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Review

Tumor microenvironment and immune-related therapies of head and neck squamous cell carcinoma

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Head and neck squamous cell carcinomas (HNSCCs) are a type of common malignant tumor, mainly manifesting as oropharyngeal, oral cavity, laryngopharyngeal, hypopharyngeal, and laryngeal cancers. These highly aggressive malignant tumors reportedly affect more than 830,000 patients worldwide every year. Currently, the main treatments for HNSCC include surgery, radiotherapy, chemotherapy, and immunotherapy, as well as combination therapy. However, the overall 5-year survival rate of HNSCC has remained 50%, and it has not significantly improved in the past 10 years. Previous studies have shown that the tumor microenvironment (TME) plays a crucial role in the recurrence, metastasis, and drug resistance of patients with HNSCC. In this review, we summarize the role of anti-tumor and pro-tumor immune cells, as well as extracellular components in the TME of HNSCC. We also discuss classical HNSCC immunotherapy and highlight examples of clinical trials using CTLA-4 inhibitors and programmed cell death 1 (PD-1)/programmed cell death ligand 1 (PD-L1)-related combination therapies. We also outline some molecules in the TME known to regulate immunosuppressive cells. Furthermore, the role and underlying mechanism of radiation therapy on the TME, immune cells, and immune response are discussed.

About 90% of head and neck cancers occur as head and neck squamous cell carcinoma (HNSCC). According to the global cancer statistics of 2018,[1] more than 830,000 new HNSCC cases and 430,000 related deaths occur worldwide each year. HNSCC incidence and mortality are very high, with the condition reportedly exacerbated by human papillomavirus infection, alcohol consumption, and tobacco smoking. Approaches for managing HNSCC, such as surgery, radiotherapy, chemotherapy, new immunotherapy, and combination therapies, have been applied, although tumor recurrence still occurs in 50% of the patients. In addition, surgical removal of the tumor will reduce the patient's postoperative physical function, but many patients still have recurrence and metastasis.[2,3] Consequently, the 5-year overall survival rate of HNSCC still has not improved.[1,4]

The tumor microenvironment (TME) comprises immune and non-immune cells, as well as extracellular components, that play a very important role in tumor recurrence and metastasis. Specifically, immune cells include myeloid-derived suppressor cells (MDSCs), regulatory T (Treg) cells, tumor-associated macrophages (TAMs), natural killer (NK) cells, and dendritic cells (DCs), whereas non-immune cells are mainly made up of cancer-associated fibroblasts (CAFs). Alternatively, extracellular components comprise cytokines, growth factors, extracellular matrix (ECM), and exosomes, among others. Generally, the TME of HNSCC harbors some unique aspects that cause a decline in anti-tumor immune function. Although our body's immune system can recognize and eliminate tumor cells in a timely manner,[5] HNSCC may hijack immune cells in the TME and use them to activate immune suppression and avoid recognition.[5] Previous studies have shown that downregulating expression of human leukocyte antigen (HLA) not only achieves immune evasion, but it also reduces recognition of cancer cells by T cells.[6] In addition, the TME of HNSCC has been found to also destroy tumor-infiltrating lymphocytes (TILs) and NK cells,[7] whereas some important immune cell subpopulation, such as MDSCs, reportedly play a crucial role in tumor growth and metastasis. A summary of mechanisms underlying the interaction between immune cells and tumor cells in the TME of HNSCC is shown in Figure 1. The tumor immune microenvironment plays an important regulatory role in tumorigenesis and development, with numerous studies implicating it in the occurrence, metastasis, diagnosis, and treatment of HNSCC.[8–12]

