Cancer Cell
Article

Evidence that Inositol Polyphosphate 4-Phosphatase Type II Is a Tumor Suppressor that Inhibits PI3K Signaling

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DOI 10.1016/j.ccr.2009.06.006

SUMMARY

We report that knocking down the expression of inositol polyphosphate 4-phosphatase type II (INPP4B) in human epithelial cells, like knockdown of PTEN, resulted in enhanced Akt activation and anchorage-independent growth and enhanced overall motility. In xenograft experiments, overexpression of INPP4B resulted in reduced tumor growth. INPP4B preferentially hydrolyzes phosphatidylinositol-3,4-bisphosphate ($PI(3,4)P_2$) with no effect on phosphatidylinositol-3.4.5-triphosphate ($PI(3,4,5)P_3$), suggesting that $PI(3,4)P_2$ and $PI(3,4,5)P_3$ may cooperate in Akt activation and cell transformation. Dual knockdown of INPP4B and PTEN resulted in cellular senescence. Finally, we found loss of heterozygosity (LOH) at the INPP4B locus in a majority of basal-like breast cancers, as well as in a significant fraction of ovarian cancers, which correlated with lower overall patient survival, suggesting that INPP4B is a tumor suppressor.

INTRODUCTION

During the generation and progression of epithelial tumors, oncogenes and tumor suppressor genes cause multiple cell-autonomous alterations, resulting in aberrant control of proliferation, apoptosis, angiogenesis, and cellular life span (Hahn and Weinberg, 2002). The cyclic regulation of the mammary gland confers vulnerability to these cancer-promoting processes, and imbalances between proliferation and apoptosis sets the scene for mammary tumorigenesis (Scheid and Woodgett, 2001). The phosphinositide 3-kinase (PI3K)-Akt pathway is deregulated in a wide spectrum of human cancers, and gain or loss-of-function mutations of several components of the pathway lead to neoplastic transformation in various model systems (Engelman et al., 2006; Vivanco and Sawyers, 2002).

In vivo activation of PI3K increases the intracellular amounts of phosphatidylinositol(3,4)bisphosphate ($PI(3,4)P_2$) and phosphatidylinositol(3,4,5)trisphosphate ($PI(3,4,5)P_3$). A variety of signaling proteins, including the Ser/Thr kinases Akt and PDK1, are able to bind to one or both of these PI3K lipid products and are thereby localized to the plasma membrane to initiate cell growth and cell survival pathways. (Plas and Thompson, 2005; Manning and Cantley, 2007).

The termination of signaling downstream of PI3K by degradation of $PI(3,4,5)P_3$ can be mediated by two different types of phosphatases. PTEN dephosphorylates the 3-position of $PI(3,4,5)P_3$ to produce $PI(4,5)P_2$, whereas SHIP family phosphatases

SIGNIFICANCE

Loss-of-function mutations or deletions of PTEN and activating mutations or amplifications of PIK3CA are among the most common events in solid tumors. Here, we show that the phosphoinositide phosphatase INPP4B plays a role similar to that of PTEN in suppressing the PI3K/AKT signaling pathway, thereby suppressing tumor growth. We also show that INPP4B is frequently deleted in a variety of solid tumors, including the majority of basal-like breast cancers. These results suggest that PI3K pathway inhibitors in current clinical trials may be effective in treating cancers where INPP4B is deleted.

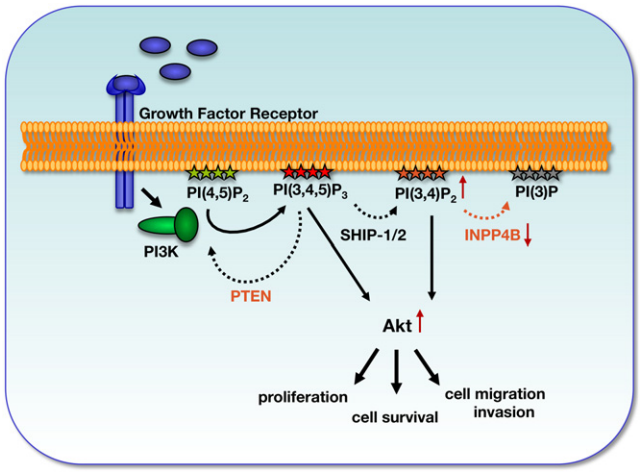

Figure 1. A Model Indicating the Roles of PTEN and INPP4B in Regulation of Signaling Downstream of Class I PI3Ks

Upon stimulation of Class 1 PI3Ks, two major phospholipid pools are generated: $PI(3,4,5)P_3$ and $PI(3,4)P_2$. PTEN hydrolyzes the 3′-phosphate of $PI(3,4,5)P_3$ to terminate PI3K signaling. However, SHIP family members hydrolyze the 5′-phosphate of $PI(3,4,5)P_3$ to generate $PI(3,4)P_2$, which, like $PI(3,4,5)P_3$, can facilitate PDK1-dependent phosphorylation and activation of AKT. INPP4B converts $PI(3,4)P_2$ to PI(3)P. Loss of PTEN or loss of INPP4B results in prolonged activation of Akt and, subsequently, in increased cell proliferation, cell migration, and invasion.

