PAX8 Mutation Disturbing Thyroid Follicular Growth: A Case Report

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Context: Heterozygous inactivating PAX8 mutations cause congenital hypothyroidism. Although more than 30 mutation carriers have been reported, no histological examination of the thyroid has been conducted.

Objective: The objective of this study was to document the histological characteristics of the thyroid tissue harboring a PAX8 mutation.

Subjects and Methods: The patient was a 40-yr-old female, whose two children had congenital hypothyroidism and an inactivating PAX8 mutation (p.K80_A84dup). She had normal thyroid function but had a thyroid nodule and received right hemithyroidectomy at age 28 yr. Mutation analyses using DNA derived from multiple sources, namely lymphocytes, nails, and laser capture microdissected thyroid samples, were performed.

Results: The PAX8 mutation was detected in the lymphocytes; however, the level of the mutant allele was significantly lower than that of the wild-type allele. This finding was compatible with her somatic mosaic state. We reviewed the histology of her resected thyroid and found a characteristic lesion in the nonneoplastic tissue: dense aggregates of thyrocytes with absent or very small follicles, resembling a fetal thyroid in the late phase of development. Mutation analyses of laser capture microdissected thyroid samples revealed that the fetal-like tissue carried the PAX8 mutation, whereas surrounding morphologically normal tissue and adenoma tissue did not.

Conclusions: In our case, the histology of PAX8 mutation-carrying thyroid tissue was characterized by the lack of follicular growth. Our observations provide the first evidence suggesting that the late phase of thyroid development is sensitive to the PAX8 gene dosage and can be disturbed by heterozygous inactivating PAX8 mutations. (J Clin Endocrinol Metab 96: E2039–E2044, 2011)

Thyroid development is orchestrated by several tissue-specific transcription factors (i.e. thyroid transcription factors) that regulate expression of thyroid-specific molecules. Paired box 8 (Pax8) is one of the thyroid transcription factors, which is expressed in the developing thyroid from the time of specification of thyroid anlage to adulthood (1). Pax8 directly regulates transcription of thyroglobulin (Tg) and thyroperoxidase (TPO) in cultured cell lines (2), indicating its essential role in the thyroid physiology. Furthermore, genetically engineered Pax8-deficient mice have severe thyroid hypoplasia (3), implying that Pax8 is also involved in the thyroid development.

In humans, heterozygous inactivating PAX8 mutations cause congenital hypothyroidism (CH) with or without...
thyroid hypoplasia (4). The mode of inheritance is dominant, which is contrasting to recessive inheritance of Pax8 knockout mice (5). To date, 31 mutation carriers belonging to 12 families have been described (4, 6–14). Their phenotypes are variable, ranging from overt CH with severe thyroid hypoplasia to subclinical hypothyroidism with a normal-sized thyroid. No histological investigation of a thyroid of the mutation carrier has been documented. Thus, little is known in humans about cellular and histological changes brought by PAX8 mutations.

We have recently reported half-siblings having CH and an inactivating PAX8 mutation (p.K80_A84dup) (13). In the present study, we extensively investigated the mother of the siblings and found that she had a somatic mosaic mutation. Of interest, her right thyroid lobe was resected due to a benign nodule, giving us a unique opportunity to examine the histology of thyroid tissue carrying a PAX8 mutation.

Subjects and Methods

Patient report

The patient, a 40-yr-old female, was a mother of two half-siblings with CH due to a PAX8 mutation (p.K80_A84dup) (Fig. 1A). A detailed description of the children has been published (13). In brief, they were diagnosed as permanent CH in the frame of newborn screening. Sequential ultrasonography revealed that their thyroid glands were normal in size in the neonatal period but showed no growth thereafter.

