

p53 protein expression in synchronously occurring dedifferentiating stages of thyroid cancer in a patient with neurofibromas: A case report

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Abstract. Poorly differentiated thyroid carcinoma (PDTC) and anaplastic thyroid carcinoma (ATC) have been hypothesized to arise from well-differentiated thyroid carcinoma (WDTC) due to frequently reported synchronous and metachronous occurrence. Loss of normal p53 function has been implicated in this dedifferentiation process. The current case report presents a 60-year-old male with multiple neurofibromas who underwent total thyroidectomy due to multiple palpable thyroid nodules. Histopathological examination revealed three foci of predominantly papillary, but also follicular carcinoma growth pattern, and two lesions with histological features of insular and trabecular variant, with the larger one showing foci of anaplastic transition. Nuclear p53 protein accumulation, corresponding to mutant abnormally stabilized p53, was higher in more aggressive variants compared with WDTC. The somatic molecular events and downstream pathways of this dedifferentiation course have not been unraveled yet. The present case report demonstrated the simultaneous presence of three divergent histological subtypes in a single thyroid gland, with progressive enhancement of nuclear p53 protein expression, associated with mutant p53 protein, in the more aggressive variants. This is a rare case of progressive enhancement of mutant nuclear p53 protein expression in multifocal thyroid tumor areas consisting of WDTC, PDTC and ATC histological types, highlighting the possibility that WDTC can

progress to PDTC and then ATC through an intricate procedure, involving loss of normal p53 function.

Introduction

Poorly differentiated thyroid carcinoma (PDTC) is a clinically and molecularly inchoate histologic variant of thyroid carcinoma. PDTC occupies an intermediate position between well-differentiated thyroid carcinoma (WDTC) and anaplastic thyroid carcinoma (ATC) with regard to both histological features and biologic aggressiveness and is often associated with locoregional recurrence and distant disease leading to compromised survival (1,2). PDTC was originally classified as a distinct clinicopathologic variant in 2004 by the World Health Organization (WHO), but it was not until 2006 that more stringent criteria, known as the 'Turin criteria', were established to allow diagnosis standardization (2,3). Turin proposal stated that PDTC diagnosis should be rendered on histologic specimens demonstrating solid/trabecular/insular growth pattern, absence of conventional nuclear features of PTC, and at least one of the following features: Convoluted nuclei, necrosis or mitotic index $\geq 3/10$ high power fields (HPFs) (2-4).

Although PDTC has distinct diagnostic criteria and can develop de novo, in a significant proportion of patients, PDTC-like features may occur simultaneously with follicular, papillary or anaplastic carcinoma stressing the possibility that PDTC may arise by dedifferentiation of pre-existing WDTC (5,6). The mutational events that orchestrate this malignant progression are ill-defined. Genetic alterations frequently detected in PDTC include TP53, RAS, BRAF and CTNNB1 genes (7,8).

Environmental factors, such as iodine deficiency and prior neck irradiation, have been implicated in the pathogenesis of PDT (9). In chorus with other thyroid carcinoma subtypes, PDTC demonstrates a female predominance (9).

TP53 gene encodes a nuclear transcription factor that regulates cell cycle by inducing arrest at the G1/S check point and promotes apoptosis, hence functioning as a tumor suppression

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gene. Inactivation of the p53 gene has often been reported in undifferentiated thyroid carcinomas (10). Wild type p53 is an unstable protein which is present at very low cellular levels due to murine double minute gene 2 (MDM2)-mediated degradation by cytoplasmic and nuclear proteasomes (11). On the contrary mutated counterpart is resistant to MDM2-mediated degradation, resulting in abnormal stabilization and immunohistochemically detectable protein accumulation (12).

The aim of this case report presentation is to clearly depict and confirm the hypothesis of the natural progression of WDTC to ATC, showing the progression of tumor dedifferentiation in a single patient, with progressive loss of p53 expression from the well differentiated to the dedifferentiated components, confirming the hypothesis that p53 dysregulation may contribute to the natural history of the disease.

Case report

A 60-year-old man presented with a palpable anterior neck mass, which was firm and fixed to underlying structures. The mass caused mild compressive symptoms including neck fullness and dysphagia. The patient also manifested multiple cutaneous lesions with histopathologic features compatible with neurofibroma. He did not fulfill though at least one more of the seven cardinal diagnostic criteria for von Recklinghausen disease, as he did not have freckling of the groin or the axilla, café-au-lait macules, skeletal abnormalities, Lisch nodules or a first-degree relative with the disease. He refused imaging control to exclude an optic glioma, although optic fields were normal with finger confrontation test. He also refused mutational analysis. The diagnosis of von Recklinghausen disease could therefore not be established. Cervical radiation exposure was not elicited from past history. Iodine status did not need to be evaluated, since the patient lives in an iodine replete country.

The patient's medical record included hypercholesterolemia, psychotic disorder, posttraumatic acute subdural hematoma and nasal polyps. Family history was unremarkable, except from a 46-year-old sister with breast cancer and total thyroidectomy due to multinodular goiter.

