

# Functional and genetic studies of isolated cells from parathyroid tumors reveal the complex pathogenesis of parathyroid neoplasia

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Parathyroid adenomas (PAs) causing primary hyperparathyroidism (PHPT) are histologically heterogeneous yet have been historically viewed as largely monotypic entities arising from clonal expansion of a single transformed progenitor. Using flow cytometric analysis of resected adenomatous parathyroid glands, we have isolated and characterized chief cells, oxyphil cells, and tumor-infiltrating lymphocytes. The parathyroid chief and oxyphil cells produce parathyroid hormone (PTH), express the calcium-sensing receptor (CASR), and mobilize intracellular calcium in response to CASR activation. Parathyroid tumor infiltrating lymphocytes are T cells by immunophenotyping. Under normocalcemic conditions, oxyphil cells produce ~50% more PTH than do chief cells, yet display significantly greater PTH suppression and calcium flux response to elevated calcium. In contrast, CASR expression and localization are equivalent in the respective parathyroid cell populations. Analysis of tumor clonality using X-linked inactivation assays in a patient-matched series of intact tumors, preparatively isolated oxyphil and chief cells, and laser-captured microdissected PA specimens demonstrate polyclonality in 5 of 14 cases. These data demonstrate the presence of functionally distinct oxyphil and chief cells within parathyroid primary adenomas and provide evidence that primary PA can arise by both clonal and polyclonal mechanisms. The clonal differences, biochemical activity, and relative abundance of these parathyroid adenoma subpopulations likely reflect distinct mechanisms of disease in PHPT.

tumor heterogeneity | endocrine neoplasia

The parathyroid glands maintain serum calcium concentration within a narrow physiological range through regulated synthesis and secretion of parathyroid hormone (PTH) (1). Parathyroid neoplasia results in inappropriate secretion of PTH by one or more glands, leading to hypercalcemia and the disease primary hyperparathyroidism (PHPT) (2). To date, research in the field has focused on histologic and molecular profiling of parathyroid tumors and the investigation of calcium sensing in dispersed cells from parathyroid tumors and normal bovine parathyroid glands. Few studies have characterized the individual cellular constituents of human parathyroid tumors, and no publications have reported live-cell functional evaluation of the different cellular subtypes observed in parathyroid tumors.

Parathyroid adenomas, the most common cause of PHPT, are considered clonal proliferations of a transformed parathyroid cell that has acquired proliferative or survival advantage due to one of several genetic abnormalities including the PRAD1 translocation or mutations in the genes encoding menin, PS3, and P27 (3-7). Regardless of type, most authors consider parathyroid tumors clonal (8, 9) although intratumoral heterogeneity has been observed and polyclonality of microdissected parathyroid adenomas has been reported (10, 11).

Although most data support mutation-driven clonal expansion of parathyroid tumors, an alternative model for the origin of parathyroid tumors is that abnormal calcium sensing by parathyroid cells leads to abnormal secretion of PTH initially, followed by proliferation of parathyroid cells in response to chronic demand for increased PTH (12, 13). An abnormal calcium-PTH set point has been well-described in aggregate dispersed cells from parathyroid adenomas, and most reports attribute the impaired set point in these cells to decreased expression of the calciumsensing receptor (CASR) (14, 15) or more recently to altered expression of downstream molecules linked to CASR signaling, including RhoGEF and RGSS (16, 17). In contrast to clonal expansion following genomic tumor-initiating events, attenuated calcium responsiveness could be expected to drive polyclonal proliferation in the parathyroid gland.

To investigate the composition of parathyroid tumors, we sought to characterize a series of parathyroid adenomas at the cellular and functional level. In this study, we report the isolation and characterization of chief cells, oxyphil cells, and lymphocytes present in parathyroid adenomas and histologically normal parathyroid glands from patients with PHPT. Our results show that parathyroid adenomas removed from patients with PHPT are composed of functionally and genetically distinct oxyphil and chief cells and have varying amounts of infiltrating lymphocytes. The chief and oxyphil cells within parathyroid adenomas have differing ability to respond to changes in ambient calcium and produce PTH although CASR expression is comparable between these parathyroid cell subtypes. Further, we show that a significant proportion of PHPT patients have polyclonal tumors. The relative abundance, functional behavior, and clonal origin of

#### Significance

Parathyroid adenomas, the main cause of primary hyperparathyroidism (PHPT), are thought to result from clonal expansion of tumor cells and to be insensitive to normal calcium feedback due to the loss of the calcium-sensing receptor (CASR). Utilizing flow cytometric analysis to isolate and individually study oxyphil cells, chief cells, and lymphocytes from resected parathyroid tumors and glands, we now report previously unrecognized heterogeneity in these tissues with respect to calcium responsiveness, CASR expression, and donal origin of parathyroid tumors. Such heterogeneity of parathyroid adenomas likely reflects the complex etiopathogenesis and clinical heterogeneity of PHPT.

Author contributions: Y.S., J.K., and J.A.O. designed research; Y.S. and J.H. performed research; Y.S., L.H., D.D., J.K., and J.A.O. contributed new reagents/sens/ytic tools; Y.S., J.K., and J.A.O. wrote the paper.

