EDITORIAL

Journal of Stem Cell Research and Regenerative Medicine

REGULATORY ROLES OF PROTEIN PHOSPHATASES IN THE APICAL-BASAL POLARITY AND THEIR IMPACT ON LUNG STEM CELL BEHAVIOR AND PROTECTION AGAINST LUNG FIBROSIS

Ahmed El-Hashash

The University of Edinburgh, United Kingdom

Keywords: Protein Phosphatase, Stem Cell, Lung Fibrosis

The balance between cell gain (self-renewal) and cell loss (apoptosis and differentiation) governs the size of the progenitors' compartment. Molecular programs regulating the balance between the self-renewal and differentiation and the balance of apoptosis versus self-renewal/differentiation of endogenous organ-specific stem/progenitor cells are likely critical both to development and to regenerating diseased and damaged tissues in different organs, including the lung. Recent studies demonstrated the importance of disruption of epithelial apical-basal polarity (mediated by Par-polarity protein complex) in epithelial cell apoptosis and proliferation. However, how epithelial polarity regulation is coupled to apoptosis and proliferation is not well understood. We find that Asp-based PTPs such as Eya1 and none-receptor PTPs are essential for balancing differentiation and proliferation, and apoptosis versus self-renewal/differentiation, respectively by controlling the activity and localization of Par polarity complex in lung distal epithelial progenitors during pre- and postnatal development. The difference of the effect of these different PTPs possibly reflects the facts they regulate the activity and localization of Par polarity complex by targeting the activity of different upstream signaling events: Eya1 controls Par polarity complex by binding to and controlling aPKCζ activity, while none receptor PTPs controls Par complex by controlling the activity of RhoGTases. Thus, Eya1 phosphatase regulates cell polarity and mitotic spindle orientation by controlling aPKCζ phosphorylation levels. Loss of apical-basal polarity in Eya1-/distal lung progenitors results in loss of asymmetric cell division, leading to increased symmetric differentiation and hence lack of stem/progenitor cell self-renewal. Conversely, none-receptor PTPs control Par complex by regulation of the activity of RhoGTases. Conditional deletion of none-receptor PTPs in lung epithelial progenitors results in disruption of Par polarity complex, and consequently inhibition of PI3K pathway leading to activation of the caspase-3 apoptotic cascade that results in increased apoptosis, but decreased cell proliferation/differentiation. Most importantly, we find that these mechanisms are recapitulated during fibrosis in alveolar epithelia undergoing apoptosis, which is a crucial early step in the development of lung fibrosis.

Epithelial tissues compartmentalize multicellular organisms and have a variety of specialized roles that rely on asymmetries in protein and lipid composition and the precise positioning of intercellular junctions along its apical-basal axis. This polarity axis is defined by conserved apical-basal polarity proteins, which segregate into distinct domains along the epithelial cell cortex . One such protein is Lethal giant larvae (Lgl), originally identified as a tumor suppressor in Drosophila and often misregulated in human cancer, Halaoui and McCaffrey. In epithelia, Lgl cooperates with Discs Large (Dlg) and Scribbled as a basolateral determinant to restrict the localization and activity of the atypical protein kinase C (aPKC) and Crumbs complexes to the apical, possibly by inhibiting aPKC activity or regulating the trafficking of apical transmembrane proteins Lgl associates with the actomyosin cytoskeleton and recent work revealed that its cortical localization is, however, primarily mediated by binding to plasma membrane phosphoinositides through a positively charged basic and hydrophobic (BH) motif. This motif is phosphorylated by aPKC to exclude Lgl from the apical cortex during interphase and by mitotic kinase Aurora A (AurA) to completely release Lgl from the cortex and promote proper mitotic spindle orientation in epithelial tissue (Bell et al., 2015, Carvalho et al., 2015). Interestingly, apical polarity proteins such as aPKC, Par-6, and Par-3 also adjust

localization during epithelial mitosis, as shown in Drosophila and during early embryogenesis of Nematostella vectensis. However, it is unknown how Lgl cortical localization is restored at the end of cell division and how this is coordinated with daughter cell polarization. Moreover, although phosphorylation controls Lgl localization and function, how (and whether) counteracting protein phosphatases actively dephosphorylate Lgl remains undetermined.

Here we report the PP1-Sds22 complex as a critical regulator of Lgl dephosphorylation, promoting its localization at the cortex and plasma membrane. Because dividing cells must deal with the complete pool of Lgl in the cytoplasmic and hyperphosphorylated form, this mechanism has foremost significance to timely polarize daughter cells and maintain the architecture of proliferating epithelia.

Apical-basal polarity relies on the asymmetric distribution of the polarity determinant Lgl to the lateral cortex of epithelial cells. Research regarding Lgl regulation has been focused on the modulation of phosphorylation via a PKC and Aurora kinases. Here we found that PP1 provides another layer of regulation, antagonizing Lgl phosphorylation by aPKC or AurA. We show that PP1-mediated dephosphorylation can control Lgl subcellular distribution in both epithelial and non-epithelial cells and is critical to maintain the monolayered organization and apical-basal polarity. Because Lgl is a general cell polarity regulator and also controls the Notch and Hippo signaling pathways, it is likely that the significance of Lgl regulation by PP1 extends to a range of processes, including asymmetric cell division, cell migration, fate specification, and growth control.

PP1 directly dephosphorylates Lgl, and we show that Lgl localization is insensitive to PP1 depletion when the aPKC and AurA phosphorylation sites are mutated. Thus, PP1 dephosphorylation promotes the phosphorylation-inhibited binding between the BH motif of Lgl and plasma membrane phosphoinositides. This mode of regulation parallels cell polarization in fission yeast, where the protein phosphatase 1 complex Tea4-Dis2 controls dephosphorylation of the polarity regulator Pom1 to expose a basic region with affinity for plasma membrane phospholipids .Furthermore, PP1 has been shown to dephosphorylate Par-3 on aPKC phosphorylation sites. PP1 is therefore a critical regulator of cell polarity and antagonizes aPKC activity over multiple substrates in epithelial tissues.

We also show that Sds22 promotes Lgl cortical localization. Sds22 has been identified previously as a regulator of epithelial organization, but this function has been mostly linked to the deleterious effect of myosin or moesin hyperphosphorylation. Interestingly, similarly to loss of function of the basolateral polarity proteins Scrib, Dlg, and Lgl, sds22 mutation also promotes neoplastic tumor development in Drosophila imaginal discs. Thus, the role of the PP1-Sds22 complex in Lgl cortical localization provides a possible mechanistic interpretation for the tumor-suppressive role of Sds22 and its human homolog PPP1R7.

Lgl becomes fully cytoplasmic because of Aur A phosphorylation at mitotic entry, and since preexisting phosphorylated Lgl is not degraded, PP1-mediated dephosphorylation is critical to reload cytoplasmic Lgl to the cortex of the daughter cells. Moreover, we show that cortically localized Lgl inhibits aPKC accumulation at the lateral domain of the newly formed interface between daughter cells, promoting its apical localization. Hence, although asymmetric furrow ingression and apical midbody positioning may assist with the establishment of apical-basal asymmetries at mitotic exit PP1-mediated cortical reloading of Lgl plays an essential role in the de novo polarization of the daughter-daughter interface. PP1 is tightly regulated throughout the cell cycle, being reactivated at mitotic exit to dephosphorylate a number of mitotic proteins This study reveals how the cell cycle-dependent control of PP1 activity also plays an essential role in the context of apical-basal polarity, coupling cell division with the post-mitotic polarization of epithelial cells.

E-mail: hashash05@yahoo.co.uk