A WAVE2-Arp2/3 actin nucleator apparatus supports junctional tension at the epithelial zonula adherens

Aiyappan Raksha, Chua Tze Hean, Darren Chong Kai Siang, Sandra Lau Hui Min

Lee Kong Chian School of Medicine, Nanyang Technological University, Headquarters & Clinical Sciences Building, 11 Mandalay Road, Singapore 308232

Introduction

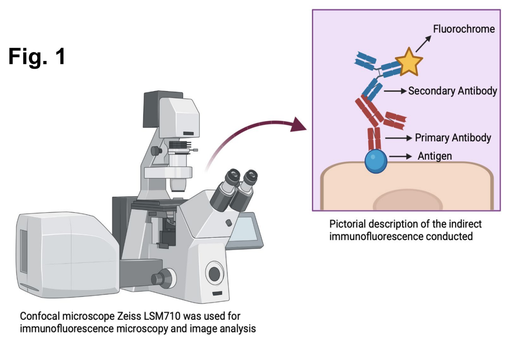
The epithelial zona adherens (ZA) is a specialised adhesive junction that contributes to tissue integrity and contractility (Sawyer et al., 2010), through a coordinated interaction between adhesion and contractile forces that creates what is known as junctional tension (Fernandez- Gonzalez et al., 2009; Ratheesh et al., 2012). Arp2/3 has been observed to generate most of the cytoskeleton at the ZA, although to an unknown effect. It is activated in response to intermediary proteins, of which include the WASP/WAVE family of nucleation promoting factors (Padrick and Rosen, 2010). These proteins control the specific site where Arp2/3 acts upon, N-WASP being shown to regulate the junctional cytoskeleton at a post nucleation stage (Kovacs et al., 2011).

Therefore in this study we aimed to clarify the role of WAVE in junctional actin regulation as the basis for testing the effect of Arp2/3-mediated nucleation upon force generation at the ZA.

Materials & Methods

Immunofluorescent confocal microscopy (Fig. 1) was performed on fixed cultured Caco-2 cells. Quantitative analysis was performed on confocal images by line scans of staining intensity at contacts.

Fig. 1



Confocal microscope Zeiss LSM710 was used for immunofluorescence microscopy and image analysis

Pictorial description of the indirect immunofluorescence conducted

Kymographs were generated based on live-cell imaging performed using the spinning-disk confocal system.

FRAP experiments was conducted on the cultured cells. Quantitative analysis was conducted on frame-to-frame tracking of recovery in photobleached areas.

Local ablation using laser nanoscissors was performed on Control and WAVE2 KD cells. Subsequent image analysis was conducted on confocal images of GFP fluorescence of cells.

Results

Fig. 2

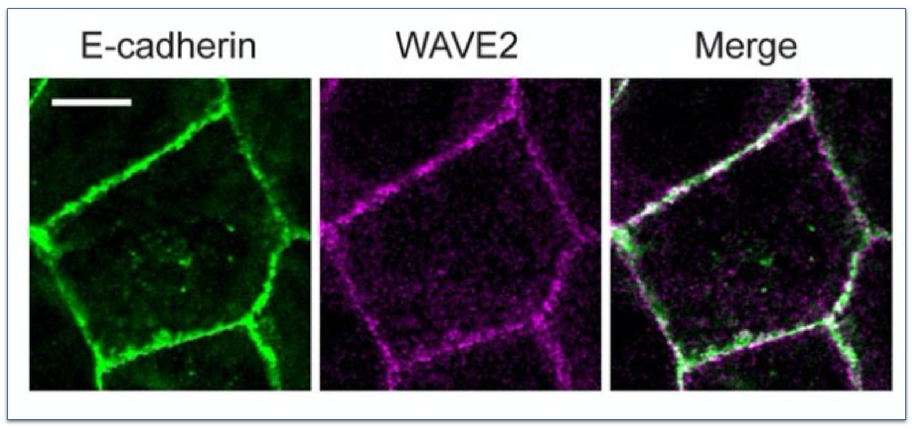


Figure 2: WAVE2 is a component of the zonula adherens. Confocal immunofluorescence microscopy demonstrated clear junctional staining for WAVE2 which accumulated with E-cadherin at the apical ring of the ZA.

Fig. 3

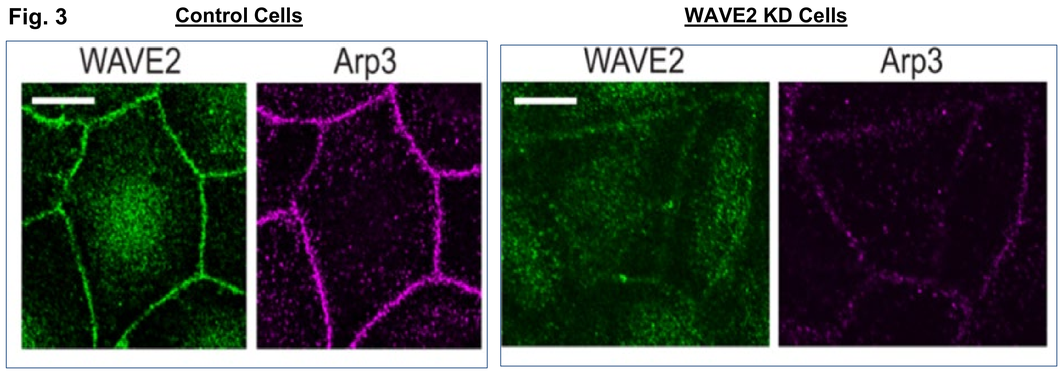


Figure 3: WAVE2 regulates junctional actin assembly. Junctional Arp2/3 was reduced in WAVE2 KD cells, and both the barbed-end labelling and actin content of the zonula adherens were reduced in cells transfected with Arp3 siRNA.

