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Epilysin (MMP-28) is deposited to the basolateral extracellular matrix of epithelial cells

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ABSTRACT

Epilysin (MMP-28) is a conserved member of the matrix metalloproteinase (MMP) family. It is expressed in various normal tissues, and induced in wounds and in developing and regenerating nerves. Epilysin induces TGF-β mediated epithelial to mesenchymal transition, but its other functions are largely unknown. We have characterized the localization of both catalytically active and mutated inactive, overexpressed epilysin in established epithelial cell lines. We found that epilysin was localized abundantly to the basolateral side of the cells and associated with the extracellular matrix (ECM) as verified by immunoblotting and confocal microscopy. Overexpression of epilysin in MDCK cells resulted in a drastic reduction of basolateral ECM, as observed by the disappearance of collagen type IV, laminin and fibronectin. Cultivation of epilysin expressing MDCK cells in defined serum free medium resulted in the restoration of these proteins to the ECM. The levels of fibronectin and collagen IV were, however, reduced in epilysin expressing cells under the serum free conditions, and degradation fragments of collagen IV were detected supporting the activation of proteolysis by epilysin. Epilysin was observed in its unprocessed 50 kDa active form in the ECM of MDCK cells under serum free conditions whereas in cells cultured in serum containing it was processed to the 48 kDa form. Current results indicate that epilysin associates with the basolateral ECM of cultured epithelial cells, where it plausibly plays a role in the regulation of matrix composition and turnover.

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1. Introduction

Matrix metalloproteinases can degrade all major structural proteins of the extracellular matrix. MT-MMPs are, in general, necessary for cell invasion through basement membranes. Cytokines, chemokines, growth factors, cell-to-cell adhesion receptors and cell-to-ECM adhesion receptors have also been identified as MMP substrates. MMP activity is essential in the control of extracellular matrix turnover and cell motility in tissues and through tissue boundaries, for example in inflammation, immune responses and during recovery from injury. Expression of MMPs is frequently elevated in pathological processes involving cell migration and tissue degradation like cancer and osteoarthritis (Hotary et al., 2006; Page-McCaw et al., 2007; Stamenkovic, 2003).

Epilysin (MMP-28) and MMP-19 are related to insect MMPs and are evolutionary conserved (Altincicek and Vilcinskas, 2008). The catalytic domain is the most conserved domain (Illman et al., 2001, 2003; Werner et al., 2007). Epilysin has a typical MMP structure consisting of pro-, catalytic-, hinge- and hemopexin like-domains. Epilysin has a furin recognition site in the end of its prodomain. This site is cleaved intracellularly by proprotein convertases, as are the corresponding sites of the MT-MMPs and MMP-11 (Bassi et al., 2005; Pei and Weiss, 1995). Epilysin is thus secreted in the 48 kDa active form and is physiologically expressed in adults in various tissues such as testis, colon, lung and heart (Lohi et al., 2001; Marchenko and Strongin, 2001). In cell culture endogenous epilysin is expressed in immortalized human keratinocytes (HaCat), primary keratinocytes (Lohi et al., 2001), developing neurons (Werner et al., 2007) and several transformed cell lines. The expression of epilysin is up-regulated in wound healing and in response to TNF-α. In cutaneous wounds, strong epilysin expression has been detected in basal keratinocytes distal from the wound edge in association with intact basement membrane (Saarialho-Kere et al., 2002).

Epilysin is expressed during pathological processes, such as in different carcinomas (Marchenko and Strongin, 2001). Among the 24 human MMPs, epilysin, MT1-MMP and MMP-2 are most highly elevated in urothelial carcinomas where their transcription levels correlate with the cancer grade (Wallard et al., 2006). Expression of epilysin has further been detected in invasive ductal cell carcinomas of the breast (Overall et al., 2004). In oral squamous cell carcinomas epilysin was widely expressed, and cells derived from these carcinomas were capable of colony formation and anchorage independent growth depending on epilysin expression (Lin et al., 2006). Reduction of epilysin mRNA by anti-sense oligonucleotides abolished these properties. Lung

