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BRIEF REPORT

Malignant transformation of salivary gland pleomorphic adenoma: proof of principle

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Abstract

Supposed risk of malignant transformation of salivary gland pleomorphic adenoma (SGPA) is an important reason for aggressive retreatment in recurrent pleomorphic adenoma (RPA). However, although the diagnostic category 'carcinoma ex-pleomorphic adenoma' suggests that malignant transformation of a pleomorphic adenoma is a regular event, this has to date not been shown to occur in sequential lesions of one patient. Here, we show the molecular events in transformation to malignancy of a pleomorphic adenoma of the parotid gland. Detailed molecular analysis revealed an *LIFR/PLAG1* translocation characteristic for pleomorphic adenoma and, next to this, a *PIK3R1* frameshift mutation and several allelic imbalances. In subsequent malignant recurrences, the same *LIFR/PLAG1* translocation, *PIK3R1* frameshift mutation, and allelic imbalances were present in addition to *TP53* mutations. Thus, this case not only shows malignant transformation of SGPA, but also demonstrates that molecular analysis can be of help in recognising malignancy in the rare instance of RPA.

Keywords: carcinoma ex-pleomorphic adenoma; pleomorphic adenoma; malignant transformation; salivary gland; *TP53*; *PIK3R1*, NGS, SNP

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No conflicts of interest were declared.

Introduction

The risk of malignant transformation from the most common salivary gland tumour, salivary gland pleomorphic adenoma (SGPA), to the fifth most frequent salivary gland carcinoma (carcinoma ex-pleomorphic adenoma; CEPA) is notorious [1,2]. It can lead to dilemmas in both pathological diagnosis and surgical/adjuvant radiation treatment of primary and recurrent pleomorphic adenoma (RPA). This risk of malignant transformation is, however, rare as only 3% of SGPAs recur at 12.5-year follow-up, of which 6% seem to show malignant transformation [1].

The hypothesis that malignant transformation of SGPA occurs is mainly based on the recognition of a pleomorphic adenoma component in malignant salivary gland tumours that are therefore classified as 'CEPA'. In these tumours, morphological as well as molecular transitions are seen from the benign pleomorphic adenoma component to the malignant carcinoma component [3–13]. At the molecular level, the pleomorphic adenoma component is recognised by the presence of *PLAG1* and *HMGA2* gene fusions [6,7,14], while the malignant component harbours additional mutations such as *TP53*, *c-MYC*, *RAS*, and *P21* [8,15–19]. These events have been shown within CEPA as well as in studies comparing SGPA and CEPA cases but not in sequential lesions from one patient [8,15–18].

CEPA can be diagnosed as a primary malignant tumour or as a recurrence after a benign earlier-resected SGPA [9,20,21]. In both cases, malignant transformation is thought to result from progression of an SGPA due to accumulation of genetic changes. SGPA itself is characterised by *PLAG1* gene

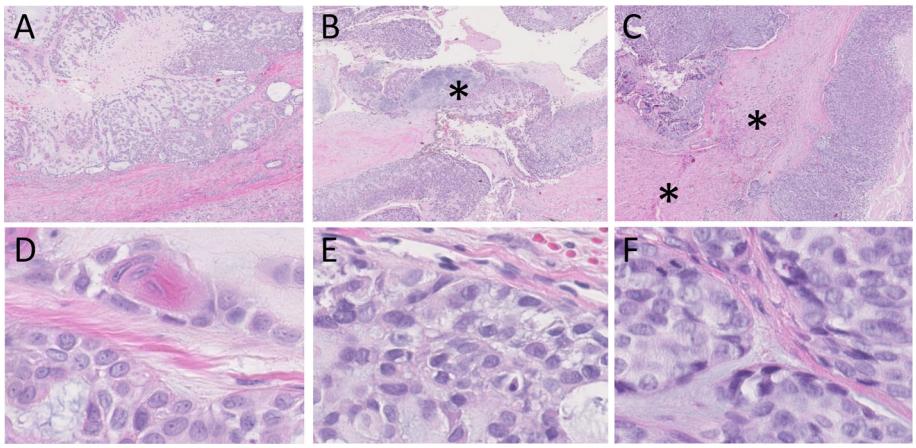

Figure 1. H&E histology (×2 and ×60 magnification) of the primary, first, and second recurrent tumours (A and D, B and E, C and F, respectively) showing progression of the pleomorphic adenoma without cytonuclear atypia to a carcinoma with more nuclear atypia and mitoses (mitosis in the left upper quadrant of F). Pre-existent pleomorphic adenoma is seen at the asterisk in (B). Infiltrative growth in skeletal muscle (asterisks) is present in (C).

overexpression frequently due to a chromosomal translocation resulting in a gene fusion with several candidate genes [10–12]. Progression to CEPA due to *HMGIC* and possibly *MDM2* amplification has been suggested [3,13].