Fig. 4

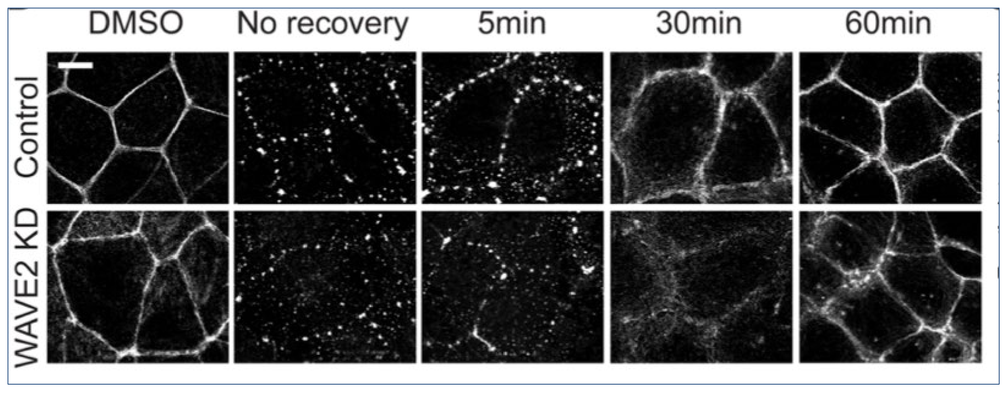


Figure 4: WAVE2 is required for actin reassembly, as WAVE2 KD impairs junctional actin recovery after latrunculin washout as compared to control.

Figure 5: Using the fluorescence recovery after photobleaching technique, we showed that half-life of recovery and mobile fraction of E-cadherin were reduced in WAVE2 KD cells.

Fig. 5

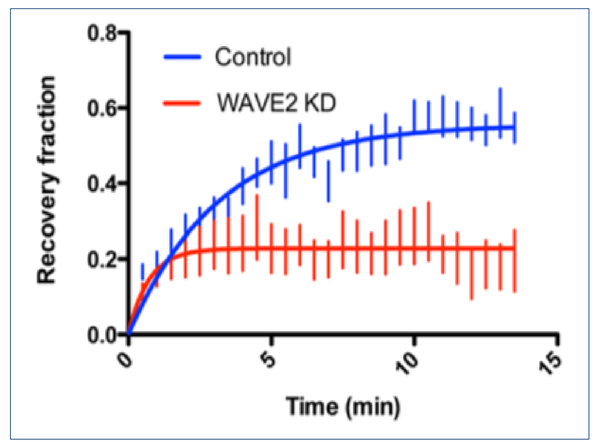
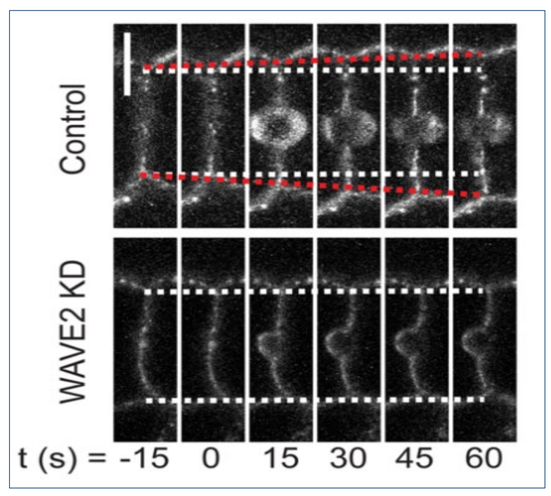


Figure 6: WAVE2-Arp2/3 regulates lateral junctional movement and tension at the zonula adherens.This kymograph shows that WAVE2 KD increases lateral (orthogonal) junctional movement at the the cell-cell junction.

Fig. 6



Discussion

Firstly, through this study we demonstrated that WAVE2 is required for localising Arp2/3 to the cell-cell junction. This ties in with previous findings that Arp2/3 is a potential nucleator for junctional actin assembly as it localises to the ZA (Helwani et al., 2004; Verma et al., 2004; Kovacs et al., 2011), possibly through interaction with the E-cadherin molecular complex (Kovacs et al., 2002). Secondly, we demonstrate that WAVE2 is required for actin reassembly. Hence we can conclude that the WAVE2–Arp2/3 apparatus is a major nucleator of actin assembly at the ZA.

This is significant because actin assembly is important for junctional tension, and therefore we have provided a new mechanism by which junctional tension is maintained. Our study therefore demonstrates that Arp2/3 and WAVE2 are necessary to support junctional tension.

References

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