

Original Article

Expression of transcription factor ZEB1 in sarcomatoid salivary duct carcinoma of the parotid gland

Keisuke Kawashima¹, Chiemi Saigo¹, Hanamatsu Yuki¹, Ayumi Niwa², Yusuke Kito¹, Ryo Kawaura³, Bunya Kuze³, Tamotsu Takeuchi¹

Departments of ¹Pathology and Translational Research, ²Tumor Pathology, ³Otolaryngology, Graduate School of Medicine, Gifu University, Gifu, Japan

Received February 1, 2017; Accepted February 26, 2017; Epub May 1, 2017; Published May 15, 2017

Abstract: Recent studies have identified the epithelial mesenchymal transition-inducing transcriptional repressor ZEB1 as promoting invasion, metastasis, and stemness in aggressive cancers and causing resistance to cancer therapy. We present a study of aberrant ZEB1 expression in a case of salivary duct carcinoma harboring sarcomatoid elements. A 60-year-old man presented with a mass, which rapidly grew in the right parotid gland; the patient underwent surgical resection. The mass extended widely, into the adipose tissue surrounding the parotid gland, with regional lymph node metastasis. Histopathological examination revealed that the tumor resembled ductal carcinoma of the breast, with comedo-like necrosis, and elements of invasive-growth adenocarcinoma. Immunohistochemical study revealed HER2/neu, androgen receptor, and mammaglobin immunoreactivity in the cancer cells. Furthermore, we found a sarcomatous element, highlighted by pleomorphic spindle-shaped cells with marked nuclear atypia, in the tumor. Based on these histopathological features, we considered the tumor to be a sarcomatoid variant of salivary duct carcinoma. Further immunohistochemical analysis showed that not only sarcomatoid tumor cells, but also epithelial tumor cells, including intraductal carcinoma-like cells, exhibited immunoreactivity with a specific antibody to ZEB1. Notably, ZEB1 immunoreactivity was also found in non-sarcomatoid salivary duct carcinoma of other patients. In contrast, intraductal components of breast cancer from other patients did not show ZEB1 immunoreactivity. Our findings might indicate that “ductal carcinoma of breast-like cells”, in both sarcomatoid variants and non-sarcomatoid type of salivary duct carcinoma, expressed ZEB1, and thus, had a greater potential to be aggressive compared to morphologically similar breast cancer cells.

Keywords: Salivary duct carcinoma, sarcomatoid variant, epithelial mesenchymal transition, ZEB1

Introduction

Although salivary duct carcinoma has a close morphologic resemblance to ductal carcinoma of the breast, clinicopathological studies show that salivary duct carcinoma exhibits more aggressive behavior than breast ductal carcinoma [1, 2]. Its aggressive behavior is characterized by rapid progression, early lymph node metastasis, a high risk of local recurrence, and distant metastasis, resulting in poor survival rates (median 3 years) [3]. The sarcomatoid variant of salivary duct carcinoma, especially, is a highly aggressive cancer, defined by a biphasic neoplasm with both salivary duct carcinoma and sarcomatoid components [4, 5]. Unraveling the molecular mechanisms that contribute to the highly malignant properties of this cancer may provide insights into regulating various aggressive cancers.

Recently, ZEB1 expression appeared to be the common link in the aggressive behavior of many cancer types [6-8]. In this study, we examined the expression of ZEB1 in a case of sarcomatoid variant of salivary duct carcinoma by comparing it to the expression of ZEB1 in breast cancer. The present findings may indicate that the cells of a sarcomatoid variant and non-sarcomatoid type of salivary duct carcinoma harbored an aberrant ZEB1 expression phenotype in a “breast intraductal carcinoma”-like element.

