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The adhesion molecules NCAM, HCAM, PECAM-1 and ICAM-1 in normal salivary gland tissues and salivary gland malignancies

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BACKGROUND: Some malignant salivary gland tumors are known for their propensity to exhibit perineural invasion and vascular metastases. It was hypothesized that alterations in the expression of cell adhesion molecules are involved in these processes.

METHODS: The expression and distribution of neural cell adhesion molecule (NCAM), HCAM (CD44), platelet-endothelial cell adhesion molecule-1 (PECAM-1), and intercellular cell adhesion molecule-1 (ICAM-1) in normal salivary gland tissues and selected salivary gland malignancies, especially adenoid cystic carcinoma (AdCyCa) and polymorphous low-grade adenocarcinoma (PMLG), were determined immunohistochemically, and their influence on histologically demonstrated perineural invasion, vascular invasion, and tumor recurrence/patient death were investigated.

RESULTS: NCAM, HCAM, and ICAM-1 were often found to be expressed by neoplastic cells, but no correlation to perineural invasion, tumor behavior, or patient prognosis was found. PECAM-1 was rarely and only focally expressed in three tumors, all of which were related to tumor metastases and patient death.

CONCLUSIONS: Immunohistochemical demonstration of NCAM, HCAM, and ICAM-1 is not related to perineural invasion or tumor behavior. PECAM-1 expression was related to vascular invasion and poor patient prognosis in three cases.

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Introduction

Adenoid cystic carcinoma (AdCyCa) and polymorphous low-grade adenocarcinoma (PMLG) of salivary glands are known to exhibit a propensity for perineural invasion (1, 2). These and unclassified salivary gland adenocarcinomas (AdenoCa) may exhibit vascular invasion. As these behavioral characteristics are reported to influence prognosis for affected patients (3), research on their pathogeneses is important if appropriate therapeutic strategies are to be developed. Perineural and vascular invasion may be related to alteration in the expression of cell adhesion molecules on tumor cells (4, 5). Our study tested four hypotheses:

1. That neural cell adhesion molecule (NCAM; human CD56 antigen), normally expressed by peripheral nerve sheath cells (6), is expressed by neoplastic cells in AdCyCa, PMLG, and AdenoCa, and is involved in homophilic binding in maintaining established perineural invasion. Toth et al. (7) showed that neoplastic myoepithelial cells in AdCyCa and PMLG have the ability to express the nerve sheath/neuronal molecules S100 protein, glial fibrillary acidic protein, and neuron-specific enolase, not normally seen in normal myoepithlial cells, indicating differentiation towards Schwann/perineural cells. Schwann/perineural cell adhesion molecules may therefore be involved in the process of perineural invasion.
2. That HCAM (human CD44 antigen), which is expressed on Schwann cells and other epithelial cells (8) exhibits increased expression or aberrant expression, facilitating perineural invasion.
3. That platelet-endothelial cell adhesion molecule-1 (PECAM-1; human CD31 antigen) (9) is expressed by neoplastic cells in salivary gland malignancies and is involved in homophilic attachment of tumor cells to endothelial cells, facilitating vascular and lymphatic metastases.
4. That intercellular adhesion molecule-1 (ICAM-1; human CD54 antigen) found on endothelial and other epithelial cells (8) exhibits increased or altered expression, facilitating vascular invasion.

Materials and methods

Antibody selection was limited to those capable of reacting with formalin-fixed, paraffin-embedded human archival tissues from pathology diagnostic services.

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The following tissues were tested:

1. Normal salivary gland tissues: 30 cases – 17 from major glands (parotid/submandibular), 13 from minor glands (intraoral).
2. Pleomorphic adenomas (PleoAd): 5 cases.
3. Malignant salivary gland tumors: 53 cases – 22 AdCyCa, 13 PMLG, 8 AdenoCa, 6 acinic cell carcinomas (ACC), and 4 mucoepidermoid carcinomas (Mucoep).

