Book Chapter

Histopathological Aspects of The Prognostic Factors for Salivary Gland Cancers

Haruto Nishida*, Takahiro Kusaba, Kazuhiro Kawamura, Yuzo Oyama and Tsutomu Daa

Department of Diagnostic Pathology, Faculty of Medicine, Oita University, Japan

***Corresponding Author:** Haruto Nishida, Department of Diagnostic Pathology, Faculty of Medicine, Oita University, Oita 879-5593, Japan

Published **May 16, 2023**

This Book Chapter is a republication of an article published by Haruto Nishida, et al. at Cancers in February 2023. (Nishida, H.; Kusaba, T.; Kawamura, K.; Oyama, Y.; Daa, T. Histopathological Aspects of the Prognostic Factors for Salivary Gland Cancers. Cancers 2023, 15, 1236. https://doi.org/10.3390/cancers15041236)

How to cite this book chapter: Haruto Nishida*, Takahiro Kusaba, Kazuhiro Kawamura, Yuzo Oyama, Tsutomu Daa. Histopathological Aspects of The Prognostic Factors for Salivary Gland Cancers. In: Prime Archives in Cancer Research: 3rd Edition. Hyderabad, India: Vide Leaf. 2023.

Author Contributions: Conceptualization, H.N.; methodology, H.N.; investigation, T.K., K.K., and Y.O.; writing—original draft preparation, H.N.; writing—review and editing, T.K., K.K., and Y.O.; supervision, T.D.; project administration, T.D. All authors have read and agreed to the published version of the manuscript.

Funding: This research received no external funding.

Data Availability Statement: The data presented in this study are available on request from the corresponding author.

Conflicts of Interest: The authors declare no conflict of interest.

Simple Summary: We describe the currently known histopathological aspects of the prognostic factors for salivary gland cancers and discuss the genetics or molecules used as diagnostic tools, which might serve as treatment targets in the future.

Abstract

Salivary gland cancers (SGCs) are diagnosed using histopathological examination, which significantly contributes to their progression, including lymph node/distant metastasis or local recurrence. In the current World Health Organization (WHO) Classification of Head and Neck Tumors: Salivary Glands (5th edition), malignant and benign epithelial tumors are classified into 21 and 15 tumor types, respectively. All malignant tumors have the potential for lymph node/distant metastasis or local recurrence. Particularly, mucoepidermoid carcinoma (MEC), adenoid cystic carcinoma (AdCC), salivary duct carcinoma, salivary carcinoma, not otherwise specified (NOS, formerly known as adenocarcinoma, NOS), myoepithelial carcinoma, epithelial–myoepithelial carcinoma, and carcinoma ex pleomorphic adenoma (PA) are relatively prevalent. High-grade transformation is an important aspect of tumor progression in SGCs. MEC, AdCC, and salivary carcinoma, NOS, have a distinct grading system; however, a universal histological grading system for SGCs has not yet been recommended.

Conversely, PA is considered benign; nonetheless, it should be cautiously treated to avoid the development of metastasizing/recurrent PA. The aim of this review is to describe the current histopathological aspects of the prognostic factors for SGCs and discuss the genes or molecules used as diagnostic tools, which might have treatment target potential in the future.

Keywords

Salivary Gland Cancers; Prognostic Factors; Histopathology; Genetics; Molecules

Introduction

In the current World Health Organization (WHO) Classification of Head and Neck Tumors: Salivary Glands (5th edition), malignant and benign epithelial tumors are classified into 21 and 15 tumor types, respectively (Table 1) [1].

The salivary gland comprises three major salivary glands (parotid, submandibular, and sublingual glands) and several minor salivary glands. Salivary gland cancers (SGCs) can arise from any salivary gland and has a morbidity rate of approximately 10–20% [2,3]. SGCs are diagnosed using histopathological examination, and histological findings can be considered the most valuable and distinct prognostic factors [2,3]. SGCs show various tumor types because a healthy salivary gland contains inner luminal/epithelial or acinar/mucous cells and outer basal/myoepithelial cells in the duct or the secretory part. The histopathological features correlate with the tumor progression, including lymph node/distant metastasis or local recurrence. In 1986, Spiro reported that the significant prognostic factors were the site of origin, histologic subtype, histologic grading, and clinical stage [4]. However, some carcinomas show mild cytological atypia, making the evaluation of tumor invasion challenging. For all malignant tumors, mucoepidermoid carcinoma (MEC), adenoid cystic carcinoma (AdCC), and salivary carcinoma, not otherwise specified (NOS) (formerly known as adenocarcinoma, NOS), validated grading systems exist; however, a universal histological grading system

for SGCs is not recommended [1]. Furthermore, novel genes or genetic components and proteins are being validated as diagnostic tools and some of them may even serve as targets for new drugs. We summarize the present SGCs classification with a focus on the prognostic factors and discuss new and potential prognostic factors from the histopathological viewpoint.

Table 1: WHO Classification of Head and Neck Tumors: Salivary Glands (5th edition).

