Cutaneous Squamous Cell Carcinoma

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KEYWORDS

• Squamous cell carcinoma • Actinic keratosis • Keratoacanthoma
• Spindle cell squamous cell carcinoma • Desmoplastic squamous cell carcinoma
• Acantholytic squamous cell carcinoma • Pathogenesis

KEY POINTS

- There is a persistent trend for an increasing incidence of cutaneous squamous cell carcinoma (cSCC).
- It is crucial to differentiate cSCC from the benign and reactive squamoproliferative lesions and report the high-risk features associated with an aggressive tumor behavior.
- Understanding the molecular mechanisms that drive the development and progression of cSCC is necessary to develop diagnostic and prognostic assays and targeted therapies.

INTRODUCTION

Epidemiology

Nonmelanoma skin cancer is the most common malignancy worldwide. Historically, cutaneous squamous cell carcinoma (cSCC) has been thought to comprise about 20% of all nonmelanoma skin cancers, thus being the second most common malignancy after basal cell carcinoma (BCC), with a ratio of BCC to SCC estimated to be 4:1.[1,2] However, recent data indicate that there is a significant shift underway in the relative proportion of nonmelanoma skin cancer, with the ratio of BCC to SCC found to be 1.0 in the US Medicare population.[3] Several other studies bear out a trend for an increasing incidence of cSCC compared with BCC, particularly in the aging population.[4–8] An accurate incidence of cSCC is not known because it is not required to be reported to national cancer registries; however, a metaanalysis of population-based studies estimated that in 2012, 186,157 to 419,543 white individuals were diagnosed with cSCC in the United States alone. Note, these estimates do not include squamous cell carcinoma in situ (SCCIS), which likely occurs more frequently.[9]

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Etiopathogenesis

Most cSCC arise in the sun-damaged skin of the elderly white individuals of European ancestry, in the background of preexisting lesions of actinic keratosis (AK).[1] Apart from ultraviolet (UV) radiation exposure, other predisposing factors include chronic immunosuppressed state (solid organ transplantation, human immunodeficiency virus infection),[10–13] chronic skin conditions (burn scars, hidradenitis suppurativa, chronic osteomyelitis, discoid lupus erythematosus, lichen plans, lichen slecrosus et atrophicus),[14–20] inherited genetic conditions (albinism, epidermolysis bullosa, xeroderma pigmentosum),[21–23] exposure to ionizing radiation,[24] chronic arsenic exposure,[25] human papillomavirus infection,[26,27] and treatment with BRAF inhibitors (vemurafenib and dabrafenib),[28] among others.

Clinical Features

AK and SCCIS are considered to be the precursor lesions of cSCC in most instances, and, frequently, patients present with cSCC in association with numerous precursor lesions. AK and SCCIS typically present as flesh-colored, pink, brown, often pigmented, scaly patches, papules, or plaques on an erythematous base. Lesions of cSCC manifest a range of clinical presentations, including papules, plaques, or indurated nodules with a smooth, scaly, verrucous, or ulcerative surface. Cutaneous SCC can be asymptomatic, pruritic, or tender. Local neuropathic symptoms such as numbness, burning, paresthesia, or paralysis are associated with perineural invasion.[29] Although cSCC typically arises on the sun-exposed areas of fair-skinned individuals and often on the sun-exposed areas of dark-skinned individuals, an involvement of the non–sun-exposed areas is more common in dark-skinned individuals.[30,31]

PRECURSOR LESIONS

Actinic Keratosis

Also known as solar keratosis, AK represents an early precursor lesion that can accumulate additional mutations and in some cases progress to SCCIS and invasive SCC.[32] Clinically, AKs often manifest spontaneous regression and approximately one-third of AKs exhibit regression in 1 year.[33]

Histologically, AK occurs as a proliferation of cytologically atypical keratinocytes that is confined to the lower levels of the epidermis. The lesional cells show loss of polarity, increased size, pleomorphic and hyperchromatic nuclei, and an increased number of mitoses. There is often an increased nuclear:cytoplasmic ratio within lesional cells. There is crowding of the basal portion of the epidermis with variable acanthosis and/or budding of the neoplastic keratinocytes in the papillary dermis, without breach of the basement membrane. By definition, the atypical proliferation does not occupy the full thickness of the epidermis. Hypogranulosis is often seen. The stratum corneum overlying the atypical keratinocytes typically shows hyperkeratosis with parakeratosis. Because the preneoplastic process usually spares the adnexal structures, this results in alternating areas of orthokeratosis and parakeratosis (flag sign). The underlying dermis almost invariably shows solar elastosis, which represents an important diagnostic clue. AKs exhibit a variety of histologic variants with a broad range of histologic patterns.[34,35]

Pigmented actinic keratosis

This variant shows hyperpigmentation of the lower epidermal layers owing to an increased amount of melanin in the basilar keratinocytes. Melanophages may be present in the superficial dermis. It is important to recognize this entity because it can be

confused clinically, as well as histologically, with melanoma in situ, particularly in the presence of severe solar elastosis. There may be mild melanocytic hyperplasia of melanocytes typical of that seen in sun-damaged skin. Melanocytes in these lesions do not manifest cytologic atypia. Immunohistochemistry with melanocytic markers is useful in difficult cases.

Lichenoid actinic keratosis
This variant is characterized by a dense, bandlike lymphocytic infiltrate at the dermal–epidermal junction with focal vacuolar alteration and necrosis of the basal keratinocytes. This entity may be confused morphologically with benign lichenoid keratosis and lichenoid regression in melanoma.

Bowenoid actinic keratosis
In this variant, the atypical keratinocytes occupy almost the full thickness of epidermis and yet do not reach the level of SCCIS. There may be palisading in the basal layer. The adnexal sparing character of AK is often helpful in distinguishing this variant from SCCIS or Bowen's disease.