Glass slide-mounted 5-μm tissue sections of formalin-fixed paraffin-embedded experimental cases were cut, deparaffinized, and rehydrated using standard techniques. Endogenous peroxidase was quenched with 3% hydrogen peroxide in methanol, and sections were rinsed in phosphate buffered saline (PBS). Sections were incubated with mouse anti-human monoclonal antibodies to NCAM (1 : 100; Novocastra Laboratories Ltd., Newcastle upon Tyne, UK), mouse anti-human monoclonal antibodies to HCAM (1 : 400; isotype unspecified; PharMingen International, San Diego, CA, USA), mouse anti-human monoclonal antibodies to PECAM-1 (1 : 100; Novocastra Laboratories Ltd., Newcastle upon Tyne, UK), and mouse anti-human antibodies to ICAM-1 (1 : 100; Chemicon International Inc., Temecula, CA, USA) for 1 h following a PBS rinse and blocking with 10% horse serum for 30 min. NCAM, HCAM, and PECAM-1 were subjected to antigen unmasking by boiling in citrate buffer (pH 6.0) for 20 min in a microwave oven, before antibody incubation. Positive control tissues were neuroblastoma for NCAM, tonsil for HCAM, breast carcinoma for PECAM-1, and tonsil for ICAM-1. Sections were then incubated with biotinylated horse anti-mouse antibody (1 : 200) followed by avidin-biotin-PAP (peroxidase-antiperoxidase) complex (1 : 50; Vector Laboratories (Canada) Ltd., Burlington, Ont., Canada), each for 30 min. Staining was visualized with 3,3-diaminobenzene before sections were counterstained with hematoxylin and cover slipped. Negative controls were carried out at each experiment by omitting the primary antibody. The monoclonal antibodies for NCAM and PECAM-1 were the same isotype, mouse IgG1, and exhibited different staining specificity in the same tissues, ruling out non-specific IgG1 tissue binding. The monoclonal antibodies for HCAM and ICAM-1 were the same isotype, mouse IgG2b, and exhibited different staining specificity in the same tissues, ruling out non-specific IgG2b tissue binding. The specific reactivity of NCAM has been reported by Watanabe et al. (10), of HCAM by Haynes et al. (11), of PECAM-1 by Kua et al. (12), and of ICAM-1 by Dustin et al. (13).

Stained sections were examined by light microscopy after confirmation of appropriate positive and negative controls. Tumors were graded on a standard scale from 0 to 4 as follows: 0, no staining; 1, focal weak positivity; 2, weak patchy or focal strong staining; 3, weak generalized of strong patchy staining; 4, generalized strong staining.

Staining pattern (cell surface and/or cytoplasmic) and cell type (ductal, myoepithelial) were noted. Nuclear staining was not considered positive. The relation of positively stained neoplastic cells to peripheral nerves and blood vessels was determined.

Demographic, treatment, and follow-up data on all patients with malignant salivary gland tumors were recorded. For AdCyCa, PMLG, and AdenoCa, statistical analysis comparing presence of perineural invasion versus recurrence/death from disease was carried out by Fisher's exact test.

For AdCyCa and PMLG, the Mann–Whitney test for non-parametric data was used to determine if there was a significant difference in immunohistochemical staining scores between tumors with or without perineural invasion, and immunohistochemical staining scores between tumors with or without recurrence. Data were considered statistically significant at $P < 0.05$.

Results

Recurrence and survival data

Table 1 lists a summary of the demographic, perineural invasion status (as determined by histologic examination), treatment, and follow-up data on the normal and neoplastic cases. Statistical analysis failed to show a difference in recurrence rate with or without the presence of histologic perineural invasion for AdCyCa ($P = 1.000$), PMLG ($P = 1.000$), or AdenoCa ($P = 0.4286$). Neither was there a significant difference in death of the patient caused by the tumor with or without the presence of histologic perineural invasion for the same three tumor types ($P = 0.6550$, $P = 0.4615$, and $P = 0.4286$, respectively). Nor was there a statistical difference for recurrence or death from disease with respect to initial treatment modality for these three tumor types. Staging data were not available and not included in these analyses.

Table 1 Demographic, treatment and follow-up data

		Demographics				*Treatment*				*Follow-up*				
Tissue type	*n*	*Gender (m/f)*	*Mean age*	*Site (maj/min)*	*pni*	*surg*	*rad*	*s + r*	*pall*	*no rec*	*rec*	*dod*	*doc*	*u*
Normal	30	13/17	52.7	17/13	–	–	–	–	–	–	–	–	–	–
PleoAd	5	3/2	52.0	2/3	–	5	–	–	–	–	–	–	–	–
AdCyCa	22	8/14	61.3	7/15	9	9	4	7	1	10	9	6	3	3
PMLG	13	5/8	56.8	0/13	6	11	1	1	0	10	2	1	1	1
AdenoCa	8	6/2	68.8	5/3	1	3	0	5	0	3	4	4	1	1
ACC	6	3/3	58.3	6/0	0	1	0	5	0	5	0	0	1	1
Mucoep	4	2/2	61.0	4/0	2	1	0	3	0	2	1	1	3	1

n, sample size; m, male; f, female; maj, major glands; min, minor glands; pni, perineural invasion; surg, surgery; rad, radiation therapy; s + r, surgery followed by therapeutic radiation; pall, palliative care; rec, recurrence; dod, died of disease; doc, died of other causes; u, lost to follow-up.

