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PRAD1 (Cyclin D1): A Parathyroid Neoplasia Gene on 11q13

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Hyperparathyroidism is a central component of multiple endocrine neoplasia type 1 (MEN 1), and both sporadic and familial forms of parathyroid disease may share certain pathogenetic features. We recently identified a gene that is clonally rearranged with the PTH locus in a subset of sporadic parathyroid adenomas. This candidate oncogene, PRAD1 (previously D11S287), appears to contribute to parathyroid tumorigenesis in a fashion analogous to activation of C-MYC or BCL-2 by rearrangement with tissue-specific enhancers of the immunoglobulin genes in B-lymphoid neoplasia. The PRAD1 gene maps to 11q13 and has been linked to the BCL-1 breakpoint locus, although not to the most tightly linked MEN 1 markers, by pulsed field gel electrophoresis. PRAD1 may, in fact, be the long-sought BCL-1 lymphoma oncogene. PRAD1 encodes a novel type of cyclin protein and thus may normally function in controlling the cell cycle, perhaps through direct interaction with cdc2 or a related kinase. PRAD1's possible primary, or more likely secondary, involvement in the pathogenesis of MEN 1-related tumors is unknown and under investigation. (Henry Ford Hosp Med J 1992;40:177-80)

Hyperparathyroidism is a central component of familial multiple endocrine neoplasia type 1 (MEN 1). Our recent identification of a cellular oncogene that appears to participate in the pathogenesis of a subset of nonfamilial or sporadic parathyroid adenomas could be relevant in MEN 1, since familial and sporadic forms of other types of tumors can share pathogenetic features. We provide a brief review of the characterization of this gene, PRAD1 (or cyclin D1).

During studies that established the monoclonality of parathyroid adenomas, we encountered two tumors with parathyroid hormone (PTH) gene rearrangements (1). Molecular dissection showed that in one case the tumor-specific clonal rearrangement separated the PTH gene's 5′ flanking region from its coding exons in a reciprocal recombination event (2). Because the allelic rearrangement appeared to be present in every cell of the tumor, and thus was apparently present in the tumor's original clonal precursor, it was likely to be pathogenetically relevant. One possibility was that the PTH gene's regulatory elements, which actively up-regulate PTH transcription in parathyroid tissue, were influencing the expression of a proto-oncogene that was placed in this vicinity by the rearrangement. Such a situation would be analogous to the juxtaposition of immunoglobulin sequences with the C-MYC or BCL-2 oncogenes in Burkitt's lymphoma or follicular lymphoma, respectively (3-5); in both examples the tissue-specific enhancer is "tricked" by the DNA rearrangement into activating transcription of the misplaced proto-oncogene.

The DNA of unknown origin that had been rearranged with the PTH locus was cloned and found to be normally located on chromosome 11; it was initially called D11S287 (2). In situ hybridization more precisely localized D11S287 to band 11q13 (2). This localization was intriguing in that 11q13 was the established location of the known or suspected oncogenes INT-2, HST-1, SEA, and BCL-1, as well as the MEN 1 gene, a still unidentified putative tumor suppressor. The cloned D11S287 sequences, however, did not appear to include the INT-2, HST-1, SEA, or BCL-1 loci, based on restriction map comparisons and DNA hybridization data. Because the PTH gene is located on the *short* arm of chromosome 11 (11p15), the simplest cytogenetic explanation for the observed molecular rearrangement is a pericentromeric inversion; however, direct cytogenetic information could not be obtained on the frozen tumor specimen.

Subsequent study of a panel of tumors at our institution and at another revealed two additional and completely independent parathyroid adenomas with similar PTH/D11S287 DNA rearrangements (6,7). In both cases, the breakpoints within the PTH gene are similar to that in the original tumor. Regarding the 11q13 breakpoints, one was approximately 15 kb from that in the first tumor (6) (Fig 1), while the other was similar to the original breakpoint in this respect (7).

Next, we showed that a subclone of D11S287 contained a transcribed sequence, detectable on blots of parathyroid RNA; this expressed sequence was called D11S287E and, later, PRAD1, for parathyroid adenomatosis-1. The normal size PRAD1 mRNA was overexpressed about 15-fold in all parathyroid tumors bearing the clonal PTH/PRAD1 region DNA rearrangements (6). This impressive tumor-specific transcriptional activation associated with a clonal DNA rearrangement further

