Original paper

Folia Neuropathologica

Thyroid carcinoma metastases to central nervous system and vertebrae

Bette Kleinschmidt-DeMasters, Carrie Marshall

Department of Pathology, University of Colorado Anschutz Medical Campus, Aurora, CO, United States

Folia Neuropathol 2022; 60 (3): 292–300 *DOI: https://doi.org/10.5114/fn.2022.118608*

Abstract

Introduction: *Metastatic thyroid carcinoma rarely involves the parenchyma of the central nervous system (CNS) or vertebral bones. While various mutations have been identified in primary thyroid carcinomas and differ based on the histological type, little is known about the molecular features of thyroid carcinoma metastases to brain or spinal column. Based on limited prior literature, we hypothesized that TERT mutations might be enriched in CNS metastatic lesions.*

Material and methods: *CNS/vertebral metastases were identified via database search, 1.01.2006 to 9.08.2021, and mutation/fusion testing performed.*

Results: *21 surgically resected lesions were identified from 16 patients: 15/21 metastases were to the vertebral bone, requiring neurosurgical intervention for cord compression and 6/21 metastases were intraparenchymal. Male : female ratio was 1 : 1, with median age at the time of CNS metastasis of 62 years. Metastases were of varied histological types, with follicular the most common; the histological subtype often matched in patients with multiple CNS metastases although 2 patients showed dedifferentiation in subsequent metastases. Diagnosis of thyroid carcinoma antedated development of CNS metastases in all but 2 patients in whom a surgically-resected bone metastasis represented their first diagnosis. Intervals for the remaining 14 patients from primary to CNS/vertebral metastasis ranged from 6 months to 41 years. Mutations were multiple in 14/15 cases, including TERT (n = 12) and NRAS (n = 9), with fewer TP53, ATM, AKT1, PTEN, NOTCH1 mutations. Two specimens had fusions involving RET.*

Conclusions: *TERT mutation occurred in a significantly higher percentage (80%) of mutations than reported for primary tumors, underscoring the need for molecular testing of the metastases, should a targeted therapy become available.*

Key words: *mutation, thyroid carcinoma, metastasis, RAS, TERT.*

Introduction

Metastases from thyroid carcinoma impacting the central nervous system (CNS) are rare, with a 1% incidence of brain metastases for differentiated thyroid carcinoma types and 20% incidence of bone metastases [9]. Not all CNS metastases require biopsy/surgical excision for diagnosis or treatment and thus, specimens for molecular studies are difficult to accrue in large numbers. The most likely metastases to come to surgery are single lesions in patients without a known primary tumor or vertebral bony/epidural masses impacting spinal cord function, necessitating emergency decompression.

Genetic features of thyroid carcinoma at its primary site have been studied, but considerably less is known about molecular features of metastatic lesions, particularly those to specific anatomical regions, including those impacting the CNS. In the primary tumor, the most prevalent reported genetic alterations are recognized to differ by histological subtype [30]. Specifically, papillary carcinoma of the thyroid has been shown to have mutations in *BRAF* (40-45% of cases), *RAS* (10-20%), *RET/PTC* (10-20%), and *TRK* (< 5%) [30]. Follicular carcinoma demonstrates mutations in *RAS* (40-50%), *PAX8/PPARγ* (30-35%), *PIK3CA* (< 10%), and *PTEN* (< 10%) [30]. As poorly differentiated and anaplastic thyroid carcinomas

Communicating author:

Bette Kleinschmidt-DeMasters, Department of Pathology, University of Colorado Anschutz Medical Campus, Aurora, CO, United States, e-mail: bk.demasters@cuanschutz.edu

most often arise from pre-existing differentiated carcinoma types, they often retain the mutations common to differentiated carcinomas, but gain additional mutations in *TP53*, *CTNNB1* and *AKT1*. Poorly differentiated thyroid carcinomas more frequently have mutations in *RAS* (20-50%), *TP53* (10-35%), *BRAF* (5-15%), *CTNNB1* (0-5%) and *PIK3CA* (0-15%) [43]. Anaplastic thyroid carcinoma shows *TP53* (40-80%), *CTNNB1* (0-5%), *RAS* (10-50%), *BRAF* (10-50%), *PIK3CA* (5-25%) and *PTEN* (10-15%) mutations [44]. Sporadic medullary carcinoma harbors mutations in *RET* (40-50%) and *RAS* (25%) [30]. As noted in one recent review, "multiple genes are implicated in the development of thyroid neoplasms, both benign and malignant ...in genes which are responsible for cell proliferation, survival and differentiation through different pathways. Approximately 90% of mutations are mutually exclusive activating mutations in oncogenes *RAS* (~13%) and *BRAF* (~60%), ...whilst the remaining 10% are loss-of-function mutations affecting tumour suppressor genes such as *PTEN*, *PPARγ* and *TP53*" [38]. A second recent review emphasizes that "the most frequent driver events can be either point mutations or gene rearrangements, mainly affecting the MAPK pathway and phosphatidylinositol-3 kinase (PI3K)/AKT pathway" [24].

Reactivation of telomerase reverse transcriptase (TERT) occurs in several types of cancer, including thyroid cancers, especially in 2 hotspots in the promotor region c.124C>T and c.146C>T [35]. In a large study of > 200 various histological types of thyroid carcinoma, *TERT* promotor mutations involving these 2 hotspots were detected in 7% of papillary, 18% of follicular, 25% of Hurthle cell, and 86% of poorly differentiated/anaplastic carcinomas at the primary site [35].

