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Generation of cells that ignore the effects of PIP3 on cytoskeleton

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In order to divide into daughter cells, to engulf nutrients, and to move, cells must deform their cortical membrane in a precisely controlled manner. A decade ago, studies of directed migration or chemotaxis in the amoebae Dictyostelium suggested that asymmetric distributions of the inositol lipid PtdIns(3,4,5)P_3 mediate changes in cell morphology.[1] PtdIns(3,4,5)P_3 is also an internal cue found at the leading edge of migrating neutrophils and T cells, as well as tumor cells, and within growth cones of neurons. In migrating cells, PtdIns(3,4,5)P_3 is asymmetrically localized, because PI 3-kinases (PI3Ks) are recruited to the front of the cell, while the enzyme that degrades PtdIns(3,4,5)P_3, PTEN is lost from the front and retained at the back.[2] Remarkably, the "PI3K in front" vs. "PTEN in back" mechanism, is also associated with other polarized cell morphologies (**Fig. 1A**). In cytokinesis, PI3K is recruited to the poles whereas PTEN is found in the furrow: Think of a dividing cell as two cells migrating away from each other.[3] In phagocytosis, PI3K is recruited to and PTEN is lost from the phagocytic cup when cells engulf particles.[4] Interestingly, in epithelial sheets, PtdIns(3,4,5)P_3 is found on the basal-lateral membrane while PTEN is localized at the apical side of the cells.[5]

In spite of the universality of this paradigm, a role of PtdIns(3,4,5)P_3 in cytoskeletal rearrangements has been questioned due to the existence of parallel pathways. Ectopic PtdIns(3,4,5)P_3 does trigger pseudopodia formation in migratory cells and interferes with proper cytokinesis.[2] In contrast, because there are compensating mechanisms, reductions in PtdIns(3,4,5)P_3 often have surprisingly minor effects in the same systems. In instances where lowering PtdIns(3,4,5)P_3 has minor consequences, deletion of its downstream effectors would not be expected to yield dramatic phenotypes. Indeed, disruption of many of the known PtdIns(3,4,5)P_3 binding proteins in Dictyostelium has only marginal effects on cell migration.

Seeking a different strategy, we found that gene disruptions which do not yield dramatic phenotypes in a wild type background nevertheless can effectively suppress the defects caused by delocalized PtdIns(3,4,5)P_3 in Dictyostelium cells lacking PTEN.[6] The *pten*$^-$ cells extend extraneous pseudopodia and spread excessively on the substratum which impairs migration and interferes with cytokinesis. Disruption of the AKT/PKB homolog PkbA completely restores cytokinesis and greatly improves chemotaxis in these cells. The defects in F-actin polymerization seen in *pten*$^-$ cells were corrected even though PtdIns(3,4,5)P_3 remained distributed uniformly along the membrane (**Fig. 1B**). Suppression was also achieved by disrupting Pianissimo (PiaA), a gene originally isolated as a chemotaxis mutant in Dictyostelium.[7] PiaA is an ortholog of Rictor, a subunit of the target of rapamycin complex 2 (TORC2), which phosphorylates the hydrophobic motif and enhances activation of PKBs.[8]

This suppression strategy led further to a critical PKB substrate that acts downstream of PtdIns(3,4,5)P_3. Among PKB targets, p21-activated kinase PakA and three others showed heavier and more persistent phosphorylation in *pten*$^-$ cells vs. wild-type or *pten*$^-$/*pkbA*$^-$ cells. Disruption of individual genes in the *pten*$^-$ background showed that loss of PakA could rescue the defects of the *pten*$^-$ cells. The ability of PakA to mediate the effects of PtdIns(3,4,5)P_3 depended on its phosphorylation, since alanine or phosphomimic glutamic acid substitutions in the critical PKB phosphorylation site either abolished or exaggerated its ability to reverse the phenotype of the *pten*$^-$ cells.[6]

