Parathyroid tumorigenesis

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Summary

Primary hyperparathyroidism (PHPT) is a common endocrinopathy, mostly caused by a monoclonal parathyroid adenoma. This review primarily summarizes current knowledge concerning molecular pathogenesis of familial forms of primary hyperparathyroidism and sporadic (non familial) parathyroid tumors. The hereditary syndromes have been recognized as exhibiting Mendelian inheritance patterns and include multiple endocrine neoplasia types 1 (MEN 1) and 2A (MEN 2A), hereditary hyperparathyroidism-jaw tumor (HPT-JT) syndrome, familial isolated hyperparathyroidism (FIHP), familial hypocalciuric hypercalcemia (FHH) and severe neonatal hyperparathyroidism (NSHPT). Inactivating mutations of *MEN1* tumor suppressor gene are responsible for MEN 1 in >90% of cases. *MEN1* gene has also an established role in the pathogenesis of sporadic parathyroid adenomas. Allelic loss (LOH) of chromosome 11q13 occurs in about 30-40% and somatic mutation of *MEN1* gene occur in about 12-20% of sporadic parathyroid adenomas. A mouse model of *MEN1* deficiency causes a phenotype that includes the same range of major endocrine tumors as in MEN 1 patients, and exhibits multistage tumor progression with metastatic potential. Hormonal disturbances, such as abnormal PTH and insulin levels, were also observed in these mice. Mutations in a newly identified tumor suppressor gene, *HRPT2*, have been recently associated with the development of HPT-JT. *HRPT2* mutations are also frequent in sporadic parathyroid carcinomas and central to their pathogenesis. *MEN1* and *HRPT2* genes mutations have also been found in a subset of FIHP families. FHH and NSHPT represent the mildest and severest variants of PHPT, respectively. Both cause hypercalcemia from birth and atypical PHPT that always uniquely persists after subtotal parathyroidectomy. Future identification of additional oncogenes and tumor suppressor genes will clarify the molecular basis of abnormalities of parathyroid proliferation and regulatory function and other specific features unique to the parathyroid tumorigenesis.

KEY WORDS: familial hyperparathyroidism, menin, CASR, FIHP, parafibromin, HRPT2, MEN1, parathyroid carcinoma, cyclin D1.

Introduction

Over the past ten years, our knowledge of the molecular basis of parathyroid neoplasia has increased substantially. Many of the well established general themes in tumor biology appear to be applicable to parathyroid tumorigenesis, in spite of the typically nonmalignant status of these tumors. Patients with primary hyperparathyroidism (PHPT) have both an increased parathyroid proliferation and a resetting of the control of PTH secretion (set-point abnormality) (1, 2). A full molecular description of parathyroid tumor biology will need to explain these phenomena and other special aspects such as the role of irradiation in predisposing to parathyroid tumors, the rarity of parathyroid cancer as compared with the abundance of benign parathyroid lesion, and the possible role of the post menopause and/or estrogen status.

It is now clear that neoplasia is a genetic disease, in which the DNA damage occurs somatically and is typically not inherited or present in the germ cells. In general, multiple mutations (accumulating within the same cell and its progeny) are required for the emergence and progression of the clonal status. Furthermore, the monoclonality of tumors implies that the needed accumulation of mutations occurs only rarely in a large population of cells in a tissue.

Some genes may contribute to tumor development of only one or a few cell types, whereas other genes may be involved in many different types of tumors. Moreover, for most tumor cell types it appears that a single gene is not always involved and that different combinations of mutated genes may result in similar cellular and clinical alteration (3, 4).

The recognition that parathyroid adenomas are monoclonal tumors generated the expectation that these general principles would indeed apply to parathyroid tumorigenesis (4). Clonal DNA damage of two groups of normal cellular genes (protooncogenes and tumor suppressor genes) contributes to the development of neoplasia (5).

Protooncogenes are often involved in the physiological control of cellular growth, differentiation and proliferation. Conversion of a protooncogene to an “oncogene” is caused by a deregulation of the expression of its protein product or by formation of an abnormal product. Proto-oncogene can be activated by a variety of mechanisms, including chromosome translocation or inversion, point mutation, proviral insertion, or gene amplification.

The products of tumor suppressor genes normally restrain proliferation and their gene inactivation contributes to neoplasia. Inactivation of tumor suppressor genes can occur by point mutation or deletion, and may be inherited or occurs somatically. The causes of this oncogenic “hits” are not well understood. Some genetic changes tend to occur more commonly in mitotically active cells, and carcinogens may act either by direct mutagenesis of DNA or through an increase of the mitotic rate,

which favors the occurrence of an oncogenic chromosome aberrancy.

Monoclonality of parathyroid adenomas

Early studies of the clonal status of parathyroid tumors assessed X-chromosome inactivation patterns by measuring isoforms of glucose-6-phopshate dehydrogenase in parathyroid adenomas of women heterozygous for the polymorphism (6, 7). These studies indicated that parathyroid adenomas were polyclonal as opposed to monoclonal growths. The issue of the clonal status of parathyroid tumors was reevaluated several years later, again by X- chromosome inactivation method, using a DNA polymorphism-based method that avoids many of the pitfalls of the previously protein polymorphism approach. It was demonstrated that most, if not all, parathyroid adenomas are monoclonal tumors (8-10). This finding is consistent with the general experience that surgical removal of such tumors is curative of the disease. Parathyroid carcinomas, as would be expected, are also monoclonal. Monoclonality is a reflection of the selective advantage gained by a parathyroid cell when a sufficient number of critical changes in protooncogenes and/or tumor suppressor genes occur within it. Progeny of such a cell grows and accumulates additional genetic changes (this is clonal evolution), ultimately forming a clinically apparent mass of cells.

