Salivary duct carcinoma in situ of the parotid gland

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Aims: To describe three cases of purely in situ salivary duct carcinoma, so as better to define the entity.
 Methods and results: Three primary tumours of the parotid gland are presented, in each case composed of cysts and ducts and lined by high nuclear grade epithelial cells. All parts of each tumour were surrounded by a myoepithelial cell rim and there was no evidence of invasion. The tumour cells expressed immunohistochemical markers seen in invasive salivary duct carcinoma of usual (high-grade) type. In two cases the androgen receptor (AR) reaction was strong, but there was no immunohistochemical expression of HER2 protein or gene amplification by in situ hybridization. In the remaining case, fewer nuclei stained for AR, but both HER2 protein and gene amplification were demonstrated.

Conclusions: Salivary duct carcinoma in situ is morphologically similar to breast ductal carcinoma in situ and, although our cases are few, salivary duct carcinoma in situ can possibly be subdivided into luminal and non-luminal cell types, as can analogous mammary neoplasms. The present study cannot determine whether low-grade cribriform cystadenocarcinoma, architecturally similar but immunohistochemically different, is part of the spectrum of salivary duct carcinoma in situ, or whether it represents a separate entity.

Keywords: immunohistochemistry, parotid gland, salivary gland neoplasms, silver in situ hybridization

Abbreviations: AR, androgen receptor; CK, cytokeratin; DCIS, ductal carcinoma in situ; EGFR, epidermal growth factor receptor; ER, oestrogen receptor; FISH, fluorescence in situ hybridization; LGCCC, low-grade cribriform cystadenocarcinoma; PASD, periodic acid–Schiff after diastase digestion; PR, progesterone receptor; SDC, salivary duct carcinoma; SDCIS, salivary duct carcinoma in situ; SISH, silver in situ hybridization; WHO, World Health Organization

Introduction

Salivary duct carcinoma (SDC) is defined in the 2005 World Health Organization (WHO) classification as “an aggressive adenocarcinoma which resembles high-grade breast ductal carcinoma”. 1 Most cases arise de novo, although some develop as the malignant component of carcinoma ex pleomorphic adenoma and a single case has been reported arising in (or in association with) a polymorphous low-grade adenocarcinoma of the palate. 2 The diagnostic clue in most cases is an intraductal component, comprising proliferating ductal cells with varying degrees of nuclear pleomorphism arranged in architectural patterns including solid, “Roman bridge”, papillary and cribriform structures and there is often central comedonecrosis.

Although purely in situ SDC was not recognized as an entity by the 2005 WHO classification, occasional cases have been described, 3-7 characterized by a pure intraductal proliferation of tumour cells, similar to intraductal carcinoma of the breast. 8 The rarity is not surprising, as SDC usually presents at an advanced and
invasive stage and, furthermore, there is no system for early detection analogous to mammographic screening programmes for breast cancer. Similarly, little is known about its treatment.

The WHO classification does include a possibly related entity, low-grade cribriform cystadenocarcinoma (so-called low-grade salivary duct carcinoma), defined as "a rare, cystic, proliferative carcinoma that

<table>
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<th>Table 1. Immunohistochemical findings</th>
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<tr>
<td><strong>Marker</strong></td>
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<td>HER2/neu</td>
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<td>Androgen receptors</td>
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<td>Oestrogen receptors</td>
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<td>Progesterone receptors</td>
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<td>p63</td>
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<td>CK5/6</td>
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<td>CK14</td>
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<td>MIB1</td>
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<td>p53</td>
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<td>Bcl-2</td>
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<tr>
<td>EGFR</td>
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<tr>
<td>Prostate-specific antigen</td>
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<tr>
<td>Prostatic acid phosphatase</td>
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<td>AMACR (racemase)</td>
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HER2 scoring as in HercepTest. Hormone receptors scored as in Harvey et al.10 (percentage of positive nuclei + intensity = total score). For other markers: 0, no significant reactivity; F+, definite reactivity, but in <10% of cells; +, 10–33% cells reactive; ++, 34–66% cells reactive; ++++, 67–100% of cells reactive. The figures are approximate and where the percentage of immunopositive cells overlaps two categories, the intensity of reaction is considered.

ND, Not done; CK, cytokeratin; EMA, epithelial membrane antigen; CEA, carcinoembryonic antigen; GCDFP, gross cystic disease fluid protein; SMMHC, smooth muscle myosin heavy chain; EGFR, epidermal growth factor receptor.