We hypothesized that if targeted therapies become available in the future that molecular assessment of the metastasis itself, rather than the primary tumor, might be optimal for patient management and that metastases might be enriched for mutations seen in a higher percentage of aggressive thyroid carcinoma types, such as *TERT* promotor mutations. In addition, we especially were interested in *TERT* promotor mutations, given the fact that their presence is definitional for the highest grade in some tumor types, such as meningiomas and astrocytic lineage tumors that are wildtype for isocitrate dehydrogenase (IDH) [7,39]. Therefore, we interrogated our databases to identify thyroid metastases resected at our institution over the past 15 years from either spinal column or CNS parenchyma. We recorded information regarding the date of first resection of the primary thyroid tumor, histological type of the primary tumor, and assessed the metastases for histological type, following which we tested the metastases for mutations and fusions. We then compared the frequency and types of mutations we identified with well-published data from the literature.

Material and methods

Computer-based database queries of our Surgical Pathology Department files were utilized to identify cases *via* text word search, 1.01.2006 to 9.08.2021, inclusive, linking the text words "thyroid" and "metastatic" with "brain" or "central nervous system". Once identified, slides were retrieved from the files and all cases were re-examined microscopically by the expert endocrine pathologist on the paper (CBM) to confirm diagnosis and classify the type of thyroid carcinoma, as most had been originally diagnosed only as "metastatic thyroid carcinoma" without further classification. Metastases were categorized as to papillary, follicular, papillary carcinoma-follicular variant, poorly differentiated, anaplastic, Hurthle cell, and medullary carcinoma subtypes.

After obtaining internal review board approval (COMIRB #21-3458), clinical history was extracted from the medical record, including anatomical location of the metastasis/metastases involving the CNS parenchyma or vertebral bodies, date of diagnosis and histological type of the primary thyroid carcinoma if known, and date of demise or survival as of 9.08.2021. The survival interval from first diagnosis of thyroid carcinoma to development of first metastasis involving the CNS parenchyma or vertebral bodies was calculated.

Unfortunately, given the retrospective nature of the study, the referral nature of patients to our tertiary care center, and the tendency for patients to receive care at multiple different hospitals, the slides or paraffin tissue blocks from the original primary thyroid carcinoma were not available for nearly all our cases. Thus, we were unable to directly compare genetic features between paired primary thyroid and metastatic samples.

The next generation sequencing (NGS) gene fusion and gene mutation assays were validated and performed for 12 cases in the Colorado Molecular Correlates Laboratory in the Department of Pathology at the University of Colorado – Anschutz Medical Campus. Total nucleic acid (TNA) was extracted from formalin fixed paraffin embedded (FFPE) processed material via the Agencourt FormaPure Kit (Beckman Coulter, Brea, CA). TNA was processed for gene fusion analysis via the Archer FusionPlex Solid Tumor library preparation kit and for gene mutation analysis by a customized version of the Archer VariantPlex Solid Tumor library preparation kit (ArcherDx, Boulder, CO). The resulting libraries were sequenced on either the Illumina MiSeq or Illumina NextSeq instruments (Illumina, San Diego, CA). Raw sequence data were analyzed using the ArcherDx Analysis software package (version 4.1.1.7 for fusions,

version 5.1.2 for mutations, ArcherDx). Bioinformatically identified fusions and mutations were manually inspected by highly trained personnel. Methods have been previously published [3,13,37,56]. Mutation analysis for three cases was ordered by the oncology team and performed prior to this study at outside national reference laboratories.

Results

Our database search identified 21 resection specimens from 16 patients, with 2 patients each having had 3 surgical resections of spinal bone lesions (cases 2, 3, 4 from a female who was 51 years of age at first resection; cases 6, 7, 8 from a female who was 63 years of age at first resection) (see Table I). Surgical excisions in these 2 women had been undertaken over a 6-year time period for the first and over a 1-year time interval for the second. One man initially presented with a pituitary mass thought preoperatively to be a non-secretory pituitary macroadenoma (case 15), but proven to be thyroid carcinoma after histological examination. He then required surgical decompression of an L1 bone mass the following year (case 16); this was the sole patient with thyroid metastases from both bone and intraparenchymal compartments. All bone lesions were to the spine; none to the skull or other bony sites were identified in our database.

Gender distribution was equal (8 males, 8 females), ages 25-71 years; excluding the youngest patient (age 25) with medullary carcinoma of the thyroid who had MEN2B syndrome, there was a median age of 62 years (Table I). Metastases were of all types, although the most frequent type was follicular thyroid carcinoma (FTC) (n = 9 patients, plus one with FTC-Hurthle cell features, case 11). There was a single case of medullary carcinoma, two cases of classical papillary thyroid carcinoma, and one case of follicular variant of papillary thyroid carcinoma. Four cases were poorly differentiated thyroid carcinoma (PDTC) and three were anaplastic thyroid carcinoma (ATC). In both patients with multiple bone resection specimens, the histologic type changed; from FTC to PDTC (cases 2, 3, 4) and from FTC to ATC (cases 6, 7, 8) (Table I). Three patients had a different histological type for their primary tumor recorded in the medical record than was found in the metastasis (cases 10, 17, 20). However, since the original slides and blocks were not available from the primary tumor,

