Genomic Aberrations in Salivary Duct Carcinoma Arising in Warthin Tumor of Parotid Gland

DNA Microarray and HER2 Fluorescence In Situ Hybridization

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Carcinoma arising from Warthin tumor is extremely rare. A 79-year-old man was admitted for a firm, well-defined, 5-cm left infra-auricular mass. Aspiration cytology showed many lymphohistiocytes and oncocyes in a proteinaceous background, compatible with Warthin tumor. A left superficial parotidectomy showed a solid mass around the cyst wall. The tumor cells of the solid area were arranged as infiltrative ducts with a few foci of malignant transformation. Virtual karyotyping disclosed a complex pattern of genetic aberrations with a focal amplification in 12q14–q21.2. This chromosomal region contains the MDM2 (murine double minute) gene, which regulates p53 inactivation. HER2 fluorescence in situ hybridization showed a focal amplification. Subsequently, the patient underwent total parotidectomy and ipsilateral neck dissection for a recurrence. To our knowledge, this is the first case of salivary duct carcinoma arising from Warthin tumor. The essential molecular pathway has not been reported, we presume an important role of MDM2 amplification–P53 inactivation.

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REPORT OF A CASE

A 79-year-old man presented with a painful left infra-auricular mass. A physical examination revealed a firm, well-defined, 5-cm tumor in the left parotid area. The patient had a history of diabetes mellitus, hypertension, and resolved pulmonary tuberculosis. No systemic neoplasm was identified. Fine-needle aspiration of the tumor was performed. Some turbid fluid was aspirated. The cytologic smear showed many lymphoid cells, histiocytes, and clusters of oncocytic cells in a proteinaceous background, suggesting typical WT (Figure 1). Few scattered atypical single cells were noted, but due to the paucity of atypical cells, it was considered benign atypia of oncocytic cells. Ten months later, the patient returned due to aggravated pain and increase in size of the mass. On head and neck computed tomographic imaging, a left parotid mass with internal low densities and many enlarged lymph nodes in left parotid, carotid, and parapharyngeal spaces were noted, suggesting WT associated with tuberculous or suppurrative lymphadenitis (Figure 2). A left superficial parotidectomy was performed. Seven months later, a recurrent mass (5.5 cm in greatest dimension) developed at the deep lobe of the left parotid gland. He underwent a total parotidectomy and ipsilateral neck dissection. Eleven of 30 cervical lymph nodes were proven to contain metastatic carcinoma.

PATHOLOGIC FINDINGS

The gross examination of the superficial parotidectomy specimen showed a firm infiltrative mass (3.8 cm in greatest dimension) around the cystic mass (2.5 cm) (Figure 3, A). The cystic cavity was filled with turbid necrotic fluid. The cyst wall was thickened up to 0.8 cm, focally showing papillary excrescences. The cut surface of the solid mass was whitish gray and granular with multifocal necrotic foci. Microscopically, the tumor cells were arranged as infiltrative ducts with comedo necrosis in a background of desmoplastic stroma (Figure 3, B). The WT portion revealed a typical morphology with the cyst wall lined by oncocytic epithelium with underlying lymphoid tissue (Figure 3, C). The inset shows 2 cell layers (basal layer and columnar oncocytic cell) of oncocytic epithelium. There were a few foci of transition from oncocytic/metaplastic to malignant cells (Figure 3, D). p63 (Biogenesis, Cambridge, Massachusetts) immunostaining was lost at the point of transition from oncocytic epithelium to salivary duct carcinoma (SDC) (Figure 3, E). Nodal metastases were identified in cervical and intraparotid lymph nodes. HER2/neu (Dako, Carpinteria, California) immunohistochemical staining revealed focal...
The preoperative aspiration cytology finding shows many lymphocytes and foam cells in a proteinaceous fluid background admixed with clusters of oncocytic epithelial cells (Papanicolaou, original magnifications ×100 and ×400 [inset]).

Neck computed tomography shows a left parotid mass (5 cm in greatest dimension) with an internal low density that infiltrates into adjacent soft tissue. There are multiple enlarged lymph nodes in the left carotid, parapharyngeal space.

