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ANAPLASTIC TRANSFORMATION OF *BRAF* AND *TERT* PROMOTER DOUBLE MUTANT PAPILLARY THYROID CARCINOMA: CLINICAL, MORPHOLOGICAL, AND MOLECULAR GENETIC FEATURES

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Abstract

Background. We report the case of a 74-year-old woman with *BRAF* and *TERT* promoter double-mutation, with an aggressive papillary thyroid carcinoma (PTC) with a focal undifferentiated component.

Case presentation. PTC was diagnosed via cytological analysis and total thyroidectomy and lymph node dissection were performed 15 months before her death. Pathological diagnosis revealed stump-positive PTC pT4aN1bM1, Stage IVB. An initial radioiodine ablative dose (150 mCi) was administered. Thereafter, the mediastinal lymph node and multiple bilateral lung metastases were observed upon computed tomography. Six months later, recurrent lesions were irradiated with external beam radiation (39 Gy/13 fr). Within the next five months, she developed multiple-organ metastases. A month before death, recurrent lesions increased rapidly and an undifferentiated cancer was diagnosed upon biopsy. The multifocal disease was rendered inoperable. After gradual progression of respiratory failure, the patient died.

During initial resection, a focal invasion component with severe nuclear atypia and spindle-shaped, giant cells were noted, thereby increasing the probability of focal undifferentiated transformation. A focal hobnail pattern and minor necrosis were observed. Upon autopsy, lung and multiple-organ metastases and massive mediastinal invasion were observed. The immunohistochemically undifferentiated lung cancer expressed vimentin, AE1/AE3, CK7, and p53, but not thyroglobulin, TTF-1, and Napsin A. Furthermore, a PTC component was observed in the lung, showing micropapillary architecture with a prominent hobnail pattern. Molecular analysis revealed a double-mutation in *BRAF* (V600E) and *TERT* (C228T) promoters. The Ki-67 labeling index of surgical papillary carcinoma tissue was 34%. *BRAF* mutations associated with p53 mutations triggered an additional *TERT* promoter mutation with upregulated Ki-67 in primary PTC, which can be a network of genetic alterations driving tumor progression and distant metastasis to the undifferentiated/anaplastic phenotype.

Conclusions. The above mentioned molecular genetic features with the histologically hobnail component should be considered and tumor recurrence should be assessed carefully.

Keywords. *Papillary thyroid carcinoma, anaplastic thyroid carcinoma, thyroglobulin, radioiodine therapy, mutation.*

Резюме

АНАПЛАСТИЧЕСКАЯ ТРАНСФОРМАЦИЯ ПАПИЛЛЯРНОЙ КАРЦИНОМЫ ЩИТОВИДНОЙ ЖЕЛЕЗЫ С ДВОЙНОЙ МУТАЦИЕЙ В ГЕНЕ BRAF И ПРОМОТЕРЕ TERT: КЛИНИЧЕСКИЕ, МОРФОЛОГИЧЕСКИЕ И МОЛЕКУЛЯРНО-ГЕНЕТИЧЕСКИЕ ОСОБЕННОСТИ

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Введение. Описан клинически случай 74-летней женщины с двойной мутацией в промоторе *TERT* и *BRAFV600E,* агрессивный папиллярный рак щитовидной железы (ПРЩЖ) с очаговым недифференцированным компонентом.

Клинический случай. ПРЩЖ был диагностирован цитологически, и за 15 месяцев до смерти была выполнена тотальная тиреоидэктомия с лимфодиссекцией. Патологический диагноз: ПРЩЖ pT4aN1bM1, стадия IVB, положительный край резекции. Была проведена начальная абляционная доза радиоактивного йода (150 мКи). На компьютерной томографии выявлены метастатические лимфатические узлы средостение и множественные метастазы в обеих легких. Через шесть месяцев на область очагов рецидива опухоли проведена дистанционная лучевая терапия (39 Гр / 13 фр). В течение следующих пяти месяцев у пациентки развились множественные метастазы. За месяц до смерти рецидивирующие поражения быстро увеличивались в размерах. Недифференцированный рак выставлен на биопсии. Мультифокальное поражение было расценено нерезектабельным. В результате прогрессирования дыхательной недостаточности наступила смерть.

