Short Review

Epigenetics Meets the Tumor Microenvironment

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Key Words

Epigenetics · Tumor · Microenvironment · Carcinogenesis

Abstract

It is known that tumor cells undergo genetic and epigenetic alterations. Recent evidence suggests that epigenetic changes in the tumor microenvironment play a significant role in carcinogenesis. In this brief review, we discuss the epigenetic changes of the microenvironment in different malignancies and their clinical significance.

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Introduction

Cancer develops due to successive multistep genomic abnormalities of the cells that drive a normal cell into a highly malignant cell through a series of changes. These genomic abnormalities may occur due to a germline mutation (inherited) or a somatic mutation (acquired). The target genes may undergo various structural alterations such as mutations, deletions, copy number abnormalities and translocations. At the time of proliferation, each mutational change provides a specific growth advantage to the cancer cell, such as uncontrolled cell proliferation, prolonged cell survival, angiogenesis, invasion and distant metastasis [1]. These genetic changes in cancer usually occur at a low frequency unless there is also a defect in the DNA repair genes. Recently, however, this tumor-centric view of carcinogenesis has been reevaluated, and the focus of attention is presently being directed to epigenetics and the tumor microenvironment (TME). It has been proven by various studies that the microenvironment surrounding tumor cells plays a crucial role in tumor initiation, progression and metastasis [2–5]. In the last few years, various cancer models of breast cancer, melanoma, gastrointestinal cancer, leukemia, etc., have been focused on the TME and highlighted the alteration of the

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complex spatial and temporal relation of the TME and epithelial cells in the process of carcinogenesis [6–8]. The term ‘epigenetics’ indicates an alteration of gene expression without any structural alteration of the DNA base-pair arrangement. In contrast to genetic changes, epigenetic changes are versatile, more flexible and can also propagate through multiple cell cycles. In epigenetic changes, there is a differential transcriptional ability of the gene due to an alteration of the chromatin package. This may occur due to an alteration in the DNA and/or histone modifications [9]. In this brief review, we discuss the epigenetic changes of the microenvironment and their role in cancer initiation and progression.

Microenvironment

The TME is a dynamic network adjacent to the tumor that predominantly consists of fibroblasts, myofibroblasts, myoepithelial (ME) cells, vascular endothelial cells, macrophages, leukocytes and the extracellular matrix (ECM). Myofibroblasts and stromal fibroblasts are morphologically indistinguishable. These cells have a moderate amount of cytoplasm with oval-to-elongated nuclei. Myofibroblasts are positive for lamin, β_4 -integrin and maspin. Macrophages, one of the important components of the TME, are derived from bone marrow precursor cells. The circulated precursor promonocytes differentiate into monocytes and finally accumulate in the tumor sites as macrophages. The ECM is composed of collagen, elastin, fibronectin and laminin. These materials are produced predominantly by fibroblasts and partly by other resident cell populations.

Epigenetic Changes in the TME

Figure 1 highlights the various aspects of epigenetic changes in the TME.

Tumor Initiation and Growth Induction

In their study on breast carcinoma, Allinen et al. [10] explored the molecular basis of carcinogenesis caused by the TME. They performed comprehensive gene expression profiles of stromal cells and epithelial cells of normal, ductal carcinoma in situ (DCIS) and invasive carcinoma of the breast by using serial analysis of gene expression. The significant changes in gene expression were noted in all cell types, including the stromal cells such as myofibroblasts, endothelial cells, fibroblasts, infiltrating lymphocytes and epithelial cells in both DCIS and infiltrating ductal carcinoma (IDC). The stromal cells showed dramatic changes in the gene expression of several proteins and receptors in DCIS and IDC. These alterations in the TME persisted even after the removal of the cells from the patients and in in vitro cultures of these cells [2, 11, 12].

It is important to note that with the help of comparative genomic hybridization, the clonally restricted genetic abnormality was detected only in neoplastic epithelial cells and could not be detected in the stromal cells [10]. This type of alteration of gene expression without any genetic change provokes the possibility of an epigenetic alteration of the stromal cells. To explore such a possibility concerning epigenetic changes, Hu et al. [4] carried out a unique study by performing methylation-based digital karyotyping to characterize the comprehensive DNA methylation profile of epithelial cells, ME cells and fibroblasts of normal breast tissue in in situ and invasive carcinoma cases. They demonstrated that numerous genes differentially methylated between normal and carcinoma-associated stromal cells. Their study highlighted the distinct epigenetic changes in the TME. Fiegl et al. [13] analyzed the DNA methylation profile of tumor epithelial cells and stromal tissue of HER-2-positive

Fig. 1. Schematic view showing epigenetic changes and their effects on tumor initiation and progression. BM = Basement membrane; EP = epithelial cells; FB = fibroblasts; PMN = polymorphonuclear neutrophils.

breast carcinoma cases by separating them with the help of laser capture microdissection. They demonstrated that the DNA methylation profiles of stromal tissue are different in different tumor subtypes.

Hanson et al. [5] studied the epigenetic changes of the TME in prostate carcinoma cases by analyzing the gene promoter methylation profile of GSTP1 and RAR β ₂ in stromal and epithelial cells in benign and carcinoma cases. They demonstrated distinct gene promoter methylation of the stromal cells. Rodriguez-Canales et al. [14] analyzed the extent and distribution of tumor and stromal cell modulation of the whole prostate by using pyrosequencing quantification of GSTP1 promoter methylation. They demonstrated a localized and distinct area of stromal methylation of the TME in the prostate. Their study indicates an epigenetically unique microenvironment within the prostatic carcinoma that may have a role in the initiation of the tumor.

