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Review

Fibroblasts and macrophages: Key players in the head and neck cancer microenvironment

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ABSTRACT

Background: The secretion of soluble factors and extracellular matrix (ECM) proteins by tumor cells and surrounding stromal cells creates a tumor microenvironment (TME). Here, we reviewed a key role of cancer-associated fibroblasts (CAFs) and tumor-associated macrophages (TAMs or M2 macrophages) in the development and metastasis of head and neck squamous cell carcinoma (HNSCC).

Highlight: Interactions between HNSCC cells and CAFs induce overexpression of TGF-β, VEGF, TNF-α, HGF, IL-1α, IL1-β, IL-6, IL33, CXCL12, and MMPs in both cell types. Elevated concentrations of these soluble factors contribute to the growth, migration, and invasion of HNSCC cells. Periostin, an ECM protein, is also upregulated in CAFs during HNSCC, and it has been shown to accelerate HNSCC progression. Macrophages are a double-edged weapon, and any imbalance in the regulatory mechanisms may cause a shift from tumoricidal to tumorigenic activity of these cells. TAMs are common infiltrated inflammatory cells in HNSCC. Such TAMs express M2 markers, including FR-β, CD206, and TGF-β. TAMs contribute to HNSCC progression through various mediators. Increased levels of TGF-β, IL-10, and macrophage inflammatory protein-3 alpha (MIP-3α/CCL20) expression were found in TAMs in HNSCC. These soluble factors play a key role in the migration and invasion of HNSCC cells.

Conclusion: CAFs can promote HNSCC progression through direct contact and/or paracrine signaling. Interactions between HNSCC cells and CAFs stimulate expression of various growth factors, cytokines, chemokines, MMPs, and periostin. TAMs in HNSCC upregulate the production of IL-1β, IL-10, and MIP-3α/CCL20, which are involved in tumorigenic processes.

Contents

1. Introduction

Head and neck cancer is a broad term that encompasses epithelial malignancies, which arise in the paranasal sinuses, nasal cavity, oral cavity, pharynx, or larynx. Almost all of these malignancies are squamous. Head and neck squamous cell carcinoma

Abbreviations: (TGF-β), transforming growth factor-beta; (VEGF), vascular endothelial growth factor; (TNF-α), tumor necrosis factor-alpha; (HGF), hepatocyte growth factor; (IL-1α), interleukin-1alpha; (IL1-β), interleukin-1beta; (IL-6), interleukin-6; (IL-33), interleukin-33; (SDF-1/CXCL12), stromal cell-derived factor-1; (MMPs), matrix metalloproteinases

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(HNSCC) ranks among the six most common cancers in the world and is a significant cause of cancer morbidity and mortality. Tobacco and alcohol consumption, betel nut chewing, and human papillomavirus infection are the most commonly cited risk factors for the development of HNSCC [1].

At present, our knowledge of the mechanisms driving transformation of normal cells into HNSCC cells is incomplete. Generally, carcinomas develop from cells that have undergone genetic mutations that impair normal growth-controlling mechanisms. Research on the origins of cancer has focused on the study of tumor cells and has considered tumorigenesis as an independent process governed by the tumor cell genes. However, the secretion of growth factors, matrix proteins, and proteases by tumor cells and surrounding stromal cells creates a tumor microenvironment (TME) that facilitates tumor growth, invasion, and metastasis. The TME comprises the extracellular matrix (ECM) and stromal cells, such as fibroblasts, endothelial cells, and inflammatory cells, which are known to collectively influence the initiation and progression of tumors [2].

Major cell types present in the HNSCC TME are tumor cells, cancer-associated fibroblasts (CAFs), tumor-associated macrophages (TAMs), endothelial cells, and inflammatory or immune cells (Fig. 1) [2,3]. Here, we discuss current research that demonstrates a crucial role for certain stromal cells during the development and metastasis of head and neck cancer. In light of the breadth and complexity of each step in the invasion-metastasis cascade and strong TME influence during each phase of tumor progression, we chose to focus our discussion on specific characteristics as well as roles and functions of CAFs and TAMs during tumor growth. We also discuss evidence supporting the extent of interactions within tumors, whereby stromal cells exchange signals not only with tumor cells but also with each other, reflecting the inherent complexity of the TME in HNSCC.

2. Cancer-associated fibroblasts

2.1. Origin and markers of CAFs

Currently, according to the concept that tumors are similar to a chronic non-healing wound, fibroblasts are thought to be commonly activated in tumors. These activated fibroblasts, termed cancer-associated fibroblasts (CAFs), share many similarities with activated fibroblasts or myofibroblasts found in wounds and inflammatory sites [4]. Different elements contribute to the heterogeneity of CAFs, including the tissue type in which the tumor grows, the local paracrine environment, and the cell type of origin. Immunohistochemical analysis showed that 60–70% of human oral squamous cell carcinoma (OSCC) tumors contained CAFs [5,6]. One significant source of CAFs is local fibroblasts and tissue-resident fibroblast precursor cells that are incorporated into the growing tumor by tumor-derived stimulants. Additionally, other cellular sources have been suggested, including bone marrow-derived mesenchymal stem cells (BM MSCs), endothelial cells that underwent epigenetic transitions, and cancer cells that went through epithelial-mesenchymal transition (EMT) [7]. However, unlike in the case with local fibroblasts, it is not yet clear whether BM MSCs and transformed endothelial and cancer cells definitely turn into CAFs in HNSCC [8,9].