Benign Epithelial Tumours	**Malignant Epithelial Tumours**
Pleomorphic adenoma	Mucoepidermoid carcinoma
Basal cell adenoma	Adenoid cystic carcinoma
Warthin tumour	Acinic cell carcinoma
Oncocytoma	Secretory carcinoma
Salivary gland myoepithelioma	Microsecretory adenocarcinoma
Canalicular adenoma	Polymorphous adenocarcinoma
Cystadenoma of salivary gland	Hyalinizing clear cell carcinoma
Ductal papillomas	Basal cell adenocarcinoma
Sialadenoma papilliferum	Intraductal carcinoma
Lymphadenoma	Salivary duct carcinoma
Sebaceous adenoma	Myoepithelial carcinoma
Intercalated duct adenoma and hyperplasia	Epithelial-myoepithelial carcinoma
Striated duct adenoma	Mucinous adenocarcinoma
Sclerosing polycystic adenoma	Sclerosing microcystic adenocarcinoma
Keratocystoma	Carcinoma ex pleomorphic adenoma
	Carcinosarcoma of the salivary glands
Mesenchymal tumours specific to the salivary glands	Sebaceous adenocarcinoma
Sialolipoma	Lymphoepithelial carcinoma
	Squamous cell carcinoma
	Sialoblastoma
	Salivary carcinoma, NOS and emerging entities

The Past and Present of SGCs

The General Histopathological Prognostic Factors for SGCs

The Histological Types

The most prevalent malignancies are MEC and AdCC, and most SGCs have a distinct histological grade (Table 2). The histological diagnosis reflects biological behavior in several cases. The current WHO classification (5th edition) describes the grading system for MEC, AdCC, salivary carcinoma, NOS [1]. Other tumors that show similar classification include carcinoma ex pleomorphic adenoma (CXPA) and intraductal carcinoma (IDC). The WHO classification detached grades from tumor names since tumors with the same features of a cancer type do not necessarily have the same severity or aggressiveness, and it allows flexibility in describing the tumor [1,2,5]. Each tumor's histological features are subsequently discussed.

High-Grade Transformation (Dedifferentiation)

Dedifferentiation is a regress from a more differentiated to a less differentiated state (stem cell-like). Particularly, in a malignant tumor, a differentiated cell loses its specific form or function [6,7]. Histopathologically, dedifferentiation is observed as the abrupt transformation of a well-differentiated tumor into high-grade morphology (poorly differentiated or anaplastic/undifferentiated), lacking the original morphology (Figure 1) [8].

Table 2: Native histopathological stratification of salivary gland malignancies.

Low-Grade Malignancy	Intermediate Malignancy	High-Grade Malignancy	Variable Grade
Acinic cell carcinoma	Myoepithelial carcinoma	Salivary duct carcinoma	Mucoepidermoid carcinoma
Basal cell adenocarcinoma	Sebaceous adenocarcinoma	Squamous cell carcinoma	Adenoid cystic carcinoma
Epithelial-myoepithelial carcinoma	Lymphoepithelial carcinoma	Small cell carcinoma	Salivary carcinoma, NOS
Secretory carcinoma		Large cell neuroendocrine carcinoma	Intraductal carcinoma

Polymorphous adenocarcinoma		Large cell undifferetiated carcinoma	Carcinoma ex pleomorphic adenoma
Hyalinizing clear cell carcinoma		Carcinosarcoma	
Mucinous adenocarcinoma		Salivary gland carcinomas with high-grade transformation	
Microsecretory adenocarcinoma			
Sclerosing microcystic adenocarcinoma			
Sialoblastoma			
(Metastasizing pleomorphic adenoma)			

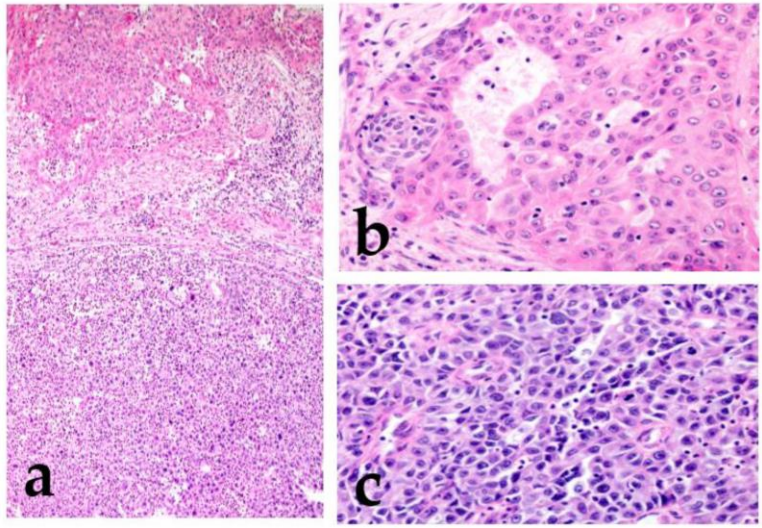

Figure 1: Mucoepidermoid carcinoma (MEC) with high-grade transformation. There is intermediate-grade MEC in the upper region, and the lower region shows high-grade transformation **(a)**. Intermediate-grade MEC forms glandular or solid structures and consists mainly of intermediate cells with moderate atypia (increased nuclear size with an obvious nucleus) **(b)**. The high-grade region shows undifferentiated features, and the cells lack the original morphology **(c)**.