Materials and methods

Ethical statements

All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or

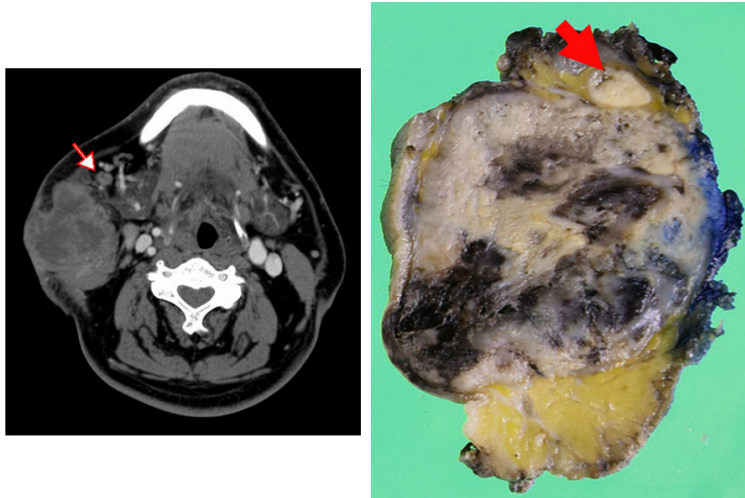


Figure 1. Left: Axial contrast-enhanced CT image showed a tumor in the deep lobe of the parotid gland. Right: The cut surfaces of tumor showed a white-gray mass containing necrotic and hemorrhagic areas. Histopathological study unraveled metastatic tumor in enlarged lymph node (arrow).

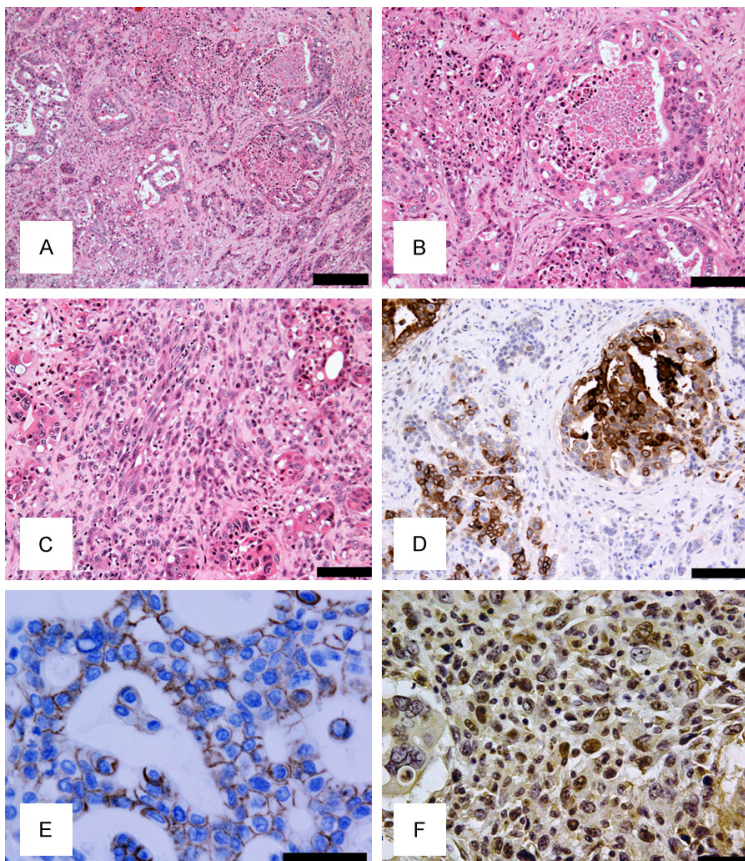


Figure 2. (A-C) Histopathologically, the tumor demonstrated a glandular growth pattern with robust necrosis in the center of the duct, mimicking high-grade intraductal carcinoma of the breast, invasive cancer nests, and sarcomatous irregular spindle-to-oval-shaped cells. (D-F) On immunohistochemical staining, tumor cells exhibit mammaglobin (D), HER2/neu (E), and AR immunoreactivity (F). Scale bars = 200 μ m in (A); 100 μ m in (B-D); and 50 μ m in (E and F).

national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. Approval to use archival pathological tissue specimens was obtained from the Institutional Review Board of the Gifu University Graduate School of Medicine (a specific approval number 24-256). Informed consent was obtained from individual participant with sarcomatoid variant of salivary duct carcinoma in the study.

Case presentation

The patient was a 60-year-old man. He presented with pain in the right parotid gland that had persisted for 6 months. The clinical examination, including imaging, indicated that the patient had a tumor in the right parotid gland (**Figure 1**). Fine needle aspiration biopsy was conducted and resulted in a diagnosis of the mass as adenocarcinoma. The patient underwent surgical resection.

Antibodies and immunohistochemical staining

The detailed procedure for preparation and characterization of a conventional rabbit antibody to ZEB1 was previously described [9]. A conventional rabbit antibody against SNAIL was purchased from Abgent (San Diego, CA, USA). Rabbit monoclonal antibodies to androgen receptor (AR) and HER2/neu were purchased from Cell Signaling technology (Beverly, MA) and Roche Diagnostics (Penzberg, Germany), respectively. Murine monoclonal antibody to mammaglobin and Gross Cystic Disease Fluid Protein-15 (GCDFP-15) were purchased from Dako-Agilent Technologies (Glostrup, Denmark) and Leica Biosystems Newcastle Ltd (Newcastle, UK), respectively.