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# **Genetic Studies Of Endocrine Abdominal Tumors**

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#### **Genetic Studies Of Endocrine Abdominal Tumors:**

Genetic Studies of Endocrine Abdominal Tumors Ola Hessman. 2001 Pathology and Genetics of Tumours of **Endocrine Organs** Ronald A. DeLellis, 2004 This vol was produced in collaboration with the International Academy of Pathology IAP This publication reflects the views of a working group that convened for an editorial and consensus conference **Molecular Genetics and Emerging Therapies for Epithelial Ovarian Cancer:** in Lyon France April 23 26 2003 Basic Research and Clinical Perspectives Stergios Boussios, Eleftherios Pierre Samartzis, Nicholas Pavlidis, J. Alejandro Perez-Fidalgo, 2023-11-06 Epithelial ovarian cancer EOC is the most lethal gynecological disorder due to a lack of effective early detection strategies Worldwide approximately 230 000 women are diagnosed annually whereas 150 000 die It represents the seventh most commonly diagnosed cancer among women in the world with 5 year survival rate of 46% More than one fifth of EOC have been related to hereditary conditions Considerable efforts have been made to implement screening of the general population to diagnose EOC early nevertheless this has been ineffective and there is no approved strategy Nowadays new approaches for early diagnosis and prevention based on molecular genomics are in development Whole genome sequencing has established the potency of the somatic genome characterised with diverse DNA repair deficiencies that can be used to stratify EOCs into distinct biological groups with predictive signatures of resistance or relapse The incorporation of next generation sequencing NGS into clinical practice remains challenging for two reasons Firstly the EOC risk is not clear for some of the included genes and secondly the variant of uncertain significance rates increase as more genes are analyzed Finally beyond germline pathogenic variants somatic mutations may also affect therapeutic choices and as such upfront tumor sequencing may be equally important to NGS particularly as we continue to challenge treatment paradigms in the first line management of EOC Intestinal Mucosa and Its Diseases -Pathophysiology and Clinics W. Domschke, R. Stoll, T.A. Brasitus, M.F. Kagnoff, 1999-07-31 These proceedings of Falk Symposium 110 on Intestinal Mucosa and its Diseases Pathophysiology and Clinics held at Titisee Black Forest Germany 16 17 October 1998 comprehensively review salient clinical as well as scientific aspects pending or settled of the main intestinal diseases In particular chapters deal with infectious and neuroendocrine diarrhoeas coeliac sprue allergic enteropathy intestinal lymphomas colorectal carcinomas and chronic inflammatory bowel diseases in terms of clinical presentation as well as underlying mechanisms This book therefore aims to bridge the gaps between basic science and clinics for the eventual treatment of patients afflicted with these diseases 
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#### Table of Contents Genetic Studies Of Endocrine Abdominal Tumors

- 1. Understanding the eBook Genetic Studies Of Endocrine Abdominal Tumors
  - The Rise of Digital Reading Genetic Studies Of Endocrine Abdominal Tumors
  - Advantages of eBooks Over Traditional Books
- 2. Identifying Genetic Studies Of Endocrine Abdominal Tumors
  - Exploring Different Genres
  - o Considering Fiction vs. Non-Fiction
  - Determining Your Reading Goals
- 3. Choosing the Right eBook Platform
  - Popular eBook Platforms
  - Features to Look for in an Genetic Studies Of Endocrine Abdominal Tumors
  - User-Friendly Interface
- 4. Exploring eBook Recommendations from Genetic Studies Of Endocrine Abdominal Tumors
  - Personalized Recommendations
  - Genetic Studies Of Endocrine Abdominal Tumors User Reviews and Ratings
  - Genetic Studies Of Endocrine Abdominal Tumors and Bestseller Lists

- 5. Accessing Genetic Studies Of Endocrine Abdominal Tumors Free and Paid eBooks
  - Genetic Studies Of Endocrine Abdominal Tumors Public Domain eBooks
  - Genetic Studies Of Endocrine Abdominal Tumors eBook Subscription Services
  - Genetic Studies Of Endocrine Abdominal Tumors Budget-Friendly Options
- 6. Navigating Genetic Studies Of Endocrine Abdominal Tumors eBook Formats
  - ∘ ePub, PDF, MOBI, and More
  - Genetic Studies Of Endocrine Abdominal Tumors Compatibility with Devices
  - Genetic Studies Of Endocrine Abdominal Tumors Enhanced eBook Features
- 7. Enhancing Your Reading Experience
  - Adjustable Fonts and Text Sizes of Genetic Studies Of Endocrine Abdominal Tumors
  - Highlighting and Note-Taking Genetic Studies Of Endocrine Abdominal Tumors
  - Interactive Elements Genetic Studies Of Endocrine Abdominal Tumors
- 8. Staying Engaged with Genetic Studies Of Endocrine Abdominal Tumors
  - Joining Online Reading Communities
  - Participating in Virtual Book Clubs
  - Following Authors and Publishers Genetic Studies Of Endocrine Abdominal Tumors
- 9. Balancing eBooks and Physical Books Genetic Studies Of Endocrine Abdominal Tumors
  - Benefits of a Digital Library
  - $\circ\,$  Creating a Diverse Reading Collection Genetic Studies Of Endocrine Abdominal Tumors
- 10. Overcoming Reading Challenges
  - Dealing with Digital Eye Strain
  - Minimizing Distractions
  - Managing Screen Time
- 11. Cultivating a Reading Routine Genetic Studies Of Endocrine Abdominal Tumors
  - Setting Reading Goals Genetic Studies Of Endocrine Abdominal Tumors
  - Carving Out Dedicated Reading Time
- 12. Sourcing Reliable Information of Genetic Studies Of Endocrine Abdominal Tumors
  - Fact-Checking eBook Content of Genetic Studies Of Endocrine Abdominal Tumors
  - Distinguishing Credible Sources
- 13. Promoting Lifelong Learning

- Utilizing eBooks for Skill Development
- Exploring Educational eBooks
- 14. Embracing eBook Trends
  - Integration of Multimedia Elements
  - Interactive and Gamified eBooks

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