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Understanding the squamous cell carcinoma immune microenvironment

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Primary cutaneous squamous cell carcinoma (cSCC) is the second most common human cancer with a rising incidence of about 1.8 million in the United States annually. Primary cSCC is usually curable by surgery; however, in some cases, cSCC eventuates in nodal metastasis and death from disease specific death. cSCC results in up to 15,000 deaths each year in the United States. Until recently, non-surgical options for treatment of locally advanced or metastatic cSCC were largely ineffective. With the advent of checkpoint inhibitor immunotherapy, including cemiplimab and pembrolizumab, response rates climbed to 50%, representing a vast improvement over chemotherapeutic agents used previously. Herein, we discuss the phenotype and function of SCC associated Langerhans cells, dendritic cells, macrophages, myeloid derived suppressor cells and T cells as well as SCC-associated lymphatics and blood vessels. Possible role(s) of SCC-associated cytokines in progression and invasion are reviewed. We also discuss the SCC immune microenvironment in the context of currently available and pipeline therapeutics.

KEYWORDS

squamous cell carcinoma, tumor microenvironment, PD-1, tumor infiltrating lymphocytes, exhausted T cells, cytokines

1 Introduction

Cutaneous SCC (cSCC) is the second most frequent skin cancer in the United States (US) with 1.8 million new cases each year, and its global incidence rate has been reported to increase 3-7% annually (1, 2). cSCC lesions appear in regions that are most exposed to ultraviolet (UV); the head and the neck are the most common sites followed by the trunk and extremities (3).

UV radiation can alter the genome of epidermal cells and cause SCC development and subsequent metastasis, usually to nearby lymph nodes. A complex network of genes (TP53, CDKN2A, NOTCH1, NOTCH2, EGFR and TERT) and molecular pathways (RAS/RAF/MEK/ERK and PI3K/AKT/mTOR) are associated with the pathogenesis of cSCC (4). Also, recent findings identified EP300, PBRM1, USP28, and CHUK as four novel genes that are mutated in greater than 10% of cSCCs (5). The top three recurrently altered genes in metastatic cSCCs are TP53, CDKN2A, and NOTCH1/2 (6–8).

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In addition to UV exposure ionizing radiation, fair skin, chronic immunosuppression, genetic conditions, the presence of chronic wounds or scars, smoking, chemical carcinogens, and human papillomavirus (HPV) infection are the other risk factors of cSCC development (9). The vast majority of cSCC cases are treated successfully by excision with clear margins (10, 11); however, these tumors can be aggressive and responsible for most of the ~15,000 non-melanoma skin cancer deaths in the United States each year (1). Patients with localized cSCC have a favorable prognosis with a 5-year survival rate of 99% following Mohs micrographic surgery (12, 13). Metastasis affects approximately 3.7%-5.2% of all SCC patients (14). The expected 5-year and 10-year survival rates in these patients decreases to 25-50% and 16%, respectively (11, 15–17).

Advanced cSCC is described as either a locally advanced disease that is untreatable by surgery or radiation therapy (RT), a metastatic disease with distant metastases, or large, multiple, and extracapsular nodal disease with a high risk of recurrence despite lymphadenectomy and radiation therapy (18). Cemiplimab, an immune checkpoint inhibitor, is the first medication approved in the United States for advanced cSCC (19). It is a human monoclonal antibody that inhibits the PD-1 pathway by blocking T-cell inactivation, thus assisting the immune system in fighting cancer cells (20) as illustrated in Figure 1. Cemiplimab exhibits an overall response rate of 50%, which is a significant improvement over conventional chemotherapy. It has been shown that cemiplimab has a significant antitumor function with long-lasting response, and acceptable safety profile in patients (19). Pembrolizumab is another PD-1 inhibitor, with a similar mechanism to cemiplimab, and has been recently approved in the United States for recurrent or metastatic cSCC that is uncurable with surgery or radiation therapy (21). A case of metastatic cSCC treated with nivolumab, another PD-1 inhibitor, has been reported, and the patient exhibited a complete response to this treatment (22). In another case report, a patient with unresectable recurrent scalp cSCC with meningeal invasion was successfully treated with nivolumab monotherapy (23).

Lymphocyte activation gene 3 (LAG3) is an inhibitory receptor that is expressed on $CD4^+$, $CD8^+$, regulatory T (T-reg) cell, natural killer cell, B cell, and other immune cells (24). LAG3 serves a negative regulatory role in cancer immunology by interacting with its ligands. Higher LAG3 expression has been reported in head and neck squamous cell carcinoma compared to normal tissues. Therefore, LAG3-targeting agents could represent another promising checkpoint inhibitor immunotherapy for these malignancies (25). Combining immunotherapy and radiotherapy is another cutting-edge method of treating cSCC (26). The trials of radiation therapy and cemiplimab in patients with skin cancer (NCT05574101) as well as radiotherapy in combination with atezolizumab (PD-L1 inhibitor) in locally advanced borderline resectable or unresectable cSCC (NCT05085496) are ongoing. Another ongoing trial is testing cetuximab (EGFR inhibitor) before surgery in the treatment of patients with aggressive locally advanced skin cancer (NCT02324608).