Immunohistochemical data

Normal tissues

Only minor differences in staining pattern or intensity between major and minor salivary glands were noted.

1. NCAM: Staining was positive in peripheral nerves found in these tissues. Staining of salivary gland parenchyma was absent (Fig. 1A), with the exception of weak focal to patchy cytoplasmic staining of intercalated duct cells in 20 of the cases.
2. HCAM: All 30 cases were positively stained. Most cases exhibited strong patchy to generalized cell membrane staining of acinar cells (but never on the luminal surface), large intercalated duct cells, excretory duct cells (with additional faint cytoplasmic staining in the basalar cytoplasm), and the inner surface of myoepithelial cells. Small intercalated duct cells were usually negative, but some exhibited faint basalar cytoplasmic staining. (Fig. 1B).
3. PECAM-1: All blood vessels in the tissues examined showed strong positive staining of endothelial cells (Fig. 1C). Weak to focally moderate, patchy cytoplasmic staining was observed in intercalated duct cells and sometimes excretory duct cells in 25 of the 30 tumors.
4. ICAM-1: All cases exhibited diffuse staining of the cytoplasm of ductal cells, ranging from a weak patchy to a strong generalized pattern (Fig. 1D). Serous acinar cells sometimes displayed faint diffuse or localized cytoplasmic staining, but no staining was detected in mucous acinar cells or myoepithelial cells.

Pleomorphic adenomas: five cases

1. NCAM: There was no staining in any of the five cases.
2. HCAM: All cases showed cell membrane staining affecting primarily neoplastic myoepithelial cells, ranging from weak to strong generalized staining, and sometimes weak focal to weak patchy staining of ductal cells. Luminal surfaces were never stained.
3. PECAM-1: There was weak focal cytoplasmic staining of ductal cells in one case only.
4. ICAM-1: There was strong patchy staining of ductal cell cytoplasm in all cases. Neoplastic myoepithelial cells exhibited weak focal to weak patchy staining in the five tumors, but these cells were unstained in most areas.

Salivary gland malignancies

Adenoid cystic carcinoma: 22 cases

1. NCAM: Patchy weak to moderate cytoplasmic and focal membrane staining of basaloid (myoepithelial) cells was present in 7 of the 22 cases (31.4%). Of these seven, three had exhibited perineural invasion histologically, and