It is well known that various forms of parathyroid hyperplasia, presumably caused by a stimulus of parathyroid cell proliferation affecting all parathyroid glands, may evolve into a monoclonal tumor. Indeed, monoclonality has been documented in the majority of parathyroid tumors from patients with sporadic PHPT and secondary (or tertiary) hyperparathyroidism of uremia (10). Monoclonality also characterizes the enlarged parathyroids of patients with multiple endocrine neoplasia type 1 (MEN 1) (11-13), but it is not known whether such outgrowth derives from a previous stage of true polyclonal hyperplasia that might be driven by the inherited haploinsufficiency for the *MEN1* gene.

Familial parathyroid diseases

Genetic studies on inherited tumor susceptibility disorders have increased our knowledge of tumorigenesis. PHPT is found in several disorders with an autosomal dominance inheritance, such as multiple endocrine neoplasia type 1 (MEN 1) and 2A (MEN2A), hereditary hyperparathyroidism-jaw tumor (HPT-JT) syndrome, familial isolated hyperparathyroidism (FIHP), familial hypocalciuric hypercalcemia (FHH) and neonatal severe hyperparathyroidism (NSHPT). The genetic characteristics of familial parathyroid diseases are shown in Table I.

Multiple endocrine neoplasia type 1

Genetic mapping studies in families with MEN 1 syndrome showed that the gene responsible is on chromosome 11q13 (14). By analogy with familial retinoblastoma, which involves inheritance of mutations in the Rb gene, it was suggested that the *MEN1* gene was a tumor suppressor gene. Inactivation of both alleles is required to completely deplete the gene's antineoplastic product. A common inactivation mechanism is a somatic deletion of a substantial proportion of chromosomal DNA that includes the relevant gene. This is revealed by a loss of heterozygosity (LOH) of DNA markers in tumor DNA relative to normal DNA of the same individual (Fig. 1). The evidence that the *MEN1* was a tumor suppressor gene was provided by the demonstration of somatic genetic abnormalities in MEN1 tumors which inactivate one allele of a gene at 11q13 and so reveal the inherited *MEN1* mutation on the other allele (Fig. 2). LOH of polymorphic marker DNAs from this region has been found in the majority of MEN 1-associated tumors including those of parathyroids (15, 16). Mutations of the *MEN1* gene have been detected in >90% of MEN1 families. The mutations are scattered through the nine translated exons of the *MEN1* gene, and approximately half the members of each family have

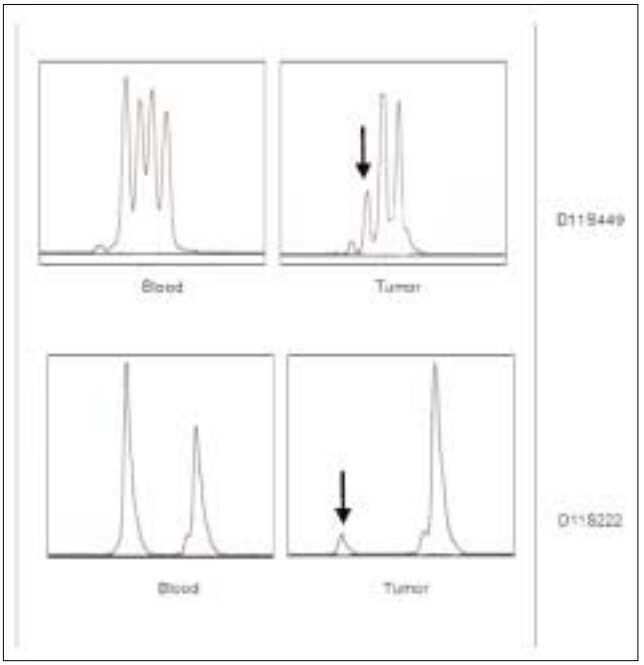

Figure 1 - Microsatellite length polymorphisms around *MEN1* locus analyzed using polymorphic DNA markers on chromosome 11q13 in MEN 1 family. Arrows denote peaks with reduced fluorescence intensity in tumor compared to blood DNA; these findings indicate loss of heterozygosity.

Table I - Genetic characteristics of familial hyperparathyroid diseases

Disease	Gene	Chromosomal location	Inheritance
MEN 1	*MEN1*	11q13	Autosomal dominant
MEN 2A	*RET*	10q21	Autosomal dominant
HPT-JT	*HRPT2*	1q24-32	Autosomal dominant
FIHP	*MEN1, HRPT2,CASR, NI**	11q13, 1q24-32, 3q21-24, ?	Autosomal dominant
FHH	*CASR, NI*	3q21-24, 19q13, 19p13.3	Autosomal dominant
NSHPT	*CASR*	3q21-24	Autosomal recessive

* Not identified.

