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Article

Tumor Suppression by Phospholipase C-β3 via SHP-1-Mediated Dephosphorylation of Stat5

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SUMMARY

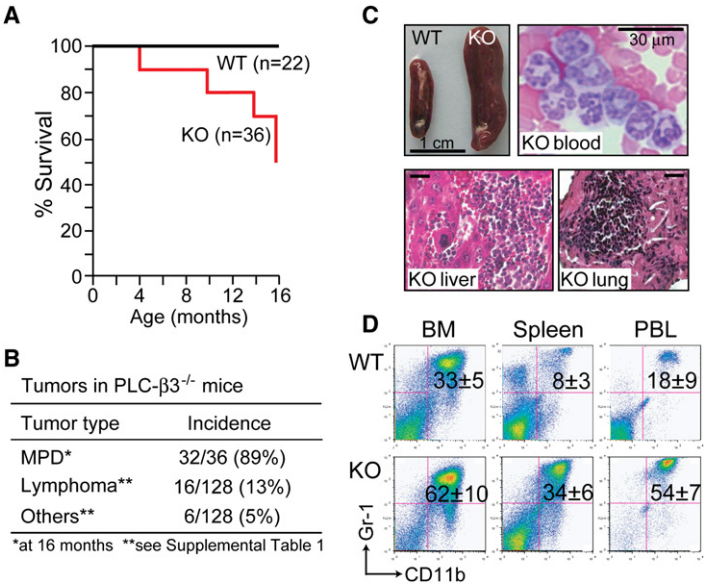
Given its catalytic activity to generate diacylglycerol and inositol 1,4,5-trisphosphate, phospholipase C (PLC) is implicated in promoting cell growth. However, we found that PLC-β3-deficient mice develop myeloproliferative disease, lymphoma, and other tumors. The mutant mice have increased numbers of hematopoietic stem cells with increased proliferative, survival, and myeloid-differentiative abilities. These properties are dependent on Stat5 and can be antagonized by the protein phosphatase SHP-1. Stat5-dependent cooperative transformation by active c-Myc and PLC-β3 deficiency was suggested in mouse lymphomas in *PLC-β3*$^{-/-}$ and in *Eμ-myc;PLC-β3*$^{+/-}$ mice and human Burkitt's lymphoma cells. The same mechanism for malignant transformation seems to be operative in other human lymphoid and myeloid malignancies. Thus, PLC-β3 is likely a tumor suppressor.

INTRODUCTION

The production and lineage commitment of hematopoietic cells is controlled by the actions of a multitude of cytokines, growth factors, and hormones (Kondo et al., 2003). Cell surface receptors bound by these ligands activate several signaling pathways including the Jak-Stat pathway. This pathway plays a crucial role in a number of biological functions by activating transcription of various target genes (Levy and Darnell, 2002; O'Shea et al., 2002; Schindler et al., 2007). Cytokine stimulation activates Jak kinases through transphosphorylation and results in tyrosine phosphorylation of receptor sites, Stats, and other substrates.

SIGNIFICANCE

Many hematopoietic malignancies depend on the activity of Stat5 transcription factor. Here, we report a novel Stat5-suppressive mechanism by which PLC-β3 augments dephosphorylating activity of SHP-1 toward Stat5 by recruiting SHP-1 and Stat5 to its C-terminal sequence. Abrogation of this suppression leads to myeloproliferative disease, lymphoma, and other types of cancer in *PLC-β3*$^{-/-}$ mice. PLC-β3 deficiency or downregulation appears to cooperate with c-Myc to induce B cell lymphoma in mice and humans. The same mechanism may be operative in human myeloid and lymphoid tumors. Therefore, the adaptor function of PLC-β3 seems essential to protect the hematopoietic and nonhematopoietic systems from tumor development.



B

Tumors in PLC-β3$^{-/-}$ mice

Tumor type	Incidence
MPD*	32/36 (89%)
Lymphoma**	16/128 (13%)
Others**	6/128 (5%)

*at 16 months **see Supplemental Table 1

Figure 1. *PLC-β3*$^{-/-}$ Mice Develop MPD, Lymphoma, and Other Tumors

(A) Survival analysis.

(B) Summary of tumors developed in *PLC-β3*$^{-/-}$ mice.

(C) Hematologic analysis of 10-month-old *PLC-β3*$^{-/-}$ mice. Splenomegaly in *PLC-β3*$^{-/-}$ mice (top left) was associated with effaced splenic architecture (data not shown). Increased mature granulocytes in *PLC-β3*$^{-/-}$ mice were shown by blood smear (top right) and hematoxylin and eosin staining of lung and liver sections (bottom). Bars in tissue sections indicate 30 μm.

(D) Flow cytometric analysis of nucleated cells in BM, spleen, and peripheral blood leukocytes (PBL) from 10-month-old mice (n = 16). Granulocytes (CD11b$^+$/Gr-1$^+$; percentages ± SD shown) were increased in these organs of aged *PLC-β3*$^{-/-}$ mice.