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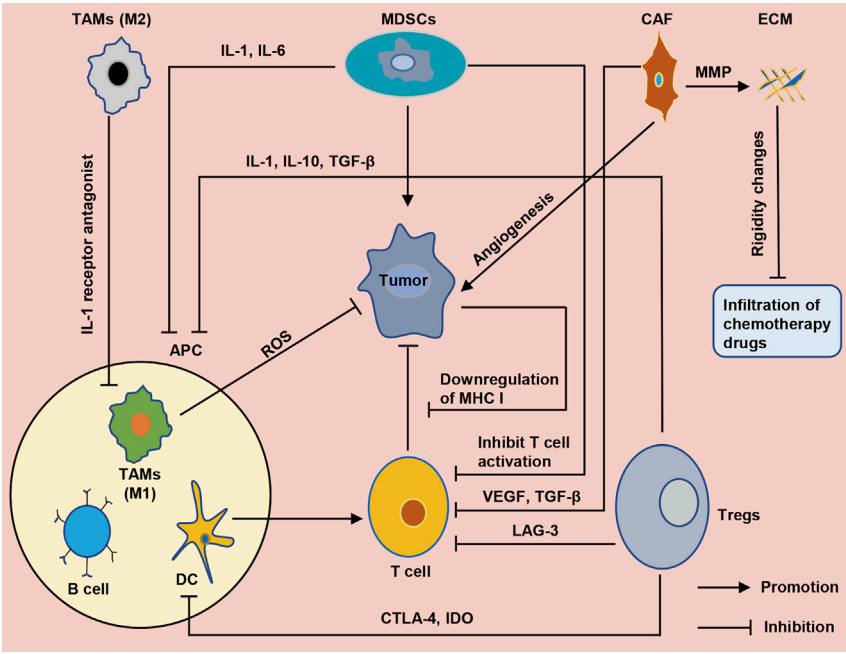

Figure 1. Schematic diagram represents the interaction between the tumor microenvironment and the tumor cells

The tumor microenvironment includes immune cells (MDSCs, Treg cells, TAMs, DCs, and B cells), non-immune cells (CAFs), and extracellular matrix (ECM).

In this review, we focus on the role of anti-tumor and pro-tumor immune cells, as well as extracellular components in the TME of HNSCC. We highlight classical TME cells in HNSCC and provide examples of clinical trials using CTLA-4 inhibitors and programmed cell death 1 (PD-1)/programmed cell death ligand 1 (PD-L1), as well as combination therapies. Finally, we outline molecules that regulate immunosuppressive cells in the TME.

Immunosuppressive cells

MDSCs

MDSCs promote angiogenesis and metastasis via multiple mechanisms.[13] Functionally, they regulate immune escape and have a negative association with overall survival rates of patients. Previous studies have shown that MDSCs not only inhibit activated T cells, but they also produce reactive oxygen species (ROS), which interact to catalyze nitrification of T cell receptors, thereby inducing T cell tolerance.[14] MDSCs present in the TME promote immunosuppression via various mechanisms, including T cell suppression and innate immune regulation.[15] In the TME, vascular endothelial growth factor (VEGF), interleukin 6 (IL-6), and other factors have been shown to induce MDSC aggregation.[16] In HNSCC, elevated MDSC levels reportedly upregulate inflammatory mediators, such as IL-1 and IL-6, making the environment unconducive for maturation of antigen-presenting cells (APCs), thereby indirectly promoting growth of tumor cells. Moreover, MDSCs can also induce development of Treg cells.[17]

Treg cells

The normal function of Treg cells is to suppress excessive immune responses and ensure that an immune balance in the body is maintained,[18] whereas in the tumor immune microenvironment, they regulate tumor progression by lowering anti-tumor immunity.[19] Treg cells are T cell subsets involved in the HNSCC immunosuppressive TME,[20] and their aggregation is regulated by chemokines and related receptors, such as CCR4-CCL17/22, CCR8-CCL1, CCR10-CCL28, and CXCR3-CCL10. In the TME, Treg cells such as effector Treg (eTreg) cells are associated with poor prognosis, just as Foxp3 has been reported in other cancers to be associated with poor prognosis.[21]

