Molecular genetic insights into sporadic primary hyperparathyroidism

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Abstract

Primary hyperparathyroidism (PHPT) is a common endocrine disorder characterized by dysregulation of parathyroid hormone release. The large majority of PHPT cases are attributable to sporadic, single-gland parathyroid adenoma, in which MEN1 and CCND1/cyclin D1 are the most well-established drivers of tumorigenesis. Sporadic parathyroid carcinoma, which appears to mostly arise through molecular pathways distinct from those causing benign parathyroid tumors, is rare and is most frequently driven by mutational inactivation of the CDC73 (HRPT2) tumor suppressor gene. Targeted investigation of suspected tumor driver genes, as well as unbiased whole-genome or exome sequencing of small cohorts, have revealed additional novel candidate tumor genes in sporadic parathyroid neoplasia, generally at modest or low mutational frequencies consistent with marked molecular genetic heterogeneity from tumor to tumor. The ability of these additional candidates to participate in the pathogenic process of driving parathyroid tumorigenesis in vivo largely remains to be demonstrated experimentally. This review will summarize the molecular genetic abnormalities identified to date in sporadic PHPT and discuss the strength of evidence for their proposed roles in parathyroid tumor formation.

Introduction

Primary hyperparathyroidism (PHPT) occurs across all age groups and genders, with an estimated incidence of 21.1–65.5 per 100,000 person-years and prevalence of 0.6–36.0 per 1000 population (Christensson et al. 1976, Mundy et al. 1980, Wermers 1997, Wermers et al. 2006, Yu et al. 2009, Yeh et al. 2013). However, PHPT disproportionately affects women who account for 74% of cases overall, and its prevalence increases markedly with age (Wermers et al. 2006). Defined as a biochemical diagnosis, PHPT is nearly universally caused by tumors of the parathyroid glands, which are classified as solitary adenoma (85%), multi-gland disease (including primary hyperplasia and double adenoma; 15%), or carcinoma (<1%). PHPT caused by ectopic secretion of parathyroid hormone (PTH) from a non-parathyroid tumor is exceedingly rare. While PHPT is mostly sporadic, it is also encountered as part of several familial syndromes, including multiple endocrine neoplasia types 1, 2a, and 4 (MEN1, MEN2A, MEN4), hyperparathyroidism-jaw tumor syndrome (HPT-JT), familial hypocalciuric hypercalcemia.
(FHH), or familial isolated hyperparathyroidism (FIHP). Some of the genes responsible for heritable forms of PHPT also contribute to sporadic PHPT, either through somatic mutation or via predisposing germline mutation despite the nonfamilial/nonsyndromic clinical presentation. This review will focus on the clonal origins of and molecular genetic events contributing to sporadic parathyroid neoplasia (summarized in Tables 1 and 2), including discussion of its clonal origins.

### Clonality

Whether a tumor comprises a ‘clone’ derived from one tumor progenitor cell or reflects the generalized growth of a tissue has long been of interest to scientists and clinicians as a descriptor of tumor origin and an indicator of its patterns of growth, both of which carry potential therapeutic implications. In the context of neoplasia, monoclonality implies that tumor outgrowth occurred due to the selective advantage conferred upon a single tumor progenitor cell by its acquisition of rare and specific oncogenic changes, such as somatic mutations, with transmission of this genetic advantage to daughter cells. Occasionally, such a rare acquired selective advantage could even be attained independently by more than one progenitor cell, resulting in a biclonal (Sklar et al. 1984, Delville et al. 2007) or oligoclonal tumor (Thirlwell et al. 2010). Clonal growth, whether monoclonal or oligoclonal, implies a fundamentally different pathogenesis than does non-clonal growth, which has often been termed ‘polyclonal’ in the literature on tumor origins. Non-clonal growth could result, for example, from the broad hyperplastic expansion of all cells in a tissue in response to an external growth stimulus or to certain germline genetic predispositions, without the need for any rare cell to gain a selective advantage (Fialkow 1979).

Evidence for clonal growth can be direct, i.e. the observation of acquired tumor-specific DNA or chromosomal defects in a major proportion of a tumor’s cells, or indirect, as in X-chromosome inactivation assays. Typically, reports of somatic mutations or tumor-specific DNA copy number alterations have understandably focused on the particular genes involved and have not explicitly pointed out that such otherwise rare mutations/changes must have been identically present in a large proportion of a tumor’s cells in order to be detected. Therefore, excepting special circumstances (e.g. mosaicism, single-cell samples etc.), such reports do provide powerful and direct evidence for clonal growth. Most of the published literature on molecular genetic lesions in human neoplasms indeed contains overwhelming evidence for their clonal pathogenesis.

### Table 1

<table>
<thead>
<tr>
<th>Gene</th>
<th>Established or hypothesized role</th>
<th>Primary mechanism</th>
<th>Role in tumorigenesis</th>
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<tbody>
<tr>
<td>MEN1 (menin)</td>
<td>Tumor suppressor</td>
<td>Somatic</td>
<td>Established driver</td>
</tr>
<tr>
<td>CCND1 (cyclin D1)</td>
<td>Oncogene</td>
<td>Somatic</td>
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<tr>
<td>CDKN1B (p27)</td>
<td>Tumor suppressor</td>
<td>Germline, Somatic</td>
<td>Predisposition; Putative driver</td>
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<td>CDKN2C (p18)</td>
<td>Oncogene</td>
<td>Germline</td>
<td>Candidate driver</td>
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<td>ZFX</td>
<td>Oncogene</td>
<td>Somatic</td>
<td>Candidate driver</td>
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<td>EZH2</td>
<td>Oncogene</td>
<td>Germline, Somatic</td>
<td>Candidate driver</td>
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<tr>
<td>CTNNB1 (beta-catenin)</td>
<td>Tumor suppressor</td>
<td>Germline</td>
<td>Candidate predisposition</td>
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<tr>
<td>CDKN2B (p15)</td>
<td>Oncogene</td>
<td>Germline, Somatic</td>
<td>Candidate predisposition</td>
</tr>
<tr>
<td>CDKN1A (p21)</td>
<td>Tumor suppressor</td>
<td>Germline, Somatic</td>
<td>Potential predisposition</td>
</tr>
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<td>GCM2</td>
<td>Oncogene</td>
<td>Germline, Somatic</td>
<td>Predisposition; Driver(^a)</td>
</tr>
<tr>
<td>CDC73 (parafibromin)</td>
<td>Tumor suppressor</td>
<td>Germline</td>
<td>Predisposition(^a)</td>
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<td>CASR</td>
<td>Tumor suppressor</td>
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\(^a\)Very rare in unselected solitary typical adenomas.