The efficacy of talimogene laherparepvec (oncolytic viral immunotherapy) and panitumumab (EGFR inhibitor) for the treatment of locally advanced or metastatic cSCC is being researched in another ongoing trial (NCT04163952).

The development and progression of non-melanoma skin cancer (NMSC) are significantly influenced by immune system function (27). An increased incidence of cSCC in immunocompromised solid organ transplant recipients indicates the critical role of the immune surveillance in host protection (28). The immune system recognizes cancer cells as abnormal and can eliminate them in some cases (29); however, tumor cells might evade immune surveillance through immunoediting processes (30). Cancer cells utilize several mechanisms to escape immune surveillance, including MHC loss and expression of immunosuppressive factors, such as IL-6, IL-10, TGF-β, prostaglandins, and Fas ligand (31, 32).

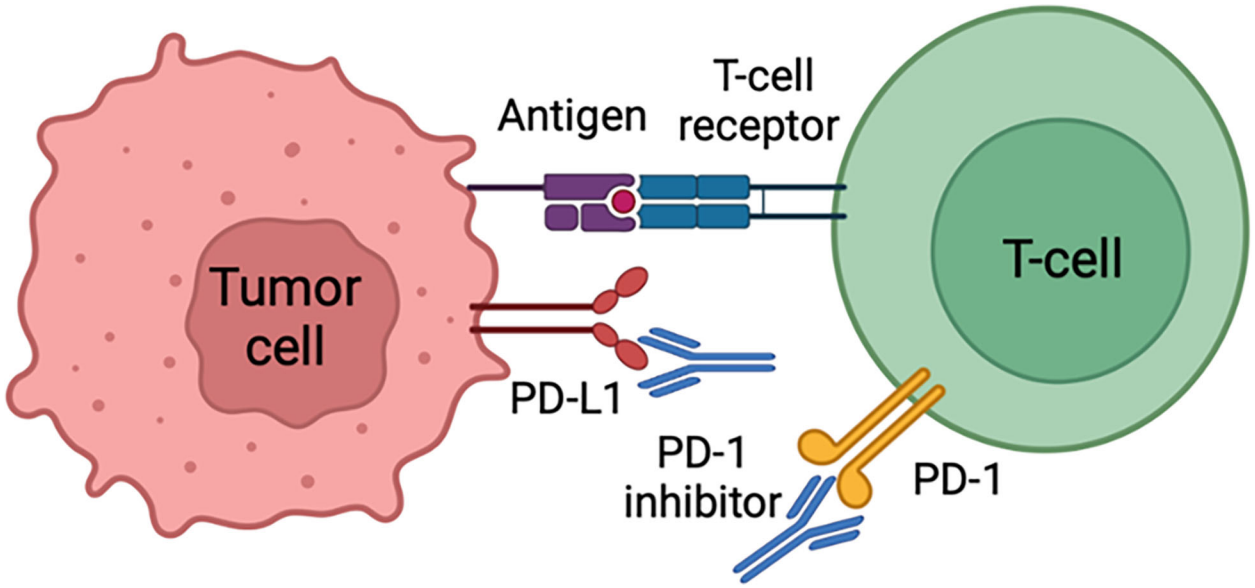

FIGURE 1
Cancer cells can evade immune surveillance by expressing PD-L1 protein that acts as a "stop sign" to inactivate T cells. PD-L1 attaches to PD-1 and B7.1 T cell receptors, both of which inactivate T cells. Cemiplimab prevents T cell inactivation and subsequently increases anti-cancer activity through PD-L1 blockade.

The tumor microenvironment is characterized as a combination of tumoral and non-tumoral cells at the dynamic interface of neoplasia (33). Although non-tumoral cells within the tumor microenvironment may have protective functions in limiting tumor progression, many studies show that they have also an important role in tumor growth and metastasis (34). Therefore, it is crucial to understand the features of the cSCC tumor-associated immune microenvironment in detail to develop reliable prognostic markers and new advanced treatments.

In this review, phenotype and functions of cSCC-associated Langerhans cells, dendritic cells, macrophages, myeloid-derived suppressor cells and T cells as well as cSCC-associated lymphatics and blood vessels are discussed. Moreover, the potential roles of cSCC-associated cytokines in progression and invasion of the tumor are described.

2 Myeloid-derived suppressor cells in SCC

Myeloid-derived suppressor cells (MDSCs) are pathologically activated neutrophils and monocytes with immunosuppressive activity. They participate in the regulation of immune responses in many pathological conditions, such as cancer, chronic infection, sepsis, and autoimmunity. Two major groups of MDSCs in humans include granulocytic/polymorphonuclear MDSCs (PMN-MDSCs) and monocytic MDSCs (M-MDSCs), which originate from the granulocytic and monocytic myeloid cell lineages, respectively (35). MDSCs are related to poor outcomes in cancer (36). It has been shown that high levels of circulating MDSC in patients with solid tumors, were related to poor overall survival (37).

In cancer patients, these cells express the common myeloid marker CD33 but not mature myeloid and lymphoid cell markers in cancer patients. In humans, MDSCs are identifiable as lineage (CD3, CD14, CD19, CD56)–negative, HLA-DR–negative, and CD33-positive or $CD33^{+}CD14^{-}$ $CD11b^{+}$ cells (38, 39).