Most studies of PI3K in oncogenesis or PTEN in tumor suppression have focused on the role of elevated PtdIns(3,4,5)P_3 in promoting cell growth and survival rather than in altering cytoskeletal activity.[9] There is little consensus on potential targets of PtdIns(3,4,5)P_3 which might mediate its effects on cell polarity during migration, cytokinesis or other events. For example, PtdIns(3,4,5)P_3 is reported to recruit to the membrane the Rac exchange factors and Dock 180,[10] which would be expected to regulate cytoskeleton but there is little causal evidence for their role as mediators of PtdIns(3,4,5)P_3. For PKB, there is acknowledgment that it plays a role in migration but the precise role has not been defined.[11] Amid a network of redundant pathways, our genetic suppression strategy allowed us to clearly show that PKB signaling, and in particular phosphorylation of PakA, is an important event in migrating Dictyostelium cells. This strategy should also be useful to identify other important substrates. An interesting implication of these studies is that interventions designed to halt the rapid growth of PTEN negative cells (i.e., inhibition of PKB signaling) might have the unintended consequence of improving their polarity and migration, and perhaps lead to increased invasion.

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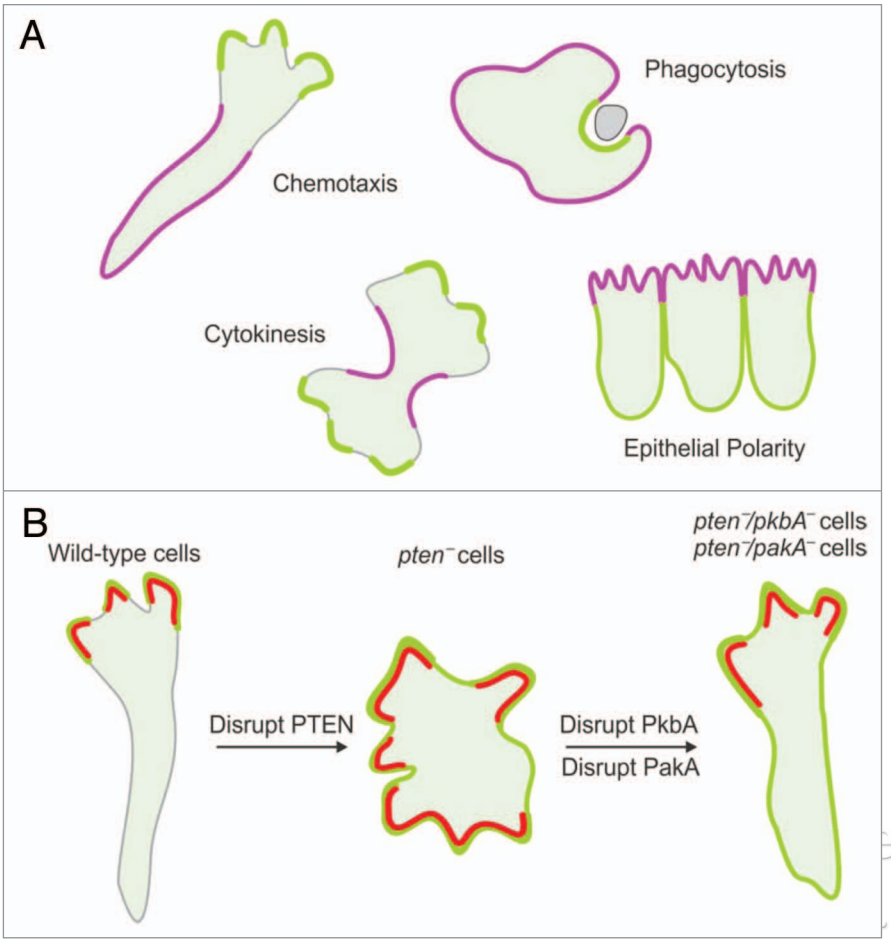

Figure 1. (A) The "PI3K in front" vs. "PTEN in back" mechanism is associated with many polarized cell morphologies. Localization of PI3K and PTEN are indicated with green and purple color, respectively. (B) Disruption of PTEN generates extraneous pseudopodia and cells lost polarity. Disruption of PKB or its substrate PakA reverses this defect. F-actin and PIP3 localization are indicated as red and green, respectively.

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