In the high-grade region, the tumor cells show anaplastic cells with large vesicular pleomorphic nuclei, prominent nucleoli, increased mitoses/Ki67 labeling index, and necrosis [8,9]. The term "dedifferentiation" is occasionally used in malignant soft tissue tumors, such as dedifferentiated liposarcoma or dedifferentiated chondrosarcoma [10,11]. However, in SGCs, a malignant tumor is rarely replaced by a completely different one, and the original morphological features usually remain. Therefore, the term high-grade transformation is used to describe this phenomenon [8,12]. This transformation is reported not only in variable low-grade malignant tumors (acinic cell carcinoma, MEC, secretory carcinoma, hyalinizing clear cell carcinoma, myoepithelial carcinoma, epithelial–myoepithelial carcinoma, and polymorphous adenocarcinoma) but also in high-grade tumors, including AdCC [12–21]. Tumors with this finding have an even worse prognosis.

Micropapillary Pattern

Invasive micropapillary carcinoma (IMPC) was first reported in the breast [22]. Neoplastic cell nests are uniformly distributed throughout a reticulated interstitium and exhibit a reverse polarity or "inside-out" growth pattern. This histological finding is observed in other malignant tumors of the urinary bladder, lung, stomach, colon, or bile duct [23–27]. IMPC, a tumor with a micropapillary pattern, frequently shows lymphatic invasion and lymph node metastasis, and its prognosis is very poor. Among SGCs, micropapillary salivary duct carcinoma (SDC) is the most prevalent; nonetheless, micropapillary AdCC and intraductal papillary mucinous neoplasm (IPMN) have also been reported (Figure 2) [21,28,29].

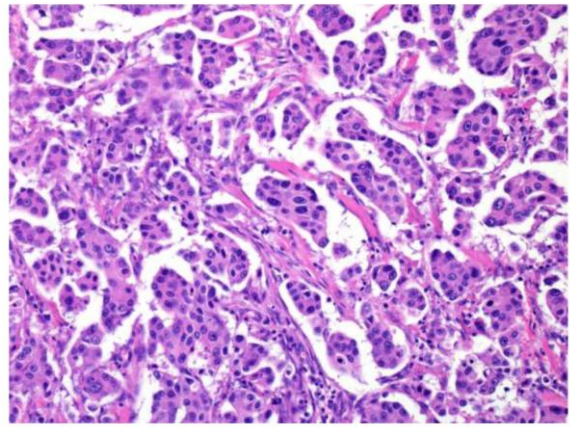

Figure 2: Micropapillary salivary duct carcinoma (SDC). The tumor forms many micropapillary nests that consist of eosinophilic cytoplasm and irregular nuclear-like SDC.

Other Histologic Findings

Strong prognostic factors prevalent in many tumors include increased cellular atypia, perineural invasion, lymphovascular invasion, increased mitoses/Ki67 labeling index, necrosis, local recurrence/distant metastasis, and poor surgical margin (Figure 3) [2–5].

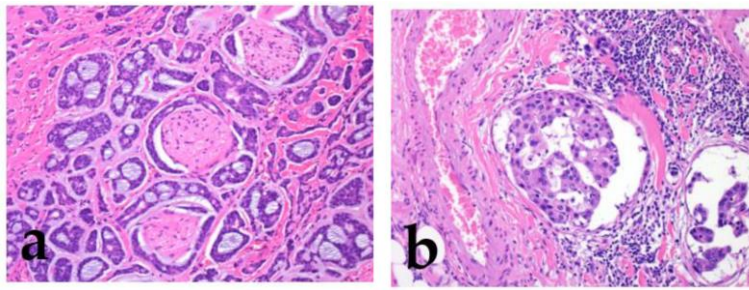

Figure 3: Perineural invasion and lymphatic invasion. Adenoid cystic carcinoma shows frequent perineural invasion **(a)**, and salivary duct carcinoma shows lymphatic invasion **(b)**.

For lymph node metastasis, important prognostic factors include the number of nodes, foci size, unilateral/bilateral involvement,

extranodal extension, and stromal reaction [30–32]. Lombardi reported that intra-parotid node metastasis implies an increased risk of lateral neck involvement and impact on the survival of patients with SGCs [33]. More specifically, the overall number (0 vs 1–3 vs ≥ 4) and diameter (<20 mm vs ≥20 mm) of the node metastasis represent major prognostic factors for overall survival [33]. Additionally, for parotid gland carcinoma, facial nerve paralysis and tumor adhesion/immobility could be the predictive factors for high-grade SGCs [34].

Other Related Factors

Although not directly involved histopathologically, the most predictive factors for tumor recurrence are advanced age, male sex, larger tumor size, and high clinical stage [2,35–37]. Concerning the site of tumor origin, small salivary glands have a high frequency of SGC occurrence, and the sublingual salivary gland has the highest frequency of malignancy [2,38].

Validated Grading Systems for Individual SGCs and Similar Diseases

This section discusses MEC, AdCC, and salivary carcinoma, NOS, which have validated grading systems in the WHO classification (5th edition), focusing on structural atypia, such as the presence or absence of tumor nest/solid part and cellular atypia. Furthermore, it describes IDC and CXPA, which are classified by the presence or absence of tumor invasion and atypical cellular morphology. Moreover, metastasizing PA (MPA) is also discussed.