Proliferative actinic keratosis
In this variant, atypical keratinocytes extend fingerlike projections in the superficial dermis. Examination of multiple, deeper level sections is often helpful in allaying a concern for superficial invasion. This variant is associated with a more aggressive behavior.[36]

Hypertrophic actinic keratosis
This variant demonstrates epidermal hyperplasia with a prominent hyperparakeratotic stratum corneum. Often, the epidermal changes suggestive of a superimposed lichen simplex chronicus are also present.

Atrophic actinic keratosis
This variant shows atrophic changes in the form of thinned out epidermis and flattened rete ridges.

Acantholytic actinic keratosis
This variant is characterized by acantholysis of atypical keratinocytes resulting in detachment from each other and intraepidermal clefting. Dyskeratosis may be present. The differential diagnosis includes benign acantholytic disorders.

Squamous Cell Carcinoma In Situ

SCCIS occupies the intermediate step in the progression from AK to invasive SCC. Although some use SCCIS and Bowen's disease terminology interchangeably, Bowen's disease typically occurs in the anogenital region and is unrelated to UV-induced AK and more often associated with human papillomavirus infection, thus being more common in young adults.[37]

Histologically, SCCIS exhibits full-thickness atypia of the epidermis, sparing the adnexal structures. The hyperparakeratosis can be minimal or exuberant and can produce a cutaneous horn. The atypical keratinocytes show nuclear pleomorphism, hyperchromasia, frequent mitoses with atypical forms, and apoptosis. The loss of polarity imparts a "windblown" appearance. Frequently, the atypical keratinocytes spare the basal layer and produce a characteristic pattern called the "eyeliner sign," a useful diagnostic clue observable on a low-power examination. By definition, there is no dermal invasion. Similar to AK, several histomorphologic variants of SCCIS have been described, including hyperkeratotic, atrophic, verrucous, psoriasiform,

acantholytic, clear cell, and pagetoid subtypes. It is important to histologically distinguish the pagetoid variant of SCCIS from extramammary Paget's disease and melanoma in situ. Immunohistochemical (IHC) markers such as CK7, CAM5.2, carcinoembryonic antigen, and epithelial membrane antigen (positive in extramammary Paget's disease, negative in SCCIS), p63 (positive in SCCIS, negative in extramammary Paget's disease), and MART1 (positive in melanoma in situ) are helpful in diagnosing difficult cases.[38,39]

INVASIVE CUTANEOUS SQUAMOUS CELL CARCINOMA

Cutaneous SCC can arise as the result of tumor progression in the sun-damaged skin or can occur de novo. It is characterized by invasion of the dermis by the neoplastic squamous epithelial cells. The invasive component can take the form of infiltrating cords, sheets, or single cells, or can present as well-circumscribed nodules, squamous islands, or cystic structures composed of malignant keratinocytes. Interestingly, in contrast with AK and SCCIS, the cytomorphology of malignant keratinocytes in cSCC can vary from a very banal appearance to a highly anaplastic one.[40,41]

Histologic Grading

Lesions of cSCC can be histologically divided into 3 grades based on their degree of differentiation: well, moderately, and poorly differentiated. The factors taken into consideration for this type of grading include the degree of keratinization, nuclear atypia, and the degree of architectural atypia (well circumscribed vs infiltrative).

A well-differentiated cSCC shows slightly enlarged keratinocytes with abundant, glassy-pink to eosinophilic cytoplasm. Intercellular bridges are generally visible. Keratinization is usually present and morphologically manifests as a central plug of keratinization within a nest of well-differentiated keratinocytes, commonly referred to as a "keratin pearl." Importantly, identifying the retention of keratinocyte nuclei (parakeratosis) within these keratin pearls is often useful in discriminating a well-differentiated cSCC from a benign squamoproliferative lesion in a superficial biopsy. Well-differentiated cSCC tends to be well-circumscribed with pushing margins and a lobulated appearance. In contrast, a poorly differentiated cSCC shows a highly infiltrative pattern and is composed of highly atypical keratinocytes with pleomorphic, hyperchromatic nuclei, numerous atypical mitotic figures, and shows little or no keratinization[40,42] (**Fig. 1**).

Histologic Variants

Several histologic variants of cSCC have been described. Knowledge of these entities has diagnostic and prognostic significance.

Acantholytic squamous cell carcinoma

This variant is also known as adenoid SCC, adenoacanthoma of sweat glands, and pseudoglandular SCC. Rare subtypes such as small cell SCC, pseudovascular SCC, and pseudoangiosarcomatous SCC have been described. Histologically, the lesional cells show a variable degree of desmosomal disruption, resulting in rounded cells with centrally placed round nuclei. Acantholysis results in various morphologic patterns, such as pseudoglandular, pseudoalveolar, or pseudovascular spaces. Based on their involvement of the follicular epithelium alone or involvement of follicular epithelium and interfollicular epidermis, these tumors have also been further subdivided as the follicular type and follicular pattern, respectively[42–47] (**Fig. 2**A).

The differential diagnoses for acantholytic SCC include adenoid BCC, eccrine carcinoma, metastatic adenocarcinoma, and, rarely, angiosarcoma. Identifying

