Oncocytic change in pleomorphic adenoma: molecular evidence in support of an origin in neoplastic cells

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J Clin Pathol 2007;60:492–499. doi: 10.1136/jcp.2005.031369

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Accepted 9 August 2005
Published Online First
7 February 2006

Background: Cells with oncocytic change (OC) are a common finding in salivary glands (SGs) and in SG tumours. When found within pleomorphic adenomas (PAs), cells with OC may be perceived as evidence of malignancy, and lead to a misdiagnosis of carcinoma ex pleomorphic adenoma (CaExPa).

Aim: To describe a case of PA with atypical OC, resembling a CaExPa. A genomewide molecular analysis was carried out to compare the molecular genetic features of the two components and to determine whether the oncocytic cells originated from PA cells, entrapped normal cells, or whether these cells constitute an independent tumour.

Materials and methods: Representative blocks were immunohistochemically analysed with antibodies raised against cytokeratin (Ck) 5/6, Ck8/18, Ck14, vimentin, p63, α -smooth muscle actin (ASMA), S100 protein, anti-mitochondria antibody, β -catenin, HER2, Ki67, p53 and epidermal growth factor receptor. Typical areas of PA and OC were microdissected and subjected to microarray-based comparative genomic hybridisation (aCGH). Chromogenic in situ hybridisation (CISH) was performed with in-house generated probes to validate the aCGH findings.

Results: PA cells showed the typical immunohistochemical profile, including positivity for Ck5/6, Ck8/18, Ck14, vimentin, ASMA, S100 protein, p63, epidermal growth factor receptor and β -catenin, whereas oncocytic cells showed a luminal phenotype, expression of anti-mitochondria antibody and reduced β -catenin staining. Both components showed low proliferation rates and lacked p53 reactivity. aCGH revealed a similar amplification in both components, mapping to 12q13.3–q21.1, which was further validated by CISH. No HER2 gene amplification or overexpression was observed. The foci of oncocytic metaplasia showed an additional low-level gain of 6p25.2–p21.31.

Conclusion: The present data demonstrate that the bizarre atypical cells of the present case show evidence of clonality but no features of malignancy. In addition, owing to the presence of a similar genome amplification pattern in both components, it is proposed that at least in some cases, OC may originate from PA cells.

Oncocytic metaplasia (OM) is a common finding in neoplastic and non-neoplastic salivary gland (SG) specimens. In normal SG, the presence of OM, also known as "oncocytosis", is regarded as a feature of an "ageing" salivary tissue.¹ Virtually all types of benign and malignant tumours as well as hyperplastic lesions of the SGs can harbour diffuse or focal areas of OM.^{1–5} Oncocytic cells have been observed in the epithelial and myoepithelial components of SG tumours,^{3–7} as well as in thyroid^{8–9} and other endocrine tissues, including the parathyroid, pituitary, adrenal cortex, pancreas, gut and lung.^{7–10–11}

Oncocytic cells characteristically have an abundant, finely granular, eosinophilic cytoplasm, with large vesicular nuclei having one or more prominent nucleoli.^{2–5–7–10} Clear cell change in oncocytic cells may occasionally be seen. Ultrastructural analysis has revealed the presence of an increased number of normal and abnormal mitochondria in the cytoplasm of oncocytic cells.^{2–5–7–10} When analysed out of context, the cytological features (ie, vesicular nuclei with prominent nucleoli) of these cells may be taken as a sign of malignancy by the unwary. In fact, pseudo malignant changes, including bizarre oncocytic cells, have been reported to be observed in approximately 3% of all pleomorphic adenomas (PAs),¹² and the possibility of such misdiagnosis has been repeatedly reported in the literature.^{5–6–13}

Malignant changes in PA can take several forms.^{6–14–15} In particular, it may be quite difficult to distinguish in situ (or

non-invasive) carcinoma ex-pleomorphic adenoma (CaExPa) from bizarre/reactive/metaplastic cells.¹⁴ Our group has recently demonstrated that HER2 overexpression and gene amplification can be used to detect early/focal malignant changes in PAs.¹⁴ Although 81% and 67% of in situ CaExPa show HER2 overexpression and amplification, respectively,¹⁴ HER2 gene amplification has not been described in reactive and/or metaplastic cells.