Treg cells have been shown to use multiple mechanisms and generate immunosuppressive effects. The first mechanism entails overexpression of IL-2, the production of inhibitory cytokines, such as transforming growth factor (TGF)-β, IL-10, and IL-35, as well as perforin and granzyme B, which directly kill effector cells or APCs.[22] The second mechanism involves Treg cell-mediated inhibition of effector T cells through major histocompatibility complex class II (MHC class II) by the ligand LAG-3,[23] whereas the third immunosuppressive mechanism involves controlling indoleamine 2,3-dioxygenase (IDO) in DCs to reduce tryptophan. Consequently, T cells are inhibited due to depletion of key substances.[24] In addition, adenosine produced by ATP metabolism, and regulated by CD39 and CD73 in activated Treg cells, causes inhibition of T cells through induction of negative signals to effector T cells and APCs. The last mechanism entails DC inhibition, via CTLA-4, which subsequently downregulates CD80 expression by combining with CTLA-4 produced by stimulated eTreg cells. Consequently, this leads to APC maturation and functional impairment.[25] Additionally, CTLA-4, PD-1, and PD-L1 regulate immunization of a variety of tumors to suppress the microenvironment.[26] Therefore, treatment approaches for HNSCC have used antibody therapy against these molecules.

TAMs

TAMs have two phenotypes, namely M1 and M2. The resulting phenotypes have different shapes and functions, with the M1 phenotype found to promote the T helper 1 (Th1) response and exhibit anti-tumor properties, whereas the M2 phenotype reportedly promotes the Th2 response, which is associated with tumor growth and migration.[27] TAMs occupy the main part of the TME.[28] Functionally, TAMs promote development of tumors into malignancies, and this has been associated with poor prognosis.[29] M1 macrophages can kill microorganisms and tumor cells by producing ROS. In addition, chemokines, such as CXCL9 and CXCL10, as well as proinflammatory

cytokines, such as tumor necrosis factor-α (TNF-α), IL-1, IL-6, and IL-12, have been shown to cause M1-polarized macrophages to absorb new Th1 cells.[30] Moreover, tumor M1 infiltration is positively correlated with prognosis of certain cancers.[31]

Alternatively, M2 macrophages initiate immunosuppression through Th2, with the M2 phenotype shown to be induced by cytokines, such as IL-4 and IL-10.[32] M2 macrophages secrete a large number of chemokines, including CCL24, IL-10, and IL-12, among others.[33,34] Previous studies have shown that activated M2 macrophages downregulate M1 by secreting anti-inflammatory cytokines, including IL-1 receptor antagonists, thereby inhibiting anti-tumor immunity.[32] When they lose the ability to present antigens, M2 macrophages have been found to regulate tissue remodeling, debris removal, and immune regulation,[35] and they have also been associated with poor prognosis of nasopharyngeal carcinoma.[36]

CAFs

CAFs are very active cellular components of the TME that play a vital role in tumor angiogenesis and invasion, as well as metastasis.[37–39] In the TME, fibroblasts are transformed into CAFs through the TGF-β and IL-1β signaling pathways.[40,41] According to other cancer reports, CAFs account for the largest tumor volume in the TME.[42] Functionally, CAFs work synergistically with tumor cells to build an immunosuppressive network, which promotes tumor escape from immune killing. In HNSCC, CAFs reportedly inhibit T cell proliferation, via VEGF and TGF-β, and subsequently induce immune suppression by inducing Treg cells.[38] In addition, CAFs have been shown to help tumor cells escape the body's immune killing effect by gathering M2 macrophages and MDSCs.[43,44] Since production of matrix metalloproteinases (MMPs) depends on CAFs, CAFs control the microenvironment by regulating remodeling and degradation of ECM, which causes increased cancer cell invasiveness.[45,46]

