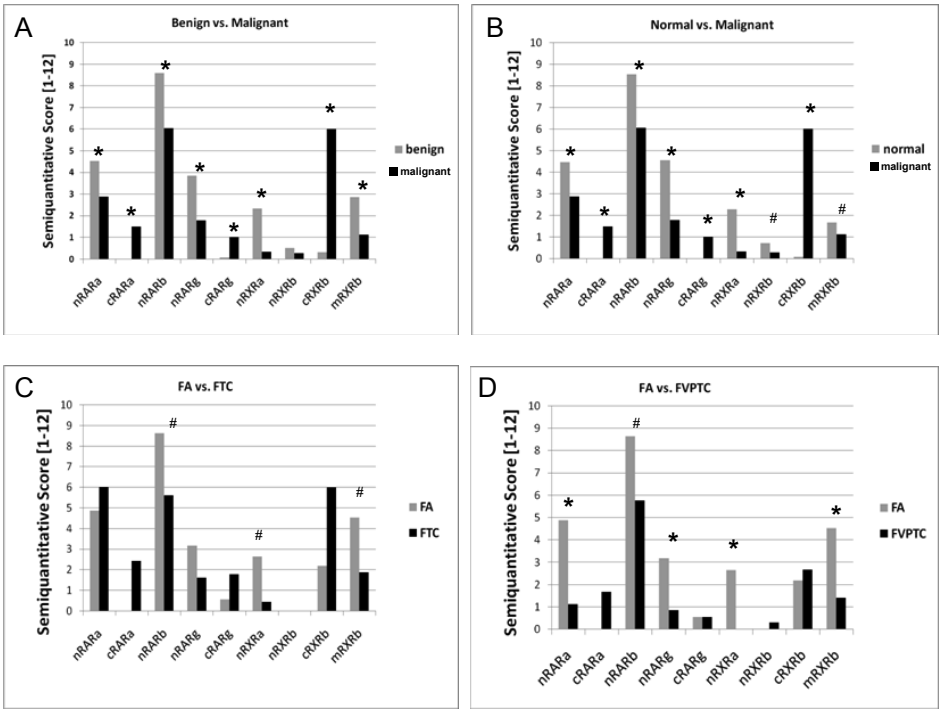


Figure 1

Immunostaining of RAR and RXR subtype antibodies in normal, benign, and malignant thyroid lesions. Immunohistochemistry scores were expressed semi-quantitatively (for explanation, see text). Data are expressed as mean ± S.D. Comparisons between (A) benign and malignant, (B) normal and malignant, follicular adenoma (FA) and follicular thyroid carcinoma (FTC), and (D) FA and follicular variant of papillary carcinoma (FVPTC) were performed with the Mann–Whitney test.
*P<0.005; # P<0.05; n= nuclear; c= cytoplasmic; m= membranous; RAR= Retinoic Acid Receptor; RXR= Retinoid X Receptor

Figure 1 shows the differences in expression patterns for different categories of thyroid tissues. All RAR and RXR subtypes appeared to be differentially expressed between malignant thyroid lesions and normal thyroid tissue.

RAR and RXR expression in thyroid lesions: follicular lesions

The differentiation between follicular lesions (FA, FTC, and FVPTC) is difficult. Therefore, we compared these subgroups separately. FTC had a significantly lower expression of nRARbeta, nRXRalpha, and mRXRbeta compared with FA. FVPTC had a significantly lower expression of nRARalpha, nRARbeta, nRARgamma, nRXRalpha, and mRXRbeta compared with FA (**Figure 1**).

ROC analyses

For each differentially expressed antibody between two categories, the optimal cut-off values for the distinction between the two histological classes were determined by ROC analysis. Only antibodies with sensitivities and specificities above 70% were used for further analyses (**Table 3**). Comparison of the expression between benign and malignant thyroid tissues revealed sensitivities and specificities >70% for nRXRalpha, cRXRbeta, and mRXRbeta, the highest sensitivity (89%) and specificity (96%) for nuclear RXRalpha (**Table 3**). NRXRalpha and cRXRbeta also discriminated reasonably between malignant and normal thyroid tissues.

In the comparison between FA and FTC, nRARbeta, nRARalpha, cRXRbeta, and mRXRbeta had sensitivities and specificities above 70%, the highest sensitivity for FTC found for nRARalpha (85%) and the highest specificity for nRARbeta (91%; **Table 3**).

In the comparison between FA and FVPTC, nRARbeta, nRARgamma, nRARalpha, nRXRalpha and mRXRbeta had sensitivities and specificities above 70%. The highest sensitivity for FVPTC was found for nRXRalpha (100%) and the highest specificities for both nuclear nRARalpha (91%) and nRARgamma (91%; **Table 3**).

