11-23-2023

Educational Case: An Invasive Salivary Gland Tumor: Adenoid Cystic Carcinoma of the Parotid Gland

Jaime Eberle-Singh
Thomas Jefferson University

Madalina Tuluc
Thomas Jefferson University

Joanna Sue Yee Chan
Thomas Jefferson University

Contents lists available at ScienceDirect

Academic Pathology 10/4 (2023) 100098

Academic Pathology

journal homepage: www.journals.elsevier.com/academic-pathology

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Educational Case: An invasive salivary gland tumor: Adenoid cystic carcinoma of the parotid gland

Jaime Eberle-Singh, MD PhD, Madalina Tuluc, MD, Joanna Sue Yee Chan, MD *

Department of Pathology and Genomic Medicine, Thomas Jefferson University Hospital, Philadelphia, PA, USA

The following fictional case is intended as a learning tool within the Pathology Competencies for Medical Education (PCME), a set of national standards for teaching pathology. These are divided into three basic competencies: Disease Mechanisms and Processes, Organ System Pathology, and Diagnostic Medicine and Therapeutic Pathology. For additional information, and a full list of learning objectives for all three competencies, see https://www.sciencedirect.com/journal/academic-pathology/about/pathology-competencies-for-medical-education-pcme.[1]

Keywords: Pathology competencies, Disease mechanisms, Neoplasia, Characteristics of neoplasia, Cellular capabilities of neoplasia, Invasion, Metastasis, Adenoid cystic carcinoma, Salivary gland tumor

Primary objective

Objective N3.2: Cellular Capabilities of Neoplasia. Discuss the cellular capabilities of neoplasms that enable them to invade tissues and to metastasize and recognize how this differentiates benign from malignant neoplasms.

Competency 1: Disease Mechanisms and Processes; Topic N: Neoplasia; Learning Goal 3: Characteristics of Neoplasia.

Patient presentation

A 48-year-old woman presents to her primary care doctor with a progressively enlarging right-sided parotid mass and associated right-sided facial paralysis. She denies fevers, chills, night sweats, and unintentional weight loss. Sixteen months earlier, the patient had sudden onset right-sided facial paralysis with no additional symptoms. At that time, she was diagnosed with Bell's palsy and referred to physical therapy, which provided some relief. The patient has no other significant past medical history.

Diagnostic findings, Part 1

Physical exam reveals the mass to be firm, fixed, and non-tender to palpation. A computed tomography (CT) scan is obtained and shows a 4.2 cm mass with an infiltrative appearance.

Questions/discussion points, Part 1

What is the differential diagnosis?

When taking into consideration the patient's history of a progressively enlarging parotid gland mass that is fixed on physical exam and appears infiltrative on CT, the differential diagnosis would favor malignant tumors of the parotid gland. Entities to consider include mucoepidermoid carcinoma, adenoid cystic carcinoma, adenocarcinoma, and acinic cell carcinoma. Even in a patient without a prior cancer diagnosis, it is necessary to rule out metastasis from an unknown primary tumor. The two most common malignancies to metastasize to the parotid are cutaneous squamous cell carcinoma and melanoma.[2]

Diagnostic findings, Part 2

After appropriate workup, including a fine needle aspiration (FNA) and genetic testing—which reveals a *MYB-NFIB* fusion, the mass is resected. The patient undergoes a right parotidectomy and mastoidectomy with neck dissection. Representative histologic images are shown in Figs. 1–3.

Questions/discussion points, Part 2

Describe the morphologic findings

Resection shows a cellular tumor comprised of small cells with a high

* Corresponding author. Department of Pathology, Anatomy and Cell Biology, Thomas Jefferson University Hospital, 132 S 10th Street, Suite 285 Main Building, Philadelphia, PA, 19107, USA.