While reviewing a series of in situ CaExPa, we came across a case of PA with OM resembling an in situ CaExPa. The molecular aspects of oncocytic changes have been extensively studied in the context of thyroid lesions^{9–16–17}; however, there are no reports on the molecular genetic features of OM in PA. Therefore, we carried out a thorough comparison of the immunohistochemical and genomewide molecular genetic features of a PA with foci of bizarre oncocytic metaplastic cells. The aim of this study was twofold: (1) to characterise the immunohistochemical and genomic profile of PA and the foci of OM, and (2) to determine whether the oncocytic cells

Abbreviations: aCGH, microarray-based comparative genomic hybridisation; ASMA, α -smooth muscle actin; BAC, bacterial artificial chromosome; CaExPa, carcinoma ex pleomorphic adenoma; CDK4, cyclin-dependent kinase 4; CISH, chromogenic in situ hybridisation; Ck, cytokeratin; EGFR, epidermal growth factor receptor; OC, oncocytic change; OM, oncocytic metaplasia; PA, pleomorphic adenoma; SDS, sodium dodecyl sulphate; SG, salivary gland; SSC, saline sodium citrate

Figure 1 Photomicrograph of pleomorphic adenoma with tubular (A) and spindle, myoepithelial-rich (B) areas, which were negative for anti-mitochondria antibody (C) and positive for cytokeratin 8/18 (D) and p63 (E). Note the low/moderate proliferation rate in the myoepithelial-rich area (F).

originated from cells of PA or, from entrapped normal SG cells, or whether these cells constitute an independent tumour.

Case report

A 44-year-old woman presented with a lump in the right parotid gland.

After a cytological diagnosis of PA, the patient underwent right superficial parotidectomy.

MATERIALS AND METHODS

Histology and immunohistochemistry

The surgical specimen was routinely fixed in 10% buffered formalin and then embedded in paraffin wax. For routine histological examination, H&E-stained sections were obtained.

Sections from representative areas were cut at 4 μ m thickness and mounted on silane-coated slides. Immunohistochemical analysis was performed according to the streptavidin-biotin-peroxidase complex method, as described previously,¹⁸ with antibodies raised against cytokeratin (Ck) 5/6 (D516B4, 1:600, Chemicon, Temecula, California, USA), Ck8/18 (NCL-5D3, 1:100, Novocastra, Newcastle-upon-Tyne, UK), Ck14 (LL02, 1:40, Novocastra), vimentin (V9, 1:500, Dako, Glostrup, Denmark), p63 (4A4, 1:50, Santa Cruz

Biotechnology, Santa Cruz, California, USA), α -smooth muscle actin (ASMA, 1A4, 1:200, Dako), S100 protein (polyclonal, 1:6000, Dako), anti-mitochondria antibody (113-1, BioGenex, San Ramon, California, USA), β -catenin (17C2, 1:100, Novocastra), HER2 (polyclonal, 1:1200, Dako), Ki67 (MIB-1, 1:300, Dako), p53 (DO7, 1:200, Dako), MDM2 (Ab-1, 1:10, Calbiochem, Darmstadt, Germany) and epidermal growth factor receptor (EGFR, 31G7, 1:50, Zymed, South San Francisco, California, USA).

Positive and negative controls were included in each slide run. For Ck14, Ck5/6, ASMA and mitochondria antibody, only cytoplasmic staining was considered as specific. For p63, p53 and MDM2 only nuclear reactivity was considered as specific, whereas for HER2 and EGFR only membrane staining was regarded as specific. For β -catenin, both membrane and nuclear expression were considered as specific.