В первичной опухоли был очаговый инвазивный компонент с высокой ядерной атипией, веретенообразными клетками, гигантскими клетками, что увеличивало вероятность очаговой недифференцированной трансформации. Наблюдались очаговые скопления клеток в виде “шляпки гвоздей” и незначительный некроз. На патологоанатомическом вскрытии были выявлены метастазы в легких и других органах, а также массивная инвазия в средостение. Иммуногистохимически недифференцированный рак легкого с экспрессией виментина, AE1/AE3, CK7 и p53, но без экспресии тиреоглобулин, TTF-1 и напсинА. Кроме того, компонент ПРЩЖ, демонстрирующий микропапиллярную архитектуру с клетками в виде “шляпки гвоздей” был обнаружен в легких. Молекулярный анализ выявил двойную мутацию в промоторе *TERT* C228T и *BRAFV600E*. Индекс Ki-67 папиллярной карциномы составил 34%. Мутация *BRAFV600E*, связанная с мутацией p53, вызвали дополнительную мутацию промотора *TERT* с усилением регуляции Ki-67 в первичной ПРЩЖ. Данные молекулярные изменения могут иметь место в цепочке генетических изменений, приводящих к прогрессированию опухолевого процесса и развитию отдаленных метастатических поражении с недифференцированным/анапластическим фенотипом.

Выводы. Следует учитывать вышеупомянутые молекулярно-генетические особенности с гистологическим компонентом в виде “шляпки гвоздей” для оценки рецидива опухоли.

Ключевые слова. Папиллярный рак щитовидной железы, анапластический рак щитовидной железы, тиреоглобулин, радиойодтерапия, мутации.

Түйіндеме

ҚОСАРЛЫ BRAF ГЕНІНІҢ ЖӘНЕ TERT ПРОМОТЕРІНІҢ МУТАЦИЯСЫ БАР ҚАЛҚАНША БЕЗІНІҢ ПАПИЛЛЯРЛЫ КАРЦИНОМАСЫНЫҢ АНАПЛАСТИКАЛЫҚ ТРАНСФОРМАЦИЯСЫ: КЛИНИКАЛЫҚ, МОРФОЛОГИЯЛЫҚ ЖӘНЕ МОЛЕКУЛАЛЫҚ-ГЕНЕТИКАЛЫҚ ЕРЕКШЕЛІКТЕРІ

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Кіріспе. 74 жастағы әйел адамда *TERT* және *BRAFV600E* промоторында екі мутациясы анықталған сараланбаған ошақты компоненті бар қалқанша безі папиллярлы қатерлі ісігінің (ҚБПҚІ) агрессивті жағдайы сипатталған.

Клиникалық жағдай. ҚБПҚІ цитологиялық тұрғыда анықталған және науқас өліміне 15 ай бұрын лимфодиссекция мен толықтай тиреоидэктомия жүргізілді. Патологиялық диагнозы: ҚБПҚІ pT4aN1bM1, IVB кезең, резекция шетінің оң болуы. Радиоактивті йодтың бастапқы абляциялық дозасы (150 мКи) жүргізілді. Компьютерлік томографияда кеуде қуысында метастаздық лимфа түйіндері және өкпе екі жағындағы көптеген метастаздар анықталған. Алты айдан соң рецидив ошақтары аймағына дистанциялық сәулелі терапия (39 Гр / 13 фр) жүргізілді. Келесі бес айда науқаста көптеген метастаздар пайда болды. Науқас өліміне дейін бір ай бұрын рецидивтік зақымданулар көлемі жылдам ұлғайып, биопсия көмегімен сараланбаған қатерлі ісік анықталды. Мультифокалды зақымдану резекция жасау арқылы емделмейтіндігі анықталды. Тыныс алу жетіспеушілігінің үдеуі науқас өліміне әкелді.

Біріншілік ісікте сараланбаған ошақты трансформация ықтималдылығын арттыратын, жоғары ядролық атипиясы бар алып жасушалар және ұршық тәрізді жасушалардың ошақты инвазивті компоненті анықталды. “Шеге қалпақшалары" тәрізді жасушалардың ошақты жиналуы және біраз некроз байқалды. Аутопсия өкпедегі және басқа мүшелердегі метастаздарды, сонымен қатар көкірек қуысындағы біршама инвазияны көрсетті. Иммуногистохимиялық тұрғыда виментин, AE1/AE3, CK7 және p53 экспрессиясы, бірақ тиреоглобулин, TTF-1 және напсинА экспрессиясы анықталмады. Сонымен қатар, “шеге қалпақшалары" тәрізді жасушалары бар микропапиллярлы архитектураны көрсететін ҚБПҚІ компоненті өкпеде анықталды. Молекулалық анализ *TERT* C228T және *BRAFV600E* промоторындағы екі мутацияны анықтады. Папиллярлы карцинома Ki-67 индексі 34% болып шықты. p53 мутациясымен байланысқан *BRAFV600E* мутациясы біріншілік ҚБПҚІ Ki-67 реттелуінің үдеуімен қосымша *TERT* промоторының мутациясын тудырды. Көрсетілген молекулалық өзгерістер ісіктің үдеуіне және сараланбаған/анапластикалық фенотипі бар басқа мүшелердегі метастаздарға әкеліп соқтыратын генетикалық өзгерістер тізбегінде орын алуы мүмкін.