Following tyrosine phosphorylation, Stats homo- or heterodimerize, translocate to the nucleus, and activate gene expression through sequence-specific response elements.

To date, seven mammalian Stat family members have been identified: Stats 1, 2, 3, 4, 5A, 5B, and 6; Stat5 is encoded by two recently duplicated genes, *Stat5A* and *Stat5B*, with ~96% sequence identity (Mui et al., 1995). Stat5 plays a crucial role in early hematopoiesis—*Stat5A* and *Stat5B* doubly disrupted mice displayed a profound defect in competitive repopulation of hematopoiesis (Bunting et al., 2002; Snow et al., 2002). The commitment of embryonic stem cells to hematopoietic cells is augmented by a Stat5-mediated signal (Kyba et al., 2003). Stat5 also plays a role in myeloid cell proliferation and differentiation (Ilaria et al., 1999). Constitutive activation of Stat5 in human cord blood CD34$^+$ cells enhances their capacity to repopulate NOD/SCID mice and promotes erythroid differentiation (Schuringa et al., 2004). Constitutively active Stat5 promotes self-renewal, proliferation, and survival of mouse hematopoietic stem cells (HSC) and induces a lethal myeloproliferative disease (MPD) in mice (Kato et al., 2005).

Stat signals are downregulated by three well-characterized mechanisms: dephosphorylation, nuclear export, and suppressor of cytokine signaling (SOCS) feedback inhibition (Shuai, 2000; Tanaka et al., 2005). SOCS proteins bind either activated Jak proteins or cytokine receptors to inhibit Jak activity (Alexander and Hilton, 2004). Several protein tyrosine phosphatases have been reported to dephosphorylate either Jak and/or Stat proteins. For example, Jak2 interacts with SHP-1 and may be dephosphorylated by SHP-1 (Jiao et al., 1996; Klingmuller et al., 1995).

Phospholipase C-β (PLC-β) is a small family of enzymes that can produce diacylglycerol and IP_3 downstream of heterotrimeric G proteins (Rhee, 2001). As diacylglycerol can activate protein kinase C (PKC), and IP_3 can mobilize Ca^{2+}, PLC-β is implicated in promoting cell proliferation. PLC-β directly interacts with GTP-bound Gα subunits, leading to its catalytic activation. The four isoforms of PLC-β (β1–β4) show different tissue expression specificity and heterotrimeric G protein regulation profiles. PLC-β1 and PLC-β3 are expressed in a wide range of tissues and cell types, while PLC-β2 and PLC-β4 are expressed only in hematopoietic and neuronal tissues, respectively. PLC-β2 and PLC-β3 can also be activated by βγ subunits of the $G\alpha_{i/o}$ family of G proteins (Camps et al., 1992; Katz et al., 1992; Lee et al., 1993). Consistent with their roles in G protein-coupled receptor signaling, chemokine-induced IP_3 production, Ca^{2+} signaling, and migration are reduced in *PLC-β2*$^{-/-}$ and *PLC-β2*$^{-/-}$*;PLC-β3*$^{-/-}$ neutrophils (Li et al., 2000) and T cells (Bach et al., 2007). However, little is known about the role of these PLC-β isoforms in hematopoiesis or tumorigenesis. Here we have studied the role of PLC-β3 in these processes using *PLC-β3*$^{-/-}$ mice.

RESULTS

PLC-β3-Deficient Mice Develop Various Tumors Including MPD and Lymphoma

PLC-β3 deficiency led to a premature death in mice (Figure 1A). Fifty percent (18 of 36 mice) of *PLC-β3*$^{-/-}$ mice died within an observation period of 16 months, in contrast with 100% survival of wild-type (WT) mice. By the age of 16 months, most *PLC-β3*$^{-/-}$ mice in this cohort exhibited splenomegaly (Figure 1B-C), the incidence of which reached 89% when prematurely dead mice with this abnormality were included. The enlarged spleens had effaced architecture characterized by markedly increased myeloid cells and some erythroid cells, indicative of extramedullary hematopoiesis (data not shown). Livers and lungs also had foci composed of myeloid cells (Figure 1C). Dramatic increases in CD11b$^+$Gr-1$^+$ mature granulocytes in bone marrow (BM), spleen, and peripheral blood from these mice were observed (Figures 1C and 1D and see Tables S1 and S2 available with this article online). Microbiological examinations showed no indications of bacterial infection in the diseased mice, and antibiotic treatments did not affect the number of granulocytes (data not shown). Therefore, these hematologic findings were consistent with the diagnosis of MPD (Kogan et al., 2002), unlike myelodysplastic syndrome that is frequently associated with anemia.

During a 2 year observation period, three in another cohort of 16 *PLC-β3*$^{-/-}$ mice with increased granulocytes developed anemia (hematocrits of 11%, 16%, and 22%) with increased numbers of blast cells in their BM (32%, 35%, and 45%, respectively). This result suggests that the MPD can evolve to accelerated and blast-crisis stages, similar to human chronic myelogenous leukemia (CML) (Sawyers, 1999). Gross and histologic