Mucoepidermoid Carcinoma (MEC)

MEC is characterized by mucous, intermediate, and epidermoid/squamoid tumor cells. However, some tumors show intermediate cells are predominant. It forms cystic and solid growth patterns, usually associated with *MAML2* rearrangement [39,40]; therefore, some reports describe the histological features for grading (Table 3, Figure 4) [39–44], which particularly include structural atypia (cystic/solid component,

border invasion pattern, lymphovascular/perineural invasion, and necrosis) and cytological atypia (nuclear anaplasia/pleomorphism and mitoses) [36–44]. According to these grading, MEC should be graded as low, intermediate, and high. The higher the grade, the greater the possibility of metastasis or recurrence [39,40].

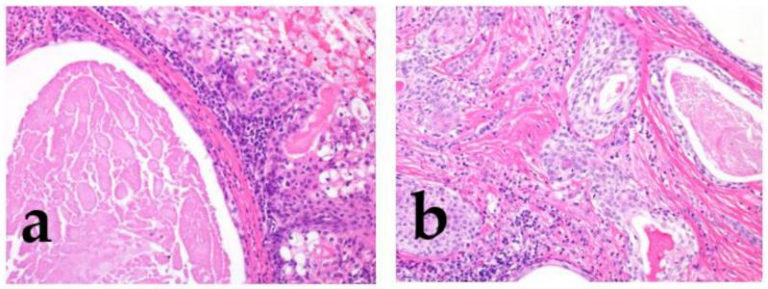

Figure 4: The morphological features of low/intermediate mucoepidermoid carcinoma (MEC). The tumor forms cystic (a; low grade) and solid growth patterns (b; intermediate). In both, tumor cell atypia is mild, but the intermediate one shows slightly different nuclear sizes with mitosis.

The Armed Forces Institute of Pathology (AFIP) grading system was previously used; however, this system may not necessarily indicate the actual degree of some aggressive cases [40,41]; hence, the Brandwein system was introduced to classify these cases from the viewpoint of anaplasia [43]. Nevertheless, high-grade MEC is very rare, and there is no difference in outcome between low and intermediate grades using any grading system. Another study has reported that mitosis and necrosis may be helpful in the classification [44].

Table 3: Comparison of the mucoepidermoid carcinoma grading systems.

Comparison of Mucoepidermoid Carcinoma Grading Systems			
Feature	**AFIP [7,8]**	**Brandwein [9]**	**Katabi [10]**
Cysts/Architecture	2 (<20% cystic)	2 (<25% cystic)	LG: predominantly cystic
			IG/HG: predominantly solid
Border/Invasive Front	n/a	2 (small nests & islands)	LG: circumscribed
			IG/HG: infiltrative
Necrosis	3	3	LG/IG: absent
			HG: present
Nuclear Anaplasia/Pleomorphism	4	2	LG/IG: not significant
Lymphovascular Invasion	n/a	3	n/a
Perineural Invasion	2	3	n/a
Mitoses	3 (4/10 HPF)	3 (5/10 HPF)	LG: 0–1/10 HPF
			IG: 2–3/10 HPF
			HG: 4+/10 HPF
Bony Invasion	n/a	3	n/a
Low Grade (LG)	0–4	0	Qualitative Assessment
Intermediate Grade (IG)	5–6	2–3	
High Grade (HG)	7–14	4–16	
LG: low grade; IG: intermediate grade; HG: high grade; n/a: not applicable; AFIP: Armed Forces Institute of Pathology			
If a pathologic feature is present, relevant points are assigned as listed in the table. Final grade is given by sum of points.			

Adenoid Cystic Carcinoma (AdCC)

AdCC consists of two main cells: ductal cells located in the inner part and myoepithelial cells located in the outer part of the duct. The ductal cells have eosinophilic cytoplasm and uniform round nuclei, while the myoepithelial cell has clear cytoplasm and hyperchromatic angular nuclei [45,46]. Perineural invasion is an AdCC hallmark, and genetically, it is characterized by *MYB* or related-gene translocations. Typically, AdCC comprises

pseudocysts and true glandular lumina. AdCC shows three growth patterns: tubular, cribriform, and solid [45,46]. Consequently, the following histological grading is used for its classification: tubular predominant as grade Ⅰ, cribriform predominant as grade Ⅱ, and solid predominant as grade Ⅲ (Figure 5) [45–47].

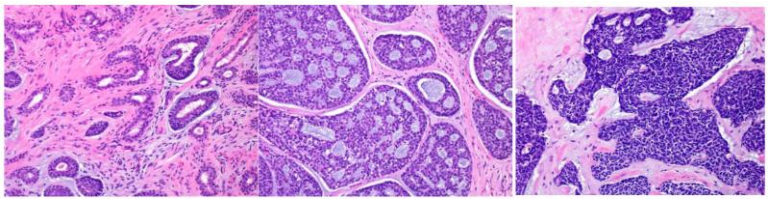

Figure 5: The histological grade of adenoid cystic carcinoma (AdCC). The tumor shows tubular (left), cribriform (middle), and solid (right) growth patterns.

