Journal of Cancer Research and Clinical Oncology (2019) 145:1977–1986
https://doi.org/10.1007/s00432-019-02945-9

ORIGINAL ARTICLE – CANCER RESEARCH

Genetic profiling as a clinical tool in advanced parathyroid carcinoma

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Received: 27 February 2019 / Accepted: 27 May 2019 / Published online: 15 July 2019

Abstract

Context Parathyroid carcinoma (PC) is a rare endocrine malignancy with no approved systemic therapies for unresectable locally invasive or distant metastatic disease. Understanding the molecular changes in advanced PC can provide better understanding of this disease and potentially help directing targeted therapy.

Objective To evaluate tumor-specific genetic changes using next-generation sequencing (NGS) panels.

Design All patients with advanced PC were tested for hot-spot panels using NGS panels including a 50-gene panel, a 409-gene panel if the standard 50-gene panel (Ion Torrent, Life Technology) was negative or a FoundationOne panel.

Setting The University of Texas MD Anderson Cancer Center, Houston, Texas, USA.

Patients or other participants 11 patients with advanced PC were selected to undergo molecular testing.

Main outcome measure(s) Genetic profiles of advanced PC.

Results Among the 11 patients, 4 patients had the 50-gene panel only, 6 had 409-gene panel after a negative 50-gene panel and 1 had FoundationOne. One patient who had 50-gene panel only also had his metastatic site (esophagus) of his tumor tested with FoundationOne. The most common mutations identified were in the PI3 K (*PIK3CA, TSC1* and *ATM*) (4/11 patients) and TP53 (3/11) pathways. Genes not previously reported to be mutated in PC included: *SDHA, TERT* promoter and *DICER1*. Actionable mutations were found in 54% (6/11) of the patients.

Conclusions Mutational profiling using NGS panels in advanced PC has yielded important potentially targetable genetic alterations. Larger studies are needed to identify commonly mutated genes in advanced PC patients. Development of novel therapies targeting these cellular pathways should be considered.

Keywords Parathyroid carcinoma · Genetic profiling · Next-generation sequencing · Targeted therapy

Introduction

Parathyroid carcinoma (PC) is a rare endocrine malignancy that has poorly understood risk factors and carries significant morbidity. PC-related death usually results from unremitting hypercalcemia and its complications

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rather than tumor burden. Surgical resection is the main modality of treatment (Cetani et al. 2016). Because no definitive diagnostic markers exist to differentiate PC from benign disease, parathyroid tumors are resected en bloc. However, initial surgical intervention is usually not curative, and PC tends to persist or recur locoregionally or metastasize to distant organs (Cetani et al. 2016; Schulte and Talat 2012; Busaidy et al. 2004). When local recurrence occurs, subsequent surgeries are hampered by scarring and fibrosis from previous operations and/or external beam radiation therapy.

Medical management using anti-resorptive therapies and/or calcimimetic agents to control hypercalcemia is often transient and unsuccessful. There is no evidence that controlling hypercalcemia with these medications modifies the outcome of the disease such as slowing the progression or prolonging survival for patients with PC (Cetani et al. 2016).

The etiology of PC is largely unknown. Due to the lack of studies and the rarity of cases, it is not known whether the disease arises de novo or from parathyroid adenoma. PC is usually a sporadic disease, but familial cases have been reported in the literature. Germline mutations in the tumor suppressor gene *CDC73* (also known as *HRPT2*) are seen in hyperparathyroidism-jaw tumor (HPT-JT) and familial isolated primary hyperparathyroidism, and somatic mutations of *CDC73* are well known to develop in sporadic cases of PC (Jackson et al. 1993). Indeed, *CDC73* mutations have been reported in 15–100% of sporadic PC cases, compelling the necessity for routine germline screening (Schulte and Talat 2012; Frank-Raue et al. 2011; Shattuck et al. 2003). Although rare, PC or atypical parathyroid neoplasia was reported in patients with MEN1 (also called multiple endocrine neoplasia I) syndrome (Agha et al. 2007; Christakis et al. 2016). Other genes and/or their encoded proteins reported to be associated with PC are *RB1* (which encodes retinoblastoma protein), *CCND1* (also known as cyclin D1 and *PRAD1*), *TP53*, and *BRCA2* (Cetani et al. 2004; Cetani et al. 2016; Schulte and Talat 2012; Agha et al. 2007; Yu et al. 2015; Cryns et al. 1994).

