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REVIEW ARTICLE

Epithelial Tumor, Invasion and Stroma

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Uncontrolled proliferation is a defining feature of the malignant phenotype. Nevertheless, the supportive network provided by the stroma is indispensable for further invasion, progression and metastasis of cancer cells. In addition, the role of inflammation in tumorigenesis is now generally accepted, and it has become evident that an inflammatory microenvironment is an essential component of tumor progression. Since skin tumors are common and easily assessable lesions with features at various stages of tumorigenesis, they provide a wide scope for research in this field to further our understanding of fundamental and clinical carcinogenesis. Some of the basic aspects of epithelial tumorigenesis, invasion and stromal reaction are reviewed in this paper. **(Ann Dermatol 23(2) 125～131, 2011)**

-Keywords-
Fibroblast, Invasion, Stroma, Tumor

INTRODUCTION

Tumor invasion consists of a series of discrete biological processes that move tumor cells from the primary neoplasm to underlying stroma. Invasion involves changes in tumor cell adherence to other cells and to the extracellular matrix (ECM), proteolytic degradation of surrounding stroma and motility to physically propel a tumor cell through the stroma. Tumor cell adherence to the ECM is mediated by integrins and other cell receptors for ECM proteins[1]. Skin tumors are a common health problem and comprise a wide range of benign and malignant features such as seborrheic keratosis, keratoacanthoma, actinic keratosis, Bowen's disease and squamous cell carcinoma. Therefore, morphological and immunobiological analysis of skin tumors can provide an important model for understanding the multi-step progression of epithelial tumorigenesis.

LOSS OF CELL-CELL ADHESION AND EPITHELIAL-MESENCHYMAL TRANSITION

Epithelial cells establish close contact with neighboring cells and an apical-basal axis of polarity through the sequential arrangement of adherens junctions, desmosomes, and tight junctions. Epithelial cell-cell adhesion is

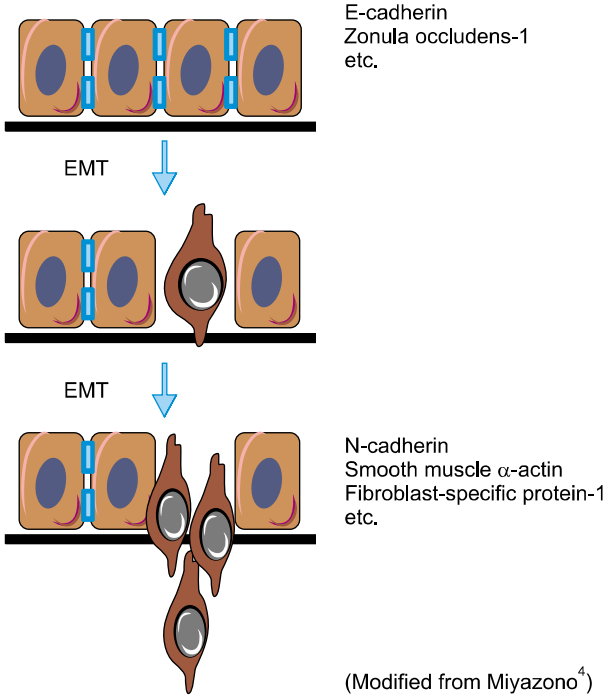

Fig. 1. Process of epithelial-mesenchymal transition (EMT).

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mediated by cadherins, which bind cells through homophilic protein-protein interactions of their extracellular domains; intracellularly, cadherins interact with catenins and the actin cytoskeleton. Invasion is accompanied by a 'switch' in tumor cell cadherin expression, for instance from E-cadherin, which promotes tumor cell-tumor cell adhesion (and blocks invasion), to N-cadherin, which is normally expressed on mesenchymal cells and facilitates tumor cell binding to the stroma during invasion (Fig. 1). In this process, certain tumor cells undergo an epithelial-mesenchymal transition (EMT)[2]. Three types of EMT are recognized depending on the phenotype of the output cells[3]. Type 1 EMT is seen when primitive epithelial cells transit into mesenchymal cells that form the diaspora of the basic body plan following gastrulation or neural crest migration. Type 2 is seen when secondary epithelial cells or endothelial cells populate interstitial spaces with resident or inflammation-induced fibroblasts, the latter during persistent injury. Type 3 is part of the metastatic process, whereby epithelial tumor cells leave a primary tumor nodule, migrate to a new tissue site, and reform as a secondary tumor nodule[3]. Type 3 EMT occurs in an orchestrated fashion; one of the earliest events involves the disruption of tight junctions that connect epithelial cells, as well as delocalization of tight junction proteins, including zonula occludens-1, claudin-1, and occludin. Adherens junction complexes, which contain E-cadherin and β-catenin, are also disrupted, and reorganization of the actin cytoskeleton from a cortical location into actin stress fibers anchored to the focal adhesion complexes is observed (Figs. 1 and 2)[3,4].