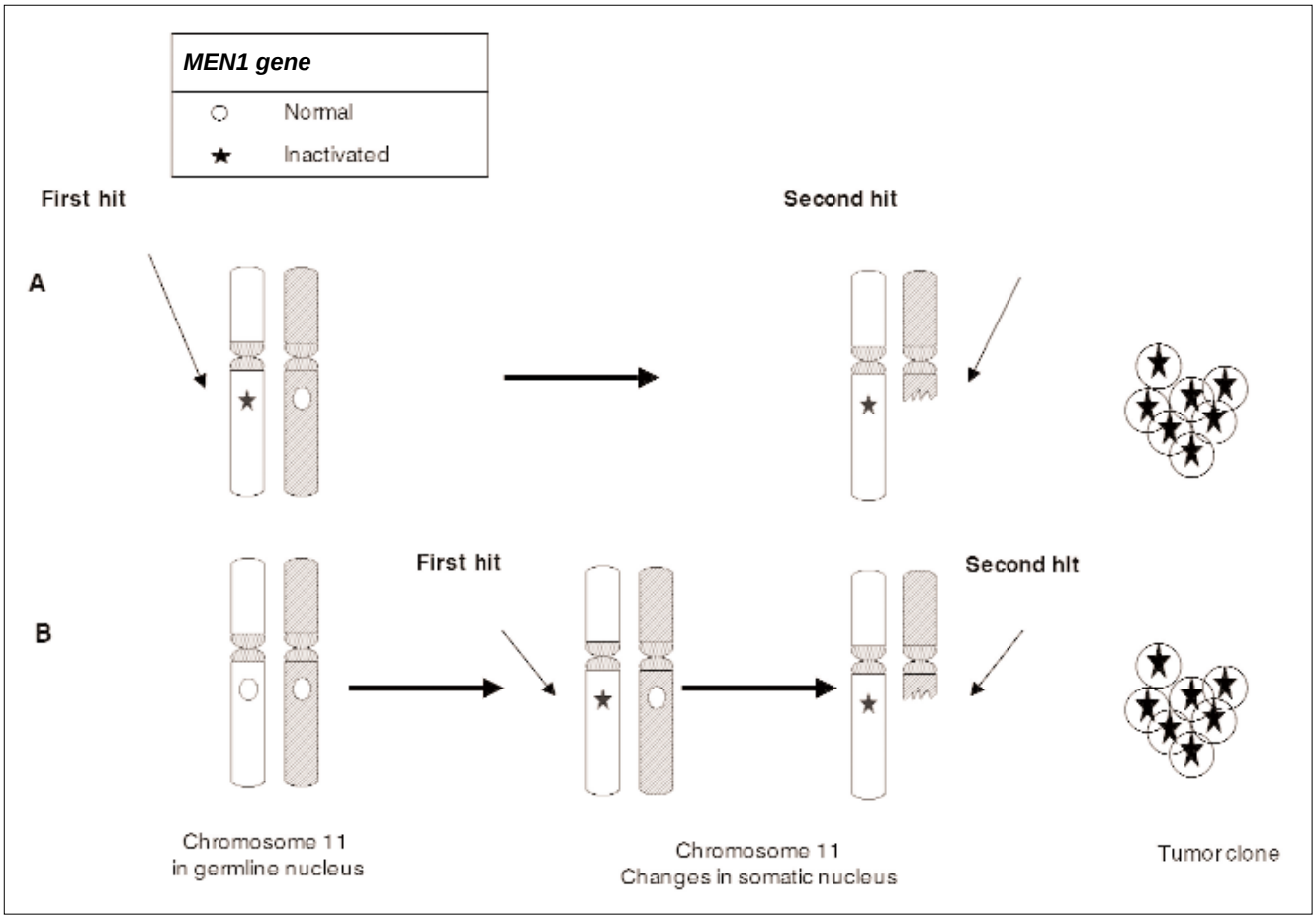

Figure 2 - Schematic representation illustrating the inactivation of *MEN1* gene in MEN 1 syndrome (panel A) and in sporadic primary hyperparathyroidism (panel B) according to Knudson's hypothesis. *Panel A.* A mutant copy of *MEN1* gene is inherited in MEN 1 and present in all parathyroid cells. A second somatic deletion/mutation of the remaining normal allele leads to a clonal progenitor cell that lacks functional gene product with the development of the tumor. *Panel B.* Mutation of one allele of the same gene may occur somatically in specific parathyroid cell(s). A second somatic deletion/mutation of the remaining normal allele leads to a clonal progenitor cell that lacks functional gene product with the development of the tumor.

a mutation unique to that family, making presymptomatic genetic testing laborious (17).

Allelic loss of chromosome 11 markers occurs in about one-third of the large number of sporadic parathyroid adenomas

Table II - Menin interacting proteins.

Interacting proteins	Functions of interacting protein
Nuclear	
JunD	Trascriptional regulation
Smad1, Smad3, Smad5, Runx2	Trascriptional regulation
NFkB	Trascriptional regulation
Pem	Trascriptional regulation
RPA2	DNA processing and repair
FANCD2	DNA processing and repair
Double-stranded DNA	DNA processing and repair
Cytoplasmic	
GFAP and vimentin	Cytoskeletal organization
NMMHC IIA	Cytoskeletal organization
Rat nm23-beta	GTPase activity
Nuclear and cytoplasmic	
Hsp70 and CHIP	Protein degradation