The patient had no remarkable medical history until when her primary physician noticed a mass in the right lobe of her thyroid at age 27 yr. She had a normal serum TSH level (1.6 mU/liter; reference 0.5–5.0), a normal free T4 level (0.9 ng/dl, reference 0.9–1.8), and a slightly high Tg level (31 ng/ml; reference <30). Ultrasonography showed a 15 × 23-mm hypoechogenic solid nodule in the mid right lobe (Supplemental Fig. 1A, published on The Endocrine Society’s Journals Online web site at http://jcem.endojournals.org). Repeated fine-needle aspiration biopsy revealed follicular cells with enlarged nuclei and condensed chromatin, suggesting malignancy. A right hemithyroidectomy was performed at age 28 yr. The pathological diagnosis was microfollicular adenoma with no invasion of the capsule or blood vessels, namely a benign neoplasm. Although she has been well with levothyroxine replacement, ultrasonography performed at age 40 yr showed three nodules in the left lobe along with scattered hypoechogenic foci in the thyroid parenchyma (Supplemental Fig. 1B).

We obtained written informed consent for molecular studies from the patient. The Institutional Review Board of Keio University School of Medicine approved the study.

Mutation detection

We extracted genomic DNA from the following: 1) peripheral lymphocytes (Gentra Puregene blood kit; QIAGEN, Hilden, Germany), 2) nails (ISOHAIR kit; Nippon Gene, Tokyo, Japan), and 3) laser capture microdissected, formalin-fixed, paraffin-embedded (FFPE) thyroid samples (QIAamp DNA FFPE tissue kit; QIAGEN). Extracted DNA were subject to PCR-based direct

FIG. 1. Demonstration of a somatic mosaic PAX8 mutation. A, A pedigree of the patient is shown along with partial sequence chromatograms of three mutation carriers. The three subjects had the 15-bp duplication mutation (c.238_252dup, p.K80_A84dup), resulting in mixed fluorescent signals of wild-type and mutant PCR fragments (indicated by the shaded area). Signals derived from the mutant fragment were weaker than those from the wild-type fragment in the patient (indicated by the asterisks), whereas the levels of the two fragments were comparable in her offspring. Cicles, Female individuals; squares, male individuals; solid symbols, individuals with congenital hypothyroidism. B, A schematic diagram showing the method of the semi quantitative mutation analysis. A PCR primer pair was designed to amplify a 103-bp fragment from the wild-type PAX8 allele. Because the patient had a 15-bp duplication mutation, PCR amplification using the primer pair yielded two fragments (wild-type, 103 bp; mutant, 118 bp) that can be separated by capillary electrophoresis. C, Results of the semiquantification of the mutant and wild-type fragments using the GeneScan analysis are shown. In this analysis, fluorescent-labeled PCR fragments were subject to capillary electrophoresis. The relative amount of the mutant fragment was assessed by the signal strength (as measured by peak height or peak area). The double peak pattern is likely caused by nontemplated addition of adenine by Taq DNA polymerase. The upper two panels show the data of the patient, whereas the lower two panels show the data of controls (a wild-type individual and a germline heterozygous mutation carrier). The arrows indicate the signals derived from the mutant fragment. Note that the signal derived from the mutant allele was slightly weaker than that derived from the wild-type allele in the germline mutation carrier, probably because the shorter wild-type fragment was amplified more efficiently than the longer mutant fragment. The analysis using the patient’s lymphocytes showed significantly weaker signal from the mutant as compared with the signal from the wild-type allele. In contrast, the levels of wild-type and mutant fragments seemed to be comparable in the patient’s nails. Each result is representative of three independent experiments that yielded same results.
sequencing and a semiquantitative GeneScan analysis with the ABI 3100xl genetic analyzer (Applied Biosystems, Foster City, CA). PCR conditions and primer sequences are available upon request.

Laser capture microdissection

We cut 5-µm sections from the FFPE thyroid block and mounted them on slides covered with polyethylene naphthalate membrane (PALM MembraneSlides; P.A.L.M. Microlaser Technologies, Bernried, Germany). After deparaffinization in xylene, sections were rehydrated in a series of graded alcohols and were subject to laser capture microdissection (PALM MicroBeam laser capture microdissection platform; Carl Zeiss MicroImaging, Bernried, Germany). For each sample, three areas were evaluated.