Anti-tumor immune cells

NK cells

NK cells are one of the most significant cells in anti-tumor immune cells. Although cancer cells and their TME inhibit NK cell activity,[47] NK cells have been shown to eliminate cancer cells through secretion of immunomodulatory cytokines.[48] Consequently, NK cell-based immunotherapies are on the rise,[49,50] and they seek to restore anti-tumor immunity by regulating immune checkpoints that regulate NK cell activity.[51] Several mechanisms, including binding tumor ligands, promoting NK cell infiltration, and targeting multiple activated NK cell receptors, have been shown to release NK cells against tumors.[51]

DCs

DCs, an outpost of the immune system, play a crucial role as a bridge between innate and adaptive immune responses.[52] Functionally, $CD4^+$ T cells support the $CD8^+$ T cell response through DCs.[53] DCs have been described as a strong APC, due to their role in activation of antigen-specific CD4 and CD8 T cells by processing and presenting antigens to initiate an adaptive immune response.[54] Several factors in the TME, such as IL-6, macrophage colony-stimulating factor (M-CSF), and IL-10,[55] have been implicated in the downregulation of DC function, which subsequently inhibits T cell activation and impairs immune checkpoint blocking therapy.[56]

$CD8^+$ T cells

$CD8^+$ T cells can fight tumors by producing cytokines and killing effects.[57,58] Some substances in the TME have been shown to induce $CD8^+$ T cell exhaustion,[59] although these cells are continuously stimulated in some cases, such as chronic inflammation and cancer, which leads to exhaustion of their function.[57] An early indication of this exhaustion entails significant reduction of IL-2 secretion,[60] which subsequently lowers production of cytokines such as TNF. In this state, T cell apoptosis may also occur, resulting in a significant decrease in virus-specific T cells.[61,62] There have been reviews comprehensively describing how to reverse T cell failure.[63]

Extracellular components

Pro-tumor effect of ECM

Massive remodeling of ECM causes changes in its density and rigidity, which are related to the malignant phenotype.[64] In fact, ECM rigidity can prevent drugs from reaching tumor cells to promote migration and growth of cancer cells, through epithelial-mesenchymal transition (EMT) and angiogenesis.[65] Despite its degradation being closely associated with tumor metastasis,[66] ECM has been implicated in tumor-related immunosuppression due to its role in regulating proliferation, localization, and function of myeloid cells.[67]

Anti-tumor effect of ECM

Under normal circumstances, drug transport in the tumor stroma mainly depends on diffusion. The ECM component reportedly acts as an effective barrier to the spread of tumor cells,[68,69] which generally increase ECM rigidity, and it blocks chemotherapeutic drugs from entering and making contact with tumor cells.[70] To enhance drug penetration, it is imperative to normalize ECM during treatment.[71,72]

Tumor-promoting cytokines

In most HNSCC cases, epidermal growth factor receptor (EGFR) has been shown to increase immature DCs in the TME, thereby causing abnormal T cell function.[73] IL-10, which is produced by Th2 cells,[74] can induce recruitment of M2 macrophages and increase the number of Treg cells, subsequently inhibiting DC function. All cytokines listed in Table 1, except those that induce the M1 phenotype, can also promote suppressive immune cells.

Anti-tumor cytokines

Type I interferons (IFNs) induce expression of MHC class I molecules in tumor cells, promote DC maturation, and increase anti-tumor immunity.[75] Alternatively, IL-12 and IL-18 stimulate Th1 immune response to initiate anti-tumor immunity.[74] In addition, CXCL9, CXCL10, TNF-α, IL-1, IL-6, and IL-12 can induce the M1 phenotype of TAMs. Some cytokines have two sides in promoting tumor or anti-tumor factors, such as IL-6. Functionally, IL-6 makes DCs immature, thereby inhibiting activation of neutrophils, macrophages, NK cells, and T cells,[76] and it is closely related to HNSCC prognosis.[77]