The signals driving MDSCs development occur in two partially overlapping phases. Expansion of immature myeloid cells occurs in phase 1, and neutrophils and monocytes convert to pathologically activated MDSCs in phase 2 (38).

MDSCs are one of the major factors responsible for immune suppression in cancers that not only cause tumor progression but also result in the failure of immunotherapy (39). Arginase, nitric oxide (NO), and reactive oxygen species (ROS) have all been shown to play a role in MDSC-mediated T-cell suppression (40). MDSCs are critical producers of NO in SCC, which suppresses E-selectin expression on tumor vessels. Subsequently, the entry of skin homing T-cells into tumors are restricted, resulting in evasion of SCC from immune detection (41).

Clearly, a successful cancer immunotherapy will be possible if the immune suppressive factors can be eliminated from the body. As MDSCs are one of the major immune suppressive factors in cancers, the challenge of effectively and selectively targeting MDSCs remains (39). Medications that diminish NO production e.g., iNOS inhibitors, may be effective in the treatment of SCCs and their premalignant precursor lesions actinic keratoses through improvement of anti-tumor immune responses (41). Based on earlier studies, all-trans retinoic acid (ATRA) promotes the differentiation of M-MDSCs into macrophages and DCs and apoptosis of PMN-MDSCs in both mice and humans (42–44). Concurrent use of ATRA therapy with CTLA-4 blockade was tested in melanoma patients and resulted in decrease in the number of circulating MDSCs. Therefore, targeting MDSCs in combination with immunotherapies may improve response rates and effectiveness in other skin cancers (45).

3 Tumor-associated macrophages

Macrophages are important tumor-infiltrating cells (46) contributing to different carcinogenesis stages, including initiation, growth, invasion, and metastasis (47, 48). More macrophages are present in SCC compared with normal skin (49). Macrophages surrounding and penetrating the tumor are termed tumor-associated macrophages (TAMS) (46).

In response to tumors, macrophages display a polarized reaction defined by two different states: classically activated macrophage (M1) and alternatively activated macrophage (M2). M1 macrophages are activated by interferon-γ (IFN-γ), bacterial lipopolysaccharide (LPS), or tumor necrosis factor-α (TNF-α) and release interleukin 12 (IL-12) to prevent tumor growth. In contrast, M2 macrophages are activated by IL-4 and release IL-10, which contributes to tumor progression (27, 50–52).

Tumor-associated macrophages have many similar characteristics to alternatively activated macrophages (M2 macrophages) (46). Based on recent studies, macrophage activation in SCC is heterogenous and there are three types of TAMs: TAMs expressing M1 markers, TAMs expressing M2 markers and TAMs simultaneously expressing M1 and M2 (49) (Figure 2). It is believed that tumors can generate a dynamic microenvironment that alters the TAMs into macrophages that help tumor growth (53). Weaker classical macrophage activation in SCC cause TAMs to produce more tumorigenic growth factors (49). Increased TAM levels are associated with poor prognosis in various human malignancies (47, 48, 54).

Heterogeneous activation of TAMs in SCC suggests potential treatment strategies contributing to the induction of a more dominant M1 activation state with anti-cancer phenotype (27).

TAMs in SCC may produce matrix metalloproteinases (MMPs) that may aid tumor invasion. A positive correlation between MMP-9 (gelatinase B) and MMP-11 (stromelysin-3) proteins and increased tumor aggressiveness has been revealed (55–58). TAMs also contribute to lymphangiogenesis through vascular endothelial growth factor-C (VEGF-C) expression (59). It has been reported that enhanced lymph vessel density is related to increased risk of metastasis in the oral cavity SCC and melanoma (60, 61).

TAM densities and functional immunophenotypes differ in human cutaneous SCCs and BCCs, which can contribute to behavioral differences between these two tumors. It has been shown that SCCs express more TAM-associated markers (MMP-9, arginase-1, CD127 and CD40) compared with BCCs, and TAMs in SCC have a higher density and polarization state. Lactic acid levels are higher in SCCs compared with BCCs, and tumor-derived lactic acid is an important factor playing a role in TAM polarization in SCCs (62).