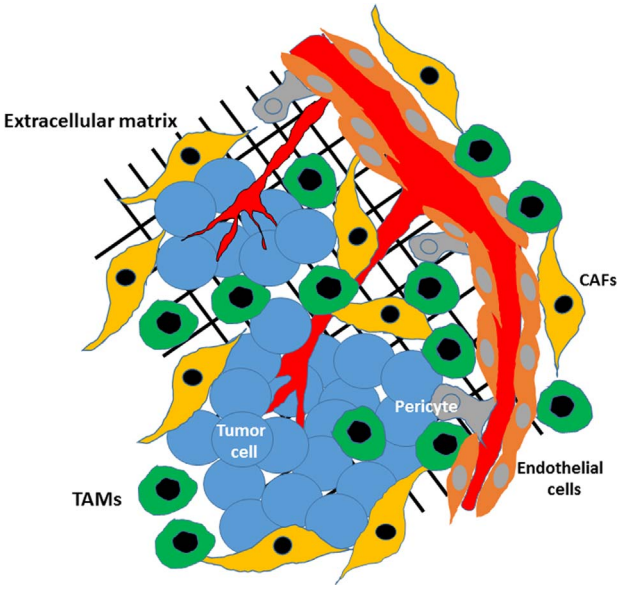

Fig. 1. Schematic representation of tumor microenvironment (TME) components. TME consists of tumor cells, stromal cells such as carcinoma-associated fibroblast (CAF), tumor associated macrophage (TAM), pericyte and endothelial cell, extracellular matrix and soluble factors.

With respect to transformation of local fibroblasts into CAFs, it should be noted that the activation of resident fibroblasts is induced by various tumor-secreted factors, including TGF-β [10–12] and CXCL12/CXCR4 [13], or by loss of suppressor genes, encoding such proteins as PTEN, CAV-1, p53, or p21 [14–19]. An immunohistochemical analysis of oral squamous cell carcinoma demonstrated that α-smooth muscle actin (α-SMA) expression was increased in the tumor stroma, whereas expression of CAV-1 was lower in fibroblast-like cells [20]. Deregulation of p21 was associated with the activation of stromal fibroblasts in oral cancers by a mechanism that involved the stimulation of α-SMA expression [21]. In HNSCC, TGF-β levels in CAFs are persistently higher compared to those in normal dermal and mucosal fibroblasts. Elevated levels of TGF-β were also identified in the stromal compartment of HNSCC tumors by immunohistochemical analysis [22]. Malignant keratinocytes from oral cancer tumors, but not their pre-malignant counterparts, produce high levels of reactive oxygen species, which, in turn, stimulate TGF-β1 expression and induce fibroblast activation [23]. *In vitro* studies demonstrated that treatment of oral fibroblasts with TGF-β1 induced myofibroblastic differentiation. Co-culture of oral fibroblasts with oral cancer cells also promoted myofibroblastic phenotypes in oral fibroblasts. Therefore, it is possible that oral cancer cells activate differentiation of normal oral fibroblasts to myofibroblasts via TGF-β1 secretion [5,24]. Additionally, it was found that myofibroblast differentiation was suppressed by αvβ6 integrin inhibition. However, inhibition of αvβ6 significantly suppressed TGF-β1 activation in oral cancer cells, but not in oral fibroblasts. Therefore, it has been suggested that αvβ6 integrin is involved in the TGF-β1 activation mechanism in oral cancer and plays an important role in myofibroblast induction in HNSCC [24].

In relation to the second source of CAFs, many previous studies suggested that CAFs may originate from BM MSCs. BM MSCs attracted much attention because they can differentiate into a variety of different stromal cells, including CAFs, depending on the factors and structures they are exposed to. When MSCs labeled with the green fluorescent protein were intravenously injected into the tail vein of tumor-bearing mice, they were recruited to xenografts derived from cancer cells of head and neck, colon, and breast cancers [25]. Once BM MSCs arrive in the tumor periphery, they are exposed to various factors secreted by cancer cells, which induce differentiation of BM MSCs into CAFs. It has been reported that at least 20% of CAFs in induced mouse gastric cancer originate in the bone marrow and are derived from MSCs [26]. One study that employed a xenograft mouse model showed that labeled MSCs localized within the tumor mass of ovarian carcinoma and differentiated into CAFs and pericytes, characterized by high expression of α-SMA,

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