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Cutaneous Squamous Cell Carcinoma: The Frontier of Cancer Immunoprevention

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Abstract

Cutaneous squamous cell carcinoma (cSCC) is the second most common cancer, with its incidence rising steeply. Immunosuppression is a well-established risk factor for cSCC, and this risk factor highlights the critical role of the immune system in regulating cSCC development and progression. Further highlighting the nature of cSCC as an immunological disorder, substantial evidence demonstrates a tight association between cSCC risk and age-related immunosenescence. Besides the proven efficacy of immune checkpoint blockade therapy for advanced cSCC, novel immunotherapy that targets cSCC precursor lesions has shown efficacy for cSCC prevention. Furthermore, the appreciation of the interplay between keratinocytes, commensal papillomaviruses, and the immune system has revealed the possibility for the development of a preventive cSCC vaccine. cSCC shares fundamental aspects of its origin and pathogenesis with mucosal SCCs. Therefore, advances in the field of cSCC immunoprevention will inform our approach to the management of mucosal SCCs and potentially other epithelial cancers.

INTRODUCTION

Cutaneous squamous cell carcinoma (cSCC) is caused by a malignant proliferation of keratinocytes originating from the epidermis and adnexal structures, including pilosebaceous units and eccrine glands, which clinically presents as an indurated crusted lesion (1, 2). cSCC is the second most common cancer in humans and represents nearly one-third of all nonmelanoma skin cancers (NMSCs), which are associated with significant morbidity, mortality, and economic burden (3–8).

Although previous estimates of lifetime incidence of cSCC (7–11%) (6, 9) are lower than those of basal cell carcinoma (BCC) (28–33%) (10), cSCC cases have been disproportionately increasing in recent years (11). The ratio of cSCC to BCC in the Medicare population increased fourfold from 1992 to 2012 (12). The national incidence rates of cSCC tripled for men and increased by fivefold for women between 1989 and 2017; these rates are estimated to keep growing at a rate of 23% for males and 29% for females in the coming years (13), highlighting the need for improved prevention and treatment of cSCC.

Despite having well-characterized risk factors, cSCC remains a substantial health challenge. Unlike most other NMSCs, 4% of patients with cSCC experience local recurrence or metastasis after complete excision of the primary tumor (14, 15). Particularly in solid organ transplant recipients (SOTRs) undergoing systemic immunosuppression, cSCC has a metastatic rate of up to 8% in comparison with 1% in the general population (8, 9) and has a mortality rate similar to that of melanoma with a 3-year mortality rate of 46% (6, 7, 16, 17). In addition, localized cSCC is associated with significant morbidity, including pain, ulceration, and disfigurement (6, 7, 16, 17).

For effective diagnosis and treatment, it is important to consider the histopathologic diversity of cSCC. In contrast to BCC, which despite its numerous subtypes has limited differences in clinical behavior or metastasis risk, cSCC has a more heterogeneous presentation. cSCC lesions can range from indolent neoplasms with low metastatic potential to highly aggressive and invasive tumors and histopathologic variants extending from keratoacanthoma with good prognosis to poorly differentiated cSCC with poor prognosis (18–21). Thus, it is critical to distinguish between these variants clinically and histologically to accurately treat high-risk tumors early and reduce risk for recurrence and metastasis (22, 23).

Recent advances in understanding the immunobiology of cSCC have created novel opportunities for preventative and therapeutic interventions. Investigating the dramatic differences in cSCC risk between immunosuppressed and immunocompetent patients has revealed the critical role of the immune system in controlling the malignant transformation in keratinocytes (24). The greater incidence of cSCC in SOTRs accompanied by worse prognosis due to the iatrogenic immunosuppression in these patients highlights this role (25, 26). cSCC serves as a unique cancer model for studying immunoprevention and immunotherapy. In contrast to internal cancers (e.g., breast cancer) that appear as a singular lesion and take years to decades to develop, several independent primary cSCCs can arise rapidly in a single patient (13, 27). In fact, the cSCC disease burden largely relates to the appearance of multiple primary lesions in high-risk patients (14, 28, 29). Evidence demonstrates that a subsequent cSCC risk is exponentially greater in patients with a history of cSCC in the past, highlighting the critical need for preventive strategies, such as vaccines, for cSCC (13, 27, 30).

In this review, recent advances in understanding cSCC epidemiology, biology, pathogenesis, clinical presentation, prevention, and therapeutics are summarized. Particularly, emerging therapeutic strategies that focus on cSCC immunobiology are highlighted.

EPIDEMIOLOGY

The non-Hispanic White population in the United States has an estimated 14–20% lifetime risk of developing cSCC (10, 31). There are approximately 1 million new cases of cSCC per year in the United States (12, 32). However, cSCC incidence differs among the reported nationwide studies. In addition, cSCC exclusion from the national tumor registries has resulted in an underreporting of cases. Thus, the true cSCC incidence rate is unclear (33, 34). In terms of health care burden and costs, it is estimated that 5 million adults are treated for skin cancer annually, with average treatment costs exceeding $8.1 billion each year ($4.8 billion for NMSCs) (35). A recent nationwide epidemiologic cohort study in the Netherlands found a substantial increase in the incidence of the first cSCC from 1989 to 2017 (40.0 to 107.6 per 100,000 person-years), which was particularly high among female patients (13.9 to 68.7 per 100,000 person-years) (13). Thus, the rising incidence of cSCC, particularly the development of multiple cSCCs per patient, highlights the substantial burden of cSCC and its impact on health care.

Numerous risk factors for cSCC have been identified, including ultraviolet (UV) radiation, immunosuppression, and previous history of cSCC, as well as ionizing radiation, old age, genodermatoses (e.g., xeroderma pigmentosum), arsenic, polycyclic aromatic hydrocarbons, pharmacological treatment with voriconazole, fair skin (Fitzpatrick skin type I, II, and III; albinism), chronic ulcer, burn wound (Marjolin ulcer) and chronic scar, and preexisting chronic dermatoses, such as dystrophic epidermolysis bullosa, epidermodysplasia verruciformis, and erosive lichen planus (36). A tumor diameter >2 cm, poorly differentiated tumor histology, perineural and lymphovascular invasion, extension into the subcutaneous tissue, and bone invasion are tumor-intrinsic risk factors associated with cSCC recurrence and metastasis (37–39). Although the mechanisms underlying each of these risk factors are not yet fully understood, each is thought to contribute to cSCC development and progression, which are often multifactorial.

UV radiation, specifically UVB (280–315 nm) and UVA (315–400 nm), is associated with the highest risk for developing skin cancer overall (40). Despite accounting for 2% of UV rays in the sunlight, UVB is primarily responsible for UV-related skin cancers (41). Through inducing DNA damage and mutations in tumor suppressor genes, such as *TP53* (42, 43), UVB is responsible for both tumor initiation and promotion, whereas UVA radiation causes skin aging and indirect DNA damage (40, 44). The effects of increased UV exposure are even more pronounced in individuals with lighter skin color, as well as in those with increased sun exposure and history of sunburns in childhood (45, 46).

In contrast to the dominance of BCC in immunocompetent patients, cSCC is the most predominant skin cancer in immunosuppressed SOTRs, with a 65–250-fold greater incidence and higher mortality rate in SOTRs compared with the general population (25). In the United States and western Europe, the incidence of cSCC dramatically increases with time in SOTRs, from 10–27% at 10 years to 40–60% at 20 years posttransplant (47). Risk of cSCC development also correlates with the degree of immunosuppression. For instance, patients with heart and lung transplants have a higher risk of cSCC compared with liver transplant patients due to their more intensive immunosuppression regimens (47, 48). SOTRs are also more likely to have aggressive cSCCs (8% risk for metastasis) (49). The 5-year overall survival rate due to aggressive cSCCs in SOTRs is 23%, while the 5-year disease-specific survival rate is 30.5% (26). Additionally, 66% of SOTRs will develop a second cSCC within 5 years of their first cSCC diagnosis (30). The risk of new cSCCs in SOTRs is markedly amplified to more than 75% within 2 years after the second cSCC diagnosis (27). Noniatrogenic immunosuppression in human immunodeficiency virus (HIV) patients and cell-mediated immunodeficiency in lymphoproliferative disorders also lead to increased risk of aggressive cSCC development (50).

A notable difference in age distribution exists between BCC and cSCC, and cSCC is more commonly found in older patients. A 19-year single-center study found that the most frequently observed age group for cSCC included those aged 80 to 89 years old (41.8%), as opposed to 70 to 79 years old for BCC (44.1%) (51). Similarly, a population-based study in Minnesota over 10 years showed a median age at incident diagnosis of 72.0 years in cSCC patients and 63.8 years in BCC patients (11). In another study in Australia, between 2011 and 2014, it was demonstrated that the BCC-to-cSCC ratio reduced by age from 11.4 for women and 8.01 for men in the 40–44-year-old age group to 2.5 for women and 2.25 for men in the 70–74-year-old age group (52).

Additional predisposing risk factors, such as genodermatoses and human papillomavirus (HPV), are discussed in subsequent sections. Understanding the underlying molecular pathways behind genetic and viral causes of cSCC is an emerging area of focus within the field, given the potential for preventative and therapeutic innovations.