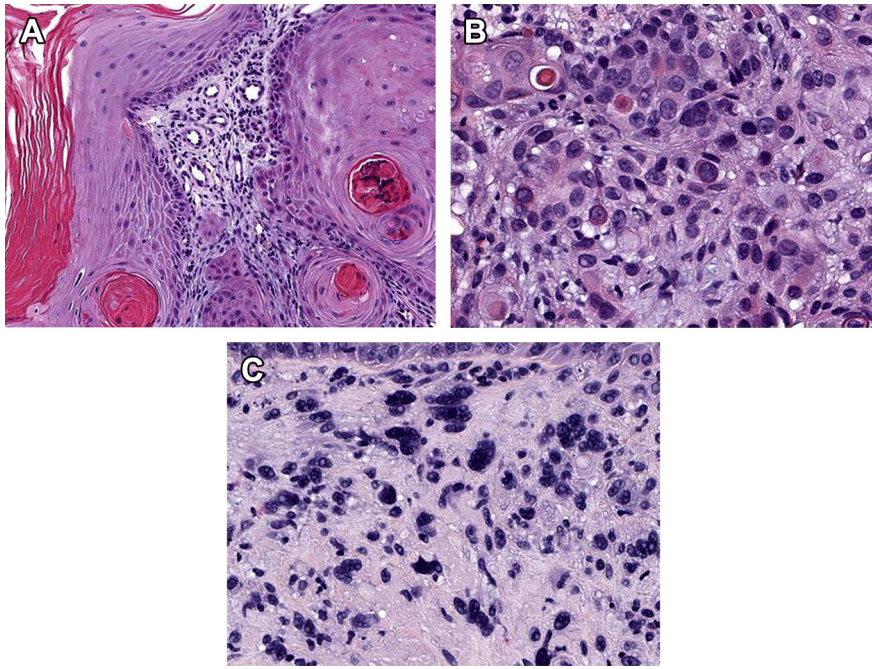

Fig. 1. Squamous cell carcinoma. (*A*) Well-differentiated. The tumor shows nests of mature keratinocytes with a low nuclear:cytoplasmic ratio and "keratin pearls" (original magnification, ×200), (*B*) Moderately differentiated. The tumor shows cellular pleomorphism, few, if any, keratin pearls and cells with more prominent cellular atypia (original magnification, ×400). (*C*) Poorly differentiated. The tumor shows infiltrative pattern and highly atypical keratinocytes with pleomorphic, hyperchromatic nuclei, and little to no keratinization (original magnification, ×400).

characteristic areas with basaloid cells, peripheral palisading, single cell necrosis, artifactual clefting, and stromal mucin would help to distinguish the adenoid BCC. Identifying ductal structures with a basal or myoepithelial layer that stains for smooth muscle actin, p63, calponin, or S100 protein, luminal borders that stain for carcinoembryonic antigen, and luminal secretions that stain with periodic acid–Schiff distase help to distinguish the eccrine carcinoma. Metastatic adenocarcinoma can be suspected from the clinical history, a multiplicity of lesions, and a lack of epidermal connection. Use of high- and low-molecular-weight cytokeratin antibodies and a battery of immunostains specific for adenocarcinomas from various sites of origin are essential in arriving at the correct diagnosis. Angiosarcoma can be suspected from blood-filled spaces and confirmed with various endothelial markers such as CD31, CD34, and ERG.

Adenosquamous carcinoma

This rare variant of cSCC is characterized by true glandular differentiation, in contrast with the pseudoglandular appearance seen in the acantholytic SCC. Histologically, the atypical squamoid cells are arranged as interconnecting nests, frequently forming keratocysts. Additionally, there are focal or diffuse areas of gland formation within the squamous nests. These glands are lined by cuboidal to low columnar epithelium that shows luminal positivity for carcinoembryonic antigen. The luminal secretions

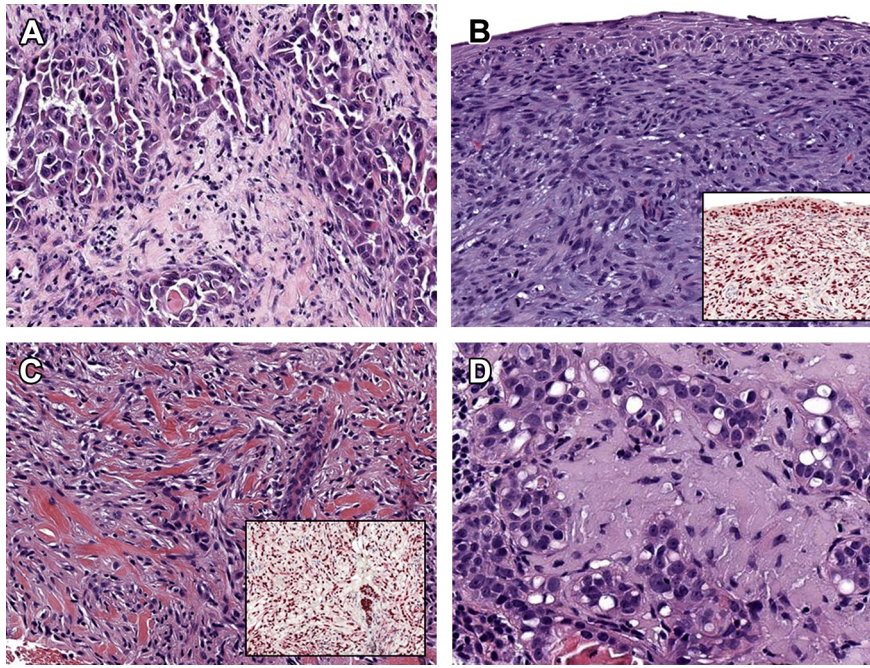

Fig. 2. Squamous cell carcinoma. (*A*) Acantholytic. Desmosomal disruption results in clefting and rounding of the tumor cells (original magnification, ×200). (*B*) Spindle cell. Haphazard growth of atypical spindle-shaped keratinocytes in the dermis. Inset: p63 immunostain confirms epithelial origin (original magnification, ×200). (*C*) Desmoplastic. Infiltrating cords of spindled tumor cells surrounded by a densely collagenous stroma. Inset: p63 immunostain (original magnification, ×200). (*D*) Signet ring cell. A variable number of tumor cells show clear cytoplasm that pushes the nucleus to the periphery imparting a signet ring appearance (original magnification, ×400).

stain with mucicarmine and Alcian blue at a pH of 2.5. The epidermal origin is evidenced by multifocal epidermal connections. The tumor commonly invades the deep dermis.[48–50]

The differential diagnosis for this variant includes primary cutaneous mucoepidermoid carcinoma and metastatic adenocarcinomas from various sites of origin. Primary cutaneous mucoepidermoid carcinoma is a controversial entity and, if it does exist, currently there is no reliable way to distinguish it from adenosquamous carcinoma.[51] Distinction from metastatic adenocarcinomas requires a thorough clinical history and imaging studies to identify a primary site, the presence of multiple lesions, histologic demonstration of a lack of epidermal connection, and, when necessary, judicious use of IHC markers.

