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CASE REPORT

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Unilateral aneuploid dedifferentiated acinic cell carcinoma associated with bilateral-low grade diploid acinic cell carcinoma of the parotid gland

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Abstract A dedifferentiated acinic cell carcinoma (AciCC) of the right parotid gland with lymph node metastases occurred in a 36-year-old woman. The tumour was associated with a bilateral well-differentiated AciCC. The two components of this tumour had different (high and low) proliferative activity measured by Mib-1 and different (aneuploid and diploid) DNA content. Despite the presence of a high-grade component, TP53 mutations, microsatellite instability (MSI) and/or loss of heterozygosity (LOH) at the *p53* locus were not detected. Although the follow-up of the patient is very short, the aggressiveness of the tumour is shown by a recurrence in the right parotid within 4 months and by the rapid development of regional metastases.

Key words Dedifferentiated acinic cell carcinoma · Parotid · Ploidy · Proliferative activity · TP53-MSI-LOH

Introduction

Acinic cell carcinoma (AciCC) is a tumour of low-grade malignancy with a 5-year survival rate of over 80% and metastases in nearly 15% of cases [7]. It is usually thought of as the least aggressive of all malignant salivary gland tumours [11]. Histologically and cytologically it shows a great variety of patterns ranging from a solid well-differentiated appearance simulating a normal gland to a microcystic-papillary-follicular pattern causing it to resemble tumours of the thyroid [11]. AciCC is also characterized by its low-grade behaviour, although it has an element of unpredictability in that it may recur many years later and metastasize. It has been said that there are no histological criteria that allow prediction of the clinical outcome, but the evaluation of proliferative activity of tumour cells has been suggested as a prognostic factor [13].

Recently a variant of AciCC has been described [14], characterized histologically by the presence of a featureless poorly differentiated component with a predictably more aggressive behaviour. This variant arises in contiguity with typical low-grade AciCC, but shows a conspicuous pleomorphism and lack of differentiation. This is therefore regarded as an example of dedifferentiation in a tumour.

We report here another example of dedifferentiated AciCC, which arose in the right parotid gland against a background of bilateral low-grade AciCC. In the present case the morphological study has been complemented by immunohistochemistry for Mib1 to detect proliferative cells, DNA ploidy by image analysis, *TP53* gene mutations by PCR-SSCP, presence or absence of microsatellite instability (MSI) and/or loss of heterozygosity (LOH) by PCR analysis both in the dedifferentiated components and in the well-differentiated AciCC of both left and right parotid glands.

Clinical history

A 35-year old woman presented with a well-circumscribed nodule 2.5 cm in diameter in the superficial lobe of the left parotid gland. Superficial parotidectomy was performed with preservation of the facial nerve. At follow-up 1 year later a similar mass was identified in the lower pole of the right parotid gland. A fine needle aspiration (FNA) performed elsewhere resulted in a cytological diagnosis of acinic cell carcinoma, and based on this a clinical decision for enucleoresection of this tumour was made.

The postoperative period was uneventful. However, 4 months later the patient re-presented with pain in the right parotid scar, and an underlying mass was identified. Ipsilateral lymph node enlargement was conspicuous. Total parotidectomy with preservation of the facial nerve and nodal neck dissection were performed. The patient is alive and well 4 months after surgery.