BIOLOGY AND PATHOGENESIS

The skin is composed primarily of the epidermis, adnexal structures, dermis, and subcutaneous tissue (**Figure 1**). The epidermis is stratified and its layers, from outermost to innermost, are the stratum corneum, stratum lucidum, stratum granulosum, stratum spinosum, and stratum basale (basal layer), which is the mitotically active layer of the epidermis (53). Stem and transiently amplifying cells lie in the basal layer, and these cells differentiate and give rise to the keratinocytes in upper differentiated layers of the epidermis. Likewise, most hair follicle stem cells reside in a niche known as the bulge region (54). Thus, given their potential for self-renewal and multilineage differentiation, both the basal layer and the hair follicle bulge have been described as potential cells of origin for keratinocyte carcinomas, including cSCC and BCC (55, 56). Of note, combined single-cell RNA sequencing with spatial transcriptomics and multiplexed ion beam imaging from cSCCs have revealed four tumor cell subpopulations, with three recapitulating normal epidermal states and a tumor-specific keratinocyte population residing within a fibrovascular niche (57). Various features of potential immunosuppression have also been noted, including regulatory T cell colocalization with $CD8^+$ T cells in tumor stroma (57). The cSCC immune landscape is further compromised in SOTRs. Gene expression and single-cell T cell receptor sequencing have shown reduced tumor-infiltrating cytotoxic $CD8^+$ T and naive T lymphocytes in cSCCs of SOTRs, while regulatory T cells maintain similar numbers in tumors of immunocompetent and immunosuppressed patients (58).

The extracellular matrix (ECM) plays an important role in cSCC development. Consisting of various structural proteins and other macromolecules that provide structural scaffolding to the skin, the ECM has been shown to interact with cSCC cells (59). For instance, laminin 332 is an ECM protein found in the basement membrane zone (BMZ) and is critical for maintaining skin integrity. Along with its ligation partner, $\alpha 6\beta 4$ integrin, laminin 332 was found to be required for human cSCC tumorigenesis in a murine xenograft model (60). In addition, small interfering RNA (siRNA)-mediated depletion of collagen VII, which comprises anchoring fibrils in the BMZ to the dermis, promotes migration, invasion, disorganized differentiation, and epithelial-mesenchymal transition of cSCC cells in a 3D organotypic skin model (61). Other mechanisms involved in the epithelial-mesenchymal transition of malignant keratinocytes include the loss of E-cadherin, desmogleins, and catenins, which are keratinocyte adhesion molecules, and activation of matrix metalloproteinases (MMPs), which result in ECM degradation and remodeling of cytoskeletal filaments (62). Cytokines such as transforming growth factor beta (TGF-β), transcription factors including Snail 1 and Snail 2, and microRNAs such as miR-21 and miR-205, which are involved in posttranscriptional regulation of gene expression, also play pivotal roles in cSCC progression (62, 63).

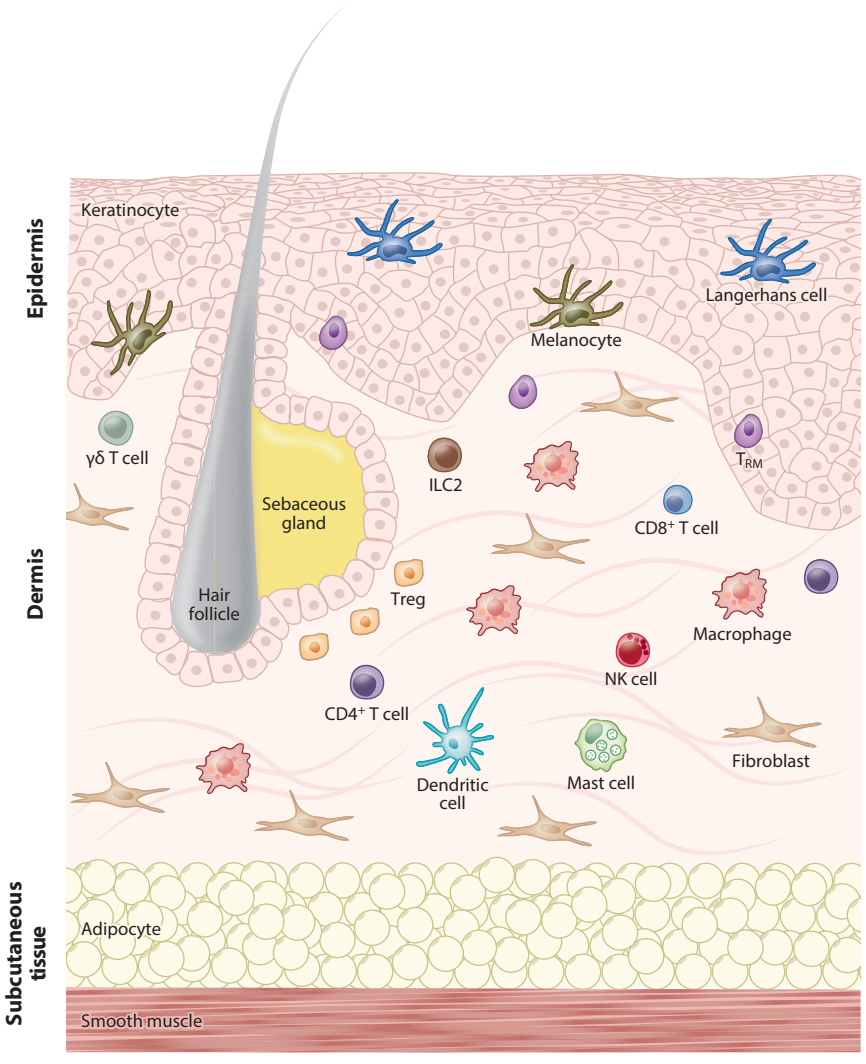

Figure 1

The skin with its resident immune cells. The epidermis and hair follicle primarily consist of keratinocytes and are infiltrated by Langerhans cells, which are tissue-resident antigen-presenting cells, as well as T_{RM} cells and melanocytes. cSCC-initiating cells are thought to exist in the bulge region of hair follicles and in the basal layer of the epidermis. In the dermis, fibroblasts predominate and are responsible for the synthesis and development of the extracellular matrix, including collagen to provide structural support to the skin. Macrophages, Tregs, T_{RM} cells, recirculating $CD4^+$ and $CD8^+$ T cells, γδ T cells, dendritic cells, NK cells, and mast cells are also found in normal skin. The subcutaneous tissue consists of the fat layer, composed of adipocytes, as well as the muscle layer below, primarily composed of smooth myocytes. Abbreviations: cSCC, cutaneous squamous cell carcinoma; ILC2, group 2 innate lymphoid cell; NK cell, natural killer cell; Treg, regulatory T cell; T_{RM} cell, tissue-resident memory T cell.

The spectrum of cSCC lesions can appear at varying stages, presenting as early as precancerous actinic keratoses (AKs) and as late as metastatic SCC (**Figure 2**). AKs are common skin lesions that have an increased risk for malignant progression to cSCC and are associated with an increased number of UV-driven mutations (64). Histologically, AKs are characterized as intraepidermal

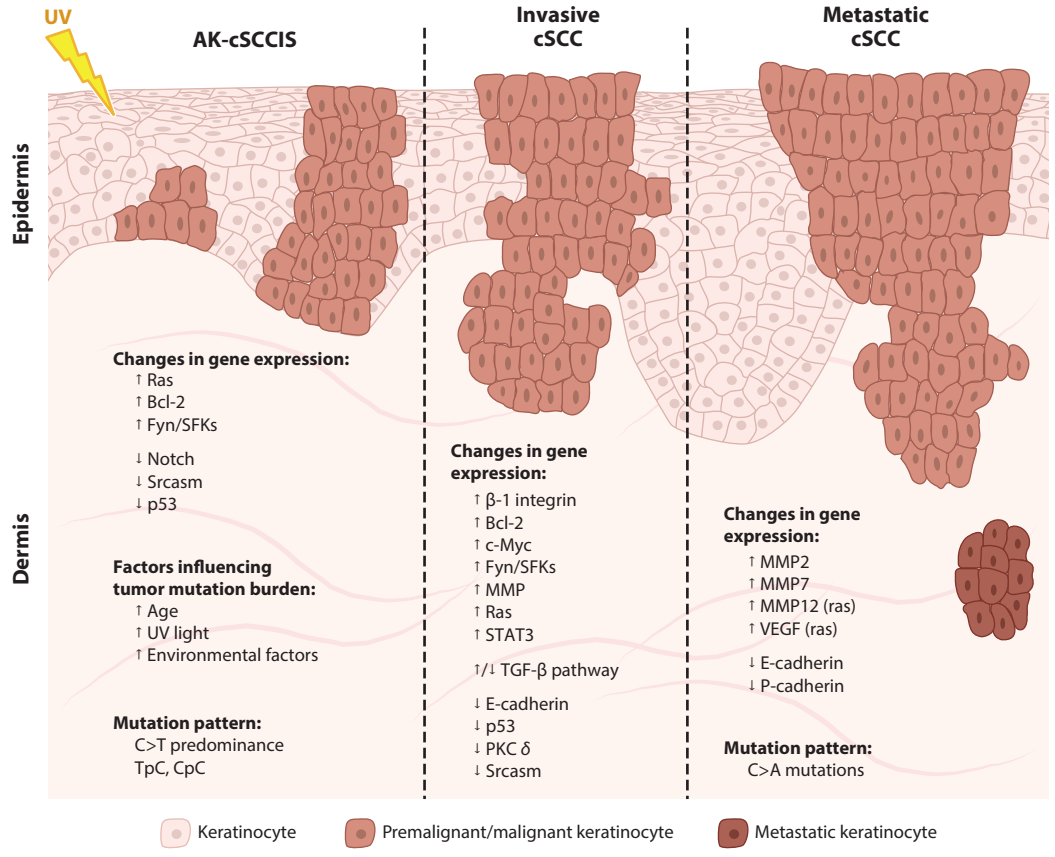

Figure 2

The molecular alterations associated with cancer progression from AK to invasive cSCC. Schematic depicting the spectrum of morphologies of AK-cSCC lesions, with relevant changes in gene expression and mutation patterns. Abbreviations: A, adenine; AK, actinic keratosis; Bcl-2, B cell lymphoma 2; C, cytosine; cSCC, cutaneous squamous cell carcinoma; cSCCIS, cutaneous squamous cell carcinoma in situ; MMP, matrix metalloproteinase; PKC, protein kinase C; SFK, Src-family tyrosine kinase; Srcasm, Src-activating and signaling molecule; T, thymine; TGF-β, transforming growth factor beta; UV, ultraviolet; VEGF, vascular endothelial growth factor.