Table 1. Immunosuppressive cells and their functions in the TME of HNSCC

Immunosuppressive cells	Substance that causes its aggregation or production	Function and mechanism
MDSCs	VEGF, IL-6, GM-CSF	induction of T cell tolerance; inhibition of activated T cells; downregulation of anti-tumor immunity through innate immune regulation
Treg cells	CCR4-CCL17/22, CCR8-CCL1, CCR10-CCL28, and CXCR3-CCL10	destruction of APCs by expressing IL-2, TGF-β, and IL-10; inhibition of effector T cells through the MHC class II by ligand LAG-3; inhibition of T cells by regulating IDO in DCs; inhibition of DCs through CTLA-4, impairs APC maturation
TAMs	(1) CXCL9, CXCL10, TNF-α, IL-1, IL-6, and IL-12 can induce the M1 phenotype; (2) IL-4, IL-10, and IL-13 can induce the M2 phenotype	M1 macrophages kill microorganisms and tumor cells by ROS; M2 macrophages initiate anti-tumor immunity through Th2; M2 macrophages lose the ability to present antigen; activated M2 macrophages downregulate M1 by secreting anti-inflammatory cytokines; M2 macrophages downregulate anti-tumor immunity by expressing IL-1β, IL-10, MMPs, and TGF-β
CAFs	fibroblasts are transformed into CAFs through TGF-β and IL-1β signaling pathways	inhibition of T cell proliferation through VEGF and TGF-β; induction of immune suppression by inducing Treg cells; combine with tumor cells to establish an immunosuppressive network; help tumor cells escape the body's immune killing effect by gathering M2 macrophages and MDSCs; regulation of the microenvironment by activating ECM remodeling and degradation

GM-CSF, granulocyte-macrophage colony-stimulating factor.

However, IL-6 can also induce production of M1 macrophages for an anti-tumor immune response. The function of cytokines in tumor immunotherapy has been extensively reviewed.[75]

"Messenger" in TME: exosomes

Communication among cancer cells, as well as between cancer and other cells in the TME, occurs through direct contact or indirectly via secretion of chemokines/cytokines. Alternatively, a special way in which extracellular vesicles act as messengers among cancer cells or between cancer and normal cells has also been described.[78] One type of these vesicles, called exosomes and produced by CAFs, increases tumor cell growth and drug resistance.[79] Cancer cells convert fibroblasts into CAFs by producing exosomes.[80] Exosomes produced by HNSCC inhibit immune cell function by promoting $CD8^+$ T cell apoptosis.[81] Future research should explore the potential for restoring the body's anti-tumor immunity via anti-exosomes.

Immune-related therapy of HNSCC

The existing therapies for HNSCC include surgery, radiotherapy, chemotherapy, immunotherapy, targeted therapy (small molecule inhibitors or antibodies), and combination therapy. Generally, either surgery or radiotherapy may be selected as the treatment of choice when HNSCC is diagnosed early. However, HNSCC patients may decline surgery, owing to the serious impacts on body functions (pronunciation, swallowing, among others) as well as tumor recurrence. The genetic heterogeneity of NHSCC presents a big challenge for specific targeted therapies. Consequently, the associated drug resistance and recurrence problems mean that the current HNSCC treatment strategies are far from enough. To solve this problem, there is a need to explore additional novel treatment strategies and identify potential treatment targets that can generate effective treatment options for HNSCC patients to improve outcomes and overall survival rates. To date, numerous studies have reviewed the use of different approaches to treat HNSCC, including the combination of surgery and immunotherapy,[82] radioimmunotherapy,[83] and a combination of chemotherapy and immunotherapy.[84] In this review, we introduce classical immunotherapy and PD-1/PD-L1-related combination therapy, as well as molecules that regulate immunosuppressive cells.