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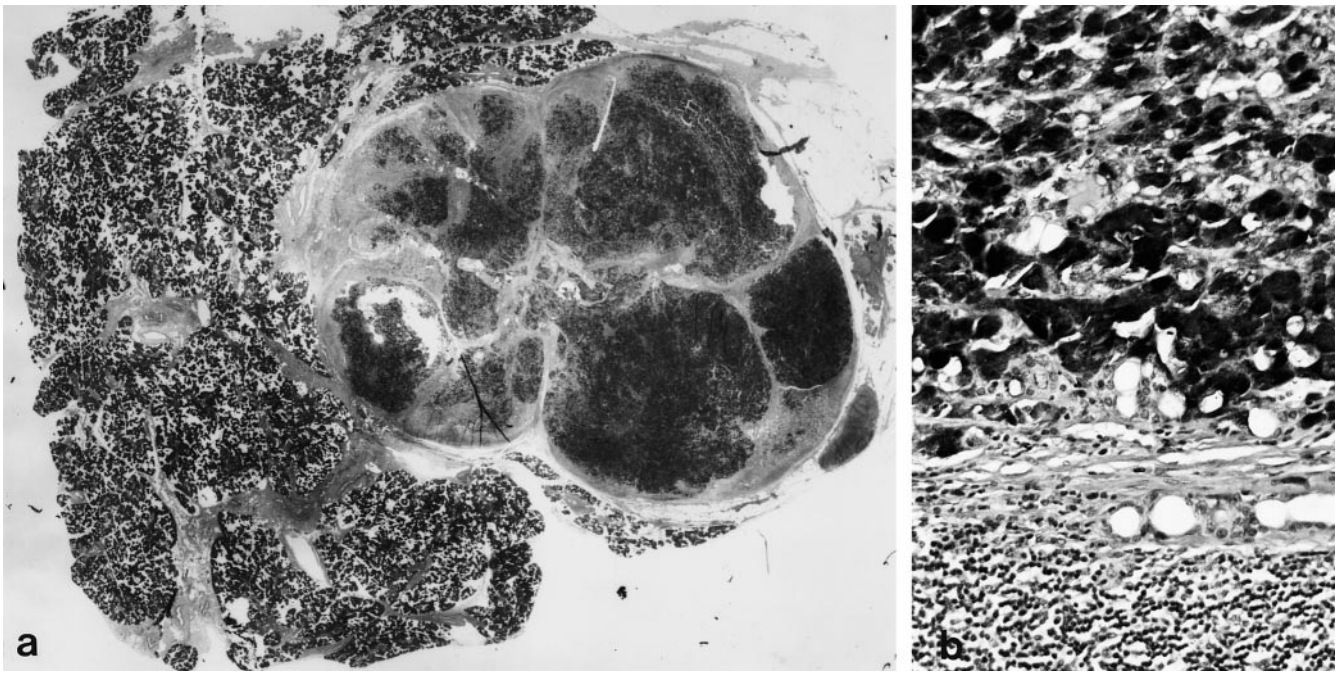

Fig. 1a Superficial left parotidectomy: 2.5 cm roundish, well-circumsbribed tumour mass with surrounding adjacent normal parotid. H&E, ×4. **b** High magnification showing well-differentiated AciCC ("blue dot tumour") with solid pattern. H&E, ×40

Materials and method

All the surgical specimens were processed routinely. Sections were prepared in the conventional manner and stained with H&E. For immunohistochemistry the following primary antibodies were employed: Mib-1 (1:100; Immunotech), p53 (DO7, 1:400; Ylem, Novocastra), mdm2 (IF 2, 1:200; Oncogene Science, S. Cruz), p21 Waf1, (1:400; S. Cruz).

Immunostaining was performed by a sensitive streptavidin–biotin–immunoperoxidase method (streptavidin HRP: horseradish peroxidase) as a modification of the avidin–biotin complex method. The antigen retrieval was carried out by pretreating sections at 95°C for 6 min in an autoclave.

For Ploidy analysis, histological sections were used. After dewaxing and hydratation the specimens were Feulgen stained according to the directions of the ESACP Consensus Meeting [2]. Ploidy analysis was performed using the Cires-Vidas imaging system (Zeiss, Kontron Elektronik, Oberkochen, Germany) as previously described [17]. The well-differentiated AciCC of the first surgical specimen (left parotid), the well- and dedifferentiated component of the second specimen (right parotid) and the dedifferentiated cells of the first recurrence were all evaluated by image analysis. Results of the cytophotometric DNA measurements, on the basis of the histograms obtained, were expressed as diploid and aneuploid.

