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Adhesion and protease activity in cell lines from human salivary gland tumors are regulated by the laminin-derived peptide AG73, syndecan-1 and β1 integrin ☆

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Abstract

We studied the induction of protease activity by the laminin α1-derived peptide AG73 in cells from adenoid cystic carcinoma (CAC2) and myoepithelioma (M1), respectively a malignant and a benign salivary gland tumors. Laminin α1 chain and MMP9 were immunolocalized in adenoid cystic carcinoma and myoepithelioma *in vivo* and *in vitro*. Cells grown inside AG73-enriched laminin-111 exhibited large spaces in the extracellular matrix, suggestive of remodeling. The broad spectrum MMP inhibitor GM6001 decreased spaces induced by AG73 in CAC2 and M1 cells. This result strongly suggests that AG73-mediated matrix remodeling involves matrix metalloproteinases. CAC2 and M1 cells cultured on AG73 showed a dose-dependent increase of MMP9 secretion, as detected by zymography. Furthermore, siRNA silencing of MMP9 decreased remodeling in 3D cultures. We searched for AG73 receptors regulating MMP9 activity in our cell lines. CAC2 and M1 cells grown on AG73 exhibited colocalization of syndecan-1 and β1 integrin. siRNA knockdown of syndecan-1 expression in these cells resulted in decreased adhesion to AG73 and reduced protease and remodeling activity. We investigated syndecan-1 co-receptors in both cell lines. Silencing β1 integrin inhibited adhesion to AG73, matrix remodeling and protease activity. Double-knockdown experiments were carried out to further explore syndecan-1 and β1 integrin cooperation. CAC2 cells transfected with both syndecan-1 and β1 integrin siRNA oligos showed significant decrease in adhesion to AG73. Simultaneous silencing of receptors also induced a decrease in protease activity. Our results suggest that syndecan-1 and β1 integrin signaling downstream of AG73 regulate adhesion and MMP production by CAC2 and M1 cells.

Keywords: Salivary gland tumors; Adenoid cystic carcinoma; Myoepithelioma; Extracellular matrix; Laminin; Matrix metalloproteinases; Syndecan; Integrins

1. Introduction

Adenoid cystic carcinoma is a frequently occurring malignant salivary gland neoplasm with recurrence and metastasis (Seifert and Sobin, 1992). This tumor exhibits solid, tubular and pseudocystic subtypes (Dardick, 1996; Seifert and Sobin, 1992). A prominent feature of adenoid cystic carcinoma is the perineural spreading, probably caused by the affinity of the neoplasm for basement membrane rich tissues, such as nerves and blood vessels (Dardick, 1996; Seifert and Sobin, 1992).

Myoepithelioma is a rare benign tumor of myoepithelial cell differentiation (Seifert and Sobin, 1992). Growth patterns may be solid, myxoid and reticular, with two main cytological subtypes: spindle-shaped and plasmacytoid (Dardick, 1995; Sciubba and Brannon, 1982; Takai et al., 1995, 1994). Myoepithelioma and adenoid cystic carcinoma share the same origin, cells from the intercalated duct of salivary gland (Batsakis, 1980).

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Both adenoid cystic carcinoma and myoepithelioma express prominent extracellular matrix (Cheng et al., 1995, 1992). It has been suggested that this matrix plays an important role as regulatory factor of phenotypic differences among salivary gland neoplasms (Capuano and Jaeger, 2004; de Oliveira et al., 2001; Franca et al., 2000, 2001; Freitas and Jaeger, 2002; Freitas et al., 2004, 2007; Jaeger et al., 1997a,b; Morais Freitas et al., 2007). We have already demonstrated that laminin regulates the phenotype of an adenoid cystic carcinoma cell line (CAC2) and myoepithelioma cell line (M1) (Capuano and Jaeger, 2004; Freitas and Jaeger, 2002; Freitas et al., 2004, 2007; Morais Freitas et al., 2007). Growth of CAC2 cells within laminin-111 created duct-like and pseudocystic structures, similar to that occur in the neoplasm *in vivo* (Freitas and Jaeger, 2002; Freitas et al., 2004). We have also reproduced myoepithelioma architecture by growing a cell line (M1) derived from this tumor inside Matrigel (de Oliveira et al., 2001).