### Table 2

<table>
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<td>Somatic</td>
<td>Established driver</td>
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<td>PIK3CA/MTOR</td>
<td>Oncogene</td>
<td>Somatic</td>
<td>Putative drivers</td>
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<td>ADCK1</td>
<td>Oncogene</td>
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<td>Candidate driver</td>
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<td>AKAP9</td>
<td>Somatic</td>
<td>Somatic</td>
<td>Candidate driver</td>
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<td>PRUNE2</td>
<td>Unknown</td>
<td>Somatic</td>
<td>Candidate driver</td>
</tr>
<tr>
<td>ZEB1</td>
<td>Tumor suppressor</td>
<td>Germline, Somatic</td>
<td>Candidate driver</td>
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\(^a\)Very rare in unselected solitary typical adenomas.
(see cancer mutation databases TCGA Research Network (cancergenome.nih.gov) and COSMIC (cancer.sanger.ac.uk); Forbes et al. 2017); sporadic parathyroid adenomas and carcinomas are no exception.

However, the clarity on assessment of clonal status achieved by direct identification of clonal defects has not always been matched in studies utilizing the indirect assays of tumor clonality based on X-chromosome inactivation. These assays depend on the assumption that certain X-linked genomic loci will be uniformly methylated or unmethylated depending on whether they are located on the cell’s inactive or active X-chromosome, and that all neoplastic cells in a monoclonal tumor (unlike normal tissue or non-clonal expansions) will share the identical nonrandom methylation pattern having been descended from a single progenitor (Vogelstein et al. 1985). Most of the common pitfalls of X-inactivation assays – admixture of non-neoplastic stroma, impaired function of methylation-sensitive nucleases, aberrant or inconsistent methylation patterns on the active or inactive X-chromosomes at the scored loci, or oligoclonal tumors – would cause potential errors in the direction of falsely indicating non-clonality. As such, a result indicating monoclonality in X-inactivation assays is much more definitive than a polyclonal/non-clonal pattern; the latter must be interpreted with particular caution and results should be supplemented and confirmed using independent direct-detection approaches (Corrado et al. 2015).

Despite the pitfalls of indirect assessment of clonality, the majority of sporadic solitary parathyroid adenomas and carcinomas have been determined to be monoclonal by X-chromosome inactivation (Arnold et al. 1988, Miedlich et al. 2000, Shi et al. 2014). These results are supported by independent and more direct evidence, which has identified tumor-specific monoclonal markers such as loss of heterozygosity and chromosomal defects (Arnold et al. 1988, 1989, 1995, Byström et al. 1990, Orndal et al. 1990, Noguchi et al. 1994, Tahara & Arnold 1997, Yi et al. 2008). More recently, and not surprisingly, techniques capable of even more robust, sensitive, and direct detection of acquired clonal changes in the tumor genome have increased the percentage of parathyroid adenomas that can be confidently scored as clonal neoplastic outgrowths; whole-exome sequencing cohorts revealed verified somatic mutations marking a dominant major clone in 23 of 24 samples (96%; see Cromer et al. 2012, Newey et al. 2012). Likewise, with apparent uniformity, parathyroid carcinomas bear direct genetic evidence for clonal pathogenesis (Cryns et al. 1994a, Shattuck et al. 2003a, Cetani et al. 2004, Pandya et al. 2017).

However, in contrast with the near-uniform finding of clonal growth in such cases using direct methods as noted above, a significant minority (25–35%) of adenomas can exhibit non-clonal patterns in X-inactivation assays (Arnold et al. 1988, Shi et al. 2014). Given the care taken to avoid most X-inactivation assay pitfalls in parathyroid tumor studies, the unavoidable possibility of aberrant X-chromosome methylation marking underlying monoclonality is a plausible scenario in this minority of cases. Indeed, tumor-associated aberrant methylation (Jones & Baylin 2002, Patra et al. 2008) has been shown on occasion to directly confound X-inactivation clonality assays (Sakurazawa et al. 2000). An intriguing hypothesis is whether non-clonal X-inactivation patterns, particularly if and when found in adenomas proven to be monoclonal by directly observed somatic mutations, might constitute a biomarker of widespread aberrant DNA methylation and thus reflect an alternative pathway of molecular pathogenesis and perhaps even different clinical characteristics.

The clonal origins of multigland parathyroid disease might be expected to be different from typical sporadic adenomas. Indeed, parathyroid tissues from patients with sporadic multi-gland primary hyperplasia showed a high proportion of non-clonal patterns by X-inactivation (Arnold et al. 1995) although, more interestingly, some were monoclonal. Shi and colleagues observed an increased proportion of cases with non-clonal X-inactivation patterns when selection criteria were broadened to include multigland disease compared with solitary adenomas (Shi et al. 2018). Finally, X-inactivation assays revealed that in the setting of end-stage renal disease, monoclonal outgrowths can evolve on a background of presumed generalized non-clonal parathyroid hyperplasia (secondary HPT; see Arnold et al. 1995). Oligoclonal parathyroid tumors are also possible; this has been observed in familial MEN1 (Lubensky et al. 1996). While a highly interesting concept, direct confirmation of oligoclonality in other forms of hyperparathyroidism remains to be obtained. In all instances of parathyroid tumor clonal growth, identification of the full array of acquired and selected driver mutations will be crucial to understanding pathogenesis. Current knowledge of such established and candidate drivers of clonal growth are discussed below.
Parathyroid adenoma

Benign, sporadic parathyroid adenoma is the most common cause of PHPT. As the use of different case selection criteria could influence findings on molecular genetic contributors, in the current discussion, we define typical sporadic adenoma as otherwise unselected cases that were clinically determined to be single gland disease, occurred in adults with no personal/family history of primary hyperparathyroidism, had no personal/family history suggestive of multiple endocrine neoplasia or any familial hyperparathyroid syndrome, and demonstrated no atypical or malignant features in either gross examination or histopathology.

Two genes, MEN1 and CCND1, a tumor suppressor and a proto-oncogene respectively, have been solidly established as primary tumorigenic drivers in parathyroid adenomas. They have not only been the target for recurrent, clonally selected inactivating (for MEN1) or activating (for CCND1) somatic alterations/mutations, but such gene alterations have also been experimentally shown to drive hyperparathyroid phenotypes in relevant \textit{in vivo} models such as genetically engineered mice. Other genes, some involved in pathways leading to CCND1 activation, MEN1 inactivation, or the parathyroid hormone (PTH) dysregulation characteristic of PHPT, have been and continue to be explored as candidate parathyroid tumor-driving genes (summarized in Table 1), and a few selected examples are also discussed below.

