Pathol. Oncol. Res. (2012) 18:607–614
DOI 10.1007/s12253-011-9483-7

RESEARCH

Molecular Profiling of Parathyroid Hyperplasia, Adenoma and Carcinoma

Kristóf Árvai · Katalin Nagy · Helga Barti-Juhász · István Peták · Tibor Krenács · Tamás Micsik · Gyula Végső · Ferenc Perner · Béla Szende

Received: 13 July 2011 / Accepted: 23 November 2011 / Published online: 24 December 2011

Abstract The objective of the study was to examine proliferation and apoptosis associated gene expression in the whole sequence parathyroid lesions to reveal specific features of carcinoma. This study was based on surgically removed parathyroid tissues, gene expression analysis was performed both at gene and protein level. First, mRNA isolation was performed from deep-frozen tissue samples, and further apoptosis pathway-specific cDNA macroarray analysis was carried out. The results were validated with real-time PCR. Subsequently, protein expression was analyzed with immunhistochemistry on Tissue Micro Array multi-blocks derived from several paraffin-embedded samples. cDNA macroarrays revealed elevated expression of both pro-apoptotic (*FAS* receptor, *TRAIL* ligand, *CASPASE8*, and *−4*) and anti-apoptotic (*cIAP1, APOLLON*) genes in benign proliferative lesions compared to that in normal gland. TMA studies showed overexpression of KI67, P53, SURVIVIN and APOLLON protein and failure of expression of P27, BCL2, BAX, CHROMOGRANIN-A, SYNAPTOPHYSIN, CYCLIND1, FLIP, TRAIL, CK8, CK18, CK19 in parathyroid carcinoma was detected. These alterations in gene expression of the investigated products could be used in differentiation between beningn and malignant proliferative processes of the parathyroid gland. Authors conclude that a series of alterations in gene expression such as overexpression of APOLLON, P53, KI67 and suppression of P27, BCL2, BAX lead to uncontrolled cell proliferation, but still not leading to increased apoptotic activity in parathyroid carcinoma.

Keywords Parathyroid lesions · Apoptosis · Gene expression profiling · Tissue micro array

K. Árvai · K. Nagy · H. Barti-Juhász · I. Peták · T. Krenács · T. Micsik · B. Szende (✉)
Department of Pathology and Experimental Cancer Research, Semmelweis University,
Üllői út 26,
1085 Budapest, Hungary
e-mail: bszende@korb1.sote.hu

G. Végső · F. Perner
Department of Transplantation and Surgery, Semmelweis University,
Budapest, Hungary

Introduction

Hyperplasia and adenoma are the most common proliferative lesions of the parathyroid gland, whereas parathyoid carcinomas are extremely rare neoplasms [1]. Hyperparathyroidism is a common disease, it's prevalance believed ~3:1000 in the general population [2]. Hyperparathyreodism could be classified into three groups: primary, secondary and tertiary disorder. Most patients with primary hyperparathyroidism have high serum parathyroid hormone and high serum calcium concentrations. Solitary and even most of the multiglandular parathyroid adenomas are monoclonal or oligoclonal tumors, probably caused by abnormal growth from somatic or germline mutations in parathyroid-tumor initiating cells. Mutations characteristic to hyperparathyroidism have been found only in a small minority of tumors. The tumor-suppressor gene multiple endocrine neoplasia type 1 (*MEN1*) is known to have somatic mutations in both alleles in parathyroid adenomas. Secondary and tertiary hyperparathyroidism is offen named as uremic hyperparathyroidism and it is caused by chronic renal failure. Secondary hyperparathyroidism can develop into a disorder with secretory dysfunction of autonomously functioning parathyroid cells causing oversecretion of parathyroid hormone with hypercalcemia (tertiary hyperparathyroidism) [3]. Furthermore, histopathological differentiation of parathyroid carcinoma from certain forms of parathyroid adenoma—

especially atypic adenoma—is difficult in some cases [4, 5]. According to our previous studies, the rate of apoptotic cells is very low througout the hyperplasia, adenoma, carcinoma series. Similarly the ratio of mitotic cells does not exceed 1–2% even in carcinoma [6]. Gene expression profiling of the parathyroid hyperplasia, adenoma and carcinoma may provide explanation for the rare occurrance of parathyroid carcinoma and help the differential diagnosis of the proliferative lesions [7–9]. Moreover, molecular genetic studies may also shed light on the very low mitotic and apoptotic activity of the proliferative lesions of parathyroid gland [6].

Since the morphological features are not always unequivocal for secure to confirm or exclude malignancy, data were already published on the expression of gene products overexpressed (KI67, P53, GALECTIN-3) [6–8] or underexpressed (BCL2, RB, P27, MDM2, P21) [7, 9] in parathyroid carcinoma. Based on these data and our earlier results [6, 9] we screened at the mRNA and protein level the expression of genes regulating cell proliferation and apoptosis through the hyperplasia, adenoma, carcinoma series in parathyroid lesions to define further differences between the various proliferative processes and to help the distinction between benign and malignant lesions. For this purpose a complex gene expression profile was determined at mRNA level and the most important results were correlated to those observed for protein expression. Useful data for differential diagnosis and on the molecular background of the hyperplasia, adenoma, carcinoma series were also expected.

