Fine Needle Aspiration of Basal Cell Adenocarcinoma of the Parotid Gland

Report of a Case with Assessment of DNA Ploidy in Aspirates and Tissue Sections by Image Analysis

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BACKGROUND: Basal cell adenocarcinoma of the parotid gland is a low grade malignant neoplasm. It has cytologic features of basal cell adenoma and a histologically infiltrative growth pattern of malignant tumors with perineural and vascular invasion.

CASE: Fine needle aspiration biopsy findings of basal cell adenocarcinoma of the parotid gland in a 77-year-old male were supplemented by DNA ploidy analysis.

CONCLUSION: No single cytologic feature was found to unequivocally distinguish this lesion from basal cell adenoma and/or solid variant of adenoid cystic carcinoma. Therefore, for diagnostic purposes, we grouped all three lesions under the term basal cell tumor. *Evaluation of DNA content of tumor cells revealed diploid histograms in both cytologic material and paraffin-embedded tissue. Infiltrative tumor nests, the histologic basis for differentiating basal cell adenocarcinoma from adenoma, showed the same diploid pattern. Though DNA quantitation may not discriminate basal cell adenoma from basal cell adenocarcinoma, it may prove useful in separating them from adenoid cystic carcinoma, which is considered to be a tumor with high malignant potential.* (Acta Cytol 1996;40:773–778)

Keywords: adenocarcinoma, basal cell; parotid neoplasms; ploidies; image analysis, computer-assisted; aspiration biopsy.

The growing list of new entities ... in the classification of salivary gland tumors imposes new diagnostic responsibilities upon cytopathologists.

Fine needle aspiration (FNA) of salivary glands is a standard diagnostic procedure that aids the surgeon in choosing the best treatment plan. The extent of surgery depends on cytologic diagnosis, often confirmed with frozen section biopsy. The diagnostic sensitivity of FNA for malignant neoplasms of the salivary gland varies from 58% in community settings to 86% at academic centers and is higher for benign lesions.[6,18] There are occasional cases, however, where the cytologic differentiation of benign from malignant salivary gland tumors is difficult, including basal cell adenoma from adenoid cystic carcinoma.[16,23]

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Health Organization monograph[21] on histologic typing of salivary gland tumors introduced several new entities, such as basal cell adenocarcinoma, polymorphous low grade adenocarcinoma, salivary duct carcinoma and malignant myoepithelioma. Recognition of these lesions on FNA becomes essential.

Image-based assessment of DNA ploidy may be helpful ... to differentiate this tumor from adenoid cystic carcinoma.

The term *basal cell adenocarcinoma of the salivary gland* was introduced in 1990 by Ellis and Wiscovitch.[9] They identified a group of neoplasms that had the growth characteristics of malignant tumors but with cytologic features of basal cell adenoma. The only reliable criterion they found to distinguish adenoma from carcinoma was an infiltrative growth pattern with perineural and intravascular invasion. However, these features are not applicable to cytologic material.

Solid variant of adenoid cystic carcinoma should also be considered in the differential diagnosis.[8,22] Seifert et al[22] include all three entities under the term *basal cell tumors* and stress the importance of differential diagnosis based on different histologic appearances and clinical prognosis.

To the best of our knowledge, this is the first case report that describes FNA findings in basal cell adenocarcinoma of the parotid gland and includes a prospective study on tumor DNA ploidy by image analysis. DNA content of tumor cells from cytologic material is compared with that from histologic sections. In addition, DNA content of infiltrative tumor nests is compared with the central parts of the adenocarcinoma in tissue sections. The possible differential utility of image-based ploidy assessment in some salivary gland tumors is discussed.

Case Report

A 77-year-old male was referred for consultation to the fine needle aspiration clinic by his otolaryngologist. The patient had noticed a left, retroauricular nodule, present for the last three months. He denied having a cough, night sweats or weight loss. He mentioned rare incidents of paresthetic pain radiating from his left ear to left shoulder. His past medical history was nonsignificant.

