Urokinase-Type Plasminogen Activator Receptor Expression in Adenoid Cystic Carcinoma of the Skull Base

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Background: Head and neck adenoid cystic carcinoma (ACC) is a malignancy of the salivary and lacrimal glands with a variable growth pattern and propensity for perineural spread. Involvement of the skull base indicates a poor prognosis. Despite surgical resection and adjuvant radiotherapy, tumor recurrence and metastases are common. The urokinase-type plasminogen activator and its receptor (uPAR) have an important role in tumor invasion and metastasis. The expression of uPAR is predictive of poor outcomes in many tumors. This study examines the expression of human uPAR in ACCs involving the skull base.

Objectives: To determine uPAR expression in ACCs of the skull base by immunohistochemical analysis and compare expression with tumor histologic findings and clinical outcomes.

Study Design: Analysis of uPAR in archival ACC specimens and a retrospective medical chart review.

Setting: Multidisciplinary cranial base program at a university medical center with tertiary referral pattern.

Results: Ten (83%) of 12 tumors stained positive for uPAR. Three of 3 patients who died of ACC and 6 of 6 patients alive with disease expressed uPAR. Only 1 of 3 patients free of disease was uPAR positive.

Conclusions: In most patients with ACC of the skull base, uPAR was expressed. Its expression seems to be a negative prognostic factor. However, the small study sample limits our observations. Additional study of uPAR expression in ACC at other anatomic sites is required.


ADENOID CYSTIC carcinoma (ACC) of the head and neck is an uncommon neoplasm that exhibits a variable growth pattern and tendency for perineural spread. Arising from salivary and lacrimal glands, these tumors generally exhibit slow but progressive growth. At other times, these tumors will behave more aggressively, directly invading and destroying surrounding tissues and spawning metastases. When tumor involves the cranial base, prognosis is poor even with aggressive surgical resection and adjuvant radiotherapy. Despite advances in skull base surgical techniques, negative margins are difficult to obtain and delayed recurrences and systemic metastases are common. Because of its unique biologic characteristics, determining prognostic variables in ACC requires extended follow-up. This has prompted investigators to seek novel methods of predicting disease behavior.

The greatest predictor of aggressive tumor behavior is the ability to invade and metastasize. Accordingly, the factors regulating these functions are widely studied. Among this group of molecules are the urokinase-type plasminogen activator (uPA) and its receptor (uPAR). The uPAR participates in several normal cellular processes. However, it also influences tumor invasion and metastasis by facilitating the destruction of extracellular matrices. Clinically, cellular expression of uPAR denotes a worse prognosis for many malignancies. To our knowledge, there are no previous studies that report the expression of uPAR in ACC.

Our current investigation examines uPAR expression in the surgical specimens from patients with ACC of the skull base. Comparisons are made among clinical outcomes, tumor histologic findings, and the immunohistochemical expression of uPAR. We propose that increased expression of uPAR facilitates extension of tumor to the skull base and is associated with a worse prognosis.

METHODS

Fourteen patients with ACC of the skull base were retrospectively identified from the database of the Cranial Base Program at the University of Michigan, Ann Arbor, since its inception in 1994. The clinical records were reviewed for the site of the primary tumor, site of skull base involvement, surgical margins, clinical management, and treatment outcomes. Two pathologists (A.F., V.E.)
The study group included 6 men and 8 women with a mean age of 52 years (range, 30-76 years). There were 12 primary and 2 recurrent cases of ACCs. The primary tumor site and pattern of staining were as follows: nasal cavity (2 cases), ethmoid sinus (1 case), maxillary sinus (4 cases), parotid gland (4 cases), lacrimal gland (4 cases), lacrimal sac (1 case), and submandibular gland (2 cases). The pattern of uPAR expression was determined for all cases. Staining intensity was scored from 0 (none) to 3+ (heavy), whereas the pattern of staining was described as either focal or diffuse. Statistical analysis was performed using the Fisher exact test with statistical significance defined as P < .05.

RESULTS

The predominant pattern in the 14 patients was cribriform in 10 (71%), tubular in 2 (14%), and solid in 2 (14%) (Table 2).

At a median follow-up of 37 months, 4 patients were alive with no evidence of disease, 5 patients were alive with disease, and 4 patients had died of disease; 1 patient with active intracranial disease was lost to follow-up and considered alive with disease. Seven (88%) of 8 patients with positive margins were either alive with disease or dead of disease. Only 2 (40%) of 5 negative margin cases had similar disease status.

In total, 10 (83%) of 12 tumors expressed uPAR. Five specimens stained strongly for uPAR (3+), 3 stained moderately (2+), and 2 were weakly (1+) positive for uPAR (Table 2). All controls were negative for uPAR. The cellular location of uPAR staining was variable. Some specimens demonstrated uPAR expression in the cytoplasm of tumor cells (Figure 1), whereas others expressed the receptor on the cell surface and along the basement membrane (Figure 2). A focal pattern of staining was seen in 4 (40%) of 10 cases, and a diffuse pattern was seen in 4 others (40%). Two cases (20%) showed diffuse background expression with focal areas of intense staining. We observed positive staining in 6 (75%) of 8 cribriform tumors, 2 (100%) of 2 solid tumors, and 2 (100%) of 2 tubular tumors. There was no difference in uPAR expression in the margin-positive and margin-negative groups (P = .49). Expression of uPAR corresponded closely with clinical disease status. In all patients dead of disease (3/3) and patients alive with active disease (6/6), uPAR staining was found. In the smaller group of patients free of disease, 1 of 3 was uPAR positive (P = .045).