Anti-PD-1/PD-L1 therapy

Under normal circumstances, the inhibitory receptor PD-1 is expressed on the surface of T cells and acts to protect normal cells in the body.[85] Functionally, this receptor binds to its ligand, PD-L1, and transmits information to T cells via downstream signaling pathways, thereby inhibiting T cell activation and proliferation.[85,86] However, tumor cells use this mechanism to escape immunity by expressing a large number of PD-L1 ligands on the surface. Currently, the US Food and Drug Administration (FDA) have approved two immunotherapeutic agents, pembrolizumab[86,87] and nivolumab,[88] for treatment of recurrent and metastatic HNSCC. In 2019, pembrolizumab was approved for treatment of unresectable recurrent/metastatic (R/M) HNSCC patients.[89] However, PD-1/PD-L1 blocking alone showed low safety and efficiency in HNSCC,[90,91] indicating that a combination with other treatments is needed (Table 2).

CTLA-4 inhibitors

Under normal circumstances, CTLA-4 plays a crucial role in maintaining a normal immune balance.[92] Tumor cells take advantage of CTLA-4's negative immune regulation, and they generate signals that inhibit T cell activation through CTLA-4.[93,94] Overall, CTLA-4 inhibitors represent an important immune checkpoint inhibitor. In the TME of HNSCC, Treg cells have been shown to suppress anti-tumor immunity by regulating CTLA-4 expression on the cell surface.[95] Therefore, CTLA-4 inhibitors can effectively reverse Treg cell-

Table 2. Clinical trials of PD-1/PD-L1-related therapy in HNSCC

PD-1/PD-L1-related combination therapy	Status	Phase	NCT no.: ClinicalTrials.gov
PD-1 + surgery	recruiting	2	NCT03355560
	recruiting	2	NCT03565783
	recruiting	2	NCT04126460
	not yet recruiting	1/2	NCT04340258
PD-1 + chemotherapy (only phase 3 and phase 4)	not yet recruiting	2/3	NCT04129320
	active, not recruiting	3	NCT02358031
	recruiting	3	NCT03855384
	recruiting	3	NCT04428333
PD-1 + radiotherapy (only phase 3 and phase 4)	recruiting	3	NCT03765918
	active, not recruiting	3	NCT03040999
PD-1 + CTLA-4	recruiting	1/2	NCT03019003
	recruiting	2	NCT 04080804
	recruiting	1	NCT04140526
	recruiting	2	NCT04326257
	recruiting	2/3	NCT03755739
PD-L1 + MDSCs	not yet recruiting	2	NCT 04262388
PD-L1 + Treg cells	recruiting	1/2	NCT 03844763
	not yet recruiting	2	NCT04262388
PD-L1 + TAMs	recruiting	2	NCT 02554812

For more information, refer to https://clinicaltrials.gov/. NCT, National Clinical Trial.

induced suppressive immunity. Some clinical trials evaluating CTLA-4 blockers in HNSCC are listed in Table 3.

Anti-EGFR

EGFR promotes cell proliferation, anti-apoptosis, and angiogenesis by activating downstream pathways such as phosphatidylinositol 3-kinase (PI3K)-AKT. Previous studies have reported a high rate of EGFR expression (90%) in HNSCC,[96] which is inversely proportional to patient survival rates.[97,98] Currently, the FDA has approved use of cetuximab, a monoclonal antibody with a specific molecular target, for HNSCC treatment. However, this drug's efficacy in treating HNSCC is only 13%.[99]

Potential therapeutic molecules targeting immunosuppressive cells

Molecules that regulate MDSCs

Liu et al.[100] revealed that JAK2/STAT3 suppression could reduce angiogenesis in tumor cells and reduce MDSCs in the HNSCC mouse model. In HNSCC, Younis et al.[101] found that knockdown of SEMA4D disables MDSCs, while Anderson et al.[102] found that STAT4 is a vital bridge for HNSCC tumor metastasis. In fact, its mechanism is related to T cell immunosuppression, enhanced MDSC activity, precancerous inflammation, and decreased cytotoxic antitumor lymphocyte activity. Moreover, Fugle et al.[103] demonstrated that low expression of CD24 in oral cancer was associated with poor prognosis. Specifically, their animal models showed that CD24 negatively regulates the number and functional characteristics of MDSCs and protects mice from oral cancer.