On examination the nodule was firm, well circumscribed and movable under the skin. Highly cellular material was aspirated and smeared on slides for immediate assessment after staining with Diff-Quik (Baxter Scientific Products, Grand Prairie, Texas, U.S.A.). Papanicolaou-stained smears and cytocentrifuge samples were also available for evaluation.

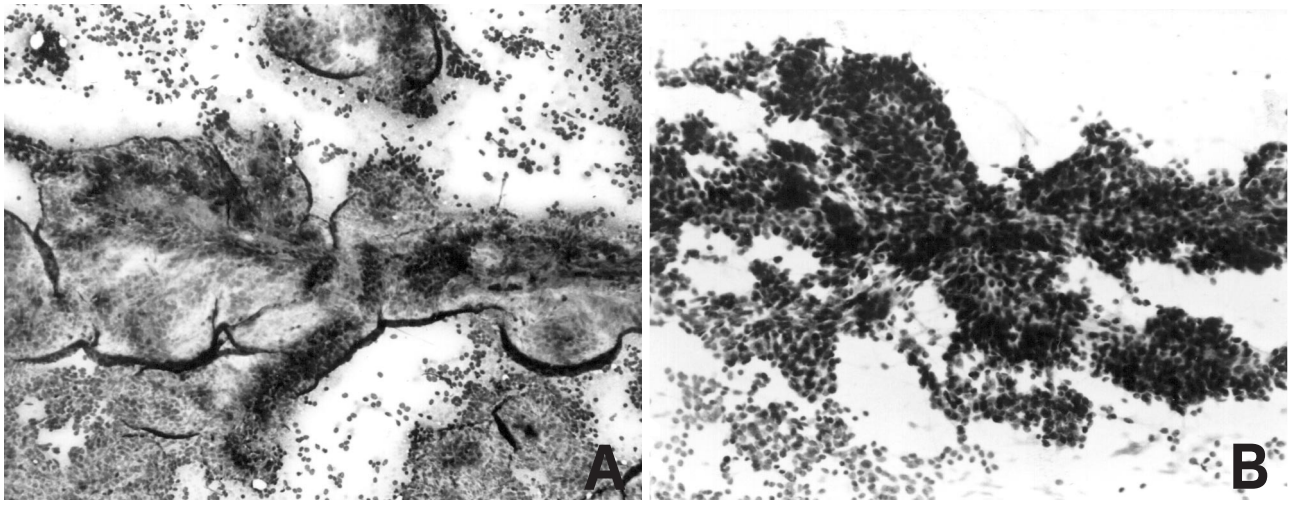

Figure 1 Basal cell adenocarcinoma of the parotid gland. (A) Tissue fragments with folded edges representing drying artifact (Diff-Quik, × 100). (B) Branching trabeculae of basaloid cells with crowding and overlapping of nuclei (Papanicolaou stain, × 200).

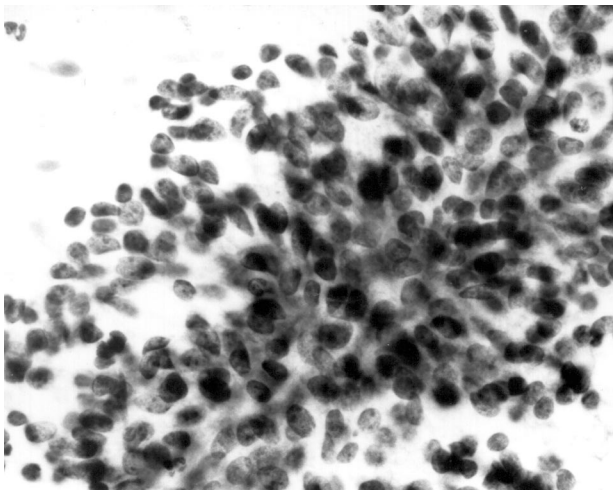

Figure 2 Oval to spindle-shaped cells with scanty cytoplasm, nuclei with fine chromatin and inconspicuous nucleoli characterize this tumor cytologically (Papanicolaou stain, × 600).