**MEN1**

The now well-established tumor suppressor gene \textit{MEN1} was discovered due to its role in the familial syndrome multiple endocrine neoplasia type 1 (MEN1), a heritable predisposition to tumors of the parathyroid glands and other tissues. Linkage analysis revealed the precise locus of \textit{MEN1} on 11q13 (Larsson \textit{et al.} 1988, Byström \textit{et al.} 1990), which was later confirmed by positional cloning (Chandrasekharappa \textit{et al.} 1997). Subsequent studies detailed the broad array of inactivating events that could negate the tumor suppressive activity of \textit{MEN1} (reviewed in Thakker 2014). The protein product of \textit{MEN1}, menin, has likewise been the subject of extensive investigation and is now understood to act in concert with a variety of transcription factors, transcriptional regulators, and signaling pathways to inhibit cellular proliferation (Wu & Hua 2011). That said, the precise functions of menin most relevant to its tumor-suppressive properties remain unclear.


In mouse models, systemic ablation of \textit{Men1} is embryonic lethal (Crabtree \textit{et al.} 2001); however, heterozygous knockouts recapitulate the phenotype observed in human MEN1 syndrome and drive tumor formation specifically in endocrine tissue, including the parathyroid glands (Crabtree \textit{et al.} 2001, Bertolino \textit{et al.} 2003, Harding \textit{et al.} 2009). This evidence of the ability...
of inactivated alleles to drive parathyroid tumorigenesis in vivo and the frequent, recurrent, and specific acquired loss and/or damaging mutation of MEN1 in human tumors solidly establish MEN1 as a key tumor suppressor gene in the parathyroid glands.

**Cyclin D1/CCND1**

Functioning as a direct-acting oncogene, CCND1, which encodes cyclin D1, is a solidly established driver of parathyroid neoplasia. CCND1 was first identified as a human oncogene via its involvement in parathyroid adenomas, of which a subset contains a pericentromeric inversion of chromosome 11. This rearrangement was found to result in juxtaposition of a specific region of chromosome 11q13 with the parathyroid hormone gene (PTH) regulatory region (Arnold et al. 1988, 1989). The gene at the 11q13 breakpoint was cloned and first called PRA1; its protein product was identified as a novel member of the cyclin family and a key regulator of cell cycle progression (Motokura et al. 1991). Subsequently called cyclin D1, the canonical pathway for its action involves its direct binding to and activation of partner cyclin-dependent kinases CDK4 and CDK6 at the G1-to-S phase transition of the cell cycle (Sherr et al. 2016), and considerable evidence points to important CDK-independent actions of cyclin D1 as well (Casimiro et al. 2015). Rearrangements involving the CCND1 locus appear to occur in up to 8% of parathyroid adenomas (Yi et al. 2008) and cyclin D1 protein overexpression occurs in 18–40% (Hsi et al. 1996, Tominaga et al. 1999, Vasef et al. 1999, Hemmer et al. 2001, Ikeda et al. 2002, Alvelos et al. 2013), suggesting the existence of additional, still unknown, genetic mechanisms that lead to cyclin D1 oncoprotein overexpression in these tumors (Fig. 1). Such mechanisms might include signal-induced transcriptional activation (Klein & Assoian 2008), alternative splicing (Knudsen et al. 2015), or increased protein stability via a breakdown in its destruction mechanism (Gong et al. 2014). In contrast to benign parathyroid adenomas, CCND1 gene amplification is frequently observed in parathyroid cancer (Zhao et al. 2014, Pandya et al. 2017), and may account for much of the observed cyclin D1 overexpression in this malignancy (Vasef et al. 1999, Zhao et al. 2014). This finding carries potential clinical significance as pharmacologic CDK4/6 inhibitors are already available for use as cancer therapeutic agents in other tumor types.

A transgenic mouse model of the PTH-CCND1 rearrangement contains a transgene with approximately 5.2kb of PTH enhancer, promoter, and noncoding exon 1 juxtaposed to genomic CCND1 and flanking sequences, causing parathyroid-specific overexpression of cyclin D1. These mice develop both biochemical PHPT and parathyroid gland hypercellularity (Imanishi et al. 2001). Increased parathyroid cell proliferation precedes biochemical abnormalities in this model system, demonstrating that a primary proliferative lesion can be the cause, rather than a consequence, of the characteristic abnormal calcium sensing and setpoint dysregulation in PHPT (Mallya et al. 2005). The strong genetic evidence of clonal CCND1 activation in human tumors, plus the ability of overexpressed CCND1 to drive hyperparathyroidism in animal models, solidly establish the gene as a tumorigenic driver in the parathyroid glands.

**Cyclin-dependent kinase inhibitor (CDKI) genes**

The inactivation of cyclin-dependent kinase inhibitors (CDKIs), the inhibitory counterparts to cyclins and cyclin-dependent kinases, also contributes to tumorigenesis in parathyroid adenoma. All members of the CDKN1 and CDKN2/INK4 families have been suspected to be tumor suppressor genes, but the amount and strength of evidence supporting the role of each of their protein products as a critical inhibitor of tumorigenesis varies from gene to gene and may be cell type specific. Germline mutations of several CDKI genes have been reported to cause MEN1 or the clinically indistinguishable syndrome MEN4 (Pellegata et al. 2006, Agarwal et al. 2009, Belar et al. 2012, Thakker 2016).

CDKN1B, which encodes P27KIP1 (p27), is the most extensively studied of the CDKN1 family in parathyroid tumors, and, like other members of its family, p27 targets both CDK2 and CDK4/6. Confirmed somatic mutations of CDKN1B are rare, occurring in about 1% or fewer cases of sporadic parathyroid adenoma; importantly, unsuspected germline mutations also occur in patients with sporadically presenting adenoma, apparently at slightly higher frequency (Costa-Guda et al. 2011, Cromer et al. 2012, Newey et al. 2012, Alvelos et al. 2013, Borsari et al. 2017). Some CDKN1B mutations have demonstrably caused protein instability, essentially eliminating expression (Costa-Guda et al. 2011). A single case with a mutation in CDKN2C, encoding P18INK4C (p18), is the only other known example of a somatic CDKI mutation in sporadic parathyroid adenoma (Costa-Guda et al. 2013a). Mutations in a subset of other CDKI genes are sometimes present in the germline of patients with sporadically presenting parathyroid adenomas.
The paucity of genetic lesions in CDKI genes suggests they are not common drivers of sporadic parathyroid tumorigenesis; however, they may act as rare predisposition alleles, and other evidence suggests they may also be downstream targets of different drivers. Aberrant methylation and low mRNA and/or protein expression of \( CDKN1A, \) encoding P21CIP1 (p21); \( CDKN1B, \) (p27); \( CDKN2A, \) encoding P16INK4A (p16); \( CDKN2B, \) encoding P15INK4B (p15); and \( CDKN2C, \) (p18) have been reported; a drastic reduction to no detectable mRNA expression of \( CDKN1A \) in 53% and of \( CDKN2C \) in 42% of one cohort further suggest that CDKI inactivation is functionally associated with parathyroid adenoma (Buchwald et al. 2004, Juhlin et al. 2010, Starker et al. 2011, Sulaiman et al. 2013, Arya et al. 2017, Borsari et al. 2017).

