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Review

Basement membrane fragments in the context of the epithelial-to-mesenchymal transition

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ABSTRACT

The epithelial-to-mesenchymal transition (EMT) enables cells of epithelial phenotype to become motile and change to a migratory mesenchymal phenotype. EMT is known to be a fundamental requisite for tissue morphogenesis, and EMT-related pathways have been described in cancer metastasis and tissue fibrosis. Epithelial structures are marked by the presence of a sheet-like extracellular matrix, the basement membrane, which is assembled from two major proteins, laminin and collagen type IV. This specialized matrix is essential for tissue function and integrity, and provides an important barrier to the potential pathogenic migration of cells. The profound phenotypic transition in EMT involves the epithelial cells disrupting the basement membrane. Matrix metalloproteinases (MMPs) are known to cleave components of basement membranes, but MMP-basement membrane crosstalk during EMT *in vivo* is poorly understood. However, MMPs have been reported to play a role in EMT-related processes and a variety of basement membrane fragments have been shown to be released by specific MMPs *in vitro* and *in vivo* exhibiting distinct biological activities. This review discusses general considerations regarding the basement membrane in the context of EMT, a possible role for specific MMPs in EMT and highlights biologically active basement membrane fragments liberated by MMPs.

Contents

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Introduction

The epithelial-to-mesenchymal transition (EMT) is a developmental process that plays an essential role in gastrulation to enable the formation of tissue layers (Hay and Zuk, 1995; Lim and Thiery, 2012; Nakaya and Sheng, 2008, 2009, 2013; Theveneau and Mayor, 2012). EMT is defined as the transition of an epithelial cell to a mesenchymal migratory phenotype facilitated by a change in the expression of key epithelial markers (Nieto, 2011; Thiery et al., 2009). Similar developmental programs have been described during cancer progression (Acloque et al., 2009; Ginnebaugh et al., 2014; Puisieux et al., 2014; Steinestel et al., 2014; Thiery, 2002; Pang et al., 2016a), and in the context of tissue fibrosis as a contributor to the accumulation of activated fibroblasts (Grande et al., 2015; Lovisa et al., 2015; Masszi and Kapus, 2010; Quaggin and Kapus, 2011; Zeisberg and Duffield, 2010). However, the significance of EMT-related processes in cancer metastasis and fibrosis *in vivo* is an ongoing debate due to conflicting reports on the source of mesenchymal phenotypes, tissue-specificity and activation of EMT-signaling pathways (Fischer et al., 2015; Kim et al., 2006; Zeisberg and Duffield, 2010; Zheng et al., 2015). Clearly, this debate depends on the criteria of how to define EMT, on the particular disease, tissue or experimental model (*e.g.* often acute fibrosis models are used which may not accurately reflect a chronic disease), and the methods to detect EMT.

Numerous studies have helped to elucidate EMT-related molecular mechanisms and to identify crucial EMT transcription factors (EMT-TFs) that are either solely capable of initiating the transformation from epithelial phenotypes or can act together with other molecules to interfere with epithelial gene expression (Aref et al., 2013; Gonzalez and Medici, 2014; Lamouille et al., 2014; Thiery and Sleeman, 2006). EMT has been described in different cell culture and *in vivo* systems, ranging from embryonic stem cells to fibrotic tissues. The activation of EMT-TFs (*e.g.* Snail1, Snail2, Zeb1/2, Twist, Prrx1) and the consequent down-regulation of E-cadherin have been identified as key molecular events (Cano et al., 2000; Mikami et al., 2011; Naber et al., 2013; Nieto et al., 1994; Vincent et al., 2009). Transforming growth factor β (TGFβ) is now known to be a major inducer of EMT *in vitro* and *in vivo*, cooperating with both smad-related and smad-independent pathways (Akhurst and Hata, 2012; Fuxe and Karlsson, 2012; Fuxe et al., 2010; Gal et al., 2007; Gonzalez and Medici, 2014; Gressner and Weiskirchen, 2006; Gui et al., 2012; Heldin et al., 2009; Jang et al., 2013; Liu, 2010; Margadant and Sonnenberg, 2010; Margetts et al., 2005; Miettinen et al., 1994; Vincent et al., 2009; Xu et al., 2009; Zeisberg et al., 2003). Additionally, integrin-linked EMT pathways have been described that correlate a change in the microenvironment to cell phenotypes (Agarwal, 2014; Bhowmick et al., 2001; Chen et al., 2013; Gilcrease, 2007; Henderson and Sheppard, 2013; Horejs et al., 2014; Kim et al., 2006, 2009a; Klinowska et al., 1999; Margadant and Sonnenberg, 2010; Zhang et al., 2003a). It is worth noting that while the role of EMT-TFs has been studied *in vivo*, EMT pathways down-stream of integrins – major basement membrane receptors – have still only been assessed *in vitro*.