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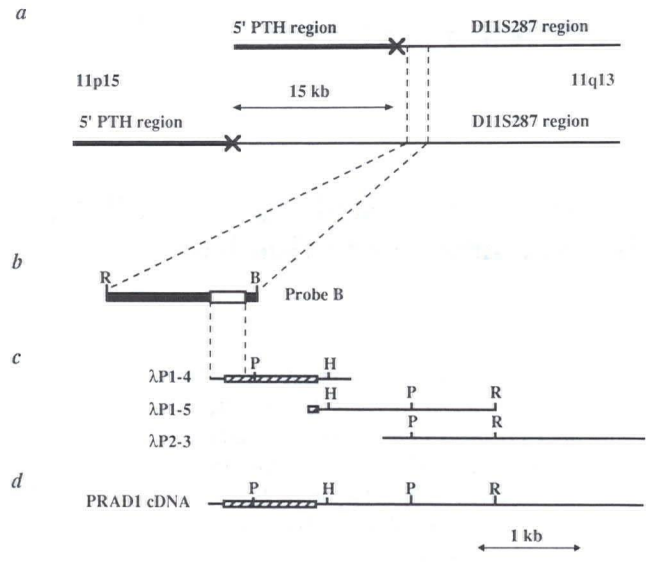

Fig 1—A) Schematic representation of PTH-D11S287 rearrangements in two parathyroid adenomas (2,6). The 5' PTH region (11p15, thick lines) was juxtaposed to the D11S287 region (11q13, thin lines). The breakpoints (X) in the D11S287 region are 15 kb apart. B) Genomic probe B (2) is shown as a darkened box, whose open area represents the first exon of PRAD1. C) Restriction maps of the inserts of three representative overlapping PRAD1 cDNA clones, λP1-4, λP1-5, and λP2-3. D) Deduced restriction map of the PRAD1 cDNA. The coding region is shown as a crosshatched box. Scale of 1 kilobase is shown as arrows. Symbols used for restriction sites are B, BamHI; R, EcoRI; H, HindIII; P, PstI. (Reprinted by permission from Nature, Vol 350, pp. 512-5. Copyright © 1991, Macmillan Magazines, Ltd.)

supported the hypothesis that PRAD1 was likely a proto-oncogene.

Additional studies have intensified interest in PRAD1 as a candidate oncogene. It has been known for a few years that 15% to 20% of human breast cancers and squamous cell cancers of the head and neck contain DNA amplification of a part of 11q13 that usually includes INT-2, HST-1, and the BCL-1 breakpoint locus, as reviewed by Lammie et al (8). However, none of these loci is invariably present on the amplicon, and none is regularly expressed in this subset of tumors. Therefore, it has been assumed that at least one additional oncogene, more central to the pathogenesis of these tumors, lies in this vicinity. PRAD1 is an excellent candidate for being such an oncogene, because it was recently shown to be consistently present on the 11q13 amplicon in this subset of breast and squamous cell cancers and, importantly, was also expressed in the tumors (8).

PRAD1 also appears to be a key B-cell lymphoma oncogene. Some B-cell tumors contain a translocation involving the immunoglobulin heavy chain locus (14q32) with a region on 11q13. The DNA on 11q13 adjacent to the breakpoint was cloned and named BCL1 with the expectation that a new oncogene would be found in this DNA and would be deregulated by the rearrangement (9). For years, extensive searching of the DNA adjacent to the original BCL1 breakpoint locus failed to reveal a transcribed gene (10,11). Recent data suggest that PRAD1 may be the elusive "BCL1 oncogene" because the BCL1 breakpoint locus is closely linked to PRAD1 (8,12-14), because PRAD1 appears to be consistently overexpressed in centrocytic lymphomas (a subtype characterized by frequent BCL-1 rearrangements) (13), and because no other CpG island lies within the 120 kb between the BCL-1 breakpoint and PRAD1 (13,14). Thus, with PRAD1 being implicated as a putative oncogene in several tumor types, it was of great interest to identify its normal structure.

We screened a human placental cDNA library with D11S287/PRAD1 genomic probe "B" (Fig 1), already known to contain transcribed sequences (6). We obtained overlapping cDNA clones that included the full 4.5 kb expected length (Fig 1C and 1D). PRAD1 cDNA (EMBL Accession #X59798) encodes a 295 amino acid protein without close homology to any previously reported protein, but with a consistent, weaker relationship (Fig 2) to the cyclin A and B families (29% to 34% identity) and yeast CLNs or G1 cyclins (19% to 20% identity) (15). Certain structural features raise the question of whether or not PRAD1 might be a human "G1 cyclin" or yeast CLN equivalent, controlling the important transition from G1 into S phase of the cell cycle. Interestingly, investigators searching for human G1 cyclins by rescue of yeast CLN mutants have recently reported isolating the PRAD1 cDNA, now often termed cyclin D1 (16, 17). Since cyclins A and B, known to function in the S or G2/M phases, can also rescue these mutants, these data do not in themselves convincingly prove that PRAD1/cyclin D1 normally functions as a G1 cyclin. In further support of this role, however, the mouse homologue of PRAD1 was identified in a search for genes that were induced in G1 by colony-stimulating factor 1 (CSF-1) in macrophages (18). Whatever its function is ultimately determined to be, PRAD1's sequence similarities with all known cyclins and the inability to readily assign it to an existing family indicate that PRAD1 represents a new cyclin family.