Cytologic Findings

Both air-dried and alcohol-fixed slides showed similar hypercellular aspirates with a predominance of tissue fragments. Crowding and overlapping of small, basaloid nuclei were observed (Figure 1). Oval to spindle-shaped cells contained scanty cytoplasm and showed moderate anisonucleosis. Finely granular chromatin and occasional inconspicuous nucleoli were seen (Figure 2). No mitoses were detected. Single, naked nuclei and palisading cells in sheets were present in the granular background (Figure 3). Cell clusters had a predominantly branching, trabecular pattern with thin, fibrovascular cores. There were occasional fragments with a peripheral tubular pattern and central hyaline material best detected on the Diff-Quik. No purple cylinders with basement membrane globules, as seen in adenoid cystic carcinoma, were found. The edges of many clusters on the air-dried smears tended to fold up, giving the impression of a thick outline. Rare foamy macrophages were identified.

The differential diagnosis included basal cell adenoma and solid adenoid cystic carcinoma. A recommendation for excisional biopsy was made.

Histologic Findings

The biopsy material revealed a 1.5-cm, unencapsulated, lobulated tumor mass. Many separate and infiltrative tumor nests were seen at the periphery. Perineural, but no vascular, invasion was identified (Figure 4). Centrally, the tumor was trabecular in appearance and contained many thin, fibrovascular cores (Figure 5). The arrangement was more solid peripherally and in the infiltrative nests. Nuclear palisading was more accentuated in the trabecular, rather than peripheral, portions of the tumor. Centrally the cells were small, spindled and hyperchromatic. This was less conspicuous toward the periphery. The predominant cell type had a basaloid nucleus with finely granular chromatin and rare, small nucleolus. Cytoplasm and cytoplasmic mem-

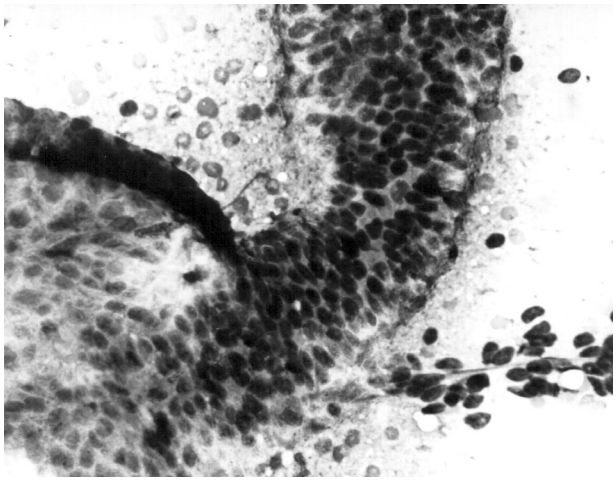

Figure 3 Cells and palisading cell clusters in a granular background (Diff-Quik, × 400).

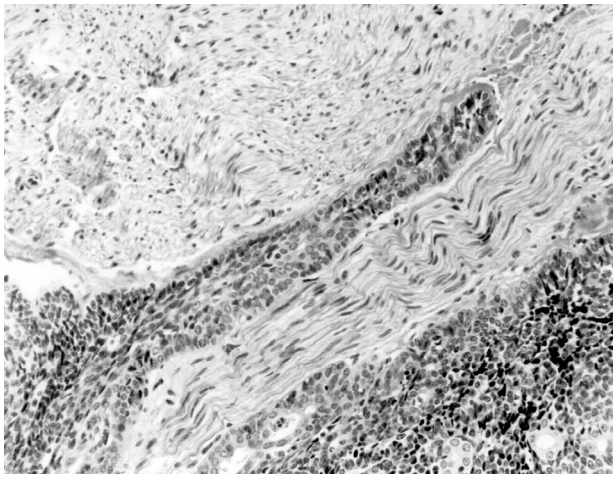

Figure 4 Basaloid nest of cells infiltrating into and around the facial nerve (hematoxylin and eosin, × 200).