neoplasms containing enlarged keratinocytes with atypical nuclei (5). UVB-induced inactivation of *TP53*, the most commonly mutated gene in AKs, is largely responsible for AK development (65). If the classic multistep model of carcinogenesis were to be applied to the progression of AK to cSCC (66), mutations in a tumor suppressor gene (i.e., *TP53*) would lead to the development of a precursor lesion (i.e., AK), associated with genetic instability and/or loss of cell cycle control (55). The major mutated genes in AKs such as *TP53*, *NOTCH1*, and *NOTCH2*, which are commonly mutated in cSCC, indicate the shared origin of AKs and cSCCs (67). In contrast, immune factor alterations including TGF-β signaling pathway mutations, which are more predominantly detected in cSCCs, highlight the role of immune dysregulation in the transition of AK to cSCC (67). Established genetic alterations in the formation of AKs result in increased oncogenic signals [e.g., Ras, Fyn/Src-family tyrosine kinases (SFKs), B cell lymphoma 2 (Bcl-2)] and decreased function of tumor suppressors [e.g., p53, Src-activating and signaling molecule (Srcasm)], which ultimately lead to the mutated keratinocytes spanning the entire epidermal thickness, as the AK progresses to cSCC (55). Although AK and cSCC are molecularly linked, it is important to note

that an individual AK rarely progresses to cSCC (68). Instead, the presence of AKs highlights the field cancerization of the sun-damaged skin and its propensity to developing de novo cSCCs (69).

Notch signaling plays a critical tumor suppressive role in cSCC development, which is blocked by inactivating UV mutations involving *NOTCH1* and *NOTCH2* genes in human AK and cSCC (67, 70). In epidermal keratinocytes, Notch signaling upregulates factors involved in differentiation, including retinoic acid and interferon regulatory factor 6 (71, 72). Retinoic acid has been shown to be protective against cSCC in mice (73), further supporting the tumor suppressive role of Notch signaling in cSCC. Notch signaling also inhibits Rho-associated kinases 1 and 2, as well as myotonic dystrophy kinase-related CDC42-binding kinase α in keratinocytes, which are implicated in neoplastic progression (74). Furthermore, loss of Notch proteins in keratinocytes generates a tumor-promoting microenvironment in the skin (75). p53 is thought to also induce Notch-mediated differentiation in keratinocytes, which activates cell cycle inhibitors (i.e., p21) and represses the inducers of immature stem/progenitor cells (i.e., p63) (76–79). Specifically for cutaneous squamous cell carcinoma in situ (cSCCIS), *NEURL1* ubiquitin ligase, which marks Notch ligands for degradation, is upregulated (80). This suggests that UV-induced increase of NEURL1 levels may result in downregulation of Notch and cancer progression (80). Collectively, aberrations in the Notch signaling pathway represent an early event in malignant transformation of keratinocytes in AK-cSCC spectrum lesions.

Various genetic alterations are associated with the progression of AK to cSCC, which is histologically marked by large, atypical keratinocytes invading into the dermis. Although the classic model of carcinogenesis requires several genetic alterations in driver oncogenes to elicit neoplastic progression, 3D models of the human epidermis have shown that as few as two proto-oncogene mutations are sufficient to drive the progression from AK to cSCC (60, 81). Genetic mutations resulting in malignant progression include oncogenes (Ras, Fyn/SFKs, c-Myc, Bcl-2, STAT-3, β-1 integrin, and MMPs) and tumor suppressors (p53, Srcasm, Notch, protein kinase C δ, and E-cadherin) (55). Relative to AKs, primary and metastatic cSCCs demonstrate increased genomic instability, resulting in chromosomal translocations, isochromosomes, gene deletions, and amplifications (55). With regard to susceptibility loci for cSCC, a recent genome-wide meta-analysis has identified 8 new single-nucleotide polymorphisms (SNPs) and corroborated 14 previously identified SNPs associated with cSCC (82–85). Further activating mutations in vascular endothelial growth factor, MMP2, MMP7, MMP12, and downregulated E-cadherin and P-cadherin highlight the epithelial-mesenchymal transition observed in the metastatic stage of cSCC (55).

CLINICAL PRESENTATION

An AK presents as an erythematous, rough papule with scale and little to no induration on sun-exposed skin (**Figure 3**) (86). Although AKs are usually asymptomatic, they may be mildly painful and are more readily diagnosed on examination with palpation. Some variants of AKs are pigmented, cutaneous horn, atrophic, Bowenoid, and lichen planus-like lesions (87). Pigmented AKs range from being pink or skin-colored to brown. cSCCIS represents another precursor lesion as part of the progression from AK to invasive cSCC (**Figure 3**) (88). cSCCIS appears as an erythematous scaly plaque, with the possibility of pigmentation, and may also present as hyperparakeratosis leading to the formation of a cutaneous horn. cSCC similarly forms on sun-exposed skin as persistent, solitary firm papules or erythematous nodules with significant scale, and the propensity for spontaneous bleeding (**Figure 3**). Other features of cSCC include induration, varying degrees of hyperkeratosis, ulceration, and tenderness. Rarely, cSCCs may manifest as multiple in-transit metastases as well. Low- to moderate-risk cSCC variants include keratoacanthomas, verrucous carcinomas, and clear cell cSCCs, and higher-risk variants include acantholytic, spindle cell, and

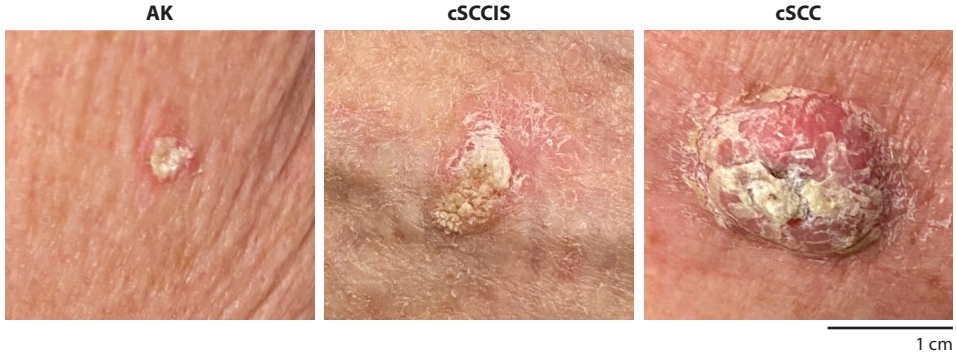

Figure 3

Clinical representation of cSCC and its precursor lesions. Abbreviations: AK, actinic keratosis; cSCC, cutaneous squamous cell carcinoma; cSCCIS, cutaneous squamous cell carcinoma in situ.

adenosquamous carcinomas (55). In addition, high-risk features of primary cSCCs include poor differentiation, perineural invasion, diameter >2 cm, depth >4 mm, history of local recurrence, and location (anogenital, ear, lip, or scar) (89).

KEY ASSOCIATIONS

Immunosuppression is highly linked to increased cSCC incidence. In SOTRs, the pathogenesis of cSCCs is related to a combination of factors. As part of their disease management, SOTRs are chronically immunosuppressed. The impaired cell-mediated immunity in the skin increases the risk for carcinogenesis by inhibition of antigen-presenting cells, T cell dysregulation, and increased inhibitory mediators such as interleukin 10 (90). The pathogenesis of cSCC in SOTRs may also relate to non-immune-mediated mechanisms including hypersensitization to UV-induced damage, resulting in suppression of p53-mediated cell death (90). Recent evidence suggests that calcineurin inhibitors such as cyclosporine used for immunosuppression can directly promote cSCC development through the calcineurin/nuclear factor of activated T cell pathway inhibition, which induces transcription factor 3 and finally leads to p53 suppression in keratinocytes (90–92). Another immunosuppressive drug, azathioprine, which induces selective UVA photosensitivity, is found to cause a unique mutational signature in cSCCs of immunosuppressed patients relative to those who are immunocompetent (93). Furthermore, it has been shown that $CD8^+$ T cells expressing high levels of CD57, an immunosenescence marker, are correlated with increased cSCC in SOTRs (94). Immunosuppression also increases the risk for viral proliferation in the skin relative to the general population, including HPV. Approximately 90% of cSCCs in SOTRs contain HPV DNA, relative to 11–32% in immunocompetent skin samples (95).