Our studies were carried out on fresh frozen and routinely archived samples of surgically excised parathyroid glands.

Materials and Methods

Collection of Samples

Deep frozen and 4% buffered formaldehyde-fixed, paraffin-embedded samples of surgically removed parathyroid tissues were investigated. The surgical excision was performed at the Department of Transplantation and Surgery, Semmelweis University, Budapest, Hungary. Two normal (accidentally surgicaly removed in calls of partial thyroidectomy), two hyperplastic and two adenomatous parathyroid glands—preserved in liquid nitrogen—were examined at the transcript level. Gene expression at protein level was investigated in routinely archived samples of excised parathyroid glands including 12 normal tissues, 12 hyperplasia, 12 adenoma and 4 carcinoma. All cases of hyperplasia were secondary hyperplasias due to chronic renal disease. Adenomas and carcinomas were primary lesions. Histopathological classification of the lesions was performed by two independent pathologists based on WHO published guidelines [10]. Diagnosis of carcinoma samples was based not only on histopathological criteria, but also the clinical behaviour of the tumor, like distant metastases and relapses.

Isolation of RNA

RNA isolation was performed using Trizol (Invitrogen, San Diego, CA) according to the manufactors manual. The quality of RNA was double-cheked by spectrophotometry and also by 1% agarose (Invitrogen, San Diego, CA) gel electrophoresis. Only samples showing sufficiant integrity and purity, i.e. at least 1.8 260/280 ratio and satisfactory gel-electrophoretic result were used.

Macroarray Study

The GEArray Q Series Human Apoptotic Gene Array (Superarray, Frederick, MD) was used in the molecular pathology studies. One hundred and twelve genes were printed on this nylon membrane in tetraspot arrangement. The length of the double-file cDNA fragments on this membrane is 250–600 base pairs, which potentiates specific binding and excludes cross-hybridisation even in

Table 1 Primer sequences used in our real-time PCR study

Gene name	Forward	Reverse	Product size
BIM	CGGAGGATGAGTGACGAGTT	GATGTGGAGCGAAGGTCACT	182 bp
DR5	AAGACCCTTGTGCTCGTTGT	AGGTGGACACAATCCCTCTG	144 bp
FAS	AGTTGGGGAAGCTCTTTCACTT	CAGTCTTCCTCAATTCCAATCC	163 bp
cIAP1	AGTGGTTTCCAAGGTGTGAGTT	ACTTTCTCCAGGTCCAAAATGA	167 bp
BOK	GTCTGAATGGAAGGGTCGAG	GTCAAAGGCGTCCATGATCT	177 bp
CASP8	CTGTTTCACCTTGTGTCTGAGC	CAAGGTTCAAGTGACCAACTCA	256 bp
GAPDH	AAGGTGAAGGTCGGAGTCAAC	ATGGGTGGAATCATATTGGAAC	153 bp
BCL-w	CAGGTCTCCGATGAACTTTTTC	CCCGTATAGAGCTGTGAACTCC	216 bp
TRAIL	GATCGTGATCTTCACAGTGCTC	ATGGTTTCCTCAGAGGTTCTCA	226 bp
BCL-X(L)	CAGGGACAGCATATCAGAGCTT	GGGTAGAGTGGATGGTCAGTGT	193 bp

Table 2 Antibodies and dilutions

Target protein	Working dilution	Manufacturer
APOLLON	1:500	BethylLaboratories
ATM	1:1000	SantaCruz
BAX	1:300	LabVision
BCL2	1:300	LabVision
CHROMOGRANIN-A	1:200	Dako
CK18	1:100	Dako
CK19	1:50	Dako
CK8	1:50	Dako
CYCLIND1	1:200	LabVision
FLIP	1:150	Abcam
KI67	1:1000	Dako
MDM2	1:800	LabVision
P27	1:1000	LabVision
P53	1:100	LabVision
PTH	1:200	Dako
SYNAPTOPHYSIN	1:200	Dako
TRAIL	1:800	Abcam

the case of closely related genes belonging to the same family. Preparation of the labelled probes was performed by Linear Polymerase Reaction (LPR), following the instruction of the manufacturer (Superarray, Frederick, MD). cDNA was prepared by reverse transcription from the isolated RNA using oligiodT primers. Traditional PCR was carried out using the cDNA, with the primer mixture specific for the genes present on the array. Biotin-16-dUTP (Roche, Basel) was added to the reaction to label the probe for subsequent detection. The membrane was pre-hybridised with salmon sperm to minimize the background of non-specific binding. The hybridisation of the membrane lasted overnight on 60C°, by permanent stirring. Enzyme-conjugated streptavidine was bound to the array, followed by addition of chemiluminescent CDP-Star™ substrate (Superarray, Frederick, MD) in the course of the processing. Photos of the membrane were taken using a cooled CCD camera (Kodak, Rochester, NY). Computer evaluation was carried out using GEArray Expression Analysis Suite software (Superarray, Frederick, MD).

