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Commentary: Next-Generation Sequence Analysis of Parathyroid Carcinoma

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Parathyroid cancer is a rare but life-threatening malignancy. Surgery is the primary treatment, and complete surgical excision at the first operation offers the best chance for cure. Despite generally slow tumor growth, locally recurrent or metastatic disease is incurable as the cumulative metabolic complications of sustained hyperparathyroidism often prove fatal. Although medical therapy with calcimimetics or other agents can aid in managing sequelae of hyperparathyroidism, no chemotherapeutic interventions aimed at reducing tumor burden have proven effective in parathyroid carcinoma. Genetic testing for predisposing *CDC73* (*HRPT2*) mutations has been an important clinical advance, aimed at early detection/treatment to prevent advanced disease [1]. There remains a substantial need to identify genetic/molecular aberrations that could serve as "actionable targets" for chemotherapeutic intervention in patients whose disease is no longer surgically curable. The study by Clarke *et al.* [2] in this issue of the *Journal of the Endocrine Society* seeks to further clarify the molecular drivers of parathyroid cancer via whole-exome next-generation sequencing (NGS) analysis.

Investigation into the molecular pathogenesis of parathyroid carcinoma has been limited by tumor rarity, but a few recent NGS studies have yielded important new data: most notably recurrent, activating mutations in *PIK3CA* and *MTOR*, as well as amplifications of *CCND1* (encoding cyclin D1), which have the potential to be targeted therapeutically by currently available drugs. They have also corroborated the high mutation frequency of *CDC73* [3–5], which, despite its diagnostic utility, is not (yet) an "actionable target." Lower frequency, but recurrent, mutations in candidate tumor genes such as *PRUNE2*, *AKAP9*, *ZEB1*, and *ADCK1*, interesting mutational signatures previously noted in other cancer types, and single-tumor mutations in a number of potential cancer genes have also been reported.

The series of carcinomas reported by Clarke *et al.* [2] used well-defined histopathologic inclusion criteria, specifically addressing the often problematic criterion of vascular invasion, which, according to World Health Organization guidelines, must be intra- or extracapsular, rather than intratumoral, to be evidence for malignancy. However, to gather a substantial number of cases, the authors made use of archival, formalin-fixed, paraffin-embedded (FFPE) tumor tissue. The use of FFPE material in sequencing studies is inherently problematic: the number of sequence variants identified in FFPE samples is higher than in matched frozen tissue, owing to sequence artifacts that can be difficult to distinguish from true calls [6].

Abbreviations: FFPE, formalin fixed, paraffin embedded; NGS, next-generation sequencing.

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Acknowledging that the most frequent type of artifactual sequence variant produced in FFPE samples is cytosine to thymine transition, the authors report a median ratio of transitions to transversions comparable to those from frozen tissue. However, as quality control metrics (including transition-to-transversion ratio and mean coverage depth) for individual tumors are not included, it is impossible for readers to interpret the potential validity of reported sequence variants. These problems are exacerbated by (i) the lack of patient-matched germline DNA, which is crucial in filtering out false-positive calls and in determining germline vs somatic status of sequence variants; (ii) the absence of independent validation of sequence variants; (iii) the reporting of sequence variants only by their predicted protein-level change; and (iv) the specific exclusion of insertion/deletion (indel) variants, thereby potentially excluding important pathogenic mutations. Thus, data from this study must be viewed cautiously, and novel findings require further validation.

Germline and/or somatic mutations of *CDC73* are widely reported in parathyroid cancer. Strikingly, Clarke *et al.* [2] observed *CDC73* mutations in only 3 of 29 carcinomas (10.3%), the lowest mutation rate reported to date. The wide range in *CDC73* mutational frequency in the literature (13% to 100%; mean, 46%) generally has been attributed to inconsistent histopathologic criteria and the possible inclusion of nonmalignant tumors. The low frequency of *CDC73* mutations in Clarke *et al.* [2] likely has a very different cause. To minimize potential false-positive variants common in FFPE samples, the authors used variant filtering that specifically excluded indel variants. Fully 68% of the 47 unique intragenic *CDC73* variants reported to date in sporadic parathyroid carcinoma and 49% of variants reported across studies (0% to 78%) are indels [7]. It is highly likely, therefore, that a substantial number of *CDC73* mutations were missed by Clarke *et al.* [2] and that the true frequency of *CDC73* mutations in their tumor series, if indels were included, is well within the range established by other studies.

