



Stromal reaction and prognosis in acinic cell carcinoma of the salivary gland

Stromalna reakcija i prognoza acinoćelijskog karcinoma pljuvačne žlezde

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Abstract

Introduction. Primary acinic cell carcinoma (ACC) is an uncommon malignant neoplasm of the salivary gland (SG), which usually presents as slow growing tumor. **Case report.** We reported a 69-year-old woman with tumor in the right parotid gland with a 5-year progress. Biopsy sections revealed a hybrid form of ACC with a low- and high-grade component and prominent lymphoid tissue in tumor stroma. Immunohistochemistry was performed to define the molecular profile of this unusual ACC, with special interest for stromal influence on to the proliferative activity of ACC with dedifferentiation. We detected that the level and the type of stromal lymphoid reaction (particularly CD8⁺/CD4⁺ ratio) had a significant influence on to Ki-67 index in the high-grade component of ACC, as well as the involvement of the CXCR4 signaling axis in the stromal reaction influence. **Conclusion.** We suggest that tumor stroma may be a source of potential new tumor biomarkers which can determine the aggressivity of this tumor.

Key words:

parotid neoplasms; carcinoma, acinar cell; ki-67 antigen; prognosis.

Apstrakt

Uvod. Primarni acinoćelijski karcinom (ACC) je redak maligni tumor pljuvačnih žlezda koji obično ima sporu progresiju. **Prikaz bolesnika.** U radu je prikazana bolesnica, stara 69 godina, sa tumorom u desnoj parotidnoj žlezdi. Na presecima tkiva otkrivena je hibridna forma ACC, sa komponentom niskog i visokog gradusa, i izraženim limfnim tkivom u stromi tumora. Tumor je pokazivao 5-godišnju progresiju. Imunohistohemijska metoda korišćena je kako bi se definisao molekularni profil ove retke forme ACC, kao i uticaj strome na proliferativnu aktivnost ACC sa njegovom dediferencijacijom. Pokazali smo da nivo i tip stromalne limfoidne reakcije (pogotovo odnos CD8⁺/CD4⁺) ima značajan uticaj na Ki-67 indeks u komponenti visokog gradusa ACC, kao i uticaj CXCR4 signalnog puta. **Zaključak.** Tumorska stroma može biti izvor potencijalno novih tumorskih biomarkera, kada imuni odgovor može uticati na agresivnost ovog tumora.

Ključne reči:

parotidne žlezde, neoplazme; karcinom acinusnog ćelija; antigen, ki-67; prognoza.

Introduction

Although acinic cell carcinomas (ACCs) represent only 2–6% of salivary gland tumors, they are the third most common epithelial malignancy after mucoepidermoid carcinoma, thus being about 10% of all malignant salivary gland tumors¹. Well-differentiated ACCs present as well circumscribed encapsulated tumors with a solid or microcystic pattern in which tumor cells are surrounded and intermingled with prominent lymphoid response. Dedifferentiated ACC presents itself with areas of low-grade ACC and areas of dedifferentiated high grade ACC, or undifferentiated carcinoma within the same tumor^{2,3}. Investigation of biological markers is very important and necessary for predicting prognosis

of salivary malignancies and better understanding the pathogenesis of salivary cancer. Ki-67 was already proven as useful prognostic markers for survival in a few studies of patients with ACC and other salivary glands tumors^{4,5}. Tumor stroma may be a source of potential new tumor biomarkers, in which the immune response is of major importance. Here we investigated stromal influence onto the proliferative activity of ACC with dedifferentiation and evolution of this parotid gland tumor.

Case report

A 69-year-old woman presented with a superficial lobulated swelling in the lower half of the right parotid

gland. The tumor had been detected 5 years before with the diameter of 1×1 cm, clinically manifested as slowly enlarging, asymptomatic, painless tumor mass. Five years later surgical resection of the lower part of the parotid gland was performed, as well as extirpation of a lymph node along the front edge of the sternocleidomastoidal muscle.

Pathological and immunohistochemical findings

In a fragment of the parotid gland (45×40 mm), gross analysis showed a well-circumscribed tan-gray tumor mass of strong consistency ($38 \times 30 \times 10$ mm), and a focus of bleeding (12 mm). Microscopically, biopsy sections revealed feature of ACC where tumor cells had basophilic cytoplasm and acinar differentiation. The tumor showed predominantly a low-grade component, but in some parts of ACC, a high-grade component was detected. Abundant lymphoid tissue with germ cell follicles was present in tumor stroma (Figure 1a). Some parts of a high-grade component showed prominent stromal lymphoid reaction with intratumoral infiltration of lymphocytes. Regional lymph node showed reactive hyperplastic change. The tumor was in pathological stage 2.