This report shows for the first time that malignant transformation is accompanied by the accumulation of mutations in tumour driver genes in sequentially occurring parotid tumours, by means of targeted next-generation sequencing (NGS) including copy number variation detection analysis using single-nucleotide polymorphisms (SNPs) on 12 chromosomes and RNA-based gene fusion analysis.

Materials and methods

Case

A 35-year-old female presented with a painful parotid tumour in the deep lobe without facial nerve palsy. Fine-needle aspiration cytology (FNAC) revealed a salivary gland tumour, possibly SGPA, uncertainly benign (Milan classification 4b). After a superficial parotidectomy, the tumour was removed with (where possible) the surrounding gland. Histology showed an SGPA (Figure 1A,D), 4 cm in diameter, with a large epithelial component, completely removed without margins. Infiltrative growth, mitotic figures, or necrosis were not observed.

Three years later, the patient presented with a painful recurrent tumour and paraesthesia of the tongue. FNAC was unsuccessful due to extreme pain. A magnetic resonance imaging showed a local recurrence, which was 5.7 cm in diameter. The tumour and remnant of the deep lobe were resected after a lateral mandibulotomy. The facial nerve was sacrificed and reconstructed. At pathology, a cellular salivary gland tumour was seen, without obvious mitosis or necrosis and with areas clearly classifying as pleomorphic adenoma (Figure 1B,E). Infiltrative growth could not be evaluated as the lesion was excised without margins. The lesion was classified as a benign recurrence reaching into the resection surface. Postoperative radiotherapy (RT, 66 Gray) was administered.

A year later, a lump medial to the sternocleidomastoid muscle was biopsied because the patient refused FNA, and this lesion was classified as suspect for adenocarcinoma. A modified radical neck dissection was performed (levels 1–5) with reconstruction of the accessory nerve and the wound defect. Pathology showed a multinodular basal cell adenocarcinoma (Figure 1C,F), reaching into the resection surface. All 24 lymph nodes were free of tumour. Postoperative RT to the neck (60 Gray) was administered.

To understand the clinical behaviour, all previous slides were reviewed and molecular analysis was performed. DNA and RNA from all the three lesions and normal tissue were isolated. Targeted NGS was performed on an Ion Torrent S5XL (Thermo Fisher Scientific, Waltham, MA, USA) prime systemusing a custom-made panel for mutation and copy number variation detection using SNPs on 12 chromosomes (for panel information, see supplementary material, Table S1) [22,23]. In addition,

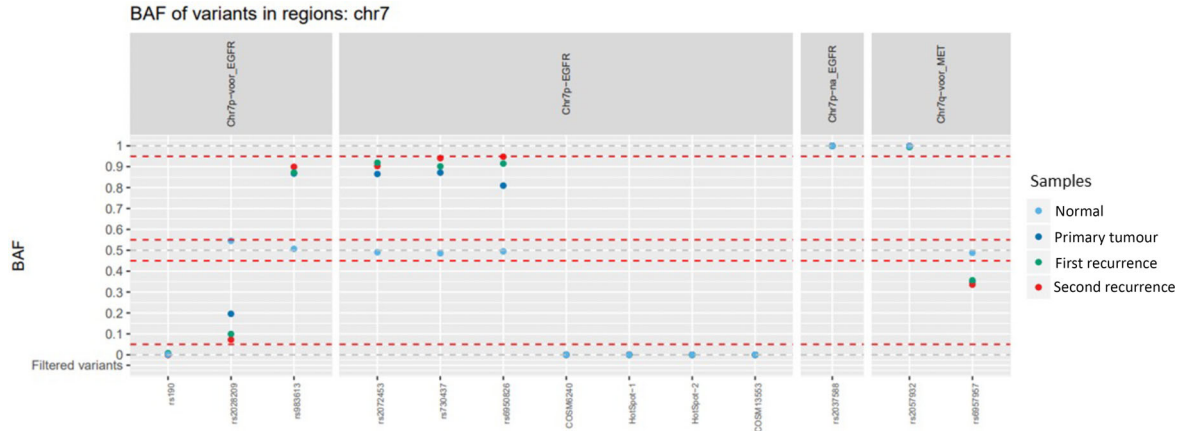

Figure 2. An identical pattern of allelic imbalance was found on chromosomes 5, 7 (shown in this figure), and 8. The imbalances are present in the primary pleomorphic adenoma, the first recurrence, and the second recurrence. BAF, B-allele frequency.

