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Chapter X

Innate Immunity and Oral Carcinogenesis

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Abstract

Oral cancer is initiated from stem cells of the basal layer of the epithelium that have acquired genetic alterations [2-5]. Accumulation of genetic alterations and environmental factors drive the development of tumors to highly malignant lesions, a process involving not only tumor cells but also many other non-neoplastic cells, particularly infiltrating immune cells, in the tumor microenvironment. In head and neck squamous cell carcinoma, infiltration of macrophages into and around cancerous tissues is significantly correlated with tumor size, aggressiveness, invasion, and poor prognosis [6-8]. However, the molecular mechanisms that underlie the relationship between tumor cells and infiltrating macrophages are largely unknown. Therefore, understanding the role of tumor cell-produced factors in regulating the phenotype and effector functions of macrophages in the tumor microenvironment is critical. Most recent studies have shown that tumor cell-produced human β-defensin-3 (hBD-3) functions as a chemoattractant to modulate tumor-associated macrophage trafficking linked with tumor development and progression. This chapter covers recent progress in understanding the role of the innate immune system in oral epithelial carcinogenesis.

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Introduction

Interactions of tumor cells with various immune infiltrates in the tumor environment can significantly influence tumor development and progression. Macrophages residing in the tumor site, collectively termed tumor-associated macrophages (TAMs), often constitute a major part of infiltrating inflammatory cells. In the tumor microenvironment, tumor cell-produced factors shift the classically activated macrophages, also known as M1 phenotype, to an alternatively activated form, referred to as the M2 phenotype [9]. In most cancers, M2 TAMs form a significant portion of immune cells in the tumor microenvironment [10-12]. TAMs possess poor antigen presenting capacity, exhibit immunosuppressive characteristics, and produce a wide array of biologically active molecules to stimulate tumor cell proliferation, angiogenesis, cancer cell invasion, and DNA breakdown [10, 11]. Clinical and experimental studies have shown that TAMs play an important role in tumor development and progression and are frequently associated with poor prognosis in over 80% of cancers, including head and neck squamous cell carcinoma (HNSCC), breast, prostate, bladder, and cervical cancers [13-17]. The tumor-related inflammation, as presented by TAM accumulation, facilitates tumor development and progression through various mechanisms and might provide opportunities for the development of new drug targets and novel therapeutic approaches.

Monocyte chemotactic protein-1 (MCP-1), also known as chemokine (C-C) ligand 2 (CCL2), is described as the most frequently expressed chemokine by tumor cells and is correlated with recruitment of TAMs in a variety of human cancers [9, 11]. However, the association of MCP-1 and TAM movement has not yet been established in HNSCC and oral cancer, suggesting that tumor cell-produced molecules, other than MCP-1, are involved in the accumulation of TAMs in oral tumor sites.

1. Oral Cancer Progression and Inflammation

The development of oral squamous cell carcinoma (OSCC) is a multistep process that involves genetic alterations related to chronic exposure to carcinogens, tobacco, alcohol, chronic inflammation, and viral infection [3]. Gene amplification and increased expression of the epidermal growth factor receptor (EGFR) and its ligand (EGF) occurs in almost all HNSCC, while inactivation of tumor suppressor genes, such as p16 and p14ARF as well as mutations of p53, are frequently observed in oral dysplastic lesions and early stage HNSCC [3, 21-26]. In addition, carcinogens can damage individual genes and chromosomes [3, 21-26]. Accumulation of those genetic alterations drives the development of dysplastic/neoplastic lesions, i.e., tumors, to highly malignant derivatives, a process involving tumor cells as well as many other non-neoplastic cells, including innate and adaptive immune cells, fibroblasts, epithelial cells, and endothelial cells, in the tumor microenvironment [27]. It is well known that infection and chronic inflammation are associated with certain types of cancers; i.e., Helicobacter pylori infection is linked to gastric carcinoma, and inflammatory bowel disease (IBD) is connected with colon cancer [10, 28-30]. These inflammatory conditions are present before dysplastic changes arise and contribute to ~15% of total cancer incidences [10, 28-30]. Comparably, oncogenic alterations can induce an inflammatory
microenvironment that exerts many tumor promoting effects [27]. This intrinsic cancer-related inflammation is attributed to immune cells, cytokines, growth factors, and tissue remodeling enzymes in the microenvironment of most, if not all, solid tumors, irrespective of the trigger for tumor development [27].