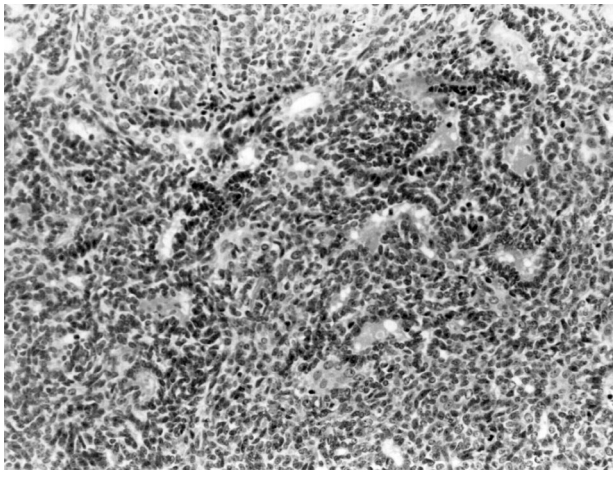

Figure 5 Central portion of the tumor showing trabecular architecture with peripheral palisading of basaloid cells (hematoxylin and eosin, × 200).

branes were not detectable. Although rare, mitoses were more prominent at the periphery and in the tumor nests. Occasional ductules with open lumina and more eosinophilic cytoplasm were seen. There was a small focus of tumor necrosis.

DNA Ploidy Findings

Air-dried, Diff-Quik–stained smears with representative tumor cells were destained[13] and then stained with the CAS DNA staining kit (Cell Analysis Systems, Elmhurst, Illinois, U.S.A.). Analysis of DNA content was performed with a CAS 200 image analyzer using the quantitative DNA analysis software program (Cell Analysis Systems). The instrument was calibrated with tetraploid rat hepatocyte nuclei (Cell Analysis Systems). As an internal control, the patient's own lymphocytes were measured to establish a mean DNA index (DI) of 0.97 and coefficient of variation (CV) of 2.49. Five hundred tumor cell nuclei were evaluated. The analysis showed diploid-range tumor cells (defined as those in which the DI was equal to that of control lymphocytes ± 10% SD), with 98.4% of cells in G0/G1 DNA peak and mean DI of 0.98, CV = 2.87. No aneuploidy or hyperdiploidy was observed.

DNA content of tumor cells was simultaneously evaluated in 4-µm sections from formalin-fixed, paraffin-embedded tissue. Numerous studies have confirmed this approach to be reliable and to have diagnostic and prognostic value.[3,5,11] Slides were deparaffinized, rehydrated and stained with the CAS DNA staining kit. As an internal control, 82 tissue lymphocytes from intraparotid lymph nodes and 103 normal acinar cell nuclei were used. The tumor cell analysis was performed for peripheral infiltrative satellite nodules exhibiting perineural invasion (313 nuclei) and separately for the central tumor mass (331 nuclei). To correct for cut nuclei, the tissue correction feature of CAS version 3.0 ploidy software (Cell Analysis System) was used for all tissue measurements. Histograms were considered diploid if the mean DNA indices of the G0/G1 were within an established diploid range, based on the internal control (DI = 1 ± 10%).

Both infiltrative and central areas of the tumor showed diploid-range histograms (Table I).

Discussion

The growing list of new entities included in the classification of salivary gland tumors imposes new diagnostic responsibilities upon cytopathologists. Basal cell adenocarcinoma is a prime example of such an entity. There are numerous cytologic publications stressing the diagnostic challenge of differentiating basal cell adenoma from solid variant of adenoid cystic carcinoma.[12,16,19,23] There is no literature, however, on cytologic findings in basal cell adenocarcinoma. Comparing detailed descriptions of adenoma and solid adenoid cystic carcinoma[16,27] with our case, we find it difficult to separate them cytologically. We suggest including all three, for

Table I *Comparison of DNA Ploidy Indices for Infiltrative Nests Versus Central Areas of the Tumor in Reference to Control Cells (G_0/G_1 and G_2/M Peaks)*