Upon EMT, apicobasal polarity is lost, and the cells exhibit a spindle-shaped morphology and express mesenchymal markers, including N-cadherin, vimentin, fibronectin, α-smooth muscle actin, and fibroblast-specific protein 1[4]. Cancer metastasis is accelerated by EMT through stimulation of cell migration and invasion, cell-substrate adhesion, intravasation, and extravasation, as well as cell survival. Indeed, cell survival is important for the transit of tumor cells in the blood and lymph systems, and reestablishment at sites of metastasis[4].

INDUCERS AND ACTORS IN TYPE 3 EMT

In Type 3 EMT, epithelial carcinoma cells undergo carcinoma-metastatic transitions forming migratory metastatic tumor cells[3]. Various kinds of stimuli are known to induce Type 3 EMT, including transforming growth factor-β (TGF-β), bone morphogenetic protein, fibroblast growth factor and epidermal growth factor (Fig. 2). In response to these stimuli, tumor cells with EMT acquire or enhance various markers including N-cadherin, $\alpha 5\beta 1$ integrins, nuclear β-catenin as well as Snail-1, an indispensable transcription factor for EMT (Fig. 3). In general, tumor cells lose their expression of E-cadherin and Type IV collagen during EMT (Fig. 3)[3]. For example, TGF-β binds to Type II and Type I serine-threonine kinase receptors, termed TβRII and TβRI, respectively. TβRII transphosphorylates TβRI, which in turn activates Smad2/3. Once activated, Smad2/3 forms a complex with Smad4 and translocates into the nucleus to regulate the transcription of target genes (Fig. 4)[4]. Meanwhile, TGF-β induces the expression of several transcription factors and regulators involved in EMT including ZEB-1 and Snail-1/2. Snail suppresses E-cadherin expression by direct binding to an E2-box sequence of the E-cadherin promoter (Fig. 4)[4-6]. The loss of E-cadherin is considered a crucial step in the progression of papilloma to invasive carcinoma[7], and it is also a fundamental event

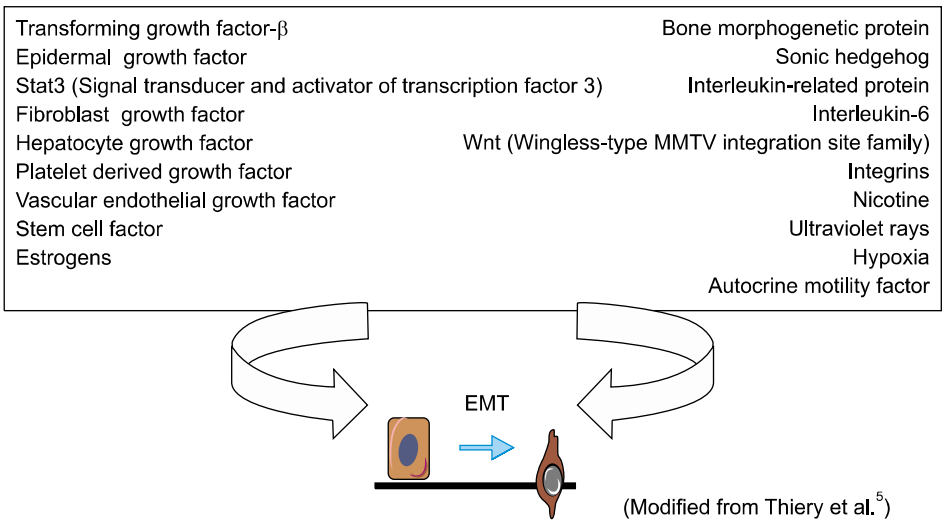

Fig. 2. Inducers of type 3 epithelial-mesenchymal transition (EMT), carcinoma-metastatic transition.