Hierarchical cluster analysis

To identify the optimal combinations of RAR and RXR subtype expression for the differential diagnosis of thyroid neoplasms, we performed an unsupervised hierarchical cluster analysis, the results of which are shown in **Table 4** and **Figure 2**. We found that 98% of thyroid lesions in cluster 2 (negative staining of mRXRbeta and positive staining for cRXRbeta) were malignant; whereas 91% of the lesions in cluster 4 (positive staining for nRXRalpha and a negative staining for cRXRbeta) were benign. The diagnostic parameters are summarized in **Table 4**. In general, the follicular lesions

Table 3: Diagnostic value of RAR and RXR differentially expressed in thyroid tissues with sensitivity and specificity above 70%.

	Malignant versus benign			**Malignant versus normal**		
	Cut-off level [a]	Sensitivity for malignancy (%)	Specificity for malignancy (%)	Cut-off level [a]	Sensitivity for malignancy (%)	Specificity for malignancy (%)
Nuclear RAR β				<8.5	72	80
Nuclear RXR α	<1	89	96	<1	89	75
Cytoplasmic RXR β	>1	71	96	>1	71	89
Membranous RXR β	<1	74	72			
	FTC versus FA			**FVPTC versus FA**		
	Cut-off level [a]	Sensitivity for FTC (%)	Specificity for FTC (%)	Cut-off level [a]	Sensitivity for FVPTC (%)	Specificity for FVPTC (%)
Nuclear RAR α	<1	85	80	<3	92	91
Nuclear RAR β	<8	73	91	<8	91	82
Nuclear RAR γ				>1	82	91
Nuclear RXR α				<1	100	73
Cytoplasmic RXR β	>2	71	82			
Membranous RXR β	<4	82	75	<1	82	82

Benign thyroid tissues= multinodular goiter, follicular adenoma and normal
RAR= retinoic acid receptor, RXR= retinoid X receptor. [a] Obtained by ROC analyses of semi-quantitative immunohistochemistry scores.

did not cluster separately, but we found that only one FA was present in cluster 2 (high positive predictive value for malignancy), whereas in cluster 4 (high positive predictive value for benign lesions) only one FTC was present (**Figure 2**).

Discussion

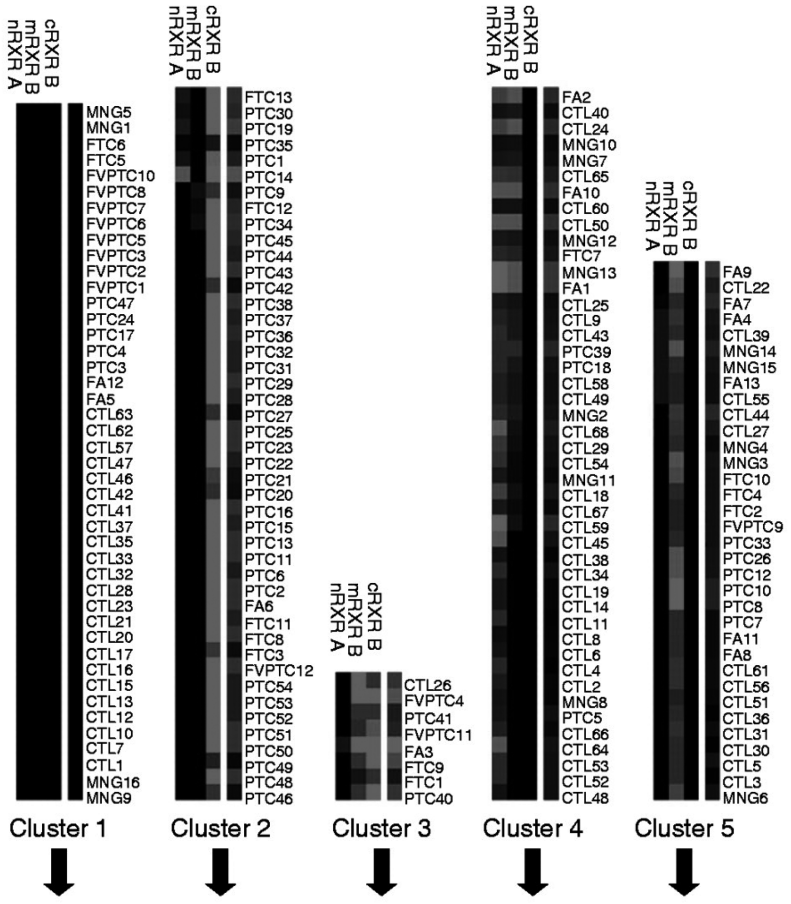
The present study was performed to evaluate the diagnostic value of the expression of RAR and RXR subtypes in a large panel of thyroid neoplasms. To our knowledge, the diagnostic value of RAR and RXR receptor expression for the differential diagnosis of thyroid neoplasms has not been published before (14–20). Our study also differed from earlier ones with regard to the identification of optimal semi quantitative cut-off levels using ROC analyses and hierarchical cluster analysis.