Spindle cell squamous cell carcinoma

This variant is also known as sarcomatoid SCC. Histologically, this tumor is characterized by a haphazard growth of atypical spindle-shaped cells in the dermis. Connection with the epidermis is not always present. The atypical spindle cells may constitute all or part of the tumor, with none or a variable component of conventional SCC forming nests, cords, and keratin pearls. Occasionally, bizarre and pleomorphic giant cells and heterologous elements with numerous mitotic figures are seen. The tumor often

infiltrates deep into the dermis, subcutis, fascia, muscle, and bone.[52–54] Importantly, there is not significant stromal desmoplasia (>30% of the tumor volume), because that would raise the diagnosis of the desmoplastic variant of cSCC[55] (see Fig. 2B).

The differential diagnosis for this variant, in the absence of an epidermal connection or an obvious evidence of keratinization, is an atypical spindle cell lesion of the dermis. This would include spindle cell/desmoplastic melanoma, leiomyosarcoma, and atypical fibroxanthoma or undifferentiated pleomorphic sarcoma, among other reactive and neoplastic dermal spindle cell proliferations. The use of the IHC markers is often required to derive a definitive diagnosis. Spindle cell SCC stains positively for p63, p40, and high-molecular-weight cytokeratins such as CK5/6.[56,57] Desmoplastic melanoma stains for S100 protein and SOX10, and leiomyosarcoma stains for smooth muscle actin and caldesmon.

Desmoplastic squamous cell carcinoma

Histologically, this variant is characterized by infiltrating cords of spindled–squamoid tumor cells surrounded by a densely collagenous (desmoplastic) stroma. In contrast with spindle cell SCC, the lesional squamous cells are oval to spindle shaped and can show single-cell keratinization. Keratin pearls are generally present, even in high-grade tumors, and the desmoplastic stromal component in this tumor should constitute greater than 30% of the tumor volume. Perineural invasion is frequent with this variant[55,58,59] (see Fig. 2C).

The differential diagnoses for this variant are entities that show sclerotic, desmoplastic stromal response with resultant infiltrative appearance. These include syringoma, desmoplastic trichoepithelioma, microcystic adnexal carcinoma, morpheaform BCC, and desmoplastic melanoma. The presence of epidermal squamous atypia and evidence of keratinization point to the diagnosis of desmoplastic SCC. Ductal differentiation points to the diagnoses of adnexal neoplasms. A diagnosis of morpheaform BCC would require identifying the typical findings of BCC, such as individual cell necrosis, mitotic figures and stromal retraction artifact in at least a focal manner. Additionally, the tumor cords in morpheaform BCC show sharp angulation that is quite characteristic. Desmoplastic melanoma is associated with the findings of in situ melanocytic lesion in the overlying epidermis and nodular lymphoid aggregates within the dermal component. Use of p63 (positive in SCC) and S100 and SOX10 (positive in desmoplastic melanoma) is helpful in difficult cases.

Clear cell squamous cell carcinoma

Also referred to as hydropic SCC or pale cell SCC, these rare tumors are subdivided into 3 categories: type I (keratinizing), type II (nonkeratinizing), and type III (pleomorphic). Type I tumors are characterized by sheets or islands of clear cells with peripherally displaced nuclei or central nuclei with bubbly cytoplasmic appearance, and focal areas of keratinization, even forming keratin pearls. Type II tumors are dermal masses without connection to the epidermis. Tumor cells show a cytoplasm with a finely reticulated clear appearance and are arranged in parallel or anastomosing cords separated by a fibrotic stroma with a heavy inflammatory infiltrate. There may be a central necrosis but, importantly, keratinization is absent. Type III tumors typically show extensive ulceration. Tumor cells are markedly pleomorphic with foci of acantholysis, dyskeratosis, keratinization, and perineural and lymphovascular invasion.[42,60]

The histologic differential diagnosis for clear cell SCC is broad: clear cell acanthoma, trichilemmoma, trichilemmal carcinoma, clear cell hidradenoma, hidradenocarcinoma, sebaceous tumors, clear cell BCC, balloon cell nevus, balloon

cell melanoma, and metastatic renal cell carcinoma, among other entities with clear cell changes.[61] A high index of suspicion, a thorough analysis of all histologic sections, and a judicious use of IHC markers are necessary to arrive at this diagnosis.

Signet ring cell squamous cell carcinoma

This is an extremely rare variant. Histologically, this tumor is composed of a variable number of signet ring cells, where a clear cytoplasm pushes the nucleus to the periphery imparting a signet ring appearance. The cytoplasm is negative for mucin and shows focal PAS positivity with diastase sensitivity[62,63] (see **Fig. 2**D).

The differential diagnosis for this variant is extensive, because several primary cutaneous neoplasms such as BCC, melanoma, histiocytoid carcinoma of the eyelid, and lymphoproliferative diseases, as well as metastatic adenocarcinomas and soft tissue tumors can demonstrate signet ring cell changes. Once the signet ring cell changes are noted, identifying a focus of conventional SCC in the histologic sections in conjunction with IHC and special stains should promote derivation of the correct diagnosis.[64,65]

Pigmented squamous cell carcinoma

This variant is characterized by a colonization of the conventional SCC by benign, heavily pigmented dendritic melanocytes. Histologically, the tumor is composed of lobules, nests, and cords of atypical squamous cells showing evidence of keratinization. Intermixed within the tumor cells are numerous darkly pigmented dendritic melanocytes that stain for melanocytic markers such as MART1, HMB45, and S100 protein, although HMB45 can be negative in rare cases. Rare focal positivity of the squamoid tumor cells for melanocytic markers is likely secondary to antigen transfer.[66–68]

The histologic differential diagnosis for this variant includes other pigmented entities such as seborrheic keratosis, melanoacanthoma, pigmented trichoblastoma, pigmented pilomatricoma, pigmented BCC, melanoma with pseudoepitheliomatous hyperplasia, and an exceedingly rare dermal squamomelanocytic tumor.[69] Of these, the one tumor that is easy to be confused with pigmented SCC with potentially serious consequences is melanoma with pseudoepitheliomatous hyperplasia, where the malignant and benign components are transposed. A careful examination and identification of atypical melanocytes is essential to avoid this pitfall.

