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ARTICLE

Expression of p53, Ki-67 and Bcl-2 in Parathyroid Adenoma and Residual Normal Tissue

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The aim of this study was to investigate the expression of Ki-67, bcl-2 and p53 in parathyroid adenomas and their residual rim of normal parathyroid tissue. Specimens from 26 parathyroid adenomas were studied by immunohistochemical analysis for Ki-67, bcl-2 and p53 expression. Positive findings were noted for p53 in 4 (15%) adenomas and none of the residual rims of normal parathyroid tissue (p = 0.055); for Ki-67 in 15 (56%) adenomas and none of the residual rims of normal parathyroid tissue (p = 0.00002); and for bcl-2 in 19 (73%) adenomas and 8 (31%) residual rims of normal parathyroid tissue (p < 0.01). The high rate of Ki-67 expression may indicate susceptibility of parathyroid adenomas to clonal proliferation. The weak immunoreactive expression of p53, combined with a relatively strong expression of bcl-2, may contribute to the characteristic slow progression of these tumors. (Pathology Oncology Research Vol 11, No 1, 45–49)

Key words: parathyroid adenoma, normal tissue, p53, Ki-67, bcl-2

Introduction

Hyperparathyroidism is a common disorder characterized by hypercalcemia due to increased parathyroid hormone (PTH) secretion. In most cases, a single benign adenomatous gland termed adenoma is found. Most of the parathyroid adenomas studied have been found to be monoclonal neoplasms,[2,3] supporting a pathogenic role of oncogenes or tumor suppressor genes.

A new class of proto-oncogenes has been defined that contributes to malignancy by inhibiting programmed cell death or apoptosis.[4,31] The bcl-2 gene, located on chromosome 18, encodes the 26 kDa bcl-2 protein, a prototype of the proteins involved in this anti-apoptotic regulatory pathway.[28] The protein resides in the nuclear envelope, endoplasmic reticulum and outer mitochondrial membrane. Its high expression in cells counteracts pro-apoptotic protein function, thereby hypothetically enhances cell survival.[16,23]

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p53 is a tumor suppressor gene located on the short arm of chromosome 17 at position 17p13.1. It is composed of 11 exons and encompasses 20 kb of DNA. The product of the p53 gene is a nuclear phosphoprotein. Normally, an excess of p53 protein at the end of G1 phase of the cell cycle will block progression to the S phase, one of the checkpoints at which the cell cycle can either slow down to allow repair of damaged DNA or stop to cause apoptosis. In this manner, p53 prevents the reproduction and proliferation of abnormal cancer cells. Sporadic mutations in the p53 gene are the single most common genetic alteration in carcinogenesis,[12,22] playing a role in more than 50% of human cancers.[8] Normal and abnormal p53 proteins can be detected immunohistochemically, even in archived tissues.

Ki-67 is a nuclear antigen expressed in all phases of the cell cycle except G0. Therefore, Ki-67 levels reflect cell division activity. The Ki-67 antigen is a bimolecular complex of 345 kDa and 395 kDa proteins. Ki-67 reactivity is now widely accepted as a marker of proliferative activity and correlates well with other cell kinetic measurements. It has proved useful for evaluating the proliferative activity of parathyroid adenoma and hyperplasia.[1,6,18]

In the present study, we compared the expression of bcl-2, p53 and Ki-67 between parathyroid adenoma and the residual rim of normal parathyroid tissue in the same histological sections.

Materials and Methods

The study sample included 26 consecutive patients with primary hyperparathyroidism, 8 males and 18 females, aged 39 to 79 years (mean 62 years). In all cases, parathyroid adenoma was clinically documented preoperatively by increased serum levels of calcium and PTH. Mean serum calcium level was 2.90 mM, range 2.6 to 3.32 mM (normal range: 2.1-2.56 mM). Following technetium 99m-methoxyisobutylisonitrile (99m Tc-MIBI) scintigraphy and ultrasound examination of the parathyroid glands, parathyroidectomy was performed. The clinical diagnosis was confirmed by histological analysis. Most of the lesions were cervical; one patient had an ectopic retrosternal tumor.

Histology

After surgical excision, the specimens were fixed in buffered formalin for 24 hours, embedded in paraffin, sectioned at 4 µm, and stained with hematoxylin and eosin. The specimens were classified as adenoma according to the histological criteria of Ghandur-Mnaymneh and Kimura.[7] The oxyphil cell content was determined by semi-quantitative assessment of the percentage of oxyphil cells in each section.