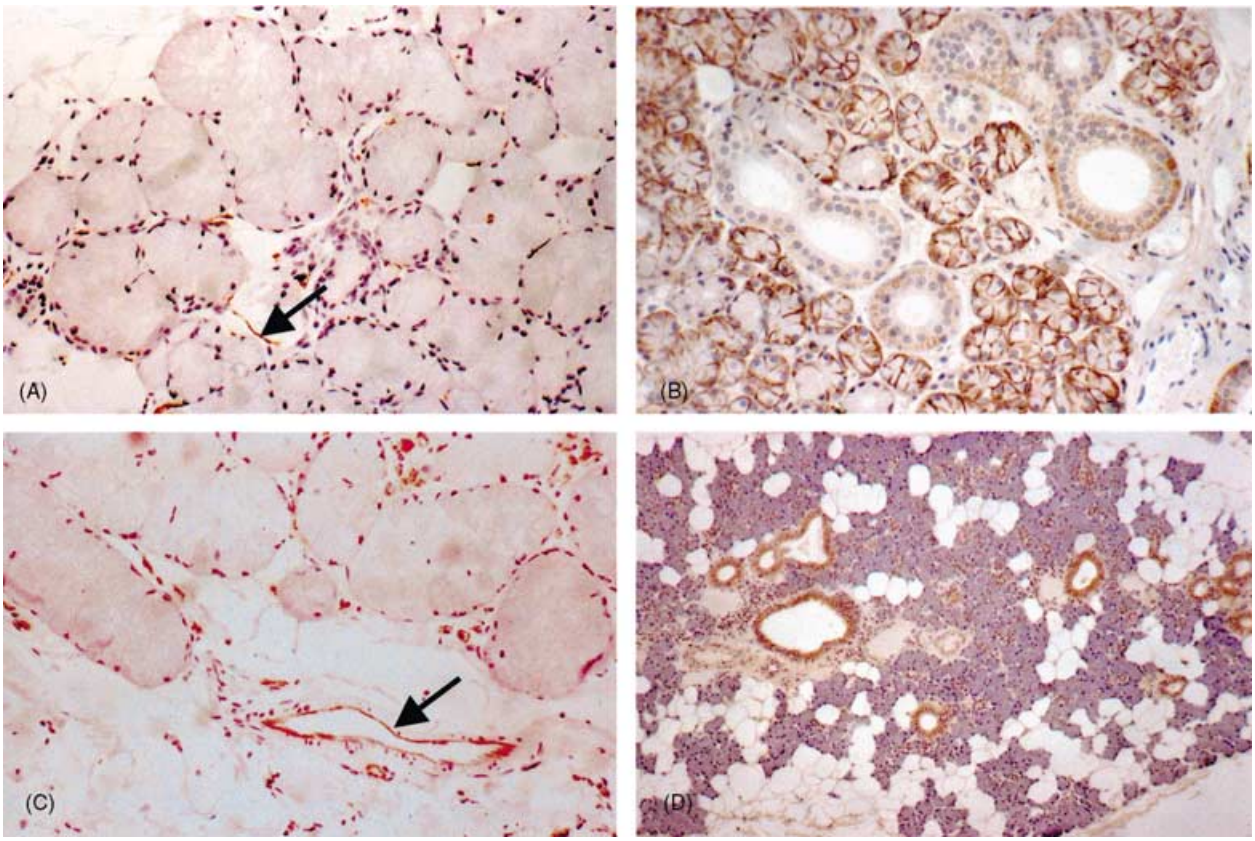

Figure 1 Normal salivary gland. (A) NCAM stained peripheral nerves (arrow), but showed only focal to patchy weak staining of intercalated duct cells (anti-NCAM/hematoxylin, magnification ×200). (B) HCAM intensely stained cell membranes of acinar cells (with the exception of luminal surfaces), myoepithelial cells, and basal layer cells, but did not or weakly stained duct luminal cells (anti-HCAM/hematoxylin, magnification ×200). (C) PECAM-1 stained endothelial cells (arrow) (anti-PECAM-1/hematoxylin, magnification ×200). (D) ICAM-1 preferentially stained duct cells (anti-ICAM-1/hematoxylin, magnification ×120).

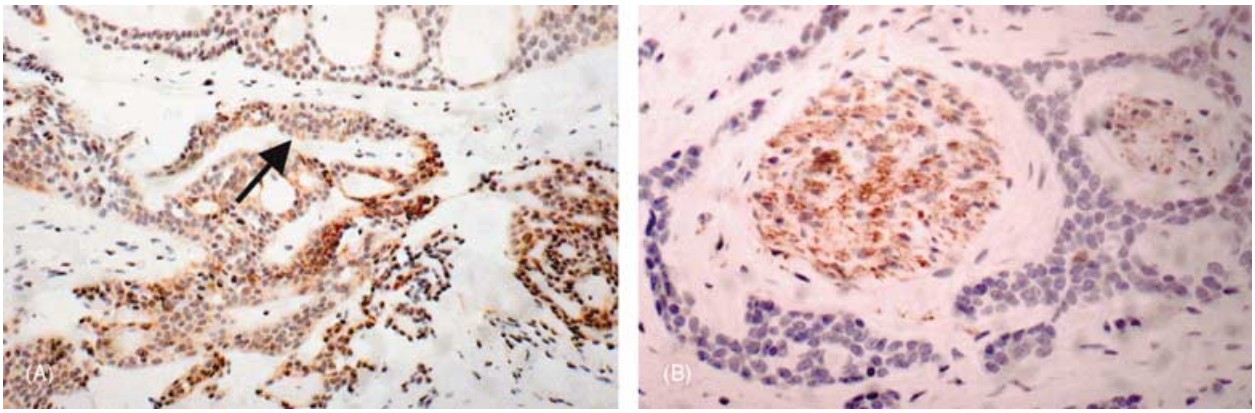

Figure 2 NCAM. (A) This PMLG exhibits typical cytoplasmic and cell membrane (arrow) staining of patches of tumor cells. A similar pattern was present in AdCyCa tumors that showed positive staining (anti-NCAM/hematoxylin, magnification ×200). (B) Areas of perineural invasion showed positive staining of peripheral nerves, but no staining of tumor cells, as illustrated in this AdCyCa (anti-NCAM/hematoxylin, magnification ×500).

in no cases were positively stained tumor cells found adjacent to the positively staining nerves (Fig. 2B). There was no significant difference in staining intensity between tumors with or without perineural invasion ($P = 0.9725$), and with or without recurrence ($P = 0.2393$).