The influence of cancer-associated inflammatory responses on tumor growth and progression is complex and multifaceted. While full activation and abundance of infiltrating adaptive immune cells, such as cytotoxic T cells, correlates with eradication of malignant cells and favorable prognosis, infiltrating and surrounding innate immune cells, such as macrophages and mast cells, can actually stimulate tumor development in most cancers [10]. For example, extensive infiltration of natural killer (NK) cells in human gastric and colorectal cancer is associated with a favorable prognosis, while tumor infiltration of TAMs and mast cells is correlated with aggressiveness and poor clinical prognosis in human breast cancer, melanoma, and lung adenocarcinoma [10, 13, 31, 32]. It has been well documented that TAM plays a critical role in cancer cell proliferation, invasion, and metastasis. For example, Lewis lung cancer (LLC) cells produce the extracellular matrix proteoglycan versican, which activates macrophages to produce IL-1β, IL-6, and TNF-α and subsequently stimulates LLC metastatic growth in vivo [33]. Qian et al. have defined a distinct macrophage population which mediates metastatic breast cancer cell extravasation, establishment, and growth using animal models of breast cancer metastasis [34]. Depletion of macrophages, through either a well-established genetic model of macrophage deficiency or transient chemical depletion, results in a rapid decrease of tumor cell numbers in metastatic lung, indicating that TAMs are critical for metastatic lesions [34]. These data suggest that cancer cells can use components of the host innate immune system, such as TAMs, to generate a prevailing inflammatory microenvironment for tumor progression and metastasis.

Elevated numbers of inflammatory cells, including macrophages, T cells, and B lymphocytes, are present in oral squamous cell carcinomas compared with normal mucosa, indicating the presence of an inflammatory tumor microenvironment [1, 6, 35-37]. Clinical studies have shown that among these cells, macrophages make up the predominant infiltrating cell type in oral cancer [6, 38, 39]. Accumulation of macrophages in response to tumor cell-derived signals, either because of tumor selection and evolution or as part of anti-tumor responses of the host, is diverted to pro-tumorigenic responses [40]. Li et al. have identified significantly higher numbers of CD68 positive (CD68+) macrophages in oral cancer samples derived from 82 patients and found that tumoral accumulation of macrophages is associated with stage of invasion, intratumoral microvessel density, and angiogenic factors, including VEGF and thymidine phosphorylase (TP) [6]. The association of TAMs with oral cancer progression has been reported in a clinical study, in which significantly increased TAMs were found in OSCCs with larger tumor sizes, positive lymph node metastasis, and more advanced clinical stages/recurrence in 92 specimens of OSCC [38]. Liu et al. also found an association of increased level of TAM infiltration inside and around oral cancer tissues correlated with angiogenic levels, lymph node metastasis, and tumor size [7]. Oral carcinoma in situ (CIS) and superficially-invasive OSCC contained more monocyte-lineage cells than those of normal tissues [41]. Our recent studies have shown that CD68+ macrophages, but not CD3+ T lymphocytes, are recruited and infiltrated into the oral CIS lesion [1, 20]. This suggests that TAMs are a major component of immune cell infiltrates of oral tumors, similar to those described in cancers of breast, cervix, lung, melanoma and others [10, 34, 42]. There is growing evidence that local and systemic antitumor immune responses are compromised in
the development of oral cancer, including apoptosis of T lymphocytes, the paucity of dendritic cells (DCs) at the tumor site, and tumoral accumulation of TAMs and regulatory T cells [10, 28-30]. Natural killer (NK) cells and T lymphocytes play critical effector functions in the host defense against neoplasia [43]. However, these effector cells are functionally inactivated in oral cancers. For example, primary oral tumors contain far fewer NK and T cells compared with regression tumor grafts of oral origin [44, 45]. In addition, patients with metastatic HNSCC have low NK and T cell activity and content [44, 45]. Host immune responses in the stroma and blood of OSCC are important to prognosis. Clinical studies have shown that the majority of CD4+/CD25+ T cells isolated from peripheral blood mononuclear cells and tumor sites express FoxP3, indicating the presence of regulatory T cells (Treg) in OSCC lesions and in the circulation [43, 44]. Particularly, T regulatory cells type 1 (Tr1), a subset of Tregs, present in the tumor and peripheral circulation of patients with HNSCC, mediate immune suppression and might contribute to tumor progression by secreting TGFβ and IL-10 [45]. These results suggest that the composition of immune infiltrates in the tumor microenvironment may be related to development and stage of oral cancer. While tumor infiltration of T lymphocytes and NK cells do not correlate with tumor differentiation, an increased number of macrophages is associated with enhanced invasion, stage, and poor prognosis of HNSCC [6, 7, 46].