PRAD1 mRNA is expressed in multiple normal tissues, including parathyroid, thyroid, placenta, lymph node, skeletal muscle, heart, liver, and breast (15). Northern blots indicate that its mRNA is also highly conserved across species from human to mouse (15). The amino acid sequence of the mouse homologue of PRAD1 is 94% identical to the human sequence (18). PRAD1 may also be part of a multigene family in the human genome as it is in the mouse; distinct cDNA clones are detectable by hybridization at reduced stringency, and we have observed additional bands that hybridize with the PRAD1 cDNA probe on genomic Southern blots washed at decreased stringency.

PRAD1 protein was purified after expression in bacteria and added to a clam embryo interphase lysate which lacks endogenous cyclins A and B and contains inactive cdc2 kinase (19). Subsequently, the PRAD1 protein could be isolated using $p13^{suc1}$ (a reagent that avidly binds cdc2-like molecules), implying but certainly not proving that PRAD1 had been complexed with cdc2 kinase or a related kinase which was directly bound by $p13^{suc1}$ (15). Addition of PRAD1 protein to the same lysate

Fig 2—Sequence homology in the "cyclin box" region between the predicted PRAD1 protein and A-type cyclins (human and clam cyclin A, 32.2% and 33.6% identity, 56.6% and 59.2% similarity), B-type cyclins (human cyclin B and Saccharomyces pombe cdc*13, 29.6% and 31.6% identity, 50% and 52% similarity), and one S. cerevisiae G1 cyclin (*cln*3, 19.1% identity, 44.1% similarity). Clam cyclin A and* S. pombe cdc*13 homologies with PRAD1 are representative of those found in their families; cln3 is better aligned with PRAD1 than is* cln*1 or 2. | = identical amino acids; * = conservative substitutions. Alignment was made with the assistance of the BESTFIT program and conservative amino acids are grouped as follows: D, E, N, Q; H, K, R; A, G, P, S, T; I, L, M, V; F, W, Y. Amino acid numbers are on the right. (Reprinted by permission from Nature, Vol 350, pp. 512-5. Copyright © 1991, Macmillan Magazines, Ltd.)*

induced phosphorylation of histone H1, a favored substrate for cdc2 kinase (15). These preliminary observations support the view that PRAD1 is indeed a new cyclin, both structurally and functionally, but multiple possibilities regarding its true physiologic role remain.

To summarize, therefore, PRAD1 is a gene that has been strongly implicated in the pathogenesis of subsets of parathyroid, B-lymphoid, breast, and epidermoid tumors, apparently as a direct-acting oncogene activated by clonal rearrangement or amplification. PRAD1 encodes a novel cyclin, whose normal function, presumably in cell cycle control, remains unclear at this early stage of investigation, but may well be important in the crucial G1-S phase transition that commits a cell to divide. Despite PRAD1's location in the genome at 11q13 and its role in sporadic parathyroid neoplasia, it seems unlikely that PRAD1 is the major predisposing gene for MEN 1. First, the 11q13 markers most closely linked to MEN 1 are centromeric to the BCL-1/PRAD1 region, probably by a few megabases (20). Second, PRAD1's involvement in neoplasia appears to occur in the context of overexpression of the normal gene product due to chromosome rearrangement or amplification, and thus as a direct-acting oncogene, while the still uncloned MEN 1 gene is likely to be a tumor suppressor, participating in neoplasia through its loss of function.

Nonetheless, PRAD1 could still play an important role in the parathyroid tumors (and possibly other tumors) in the MEN 1 or even MEN 2 syndromes. Such a potential role could be envisioned to involve a somatic DNA change (genetic "hit") that contributes to tumor development in a secondary fashion (i.e., beyond the required lesion in the MEN 1 gene itself). In this regard, the issue of whether PRAD1's location at 11q13 is simply a coincidence or whether some 11q13 deletional rearrangements might activate PRAD1 in the process of deleting the MEN 1 gene needs to be explored.

The precise mechanisms by which deregulated expression of PRAD1 is related to tumor development are unknown. It is even

possible that some of its sites of action in neoplasia could be qualitatively different from those involved in its normal function and therefore much study will be required. Such study is likely to strengthen our knowledge of the links between the cell cycle control apparatus and oncogenic processes in parathyroid and other tissues and could well have implications for illuminating fully the pathogenetic mechanisms in familial endocrine neoplasia syndromes.

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