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Parathyroid

Polyclonal origin of parathyroid tumors is common and is associated with multiple gland disease in primary hyperparathyroidism

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Background. Parathyroid tumors are mostly considered monoclonal neoplasms, the rationale for focused parathyroidectomy in primary hyperparathyroidism. We reported that flow sorting parathyroid tumor cells and methylation-sensitive polymerase chain reaction (me-PCR) of polymorphic human androgen receptor gene and phosphoglycerate kinase gene alleles in deoxyribonucleic acid reveals that ≤35% of parathyroid tumors are polyclonal. We sought to confirm these findings and assess for clinical relevance.
Methods. Parathyroid tumors from 286 female primary hyperparathyroidism patients were analyzed for clonal status. Tumor clonal status was compared with clinical variables and operative findings. Statistical analysis was performed and significance was established at $P < .05$.
Results. In the study, 176 (62%) patients were informative for human androgen receptor gene and/or phosphoglycerate kinase gene. Assignment of clonal status was made in 119 (68%) tumors, of which 64 (54%) were monoclonal and 55 (46%) were polyclonal. Comparison of tumor clonal status to clinical variables in patients with complete operative data ($N = 82$) showed that while clinical features were the same between tumor types, patients with polyclonal tumors more often had multiple gland disease (risk ratio 4.066, confidence interval, 1.016–16.26; $P = .039$) potentially missed at unilateral neck exploration.
Conclusion. This work confirms that primary hyperparathyroidism is often the result of polyclonal tumors and that parathyroid tumor clonal status may be associated with multiple gland disease.

Parathyroid adenoma originating from a single parathyroid gland is the most common cause of nonfamilial primary hyperparathyroidism (PHPT).[1] Less commonly, PHPT patients have primary chief cell hyperplasia or multiple adenomas as the cause of their disease. These processes of parathyroid neoplasia cannot be predicted on clinical grounds and can be difficult to distinguish on pathologic examination. Their importance lies in their relationship with multiple gland disease and its impact on approach to parathyroidectomy (PTX) and results of surgery. Removal of single adenoma by either a focused (i.e., unilateral) or bilateral exploration and PTX is likely curative; however, cure of PHPT due to multiple gland hyperplasia can be less reliable after surgery.[2]

The somatic mutation theory of cancer holds that a finite set of somatic mutations in deoxyribonucleic acid (DNA) result in the transformation of cells and their progression to malignancy.[3] According to this framework, parathyroid adenomas in nonfamilial PHPT are predicted to be monoclonal expansions of a single transformed parathyroid cell, whereas hyperplasias may be the result of poly- or oligoclonal expansions of multiple cells due to exogenous stimuli. Tumor clonal status then may be viewed as a potential surrogate for both underlying etiology and type of parathyroid neoplasia. The topic of parathyroid tumor clonal status has been the subject of several studies with mixed and controversial results.[4-7] In particular, the finding of parathyroid tumor polyclonal status by several investigators has been questioned due to methodologic approach (e.g., use of microdissection to remove polyclonal stroma) and the assays used to assign tumor clonal status.

We previously conducted a study of parathyroid tumors from patients with nonfamilial PHPT due to single gland disease in which cells isolated from these tumors were dispersed and flow sorted to yield purified populations of oxyphil and chief cells. These isolated cells were analyzed both functionally and genetically, and our