E-mail address: joanna.chan@jefferson.edu (J.S.Y. Chan).

https://doi.org/10.1016/j.acpath.2023.100098

Received 7 October 2022; Received in revised form 31 July 2023; Accepted 24 September 2023, Available online 23 November 2023

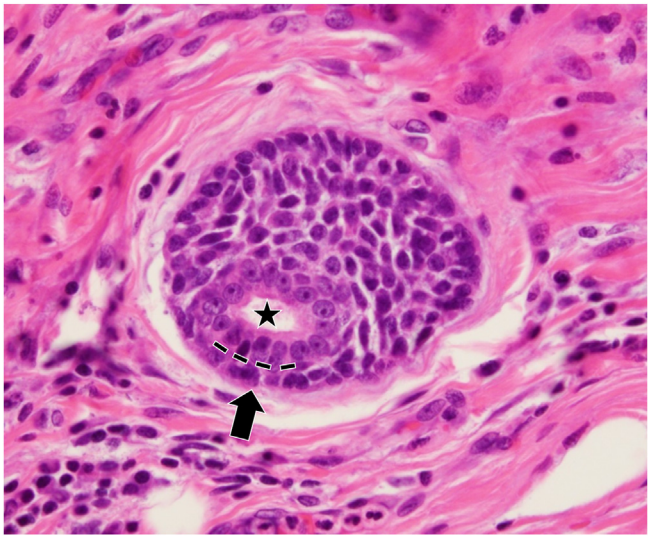

Fig. 1. Histology of the resected mass shows ductal cells (encircling the star) and surrounding myoepithelial cells (arrow). The dotted line marks the border between cell populations. Hematoxylin and eosin; original magnification: x400.

nuclear to cytoplasmic (N/C) ratio. The nuclei are small and hyperchromatic, and the cytoplasm is scant and eosinophilic. The tumor itself is comprised of two distinct populations: ductal cells surrounded by myoepithelial cells, which appear slightly darker on histology (Fig. 1). The patient's tumor shows both a tubular pattern (white arrow) and a cribriform pattern (black arrow), as can be seen in Fig. 2. Tumor cells are also seen in close proximity to, and in some cases surrounding, multiple nerves (Fig. 3).

What is the diagnosis?

The mass was determined to be adenoid cystic carcinoma based on the patient's clinical history, tumor location, cellular morphology, and the identification of a fusion of the *MYB* oncogene and the *NFIB* transcription factor.[3]

Describe the unique histology of this tumor and explain how these characteristics contribute to tumor grade

Adenoid cystic carcinoma can take on multiple growth patterns

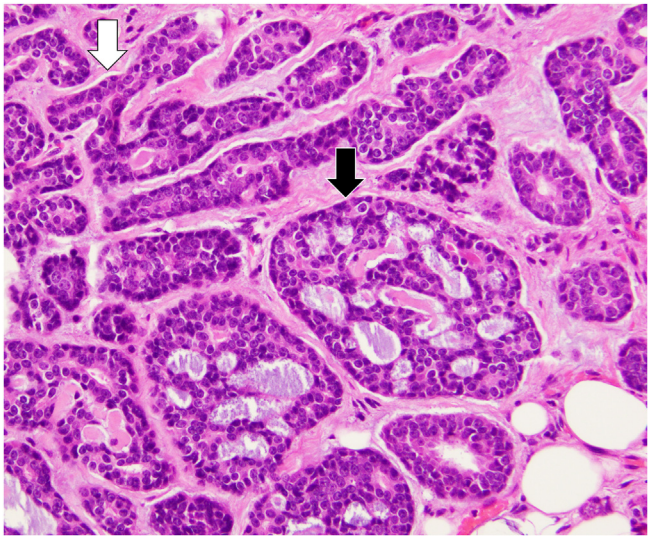

Fig. 2. The tumor shows tubular (white arrow) and cribriform (black arrow) patterns of growth. Hematoxylin and eosin; original magnification: x200.

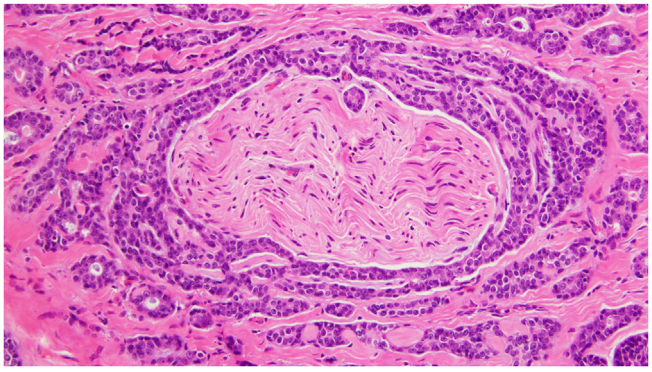

Fig. 3. Tumor cells can be seen completely surrounding the large peripheral nerve (wavy nuclei) seen in the center of the image. Hematoxylin and eosin; original magnification: x200.