Due to the association of cutaneotropic HPVs with cSCC in SOTRs, a causative role for HPVs in cSCC development has been proposed. According to this so-called hit-and-run hypothesis, HPV acts as a cofactor in facilitating UV-driven tumorigenesis in cSCC (96, 97). As such, β-HPVs initiate the development of cSCC but are not necessary later in the process of carcinogenesis (98). In immunocompetent individuals, high-risk α-HPVs are associated with cervical, anal, and oropharyngeal SCCs (99). While β-HPV types predominate in cSCCs of immunosuppressed SOTRs, their relationship with cSCC in healthy individuals is less characterized (98). Thus, immunosuppressed patients are thought to experience the hit-and-run mechanism of UV plus β-HPV exposure in driving oncogenic mutations and cSCC development. Recently, we proposed an

alternative hypothesis positing that T cell immunity against commensal papillomaviruses cross-protects the skin from cSCC development in an immunocompetent host. The loss of anti-HPV immunity rather than the oncogenic effect of HPVs accounts for the increased risk for cSCCs in immunosuppressed patients. This new emerging understanding of cSCC immunology is discussed in the section titled Frontier of Cancer Immunoprevention.

Germline mutations are associated with increased risk for cSCC development, namely xeroderma pigmentosum (XP). XP is a rare autosomal recessive disorder usually detected at the age of 1–2 years old, in which patients are highly photosensitive as a result of a defect in the nucleotide excision repair (NER) pathway (100, 101). UV light and environmental mutagens can cause helix-distorting DNA lesions including pyrimidine dimers that are typically repaired through the NER pathway (102). However, patients with XP are unable to reconcile UV-induced mutagenesis, severely predisposing them to UV mutations and cSCC (102). Interestingly, defects in cell-mediated immunity, fibroblast hyperactivation, and natural killer cell dysfunction have been also reported to contribute to skin cancer risk in XP (103).

Epidermodysplasia verruciformis (EV) is another genodermatosis associated with increased cSCC risk (104). EV is an autosomal recessive skin disorder caused by inactivating mutations in *TMC6* (encoding EVER1) or *TMC8* (encoding EVER2) genes (105). Loss of these genes results in hyperproliferation of β-HPVs, which leads to a confluent pattern of wart development and pityriasis versicolor–like lesions on the skin starting in childhood, which is thought to be due to a defect of keratinocyte-intrinsic immunity to β-HPV (104). Risks for cSCC and other NMSCs are elevated in EV patients as young as 20 to 30 years old (104).

Epidermolysis bullosa (EB) is a rare inherited bullous disorder characterized by blister formation and skin fragility caused by a defect at various compartments of the BMZ in response to minor mechanical trauma (106). Patients with recessive dystrophic EB are prone to cSCC, which is the main cause of mortality among them. Defects in genes responsible for skin wound healing result in chronic erosive wounds, fibrosis, and cSCC development.

PREVENTION AND TREATMENT

Primary prevention for the development of AKs and cSCCs typically focuses on minimizing UV exposure (107). Counseling patients on sun-protective behaviors, including applying sunscreen, wearing long-sleeved clothing, and avoiding indoor tanning, is important particularly in patients at increased risk for cSCCs. In addition, chemoprevention for cSCCs has been widely explored, with several promising agents demonstrating efficacy in preliminary studies. These agents include vitamin B_3 (nicotinamide), acitretin (vitamin-A derivative), difluoromethylornithine, and nonsteroidal anti-inflammatory drugs (108–112).

Secondary prevention primarily focuses on treating existent precursor AK lesions. Mainstay treatments for AKs include cryotherapy and field therapy, including topical 5-fluorouracil (5-FU), ingenol mebutate, and diclofenac as well as photodynamic therapy (PDT), which may be particularly advantageous for more diffuse areas of actinic damage (108, 113, 114). Despite the efficacy of cryotherapy and field therapies for AK treatment, only topical 5-FU has shown efficacy in reducing the risk of new cSCC development on the face and ears within 1 year after treatment (115). However, this protective effect is completely lost by 2 years after therapy (115). Of note, topical 5-FU in combination with calcipotriol is a novel immunotherapy for the treatment of AKs (see the next section for details) (114, 116).

For the most invasive, high-risk cSCCs, surgical excision is necessary, and this can include either conventional wide local excision or Mohs micrographic surgery, depending on the tumor

features and the anatomical location (117). Adjuvant radiation therapy may also be considered in cSCC cases with perineural invasion or high metastatic risk. For cSCCIS and low-risk cSCC, nonsurgical treatment options include electrodesiccation and curettage, PDT, topical imiquimod, and 5-FU (118).

Systemic therapies for advanced cSCC include platinum-based chemotherapies, capecitabine, and epidermal growth factor receptor inhibitors (cetuximab, panitumumab), all of which have modest efficacy (119–121). Recently, the first immune checkpoint inhibitor (ICI) targeting PD-1 (programmed cell death protein 1), cemiplimab (48), was approved for use in advanced and metastatic cSCC, with treatment response in approximately 50% of the patients (122). Other immunotherapeutics including anti-PD-L1 (anti–programmed death ligand 1) antibodies may also show high efficacy for cSCC treatment given the large tumor mutational burden and lymphocytic infiltration in cSCC (123). In particular, ICI response is shown to associate with high expression levels of PD-L1 and the presence of interferon-γ gene signature in cSCC (124).

FRONTIER OF CANCER IMMUNOPREVENTION

Recent advances in our understanding of cSCC tumorigenesis suggest that cSCC is a highly immune-regulated disease. As has been widely established, immunosuppression or immune dysregulation confers significant risk for cSCC development. In SOTRs, HIV/AIDS, and chronic lymphocytic leukemia patients with suppressed immunity, preventing and treating cSCCs remain challenging (125). The cSCC burden is markedly higher in these high-risk populations, especially when compared with melanoma and BCC. Although increased age is associated with greater risk of skin cancer overall, particularly due to increased UV-induced mutations, evidence suggests that aging may confer an additional risk for cSCC development. This is highlighted by the fact that BCC, with the highest mutation rate among all cancers [65 mutations/Mb (126)], has an earlier age of onset compared with cSCC, which has a lower mutational burden [50 mutations/Mb (93)]. Thus, the age-associated increase in the risk of cSCC likely points to the importance of immunosenescence in addition to cumulative UV damage as drivers of cSCC development with age (127).

New evidence suggests an increasingly important role of immunosenescence, the gradual deterioration of the immune system with age, in cSCC pathogenesis. Healthy, older individuals with age-related declines in immunity have been found to be substantially more permissive to cSCC development. This has been demonstrated by a comprehensive, single-site study over 19 years, in which patients with cSCC were more likely to present later than those with BCC (mean age 70.8 years for BCC and 79.9 years for cSCC), with the most frequent age group being 70–79-year-olds for BCC and 80–89-year-olds for cSCC (51). Similarly, a population-based study in Minnesota over 10 years found a disproportionate increase in cSCC later in life relative to BCC (11).

The case for the contribution of age-associated immunosenescence to cSCC risk is also supported by evidence observed in pediatric patients, who do not experience the biological effects associated with aging. A recent multicenter retrospective case-control study of pediatric patients from 1995 to 2015 sought to investigate risk factors in children and young adults with NMSCs (128). The study authors demonstrated that pediatric patients with prior chemotherapy and radiation therapy primarily developed BCCs, while children experiencing immunosuppression (i.e., transplant recipients) almost exclusively developed cSCCs. Thus, this study's findings are reflective of the age-related immunocompetency that is inversely associated with cSCC risk.

Considering cSCC as a disease of immune dysregulation rather than simply immunosuppression allows for the development of novel therapeutic strategies for cSCC prevention. Studying the role of the immune system in controlling the early stages of cSCC development in experimental

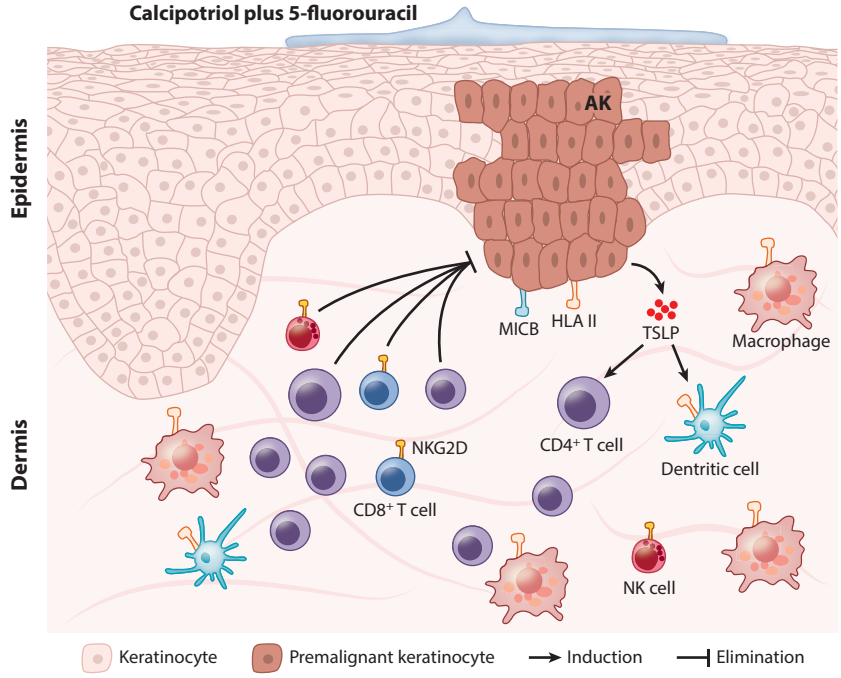

Figure 4

Topical calcipotriol plus 5-fluorouracil immunotherapy induces a potent tumor-directed immunity against AKs. Application of topical calcipotriol plus 5-fluorouracil induces TSLP cytokine expression by the keratinocytes, which activates $CD4^+$ T cell immunity against AKs. HLA class II and MICB induction on the transformed keratinocytes leads to the direct activation of $CD4^+$ T cells against AKs. In addition, $CD8^+$ T cells and NK cells can recognize NKG2D ligands expressed on the premalignant keratinocytes, which can further suppress skin cancer development. Abbreviations: AK, actinic keratosis; HLA, human leukocyte antigen; MICB, MHC class I polypeptide-related sequence B; NK cell, natural killer cell; TSLP, thymic stromal lymphopoietin.