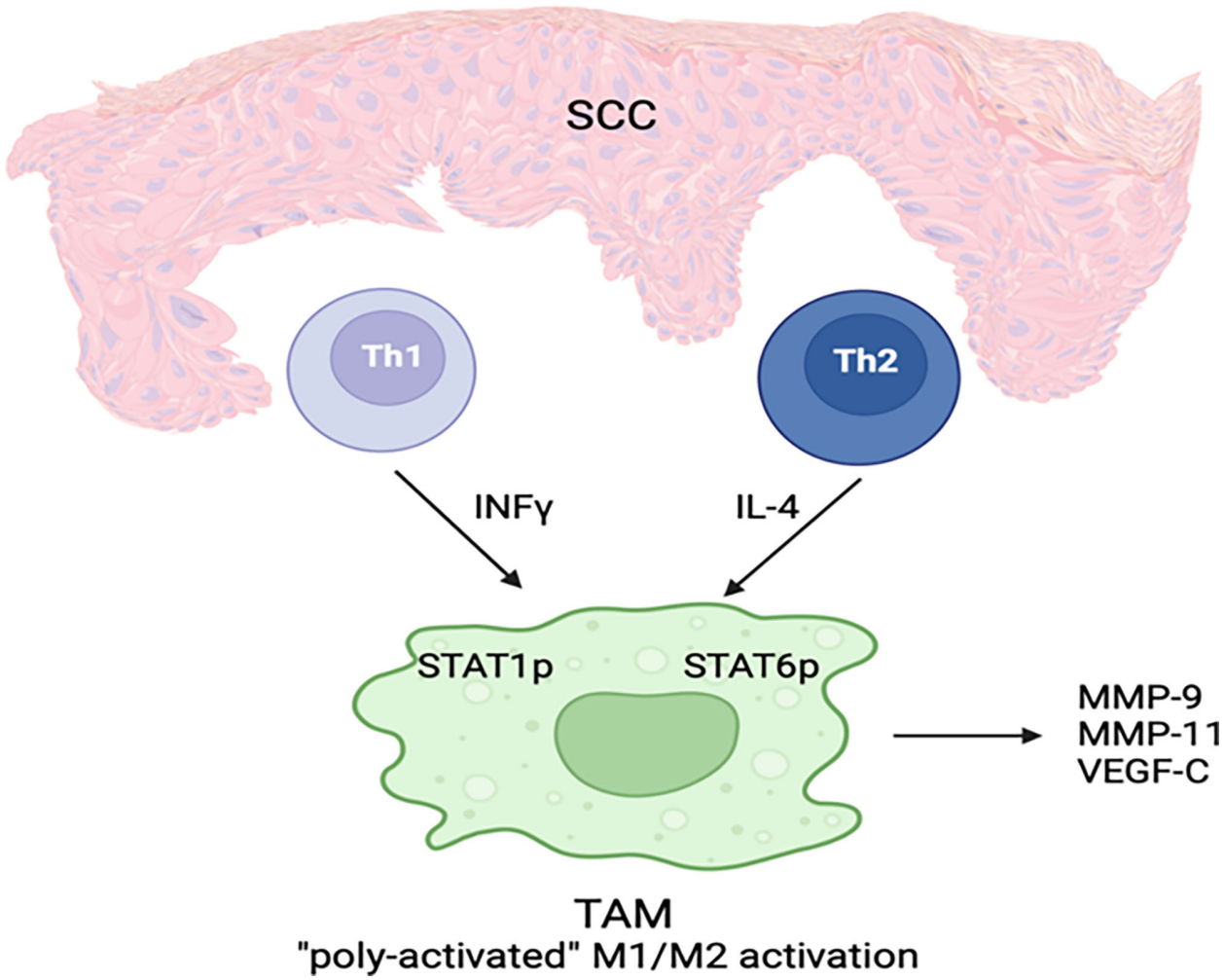

FIGURE 2
A subset of TAMs in cSCC displays both classical and alternative activation features simultaneously. IFN-γ and IL-4 are secreted by Th1 and Th2 cells, respectively, in the cSCC microenvironment. As a result of these cytokines, which activate M1 classic and M2 alternate phenotypes, poly-activated TAMs are generated. STAT1 and STAT6 phosphorylation as well as MMP-9, MMP-11, and VEGF-C expression are characteristic features of TAMs.

In fact, TAMs in SCC, due to weaker classical macrophage activation and higher production of tumorigenic growth factors, are unable to prevent tumor genesis and in fact they can even facilitate tumor growth; however, they contribute to tumor invasion and metastasis through production of high levels of MMPs, more dominant M2 activation and lymphangiogenic mediator (VEGF-C) expression (27).

CD200 (a known immunosuppressive surface protein) is overexpressed in stroma around cSCC, mainly by blood vessel endothelia. CD200 is also expressed on cSCC tumor cells (63). In addition, more $CD200R^{+}$ cells are located in the cSCC microenvironment than normal skin, and CD200R was detected on macrophages and dendritic cells (28). Increased CD200 expression on tumor cells is associated with tumor progression and decreased patient survival (63, 64). Endothelial CD200 may inhibit aberrant diapedesis of macrophages during inflammation partly through downregulation of macrophage adhesion molecules. Hence, through this mechanism, CD200 may play a role in suppression of macrophage function (65). Moreover, binding of endothelial CD200 to CD200R on macrophages and dendritic cells inhibits proinflammatory activation (66–70) and suppresses classic activation of macrophages; therefore, M2 cells become the predominant macrophage polarized state (71).

Anti-CD200 antibody (through blocking the CD200-CD200R interaction) has been shown to improve antitumor activity against CD200-expressing human tumors in a mouse model (72, 73). Thus, anti-CD200 therapies could represent effective treatments for aggressive SCCs (28).

4 Dendritic cells and Langerhans cells

Dendritic cells (DC) are antigen-presenting cells (APCs) that play an important role in linking the innate and adaptive immune systems (74). The ability of DCs to induce tumor-specific T-cell responses facilitate their vital role in cancer immune surveillance (75).

Three main subsets of cutaneous DCs in humans include Langerhans cells (LCs), myeloid DCs (mDCs), and plasmacytoid DCs (pDCs) (76). As Langerhans cells are found in the epidermis, they are the first APCs to encounter SCC (77). LCs from human SCC

can stimulate $CD8^+$- or NK-cell-mediated response more efficiently than other DC subsets, resulting in a more robust proliferation of naive $CD8^+$ T cells (78).