*Basal-myoepithelial cells positive.

resembles the spectrum of breast lesions from atypical ductal hyperplasia to micropapillary and cribriform low-grade ductal carcinoma in situ. However, its relationship to SDC is not clarified.

The aim of this study was to describe three further cases of SDC in situ, so as better to define the entity, to suggest any subtypes and to discuss its relationship to low-grade cribriform cystadenocarcinoma.

Materials and methods

The surgical pathology archives and consultation files of the Royal Devon and Exeter, the West Middlesex and Royal Surrey Hospitals contained paraffin-embedded tissue of approximately 50 tumours that had the microscopic features of SDC. Of these, three showed no evidence of invasion. Sections were cut and prepared in the conventional manner, and stained with haematxylin and eosin and by other methods.

A panel of commercially available antibodies was applied to sections using a Bond-max staining machine and the Bond Polymer Refine Detection technique. Appropriate positive and negative controls were employed. The sources and dilutions of the antibodies are listed in Table 1. Scoring for hormone receptors was performed using the quick (Allred) score. Immunohistochemistry with the rabbit monoclonal antibody for HER2 protein was performed using automated Ventana HER2 4b5 protein (Ventana Medical Systems, Tucson, AZ, USA). HER2 immunohistochemistry was scored according to the modified HercepTest, as set out in the recommendations of the American Society of Clinical Oncologists and the College of American Pathologists: 3+, >30% of cells showed strong and complete cytoplasmic membranous immunoreactivity, 2+, incomplete or in <30% of cells, and 0/1+, no or weak reactivity. Although the scoring is for use in invasive breast carcinoma, the same method was used for the purposes of this study.

Automated silver in situ hybridization (SISH) (Ventana Inform HER2 DNA probe, Ventana Medical Systems, Tucson, Arizona, USA) was performed on paraffin-embedded 5-µm thick sections. Probes for HER2 and Chr17 centromere were applied following the manufacturer’s instructions. Scoring was performed on approximately 30 nuclei. Cases were assessed as positive in the presence of >6 HER2 gene copy number and or small/large clusters.

Results

Clinical history

The clinical data are summarized in Table 2. Two of the three patients were women and the age range was 66–86 years. All tumours were in the parotid glands; in cases 1 and 3 the mass was in the superficial lobe and in case 2 it was in the deep lobe. Patient 1 reported some intermittent pain, but there were no other relevant clinical features. None of the patients had had any other primary malignancy at another site.

Treatment and follow-up

Patients 1 and 3 were treated by superficial parotidectomy and the other by a nerve-sparing deep lobe parotidectomy. Radiotherapy was given in cases 1 and 3, but not in case 2. All patients are alive with no evidence of recurrence 4 years, 14 months and >8 years later.

Pathological findings

In each case, macroscopic examination revealed a partly cystic mass 15, 22 and 20 mm in largest dimension, respectively; all were well circumscribed and there was no macroscopic evidence of invasion. All three specimens (including tumour and uninvolved parotid) were completely sampled into 10, nine and six cassettes respectively.

Each of the lesions comprised a single mass; that in case 2 was a well-circumscribed, approximately spherical cyst, 22 mm in diameter, surrounded by a fibrous wall within which were atypical ducts and there was

Table 2. Clinical findings

<table>
<thead>
<tr>
<th>Case 1</th>
<th>M 66</th>
<th>L parotid</th>
<th>15 x 10 x 8</th>
<th>Cystic fluctuating mass; pain at times, 12 months</th>
<th>NED 4 years</th>
</tr>
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<tbody>
<tr>
<td>Case 2</td>
<td>F 70</td>
<td>R parotid</td>
<td>22 diameter</td>
<td>Mass 6 months</td>
<td>NED 1 year 2 months</td>
</tr>
<tr>
<td>Case 3</td>
<td>F 86</td>
<td>R parotid</td>
<td>20 x 17 x 10</td>
<td>Hard mass, “present many years”</td>
<td>NED 8 years 8 months</td>
</tr>
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NED, No evidence of disease.
clear-cut separation from the salivary gland tissue. The other two lesions were partly circumscribed, but more multifocal, with some intervening normal salivary acini (Figure 1). They comprised cysts up to 10 mm in diameter, but also smaller ducts. In each case, the lumina contained a mixture of periodic acid–Schiff after diastase digestion (PASD)-positive epithelial mucus and necrotic debris; this was scanty in case 1, but there were foci of true comedo-carcinoma in cases 2 and 3 (Figure 2A). Calcification was prominent in case 3, more patchy in case 2, but absent in case 1.

