Case Report
Primary parotid adenocarcinoma metastasis to the spleen with *PIK3CA* mutation: cytological findings and review of the literature

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Abstract: Background: Primary solid tumor metastasis to the spleen is a rare event, and often presents as an incidental finding without clinical symptoms of the patient. The most common primary tumors that metastasize to the spleen are colorectal, ovarian, and lung carcinomas. Parotid tumor metastasis to the spleen is extremely rare. We report an unusual case of metastatic parotid adenocarcinoma NOS (not otherwise specified) to the spleen. Case report: The patient presented with primary parotid carcinoma and underwent left parotidectomy. On pathological examination of the primary parotid tumor, no vascular or perineural invasion was found; all surgical resection margins and neck lymph nodes were also uninvolved by the tumor. No other therapy was given after the surgery. Four years later, the patient developed a solitary splenic lesion detected by a routine follow-up computed tomography (CT) scan. The subsequent fine needle aspiration (FNA) and splenectomy showed a metastatic adenocarcinoma consistent with the parotid primary. Immunohistochemical (IHC) staining of the metastatic tumor also showed a similar pattern as that of the primary tumor, including positivity for pancytokeratin, S-100 and SOX10, supporting the diagnosis. Furthermore, A *PIK3CA* (phosphatidylinositol 3-kinase catalytic subunit) mutation was also detected in the splenic metastasis. Conclusion: Based on our review of the literature, we believe that this is the first report of such a case. Accurate diagnosis and molecular characterization of the splenic metastasis have a critical impact on the clinical management of the patient.

Keywords: Parotid adenocarcinoma, splenic metastasis, metastatic carcinoma

Introduction

Solid tumor metastasis to the spleen is a rare clinical entity [1-3]. Several previous large scale autopsy studies have shown that the frequency of splenic metastasis ranges from 2.3% to 7.1% in cancer patients [1, 2]. The largest autopsy series was reported by Berge T [3]. In the study, 4.4% of cancer patients (312 of 7165 autopsies) had splenic metastasis. More interestingly, he also found that microscopic splenic metastases were identified in 50% of patients who had metastases in at least 5 organs. In addition to autopsy studies, a study of 1280 splenectomy cases found that the prevalence of splenic metastasis was 1.3% (17 of 1280 cases) [4]. In the same study, 9.8% (12 of 122 cases) of diagnostic splenectomies contained metastases [4]. Based on Compérat et al.'s review of the literature published up to year

2006, the incidence of splenic metastasis, diagnosed by imaging and/or histology, ranged from <1% to 10% [1]. Taken together, the true incidence of metastasis of the spleen may have been underestimated in cancer patients due to the fact that most patients are clinically asymptomatic.

Splenic metastasis may occur from a number of primary sites. The top ten solid tumors, in descending order, that have been shown to metastasize to the spleen are colorectal, ovarian, lung, endometrial, kidney, stomach, cervical, breast, prostate and esophageal carcinomas [1]. Metastasis of primary parotid tumors to the spleen is extremely rare. Overall, primary salivary gland tumors are rare, with an incidence of 2.5 to 3.0 cases per 100,000 per year [5]. The majority of these tumors are benign and only 20% of them are malignant [6].
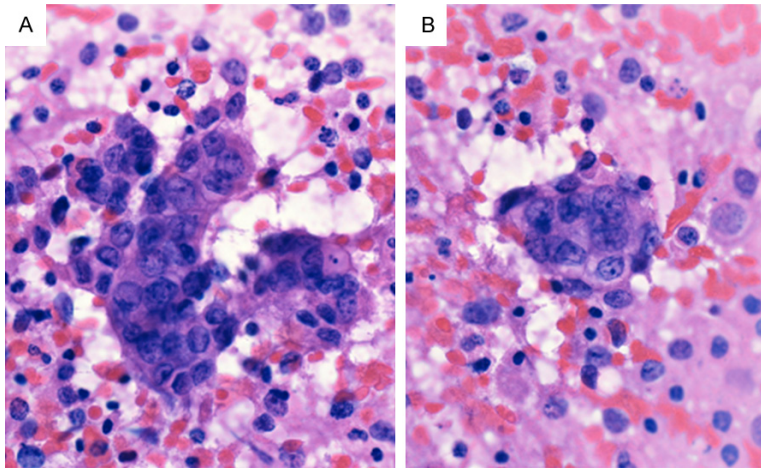
Figure 1. The FNA smears of the splenic mass. A: The tumor cells are arranged in tubulopapillary and/or two-dimensional fragments. B: The tumor cells have enlarged round-to-oval nuclei, coarse chromatin, irregular nuclear membranes, prominent nucleoli, and occasional small cytoplasmic vacuoles.