2. Association of Macrophages with Tumor Growth and Progression

Macrophages are multifunctional immune cells and their effector function is largely influenced by the stimuli they receive in the environment. TAMs often make up a significant part of infiltrating immune cells in the tumor microenvironment and direct tumor cell-mediated immune responses in this milieu [10-12]. TAMs have poor antigen presenting capacity, exhibit an immunosuppressive phenotype, and release pro-tumor substrates, such as IL-6, TNFα, epidermal growth factor (EGF), platelet-derived growth factor (PDGF), transforming growth factor β (TGFβ), fibroblast growth factor (FGF), vascular endothelial growth factor (VEGF), and metalloproteinases (MMPs) [9-11]. Clinical and animal studies have shown that TAMs play crucial roles in tumor development, invasion, angiogenesis, and metastasis and, therefore, are associated with poor prognosis in breast, prostate, bladder, and cervical cancers [13-17, 47]. TAM-produced MMPs and other enzymes digest extracellular matrix, releasing heparin-bound growth factors and enhancing angiogenesis [11, 48, 49]. In a gastric cancer animal model, infiltrating macrophages induce nuclear translocation of β-catenin, an integral component of the Wnt signaling pathway that regulates cell proliferation, to stimulate intestinal tumorigenesis [50]. Tumor cell-derived chemokines, particularly MCP-1, have been implicated in macrophage accumulation at the tumor site in several types of adenocarcinomas, including ovarian, breast, and pancreas [11]. Other tumor cell-produced growth factors and cytokines, such as VEGF, TGFβ, FGF, and PDGF, are also described as chemotactic for inflammatory and other types of cells during tumor development [11]. For example, the pro-inflammatory peptide LL-37, the 37-aa C-terminus of human cationic antimicrobial protein 18 (hCAP-18), is produced by ovarian cancer cells and recruits mesenchymal stem cells (MSC) to the tumor site, resulting in increased production of pro-
tumor cytokines, growth factors, and enhanced vascularization [51]. In some cancers, however, the accumulation of inflammatory cells at the tumor site has not been attributed to those molecules. In the development of HNSCC and oral cancer, recruitment and infiltration of TAMs, but not T lymphocytes, into and around cancer tissues are often associated with tumor aggressiveness and poor prognosis [7, 8, 46]. However, it is unknown to what extent MCP-1 contributes to the migration of inflammatory cells to the tumor site, since MCP-1 expressing tumor cells are rare in HNSCC biopsy samples [18, 19]. We have shown that tumor cells in oral CIS lesions do not produce MCP-1 and that TAM trafficking is not correlated with MCP-1 expression in the lesions [20]. However, tumor cells in CIS predominantly produce hBD-3, which correlates well with tumor macrophage recruitment and infiltration [1, 20].