The spaces were lined throughout by atypical cells varying in thickness from one to several layers of cells. In some of the smaller ducts the proliferation filled the whole lumen, but in the larger cysts there was a variety of architectural patterns, including “Roman bridges, papillary and cribriform structures (Figure 2B). In all three tumours, the lining cells had variably sized nuclei, often with prominent central nucleoli (Figure 3A). The nuclear pleomorphism was more pronounced in case 2, but was also significant in cases 1 and 3. The cytoplasm was generally plentiful and eosinophilic, occasionally vacuolated and some cells displayed apocrine snouts; these were particularly prominent in cases 1 and 3, but less frequent in case 2. A few mitotic figures were noted in all tumours. All three tumours showed extensive luminal PASD+ mucus, and some cells in each case contained intracytoplasmic mucus also, but true goblet cells were not seen.

Throughout all three lesions, the lining cells of both cysts and smaller ducts were surrounded by a mantle of small-cells, which were usually flat and inconspicuous. No evidence of invasion was seen anywhere, but foci of cancerization of acini were present in

Figure 1. Multiple cystic spaces with normal salivary tissue in between (case 1).

Figure 2. Different growth patterns, including A, comedocarcinoma (case 2), and B, “Roman bridges” and papillary structures (case 3).
case 3 (Figure 4). There was no evidence of a pleomorphic adenoma or other neoplasm in any of the three specimens. Surgical margins were involved in case 3, but not in cases 1 or 2, although the margin was only 0.2 mm in the latter.

**IMMUNOHISTOCHEMISTRY**

The findings are summarized in Table 2. The results are expressed semiquantitatively, based on the proportion of immunoreactive cells and the intensity of reactivity. The atypical ductal cells in all three tumours showed strong reactivity of all cells for cytokeratin (CK) 7 and CK19; CK20 was negative, except for occasional cells in case 3. Epithelial membrane antigen showed strong positivity of cell membranes and cytoplasm, and gross cystic disease fluid protein 15 (BRST-2) was expressed in all cases (Figure 5A), although in only about 5–10% of cells in case 2. A nuclear reaction for androgen receptor (AR) was seen in all cases, but whereas almost every nucleus in cases 1 and 3 was intensely positive (Figure 5B), only about 10–20% of them in case 2 showed moderate reactivity; no nuclear expression of oestrogen or progesterone receptors was identified in any of the tumours. Staining for HER2 rabbit monoclonal 4b5 showed strong and continuous cytoplasmic membranous immunoreactivity in the tumour cells (i.e. 3+) of case 2 only (Figure 6A). The MIB1 index averaged 13–30%, but in each case there was considerable variation from area to area (Figure 3B). Apart from a rare cell in case 2, there was no reaction for S100 protein and none of the epithelial cells expressed any prostatic, basal or myoepithelial cell markers. In contrast, every cyst and duct in all three tumours was

**Figure 3.** Epithelial cells showing high-grade nuclear atypia and increased proliferation, surrounded by flattened myoepithelial cells. A, H&E (case 3). B, MIB1 (case 2).

**Figure 4.** Cancerization of acini (case 3).
Figure 5. Markers typically expressed by high-grade salivary duct carcinoma. A, Androgen receptors (case 1), and B, gross cystic disease fluid protein-15 (case 3).

Figure 6. HER2/neu. A, Immunohistochemical expression of the protein (case 2), and B, silver in situ hybridization showing a combination of dots and clusters in the nuclei of the tumour cells indicating a high level of amplification of the HER-2 gene (case 2).
surrounded by a layer of cells expressing CK5/6 and CK14 along with p63 and smooth muscle myosin heavy chain (Figure 7A,B). In all three cases, most of these cells showed weak to moderate reactivity for epidermal growth factor receptor (EGFR), but no reaction was seen in the neoplastic ductal cells.

**IN SITU HYBRIDIZATION FOR HER2/neu**

The HER2 status of salivary duct carcinoma in situ (SDCIS) in cases 1 and 3 was scored as “no amplification” of the gene, as there were only two to three HER2 signals in the nuclei of the tumour cells. By contrast, in case 2 a mixture of large clusters and dots of HER2 gene were present in almost all of the malignant nuclei. The mean copy number of HER2 gene was 18.3, classifying case 2 as displaying a high level of amplification (Figure 6B). SISH chromosome 17 centromere probe revealed two to three signals in all three cases.