In addition to the primary role of DCs in initiating the cellular immunity, they are also involved in polarizing the naive $CD4^+$ T cells towards a Th2 immune response through releasing type II cytokines, such as IL-4, IL-5, and IL-13 (79). Furthermore, it has been reported that LCs from SCC were more powerful inducers of allogeneic $CD4^+$ and $CD8^+$ T-cell proliferation and IFN-γ production compared to those from normal skin and eventually more potent in activating type 1 T-cell responses (77).

Tumor-induced dendritic cells dysfunction (29) and tumor-induced DC apoptosis (80–82) are two of major strategies used by tumors to escape immune surveillance.

Several studies have revealed that the number of both LCs and $CD11c^+$ dermal DCs is markedly reduced in SCC lesions (83, 84) and the ability of the dermal myeloid DCs to activate T cells and stimulate the production of interferon (IFN)-γ is diminished (83, 85).

Higher levels of immunosuppressive cytokines, such as TGF-*β*, IL-10, IL-6 and VEGF-A, in the microenvironment of SCCs are believed to be possible causes of mDCs suppression (83). IL-10 has the potential to inhibit the differentiation of monocytes to DC (86), weaken APC function of DCs (87, 88), suppress DCs' ability to activate T cells, and cause induction of antigen-specific anergy (89). Increased VEGF levels are related to decreased number of DCs in tumor lesion and in the peripheral blood of patients with various malignant tumors. This finding demonstrates the ability of VEGF to inhibit DC differentiation (90–92).

The presence of large numbers of pDCs is another distinguishing feature of the SCC tumor microenvironment (83). These cells facilitate tumor eradication through production of large quantities of IFN-α in response to foreign antigen. Moreover, pDCs can recognize, process, and cross-present foreign antigen to $CD8^+$ T lymphocytes (93, 94). Despite lower antigen uptake by pDCs compared to mDCs, pDCs may still be effective in anti-tumor immune response (Figure 3) (95).

It can be concluded that DCs are desirable targets for tumor immunotherapy due to their capacity to link the innate and adaptive immune systems as well as their ability to initiate the immune response (74). In addition, human LCs have been shown to be