ranging from cribriform to tubular to solid. More than one pattern can be present within a single tumor. The cribriform pattern is very characteristic of this tumor. Despite these varying patterns of growth, the tumor cells themselves are relatively uniform in appearance.[4,5]

Three pathological grading systems are used for adenoid cystic carcinoma (Perzin/Szanto, Spiro, and van Weert). While these three systems differ slightly from one another, they are unified by a focus on the percentage of tumor that displays a solid growth pattern.[6] It has been shown that tumors with a higher percentage area of solid growth patterns are correlated with a worse prognosis.[4,7] At the time of resection, this patient's tumor did not show a solid growth pattern. However, given their invasive nature, adenoid cystic carcinomas are difficult to eradicate completely and generally have a poor long-term prognosis.[3]

What is the behavior and natural history of adenoid cystic carcinoma?

Adenoid cystic carcinoma is a slow-growing tumor that comprises 5% of major salivary gland tumors and 20% of minor salivary gland tumors.[3] In addition to the salivary glands, they can also arise in the lacrimal glands, nasal cavity, nasopharynx, paranasal sinuses, and lower respiratory tract. While the 5-year survival rate for this tumor is estimated at greater than 90%, long-term survival at fifteen years is approximately 10%—even with the use of postoperative radiotherapy—as adenoid cystic carcinoma usually recurs.[4,8]

What finding from the patient's initial presentation was suggestive of the tumor's local invasion and likely delayed her cancer diagnosis?

The patient's isolated right facial paralysis led her primary care physician to diagnose her with Bell's palsy: an idiopathic facial paralysis of a single nerve, that often resolves on its own within a few months.[9] Unfortunately, the patient was either lost to follow-up or was not referred for further work up when her facial paralysis failed to resolve. When adenoid cystic carcinoma was originally reported in the 1850s, it was described as able to spread along nerves.[10] More than a century later, this tumor is well-known for its predilection for nerves and is a classic example of perineural invasion (PNI) (see Fig. 3).[5]

What are the mechanisms underlying perineural invasion?

The process of PNI is still poorly understood, but it is clearly associated with tumor invasiveness and poor prognosis for patients, particularly when major nerves are involved.[4,11] In 2009, Liebig and colleagues completed a review of the existing literature on PNI and updated its definition to include the most recent understanding of the process.[12] They defined it as, "Tumor in close proximity to nerve and involving at

least 33% of its circumference or tumor cells within any of the three layers of the nerve sheath." PNI has been identified in many cancer types in addition to head and neck cancers—including pancreatic cancer, colorectal cancer, and prostate cancer.[11]

PNI is a separate process from vascular or lymphatic invasion and can be observed independently of both, even as a tumor's only form of metastasis.[12] The original mechanistic theories hypothesized that PNI was an extension of metastasis through the lymphatics or that neural sheaths were a path of low resistance for tumor spread.[13] Both of these theories have since been disproven.

The most current understanding of the mechanisms underlying this phenomenon points towards a complex crosstalk between tumor cells and nerves that involves regulation of the tumor microenvironment and the perineural niche.[14] A wide range of involved cell types are involved in PNI—including Schwann cells, stellate cells, macrophages, and fibroblasts. Roles for a variety of proteins have been identified, including neurotrophic factors, tumor growth factor, matrix metalloproteinases, axon guidance molecules, chemokines, and cell adhesion molecules.

In pancreatic ductal adenocarcinoma, for example, it was recently discovered that tumor cells can activate Schwann cells to form linear tracts that allow for tumor cell migration and invasion. Activation of c-JUN in Schwann cells was found to play an important role in this process.[15] While there is still much to be understood about PNI, it is likely that a tumor's proximity to nervous tissue and the unique gene expression profiles of both a tumor and its microenvironment all play an important role in determining whether PNI will occur.[11]

Diagnostic findings, Part 3

Pathology of the surgical specimen shows adenoid cystic carcinoma of the right parotid gland with invasion into the facial nerve, skin, ear canal, and temporal bone. Multiple surgical margins are positive and three of thirteen regional lymph nodes examined show evidence of tumor. The tumor stage is pT4aN2bM0 and the patient receives postoperative radiotherapy.