Molecular analysis of TP53 and p53-microsatellite marker were performed as described by Lavarino et al. [10] and Thibodeau et al. [16] respectively, following microdissection under the microscope of methylene-blue stained sections obtained from formalin-fixed paraffin-embedded tissues that allowed precise separation of dedifferentiated and well-differentiated tumour components from normal tissue, which was used as a source of control DNA with minimal reciprocal contamination.

Results

The superficial left parotidectomy specimen showed a roundish, well-demarcated tumour 2.5 cm in diameter (Fig. 1a). Histology revealed a well-differentiated AciCC sometimes known as "blue dot tumour" (Fig. 1b), with a predominantly solid but focally microcystic pattern at the periphery without pleomorphism**.** It appeared completely excised with a satisfactory margin of clearance.

The right parotidectomy specimen removed 1 year later consisted of parotid tissue of 2×1.5×1 cm containing a 1.5-cm well-circumscribed roundish nodule close to the margin of resection. Histologically (Fig. 2a, b) the central part of the tumour was necrotic and surrounded by a rim of viable tumour. The latter was composed (Fig. 2c) partly of well differentiated acinar-type cells with dark basophilic cytoplasmic granules and partly of syncytial-like sheets of cells with vesicular nuclei, prominent nucleoli frequent mitoses and necrosis. In this latter component most tumour cell nuclei were immunostained by Ki-67-related antigen (Mib-1) while in the former only occasional nuclei were labelled (Fig. 2d, e). There was no evidence of intermixture or transition between the two components (Fig. 2c). Occasionally at the periphery of the sheets there were densely granulated cells closely resembling acinic cells. There were also small cells with pyknotic nuclei suggesting apoptosis.

The third surgical specimen removed 4 months later (and 16 months after that shown in Fig. 1) comprised a 4×1 cm ellipse of skin with a scar and underlying right parotid gland, which contained a tumour 2.3 cm in diameter with poorly circumscribed margins and a haemorrhagic, partly yellow, cut surface that involved both the superficial and the deep lobe of the right parotid gland.

The tumour was composed of solid sheets with an occasional cribriform pattern of cells showing vesicular nu-