Although AdCCs with > 30% solid component have been shown as being more aggressive, any solid tumor component may be a high-grade tumor, as described in the minAmax system [48–50]. Necrosis, marked pleomorphism, or high levels of mitoses are only seen in the solid pattern, and are not utilized in the grading system [46].

Salivary Carcinoma, NOS [Former Adenocarcinoma, not otherwise Specified (NOS)]

In the current WHO classification (5th edition), adenocarcinoma, NOS, is renamed as salivary carcinoma, NOS, and we have used the same term herein [51]. It includes the subtypes oncocytic and intestinal-type adenocarcinoma. The term salivary carcinoma, NOS, should be used for the tumors arising in major/minor salivary glands; however, this category is a heterogeneous spectrum of carcinomas showing ductal and/or glandular differentiation. It represents an exclusive diagnosis of otherwise defined salivary gland carcinoma entities (nonspecific-appearing adenocarcinoma) [51–53]. Due to differences in this carcinoma's interpretation, the percentage or case numbers varied in previous reports, and the pure entity adenocarcinoma, NOS, now accounts for approximately 10% of SGCs [36,54–58]. Adenocarcinoma, NOS, was considered as a

heterogeneous group of tumors; however, the strictly selected cases were considered as the pure group [59]. The histological grading system was based on a previous report in 1982 by Spiro et al., and the tumors were classified as low, intermediate, and high grade [51,52,57,58]. They defined anaplastic or high-grade lesions as grade Ⅲ, which are arranged in close clumps and broad bands composed of small glandular tumor cells. The tumor cell nests are separated by collagen connective tissue stroma, similar to that in seen during scirrhous formation. In addition, they are divided into low (grade Ⅰ) or intermediate grade (grade II), and the low-grade variant shows no stromal invasion, whereas the intermediate grade shows definite stromal infiltration. Grade Ⅱ lesions have prominent sheets or cords of some polymorphic glandular cells with a pale cytoplasm [57]. Although this classification reflected the tumor prognosis at that time, it is still somewhat reasonably useful today; however, it should be revised owing to the differences in diagnostic criteria currently.

Carcinoma Expleomorphic Adenoma (CXPA)

CXPA is an epithelial and/or myoepithelial malignancy arising in a primary or recurrent pleomorphic adenoma (PA) [60,61]. Reflecting the presence of PA, the typical clinical presentation is a long-term painless mass with recent rapid progression or previous PA diagnosis. PA presents as ductal and myoepithelial cells arranged in bilayered tubular structures, while the stroma is typically mucoid, myxoid, hyalinized, or chondroid. Usually, the transition from PA to the malignant component is distinct; however, some cases may have heavy stromal/hyalinized collagen bundles or chondroid/myxoid stroma only. The common malignancies are SDC, epithelial–myoepithelial carcinoma, salivary carcinoma, NOS, and myoepithelial carcinoma; by contrast, carcinosarcoma is rare [62]. Most carcinosarcomas arise from PA through the intraductal or myoepithelial pathway, the multistep adenoma-carcinoma-sarcoma-sequence [63]. The relevant observations are the histological subtype/grade, the proportion of carcinoma (> 50%), and the extent of invasion [61–64]. The malignant component progresses from encapsulated neoplasm to extracapsular invasion. The term “encapsulated” is described by various

alternatives as “intracapsular”, “in situ”, “preinvasive”, “intramural”, or “noninvasive”; however, the term intracapsular is preferred. CXPA is sub-classified based on the extent of invasion beyond the PA, as follows: intracapsular, minimally invasive (the carcinoma invades <4–6 mm beyond the PA borders), and invasive (invasion beyond the PA capsule ≥6 mm) [60]. However, evaluating the fibrous capsule is occasionally challenging because the tumor forms the capsule or the capsule outline is vague, especially in primary minor salivary glands. Several specimen preparations are needed for a precise diagnosis.

Intraductal Carcinoma (IDC)

IDC is a salivary gland malignancy located entirely or predominantly intraductally [65,66]. It has papillary, cribriform, and solid structures mimicking atypical ductal hyperplasia or ductal carcinoma in situ of the breast [65,66]. It shows four subtypes based on the tumor cells: intercalated duct, apocrine, oncocytic, and mixed IDC; hence this category’s tumors are heterogeneous. Usually, intercalated duct and oncocytic IDC are low-grade, whereas apocrine and mixed IDC are low or high grade [65,66]. Despite any cellular subtypes, pure IDC behaves indolently, though invasive carcinomas ex-IDC (arising from IDC) can behave aggressively [67,68]. Therefore, these tumors may be reassessed as “IDC, noninvasive (low-grade IDC)”, “IDC, noninvasive (high-grade IDC)”, or “IDC, invasive”, similar to intraductal papillary mucinous neoplasm (IPMN) of the pancreas or CXPA of the salivary gland.