Rodent models of CDKI knockout support the hypothesis that inactivating members of this class of proteins contributes to tumorigenesis by enabling proliferative pathways. A spontaneous rat model harboring a \( Cdkn1b \) frameshift exhibits neoplasia of multiple endocrine tissues, including parathyroid hyperplasia, bilateral pheochromocytomas, bilateral medullary thyroid neoplasia, paragangliomas, and endocrine pancreas hyperplasia (Fritz et al. 2002, Pellegata et al. 2006). Homozygous null \( Cdkn1b/p27 \) mice can develop pituitary intermediate lobe hyperplasia, which may progress to adenoma as the mice age (Franklin et al. 1998), but parathyroid neoplasia has not been described (Fero et al. 1998, Lin et al. 2003). Homozygous knockout of \( Cdkn2c \) (p18) may cause low-penetration parathyroid gland neoplasia, which is enhanced when in combination with homozygous loss of \( Cdkn1a \) (p21) or heterozygous loss of \( Cdkn1b \) (p27) or \( Men1 \) (Franklin et al. 2000, Bai et al. 2007).

Some features of both human and murine evidence suggest that hindrance of CDKI tumor-suppressive function alone may not be sufficient to drive tumorigenesis in the parathyroid glands. For example, inactivation of at least one \( MEN1 \) allele has been found in some cases with \( CDKN1B \) mutation or underexpression (Costa-Guda et al. 2011, Borsari et al. 2017), suggesting functional non-redundancy despite the prior observation that menin can regulate expression of p27 (Wu & Hua 2011). In summary, CDKI variants appear to act as rare predisposition alleles for sporadic parathyroid adenomas; and CDKI variants,
especially in p27, are putative tumorigenic drivers of parathyroid adenomas as evidenced by recurrence of somatic variants, the induction of PHPT in rodent models, and its rare role in causing human familial PHPT in the context of MEN1-like phenotypes.

**β-catenin/CTNNB1 and other Wnt pathway components**

Aberrant activation of the canonical Wnt signaling pathway, which controls transcription of proliferation-and differentiation-related gene sets, has been implicated in several tumor types (Zhan et al. 2017) and hypothesized to contribute to parathyroid tumorigenesis. Because CCND1 is among the transcriptional targets of Wnt signaling, the tumor suppressors and protooncogenes involved in Wnt signaling have been appealing candidate tumor genes in parathyroid neoplasia; however, current evidence does not support a prominent ‘driver’ function for Wnt-related genes in sporadic parathyroid adenoma.

The gene CTNNB1 encodes β-catenin, the primary signal propagator of canonical Wnt signaling, which acts as a translational activator when Wnt signaling is activated (Clevers 2006). In the absence of the Wnt signal, excess β-catenin beyond that involved in adherens junctions is phosphorylated and targeted by a destruction complex (Ilyas 2005). One group identified a single, identical homozygous somatic CTNNB1 mutation, S37A, affecting a key phosphorylation site required for β-catenin degradation in a substantial proportion of parathyroid adenomas (Björklund et al. 2007a, 2008), suggesting a direct-acting driver oncogene mechanism for constitutive Wnt transcriptional activation; the surprising finding of homozygosity for what would typically be expected to be a heterozygous ‘dominant’ mutation has not been explained. The concept was supported by apparent stabilization and nuclearization of β-catenin (Björklund et al. 2007a, 2008); however, the observation of somatic S37A mutation, homozygous or heterozygous, has not been replicated in numerous other studies, collectively examining over 600 adenomas (Semba et al. 2000, Ikeda et al. 2002, Costa-Guda & Arnold 2007, Cetani et al. 2010, Haglund et al. 2010, Newey et al. 2012, Guarnieri et al. 2012a, Starker et al. 2012b). Subsequently, two investigations found a different somatic stabilizing mutation, S33C, as a heterozygous rather than a homozygous change in one adenoma each (Guarnieri et al. 2012a, Starker et al. 2012b), suggesting an incidence of less than 1%. However, S33C was not associated with increased β-catenin expression (Guarnieri et al. 2012a).

None of these reported β-catenin mutations have yet been found to drive hyperparathyroidism in relevant *in vivo* models such as transgenic mice. Overexpression and aberrant nuclear localization of β-catenin were also rare and the subject of some debate (Semba et al. 2000, Ikeda et al. 2002, Björklund et al. 2007a, 2008, Cetani et al. 2010, Guarnieri et al. 2012a, Starker et al. 2012b, Alvelos et al. 2013).

Other components of the Wnt signaling pathway have also been investigated in parathyroid neoplasia: evidence suggests, to varying degrees, that LRP5, APC, members of the SFRP family, and RASSF1A may have some relevance in Wnt-mediated tumorigenesis in the parathyroid glands. LRP5 associates with Wnt receptor Frizzled in the presence of Wnt, then acts to sequester the β-catenin destruction complex (Clevers 2006, Zhan et al. 2017). LRP5 was found to be expressed as an alternatively spliced transcript in 86% of sporadic parathyroid adenomas and 100% of secondary parathyroid hyperplasias in one cohort (Björklund et al. 2007b). In the same study, a hyperplasia-derived cell line expressing the truncated LRP5 exhibited increased Wnt-mediated transcriptional activity, suggesting that the alternate transcript may be more effective in sequestering the β-catenin destruction complex. A single case within a whole-exome sequencing cohort exhibited biallelic LRP5 loss, attributable to a heterozygous germline mutation and acquired LOH in the tumor (Cromer et al. 2012); however, somatic intragenic inactivating mutations of LRP5 have not been described in parathyroid tumors and a potential tumor suppressor role for this gene in parathyroid neoplasia remains uncertain. Further study of this issue, especially in suitable *in vivo* animal models, may shed additional light on the tumorigenicity and penetrance of various Wnt pathway aberrations.

Some negative regulators of Wnt signaling have been reported to be transcriptionally repressed via promoter methylation; while this alone is insufficient evidence for a tumor-driver role, it may prove to be useful in identifying upstream candidate tumor genes. APC, an important tumor suppressor gene in colorectal and other cancers, is an essential component of the β-catenin destruction complex. One study found APC to be among the top 50 differentially methylated genes in parathyroid adenoma compared to normal parathyroid; expression data accordingly showed decreased APC expression (Starker et al. 2011). APC promoter 1A was found to be hypermethylated in 56–71% of typical parathyroid adenomas (Juhlin et al. 2010, Sulaiman et al. 2013). RASSF1A and three members of the family of secreted frizzled-related proteins (SFRPs), all of which are antagonistic to
the Wnt/β-catenin signaling pathway, were also found to be hypermethylated and their expression downregulated in a similar fashion (Juhlin et al. 2010, Starket et al. 2011, Sulaiman et al. 2013, Arya et al. 2017). Additional genetic and epigenetic evidence will be required to demonstrate a causative role (or a crucial downstream role) for APC, RASSF1A, SFRPs, and/or their transcriptional regulators in parathyroid tumors.