Table I. Spinal and parenchymal thyroid metastases impacting CNS

Case #	Age, gender, metastatic site	Date of neurosurgical excision of metastasis/ type	First diagnosis of thyroid carcinoma, type	Survival after first surgically resected metastasis	Interval from initial diagnosis to CNS metastasis	Mutation result	Fusion results
BONY METASTASES (n = 16 surgical specimens from 10 patients; case 2, 3, 4 are from one individual and case 6, 7, 8 are from one individual)							
1	62 y, male, C2 bone	2014 PDTC	2013 PDTC	DOD 4 y 2 months	13 months	*PTEN*, *TERT*, *FANCC*	Not performed
2	51 y, female, C5-C6 bone	2012 FTC	2009 FTC	DOD 6 y 7 months	3 y 3 months	Not performed (decal)	Not performed (decal)
3	56 y, female, C6 bone	2017 FTC	2009 FTC	DOD 6 y 7 months	3 y 3 months	Not performed (decal)	Not performed (decal)
4	57 y, female, C4-C7 epidural	2018 PDTC	2009 FTC	DOD 6 y 7 months	3 y 3 months	*NRAS*, *TERT*	Not performed
5	69 y, male, T7-T8 bone	2019 PTC	1978 PTC	DOD 1 y 9 months	41 y 1 months	Not performed	NCOA4-RET
6	62 y, female, T9 bone	2007 FTC	2007 FTC	DOD 8 months	Bone metastasis first diagnosis of thyroid carcinoma	Not performed (decal)	Not performed (decal)
7	62.5 y, female, T8 bone	2008 FTC	2007 FTC	DOD 8 months	Bone metastasis first diagnosis of thyroid carcinoma	*NRAS*, *PTEN*, *TERT*	Failure

Table I. Cont.

Case #	Age, gender, metastatic site	Date of neurosurgical excision of metastasis/ type	First diagnosis of thyroid carcinoma, type	Survival after first surgically resected metastasis	Interval from initial diagnosis to CNS metastasis	Mutation result	Fusion results
8	62.5 y, female, T8 bone (repeat surgery)	2008 ATC	2007 FTC	DOD 8 months	Bone metastasis first diagnosis of thyroid carcinoma	*NRAS*, *PTEN*, *TERT*	Failure
9	51 y, male, T2 bone	2014 PTC (tall cell)	2013 PTC	AWD 4 y 9 months	1 y 3 months	Not performed (decal)	Not performed (decal)
10	55 y, female, T8-T10 bone	2018 FTC	2008 Follicular adenoma	DOD 10 months	10 y 2 months	*NRAS*, *GNAS*, *TERT*	Not performed
11	62 y, female, T12 bone	2015 FTC-HC	2013 HC	DOD 5 y 5 months	2 y 4 months	*TERT*	Fusion negative
12	47 y, male, L5 bone	2013 ATC	2012 ATC	DOD 5 months	1 y 11 months	*NRAS*, *TP53*	Fusion negative
13	70 y, female, T12-L1 bone	2020 FTC	2020 FTC	AWD 1 y 5 months	Bone metastasis first diagnosis of thyroid carcinoma	*NRAS*, *TERT*	Failure
14	55 y, male, L1 bone	2020 FTC	2017 FTC	AWD 11 months	3 y 8 months	*NRAS*, *TERT*	Fusion negative
15	56 y, male, L1 bone	2014 FTC	2005 FTC	DOD 1 y 7 months	7 y 8 months	*NRAS*, *TERT*, *PIK3CA*	Fusion negative
INTRAPARENCHYMAL METASTASES (*n* = 6 surgical specimens, 1 patient with both bone metastasis (case 15) and parenchymal metastasis (case 16))							
16	54 y, male, pituitary	2013 FTC	2005 FTC	DOD 1 y 7 months	7 y 8 months	Not performed (tissue exhausted)	Not performed (tissue exhausted)
17	59 y, male, right intraventricular/ choroid plexus	2010 PDCA	1997 PTC-FV	DOD < 1 month	13 y 9 months	*NRAS*, *TP53*	Fusion negative
18	71 y, female, right frontal	2018 FV-PTC	No exact date found in chart Unknown	AWD 2 y 9 months	"decades" thought to be > 30 years	*ATM*, *TERT*	CCDC6(ex1)-RET(ex12)
19	69 y, female, left temporal	2011 PDCA	2003 Unknown	DOD 11 months	7 y 11 months	*AKT1*, *NOTCH1*, *NRAS*, *TERT*	Fusion negative
20	59 y, female, left parieto-occipital	2014 ATC	2014 PTC	DOD 1 y	6 months	*ATM*, *TERT*, *TP53*	Fusion negative
21	25 y, female, left occipital	2016 MTC	2012 MTC	AWD 4 y 10 months	4 y 5 months	*PDGFRA*, *RET*	Fusion negative

y – year, PDTC – poorly differentiated thyroid carcinoma, FTC – follicular thyroid carcinoma, PTC – papillary thyroid carcinoma, HC – Hurthle cell carcinoma, ATC – anaplastic thyroid carcinoma, MTC – medullary thyroid carcinoma, FV – follicular variant, C – cervical, T – thoracic, L – lumbar, DOD – died of disease, AWD – alive with disease

this possible change in type could be neither confirmed nor refuted.

Spinal bone lesions involved all levels in similar numbers, i.e. cervical, thoracic, and lumbar. Intraparenchymal sites constituted 6/21 lesions, with 1 pituitary lesion, 1 in intraventricular/choroid plexus, and 1 each located in frontal, temporal, parieto-occipital and occipital lobes.

Most patients were known to have thyroid carcinoma antecedent to developing their CNS lesion, although in 2 persons, the surgically resected bone metastasis represented their first diagnosis. Intervals for the remaining 14 patients from the time of primary thyroid carcinoma diagnosis to the time of surgical resection of the CNS metastasis ranged from 6 months to 41 years (Table I).

Intervals to demise for those known to have died from disease ranged from < 1 month to over 6 years. A significant number (5/16) are known to be alive with disease at the closure of the study (9.08.2021), with the longest survival interval of 4 years, 10 months, including 2 of the 7 patients with parenchymal brain metastases (both patients are receiving targeted therapy as their tumors have *RET* mutations). Thus, although the case numbers in our cohort are too limited for a meaningful epidemiological study, we did observe that diagnosis of CNS metastasis did not imply imminent demise (Table I).