An epithelial structure is marked by the presence of a basement membrane, which is a specialized sheet-like extracellular matrix assembled from two major proteins: laminin and collagen type IV. Basement membranes permeate the body, overlie all tissues, reside in the walls of all tubular organs, and they play crucial roles in providing survival signals for cells. They also constitute an important barrier to the potential pathogenic migration of cells (LeBleu et al., 2007; Yurchenco, 2011; Yurchenco and Schittny, 1990). In the course of EMT, epithelial cells disrupt this barrier to facilitate migration (Fig. 1a). The molecular mechanisms by which epithelial cells remodel this dense matrix are the subject of considerable debate, owing to difficulties in both the examination of cell-basement membrane interactions *in vivo* and the isolation of insoluble, cross-linked, authentic basement membranes for *ex vivo* studies (Glentis et al., 2014; Kelley et al., 2014; Rowe and Weiss, 2008). Therefore, most of our understanding of basement membrane remodeling and basement membrane-cell crosstalk to date is derived from *in vitro* experiments using limited basement membrane mimics, such as Matrigel.

Recent *in vivo* studies revealed that cells pursue a diverse range of strategies to transmigrate through the basement membrane including mechanical force and local remodeling events, depending on cell and tissue type (Kelley et al., 2014; Matus et al., 2010; McNiven, 2013). Trafficking mechanisms through basement membranes might not be consistent between different cell types and migration processes, *e.g.* leukocytes continuously traverse the basement membrane without substantially altering its structure. The mechanisms that cause epithelial cells to disrupt the basement membrane in EMT remain unclear, but there is evidence that they involve matrix degradation by matrix metalloproteinases (MMPs) and complex signaling between the cells and the basement membrane (Chen et al., 2007; Cheng et al., 2006; Coyle et al., 2008; Du et al., 2012; Eastham et al., 2007; Egeblad and Werb, 2002; Giannandrea and Parks, 2014; Hotary et al., 2006; Kinoh et al., 1996; Lemaître and D'Armiento, 2006; Mogi and Toyoizumi, 2010; Murphy and Nagase, 2008; Nisticò et al., 2012; Orlichenko and Radisky, 2008; Ota et al., 2009; Poincloux et al., 2009; Rowe and Weiss, 2008; Sato et al., 1994; Takahara et al., 1997). Up-regulation and increased activity levels of MMPs have been demonstrated in a variety of different EMT types and stages (Chen et al., 2007; Du et al., 2012; Giannandrea and Parks, 2014; Hirahara et al., 2007; Kargozaran et al., 2007; Kinoh et al., 1996; Lemaître and D'Armiento, 2006; Nisticò et al., 2012; Orlichenko and Radisky, 2008; Wiseman et al., 2003), and correlations between MMPs and EMT-programs have been described (Krantz et al., 2011; Pang et al., 2016b; Shields et al., 2011). Moreover, proteolytic laminin and collagen type IV fragments have been shown to be liberated by MMPs and to modulate cell adhesion and migration once released from the basement membrane (Brassart-Pasco et al., 2012; Colorado et al., 2000; Hamano et al., 2003; Kamphaus et al., 2000; Koshikawa et al., 2003; Luo et al., 2010; Maeshima et al., 2000; Ortega and Werb, 2002; Pasco et al., 2000; Sand et al., 2013; Schenk et al., 2003; Yong et al., 2015). Recent studies have further highlighted the contribution of such fragments to EMT signaling events and a possible role in EMT regulation (Horejs et al., 2014; Monboisse et al., 2013). However, the molecular mechanisms of MMP-basement membrane crosstalk in EMT are still poorly understood partly due to the ongoing discussion about the importance of disease-related EMT-programs. This review summarizes a few key EMT processes and models, and highlights which MMPs have been reported to be expressed in the context of EMT, their possible role in basement membrane processing, and the release of biologically active basement membrane components by specific MMPs.

2. Basement membrane remodeling in EMT

2.1. Basement membrane structure, assembly and integrin interactions

Basement membranes are thin, highly cross-linked, dense sheets of extracellular matrix at the basal side of epithelial cells, vascular endothelial cells, muscle fibers, and Schwann cells (Yurchenco, 2011; Yurchenco and Schittny, 1990). Basement membranes are assembled by a laminin heterotrimer isoform and collagen type IV interconnected by nidogen and the heparan sulfate proteoglycan perlecan or agrin (Kalluri, 2003; LeBleu et al., 2007) (Fig. 2). Laminins are glycosylated, multidomain proteins of about