Verrucous carcinoma

This variant has a very distinctive silhouette owing to its endo-exophytic growth, and prominent acanthosis, papillomatosis, and hyperkeratosis. One key histologic feature is the blunt, broad, squamous epithelial projections that push into the dermis, rather than infiltrate the dermis. The tumor cells show a bland cytomorphology and the human papillomavirus-related cytopathic changes are not obvious. Rabbit burrow–like sinuses and keratocysts, and a dense inflammatory infiltrate are typically seen in carcinoma cuniculatum, a subtype of verrucous carcinoma localized to the plantar surface[42,70,71] (**Fig. 3**A).

The histologic differential diagnosis for this variant includes condyloma acuminatum, verruca vulgaris, keratoacanthoma, prurigo nodularis, and pseudoepitheliomatous hyperplasia. Clinicopathologic correlation and the availability of adequate biopsy material that includes the base of the tumor are essential for arriving at the correct diagnosis.

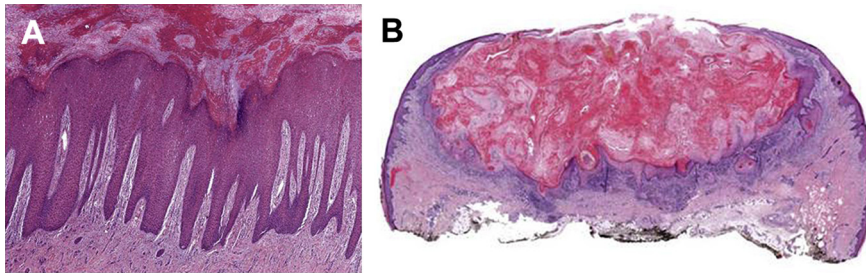

Fig. 3. (*A*) Verrucous carcinoma. Blunt, broad-based, squamous epithelial projections that push, rather than infiltrate, into the dermis (original magnification, ×20). (*B*) Keratoacanthoma. Dome-shaped nodule with a central keratin-filled crater (original magnification, ×20).

Keratoacanthoma

KA commonly presents as a rapidly growing, solitary, dome-shaped nodule with a central keratin-filled crater. The fact that it undergoes spontaneous resolution has led to a decades-long debate and uncertainty over the classification of this lesion with views ranging from KA being a benign squamoproliferative lesion, a continuum between benign and malignant proliferation, to an outright cSCC that has the biological capacity to regress. We have incorporated this entity here to enable its recognition from conventional SCC. Several clinical variants of KA are recognized including giant KA, mucosal KA, subungual KA, keratoacanthoma centrifugum marginatum, and multiple KAs associated with Ferguson-Smith disease, generalized eruptive keratoacanthomas of Grzybowski, multiple familial keratoacanthoma of Witten and Zak, Muir-Torre syndrome, and subungual tumors associated with incontinentia pigmenti[42,72–74] (see **Fig. 3**B).

Histologically, KAs are composed of mature-appearing keratinocytes that form a large, symmetric, exo-endophytic mass with a central crateriform invagination filled with a keratin plug. Typically, there is buttressing of the surrounding normal epidermis around the mass. The tumor cells have a characteristic pink, glassy cytoplasm and lack the pleomorphism and atypia seen in conventional SCC. Most KAs show scattered neutrophils and eosinophils, occasionally forming microabscesses. Perforating elastic fibers are a characteristic finding.[75] Mixed inflammatory infiltrate and small islands of tumor cells may be present in the underlying dermis, and the lesions lack infiltrative features. The histologic differential diagnosis for KA includes well-differentiated conventional SCC and pseudoepitheliomatous hyperplasia found in association with inflammatory or reactive conditions.

High-Risk Features

Although the vast majority of cSCCs are cured with complete excision, a subset of cSCCs with certain histologic and clinical features exhibits a significantly increased risk of local recurrence and metastasis, and resultant poorer prognosis.[76–78] The incidence of regional or distant metastases is estimated to be as high as 2% to 6% in such cases.[79,80] Several staging systems have been proposed to stratify the cSCC prognosis based on a number of known risk factors. These include the 2002 TNM staging system proposed by the American Joint Committee on Cancer,[81] the revised American Joint Committee on Cancer and International Union Against Cancer staging systems,[77,82] Brigham and Women's Hospital tumor staging system,[83] National Comprehensive Cancer Network guidelines[84] (**Table 1**), and European Organization for Research and Treatment of Cancer guidelines[85] (**Table 2**).

Table 1
National Comprehensive Cancer Network clinical practice guidelines, version I.2017: risk factors for local recurrence or metastasis

History and Physical Examination	Low Risk	High Risk
Location/size[a]	Area L <20 mm Area M <10 mm[d]	Area L ≥20 mm Area M ≥10 mm Area H[e]
Borders	Well defined	Poorly defined
Primary vs recurrent	Primary	Recurrent
Immunosuppression	(–)	(+)
Site of prior RT or chronic inflammatory process	(–)	(+)
Rapidly growing tumor	(–)	(+)
Neurologic symptoms	(–)	(+)
Pathology	**Low Risk**	**High Risk**
Degree of differentiation	Well or moderately differentiated	Poorly differentiated
Adenoid (acantholytic), adenosquamous (showing mucin production), desmoplastic, or metaplastic (carcinosarcomatous) subtypes	(–)	(+)
Depth[b,c]: Thickness or Clark level	<2 mm or I, II, III	≥2 mm or IV, V
Perineural, lymphatic, or vascular involvement	(–)	(+)

Area H = "mask areas" of face (central face, eyelids, eyebrows, periorbital, nose, lips [cutaneous and vermilion], chin, mandible, preauricular and postauricular skin/sulci, temple, ear), genitalia, hands, and feet. Area M = cheeks, forehead, scalp, neck, and pretibia. Area L = trunk and extremities (excluding pretibia, hands, feet, nail units, and ankles).

