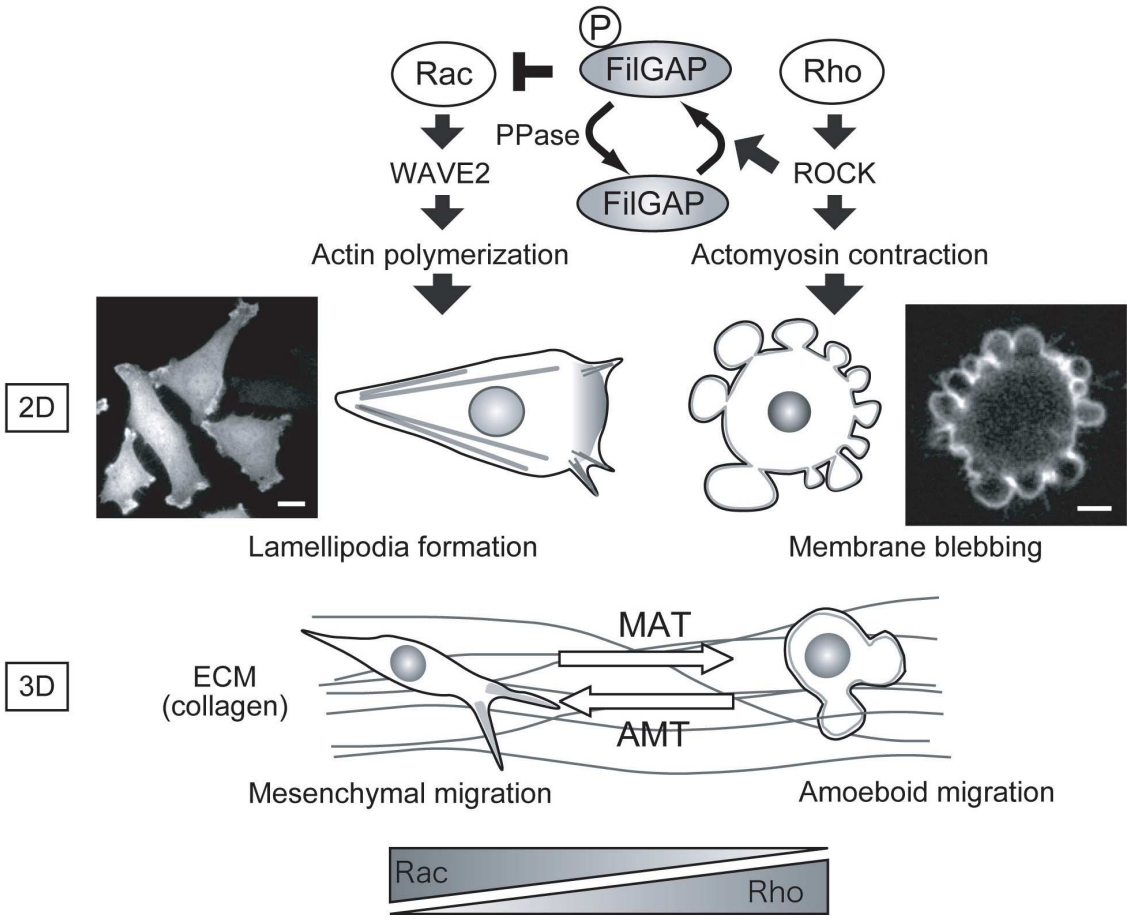

Fig. 3. FilGAP regulates cell morphology and migration by mediating the Rho-Rac antagonism. FilGAP is phosphorylated by ROCK downstream of Rho. Phosphorylated FilGAP is in an active state and suppresses Rac. Protein phosphatase (PPase) dephosphorylate FilGAP and inactivate FilGAP. In 2D environment, FilGAP suppresses Rac-dependent lamellae formation. Because Rac and Rho antagonize each other, inhibition of Rac results in activation of Rho. Rho stimulates membrane blebbing through acto-myosin contraction. Rac-dependent actin polymerization observed in membrane ruffles is visualized by lifeact-EGFP (F-actin marker, left) and membrane blebbing is visualized by fusionRed-f-mem (membrane marker, right). Scale bar, 20 μm (left) and 5 μm (right). In 3D ECM, tumor cells migrate using two types of migration mode, mesenchymal and amoeboid movement. In mesenchymal migration, cells show elongated morphology, extend thin protrusions. In amoeboid migration, cells show round morphology. In ECM, tumor cells plastically switch mesenchymal migration and amoeboid migration according with their surround microenvironment. Rac and Rho promotes mesenchymal and amoeboid migration respectively. FilGAP promotes amoeboid movement downstream of Rho.

However, it may only reflect some aspects of the cell migration *in vivo*. In 3D ECM, cells move switching the several migration modes depending on extracellular environment (Petrie and Yamada, 2016). Tumor cells in 3D ECM often switch two types of cell migration mode, mesenchymal cell migration and amoeboid cell migration (Sanz-Moreno *et al.*, 2008) (Fig. 3). The mesenchymal cell migration is characterized as an elongated cell shape and high Rac activity. In amoeboid migration, cells have round morphology and high Rho activity. Depletion of FilGAP in breast carcinoma cell (MDA-MB-231) induced Rac-dependent elongated mesenchymal morphology in collagen fibers (Saito *et al.*, 2012). Forced expression of FilGAP induced round amoeboid morphology. Importantly induction of round amoeboid morphology induced by FilGAP required the phosphorylation by ROCK. Depletion of FilGAP inhibits invasion to the collagen matrices, suggesting FilGAP may mediate Rho-dependent contraction and contribute to cell invasion to ECM. ARHGAP22 also induces amoeboid morphology in melanoma cells by inhibiting Rac (Sanz-Moreno *et al.*, 2008). ARHGAP22 also induce amoeboid morphology downstream of ROCK, however it is not phosphorylated by ROCK. ARHGAP22 shows different cellular localization from FilGAP (Mori *et al.*, 2014), it may be activated by another mechanism downstream of ROCK. Recently, Thuault *et al.* reported that ARHGAP25 positively regulates invasion to the ECM downstream of ROCK in Rhabdomyosarcoma (Thuault *et al.*, 2016). Thus all FilGAP family proteins appear to mediate Rho-Rac antagonism and regulate invasion to the ECM downstream of ROCK in different type of tumor cells.

4. Conclusions and Perspective

We've analyzed the role of FilGAP in 2D and 3D cell morphology and migration. However there remain several problems to be solved. PH domain of FilGAP binds to PIP3 *in vitro*, suggesting its involvement in phospholipids signaling mediated by PI3K (Kawaguchi *et al.*, 2014), however its role in the cell migration is unclear. PIP3 is produced in front of the migrating cells and stimulates actin polymerization through the activation of Rac GEFs (Rossman *et al.*, 2005). PIP3 binding to PH domain of GEFs not just re-