Samples of hyperplasia and adenoma were compared with normal parathyroid tissue, furthermore, samples of

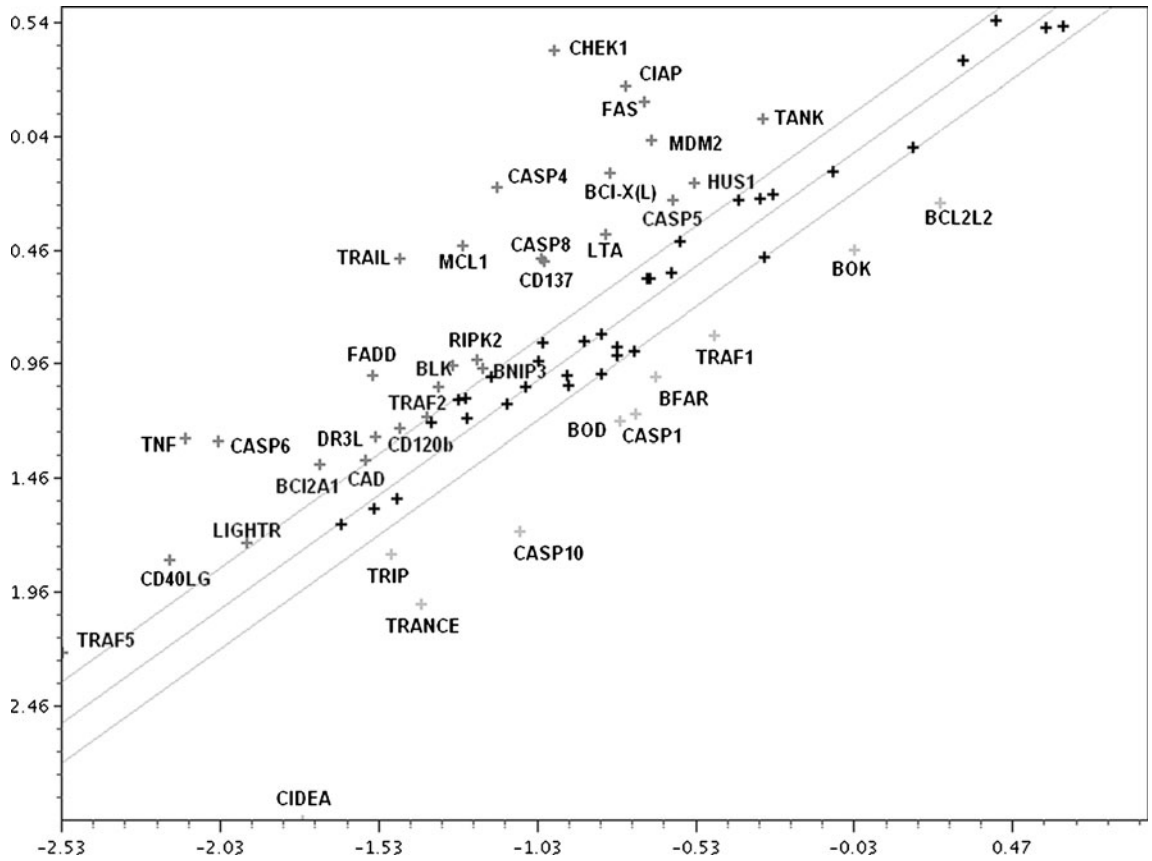

Fig. 1 Gene expression levels of samples of hyperplasia (*y-axis*) compared with normal parathyroid tissue (*x-axis*). A minimum of 1.5-fold increase or decrease in expression compared to the control tissue was considered as significant. Each cross represents one gene. The distance from middle line is proportional to the differences in gene expression level

hyperplasia and samples of adenoma were compared with each other, regarding gene expression. A minimum of 1.5-fold increase or decrease in expression compared to the control was considered as significant. Correction of the background of the arrays was carried out before analysis, utilising the values of the empty spots. The densitometric values were normalized, using the densitometric data of the housekeeping genes *ACTB* (actin beta) and *GAPDH* (glyceraldehyde-3-phosphate dehydrogenase) applying the formula:

$$N_I = \frac{V_I}{(\sum_{k=1}^{K} V_k)/K}$$

Where K is the number of the chosen genes, N is the brightness of the normalized spot, V is the value of the obtained spot.