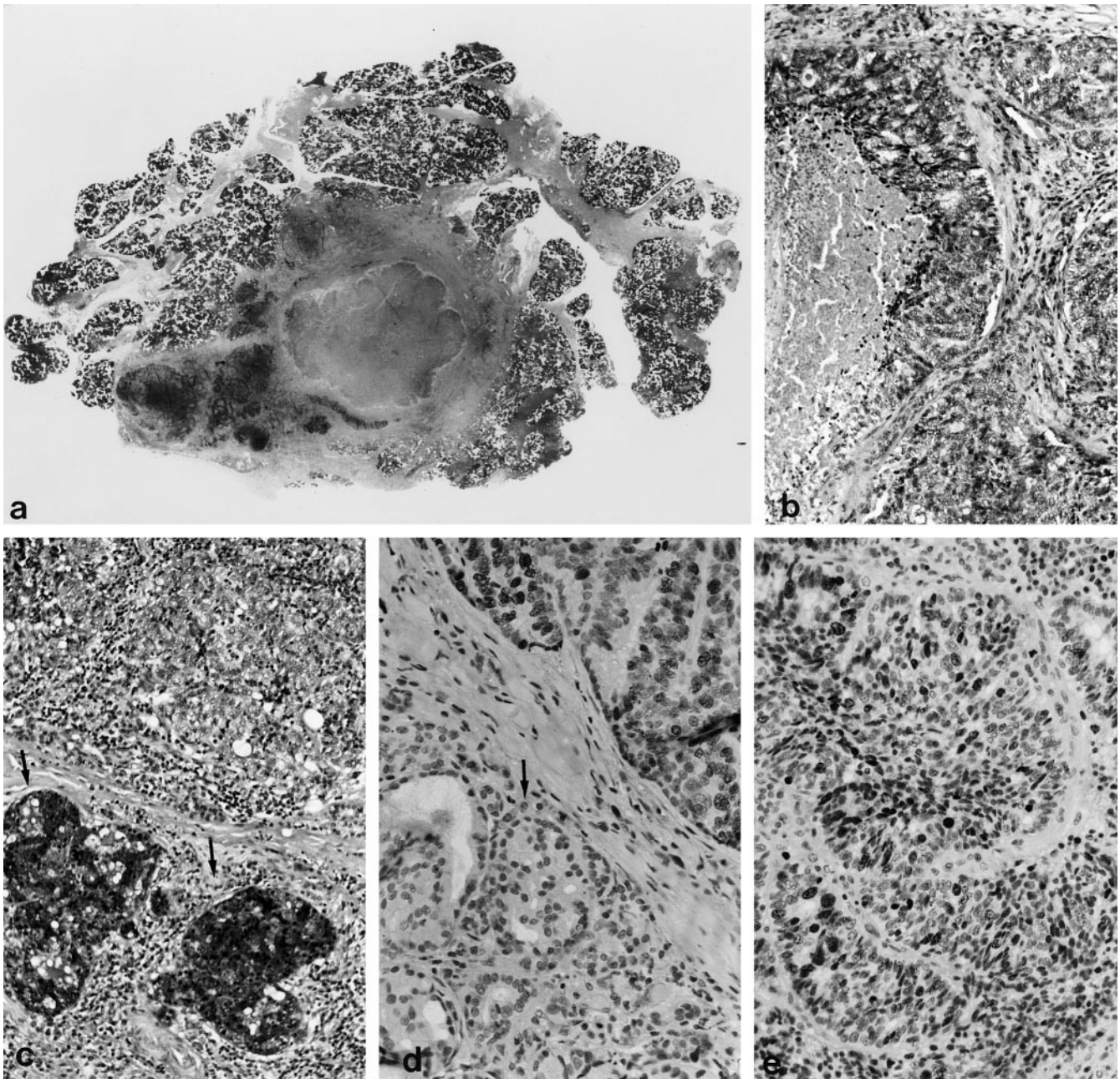

Fig. 2a–e Surgical specimen from right parotidectomy resected 1 year after the tumour shown in Fig. 1. **a** The central part is necrotic, bordered by a rim of **b** viable poorly differentiated high-grade carcinoma showing syncytial-like and cribriform patterns. **c** Areas of well-differentiated AciCC adjacent to dedifferentiated carcinoma with lymphocytic infiltrate (*arrows*). **d**, **e** Most tumour cells show nuclear Mib-1 immunostaining in the dedifferentiated component. In contrast, only scattered immunostained nuclei are present in the well-differentiated component (d, *arrow*) **a** ×4, **b** ×25, **c–e** ×40

clei, prominent nucleoli and irregular chromatin. There was severe nuclear atypia with mitoses and a great deal of necrosis (Fig. 3a). The cells occasionally had more basophilic cytoplasm mimicking rudimentary acinar formation. Only in these rudimentary acini was PAS-D positivity identified (Fig. 3b).

The previous specimens from the left parotid and the earlier right parotid resection were reviewed. The tumour in the left parotid was confirmed as a low-grade AciCC. The tumour in the first right parotid specimen on review was seen to be made up of two components corresponding to a typical low-grade AciCC and a second tumour showing features identical to those seen subsequently in the total parotidectomy specimen. Three of 14 lymph nodes contained metastatic poorly differentiated carcinoma with the same features as the recurrent tumour of the total parotidectomy specimen. The largest metastasis was 3.5 cm in diameter.

Ki-67-related antigen (Mib-1) stained the nuclei of most tumour cells in the dedifferentiated component (Fig. 2c) and only occasional cells in the well-differentiated components, but they were not labelled by p53