For the mutation/fusion testing performed for this study, only cases with FFPE material that had not been subjected to decalcification could be used, as exposure to the acid solution degrades the genetic material. This excluded from analysis 4 vertebral body/bone cases where all material had been decalcified (cases 2, 3, 6, 9). One parenchymal case could not be tested as there was no tumor material remaining in the block (case 16). In total, mutational analysis was completed in 10 of 15 bone metastases and 5 of 6 parenchymal metastases. Fusion analysis was attempted in 13 specimens, with 3 cases (all bone specimens) found to be "uninformative' for fusion results because the RNA quality needed for fusion testing was determined to be too poor to trust a negative result. The metric that guided this decision was based on a specific sequencing metric utilized in our laboratory. Two cases were found to have a fusion and eight were negative for fusions.

In terms of informative mutation results, the most frequently identified mutations were in *TERT* promotor, with 80% of all metastases having this mutation (n = 12, only one case with sole *TERT* mutation, case 11). In our study, of the 10 bony vertebral metastases that could be assessed for the mutation (i.e., not decalcified), 9 of 10 had *TERT* promotor mutation and of the 5 assessable CNS parenchymal metastases, 3 showed *TERT* promotor mutation (Table I).

The next most frequent were *NRAS* with 66.6% having this mutation (n = 10), and *TP53* with 20% having this mutation (n = 3); fewer *ATM*, *AKT1*, *PTEN*, *NOTCH1* mutations were also identified. The latter 4 mutations were all found in concert with other mutations known to be more frequent at the primary site for thyroid carcinoma.

Specifically, papillary thyroid carcinoma (PTC) has been shown to have mutations in *BRAF* (40-45% of cases), *RAS* (10-20%), *RET/PTC* (10-20%), and *TRK* (< 5%) [30]. In our study, we had only a single papillary carcinoma of the thyroid (case 5, vertebral body metastasis) that unfortunately could not be assessed for mutation. Of the parenchymal metastases, 1 was a follicular variant-PTC but did not show *BRAF* mutation (case 18). Thus, we did not identify *BRAF* mutation in the 2 PTCs in our cohort.

Follicular thyroid carcinoma (FTC) demonstrates mutations in *RAS* (40-50%), *PAX8/PPARγ* (30-35%), *PIK3CA* (< 10%), and *PTEN* (< 10%) [30]. We had 9 vertebral body metastases with a FTC component, of which 6 could be assessed; 6/6 had a *RAS* mutation (cases 7, 8, 12, 13, 14, 15), 2/6 had a *PTEN* mutation (cases 7, 8), and 1/6 had a *PIK3CA* mutation (case 15) (Table I). Of the parenchymal metastases, 0 of the 5 assessable cases were FTCs (Table I). Mutations in *TP53* were only detected in PDTC or ATC cases.

Two of our patients each had undergone multiple (n = 3) surgical resections of spinal bone lesions (cases 2, 3, 4 from a female who was 51 years of age at first resection; cases 6, 7, 8 from a female who was 63 years of age at first resection) (Table I). Of these 2 patients, only 1 of 3 bony lesions could be assessed in one patient (i.e., cases 2, 3 not informative; case 4 with *NRAS, TERT* mutation) and 2 of 3 assessable bony lesions from the other patient shared identical mutations in *NRAS, PTEN, TERT* (cases 7, 8).

The sole medullary carcinoma in the cohort had *PDGFRA* and *RET* mutations in the parenchymal brain metastasis (case 21). 5/5 parenchymal cases had more than 1 mutation identified, and 9/10 bone cases had more than 1 mutation identified. Only 2 cases had fusions detected: one *NCOA4-RET* fusion (mutational testing not performed) and one *CCDC6-RET* fusion in a tumor that also contained mutations in *TERT* and *ATM* (Table I). Thus, only a single metastasis (case 11) had a solo/isolated genetic aberration.

Discussion

Our group has a longstanding interest in metastases impacting the CNS [12,21,22,32,40,41,46,53,57] and in this study, we turned our attention to investigat-

ing thyroid metastases involving the CNS parenchyma and vertebral bodies. The most interesting finding in our study was the extremely high percentage of *TERT* promotor mutations in our metastases, at a rate higher than has been reported for primary tumors in several studies. Specifically, our study identified *TERT* promotor mutations in 80% overall of thyroid metastases involving the CNS parenchyma and vertebral bodies, suggesting that *TERT* promotor mutation is enriched in metastasis, regardless of the histological type of the metastasis. In our study, of the 10 bony vertebral metastases that could be assessed for the mutation (i.e., not decalcified), 9 of 10 had *TERT* promotor mutation and of the 5 assessable CNS parenchymal metastases, 3 showed *TERT* promotor mutation (Table I).

Previously published studies examining molecular/genetic changes in thyroid carcinoma have focused primarily on the more common diagnosis of PTC [27,49]. Masoodi *et al*. reported that mutations in the driver genes were preserved between primary PTC and metastases (including bone and brain metastases), while variants of genes involved in DNA methylation and transcriptional repression signaling were restricted to metastases [27]. Osborne *et al*. undertook molecular assessment on 20 metastases, a number very comparable to our cohort of 21 metastases. However, only 3 of these had been brain metastases; most metastases were from lung or lymph node sites [33]. Overall, 16/20 (80%) showed *TERT* promotor mutation, 55% were *BRAF* V600E mutated, 10/20 (50%) had concurrent *TERT* promotor and *BRAF* V600E mutation, 4 (20%) had *TP53* mutation, 2 had both *TERT* and *TP53*, and one had *NRAS* mutation [27]. Of note, the larger percentage of *BRAF* mutations in their study compared to ours stems from a larger percentage of PTC (54%) in their study, a histological thyroid carcinoma type known to show a higher percentage of *BRAF* mutation [30]. Our study only contained a single case of the follicular variant of PTC.

