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Review Article

Adenoid Cystic Carcinoma: Participation of Urokinase-type Plasminogen Activator (uPA) and its Receptor (uPAR) in Tumor Invasion

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Abstract: Adenoid cystic carcinoma (AdCC) is one of the most common malignant tumors of the salivary glands and has unique clinical features and behavior. AdCC grows slowly, but spreads relentlessly into adjacent tissues, with a proclivity for invading nerve and endothelial sheaths. Moreover, the frequency of recurrence and distant metastasis of AdCC is very high. *In vivo* and *in vitro*, AdCC produces a large amount of extracellular matrix (ECM), including basement membrane (BM) components, elastin, and mucopolysaccharides. The accumulation of ECM components in intercellular spaces results in the formation of a pseudocyst, which is the characteristic architecture of AdCC. AdCC cells degrade considerable amounts of mesenchymal-elaborated ECM through the urokinase-type plasminogen activator (uPA)-plasmin system. By contrast, tumor-produced ECM is resistant to degradation, because it contains plasminogen activator inhibitor type 1 (PAI-1). The migration response of AdCC cell lines to ECM, especially type I and type IV collagens, is much stronger than that of oral squamous cell carcinoma (SCC) cell lines, while both cell types generally show similar patterns of integrin subunit expression. The AdCC cell response to collagens is largely and exclusively inhibited by anti-α_2 integrin antibody. Surface uPA receptor (uPAR) expression by AdCC cell lines is greater than that by SCC cell lines and increases in response to collagen stimulation. This is accompanied by the assembly of numerous focal adhesions, consisting of the adapter proteins uPAR, α_2 integrin, vinculin, and paxillin. A role for uPAR in cell migration and assembly of adaptor proteins was also demonstrated by transfecting AdCC cells with an antisense uPAR RNA, which strongly reduced both responses. Therefore, the proclivity of AdCC cells to migrate to type I and IV collagens might be due to the overexpression of uPAR, which also plays a key role in focal adhesion assembly. In conclusion, the invasiveness of AdCC cells might be regulated by the interaction of uPA-uPAR with integrin.

Key words: adenoid cystic carcinoma, recurrence, invasion and metastasis, urokinase-type plasminogen activator (uPA), uPA receptor, plasminogen activator inhibitor type 1, matrix degradation, integrins, cell migration, focal adhesion

1. Introduction

Adenoid cystic carcinoma (AdCC) is one of the most common malignant tumors of the salivary glands. The tumor grows slowly, but spreads relentlessly into adjacent tissues, so recurrence is common and distant metastasis, especially to the lungs, is very frequent[1]. Since AdCC is resistant to radiotherapy and chemotherapy,

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effective forms of treatment are required. Histologically, the tumor is characterized by a cylindrical or cystic stroma surrounded by anastomosing cords of epithelial tumor cells. The cystic stroma and the interstitium surrounding the tumor islands are often hyalinized and contain various substances, including collagen-like fibers, elastin, basement membrane constituents, and mucopolysaccharides[1].

Invasion and metastasis are characteristic features of malignant tumors. Although the molecular mechanisms underlying metastasis are complex, it is clear that tumor cells must migrate through the extracellular matrix (ECM) in order to invade local tissues and metastasize to distal sites. Migrating cells use both adhesion molecules and proteolytic enzymes to regulate their interaction with and response to the ECM, and cooperation between these components operates at several levels: integrin signaling induces proteases[2], proteases co-localize with integrins[3], and proteases regulate the interface between integrins and the cytoskeleton[4]. A protease system intimately connected to integrins comprises urokinase-type plasminogen activator (uPA), the uPA receptor (uPAR), and plasmin[5].

The plasminogen activation system, which is made up of plasminogen activators, their inhibitors, plasminogen, and the respective cell-surface-binding proteins and receptors, plays a central role in cell invasion. Along with tissue-type plasminogen activator, uPA promotes the formation of plasmin, the key protease in fibrinolysis. Plasmin activates matrix-metalloproteases, which constitute a proteolytic system for cell migration and tissue remodeling[6, 7]. Together, these proteases facilitate cell invasion by (1) activating latent growth factors or releasing them from their ECM-binding sites, and (2) degrading a variety of proteins in the ECM. Different plasminogen activator inhibitors (PAIs) neutralize plasminogen activator, primarily fast-acting PAI-1[8]. PAI-1 is a serine protease inhibitor, but, unlike other proteases, its active conformation is stabilized by high-affinity binding to ECM-associated vitronectin. In addition to producing uPA, malignant cells also synthesize the uPA inhibitor PAI-1, thereby regulating PA activity levels in the cellular microenvironment[8, 9]. Therefore, tumor-cell-mediated proteolysis may be controlled by the fine regulation of tumor-cell-secreted proteases and protease inhibitors.

uPA binds to a glycosyl phosphatidylinositol (GPI)-anchored uPAR, which consists of three homologous domains (D1, D2, and D3). The binding of proteins such as uPA, vitronectin, and PAI-1 to uPAR involves a direct interaction with D1, but requires the integrity of the full-length receptor[7]. uPA bound to uPAR exhibits enhanced proteolytic activity and directly activates plasminogen and matrix metalloproteases, which, in turn, enhance ECM degradation[7]. The receptor-binding domain of uPA is located in the amino-terminal fragment (ATF; residues 1-135) of the uPA molecule and does not involve the protease domain. Independent of its proteolytic function in matrix degradation, the uPA-uPAR interaction 1) mediates several signaling events, 2) regulates cell adhesion on vitronectin-coated surfaces, 3) modulates integrin activity, 4) triggers cell migration, and is strongly correlated with the metastatic potential of various tumors[10].

Integrins are ubiquitous, heterodimeric transmembrane receptors that anchor the cell to the ECM and the cytoskeleton to the plasma membrane[11]. Integrin receptors mediate cell adhesion, migration, and bidirectional signal transduction via interactions with ECM proteins[11, 12]. In particular, the ECM-integrin interaction leads to the reorganization of the actin cytoskeleton, initiates signal transduction cascades, and coordinates the response to growth factors[12]. Recent work points to important interactions between integrins, cytoskeletal components, and signaling molecules[13].

Focal adhesions are specialized cell adhesion sites that transduce signals from the ECM intracellularly. The assembly and disassembly of focal adhesions result in the formation and displacement of cellular stress fibers. On ligand activation, transmembrane ECM receptors, such as integrins, cluster at focal adhesions, which are physically connected to the cytoskeleton by stable and transient interactions[14]. A characteristic property of most focal adhesion proteins is their multi-domain structure. For example, vinculin can interact with at least ten other focal adhesion proteins, including actin, tensin, paxillin, and talin, although probably not simultaneously[15]. In addition to recruiting non-catalytic components, protein kinases also cluster at focal adhesions in response to cell adhesion, which leads to increased phosphorylation of focal adhesion proteins[15]. Paxillin, another important multi-domain adaptor protein, functions in recruiting both signaling and structural molecules to focal adhesions[16]. The COOH terminus of paxil-