Қорытынды. Жоғарыда көрсетілген “шеге қалпақшалары" тәрізді гистологиялық компоненті бар молекулалық-генетикалық ерекшеліктерді ісік рецидивін бағалау үшін қарастыру қажет.

Негізгі сөздер. *Қалқанша безінің папиллярлы қатерлі ісігі, қалқанша безінің анапластикалық қатерлі ісігі, тиреоглобулин, радиойодтерапия, мутациялар.*

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Тарғынова А.Т., Мусажанова Ж.Б., Йеки Н., Болсынбекова С.О., Елеубаева Ж.Б., Калматаева Ж.А., Исаева Р.Б., Сарсенова Л.К., Умирова Р.У., Серикбайулы Д., Накашима М., Муканова А.К., Мадиева М.Р. Қосарлы BRAF генінің және TERT промотерінің мутациясы бар қалқанша безінің папиллярлы карциномасының анапластикалық трансформациясы: клиникалық, морфологиялық және молекулалық-генетикалық ерекшеліктері // Ғылым және Денсаулық сақтау. 2021. 5 (Т.23). Б. 248-256. *doi 10.34689/SH.2021.23.5.026*

Papillary thyroid carcinoma (PTC) is the most common thyroid malignancy, generally with an indolent clinical course and patient survival in stage I is approximately 100% [1]. Aggressive subtypes of PTC include tall cell, columnar cell, diffuse sclerosing variant, and hobnail variant. These variants have been associated with higher rates of extrathyroidal extension, multifocality, nodal and distant metastases, recurrence, and resistance to radioiodine therapy [2,3].

Radiation exposure is a well-known risk factor for thyroid carcinoma (American Cancer Society, Thyroid Cancer, American Cancer Society, 2016). Well-differentiated thyroid carcinomas can dedifferentiate via a multistep process involving genetic and epigenetic changes, ultimately culminating in a poorly differentiated or undifferentiated/anaplastic carcinoma [4]. Anaplastic thyroid carcinoma (ATC) is the most aggressive form of thyroid cancer and accounts for less than 5% of thyroid cancers, with a mortality rate greater than 90% and median survival of six months after diagnosis [5]. Anaplastic transformation of PTC is well documented and, in most cases, transformations occur

within the thyroid gland itself or in surrounding lymph nodes [6]. However, few cases of PTC transforming to poorly differentiated/ATC at sites other than the neck, including the lungs, and in one particular case, the shoulder, thereby mimicking a sarcoma [6,7]. In fact, a study of a series of autopsies revealed that the most common sites of distant metastasis in ATC include, in descending order of frequency, the lungs (78%), intrathoracic lymph nodes (58%), neck lymph nodes (51%), pleura (29%), adrenal glands (24%), liver (20%), brain (18%), and retroperitoneal lymph nodes (18%) [8].

Analysis of molecular markers including *BRAF* has reported an association between the *BRAF* V600E mutation and poor prognosis of PTC patients; however, clinical application of the *BRAF* V600E mutation has limitations, especially in areas with a high frequency of this mutation [9–13].

Recently, telomerase reverse transcriptase (TERT) promoter mutations, C228T and C250T, have been proposed as robust prognostic biomarkers and are reportedly associated with aggressive clinicopathological characteristics, thereby rendering biomarker research as a newly emerged field in cancer research [14]. Both mutations generate a consensus binding site in the *TERT* promoter for E-twenty-six (ETS) transcription factors, which confers increased transcriptional activity at the *TERT* promoter [15–17]. The *TERT* C228T mutation is more common than the *TERT* C250T mutation and have been reported, on average, in 0%, 11.3%, 17.1%, 43.2%, and 40.1% of benign thyroid tumors, PTC, follicular thyroid cancer, poorly differentiated thyroid cancer (PDTC), and undifferentiated/ATC, respectively, thereby displaying an association with aggressive thyroid cancers [18]. *TERT* promoter mutations are suggested to be associated with the aggressiveness of thyroid tumors, tumor recurrence, and patient mortality and are probably strong predictors for poor clinical outcomes in thyroid cancer. Coexisting *BRAF* V600E and *TERT* promoter mutations have a prominent synergistic impact on PTC aggressiveness, including increased tumor recurrence and patient mortality, while either mutation alone reportedly displayed a modest effect [19].

The molecular mechanism underlying the synergistic effects of the two mutations involves the upregulation of ETS transcription factors via the *BRAF* V600E-activated mitogen-activated protein kinase pathway. Upregulation of ETS transcription factors in turn upregulates *TERT* by binding to the binding site in the *TERT* promoter, generated via C228T or C250T mutation. *TERT* overexpression promotes tumorigenesis and malignant transformation in thyroid cancer [20]; however, the underlying mechanism is unclear owing to limited data regarding individual mutations and their coexistence.