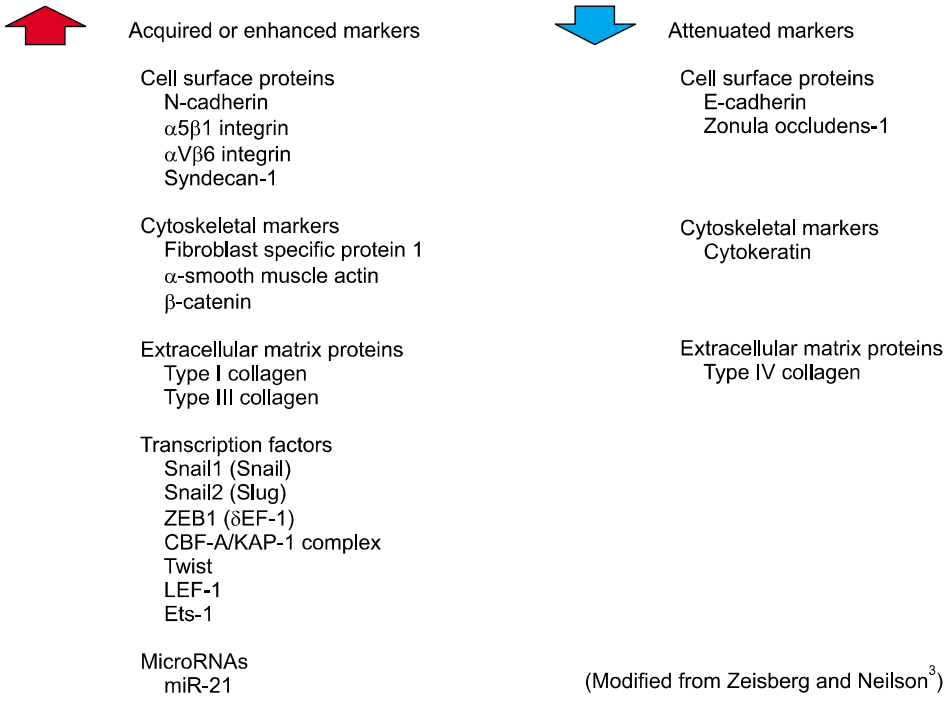

Fig. 3. Markers of epithelial-mesenchymal transition (EMT).