Immunohistochemistry

Representative paraffin blocks were retrieved for immunostaining. Sections were deparaffinized with xylene and rehydrated in a graded series of ethanol. For antigen unmasking we heated the sections in 10 mM sodium citrate buffer (pH 6) for 20 minutes in a microwave oven at high power. Immunohistochemical staining was performed with an automated immunohistochemical processor (Ventana). Details of primary antibodies used are summarized in *Table 1*.

For each antibody, negative control studies were performed in which normal serum was used instead of the primary antibody. All immunostained slides were analyzed and scored. The fraction of positively stained tumor cells was separately scored for each marker after having examined on at least 10 high-power fields (HPFs [x400]) of one section for each sample.

The immunohistochemical results for bcl-2 were scored semi-quantitatively using a four-grade scale: 0 = no immunoreaction; 1 = faint or equivocal immunoreaction in less than 10% of cells; 2 = unequivocal, strong immunoreaction in less than 30% of cells; 3 = unequivocal, strong immunoreaction in more than 30% of cells. The mean percentage of p53-positive nuclei was the average of positive nuclei of the HPFs examined.

The cut-off values for tumor cell staining used in this study were defined as follows: p53 and Ki-67 nuclear staining was considered positive when at least 5% of tumor nuclei stained positive; bcl-2 overexpression was considered when more than 30% of tumor cells showed cytoplasmic or/and membrane staining. The total number of stained cells for each immunohistochemical stain on each slide was recorded in the areas of adenoma and in the rim of normal tissue outside the adenoma capsule.

Statistical analysis

The difference in the percentage of positive p53, Ki-67 and bcl-2 immunostaining between the adenoma cells and the residual rim of normal parathyroid residual tissue for

Table 1. **Details of primary antibodies used**

Antibody against	*Monoclonal*	*Source*	*Dilution*	*Positive control*
p53	Monoclonal	ZYMED (San Francisco, CA)	ready for use	colon adenocarcinoma
Ki-67	Monoclonal	ZYMED (San Francisco, CA)	1:100	tonsil
bcl-2	Monoclonal	ZYMED (San Francisco, CA)	1:200	tonsil

Table 2. **p53, Ki-67 and bcl-2 immunostaining in 26 parathyroid adenomas and residual normal parathyroid tissue**

Parathyroid zones	*p53 positive*		*Ki-67 positive*		*bcl-2 positive*	
	No	*%*	*No*	*%*	*No*	*%*
Adenoma	4	15	15	56	19	73
Normal rim tissue	0	0	0	0	8	31
Significance	p* = 0.055		p*=0.00002		p**<0.01	

p* Fisher's exact test; p** chi square test

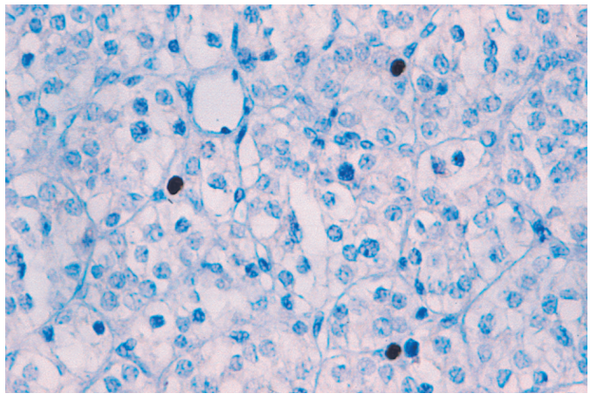

Figure 1. *Nuclear p53 immunoreactivity in parathyroid adenomas (x400, counterstained with hematoxylin)*

the 26 adenomas was tested for statistical significance by chi-square test or Fisher's exact test; $p < 0.05$ was considered statistically significant.

Results

Serum levels of calcium and PTH decreased to normal after parathyroidectomy in all patients. The pathological diagnosis was parathyroid adenoma. In the specimens, adjacent to adenomas, a residual rim of normal parathyroid tissue was seen.

