A Case of Estrogen Receptor Positive Secretory Carcinoma in a 9-Year-old Girl With ETV6–NTRK3 Fusion Gene

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The patient was a 9-year-old premenarcheal pediatric female, whose chief complaint was a well-circumscribed palpable right breast mass without nipple discharge. Although the patient had noticed the lump 2 years prior to hospital admission, its size (1.5 \times 1.3 cm) had been stable. There was no family history or previous history of malignancies. Physical examination showed a well-delimited, elastic-firm and movable tumor just beneath the nipple and areolar complex. Regional lymph nodes were not palpable. Ultrasonography and breast computed tomography revealed a subareolar oval-shaped tumor exhibiting homogeneous echogenicity with clear margins. Distant metastases could not be detected using whole-body computed tomographic scans. A fine-needle aspiration cytology specimen showed atypical cells with prominent nucleoli and abundant intracellular secretory material, suggesting the possibility of secretory carcinoma. Histopathological analysis of the core needle biopsy specimen revealed that the tumor was a secretory carcinoma. The patient underwent total mastectomy with sentinel lymph node biopsy. Metastases were not observed in the removed lymph nodes. Estrogen receptor was weakly positive and progesterone receptor was negative. Human epidermal growth factor receptor 2 expression was also negative. In addition, the ETV6 (exon 5) and NTRK3 (exon 13) fusion gene was detected using the reverse transcription–polymerase chain reaction method. This gene is considered specific for secretory carcinoma. Immunohistochemistry revealed weak basal differentiation [cytokeratin 5/6(CK5/6)(+), vimentin(+)] and epidermal growth factor receptor(+). The patient has received no adjuvant therapy and is currently disease free at 12 months after surgery.

Key words: juvenile breast cancer – secretory carcinoma – estrogen receptor – ETV6–NTRK3 fusion gene – basal marker

INTRODUCTION

Breast cancer is extremely rare in children and adolescents. It has been reported to comprise <0.1% of breast cancers and <1% of pediatric cancers (1–3). Secretory carcinoma (SC) occurs with an incidence of 0.1–0.3% of all breast cancers. Although SC is known to occur more frequently in adults, the majority of breast cancers in the pediatric population are SC (4). The typical genomic profile of SC involves the harboring of the ETV6–NTRK3 fusion gene (5,6). Immunohistological examination has revealed that SCs frequently show triple-negative features and basal-like differentiation (7). We herein report on a case involving a 9-year-old girl with SC which
had an ETV6–NRK3 fusion gene with weak basal-like differentiation, who underwent mastectomy with sentinel node biopsy. In children with clinically node-negative breast cancer, we believe that fine-needle aspiration cytology (FNAC) is needed, even if the lesion appears to be benign, and that to avoid life-long complications, sentinel node biopsy is an appropriate alternative to axillary dissection.

**CASE REPORT**

The patient was a 9-year-old premenarcheal pediatric female, whose principal complaint was a well-circumscribed palpable right breast mass. The lump had been noticed by the patient 2 years previously and its size had been stable. There was no prior or family history of malignancies. The patient’s development was normal for her age. Physical examination showed a well-delimited, elastic-firm and movable tumor just beneath the nipple and areolar complex. Nipple discharge or retraction was not observed. The patient did not feel spontaneous pain but felt tenderness at the lesion. The size of the tumor was $1.5 \times 1.3$ cm and it was not fixed to the muscle. The axillary or supraclavicular lymph node was not palpable. The left breast was unremarkable. Ultrasonography revealed a subareolar oval-shaped tumor showing homogeneous echogenicity with clear margins ($15.9 \times 13.9$ mm in size). The ultrasonographic findings related to the tumor were defined as Category 3b (8). The lactiferous duct or mammary gland was not observed. Mammography depicted the tumor as a partially lobulated mass. The margin of the mass was almost clear. Breast computed tomographic (CT) scans showed a homogeneously enhanced lesion ($17 \times 16$ mm in size) with a clear margin. Regional lymph nodes or distant metastases were not detected by whole-body CT scans. The aspiration specimen taken from the lesion showed atypical cells with prominent nucleoli and abundant intracellular secretory material, suggesting that the tumor was possibly an SC. A core needle biopsy (CNB) specimen revealed that the neoplastic cells formed glands and nests with abundant clear intracytoplasmic material, which was positive for the periodic acid Schiff (PAS) reaction. The background of the lesion was associated with prominent hyalinized fibrous tissue. Another characteristic feature was the presence of extracellular Alcian blue-positive secretory mucosubstance. The patient was subsequently given a definitive diagnosis of SC (T1N0M0 Stage I).

Using mammography, we confirmed that the extent of the patient’s mammary gland was comparable with that of adults. She underwent total mastectomy with sentinel lymph node biopsy by means of a double-mapping method. Total mastectomy was performed in an identical manner to that for adults. We removed two hot nodes as sentinel nodes and two swollen lymph nodes which were adjacent to the sentinel nodes.