RNA-based gene fusion detection using the Archer FusionPlex Sarcoma panel (ArcherDx, Boulder, CO, USA) was performed. Immunohistochemistry (IHC) for Ki-67 and p53 was performed. The medical ethical committee of the Erasmus Medial Center Rotterdam, The Netherlands, approved this study (MEC-2020-0270) on 4 May 2020.

Results

All three lesions showed the same *LIFR/PLAG1* gene fusion (see supplementary material, Figure S1), an identical somatic frameshift mutation in the *PIK3R1* gene (see supplementary material, Figure S2), and identical patterns of allelic imbalance on chromosomes 5, 7, and 8 (Figure 2 and supplementary material, Figure S3), confirming their clonal relation.

In addition, the first recurrent tumour showed a *TP53* p.R248Q (c.743G>A) mutation with a variant allele frequency (VAF) of 46% (see supplementary material, Figure S2). The second recurrent tumour showed a different *TP53* mutation: *TP53* p.Y220C (c.659A>G) with a VAF of 51% (see supplementary material, Figure S2).

The low-level/subclonal presence of both *TP53* mutations found in the two recurrences was investigated in the three tumour samples with a very sensitive NGS approach using unique molecular identifiers (ThermoFisher Oncomine™ Lung cfDNA Assay V1; Thermo Fisher Scientific, Waltham, MA, USA). With a limit of detection down to 0.3%, no indication was obtained for subclonal presence of these *TP53* mutations in the three tumour samples.

Additional IHC on the three tumours showed a Ki-67 index of 2, 15, and 40%, respectively (Figure 3). p53 IHC was scored as described by Köbel *et al* [24] and

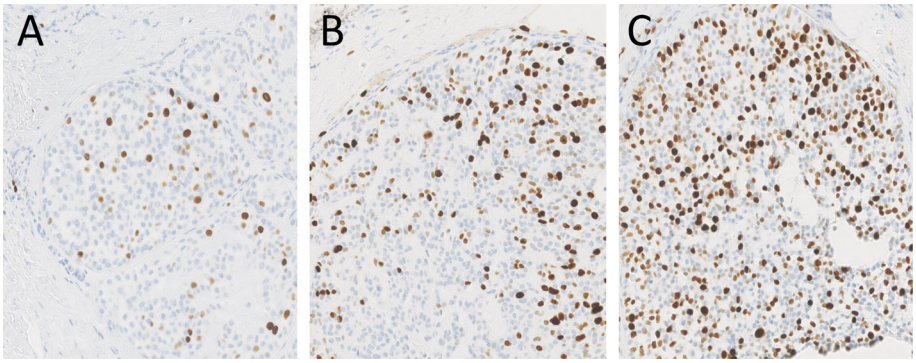

Figure 3. Ki-67 IHC (×14 magnification of digital image) showing increasing expression of Ki-67 over time (A, primary tumour; B, first recurrence; C, second recurrence).

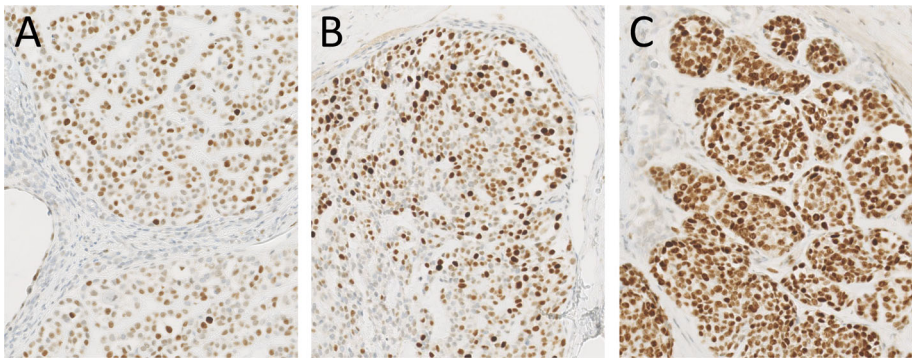

Figure 4. p53 IHC (×14 magnification of digital image) (Ventana BP53/11; BenchMark Ultra Stainer Module, Ventana Medical Systems Inc., Tucson, AZ, USA) showing (A) wild-type, (B) equivocal, and (C) mutant expression of p53 in the primary tumour, first recurrence, and second recurrence, respectively.

showed wild-type expression in the first lesion (Figure 4A) and mutant expression in the second recurrence (Figure 4C), but p53 IHC was equivocal in the first recurrence (Figure 4B). Resected tumour 11 months after the second recurrence showed the known *LIFR/PLAG1* and *PIK3R1* clonal fingerprint and the *TP53* p.R248Q (c.743G>A) mutation of the first recurrence.