(18-24), and usually involves loss of the region to which the *MEN1* gene has been mapped. By analogy to the Rb tumor suppressor gene mode, in which the sporadic counterpart of the Rb tumor results from somatic genetic events leading to loss or inactivation of both normal Rb gene alleles (Fig. 2), some sporadic parathyroid adenomas may evolve from a clonal precursor with biallelic inactivation of the *MEN1* gene. Biallelic-acquired inactivating *MEN1* mutations have been described in 12-20% of sporadic parathyroid adenomas (18-24).
The *MEN1* gene encodes a 610 amino acid protein, named menin, with no strong homology to known proteins. Menin is expressed ubiquitously and found predominantly in the nucleus (25). Sequence analyses do not reveal motifs of known function other than two nuclear localization sequences. Menin has been found to partner *in vitro* with a variety of proteins that comprise transcription factors, DNA processing factors, DNA repair proteins, and cytoskeletal proteins (Table II). The different functions of menin interactors suggest roles for menin in multiple biological pathways. Inactivation of menin switches its JUND partner from downstream action of growth suppression to growth promotion (25-27).
To examine the role of menin in tumor development, two mouse models of *MEN1* deficiency have been generated (28, 29). Heterozygous MEN1 mutant mice develop the same range of major endocrine tumors as seen in MEN 1 patients, affecting parathyroid, pancreatic islets, pituitary and adrenal glands, and exhibiting multistage tumor progression with metastatic potential (28). Hormonal disturbances (29), such as abnormal PTH and insulin levels, have also been observed in these mice. These tumors were associated with LOH of the wild type *MEN1* allele, suggesting that menin is involved in suppressing the development of these endocrine tumors. All of these features are reminiscent of MEN 1 symptoms in humans and establish heterozygous MEN 1 mutant mice as a suitable model for this disease.

Multiple endocrine neoplasia type 2 A

Germline gain-of-function mutations in the *RET* protooncogene cause MEN 2A (30). The RET protein is a receptor tyrosine kinase that normally transduces growth and differentiation signals in developing tissues including those derived from the neural crest. This makes the *RET* gene, which encodes a tyrosine kinase receptor, a candidate gene involved in nonfamilial hyperparathyroidism. There are both differences and much overlap in the specific *RET* gene mutations underlying MEN 2A and familial medullary thyroid cancer (FMTC); in contrast MEN 2B is caused by entirely distinct *RET* mutations (31). The reason for which parathyroid disease fails to develop in FMTC patients who can bear identical *RET* mutations as found in MEN 2A remains unclear. Unlike the several different inactivating mutations of *MEN1*, which are typical of a tumor suppressor mechanism, *RET* mutations in MEN 2A are limited in number, reflecting the need for specific gain-of-function changes to activate this oncogene (32). RET mutation at codon 634 seems to be highly associated with the expression of PHPT in MEN 2A. MEN2 type *RET* mutations have been implicated in the pathogenesis of some sporadic medullary thyroid carcinomas and pheocromocytomas (33). Up to date no RET mutations have been found in sporadic parathyroid adenomas (34, 35). *RET* is expressed in both MEN 2A parathyroid adenomas and in sporadic adenomas (31). This suggests that parathyroid disease is an integral part of the MEN 2A, but that *RET* do not play a role in the pathogenesis of sporadic parathyroid adenomas.

Hereditary hyperparathyroidism-jaw tumor syndrome

Mutations in a newly identified tumor suppressor gene, *HRPT2*, have been recently associated with HPT-JT syndrome and with sporadic parathyroid carcinoma (36-39). The HPT-JT syndrome is a rare autosomal disorder consisting of parathyroid tumors, ossifying fibromas of the mandible and maxilla, and occasionally renal hamartomas and cystic kidney disease. The *HRPT2* gene maps on chromosome 1q24-32 and it functions as a tumor suppressor gene. For its inactivation two "hits" must occur: first an inactivating mutation on one allele, then a second hit, either a mutation or deletion, in the remaining normal allele (identified in the tumor as LOH of polymorphic markers at *HRPT2* locus). *HRPT2* encodes a protein of 531 amino acid called parafibromin and is present in other tissues in addition to the parathyroids, such as kidney, heart, adrenal and skeletal muscle (36). Subcellular fractionation and laser confocal microscopy of normal human parathyroid gland demonstrated expression of parafibromin in both cytoplasmic and nuclear compartments. Parafibromin is a member of the human Paf1 complex which has been proposed to regulate transcription and chromatin modification (40).
Currently there is convincing evidence that *HRPT2* mutations are associated with parathyroid carcinomas. *HRPT2* is the target for germline mutation in the majority of families with the rare HPT-JT, in which parathyroid carcinoma occurs at higher frequency than in sporadic PHPT (15% vs <1%). Indeed, parathyroid carcinoma occurs frequently in HPT-JT cases than in sporadic PHPT. Moreover, recent studies identified *HRPT2* somatic or germline mutations in sporadic parathyroid cancer (37-39) (see below).

Familial isolated hyperparathyroidism

FIHP is a clinically defined entity, based on the absence of expression of the extra-parathyroid manifestations that characterize other familial HPT syndromes. FIHP is genetically heterogeneous, and can be caused by variant expressions of germline mutations in *MEN1*, *HRPT2*, *CASR*, and probably other genes (41-55).
One of the puzzling aspects of FIHP is the absence of some of the manifestations of the syndromic forms despite the fact that the same gene is affected. Several possible mechanisms might account for this finding: i) incomplete penetrance of some of the manifestations, as the gnathic and renal features in HPT-JT (41, 43); ii) difference in the spectrum of mutations: it has been suggested that missense/in-frame deletion mutations may lead to incomplete MEN 1 phenotype, whereas truncating or nonsense mutations are more frequently observed in the full-blown syndrome (15). However, recent results from our (39, 55) and other groups (37, 41-55) have ruled out this possibility; iii) different mutations of the same gene may result in various degree of structure change and, accordingly, the capability of the mutated protein of interacting with other proteins may be variously affected. Indeed, either naturally occurring or engineered *MEN1* gene mutations have been shown to affect differently binding of menin with JunD (25); iv) influences from environmental factors and the presence of modifier genes that may contribute to phenotypic variations, as reported in familial adenomatous polyposis (56).