Lubitz CC et al. First reported that the hobnail variant of PTC displays aggressive behavior, with a high incidence of infiltrative tumors and metastasis, harbors a *BRAF*^{V600E} mutation (80%) or a *RET/PTC1* rearrangement (20%). Furthermore, a few patients with an aggressive hobnail variant reportedly had very poor disease-specific survival (43–66%) [21–23].

To our knowledge, we describe the case of a patient harboring a double promoter mutation in *BRAF* and *TERT*, with an aggressive disease course of PTC with multifocal distant metastases, and transformation to anaplastic carcinoma.

Case presentation

A 74-year-old woman presented with a thyroid tumor and lymphadenopathy diagnosed via preoperative clearance single-photon emission computed tomography (SPECT-CT) (Fig. 1).

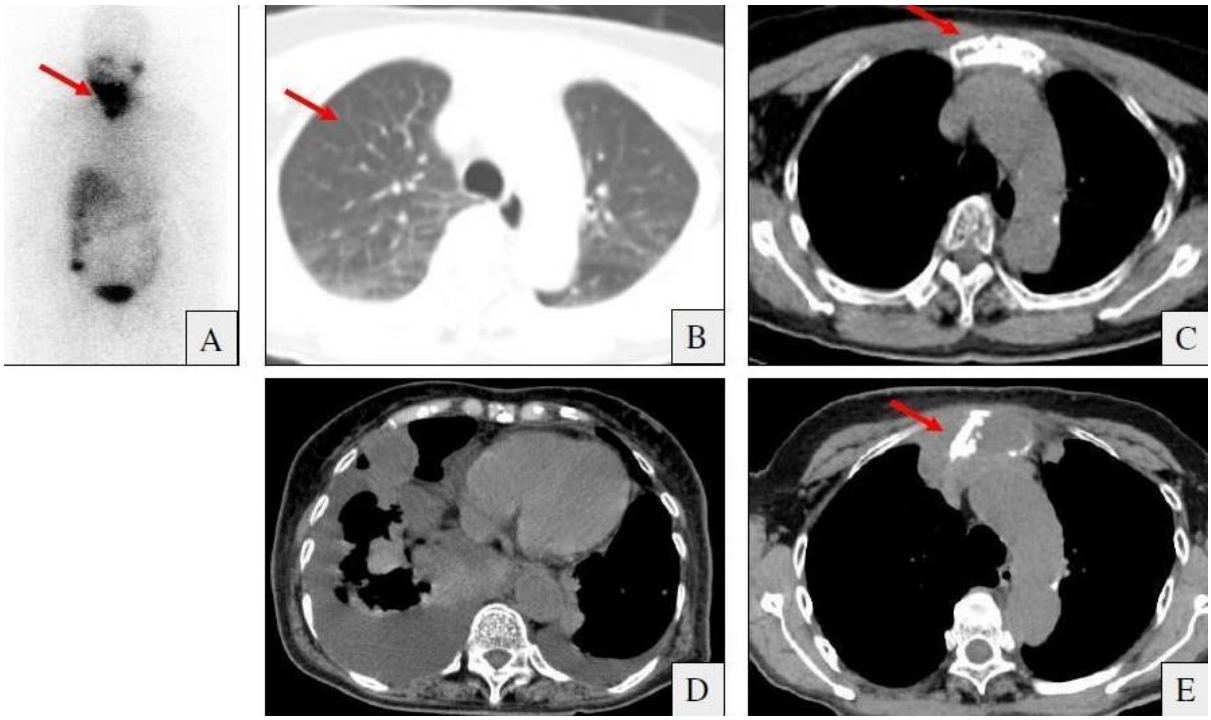

Figure 1. Eleven months before death (A-C). (A) Single-photon emission computed tomography (SPECT-CT): uptake by the in the right paratracheal area, near the medial hyoid bone (red arrow). No accumulation in the lungs. Computed tomography (CT): (B) scattered multiple granular shadows in both lungs; (C) osteolytic lesions with sternal destruction. One month before death (2). CT: (D) multiple lung metastases, increased in size. (E) Stenotic osteolytic mass. Rapid increase.

The thyroid mass was subjected to biopsy via fine-needle aspiration and a PTC was detected. Fifteen months before her death, she underwent total thyroidectomy with lymph node dissection for definitive surgical management. The surgical pathological diagnosis was reported as pT4aN1bM1, stage IVB. The initial radioiodine ablative dose (150 mCi) was administered. Approximately two months later, thyroglobulin levels elevated slightly to 54.8 ng/mL, which normalized a month later. Thereafter, the mediastinal lymph node and multiple metastases in both lungs were observed on CT. The patient's lung nodules, at that time, were not assessed via biopsy and followed up. Six months later, recurrent lesions were irradiated with external beam radiation (39 Gy/13 fr). Within the next five months, the patient developed multiple-organ metastases, including both lungs, the heart, right kidney, clavicle, sternum, thymus, diaphragm, peritoneum, lymph node masses in the mediastinum, and upper mediastinum. Owing to difficulties of resection in the lung and other lesions, respiratory symptoms such as shortness of breath and dry cough increased. Her thyroglobulin levels normalized at that time. She was transferred to the medical intensive care unit the day before her death owing to an altered mental status and exacerbated symptoms, which finally resulted in her death.