Recent findings of whole-exome sequencing in patients with sporadic PC and local invasion or distant metastasis revealed previously unrecognized mutations. Mutations were reported in the following genes *PRUNE2*, *ADCK1*, *APOBEC*, *FAT3*, and *TNRC6A* (Yu et al. 2015; Pandya et al. 2017). *NF1, KDR,* and *PTEN* mutations have also been recently reported in PC (Kang et al. 2017). However, larger sample cohorts are clearly required to validate these findings.

Owing to the rarity and low prevalence of PC and the lack of recognized targetable molecular drivers for the disease, no data exist from therapeutic trials to guide therapy for advanced cases. Cytotoxic chemotherapy is ineffective, and external beam radiation therapy only adds value in very select cases of PC following surgery (Cetani et al. 2016; Schulte and Talat 2012; Christakis et al. 2017). When PC becomes widely metastatic and surgical options are no longer available, clinical management of hypercalcemia becomes the main line of therapy. Molecular profiling of advanced PC cases may enable a more precise diagnosis of the disease and perhaps more importantly provide novel treatment opportunities through targeted therapy. Next-generation sequencing (NGS) panels are now routinely used to screen for mutations to help diagnose and guide the treatment of thyroid, colorectal, lung, brain, and ovarian cancers as well as melanoma and other solid cancers (Singh et al. 2013, 2014).

Understanding the molecular aberrations in PC is crucial to inform the selection of targeted therapy and develop new targeted chemotherapeutic agents. The objective of our study is to analyze genetic profiles and identify mutations that may be targeted in recurrent or persistent and/or metastatic PC. We also present two cases with their mutation profile, choice of systemic therapy based on mutated pathways and their response to demonstrate the importance of understanding PC at the molecular and genetic level.

Patients and methods

This study was approved by the Institutional Review Board of the University of Texas MD Anderson Cancer Center (UTMDACC). All patients consented to participate in the study. Patients with an unequivocal diagnosis of PC based on World Health Organization criteria (DeLellis 2004) and with evidence of local recurrence or distant metastatic disease requiring further therapy at the UTMDACC between July 2014 and January 2016 were selected to undergo molecular testing. All laboratory and molecular testing was performed a part of their routine medical care.

Somatic mutation of tumors was performed in a stepwise manner to increase its ability to find mutational signatures present in few samples as well as mutational signatures exhibiting a low mutational burden (Fig. 1). In 10 of the 11 patients, the tumor was initially sequenced using a 50-gene panel (Ion Torrent, Life Technology). Of individuals who initially tested negative on the 50-gene panel, more comprehensive testing was performed. Six out of the seven patients with no mutation detected on 50-gene panel consented and enrolled for more comprehensive gene studies in which their tumor and paired germline were sequenced with a 409-full-length gene panel (Ion Proton) in the setting of an institutional wide study (Kopetz et al. 2015). Among the ten patients who initially had testing with 50-gene panel, a single patient had follow up tumor testing performed using