dephosphorylate the 5-position to produce $PI(3,4)P_2$ (Figure 1). Loss of PTEN protein expression or function is frequently observed in human cancers (Vazquez and Sellers, 2000), and deletion of PTEN in mice results in a variety of tumors (Di Cristofano et al., 1998; Kishimoto et al., 2003), indicating that uncontrolled signaling through PI3K contributes to cancer formation and metastasis. In contrast, loss of genes for SHIP family members has not been correlated with solid tumor formation; thus, the role of these phosphatases in regulating PI3K signaling is complex and controversial. Some studies have indicated that SHIP family members suppress PI3K signaling (Scheid et al., 2002; Sharrard and Maitland, 2007; Sleeman et al., 2005), whereas other studies suggest that the product of SHIP family members, $PI(3,4)P_2$, is a positive regulator of Akt (Franke et al., 1997; Frech et al., 1997; Klippel et al., 1997; Scheid et al., 2002). The PH-domains of Akt and PDK1 bind both $PI(3,4)P_2$ and $PI(3,4,5)P_3$ in vitro, suggesting these lipids may be redundant in their ability to mediate Akt activation (Cantley, 2002). The possibility that both $PI(3,4,5)P_3$ and $PI(3,4)P_2$ may be necessary for optimal growth signaling downstream of PI3K has not been adequately addressed.

The inositol polyphosphate 4-phosphatase type II (INPP4B) was initially isolated from rat brain as an enzyme that hydrolyzes the 4-position phosphate of $PI(3,4)P_2$ and, to a lesser degree, inositol(3,4)bisphosphate ($Ins((3,4)P_2$) and $Ins(3,4,5)P_3$, in vitro (Norris et al., 1997; Norris et al., 1995). The ability of INPP4B to hydrolyze $PI(3,4,5)P_3$ and its effects on phosphoinositides in intact cells was not investigated; thus, the in vivo function of INPP4B remains elusive. However, INPP4B expression was recently shown to be silenced in malignant proerythroblasts. These cells displayed increased phospho-Akt levels, which could be reduced by re-expression of INPP4B (Barnache et al., 2006). In addition, recent studies described a frequently deleted region in breast cancer cell lines and basal-like, high-grade breast tumors, using high-resolution comparative genomic hybridization analysis (chromosome location 4q31.1-31.21 and chromosome location 4q31.3-q32.1, respectively), comprising 6 genes, including INPP4B (Naylor et al., 2005; Bergamaschi et al., 2006; Chin et al., 2007). Johannsdottir et al. (2004) also found LOH in this region in breast cancers. Furthermore, INPP4B was identified out of a collection of RNAis to give rise to anchorage-independent growth in human mammary epithelial cells (HMEC) (Westbrook et al., 2005).

These observations raise the possibility that INPP4B might be affecting PI3K signaling in vivo and that it might function as a tumor suppressor. In this study, we investigate this possibility with retroviral knockdown of INPP4B in human epithelial cell systems.

RESULTS

Knockdown of INPP4B Results in Anchorage-Independent Growth

To investigate the role of INPP4B on cell transformation in the human mammary epithelial cell line HMEC, retroviral hairpin constructs (shRNA) were used to knockdown its expression. An shRNA construct directed against Renilla luciferase (shRNA-Renilla) was used as negative control for the experiments. Two shRNA constructs (shRNA-INPP4B-1 and shRNA-INPP4B-2) were found to effectively reduce expression of INPP4B in several cell lines, as judged by decreased mRNA levels (Figure 2A). In MCF-7 cells, where INPP4B protein levels were high enough to be detected by western blotting, these constructs were shown to reduce INPP4B protein to the same extent (Figure 2B). Infection of HMEC cells with either shRNA-INPP4B-1 or shRNA-INPP4B-2 resulted in anchorage-independent growth comparable to that observed with an shRNA directed against PTEN (Figures 2C and 2D). Knocking down INPP4B or PTEN also increased the rate of proliferation of HMEC cells and the effect of knocking down INPP4B could be reversed by expression of a knockdown resistant INPP4B, indicating that the shRNA was acting on target (Figure 2E).

INPP4B Knockdown Results in Increased Migratory and Invasive Behavior of Human Mammary Epithelial Cells

Migration and invasion are hallmarks of tumor cells with the potential to metastasize to new locations in the body. To analyze the migratory behavior of human mammary epithelial cells, HMEC cells expressing the various shRNA constructs were grown until confluency, and a scratch wound was introduced with a pipette tip. Pictures were taken at time points t = 0 hr and t = 8 hr, as indicated (Figure 3A). Cells expressing shRNA directed against PTEN and INPP4B closed the wound more rapidly than did control cells (Figure 3A; quantified in Figure 3B). The increased rate of wound closure due to INPP4B knockdown was partially rescued by expressing a wild-type INPP4B construct that circumvents the knockdown ($INPP4B^R$) but was not affected by expressing the catalytically inactive mutant of INPP4B (INPP4B-CD) (Figure 3C; expression of $INPP4B^R$ and INPP4B-CD is presented in Figure S1, which is available with this article online). The PI3K inhibitor LY294002 also reduced