Pathological analysis (postoperative)

Pathological analysis revealed a 4 × 2.5 cm^2-sized white solid tumor, an unclear border with calcifications, and invasion to the surrounding tissue. An extrathyroidal extension was observed during resection, margins were positive, and lymph node metastases were observed, along with venous/lymphatic invasion. Histological analysis revealed a typical papillary carcinoma pattern with nuclei showing ground glass opacity, groove lesions, and pseudo-inclusions. Small foci of undifferentiated components of the invading tumor with nuclear atypia and spindle-shaped and giant cells were observed (Fig. 2).

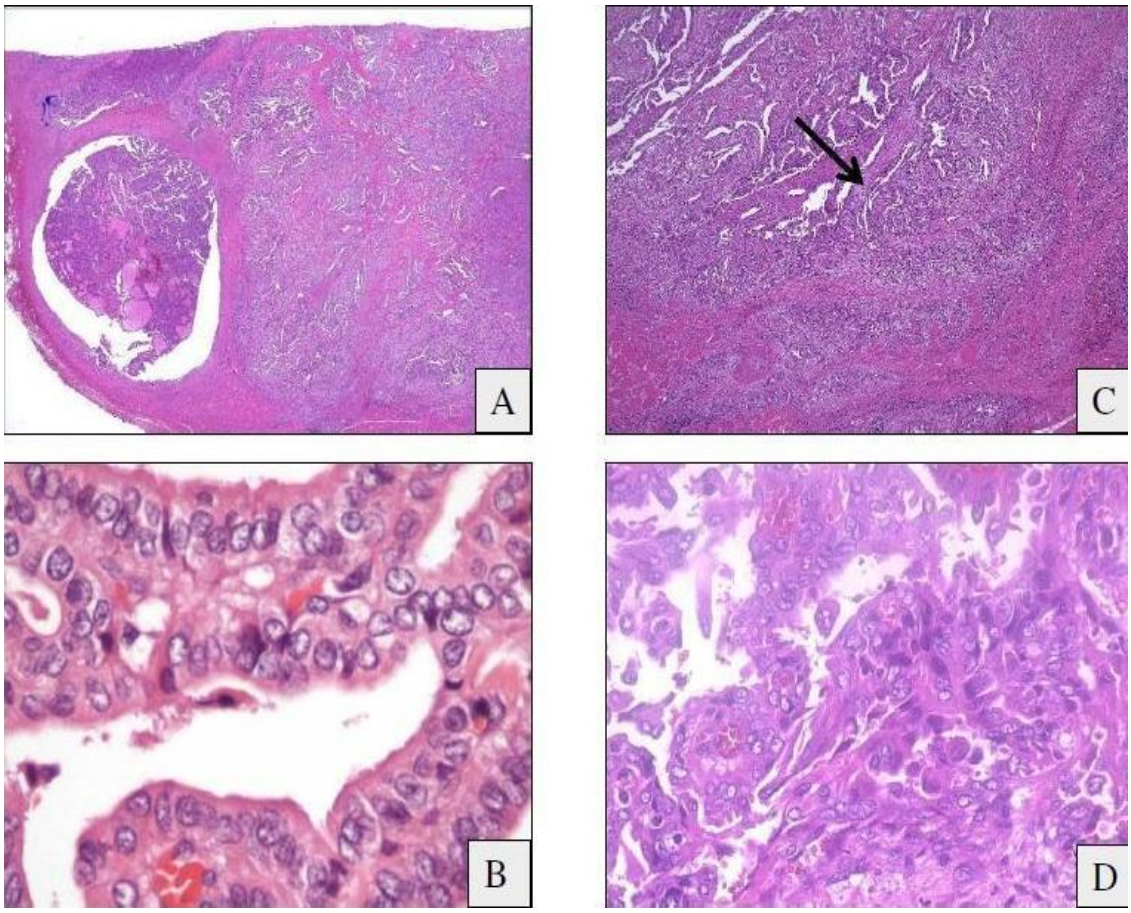

Figure 2. Representative histological images of (A and B) primary papillary thyroid tumor nuclei showing ground glass opacity and groove lesions, (C) black arrow indicating the undifferentiated component, and (D) high-power view of the focal undifferentiated component in the tumor with large, hyperchromatic and bizarre-looking nuclei, spindle-shaped, and giant cells.