**GENETIC ABBERATIONS IN FORMALIN-FIXED PARAFFIN-EMBEDDED TISSUE**

HER2 fluorescence in situ hybridization (FISH) examination revealed an increased ratio of ERBB2 (HER2) gene when compared with CEP17 (Figure 4, A). FISH analysis was performed as previously described. The virtual karyotyping with single nucleotide polymorphism (SNP) microarray was separately performed for the oncocytic epithelium of WT and the SDC component. DNA was separately extracted from manually microdissected tissue from 10-μm paraffin sections. DNA extraction from formalin-fixed paraffin-embedded tissue, SNP microarray assay, and data analysis were done as previously described. The virtual karyotype from the carcinoma showed multiple genetic aberrations: −2(q24.3–q37.3), −4, −5(p15.33–q23.1), −5(q11.2–q12.1), −6, −8, −9(p24.3–q21.33), −9(p21.3–21.1), −10, −10 (p11.23–q26.3), −11, −12,amp12(q14.3–q21.2), −15, +16p, −17, −17(p13.3–q11.2), −18, −18, −19, −22 (Figure 4, B). The SNP array virtual karyotype confirmed the imbalance between the ERRB2 locus (17q11.2–q12LSI) and CEP17 (17p11.1–q11.1). However, because the virtual karyotype is not quantitative, it only indicates lower abundance of 17p (including the centromere and a portion of the q arm), compared with the rest of 17q (including the ERBB2 locus). It is important to note the virtual karyotype results suggested polyploidy, based on the pattern of losses (losses at multiple levels) and this was confirmed by the FISH results. Interestingly, the region of amplification in chromosome 12 contains the MDM2 (murine double minute 2) gene, which is a known candidate of carcinogenesis in salivary gland tumors. In addition, this tumor showed homozygous deletion of the p16/p15 (CDKN2A/CDKN2B) locus. Loss of heterozygosity at the p16 locus has been shown to be more frequent in SDCs than in other salivary gland tumors. Single nucleotide polymorphism microarray from the oncocytic epithelium showed no detectable aberration (normal karyotype). When microdissection was performed, care was taken to select an area with predominant epithelial component. The areas selected for virtual karyotyping contained less than 10% lymphocytic stroma.

**COMMENT**

Warthin tumor is a benign lymphoepithelial neoplasm representing 10% of all parotid gland tumors. Malignant transformation of a WT is an extremely rare event. We found only 33 cases of carcinoma arising from WT in the English literature. The reported types of carcinoma arising within a WT are mucoepidermoid carcinoma (10 cases), squamous cell carcinoma (9 cases), adenocarcinoma (8 cases), undifferentiated (2 cases), poorly differentiated carcinoma (2 cases), and anaplastic carcinoma (1 case).

The differential diagnosis of a malignant neoplasm in a WT should include a metastatic tumor. The most common metastases are squamous cell carcinoma and melanoma from the head and neck. Other primary sites include lung, breast, renal, gastrointestinal, and colon carcinomas. To support a primary malignant transformation, the medical history and physical examination must exclude a metastatic source.

Seifert proposed 4 criteria that should be present for the diagnosis of malignant transformation in a WT. These include (1) the presence of a preexisting benign WT, (2) the presence of transition zones from benign oncocytic to malignant epithelia, (3) infiltrating growth in the surrounding lymphoid tissue, and (4) exclusion of metastases to the lymphoid stromal component of a primary extrasalivary tumor; our case fulfilled all 4 criteria.