models (116) has led to the development of a novel topical immunotherapy with proven efficacy in eliminating AKs (114). Specifically, we have discovered the high potency of antitumor $CD4^+$ T cell immunity induced by thymic stromal lymphopoietin (TSLP) cytokine in blocking skin cancer development (116). The combination of topical calcipotriol, a low-calcemic vitamin D analog that induces TSLP expression by keratinocytes (129), with 5-FU results in a potent tumor-directed immunity against AKs (**Figure 4**) (114). We conducted a randomized double-blind clinical trial demonstrating that the combination of topical calcipotriol synergizes with 5-FU to induce robust T cell–mediated immunity and markedly reduce the number of AKs relative to 5-FU-treated controls (87.8% versus 26.3 mean reduction) (114). Further analysis of AK samples from the subjects treated with calcipotriol plus 5-FU revealed the induction of $CD4^+$ and $CD8^+$ T_{RM} cell (tissue-resident memory T cell) formation in the skin lesions, which persisted for more than 3 years posttreatment (130). This immunity and T_{RM} cell induction correspond to a significantly lower risk of cSCC development in the group treated with calcipotriol plus 5-FU compared with the group treated with Vaseline plus 5-FU within 3 years of treatment [7% versus 28% in control group, hazard ratio 0.215 (95% confidence interval: 0.048–0.972)]. Importantly, calcipotriol plus 5-FU did not reduce the risk of BCC in subjects, highlighting the specific regulation of cSCC development by the immune system.

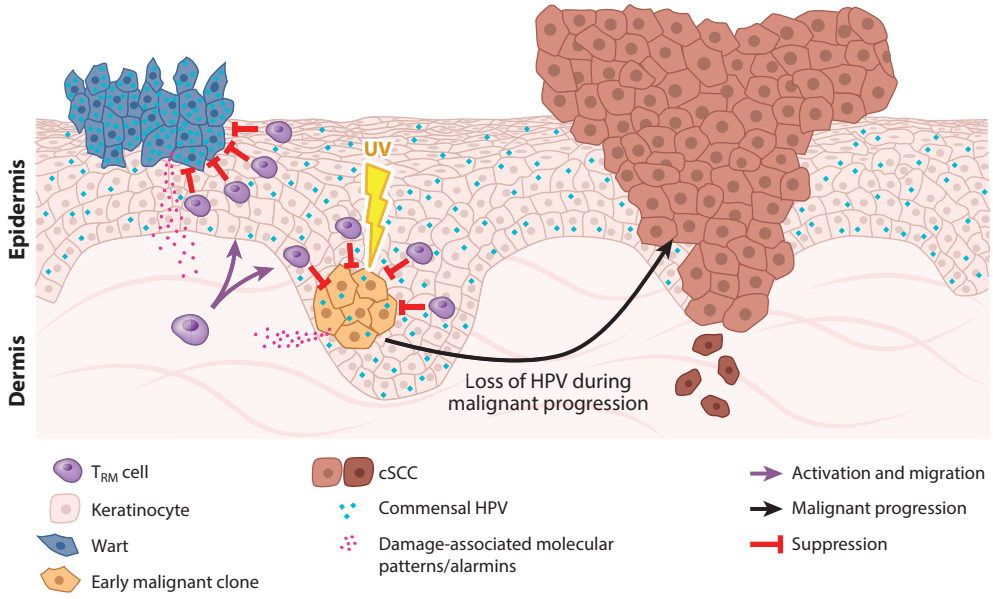

Figure 5

Immunity against skin-resident commensal HPVs protects the skin from cSCC. Commensal HPVs cause cutaneous warts in the absence of a competent immune response in the skin. In immunocompetent individuals, immune cells including T_{RM} cells block the development of warts and the expansion of UV-induced malignant clones by recognizing HPV antigens in conjunction with immunogenic factors released by the proliferating keratinocytes. Accordingly, malignant keratinocytes lose HPV gene expression to evade antiviral immunity and progress to invasive cSCC. Abbreviations: cSCC, cutaneous squamous cell carcinoma; HPV, human papillomavirus; T_{RM} cell, tissue-resident memory T cell; UV, ultraviolet.

With regard to secondary prevention, our work has also uncovered a critical role for cutaneotropic viruses in immunity against cSCC development, highlighting the potential for a novel cSCC vaccine. To understand the role of commensal HPVs in cSCC pathogenesis, we colonized mice with murine papillomavirus type 1 (MmuPV1) (97). Confluent warts caused by papillomavirus infection were observed in immunodeficient mice; however, immunocompetent mice demonstrated no skin lesions. Mice with both natural anti-MmuPV1 immunity following colonization and acquired immunity from T cell transfer or MmuPV1 vaccination were found to be protected from chemical and UV-induced skin carcinogenesis in a $CD8^+$ T cell–dependent manner. When trying to determine if β-HPVs were similarly protective in human skin, we found a significant reduction in viral activity in human skin cancer cells relative to normal adjacent skin and discovered β-HPV-specific $CD8^+$ T cells in sun-exposed normal human skin (97). Thus, we propose that the loss of immunity against skin-resident commensal HPVs, which cross-protect the skin against cSCC, is the primary reason for dramatically increased risk of cSCC upon immunosuppression (**Figure 5**). Consequently, a T cell–directed commensal HPV vaccine, which can restore anti-HPV immunity in the skin, will provide an effective strategy for cSCC immunoprevention.

Our novel findings suggest that developing cutaneotropic HPV vaccines and other immune-based therapeutics against cSCC precursor lesions will most directly deliver cSCC prevention in immunosuppressed patients and in older, high-risk populations with compromised immunity and immunosenescence. These results also suggest that the aforementioned hit-and-run hypothesis (96), in which β-HPVs serve solely to augment UV mutagenesis and cSCC promotion, may be

overly simplistic by failing to capture the inherent role of immunity in regulating the interactions between commensal virome and epithelial cells. Anti-HPV T cell immunity has a significant impact on cSCC development and β-HPV load/activity in the cancer cells. Currently licensed vaccines against HPV are B cell directed and primarily cover high-risk α-HPVs; thus, they are distinct from our proposed β-HPV vaccine (131). Advancements in boosting tumor-infiltrating T lymphocytes in established cancers by the use of tumor-associated and neoantigen vaccines can guide the development of an effective commensal HPV vaccine for cSCC immunoprevention (132).

As cSCC remains a significant public health challenge, employing immunologic strategies and reframing our understanding of how the immune system contributes to cSCC development are critical for the prevention and treatment of this disease. Ultimately, the advancement in the field of cSCC immunoprevention will inform novel strategies for the prevention of mucosal SCCs, other epithelial cancers, and beyond.

DISCLOSURE STATEMENT

S.D. is a coinventor on a filed patent for the use of calcipotriol plus 5-fluorouracil for the treatment of precancerous skin lesions (PCT/US2015/049434). S.D. is an inventor on a filed patent for the use of commensal HPV vaccine for skin cancer prevention and therapy (PCT/US2019/063172).