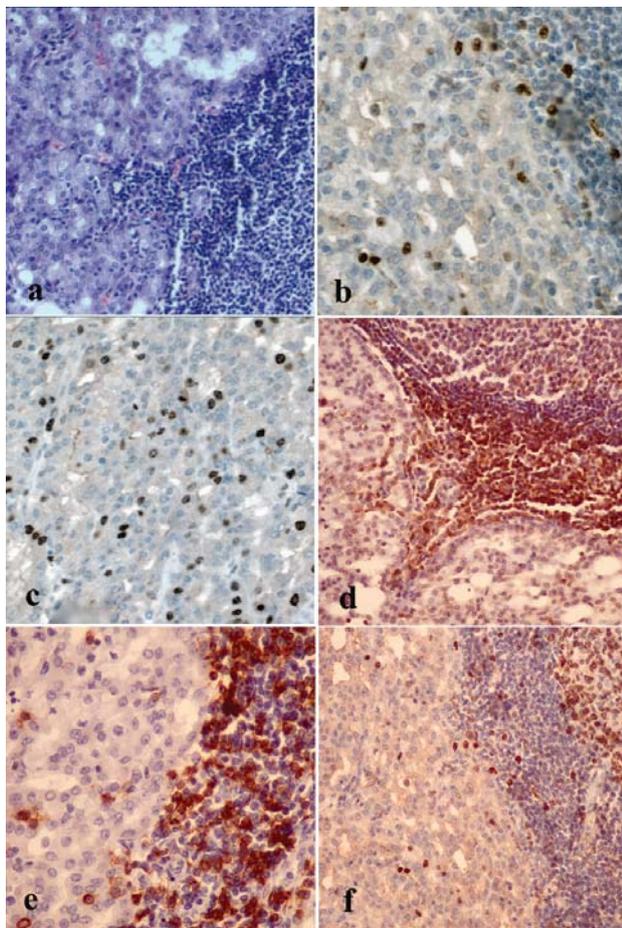


Fig. 1 – a) Acinic cell carcinomas (ACC) of the parotid gland with abundant stromal lymphoid tissue in high grade component (HE staining, $\times 40$); **b)** Immunoreactivity in high grade ACC with stromal reaction and low Ki-67 index ($\times 40$); **c)** prominent Ki-67 index in part of tumor with scanty stromal reaction ($\times 40$); **d)** with infiltration of tumor with $CD8^+$ cell ($\times 40$); **e)** and $CD4^+$ cells ($\times 40$); **f)** CXCR4 positivity in both tumor cells and lymphocytes ($\times 40$).

Broad spectrum of monoclonal antibodies was applied to define the molecular profile of low and high-grade components of ACC, and surrounding lymphoid stromal influence on to Ki-67 index. Immunohistochemical analysis was performed on the following 3 different tumor regions: low-grade, high-grade near prominent stromal reaction, and high-grade with a low stromal reaction. The tumor was analyzed using the mouse monoclonal antibody against p53, p21^{WAF1/Cip1}, HER-2, Ki-67, Bcl-2, Survivin, Bax, Fas, Caspase-3, CD4, CD8, CXCR4, GFAP, EMA, S-100, CEA, and CK (AE1/AE3). We defined indexes of Ki-67, p53, p21 and Survivin. On the literature data scoring system was performed to p21⁶; Bcl-2, Bax, Fas, and Caspase 3⁷, and CXCR4⁸. For testing the HER-2 (C-erbB2) status we used the HercepTest scoring system devised by DAKO. Characterization of the stromal reaction and a mononuclear cell infiltrate was determined based on the hot spot technique, which means that, in each studied tumor area, density was measured at the region of the highest tumor-infiltrating lymphocytes (TILs) density⁹. The TILs were further characterized by CD4 and CD8 markers expression and the percentage of CD4 and CD8 positive TILs was scored as: score 0, no immunoreactive cells; score +1, positivity in $< 10\%$ cells; score +2, positivity in $10\text{--}30\%$ cells; and score +3, positivity in $> 30\%$ of cells. Pathology data regarding proliferative index Ki-67, p21, CD4 and CD8 positivity were analyzed by the analysis of variance (ANOVA), single-factor analysis and the χ^2 test, and $p < 0.05$ was considered to be statistically significant.

Mitotic activity increased in the part of tumor with a high-grade component (Figure 1b, 1c), but it was significantly higher ($p < 0.05$) in high-grade AAC with scanty stroma. TILs showed the specific pattern of immunoreactivity. In high-grade tumor with strong lymphoid stromal reaction, $CD8^+$ cells infiltrating the tumor outnumbered the $CD4^+$ cells (Figure 1d, 1e), and as a result, the $CD4^+/CD8^+$ ratio was below 0.5 (Figure 1d, 1e). In the low grade tumor and normal immune response this ratio is between 0.9 and 1.9. In the same area both TILs and tumor cells showed an increase in the density of CXCR4 positive cells (Figure 1f) and a decrease in the tumor cells mitotic activity (Figure 1b). A similar trend was seen in the case of stromal $CD4^+$ and $CD8^+$ cells.

Ki-67 index and quantification of $CD4^+$ cells and $CD8^+$ cells was shown in Figure 2. The molecular profile of investigated components in ACC is presented in Table 1.