These findings increase the probability of focal undifferentiated transformation during initial resection. Focal hobnail patterns and small necrosis were observed. Immunostaining analyses revealed that thyroglobulin, p53, TTF-1, AE1/AE3, and CK7 were expressed with a Ki-67 labelling index (LI) of 34% (Fig. 3). Molecular genetic analysis revealed a double mutation in *BRAF* and *TERT* promoters (Fig. 5).

Pathology analysis (autopsy). Metastatic PTC and coexistent multifocal undifferentiated thyroid carcinoma were identified upon autopsy in both lungs, the heart, right kidney, clavicle, sternum, thymus, diaphragm, peritoneum, lymph node masses in the mediastinum, and upper mediastinum. The majority of tumor mass was located in the lungs, measuring up to 4 cm. Microscopic examination revealed masses with diffuse proliferation of spindle-shaped

cells with multinucleated giant cells. The nuclei of spindle cells were polymorphic and displayed severe atypia (Fig. 4). Furthermore, masses with a PTC pattern showing micropapillary architecture with a hobnail pattern were observed (Fig. 4).

The hobnail component was more prominent in post-autopsy specimens than in initial tumors. Tumor cells displaying an undifferentiated pattern expressed vimentin, CK7, AE1/AE3, p53, but not TTF-1, Napsin A, thyroglobulin (Fig. 3).

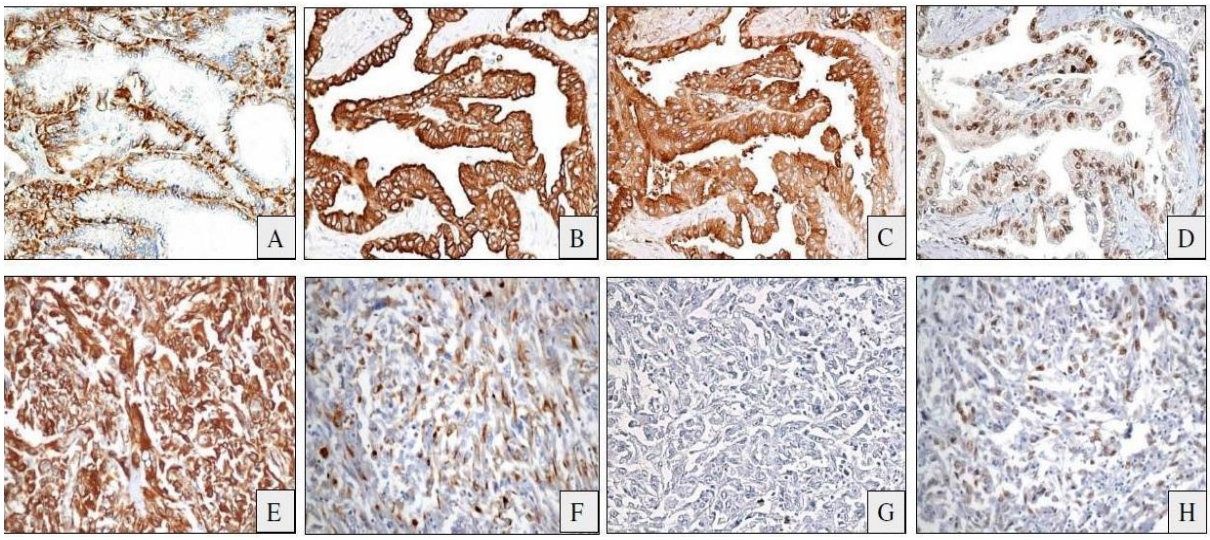

Figure 3. Images of primary thyroid tumor, papillary thyroid carcinoma, immunohistochemical stain (A-D). Images of (A) vimentin (-), (B) AE1/AE3 (++), (C) thyroglobulin (+), (D) p53 (+). Images of lung metastasis, undifferentiated carcinoma, immunohistochemical stain (E-H). Images of (E) vimentin (++), (F) AE1/AE3 (+), (G) thyroglobulin (-), (H) p53 (+). Magnification, x100.