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LITERATURE CITED

1. Kallini JR, Hamed N, Khachemoune A. 2015. Squamous cell carcinoma of the skin: epidemiology, classification, management, and novel trends. *Int. J. Dermatol.* 54:130–40
2. Jones OT, Ranmuthu CK, Hall PN, Funston G, Walter FM. 2020. Recognising skin cancer in primary care. *Adv. Ther.* 37:603–16
3. Que SKT, Zwald FO, Schmults CD. 2018. Cutaneous squamous cell carcinoma: incidence, risk factors, diagnosis, and staging. *J. Am. Acad. Dermatol.* 78:237–47
4. Am. Cancer Soc. 2019. *Cancer Facts & Figures 2019*. Atlanta: Am. Cancer Soc.
5. Cakir BO, Adamson P, Cingi C. 2012. Epidemiology and economic burden of nonmelanoma skin cancer. *Facial Plast. Surg. Clin. N. Am.* 20:419–22
6. Nehal KS, Bichakjian CK. 2018. Update on keratinocyte carcinomas. *N. Engl. J. Med.* 379:363–74
7. Clayman GL, Lee JJ, Holsinger FC, Zhou X, Duvic M, et al. 2005. Mortality risk from squamous cell skin cancer. *J. Clin. Oncol.* 23:759–65
8. Blomberg M, He SY, Harwood C, ST Arron, Demehri S, et al. 2017. Research gaps in the management and prevention of cutaneous squamous cell carcinoma in organ transplant recipients. *Br. J. Dermatol.* 177:1225–33
9. Karia PS, Han J, Schmults CD. 2013. Cutaneous squamous cell carcinoma: estimated incidence of disease, nodal metastasis, and deaths from disease in the United States, 2012. *J. Am. Acad. Dermatol.* 68:957–66
10. Miller DL, Weinstock MA. 1994. Nonmelanoma skin cancer in the United States: incidence. *J. Am. Acad. Dermatol.* 30:774–78
11. Muzic JG, Schmitt AR, Wright AC, Alniemi DT, Zubair AS, et al. 2017. Incidence and trends of basal cell carcinoma and cutaneous squamous cell carcinoma: a population-based study in Olmsted County, Minnesota, 2000 to 2010. *Mayo Clin. Proc.* 92:890–98

12. Rogers HW, Weinstock MA, Feldman SR, Coldiron BM. 2015. Incidence estimate of nonmelanoma skin cancer (keratinocyte carcinomas) in the US population, 2012. *JAMA Dermatol.* 151:1081–86
13. Tokez S, Hollestein L, Louwman M, Nijsten T, Wakkee M. 2020. Incidence of multiple versus first cutaneous squamous cell carcinoma on a nationwide scale and estimation of future incidences of cutaneous squamous cell carcinoma. *JAMA Dermatol.* 156:1300–6
14. Brantsch KD, Meisner C, Schönfisch B, Trilling B, Wehner-Caroli J, et al. 2008. Analysis of risk factors determining prognosis of cutaneous squamous-cell carcinoma: a prospective study. *Lancet Oncol.* 9:713–20
15. Schmults CD, Karia PS, Carter JB, Han J, Qureshi AA. 2013. Factors predictive of recurrence and death from cutaneous squamous cell carcinoma: a 10-year, single-institution cohort study. *JAMA Dermatol.* 149:541–47
16. Eigentler TK, Leiter U, Häfner H-M, Garbe C, Röcken M, Breuninger H. 2017. Survival of patients with cutaneous squamous cell carcinoma: results of a prospective cohort study. *J. Investig. Dermatol.* 137:2309–15
17. Silberstein E, Sofrin E, Bogdanov-Berezovsky A, Nash M, Segal N. 2015. Lymph node metastasis in cutaneous head and neck squamous cell carcinoma. *Dermatol. Surg.* 41:1126–29
18. Lohmann CM, Solomon AR. 2001. Clinicopathologic variants of cutaneous squamous cell carcinoma. *Adv. Anat. Pathol.* 8:27–36
19. Cassarino DS, DeRienzo DP, Barr RJ. 2006. Cutaneous squamous cell carcinoma: a comprehensive clinicopathologic classification. Part one. *J. Cutan. Pathol.* 33:191–206
20. Cassarino DS, DeRienzo DP, Barr RJ. 2006. Cutaneous squamous cell carcinoma: a comprehensive clinicopathologic classification. Part two. *J. Cutan. Pathol.* 33:261–79
21. Motaparthi K, Kapil JP, Velazquez EF. 2017. Cutaneous squamous cell carcinoma: review of the eighth edition of the American Joint Committee on cancer staging guidelines, prognostic factors, and histopathologic variants. *Adv. Anat. Pathol.* 24:171–94
22. de Leeuw J, van der Beek N, Neugebauer WD, Bjerring P, Neumann HM. 2009. Fluorescence detection and diagnosis of non-melanoma skin cancer at an early stage. *Lasers Surg. Med.* 41:96–103
23. Dinnes J, Deeks JJ, Chuchu N, Matin RN, Wong KY, et al. 2018. Visual inspection and dermoscopy, alone or in combination, for diagnosing keratinocyte skin cancers in adults. *Cochrane Database Syst. Rev.* 2018(12):CD011901
24. Bottomley MJ, Thomson J, Harwood C, Leigh I. 2019. The role of the immune system in cutaneous squamous cell carcinoma. *Int. J. Mol. Sci.* 20:2009
25. Jensen P, Hansen S, Moller B, Leivestad T, Pfeffer P, et al. 1999. Skin cancer in kidney and heart transplant recipients and different long-term immunosuppressive therapy regimens. *J. Am. Acad. Dermatol.* 40:177–86
26. Lanz J, Bouwes Bavinck JN, Westhuis M, Quint KD, Harwood CA, et al. 2019. Aggressive squamous cell carcinoma in organ transplant recipients. *JAMA Dermatol.* 155:66–71
27. Wehner MR, Linos E, Parvataneni R, Stuart SE, Boscardin WJ, Chren M-M. 2015. Timing of subsequent new tumors in patients who present with basal cell carcinoma or cutaneous squamous cell carcinoma. *JAMA Dermatol.* 151:382–88
28. Rowe DE, Carroll RJ, Day CL Jr. 1992. Prognostic factors for local recurrence, metastasis, and survival rates in squamous cell carcinoma of the skin, ear, and lip: implications for treatment modality selection. *J. Am. Acad. Dermatol.* 26:976–90
29. Euvrard S, Kanitakis J, Claudy A. 2003. Skin cancers after organ transplantation. *N. Engl. J. Med.* 348:1681–91
30. Euvrard S, Kanitakis J, Decullier E, Butnaru AC, Lefrancois N, et al. 2006. Subsequent skin cancers in kidney and heart transplant recipients after the first squamous cell carcinoma. *Transplantation* 81:1093–100
31. Stern RS. 2010. Prevalence of a history of skin cancer in 2007: results of an incidence-based model. *Arch. Dermatol.* 146:279–82
32. Waldman A, Schmults C. 2019. Cutaneous squamous cell carcinoma. *Hematol. Oncol. Clin. North Am.* 33:1–12

33. Wehner MR. 2020. Underestimation of cutaneous squamous cell carcinoma incidence, even in cancer registries. *JAMA Dermatol.* 156:1290–91
34. Fania L, Didona D, Di Pietro FR, Verkhovskaia S, Morese R, et al. 2021. Cutaneous squamous cell carcinoma: from pathophysiology to novel therapeutic approaches. *Biomedicines* 9:171
35. Guy GP Jr., Machlin SR, Ekwueme DU, Yabroff KR. 2015. Prevalence and costs of skin cancer treatment in the US, 2002–2006 and 2007–2011. *Am. J. Prev. Med.* 48:183–87
36. Johnson TM, Rowe DE, Nelson BR, Swanson NA. 1992. Squamous cell carcinoma of the skin (excluding lip and oral mucosa). *J. Am. Acad. Dermatol.* 26:467–84
37. Rahimi-Nedjat RK, Tuettenberg A, Sagheb K, Loquai C, Rybczynski B, et al. 2021. Factors accelerating recurrences and secondary tumors in cutaneous squamous cell carcinoma. *J. Cranio-Maxillofac. Surg.* 49:317–22
38. Schmitz L, Oster-Schmidt C, Stockfleth E. 2018. Nonmelanoma skin cancer—from actinic keratosis to cutaneous squamous cell carcinoma. *J. Dtsch. Dermatol. Ges.* 16:1002–13
39. Thompson AK, Kelley BF, Prokop LJ, Murad MH, Baum CL. 2016. Risk factors for cutaneous squamous cell carcinoma recurrence, metastasis, and disease-specific death: a systematic review and meta-analysis. *JAMA Dermatol.* 152:419–28
40. Watson M, Holman DM, Maguire-Eisen M. 2016. Ultraviolet radiation exposure and its impact on skin cancer risk. *Semin. Oncol. Nurs.* 32:241–54
41. Laikova KV, Oberemok VV, Krasnodubets AM, Gal'chinsky NV, Useinov RZ, et al. 2019. Advances in the understanding of skin cancer: ultraviolet radiation, mutations, and antisense oligonucleotides as anticancer drugs. *Molecules* 24:1516
42. Ouhtit A, Nakazawa H, Yamasaki H, Armstrong BK, Kricker A, et al. 1998. UV-radiation-specific p53 mutation frequency in normal skin as a predictor of risk of basal cell carcinoma. *J. Natl. Cancer Inst.* 90:523–31
43. Ziegler A, Jonason AS, Leffellt DJ, Simon JA, Sharma HW, et al. 1994. Sunburn and p53 in the onset of skin cancer. *Nature* 372:773–76
44. Setlow RB. 1974. The wavelengths in sunlight effective in producing skin cancer: a theoretical analysis. *PNAS* 71:3363–66
45. Thomas RF, Scotto J. 1983. Estimating increases in skin cancer morbidity due to increases in ultraviolet radiation exposure. *Cancer Investig.* 1:119–26
46. English DR, Armstrong BK, Kricker A, Winter MG, Heenan PJ, Randell PL. 1998. Demographic characteristics, pigmentary and cutaneous risk factors for squamous cell carcinoma of the skin: a case-control study. *Int. J. Cancer* 76:628–34
47. Zwald FOR, Brown M. 2011. Skin cancer in solid organ transplant recipients: advances in therapy and management: part II. Management of skin cancer in solid organ transplant recipients. *J. Am. Acad. Dermatol.* 65:263–79
48. Medina EM, Romero CJ, de la Cámara AG, Bernal AR, Municio AM, González EM. 2009. Malignancy after liver transplantation: cumulative risk for development. *Transplant. Proc.* 41:2447–49
49. Sheil AR, Disney AS, Mathew T, Amiss N. 1993. De novo malignancy emerges as a major cause of morbidity and late failure in renal transplantation. *Transplant. Proc.* 25:1383–84
50. Wilkins K, Turner R, Dolev JC, LeBoit PE, Berger TG, Maurer TA. 2006. Cutaneous malignancy and human immunodeficiency virus disease. *J. Am. Acad. Dermatol.* 54:189–206
51. Kang KW, Lee DL, Shin HK, Jung GY, Lee JH, Jeon MS. 2016. A retrospective clinical view of basal cell carcinoma and squamous cell carcinoma in the head and neck region: a single institution's experience of 247 cases over 19 years. *Arch. Craniofac. Surg.* 17:56–62
52. Pandeya N, Olsen CM, Whiteman DC. 2017. The incidence and multiplicity rates of keratinocyte cancers in Australia. *Med. J. Aust.* 207:339–43
53. Yousef H, Sharma S. 2018. *Anatomy, Skin (Integument), Epidermis*. St. Petersburg, FL: StatPearls Publ.
54. Veijouye SJ, Abazar Y, Heidari F, Sajedi N, Moghani FG, Nobakht M. 2017. Bulge region as a putative hair follicle stem cells niche: a brief review. *Iran. J. Public Health* 46:1167–75
55. Ratushny V, Gober MD, Hick R, Ridky TW, Seykora JT. 2012. From keratinocyte to cancer: the pathogenesis and modeling of cutaneous squamous cell carcinoma. *J. Clin. Investig.* 122:464–72