Questions/discussion points, Part 3

What changes do cells undergo in order to become locally invasive? How does this affect the tumor's behavior?

In general, in its initial stages, a carcinoma is contained by the basement membrane of the epithelial tissue it arises in. As a tumor acquires an invasive phenotype, it undergoes a series of changes at the cellular and molecular level that allow it to break down the basement membrane and invade into adjacent structures. This step of local invasion is what separates an *in-situ* lesion from an invasive carcinoma. This process is known as the epithelial–mesenchymal transition (EMT) and encompasses the first steps a tumor takes on its journey to metastasis. In order for cells to undergo EMT, they must forego the epithelial characteristics that contain them within the basement membrane and adopt a mesenchymal phenotype.[16] This requires major alterations in gene expression that lead to morphological and behavioral changes, which support cellular motility.

Among the most critical changes associated with EMT is the suppression of E-cadherin. In benign epithelial tissues, this transmembrane protein links epithelial cells together, allowing them to form cell sheets. In addition to the loss of cell junctions that require E-cadherin, the loss of cytokeratin expression, cell polarity, and epithelial gene expression programs are seen as well. In turn, cells undergoing EMT take on a fibroblastic shape and become motile and invasive. They have an increased resistance to apoptosis and upregulate mesenchymal gene expression programs. Instead of expressing E-cadherin, these cells express N-cadherin—along with vimentin, fibronectin, proteases, and other proteins required for invasion.[16] See Table 1 for examples of some of the cellular and histologic changes that occur as preinvasive cells acquire invasive properties.[16–18]

Table 1
Cellular changes and histologic changes seen in a benign versus a malignant tumor.

	Cellular changes[16,17]	Histologic changes[18]
Preinvasive ⇨ Invasive	• Epithelial gene expression ⇨ Mesenchymal gene expression ('EMT program') • E-cadherin ⇨ N-cadherin • Loss of cell polarity ⇨ fibroblast shape • Increased motility • Resistance to apoptosis	• Cellular atypia (anaplasia) - *high N/C ratio* - *enlarged, hyperchromatic nuclei with abnormal contours* - *prominent nucleoli* - *clumped chromatin* - *atypical mitoses* • Increased mitotic activity • Disorganized growth pattern • Invasion into adjacent structures • Presence of metastases

Extensive study of the tumor microenvironment in multiple tumor types supports the theory that signals from the surrounding stroma induce tumor cells to undergo EMT. While this is true, more recent research in this rapidly evolving field uncovered a complex crosstalk between the tumor and stroma, with the discovery that the stroma also has a role in restraining tumor invasion and metastasis.[19–21]

Adenoid cystic carcinomas are known for their invasiveness.[4] However, it is important to note that preinvasive lesions have not been identified for all tumor types—such is the case for adenoid cystic carcinoma. These tumors have only been identified in the invasive form, in which the cells have already undergone EMT and local invasion. This is in contrast to other tumor types that have clearly defined preinvasive lesions: such as ductal carcinoma in situ giving way to invasive breast carcinoma, pancreatic intraepithelial neoplasia giving way to pancreatic ductal adenocarcinoma, or an adenoma in the colon giving way to colon adenocarcinoma. While this does not mean that adenoid cystic carcinoma definitively does not have a preinvasive form, there is not yet evidence to support this.

What is the difference between regional metastasis and distant metastasis? How does this contribute to tumor stage?

Regional (or local) metastasis refers to a tumor that has invaded local tissues or has been identified in nearby lymph nodes, while distant metastasis refers to the presence of a tumor in an area of the body distant from the site of the primary tumor. Adenoid cystic carcinoma uses the TNM staging system, in which "T" refers to the size of the primary tumor and whether it has invaded local structures, "N" refers to the presence or absence of tumor in regional (head and neck) lymph nodes, and "M" refers to the presence or absence of distant metastasis. This patient's tumor stage at the time of diagnosis was pT4aN2bM0, meaning that the primary tumor was greater than 4 cm in size (T4) and had invaded the skin, jawbone, ear canal and/or facial nerve (T4**a**). She was found to has multiple positive lymph nodes on the same side of her head/neck as the primary tumor (N2b), and there was no evidence of distant metastasis (M0).[22]

Diagnostic findings, Part 4

Six years later, the patient presents to her oncologist with a two-month history of fatigue and an unintentional weight loss of twelve pounds. A fluorodeoxyglucose (FDG)-positron emission tomography (PET)/CT scan shows a region of high FDG uptake in the right upper lobe of the lung, indicating lung metastasis. The mass is biopsied and histology reveals metastatic adenoid cystic carcinoma.