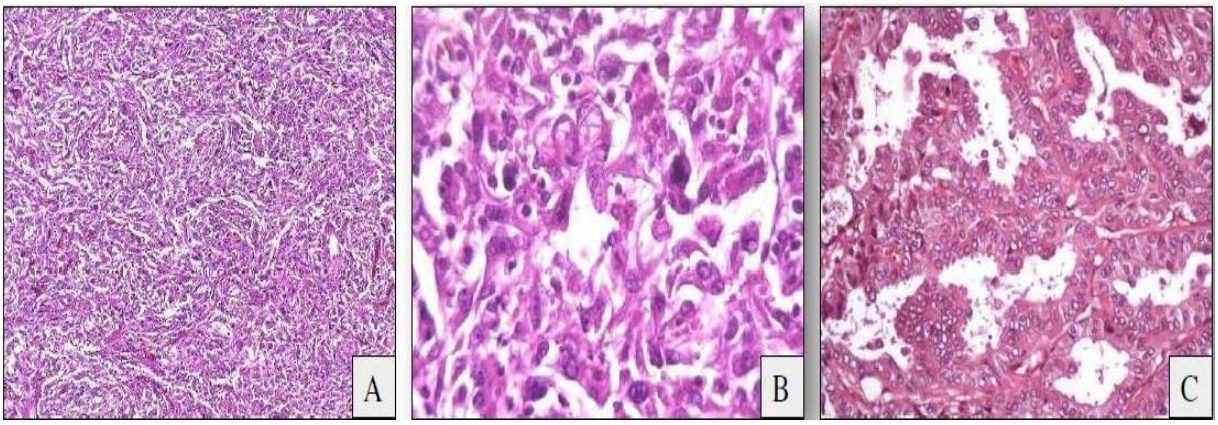

Figure 4. Representative histological images of lung metastasis. (A) Undifferentiated carcinoma with (B) diffuse proliferation of spindle-shaped cells with multinucleated giant cells. The nucleus of spindle cells is polymorphic, with severe atypia. (C) Lung metastasis component showing typical papillary thyroid pattern.

Discussion

Transformation of PTC to more aggressive undifferentiated/ATC is well known and reported recently; however, the transformation of metastatic PTC in a distant location other than the neck and cervical lymph node metastases, including the lungs, similar to that in the present case, is uncommon and such cases have been reported previously [6,24–28]. Immunostaining for TTF-1, thyroglobulin, and Napsin A, diagnostic markers differentiating PTC from primary lung adenocarcinoma, were not expressed in the present case. However, different reports reveal challenging results for immunohistochemistry staining in undifferentiated thyroid carcinomas/anaplastic carcinoma and metastases among tumor cells expressing TTF-1, thyroglobulin, Napsin A, and CK7, which confounds the diagnosis (if thyroglobulin is negative), when it is required to differentiate from a primary lung adenocarcinoma [3,29]. The tumor in the present case was primarily a well-differentiated PTC, and the undifferentiated component (noted to have severely atypical nuclear features, and spindle-shaped and giant cells) included small foci upon initial findings. Upon autopsy, multiple nodules were observed in both lungs, showing papillary and undifferentiated components, coupled with the aforementioned immunohistochemistry findings for negative TTF-1, thyroglobulin, and Napsin A expression, which would most prominently indicate a metastatic papillary thyroid carcinoma with undifferentiated/ anaplastic transformation in the lung (multifocal in this case).

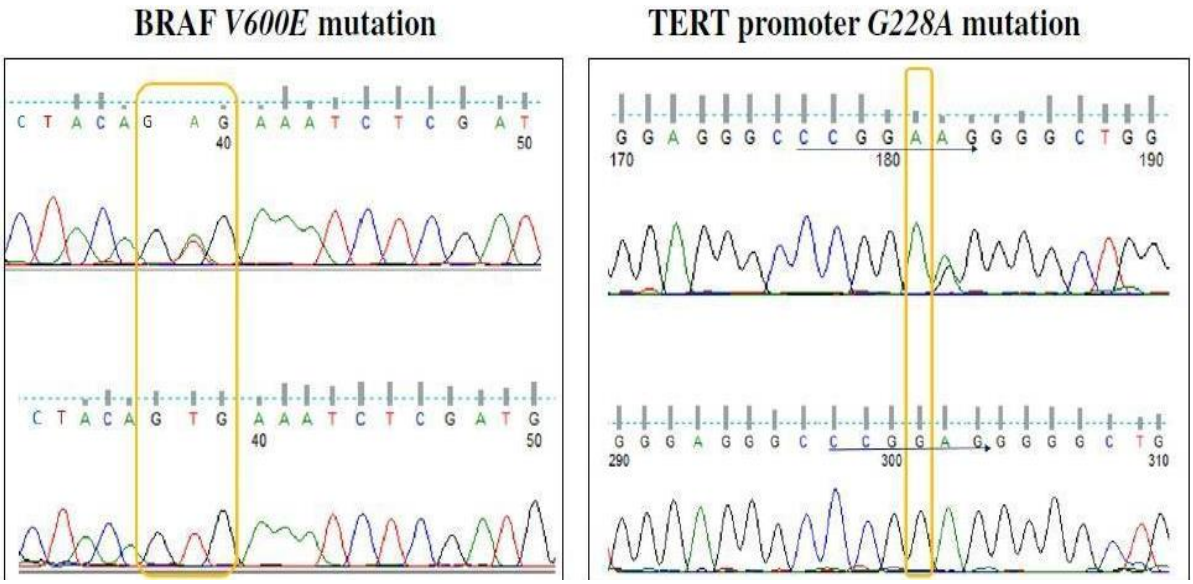

Figure 5. Molecular analysis. Double mutation of *BRAF* V600E and *TERT* promoter C228T. Polymerase chain reaction- based sequence analysis.