Immunohistochemistry

Immunohistochemical staining was done on 5-µm sections of the FFPE thyroid tissue. Tissue was deparaffinized in xylene and was rehydrated in graded concentrations of alcohol. Endogenous peroxidase activity was blocked by incubation in 1% H2O2 to unmask antigens, and the slides were microwaved in 10 mm citrate buffer (pH 6) for 15 min. After blocking nonspecific staining with horse serum, the slides were incubated with primary antibodies [rabbit anti-Tg polyclonal antibody (Dako, Kyoto, Japan) or mouse anti-TPO monoclonal antibody (ALEXIS Biochemicals, San Diego, CA)]. The dilution of each antibody was 1:5000 and 1:4, respectively. Sections were then incubated with biotin-labeled secondary antibodies (dilution 1:500). Subsequent reactions with the ENVISION system (Dako) and diaminobenzidine (Sigma-Aldrich, St. Louis, MO) were followed by counterstaining with hematoxylin.

Results

Demonstration of a somatic mosaic mutation

Using PCR-based sequencing of lymphocyte-derived DNA, we detected the PAX8 mutation (c.238_252dup, p.K80_A84dup), but the chromatogram was atypical (Fig. 1A): signals derived from the mutant fragment seemed to be weaker than those from the wild-type product. Semi-quantification of the mutant and wild-type fragments with the GeneScan analysis (Fig. 1B) gave a consistent result (Fig. 1C). We also analyzed nail-derived DNA and found that the amount of mutant and wild-type fragments were comparable in the nails. These findings indicate that she had the mutation in a somatic mosaic state.

Thyroid histology

Histological sections of the thyroid were reviewed, paying particular attention to the nonneoplastic tissue. We found hypercellular lesion surrounded by morphologically normal thyroid tissue outside the follicular adenoma (Fig. 2A). The cell aggregates consisted of thyroid follicular cells that had absent or very small follicles (Fig. 2B), resembling fetal thyroid tissue in the follicular growth stage (15). Immunostaining for Tg and TPO showed comparable distribution patterns between the morphologically normal tissue and the fetal-like tissue (Fig. 2B): follicles were stained with the anti-Tg antibody, and the cytoplasm of thyrocytes was stained with the anti-TPO antibody. As for adenoma cells, Tg was chiefly stained in the cytoplasm, and TPO was not stained (Fig. 2B).

Detection of the mutation in the fetal-like thyroid tissue

The patient’s thyroid had three distinct cell populations: morphologically normal tissue, fetal-like tissue, and adenoma tissue. To clarify the association between the characteristics of tissue and the PAX8 mutation, pure populations of the three tissues were obtained with the laser capture microdissection technique. The GeneScan analysis (Applied Biosystems) with use of microdissected samples revealed that the fetal-like tissue carried the mutation in a heterozygous state, whereas the morphologically normal tissue and adenoma tissue did not (Fig. 2B).

Discussion

To date, 11 PAX8 mutation-carrying pedigrees with familial occurrence have been reported (4, 6–14). Although nine families showed parent-to-offspring transmission (4, 6–9, 11, 12, 14), two families had affected siblings from unaffected parents (10, 13), erroneously suggesting a recessive trait. Incomplete penetrance has been proposed for an explanation of the pseudorecessive inheritance (10). Here we showed that somatic mosaicism would be another mechanism. We stress that clinicians should consider a PAX8 mutation in sibling cases with nongoitrous CH, even though their parents are apparently unaffected.

In the patient, the mutation was detected in cells derived from three germ layers [the thyroid (endoderm), blood (mesoderm), and nails (ectoderm)] and was present in germ cells. This implies that the mutation occurred in early embryonic life (i.e. between the two cell stage and blastocyst stage; Supplemental Fig. 2). In the thyroid, a large part of the tissue had normal histology, whereas the mutant cells formed small foci. Two factors would explain the skewed histological appearance. One is the lack of follicles in mutant tissue, causing a drastic reduction in tissue volume per cell. The other is the difference in survival and/or proliferating capacity between the mutant and nonmutant tissue, resulting in skewed proportion of cell number.

