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Local and global dynamics of the basement membrane during branching morphogenesis require protease activity and actomyosin contractility

Jill S. Harunaga, Andrew D. Doyle, Kenneth M. Yamada*

Cell Biology Section, Laboratory of Cell and Developmental Biology, National Institute of Dental and Craniofacial Research, National Institutes of Health, 30 Convent Drive, Building 30, Room 403, Bethesda, MD 20892, USA

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ABSTRACT

Many epithelial tissues expand rapidly during embryonic development while remaining surrounded by a basement membrane. Remodeling of the basement membrane is assumed to occur during branching morphogenesis to accommodate epithelial growth, but how such remodeling occurs is not yet clear. We report that the basement membrane is highly dynamic during branching of the salivary gland, exhibiting both local and global remodeling. At the tip of the epithelial end bud, the basement membrane becomes perforated by hundreds of well-defined microscopic holes at regions of rapid expansion. Locally, this results in a distensible, mesh-like basement membrane for controlled epithelial expansion while maintaining tissue integrity. Globally, the basement membrane translocates rearward as a whole, accumulating around the forming secondary ducts, helping to stabilize them during branching. Both local and global dynamics of the basement membrane require protease and myosin II activity. Our findings suggest that the basement membrane is rendered distensible by proteolytic degradation to allow it to be moved and remodeled by cells through actomyosin contractility to support branching morphogenesis.

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Introduction

During embryonic development, expanding epithelial tissues generally remain tightly associated with a basement membrane (BM), a dense, sheet-like type of specialized extracellular matrix composed primarily of laminin, collagen IV, nidogen, and the heparan sulfate proteoglycan, perlecan. A network of laminin self-assembles via cell surface interactions to initiate the formation of a BM (Yurchenco, 2011; McKee et al., 2007). Collagen IV then polymerizes to form a second covalently crosslinked network, which provides the bulk of the mechanical strength (Poschl et al., 2004); the two networks are linked together by nidogen and perlecan (Kelley et al., 2014; Hohenester and Yurchenco, 2013). The morphology and composition of the BM varies between tissue types, and it can also change with age, e.g., the BM of the cornea thickens and increases in stiffness over the course of adult human aging (Halfter et al., 2013; Candiello et al., 2010). These findings suggest that the BM is not a static structure and can change throughout the lifetime of the organism.

Rapid expansion of the epithelium occurs during certain stages of branching morphogenesis of lung, submandibular salivary gland (SMG), kidney, mammary gland, and other organs. This process of epithelial bud expansion and branching by cleft formation is a key developmental process to maximize organ epithelial surface area for secretion and adsorption (Patel et al., 2006). Although the morphology of these embryonic organs differ (Fig. 1A), all such epithelia are encapsulated by a BM that separates the epithelium from the surrounding mesenchyme (Patel et al., 2006; Kim and Nelson, 2012; Andrew and Ewald, 2010). BMs play multiple important roles in morphogenesis, with functions that include providing tissue structural support and boundaries, mediating growth factor signaling, and providing polarity cues (Rozario and DeSimone, 2010). Because the BM provides such important cues while the epithelium expands rapidly during development, the BM must be rapidly remodeled to accommodate the expanding tissue while continuing to surround the epithelium. While many laboratories have investigated how cells invade through a BM, particularly in pathological processes such as cancer metastasis (Rowe and Weiss, 2009), the fundamental question of how normal embryonic epithelia can expand rapidly while remaining encapsulated by a BM is poorly understood.

Recent studies in *Drosophila* egg chamber, mammary and salivary glands have shown that cells can orient, translocate, and accumulate the surrounding BM to help shape the architecture of the tissue (Daley and Yamada, 2013; Haigo and Bilder, 2011; Fata et al., 2004; Larsen et al., 2006; Wang et al., 2013). Proteolytic remodeling of the BM is required for branching morphogenesis in

* Corresponding author.
E-mail address: kenneth.yamada@nih.gov (K.M. Yamada).

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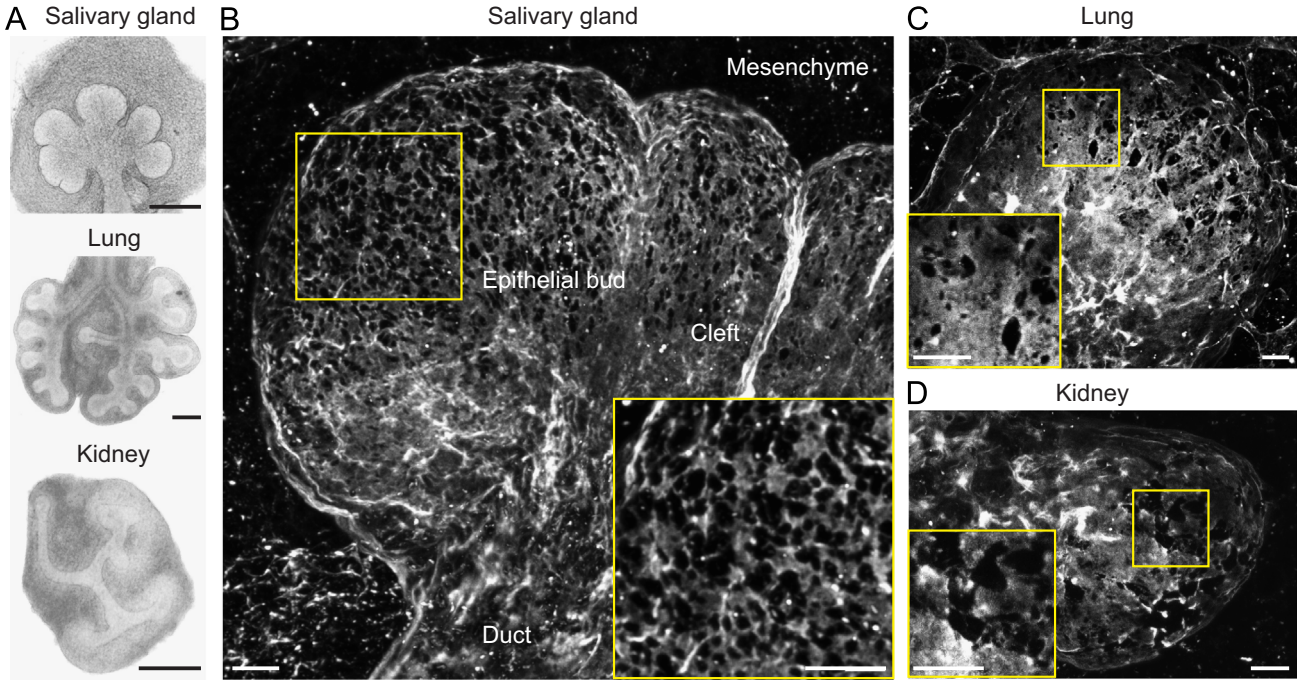