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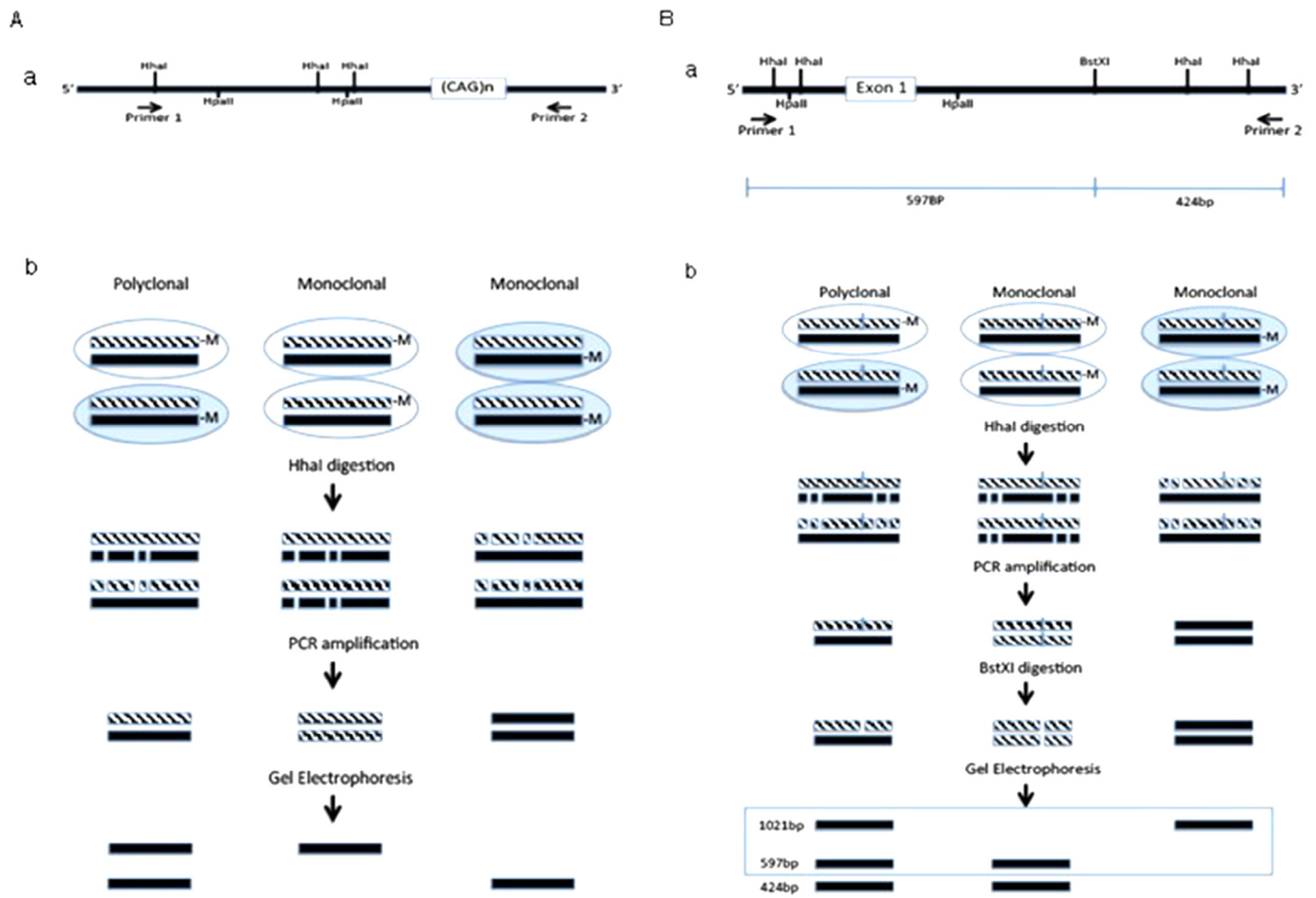

Fig 1. (*A*) The HUMARA me-PCR-based method of clonal analysis with map of the region amplified in the first exon of the HUMARA gene on X chromosome that includes the variably methylated HhaI restriction site. (*B*) The PGK PCR-based method of clonal analysis with map of PGK gene in the vicinity of the variably methylated HhaI or HpaII sites and the BstXI polymorphism.

results showed that a significant proportion (9/14, 36%) of apparent adenomas were in fact polyclonal.[8] We have examined an expanded cohort of 119 patients and have found that a significant proportion (≤46%) of patients have polyclonal parathyroid tumors as the cause of their disease and that these patients are otherwise indistinguishable based on clinical and biochemical criteria. In addition, among 82 patients well-characterized in terms of demographic, biochemical, operative, and pathologic data we found that polyclonal tumor status is associated with the presence of multiple gland disease that may be missed with unilateral exploration. Our findings indicate that the etiology of PHPT is heterogeneous and that underlying parathyroid tumor clonal status may be important to disease outcome after PTX.

Methods

Primary parathyroid tumors and clinical data were obtained from consenting PHPT patients under institutional review board-approved protocols at The University of Maryland Baltimore (UMB; $N = 135$, 2012–2016) and Duke University ($N = 151$, 2001–2012). De-identified tumor samples were transferred from Duke to UMB under a materials transfer agreement between the 2 institutions. Tumor samples were collected from resected tumors in the operating room and immediately placed in liquid nitrogen. Duplicate samples were fixed in buffered formalin, embedded, sectioned, stained with hematoxylin and eosin, and examined to ensure parathyroid tumor identity and cellularity. Parathyroid tumor samples were kept at –80°C until use. Peripheral blood lymphocytes (PBLs) were isolated from patient-matched whole blood using RBC Lysis Buffer (Biolegend, CA).

Genomic DNA extraction from peripheral blood lymphocytes and parathyroid adenoma tissue was performed using DNeasy Blood & Tissue Kit (Qiagen, Hilden, Germany). Clonal status at the human androgen receptor gene (HUMARA) locus was determined via restriction enzyme (HhaI) digestion and PCR amplification following the procedure described by Allen et al.[9] HUMARA primer sequences were: primer 1, 5'- TCCAGAATCTGTTCCAGAGCGTGC -3'; and primer 2, 5'- GCTGTGAAGGTTGCTGTTCCTCAT-3'. The PGK clonality assay was modified based on the method described by Gilliland et al.[10] The PGK primer sequences were: primer 1, 5'-TGTGGGGCGGTAGTGTGGGCCCTGTTCCTG-3'; and primer 2, 5'-AACCGTGTTGGCAAGTGACTAGAGATCCAC-3'. A schematic diagram depicting the me-PCR assays for HUMARA and PGK is shown in Fig 1.

De-identified clinical and research data were maintained in separate password-protected databases linked by a study code. Clonal status from de-identified tumor samples and clinicopatholofic data were analyzed for significant association using SPSS software (International Business Machines, Armonk, NY).

Results

PBL samples from 286 PHPT patients were analyzed for clonal status by me-PCR at both the HUMARA and PGK loci. Of these