SUMMARY

Trans-membrane E-cadherin receptors mediate cell-cell adhesion via a complex called the Adherens Junctions (AJ) that is crucial for maintenance of epithelial integrity in homeostasis, during development, morphogenesis and tissue repair. Linkage of the cytoplasmic tail of E-cadherin to the actomyosin cytoskeleton is essential for the stability of cadherin clusters, as well as to confer on the AJ force-sensing and force-generating capabilities. Actin polymerization that provides the driving force for AJ formation and remodeling is predominantly mediated by two classes of actin nucleators – the Arp2/3 complex and formins. The Arp2/3 complex has a well-characterized role in the turnover of actin at the AJ, while the role of formins is less clear. In this study, we investigated the function of formin-dependent actin polymerization at the AJ, in both quiescent and collectively migrating monolayers.

Using siRNA-mediated knockdown (KD) and live-cell imaging approaches, we identified Diaphanous-related formin-1 (mDia1) and Formin-like 3 (Fmnl3) as key regulators of junctional actin turnover in cultured mammary epithelial cells. Knockdown of either mDia1 or Fmnl3 resulted in several striking phenotypes including: (a) ~30% reduction in F-actin and E-cadherin at the AJ, (b) 2-fold increase in cell area and loss of columnar epithelial architecture, and (c) weaker cell-cell adhesion strength. Fluorescence Recovery After Photobleaching measurements of E-cadherin and F-actin at the AJ revealed increased stability of E-cadherin facilitated by formin-polymerized actin. Endogenous mDia1 exhibited diffuse localization in epithelial cells, while both endogenous and exogenous Fmnl3 localized prominently to cell-cell contacts. Expression of exogenous Fmnl3 led to a ~25% increase in F-actin and E-cadherin at the AJ. Further, activation of Fmnl3 was dependent on Cdc42 binding, which in turn was found to function downstream of Src-kinase at the AJ.

In monolayers subjected to an *in vitro* scratch assay, depletion of Fmnl3 resulted in poor cohesion and dispersion of leader cells during migration, while double KD (mDia1 and Fmnl3) led to complete failure in migration. Importantly, time-course analysis during migration revealed up-regulation of *Fmnl3* expression and increased junctional localization of the protein, implicating Fmnl3 in AJ reinforcement under conditions of increased strain. Indeed, using drug treatments to alter cellular contractility, we show that Fmnl3 is recruited to the AJ in a force-dependent manner.

In summary, this study identifies essential roles for mDia1 and Fmnl3 in reinforcing cell-cell junctions. We also show, for the first time, loss of epithelial cohesion in a collective migration model and weakening of cell-cell adhesion strength associated with perturbation of formin activity. Overall, we demonstrate the importance of formin-dependent actin polymerization in supporting adhesion and cohesion, which is vital for dynamic processes such as wound repair.