**EZH2**

Enhancer of zeste homolog 2 (EZH2) encodes an H3K27 histone methylase of the same name that acts as an epigenetic silencer and has been found to be mutated and/or upregulated in a number of cancers (Simon & Lange 2008). Heterozygous mutations corresponding to the EZH2 catalytic domain at p.Y641 and p.A677 lead to hypertrimethylation of its epigenetic targets and are found to correlate with pro-tumor activity (Yap et al. 2011, Wu et al. 2013). As a candidate driver event, apparent mutational activation of EZH2 p.Y641N (also reported as p.Y646N, referencing the same position in a different transcript) appears to be very rare in parathyroid adenomas (Cromer et al. 2012, Sanpao et al. 2016, Romano et al. 2017). In one study, universal overexpression and frequent gene amplification of EZH2 was noted; however, as the binding partners for EZH2 that are needed for its methylase activity were not similarly upregulated, it was not immediately clear how EZH2 overexpression favored proliferation in these tumors (Svedlund et al. 2014). In addition, a later study did not observe EZH2 gene amplification and pointed out potentially important methodologic pitfalls in such analyses (Romano et al. 2017).

Consequently, EZH2 overexpression in parathyroid adenomas is likely a downstream effect of a lesion in a different driver oncogene or tumor suppressor gene. Interestingly, EZH2 has been implicated in pathways that regulate several other proven and candidate parathyroid tumor genes, and evidence suggests that the involvement of EZH2 in tumorigenesis may extend beyond its role as a histone methylase. Specifically, (a) menin, a parathyroid tumor suppressor, recruits EZH2 to gene promoters to inhibit cellular proliferation (Wu & Hua 2011); (b) EZH2 may silence Wnt pathway antagonists (Cheng et al. 2011), a possibility also suggested by epigenetic silencing and low transcription of some of the genes previously discussed in this review; and (c) independent of its histone methylase activity, EZH2 may interact with β-catenin to influence Wnt signaling (Jung et al. 2013).

**ZFX**

Zinc finger X-linked (ZFX) encodes a transcription factor that plays an important role in maintaining self-renewal capacity and other stem cell-like qualities both in adult stem cells and during development (Galan-Caridad et al. 2007, Harel et al. 2012). Novel ZFX mutations at residues p.R786 and p.R787 were discovered in 4.6% of a parathyroid adenoma cohort (Arnold & Soong 2014, Soong & Arnold 2014). The substitution of non-charged amino acids for arginine at these residues may confer neomorphic function by altering the ZFX DNA-binding motif, and thereby drive tumorigenesis – a phenomenon observed in a similar transcription factor in pancreatic endocrine tumors (Cromer et al. 2015). While overexpression of wild-type ZFX is associated with poor prognosis in other tumor types (Yan et al. 2014, 2016, Li et al. 2015, Weng et al. 2015, Yang et al. 2015) and plays a role in maintaining tumorigenic phenotypes (Fang et al. 2014, Lai et al. 2014, Palmer et al. 2014, Weisberg et al. 2014), no studies have yet definitively characterized the capacity of this candidate oncogene to drive tumorigenesis, nor determined the effects of point mutations in its motif-specific zinc finger domain. Interestingly, somatic mutations at the same or adjacent amino acid residues have been reported in other tumor types, further suggesting that ZFX DNA-binding domain mutations may be activating and oncogenic (see COSMIC (cancer.sanger.ac.uk); Forbes et al. 2017). Functional studies to show that mutant ZFX can induce hyperparathyroidism, e.g. in genetically engineered mice, would crucially complement this intriguing genetic evidence and will be required to solidly establish ZFX as a parathyroid oncogene.

**Additional genetic considerations**

**GCM2**

GCM2, also known as GCMB, is a parathyroid-specific transcription factor known to be crucial for parathyroid gland development (Günther et al. 2000), and germline inactivating mutations in the GCM2 gene cause human hypoparathyroidism (Ding et al. 2001). Following contradictory reports of increased (Kebebew et al. 2004) or diminished (Correa et al. 2002) GCM2 expression in parathyroid tumors, subsequent efforts to implicate GCM2 in parathyroid tumorigenesis have turned towards interesting variants originally identified and studied in the context of familial isolated hyperparathyroidism (FIHP). In contrast to the inactivating and dominant-negative
variants identified in familial isolated hypoparathyroidism (Canaff et al. 2009), a subset of FIHP kindreds were associated with other GCM2 germline variants, some of which activated GCM2 transcriptional function in vitro (Guan et al. 2016). An enhanced association of such alleles with FIHP families of Ashkenazi Jewish descent has been reported (Guan et al. 2017). However, the relevance to tumorigenesis of this in vitro transcription assay is unclear, and the penetrance of these variants (representing the level of their potential contribution to the phenotype) remains an unresolved question, especially given the substantial frequency of these alleles in the general (and Ashkenazi) population (see gnomAD (gnomad.broadinstitute.org); Lek et al. 2016).

Some GCM2 germline variants have been proposed as potential predisposition alleles in sporadic parathyroid tumors (D’Agruma et al. 2014, Guan et al. 2017, Marchiori et al. 2017). One of these in vitro-activating variants, Y282D, was found to be enriched among a cohort with PHPT (D’Agruma et al. 2014). Other activating GCM2 variants have been identified in sporadic parathyroid adenoma: V382M (Mannstadt et al. 2011) and Y394S (Guan et al. 2017), the latter especially in Ashkenazi populations. Again, however, the overall penetrance or positive predictive value of such alleles to cause parathyroid adenomas (among Ashkenazis or the general population) is uncertain and requires additional investigation. Finally, potential evidence for a driver oncogene role of GCM2 variants, including recurrent somatic mutations and their ability to drive parathyroid tumorigenesis in an animal model, have not yet been reported.