Discussion

This report shows for the first time the chronological genetic steps of progression of SGPA to a malignant salivary gland tumour, accompanied by the occurrence of *TP53* mutations. The two lesions with different clonal *TP53* mutations probably represent two different recurrences from the same tumour, as multinodular recurrence is common. *TP53* mutations have been reported as the most frequent mutations in salivary gland carcinomas in general, followed by abnormalities in the cyclin and PI3K pathways (including PIK3R1) [25].

All lesions showed the same *LIFR/PLAG1* gene fusion, *PIK3R1* mutation, and allelic imbalance pattern, revealing their origin from the primary SGPA. It is tempting to speculate that the combination of the *LIFR/PLAG1* gene fusion and the *PIK3R1* mutation with loss of the other allele were drivers for proliferation, whereas the additional *TP53* mutations initiated the malignant transformation. Additional support for this comes from a mouse model with overexpression of *Plag1* targeted to salivary gland and hyperactivation of the PI3K pathway by a conditional salivary gland *Pten* knockout mouse model, both of which resulted in pleomorphic adenoma formation [26,27].

The occurrence of new *TP53* mutations in the recurrences does not confirm a causal role for these mutations in malignant transformation as the specificity of *TP53* mutation for this process is unknown. However, newly present *TP53* mutations in genetically related subsequent recurrent tumours were accompanied histologically by malignancy; thus, a driver role seems likely, as *TP53* is a well-known tumour suppressor gene [28]. Loss of *TP53* function is the most common molecular aberration in human malignancies and is involved in the initiation and progression of many malignant disorders. Based on the results from DNA mutation, SNP, and DNA amplicon coverage analyses, all three lesions are probably bi-allelic for the *TP53* locus (see supplementary material, Figure S3) and therefore have one functional intact *TP53* allele. The identified *TP53* mutations p.R248Q and p.Y220C, however, are known to exert a dominant-negative effect leading to complete inactivation of *TP53* without a second hit mutation (e.g. loss of the wild-type *TP53* allele) [29].

In this case, the first recurrence was not originally recognised as malignant, although the proliferation rate of 15% was relatively high [30]. In the second recurrence, morphology and proliferation rate were obvious clues for malignancy. In retrospect, p53 and Ki-67 IHC might have raised the suspicion of malignancy, warranting molecular analysis.

Several aspects complicate salivary gland tumour diagnosis in general, such as their rarity and the wide morphological spectrum. Although SGPA is the most frequent primary salivary gland tumour, it is still a rare tumour (European standardised rate: 4.5/100,000 as opposed to 62/100,000 for breast cancer) [31]. Some of its characteristics can make correct diagnosis challenging, e.g. the morphological overlap with features of some of the 22 salivary gland carcinomas [20,32].

Furthermore, malignant transformation of SGPA occurs only occasionally at a frequency of 6% of first RPAs [1]. And as shown in this case, malignant transformation can be difficult to recognise as the first recurrence already harboured a *TP53* mutation but was not identified as malignant morphologically. This shows that, in the rare case of an RPA, molecular analysis can be of value in recognising early malignant transformation.

Clinically, the risks of infiltrative growth and malignant transformation have been important arguments for aggressive surgical treatment of RPA, which warrants the sacrifice of vital structures in selected cases. This case for the first time illustrates that malignant transformation of SGPA occurs and that molecular analysis can help to recognise malignancy. The combination of histology and molecular analysis can make a more solid diagnosis possible, which is imperative for appropriate clinical decision-making.

Author contributions statement

MHV contributed conceptualisation, data curation, investigation, methodology, project administration, resources, visualisation, and writing (original draft) of the manuscript. HM contributed investigation, visualisation, and writing (review and editing) of the manuscript. ItH was involved in investigation, visualisation, and writing (review and editing) of the manuscript. LRM contributed formal analysis, investigation, methodology, visualisation, and writing (review and editing) of the manuscript. AJMB and LES were involved in conceptualisation and writing (review and editing) of the manuscript. SK contributed formal analysis, investigation, methodology, and writing (review and editing) of the manuscript. WNMD was involved in conceptualisation, data curation, formal analysis, investigation, methodology, resources, visualisation, and writing (review and editing) of the manuscript. M-LFvV contributed conceptualisation, data curation, formal analysis, investigation, methodology, project administration, resources, visualisation, supervision, and writing (review and editing) of the manuscript.

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SUPPLEMENTARY MATERIAL ONLINE

Figure S1. Analysis with the Archer FusionPlex Sarcoma panel

Figure S2. NGS analysis of *TP53* and *PIK3R1* mutations in the primary tumour and the first and second recurrences

Figure S3. NGS analysis of allelic imbalances in the primary tumour and the first and second recurrences

Table S1. Custom-made NGS panel information