3. The Role of Human β-Defensins in Oral Cancer-related Inflammation and Tam Trafficking

Human β-defensins are small peptides originally identified from the plasma of patients with renal disease and from psoriatic skin lesions, act as antimicrobial molecules of the innate system [52, 53]. All β-defensin genes, including hBD-1, hBD-2, and hBD-3, are clustered in the short arm of the chromosome 8 (8p). Normal human oral epithelia express hBD-1, -2, and -3 peptides spatially; hBD-1 and hBD-2 are mainly localized in the stratum granulosum and spinosum, whereas hBD-3 expression occurs in the less differentiated stratum basale [1, 20, 54]. While hBD-1 is considered to be constitutively produced by oral epithelial cells, the expression of hBD-2 is NFκB dependent and can be induced by pro-inflammatory cytokines, such as interleukin 1 (IL-1) and tumor necrosis factor α (TNFα), and microbial reagents, such as lipopolysaccharides (LPS), in human oral epithelial cells [55-62]. In keratinocytes and the respiratory epithelium, expression of hBD-3 is induced by TNFα, IL-1, interferon-γ (IFNγ), various bacteria, and yeast [62]. Similarly, IL-1β and INFγ induce hBD-3 gene expression in primary human oral epithelial cells in culture [1]. However, whether these factors stimulate hBD-3 expression at the transcriptional level is still unknown. Kawar et al. have shown that the expression of hBD-3 in primary human oral epithelial and squamous cancer cells is significantly induced by EGF, even in the presence of the protein synthesis inhibitor cycloheximinde [1]. EGF activates extensive networks of intracellular signaling pathways that lead to activation or inhibition of a wide array of transcription factors, which directly modulate expression of genes involved in cell proliferation, differentiation, and apoptosis [2]. Increased expression and/or gene amplification of EGFR and EGF family ligands occur frequently in majority of HNSCC [2, 3, 63]. Proteins of the EGF family are produced as transmembrane glycosylated precursors and then processed by proteolysis to become soluble growth factors. All EGF family members, including EGF and transforming growth factor α (TGFα), share a conserved EGF-like domain that confers receptor binding specificity to their cognate receptors [64, 65]. EGF and TGFα specifically interact with the cognate receptor EGFR leading to receptor dimerization, which in turn stimulates autophosphorylation of several key tyrosine residues in their C-terminal domain. Phosphorylated cytoplasmic domains then serve as docking sites for association with several signaling molecules, such as SHC, GRB2, phospholipase Cγ (PLCγ), and phosphotidylinositol 3-kinase (PI3K) [64, 66,
Subsequent activation of these proteins triggers four major intracellular signaling pathways, which include: (1) Ras-mitogen-activated protein kinase (MAPK), (2) PI3K-Akt, (3) PLCγ and protein kinase C (PKC), and (4) Janus kinase (JAK)/signal transducers and activators of transcription (STATs) [2, 68-70]. It has been shown that actively proliferating cells, as defined by proliferating cell nuclear antigen (PCNA) expression, produce hBD-3 in the basal layer of oral epithelia [1]. Moreover, expression of hBD-3 induced by EGF requires MEK1/MEK2, p38 MAPK, PI3K and PKC, but not JAK/STAT [1]. Overexpression of intracellular signaling mediators, such as MEKK1, Akt, JUN, and JNK, also stimulates hBD-3 expression [1]. These results imply that hBD-3 is produced by actively proliferating cells under the control of cell growth signals [1]. Most recently, mouse β-defensin 14 (mBD14) was identified as the orthologue of hBD-3 [71]. This peptide can be induced by Toll-like receptor agonists such as LPS and double-stranded polynucleotide polyinosinic polycytidylic acid (poly(I):poly(C)) and by pro-inflammatory cytokines TNFα and IFNγ [71]. Since the expression of hBD-3 in oral epithelial cells and mBD14 uses distinct singling pathways, the production patterns, tissue distribution, and disease association of mBD14 need to be further elucidated.