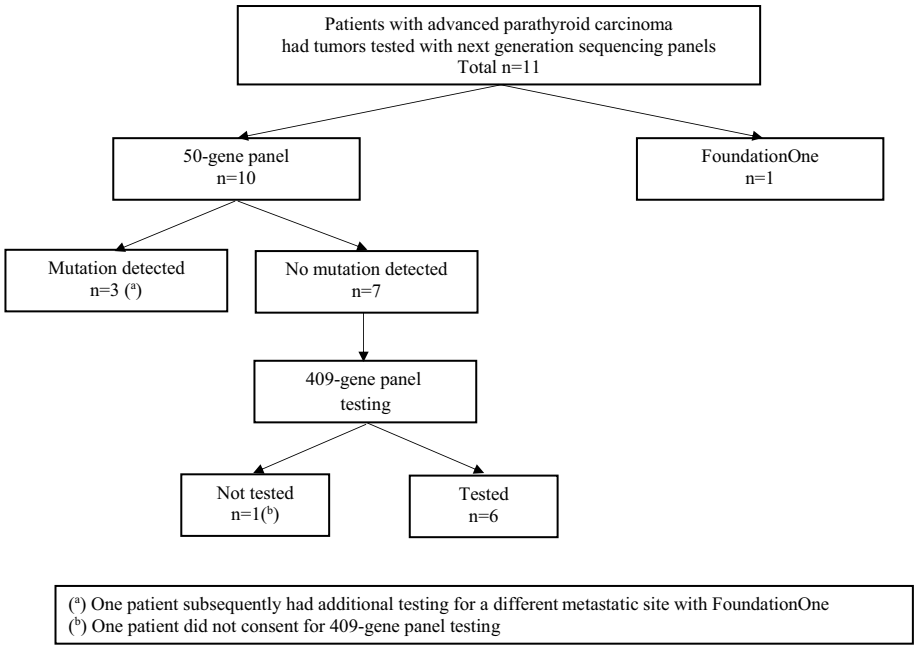

Fig. 1 Study design and method

the FoundationOne NGS panel as part of his clinical work up. One patient out of the total 11 had his tumor testing with FoundationOne as the only gene panel. All three gene panels in this study including 50-gene, 409-gene and FoundationOne were CAP accredited and CLIA approved.

The 409-gene panel is a comprehensive cancer gene panel of 409 full exomes of oncogenes and tumor suppressor genes that are frequently cited in cancer research. Genes tested in the standard 50-gene panel are also included in the 409-gene panel (https://www.thermofisher.com/order/catalog/product/4475346; https://www.thermofisher.com/order/catalog/product/4477685). The 409-gene panel is typically used if no targetable mutations were identified by the standard 50-gene panel and the patient experiences recurrence or residual disease with standard therapy (Singh et al. 2014). It is also known as the Cancer Mutational Scan 400. The genomic reference sequence used is genome GRCh37/hg19. The presence of visualized variants is read against the reference genome to denounce strand biases and sequencing errors.

The 50-gene and 409-gene panels only detect single nucleotide substitutions and small insertions or deletions. They do not cover gene rearrangements or copy number variations. FoundationOne NGS panel, on the other hand, provides data on single nucleotide substitutions, gene rearrangements, copy number variations, insertions or deletions and also information on microsatellite instability/stability and tumor burden (https://assets.ctfassets.net/vhribv12lmne/6YRrchSINOeSu48YwuesoY/0c3651c8421fa3647ccede76de9dce61). The 50-gene and FoundationOne panels did not have normal paired control samples from the same patient, while the 409-gene panel did include normal paired control samples in each patient. Therefore, germline mutations cannot be excluded in the patients who only had their tumor tested using the 50-gene or FoundationOne panel.

For clinical purposes, the effective lower limit of detection of the assay for single nucleotide variations (analytical sensitivity) was in the range of 5–10% by taking into consideration the depth of coverage at a given base and the ability to confirm low-level mutations using independent conventional platforms. All detected mutations were cross-checked with COSMIC and dbSNP genomic databases.

Pathologists in MD Anderson's Tissue Qualification Laboratory identified the optimal formalin-fixed, paraffin-embedded tissue blocks for the study. Tumor samples could be from primary and recurrent disease. From each paraffin block, a hematoxylin- and eosin-stained slide and unstained section were prepared. The tumor tissue was dissected from unstained section using the stained slide as template. DNA was then extracted from dissected tumor tissue using a QIAamp DNA FFPE Tissue Kit (Qiagen) and used for sequencing of genes in a 409-gene panel. All procedures were well established for the testing of solid tumors.

Results

Patient characteristics

Eleven patients with advanced PC requiring therapy were included in the study. Their demographic and clinical characteristics are shown in Table 1. The median patient age