Growth Induction

Transforming growth factor (TGF)- β ₁ is a soluble factor and a potent inhibitor of epithelial cell growth [15]. TGF- β receptors are present in both the epithelial and stromal cells and bind with TGF- β on the epithelial cells, causing cell death by apoptosis and cell cycle inhibition. It has been demonstrated that alteration of TGF- β type II receptors in mouse mammary fibroblasts promotes the growth and invasion of mammary carcinoma [16]. Yamashita et al. [17] demonstrated a marked downregulation of the TGFBR2 protein in high-grade prostatic intraepithelial neoplasia and prostate cancers. The decreased expression of TGF- β receptors was mainly due to decreased transcription activity, related to histone deacetylation and H3

lysine 27 trimethylation [17]. Chromatin remodeling of the TGF- β or TGF- β receptor genes was most likely responsible for the tumor development. However, since no such study was done on stromal fibroblasts, the possibility of epigenetic changes of TGFBR in stromal fibroblasts cannot be excluded. Allinen et al. [10] noted overexpression of CXCL14 and CXCL12 chemokines in the stromal cells of patients with DCIS and IDC in the breast. These chemokines bind to receptors on epithelial cells and help in cell proliferation, migration and invasion by the paracrine effect [10]. Similarly, Ma et al. [18] conducted a comparative analysis of global gene expression changes in the stromal and epithelial cells during the progression of breast carcinoma cases from normal to DCIS to invasive ductal carcinoma. They showed extensive genetic expression of cell cycle-related genes in tumor-associated stromal cells during the progression of cancer.

Tumor Invasion and Metastasis

The transformation from in situ to invasive carcinoma is one of the most crucial events in cancer progression. Most studies have been conducted on DCIS and invasive breast carcinoma cases [10, 18]; however, to date no specific molecular signature has been demonstrated that distinguishes in situ from invasive carcinoma. It is well known that in the process of transition from DCIS to IDC, the ME cell layer is broken down and the basement membrane is disrupted. This is followed by the infiltration of tumor epithelial cells into the adjacent stromal tissue. Studies have shown that the ME cells in DCIS are the main players in the process of invasion (fig. 1).

ME cells show altered gene expression and DNA methylation profile. These epigenetic changes are responsible for the liberation of the increased amount of proangiogenic factors, basement membrane-degrading factors such as matrix metalloproteinase (MMP) and plasminogen activator inhibitor type 1. Excess plasminogen activator inhibitor type 1 liberated from the myoepithelium causes the detachment of ME cells from the basement membrane and disorganization of the ductal cells [19].

Several MMPs (MMP-2, MMP-11 and MMP-14) are overexpressed by myofibroblasts [20] that break down the basement membrane and simultaneously remodel the ECM. While MMP-3 disrupts the E-cadherin intercellular junction, MMP-2 and MMP-9 break down the collagen fibers of ECM and promote the entry of the epithelial cells into the stroma [20]. These MMPs are also involved in tumor growth, angiogenesis, invasion and metastasis. The proteolytic degradation products of MMP promote activation of TGF- β_1 that causes cell proliferation. MMP also increases the bioavailability of vascular endothelial growth factor, an angiogenic growth factor [21].

Epigenetic changes in the microenvironment have a significant impact on distant metastasis. Carcinoma cells in metastatic sites often induce epigenetic changes of the stromal cells and bone marrow-derived cells to create a favorable local environment for cell proliferation [22, 23]. It has also been shown that the primary tumors often mobilize the bone marrow-derived cells to the metastatic sites before the actual metastasis. The mobilized bone marrow cells create a suitable microenvironment for metastasis [24].

Clinical Implications

The TME plays a major role in the initiation, progression, metastasis and clinical behavior of tumors. The metastatic potential of carcinoma may depend on its expression of ECM components [25]. Even chemotherapeutic drug resistance partly depends on the TME rather than

the tumor itself [26]. Therefore, the combined approach of treating both the tumor and the TME may be a wiser and more efficient way to fight cancer. Since tumor macrophages are the important part of the TME, the destruction of such cells can be done by a ‘vaccine-based approach’. Using a murine model, Luo et al. [27] showed that the elimination of tumor-associated macrophages decreased tumor growth and invasion. Due to epigenetic changes in the TME, several chemokines are overexpressed by the TME. Blockage of the receptors of these chemokines may also be a potential target in carcinoma cases [28].

Unlike genetic changes, epigenetic alterations are essentially reversible and allow plasticity. The epigenetic changes of the TME can be altered by chemotherapeutic drugs. Hellebrekers et al. [29] applied DNA methyltransferase and histone deacetylase inhibitors to upregulate the epigenetically silenced ICAM-1 in tumor endothelial cells. This epigenetic reversal therapy has potential in the future.

Conclusion

Epigenetic changes in the TME have major roles in tumor initiation, progression and metastasis. In the future, there may be a possibility to halt the disease process in its early stage by manipulating the TME. Epigenetic reversal or a targeted chemotherapeutic approach may be helpful in this aspect.

Disclosure Statement

There is no conflict of interest in this paper.

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