Interestingly, Clarke *et al.* [2] noted *MEN1* variants in two carcinomas; it is unclear from their selection criteria if patients with MEN1 syndrome may have been included and germline DNA was not analyzed, complicating interpretation of this finding. *MEN1* variants in parathyroid cancer are rare: only four *MEN1* mutation-positive sporadic parathyroid carcinomas have been reported, none of which (as described) unambiguously meet current World Health Organization criteria for parathyroid cancer [8, 9]. Including the current study, the estimated *MEN1* intragenic mutation frequency in carcinomas is 6.7%, significantly lower ($P < 0.0001$) than the 26.6% seen in benign parathyroid adenomas across studies (reviewed in Brewer *et al.* [10]). Similarly, although parathyroid carcinoma arising in MEN1 syndrome has been reported (reviewed in Di Meo *et al.* [11]), fewer than 1% of patients with MEN1 appear to develop parathyroid carcinoma in the course of their lifetime (in contrast to up to 37.5% of patients with hyperparathyroidism–jaw tumor syndrome). Although inactivation of *MEN1* has been unequivocally demonstrated to drive benign parathyroid tumorigenesis, its role as a potential driver of malignant parathyroid carcinoma is less clear and merits further study.

To aid in prioritizing candidate genes, the authors used the evolutionary action equation to assess effects of an individual sequence variant on fitness, assigning higher scores to loss-of-function variants. In the context of cancer-associated variants, this skews toward prioritization of tumor suppressor genes and would largely miss gain-of-function mutations in oncogenes that are more likely to be "actionable targets" of currently available therapeutic agents. Indeed, a number of genes previously established as tumor suppressors in other tumor types were noted by Clarke *et al.* [2] to harbor sequence variants, including *TP53* and *BRCA2*, which previously had been ruled out as major contributors to parathyroid cancer. Interestingly, a mutation in *NF1*, the neurofibromatosis type 1 tumor suppressor gene, was also noted, albeit in a single tumor. Owing to its large size, sequence variants are frequently detected in *NF1* by NGS, and false positives and passenger mutations are common. Parathyroid carcinoma (and atypical adenoma) arising in patients with neurofibromatosis type 1 has been previously reported (reviewed in Triggiani *et al.* [12]), and the co-occurrence of these two rare pathologies suggests a possible causative link. Furthermore, *NF1* is a known regulator of mTOR, and alterations of PI3K-MTOR signaling pathway genes recently have been reported in a substantial subset of parathyroid carcinomas. As this signaling pathway can be targeted by currently available chemotherapeutic agents, the

potential role of *NF1* (and other identified PI3K-MTOR pathway) mutations as drivers of parathyroid carcinoma should be a high priority for further investigation.

Notwithstanding the caveats of methodologic and analytic limitations, Clarke *et al.* [2] provide some new potential clues in the search for molecular drivers of parathyroid carcinoma. Novel candidate parathyroid cancer genes reported in this hypothesis-generating study will need to be confirmed within the present series of tumors and interrogated for involvement in other parathyroid carcinomas. Furthermore, these candidates must be validated experimentally for their ability to drive parathyroid neoplasia and biochemical hyperparathyroidism in relevant *in vivo* model systems, such as genetically engineered mice. The presence of potentially actionable targets in this and all previous parathyroid cancer NGS studies is promising; patients with surgically incurable parathyroid cancer should be strongly considered for DNA sequencing, which may uncover tumor-specific sequence variants with the potential for treatment with specific therapeutic agents.

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