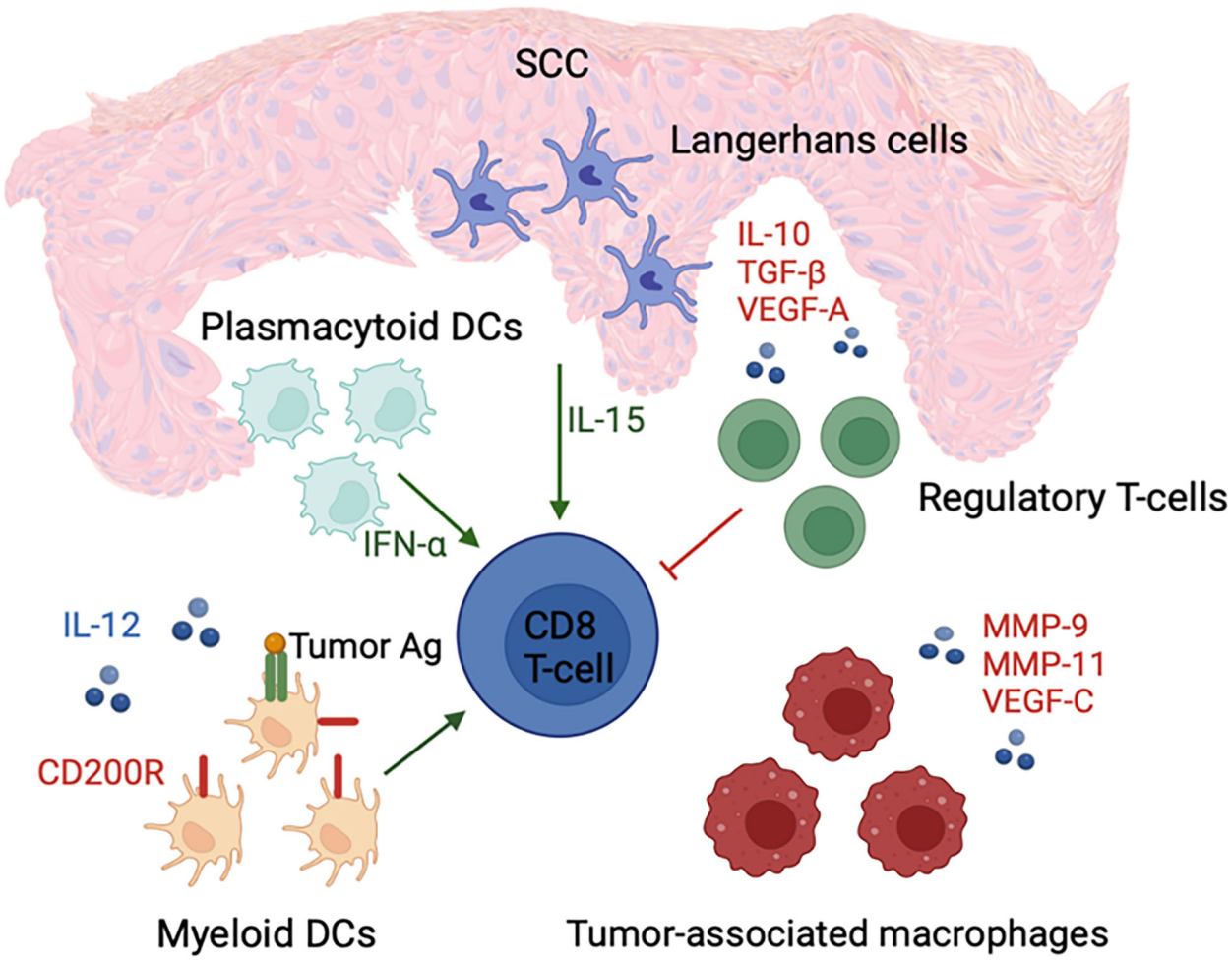

FIGURE 3
cSCC microenvironment is associated with an increased number of IFN-α-secreting pDCs and LCs with enhanced ability to activate CD8+ T cells, which potentially promote immunosurveillance. In contrast, an increased number of regulatory T cells; tumor-associated macrophages; and immune suppressive cytokines, such as IL-10, TGF-β, and VEGF-A, are present in the tumor microenvironment. These factors contribute to tumor growth and immune dysfunction through suppression of mDC and CD8+ T cell activity.

more potent inducers of type 1 T-cell response in the cSCC microenvironment. Hence, LCs can be used in DC-based cancer immunotherapy as a promising novel strategy in the treatment of skin malignancies (77).

5 T-lymphocytes

Numerous immune cells, including T-cells, are found in SCC lesions (96–98). Despite T cell infiltration into cutaneous SCC (cSCC), these cells are incapable of eradicating the tumor (99, 100).

It has been demonstrated that SCC and transplant-associated SCC (TSCC) microenvironments have significantly greater numbers of $CD3^+$ and $CD8^+$ T cells than normal skin. These cells accumulate predominantly in the peritumoral region and are less frequently noted within the tumoral region. The number of $FOXP3^+$ T reg cells is increased in both SCC and TSCC compared to normal skin (101). Approximately more than 50% of the T cells infiltrating cSCCs from both immunocompetent and immunosuppressed patients are $FOXP3^+$ T reg cells (97). These cells are $CD4^+$ and lack CLA, CCR4, and CCR6 (skin resident T reg markers) (102). Moreover, these cells express markers of central memory T cells, such as L-selectin and CCR7. Given that T reg cells do not proliferate locally in tumors, recruitment from the blood may be the main mechanism responsible for significant presence of these cells in tumors (97).

Although $FOXP3^+$ T reg cells contribute to immune tolerance (103), which is important for preventing autoimmune diseases (104), they may suppress antitumor immunity (105, 106) and play a role in immune evasion. Particularly, the immune response can be regulated by T reg cells by suppressing the proliferation and cytokine production of effector T cells (107, 108).

Based on several studies, the greater number of tumor infiltrating T regs is related to poor prognosis and lower survival rates in breast (109), ovarian (110, 111) and gastric carcinomas (105). T regs may contribute to cSCC metastasis and thus have potential prognostic significance (100). Some recent studies have identified $CD8^+$ Tregs in cSCC (112) and other tumors (113) that exhibit even stronger regulatory activities compared to $CD4^+$ Tregs (114). Given its ability to decrease the number of $FOXP3^+$ T reg cells and inhibit T reg cell function, imiquimod could effectively inhibit the immunological destruction of cSCC (97).

TSCC has a distinct immune microenvironment that promotes tumor growth. There are fewer T cells, especially $CD8^+$ T cells, in TSCC lesions in comparison to SCC lesions (101), and a decreased Tc/Treg ratio in TSCC has also been reported (112). Furthermore, an increased number of IL-22 producing $CD8^+$ T cells and decreased number of $CD4^+$ Th1 T cells have been revealed in TSCC lesions. Higher T regs and lower $CD8^+$ T cells, which result in decreased immune surveillance, and increased exposure to IL-22, which enhances tumor proliferation, represent two main factors that contribute to the aggressive nature of TSCC (101) (Figure 4).

Compared to photodamaged skin, SCCs are associated with an increased number of $CD4^+$ T-cells. However, compared to premalignant lesions, including intraepidermal carcinoma (IEC), SCCs may also be associated with fewer numbers of $CD8^+$ T-cells. The ratio of $CD4^+$ to $CD8^+$ T-cells is significantly increased in SCC compared to IEC (115).