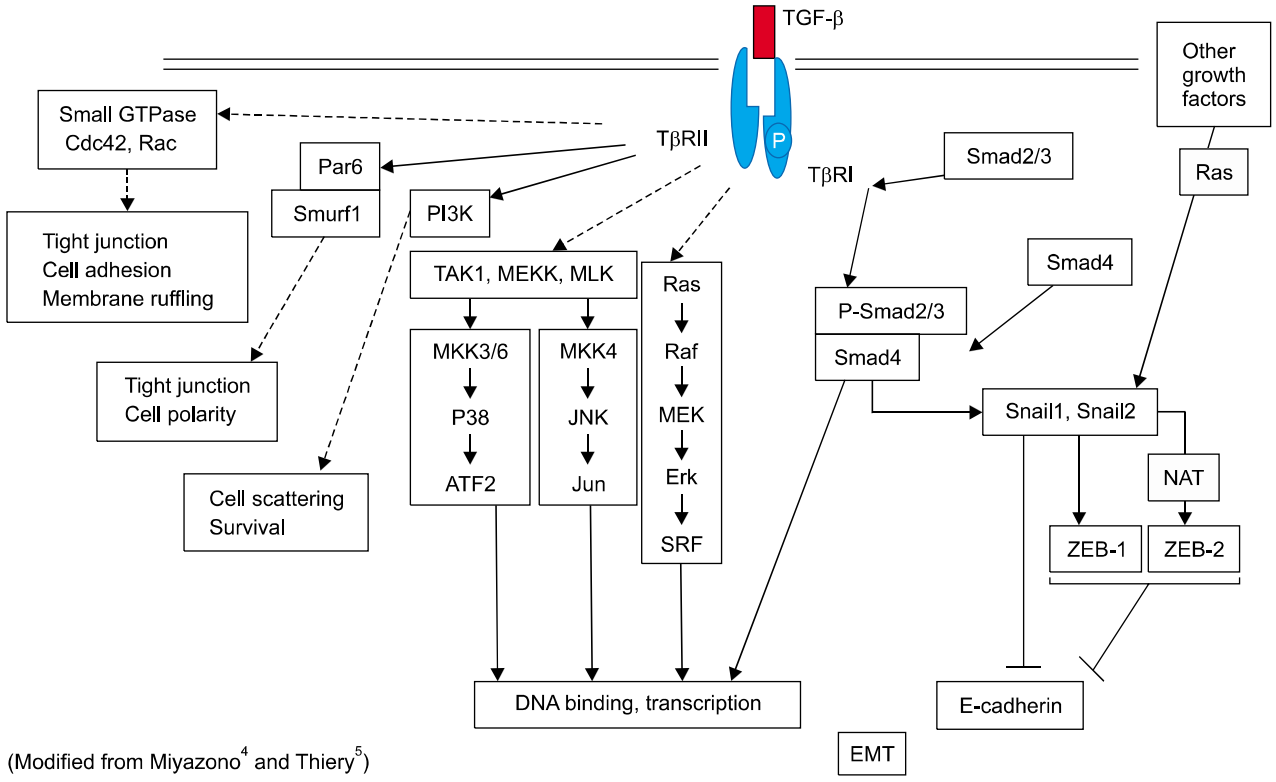

Fig. 4. TGF-β signaling and epithelial-mesenchymal transition (EMT).

in EMT[5].

During migration and invasion of cancer cells through ECM, integrins transmit both mechanical and chemical signals. Each integrin consists of two Type-I transmembrane subunits: α and β. In mammals, 18 and 8 subunits associate with various combinations to bind to collagen, laminin or the RGD motif in fibronectin (Fig. 5). Most integrins activate focal adhesion kinase (FAK) and also