Validation With Real-Time PCR

The results obtained with macroarray were validated by our Line Gene II (Bioer, Hangzhou, China) quantitative PCR instrument, using a master mix containing fluorescent SYBR Green dye (Applied Biosystems, Carlsbad, CA).

The template of cDNA was produced from the isolated RNA samples, applying High Capacity cDNA reverse Transcription Kit (Applied Biosystem, Carlsbad, CA), using gene-specific primer pairs. The primers were designed by Primer 3 software (Howard Hughes Medical Institute), on exon-exon boundaries, in order to prevent amplification due to potential genomial contamination. Specificity of primer sequences was always controlled, with the aid of BLAST algorythm and the specificity of PCR was double-checked with melting curve analysis and 1% agarose (Invitrogen, San Diego, CA) gel electroforesis. Table 1. contains the primer sequences (IDT Technologies, San Jose, CA) used in our studies. Evaluation was performed by relative quantification using *GAPDH* Ct values for normalization.

Tissue Microarray

Table 2. summarises the antibodies and dilutions used for targeting proteins in our tissue microarray (TMA) samples. Our Institute offered us an access to routine diagnostic immunhistochemistry facility, that is the reason why we could investigate much more proteins than inidicated by our mRNA results. Altogether 70 samples (normal,

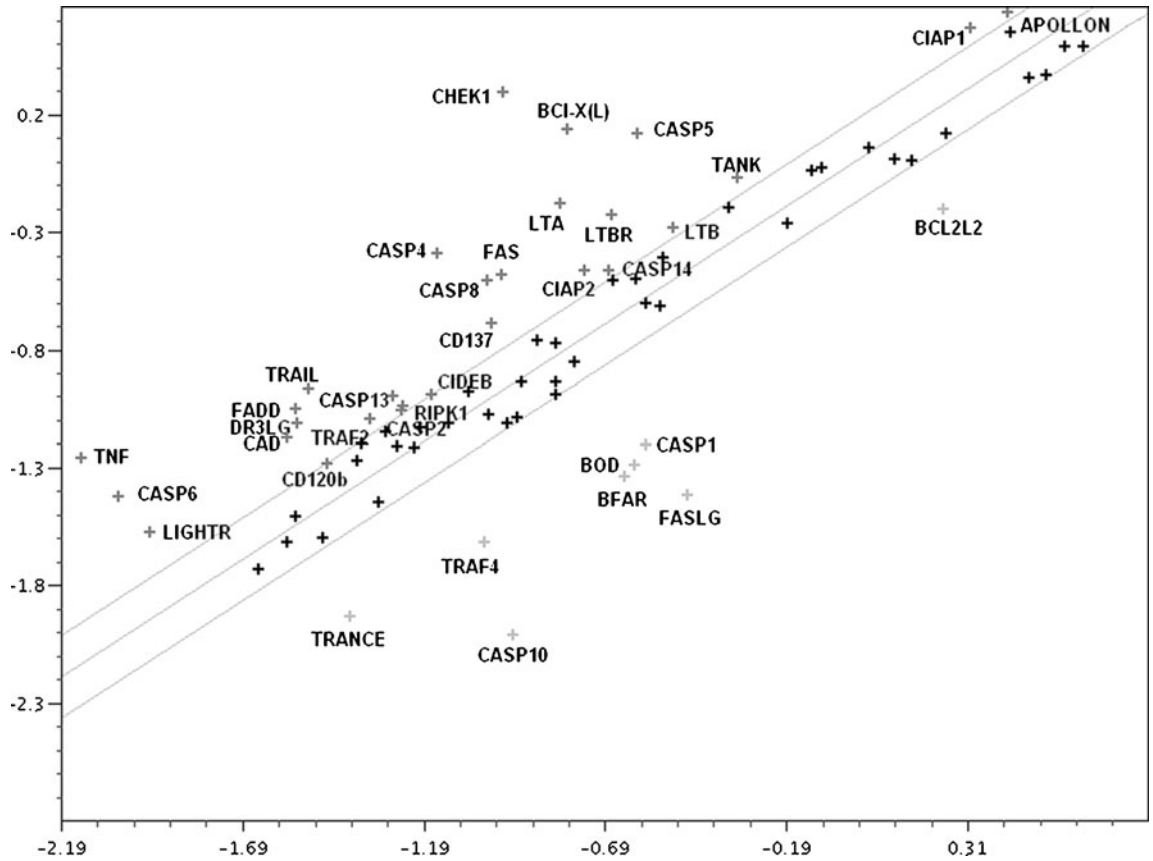

Fig. 2 Gene expression levels of samples of adenoma (*y-axis*) compared with normal parathyroid tissue (*x-axis*)