Hormonal evaluation was conducted to evaluate thyroid function: TSH was 2.5 μ IU/ml (normal values 0.27-4.2) and calcitonin 4.2 pg/ml (normal values <10 pg/ml). The patient was also subjected to 24 h urine collection to rule out the presence of a pheochromocytoma, on grounds of unproven von Recklinghausen disease, at the prospect of surgery: Vanillylmandelic acid (VMA) 2.1 μ g/24 h (normal values 1-11), catecholamines 220 μ g/24 h (normal values 80-515), metanephrines 108 μ g/24 h (normal value 52-341), adrenaline 6 μ g/24 h (normal values 4-25), noradrenaline 46.1 μ g/24 h (normal values 20-105), dopamine 168.6 μ g/24 h (normal values 60-440).

Ultrasound revealed a 5.84x2.81 cm hypoechogenic nodule on the left lobe with peripheral vascularity, which comprised a 2.24x1.28 cm area with suspicious calcifications. Three smaller hypoechogenic nodules with peripheral vascularity, measuring 3.26, 3.18 and 0.83 cm respectively, were also observed on the same lobe. On the right lobe three isoechogenic nodules with cystic areas and no remarkable vascularity, dimensions 2.19, 1.47 and 0.73 cm, were found. No suspicious lymph nodes were detected by ultrasound.

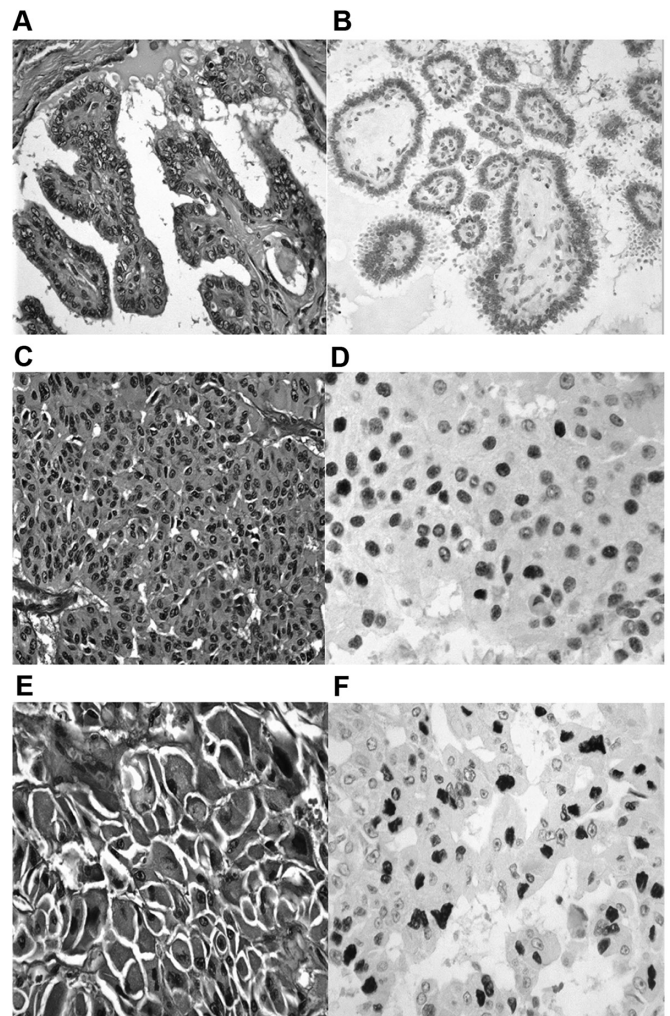


Figure 1. (A) Well differentiated papillary carcinoma (H&E; magnification, x400). (B) Weak nuclear p53 protein expression in well differentiated papillary carcinoma (magnification, x400). (C) Poorly differentiated tumor area with insular growth pattern (H&E; magnification, x400). (D) Diffuse, moderate to strong nuclear p53 protein accumulation (magnification, x400). (E) Tumor area with tendency for dedifferentiation and nuclear anaplasia (H&E; magnification, x400). (F) Diffuse strong nuclear p53 protein accumulation in dedifferentiated area (magnification, x400). H&E, hematoxylin and eosin.

Cytological examination of a fine needle aspiration (FNA) biopsy specimen was omitted from the diagnostic workup due to patient refusal, and consequently the patient underwent total thyroidectomy. Postoperative course was uneventful. The specimens underwent histopathological examination by classic H&E staining and immunohistochemical detection of p53 protein, as previously described (13). Briefly, H&E staining was performed according to routine hospital procedures, while p53 staining was performed using a mouse monoclonal anti-p53 antibody (1:3,000; DO-7; Dako) as the primary antibody. The staining intensity was graded as negative, weak, moderate and strong. Histopathological examination revealed three foci at the right lobe, dimensions 0.6, 0.8 and 2.4 cm respectively, which showed predominantly papillary but also follicular growth pattern with infiltrating margins and foci of extrathyroidal extension.