2. HCAM: All 22 cases (100%) exhibited patchy to generalized, moderate to strong staining, cytoplasmic but more intensely cell membrane, usually of the myoepithelial component (Fig. 3A) but occasionally ductal cells as well. Cells abutting and within peripheral nerves stained intensely, while the nerve fibers were not stained. There was no significant difference in staining intensity between tumors with or without perineural invasion ($P = 0.9191$) and with or without recurrence ($P = 0.1993$).
3. PECAM-1: One case of the 22 (4.5%) showed focal weak cell membrane staining. Endothelial cells stained with uniform intensity. The one case did not show PECAM-1 positivity in an area of intravascular invasion. No statistical analysis was carried out because of the small number of positive cases.
4. ICAM-1: All 22 cases (100%) showed moderate to strong, patchy to generalized cytoplasmic staining, more commonly and intensely in ductal cells than myoepithelial cells (Fig. 5). There was no statistical difference in staining intensity between tumors with or without perineural invasion ($P = 0.8371$) and with or without recurrence ($P = 0.9001$).

Polymorphous low-grade adenocarcinoma: 13 cases

1. NCAM: Eleven of the 13 stained cases (84.6%) showed patchy cytoplasmic and/or cell membrane staining usually of myoepithelial cells but including some ductal cells (Fig. 2A). Five of six cases with perineural invasion were positively stained. In no case were tumor cells adjacent to nerves found to be positive. There was no statistical difference between tumor with or without perineural invasion ($P = 0.9999$).
2. HCAM: All 13 cases (100%) showed strong patchy to generalized cytoplasmic and especially membranous staining, on most myoepithelial cells and some ductal cells. Cells around and within peripheral nerves were strongly stained, although nerve tissue was not (Fig. 3B). There

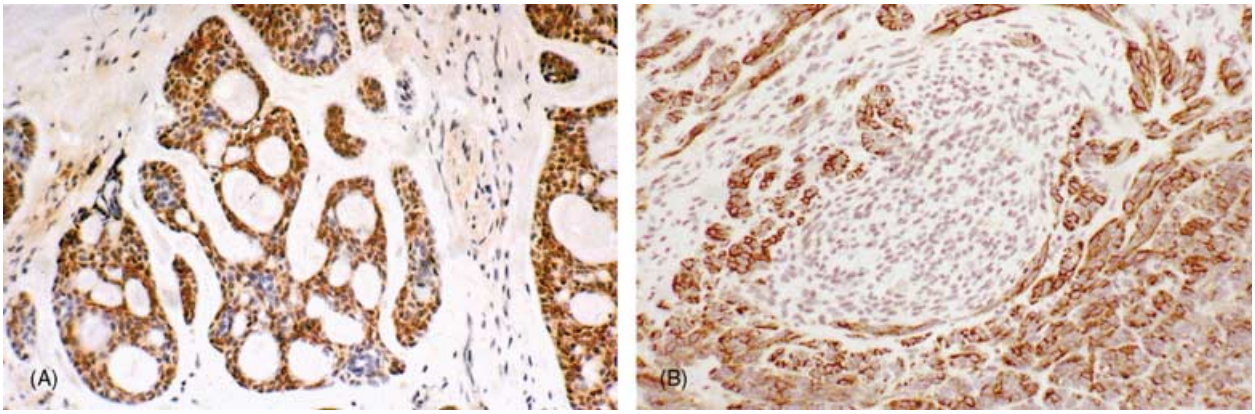

Figure 3 HCAM. (A) Preferential staining of neoplastic myoepithelial cells with sparing of ductal cells is illustrated in this photomicrograph. This was a common but not universal pattern in tumors that showed both ductal and myoepithelial differentiation (anti-HCAM/hematoxylin, magnification ×200). (B) This PMLG shows intense staining of myoepithelial cells surrounding a non-stained peripheral nerve (anti-HCAM/hematoxylin, magnification ×200).