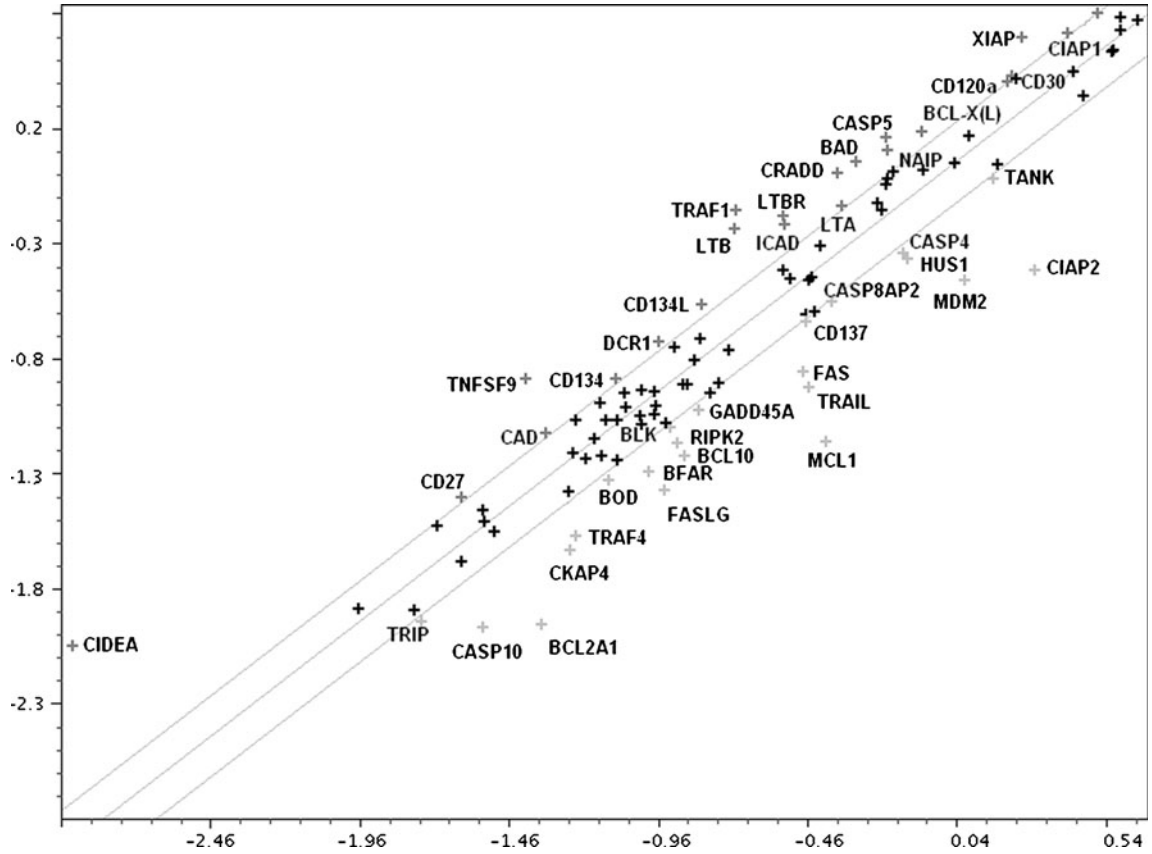

Fig. 3 Gene expression levels of samples of adenoma (*y-axis*) compared with parathyroid tissue showing hyperplasia (*x-axis*)

hyperplasias, adenomas, carcinomas) of 2.0 mm diameter cores were arranged in a recipient paraffin block. Five μm thin sections were immunostained according to the protocol given to the Novolink Detection System (Novocastra, Newcastle, UK). Briefly, dewaxing was followed by blocking of endogenous peroxidases with hydrogen-peroxide containing methanol. Antigen retrival was performed with TRS modified citrate buffer, pH 6.1 (DAKO, Glostrup, Denmark) for 40 min at boiling temperature. Primary antibodies were used for 60 min, the post-primary blocking step for 20 min and incubation with Novolink-peroxidase polymer for 30 min, at room temperature. Peroxidase activity was revealed with the DAB-hydrogen peroxide chromogen-substrate kit, resulting in brown reaction. Finally, cell nuclei were counterstained with hematoxylin. Immunostained slides were digitalized with a pannoramic slide scanner (3DHistech Ltd, Budapest, Hungary) and evaluated by two independent pathologists. Percent of positively stained parenchymal cells of the various parathyroid