It has been described that hBDs exhibit broad-spectrum antimicrobial activities in vitro and may function as host defense molecules of the innate immune system against microbial infections [72]. It is reported that β-defensin peptides display a variety of biological activities, particularly an involvement in the chemoattraction of immune cells [1, 20, 73-75]. More recently, hBDs have also been shown to display “cross-talk” activity with the adaptive immune system; potentially influencing various physiological and pathological processes, including tumorigenesis [76-78]. Moreover, hBD-3 activates professional antigen-presenting cells via TLR-1/2 to induce expression of costimulatory molecules CD80, CD86, and CD40 on monocytes and myeloid dendritic cells (mDCs) [79]. It can also antagonize stromal cell-derived factor-1α (SDF-1α)/CXCL12 for interaction with its cognate CXC chemokine receptor 4 (CXCR4) [80]. The chemotactic property of hBDs was first revealed by Yang et al. using an in vitro cell migration system, in which human immature dendritic cells (iDCs) and memory T cells migrate towards purified hBD-2 in a dose-dependent fashion [81]. CD34+ progenitor-derived iDCs express only four of the chemokine receptors, including CXCR4, CCR1, CCR5, and CCR6 [81]. Yang et al. have shown that hBD-1 and hBD-2 induced the migration of CCR6-expressing HEK293 cells but did not induce migration of HEK293 cells overexpressing CXCR4, CCR1, or CCR5 [81]. Further studies by various research groups have reported that hBDs are capable of chemoattracting a variety of immune cells, including monocytes, macrophages, iDCs, memory T cells, and mast cells [1, 20, 73, 75, 81]. Most recently, our laboratory have shown that hBD-3 induces directional migration of monocytes, including monocytic cell lines THP-1 and Mono-Mac-1 cells as well as peripheral blood monocytes (PBMs), via the chemokine receptor CCR2 [1, 20]. The role of CCR2 in mediating monocyte migration in response to hBD-3 has also been described by a study using the fusion protein of human Fc region of IgG1 with hBD-3 (hBD3-Ig), which induces migration of HEK293 cells overexpressing CCR2 but fails to attract peritoneal exudate cells from Ccr2-deficient (Ccr2−/−) mice [82]. It has been shown that CCR2 plays a non-redundant role as a major mediator of macrophage recruitment and that CCR2 ligands are effectors of those functions [83-85]. Therefore, interaction of oral tumor cell-derived hBD-3 with macrophages may play a crucial role in TAM trafficking [1, 20].
4. The Role of HBD-3 in Oral Cancer Development and Progression

The association of antimicrobial peptides with tumorigenesis has been described in ovarian cancer, renal cell carcinoma, cervical cancer, basal cell carcinoma, and prostate cancer [51, 62, 77, 78, 86-89]. In a gene expression profiling study, human β-defensin 1 (hBD-1) was found to be significantly down-regulated in conventional renal carcinoma and prostate cancer [90]. It has been shown that loss of hBD-1 expression is frequently observed in vivo in conventional clear cell (renal) carcinoma and prostate cancer and that hBD-1 stimulation induces cytolysis and caspase-induced apoptosis of ectopic androgen receptor expression in prostate cancer cells, suggesting that hBD-1 may function as a candidate tumor suppressor [89, 91]. However, whether antimicrobial peptides contribute to tumorigenesis in these cancers in vivo is still unknown.

Coffelt et al. have demonstrated that ovarian cancer cells produce the antimicrobial peptide LL-37, which recruits MSCs to the tumor site, resulting in the increased production of pro-tumor cytokines, growth factors, and enhanced vascularization [51]. In addition, we have shown that the expression of hBD-2 is exclusively associated with intratumoral vascular endothelium in oral carcinoma and Kaposi’s sarcoma and induced by TGFβ in human umbilical vascular endothelia cells (HUVECs) [92].