**CASR**

Mutations in the calcium sensing receptor gene (CASR) are associated with the hereditary syndromes familial hypocalciuric hypercalcaemia (FHH) and neonatal severe hyperparathyroidism (NSHPT), the former of which is associated with hemizygous CASR inactivation and the latter with homozygous mutation (Pollak et al. 1993, Hendy et al. 2000). In addition, CASR was suspected to play a driver role in sporadic parathyroid tumorigenesis due to the decreased sensitivity to serum calcium in patients with parathyroid adenomas. However, investigation of human parathyroid adenomas for inactivating mutations in, and allelic loss of, CASR suggest that the gene virtually never serves as a driver of sporadic hyperparathyroidism. Specifically, the only CASR abnormality identified to date in an apparently sporadic solitary parathyroid adenoma was found to be a germline variant (Guarnieri et al. 2010), and other studies did not find any sequence variants or mutations (Hosokawa et al. 1995, Cetani et al. 1999). Similarly, allelic loss at the CASR locus was found in very few cases (Thompson et al. 1995, Farnebo et al. 1997, Cetani et al. 1999). Therefore, although impaired CASR expression or signaling may play a role in the dysregulation of calcium-PTH homeostasis central to the phenotype of PHPT (Farnebo et al. 1997, Imanishi et al. 2001, Sulaiman et al. 2013, Varshney et al. 2013), the lack of clonally selected somatic inactivating genetic lesions at its locus strongly suggests that its dysfunction is generally a secondary effect rather than a cause of sporadic parathyroid tumorigenesis. This concept is supported by an animal model of primary hyperparathyroidism, in which oncogene-driven parathyroid proliferation abnormality temporally precedes the advent of PTH-calcium setpoint dysregulation (Mallya et al. 2005). That said, as acquired mutations in other calcium signaling-related genes (Koh et al. 2011, Nesbit et al. 2013a,b, Balenga et al. 2017) might be discovered in the future, it remains possible that a primary acquired defect in the calcium sensing/PTH response pathway could potentially drive parathyroid tumorigenesis in a subset of cases.

**Other genes**

Other genes have been proposed as possible contributors to sporadic parathyroid adenoma development based on putative oncogenic activation and/or tumor suppressor inactivation found in human tumors at a very low frequency, or epigenetic and/or expression data that indicate differential gene expression in parathyroid adenomas, but which in the absence of genetic evidence suggests that these genes are downstream targets of driver mutations rather than oncogenic drivers themselves.

**CDC73** (originally called HRPT2) is the causative gene for the familial hyperparathyroidism-jaw tumor syndrome (HPT-JT) and the benign and malignant parathyroid tumors associated with it. Inactivating **CDC73** mutation is a major driver of parathyroid carcinoma; in contrast, such mutations are extremely rare in sporadic parathyroid adenomas (Howell et al. 2003, Cetani et al. 2004, Krebs et al. 2005, Bradley et al. 2006, Guarnieri et al. 2012a, b). When found, they were typically associated with uncommon histologic and/or clinical features, such as cystic appearance (Carpten et al. 2002, Domingues et al. 2012), large gland weight (Sulaiman et al. 2012a), young age at presentation (Starker et al. 2012a,b), or recurrence (Cetani et al. 2007, Shibata et al. 2015), and are often found in the germline.
A limited number of other genes have been found to be somatically altered at extremely low rates (i.e. in single cases, not yet meeting the criterion of recurrence for candidate driver genes) in parathyroid adenoma. As a result of whole-exome sequencing, tumor-associated genes such as protection of telomeres 1 (POT1; see Newey et al. 2012) and Ras-related protein 1b (RAP1B; see Cromer et al. 2012) were found to harbor mutations in one case each; additional alterations were reported in a number of other genes, several of which have unknown connections to tumorigenesis and may constitute passenger mutations. Aberrant methylation and/or expression of many other genes, such as TBX1 (Verdelli et al. 2017), MYC (Björklund et al. 2007a), RBL1, PRDM2, and WT1 (Starker et al. 2011), have been reported in parathyroid adenoma; however, since the molecular events responsible for this dysregulation have not yet been identified, it remains possible that these are secondary events rather than drivers of tumorigenesis.

**Parathyroid carcinoma**

Parathyroid carcinoma is a rare but life-threatening cause of primary hyperparathyroidism. In addition to its rarity, inconsistencies in the application of diagnostic criteria have further complicated study of the genetic basis of this tumor. Unequivocal diagnosis of parathyroid carcinoma can only be made based on the presence of invasion into adjacent structures and/or distant metastasis, and in some studies the diagnosis may have been overcalled, resulting in the inclusion of atypical adenomas that might not have true malignant potential. The criterion for vascular invasion may be particularly problematic; under current WHO guidelines, only vascular invasion within or beyond the capsule meets this criterion, and intratumoral vaso-invasion is insufficient for a diagnosis of parathyroid carcinoma (DeLellis et al. 2017). Notwithstanding this need for caution in interpreting the published literature, major advances have been made in understanding the genetic underpinnings of parathyroid carcinoma.

In many tissues, clinically and histologically apparent stages of tumorigenesis can be seen, progressing from normal to hyperplastic/dysplastic and clinically apparent benign neoplasia stages, via incremental accumulation of acquired genetic abnormalities, before becoming malignant. While there is significant evidence for a malignant progression model in a number of solid tumors, genetic evidence argues against such a progression in most instances of parathyroid carcinoma. In malignant progression models, genetic alterations observed in early/benign disease are found at equal or greater frequencies in advanced/malignant disease, and additional alterations, playing an important role in progression, develop in the malignant tumors (Vogelstein et al. 2013). If such a progression model were applicable to parathyroid cancer, the same primary, clonal, genetic driver events (‘trunk mutations’) commonly present in parathyroid adenomas should be represented at least equally in parathyroid carcinoma. The most common genetic alterations in benign parathyroid tumors, loss of 11q and/or mutation of MEN1, occur in about 35% of parathyroid adenomas (Tahara et al. 1996, Agarwal et al. 1998, Farnebo et al. 1999, Hunt et al. 2005, Cromer et al. 2012, Newey et al. 2012, Costa-Guda et al. 2013b, Pardi et al. 2013). A progression model therefore would predict that 11q loss and/or MEN1 mutation would be found in at least 35% of carcinomas; however, these changes are much less frequently seen in parathyroid cancer (Agarwal et al. 1998, Farnebo et al. 1999, Kytola et al. 2000, Haven et al. 2007, Enomoto et al. 2010, Costa-Guda et al. 2013b). These observations suggest that rather than evolving from a preexisting typical benign adenoma, most parathyroid cancers arise de novo (Costa-Guda et al. 2013b), the genetic drivers of which are discussed in this section (summarized in Table 2).

**CDC73**

Somatic, intragenic, inactivating mutations of CDC73 (HRPT2) are the most common genetic alteration seen in parathyroid carcinoma, in contrast to their rarity in sporadically presenting benign parathyroid adenomas. Although a wide range of mutation frequencies (13–100%) have been reported across studies (Howell et al. 2003, Shattuck et al. 2003a, Cetani et al. 2004, 2013, Haven et al. 2007, Guarnieri et al. 2012b), likely related to sample size issues and/or inconsistencies in inclusion criteria, CDC73 mutations have generally been detectable in a substantial majority of clearly malignant parathyroid carcinomas (Gill 2014). Biallelic CDC73 inactivation, through a combination of mutation plus large genomic deletion or independent, intragenic deletion of both alleles, can be seen in many tumors (Howell et al. 2003, Shattuck et al. 2003a, Cetani et al. 2004, Cascon et al. 2011, Domingues et al. 2012, Bricaire et al. 2013), and various deletions and/or noncoding mutations would be expected to have evaded detection in most studies. Loss of immunohistochemical expression of the protein product of CDC73, parafibromin, is also seen in a majority of
sporadic parathyroid carcinomas but is rare in sporadic adenomas. Parafibromin staining may be considered as a diagnostic aid in clinically equivocal cases (Tan et al. 2004, Gill et al. 2006, Cetani et al. 2007) but has been considered insufficient to serve as a reliable diagnostic marker of parathyroid cancer on its own (DeLellis 2011).