Abbreviations: BSA, bovine serum albumin; CHO, Chinese hamster ovary; ECM, extracellular matrix; FCS, fetal calf serum; GndHCl, guanidine-HCl; HAc, acetic acid; MDCK, Madin–Darby canine kidney; MEM, Eagle's minimal essential medium; MMP, matrix metalloproteinase; MT-MMP, membrane-type matrix metalloproteinase; PBS, phosphate buffered saline; SDS, sodium dodecyl sulfate; TBS, Tris buffered saline; pex, hemopexin like domain; TGF-β, transforming growth factor-β.

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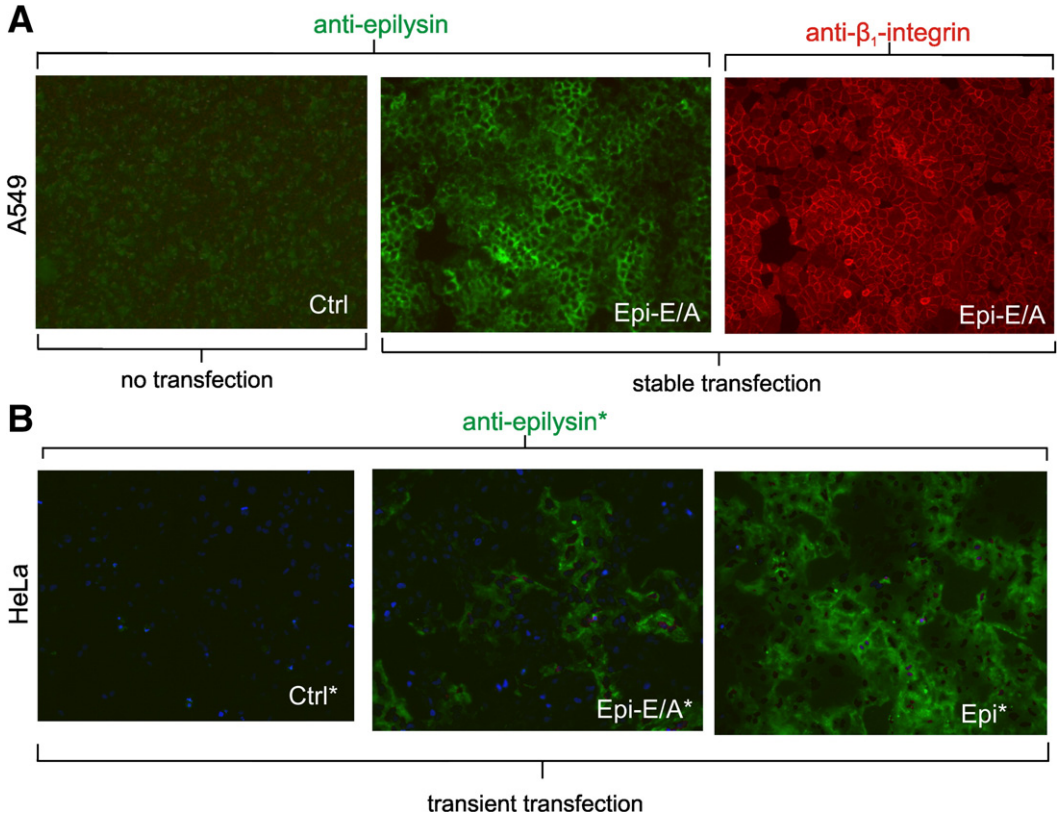