Table 1 Patient characteristics

Patient	Sex	Ethnicity	Age at diagnosis, (years)	Vital status	Family history of parathyroid disease	Highest PTH level[a], pg/mL	Highest calcium level[a], mg/dL	Persistent disease	Metastasis sites	Neck surgeries (#)	Radiation therapy to neck after first surgery	First chemotherapy regimen	Second chemotherapy regimen
1	Male	White	27	Died of PC	None	11,403	14.6	Yes	Lung	4	Yes	Procarbazine and adriamycin[b]	Sorafenib
2	Female	Hispanic	44	Died of PC	None	8835	16.8	Yes	–	4	No	Sirolimus and cetuximab	Sorafenib
3	Female	White	62	Alive	None	614	12.5	Yes	Lung	2	No	–	–
4	Male	White	49	Alive	None	7050	14.6	Yes	Liver and bone	3	No	Sirolimus and hydroxychloroquine	–
5	Female	White	47	Alive	None	476,622	13.6	Yes	Liver, lung and bone	2	No	Vandetanib and everolimus	–
6	Female	White	42	Alive	Unknown type of neck tumor in maternal grandmother	218	12.5	Yes	–	4	No	–	–
7	Male	White	57	Alive	Parathyroid adenoma in daughter s/p parathyroidectomy, benign etiology	1581	12.8	Yes	Lung	4	No	Sorafenib	Lenvatinib
8	Female	Hispanic	62	Alive	None	119	16.7	Yes	–	2	No	–	–
9	Female	White	79	Alive	None	533	12.3	Yes	–	3	No	–	–
10	Male	White	51	Alive	None	855	14.6	Yes	–	4	No	Sorafenib	–
11	Male	White	74	Alive	None	260.8	11.4	Yes	Lung	2	Yes	–	–

PC parathyroid carcinoma, *PTH* parathyroid hormone, *s/p* status post

[a]The levels shown are the highest measured for each patient at our institution

[b]Patient 1 received multiple systemic chemotherapy regimens, each for a short period: procarbazine and adriamycin, sorafenib, lenalidomide and valproic acid, and lenvatinib

was 51 years and six patients were women. The median highest serum parathyroid hormone level detected was 855 pg/mL [range 119–476,622 pg/mL (normal range (nl): 9–80)], and the median highest serum calcium level was 13.6 mg/dL (range 11.4–16.8 mg/dL (nl: 8.4–10.2). All 11 patients had neck recurrence or persistent neck disease; however, six patients had distant metastasis, namely in the lung, liver, and/or bone. All the patients underwent multiple neck surgeries (median 3; range 2–4); however, only two patients received radiation therapy to the neck after initial surgery. Six patients received systemic chemotherapy, of which three had more than one chemotherapeutic regimen. At last follow-up, nine of the patients were still living with disease, and two patients had died of PC. One patient reported a family history of benign parathyroid disease, and another patient reported a family history of an unknown type of neck tumor.

Mutational analysis

Mutation analysis results of the 11 patients are summarized in Table 2. All of the specimen sites sent for molecular studies were from sites with recurrent or persistent disease. In 8 out of 11 patients, mutations were detected. *TP53* and *PIK3CA* were the only genes found to be recurrently mutated in PC patients, 3 and 2, respectively. When pathways were considered, four patients had mutations within PI3 K pathway (*PIK3CA, TSC1* and *ATM*). Three patients had mutations involving multiple genes. In one of these patients, somatic mutations were found in the *MEN1*, *TERT* promoter and *DICER1* genes. A second patient had multiple genetic alterations in several genes (*TP53, TSC1*, *BCL2L1, DCC, EP300, EPHB4, GUCY1A2, KDM6A, MAGI1, POT1, RNASEL)*. A distinct somatic mutation in the tumor suppressor gene *CDC73* was detected in one patient who had familial hyperparathyroidism-jaw tumor syndrome. His *CDC73* germline mutation was R76X. This patient's tumor harbored a different pathogenic somatic mutation in the *CDC73* gene which was Y55C. No other patients in our cohort had somatic or germline *CDC73* mutations. In this study, we also did not find any other familial gene mutations linked to PC (i.e., *MEN1* or *RET* mutations). One patient had a mutation detected in the *ATM* gene, labeled a variant of unknown significance in 50-gene panel (left thyroid bed tissue) and subsequently had his metastatic tumor (esophagus) test positive *SDHA* gene mutation in FoundationOne platform. A single patient had no mutation detected in 50-gene panel but declined to enroll in the 409-panel study.

Case studies

Two case examples illustrate how mutational profiles can direct the use of systemic therapies. Described below are the biological reasons of choosing targeted therapy options and the clinical course and outcomes derived from those choices.