COMMENT

Adenoid cystic carcinoma involving the skull base presents a major challenge to the head and neck surgeon. Despite advanced skull base surgical techniques, the tendency for submucosal and perineural spread frustrates attempts to achieve a negative margin, en bloc resection. The current treatment for advanced ACC is surgical resection followed by postoperative radiotherapy. However, ACC tumor behavior leads to high rates of local recurrence and eventual metastases. In our clinical experience, we observed ACC behaving in 2 distinct ways. Most tumors grow slowly but steadily, often along cranial nerves. The 5-year survival for this group is acceptable, but patients eventually develop local recurrence and/or distant metastases. In contrast, we have seen a subset of ACC that behaves much more aggressively, exhibiting rapid growth with bone destruction and early metastases. These tumors invariably transgress the skull base at the time of diagnosis.

Previous investigators have sought to identify the clinical and histologic features that best predict ACC behavior. Using data from large retrospective studies, advanced tumor stage, solid tumor type, paranasal sinus sites, positive surgical margins, and nerve involvement all predict a worse outcome.1,13-16

Other predictors of ACC clinical behavior are described; several of these studies focus on measures of cellular proliferation. DNA ploidy,17 S-phase fraction,18 Ki-67,19...
proliferating cell nuclear antigen,20 and nucleolar organizer regions all are reported to correlate with clinical outcomes.21,22 Although promising, these studies are at times contradictory. Recent work examining alterations in tumor suppressor genes and oncogene function may provide more accurate prognostic information.23,24

The rationale for studying the uPA system is compelling. The uPA is secreted as an inactive precursor that binds to its receptor (uPAR) on the cell surface. Then uPA is activated by local proteases to an active form that rapidly converts plasminogen to plasmin. This is a key step in extracellular matrix degradation.

Although the uPA-uPAR system is integral to many controlled processes, including wound healing, tissue remodeling, and angiogenesis, it is also implicated in tumor invasion. By localizing the proteolytic enzymes needed to destroy the matrix components, uPAR can permit the uncontrolled translocation of neoplastic cells across host cellular and extracellular barriers.5

The expression of uPAR in both tumor tissue and blood is associated with a poorer prognosis in several cancers. Previous studies6–9 have shown that uPAR is a negative prognostic indicator in cancers of the breast, lung, colon, liver, prostate, and ovary. In the head and neck, uPAR is associated with worse outcomes in oral squamous cell carcinoma,10 cutaneous melanoma,23 and thyroid carcinoma.7

In this study, uPAR was expressed in 83% of patients with skull base carcinomas. There are no other data of uPAR expression for ACC with which to compare our findings. The expression of uPAR expression in other tumors varies widely and is influenced by the detection method. Nonetheless, the high rate of receptor expression is not unexpected. This study focuses on a series of advanced cases involving the cranial base. It is reasonable to expect that these tumors that have clinically demonstrated local invasion would possess the requisite cellular machinery.

Most noteworthy in this study is the correlation between clinical outcome and uPAR expression. Indeed, every patient dead of disease or alive with disease positively demonstrated uPAR. By contrast, only 1 patient without clinical disease was uPAR positive. Our study sample is small, limiting its statistical power, but these findings certainly warrant further investigation. Based on these preliminary observations, uPAR may help predict disease behavior.

The importance of basement membrane integrity in ACC progression and invasion is well recognized. Previous studies25–27 report the influence of several extracellular matrix components on ACC behavior. Shintani et al25

Table 2. Histopathologic and Immunohistochemical Staining Results for Patients With Adenoid Cystic Carcinoma of the Skull Base