The results of immunostaining for p53, Ki-67 and bcl-2 are summarized in *Table 2*. Nuclear p53 immunoreactivity (*Figure 1*) was detected in only 4 (15%) of the 26 parathyroid adenomas ($p = 0.055$, borderline significance); in 10% of the adenoma cells in one tumor and in 5% in the other 3 tumors. The residual rim of normal parathyroid gland did not show any positive staining (*Table 2*). Nuclear Ki-67 immunoreactivity was demonstrated in 15 (56%) parathyroid adenomas in 5 to 15% of tumor cell nuclei (*Figure 2*). Again, the residual rim of normal parathyroid tissue did not show any positive staining ($p = 0.00002$). Bcl-2 immunostaining was noted in 19 (73%) adenomas showing cytoplasmic or/and membrane staining (*Figure* 3). The remnant rim of normal parathyroid tissue contained immunoreactivity staining in 8 (31%) of cases ($p < 0.01$).

Discussion

Parathyroid neoplasms are comprised of adenomas and carcinomas. These two neoplasms have disparate natural histories, but they can be difficult to differentiate on the basis of histopathologic findings alone. Although thick fibrous bands, mitotic activity, trabecular growth pattern, and capsular, vascular, or adjacent soft-tissue invasion are considered characteristic of parathyroid carcinoma, morphological features such as fibrous bands, mitotic activity, and trabecular growth have been identified in parathyroid adenoma as well.[7,25]

According to Szende et al,[26] apoptosis and mitosis were rarely seen in hyperplasias and adenomas (under 2%), whereas in carcinomas 3% of the tumor cells were apoptotic and 4% showed mitosis. The very low rate of malignant tumors in the parathyroid glands may also have some relation to the low mitotic and apoptotic activities.

The tumor suppressor gene p53 is important for maintaining the integrity of the cellular genome and protecting the cell from malignant transformation. Mutation of the gene may produce a more stable protein that can be detected immunohistochemically.

Our data revealed p53 immunoreactivity in 15% of the adenomas and in none of the residual rims of normal parathyroid tissue. The antibody used by us labels predominantly mutant p53 by immunostaining. These findings agree with previous reports on p53 gene abnormalities in only a minority of parathyroid adenomas and in

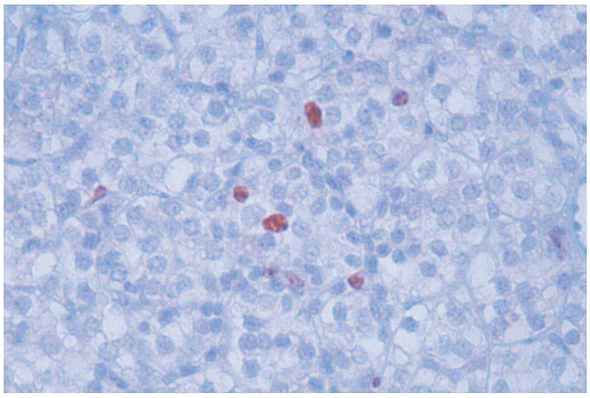

Figure 2. *Nuclear immunoreactivity of Ki-67 in parathyroid adenomas (x400, counterstained with hematoxylin)*

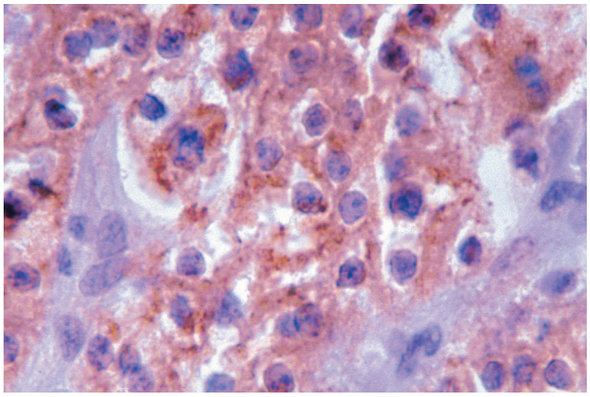

Figure 3. *Cytoplasmic or/and membrane bcl-2 immunostaining of parathyroid adenomas (x400, counterstained with hematoxylin)*

some parathyroid carcinomas,[5,10] although the range in the literature is 0% to 52.4% (*Table 3*).[9,14,15,20,21,24,25,30] Abnormalities in only one p53 allele may play a role in the pathogenesis of a small subset of parathyroid adenomas, but in general such mutations, together with loss of the wild-type p53 allele, appear in parathyroid carcinomas.[5]