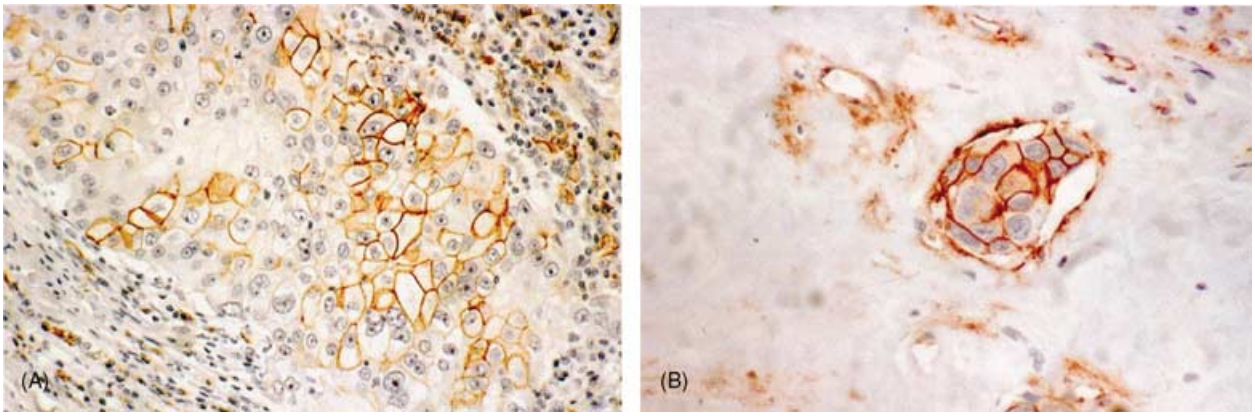

Figure 4 PECAM-1. (A) Rarely, individual cells of focal groups of tumor cells exhibited membrane staining such as those in this poorly differentiated cluster in an AdenoCa. (anti-PECAM-1/hematoxylin, magnification ×320). (B) The same tumor showed a cluster of positively stained cells attached to the interior of a small vessel. The patient developed ocular and brain metastases and died within 2 years of this biopsy (anti-PECAM-1/hematoxylin, magnification ×500).

was no significant difference in staining intensity between tumors with or without perineural invasion ($P = 0.7647$).
3. PECAM-1: No tumor cell staining was found.
4. ICAM-1: Twelve of the 13 cases (92.3%) exhibited strong patchy to generalized cytoplasmic staining, especially of ductal cells. There was no significant difference in staining intensity between tumors with or without perineural invasion ($P = 0.2335$).

Adenocarcinoma: eight cases

1. NCAM: Four of the eight cases exhibited patchy moderate to strong cytoplasmic staining, including both cases with perineural invasion.
2. HCAM: All eight cases exhibited moderate to strong patchy membranous staining.
3. PECAM-1: One case exhibited focal, strong cell membrane positivity (Fig. 4A), which was associated with apparent intravascular invasion (Fig. 4B).
4. ICAM-1: All eight cases exhibited moderate to strong patchy cytoplasmic staining, more prominent in ductal cells.

Acinic cell carcinoma: six cases. Only two cases exhibited weak focal and patchy cytoplasmic/membranous staining for NCAM. Of interest was HCAM, which displayed patchy but strong membranous staining mimicking the staining pattern of normal serous acinar cells. PECAM-1 was uniformly negative while ICAM-1 usually showed weak to moderate generalized cytoplasmic staining. No cases were found to exhibit perineural invasion.

Mucoepidermoid carcinoma: four cases. One case showed weak patchy staining for NCAM. HCAM was found to exhibit weak to strong patchy membranous staining. PECAM-1 was found in a patchy distribution in one case at the site of intravascular invasion. ICAM-1 showed weak generalized to strong patchy cytoplasmic staining.

Discussion

Follow-up data

Surprisingly, our data showed no statistical difference in recurrence and death rate between AdCyCa exhibiting or not exhibiting perineural invasion histologically ($P = 1.000$ and $P = 0.6550$, respectively). Other studies (1, 3, 14) have revealed the same finding, although Fordice et al. (3) found that major nerve involvement was significant. Similarly, we found no statistical difference in recurrence or death rate based on the presence or absence of perineural invasion for PMLG ($P = 1.000$, $P = 0.4615$, respectively) or AdenoCa ($P = 0.4286$, $P = 0.4286$, respectively). Neither did we find a significant difference in treatment modalities with respect to recurrence and survival rates. Surgery remains the primary treatment of choice for salivary gland malignancies, but the need for adjunctive radiation therapy is not well defined. In this study, in which follow-up data were available for 19 cases of AdCyCa, there was no statistical difference in the recurrence ($P = 1.000$) or survival ($P = 0.4909$), with the addition of therapeutic radiation into the patient's treatment. However, staging data were not available and may represent a confounding variable. A similar situation existed for PMLG where surgery is the treatment of choice.