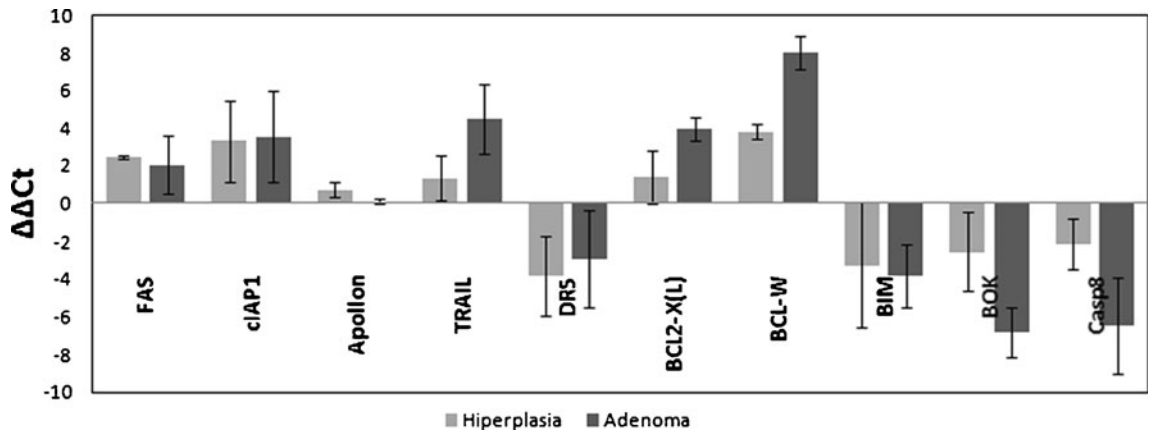

Fig. 4 Relative mean gene expression levels compared to normal parathyroid tissue. Gene names are in the x-axis and relative mean expression levels compared to normal parathyroid tissue are in the y-axis

samples was determined and scored on a 5-grade empirical scale including negative (−), equivocal (−/+), weak (+), moderate (++) and strong (+++) categories. Individual tresholds were set up for each marker to fit within the range of the scoring scheme.

Results

mRNA Expression, Macroarray and PCR Studies

Expression of several pro- and anti- apoptotic genes of hyperplastic and adenomatous parathyroid glands differed from that of the controls. Differences were also found when the mean expression of genes in hyperplasia and adenoma was compared with each other (Figs. 1, 2 and 3).

Ten genes were selected to validate these findings with real-time PCR. The results confirmed that the expression of *FAS* receptor (mean ΔΔCt=2,5 in hyperplasias and 2,07 in adenomas) and *TRAIL* ligand (mean ΔΔCt=1,34 and 4,53) increased both in hyperplasia and adenoma. *CIAP1* and *APOLLON* were overexpressed in hyperplasia (mean ΔΔCt= 3,32 and 0,74) and adenoma (mean ΔΔCt=3,55 and 0,14). Our study shows the decreased expression of the *TRAIL* ligands *DR5* receptor in both alterations (mean ΔΔCt= −3,82 in hyperplasia and −2,94 in adenoma samples). *CASPASE-8* is also underexpressed in hyperplasia (mean ΔΔCt= −2,14) and adenoma (mean ΔΔCt=−6,44).

Real-time PCR study on the gene *MDM2* could not confirm for sure the increase in expression obtained by macroarray (data not shown), but our TMA study could.

Among the members of the BCL2 family, expression of *BCL-X(L)* (mean ΔΔCt=1,41 and 3,97 in hyperplasia and adenomas samples respectively) and *BCL-w* increased (mean ΔΔCt=3,84 in hyperplasias and mean ΔΔCt=8,04 in adenomas), whereas expression of *BIM* and *BOK* decreased in hyperplasia (mean ΔΔCt=−3,24 and mean ΔΔCt=−2,54) and adenoma (mean ΔΔCt=−3,8 and mean ΔΔCt=−6,81). Our findings using real time PCR are summarised in Fig. 4.

Protein Expression

Apoptosis-pathway related proteins, like BCL2 showed moderate positivity in 19% of hyperplasias and 64% in adenomas, but no detectable staining in normal samples and carcinomas. BAX staining was moderately positive in 33% of hyperplasias, 45% of adenomas and showed weak positivity in 22% of carcinomas. Most interestingly, expression of APOLLON, while week in normal parathyroid tissue (4%), moderate in hyperplasia (18%), was equally strong in adenomas and carcinomas (68%). For further results see Table 3. which summarizes the protein expression data in our TMA studies.

Discussion

Differential diagnosis between hyperplasia, adenoma and carcinoma of the parathyroid gland is routinely based on histopathologic features [11–14]. This may also be applied

Table 3 Expression of various gene products in proliferative lesions of the parathyroid gland. Colors are indicating the levels of staining and numbers are representing the percentage of the positive cells

	normal	hyperplasia	adenoma	carcinoma
BCL2		19	64	
BAX		33	45	
APOLLON	4	18	68	68
TRAIL			9	
FLIP		10	14	
CYCLIN D1		14	18	
P53				16
MDM2	8	28	36	
KI67				64
P27	16	43 19	36 23	
ATM			32	32
CK8		10	5 27	
CK18	25	38	50	11
CK19	25	66	59	33
SYNAPTOPHYSIN		5	18	
CHROMOGRANIN-A	16	33	54	
PARATHORMONE	8	23	32	22

negative (-)
equivocal positivity (-/+)
weak positivity (+)
moderate positivity (++)
strong positivity (+++)

19 percentage of positive cells

for the rare proliferative lesions such as atypical adenoma and parathyromatosis [1]. However, differentiation between benign and malignant proliferative lesions and understanding the genetic background of these alterations could be difficult.