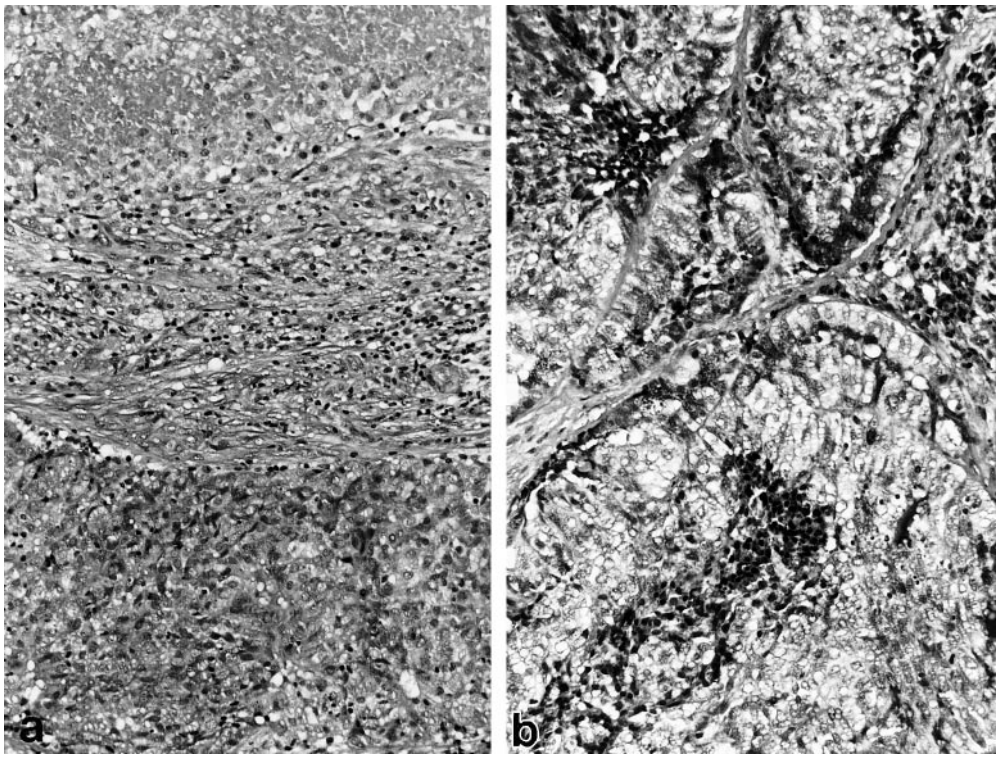

Fig. 3a, **b** Histology of the right total parotidectomy specimen removed 4 months after the tumour shown in Fig. 2 and 16 months after that shown in Fig. 1. **a** The tumour cells are mostly composed of poorly differentiated carcinoma showing extensive necrosis. H&E, ×50 **b** Tumour cells with PAS-D-positive cytoplasmic granules mimicking rudimentary acinary formation. PAS-D, ×50

(D07). There was focal positive immunostaining with mdm2 and p21.

The well-differentiated AciCC of the first surgical specimen (left parotid) had a diploid DNA content; the same was observed in the well-differentiated component of the second specimen (right parotid), while aneuploid DNA was present in the dedifferentiated component of the same specimen and in the tumour cells of the first recurrence, which was composed entirely of dedifferentiated cells.

No evidence of *TP53* mutation was observed in the exons analysed (exon 5–8), and nor was the presence of MSI and/or LOH *p53*- dinucleotide alteration detected in either the well-differentiated or the dedifferentiated components of the tumour.

Discussion

The essentially nonaggressive behaviour of AciCC is well documented [7]. In the past AciCC were thought to be mostly benign, with only occasional cases showing recurrences or metastasis, but Buxon [3] emphasized the malignant potential and they have recently all been classified as malignant tumours [11]. It is difficult to explain why most cases of AciCC behave like benign tumours, whilst similarly classified cases behave aggressively.