[a] Must include peripheral rim of erythema.

[b] If clinical evaluation of incisional biopsy suggests that microstaging is inadequate, consider narrow margin excisional biopsy.

[c] A modified Breslow measurement should exclude parakeratosis or scale crust, and should be made from base of ulcer if present.

[d] Location independent of size may constitute high risk.

[e] Area H constitutes high risk based on location, independent of size. Narrow excision margins owing to anatomic and functional constraints are associated with increased recurrence rates with standard histologic processing. Complete margin assessment, such as with Mohs micrographic surgery, is recommended for optimal tumor clearance and maximal tissue conservation. For tumors less than 6 mm in size, without other high-risk features, other treatment modalities may be considered if at least 4-mm clinically tumor-free margins can be obtained without significant anatomic or functional distortions.

From Bichakjian CK, Farma JM, Schmults CD, et al. NCCN Clinical Practice Guidelines in Oncology (NCCN Guidelines) Squamous Cell Skin Cancer. Fort Washington (PA): National Comprehensive Cancer Network, Inc; 2016; with permission.

Clinical high-risk features

Tumor location Tumors arising in head and neck locations (eg, forehead, temporal region, scalp, lips, ears) show increased rates of local recurrence and metastasis.[79,86,87] A recent metaanalysis showed that the anatomic locations of lips, ears, and temple are independent predictors of metastasis, although, in this analysis, tumor location on lips or ears did not independently predict local recurrence.[88] Additionally, tumors developing in chronic wounds or scars or at the site of prior burns or radiation therapy

Table 2
European Organization for Research and Treatment of Cancer Guidelines: prognostic risk factors in primary cutaneous squamous cell carcinoma

	Tumor Diameter	Location	Depth/Level of Invasion	Histologic Features	Surgical Margins	Immune Status
Low risk	<2 cm	Sun exposed sites (except ear/lip)	<6 mm/invasion above subcutaneous fat	Well-differentiated common variant or verrucous	Clear	Immunocompetent
High risk	>2 cm	Ear/lip Non–sun-exposed sites (sole of foot) SCC arising in radiation sites, scars, burns or chronic inflammatory conditions Recurrent SCCs	>6 mm/invasion beyond subcutaneous fat	Moderately or poorly differentiated grade Acantholytic, spindle, or desmoplastic subtype Perineural invasion	Incomplete excision	Immunosuppressed (organ transplant recipients, chronic immunosuppressive disease or treatment)

Abbreviation: SCC, squamous cell carcinoma.

From Stratigos A, Garbe C, Lebbe C, et al. Diagnosis and treatment of invasive squamous cell carcinoma of the skin: European consensus-based interdisciplinary guideline. Eur J Cancer 2015;51(14):1989–2007; with permission.

are more likely to behave aggressively in terms of local recurrence and increased rate of metastasis.[89–91]

Recurrence status Not surprisingly, tumor recurrence itself is a high-risk feature.[88] Recurrent tumors tend to be larger, are more likely to manifest perineural invasion, lymphovascular invasion, subcutaneous invasion, and lymph node metastasis, and are associated with poorer disease-specific survival.[86,87,92,93]

Number of tumors Multiple cSCC are associated with an increased risk of local recurrence and lymph node metastasis. In 1 study, having more than 1 tumor increased the risk of local recurrence and nodal metastasis by 2- to 4-fold and 3- to 4-fold, respectively.[94]

Immunosuppression Solid organ transplant recipients on immunosuppressive therapy develop aggressive tumors at an increased frequency. This increased incidence is estimated to be as high as 65- to 250-fold as compared with the general population.[11,95] These tumors also exhibit rapid growth, an increased rate of local recurrence, and metastasis.[96,97] Thus, the immunosuppressed state is an independent predictor of poor outcomes.[98]

Histopathologic high-risk features

Tumor size Cutaneous SCC tumors with a 2.0-cm or greater maximum diameter are more likely to metastasize.[79,86] A metaanalysis has shown that a tumor diameter of 2.0 cm or greater is independently predictive of recurrence and metastasis.[88] The increased recurrence and metastasis rates for an SCC 2.0 cm or greater in size arising on lips and skin were 2-fold and 3.3-fold, respectively, when compared with tumors less than 2.0 cm in size.[87]

Tumor thickness and depth of invasion Tumor thickness and depth of invasion are independent predictors of both local recurrence and metastasis.[79,88] The American Joint Committee on Cancer and National Comprehensive Cancer Network guidelines consider an invasion depth of 2.0 mm or greater or Clark level IV or higher as the high-risk factor.[77,84] A corollary of the prognostic significance of the depth of invasion could be that cSCC tumors of identical thickness may show different clinical behavior based on their body location, owing to the varying thickness of dermis and subcutaneous tissue.