The pathogenesis of papillary thyroid carcinoma transformation in poorly differentiated papillary and undifferentiated/anaplastic thyroid carcinoma remains unknown. Recently, molecular analysis has provided some insights into undifferentiated/anaplastic transformation. For instance, *BRAF* and *RAS* mutations are well-known drivers of thyroid carcinoma with poor prognosis and transformation in PDTC and ATC [30]. Recent molecular evidence suggests that distant metastatic PTC harbors additional (including double *BRAF* and/or *RAS* mutations) genetic alterations. Mutations in tumor protein p53 (TP53) occur with increasing frequency in more clinically aggressive subsets of thyroid cancers including PDTC and ATC, the highest frequency of *TP53* mutations being in ATC, at a lower frequency than that in PDTC, and uncommon in PTC [30–32].

ATC may progress spontaneously from well-differentiated thyroid carcinomas and based on the former scenario, are believed to frequently harbor *BRAF* mutations. This supports the hypothesis that *BRAF* mutations and loss of p53 coordinate in vivo to facilitate tumor progression to ATC. However, additional somatic genetic or epigenetic alterations driving tumor progression and conversion to the anaplastic phenotype may be required for ATC [33–36]. Furthermore, this could be a *TERT* promoter mutation, based on recent reports, the most prominent mutation in undifferentiated/anaplastic transformation with aggressive clinicopathological features and disease recurrence [19]. Matsuse et al. reported that recurrence of PTC was 44.4% (4/9) when the *TERT* promoter mutation and Ki-67 labelling index (LI) was 10% or greater, thereby suggesting that Ki-67 LI may be an additional promising marker to predict PTC recurrence in patients harboring a combination of *TERT* promoter/*BRAF* V600E mutations [37].

Radiation exposure is a well-known risk factor for thyroid carcinoma [1]. However, our patient cannot be considered to have been affected by radiation-induced transformation, since the patient died in the short period after the second exposition, thereby suggesting that genetic abnormalities of primary papillary cancer are involved, rather than radiation exposure.

Recently, the hobnail variants or other PTC variants with hobnail features have received increasing attention and recently, several reports about aggressive behavior, with a high incidence of infiltration and metastasis, are available. Furthermore, only one study reported genetic abnormalities such as *BRAF* V600E mutation (80%) or a RET/PTC1 rearrangement (20%) associated with PTC hobnail variants [21–23]. Our case includes a hobnail component in initial PTC and in PTC showing micropapillary architecture after autopsy. The hobnail component was more prominent in autopsy specimens than in the initial tumor.

Conclusion

Our patient experienced an aggressive disease course of PTC with a focal undifferentiated component after surgery, with a p53 mutation harboring a *TERT* promoter mutation in combination with a *BRAF* V600E mutation and high Ki-67 LI with distant metastatic transformation to undifferentiated/anaplastic carcinoma; this is the first case of extremely short survival rate. The present results also support previous suggestions that TERT promoter with BRAF V600E mutations, high Ki-67 LI, and p53 immunohistochemistry constitute a promising new set of diagnostic and prognostic genetic markers representing prove to be clinically useful for the management of thyroid cancer. Moreover, a histologic component such as hobnail with necrosis and a focal undifferentiated component of PTC could be considered for the future study, and patients could be observed for poor prognosis.

Abbreviations

PTC: Papillary thyroid carcinoma
ATC: Anaplastic thyroid carcinoma

TERT: Telomerase reverse transcriptase
ETS: E-twenty-six
PDTC: Poorly differentiated thyroid cancer
SPECT-CT: Single-photon emission computed tomography
TP53: Tumor protein p53

Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication: Written informed consent for publication of their clinical details and/or clinical images was obtained from the patient. A copy of the consent form is available for review by the Editor of this journal.

Availability of data and materials: The data sets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Competing interests: The authors declare that they have no competing interests.

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Authors' contributions

Akbota Targynova, Zhanna Mussazhanova, *Nozomi Ueki* carried out the molecular genetic studies.

Akbota Targynova, Zhanna Mussazhanova drafted the manuscript.

Akbota Targynova, Nozomi Ueki, Zhanna Mussazhanova, Saltanat Bolsynbekova, Zhanar Yeleubayeva, Zhanna Kalmatayeva, Raushan Issayeva, Lazzat Sarsenova, Raushan Umirova, Dulat Serikbaiuly, Mukanova A.K., Madiyeva M.R., Masahiro Nakashima participated in the diagnosis and interpretation of immunoassays.

Masahiro Nakashima conceived of the study and participated in coordination and helped to draft the manuscript.

All authors have read and approved the final version to be published.

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