Developmental Biology 419 (2016) 285–297

Contents lists available at ScienceDirect

Developmental Biology

journal homepage: www.elsevier.com/locate/developmentalbiology

Original research article

Guidance signalling regulates leading edge behaviour during collective cell migration of cardiac cells in *Drosophila*

Qanber Raza, J. Roger Jacobs *

Department of Biology, McMaster University, 1280 Main St W, Hamilton, ON, Canada L8S 4L8

ARTICLE INFO

Article history:
Received 10 May 2016
Received in revised form
4 September 2016
Accepted 5 September 2016
Available online 9 September 2016

Keywords:
Dorsal vessel
Lumen
EMT
Cell migration
Time-lapse
Filopodia

ABSTRACT

Collective cell migration is the coordinated movement of cells, which organize tissues during morphogenesis, repair and some cancers. The motile cell membrane of the advancing front in collective cell migration is termed the Leading Edge. The embryonic development of the vertebrate and *Drosophila* hearts are both characterized by the coordinated medial migration of a bilateral cluster of mesodermal cells. In *Drosophila*, the cardioblasts form cohesive bilateral rows that migrate collectively as a unit towards the dorsal midline to form the dorsal vessel. We have characterized the collective cell migration of cardioblasts as an *in vivo* quantitative model to study the behaviour of the Leading Edge. We investigated whether guidance signalling through Slit and Netrin pathways plays a role in cell migration during heart development. Through time-lapse imaging and quantitative assessment of migratory behaviour of the cardioblasts in loss-of-function mutants, we demonstrate that both Slit and Netrin mediated signals are autonomously and concomitantly required to maximize migration velocity, filopodial and lamellipodial activities. Additionally, we show that another Slit and Netrin receptor, Dscam1, the role of which during heart development was previously unknown, is required for both normal migration of cardioblasts and luminal expansion. Leading edge behaviour analysis revealed a dosage dependent genetic interaction between Slit and Netrin receptors suggesting that downstream signalling through these receptors converge on a common output that increases leading edge activity of the cardioblasts. Finally, we found that guidance signalling maintains the balance between epithelial and mesenchymal characteristics of the migrating cardioblasts.

1. Introduction

Collective cell migration (CCM) is a process by which cells or tissues migrate as a group towards a mutual destination. Models of CCM include migration of border cells during oogenesis (Bianco et al., 2007) and migration of tracheal progenitors in *Drosophila* (Schottenfeld et al., 2010), lateral line primordia migration in Zebrafish (Dona et al., 2013), formation of mammary ducts in mice (Affolter et al., 2003) and *in vivo* and *ex vivo* cancer invasion strands (Alexander et al., 2008; Friedl and Gilmour, 2009). Studies conducted on these models have enhanced our understanding of how this process occurs during morphogenesis and disease related processes such as cancer metastasis. Although new modes of CCM are still emerging (Aman and Piotrowski, 2010), three main features define this process: maintenance of a common direction of migration, adhesion between cells in a cluster and the ability to structurally modify the surrounding ECM (Friedl and Gilmour, 2009). All of these criteria apply to embryonic cardioblast (CB) migration in Drosophila, a process also conserved in vertebrates (Bier and Bodmer, 2004; Bodmer and Venkatesh, 1998). Although the role of cell polarisation and lumen formation has been studied extensively for the CBs, analysis of CCM has received only passing attention (Haack et al., 2014; Vanderploeg and Jacobs, 2015; Vanderploeg et al., 2012; Vogler et al., 2014).