Patient No. 5 is a female who was diagnosed with PC at age of 47 years. She initially presented with hypercalcemia (serum calcium 12–14 mg/dl (nl: 8.4–10.2)) and hyperparathyroidism (PTH 320–350 pg/ml (nl: 9–80)). The patient underwent parathyroidectomy and hemithyroidectomy which initially resolved her hypercalcemia. She had a recurrence of her PC (calcium ranging between 12.6 and 13.7 mg/dl) which was treated with neck dissection. Subsequently, she was diagnosed with liver metastasis. Tumor testing identified somatic mutations in *TP53* and *TSC1*, and several other genes (*BCL2L1, DCC, EP300, EPHB4, GUCY1A2, KDM6A, MAGI1, POT1, RNASEL)* (Table 2). The presence of the *TSC1* mutation, a known regulator of the PI3 K/mTOR pathway, provided support for systemic therapy with an mTOR inhibitor. Additionally, because VEGF has been shown to be overexpressed in parathyroid tumors in general, and thought to be a driver (Lazaris et al. 2006), vandetanib (an antiangiogenic drug) and everolimus (mTOR inhibitor) were started. The patient's best response to treatment was stable disease (observed at two and a half months) and her serum calcium levels were better controlled (10–11 mg/dL) while on drug. She voluntarily withdrew from drug use to attempt surgical metastasectomy of her liver and neck lesions (her only two sites of disease). She also had percutaneous radiofrequency ablations for liver metastases as well as reoperation in the neck with superior mediastinal lymph node and thymus resection, which helped control her disease structurally and biochemically.

Patient No. 7 is a male who was diagnosed with HPT-JT syndrome at age of 57 years. At diagnosis, his serum calcium level was 13 mg/dl with an elevated PTH level of 698 pg/ml. He initially underwent parathyroidectomy of 1 gland and followed by thyroidectomy and removal of other parathyroid glands due to persistent hyperparathyroidism and hypercalcemia. He was later found to have repeat calcium levels in the 15–17 mg/dL range and renal insufficiency (GFR 53) which was linked to recurrent neck disease and pulmonary metastases. These were treated with lung segmentectomy and multiple wedge resections. His hypercalcemia, despite treatment with cinacalcet, intermittent doses of bisphosphonate therapy and intravenous fluids, remained high (12–15 mg/dl with PTH ranging between 710 and 807 pg/ml). In an attempt to identify targets for systemic therapy, mutational profiling was ordered. The 50- and 409-gene panels identified a pathogenic somatic mutation in the *CDC73* gene (Y55C). His germline testing was performed as a routinely recommended procedure in our clinic. He was

Table 2 Summary of mutational analysis

ID	Gene panels	Specimen site	Gene	CDNA change	Protein change	Reference sequence	DbSNP_ID	COSMIC_ID
1	50-gene	Left thyroid bed	*ATM*	c.5071A>C	S1691R	NM_000051.3	rs1800059	COSM6005491
	FoundationOne	Esophagus	*SDHA*	Splice site 1432_1432 + 1delGG				
2	50-gene	Soft tissue (neck)	*TP53*	c.742C>T	R248W	NM_000546.5	rs121912651	COSM10656
3	50-gene[a]		No mutation					
4	50-gene[b]		*PIK3CA*			E545 K	rs104886003	COSM763
5	409-gene	Soft tissue (midline suprasternal neck)	*TP53*	c.916C>T	R306*	NM_000546.5	rs121913344	COSM10663
			TSC1	c.682C>T	R228*	NM_000368.4	rs118203427	COSM5711175
			TSC1	c.73G>A	V25M	NM_000368.4		
			BCL2L1	c.3G>A		NM_138578.1		
			DCC	c.2796C>A	S932R	NM_005215.3		
			EP300	c.5332G>A	G1778R	NM_001429.3		
			EP300	c.5356A>G	I1786V	NM_001429.3		
			EPHB4	c.2198A>G	E733G	NM_004444.4		
			GUCY1A2	c.769G>A	A257T	NM_000855.2		
			KDM6A	c.1849C>G	L617V	NM_021140.2		
			MAGI1	c.3061G>A	E1021K	NM_004742.2	rs397518423	COSM1692978
			POT1	c.767A>G	Q256R	NM_015450.2		
			RNASEL	c.662T>C	I221T	NM_021133.3		
6	409-gene	Soft tissue (neck)	*TP53*	c.974_983del	G325fs	NM_000546.5		COSM6925016
			NF1	c.2251G>T	G751*	NM_001042492.2		COSM560399
7	50-gene	Soft tissue (neck)	*APC*	c.3949G>C	E1317Q	NM_000038.5	rs1801166	COSM19099
	409-gene	Lung	*CTNNB1*	c.214C>T	Q72*	NM_001904.3		
			CDC73	c.164A>G	Y55C	NM_024529.4		
			KDM5C	c.1606G>A	G536R	NM_004187.3		
	Germline		*CDC73*	c.226C>T	R76X (germline)			rs886041158
8	409-gene	Lymph node (neck)	*TLR4*	c.2008G>T	G670C	NM_138554.4		
9	409-gene	Central neck	No mutation					
10	409-gene	Mediastinal	No mutation					
11	FoundationOne	Trachea	*PIK3CA*	Not available	Q546R	Not available	rs397517201	COSM12459
			PTEN	Not available	D107Y	Not available	rs57374291	COSM5212
			MEN1	Not available	S15fs*104	Not available		
			DICER1	Not available	Y1335fs*12	Not available		
			TERT promoter	124 C>T		Not available		COSM1717360