The case we have described showed the typical features of a low-grade AciCC with clear acinar formation and PAS-D-positive cytoplasmic granules and bilaterality. It is known to be the second most commonly bilateral tumour after Warthin's tumour [5]. In addition, in the right parotid gland it also showed a clear-cut severely pleomorphic contiguous component with the features of an aggressive tumour, which was interpreted as dedifferentiated AciCC. Such dedifferentiated AciCC have been described by Stanley et al. [14], who reported 6 cases of an otherwise well-differentiated AciCC that harboured a synchronous (5 cases) or metachronous (1 case) dedifferentiated carcinoma. The clinical characteristics were different from those of an ordinary low-grade AciCC, but were very similar to the present one in that there was pain, the deep and the superficial lobe were involved, the well-differentiated and poorly differentiated components were synchronous, and the tumour mass developed rapidly in the right parotid gland. However none of Stanley et al.'s 6 cases had bilateral AciCC. As in the present case, only 1 patient had cervical lymph node involvement. More recently Henley et al. [9] reported another case of dedifferentiated AciCC in a 46-year-old man with cervical lymphadenopathy similar to that seen in our case, but without bilaterality. The histological description in both papers underlies the clear-cut separation between the low- and the high-grade components of AciCC [9, 14], with no evidence of intermixture or transition between the two tumour cell populations. This finding was also observed in our case, particularly in the first surgical specimen of right parotid gland, where the dedifferentiated component was already present, thus fulfilling the criteria for dedifferentiated AciCC. In addition, most of the tumour was necrotic. This may have been the result of an earlier FNA procedure as it is known that total or subtotal tumour infarction can follow FNA [4]. However, in the first recurrence 4 months later the tumour was composed predominantly of poorly differentiated carcinoma although rudimentary serous acini were intermixed with the dedifferentiated component and necrosis was still conspicuous, suggesting that the

latter was an intrinsic feature of this tumour, rather than a response to the FNA. In a study by Batsakis at al. [1] the presence of necrosis was found to be associated with aggressive behaviour. In the present case there are numerous features supporting the view that AciCC is a highly aggressive poorly differentiated tumour. In addition to the poorly differentiated pleomorphic appearance with necrosis and numerous mitoses, the tumour cells also showed aneuploidy, whilst the well-differentiated component was diploid. For ploidy evaluation, our results, obtained by image analysis, were superimposable on results previously obtained by flow cytometry [8], when El-Naggar et al. indicated that aneuploid AciCC was associated with a poor clinical outcome. Four of their patients with aneuploid tumours had died of their disease. Moreover, as in our case, two of these had tumours that contained a dedifferentiated component.

The high proliferative activity measured by Mib-1 nuclear immunostaining also matches in very well with the data presented by Skalova et al. [13], who have shown a consistent high proliferative activity in AciCC that behaved aggressively.

We found it appropiate to investigate the possible role of *TP53* gene status, MSI and/or LOH of 17p13.1 chromosomal region in the mechanism of dedifferentiation or development of a high-grade component. Recently it has been demonstrated that dedifferentiated chondrosarcomas [12, 15], dedifferentiated carcinoma of the thyroid [6], high-grade adenoid cystic carcinomas [19] and pleomorphic adenoma with malignant progression [18] show *TP53* mutations that are not present in their well-differentiated, low-grade and benign counterparts.

However, in the present case, the absence of *TP53* mutations, along with normal pattern of dinucleotide repeat located at chromosome region 17p13.1, flanking p53 primers, demonstrated the integrity of this chromosomal region, as already suggested by the *p53-/mdm2+/p21+* immunophenotype. These data further supports the observation by Henley et al. [9] that the *TP53* gene is not implicated in the dedifferentiation process of AciCC and that other tumour suppressor genes than *TP53* might be involved.

In summary, the patient reported here had a very short clinical history with rapid local recurrence and development of regional nodal metastases. This is not the profile of an ordinary AciCC. Therefore, dedifferentiation and proliferation appear to play a major part in the aggressive clinical behaviour of AciCC. A histological diagnosis of low-grade AciCC with low proliferative activity and without any atypical elements would imply a very favourable, if not completely benign, prognosis. This type of tumour could be managed conservatively. On the other hand, the identification of any form of severe atypia in an otherwise low-grade AciCC implies the probable development of a dedifferentiated and highly malignant component, which always behaves aggressively. This should be recognized as indicating the need for more aggressive therapy, including total parotidectomy and possibly regional node dissection.