Familial hypocalciuric hypercalcemia and neonatal severe hyperparathyroidism

FHH is an autosomal dominant syndrome characterized by lifelong moderate hypercalcemia, inappropriate serum PTH levels,

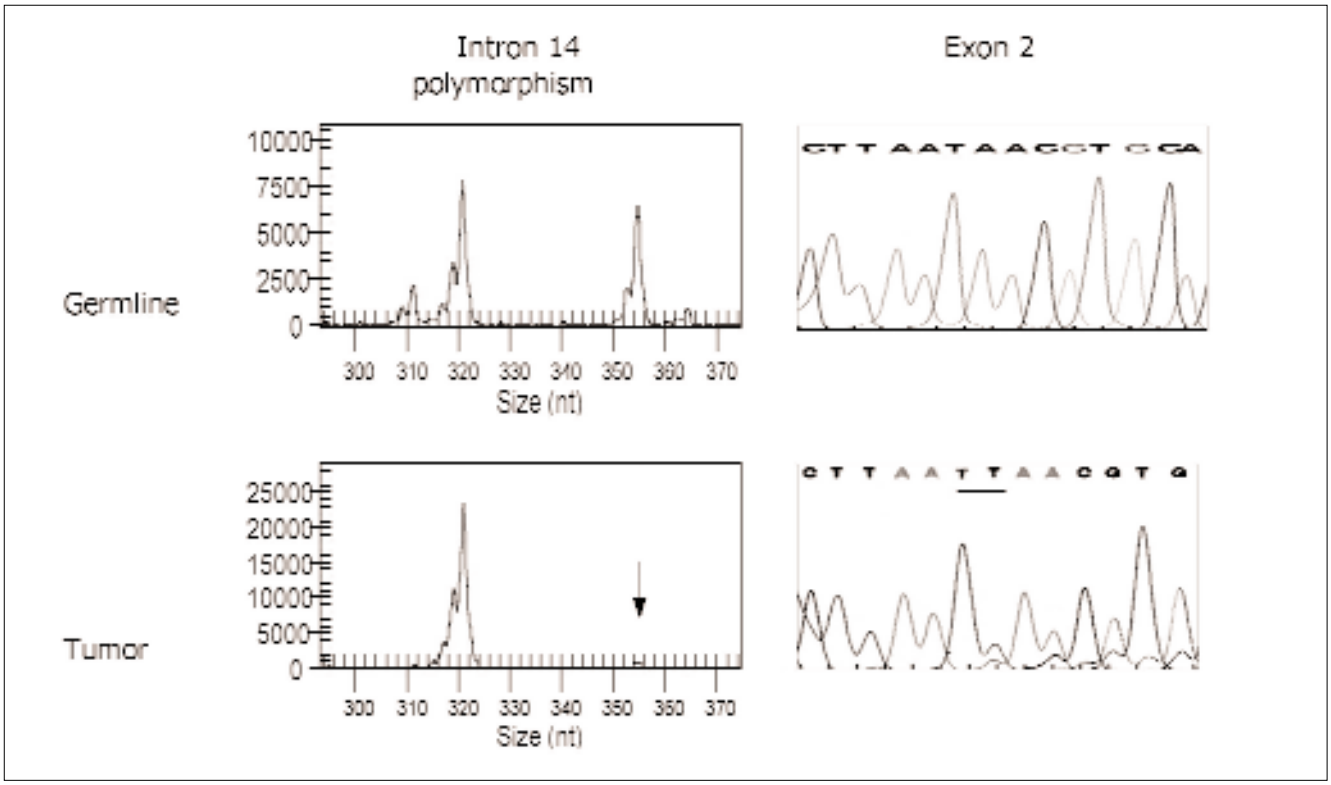

Figure 3 - Loss of heterozygosity (LOH) and mutation of the *HRPT2* gene in a patient with sporadic parathyroid carcinoma. Tumor DNA shows a biallelic inactivation of the gene due to LOH (left panel, arrow), associated with a somatic mutation (195insT) in exon 2 (right panel) (Modified from reference 39).

and relative hypocalciuria. Parathyroid gland are normal in most patients with FHH (57, 58). In some families, however, PTH is moderately elevated. The biochemical abnormality of FHH has been attributed to the increased renal retention of calcium that is not quite enough to overcome the decreased parathyroid sensitivity to calcium, so that PTH concentration is inappropriately normal. In rare cases PTH is moderately elevated, suggesting that decreased parathyroid sensitivity to calcium is accompanied by a moderate generalized parathyroid hyperplasia (59). This observation suggests that the CASR may be involved in the control of parathyroid proliferation (see below).

FHH mostly maps to *CASR* gene locus on chromosome 3q21-24, but genetic heterogeneity has been substantiated with two kindreds that show linkage to either 19p13.3 (FHH $_{19p}$) or 19q13 (FHH $_{19p}$) also known as Oklahoma variant (60-62). Heterozygous and homozygous loss-of-function mutation of CASR gene are responsible for FHH and NSHP, respectively. CASR is ubiquitous but it is most heavily expressed in the parathyroid cells and the thick ascending loop of the kidney (59). It has three main structural domains: a large extracellular amino-terminal domain, a seven transmembrane spanning domain, which characterizes the superfamily of G-protein coupled receptors, and an intracytoplasmic carboxy terminal tail. *CASR* interacts with extracellular calcium ions, transducing it into an intracellular signal, its protein category products coupling to a cytoplasmic guanyl nucleotide-binding protein. No correlation between the *CASR* mutation spectrum and the clinical phenotype has been apparent in FHH families. CASR inactivating mutations may interfere with normal function of the wild-type receptor through the following mechanisms: a) reduced affinity of the receptor for its agonists including calcium; b) inability of the receptor to reach the cell surface; c) failure of the receptor to couple with its appropriate signal transduction pathways; and d) interference of the mutated receptor with the function of the wild type receptor, known as a "dominant negative" effect or production of a truncated, inactive receptor.