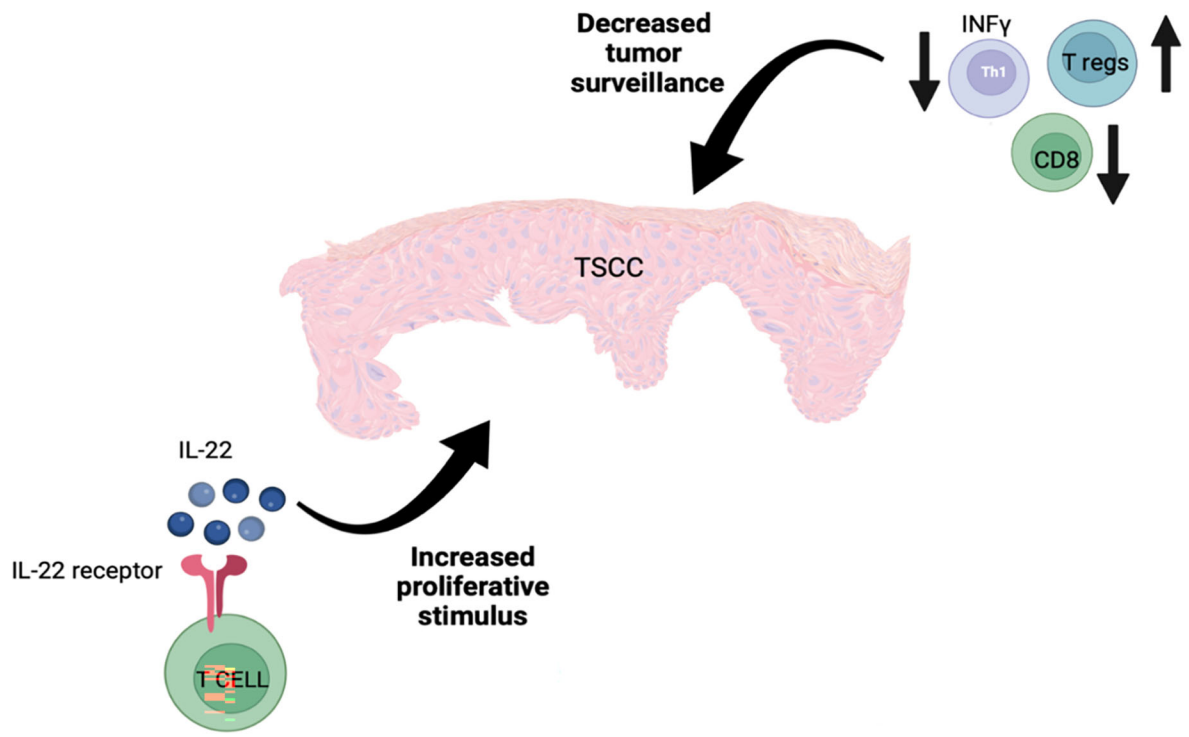

FIGURE 4
The aggressive nature of TSCC is potentially explained by the presence of increased numbers of T regs along with reduced numbers of CD8+ and IFN-γ-producing T cells, resulting in reduced tumor surveillance as well as an increase in IL-22-producing T cells, which stimulate tumor cell proliferation.

6 Lymphatic and blood vessels

The lymphatic vascular system is the main pathway for metastatic spread in SCCs. Various cancers can cause lymphangiogenesis, which is associated with increased expression of vascular endothelial growth factors as well as increased relative lymphatic vessel area (LVA) or lymphatic vessel density (LVD) (59, 116, 117) In this context, overexpression of genes related to lymphangiogenesis and increased LVD has been shown in cSCC compared to normal skin (118).

The risk of metastasis in SCCs is related to several variables, including tumor thickness, horizontal tumor size, and desmoplastic growth (11, 15–17). Tumor thickness has been shown to be the most accurate predictive factor for metastasis in SCCs. Metastatic SCCs are associated with increased lymphangiogenesis; however, the extent depends on the thickness of the tumor. It has been shown that greater tumor thickness in SCCs is accompanied by an increase in relative lymphatic vessel area and lymphatic vessel density (118). Despite clear excision margins in SCCs, increased dermal lymphangiogenesis can facilitate metastatic spread (59).

VEGF-C is a key lymphangiogenesis mediator (119). Increased VEGF-C levels in the tumor and the juxtatumoral dermis of cSCC compared with normal skin have been reported, and it has been suggested that tumor-associated macrophages may play an important role in lymphangiogenesis through production of VEGF-C (59).

Podoplanin is a distinctive immunohistochemical marker of lymphatic endothelial cells. Overexpression of podoplanin in both tumor cells and stroma of cSCC have been reported (120). Additionally, a positive correlation is noted between the expression of podoplanin in intratumoral and peritumoral regions of cSCC and the Broder's tumor differentiation grades (121–123) as well as the depth of tumor invasion to the dermis based on the Clark's scale (124). According to several studies, increased podoplanin expression is associated with a higher mean of LVD in the SCC microenvironment (120, 124–126) and presence of LN metastasis in SCC patients (120, 121, 127, 128). Therefore, podoplanin could be used as a predictor of SCC prognosis given that increased podoplanin expression is related to poor prognosis and decreased survival in cSCC patients (120).

Most immune cells have their first contact with a tumor through endothelial cells of the local blood vessels (28). Endothelial cell integrity is believed to play an important role in tumors. Normal endothelial cells promote homeostasis, but dysfunctional endothelial cells can lead to cancer growth (129). Abnormal angiogenesis also contributes to tumor growth and promotes metastatic spread. The density of neovascularization in cSCC is positively correlated with deeper invasions and poorer tumor differentiation. As a result, SCC tumors with high angiogenic activity are classified as aggressive with poor prognosis (130). Podoplanin represents a potential target for antimetastatic therapy in cSCC. A cancer-specific monoclonal antibody against human podoplanin has been demonstrated to be an effective treatment strategy particularly in podoplanin-expressing malignancies (131).