56. Kamstrup MR, Gniadecki R, Skovgaard GL. 2007. Putative cancer stem cells in cutaneous malignancies. *Exp. Dermatol.* 16:297–301
57. Ji AL, Rubin AJ, Thrane K, Jiang S, Reynolds DL, et al. 2020. Multimodal analysis of composition and spatial architecture in human squamous cell carcinoma. *Cell* 182:497–514.e22
58. Frazzette N, Khodadadi-Jamayran A, Doudican N, Santana A, Felsen D, et al. 2020. Decreased cytotoxic T cells and TCR clonality in organ transplant recipients with squamous cell carcinoma. *NPJ Precis. Oncol.* 4:13
59. Marinkovich MP. 2007. Laminin 332 in squamous-cell carcinoma. *Nat. Rev. Cancer* 7:370–80
60. Dajee M, Lazarov M, Zhang JY, Cai T, Green CL, et al. 2003. NF-κB blockade and oncogenic Ras trigger invasive human epidermal neoplasia. *Nature* 421:639–43
61. Martins VL, Vyas JJ, Chen M, Purdie K, Mein CA, et al. 2009. Increased invasive behaviour in cutaneous squamous cell carcinoma with loss of basement-membrane type VII collagen. *J. Cell Sci.* 122:1788–99
62. Fernandez-Figueras M-T, Puig L. 2020. The role of epithelial-to-mesenchymal transition in cutaneous squamous cell carcinoma. Epithelial-to-mesenchymal transition in cutaneous SCC. *Curr. Treat. Options Oncol.* 21:47
63. Stojadinovic O, Ramirez H, Pastar I, Gordon KA, Stone R, et al. 2017. MiR-21 and miR-205 are induced in invasive cutaneous squamous cell carcinomas. *Arch. Dermatol. Res.* 309:133–39
64. Berman B, Cockerell CJ. 2013. Pathobiology of actinic keratosis: ultraviolet-dependent keratinocyte proliferation. *J. Am. Acad. Dermatol.* 68:S10–19
65. McGregor J, Yu CW, Dublin E, Levison D, MacDonald D. 1992. Aberrant expression of p53 tumour-suppressor protein in non-melanoma skin cancer. *Br. J. Dermatol.* 127:463–69
66. Fearon ER, Vogelstein B. 1990. A genetic model for colorectal tumorigenesis. *Cell* 61:759–67
67. Thomson J, Bewicke-Copley F, Anene CA, Gulati A, Nagano A, et al. 2021. The genomic landscape of actinic keratosis. *J. Investig. Dermatol.* 141:1664–74.e7
68. Marks R, Rennie G, Selwood T. 1988. Malignant transformation of solar keratoses to squamous cell carcinoma. *Lancet* 331:795–97
69. Fernandez Figueras M. 2017. From actinic keratosis to squamous cell carcinoma: pathophysiology revisited. *J. Eur. Acad. Dermatol. Venereol.* 31:5–7
70. Nowell CS, Radtke F. 2017. Notch as a tumour suppressor. *Nat. Rev. Cancer* 17:145–59
71. Vauclair S, Majo F, Durham A-D, Ghyselinck NB, Barrandon Y, Radtke F. 2007. Corneal epithelial cell fate is maintained during repair by Notch1 signaling via the regulation of vitamin A metabolism. *Dev. Cell* 13:242–53
72. Collins CA, Watt FM. 2008. Dynamic regulation of retinoic acid-binding proteins in developing, adult and neoplastic skin reveals roles for β-catenin and Notch signalling. *Dev. Biol.* 324:55–67
73. Syed Z, Cheepala SB, Gill JN, Stein J, Nathan CA, et al. 2009. All-trans retinoic acid suppresses Stat3 signaling during skin carcinogenesis. *Cancer Prev. Res.* 2:903–11
74. Lefort K, Mandinova A, Ostano P, Kolev V, Calpini V, et al. 2007. Notch1 is a p53 target gene involved in human keratinocyte tumor suppression through negative regulation of ROCK1/2 and MRCKα kinases. *Genes Dev.* 21:562–77
75. Demehri S, Turkoz A, Kopan R. 2009. Epidermal Notch1 loss promotes skin tumorigenesis by impacting the stromal microenvironment. *Cancer Cell* 16:55–66
76. Giachino C, Boulay J-L, Ivanek R, Alvarado A, Tostado C, et al. 2015. A tumor suppressor function for Notch signaling in forebrain tumor subtypes. *Cancer Cell* 28:730–42
77. Parsa R, Yang A, McKeon F, Green H. 1999. Association of p63 with proliferative potential in normal and neoplastic human keratinocytes. *J. Investig. Dermatol.* 113:1099–105
78. Rocco JW, Leong C-O, Kuperwasser N, DeYoung MP, Ellisen LW. 2006. p63 mediates survival in squamous cell carcinoma by suppression of p73-dependent apoptosis. *Cancer Cell* 9:45–56
79. Rangarajan A, Talora C, Okuyama R, Nicolas M, Mammucari C, et al. 2001. Notch signaling is a direct determinant of keratinocyte growth arrest and entry into differentiation. *EMBO J.* 20:3427–36
80. Zheng Q, Capell BC, Parekh V, O'Day C, Atillasoy C, et al. 2020. Whole-exome and transcriptome analysis of UV-exposed epidermis and carcinoma in situ reveals early drivers of carcinogenesis. *J. Investig. Dermatol.* 141:295–307.e13