Salivary malignancies comprise less than 0.5% of all malignancies and about 5% of cancers of the head and neck region [5-8]. Primary parotid tumors usually spread by direct extension and/or via lymphovascular channels to local lymph nodes. When distant metastases occur, they typically involve the lung and liver [7-9]. Based on our literature search for metastatic primary parotid neoplasms to the spleen, only one case of carcinoma ex pleomorphic adenoma of the parotid gland with splenic metastasis was found [10]. Here we present a case of splenic metastasis from a parotid adenocarcinoma. The accurate diagnosis of the splenic metastasis has a critical impact on the clinical management of the patient.

Clinicopathological findings

Clinical information

A 61-year-old male presented with a left facial mass for a period of a few years. A CT scan revealed a 2.6×2.3 cm mass in the left parotid gland. The patient underwent surgical resection of the mass with ipsilateral cervical lymphadenectomy. Microscopic examination of the tumor revealed a salivary gland adenocarcinoma NOS with pathological stage of pT1 N0. No vascular or perineural invasion was found; all surgical resection margins and neck lymph nodes were also uninvolved by the tumor. The patient recovered well after the surgery. No other therapy was given after the surgery. Four

years later, a follow-up routine CT scan revealed a 6×8 cm mass in the spleen, with close proximity to the wall of the stomach. There was no indication of other lesions in the abdomen or evidence of lymphadenopathy. Fine needle aspiration cytology of the splenic mass was performed, followed by splenectomy. Pathological examination confirmed the diagnosis of metastatic adenocarcinoma, consistent with the parotid primary. Mutational analysis of the splenic metastasis revealed the *PIK3CA* (I391M), *KDR* (Q472H), and *TP53* (P72R) mutations. The patient was treated with the PIK3 inhibitor Buparlisib. However, he developed multiple metastatic lesions in his lung, liver and peritoneum a few months following the targeted therapy.

Cytological and histological findings

The FNA smears of the splenic mass were fixed in alcohol and stained with Papanicolaou methods. The smears were cellular and adequate, and showed malignant epithelioid cells in a background of blood and admixed with inflammatory cells (**Figure 1**). The atypical cells were arranged in tubulopapillary and/or two-dimensional fragments. These cells had scant cytoplasm with ill-defined cellular borders, enlarged round-to-oval nuclei, irregular nuclear membranes, coarse chromatin and prominent nucleoli. The majority of the cells showed hyperchromatic nuclei with prominent nucleoli. Occasional tumor cells contained prominent cytoplasmic vacuoles and eccentric nuclei. Some of the tumor cells also contained small intracytoplasmic vacuoles. Over-all, the tumor cells were intermediate in size, with a high nuclear/cytoplasmic (N/C) ratio and marked nuclear atypia. Tumor necrosis was also present.