The distribution of Ck5/6, Ck8/18, Ck14, vimentin, p63, ASMA, S100 protein and β -catenin was evaluated semiquantitatively and scored into four categories: negative (–), $\leq 5\%$ of cells stained; (+/–), 5–10% of cells stained; (+), 10–50% of cells stained; (++), $> 50\%$ of cells stained. For HER2/neu and EGFR, the Herceptest (Dako) scoring system was adapted for non-invasive lesions: negative, no membrane staining or $< 10\%$ of

Figure 2 Photomicrograph of foci of oncocytic changes (A). Note the presence of scattered bizarre cells (B). These cells were positive for anti-mitochondria antibody (C) and positive for cytokeratin 8/18 (D). No p63 staining was observed in these cells (E). Despite the worrisome nuclear features, these cells showed a low proliferation rate (F).

cells stained; 1+, incomplete membrane staining in >10% of cells; 2+, >10% of cells with weak to moderate complete membrane staining; and 3+, strong and complete membrane staining in >10% of cells. For p53 and MDM2, a threshold of >10% of neoplastic cells of each component was adopted. Ki67 proliferation index was assessed in 200 cells at hot spot areas.

Microdissection and DNA extraction

For array-based comparative genomic hybridisation (aCGH) analysis, the bona fide PA and the areas with OM were microdissected with a sterile needle under a stereomicroscope (Olympus SZ61, Tokyo, Japan) from two consecutive 5 μ m sections stained with nuclear fast red. The estimated purity of both components was 90%. DNA was extracted in 200 μ l digestion buffer (100 mM NaCl, 10 mM Tris-HCl (pH 8.0), 25 mM EDTA (pH 8.0), 0.5% sodium dodecyl sulphate (SDS), 0.5 mg/ml proteinase K (Invitrogen Life Technologies, Paisley, UK)), and was purified by phenol/chloroform extraction in Phase Lock Gel Light tubes (Eppendorf, Hamburg, Germany). The purified DNA samples were precipitated in ethanol and the DNA resuspended in Tris EDTA buffer (pH 7.5). The DNA yield was assessed by spectrophotometry and the DNA quality (DNA fragment size range) by agarose gel electrophoresis.

Microarray-based comparative genomic hybridisation

aCGH was performed using an array of 5623 bacterial artificial chromosomes (BACs) spaced at approximately 0.9 Mb intervals throughout the genome as described previously.¹⁸ This array was produced by standard degenerate oligonucleotide primer-PCR of BAC clones that were spotted in triplicate on gamma-aminopropyl silane II aminosilane slides. BAC clones were mapped to the latest human genome assembly (hg17) on the basis of in-house re-sequencing ($n = 700$), publicly available sequence data ($n = 2500$), and both clone ID and sequence-tagged site marker accessions.

DNA labelling and array hybridisation

Reference (normal DNA extracted from blood lymphocytes pooled from five females) and tumour DNAs, each 1 μ g, were labelled with Cy3- or Cy5-conjugated deoxycytidine triphosphate (Amersham Biosciences, Buckinghamshire, UK) using random primed BioPrime DNA labelling (Invitrogen Life Technologies) according to the manufacturer's protocol modified to incorporate 1.0 mM Cy dye, 0.6 mM deoxycytidine triphosphate and 1.2 mM deoxyadenosine triphosphate, deoxyguanosine triphosphate and deoxythymidine triphosphate. After labelling at 37°C for 16 h, non-incorporated reaction

Table 1 Summary of the immunohistochemical and chromogenic in situ hybridisation findings

Component	Pleomorphic adenoma		
Antibody	Spindle	Epithelioid	Oncocytic change
Anti-mitochondria	—	—	++
Ck5/6	+	+	—
Ck8/18	++	++	++
Ck14	+	+	—
Vimentin	++	+	—
p63	++	+	+/-
ASMA	++	+	—
S100 protein	++	+	+/-
EGFR	++	++	—
β-Catenin	++	++	+/-
HER2	—	—	1+†
P53	—‡	—	—‡
Ki67	8% (16/200 cells)	4% (8/200 cells)	4.5% (9/200 cells)
MDM2	—§	—§	+/- (10–25%, weak)
CISH 12q14.1	Amplified	Amplified	Amplified

ASMA, α-smooth muscle actin; CISH, chromogenic in situ hybridisation; Ck, cytokeratin; EGFR, epidermal growth factor receptor.