Immunohistochemical staining data

NCAM

Hutcheson et al. (15), Gandour-Edwards et al. (16), and Franca et al. (17) have investigated the presence of NCAM in AdCyCa. Hutcheson et al. (15) found positive staining of at least 50% of tumor cells in all 37 cases tested (100%). Photomicrographs show cytoplasmic staining, including cells exhibiting perineural invasion. Gandour-Edwards et al. (16) found diffuse positive cell membrane and cytoplasmic staining in 16 of 18 cases studied (89%), including tumor cells exhibiting perineural and intraneural invasion. Franca et al. (17) studied NCAM expression in one case of AdCyCa, including cell culture experiments examining NCAM's relationship to basement membrane proteins. *In situ* immunohistochemistry of their case showed diffuse cytoplasmic staining including cells adjacent to peripheral

nerve tissue. Cultured cells also expressed this protein (17). Our study confirms the presence of cytoplasmic and membrane staining, but we found positive staining in only seven of the 22 cases of AdCyCa (31.8%), and three of the nine cases (33.3%), which exhibited perineural invasion histologically. We found the same cell type to be positive in 11 of 13 PMLG. We agree with Hutcheson et al. (15) that there is no statistical difference in the staining scores for those AdCyCa exhibiting and not exhibiting perineural invasion ($P = 0.9725$). Nor could we find a statistical correlation of tumor recurrence and the expression of NCAM ($P = 0.2393$). These data are in contrast to the findings for squamous cell carcinoma where both Vural et al. (18) and McLaughlin et al. (19) found a positive correlation between NCAM immunostaining and perineural invasion and biological behavior.

Neoplastic myoepithelial cells are the dominant cell type to express NCAM in these malignancies. This appears to be a result of neoplastic transformation, similar to the expression of S100 protein, GFAP, and NSE, as normal myoepithelial cells were not found to stain positively. Our results, and those of others (15–17), support the proposal by Toth et al. (7) that neoplastic myoepithelium may exhibit peripheral nerve sheath cell differentiation. However, we were unable to observe simultaneous expression of NCAM in nerves and in tumor cells exhibiting contact with the nerves, suggesting that the involvement of NCAM is not by homophilic binding in established perineural invasion. Further investigation involving other peripheral nerve sheath proteins in the process of perineural invasion by these tumors is warranted.

HCAM

Fonseca et al. (20) studied the distribution of isotypes of HCAM (CD44) in tissues from 26 normal salivary glands, both immunohistochemically and by electron microscopy. Our observations of strong cell membrane staining in normal salivary gland tissues echoes their results for CD44v3 and CD44v6, involving the basal and lateral walls of serous acinar cells, the inner (juxta-acinar) surface of myoepithelial cells, and the lack of staining of intercalated duct cells. Additionally, we found some staining of mucous acinar cells and excretory duct cells. Xing et al. (21) documented HCAM staining in salivary gland neoplasms including all of 9 pleomorphic adenomas, 7 of 8 AdCyCa, 8 of 10 PMLG, and all of 10 Mucoep. Similarly, we found membranous staining, especially of myoepithelial cells in all 5 of our pleomorphic adenomas, in all 22 of our AdCyCa, all 13 of our PMLG, and all 4 of our Mucoep. Peripheral nerves in tissue sections were uniformly negative for HCAM.

The general presence of HCAM in normal and neoplastic tissues in this study and the decreased staining of neoplastic ductal cells mimicks the staining pattern of normal salivary gland tissue, suggesting that alteration in the expression of this protein does not occur with neoplastic transformation. It was found in tumor cells adjacent to nerve sheath cells, but not in peripheral nerve tissue, indicating that homophilic binding in the process of perineural invasion is unlikely.