**Discussion**

Because the diagnosis of invasive SDC implies a poor prognosis and the need for aggressive therapy, the identification of pure SDCIS carries significant clinical implications. The diagnosis requires strict criteria, such as the absence of metastases or local invasion, the latter determined by the presence of an intact myoepithelial layer around all tumour islands, ideally confirmed by immunohistochemistry. The exclusion of any invasive component is largely dependent on adequate sampling of the whole tumour.

All three cases in the present study were well sampled and no evidence of invasion was identified. Therefore, they satisfy the diagnostic criteria for pure SDCIS. All were composed of ducts lined by a proliferation of cells with architectural patterns of Roman bridges, papillary and cribriform structures and there was focal comedonecrosis in cases 2 and 3. By using the criteria of ductal carcinoma in situ (DCIS) of the breast, there were nuclei in all three lesions atypical enough to be considered as high grade, although there were also intermediate-grade nuclei in cases 1 and 3.

In major salivary glands, the only previously published cases apparently identical to the present ones were three reported by Anderson et al. They described two women and a man, each of whom had a parotid tumour composed of “numerous smoothly contoured ducts of varying size containing rigid cribriform proliferations of malignant epithelial cells”. These were confined to ducts surrounded by a rim of actin-positive myoepithelial cells. The epithelial cells were described
as moderately or markedly atypical and in one case comedonecrosis was noted.

In minor salivary glands, a few purely in situ high-grade SDCs have been described: Cheuk et al. reported a lesion of the buccal mucosa composed of intermediate-grade epithelial cells surrounded by a rim of actin and p63+ myoepithelial cells. Unusually for SDC, many cells were S100+ and the Ki67 index was only 5%. A palatal lesion described by Tatemoto et al. was a non-invasive proliferation of epithelial cells with atypical nuclei. Two lesions in the tongue could have been further examples, but no myoepithelial cell rim was described and no immunohistochemical findings were reported. An example of Paget’s disease of the oral mucosa associated with a largely in situ carcinoma of minor salivary glands was probably similar, but it also had an area of invasion at the time of diagnosis.

The morphology of SDCIS as seen in the current cases is identical to the in situ lesions seen in examples of invasive SDC, in which, according to the WHO fascicle, the “ductal lesion” comprises pleomorphic, epithelioid tumour cells with a cribriform growth pattern, “Roman bridge” formation, and intraductal comedonecrosis. The immunohistochemical pattern in the in situ and invasive components is usually identical, and it is therefore reasonable to suppose that pure SDCIS is a precursor lesion of invasive SDC. As the WHO definition of SDC notes the morphological similarity to intraductal and infiltrating breast cancer, it is also reasonable to suppose that they could be analogous in other ways, if not quite identical. In particular, SDCIS shares a range of architectural characteristics with DCIS of the breast, including features such as cancerization of acini (described here for the first time) and Paget’s disease.

Mammary DCIS has been divided into two main groups (luminal and non-luminal cells), based on various markers, in particular nuclear immunoreactivity of receptors for progesterone (PR) and oestrogen (ER)—the latter is mainly the α isoform, with a subset of cancers expressing the β isoform. The luminal cell group were mostly of low or intermediate nuclear grade (75%) and typically ER+, PR+, Bcl-2+, HER2– and p53–. In contrast, the non-luminal cell group were mostly high nuclear grade (80%) and typically ER–, PR–, Bcl-2–, HER2+ and p53+. Both groups were negative for S100 protein. In addition, a third form of DCIS with a basal phenotype has been described; it usually expresses basal CKs (CK5/6, CK14 and CK17), EGFR and P-cadherin, but not ER, PR or HER2 protein—i.e. triple negative.

As so few cases of SDCIS have been described, its biology is not as well known as that of breast DCIS. In particular, it is more difficult to classify SDCIS as either luminal, non-luminal or basal phenotype, because ER α isoform (ERα) staining is exceptional in both in situ and invasive SDC. However, several studies have demonstrated AR expression in invasive SDC; two further large series found that 83 and 67% of cases were positive. We therefore speculate that AR expression in SDC is analogous to ERα reactivity in breast carcinoma. In addition, in the more recent of these series, Williams et al. showed that 73% of SDCs expressed AR β isoform (ERβ), a receptor in which androgens participate in its regulation – it is of interest that androgens participate in the regulation of this receptor. On the basis of these and other markers, the authors suggested that there are several subsets of SDC, for which different therapies could be targeted. Tumours negative for both AR and ERβ were more aggressive than SDCs that expressed one or both of these markers. Similarly, carcinomas which were HER2 protein 3+ had a worse outcome than those which were HER2 protein 0–2+. Skálová et al. have shown in an immunohistochemical study of several different HER2 protein antibodies, together with fluorescence in situ hybridization (FISH) gene analysis, that protein overexpression is usually, but not always (even when 3+) associated with gene amplification. In the present study, gene analysis was performed using SISH, a modified chromogenic in situ hybridization, which in breast cancer has been shown to be as sensitive and as specific as FISH.