Table 3. Clinical trials evaluating CTLA-4 inhibitors for HNSCC treatment

NCT no.: ClinicalTrials.gov	Status	Intervention/treatment	Phase
NCT02812524	recruiting	Ipilimumab	1
NCT02919683	active, not recruiting	Nivolumab	2
		Ipilimumab	
NCT02741570	active, not recruiting	nivolumab, ipilimumab	3
		cetuximab/Erbitux, cisplatin, carboplatin, fluorouracil	
NCT02823574	active, not recruiting	Nivolumab	2
		Ipilimumab	
NCT04080804	recruiting	nivolumab, relatlimab, ipilimumab	2
NCT03690986	recruiting	VX15/2503, ipilimumab, nivolumab	1
NCT03700905	recruiting	surgery + radiotherapy and chemotherapy	3
		neoadjuvant nivolumab, adjuvant nivolumab, ipilimumab	
NCT03162731	active, not recruiting	nivolumab, ipilimumab	1
NCT01935921	active, not recruiting	cetuximab, ipilimumab + radiotherapy	1
NCT03003637	recruiting	nivolumab	2
		ipilimumab	
NCT03098160	recruiting	Evofosfamide	1
		ipilimumab	
NCT04290546	recruiting	IL-15 superagonist (N-803)	1
		CIML NK cell infusion	
		Ipilimumab	
NCT04326257	recruiting	nivolumab + relatlimab	2
		nivolumab + ipilimumab	
NCT03620123	recruiting	nivolumab and ipilimumab	2
		docetaxel	
NCT03058289	recruiting	anti-CTLA-4 antibody	1
			2

For more information, refer to https://clinicaltrials.gov/.

Molecules that regulate Treg cells

Wu et al.[104] found that anti-TIGIT treatment significantly delays growth of tumors in transgenic HNSCC mice, and it inhibits tumor growth by activating $CD8^+$ T cell effector functions and reducing the number of Treg cells. Mao et al.[105] demonstrated that targeting

Table 4. Potential therapeutic molecules regulating immunosuppressive cells in TME of HNSCC

Potential target and mechanism	Effect	Reference
Inhibition of JAK2/STAT3	reducing tumor-induced angiogenesis and MDSCs	100
Blocking activation of NLRP3	significant decrease in production of IL-1β and MDSCs	109
Blocking COX-2	reduces induction and function of MDSCs and inhibits tumor growth	110
Downregulation of Sema4D	reduces tumor-promoting cytokines produced by MDSCs	101
Activating the STAT4 pathway	decreases T cell immunosuppression and MDSC activity	102
Upregulation of CD24	decreases the number and function of MDSCs	103
Anti-TIGIT treatment	enhances anti-tumor immune response by activating the effector function of $CD8^+$ T cells and reducing the number of Treg cells	104
Targeting Notch1	reduces the number of MDSCs and Treg cells, as well as expression of inhibitory immune checkpoint molecules, such as PD-1 and CTLA-4	105
Anti-CD47 treatment	stimulates cytotoxic T cells and reduces MDSCs	106
Blocking A2AR	reduces the number of $CD4^+Foxp3^+$ Treg cells and enhances the anti-tumor response of $CD8^+$ T cells	107
Blocking TIM3	enhances anti-tumor immune response by reducing tumor cytotoxicity	108
Blocking CD73	downregulates total expression of PD-1 and CTLA-4 on T cells	111