Metastasizing Pleomorphic Adenoma (MPA)

PA is a benign tumor composed of benign ductal and myoepithelial cells, having a chondromyxoid or sclerosing fibrous component in the background [69,70]. In peculiar cases, benign-looking PA metastasizes to the bone, lung, and neck lymph nodes with some local recurrence [71,72]. Knight et al. reported that 41 patients (80.4%) with MPA were alive at the 1-year follow-up. However, survival was poor, and 17.6% (9/51 cases) died from MPA [72]. Although the term includes “PA”, considering the clinical course, it was managed at least as a low-grade malignancy. There are no histological or molecular

features to predict metastasis, and it is not distinguishable from a benign tumor at the primary site [72,73]. Thus, first-time surgery for PA must be performed cautiously if the patient is young, the PA is multinodular, or there is a tumor rupture. Furthermore, a post-operative status of incomplete tumor excision, incomplete pseudocapsule, extracapsular extension (microscopic, pseudopods, skip/satellite lesion beyond the pseudocapsule), or poor margin should also be considered due to the possibility of recurrent PA [74–77].

Future Perspectives on SGCs

This section discusses SGC-specific genes or characteristic proteins, including their immunohistochemistry. These are emerging as diagnostic tools in recent years and could be clinicopathological predictors of SGCs. Although many drugs are not included in the usual regimens, drug-targetable proteins and genes, such as hormone receptors in breast cancers, have been shown to alter SGCs prognosis. Although various methods are used for investigating SGCs or target genes, in several cases, immunohistochemistry (IHC), reverse transcription polymerase chain reaction (RT-PCR), fluorescence in situ hybridization (FISH), and Sanger sequencing/next-generation sequencing (NGS) are commonly used. IHC, RT-PCR, and FISH detect protein expression, fusion genes and gene translocation, and gene translocation and amplification, respectively. Furthermore, Sanger sequencing detects point mutations or minor genetic alterations, and NGS can be used for whole-genome sequencing; nevertheless, IHC is the most commonly performed investigation as it is economic and convenient. Recently, targeted therapies have been developed to target the signaling pathways involving the molecular signatures detected by these techniques. For example, in case the detected oncogene signature is *VEGF/ANG2*, *VEGFR/FGFR/PDGFR*, *HER2*, *EGFR*, or *TrkB/BDNF*, the targeted therapy is sorafenib, nintedanib, trastuzumab, lapatinib, or ANA-12, respectively, for MEC [78].

Genetics as a Diagnostic Tool

SGCs are heterogeneous tumors, and genetics aids in understanding the molecular biology of each tumor. All

malignant tumors listed in the WHO classification (5th edition) and related genes are summarized in Table 4 [1,2,79–84].

These gene alterations are used as diagnostic tools and represent the tumor's specific characteristics. Some of these genes are druggable, and HER2 or tropomyosin receptor kinase (TRK) inhibitors have been used to treat salivary duct or secretory carcinoma, respectively [82–84]. Some of these drugs significantly change patients' prognoses, and they are discussed in the following section.

Table 4: Comparison of the histological diagnoses and gene alterations.

Tumour Type	Chromosomal Region	Gene Alterations
Mucoepidermoid carcinoma	t(11;19) (q21;p13) t(11;15) (q21;q26)	CRTC1::MAML2 CRTC3::MAML2
Adenoid cystic carcinoma	9p21.3 6q22-23 8q13 9q34.3	CDKN2A deletion MYB fusion/activation/amplification MYBL1 fusion/activation/amplification NOTCH mutations
Acinic cell carcinoma	9q31 9q31.1	NR4A3 fusion/activation MSANTD3 fusion/amplification
Secretory carcinoma	t(12;15) (p13;q25) t(12;10) (p13;q11) t(12;7) (p13;q31) t(12;4) (p13;q31) t(10;10) (p13;q11)	ETV6::NTRK3 fusion ETV6::RET fusion ETV6::MET fusion ETV6::MAML3 fusion VIM::RET fusion
Microsecretory adenocarcinoma	t(5q14.3) (18q11.2)	MEF2C::SS18 fusion
Polymorphous adenocarcinoma		
Classic subtype Cribriform subtype	14q12 14q12 19q13.2 2p22.2	PRKD1 mutations PRKD1 fusions PRKD2 fusions PRKD3 fusions
Hyalinizing clear cell carcinoma	t(12;22) (q21;q12)	EWSR1::ATF1 fusions EWSR1::CREB1 fusions EWSR1::CREM fusions
Basal cell adenocarcinoma	16q12.1	CYLD mutations CTNNB1 mutation

Intraductal carcinoma		
Intercalated duct subtype	10q11.21	RET fusions TRIM27::NCOA4 fusions
Apocrine subtype	3q26.32 11p15.5	PIK3CA mutations HRAS mutations
Salivary duct carcinoma	17q21.1 8p11.23 17p13.1 3q26.32 11p15.5 Xq12 10q23.31 9p21.3	HER2 amplification FGFR1 amplification TP53 mutation PIK3CA mutation HRAS mutation AR copy gain PTEN loss CDKN2A loss
Myoepithelial carcinoma	8q12 t(12,22) (q21;q12)	PLAG1 fusions EWSR1::ATF1 fusions
Epithelial-myoepithelial carcinoma	11p15.5	HRAS mutations PLAG1 fusion HMGA2 fusion
Mucinous adenocarcinoma	14q32.33 17p13.1	AKT1 p.E17K mutations TP53 mutations
Sclerosing microcystic adenocarcinoma	1p36.33	CDK11B mutation
Sebaceous adenocarcinoma	2p21	MSH2 loss
Carcinosarcoma	none specific	
Lymphoepithelial carcinoma	Not reported	
Squamous cell carcinoma	Not reported	
Sialoblastoma	Not reported	
Carcinoma ex pleomorphic adenoma	8q12 12q13-15 17p13.1	PLAG1 fusions/amplification HMGA2 fusions/amplification TP53 mutations
(Pleomorphic adenoma)	8q12 12q13-15	PLAG1 fusions/amplification HMGA2 fusions/amplification