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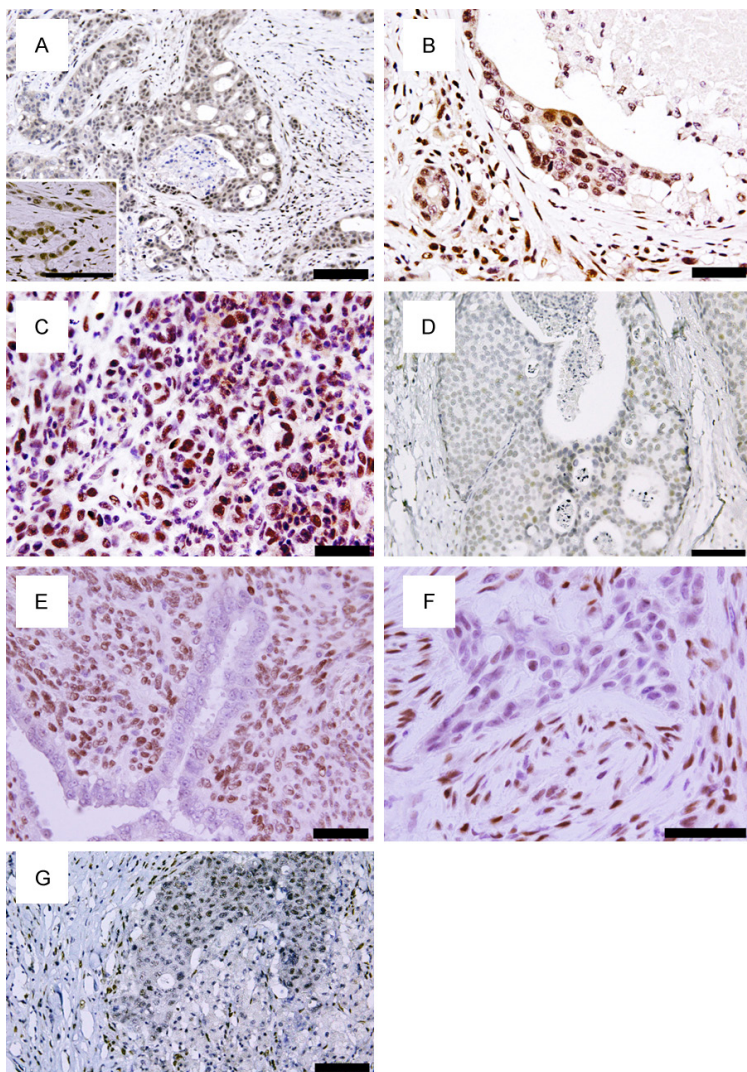


Figure 3. Representative immunohistochemical staining using the specific antibody to ZEB1 in the present tumor (A-C), intraductal carcinoma components in the breast cancer (D), carcinosarcoma of the uterus (E), metaplastic breast cancer (F), and non-sarcomatoid salivary duct carcinoma cells (G). In the present case, ZEB1 immunoreactivity was detected in the intraductal cancer cells (A and B; inserted figure of A shows invasive cancer) and sarcomatoid cancer cells (C). By contrast, little or no ZEB1 immunoreactivity was found in ductal carcinoma cells of the breast cancer (D). ZEB1 immunoreactivity was also identified in the sarcomatoid cells, but not in the epithelial cancer cells of the uterine carcinosarcoma (E) and metaplastic breast cancer (F). Note ZEB1 immunoreactivity in non-sarcomatoid salivary duct carcinoma cells in another case. Scale bars = 100 μ m in (A, D, and G); 50 μ m in (B, C, E, and F).

Archived pathological tissue specimens from non-sarcomatoid salivary duct carcinoma and breast cancer were also used in this study. All tissue specimens were obtained surgically, fixed in 10% buffered formalin, and embedded in paraffin. Tissues were immunostained with antibodies using the ImmPRESS™ polymerized

reporter enzyme staining system (Vector laboratories, Inc. Burlingame, CA, USA) as previously reported [10].