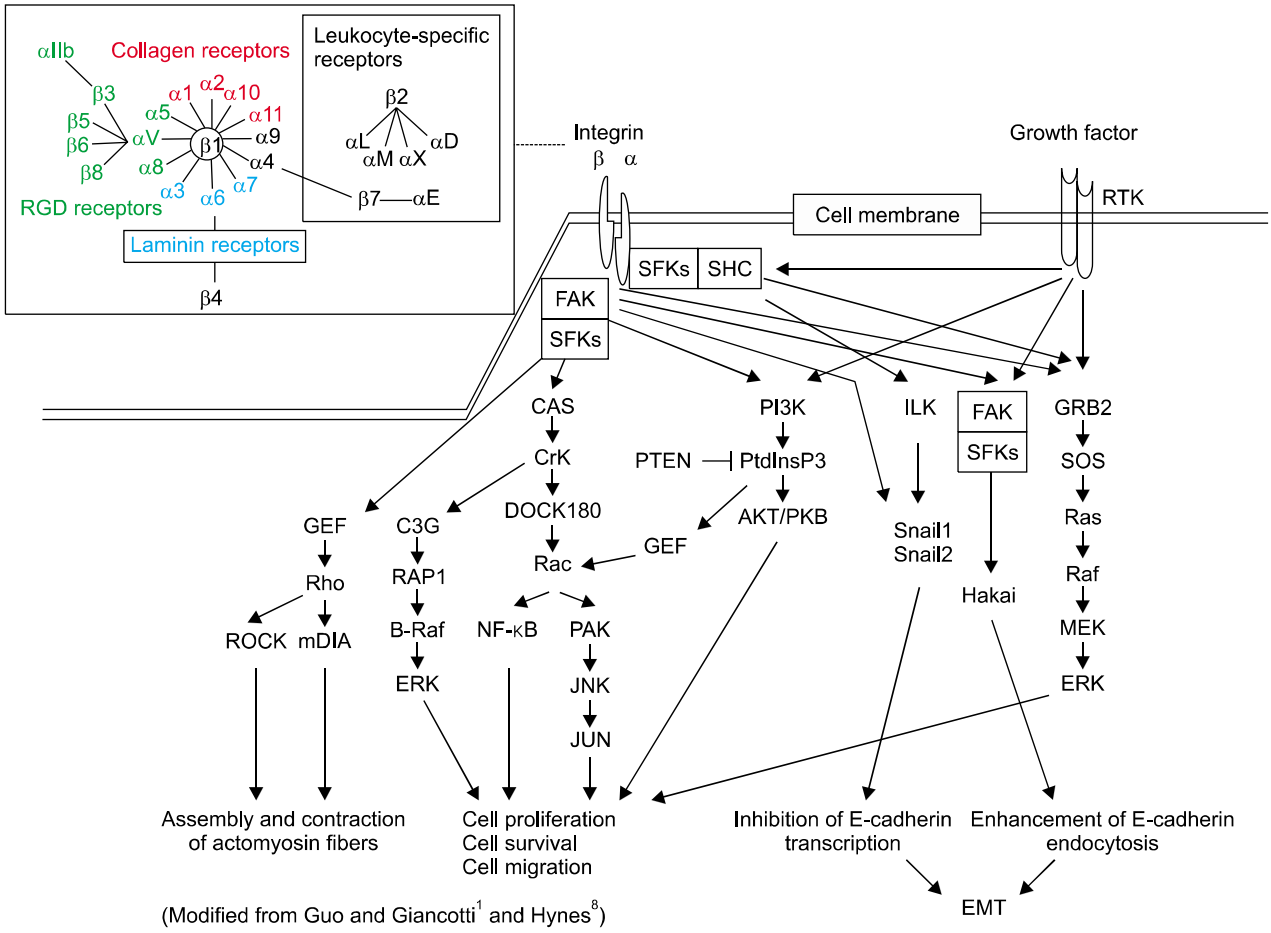

Fig. 5. Integrin signaling.

Src-family kinases, which induces the phosphorylation of CAS and paxillin. Various growth factors and their receptor tyrosine kinases (RTK) activate Ras/ERK signaling together with PI3K and FAK activation. Excessive joint integrin/RTK signaling disrupts cell-cell adhesion by inhibiting E-cadherin transcription through Snail cascade and by enhancing E-cadherin endocytosis via Hakai (Cbl-like E3 ubiquitin protein ligase) recognition, leading to EMT (Fig. 5)[1,8].

TUMOR PROLIFERATION AND INTRACELLULAR SIGNALING

All cancers are thought to share a common pathogenesis. DNA damage and genomic instability are underlying features of human cancer from the earliest stages of tumorigenesis. Damage to genomic DNA is evident even in apparently normal cells and becomes amplified as tumors emerge[9]. The inappropriate proliferation of cells harboring oncogenic lesions is challenged by multiple layers of mechanisms that suppress tumor formation. Several of these barriers are cell intrinsic, such as the genotoxic stress induced by oncogenes, the expression of growth inhibitory, apoptotic and senescence pathways, and telomere attrition. Evasion of these tumor-suppressive pathways is a hallmark of primary tumors[9]. However, an entirely distinct class of pressures comes from sources that are extrinsic to the cancerous cells. Factors in the tumor microenvironment that limit tumor progression include extracellular matrix components, basement membranes, reactive oxygen species, limited availability of nutrients and oxygen, and attack by the immune system (Fig. 6)[9].

In tumors, hypoxia is a strong selective pressure that promotes the outgrowth of malignant cells with a diminished susceptibility to apoptosis. The cellular response to low oxygen tension involves the stabilization of a hypoxia-inducible factor-1 (HIF-1) transcriptional complex that activates genes that promote angiogenesis, anaerobic metabolism, cell survival, and invasion[9,10]. Tumors that exhibit abundant HIF-1 stabilization have a greater likelihood of developing metastatic relapse and correlate with a shorter survival time[9,11]. Accordingly, by analyzing