the Notch1 signal reduces the number of MDSCs and Treg cells, thereby downregulating inhibitory immune checkpoint molecules, such as PD-1 and CTLA-4. Results from survival analysis in their work revealed overexpression of CD47 in HNSCC. In addition, CD47 upregulates expression of inhibitory markers PD-1 and PD-L1, thereby accumulating Treg cells and MDSCs. Blocking CD47 was found to effectively stimulate cytotoxic T cells, reduce immunosuppressive cells, and improve the immunosuppressive environment.[106] Apart from these, Ma et al.[107] found that A2AR is positively correlated with HIF-1α, CD73, and Foxp3. Blocking A2AR resulted in significant reduction in the number of $CD4^+Foxp3^+$ Treg cells and upregulated the killing of $CD8^+$ T cells in tumor cells. Alternatively, Liu et al.[108] showed that blocking TIM3 in HNSCC significantly enhanced the anti-tumor immune response by alleviating tumor cell toxicity and reducing the number of Treg cells (Table 4). All of these molecules are constrained by various factors, including the need to identify target molecules that must only be expressed in tumor cells and marked side effects associated with the treatment. Consequently, there is a need for comprehensive studies to validate these targets for effective clinical conversion.

Conclusions and perspectives

Previous studies have focused on changes in gene expression, as well as abnormal genetic and epigenetic mutations in tumor cells. In recent years, numerous studies have focused on the tumor-promoting functions of cellular components in the HNSCC tumor microenvironment, and these are expected to better our understanding of the immunosuppressive mechanism underlying tumor recurrence and drug resistance. Overall, these studies are expected to reveal novel targets to guide future treatment options.

In this review, we mainly described the role of anti-tumor and pro-tumor immune cells as well as extracellular components in the TME of HNSCC. We also outlined classical HNSCC immunotherapies, with focus on clinical trials using CTLA-4 inhibitors and a PD-1/PD-L1-related combination. Finally, we listed some molecules that target immunosuppressive cells. In addition, we reviewed literature on the FDA's approved anti-EGFR therapy (cetuximab) and anti-PD-1 therapy (pembrolizumab and nivolumab) for immunotherapeutic management of HNSCC, and further listed some potential treatments that are not yet approved by the FDA but are under clinical trials. Results from these clinical trials and potential therapeutic targets are expected to guide development of effective therapies to improve overall survival rates of R/M HNSCC patients.

Radiation therapy (also known as radiotherapy) is one of the primary treatment methods for patients with HNSCC, particularly for nasopharyngeal carcinoma.[90] Recent studies revealed that radiotherapy plays an important role in the TME and a tumor's response to immunotherapy. Increasing evidence has implicated TME as an essential mediator of radiation responses both locally and systemically, and radiotherapy functions as an immunomodulatory tool that facilitates recruitment and activation of the immune system to fight tumors.[112] The main mechanisms of radiotherapy's effect on antitumoral immunity are: (1) increasing the release of tumor antigens and their availability for APCs;[113,114] (2) enhancing activation of T cells and destroying the immune inhibitory TME;[115,116] (3) promoting T cell homing into the tumor bed through modulating chemokine expression levels, macrophage polarization, and expression of adhesion molecules on tumor vasculature;[117,118] and (4) regulating the expression levels of immune checkpoint molecules on the surfaces of both cancer cells and immune cells.[119,120] Further work is required to understand the effects and exact mechanisms of radiotherapy on the TME, immune cells, and immune response in HNSCC. Moreover, it is necessary to optimize combinations and timing of radiotherapy with immunotherapy for HNSCC. We think that the combination of immunotherapy with radiotherapy is a promising strategy for the treatment of HNSCC in the future.

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AUTHOR CONTRIBUTIONS

B.W., Y.W., and W.G. designed the review and contributed to manuscript preparation. Y.Q. and X.Z. wrote the manuscript. Y.W. and W.G. performed technical and administrative support. All authors reviewed and approved the final version of the manuscript.

DECLARATION OF INTERESTS

The authors declare no competing interests.

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