We are currently studying the effect of particular domains of laminin on CAC2 and M1 cells. We have started this approach using the laminin-derived peptide SIKVAV (Freitas and Jaeger, 2002; Freitas et al., 2004, 2007). This peptide regulates morphology and protease activity of CAC2 cells (Freitas and Jaeger, 2002; Freitas et al., 2004, 2007). Among the laminin-derived bioactive peptides the RKRLQVQLSIRT sequence (designated as AG73 and located at the LG4 globular domain of α1 chain) is the most effective in many biological assays (Nomizu et al., 1995; Suzuki et al., 2003a). AG73 has been tested both *in vitro* and *in vivo* in different cells and systems (Engbring et al., 2002, in press; Hoffman et al., 2001, 1998). It promotes attachment of numerous cell types (Nomizu et al., 1995, 1998), induces salivary acinar cell differentiation (Hoffman et al., 1998), inhibits branching morphogenesis of embryonic salivary glands (Kadoya et al., 2003, 1998; Kadoya and Yamashina, 2005), stimulates neurite outgrowth (Richard et al., 1996), stimulates matrix metalloproteinase secretion by PC12 cells (Weeks et al., 1998).

AG73 has a striking relevance in tumor biology (Engbring et al., 2002, in press; Kim et al., 1998; Mochizuki et al., 2007; Song et al., 1997; Suzuki et al., 2003b, 2005). It caused liver metastasis and increased lung colonization of B16F10 melanoma cells (Kim et al., 1998; Song et al., 1997). Ovarian cancer cells increased metastasis in the presence of AG73 (Yoshida et al., 2001). This peptide is also related to angiogenesis in different systems, such as aortic ring sprouting, endothelial tube formation, and chick chorioallantoic membrane assays (Mochizuki et al., 2007). This evidence strongly suggests that AG73 may have different roles in promoting tumor growth and metastasis. Because of its potential AG73 has been proposed as therapeutic material for tissue regeneration and engineering (Ikemoto et al., 2006; Mochizuki et al., 2003). Despite its biological significance, the mechanisms regulating the bioactivity of AG73 are not fully understood.

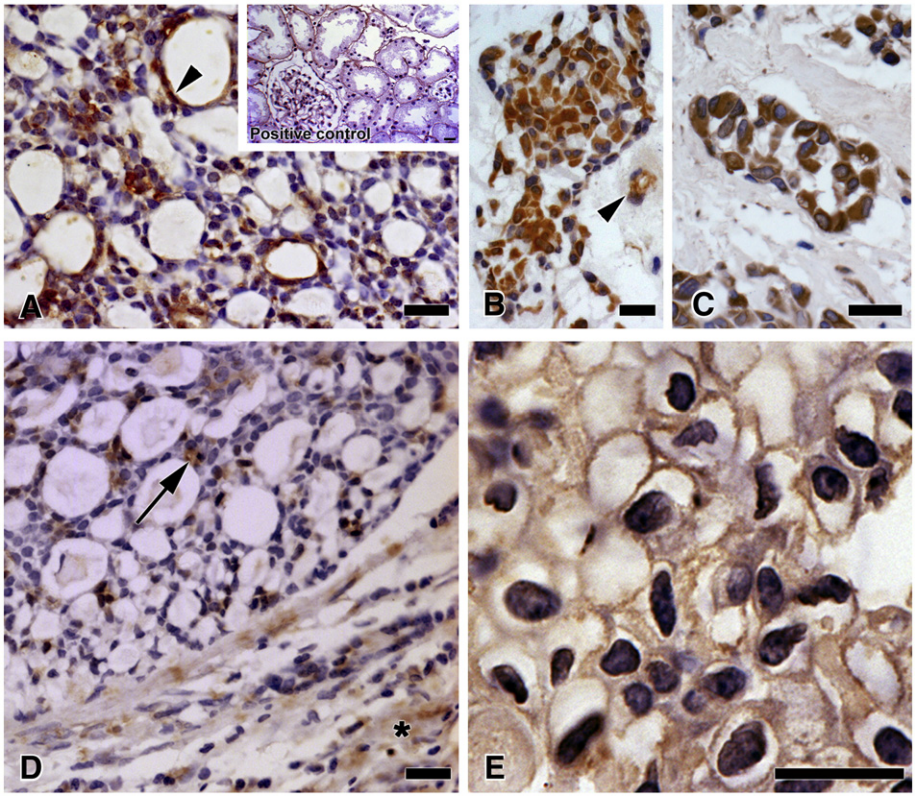

Fig. 1. Laminin α1 and MMP9 are detected in adenoid cystic carcinoma and myoepithelioma. In adenoid cystic carcinoma this protein is observed either as a diffuse pattern or a basement membrane-like linear structure (A, arrowhead). In myoepithelioma laminin α1 chain shows cytoplasmic localization (B, C). Laminin is also found staining blood vessels (B, arrowhead). Human kidney positive control exhibits laminin α1 chain in proximal tubules (A, inset). MMP9 is observed in adenoid cystic carcinoma (D) and myoepithelioma (E). In adenoid cystic carcinoma this enzyme is present in the cribriform subtype (D, arrow) and in stromal regions (D, asterisk). Myoepithelioma expresses MMP9 at cell edges (E). Bars: 20 μm.