Druggable Genes and Proteins (including Drug Repositioning/Drug Repurposing)

Human Epidermal Growth Factor Receptor 2 (HER2)

HER2 is a proto-oncogene that is expressed or overexpressed in a variety of epithelial malignancies, including, breast, stomach, colon, rectum, biliary tract, and lung cancer [85-93]. Its overexpression is associated with *HER2* gene amplification or

mutation. That is, the *HER2* gene is amplified in 20% to 25% of primary breast cancers. Accordingly, HER2 inhibitors are used to treat HER2-positive breast and gastric cancers [86]. In SGCs, the overall frequency of HER2 overexpression is 17% and is predominantly seen in SDCs [94]. Other HER2-high expression tumors are CXPA, adenocarcinoma, NOS, squamous cell carcinoma, and MEC [95]. HER2-positive tumors, for example, breast carcinoma, have been treated with trastuzumab (Herceptin), and its use is expanding to gastric or colon cancers [87,88]. Recently, its use has been explored for the management of SDC, urothelial carcinoma, and bile duct adenocarcinoma, and so on [89–93]. Trastuzumab treatment for HER2-positive patients is correlated with good response and long-term survival [90]. Notably, an advanced anti-HER2 antibody, trastuzumab deruxtecan (T-Dxd, Enhertu), was developed, which is used for HER2-low breast cancers (HER2 IHC score of 1+, or 2+ without gene amplification) [96]. T-Dxd is an antibody-drug conjugate combination of trastuzumab and topoisomerase I inhibitor, which implies that its use could be expanded to treat a variety of tumors, including HER2-positive SDCs, in the future [97,98].

Androgen Receptor (AR)/NK3 Homeobox 1 (NKX3.1)

AR expression is mainly characteristic of SDC among all SGCs [93,99]. Owing to AR copy number gain, ligand-independent splice variants, and mutations, AR is overexpressed in typical SDCs [99]. Some SDCs are also positive for NKX3.1, α-methylacyl-CoA racemase (AMACR), and prostatic acid phosphatase (PAP), and the positivity of these androgen hormone-related proteins was reported in prostatic cancer [100,101]. Hence, androgen deprivation therapy is part of the standard of care for advanced and metastatic prostate cancer [102]. Similar to that for prostatic cancer, androgen deprivation therapy has been performed, and some reports have stated that it is effective for SDC patients [103]. However, the prognosis of patients with AR-, AMACR-, or PAP-negative SDC remains poor [93,100]. In particular, AR negativity is associated with significantly worse overall survival as splice variants and increased gene copy number may reduce the drug response and increase therapeutic resistance [104, 105].

Protein Receptor Kinase/Protein Kinase

Various genetic alterations have been reported for SGCs; among them are some oncogenic driver alterations (for example, *EGFR* mutation and *ALK* translocation; Table 4) [1,2,106]. These alterations accelerate tumorigenesis. For example, *RET* or *MET* acts on tumors with activating alterations as proto-oncogenes, such as point mutations or fusions. Therefore, these alterations are an easy therapeutic target. Alterations in *MET* and *RET* have been reported in 1.2% and 0.8% of non-small cell lung cancers, respectively [107-109]. Meanwhile, MET and RET inhibitors exhibit high efficacy rates and good tolerability [107–110]. Particularly for SGCs, the use of tyrosine kinase inhibitor (TKI)/TRK inhibitor is expected to be beneficial because these protein-coding gene alterations are detectable. Representative TKIs include those against vascular endothelial growth factor receptors (VEGFR), fibroblast growth factor receptors (FGFR), platelet-derived growth factor receptors (PDGFR), and so on [111]. These TKIs are related to cell regulation and survival as they influence angiogenesis and lymphangiogenesis. VEGFR inhibitors have been successfully used for the treatment of lung, stomach, liver, and kidney cancer [112]. Additionally, PDGFR inhibitors target gastrointestinal tumors, glioblastomas, sarcomas, leukemias, and dermatofibrosarcoma protuberans [113]. TRK is encoded by the *NTRK* gene family (*NTRK1*, *NTRK2*, and *NTRK3*) [114]. This proto-oncogene is responsible for cancer cell transformation, tumor cell proliferation, migration, and invasion. NTRK expression is detectable in approximately 90% of certain cancer types, including secretory breast carcinoma, secretory carcinoma in the salivary gland, and congenital infantile fibrosarcoma, while it is reported in less than 1% of common cancers such as non-small cell lung, colorectal, thyroid, and salivary gland cancers [115]. Currently, numerous protein inhibitors are being developed for various tumors and are expected to be effective against SGCs as many carry genetic alterations associated with tumorigenesis [63,83,87,116,117,118]. Among SGCs, the overall response rates to protein inhibitors and disease control rates (0–46.2% and 59.7–100.0%, respectively) are similar to those reported in chemotherapy trials [119].