One large family has been described with a phenotype different from FHH and FIHP. These patients had hypercalcemia, hypercalciuria, and serum PTH levels in the upper part of the normal range. Some family members had parathyroid adenoma or hyperplasia and, unlike FHH, had postoperative reversal of hypercalcemia. Genetic studies identified an atypical inactivating mutation in the intracellular part of the *CASR* (63).

NSHPT is often diagnosed within a week of birth and may carry a very high mortality rate unless recognized and treated promptly. The severity of the hypercalcemia, bone disease and high PTH levels is mostly explained by the absence of *CASR* in the homozygous and compound heterozygous forms of NSHPT. The dramatic increase of parathyroid gland volume suggests a negative trophic effect of the normal CASR on cell growth and proliferation.

Gene knockout model (64) provided the definitive proof for the central role for the normal *CASR* in calcium metabolism and of inactivating mutations in causing the FHH and NSHPT phenotypes. Heterozygous mice had about a 50% reduction in the expression of the *CASR* protein both in the parathyroid gland and the kidney compared with the wild type mice. They were similar to wild type mice with normal skeletal films and parathyroid gland histology. They had mild hypercalcemia with normal PTH levels, hypocalciuria and mildly elevated serum magnesium compared with the wild-type mice. In the mutant mice there was a mild elevation of the set point for calcium-regulated PTH release, similar to what has been reported in FHH families. Ho-

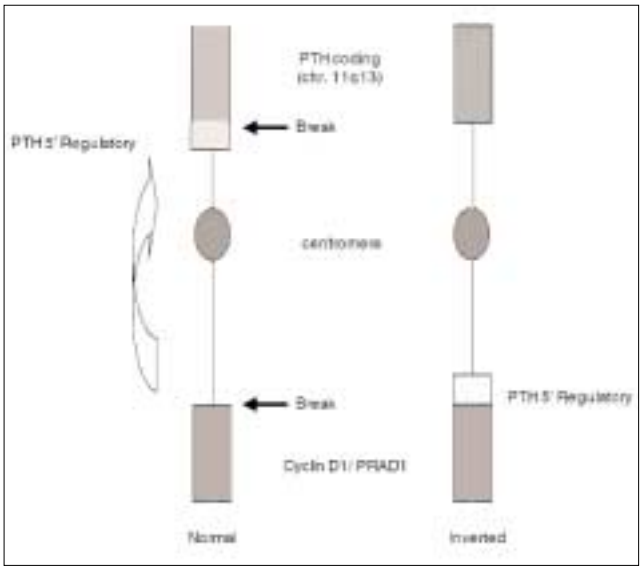

Figure 4 - Schematic diagram illustrating the pericentromeric chromosomal inversion that occurred in parathyroid adenomas. The PTH gene's 5'-regulatory region is rearranged upstream of cyclin D1/PRAD1, and the cyclin D1 gene is overexpressed by the PTH promoter.

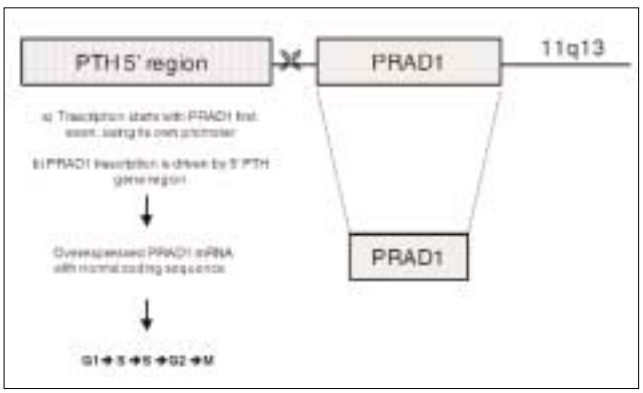

Figure 5 - Schematic diagram of the molecular structure of the PTH/PRAD1 DNA rearrangement and its functional consequences. The sign X represents the chromosomal breakpoint between the PTH gene regulatory region, plus PTH 5' region.

mozygous mice had a complete absence of CASR protein in the parathyroid and kidney. The phenotypic and biochemical profiles of these mice was comparable to that of NSHPT.

Genetic abnormalities in sporadic parathyroid adenomas

To date cyclin D1/parathyroid adenomatosis gene 1 (PRAD1) together with the *MEN1* is the only gene with an established role in the development of sporadic (nonfamilial) parathyroid adenomas.

The cyclin D1/parathyroid adenomatosis gene 1 oncogene

The *cyclin D1/PRAD1* gene was identified as a parathyroid oncogene on chromosome 11q13, clonally activated in a subset of parathyroid adenomas by tumor-specific DNA rearrangement with the parathyroid hormone (*PTH*) gene locus (65-67). The rearrangement separated the 5' regulatory region and the noncoding exon 1 of the PTH gene from its coding exons, with different, non-PTH DNA, placed adjacent to each *PTH* gene section with a pericentromeric inversion of chromosome 11, bringing the PRAD1 (normally on 11q) under the control of the *PTH* gene 5' flanking region (on 11p) (Fig. 4). (The tumor cells has one intact PTH gene that accounted for expression of PTH by the tumor). This rearrangement causes transcriptional activation and overexpression of the PRAD1 gene (Fig. 5) (68, 69). Consequent to its discovery as a parathyroid oncogene, *cyclin D1* has become established as a major and broad contributor to other neoplasia such as breast cancer, multiple mieloma, B-cell lymphomas and others (70, 71).