7 Cytokines

Cytokines play an important role in tumor biology. It was previously thought that IFN-γ and other Th1 cytokines exhibit antitumor activity, whereas IL-4 and other Th2 cytokines have protumor function (132). However, based on recent studies, some cytokines, such as IFN-γ, have been shown to have pro-tumor or anti-tumor functions depending on the tumor type and tumor microenvironment (133).

High serum levels of proinflammatory cytokines, such as interleukin (IL)-1, IL-6, IL-8, and TNF-α, are often related to tumor growth and poor clinical prognosis in cancer patients (134–137). It has been suggested that the balance between multiple cytokines may contribute to the SCC pathogenesis (138). Several cytokines, including IL-6, IFN-γ, TGF-β and GM-CSF, play a role in keratinocyte proliferation and SCC development (139–143).

Significantly elevated serum IFN-γ levels have been reported in SCC patients compared with normal subjects, and higher IFN-γ levels in SCC patients are corelated with more advanced cancer stages. The combination of serum IFN-γ and TGF-β levels is more reliable for diagnosis of SCC, whereas measurement of serum IFN-γ alone is helpful in evaluating the SCC progression from early to middle stages (138).

Elevated serum IL-6 levels are associated with increased malignancy and poor prognosis in different types of tumors (144–146). It has been demonstrated that IL-6 is important in transforming benign tumors into malignant, invasive SCCs in the HaCaT cell model of skin carcinogenesis. A complex, reciprocally regulated cytokine network induced by IL-6 in the tumor cells, including inflammatory cytokines (MCP-1, GM-CSF, and IL-8) and angiogenic factor (VEGF), results in malignant and invasive tumor growth *in vivo* and stimulates tumor cell proliferation and migrations. These findings indicate that IL-6 could represent a great target for effective cSCC treatment (147).

IL-24 overexpression has been noted in invasive cSCC. IL-24 facilitates cSCC invasion (132) by increasing focal MMP-7 expression, and MMP-7 promotes cancer cell proliferation, migration, and invasion (148).

According to several reports, constitutive expression of G-CSF and GM-CSF together has been shown in SCCs (149–151). Through induction of cell proliferation, migration, and angiogenesis in cSCCs, G-CSF and GM-CSF contribute to tumor growth, invasion, and metastasis (149, 150, 152).

Transforming growth factor-β (TGF-β) signaling is mediated by several downstream proteins, such as Smad family proteins. This signaling pathway has a paradoxical role by acting as a tumor-suppressor or tumor-promoting factor in many types of cancers, such as SCC. In the early stages of SCC, TGF-β1 and TGF-βRI act as tumor suppressors. However, in later stages, these proteins promote tumor growth. Smad2, TGF-βRII, and Smad4 are typically considered tumor suppressors in SCC (153).

IL-22 is produced by $CD4^+$ helper T lymphocytes (Th), such as Th1, Th17, and Th22 as well as a subset of $CD8^+$ cytotoxic T cells

(Tc22) (154–157). Significantly increased IL-22 is noted in the peritumoral regions of SCC and TSCC compared to normal skin. In transplant patients, overexpression of IL-22 and IL-22R facilitate tumor growth (101) and result in poorer prognosis (158). In addition to the role of IL-22 in cell proliferation, it can reduce IFN-γ production by Th1 cells as well as increase the production of immunosuppressive cytokines (159). It has been proposed that treating highly aggressive forms of SCCs in transplant patients by targeting the IL-22 pathway could represent an important, life-saving strategy (101).

8 Discussion

Skin malignancies are the most prevalent human cancers, and the immune system plays an important role in their development, progression, and eradication (160). There are approximately 1 million memory T cells/cm^2 in normal human skin, which is approximately twofold the number of T cells that exist in the entire circulation (161), indicating the importance of cutaneous immune surveillance as part of the immune system.

The immune microenvironment surrounding the cSCC is dynamic and contains contradictory forces that promote and suppress tumor growth (72, 162–165).

To summarize, the cSCC microenvironment has more Tregs and myeloid-derived suppressor cells that suppress immune responses and fewer mDCs with poor antigen-presenting function. The macrophages present in the cSCC microenvironment predominantly exhibit the M2 phenotype and promote tumor invasion and metastasis through producing MMPs and lymphangiogenic mediators. The SCC microenvironment is rich in IL-6, IFN-γ, TGF-β, GM-CSF, and IL-24, which induce tumor growth and invasion. Moreover, increased dermal lymphangiogenesis facilitates metastatic spread. Overexpression of IL-22 and IL-22R accelerate tumor proliferation and subsequently result in poorer prognosis in transplant patients with cSCCs.

Author contributions

VS performed literature searches and composed initial draft of the manuscript. ND co-wrote initial draft and participated in all revisions. JAC conceived the original concept and provided multiple revisions of the manuscript. All authors contributed to the article and approved the submitted version.

Conflict of interest

JC has been the recipient of funding for investigator initiated basic science research from Regeneron and GlaxoSmithKline.

The remaining authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

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