*Ck 14, 5/6, vimentin, p63 and S100 protein showed positivity in the outer layer of the tubular structures and in solid epithelioid areas, whereas ASMA was restricted to the outer layer of tubular structures.

†Incomplete membrane staining was observed in >10% of neoplastic cells.

‡<10% of strongly positive cells were observed.

§<10% of weakly positive cells.

constituents were removed by MinElute Reaction Cleanup (Qiagen, Crawley, UK). For dye bias correction, in separate reactions, the same reference and sample DNAs were labelled with the opposite dye (dye swap).

Labelled reference and tumour DNAs were co-ethanol precipitated with 100 µg of human Cot-1 DNA (Invitrogen Life Technologies) and resuspended in 50 µl hybridisation buffer (50% deionised formamide, 10% (w/v) dextran sulphate, 2× saline sodium citrate (SSC), 2% SDS and 20 µg yeast transfer RNA). Labelled DNA was denatured at 70°C for 15 min, followed by a 30 min incubation at 37°C to allow blocking of repetitive sequences by human Cot-1 DNA. Denatured DNA samples were applied to the microarray under a coverslip and hybridised at 37°C for 18 h. After hybridisation, the coverslips were removed by washing slides in 2× SSC/1% SDS for 15 min at 45°C. The slides were then washed in 50% de-ionised formamide/2× SSC for 15 min at 45°C, followed by 2× SSC/1% SDS for 30 min at 45°C and twice in 0.2× SSC for 15 min at room temperature. After washing, the arrays were dried by centrifugation at 2000 rpm for 2 min.

Arrays were scanned with a GenePix 4000A scanner (Axon Instruments, Union City, California, USA); fluorescence data were processed using GenePix 3.0 image analysis software (Axon Instruments).

Data analysis

Data analysis was performed as described previously.¹⁸ Briefly, the log₂ ratios were normalised for spatial and intensity-dependent biases using a row-based local regression. BAC clone replicate spots were averaged across dye-swap experiments, after exclusion of poorly reproducible replicates (SD>0.2) and excessively flagged clones (>70% of samples). This left a final dataset of 4517 clones with unambiguous mapping information, according to the May 2004 build of the human genome (hg17). Data were smoothed using a local polynomial adaptive weights smoothing procedure for regression problems with additive errors.¹⁹ A categorical analysis was applied to the BACs after classifying them as representing gain, loss or no change according to their smoothed log₂ ratio values. Log₂ ratio values

<−0.09 were categorised as losses, those >0.09 as gains and those in between as unchanged. These threshold values were chosen to correspond to 3 standard deviations of the normal ratios obtained from the filtered clones mapping to chromosomes 1–22, assessed in comparison between DNA extracted from a pool of male and that from a pool of female blood donors (data not shown). All data transformation and statistical analysis was carried out in R 2.0.1

(<http://www.r-project.org/>) and BioConductor 1.5 (<http://www.bioconductor.org/>), making extensive use of modified versions of the packages, in particular aCGH, marray and aws.

Each of the 4517 BACs defines a genomic region starting from the end position of the previous non-overlapping clone on the array to the start position of the next contiguous, non-overlapping array clone. These genomic regions were defined because any deletion or gain/amplification seen for a clone may extend beyond the clone, and thus the extent of change was determined by the next unaffected clone on the array in both directions.