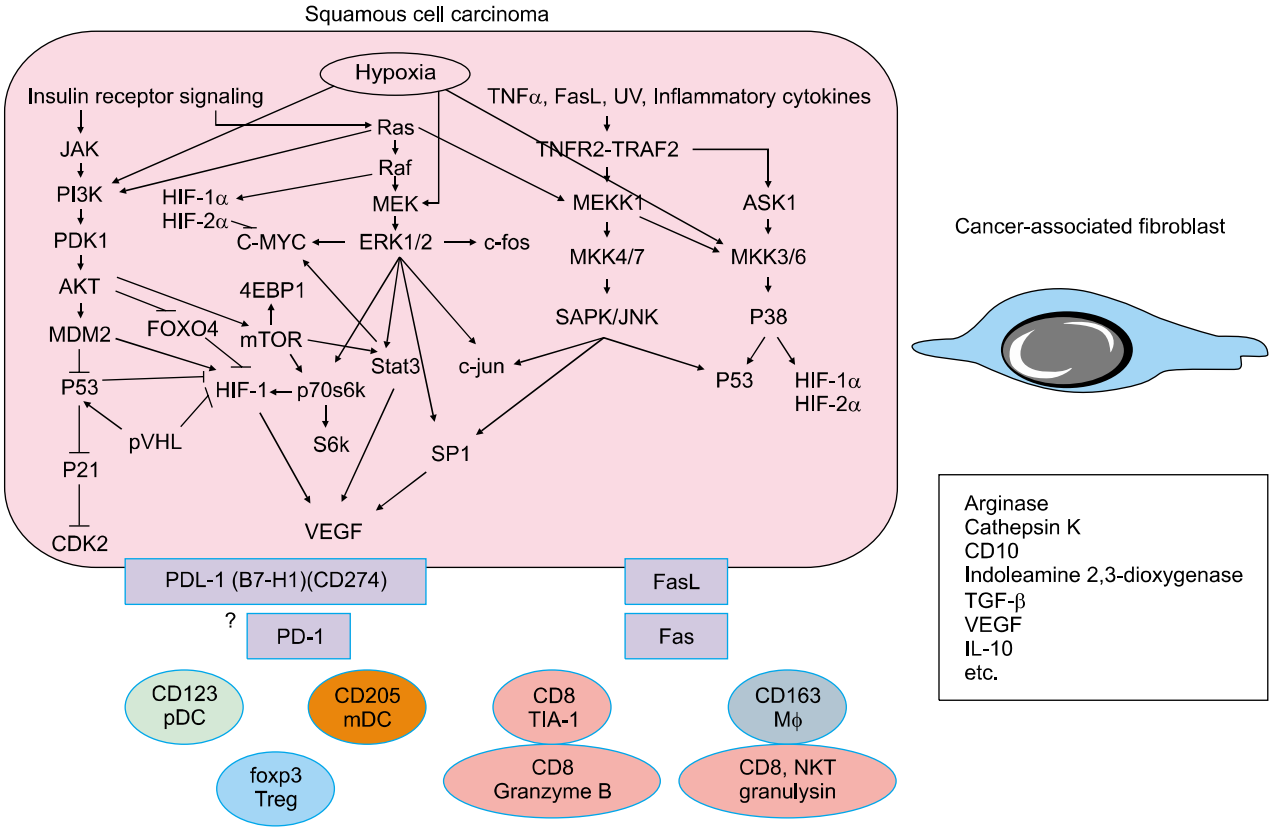

Fig. 6. Intracellular signaling by hypoxia and stromal reaction.

global transcript levels, an "epithelial cell hypoxia signature" has been established as an independent predictor of metastatic risk for breast and ovarian carcinomas[9,12]. A hypoxia-induced signaling pathway is, at least in part, depicted in Fig. 6. Overexpression of HIF-1 and downstream signal molecules such as p-STAT3, p-AKT and p-ERK was detected in malignant keratinocytic tumors[13-15]. A subset of HIF-1 target genes may act as a mediator of metastatic progression. HIF-1 induces the expression of the chemokine receptor CXCR4 in renal cell carcinoma cells, which may promote organ-specific metastatic dissemination[9,16].

INVASION, ECM AND CANCER-ASSOCIATED FIBROBLASTS

Cancer cells acquire cell-autonomous capacity for limitless proliferation and survival through the activation of oncogenes and inactivation of tumor suppressor genes. Nevertheless, the formation of a clinically relevant tumor requires support from the surrounding normal stroma, also referred to as the tumor microenvironment. Carcinoma-associated fibroblasts, bone marrow derived cells, and vascular/lymphatic endothelial cells present within the tumor microenvironment contribute to tumor progression[17,18]. Tumor-associated macrophages and other immune cells participate in the regulation of tumor growth and apoptosis by expressing PD-1/PDL-1 and Fas/FasL (Fig. 6)[19,20]. Growth, invasion and metastatic potential of tumor cells are influenced through tumor-stroma interaction in which tumor cells and mesenchymal cells including inflammatory cells surrounding the tumor cells interact with each other[2].