Histological examination of the resected splenic mass revealed an infiltrating, poorly differentiated adenocarcinoma comprised of sheets and clusters of cells with focal glandular architecture and extensive tumor necrosis (**Figure 2**). Tumor cells also revealed similar cytological
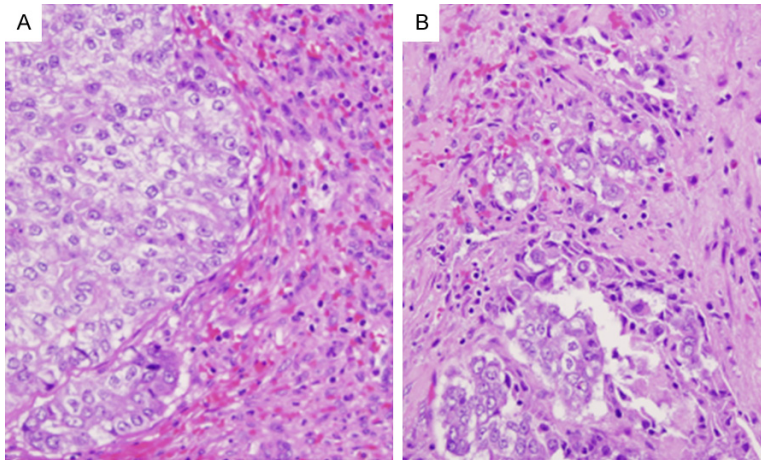
Figure 2. Histomorphology of the surgical resected splenic metastasis. A: The tumor infiltrates into the splenic parenchyma without capsular formation. Tumor cells are arranged in sheets and clusters. B: Tumor cells have large hyperchromatic nuclei, coarse chromatin, irregular nuclear membranes and prominent nucleoli. Numerous mitotic figures are identified.
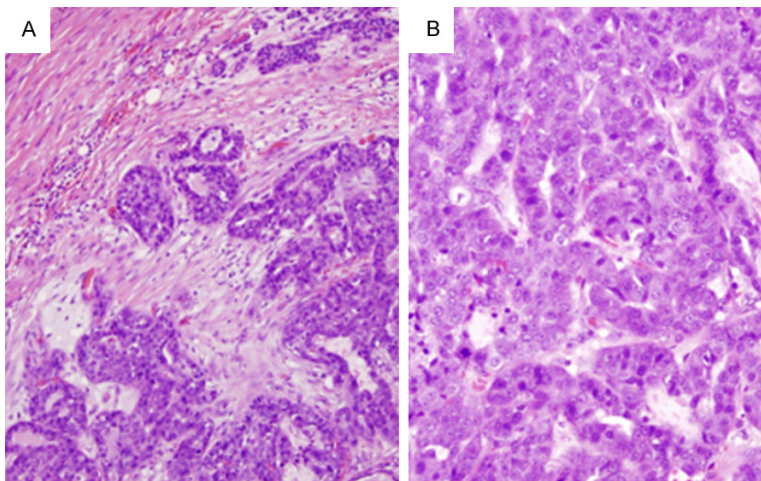
Figure 3. Histomorphology of the parotid adenocarcinoma. A: The parotid parenchyma is replaced by infiltrating clusters of malignant epithelioid cells. Most of the tumor demonstrates small glandular architecture separated by fibrous bands. B: The tumor cells are intermediate in size, with high nuclear/cytoplasmic (N/C) ratio and marked nuclear atypia.

features, including findings of hyperchromatic nuclei with prominent nucleoli, eccentric nuclei, and prominent cytoplasmic vacuoles.

We also reviewed the primary parotid tumor. The tumor showed an infiltrative growth pattern with clusters of malignant neoplastic cells, consisting of glandular structures which were separated by fibrous bands (**Figure 3**). There was no microfollicular or basaloid growth pattern or nuclear palisading of the tumor. The tumor cells morphologically had large hyper-

chromatic nuclei with coarse granular chromatin, irregular nuclear membranes, and prominent nucleoli. Rare cytoplasmic mucin production was seen in cells forming glandular structures. Numerous mitotic figures were readily identified. No vascular or perineural invasion was found on the primary tumor. All surgical resection margins of the primary tumor and 29 neck lymph nodes were uninvolved by the tumor. The immunostains of the tumor revealed that tumor cells were diffusely positive for pancytokeratin, S-100 and SOX10, but negative for GCDFP (gross cystic disease fluid protein), androgen receptor, PAX8, thyroglobulin, napsin and TTF-1.
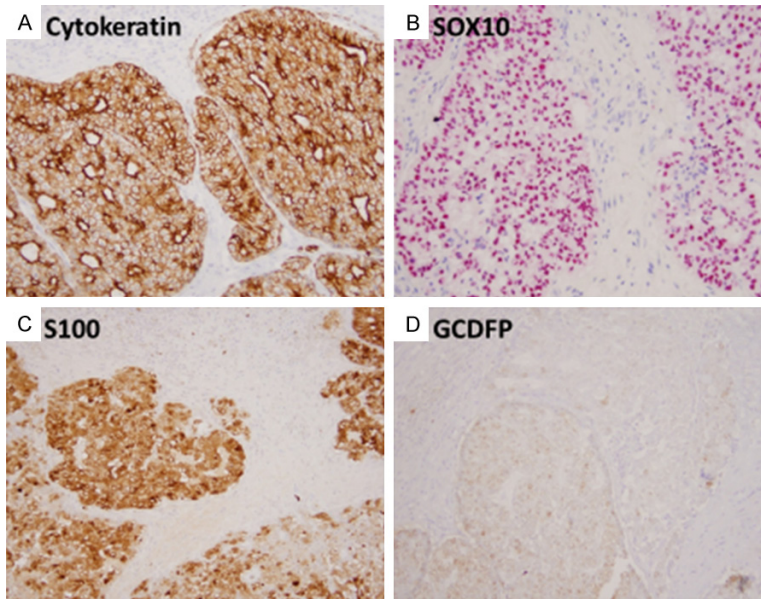
IHC and molecular analysis of the metastatic tumor