	G_0/G_1 peak				G_2/M peak	
Index	Tissue lymphocytes	Acinar nuclei	Central tumor	Tumor nests	Central tumor	Tumor nests
Mean DI	1.00	1.01	0.99	0.99	1.95	2.01
Modal DI	1.02	1.00	0.93	0.92	1.88	2.15
SD	0.06	0.05	0.05	0.06	0.11	0.12
CV	5.56	5.21	5.54	5.75	5.54	4.47

routine diagnostic purposes, under the term basal cell tumor. The clinician should be informed of the differential diagnoses. Clinical symptoms of perineural invasion, thought to be in favor of adenoid cystic carcinoma, are no longer helpful as the process is seen in a variety of other salivary gland tumors including basal cell adenocarcinoma,[9] polymorphous low-grade adenocarcinoma[4] and salivary duct carcinoma.[10] Also, identification of hyaline globules of reduplicated basement membrane is of no differential help. They are often present in cribriform and tubular variants of adenoid cystic carcinoma[22] as well as membranous types of basal cell adenoma and adenocarcinoma.[8] On the other hand, these globules are often absent in solid variants of all three tumors.[16,23] Immunocytochemistry also does not help to solve that differential diagnostic dilemma. There is an overlap of reactivity with a variety of antibodies between basal cell adenoma and adenocarcinoma[26] and similar staining immunoprofiles with adenoid cystic carcinoma.[24,25]

The purpose of our image-based quantitation of nuclear DNA content was to provide more detailed data on the biologic potential of this tumor. Numerous studies have shown that DNA analysis may provide useful prognostic information on many neoplasms.[1,5,17] Data on the value of DNA ploidy in salivary gland lesions are too scarce to warrant a generalization.

According to Hamper et al,[15] diploidy in mucoepidermoid carcinoma does not necessarily mean a good prognosis; however, it is a better discriminator of a favorable disease course than is histologic grade alone. In contrast, atypical DNA pattern, regardless of the histologic type of tumor, is an unfavorable sign.

Gustafsson et al,[14] in a study of 13 acinic cell carcinomas, showed that all tumors had diploid or near-diploid DNA patterns. This was not of prognostic value since there was no correlation with survival time and / or potential for metastases.

Schimmelpenning et al[20] assessed 98 salivary gland tumors for DNA ploidy. The primary goal of their retrospective study was to compare DNA content in tissue sections with cytocentrifuge samples from the same paraffin-embedded specimens. A high correlation was found in acinic cell carcinomas, mucoepidermoid carcinomas and adenocarcinomas. A lower degree of correlation was seen in cases of adenoid cystic carcinoma. The authors did not correlate ploidy with the biologic behavior of these tumors.

We prospectively assessed nuclear DNA in both FNA material and paraffin sections in our case of basal cell adenocarcinoma of the parotid gland. The problem of cell disaggregation faced by Schimmelpenning et al[20] and related to this technical bias was eliminated in our approach. To better characterize the biology of basal cell adenocarcinoma, we examined infiltrative nests as compared to the rest of the tumor. The presence of these infiltrative nests differentiates the lesion from its benign counterpart, basal cell adenoma.[9]

There was no difference in ploidy pattern and no intratumor heterogeneity in our case. These findings may suggest the low malignant potential of this tumor and its *de novo* origin rather than transformation from adenoma. More studies, however, with long-term follow-up are needed to determine its true biologic behavior. Ellis and Auclair[8] consider this tumor to be a low grade adenocarcinoma with a relatively good prognosis. Therefore, it becomes important to distinguish it from solid adenoid cystic carcinoma, which can be unpredictable and is considered highly malignant. Of 28 adenoid cystic carcinomas studied with flow cytometry by Eibling et al,[7] 22 (78%) were aneuploid. Considering the diploid DNA pattern in our case of basal cell adenocarcinoma and in that reported by Atula et al,[2] we suggest that image-based assessment of DNA ploidy may be helpful in diagnostically difficult cases to differentiate this tumor from adenoid cystic carcinoma.

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