Margin status Margin-positive reexcision is recently identified as an independent risk factor for locoregional recurrence, whereas margin-negative reexcision is associated with a low-risk prognosis (29% vs 5% local recurrence). Hence, while evaluating a reexcision specimen, patients with a positive margin should be considered at high risk for recurrence.[85,99]

Histologic grade Tumor differentiation grade is an independent predictor of recurrence, metastasis, and patient survival.[79,86,88] Indeed, in 1 study, well-differentiated cSCC showed a local recurrence rate of 13.6%, and a 5-year cure rate of 94.6%, whereas the poorly differentiated cSCC showed a recurrence rate of 28.6% and 5-year cure rate of 61.5%.[87] A more recent metaanalysis showed that the 5-year metastasis-free and overall survival rates were significantly higher in well-differentiated tumors (70%) as compared with moderately differentiated (51%) and poorly differentiated (26%) tumors.[88]

Histologic subtype Although it is customary to think of several cSCC histologic subtypes as being associated with an aggressive tumor behavior, for the most part, there

are insufficient data in this regard. For example, acantholytic SCC is thought to be highly aggressive, but this is not convincingly supported by published literature.[47,100] In contrast, desmoplastic SCC or tumors with infiltrative and desmoplastic growth patterns are associated with aggressive behavior in terms of local recurrence and metastasis.[59,101] The 2016 National Comprehensive Cancer Network Clinical Practice Guidelines for cSCC designates acantholytic, adenosquamous, and desmoplastic SCC subtypes as high-risk factors.[84] The current European Organization for Research and Treatment of Cancer guidelines list acantholytic, spindle, and desmoplastic subtypes as the high-risk prognostic factors.[85] KA, when identified with certainty based on clinical and histologic features, is not regarded as a subtype of cSCC and that is borne out by a recent metaanalysis of 445 cases of KA with reported follow-up; none of these cases resulted in death or distant metastases.[102]

Perineural invasion Perineural invasion independently predicts increased rate of local recurrence and metastasis. In particular, perineural invasion of large-caliber nerves ($\geq$0.1 mm) is associated with an increased likelihood of lymph node metastasis and higher mortality rate.[92,103,104] In cSCC of the head and neck region, 1 study found perineural invasion in 14% of all cases, which was associated with increased incidence of cervical lymphadenopathy, distant metastasis, and a significantly reduced survival.[105]

Lymphovascular invasion Lymphovascular invasion is an independent predictor of lymph node metastasis[106,107] and disease-specific death.[103]

Our recommendations for pathology reporting

Based on this discussion of the current evidence and guidelines, we recommend that a pathology report includes a comment on the following features:

- *Tumor size* - particularly when approaching or more than 2 cm
- *Tumor thickness* - particularly when approaching or more than 2 mm
- *Tumor depth* - particularly when approaching or more than Clark level IV
- *Margin status* - particularly in the reexcision specimens
- *Histologic grade* - particularly when poorly differentiated
- *Histologic subtype* - particularly when acantholytic, adenosquamous, spindle cell, or desmoplastic
- *Perineural invasion* - particularly when involving a nerve approaching or greater than 0.1 mm in diameter
- *Lymphovascular invasion*

MOLECULAR PATHOGENESIS

Importance

The high burden of cSCC produces significant morbidity and mortality around the world; therefore, diagnosing and treating cSCC early in its development is crucial and will minimize morbidity and conserve health care resources. Unfortunately, owing to their cosmetic or functional consequences, dermatologic biopsies are often small and superficial, and hence the entire lesion is frequently not available for examination. This often leads to 1 of 3 undesirable consequences: (1) repeat biopsy, which increases health care costs, (2) overdiagnosis, which leads to an unnecessary reexcision, increased morbidity, and an increased health care costs, or (3) underdiagnosis, which results in a missed opportunity to diagnose cSCC early and may result in increased morbidity and mortality. Therefore, identifying the unique molecular alterations associated with cSCC development, and developing assays that use these molecular alterations as markers of malignancy is of paramount importance. Ideally, such

assays would significantly increase the diagnostic yield even with a limited biopsy specimen. Moreover, in regard to cSCC treatment, there is no standard of care beyond complete surgical excision of the lesion, and the therapeutic options for locally advanced and metastatic disease are limited. Consequently, identifying molecular targets and pathways that drive cSCC development is imperative. We provide a brief overview of the current state of knowledge.

Chromosomal Aberrations, Instability, and Epigenetic Changes

Cytogenetic studies in cSCC have revealed a large number of complex allelic alternations such as deletions, insertions, and translocations.[108] The chromosomes most commonly affected include chromosomes 1, 11, 8, 9, 5, 3, and 7. The most frequently rearranged chromosomal sites are pericentromeric, such as 8q10-q11, 1p10-q12, 5p10-q11, 11p15, and 9p10-q10. Recurrent anomalies such as i(1q), i(8q), i(5p), i(1p), i(9p), and i(9q); losses of part of or the entire chromosomes 2, 4, 8, 9, 11, 13, 14, 18, 21, X and Y, and overrepresentation of 1q, chromosome 7, and 8q have been identified as most frequent cytogenetic aberrations.[109] A large-scale genome-wide association study of cSCC has identified 10 single nucleotide polymorphism (SNP) loci, 6 of which encompass pigmentation genes associated with skin cancer risk. These include nonsynonymous SNPs in SLC45A2 gene on chromosome 5p13 and in TYR gene on chromosome 11q14, as well as a functional intronic SNP in IRF4 gene on chromosome 6p25. Three more previously unreported SCC-associated SNPs were identified in HERC2/OCA2 genes at 15q13, DEF8 gene at 16q24, and RALY gene at 20q11.[110]

A genome-wide SNP microarray analysis showed that well-differentiated cSCCs are a genetically distinct subpopulation among all cSCCs. Extensive loss of heterozygosity were seen at 3p and 9p. Loss of 9p could result in inactivation of protein tyrosine phosphatase delta, proposed as a candidate tumor suppressor gene in cSCC. Protein tyrosine phosphatase delta microdeletions were also demonstrated in a subset of cSCCs. Fragile histidine triad, a recognized tumor suppressor gene on 3p14.2 was proposed as another candidate gene that undergoes inactivation.[111] Another study demonstrated that there were 2 distinct telomere phenotypes in cSCCs (and AKs), suggesting 2 modes of initiation of chromosomal instability in cSCCs. One of the telomere phenotypes was associated with a higher degree of aberrant p53 and cyclin D1 expression as well as a more complex karyotype.[112]

Specific Gene Mutations

A unique aspect of the skin biology is the presence of a high number of cancer driver gene mutations in the histologically normal sun-exposed skin. It has been shown that there are thousands of evolving cellular clones in the aged, sun-exposed, physiologically normal skin with more than one-quarter of cells carrying cancer-causing mutations in genes such as TP53, NOTCH1, NOTCH2, and FAT1.[113] Although this intriguing observation has the potential to provide insights into the early stages of squamous carcinogenesis, it also points to a potential impediment in being able to use these genes as diagnostic or prognostic biomarkers, or therapeutic targets. Another study, despite the high mutational background caused by UV exposure, has identified 23 candidate driver genes in aggressive cSCC that include TP53, CDKN2A, NOTCH1, NOTCH2, AJUBA, HRAS, CASP8, FAT1, KMT2C (MLL3), PARD3, and RASA1.[114] We discuss in detail some of the genes frequently found to be mutated in cSCC.