Immunohistochemical studies were performed on the neoplasm in the spleen. The sections were cut at 4 µm in thickness and deparaffinized before incubation with primary antibodies. Heat antigen retrieval at 70°C for 40 minutes was also used to enhance antigen detection. The IHC was performed using a Dako autostainer. The dilutions of primary antibodies were used according to the manufacturer's suggestions and standard protocols.

The tumor was diffusely and strongly positive for pancytokeratin AE1/AE3 and SOX10, focally positive for S100, and predominately negative for GCDFP (**Figure 4**). The tumor was also negative for TTF1, Napsin A, androgen receptor, PAX8 and thyroglobulin. Mucicarmine and periodic acid-Schiff (PAS) special stains were performed, and both stains demonstrated intracytoplasmic mucin.

Figure 4. IHC study of the splenic metastasis. A and B: The tumor is diffusely positive for pancytokeratin AE1/AE3 and SOX10. C: The tumor is focally positive for S100. D: GCDFP is predominantly negative.

Molecular analysis of the tumor was performed at our hospital's clinical molecular laboratory using next generation sequencing (NGS) technique. Briefly, DNA from tumor cells was harvested from unstained slides. NGS was conducted using AmpliSeq Cancer Hotspot Panel (v2) for targeted multi-gene amplification. The Ion AmpliSeq Library Kit 2.0 for library preparation and the Ion Personal Genome Machine 200 Sequencing Kit with the Ion 318 Chip and Personal Genome Machine as the sequencing platform (Life Technologies, Carlsbad, California) were used in the assay. It revealed *PIK3CA* (I391M), *KDR* (Q472H) and *TP53* (P72R) mutations.

Discussion

Splenic metastasis is usually a manifestation of late stage disease in cancer patients [7-9]. The most common neoplasms reported in the literature that spread to the spleen are colorectal, ovarian and lung carcinomas [1]. Splenic involvement by metastatic head and neck cancers is exceedingly rare. Lam et al. reviewed autopsies over a 25-year period and found only five cases of splenic metastases originating from the nasopharynx and one from the larynx [2]. There was one report of a patient with squamous cell carcinoma of the tonsil which metastasized to the spleen, causing splenic rupture [11], and another report of a patient with squamous cell carcinoma of the neck and metastasis to the spleen that was treated with laparoscopic splenectomy [12].

Parotid adenocarcinoma NOS is an aggressive malignant salivary tumor with poor prognosis and high mortality [7-9]. The term "salivary adenocarcinoma NOS" encompasses a heterogeneous group of salivary carcinomas with gland and/or duct formation that do not meet criteria for recognized salivary carcinoma entities [7]. Important prognostic factors for malignant salivary tumors include stage, lymph node involvement, tumor type and histological grade, and perineural invasion [7-9]. More specifically, distant metastases have been associated with the tumor stage, sex, skin involvement, perineural invasion, and histological type [13]. Speight et al. also suggested that a tumor size greater than 4 cm indicates a poor prognosis [14]. Surgical resection is the primary treatment for salivary malignancies, and adjuvant radiation therapy may be used in patients with adverse risk factors. Patients who present with unresectable disease or who are not fit for surgery will often get radiation therapy alone. Systemic chemotherapy has been predominantly reserved for palliative treatment, as it has not been shown to prolong survival, but can aid tumor shrinkage [9].

Malignant parotid tumors typically spread by direct extension, through lymphatics to cervical lymph nodes, and rarely by hematogenous spread to distant sites. When distant metastases are identified, they have been reported to mainly involve the lung, liver and bone [8]. Three patterns of splenic involvement by metastatic tumors have been described, including macronodular, micronodular, and diffuse patterns [1]. The macronodular pattern is characterized by involvement of the splenic parenchyma by a solitary nodule or multiple large nodules of varying sizes, and the micronodular pattern is characterized by the involvement by re-

latively uniform small nodules. In the diffuse microscopic pattern, the splenic parenchyma is diffusely infiltrated by metastatic tumor cells that do not form a discreet lesion; the tumor cells can be identified within the venous sinuses, the red pulp, the white pulp, or the trabecular vessels [1].