Chromogenic in situ hybridisation

Chromogenic in situ hybridisation was used to allow a direct evaluation of the copy number changes in non-neoplastic, typical PA and oncocytic cells. Probes used included three contiguous fluorescent in situ hybridisation mapped BACs, RP11–66N19, RP11–277A02 and RP11–549D07, which map to 12q14.1 and span the region comprised between approximately 56 360 and 56 790 kb on the long arm of chromosome 12. BAC DNA was extracted using a Qiagen plasmid mini kit (Qiagen, Valencia, California, USA), according to the manufacturer's protocol, and independently amplified with GenomiPhi (Amersham Biosciences, Bucks, UK) whole genome amplification kit. A pool of the three amplified products was then biotin labelled with the Bioprime kit (Invitrogen Life Technologies) according to the manufacturer's protocol. Biotin-labelled probes were precipitated with 60 µg human Cot-1 DNA (Invitrogen Life Technologies) and 10 µg salmon sperm DNA (Invitrogen Life Technologies), and re-suspended in 20 µl of hybridisation buffer (50% deionised formamide; 20% w/v dextran sulphate; 2× SSC; 0.1 mM EDTA, pH 8.0; 0.2 mM Tris-HCl, pH 7.6). Two deparaffinised sections were pre-treated with heat for 15 min at 98°C in a coplin jar containing chromogenic in situ hybridisation pretreatment buffer (SPOT-light tissue pretreatment kit, Zymed) and digested with pepsin for 5 min at room temperature according to the manufacturer's instructions. Subsequently, the sections were washed with water, dehydrated with graded ethanol and air-dried. The re-suspended probes were applied to the centre of 24×50 mm coverslips and placed on respective tissue sections. After sealing the edges of the coverslip with rubber cement, the tissue sections and probes were denatured for 5 min at 95°C on a hot plate. Hybridisation was performed overnight in a humid chamber at 37°C. Slides were rinsed in 0.5× SSC at room temperature and then washed in 0.5× SSC for 5 min at 78°C. Endogenous peroxidase activity and non-specific protein binding were blocked by incubation with 3% H₂O₂ in methanol for 10 min and in CAS block (Zymed) for 10 min, respectively. The sections were incubated with horse radish peroxidase–streptavidin (Zymed) for 30 min at room temperature and washed in phosphate-buffered saline/0.025% Tween 20. Development of the staining was completed using diaminobenzidine (Zymed) as the chromogen. Tissue sections were counterstained with haematoxylin and mounted. An appropriate gene-amplified breast tumour control was included in the slide run. The section was analysed by two of the authors (JSR-F and SDP) on a multi-headed microscope. Only unequivocal signals were counted. Signals were evaluated at ×400 and

Figure 3 Array comparative genomic hybridisation genome plots of the pleomorphic adenoma and the foci of oncocytic change (y axis: sample:reference ratios; x axis: chromosomes; continuous vertical lines: chromosome boundaries; dotted vertical lines: centromeres; Black dots: $\log_2$ ratios; blue dots: aws ratios).

$\times 630$, and 60 cells were counted for the presence of the 13q14.1 signals. A given area was considered to be amplified for 12q14.1 when $>50\%$ of the neoplastic cells harboured large gene copy clusters and/or >5 signals per nuclei.¹⁸ The signals per nucleus ratio were calculated in both the typical PA areas and the foci of OM, as well as in normal luminal and myoepithelial cells and endothelial cells.

RESULTS

Histological findings

The superficial parotidectomy specimen measured $45 \times 35 \times 15$ mm. Slicing revealed a well-circumscribed nodule measuring 30 mm in diameter, surrounded by a fibrous capsule with an area of calcification. Histological examination revealed parotid gland harbouring a well-circumscribed tumour, which

Table 2 Molecular genetic changes detected by microarray-based comparative genomic hybridisation