Pozdeyev *et al*. studied 779 specimens from advanced differentiated (i.e., with distant metastases) and anaplastic thyroid cancers, including metastatic specimens at lung (n = 67), bone (n = 33) and brain (n = 14) sites and found a high percentage with *TERT* mutations, although results were not detailed as to presence of *TERT* mutation at a specific metastatic site, such as brain [36]. Song *et al*. found that while differentiated thyroid carcinoma at the primary site had *TERT* promoter mutations in 4.5% of all cases, the mutation was enriched in cases where the patient had distant metastases (24%) or died from disease (20%) [47]. Thus, our results are concordant with those from prior limited studies in the literature and our original hypothesis was confirmed.

An acknowledged limitation of our study was that we did not have the paired primary thyroid carcinoma and CNS metastases from the same patient in our cohort. However, it cannot be emphasized strongly enough that paired samples were also limited in the study by Osborne *et al*. [33]. Indeed, in their study, only 3 of their 20 patients had both the primary thyroid carcinoma and a metastasis for testing and comparison [33]. This reflects the fact that many patients in North America receive care at several different medical facilities, making it significantly less likely that both the primary tumor and the metastasis had been surgically resected at the same hospital. In addition, many thyroid carcinoma patients in our study had had a long time interval between their diagnosis of primary thyroid carcinoma (Table I) and their CNS metastasis, and often the medical record did not have information regarding where the first tumor had been operated, further hampering retrieval of tissue blocks from the primary thyroid tumor for mutational/fusion testing. It is also worth emphasizing that even if the primary tumor had been available for mutation testing, intratumoral heterogeneity mutations occurring in a small subclone of tumor cells might well have made testing inaccurate.

In terms of types of other mutations found in our metastases, all mutations in metastases identified in the study of Osborne *et al*. were also found in our cohort, except for *BRAF* V600E. In the study by Nikiforov and Nikiforova, mutations typical of FTCs in primary thyroid carcinomas, including *PTEN*, *NRAS*, and *PIK3CA*, were identified in the FTC metastases in our cohort [30]. The sole medullary carcinoma in our cohort had *PDGFRA* and *RET* mutations in the parenchymal brain metastasis (case 21), but not *TERT* promoter mutation. This is in keeping with prior studies that have not identified *TERT* promoter mutations in MTC [52]. Thus, as might be expected, driver mutations were preserved, based on the histological type.

In our study, even if the metastases were not classifiable as "poor differentiated/anaplastic", they still had *TERT* promotor mutation in an overall high percentage of cases (80%). The percentage of *TERT* promotor mutations detected at the primary site does differ based on this histological subtype, with one of the largest studies showing *TERT* in 7% of papillary, 18% of follicular, 25% of Hurthle cell, and 86% of poorly differentiated/anaplastic carcinomas [38]. Vinagre *et al*. report *TERT* mutations in 3/14 poorly differentiated thyroid (21%), 9/169 follicular (14%), 2/16 anaplastic (13%), 13/169 papillary (8%) and 0/28 medullary carcinomas [52]. Tanaka *et al*. reported that *TERT* promoter mutations were associated with transformation from papillary to anaplastic types [49], although in our study anaplastic

transformation was not essential to *TERT* promotor mutation identification.

The possibility that CNS metastases may manifest evolving or acquired features compared to the primary tumor has precedent in many studies, including several by our group. Specifically, we recently investigated prostatic adenocarcinoma CNS parenchymal and dural metastases for *ERG*, *CHD1*, and *MAP3K7* expression and reported that immunohistochemical markers previously shown to be downregulated in aggressive prostatic carcinomas at their primary site also showed reduced expression in prostatic metastases impacting the CNS [32]. In addition, our group as long ago as 2004 had an interest in assessing possible differences between primary tumors and brain metastases from breast carcinoma [16]. Discordance in epidermal growth factor receptor (EGFR) status in 18% of brain metastases implied that drug therapies should be individualized for patients based on test results of the metastases, not simply extrapolated from the primary tumor [16]. Recent reports comparing primary tumors and brain metastases in breast carcinoma have also shown discordance for various parameters [50].

Groups investigating this topic note that there are "unique biological features of each metastatic site" and emphasize "the need to biopsy metastatic disease in patients with advanced breast cancer" since "understanding the biology of each metastatic site can potentially impact the design of new therapies and ultimately improve patient outcomes" [6]. This same message is appropriate for CNS thyroid metastases.

While clinical features were not our main focus for this study, we did review demographics, tumor location, patient age, and survival prior to conducting molecular testing (Table I). Our cohort included 21 specimens from 16 patients, several of which were located in uncommon, but well-reported, intraparenchymal locations including pituitary [2,4,5,8,10,11,14,23,26,28,29,31,43,48,55,58] and intraventricular/choroid plexus [17,20,25,42,51,53,59]. Bone involvement occurred at cervical, thoracic, and lumbar levels and 2 patients required multiple surgical resections of bone lesions due to symptoms (Table I). None were from the skull.

The numbers in our cohort compare favorably to several prior clinical studies. Slutzky-Shraga *et al*. reported 10 patients with brain metastases from thyroid carcinoma [45], Hong *et al*. reported 25 patients with thyroid metastases to CNS and nearby bony sites [19], and Choi *et al*. detailed clinical features in 37 patients [9]. Several of these studies differ in several aspects from ours in that they either excluded medullary thyroid cancer [45] or included skull metastases [19]. All were from differing patient populations/geographical areas than ours, namely Israel [45], Taiwan [19,51], and Korea [9] and all contained a slightly different proportion of thyroid carcinoma types. Nevertheless, these 3 studies showed a nearly identical median age for patients with thyroid metastases to CNS to ours of 62 years, with 53.5 years, 63 years, and 63 years, respectively [9,19,45]. Only one included information regarding interval from initial diagnosis of the primary thyroid carcinoma to development of the CNS metastasis, i.e., Slutzky-Shraga *et al*., who recorded an interval range of 9 months-17 years 3 months [45]. In comparison, 2 of our patients had their first diagnosis of thyroid carcinoma at the time of bone metastasis and one of our patients had a 41-year interval from initial diagnosis of thyroid carcinoma to CNS-impacting metastasis.