In our case, the primary tumor did not reveal vascular or perineural invasion, and all surgical resection margins and 29 neck lymph nodes were uninvolved by the tumor. The patient was found to have a macronodular pattern of splenic metastasis four year after the resection of the primary tumor. The splenic metastasis presented as a solitary mass with morphologic features of the primary parotid adenocarcinoma, such as tight cellular clusters and acinar structures, intracytoplasmic mucin, irregular nuclear contours, coarse chromatin and prominent nucleoli. However, these features are nonspecific and can be seen in nearly all types of adenocarcinomas from various anatomic sites.

The differential diagnosis of a metastatic adenocarcinoma, therefore, is broad and includes metastatic adenocarcinoma of the gastrointestinal tract, lung, and kidney; prostate carcinoma in males; and gynecological tract and breast carcinomas in females. A comprehensive approach using clinical, radiologic, and histomorphological findings is necessary for accurate diagnosis.

Immunohistochemistry can be a useful ancillary adjunct to morphologic examination in determining site of origin for poorly differentiated adenocarcinomas. For example, lung adenocarcinomas are frequently positive for CK7, Napsin A and TTF-1; colorectal adenocarcinomas are often positive for CK20 and CDX2, while being negative for CK7; and prostatic carcinomas are often positive for prostate specific antigen (PSA) and NKX3.1. While certain immunoprofiles are strongly suggestive of particular sites of origin, many sites are associated with a relatively nonspecific immunoprofile (e.g., CK7 positivity in upper gastrointestinal tract adenocarcinomas and many other adenocarcinomas). In addition, poorly differentiated neoplasms will not infrequently lose expression of their site-specific markers, making attention to subtle morphologic findings and clinical history increasingly important.

Currently, immunohistochemical staining is used sparingly to aid in distinguishing different salivary gland neoplasms, and classification relies heavily on morphology due to the lack of specificity for distinct salivary tumor types [15]. In addition, there are no widely used immunohistochemical markers that are entirely specific for salivary origin, which would be useful in the workup of a metastatic poorly differentiated neoplasm suspicious for salivary origin. However, although classically used as a marker for melanocytic and Schwannian tumors [16], SOX10 has recently been described as a useful marker of salivary acinus and intercalated duct differentiation, with expression in a variety of salivary neoplasms [17-19]. Ohtomo et al. examined different types of salivary gland tumors and found SOX10 expression in acinic cell carcinomas, adenoid cystic carcinomas, epithelial-myoepithelial carcinomas, myoepithelial carcinomas, and pleomorphic adenomas; and absence of SOX10 expression in salivary duct carcinomas, mucoepidermoid carcinomas, oncocytomas, and Warthin tumors [17]. Furthermore, 5 of 22 (23%) cases of carcinoma NOS were positive for SOX10 immunostaining. The strong and diffuse SOX10 expression in our case was, therefore, in keeping with salivary origin versus a second primary site, especially given the patient's history of salivary adenocarcinoma. SOX10 has also been shown to be expressed in myoepithelial tumors of soft tissue and carcinomas with basal/myoepithelial features, particularly triple-negative and metaplastic breast carcinomas [19-21]. Although SOX10 is often positive in melanoma, the glandular architecture, intracytoplasmic mucin, and strong cytokeratin positivity in our case argues against this diagnosis.

Finally, mutational analysis of the metastatic splenic tumor revealed *PIK3CA* (I391M), *KDR* (Q472H) and *TP53* (P72R) mutations. Molecular studies are becoming increasingly more important for the implementation of targeted therapy [9]. The finding of *PIK3CA* mutation has not been described in parotid adenocarcinomas [14-24]. In our case, the patient was treated by buparlisib (PI3K inhibitor) following surgical resection, but his disease still progressed, with metastasis to the lung, liver and peritoneum several months after therapy.

In summary, our case demonstrates a rare occurrence of metastatic salivary adenocarcinoma to the spleen with a targetable *PIK3CA* mutation. Although rare, salivary cancers should

be considered in the differential diagnosis of splenic metastasis, especially in a patient with a history of a salivary neoplasm. Salivary adenocarcinoma NOS has nonspecific morphologic features which overlap with many adenocarcinomas from other sites, and SOX10 expression may be useful in confirming salivary origin, following exclusion of melanoma and carcinomas with basal/myoepithelial features.

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Disclosure of conflict of interest

None.

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