The involvement of antimicrobial peptides in OSCC has been controversial, mainly due to the approaches (reverse transcription-polymerase chain reaction (RT-PCR) vs. immunohistochemistry) and materials (OSCC biopsies vs. cultured oral cancer cell lines) used in published studies. Whereas some reported that OSCC lesions showed only modest or even no hBD production compared to normal oral epithelia [78], others have observed a significant expression of hBD-2 in OSCC [93-95]. Using immunofluorescence microscopy, we have shown that expression of hBD peptides is spatiotemporally modulated in normal oral epithelia and at various stages of oral carcinoma [96]. In normal oral mucosa, hBD-1 and hBD-2 are co-localized in the superficial epithelial layers [1, 20], where differentiated stratum spinosum cells and flattened cells in the upper spinous and subsurface layers are located [97]. However, hBD-3, but not hBD-1 or hBD-2, is exclusively produced by basal and suprabasal cells [1, 20] (Figure 1), similar to what was reported by Lu et al. in normal gingival epithelium [98].

The basal layer contains stem cells and transit-amplifying cells that are continuously dividing and provide a reservoir of cells for the suprabasal layers [97]. HBD-3-producing cells in the basal layer of oral epithelia are stained by PCNA [1]. These results indicate that expression of both hBD-1 and hBD-2 is associated with differentiated epithelial cells of oral mucosa and that the production of hBD-3 is a signature of undifferentiated proliferating cells.
Figure 1. Expression of hBD-2 (green) and hBD-3 (red) in normal, moderate dysplasia, CIS, and OSCC oral epithelial biopsies. N, normal region adjacent to the CIS lesion; CIS, carcinoma in situ lesion; arrows, the leading edge of OSCC that express hBD-3; nuclei, blue (DAPI) (modified from Kawsar et al. [1]).