In conclusion, we show that that *TERT* mutation occurs in a higher percentage of metastases (80%) than primary tumors. This study indicates that testing of the metastasis itself will be necessary, should targeted anti-*TERT* mutation therapies become available in the future. The study also adds to the literature new molecular information on CNS metastases, an area of research focus that is relatively "underserved" compared to that for primary brain tumors. Of note, *TERT* promotor mutations are also frequent in several types of solid tumors, including hepatocellular carcinoma, in which *TERT* promotor mutations have been assessed in plasma as a biomarker for prognosis [1,18,34]. In addition, in melanoma, TERT-mutated patients had a significantly worse overall survival than those with wild-type status [15]. Thus, there are already precedents in other solid tumor types for using *TERT* promotor mutation assessment in prognosis.

Acknowledgements

The authors thank Ms. Jennifer Platte for manuscript preparation, Ms. Lisa Litzenberger for photographic expertise, Dr. Kurt Davies for advice regarding molecular testing, and Dr. Bryan Haugen for review of the paper and critical comments. This work was supported by the Pathology Shared Resource and the University of Colorado Cancer Center Support Grant (P30CA046934).

Disclosure

The authors report no conflict of interest.

References

1. Akuta N, Kawamura Y, Kobayashi M, Arase Y, Saitoh S, Fujiyama S, Sezaki H, Hosaka T, Kobayashi M, Suzuki Y. TERT promoter mutation in serum cell-free DNA is a diagnostic marker of primary hepatocellular carcinoma in patients with nonalcoholic fatty liver disease. Oncology 2021; 99: 114-123.
2. Bell CD, Kovacs K, Horvath E, Smythe H, Asa S. Papillary carcinoma of thyroid metastatic to the pituitary gland. Arch Pathol Lab Med 2001; 125: 935-938.