Pleomorphic adenoma					Oncocytic metaplasia				
Chrom	Start (kb)	End (kb)	Cytoband	Type	Chrom	Start (kb)	End (kb)	Cytoband	Type
12	55 277	74 109	12q13.3–q21.1	Gain	12	52 292	74 109	12q13.13–q21.1	Gain
13	110 344	113 805	13q34	Gain	6	2334	36 174	6p25.2–p21.31	Gain

was composed of a complex admixture of spindle, epithelioid and stellate cells arranged in solid sheets and cords, as well as forming bilayered glandular structures (figs 1A,B). The neoplastic cells were immersed in myxoid and/or collagenous matrix. Multiple foci composed of tightly packed large cells with granular eosinophilic-to-pale cytoplasm, and large vesicular nuclei with conspicuous, prominent nucleoli were identified (fig 2A). Scattered bizarre cells were also observed (fig 2B). Mitotic figures were rare in PA and OC areas (~1/10 high-power fields); however, a higher mitotic index was observed in myoepithelial cell-rich areas (4/10 high-power fields).

Immunohistochemistry

Table 1 summarises the immunohistochemical findings. Briefly, the oncocytic component was strongly and diffusely positive for the anti-mitochondria antibody (fig 2C), whereas the remaining areas were negative (fig 1C). Both PA and oncocytic cells were positive for Ck8/18 (figs 1D, 2D). Ck5/6 and 14 were positive in the epithelioid components of PA and to a lesser extent in spindle cells, whereas ASMA showed the opposite pattern. No Ck5/6 and/or Ck14 reactivity was identified in the oncocytic cells. S100 protein, p63 and EGFR were diffusely positive in PA (fig 1E), but largely negative in the foci of OM (fig 2E), demonstrating the lack of myoepithelial differentiation in oncocytic cells.

β -Catenin showed diffuse and strong complete membrane staining in PA, whereas membranes were weakly and focally positive in the oncocytic cells. No nuclear β -catenin staining was observed. Scattered p53 strongly positive cells were observed in both components; however, these cells accounted for <5% of each component. MDM2 was negative (<10%) in PA and weakly positive in 10–25% of the cells with OC. No HER2 expression was detected in PA, whereas >10% of oncocytic cells showed incomplete membrane staining. Ki67 labelling index was low in both components: 8% in PA and 4.5% in the foci of OM (figs 1F, 2F).

Taken together, the above histological and immunohistochemical findings are consistent with a diagnosis of PA showing multiple foci OM.

Array comparative genomic hybridisation

Figure 3 and table 2 summarise the aCGH findings for each component. As expected, few molecular genetic changes were identified in both components. However, importantly, high-level gain/ amplification of 12q13.3–q21.1 was observed in both components. This region comprises multiple genes of interest, including *MDM2*, cyclin-dependent kinase 4 (*CDK4*), sarcoma amplified sequence, high mobility group AT-hook 2 (*HMG2*, aka *HMGIC*), glioma-associated antigen (*GLI1*), ras association domain family protein 3 (*RASSF3*), dual-specificity tyrosine phosphorylation-regulated kinase 2 (*DYRK2*), ras-related protein (*RAP1B*), fibroblast growth factor receptor substrate 2 (*FRS2*) and RAB3A-interacting protein (*RAB3IP*).

The PA harboured an additional gain spanning an approximately 3.5 Mb region on 13q34. This gene-rich region comprises interesting gene candidates, including Rho guanine nucleotide exchange factor 7 (*ARHGEF7*), SRY-related HMG-box gene 1 (*SOX1*), cullin 4A (*CUL4A*), lysosomal-associated membrane protein 1 (*LAMP1*), transcription factor Dp-1 (*TFDP1*), G protein-coupled receptor kinase 1 (*GRK1*) and growth arrest-specific 6 (*GAS6*). The oncocytic component also showed low-level gain of 6p25.2–p21.31, which is a region reported to be altered in several human tumours and involved in chromosomal translocations. This region encompasses high mobility group AT-hook 1 (*HMG1*, aka *HMGII*), a gene occasionally rearranged in PAs of the SGs.²⁰

Chromogenic in situ hybridisation

Both components showed increased copy number gains (>5 copies/nucleus) of 12q14.1 (figs 4A, B), whereas endothelial cells, and normal SG epithelial and myoepithelial cells showed an average of approximately two copies/nucleus.