Vargas et al[29] found p53 immunoexpression only in parathyroid carcinomas and not in adenomas. Ricci et al[24] observed nuclear p53 immunoreactivity in 11 of 21 (52.4%) adenomas and Kayath et al[14] in 10 of 28 (36%) adenomas. Our study showed Ki-67 positivity in 56% of the adenoma specimens and in none of the rims of normal parathyroid tissue. The Ki-67 antibody has been widely used to evaluate cell proliferation in various types of tumors and other lesions. It is expressed during all cell phases of the cell cycle except G0. A higher tumor proliferating fraction detected by Ki-67 immunostaining is associated with aggressive neoplasms. Some authors found that the cell proliferation rate is consistently higher in hyperplastic and adenomatous parathyroid glands than in normal parathyroid tissue.[1,30] Wang et al[30] found that all parathyroid adenomas were immunoreactive to Ki-67, while only in 2 out of 15 (13.3%) demonstrated positivity for Ki-67 in the residual rim of normal parathyroid tissue. Abbona et al[1] demonstrated that parathyroid carcinomas have a higher tumor proliferating fraction than parathyroid adenomas and hyperplasias. Stojadinovic et al[25] and Karak et al[13] found that parathyroid adenomas were immunoreactive for Ki-67 only in 2% and 1.36% of the cases, respectively.

Table 3. Immunoreactivity of p53, Ki-67 and bcl-2 in parathyroid adenoma and normal parathyroid tissue

Author	*Tissue*	*No. of cases*	*p53 positive (%)*	*Ki-67 positive (%)*	*bcl-2 positive (%)*
Loda et al, 1994[18]	adenoma	21	–	32	–
Abbona et al, 1995[1]	normal	9	–	0.1	–
	adenoma	11	–	3.3	–
Lloyd et al, 1995[17]	adenoma	35	–	2.4	–
Wang et al, 1996[30]	normal	15	0	13	100
	adenoma	20	15	100	95
Karak et al, 1997[13]	normal	14	–	0.03	–
	adenoma	22	–	1.36	–
Vargas et al, 1997[29]	adenoma	10	–	2.3	70
Naccarato et al, 1998[21]	normal	33	0	LP	100
	adenoma	43	0	9	55
Kayath et al, 1998[14]	normal	14	0	–	–
	adenoma	28	36	–	–
Martin et al, 1998[20]	normal	12	0	–	–
Kishikawa et al, 1999[15]	adenoma	32	12.5	–	–
Gulkesen et al, 2001[9]	adenoma	12	0	–	–
Ricci et al, 2002[24]	adenoma	21	52.4	57.2	76.2
Stojadinovic et al, 2003[25]	adenoma	53	0	2	98
Present study	normal	26	0	0	31
	adenoma	26	15	56	73

LP= low proliferation rate

We found bcl-2 immunoreactivity in 73% of the adenomas and in 31% of the rims of normal parathyroid tissue, detected immunohistochemically by diffuse cytoplasmic or nuclear envelope staining. Bcl-2 is critical for the regulation of apoptosis. Its overexpression may result in cell proliferation, as cells normally scheduled for death may undergo further mutations. Bcl-2 overexpression was first characterized in B-cell lymphomas and has since been noted in several epithelial malignancies.[11,19] In parathyroid adenoma, findings of positive bcl-2 staining have been reported in 55% to 95% of parathyroid adenomas and in 100% of rims of normal parathyroid tissue. Although our results differ from previous reports, there are few immunohistochemical studies on p53, Ki-67 and bcl-2 expression in parathyroid tissues. Our results showing a low expression of bcl-2 (31%) in normal parathyroid tissue versus high expression (73%) in parathyroid adenomas suggest that it could be an important marker for tumor progression. Moreover, expression of genes influencing apoptosis, such as bcl-2, p53 and bax, may be of importance from the point of view of histological differential diagnosis.[27]

In conclusion, our findings suggest that parathyroid adenomas contain cell populations with immunohistochemical expression of Ki-67 (56%), which are at risk of developing genetic abnormalities that may lead to clonal proliferation. The weak immunoreactivity for p53 (15%) combined with the relatively strong expression of bcl-2 (73%) might contribute to the slow progression that characterizes most parathyroid adenomas and is not exclusive to malignant transformation.

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