VUS variant of undetermined significance, *SNV* single nucleotide difference

* stop codon

[a]Patient did not give consent for the 409-gene panel

[b]Patient 4′s 50-gene analysis was done at an offsite facility and not repeated at our institution

found to have a distinct germline mutation in the *CDC73* gene (R76X). The identification of this mutation confirmed his HPT-JT syndrome diagnosis and also supported the two-hit hypothesis in oncogenesis (Knudson 2001). Additional somatic mutation of *KDM5C* gene was also found. Given evidence that in renal cell carcinoma patients with *KDM5C* mutations have positive responses with first-line sunitinib, a potent inhibitor of all VEGF and PDGF receptors (Hsieh et al. 2017; Le Tourneau et al. 2007), an antiangiogenic drug was considered for therapy. The patient was treated with sorafenib, which is an antiangiogenic multi-targeted tyrosine kinase inhibitor with a target profile similar to sunitinib (Nexavar prescibing information 2018). Initially, the patient's hypercalcemia and hyperparathyroidism were well controlled. He became hypocalcemic after 3 months so his cinacalcet was discontinued and remained normocalcemic despite radiography that his neck and lung tumors showed a stable rather than reducing response to treatment. After 3 years on sorafenib therapy, however, his calcium and parathyroid hormone levels started to rise again with progression of disease in the lungs. At this time, his serum calcium was 12.8 mg/dl and PTH was markedly elevated to 1471 pg/ml. Sorafenib was discontinued and a second more potent antiangiogenic inhibitor lenvatinib was initiated (Matsui et al. 2008). The patient has been on lenvatinib for 20 months with radiographically stable disease. Furthermore, his

calcium level is well controlled at 9–10 mg/dL without any calcimimetic medication.

Discussion

In this study, we used NGS panels to identify mutations in patients with advanced persistent or recurrent and/or metastatic PC. The majority of the mutations found in our study were loss of function of tumor suppressor genes rather than gain of function mutations of oncogenes (Table 2). Genetic changes in PI3 K and TP53 pathways, which are the common mutations found in advanced PC and similar to what has been reported in other published studies were found in our studies. In addition, new mutations that have not been reported in PC were also identified.

Mutation of *TP53*, which encodes p53 transcription factor, is the most studied cancer-causing somatic mutation and is associated with many types of cancer, including PC (Stracquadanio et al. 2016). It has been shown to play an important role in multiple cellular processes such as transcriptional regulation, microRNA processing, cell cycle control, and apoptosis (Stracquadanio et al. 2016). We found *TP53* somatic mutations in three patients in our cohort. Despite many efforts and ongoing research, targeted therapy for p53 has not yet been identified (Khoo et al. 2014).