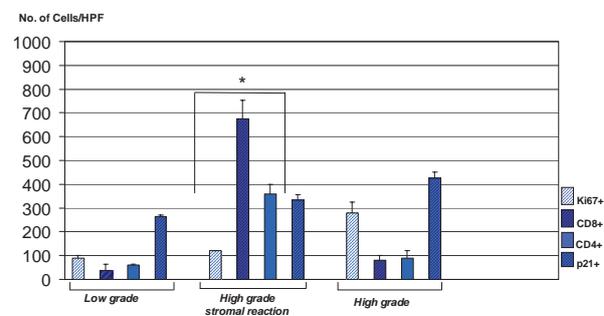


Fig. 2 – Lymphoid stromal reaction, Ki-67 and p21 expression in acinic cell carcinoma.

HPF – high power field.

Table 1
Expression of molecular markers in acinar cell carcinoma

Molecular markers	Low grade	High-grade stromal reaction	High grade
p53	–	–	–
p21%	–	Score 1	Score 2
HER-2	–	Score 2	Score 2
Ki-67	Score 1	Score 2	Score 1
Bcl-2	–	altered	altered
Survivin	–	–	–
Bax	normal	altered	altered
Fas	altered	altered	altered
Caspase-3	altered	–	–
CD4	Score 1	Score 3	Score 1
CD8	Score 1	Score 3	Score 1
CXCR4	+	+	–
GFAP	–	–	–
EMA	–	–	–
S-100	+	+	+
CEA	–	–	–
Cytokeratin	+	+	+

Discussion

Prognosis of salivary tumors depends mostly on the microscopic grade and the tumor type, as well as the stage of the disease and localization¹⁻³. Investigation of proliferative activity in ACCs and other malignant salivary gland tumors, evaluating MIB-1 or Ki-67 index, shows that this is highly effective tool in patient follow-up and prognosis. Patients with MIB-1-negative ACCs had significantly better survival than patients with MIB-1-positive tumors. This is an independent prognostic factor for survival in patients with ACCs⁴.

Furthermore, TILs showed the specific pattern of immunoreactivity regarding the tumor grade as well as the stroma/tumor ratio. In high-grade tumor with strong lymphoid stromal reaction, CD8⁺ cells infiltrating the tumor were a dominant component of immune infiltrate. CD4⁺ also revealed strong expression, although CD8⁺ cytotoxic lymphocytes highly outnumbered CD4⁺ TILs. This would suggest a later phase of the T-cell activation process¹⁰⁻¹² and may be a result of a relatively long period of tumor evolution.

In view of clinical and pathological data, it is speculated that the tumor foci lacking lymphoid stroma possibly represented a clone of high-grade malignancy arising within low-grade acinic cell carcinoma with lymphoid stroma. Several studies on various carcinomas have shown that a high CD8⁺/CD4⁺ T cell ratio is associated with favorable prognosis and *vice versa*^{11,13,14}. The same area showed an increase in the density of CXCR4 positive cells in both TILs and tumor cells shown, which also correlated with low prolifera-

tion index. CXCR-4 is an alpha-chemokine receptor specific for stromal-derived-factor-1 (SDF-1 also called CXCL12), a molecule endowed with a potent chemotactic activity for lymphocytes. The CXCR4 signaling pathway is a key regulator of many essential biological processes, such as cell motility, differentiation switch, apoptosis and lymphocyte homing^{15,16}. However, data regarding the role of CXCR4 is sometimes dubious. Until recently, SDF-1 and CXCR4 were believed to be a relatively “monogamous” ligand-receptor pair correlated to other chemokine receptors. Recent evidence demonstrates ubiquitin (a well-known anti-inflammatory immune modulator and endogenous opponent of proinflammatory damage) may also be a natural ligand of CXCR4^{17,18}. There is still no data on the role of this signaling axis in the ACC pathogenesis.

It is well-known that salivary gland cancer may resist programmed cell death with altered expression of both proapoptotic and antiapoptotic proteins^{4,5,19}, but this process can be regulated by expression of HER-1 and p21²⁰. So, a weak bcl-2 expression in SG tumors is associated with a high frequency of apoptosis, but strong Bax expression has no influence¹⁹. Some authors suggest that HER2/neu overexpression in cancer cells, in addition to stimulating tumor cell proliferation, acts as an antiapoptotic cell survival factor²⁰. On the other hand, cancer cells lacking p21 are more sensitive to apoptosis, by arresting cell cycle progression or p21 could interact with and inhibit proapoptotic molecules, such as procaspase-3, caspase-8, and apoptosis signal-regulating kinase 1²¹.

This article describes a very unusual hybrid form of ACC with slow progression. In this case, a high-grade component of ACC showed deregulation of cell cycle, with high expression of HER-2, p21, and Bcl-2. Proapoptotic markers were effective in low grade ACC, but with dedifferentiation of ACC, tumor cells expressed Bax and Fas, however without Caspase-3 activity. Stromal lymphoid tissue had a significant influence on to Ki-67 index in a high-grade component of ACC, as well as involvement of the CXCR4 signaling axis in the stromal reaction influence.

Conclusion

Stromal lymphoid reaction (particularly CD8⁺/CD4⁺ ratio) has a significant influence onto Ki-67 index in high-grade ACC of the parotid gland, which can determine the aggressivity of this tumor, and therefore may be used as a novel prognostic biomarker.

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