With complex expresion profiling of genes and their related proteins regulating the cell cycle and apoptosis, we revealed new features along the series of parathyroid proliferative lesions. In one hand, both alterations showed an increased sensitivity to pro-apoptotic signals with overexpressing the *FAS* and *TRAIL* genes, but in the other hand, we found a strong anti-apoptotic effect with increased expression of *cIAP1, APOLLON, BCL-X(L and BCL-w.* Adenomas showed even higher expression of *cIAP1* and *BCL-X(L)* genes compared to hyperplasias, and overexpressed further genes with anti-apoptotic effect, like *XIAP* and *NAIP*. The expression of *FAS* gene are still increased in adenomas compared to normal tissue, but compared to hyperplasias, the expression levels of these genes are lowered. The pro-apoptotic members of the BCL2-family, like *BIM* and *BOK*, showed decreased expression in both alterations. In addition, the *BCL-X(L)* and *BCL-w* overexpression suggest that, the BCL2 genes are off-balanced to the anti-apopototic phase.

In protein level, we found both normal parathyroid tissue and carcinoma lacking BAX, BCL2, CYCLIND1, CK8, MDM2 and SYNAPTOPHYSIN, while hyperplasia and adenoma expressed these markers to some extent. Also, CK18 and CK19 expression in normal and carcinomatous parathyroid tissue was much lower compared to hyperplasia and adenoma of the parathyroid gland. P53 and KI67 was expressed only in carcinomas, whereas P27 and CHROMOGRANIN-A were not detectable only in carcinomas. ATM showed moderate expression in both adenoma and carcinoma. The expression of the anti-apoptotic APOLLON protein in hyperplasia, adenoma and carcinomas indicates the pro-proliferative activity in these alterations, which is apparently balanced by pro-apoptotic factors in hyperplasia and adenoma but not in carcinoma. It seems that the most active lesions in to cell proliferation are hyperplasia and adenoma, which may also help in distinction between benign and malignant lesions. In accordance with the findings of Haven et al. the hyperplasia, adenoma to carcinoma model is not suggested [15], i.e. our findings also argue against a molecular sequence in parathyroid carcinogenesis.

Taking into consideration the various steps of proliferation such as hyperplasia, adenoma and carcinoma, our macroarray and real time PCR studies on such lesions of the parathyroid gland showed that in hyperplasia and adenoma a certain balance developed between the expression of pro-apoptotic and anti-apoptotic genes. The overexpression of pro-apoptotic factors may have no effect on the cell survival, because of the overexpression of caspase inhibitor genes and the lowered expression of *CASP8*. This balance seems to be not established in case of carcinoma, shown by failure of expression of FLIP, P27, BCL-2, CYCLIND1, and overexpression of P53 and APOLLON according to our TMA studies. These changes may allow increased and uncontrolled cell proliferation, but do not lead to increased apoptotic activity [6].

Immunohistochemical studies on various gene products affecting cell proliferation were carried out in several laboratories. Parathyroid carcinomas showed increased expression of KI67 (MIB1), compared to adenomas [6–8]. Decrease or failure of expression of BCL-2 [7, 9], RB, P27, MDM2 and P21 [7] and slight increase of P53 expression was reported in case of parathyroid carcinoma. Fernandez-Ranvier et al. [4] found complete loss of PARAFIBROMIN expression in 5 of 16 parathyroid carcinomas, whereas all benign prolifrative lesions investigated by this group stained positive. Loss of RB expression was seen in 5 of 15 carcinomas and in one of 14 hyperplasias, otherwise all benign lesions showed positive reaction. GALECTIN-3 stained strongly positive in 14 of 15 carcinomas, in 3 of 16 cases of parathyromatosis, in 2 of 2 atypical adenomas, 1 of 18 adenomas and 2 of 14 hyperplasias. The role of PARAFIBROMIN underexpression in the development and also diagnosis of parathyroid carcinoma became increasingly important after the discovery of the role of *HPRT2* gene mutations in the hyperparathyreodism-jaw tumor symptone [16–19]. Other genetic alterations [20, 21] may also reveal data on pathogenesis of unlimited proliferation of the parathyroid cells. It seems that a series of gene expression alterations leads to the relatively rare occurrance of parathyroid carcinoma, rather than overexpression or suppression of one single gene. From practical point of view, the diagnostic panel, including RB, BCL2, BAX, PARAFIBROMIN (underexpressed in carcinoma) as well as KI67, GALECTIN-3 and APOLLON (overexpressed in carcinoma) can be recommanded for this purpuse.

Our findings on expression of gene products—together with the already published data [9, 11, 22, 23]—may provide a battery of immunohistochemical reactions which could safely define the benign or malignant nature of a given proliferative lesion of the parathyroid gland.