DISCUSSION

Here, we demonstrate that oncocytic cells arising in PA harbour similar molecular genetic changes as those seen in typical cells of PA. The presence of 12q13.3–q21 amplification in both components of the present case suggests a clonal origin for both, with the subsequent acquisition of an oncocytic phenotype in a subpopulation of the cells. Given that the oncocytic cells harbour only one additional genetic change on 6p, the

Figure 4 Chromogenic in situ hybridisation demonstrating >5 signals/nucleus in typical areas of pleomorphic adenoma (A) and oncocytic change (B). Note the presence of 1 or 2 signals in normal endothelial cells (arrows). B (inset): Note the oncocytic cell with six signals.

oncocyctic cells of the present case should be seen as a new clone emerging from cells of an otherwise typical PA. Although several mechanisms for the acquisition of oncocyctic phenotypic features have been put forward,^{9–16} the molecular genetic features of this case suggest that, at least in some PAs with oncocyctic changes, the acquisition of neoplastic biological properties precedes the process of OM.

Rearrangements and amplifications of 12q13 have been documented before in bona fide PAs,^{21–22} malignant mixed tumours²³ and CaExPa.²⁴ In the present case, the minimal region of amplification observed in both components harbours several interesting candidate genes, including *MDM2*, *CDK4* and *HMG2*. *MDM2*, a target gene of the transcription factor and tumour suppressor gene *TP53*, encodes a nuclear phosphoprotein that binds and inhibits transactivation by tumour protein p53.²⁵ Owing to its E3 ubiquitin ligase activity, which targets the p53 protein for proteasomal degradation, *MDM2* protein modulates p53 activity through an autoregulatory negative feedback loop.²⁵ Amplification of *MDM2* has been described in several neoplasms^{23–26} as an alternative mechanism for p53 inhibition. In fact, overexpression of *MDM2* protein can result in excessive inactivation of tumour protein p53, diminishing or abrogating its tumour suppressor function. The *CDK4* gene encodes a serine/threonine protein kinase, which plays a major role in cell cycle G1 phase progression. The activity of this kinase is tightly controlled by D-type cyclins and CDK inhibitor p16, and restricted to the G1-S phase. *CDK4* gene amplification^{23–26–27} has been found to be associated with tumorigenesis of a variety of cancers. Recurrent *HMG2* gene rearrangements have been reported in PAs,^{20–28–30} and in a plethora of benign mesenchymal tumours.³¹ *HMGIC* encodes an architectural transcription factor that promotes activation of gene expression by modulating the conformation of DNA. The protein has three DNA-binding domains (AT-hook motifs) that bind to the minor groove of AT-rich DNA.³¹ Although we could not identify the amplicon driver in this particular case, as commercially available antibodies for *CDK4* and *HMG2* were unsuitable for immunohistochemical analysis on formalin-fixed paraffin-wax-embedded tissue sections in our study (data not shown), our data suggest that *MDM2* is unlikely to be the amplicon driver in this particular tumour, as no expression of *MDM2* was observed in PA cells.

Chromosomal rearrangements involving 6p have been described in PAs.²⁰ This region encompasses another member of the high mobility group family, *HMG1*. Rearrangements 6p21–p23, encompassing the locus of *HMG1*, have been described in about 1.5% of PAs. However, Rohen *et al*²⁰ suggested that *HMG1* should not be considered the target gene of 6p21–p23 rearrangements, as the genomic locus of this gene (6p21) is very rarely affected in PAs and most rearrangements involving 6p affect a region distal to *HMG1*. Further analysis of this region is warranted.