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References

1. Batsakis JG, Chinn EK, Weimert TA, Work WP, Krause CJ (1979) Acinic cell carcinoma: a clinicopathologic study of thirty-five cases. J Laryngol Otol 93:325–340
2. Bocking A, Giroud F, Reith A (1995) Consensus report of the ESACP task force on standardization of diagnostic DNA image cytometry. Anal Cell Pathol 8:67–74
3. Buxon RW, Maxwell JH, French AJ (1953) Surgical treatment of epithelial tumour of the parotid gland. Surg Gynecol Obstet 97:401–416
4. Chan JKC, Tang SK, Tsang WYW, Lee KC, Batsakis JG (1996) Histologic changes induced by fine-needle-aspiration. Adv Anat Pathol 3:71–90
5. Colmenero C, Paron M, Sierra I (1991) Acinic cell carcinoma of the salivary glands. A review of 20 new cases. J Craniomaxillofac Surg 19:260–266
6. Donghi R, Longoni A, Pilotti S, Michieli P, Della Porta G, Pierotti MA (1993) Gene *p53* mutations are restricted to poorly differentiated and undifferentiated carcinoma of the thyroid gland. J Clin Invest 91:1753–1760
7. Ellis GL, Corio RL (1983) Acinic cell adenocarcinoma. A clinicopahologic analysis of 294 cases. Cancer 52:542–549
8. El-Naggar AK, Batsakis JG, Luna MA, McLemore D, Byers RM (1990) DNA flow cytometry of acinic cell carcinomas of major salivary glands. J Laryngol Otol 104:410–416
9. Henley JD, Geary WA, Jackson CL, Daniel C, Gnepp DR (1997) Dedifferentiated acinic cell carcinoma of the parotid gland: a distinct rarely described entity. Hum Pathol 28:869–863
10. Lavarino C, Corletto V, Mezzelani A, Della Torre G, Bartoli C, Riva C, Pierotti MA, Rilke F, Pilotti S (1998) Detection of TP53 mutation, loss of heterozygosity and DNA content in fine-needle aspirates of breast carcinoma. Br J Cancer 77:125–130
11. Seifert G (1991) Histological typing of salivary gland tumours. Springer, Berlin Heidelberg New York, pp 13–14
12. Simms WW, Ordònez NG, Johnston D, Ayala AG, Czerniak B (1995) p53 expression in dedifferentiated chondrosarcoma. Cancer 76:223–227
13. Skalova A, Leivo I, von Boguslawsky K, Saksela E (1994) Cell proliferation correlates with prognosis in acinic cell carcinomas of salivary gland origin. J Pathol 173:13–21
14. Stanley RJ, Weiland LH, Olsen KD, Pearson BW (1988) Dedifferentiated acinic cell (acinous) carcinoma of the parotid gland. Ololaryngol Head Neck Surg 98:155–161
15. Terek RM, Healey JH, Garin-Chiesa P, Mak S, Huvos A, Albino AP (1998) p53 mutations in chondrosarcoma. Diagn Mol Pathol 7:51–56
16. Thibodeau SN, Bren GB, Schaid D (1993) Microsatellite instability in cancer of the proximal colon. Science 260:816–819
17. Verderio P, Corletto V, Boracchi P, Gambacorta M, Giardini R (1996) Evaluation of the reproducibility of Auer's classification of DNA histogram in breast carcinoma. Anal Cell Pathol 11:97–106
18. Yamamoto Y, Kishimoto Y, Wistuba II, Virmani AK, Vuitch F, Gazdar AF, Albores-Saavedra J (1998) DNA analysis at *p53* locus in carcinoma arising from pleomorphic adenomas of salivary glands: comparison of molecular study and p53 immunostaining. Pathol Int 48:265–272
19. Yamamoto Y, Wistuba II, Kishimoto Y, Virmani AK, Vuitch F, Albores-Saavedra J, Gazdar AF (1998) DNA analysis at p53 locus in adenoid cystic carcinoma: comparison of molecular study and p53 immunostaining. Pathol Int 48:273–280