The pattern of hBD expression varies in the evolution of OSCC. In a moderate dysplasia biopsy, where dysplastic cells occupied over one third of the epithelium, hBD-3 is expressed in dysplastic cells, while hBD-2 is restricted to the mature flattened cells in the subsurface layer [1] (Figure 1). In terminally differentiated OSCC, however, hBD-2 expression is confined to cancerous cells surrounding the “keratin pearls”, whereas cancer cells in the leading edges of OSCC only express hBD-3 [1] (Figure 1). Kesting et al. reported that hBD-3 is significantly overexpressed in oral cancer tissues using RT-PCR and immunohistochemistry analysis in paired cancerous and noncancerous specimens derived from 45 patients [99]. Most importantly, we have shown that tumor cells in the CIS lesion only produce hBD-3, thereby generating an hBD-3-rich tumor microenvironment [1, 20]. HBD-3-producing tumor cells in the CIS site are also positive in PCNA staining and overexpress β-catenin associated with the nuclear translocation of the protein [1]. β-catenin is a transcription modulator that participates in tumorigenesis [100, 101]. An oral CIS lesion is a histopathologic entity clinically classified as stage 0 of oral cancer, in which dysplastic cells, arising from the basal layer, occupy the full thickness of the epithelium from the basement membrane to the surface and, in all likelihood, will progress to invasive carcinoma [102, 103]. We have concluded the hBD-3-production by cells in oral CIS lesions suggests that hBD-3 may participate in the development and progression of oral carcinogenesis.
We further investigated the relationship between tumor cell-derived hBD-3 and infiltrating immune cells into the tumor microenvironment. We showed that the significant increase in hBD-3 expression in oral CIS is correlated with the accumulation and infiltration of CD68+ macrophages to the lesion site, but not CD3+ lymphocytes (Figure 2). In stark contrast, there was no migration of macrophages into the oral epithelium derived from non-cancer biopsies or in the normal region adjacent to the CIS lesion [1, 20]. Importantly, we reported that MCP-1 is not correlated with TAM trafficking and that tumor cells do not produce MCP-1 in oral CIS biopsies [20], suggesting that hBD-3 plays an important role in mediating TAM trafficking (Figure 2). We tested our hypothesis by using an animal model, in which wild type tumorigenic HEK293 cells or HEK293 cells overexpressing hBD-3 were inoculated into nude mice [20]. These xenograft tumor models resulted in massive host macrophage infiltration by cells overexpressing hBD3 when compared to wild type HEK293 cells [20]. In addition, the chemoattracted cells were CCR2 positive, suggesting that hBD-3 regulates macrophage migration via this chemokine receptor [20]. Inoculation of hBD-3 overexpression tumorigenic cells significantly increases tumor incidences and rates of tumor growth in nude mice [20]. We found that hBD-3 overexpressing tumorigenic cells formed tumors in all inoculated sites (n=8); however, only half of inoculations (n=8) generated tumors in mice injected with parent HEK293 cells [20]. The average volumes for hBD-3 overexpressing tumors were more than two-fold than those established using parent cells [20].
To examine whether hBD-3 affects tumor angiogenesis, we stained the xenograft sections with the antibody to CD34, an endothelial cell marker. Our results show that hBD-3 overexpressing tumors exhibited significantly higher density of CD34+ cells than tumors not expressing hBD-3, suggesting that hBD-3 is able to enhance tumor angiogenesis in vivo (Jin, unpublished data). Importantly, hBD-3 stimulated expression of pro-tumor cytokines in macrophages differentiated from monocytic THP-1 cells and peripheral blood monocytes (PBMs) [20]. Treatment of macrophages with hBD-3 for 16 h significantly induced the expression of IL-1α, IL-8, IL-6, and CCL18 [20]. In addition, hBD-3 stimulated TNFα gene expression in macrophages derived from PBMs [20]. Grevennikov et al. noted that most tumor-promoting cytokines, such as IL-1, IL-8, and TNFα are “M1 cytokines;” even most TAMs are considered as M2 type macrophages, suggesting the functional plasticity of macrophage infiltrates [104]. IL-6 is a potent inflammatory cytokine that is a key tumor-promoting and antiapoptotic factor [28, 105]. This cytokine contributes to the induction of skin tumors [105], triggers malignant features in breast tumor mammospheres [106], and participates in suppression of antigen-specific anti-tumor immunity through up-regulation of macrophage B7-H4 expression [107]. TAM expression of IL-8 and a number of molecules, such as VEGF and TNFα, have been implicated in enhanced angiogenesis [108, 109], while TAM-produced CCL18 can recruit naïve T cells to the microenvironment dominated by TAMs for possible T cell anergy [108]. These data collectively indicate that, in the development and progression of oral cancer, hBD3 overproduction serves as a chemotactic factor in recruiting circulating monocytes to the tumor site and subsequently functions as a stimulator to activate TAMs to produce tumor-promoting cytokines and growth factors, leading to the development and progression of oral cancer (Figure 3).

Figure 3. hBD-3 activates TAMs to enhance tumor growth and progression. Tumor cell-derived hBD-3 stimulates TAMs to produce pro-tumor cytokines and growth factors, which in turn promote tumor cell proliferation, invasion, angiogenesis, and suppression of anti-tumor immunity.
Conclusion

A big question in oral tumorigenesis is how tumor-promoting inflammation is induced. Recent studies in oral cancer have identified the innate antimicrobial peptide, hBD-3, as an effector molecular that links oncogene activation with initiation of tumor-related inflammatory responses. HBD3 related tumorigenic inflammation can direct tumor specific immune cells to become pro-tumorigenic; i.e., production of pro-tumor cytokines and chemokines in TAMs, and stimulate tumor growth for further invasion and metastasis. Therefore, hBD-3 may play a crucial role in tumor initiation by recruiting macrophages and stimulating cytokine production in these cells. TAMs can stimulate tumor growth and invasion through enhanced cell proliferation mediated through the production of TNFα, IL-6, and other cytokines, a process involving tumor cell-derived hBD-3. Further research of hBD-3-mediated suppression of immune responses and tumor progression will aid in a better understanding the mechanisms that cancer cells use to evade host anti-tumor immune responses and, thereby, could identify novel therapeutic targets to suppress tumorigenesis.

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References