Fig. 1. Micro-perforated basement membranes are present in multiple embryonic organs. (A) Brightfield images of an E13 SMG, E11 lung, and E11 kidney show the differences in morphology among developing branched organs; scale bars: 200 μm. (B) Maximum-intensity projection of an E13 SMG immunostained for collagen IV with labels indicating an epithelial bud with a developing cleft and its duct, surrounded by mesenchyme. Maximum projection images of confocal slices of E11 lung (C) and kidney (D) immunostained for laminin. Scale bars: 20 μm and 10 μm for the insets, respectively.

several organs (Wessells and Cohen, 1968; Banerjee et al., 1977; Nakanishi et al., 1986). Specifically, matrix metalloproteases are necessary for lung, salivary and mammary gland branching, allowing outgrowth (Fata et al., 2004) and growth factor release (Rebustini et al., 2009) that contribute to branching. During chick development, prior to gastrulation and EMT invasion, the BM moves along with the epiblast cells in primitive streak formation (Zamir et al., 2008), suggesting either that the cells and matrix move together as a tissue, or that the cells tow their underlying matrix with them as the migrate. Normal cells can also traverse the BM under certain circumstances. During *Caenorhabditis elegans* uterine-vulva formation, a break in the BM is initiated by anchor invadopodia, and this gap is widened by cell-mediated mechanical displacement and sliding of the BM (Kelley et al., 2014; Ihara et al., 2011; Hagedorn et al., 2013). Leukocytes also must cross BMs to enter and exit lymph vessels; they do so through preexisting holes in the BM that become mechanically dilated as the cell squeezes through (Pflicke and Sixt, 2009). In the present study, we report that the BM surrounding branching organs is highly dynamic, exhibiting both local and global remodeling via protease activity and actomyosin contractility, while still surrounding an intact epithelial compartment.

Materials and methods

Dissection

SMGs were dissected from the following mouse strains at embryonic day 12.5 (E12.5; E0 is defined as the day of conception with a vaginal plug): wild-type ICR (Harlan), homozygous eGFP-myosin IIA knock-in transgenic (B6, 129, BALB) (Zhang et al., 2012) or homozygous eGFP-myosin IIB knock-in transgenic (C57BL6 and 129Sv, MMRRC, ID# 37053) (Fischer et al., 2009). Myosin II knock-in mice were generated in the laboratory of Robert Adelstein (NHLBI) and bred in-house. All mice were housed, bred, and euthanized according to an approved NIDCR animal study protocol.

Live imaging

Collagen IV antibody was labeled as previously described (Hsu et al., 2013). Mouse SMGs were dissected and cultured on 0.2 μm filters overnight; the filter and glands were inverted so that the glands faced the cover glass of a MatTek dish; the filter was secured to the glass with vacuum grease along the periphery of the filter. Glands were cultured in media plus OxyFluor (Oxyrase) and 5 μg/ml DyLight-649-labeled collagen IV antibody for 2–5 h. Live-organ imaging was performed using a CSU-Z1 spinning disc confocal (Yokogawa) on an Axiovert 200 M microscope (Zeiss) with either a EM-CCD camera (Photometrics) or a sCMOS camera (Hamamatsu) using a 40X C-Apochromat water objective (NA 1.2) or a 63X Plan-Apochromat oil objective (NA 1.4). The lasers, stage, chamber, and software were described previously (Hsu et al., 2013). Laser power settings were set to 15% for 488 nm and 10% for 647 nm. Exposure times were 500–800 ms for each channel. Glands were imaged for 20 min to 12 h, and intervals varied between 5 s to 10 min depending on the experiment.

For photo-bleaching of the BM, an iLAS FRAP module (Roper Scientific Europe) was used together with a 50 mW 405 nm diode laser (CrystaLaser) on the spinning disc confocal microscope. A 7 × 60 pixel box was drawn parallel to the tip of the bud and bleached at 55% power for 3 s.

See Supplementary material for more detailed methods.

Results and discussion

The basement membrane surrounding several embryonic branched organs becomes transiently perforated

During branching of the lung, mammary gland, and SMG, the BM is known to become thinner or display increased remodeling at the tips of expanding epithelial buds compared to cleft and duct regions (Bernfield and Banerjee, 1982; Mollard and Dziadek, 1998;

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