Table 4: Diagnostic value of combinations of retinoid receptor staining for benign *vs*. malignant thyroid lesions, based on hierarchical cluster analysis.

	Combination mRXRβ- and cRXRβ+	**Combination not present**	**Total**
(a) Diagnostic value of combinations of retinoid receptor staining for benign versus malignant thyroid lesions, based on cluster 2 in hierarchical cluster analysis			
Malignant	44	35	79
Benign	1 (FA)	96	98
Total	45	131	176
	PPV malignancy = 98%	NPV malignancy = 72%	LR malignant 56
	Combination nRXRα+ and cRXRβ -	**Combination not present**	**Total**
(b) Diagnostic value of nRXRα and cRXRβ staining for benign versus malignant thyroid lesions, based on cluster 4 in hierarchical cluster analysis			
Benign	41	56	97
Malignant	4 (3 PTC and 1 FTC)	75	79
Total	45	130	175
	PPV benign = 91%	NPV benign = 57%	LR benign 8.6

RAR= retinoic acid receptor, RXR= retinoid X receptor, PPV= positive predicting value, NPV= negative predicting value, LR= likelihood ratio

In general, we found an increased expression of cRARalpha, cRARgamma, cRXRbeta, and a decreased expression of nRARbeta, nRARgamma, and nRARalpha in thyroid carcinomas compared with benign thyroid tissue. The most challenging pathological differential diagnosis is between FA, FTC, and FVPTC. We found three proteins differentially expressed between FA and FTC and five proteins differentially expressed between FA and FVPTC. In the comparison between FA and FTC the highest sensitivity for FTC was found for nRARalpha and the highest specificity for nRARbeta. In the comparison between FA and FVPTC, the highest sensitivity for FVPTC was found for nRXRalpha and the highest specificities for nRARalpha and nRARgamma. Some of these observations are in line with other studies that investigated RAR and/or RXR expression in thyroid tissue samples (**Table 1**). Rochaix *et al*. (14) (Immunohistochemistry), Haugen *et al*. (18) (RT-PCR), and Elisei *et al*. (19) (RT-PCR) also found reduced RARbeta expression in PTC, compared with normal tissue. Rochaix *et al*. (14) only investigated two FTC samples of which one sample showed moderate RARbeta expression and the other did not. Our finding of higher nuclear and lower cytoplasm expression of RARgamma in malignant thyroid tissues was not reported before. Nuclear RXRalpha expression was low or absent in thyroid carcinomas in our study. This finding is confirmed by a paper by Takiyama *et al*. (16). We did not find positive RXRgamma staining in thyroid tissues, which is unexpected, given the results of Haugen *et al*. by western blot (18).



	Cluster 1	Cluster 2	Cluster 3	Cluster 4	Cluster 5
nRXRA	–	+/–	–	+	–
mRXRB	–	–	+	+/–	+
cRXRB	–	+	+	–	–
Malignant	15	44	6	4	10
Benign	29	1	2	41	24

Figure 2

Hierarchical cluster analyses using RAR and RXR subtype antibodies in thyroid tissues. Nuclear RXRalpha, cytoplasmic RXRbeta, and membranous RXRbeta were identified as the best predictors of benign or malignant thyroid lesions. The absence of membranous (m) RXRbeta and the presence of cytoplasmic (c) RXRbeta had a high positive predictive value for malignancy (98%, cluster 2). The presence of nuclear (n) RXRalpha and absence of cytoplasmic RXRbeta had a high positive predictive value for benign lesions (91%, cluster 4). CTL= normal thyroid, FA= follicular adenoma, FTC= follicular thyroid carcinoma, PTC= papillary thyroid carcinoma, FVPTC= follicular variant PTC.

There are two studies on RXRbeta expression in thyroid neoplasms (15,16). They both found decreased or absent expression of RXRbeta in carcinomas. One of these studies, however, (15) used RT-PCR and contained only 12 human thyroid carcinoma samples. In our study, we differentiated between nuclear, cytoplasmic, and membranous staining. The only study that also differentiated between nuclear and cytoplasm staining pattern, only investigated RXR isoform expression (16).