Tumor-Infiltrating Lymphocytes (TILs)/Immunotherapy-Related Proteins

SGCs have shown lymphocytic infiltrations with or without lymphoid follicles, and certain cases are called tumor-associated lymphoid proliferation (TALP) [120]. This may be misdiagnosed as lymph node metastasis by pathologists if they are not aware; nonetheless, its relationship with the prognosis remains unclear [120]. In addition, the special SGC type, lymphoepithelial carcinoma, presents non-keratinizing poorly differentiated squamous cell-like carcinoma with a predominant lymphoid stroma [121]. Most cases show Epstein-Barr virus (EBV) infection, and this tumor has a relatively good prognosis [122]. Moreover, in tumor immune environment, TILs are related to the prognosis of breast cancer [123]. Although reports are conflicting, the number of TILs is associated with a better prognosis. Moreover, therapeutic effect is frequently correlated with the number of TILs and tumor mutation burden (TMB) in many tumors, such as malignant melanoma, colon cancer, pancreatic cancer, or biliary tract cancer [124–127]. TMB is the total number of DNA alterations in cancer cells and is measurable using NGS. Moreover, mismatch repair deficiency (dMMR) or microsatellite instability (MSI-H) causes high TMB; colorectal cancer includes two subtypes (Lynch syndrome and sporadic MSI-H cancer) [128]. Approximately 20% of colon cancer patients are MSI-H, and approximately 3% of patients with MSI-H colon cancer are diagnosed with Lynch syndrome [129]. The US Food and Drug Administration approved immune checkpoint inhibitors for metastatic MSI-H colon cancer and solid tumors with dMMR/MSI-H [130]. In addition, anti-cytotoxic T lymphocyte-associated antigen 4 (CTLA-4) agents and anti-PD-1 agent combination therapy have exhibited acceptable antitumor efficacy in MSI-H/dMMR metastatic colorectal cancers [130]. However, research on TILs related to global SGCs showed no significant difference in $CD8^+$ TIL density between disease-free survival and overall survival [131]. This may be owing to the limitation of not treating the tumors individually or the low case numbers. Hence, further examination is required.

Programmed death (PD)-1/ programmed death-ligand 1 (PD-L1) exists in the tumor microenvironment. Immunotherapy can kill tumor cells by activating antitumor immunity against tumor antigens. In particular, PD-1 is the most important receptor responsible for activating T-cells and mediating immunosuppression. However, PD-L1 is also involved in PD-1-related pathways, leading to the induction of T cell apoptosis or anergy [132]. The PD-1/PD-L1 pathway is the most notable checkpoint inhibitor pathway. Moreover, CTLA-4 is also the immune checkpoint target in clinical practice for many tumors [132,133]. When CTLA-4 translocates to the cell surface, CTLA-4 mediates inhibitory signaling into the T cell, and arrests cell proliferation and activation [134]. Hence, anti-CTLA-4 agents are expected to exhibit beneficial therapeutic effects. In particular, a monoclonal antibody against CTLA-4 effectively amplifies immune stimulation and boosts tumor annihilation [135]. This antibody has been applied for the treatment of non-small or small cell lung cancer, renal cell carcinoma, urothelial carcinoma, pancreatic cancer, gastric cancer, and malignant melanoma [135]. However, limited data are available regarding the therapeutic potential of immune checkpoint inhibitors for SGCs, especially MEC and AdCC [136]. In particular, PD-L2 may be an important biomarker in SGCs (for example, MEC, AdCC, and SDC) [133,137].

Other Targetable Genes and Proteins

In addition, though rare, the following tumors have specific proteins or gene alterations involving tumorigenesis: (i) nuclear protein in testis (NUT); NUT carcinoma, (ii) subfamily of ATP-dependent chromatin remodeling complexes SWI/SNF (switch/sucrose non-fermentable); SWI/SNF related, matrix associated, actin dependent regulator of chromatin, subfamily B, member 1 (SMARCB1)-deficient high-grade transformed/dedifferentiated acinic cell carcinoma, (iii) BRAF; IPMN, (iv) neuroendocrine granules; small cell neuroendocrine carcinoma “Merkel type” (SNECM), large cell neuroendocrine carcinoma, and (v) salivary gland carcinoma with viral infection (human papillomavirus, EBV, polyomaviruses, and so on) [138–149]. These unique proteins or gene alterations might be related

to tumorigenesis and, thus, may represent novel therapeutic targets.

Conclusions

We discussed the prognostic factors, focusing on the histopathological findings for SGCs, and described the current scenario and future perspectives. The interaction between clinicians and pathologists is essential since the pathological report includes many prognostic factors for patients and should be read carefully. Furthermore, genetics and molecular pathology are continuously advancing. Thus, novel information is continuously emerging, requiring further exploration.

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