One situation which is likely an exception to the predominant process of de novo parathyroid carcinogenesis is that of germline CDC73 mutation. Germline CDC73 mutation causes the autosomal dominantly inherited HPT-JT syndrome, which predisposes to the development, with variable penetrance, of parathyroid tumors, benign ossifying fibromas of the jaw (distinct from the ‘brown tumors’ that are a consequence of severe hyperparathyroidism), and a variety of uterine and/or kidney lesions and tumors. While the majority of parathyroid tumors in this syndrome are benign, parathyroid carcinoma is greatly overrepresented, with up to 37.5% of affected individuals developing parathyroid carcinoma (Iacobone et al. 2009, Frank-Raue et al. 2011, Mehta et al. 2014). These patients may develop parathyroid carcinomas that have evolved from preexisting benign or atypical adenomas, and likely explain some rare reports of apparent progression. A substantial minority of patients with sporadically presenting parathyroid carcinoma possess unexpected germline CDC73 mutations. It is likely that these patients represent new index cases of HPT-JT or a phenotypic variant, such as FIHP. The presence of such germline CDC73 mutations has important implications for their long-term management and for their families (Shattuck et al. 2003a, Cetani et al. 2004, Guarnieri et al. 2006, Kelly et al. 2006), and diagnostic DNA testing is now widely available for this purpose (El-Hajj Fuleihan & Arnold 2017).

The precise mechanisms through which inactivation of CDC73, and subsequent loss of parafibromin, promote tumorigenesis remain unclear. Parafibromin is ubiquitously expressed, evolutionarily conserved, and predominantly a nuclear protein. Further localization to the nucleolus appears to be central to parafibromin’s tumor-suppressive function; among the few known CDC73 tumor-promoting missense mutations, at least two have been experimentally demonstrated to impair nucleolar localization (Panicker et al. 2010, Masi et al. 2014). Cytoplasmic parafibromin may have distinct functions from nuclear parafibromin (Agarwal et al. 2008, Jo et al. 2014).

Most of parafibromin’s known functions are related to its similarity to the Saccharomyces cerevisiae protein Cdc73p, a component of the yeast RNA polymerase II-associated-associated factor 1 complex (Paf1c; see Carpten et al. 2002). The human PAF1 complex (hPAF1C) includes homologs of most of the same subunits and shares similar functions with the yeast Paf1c. Cdc73p homologs in higher-level organisms contain a metazoan-specific N-terminal domain, which functions in Wnt signaling; parafibromin can directly bind β-catenin (Mosimann et al. 2006). The involvement of parafibromin in canonical Wnt/β-catenin signaling provides one potential mechanism for its role in tumourigenesis; however, parafibromin’s precise role in Wnt signaling remains unclear. Accumulation of β-catenin (Svedlund et al. 2010) and loss of the Wnt pathway components APC and GSK3β (Juhlin et al. 2009) and have been described in parathyroid cancer. Wnt signaling is also known to regulate, in part, expression of cyclin D1, an important parathyroid oncogene (Shutman et al. 1999, Tetsu & McCormick 1999). Parafibromin’s in vitro ability to inhibit cancer cell growth and cause G1 phase arrest is effected in part through regulation of cyclin D1 (Woodard et al. 2005, Lin et al. 2008).

To elucidate the functions of parafibromin in a complex, biologically relevant system, conventional and conditional transgenic mouse knockouts of Cdc73 have been developed. Homozygous germline deletion of Cdc73 was embryonic lethal by embryonic day 6.5 (Wang et al. 2008). To overcome the embryonic lethality of a conventional Cdc73 knockout, floxed-Cdc73 mice were mated to mice containing a ubiquitously expressed, tamoxifen-inducible Cre recombinase. Germline deletion of Cdc73 by tamoxifen administration at later stages of development led to growth retardation, severe cachexia and death within 20 days (Wang et al. 2008). Loss of parafibromin was associated with increased apoptosis in many tissues, consistent with in vitro findings (Lin et al. 2007, Jo et al. 2014). Initially, no parathyroid gland abnormalities were described in either the conventional or conditional Cdc73 knockout (Wang et al. 2008). However, in a follow-up study which assessed the conventional heterozygous Cdc73-knockout mice out to 21 months of age, increased proliferation was noted in the parathyroid glands; histologic abnormalities such as nuclear pleomorphism and/or fibrous septation, features commonly observed in atypical parathyroid adenomas and parathyroid carcinomas in humans, were also noted (Walls et al. 2017). Parathyroid-targeted deletion of Cdc73, by crossing floxed-Cdc73 mice with PTH-Cre mice, resulted in similar parathyroid gland abnormalities;
both heterozygous and homozygous mice were affected (Walls et al. 2017). Further studies are needed to determine how loss of CDC73 expression promotes tumorigenesis in humans.

**PI3K/mTOR**

Gain-of-function mutations of two genes in the phosphatidylinositol 3-kinase (PI3K)/mammalian target of rapamycin (mTOR) pathway, PIK3CA and MTOR, have been identified in parathyroid carcinoma (Kasaian et al. 2013, Pandya et al. 2017). The PI3K/mTOR pathway is a key regulator of cell growth and survival and cell cycle progression. PIK3CA, which encodes the p110-alpha subunit PI3K, is a recognized driver oncogene in many human malignancies, as is MTOR (Saxton & Sabatini 2017). Known oncogenic PIK3CA hotspot mutations p.K111E, p.E545A, p.E545K, and p.H1047R were observed in two recent next-generation sequencing studies; activating mutations of MTOR were also seen recurrently (Kasaian et al. 2013, Pandya et al. 2017). Interestingly, PIK3CA and CDC73 mutations tended to be mutually exclusive but MTOR mutations generally overlapped with mutations in either PIK3CA or CDC73. While the complete establishment of PIK3CA and/or MTOR as parathyroid tumor drivers will require demonstration that such mutations can induce experimental hyperparathyroidism, they (especially PIK3CA) must be considered strong putative parathyroid oncogenes given the weighty genetic evidence. As these oncogenes are the targets of current and future cancer therapeutic agents, appropriately selected patients with surgically incurable parathyroid cancer should be considered for DNA sequencing and potential use of such agents.