Questions/discussion points, Part 4

What are the steps required for tumor cells to undergo metastasis?

Adenoid cystic carcinomas often do not metastasize for years, as was the case with this patient. When metastasis does occur, cancer cells must

succeed in undergoing a complex sequence of events known as the *invasion-metastasis cascade*.[16] The earliest tenets of this theory were described in 1889 by the English surgeon, Stephen Paget, in what he coined as the "seed-and-soil hypothesis."[23]

The process begins when locally invasive cells that have undergone EMT intravasate into blood and lymphatic vessels, where they are transported to various organs throughout the body. During this phase, the cell must avoid death by anoikis—a form of apoptosis that occurs when cells are detached from extracellular matrix—and are made further vulnerable by the lack of a supportive stroma. Cells that survive until this point will become lodged in the microvessels of another organ. Once here, they begin the process of extravasation where they will burrow their way through the vessel wall and take up residence in their new home, forming a micrometasasis. In order to achieve the final step of macrometastasis, the cells must colonize this distant tissue by successfully adapting to their new microenvironment. This often involves a reversion back to the tumor's original epithelial phenotype via the process of mesenchymal–epithelial transition (MET). It is thought that the absence of EMT-inducing signals in their new microenvironment allows the cells to return to their original state and explains why metastases often resemble the primary tumor.[15]

The processes of local invasion, intravasation, extravasation, and colonization all require the tumor cell to undergo major changes at the cellular and molecular level and to rapidly adapt to a range of environments. Only a few cells of the tumor are believed to successfully complete this process.[16] The biological underpinnings of the invasion-metastasis cascade are still the subject of multidisciplinary research endeavors, the findings of which will undoubtedly reveal susceptibilities that can be leveraged into novel cancer therapies.

Teaching points

- Adenoid cystic carcinoma is a slow-growing, invasive tumor known for high rates of perineural invasion (PNI) and poor long-term survival. The tumor is comprised of epithelial and myoepithelial cells that can grow in cribriform, tubular, and solid patterns—with the percentage of tumor growing in a solid pattern determining the tumor grade.
- The primary distinguishing feature between an *in-situ* and invasive neoplasm is its ability to invade through the basement membrane and into adjacent tissues. To do this, the tumor cell must undergo the process of epithelial–mesenchymal transition (EMT).
- Table 1 outlines both the cellular changes and histologic changes that help distinguish between a preinvasive and invasive tumor. Cellular changes include transitioning from the expression of epithelial genes to the expression of mesenchymal genes (including N-cadherin), loss of polarity, increased motility, and resistance to apoptosis. Histologic changes that can be seen include cellular atypia, increased mitotic activity, disorganized growth pattern, invasion into adjacent structures, and the presence of metastases. It is important to keep in mind that these changes occur along a spectrum and may not apply to all tumor types.
- The role of the tumor microenvironment and its contribution to a tumor's capacity for invasion and metastasis is still being determined; however, the literature supports a complex role for tumor stroma that may act to both promote and restrict EMT.
- The mechanisms of PNI are also not well understood. Similar to the cross-talk present in the tumor microenvironment, research suggests a complex interplay between the tumor microenvironment and the perineural niche.
- Once a tumor undergoes EMT, it must successfully complete all steps in the invasion-metastasis cascade in order to metastasize. Following colonization of a distant tissue, the metastatic cells may undergo mesenchymal–epithelial transition (MET) and revert back to the original phenotype of the primary tumor.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Funding

The article processing fee for this article was funded by an Open Access Award given by the Society of '67, which supports the mission of the Association of Pathology Chairs to produce the next generation of outstanding investigators and educational scholars in the field of pathology. This award helps to promote the publication of high-quality original scholarship by authors at an early stage of academic development.

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