The *cyclin D1* gene encodes a 295 amino acid protein homologous to members of cyclin class of proteins (70). Cyclins play an important role in the regulation of cell cycle progression, and human cyclins have been grouped in different types according to sequence similarity (70). The C, D and E type cyclins appear to be G1 cyclins which regulate the progression throught G1 phase and the G1-S transition, determining whether initiation of a new cell cycle occurs. During G1, cyclin D1 complexes with and activates its kinase partner, cyclin-dependent kinase (CDK) CDK4 or CDK6, depending on tissue type. The activated kinase is involved in the phosphorylation and inactivation of retinoblastoma protein (pRb), determining progression toward S-phase. It is thought, therefore, that overexpression or deregulated expression of cyclin D1 could quite conceivably accelerate the cell's progress through G1 into S phase, bypassing normal regulatory controls in committing to divide, and also be well tolerated by the cell during the remainder of the cycle. Such a mechanism would provide an appealing explanation for the benign nature of parathyroid adenomas, because it could yield excessive cellular proliferation without necessarily conferring the phenotype of invasiveness or metastasis to the tumor cell.

Functional studies have shown that the effects of cyclin D1 on proliferation are mediated throught its ability to phosphorylate and thereby inactivate pRb. It appears that the cyclin D1-CDK4/6-p16-Rb pathway has become aberrant in virtually every human tumor. In recent years, our understanding of the mechanisms by which cyclins regulate proliferation and differentiation has evolved and accumulating evidence suggests that, in addition to its original description as a CDK-dependent regulator of the cell cycle, cyclin D1 also conveys cell cycle or CDK-independent functions. Cyclin D1 regulates activity of transcription factors, coactivators and corepressors that govern histone acetylation and chromatin remodeling proteins. The recent findings that cyclin D1 regulates cellular metabolism, fat cell differentiation and cellular migration have refocused attention on novel functions of cyclin D1 and their possible role in tumorigenesis (70).

In different parathyroid adenomas, the 11q13 chromosome breakpoint can be positioned within 1-2 kb of *cyclin D1*, or as much 300 kb upstream and further. Because these gene break points could vary widely they can be missed with traditional approaches (i.e. Southern blotting) and we may not have therefore a precise percentage of the rearrangements in parathyroid neoplasia. The best estimate to date of the frequency of the involvement of *cyclin D1* expression come from assessment of expression at the protein level. Immunohistochemical studies have shown a *cyclin D1* overexpression in 20-40% of parathyroid adenomas (68, 69).

To define the role of *cyclin D1* in parathyroid neoplasia, and to investigate the relationship between proliferative and hormonal regulatory abnormalities in this disease, a transgenic mouse model with parathyroid-targeted overexpression of *cyclin D1*

has been developed (72). These mice carry a transgene in which the *cyclin D1* gene is placed adiacent to a 5.2 kb fragment of the PTH regulatory region, thereby mimicking the DNA rearrangement and *cyclin D1* overexpression observed in human tumors. The phenotype of these mice was very similar to that of patients with PHPT. The parathyroid glands were hypercellular with increased proliferative rates (72) and, in some cases, they develop parathyroid adenomas. *PTH-cyclin D1* mice developed not only abnormal parathyroid cell proliferation but also biochemical hyperparathyroidism, with the characteristic bone abnormalities. The transgenic mice show decreased bone volume and increased bone turnover, with increased numbers of osteoclasts and reduced bone formation. This high turnover phenotype has marked similarities with the human bone under the influence of PTH excess.

Notably, these mice had an increase in the PTH-calcium set point, similar to that observed in the human disease. Thus, *cyclin D1* may not only control cellular proliferation but also contribute to abnormal hormonal secretion. In a recent article (73), the same authors, using this animal model, analyzed the temporal sequences of proliferative and set point abnormalities that occur in parathyroid tumorigenesis. They demonstrate that abnormal parathyroid proliferation regularly precedes dysregulation of the calcium-PTH axis, supporting the concept that disturbed parathyroid proliferation is the crucial primary initiator leading to the development of the biochemical phenotype of PHPT. In addition, they observed that decreased expression of the CASR in the parathyroid glands occurs several months before development of biochemical PHPT, suggesting that decreased *CASR* may not be sufficient to cause PTH dysregulation in this animal model of PHPT. In contrast, mice that exhibit a similarly decreased level of *CASR* but, as a result of a germline heterozygous knockout of the *CASR* gene, demonstrate a clear rightward shift in the set point curve with hypercalcemia and inappropriate PTH level. It is possible, that the stage of development at which alteration is imposed (embryonic/germline vs. postnatal/acquired) may lead to different phenotype. These data suggest that the typical reduction in *CASR* expression in parathyroid adenoma may not be the only determinant of the altered set point of the tumor cells. It has been also hypothesized that somatic mutations that inactivate the *CASR* gene play an important role in parathyroid tumorigenesis. However, *CASR* mutations are not observed in sporadic parathyroid adenomas (74, 75). It has been suggested that other factors such as functional activity of the CASR or oscillations of intracellular Ca^{2+} may play a role in the *CASR*-mediated signaling. Furthermore, the primary *cyclin D1* abnormalities, may have a more direct role in regulating the expression of *CASR*.