The most noteworthy observation of the present study is the histology of the PAX8 mutation-carrying thyroid tissue, which was characterized by absent or very small follicles. Histological differentiation of the human thyroid
FIG. 2. Histology of the resected thyroid. A, Semimacroscopic appearance of the thyroid section [left panel; hematoxylin and eosin (H&E) stain, scale bar, 500 μm] and a schema of the section (right panel) are shown. Normal thyroid tissue has large follicles with abundant colloid, and appears pink in the H&E-stained section. Contrastingly, the adenoma tissue (indicated by A in the right panel) and the fetal-like tissue (indicated by F in the right panel) appear purple because of their high cellularity. B in the right panel represents a blood vessel. B, Microscopic images of H&E staining and immunostaining (×400, scale bars, 100 μm). Images and results of the GeneScan analysis are aligned with each cell population, namely morphologically normal tissue, fetal-like tissue, and adenoma tissue. Although the fetal-like tissue had absent or very small follicles, each thyrocyte in the tissue showed normal microscopic appearance. The colloid was immunoreactive for Tg as in the normal tissue. TPO staining also showed comparable cytoplasmic immunoreactivity with the normal tissue. As for adenoma, anti-Tg antibody stained the colloid and cytoplasm, but TPO staining showed no immunoreactivity. Semiquantitative GeneScan analyses using microdissected thyroid samples showed that the fetal-like tissue carried the mutation in the heterozygous state, whereas the morphologically normal cells and adenoma cells did not. Each result is representative of three experiments (using independent microdissected samples) that yielded same results. The peak shapes were somewhat different among the sources of DNA. This was reproducible, but we could not clarify the mechanism.
can be divided into three stages according to cell morphology and follicle size (15): the precolloid stage (7–13 gestational weeks in humans), the beginning colloid stage (13–14 gestational weeks), and the follicular growth stage (after 14 gestational weeks). The histological characteristics of the PAX8 mutation-carrying thyroid tissue seemed to be comparable with those of fetal thyroid in the follicular growth stage. Immunostaining patterns for Tg and TPO, which showed comparable distribution patterns between the mutation-carrying thyrocytes and normal thyrocytes, also support this assumption (16). The histological characteristics described above imply that the follicular growth stage is sensitive to PAX8 gene dosage and can be disturbed by heterozygous inactivating PAX8 mutations in humans.

In the two children of the patient (germline mutation carriers), peculiar chronological change of the thyroid morphology was observed (13). Their thyroids were normal in size in the neonatal period but showed no growth thereafter. Meeus et al. (9) has also described similar chronological changes previously. We suppose that these chronological changes can be explained by limited follicular growth capacity of the mutation-carrying thyroids because thyroid growth is determined by increase in follicle size (but not by increase in the number of follicles) (17). Moreover, one child of the patient (subject II-1 in Fig. 1A) showed low thyroid echogenicity at age 15 yr (13). This finding seems compatible with the presumed histological changes (i.e. small follicles) because low thyroid echogenicity suggests decreased follicle size (18). Based on our experience, we propose two subtypes of thyroid hypoplasia: hypoplasia with a quantitative abnormality (i.e. decrease in absolute number of thyrocytes) and one with a qualitative abnormality (i.e. decrease in follicle size). These two types may have distinct cellular and developmental defects, for example, reduced progenitor cell population in the former and lack of terminal differentiation in the latter.

The patient had multiple thyroid adenomas. It is unlikely that the mutation facilitated tumorigenesis directly because at least one adenoma arose from a thyrocyte without the mutation. However, we cannot exclude the possibility that mutation-carrying cells induced neoplastic transformation of neighboring cells.

In summary, we identified the first individual who had a somatic mosaic PAX8 mutation. Her thyroid was a mosaic for thyrocytes with or without the mutation. The PAX8 mutation-carrying thyroid tissue showed fetal-like appearance, which was comparable with that of fetal thyroid tissue in the follicular growth stage. Collectively, we showed the first evidence suggesting that a subset of PAX8 mutation carriers may have thyroid hypoplasia with a qualitative abnormality.

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References