**Cyclin D1/CCND1**

In contrast to benign parathyroid adenomas, CCND1 gene amplification is frequently observed in parathyroid cancer (Zhao et al. 2014, Pandya et al. 2017), and may well account for much of the observed cyclin D1 overexpression (Vasef et al. 1999, Zhao et al. 2014) in this disease. This finding carries potential clinical significance as cyclin D1’s partner kinases are ‘actionable targets’ and pharmacologic CDK4/6 inhibitors are already available for use as cancer therapeutics in other tumor types (Sherr et al. 2016). These drugs have yet to be tested in parathyroid cancer but certainly hold promise for appropriately selected patients.

**Additional genetic considerations**

Many early studies seeking to identify genetic contributors to parathyroid cancer focused on identification of locations of allelic imbalance. Such studies of allelic imbalance have identified recurrent regions of loss, each likely to contain one or more key tumor suppressor genes, on chromosomes 1p, 3, 13q, and 14; and recurrent regions of gain, each likely to contain one or more driver oncogenes, on chromosomes 1q and 16 (Agarwal et al. 1998, Farnebo et al. 1999, Kytola et al. 2000, Hunt et al. 2005, Sulaiman et al. 2012b, Costa-Guda et al. 2013b). Whole genome and whole exome sequencing analyses have thus far failed to uncover recurrently altered target tumor genes within those predicted genomic locations (Kasaian et al. 2013, Yu et al. 2015, Pandya et al. 2017), although observed preferential amplification of mutant CDC73 might account, at least in part, for allelic gains noted in 1q (Yu et al. 2015). As noted above, CCND1 gene amplification (Zhao et al. 2014, Pandya et al. 2017) and cyclin D1 overexpression (Vasef et al. 1999, Zhao et al. 2014) are frequently observed in parathyroid cancer. Several additional studies sought to interrogate candidate genes for sequence and/or expression abnormalities in parathyroid cancer, showing that such important human cancer genes as p53 (Cryns et al. 1994b, Hakim & Levine 1994), RB1, BRCA2 (Shattuck et al. 2003b), and others (Yoshimoto et al. 1992, Cryns et al. 1994a) were unlikely to make a major contribution. The absence of alterations in such common ‘cancer genes’ was further confirmed by next-generation sequence analyses (Kasaian et al. 2013, Yu et al. 2015, Pandya et al. 2017).

A small subset of sporadic parathyroid carcinomas has been reported to harbor somatic MEN1 mutations. However, owing to inconsistencies in diagnostic histopathologic criteria or in specific criteria stated for inclusion as a sporadic case, the published literature is not entirely clear on the frequency of this finding (Haven et al. 2007, Enomoto et al. 2010, DeLellis et al. 2017). That said, even if all such cases met rigorous criteria, the frequency of MEN1 mutation in parathyroid cancer is significantly lower (6.7%; P<0.01) than in parathyroid adenoma, and biallelic inactivation of MEN1 has only been reported in two carcinomas (4.2%; see Carling et al. 1998, Scarpelli et al. 2004, Haven et al. 2007, Enomoto et al. 2010, Kasaian et al. 2013, Yu et al. 2015, Pandya et al. 2017). Further, in the setting of germline MEN1 mutation, parathyroid carcinoma is rare, with only 17 cases reported to date (reviewed in Di Meo et al. 2018). In contrast to the high rate of parathyroid cancer in patients harboring germline...
CDC73 mutations, fewer than 1% of MEN1 syndrome patients appear to develop parathyroid carcinoma in the course of their lifetimes. While the potential for familial/syndromic MEN1 patients to develop parathyroid cancer is a legitimate consideration for management, it does not appear that having a germline MEN1 mutation significantly increases an individual’s risk of developing parathyroid carcinoma.

In addition to the genes recurrently altered in parathyroid cancer, described above, next-generation sequence analyses have provided important, novel insights into other potential genetic contributors to parathyroid cancer. One such gene, PRUNE2 (Yu et al. 2015, Pandya et al. 2017) functions to suppress Ras homolog family member A (RhoA) activity, thereby suppressing oncogenic cellular transformation. This functional framework is consistent with its mutational pattern that raises the possibility of a tumor suppressor role in parathyroid carcinoma: germline missense variant accompanied by LOH was found in one parathyroid cancer and two somatic nonsense mutations were found in another parathyroid carcinoma subjected to whole exome sequencing. Two additional somatic missense variants in PRUNE2 were revealed by Sanger sequencing (Yu et al. 2015). The functional consequences of the observed missense variants warrant investigation, as does the potential for the observed DNA changes to induce experimental hyperparathyroidism in vivo.

Next, recurrent mutations in AKAP9, a gene involved in regulation of protein kinase A, and frequently altered in several epithelial cancers, were observed in parathyroid carcinoma (Pandya et al. 2017). ZEB1, a transcriptional regulator involved in epithelial-mesenchymal transition, and the putative kinase gene ADCK1 were also recurrently mutated (Pandya et al. 2017), the former involving different missense changes, and the latter with a singular missense alteration shared among distinct tumors suggestive of a gain-of-function/direct-acting oncogene role. Mutations in NOTCH1, which has been described as an oncogene in some malignancies and a tumor suppressor gene in others, were seen in two tumors (Pandya et al. 2017). Three carcinomas in one study displayed mutational spectra consistent with the apolipoprotein B mRNA editing enzyme, catalytic peptide-like (APOBEC) mutational signature, in which C>T and C>G at TpC are overrepresented; this mutational signature has described in several other cancer types and has been associated with poor prognosis (Yu et al. 2015). The oncogenic potential of the observed mutations remains unclear and merit further study. Mutations affecting only a single tumor but strongly associated with other human cancers were also observed. Of special note, mutations were identified in MLL2, a putative tumor suppressor gene known to interact with MEN1, and THRAP3, a member of the SNRP complex that can regulate cyclin D1 expression (Kasaian et al. 2013). A mutation was also identified in CDKN2C/p18 (Kasaian et al. 2013), interestingly affecting the same amino acid residue as a mutation previously identified in a parathyroid adenoma (Costa-Guda et al. 2013a). Mutations were noted in canonical Wnt pathway genes APC and RNF43. Additional mutations were identified in several kinase genes potentially involved in cell migration and invasion, including MAP3K11, JAK1, and RIOK3 (Yu et al. 2015), and in genes involved in regulating chromatin structure, including ARID2, ARID4A, KDM5C, KDM4C, KDM4E, JMJD1C, and SETD1B (Pandya et al. 2017). Owing to their non-recurrence and absence of functional studies, it is impossible to predict which of the mutated genes identified by next-generation sequence analysis may emerge as drivers, rather than passengers, in parathyroid cancer. However, the identification of such mutations is a potentially important step in expanding our understanding of the molecular pathogenesis of parathyroid cancer.

Declaration of interest
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Primary hyperparathyroidism


Primary hyperparathyroidism


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