The initial diagnosis of the tumor using FNAC and CNB was confirmed on permanent histopathological examination (Fig. 1a). The lesion was surrounded by a thick wall and its margin status was negative. Metastases were not observed in the sentinel lymph nodes and two adjacent nodes. The estrogen receptor (ER) was weakly positive (Fig. 1b) and the progesterone receptor was negative. Human epidermal growth factor receptor 2 (HER-2) expression was also negative. Immunohistochemistry revealed weak basal differentiation [CK5/6(+), vimentin(+) and epidermal growth factor receptor (EGFR) (+)] (Fig. 2a–c). The antibodies that we used were as follows: anti-ER (SP1, Predilute: Roche, Basel, Switzerland); anti-PgR (1E2, Predilute: Roche); anti-Her2 (Rabbit polyclonal 1:300: Dako, Carpinteria, CA, USA); anti-CK5/6 (D5/16B4, 1:100: Dako); anti-vimentin (V9, 1:100: Dako); anti-EGFR (EGFR25, 1:50: Leica Microsystems, Bannockburn, IL, USA).

In order to confirm the diagnosis, we needed to detect the ETV6 (exon 5) and NTRK3 (exon 13) fusion gene t(12;15)(p13;q25) of 511 bp using the reverse transcription–polymerase chain reaction (RT–PCR) method. This gene is specific to SC. We extracted total RNA from snap-frozen tissue. PCR was carried out to amplify the ETV–NTRK3 fusion gene using 5’-TCCTCGAGTCCCCACCCGAAG-3’ as a forward primer and 5’-CATCGCCGCACACTCCATA GAA-3’ as a reverse primer. We detected the PCR products
of 511 bp using agarose gel electrophoresis. PCR products of 511 bp can be seen between the marker fragments of 400 and 600 bp. We then confirmed the PCR product as the fusion of the ETV6 (exon 5) and NTRK3 (exon 13) genes by direct sequence using Big Dye Terminator ver. 3.1 (Life Technologies Japan, Tokyo, Japan) (Fig. 3b).

The patient received no adjuvant therapy including radiotherapy and is currently disease free at 12 months after surgery. Genetic testing was not performed because her mother refused permission. The parents have discussed the timing of plastic surgery with us. We are planning two future plastic surgeries, the first during the patient’s adolescence and the second at adulthood. We intend to follow the patient up for at least 20 years.

**DISCUSSION**

Age is an important factor in the diagnosis and management of breast tumors. The majority of breast masses in children, such as fibroadenoma in girls and gynecomastia in boys, are benign. Murphy et al. (9) reported that the average age of children with primary breast cancer was 11 years, with a range of 3–19 years. Tanimura and Konaka documented breast cancer in a 5-year-old Japanese girl (10). Approximately 80% of breast cancers in children are SC (10). Age at presentation with SC varies from 3 to 87 years (11,12). The present patient was one of the youngest SC cases reported in Japan. SC is often located near the areola and has an indolent clinical course. One of the characteristic biological features of juvenile SC is negative hormone receptor status, suggesting that this type of tumor is not influenced by sex hormones in juvenile patients (9,13,14). ER-positive cases have been reported in invasive ductal carcinoma and invasive lobular carcinoma (14). However, the majority of SCs tend to be ER- and PR-negative and do not show HER-2 overexpression (15). The present case exhibited weakly positive ER expression, although the patient had not gone through menarche. The association between sex hormones and juvenile SCs should be investigated in the future. Typical pathologic features for this cancer have been reported previously (14). In the present
Distant metastases from SC are extremely rare and only five juvenile patients with SC over at least this time period (24). Even though there are few cases of juvenile SC (10,14). Even though there are node biopsy procedures have been successfully applied in a fusion gene which is considered to be specific to SC. and direct sequencing to diagnose the tumor as SC, due to study. It did not exhibit pathological features that are typical of SC. However, it was possible using the RT–PCR method and direct sequencing to diagnose the tumor as SC, due to detection of the ETV6 (exon 5) and NTRK3 (exon 13) fusion gene which is considered to be specific to SC.

Juvenile SC usually follows a favorable prognosis. The overall incidence of axillary lymph node infiltration is around 30% in children and adults regardless of gender (16,17). Although lymph node metastases are rarely observed in female patients with SC tumors of <2 cm in diameter, nodal metastases might occur more frequently in male patients with smaller tumors (18). Axillary metastases rarely involve more than three lymph nodes (19). Lymphedema, which is a serious life-long problem, occurs in 6–30% of the patients treated by axillary dissection (17,20). Sentinel node biopsy procedures have been successfully applied in a few cases of juvenile SC (10,14). Even though there are currently no data on sentinel node biopsy followed by back-up axillary dissection in children, it is likely that sentinel node biopsy would be a valuable tool for breast cancer, just like it is in adults. Post-operative radiotherapy (17,21) and adjuvant chemotherapy (21,22) have been used on at least two occasions. However, there is at present insufficient evidence to recommend either approach in the management of SC (23). Post-operative radiotherapy is not advised for children due to possible secondary effects such as fibrosis of the lung, rib damage and the consequent asymmetry of the rib cage (21,22).

Adverse prognostic features previously reported were tumors that were larger than 2 cm in diameter, lack of gross circumscription and infiltrative margins (16).

Recurrence has been reported to occur at up to 20 years after the initial treatment (24). Thus, it is desirable to follow juvenile patients with SC over at least this time period (24). Distant metastases from SC are extremely rare and only five cases have been reported (23).

In conclusion, even though breast cancer is extremely rare in children and adolescents, it is important to confirm the pathology of the breast tumors that are found. Immunohistochemical examination demonstrated that ER was weakly positive in the tumor of the case involved in our study. It did not exhibit pathological features that are typical of SC. However, it was possible using the RT–PCR method and direct sequencing to diagnose the tumor as SC, due to detection of the ETV6 (exon 5) and NTRK3 (exon 13) fusion gene which is considered to be specific to SC.

Conflict of interest statement
None declared.

References