We performed a cluster analysis including all studied tissues and antibodies. Our findings showed that the combination of negative staining of mRXRbeta and a positive staining for cRXRbeta had a high accuracy for the detection of malignant thyroid tissues, whereas the combination of a positive staining for nRXRalpha and a negative staining for cRXRbeta was present in most benign tissues.

There are some limitations to our study. Although we were able to distinguish between follicular lesions, the number of follicular lesions was relatively small. Therefore, additional studies should be performed with larger numbers of follicular lesions, also including histological subtypes of follicular lesions. Moreover, the findings of our study and the clinical usefulness of hierarchical cluster analysis have to be validated in subsequent studies and most importantly in cytological preparations. Also, other difficult-to-classify thyroid neoplasms such as minimally invasive follicular carcinomas as well as FA subclasses should be included in subsequent studies. The biological mechanisms responsible for the differential expression of RAR and RXR between thyroid tissues also remain to be elucidated. In conclusion, differences in RAR and RXR subtype protein expression as studied by immunohistochemistry may be of additional value in the differential diagnosis of thyroid neoplasms.

References

1. Huang Y, Prasad M, Lemon WJ, Hampel H, Wright FA, Kornacker K, LiVolsi V, Frankel W, Kloos RT, Eng C, Pellegata NS, de la Chapelle A. Gene expression in papillary thyroid carcinoma reveals highly consistent profiles. PNAS 2001 98 15044–15049.
2. Zhao J, Leonard C, Gemsenjager E, Heitz PU, Moch H, Odermatt B. Differentiation of human follicular thyroid adenomas from carcinomas by gene expression profiling. Oncology Reports 2008 19 329–337.
3. Zhao J, Leonard C, Brunner E, Gemsenjager E, Heitz PU, Odermatt B. Molecular characterization of well-differentiated human thyroid carcinomas by cDNA arrays. International Journal of Oncology 2006 29 1041–1051.
4. Lubitz CC, Fahey TJ III. Gene expression profiling of thyroid tumors – clinical applicability. Nature Clinical Practice. Endocrinology, Metabolism 2006 2 472–473.
5. Fryknas M, Wickenberg-Bolin U, Goransson H, Gustafsson MG, Foukakis T, Lee JJ, Landegren U, Hoog A, Larsson C, Grimelius L, Wallin G, Pettersson U, Isaksson A. Molecular markers for discrimination of benign and malignant follicular thyroid tumors. Tumour Biology 2006 27 211–220.
6. Eszlinger M, Wiench M, Jarzab B, Krohn K, Beck M, Lauter J, Gubala E, Fujarewicz K, Swierniak A, Paschke R. Meta- and reanalysis of gene expression profiles of hot and cold thyroid nodules and papillary thyroid carcinoma for gene groups. Journal of Clinical Endocrinology and Metabolism 2006 91 1934–1942.
7. Fujarewicz K, Jarzab M, Eszlinger M, Krohn K, Paschke R, Oczko- Wojciechowska M, Wiench M, Kukulska A, Jarzab B, Swierniak A. A multi-gene approach to differentiate papillary thyroid carcinoma from benign lesions: gene selection using support vector machines with bootstrapping. Endocrine-Related Cancer 2007 14 809–826.
8. Rosen J, He M, Umbricht C, Alexander HR, Dackiw AP, Zeiger MA, Libutti SK. A six-gene model for differentiating benign from malignant thyroid tumors on the basis of gene expression. Surgery 2005 138 1050–1056.
9. Stolf BS, Abreu CM, Mahler-Araujo MB, Dellamano M, Martins WK, de Carvalho MB, Curado MP, Diaz JP, Fabri A, Brentani H, Carvalho AF, Soares FA, Kowalski LP, Hirata R Jr, Reis LF. Expression profile of malignant and non-malignant diseases of the thyroid gland reveals altered expression of a common set of genes in goiter and papillary carcinomas. Cancer Letters 2005 227 59–73.
10. Finley DJ, Lubitz CC, Wei C, Zhu B, Fahey TJ III. Advancing the molecular diagnosis of thyroid nodules: defining benign lesions by molecular profiling. Thyroid 2005 15 562–568.
11. Wasenius VM, Hemmer S, Kettunen E, Knuutila S, Franssila K, Joensuu H. Hepatocyte growth factor receptor, matrix metalloproteinase-11, tissue inhibitor of metalloproteinase-1, and fibronectin are up-regulated in papillary thyroid carcinoma: a cDNAand tissue microarray study. Clinical Cancer Research 2003 9 68–75.
12. Liu YY, Morreau H, Kievit J, Romijn JA, Carrasco N, Smit JW. Combined immunostaining with galectin-3, fibronectin-1, CITED- 1, Hector Battifora mesothelial-1, cytokeratin-19, peroxisome proliferator-activated receptor-gamma, and sodium/iodide symporter antibodies for the differential diagnosis of non-medullary thyroid carcinoma. European Journal of Endocrinology 2008 158 375–384.
13. Smith MA, Parkinson DR, Cheson BD, Friedman MA. Retinoids in cancer therapy. Journal of Clinical Oncology 1992 10 839–864.