Therefore, we suggest that AR expression may be used as a marker of the luminal phenotype in invasive SDC, which can be classified into luminal, HER2 and basal-like phenotypes. This concept could also be applied to in situ SDC and evidence of the luminal phenotype was found in our cases 1 and 3, in which there was strong immunopositivity for AR and CK19, together with no HER2 protein overexpression or gene amplification. Our case 2 could represent an example of the non-luminal HER2 phenotype, as there was AR expression in only 10–20% of nuclei, but strong (3+) overexpression of HER2 protein and a high level of amplification of the HER2 gene. None of our cases showed any tumour cell reactivity for basal phenotype markers such as EGFR, p63, CK14 or CK5/6. This is not surprising, as the basal phenotype is rare even in breast DCIS.

As so few cases of purely in situ SDC have been described up to now, only a few conclusions can be drawn. The epithelial cells in the cases of Tatemoto et al. and Cheuk et al. were negative for p53 and HER2 proteins, and also for p63 in the latter report. This may indicate a luminal phenotype, but the AR
status was not noted. The case of SDCIS and Paget’s disease was negative for a basal CK cocktail.

Architecturally similar to SDCIS is the entity of low-grade cribriform cystadenocarcinoma (LGCCC). This has been described in two large series as low-grade SDC, and it arises in major glands. It is usually composed of solid, cribriform and cystic islands of cells with anastomosing micropapillae within the cystic spaces and occasional “Roman bridges”, but no comedonecrosis. In most cases, the cells are bland with low-grade nuclei showing little atypia or mitotic activity, and in most cases the islands are surrounded by a rim of myoepithelial cells. In the second of these series, focal limited invasion was seen in four tumours, characterized by small solid islands of carcinoma present in the surrounding stroma. Two of them also demonstrated transition from low-grade to high-grade, oncocytoid cytology, with scattered mitotic figures and focal necrosis. The immunohistochemical profile differed from the tumours in the present study in that LGCCC was strongly positive for S100. Nine cases were negative for HER2 protein, and one case was stained for ARs and was negative, as were two subsequent LGCCCs in the personal experience of one of us (R.H.W.S.).

A further series of three tumours reported by Weinreb et al. in 2006 has raised very interesting questions. Each was an intraductal neoplasm surrounded by a continuous myoepithelial layer and these were considered to be examples of LGCCC. There was more nuclear atypia than is usual in LGCCC and all three tumours expressed AR and BRST-2. In addition, two were S100+; one tumour later became invasive. CK14, p63 and HER2 protein were negative. The authors were of the opinion that LGCCC shares architectural and cytological features with conventional SDC and may show progression to intermediate or high-grade cytological features. They concluded that LGCCC has invasive potential and may transform to high-grade carcinoma and recommended that the terms low-grade SDC and LGCCC should be changed to low-grade intraductal carcinoma of salivary gland. However, from their illustrations it is not entirely clear whether some or all were examples of LGCCC, intermediate/high-grade SDCIS, or represented an overlap.

Our view is that at this stage, it is not clear what the relationship of LGCCC is to SDCIS. It could be a separate entity based on significant immunohistochemical differences (i.e. it is S100+, AR− and HER2−), but equally, it might well represent the extreme low-grade end of the spectrum of salivary DCIS, in which AR development is not yet apparent. In favour of the latter is the overlap of architectural patterns with SDCIS, together with the occasional case showing progression to higher grade cytology. We do not feel we have sufficient evidence to answer this question either way, but hope that this will be elucidated by further studies.

The outcome for pure in situ SDC should be good, provided it is completely excised, but two of the cases of Anderson et al. recurred after subtotal parotidectomy, presumably due to multifocal disease in the remaining parotid. Consequently, they advocated total parotidectomy with sparing of the facial nerve. So far, our patients have done well after more limited surgery, with no evidence of recurrence after 1, 4 and 8 years.

In summary, we believe that SDCIS is a precursor lesion for invasive SDC. There is evidence that there are at least two subsets of SDCIS (luminal and non-luminal cell and HER2), which may have different degrees of aggressiveness, as recently shown in their invasive counterparts. A third subtype with a basal phenotype probably exists also, but has not yet been described.

Acknowledgements

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References