PECAM-1

At the time of writing, we were unaware of other published studies on the expression of PECAM-1 in salivary gland tumors and its association to vascular invasion. Horak et al. (22) used PECAM-1 to assess relative vascularity in the biological behavior of breast carcinoma, but we wished to determine if neoplastic cells expressed PECAM-1, which could be involved in homophilic binding to endothelium in the process of vascular metastasis. In all tissues examined, PECAM-1 showed intense staining of endothelium. In only 3 of the 53 malignant tumors (5.7%) was there focal cytoplasmic or cell membrane staining of individual or small focal groups of tumor cells. One case was a 79-year-old female with advanced AdCyCa for which the patient was given only palliative care and died 2 months following diagnosis. The second case was an AdenoCa in which intravascular tumor cell clusters expressing PECAM-1 were observed bound to the inner side of a blood vessel wall (Fig. 4A,B). The patient was a 71-year-old male who was found to have ocular and brain metastases 1 year after combined surgery and radiation treatment, and who died within 2 years. The third case was a Mucoep in a 71-year-old male's parotid gland. Three years after surgery and radiation, he was found to have iliac crest metastases and the patient died within the following year. These cases suggest that PECAM-1 expression by malignant salivary gland tumor cells is rare, can be involved in vascular metastasis, and is associated with a poor prognosis. More cases are needed to confirm these preliminary observations.

ICAM-1

Studies have shown the presence of ICAM-1 in cancer cells of other tissues (4, 5, 23), but we were unable to find previous studies investigating the presence of ICAM-1 in malignant salivary gland neoplasms. We found it to be moderate to intense in 52 of the 53 malignant tumors (98.1%). ICAM-1 exhibited a propensity to stain ductal cells rather than myoepithelial cells, mimicking the staining pattern observed for normal salivary gland tissues. Like HCAM, its general presence and distribution suggests that its expression is not significantly altered by neoplastic transformation. We were unable to find any evidence that altered ICAM-1 expression was related to perineural or vascular invasion.

Bilayer theory of salivary gland neoplasm morphogenesis

Dardick (24) proposed that salivary gland tumors develop morphologically from either luminal cells, abluminal cells, or a combination of both, based in part on his finding of differential staining of cytokeratin 14. Immunohistochemical staining patterns of HCAM and ICAM-1 in neoplasms composed of cells from both layers (AdCyCa, PMLG, PleoAd) support this concept. HCAM showed preferential immunoreactivity in neoplastic myoepithelial cells (outer layer cells; Fig. 3A) while ICAM-1 preferentially stained ductal (luminal) cells (Fig. 5).

Conclusions

NCAM may be expressed by neoplastic modified myoepithelial cells. This expression was not found to correlate with the histologic presence of perineural invasion or with tumor behavior.

HCAM is widely expressed in normal salivary glands and exhibits a predilection for neoplastic myoepithelial cells in salivary gland tumors. Neither is its expression

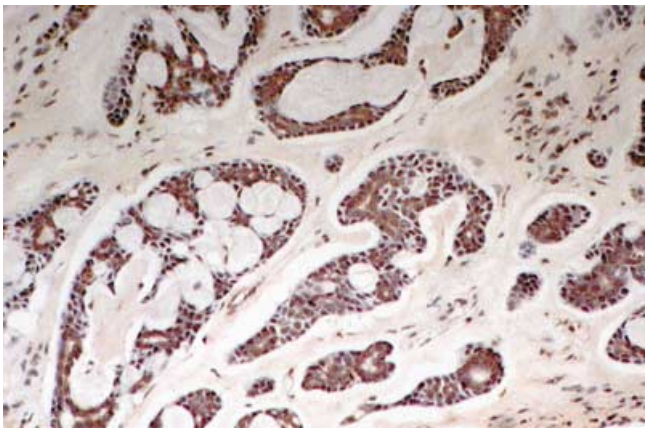

Figure 5 ICAM-1. ICAM-1 stained most cells with preferential staining of ductal cells, as demonstrated in this AdCyCa. This is the inverse of staining seen for HCAM (anti-ICAM-1/hematoxylin, magnification ×200).

related to perineural invasion, nor does it alter biological behavior.

PECAM-1 is rarely expressed by neoplastic salivary gland tumor cells, but was observed to be involved in the process of vascular metastasis in one case. Its presence in three cases was associated with metastases and a poor prognosis for the patient. Further study is needed to confirm these observations.

ICAM-1 is widely expressed in salivary gland ductal cells in both normal and neoplastic tissues. No significant relation to perineural invasion, vascular invasion, or biological behavior was found.

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