Drosophila heart development initiates after progenitor cells from the lateral mesoderm are specified to become CBs. The CBs then undergo mesenchymal to epithelial transition (MET) to form bilateral rows, with a medially extended Leading edge (LE), and collectively migrate towards the dorsal midline in tandem with the dorsal ectoderm to meet their contralateral partners and form the dorsal vessel (DV) (Haack et al., 2014; Tao and Schulz, 2007). Guidance signalling through Slit-Robo and Netrin-Frazzled-Unc5 pathways orchestrates the assembly of the DV. Robo, a Slit receptor, and Uncoordinated-5 (Unc5), a Netrin receptor, localize to the luminal domain of CBs and are required for formation of the lumen (Albrecht et al., 2011; MacMullin and Jacobs, 2006; Medioni et al., 2008; Qian et al., 2005; Santiago-Martinez et al., 2008). Frazzled (Fra), a Netrin receptor, localizes to the medial adhesive (non-luminal) domain and is required for the formation of apical outgrowths of CBs (Macabenta

* Corresponding author.
E-mail address: jacobsr@mcmaster.ca (J.R. Jacobs).

http://dx.doi.org/10.1016/j.ydbio.2016.09.005
0012-1606/

et al., 2013). During heart development, apicalization of polarity markers such as Integrin and it's intracellular adaptor Talin, which collectively localize Slit-Robo signalling, have been shown to modulate migratory behaviour and lumen formation in the migrating CBs (Vanderploeg and Jacobs, 2015; Vanderploeg et al., 2012; Vogler et al., 2014). However, the role of the Slit, Netrin and their respective receptors during CCM is less characterized.

We employed an *in vivo* quantitative approach to study the role of guidance signalling molecules during CCM of CBs. With live imaging and non-invasive embryo handling techniques (Reed et al., 2009), we tracked the migration of CBs in loss-of-function guidance signalling mutants and quantitatively evaluated their migratory behaviour. Here we report that LE activity of the CB increases as the distance between the contralateral rows decreases. This increase in filopodial and lamellopodial activity is dependent on Slit-Robo-Robo2 and Netrin-Frazzled-Unc5 mediated signalling. We demonstrate that another Slit and Netrin receptor, Down syndrome cell adhesion molecule (Dscam1), contributes to cell migration and luminal expansion during heart development. Our results suggest that signalling downstream of Robo, Fra, Unc5 and Dscam1 converges to activate a common cellular mechanism which regulates the protrusive activity of the CB LE. Lastly, we identify a role for guidance signals in maintaining epithelial aspects of MET during CCM.

2. Results

2.1. Filopodial activity of the LE increases as the CBs approach the midline

Actin based cell surface protrusions are of 2 major types: filopodia - thin finger-like structures comprised of parallel bundles of actin, and lamellopodia - thin sheet-like structures filled with branched networks of actin (Mattila and Lappalainen, 2008). To visualize LE protrusions of CBs we expressed a mCherry tagged C-terminal actin binding domain of Moesin (*moesin-mCherry*) which co-localizes with Actin at the filopodia and lamellopodia (Millard and Martin, 2008). We also tested GFP tagged actin binding molecule, lifeAct, and determined that the sensitivity and distribution of both tags is identical (Supplemental Fig. 1A,A′ arrows). We validated earlier reports that expression of *moesin-mCherry* does not generate dominant effects (Millard and Martin, 2008). We generated time-lapse movies of wild-type embryos expressing *tailup-GFP* (*tup-GFP*) and *UAS-moesin-mCherry* driven by *dmef-GAL4* to identify CB nuclei and actin cytoskeleton respectively. At stage 14 when CBs migrate along with the ectoderm towards the dorsal midline (Haack et al., 2014; MacMullin and Jacobs, 2006), few filopodia are visible at the LE (Fig. 1A). Cross sectional images further revealed that apical extensions were short and CBs had a rounded morphology (Fig. 1A′). At stage 15, we