81. Lazarov M, Kubo Y, Cai T, Dajee M, Tarutani M, et al. 2002. CDK4 coexpression with Ras generates malignant human epidermal tumorigenesis. *Nat. Med.* 8:1105–14
82. Sarin KY, Lin Y, Daneshjou R, Ziyatdinov A, Thorleifsson G, et al. 2020. Genome-wide meta-analysis identifies eight new susceptibility loci for cutaneous squamous cell carcinoma. *Nat. Commun.* 11:820
83. Chahal HS, Lin Y, Ransohoff KJ, Hinds DA, Wu W, et al. 2016. Genome-wide association study identifies novel susceptibility loci for cutaneous squamous cell carcinoma. *Nat. Commun.* 7:12048
84. Siiskonen SJ, Zhang M, Li W-Q, Liang L, Kraft P, et al. 2016. A genome-wide association study of cutaneous squamous cell carcinoma among European descendants. *Cancer Epidemiol. Prev. Biomark.* 25:714–20
85. Asgari MM, Wang W, Ioannidis NM, Itnyre J, Hoffmann T, et al. 2016. Identification of susceptibility loci for cutaneous squamous cell carcinoma. *J. Investig. Dermatol.* 136:930–37
86. Reinehr CPH, Bakos RM. 2019. Actinic keratoses: review of clinical, dermoscopic, and therapeutic aspects. *An. Bras. Dermatol.* 94:637–57
87. Moy RL. 2000. Clinical presentation of actinic keratoses and squamous cell carcinoma. *J. Am. Acad. Dermatol.* 42:S8–10
88. Parekh V, Seykora JT. 2017. Cutaneous squamous cell carcinoma. *Clin. Lab. Med.* 37:503–25
89. Jennings L, Schmults CD. 2010. Management of high-risk cutaneous squamous cell carcinoma. *J. Clin. Aesthet. Dermatol.* 3:39–48
90. Mittal A, Colegio O. 2017. Skin cancers in organ transplant recipients. *Am. J. Transplant.* 17:2509–30
91. Abikhair M, Mitsui H, Yanofsky V, Roudiani N, Ovits C, et al. 2016. Cyclosporine A immunosuppression drives catastrophic squamous cell carcinoma through IL-22. *JCI Insight* 1(8):e86434
92. Wu X, Nguyen BC, Dziunycz P, Chang S, Brooks Y, et al. 2010. Opposing roles for calcineurin and ATF3 in squamous skin cancer. *Nature* 465:368–72
93. Inman GJ, Wang J, Nagano A, Alexandrov LB, Purdie KJ, et al. 2018. The genomic landscape of cutaneous SCC reveals drivers and a novel azathioprine associated mutational signature. *Nat. Commun.* 9:3667
94. Bottomley MJ, Harden PN, Wood KJ. 2016. $CD8^+$ immunosenescence predicts post-transplant cutaneous squamous cell carcinoma in high-risk patients. *J. Am. Soc. Nephrol.* 27:1505–15
95. Nindl I, Gottschling M, Stockfleth E. 2007. Human papillomaviruses and non-melanoma skin cancer: basic virology and clinical manifestations. *Dis. Markers* 23:247–59
96. Hufbauer M, Akgül B. 2017. Molecular mechanisms of human papillomavirus induced skin carcinogenesis. *Viruses* 9:187
97. Strickley JD, Messerschmidt JL, Awad ME, Li T, Hasegawa T, et al. 2019. Immunity to commensal papillomaviruses protects against skin cancer. *Nature* 575:519–22
98. Weissenborn SJ, Nindl I, Purdie K, Harwood C, Proby C, et al. 2005. Human papillomavirus-DNA loads in actinic keratoses exceed those in non-melanoma skin cancers. *J. Investig. Dermatol.* 125:93–97
99. Hübbers CU, Akgül B. 2015. HPV and cancer of the oral cavity. *Virulence* 6:244–48
100. Gillet LC, Schärer OD. 2006. Molecular mechanisms of mammalian global genome nucleotide excision repair. *Chem. Rev.* 106:253–76
101. Zebian A, Shaito A, Mazurier F, Rezvani HR, Zibara K. 2019. XPC beyond nucleotide excision repair and skin cancers. *Mutat. Res. Rev. Mutat. Res.* 782:108286
102. Kusakabe M, Onishi Y, Tada H, Kurihara F, Kusao K, et al. 2019. Mechanism and regulation of DNA damage recognition in nucleotide excision repair. *Genes Environ.* 41:2
103. Gonçalves-Maia M, Gache Y, Basante M, Cosson E, Salavagione E, et al. 2020. NK cell and fibroblast-mediated regulation of skin squamous cell carcinoma invasion by CLEC2A is compromised in xeroderma pigmentosum. *J. Investig. Dermatol.* 140:1723–32
104. de Jong SJ, Imahorn E, Itin P, Uitto J, Orth G, et al. 2018. Epidermodysplasia verruciformis: inborn errors of immunity to human beta-papillomaviruses. *Front. Microbiol.* 9:1222
105. de Jong SJ, Crequer A, Matos I, Hum D, Gunasekharan V, et al. 2018. The human CIB1-EVER1-EVER2 complex governs keratinocyte-intrinsic immunity to β-papillomaviruses. *J. Exp. Med.* 215:2289–310
106. Condorelli AG, Dellambra E, Logli E, Zambruno G, Castiglia D. 2019. Epidermolysis bullosa-associated squamous cell carcinoma: from pathogenesis to therapeutic perspectives. *Int. J. Mol. Sci.* 20:5707

107. Green AC, Olsen C. 2017. Cutaneous squamous cell carcinoma: an epidemiological review. *Br. J. Dermatol.* 177:373–81
108. Mounessa J, Qin R, Dunnick CA, Dellavalle RP. 2016. Chemoprevention of keratinocyte carcinomas: an updated review. *Am. J. Clin. Dermatol.* 17:475–84
109. Chen AC, Martin AJ, Choy B, Fernández-Peñas P, Dalziell RA, et al. 2015. A phase 3 randomized trial of nicotinamide for skin-cancer chemoprevention. *N. Engl. J. Med.* 373:1618–26
110. Kreul SM, Havighurst T, Kim K, Mendonça EA, Wood GS, et al. 2012. A phase III skin cancer chemoprevention study of DFMO: long-term follow-up of skin cancer events and toxicity. *Cancer Prev. Res.* 5:1368–74
111. Muranushi C, Olsen CM, Pandeya N, Green AC. 2015. Aspirin and nonsteroidal anti-inflammatory drugs can prevent cutaneous squamous cell carcinoma: a systematic review and meta-analysis. *J. Investig. Dermatol.* 135:975–83
112. George R, Weightman W, Russ GR, Bannister KM, Mathew TH. 2002. Acitretin for chemoprevention of non-melanoma skin cancers in renal transplant recipients. *Australas. J. Dermatol.* 43:269–73
113. Lebwohl M, Swanson N, Anderson LL, Melgaard A, Xu Z, Berman B. 2012. Ingenol mebutate gel for actinic keratosis. *N. Engl. J. Med.* 366:1010–19
114. Cunningham TJ, Tabacchi M, Eliane JP, Tuchayi SM, Manivasagam S, et al. 2017. Randomized trial of calcipotriol combined with 5-fluorouracil for skin cancer precursor immunotherapy. *J. Clin. Investig.* 127:106–16
115. Weinstock MA, Thwin SS, Siegel JA, Marcolivio K, Means AD, et al. 2018. Chemoprevention of basal and squamous cell carcinoma with a single course of fluorouracil, 5%, cream: a randomized clinical trial. *JAMA Dermatol.* 154:167–74
116. Demehri S, Turkoz A, Manivasagam S, Yockey LJ, Turkoz M, Kopan R. 2012. Elevated epidermal thymic stromal lymphopoietin levels establish an antitumor environment in the skin. *Cancer Cell* 22:494–505
117. Combalia A, Carrera C. 2020. Squamous cell carcinoma: an update on diagnosis and treatment. *Dermatol. Pract. Concept.* 10:e2020066
118. Alam M, Armstrong A, Baum C, Bordeaux JS, Brown M, et al. 2018. Guidelines of care for the management of cutaneous squamous cell carcinoma. *J. Am. Acad. Dermatol.* 78:560–78
119. Maubec E, Petrow P, Scheer-Senyarich I, Duvillard P, Lacroix L, et al. 2011. Phase II study of cetuximab as first-line single-drug therapy in patients with unresectable squamous cell carcinoma of the skin. *J. Clin. Oncol.* 29:3419–26
120. Foote M, McGrath M, Guminski A, Hughes B, Meakin J, et al. 2014. Phase II study of single-agent panitumumab in patients with incurable cutaneous squamous cell carcinoma. *Ann. Oncol.* 25:2047–52
121. Jarkowski A III, Hare R, Loud P, Skitzki JJ, Kane JM III, et al. 2016. Systemic therapy in advanced cutaneous squamous cell carcinoma (CSCC): the Roswell Park experience and a review of the literature. *Am. J. Clin. Oncol.* 39:545–48
122. Migden MR, Rischin D, Schmults CD, Guminski A, Hauschild A, et al. 2018. PD-1 blockade with cemiplimab in advanced cutaneous squamous-cell carcinoma. *N. Engl. J. Med.* 379:341–51
123. Seiwert TY, Burtness B, Mehra R, Weiss J, Berger R, et al. 2016. Safety and clinical activity of pembrolizumab for treatment of recurrent or metastatic squamous cell carcinoma of the head and neck (KEYNOTE-012): an open-label, multicentre, phase 1b trial. *Lancet Oncol.* 17:956–65
124. Patel R, Chang ALS. 2019. Immune checkpoint inhibitors for treating advanced cutaneous squamous cell carcinoma. *Am. J. Clin. Dermatol.* 20:477–82
125. Willenbrink T, Jambusaria-Pahlajani A, Arron S, Seckin D, Harwood C, Proby C. 2019. Treatment approaches in immunosuppressed patients with advanced cutaneous squamous cell carcinoma. *J. Eur. Acad. Dermatol. Venereol.* 33:57–60
126. Bonilla X, Parmentier L, King B, Bezrukov F, Kaya G, et al. 2016. Genomic analysis identifies new drivers and progression pathways in skin basal cell carcinoma. *Nat. Genet.* 48:398–406
127. Chitsazzadeh V, Coarfa C, Drummond JA, Nguyen T, Joseph A, et al. 2016. Cross-species identification of genomic drivers of squamous cell carcinoma development across preneoplastic intermediates. *Nat. Commun.* 7:12601
128. Huang JT, Coughlin CC, Hawryluk EB, Hook K, Humphrey SR, et al. 2019. Risk factors and outcomes of nonmelanoma skin cancer in children and young adults. *J. Pediatr.* 211:152–58

129. Li M, Hener P, Zhang Z, Kato S, Metzger D, Chambon P. 2006. Topical vitamin D3 and low-calcemic analogs induce thymic stromal lymphopoietin in mouse keratinocytes and trigger an atopic dermatitis. *PNAS* 103:11736–41
130. Rosenberg AR, Tabacchi M, Ngo KH, Wallendorf M, Rosman IS, et al. 2019. Skin cancer precursor immunotherapy for squamous cell carcinoma prevention. *JCI Insight* 4:e125476
131. Gupta R, Rady PL, Doan HQ, Tyring SK. 2020. Development of a β-HPV vaccine: updates on an emerging frontier of skin cancer prevention. *J. Clin. Virol.* 126:104348
132. Finn OJ. 2018. The dawn of vaccines for cancer prevention. *Nat. Rev. Immunol.* 18:183–94