At the left lobe two lesions of 6.0 and 6.5 cm with PDTC-like histologic features of insular and trabecular

variant of PDTC were described. The tumors were composed of small uniform cells and of cells with large irregular nuclei, with partially acidophil characteristics, and were surrounded by fibrous stroma. The larger tumor showed foci of anaplastic transition, with diffuse growth pattern, nuclear pleomorphism and areas of necrosis. Both tumors invaded thyroid capsule and displayed extrathyroidal extension reaching the inked surgical margins. Vascular emboli were also identified. A dissected isthmus lymph node was free of disease.

WDTC carcinoma demonstrated weak nuclear p53 protein expression (Fig. 1A and B). PDTC appeared with diffuse, moderate to strong nuclear p53 protein accumulation (Fig. 1C and D). ATC showed diffuse, strong nuclear p53 protein accumulation (Fig. 1E and F). The patient was referred for radioiodine remnant ablation.

Discussion

PDTC and ATC have been conjectured to arise from WDTC due to frequently reported synchronous and metachronous occurrence. The somatic molecular events and downstream pathways of this dedifferentiation course have not been unraveled yet. In this paper we demonstrate the simultaneous presence of the three divergent histological subtypes in a single thyroid gland, with progressive enhancement of nuclear p53 protein expression, correlating with mutant p53 protein, in the more aggressive variants.

PDTC is a rare thyroid carcinoma histotype that occupies an intermediate position between WDTC and ATC in regards of both morphological features and biologic behavior (1,2). PDTC was initially designated as a distinct entity in 2004 by WHO, but more refined criteria were established in the 2006 Turin proposal (2-4). In juxtaposition to WDTH, which usually has an indolent course, PDTC diagnosis signals a steep increase in mortality and accounts for a disproportionate number of deaths due to non-ATC (9).

The coexistence of WDTC or ATC with PDTC-like tumor components has frequently been observed, suggesting that PDTC could represent a bridge in the stepwise dedifferentiation from WDTC to ATC (14). Although the molecular pathways underlying this intricate procedure have not been elucidated yet, loss of p53 tumor suppressor gene has been assumed to convey to this anaplastic transformation, since p53 is well expressed in WDTC but abated in PDTC and ATC (15).

Genetic analysis of thyroid malignancies has shown varying correlations of specific mutations with tumor aggressiveness. Although rearrangement of the RET proto-oncogene are frequent even in the early stages of thyroid tumorigenesis, RAS oncogene family mutations and BRAF600 gene mutations have failed to date to show a distinct prognostic significance for recurrence and overall survival. In the other hand, mutations in the TERT promoter gene (especially in combination with BRAF600 gene mutations) and p53 tumor suppressor gene mutations, have been associated with poor prognosis and have been implicated in the progression of WDTC to dedifferentiation (16). In detail, mutations in p53 gene leads to either the expression of mutant protein or to complete absence of expression. These mutations are seen in up to 25% of patients with

PDTC and in over 60% of patients with ATC (17). However, various studies have shown mutations in well differentiated tumors as well (18,19). This reveals that p53 mutation is an early event in the sequence of tumor dedifferentiation and transformation to ATC.

There is no explicit consensus regarding the optimal management of PDTC, partially due to its infrequent occurrence and the previous heterogeneity of inclusion criteria (1,20,21). The mainstay of treatment usually comprises total thyroidectomy plus regional lymph dissection when required whereas the efficacy of other modalities, such as radioactive iodine ablation (RAI), external beam radiation therapy and chemotherapy is still questionable (22). Although RAI treatment is commonly implemented, PDTC may exhibit decreased radio avidity and thus be refractory (6,22).

Finally, although our patient did not typically fulfill the diagnostic criteria for von Recklinghausen disease, the coexistence of neurofibromas is intriguing due to the scarcity of reported coexistence of thyroid carcinoma with neurofibromatosis in the literature (23-25).

Both PDTC and ATC demonstrate aggressive behavior and forecast a robust increase in coherent mortality. It is consequently of key interest to illuminate the mechanisms of the dedifferentiation process. Herein, we present a rare case of gradual enhancement of nuclear mutated p53 protein expression in multifocal thyroid tumor areas consisting of WDTC, ATC histology and PDTC-like features. This observation strengthens the postulation that ATC and PDTC could arise from progenitor foci of WDTC through a multifactorial process involving loss of normal p53 function.

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Availability of data and materials

Data sharing is not applicable to this article, as no datasets were generated or analyzed during the current study, as this is a report of an interesting case. Patient records are available upon request from the corresponding author.

Authors' contributions

GB, KN, EK and EPi contributed to conception and design of the manuscript. EPa, AL, DP, AnaP and AndP acquired, analyzed and interpreted the data, and drafted the manuscript. GB, KN and EPi revised the manuscript. DP, KN and EK confirm the authenticity of all the raw data. All authors have read and approved the final version of this manuscript.

Ethics approval and consent to participate

No ethics approval was obtained as the patient received standard treatment. Consent for treatment was obtained by the patient per protocol.

Patient consent for publication

The patient has provided written informed consent for the publication of this case report.

Competing interests

The authors declare that they have no competing interests.

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