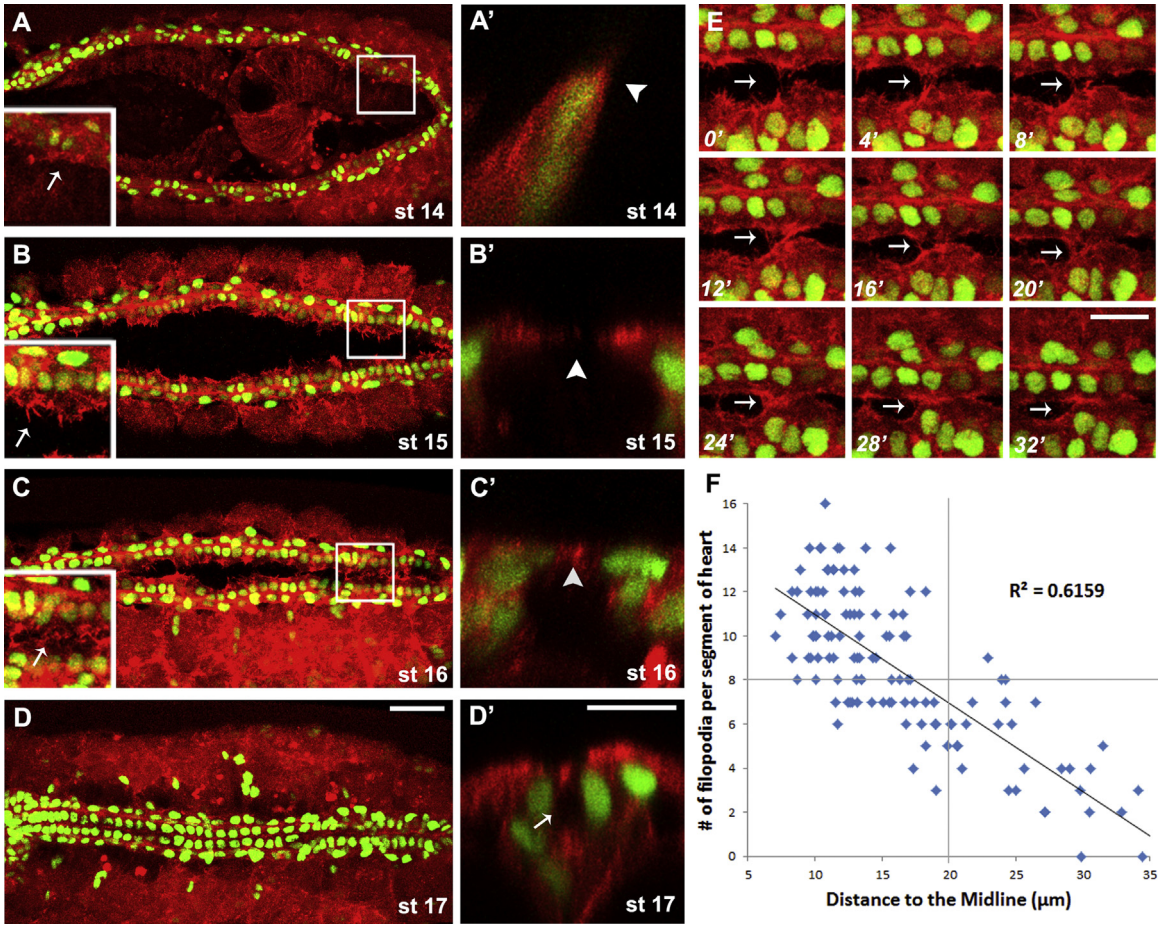

Fig. 1. Filopodia and lamellopodia activity increases during migration. In this and subsequent figures, images of live embryos expressing *tailup-GFP* and *UAS-moesin-mCherry* under the control of *mef2-GAL4* driver are visualised. Dorsal (A-D) and posterior cross-sectional (A′-D′) views of heart development at stage 14, 15, 16 and 17 are shown. At stage 14, the LE of CBs is minimally active with few filopodia and lamellopodia extended extending towards the midline (A arrow). As the CBs approach the midline at stage 15, LE activity increases at the most posterior and anterior domains of the bilateral rows (B arrow). CBs adopt a pear-shaped morphology and extend cytoplasmic processes towards the midline (B′ arrowhead). At stage 16, CBs make the contralateral contact (C arrow, C′ arrowhead). At stage 17, CBs form a medial lumen (D′ arrow). (E) Magnified time-lapse images of a stage 16 posterior heart are shown. Contralateral CB adhesion is stabilized upon contact (arrows). (F) A scatter plot demonstrates the inverse relationship between 'distance to the midline' and 'number of filopodia' extended by cells within heart segments. In this and subsequent figures, posterior is to the right. Calibration is 25 μm for (A-D) and 10 μm for (A′-D′, E).