The *PIK3CA* gene encodes the 110 kDa subunit of phosphatidylinositol 3-kinase (PI3 K). The PI3 K/AKT/mTOR pathway is a well-characterized cancer driver pathway. It has crucial roles in cell growth and proliferation and cell survival and is regulated through phosphorylation of key pathway components (Cantley 2002). Mutations of *PIK3CA* have been studied extensively in breast cancer, colorectal cancer, non-small cell lung cancer, ovarian cancer, thyroid cancer and other solid tumors (Yuan and Cantley 2008;Liu et al. 2008; Shayesteh et al. 1999). It also has been reported in PCs (Pandya et al. 2017; Kasaian et al. 2013). Interestingly, in a single case study by Kasaian et al. (2013), *PIK3CA* mutation was found in the primary but not recurrent tumor which suggests the role of *PIK3CA* mutation in tumor initiation. However, the mechanism and meaning of the loss of activating mutation of *PIK3CA* in recurrent tumor like in this case is not clear. Several inhibitors of PI3 K and AKT are currently being studied in clinical trials (Owonikoko and Khuri 2013).

A distinct somatic mutation in the tumor suppressor gene *CDC73* was detected in one patient who had familial HPT-JT syndrome. *CDC73* mutations have been previously detected in patients with HPT-JT and also reported in 20–29% of individuals with apparently sporadic PC (Jackson et al. 1993). Interestingly, no other patients in our cohort had somatic or germline *CDC73* mutations. In this study, we also did not find any other familial gene mutations linked to PC (i.e., *MEN1* or *RET* mutations).

In this cohort, we identified two patients (patient 5 and 7) with alterations in histone lysine demethylases (*KDM* genes). KDMs have been observed to be overexpressed/amplified or mutated in other cancers such as hepatocellular carcinoma and kidney cancer (Ji et al. 2015; Hakimi et al. 2013) and have been linked to worse prognosis in some cases (Paolicchi et al. 2013). *KDM5C* gene is located on chromosome X (Wu et al. 1994). *KDM5C* loss of function mutations have been reported to be more common in males by escaping X-inactivation (Dunford et al. 2017). Single-allele mutations of *KDM5C* gene might be more relevant in male than female patients with regard to clinical presentation and therapeutic consideration (Hsieh et al. 2017). Patient 7 who harbored a *KDM5C* mutation in our study was also a male. He demonstrated stabilization of their tumor growth and good control of their hypercalcemia from anti-VEGF therapy as presented above. This single patient observation will require studies in an expanded population to determine if *KDM* gene mutation should be used to drive therapy selection or if the antiangiogenic agents should be broadly considered to treat PC.

A single patient within our cohort had a *TSC1* gene mutation. This tumor suppressor gene that encodes hamartin, forms the harmartin–tuberin complex that is responsible for the mTOR activity (Curatolo and Maria 2013). Consequently, mTOR increases if the hamartin–tuberin complex is impaired, resulting in dysplasia, angiogenesis, and tumorigenesis (Huang and Manning 2008). The role of *TSC1* in tumorigenesis is highlighted in several cases of bladder cancers (Guo et al. 2013; Hornigold et al. 1999). To our knowledge, *TSC1* mutation has not been reported in PC. Recently, mTOR inhibitors have been reported to be effective for the treatment of tumors with *TSC1* mutations (Iyer et al. 2012) and *TSC2* mutations (Huynh et al. 2015). However, despite the promising results attained with everolimus, the occurrence of resistance to mTOR inhibitors is not uncommon (Bihani et al. 2015).

Mutations in *SDHA, TERT* promoter and *DICER1* genes have been reported and studied in other endocrine neoplasia disorders and for the first time reported in PC in this study.

SDHA gene is known as a tumor suppressor gene which plays an important role in the tumorigenesis of pheochromocytoma and paraganglioma (Burnichon et al. 2010). *SDHA* mutations can also be associated with pituitary adenoma and gastrointestinal stromal tumors (Dwight et al. 2013a, b). SDHA mutations have not been reported in parathyroid carcinoma. In this study, the SDHA mutation was found in a metastatic site (esophagus) of the tumor. SDHA gene is not included in the 50-gene panel, therefore it is not clear if this was a secondary mutation acquired during his disease progression or had been present in the original tumor.