The pathogenesis of malignant transformation in WT is unclear. However, Damjanov et al hypothesized that squamous carcinoma might arise from foci of squamous metaplasia. In their studies, electron microscopy was used to demonstrate a transition from mitochondrial-rich cylindrical cells to squamous cells, although the exact histogenetic sequence could not be reconstructed. Transition from cylindrical cells to squamous cells may be due to infection, ischemia, or necrosis.
Figure 3.  
A, The gross specimen shows a protruding mass from the superficial lobe of parotid gland, measuring 5 cm in greatest dimension. It is partly solid and partly cystic. The cyst wall (yellow rectangle) is covered with thick turbid fluid with partial papillary excrescences (black star). The inner part reveals infiltrative firm tumor (red square), measuring 3 cm in greatest dimension.  
B, The tumor cells are arranged as infiltrative ducts with comedo necrosis. The nuclei are enlarged with marked pleomorphism and prominent nucleoli, suggesting high nuclear grade.  
C and inset, Grossly papillary excrescent part shows typical Warthin tumor, composed of bilayered oncocytic tubules in the lymphoid background.  
D, A focus of transition from oncocytic cells to malignant cells is identified at the cyst wall.  
E, p63 immunohistochemical staining shows continuous linear pattern along the basal layer in oncocytic epithelium but is abruptly lost at the salivary duct carcinoma portion.  
F, HER2/neu immunohistochemistry discloses a diffuse membranous staining.  
G, Gross cystic disease fluid protein 15 reveals diffuse and strong positivity of tumor cells.  
H, Androgen receptor shows nuclear staining of tumor cells (hematoxylin-eosin, original magnifications $\times$100 [B], $\times$200 [C, inset], and $\times$400 [D]; original magnification $\times$40 [E]; original magnification $\times$400 [F]; original magnification $\times$200 [G]; original magnification $\times$200 [H]).

Figure 4.  
A, HER2 fluorescence in situ hybridization shows a definitive amplification, at least 2-fold increase of HER2 signal (orange signals) rather than CEP17 green signal (centromere).  
Salivary duct carcinoma has been a first reported type among malignant transformations from WT. Salivary duct carcinoma is always a high-grade malignancy with a male to female ratio of 4:1. Some SDCs arise as the malignant component of a carcinoma ex pleomorphic adenoma. Most SDCs show positive distinct membrane staining for HER2/neu protein. In this case, the carcinoma component also shows 2+ to 3+ membranous staining pattern. The overexpression of this protein is well correlated with an amplification of ERBB2 (HER2 gene) on FISH. Patients with ERBB2-positive tumors are potential candidates for treatment with trastuzumab (Herceptin, Genentech, San Francisco, California). HER2/neu overexpression is considered a prognostic marker for SDC, as well as tumor size and distant metastasis.

For evaluation of tumor progression, we performed SNP microarray analyses from the oncocytic epithelium of the WT component and the SDC component. The oncocytic epithelium showed a normal diploid chromosomal complement. The SDC tumor showed a complex chromosomal profile with multiple losses including homozygous loss of the CDKN2A/CDKN2B (p16/p15) locus and amplification of a region in 12q that includes the MDM2 gene, which is a key regulator of p53 activity by inducing its degradation. Overexpression of MDM2 mRNA has been reported in most salivary gland tumor cell lines (pleomorphic adenoma; epithelial-myoepithelial carcinoma; carcinoma ex pleomorphic adenoma; adenoid cystic carcinoma; adenocarcinoma, not otherwise specified). MDM2 overexpression is capable of inactivating the p53 function, leading to tumorigenesis. The virtual karyotype results also confirmed the increased ratio of the ERBB2 locus compared with the chromosome 17 centromere, thus confirming the increased ERBB2:CEP17 ratio detected by FISH.

In a recent report, Persson and colleagues have postulated that 12q genes (in particular MDM2) may be a main target gene for malignant transformation in carcinoma ex pleomorphic adenoma. In addition, HMG2A has been shown to be the target gene in pleomorphic adenoma with translocations involving 12q14–15 and is frequently coamplified with MDM2. Stenman concluded that pleomorphic adenoma with coamplification of HMG2A and other 12q genes (especially MDM2) may have an increased risk of malignant transformation.

In this study, we have reported a case of SDC arising from WT. This tumor showed genetic aberrations in transformation of WT to SDC. However, this is the first report for genetic aberrations that overlap with those reported in other salivary gland tumors such as carcinoma ex pleomorphic adenoma. Further studies on the role of MDM2 and HER2 in SDC should be carried out to elucidate their pathophysiologic role and to enable targeted therapies for this neoplasm.

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References