Genetic abnormalities in sporadic parathyroid carcinoma

Parathyroid carcinoma is usually associated with more severe clinical manifestations of PHPT than parathyroid adenomas. The incidence of parathyroid cancer does not favor women but is matched between the sexes, and the age of onset is approximately earlier than in benign disease (mid-40yr instead of the mid-50yr). The principal histological features of parathyroid carcinoma include mitoses, tick fibrous bands, and capsular and blood vessel invasion (76). However, the distinction between benign and malignant parathyroid tumors cannot be definitively established in the absence of local invasion or metastases (77). Nonetheless, these features are not always clearly present and certainly identify a late stage of the disease with a poor prognosis and low cure rate.

In 1994 Cryns et al. (78) reported that inactivation of the Rb1 gene might be involved in the pathogenesis of parathyroid carcinoma and that this finding might be a useful tool for the diagnosis of parathyroid malignancy; since then, contradictory results have been obtained by other investigators (79). Our group, recently, further investigated the role of the Rb1 gene in the differential diagnosis between benign and malignant parathyroid tumors by evaluating LOH at this locus and pRb immunohistochemistry (79). We show that Rb1 gene alterations are not specific for parathyroid cancer. Retention of Rb heterozygosity excludes such diagnosis, which is suggested by the combined finding of LOH and lack of pRb expression. However, the same authors who previously reported that inactivation of Rb1 is a key factor in the pathogenesis of many or most parathyroid carcinomas, showed no microdeletion, insertions or point mutations in the coding regions and promoter of the Rb1 gene in a small series of parathyroid carcinomas (80).

Table III - Prevalence of HRPT2 mutations in sporadic parathyroid carcinomas.

Authors	N. samples	Mutations
Howell et al. ()	4	4/4
Shattuck et al. ()	15	10/15[a]
Cetani et al. ()	7	6/7[b]
Totals	26	20/26

[a] Three of ten patients had germline mutations.
[b] Two of six patients had germline mutations.

Recent studies (37-39) have found an involvement of *HRPT2* gene in the pathogenesis of sporadic parathyroid cancer. Shattuck et al. (38) found *HRPT2* mutations in 10 of 15 patients with apparently sporadic parathyroid carcinoma; three of the patients had *HRPT2* germline mutations. Howell et al. (37) detected *HRPT2* somatic mutation in four of four parathyroid carcinoma. Our group (39) identified HRPT2 mutations in six of seven parathyroid carcinomas. Thus HRPT2 mutation is pathogenic for most sporadic parathyroid carcinomas. Combining all the results the prevalence of HRPT2 mutations in sporadic parathyroid carcinomas is 77% (Table III). It is conceivable that inactivating mutation of non coding or regulatory regions could be also implicated in the pathogenesis of sporadic parathyroid cancer. Thus, *HRPT2* mutation is central to the pathogenesis of most, and perhaps virtually all sporadic parathyroid carcinoma.

Preliminary reports of immunostaining for parafibromin (81), have shown that the loss of immunoreactivity to parafibromin has a high sensitivity and specificity for diagnosing parathyroid carcinoma.

The high rate of *HRPT2* mutation in carcinomas also suggests the potential diagnostic utility of *HRPT2* mutation status. However, this diagnostic potential hinges on the frequency of mutation in benign parathyroid adenomas. The prevalence of PHPT is one to five per 1000 in the general population and approximately 2% in postmenopausal women. It can be attributed to a benign, single adenoma in 80% of cases and occasionally <1% to parathyroid carcinoma. Thus, even a low detection rate of *HRPT2* mutation in parathyroid adenomas could adversely impact the diagnostic specificity and positive predictive value of *HRPT2* mutation status to differentiate between adenomas and carcinomas. Carpten et al. (36), detected mutations in two of 47 parathyroid adenomas that were selected for their cystic features, a specific characteristic of tumors of HPT-JT. A study by our group found a single mutation among 35 sporadic adenomas selected to exclude cases containing LOH at 11q13. In

contrast two studies of unselected parathyroid adenomas (37, 84) did not detected *HRPT2* mutations. Combining the data from the latter two groups (85 tumors) the prevalence of HRPT2 mutation was zero. If all the data of these studies (selected and unselected adenomas, n= 167) were combined a prevalence of 1.8% was found. However, as suggested by Krebs et al. (82), a better estimate may be 0.8% (1/120) derived from the study of Krebs, Howell (37) and our group (39). These observations indicates that *HRPT2* mutations are not an important participant in the pathogenesis of typical sporadic adenomas.

Up to date tests for *HRPT2* or parafibromin are not commercially available, but these tools could be a way in the future to distinguish between parathyroid carcinoma and adenoma at the time of initial surgery. Will *HRPT2* be a useful screening test? As suggested by Rubin et al. (83), the screening test could be performed in the following conditions: a) patients with severe benign PHPT, i.e. patients with clinical manifestations similar to parathyroid carcinoma; b) patients with atypical adenomas, i.e tumors containing features such as fibrous bands, mitotic figures, or microscopic invasion, but without distant metastases or gross invasion of surrounding structures and thus lacking the diagnostically definitive features of carcinoma; c) patients with parafibromatosis, in which multiple recurrent nodules of hyperfunctioning parathyroid tissue are found in the neck and mediastinum; d) patients in whom one has a clinical suspicious for parathyroid cancer before or after initial surgery. If the mutation is found, the likelihood of malignancy is certainly increased and a more aggressive surgery and family screening would be indicated. We agree with Rubin et al. (83), that *HRPT2* status would be a valuable piece of information to add to conventional clinical parameters of parathyroid carcinoma. This test will be very helpful if it directs us to an aggressive surgical approach when it is needed, because this is the only cure for this disease.

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