Acknowledgements This work was supported by a grant of the Hungarian National Science and Research Foundation (OTKA T46665) grant. The authors would like to thank Tibor Vaszkó for his valuable comments to improve the manuscript.

Declaration of interest The authors confirm that there are no conflicts of interest.

References

1. Fernandez-Ranvier GG, Khanafshar E, Jensen K et al (2007) Parathyroid carcinoma, atypical parathyroid adenoma, or parathyromatosis? Cancer 110(2):255–264
2. Adami S, Marcocci C, Gatti D. Epidemiology of primary hyperparathyroidism in Europe. J Bone Miner Res 17 Suppl 2:N18-23

3. Marx SJ (2000) Hyperparathyroid and hypoparathyroid disorders. N Engl J Med 343:1863–1875
4. Fernandez-Ranvier GG, Khanafshar E, Tacha D et al (2009) Defining a molecular phenotype for benign and malignant parathyroid tumors. Cancer 115(2):334–344
5. Stojadinovic A, Hoos A, Nissan A et al (2003) Parathyroid neoplasms: clinical, histopathological, and tissue microarray-based molecular analysis. Hum Pathol 34(1):54–64
6. Szende B, Farid P, Végso G et al (2004) Apoptosis and P53, Bcl-2 and Bax gene expression in parathyroid glands of patients with hyperparathyroidism. Pathol Oncol Res 10(2): 98–103
7. Vargas MP, Vargas HI, Kleiner DE et al (1997) The role of prognostic markers (MiB-1, RB, and bcl-2) in the diagnosis of parathyroid tumors. Mod Pathol 10(1):12–17
8. Hadar T, Shvero J, Yaniv E et al (2005) Expression of p53, Ki-67 and Bcl-2 in parathyroid adenoma and residual normal tissue. Pathol Oncol Res 11(1):45–49
9. Szende B, Arvai K, Peták I et al (2006) Changes in gene expression in the course of proliferative processes in the parathyroid gland. Magy Onkol 50(2):137–140
10. Solcia E, Kloppel G, Sobin LH (2000) Histological typing of endocrine tumours; international histological classification of tumours. WHO Ed. 2. Springer; p.48–55
11. Shane E, Bilezikian JP (1982) Parathyroid carcinoma: a review of 62 patients. Endocr Rev 3(2):218–226
12. Favia G, Lumachi F, Polistina F et al (1998) Parathyroid carcinoma: sixteen new cases and suggestions for correct management. World J Surg 22(12):1225–1230
13. Hundahl SA, Fleming ID, Fremgen AM et al (1999) Two hundred eighty-six cases of parathyroid carcinoma treated in the U.S. between 1985–1995: a National Cancer Data Base Report. Cancer 1;86 (3):538–44
14. Busaidy NL, Jimenez C, Habra MA et al (2004) Parathyroid carcinoma: a 22-year experience. Head Neck 26(8):716–726
15. Haven CJ, Howell VM, Eilers PH et al (2004) Gene expression of parathyroid tumors: molecular subclassification and identification of the potential malignant phenotype. Cancer Res 64(20):7405–7411
16. Woodard GE, Lin L, Zhang JH et al (2005) Parafibromin, product of the hyperparathyroidism-jaw tumor syndrome gene HRPT2, regulates cyclin D1/PRAD1 expression. Oncogene 10;24(7):1272–6
17. Cetani F, Pardi E, Ambrogini E et al (2007) Different somatic alterations of the HRPT2 gene in a patient with recurrent sporadic primary hyperparathyroidism carrying an HRPT2 germline mutation. Endocr Relat Cancer 14(2):493–499
18. Iacobone M, Masi G, Barzon L et al (2009) Hyperparathyroidism-jaw tumor syndrome: a report of three large kindred. Langenbecks Arch Surg 394(5):817–825
19. Yang Y-J, Han J-W, Youn H-D et al (2010) The tumor suppressor, parafibromin, mediates histone H3 K9 methylation for cyclin D1 repression. Nucleic Acids Res 38:382–390
20. Masi G, Barzon L, Iacobone M et al (2008) Clinical, genetic, and histopathologic investigation of CDC73-related familial hyperparathyroidism. Endocr Relat Cancer 15(4):1115–1126
21. Shih RY, Fackler S, Maturo S et al (2009) Parathyroid carcinoma in multiple endocrine neoplasia type 1 with a classic germline mutation. Endocr Pract 15(6):567–572
22. Cetani F, Ambrogini E, Viacava P et al (2007) Should parafibromin staining replace HRTP2 gene analysis as an additional tool for histologic diagnosis of parathyroid carcinoma? Eur J Endocrinol 156(5):547–554
23. Howell VM, Gill A, Clarkson A et al (2009) Accuracy of combined protein gene product 9.5 and parafibromin markers for immunohistochemical diagnosis of parathyroid carcinoma. J Clin Endocrinol Metab 94(2):434–441