Results

Pathology findings

The tumor was a firm, ill-defined mass, infiltrating surrounding soft tissue and measuring approximately 60 \times 80 \times 55 mm. The cut surfaces showed a white-gray mass containing necrotic and hemorrhagic areas (**Figure 1**). Histopathological examination of the resected tissue specimens showed well-defined nests of cancer cells exhibiting a cribriform pattern and comedo necrosis, resembling ductal carcinoma of the breast with an overtly infiltrative growth pattern. Furthermore, we observed sarcomatoid components, which comprised anaplastic fusiform and pleomorphic cells. Many proliferating spindle cells exhibited marked nuclear atypia. Representative features are shown in **Figure 2A-C**.

In the immunohistochemical analysis, epithelial components exhibited HER2/neu, AR, GCDFP-15, and mammaglobin immunoreactivity, while most of the proliferating spindle cells stained positive for vimentin. The representative immunohistochemical stains are shown in **Figure 2D and 2E**.

Based on these histopathological features, we diagnosed the present case as a sarcomatoid variant of salivary duct carcinoma.

ZEB1 expression in the present case and breast cancers

Subsequently, we investigated whether the cancer cells expressed the epithelial mesen-

chymal transition (EMT)-related molecules, ZEB1 or SNAIL. We previously reported generation and characterization of rabbit antibody to ZEB1, which had no cross-reactivity to ZEB2 [9]. Representative staining is shown in **Figure 3**. Surprisingly, not only invasive cancer cells, but also intraductal-carcinoma like cells exhibited ZEB1 immunoreactivity (**Figure 3A** and **3B**). We also found ZEB1 immunoreactivity in sarcomatous cells of the present tumor (**Figure 3C**). We did not identify significant ZEB1 immunoreactivity in intraductal carcinoma cells of the breast, which is consistent with the results of previous studies [11, 12] (**Figure 3D**). Notably, we detected ZEB1 immunoreactivity in sarcomatoid tumor cells, but not in epithelial tumor cells in a case of carcinosarcoma of the uterus (**Figure 3E**) and a case of metaplastic breast cancer (**Figure 3F**). ZEB1 immunoreactivity was also found in non-sarcomatoid salivary duct carcinomas, examined (**Figure 3G**). We did not detect SNAIL immunoreactivity in the present tumors.

We believe that ductal carcinoma-like cancer cells, including the intraductal-like component, as well as sarcomatoid cancer cells of the present case, might display potent, highly aggressive activity because they harbor the ZEB1 protein. This is in contrast to intraductal carcinoma cells of the breast.

Discussion

Herein, we report a case of sarcomatoid variant of salivary ductal carcinoma, focused on the effects of the expression of ZEB1. ZEB1 is well-characterized as an EMT-activator, which is a crucial promoter of cancer invasion and metastasis [13]. The clinical importance of ZEB1 expression is also based on ZEB1-mediated acquired chemoresistance and radioresistance of cancer cells. Furthermore, ZEB1 is a main regulator of breast cancer cell plasticity, enabling the reversible conversion of non-cancer stem cells into cancer stem cells [14].

Interestingly, the present case study indicated that ZEB1 was aberrantly expressed in the sarcomatoid variant of salivary duct carcinoma, both in the breast ductal carcinoma-like elements and in the intraductal carcinoma-like region and sarcomatoid cells. Moreover, we observed ZEB1 immunoreactivity in non-sarcomatoid salivary duct carcinoma cells. In contrast, breast intraductal-carcinoma cells did

not exhibit ZEB1 immunoreactivity as previously reported [11, 12]. We speculate that aberrant ZEB1 expression may be the reason for the highly aggressive potential of both non-sarcomatoid and sarcomatoid salivary duct carcinoma.

To the best of our knowledge, this is the first report that describes ZEB1 expression in salivary duct carcinoma. Although additional case studies would be necessary to understand fully the expression of ZEB1 in salivary duct carcinoma, the vast majority of salivary duct carcinoma cells express AR, similar to the present tumor. ZEB1 binds directly to the E-box located in the AR promoter, and increases transcription of the AR gene [15]. Thus, ZEB1 might be expressed in many salivary duct carcinomas.

In summary, the present study suggested an important role for ZEB1 in explaining the aggressive behavior of salivary duct carcinoma. Developing an appropriate molecular therapy to limit or address aberrant expression of ZEB1 is recommended.

Acknowledgements

This study was supported by grants from the Ministry of Education of Japan (KAKEN 15K-08361, 15K19051).

Disclosure of conflict of interest

None.

Address correspondence to: Tamotsu Takeuchi, Department of Pathology and Translational Research, Graduate School of Medicine, Gifu University, Gifu, Yanagido 1-1, Gifu, Japan. E-mail: takeutit08@gmail.com

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