TERT promoter mutations are relatively common with high frequency in many cancers including the central

nervous system, thyroid, bladder and melanoma (Vinagre et al. 2013). The coexistence of *TERT* mutations with others or their polymorphisms can serve as prognostic factors in certain malignancies, for example, thyroid cancer and primary glioblastoma (Xing et al. 2014; Mosrati et al. 2015). It has been rarely reported in atypical parathyroid adenoma (Haglund et al. 2015) but has never been reported in parathyroid carcinoma. Given the potential therapeutic effects of inhibiting overexpressed telomerase activity in tumor growth, several agents targeting telomerase enzyme in *TERT* mutated cancers are being studied (Celeghin et al. 2016; Burchett et al. 2014).

DICER1 gene is involved in the modulation of gene expression at the posttranscriptional level through the regulation of microRNAs. Mutations in this gene have been identified in various cancers. Germline loss of function mutations in *DICER1* is the hallmark of a tumor susceptibility syndrome, in which individuals carrying the mutations are at higher risk of developing cancers such as cystic nephroma, pleuropulmonary blastoma, pinealblastoma and ovarian sex-cord stromal tumors (Foulkes et al. 2014). *DICER 1* mutations have not been described in PC.

Our study is the first to report mutations in *SDHA*, *TERT* promoter and *DICER1* genes in patients with advanced PC. In our cohort, we also found other gene mutations such as *BCL2L1, DCC, EP300, EPHB4, GUCY1A2, MAF11, POT1, RNASEL, ATM,* and *TLR4*. Each has been reported elsewhere to have a role in certain malignancy such as colorectal cancer (*DCC*) (Gotley et al. 1996), melanoma (*POT1*) (Robles-Espinoza et al. 2014), lymphoma (*ATM*) (Schaffner et al. 2000), and prostate cancer (*RNASEL*) (Rokman et al. 2002). Given that in our study, these mutations were found in a single tumor, the significance of these gene mutations in PC is not quite understood at this point. It will be helpful to perform further studies to elucidate the molecular mechanisms of these mutations in PC tumorigenesis.

Identifying genetic mutations responsible for tumor formation and growth could be crucial in understanding cancer biology and subsequently choosing a systemic therapy for PC patients. In our study, potential actionable mutations were identified in 6/11 (54%) patients (patient 1, 4, 5,6, 7 and 11). *ATM, PIK3, TSC, NF1* genetic mutations were potentially targetable in the mTOR/PIK3 pathways. As in the above discussion, there are targeted therapies which have been known to be effective in non-PC tumors with similar mutated genetic pathways. None of these targeted therapies have been approved by the Food and Drug Administration to use for PC. However, in the setting of no established systemic therapies in advanced PC, these therapies can be considered. Genetic testing can yield important information in understanding tumor formation and development as well as guiding the selection of targeted therapies. In this manuscript, we described two case examples of using NGS as a clinical tool to guide targeted therapies. Using this approach, our group now has treated other patients with advanced PC with some success.

It is important to note that our study has limitations. First of all, it is a small study. Given that we examined 11 patients, the identified genes may not have been the driver genes of PC in all cases. However, PC is such a rare disease that conducting a study with a large number of patients is a challenging task. These data are useful, but genetic screening should be done in larger groups of patients. Secondly, the 50-gene and 409-gene panels, despite being extensive panels at this current time point, do not cover all potential genetic defects and most notably do not test for gene rearrangements or copy number variations.

Conclusion

In conclusion, this is the first study to use 50- and 409- gene panels to identify actionable mutations in patients with persistent recurrent and/or metastatic PC that require systemic therapy. Gene analysis may be a useful clinical tool for directing targeted therapy for advanced PC patients. Larger studies are needed to validate these findings and identify targetable mutations in PC patients. Clinical trials targeting these mutations are warranted including the development of new therapies targeting identified mutations.

Acknowledgements We wish to thank Mrs. Jennifer Jordan and Mr. Len Jordan for their generous contributions that made this study possible. We thank Bryan Tutt from the Scientific Publications Department at MD Anderson Cancer Center for editing our manuscript.

Funding Supported by the NIH/NCI under award number P30CA016672 and philanthropic donation from patients

Compliance with ethical standards

Conflict of interest NLB has received grant funding from Novartis and Bayer and consulting fees from Eisai. MEC has received grant funding from Eisai. MK, HTN, LK, CC, AS, EI, SGW, CJ, MH, SS, SK, RB, RD, KW, MW, MZ, NP: nothing to disclose

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