14. Rochaix P, Monteil-Onteniente S, Rochette-Egly C, Caratero C, Voigt JJ, Jozan S. Reduced expression of retinoic acid receptor beta protein (RAR beta) in human papillary thyroid carcinoma: immunohistochemical and western blot study. Histopathology 1998 33 337–343.
15. Schmutzler C, Brtko J, Winzer R, Jakobs TC, Meissner-Weigl J, Simon D, Goretzki PE, Kohrle J. Functional retinoid and thyroid hormone receptors in human thyroid-carcinoma cell lines and tissues. International Journal of Cancer 1998 76 368–376.
16. Takiyama Y, Miyokawa N, Sugawara A, Kato S, Ito K, Sato K, Oikawa K, Kobayashi H, Kimura S, Tateno M. Decreased expression of retinoid X receptor isoforms in human thyroid carcinomas. Journal of Clinical Endocrinology and Metabolism 2004 89 5851–5861.
17. Schmutzler C, Hoang-Vu C, Ruger B, Kohrle J. Human thyroid carcinoma cell lines show different retinoic acid receptor repertoires and retinoid responses. European Journal of Endocrinology 2004 150 547–556.
18. Haugen BR, Larson LL, Pugazhenthi U, Hays WR, Klopper JP, Kramer CA, Sharma V. Retinoic acid and retinoid X receptors are differentially expressed in thyroid cancer and thyroid carcinoma cell lines and predict response to treatment with retinoids. Journal of Clinical Endocrinology and Metabolism 2004 89 272–280.
19. Elisei R, Vivaldi A, Agate L, Ciampi R, Molinaro E, Piampiani P, Romei C, Faviana P, Basolo F, Miccoli P, Capodanno A, Collecchi P, Pacini F, Pinchera A. All-trans-retinoic acid treatment inhibits the growth of retinoic acid receptor beta messenger ribonucleic acid expressing thyroid cancer cell lines but does not reinduce the expression of thyroid-specific genes. Journal of Clinical Endocrinology and Metabolism 2005 90 2403–2411.
20. Koh CS, Ku JL, Park SY, Kim KH, Choi JS, Kim IJ, Park JH, Oh SK, Chung JK, Lee JH, Kim WH, Kim CW, Cho BY, Park JG. Establishment and characterization of cell lines from three human thyroid carcinomas: responses to all-trans-retinoic acid and mutations in the BRAF gene. Molecular and Cellular Endocrinology 2007 264 118–127.
21. Havekes B, Schroder Van Der Elst JP, van der PG, Goslings BM, Romijn JA, Smit JW. Beneficial effects of retinoic acid on extracellular matrix degradation and attachment behaviour in follicular thyroid carcinoma cell lines. Journal of Endocrinology 2000 167 229–238.
22. Simon D, Korber C, Krausch M, Segering J, Groth P, Gorges R, Grunwald F, Muller-Gartner HW, Schmutzler C, Kohrle J, Roher HD, Reiners C. Clinical impact of retinoids in redifferentiation therapy of advanced thyroid cancer: final results of a pilot study. European Journal of Nuclear Medicine and Molecular Imaging 2002 29 775–782.
23. Liu YY, Stokkel MP, Pereira AM, Corssmit EP, Morreau HA, Romijn JA, Smit JW. Bexarotene increases uptake of radioiodine in metastases of differentiated thyroid carcinoma. European Journal of Endocrinology 2006 154 525–531.
24. Kononen J, Bubendorf L, Kallioniemi A, Barlund M, Schraml P, Leighton S, Torhorst J, Mihatsch MJ, Sauter G, Kallioniemi OP. Tissue microarrays for high-throughput molecular profiling of tumor specimens. Nature Medicine 1998 4 844–847.
25. Toronen P. Selection of informative clusters from hierarchical cluster tree with gene classes. BMC Bioinformatics 2004 5 32.