3. Benelli M, Pescucci C, Marseglia G, Severgnini M, Torricelli F, Magi A. Discovering chimeric transcripts in paired-end RNA-seq data by using EricScript. Bioinformatics 2012; 28: 3232-3239.
4. Besic N, Gazic B. Sites of metastases of anaplastic thyroid carcinoma: autopsy findings in 45 cases from a single institution. Thyroid 2013; 23: 709-713.
5. Bhatoe HS, Badwal S, Dutta V, Kannan N. Pituitary metastasis from medullary carcinoma of thyroid: case report and review of literature. J Neurooncol 2008; 89: 63-67.
6. Braso-Maristany F, Pare L, Chic N, Martinez-Saez O, Pascual T, Mallafre-Larrosa M, Schettini F, Gonzalez-Farre B, Sanfeliu E, Martinez D, Galvan P, Barnadas E, Salinas B, Tolosa P, Ciruelos E, Carcelero E, Guillen C, Adamo B, Moreno R, Vidal M, Munoz M, Prat A. Gene expression profiles of breast cancer metastasis according to organ site. Mol Oncol 2022; 16: 69-87.
7. Brat DJ, Aldape K, Colman H, Holland EC, Louis DN, Jenkins RB, Kleinschmidt-DeMasters BK, Perry A, Reifenberger G, Stupp R, von Deimling A, Weller M. cIMPACT-NOW update 3: recommended diagnostic criteria for "Diffuse astrocytic glioma, IDH-wildtype, with molecular features of glioblastoma, WHO grade IV". Acta Neuropathol 2018; 136: 805-810.
8. Chikani V, Lambie D, Russell A. Pituitary metastases from papillary carcinoma of thyroid: a case report and literature review. Endocrinol Diabetes Metab Case Rep 2013; 2013: 130024.
9. Choi J, Kim JW, Keum YS, Lee IJ. The largest known survival analysis of patients with brain metastasis from thyroid cancer based on prognostic groups. PLoS One 2016; 11: e0154739.
10. Chrisoulidou A, Pazaitou-Panayiotou K, Flaris N, Drimonitis A, Giavroglou I, Ginikopoulou E, Vainas I. Pituitary metastasis of follicular thyroid carcinoma. Horm Res 2004; 61: 190-192.
11. Conway A, Wiernik A, Rawal A, Lam C, Mesa H. Occult primary medullary thyroid carcinoma presenting with pituitary and parotid metastases: case report and review of the literature. Endocr Pathol 2012; 23: 115-122.
12. Dahl NA, Luebbert T, Loi M, Neuberger I, Handler MH, Kleinschmidt-DeMasters BK, Mulcahy Levy JM. Chordoma occurs in young children with tuberous sclerosis. J Neuropathol Exp Neurol 2017; 76: 418-423.
13. Davies KD, Le AT, Sheren J, Nijmeh H, Gowan K, Jones KL, Varella-Garcia M, Aisner DL, Doebele RC. Comparison of molecular testing modalities for detection of ros1 rearrangements in a cohort of positive patient samples. J Thorac Oncol 2018; 13: 1474-1482.
14. Fabiano AJ, Qiu J, Rigual N. Thyroid Hurthle cell carcinoma metastatic to the pituitary gland. Thyroid 2012; 22: 442.
15. Gandini S, Zanna I, De Angelis S, Palli D, Raimondi S, Ribero S, Masala G, Suppa M, Bellerba F, Corso F, Nezi L, Nagore E, Caini S. TERT promoter mutations and melanoma survival: A comprehensive literature review and meta-analysis. Crit Rev Oncol Hematol 2021; 160: 103288.
16. Grupka NL, Lear-Kaul KC, Kleinschmidt-DeMasters BK, Singh M. Epidermal growth factor receptor status in breast cancer metastases to the central nervous system. Comparison with HER-2/neu status. Arch Pathol Lab Med 2004; 128: 974-979.
17. Heery CR, Engelhard HH, Slavin KV, Michals EA, Villano JL. Unusual CNS presentation of thyroid cancer. Clin Neurol Neurosurg 2012; 114: 1107-1109.
18. Hirai M, Kinugasa H, Nouso K, Yamamoto S, Terasawa H, Onishi Y, Oyama A, Adachi T, Wada N, Sakata M, Yasunaka T, Onishi H, Shiraha H, Takaki A, Okada H. Prediction of the prognosis of advanced hepatocellular carcinoma by TERT promoter mutations in circulating tumor DNA. J Gastroenterol Hepatol 2021; 36: 1118-1125.
19. Hong YW, Lin JD, Yu MC, Hsu CC, Lin YS. Outcomes and prognostic factors in thyroid cancer patients with cranial metastases: A retrospective cohort study of 4,683 patients. Int J Surg 2018; 55: 182-187.
20. Kitagawa Y, Higuchi F, Abe Y, Matsuda H, Kim P, Ueki K. Metastasis to the choroid plexus from thyroid cancer: case report. Neurol Med Chir (Tokyo) 2013; 53: 832-836.
21. Kleinschmidt-DeMasters BK. Dural metastases. A retrospective surgical and autopsy series. Arch Pathol Lab Med 2001; 125: 880-887.
22. Lillehei KO, Kleinschmidt-DeMasters B, Mitchell DH, Spector E, Kruse CA. Alveolar soft part sarcoma: an unusually long interval between presentation and brain metastasis. Hum Pathol 1993; 24: 1030-1034.
23. Lim W, Lim DS, Chng CL, Lim AY. Thyroid carcinoma with pituitary metastases: 2 case reports and literature review. Case Rep Endocrinol 2015; 2015: 252157.
24. Lloyd RV OR, Klöppel G, Rosai J. WHO classification of tumours of endocrine organs: IARC, 2017.
25. Manzil FF, Bender LW, Scott JW. Evaluation of rare choroid plexus metastasis from papillary thyroid carcinoma with multimodality imaging. Clin Nucl Med 2014; 39: 551-553.
26. Masiukiewicz US, Nakchbandi IA, Stewart AF, Inzucchi SE. Papillary thyroid carcinoma metastatic to the pituitary gland. Thyroid 1999; 9: 1023-1027.
27. Masoodi T, Siraj AK, Siraj S, Azam S, Qadri Z, Albalawy WN, Parvathareddy SK, Al-Sobhi SS, Al-Dayel F, Alkuraya FS, Al-Ku-raya KS. Whole-exome sequencing of matched primary and metastatic papillary thyroid cancer. Thyroid 2020; 30: 42-56.
28. 28. Matyja E, Zielinski G, Witek P, Kaminski G, Grajkowska W, Maksymowicz M. Pituitary metastases from the oncocytic variant of follicular thyroid carcinoma: a case report and diagnostic dilemmas. Folia Neuropathol 2013; 51: 261-268.
29. Mota JS, de Sá Caldas A, Nascimento AGdAC, dos Santos Faria M, Sobral CSP. Pituitary metastasis of thyroid carcinoma: a case report. Am J Case Rep 2018; 19: 896-902.
30. Nikiforov YE, Nikiforova MN. Molecular genetics and diagnosis of thyroid cancer. Nat Rev Endocrinol 2011; 7: 569-580.
31. Ochiai H, Nakano S, Goya T, Wakisaka S, Kinoshita K. Pituitary metastasis of thyroid follicular adenocarcinoma – case report. Neurol Med Chir (Tokyo) 1992; 32: 851-853.
32. Ormond DR, Kleinschmidt-DeMasters BK, Cavalcante D, Smith EE, Cramer SD, Lucia MS. Prostatic adenocarcinoma CNS parenchymal and dural metastases: alterations in ERG, CHD1 and MAP3K7 expression. J Neurooncol 2019; 142: 319-325.
33. Osborne JR, Kondraciuk JD, Rice SL, Zhou X, Knezevic A, Spratt DE, Sabra M, Larson SM, Grewal RK. Thyroid cancer brain metastasis: survival and genomic characteristics of a large tertiary care cohort. Clin Nucl Med 2019; 44: 544-549.
34. Oversoe SK, Clement MS, Pedersen MH, Weber B, Aagaard NK, Villadsen GE, Grønbæk H, Hamilton-Dutoit SJ, Sorensen BS, Kelsen J. TERT promoter mutated circulating tumor DNA as a biomarker for prognosis in hepatocellular carcinoma. Scand J Gastroenterol 2020; 55: 1433-1440.
35. Panebianco F, Nikitski AV, Nikiforova MN, Nikiforov YE. Spectrum of TERT promoter mutations and mechanisms of activation in thyroid cancer. Cancer Med 2019; 8: 5831-5839.
36. Pozdeyev N, Gay LM, Sokol ES, Hartmaier R, Deaver KE, Davis S, French JD, Borre PV, LaBarbera DV, Tan AC, Schweppe RE, Fishbein L, Ross JS, Haugen BR, Bowles DW. Genetic Analysis of 779 Advanced Differentiated and Anaplastic Thyroid Cancers. Clin Cancer Res 2018; 24: 3059-3068.