<table>
<thead>
<tr>
<th>Patient No.</th>
<th>Tumor Margin</th>
<th>Perineural Involvement</th>
<th>Tumor Type</th>
<th>uPAR Expression</th>
<th>Pattern</th>
<th>Follow-up, mo</th>
<th>Disease Status</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>+</td>
<td>+</td>
<td>Solid</td>
<td>3+</td>
<td>F/D</td>
<td>2</td>
<td>AWD</td>
</tr>
<tr>
<td>2</td>
<td>–</td>
<td>–</td>
<td>Cribriform</td>
<td>Not stained</td>
<td>NA</td>
<td>71</td>
<td>NED</td>
</tr>
<tr>
<td>3</td>
<td>+</td>
<td>–</td>
<td>Cribriform</td>
<td>2+</td>
<td>F</td>
<td>14</td>
<td>AWD</td>
</tr>
<tr>
<td>4</td>
<td>+</td>
<td>–</td>
<td>Cribriform</td>
<td>3+</td>
<td>D</td>
<td>102</td>
<td>AWD</td>
</tr>
<tr>
<td>5</td>
<td>+</td>
<td>–</td>
<td>Cribriform</td>
<td>0</td>
<td></td>
<td>39</td>
<td>NED</td>
</tr>
<tr>
<td>6</td>
<td>+</td>
<td>+</td>
<td>Cribriform</td>
<td>3+</td>
<td>F</td>
<td>45</td>
<td>AWD</td>
</tr>
<tr>
<td>7</td>
<td>–</td>
<td>+</td>
<td>Cribriform</td>
<td>0</td>
<td></td>
<td>18</td>
<td>NED</td>
</tr>
<tr>
<td>8</td>
<td>+</td>
<td>+</td>
<td>Cribriform</td>
<td>3+</td>
<td>F</td>
<td>39</td>
<td>AWD</td>
</tr>
<tr>
<td>9</td>
<td>+</td>
<td>+</td>
<td>Cribriform</td>
<td>Not stained</td>
<td>NA</td>
<td>24</td>
<td>DOD</td>
</tr>
<tr>
<td>10</td>
<td>–</td>
<td>+</td>
<td>Cribriform</td>
<td>2+</td>
<td>F/D</td>
<td>39</td>
<td>NED</td>
</tr>
<tr>
<td>11</td>
<td>+</td>
<td>+</td>
<td>Tubular</td>
<td>1+</td>
<td>F</td>
<td>26</td>
<td>AWD</td>
</tr>
<tr>
<td>12</td>
<td>+</td>
<td>+</td>
<td>Tubular</td>
<td>1+</td>
<td>D</td>
<td>20</td>
<td>DOD</td>
</tr>
<tr>
<td>13</td>
<td>NA</td>
<td>+</td>
<td>Tubular</td>
<td>1+</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>14</td>
<td>–</td>
<td>+</td>
<td>Solid</td>
<td>2+</td>
<td>D</td>
<td>37</td>
<td>DOD</td>
</tr>
</tbody>
</table>

Abbreviations: AWD, alive with disease; D, diffuse; DOD, dead of disease; F, focal; minus sign, negative; NA, not applicable; NED, no evidence of disease; plus sign, positive; and uPAR, urinokinase-type plasminogen activator receptor.

Figure 1. A 3+ diffuse urokinase-type plasminogen activator receptor expression with prominent nuclear staining (hematoxylin-eosin, original magnification ×200).

Figure 2. A 3+ diffuse urokinase-type plasminogen activator receptor expression with staining along cell surface and basement membrane (hematoxylin-eosin, original magnification ×100).
have shown that tenasin, a glycoprotein component of the matrix normally produced by fibroblast and muscle cells, is increased in invasive areas of ACC and associated with tumor progression. These authors further demonstrated that laminin and type IV collagen, key components of basement membrane integrity, are absent in areas of invasion. Related work by Franchi et al27 showed e-cadherin, a mediator of cell-cell adhesion in glandular and surface epithelia, is associated with increased invasiveness and metastatic potential and is down-regulated in advanced ACC.

One aim of this study was to determine if uPAR expression could aid in determining tumor margins and predicting tumor resectability. Because of the unique growth pattern of ACC and the complex 3-dimensional anatomy of the skull base, it is difficult to achieve negative margin resection in patients with ACC. Historically, involvement of the cranial base categorized tumors as unresectable. With advances in skull base surgery, safe resection of tumors extending up to and even into the cranium is now possible. The long-term benefits of such efforts are not known. Pitman et al2 reported that despite extended resections, tumor recurred in 71% of sinonasal ACC cases. They concluded that although skull base techniques allow gross tumor excision in cases previously deemed inoperable, such efforts do not appear to improve disease-free survival. In our own series, 8 (62%) of 13 patients had positive surgical margins despite aggressive surgical management. These results clearly illustrate the frustrations in obtaining clear margins in these complex anatomic sites. We had hoped that uPAR expression could be used to help determine surgical margins. However, in this series the ability to obtain negative margins did not correlate with uPAR expression. Rather, uPAR was expressed in most negative-margin and positive-margin specimens.

Studies that focus on ACC of the skull base are few. Gandour-Edwards et al3 studied expression of Ki-67 and neural cell adhesion molecule in 18 patients with skull base ACC. Neural cell adhesion molecule was seen in 89% and Ki-67 in 100% of specimens. These authors reasoned that the high degree of neural cell adhesion molecule staining explained the tumor’s tendency to extend along neural structures into the cranium. These results and our own observations for uPAR suggest that there are a number of factors of cellular control that are suppressed or absent in these aggressive tumors.

In conclusion, the results of this study of uPAR expression are informative, but further work is needed to define the clinical significance of uPAR expression. We found a high rate of expression in clinically aggressive skull base tumors. However, we lack data about its expression in locally controlled ACC. Our limited sample and relatively short follow-up period constrain our clinical conclusions. Nonetheless, uPAR was expressed in most of our patients and correlated with clinical behavior. A greater understanding of the uPA-uPAR system in ACC disease spread and metastases offers a potential opportunity to predict behavior and possibly to direct treatment.

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REFERENCES