Gain of genomic material on 13q34 was observed only in the PA, suggesting that this may be a late genomic event in this particular tumour. Although genomic aberrations in this region have not been described in PAs, 13q34 is reported to be altered in a number of human neoplasms.^{32–34} Moreover, in the present case, the amplified region harbours genes that encode transcription factors (*SOX1*, *TFDP1*), a GTPase (*ARHGEF7*), a serine/threonine kinase (*GRK1*), a cell cycle associated gene (*GAS6*) and a gene related to the ubiquitin conjugating system (*CUL4A*). Interestingly, *CUL4A* ubiquitin ligase has been implicated in the ubiquitination and proteolysis of *DDB2*, *CDT1*, c-jun and *STAT* proteins, and is reported to associate with *MDM2* protein and participate in p53 proteolysis.³³ In addition, amplification of *CUL4A* has been described in liver hepatocellular carcinomas³² and breast cancer.³⁴ Taken together, the

present findings suggest that rearrangements of this region may play a role in the genetic evolution of PAs.

The identification of molecular genetic changes in the oncocyctic cells by aCGH in the present case supports the idea that oncocyctic cells arising in this tumour are clonal and neoplastic, but not necessarily malignant. In fact, despite the worrisome nuclear features, these bizarre cells (fig 2B) showed low proliferative ratios and lacked p53 nuclear staining. HER2 protein overexpression and gene amplification have been well documented as an evidence of early malignant changes in PAs.¹⁴ The bizarre oncocyctic cells of the present case showed neither genomic gains mapping to 17q11.2–q12 (*ERBB2* locus) nor HER2 protein overexpression. Taken together, the above findings support the idea that the oncocyctic cells in this tumour are not malignant.

In conclusion, our data suggest that, in at least a proportion of myoepithelial tumours/tumours with myoepithelial participation of the SGs, the acquisition of an oncocyctic phenotype occurs after neoplastic transformation. This study also highlights the potential use of molecular genetic methods for differentiating pseudo-malignant clonal changes from malignancy in PAs.

ACKNOWLEDGEMENTS

This study was supported by Breakthrough Breast Cancer. JSR-F is supported in part by PhD grant SFRH/BD/5386/2001 from the Fundação para a Ciência e a Tecnologia, Portugal, and grant POCTI/CBO/45157/2002 from Programa Operacional Ciência, Tecnologia e Inovação, Fundação para a Ciência e a Tecnologia, Portugal.

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Competing interests: None declared.

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ECHO.....

Diagnosing temporal arteritis becomes easier

Please visit the *Journal of Clinical Pathology* website [www.jclinpath.com] for a link to the full text of this article.

New diagnostic criteria for a disease that can cause later blindness will reduce false negative results arising from corticosteroid treatment. General pathologists will now be able to diagnose temporal arteritis (TA) (or giant cell arteritis (GCA)) after treatment has been started, if recent results are replicated in a larger multicentre randomised study.

The most reliable criteria for TA in its healing phase during treatment are a cuff of lymphocytes and epithelioid histiocytes where the outer muscle layer of the temporal artery and adventitia meet; large circumferential defects in, or absence of, the elastic lamina; and rather sparse, small multinucleate giant cells. This contrasts with the typically uniform granulomatous inflammation through the entire arterial wall in biopsy specimens taken before treatment.

Special stains are strongly recommended and immunohistochemical staining for CD-68 to show epithelioid histiocytes; clinicians should inform pathologists about dosage and duration of treatment as these influence histological appearance.

The criteria were drawn up by ophthalmic pathologists from in depth analysis of 35 cases of TA in which biopsy specimens were obtained after corticosteroid treatment had started. The cases were drawn from a retrospective review of all cases of TA recorded over five years at the pathology laboratory of one eye institute in the United States.

The best treatment for TA is to give corticosteroids early, even before biopsy. As identification according to histological appearance of biopsy specimens is the diagnostic standard, there was a need to define cardinal histological features in the healing phase.

▲ Font RL, *et al.* *British Journal of Ophthalmology* 2007;**91**:204–209.