TP53

It has been well-established that a large proportion of cSCC and precursor lesions harbor UV radiation–induced TP53 mutations. These UV signature mutations are found in up to 90% of all cSCC. The fact that TP53 mutations have been found in precursor lesions suggests that this might be an early event in squamous carcinogenesis.[115–117] However, the increased expression levels of mutant p53 also predict aggressive tumor behavior. One study showed that the IHC scores of p53 protein expression had a strong association with histologic grades and TNM stages of cSCC, with tumors expressing high score of p53 being more aggressive as compared with tumors having low score of p53.[118]

CDKN2A

Tumor suppressor genes p16INK4a and p14ARF are the alternative reading frames of CDKN2A locus on 9p21, which is frequently deleted in cSCC. Deletion of p16INK4a is thought to correlate with progression from AK to cSCC.[119,120]

NOTCH

NOTCH is a direct target of p53 that plays a role in the differentiation of epidermal keratinocytes. NOTCH1 is expressed in full thickness of the epidermis, whereas NOTCH2 expression is localized mainly to the basal layer of epidermis. Loss of function NOTCH1 and NOTCH2 mutations are identified in more than 75% of cSCC. NOTCH1 mutation is considered an early event in squamous carcinogenesis of the skin and has been demonstrated to lead to a patchy loss of expression in the normal epidermis.[117–121] Precise exomic sequencing of UV-exposed epidermis and SCCIS also implicates NOTCH1, NOTCH2 and multiple nulceoporins in the early stages of UV-induced carcinogenesis (Seykora and colleagues, unpublished data, 2017).

RAS

The dysregulation of the RAS protooncogene has been implicated in the cSCC initiation in mouse models of chemical carcinogenesis. Recent studies have shown activating mutations of RAS in 12% to 20% of cSCCs.[122,123] The use of targeted BRAF inhibition in melanoma has led to additional insights into the role of RAS in cSCC. About 25% of patients receiving vemurafenib develop squamoproliferative lesions, including well-differentiated cSCC, which have an increased frequency of gain of function RAS mutations (35%–60%) compared with sporadic cSCC.[28]

KNSTRN

UV radiation signature mutations in KNSTRN, a kinetochore protein have been detected in 19% of cSCC in 1 study. Point mutations of KNSTRN disrupt sister chromatid cohesion and chromosome segregation, leading to aneuploidy. KNSTRN mutations are also identified in the normal epidermis in addition to AK and cSCC, suggesting that KNSTRN dysregulation can be an early event in squamous carcinogenesis.[124]

p300

Higher expression of the transcriptional coactivator p300 has been found in cSCC compared with the adjacent histologically normal skin. Moreover, high p300 expression has been correlated positively with lymph node metastasis, advanced clinical stage, and poor patient outcomes in terms of recurrence-free survival and overall survival, leading to a suggestion that p300 expression can be a biomarker for predicting clinical outcomes in cSCC patients.[125]

TERT

TERT promoter mutations are frequent in cSCC. Heterogeneity of TERT promoter mutations were found in SCC of different anatomic sites, giving rise to the hypothesis that different tumor development mechanisms are operational in SCC of different sites.[126,127]

CARD11

In 1 study, CARD11 was found to be mutated in more than 38% of 111 cSCCs. CARD11 regulates nuclear factor κB signaling cascade and point mutations of CARD11 can lead to constitutive activation of the nuclear factor κB pathway, which in turn can lead to the transformation of keratinocytes. Consistent with that, CARD11 messenger RNA and protein expression were detectable in normal skin and increased in cSCC. CARD11 mutations are also identified in the peritumoral and sun-exposed skin, suggesting that these mutations may also be early events in tumor development as with TP53, NOTCH1, and KNSTRN.[128]

MicroRNA Alterations

MicroRNAs (miRNA) are small noncoding RNAs that negatively regulate protein expression. Several studies have found that altered expression of miRNAs contribute to the initiation and progression of cSCC. In cSCC, miRNAs that are downregulated include miR1, miR-34a, MiR-124a, miR-125b, miR-155, miR-193b/365a, MiR-199a, MiR-361-5p, and miR-483-3p. The miRNAs that are specifically upregulated in cSCC include miR-21, miR-31, miR-135b, miR205, miR-223, miR-365, miR-424, and miR-766.[129,130] One study has found significant upregulation of miR-4286, miR-200a-3p, and miR-148-3p, and down-regulation of miR-1915-3p, miR-205-5p, miR-4516, and miR-150-5p in metastatic cSCC as compared with nonmetastatic primary cSCC.[131]

SUMMARY

Cutaneous SCC is one of the most common malignancies worldwide, with a trend toward an increasing incidence. It is important to distinguish well-differentiated cSCC from several other benign and reactive squamoproliferative lesions, identify the common histologic variants to avoid diagnostic pitfalls, as well as to detect and report the well-known high-risk histologic features predictive of an aggressive tumor behavior. A better understanding of the molecular pathways that drive the development and progression of cSCC would provide us with new markers for the diagnostic and prognostic assessment, and molecular targets for newer therapeutic modalities.

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