Fig. 1. Immunolocalization of epilysin in nonpermeabilized epithelial cells. (A) Cell surface distribution of epilysin resembles that of β_1-integrin. Nonpermeabilized A549 cells stably transfected with pEF-IRES-P vector coding the inactive epilysin mutant (Epi-E/A; epilysin is abbreviated as Epi) were co-stained with antibodies against epilysin (produced by immunization with purified recombinant hemopexin like domain (pex*; in Supplementary Fig. 1) and β_1-integrin. (B) Cell surface distribution of V5 epitope-tagged active epilysin in HeLa cells is similar to distribution of inactive epilysin. Cells were transiently transfected with pEF1/V5-His vector coding either active epilysin (Epi*) or inactive mutant Epi-E/A* or control (C*) tagged with V5-epitope at C-terminus. Epilysin was visualized with anti-V5 epitope antibody (denoted anti-epilysin*). The cells were fixed 60 h after transfection.

adenocarcinoma A549 cells undergo TGF-beta mediated epithelial-to-mesenchymal transition (EMT) when stably transfected with epilysin, and adopted an invasive phenotype. The expression of MMP-9 was up-regulated and the expression of MMP-2 was down-regulated in this process in the culture medium. Interestingly, the level of MT1-MMP in cell lysates was also increased (Illman et al., 2006, 2008).

We report here that overexpressed epilysin is deposited abundantly and tightly to the insoluble basolateral extracellular matrix (ECM) of different cultured epithelial cell lines grown to confluency, and that the content of ECM proteins is reduced in epilysin overexpressing MDCK cells, especially when cultured in serum containing medium. The extent of this down-regulation of ECM was dependent on the factors present in the microenvironment, as exemplified by the amplifying effect of serum. Epilysin seemed to be crucially involved in the initiation of a proteolytic cascade. Under serum free conditions both degradation fragments of type IV collagen and only the 50 kDa form of epilysin could be detected from the basolateral ECM of MDCK cells. Under serum-containing culture conditions the basement membrane proteins type IV collagen, laminin-1 and fibronectin were missing from the ECM, and, in addition to the 50 kDa, also the 48 kDa processed form of epilysin was detected. Epilysin could thus have a major role in the regulation of connective tissue degradation during cell invasion.

2. Results

2.1. Epilysin is deposited to the basolateral side of cultured epithelial cells and associates with basolateral cell surface

The distribution of epilysin was analyzed in three different epithelial cell lines with specific antibodies to determine the cellular localization of epilysin in epithelial cell cultures in general. Epilysin expression has been detected in adenocarcinoma cell lines of epithelial origin (Marchenko and Strongin, 2001). The distribution of overexpressed epilysin in two well-known cancer epithelial cell lines, A549 lung adenocarcinoma and HeLa cervical carcinoma was analyzed. As control we analyzed epilysin distribution in the widely used non-malignant epithelial cell model, MDCK. This canine kidney epithelial cell line produces prominent basement membrane in culture, and was of major interest. A549 cells were stably transfected with inactive, mutated epilysin (Epi-E/A; Figs. 1A, 2 and 3B) and MDCK cells with active, wild-type epilysin (Epi; Figs. 4 and 5) in pEF-IRES-P vector. The transfected cells were selected for continuous production of recombinant epilysins with puromycin (Illman et al., 2003, 2006). In this way, we obtained stably transfected cell pools derived from a number of cells instead of a single cell. Stable expression of active epilysin in A549 cells leads to loss of polarized epithelial morphology through EMT and loss of epilysin binding on cell surface (Illman et al., 2006). Therefore, these cells could not be used to study epilysin in polarized epithelial cells. Stably active epilysin expressing MDCK cells also show a rounded morphology, but grow in monolayer. A549 cells expressing inactive (E/A) epilysin retain their epithelial morphology and are suitable for studies of polarized secretion of epilysin in epithelial cells. To compare the localization of active and mutated epilysin, A549 and HeLa cells were transiently transfected with Epi* and Epi-E/A* constructs (Figs. 1B, 3A, C and 6; * denotes V5-his epitope tag in the C-terminus of the recombinant protein). Antibodies against the C-terminal region (RP4) of epilysin (staining not shown), against recombinant hemopexin like-domain of epilysin (pex*) produced during this work (Supplementary Fig. 1; denoted anti-epilysin in Figs. 1–4; see Experimental procedures) and against anti-V5 tag (used against the

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