The engagement of integrins and other attachment molecules is accompanied by the recruitment of proteases to degrade the ECM, providing a pathway for invasion[21]. Matrix metalloproteinases (MMPs), plasmins, urokinase-type plasminogen activators, cathepsins and heparanases, when transfected into a tumor cell line, augment invasion. Most tumor cell movement in invasion is dynamic, involving the formation of adhesions to the ECM at the leading edge of the cell, detachment from the ECM at the trailing edge and ratcheting of the cell in the forward direction. Virtually every 'growth factor' stimulates tumor cell motility *in vitro*. Chemokines, chemotactic cytokines that bind G-protein-coupled receptors, represent another important class of motility-inducing proteins[2,22]. Chemokines also contribute to tumor cell invasion by inducing

the infiltration of tumors by macrophages and lymphocytes, which release proteases and other inflammatory stimuli. Growth factors can stimulate motility and invasion by mechanisms distinct from those involved in mitogenesis. In the case of RTK activation, proliferation is mediated by the phosphatidylinositol-3 kinase and ERK pathways, whereas invasion can occur by RTK interaction with integrins to stimulate formation of an FAK-Src complex[2,23]. Sequential binding of protein cascades to FAK triggers many of the downstream cellular changes in invasion. FAK binding of GEF leads to Rho activation, the formation of cytoplasmic actin stress fibers and mature focal adhesions, and stabilization of the nascent adhesion. Adhesion at the leading edge must be coupled with de-adhesion at other sites so that the cell can be pulled forward by cytoskeletal contraction (Fig. 5)[2,23,24].

Cancer-associated fibroblasts are essential for tumor progression providing both a functional and structural supportive environment, and the presence of fibroblast populations within human tumors is usually associated with poor outcome and an increase in metastatic potential by enhancing matrix degradation and angiogenesis through the production of MMPs and various cytokines or growth factors[25-27]. At the invasion front, the cancer-associated fibroblasts are known to produce large amounts of MMPs and cathepsin K[28,29]. Cathepsin K, a cysteine protease with strong collagenolytic and elastolytic properties involved in extracellular matrix turnover, plays an essential role in osteoclast-mediated bone resorption[30]. Cathepsin K is expressed in the stroma of basal cell and squamous cell carcinomas, especially in invasive cases, but not in normal fibroblasts[31]. A high expression level of cathepsin K together with cathepsin B is significantly correlated with shorter recurrence-free interval and overall survival time in pulmonary adenocarcinomas[32].

CD10 is also an interesting molecule produced from cancer-associated fibroblasts. CD10 is a 90- to 110-kDa cell-surface zinc-dependent metalloproteinase also referred to as a neutral endopeptidase (EC 3.4.24.11), enkephalinase, neprilysin and common acute lymphoblastic leukemia antigen, which cleaves various peptide substrates including substance P[33]. CD10 expression has been detected in peritumoral fibroblasts within the invasive area of various cancers such as prostate, breast, colorectal, and skin carcinomas, suggesting that CD10 expression in peritumoral cells may contribute or correlate to malignant tumor progression[34-38]. The fact that CD10+ fibroblasts are frequently seen at the invasive front suggests that tumor-stromal interaction exists between cancer cells and CD10+ fibroblasts. CD10 is a potent degrading enzyme for substance P, which is known to promote motility of epithelial cells[39]. Therefore, CD10-bearing fibroblasts may play a protective role in tumor invasion. The tumor-stroma cellular interaction includes direct and/or indirect cross-talk because the active interplay results in the secretion of autocrine and/or paracrine soluble factors. However, the nature and regulatory mechanisms of cancer-associated fibroblasts still remain largely unknown.

CONCLUSION

Uncontrolled proliferation is a defining feature of the malignant phenotype. Nevertheless, the supportive network provided by the stroma is indispensable for further invasion, progression and metastasis. In addition, the role of inflammation in tumorigenesis is now generally accepted, and it has become evident that an inflammatory microenvironment is an essential component of all tumors[40]. For example, inflammatory bowel disease greatly increases the risk of colorectal cancer[41]. However, not all chronic inflammatory diseases increase cancer risk, and some of them, such as psoriasis, may even reduce it[42]. It is not clear what kinds of external and internal stimuli drive or suppress tumorigenesis.

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