37. Robinson JT, Thorvaldsdóttir H, Winckler W, Guttman M, Lander ES, Getz G, Mesirov JP. Integrative genomics viewer. Nat Biotechnol 2011; 29: 24-26.
38. Romei C, Elisei R. A narrative review of genetic alterations in primary thyroid epithelial cancer. Int J Mol Sci 2021; 22: 1726.
39. 39. Sahm F, Brastianos P, Claus E, Mawrin C, Perry A, Santagata S. Meningiomas. World Health Organization Classification of Central Nervous System Tumours. International Agency for Research on Cancer, Lyon, France 2021.
40. Schowinsky JT, Ormond DR, Kleinschmidt-DeMasters BK. Tibial adamantinoma: late metastasis to the brain. J Neuropathol Exp Neurol 2015; 74: 95-97.
41. Seinfeld J, Wagner AS, Kleinschmidt-DeMasters BK. Brain metastases from hepatocellular carcinoma in US patients. J Neurooncol 2006; 76: 93-98.
42. Sharifi G, Bakhtevari MH, Alghasi M, Nosari MA, Rahmanzade R, Rezaei O. Bilateral choroid plexus metastasis from papillary thyroid carcinoma: case report and review of the literature. World Neurosurg 2015; 84: 1142-1146.
43. Simmons JD, Pinson TW, Donnellan KA, Harbarger CF, Pitman KT, Griswold R. A rare case of a 1.5 mm papillary microcarcinoma of the thyroid presenting with pituitary metastasis. Am Surg 2010; 76: 336-338.
44. Singh A, Ham J, Po JW, Niles N, Roberts T, Lee CS. The genomic landscape of thyroid cancer tumourigenesis and implications for immunotherapy. Cells 2021; 10: 1082.
45. Slutzky-Shraga I, Gorshtein A, Popovitzer A, Robenshtok E, Tsvetov G, Akirov A, Hirsch D, Benbassat C. Clinical characteristics and disease outcome of patients with non-medullary thyroid cancer and brain metastases. Oncol Lett 2018; 15: 672-676.
46. Somerset H, Witt JP, Kleinschmidt-Demasters BK. Hepatocellular carcinoma metastases to the epidural space. Arch Pathol Lab Med 2009; 133: 1975-1980.
47. Song YS, Lim JA, Choi H, Won JK, Moon JH, Cho SW, Lee KE, Park YJ, Yi KH, Park DJ, Seo JS. Prognostic effects of TERT promoter mutations are enhanced by coexistence with BRAF or RAS mutations and strengthen the risk prediction by the ATA or TNM staging system in differentiated thyroid cancer patients. Cancer 2006; 122: 1370-1379.
48. Sziklas JJ, Mathews J, Spencer RP, Rosenberg RJ, Ergin MT, Bower BF. Thyroid carcinoma metastatic to pituitary. J Nucl Med 1985; 26: 1097.
49. Tanaka A, Matsuse M, Saenko V, Nakao T, Yamanouchi K, Sakimura C, Yano H, Nishihara E, Hirokawa M, Suzuki K, Miyauchi A, Eguchi S, Yoshiura KI, Yamashita S, Nagayasu T, Mitsutake N. TERT mRNA Expression as a novel prognostic marker in papillary thyroid carcinomas. Thyroid 2019; 29: 1105-1114.
50. Thulin A, Andersson C, Werner Rönnerman E, De Lara S, Chamalidou C, Schoenfeld A, Kovács A, Fagman H, Enlund F, Linderholm BK. Discordance of PIK3CA and TP53 mutations between breast cancer brain metastases and matched primary tumors. Sci Rep 2021; 11: 1-9.
51. Umehara T, Okita Y, Nonaka M, Mori K, Kanemura Y, Kodama Y, Mano M, Kudawara I, Nakajima S. Choroid plexus metastasis of follicular thyroid carcinoma diagnosed due to intraventricular hemorrhage. Intern Med 2015; 54: 1297-1302.
52. Vinagre J, Almeida A, Populo H, Batista R, Lyra J, Pinto V, Coelho R, Celestino R, Prazeres H, Lima L, Melo M, da Rocha AG, Preto A, Castro P, Castro L, Pardal F, Lopes JM, Santos LL, Reis RM, Cameselle-Teijeiro J, Sobrinho-Simoes M, Lima J, Maximo V, Soares P. Frequency of TERT promoter mutations in human cancers. Nat Commun 2013; 4: 2185.
53. Wagner AS, Fleitz JM, Kleinschmidt-Demasters BK. Pediatric adrenal cortical carcinoma: brain metastases and relationship to NF-1, case reports and review of the literature. J Neurooncol 2005; 75: 127-133.
54. Wasita B, Sakamoto M, Mizushima M, Kurosaki M, Watanabe T. Choroid plexus metastasis from papillary thyroid carcinoma presenting with intraventricular hemorrhage: case report. Neurosurgery 2010; 66: E1213-1214.
55. Williams MD, Asa SL, Fuller GN. Medullary thyroid carcinoma metastatic to the pituitary gland: an unusual site of metastasis. Ann Diagn Pathol 2008; 12: 199-203.
56. Wu TD, Nacu S. Fast and SNP-tolerant detection of complex variants and splicing in short reads. Bioinformatics 2010; 26: 873-881.
57. Yang MJ, Whelan R, Madden J, Mulcahy Levy JM, Kleinschmidt-DeMasters BK, Hankinson TC, Foreman NK, Handler MH. Intracranial Ewing sarcoma: four pediatric examples. Childs Nerv Syst 2018; 34: 441-448.
58. Yilmazlar S, Kocaeli H, Cordan T. Sella turcica metastasis from follicular carcinoma of thyroid. Neurol Res 2004; 26: 74-78.
59. Zhang YA, Kavar B, Drummond KJ. Thyroid carcinoma metastasis to the choroid plexus of the lateral ventricle. J Clin Neurosci 2009; 16: 118-121.