The SPS Affair: A Complex Tale of Illicit Proliferation

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In this issue of *Cancer Cell*, Xiao et al. report that PLC-β3 mutant mice develop myeloprolfierative neoplasms and show that tumor suppressor activity does not require PLC-β3 catalytic activity. Instead, PLC-β3 forms a complex with SHP-1 and Stat5 that facilitates Stat5 dephosphorylation. A similar mechanism may be operative in some human leukemias.

Subversion of growth factor signaling is a hallmark of oncogenesis. This has immediate therapeutic implications because specific classes of signal transduction molecules, such as tyrosine kinases, have proven amenable to inhibition by small molecules. Whereas studies in which oncoproteins were overexpressed in immortalized cell lines and heterologous tissues defined the basic architecture of canonical cancer signaling pathways, it has become increasingly clear that the biochemical wiring of primary neoplastic cells is more nuanced and complicated. This is due to both the inherent ability of cells to remodel signaling networks in response to stress and to the profound complexity of signaling networks. One factor underlying this complexity is the sheer number of molecules involved in the response to many stimuli. This is exacerbated by the coexpression of structurally distinct isoforms of many enzymes, generated from related genes or through alternative splicing of a single gene. Furthermore, interlocking feedback loops create highly dynamic and nonlinear response characteristics in networks. Finally, the repertoire of growth factor receptors, adaptors, and downstream network components varies widely among distinct cell types.

In this issue, Xiao et al. use mouse genetics as a starting point for investigating the consequences of disrupting the β3 isozyme of phospholipase C (PLC-β3) (Xiao et al., 2009). They unexpectedly found that PLC-β3 functions as a tumor suppressor in hematopoietic cells, performed incisive biochemical studies that implicate PLC-β3 as a negative regulator of Stat5 signaling, and reported intriguing preliminary observations in human cells.

Like the other 12 members of the phospholipase C family, PLC-β3 catalyzes the conversion of phosphatidylinositol 4,5-bisphosphate (PIP_2) to inositol-1,4,5-trisphosphate (IP_3) and diacylglycerol (DAG) (Figure 1A) (Suh et al., 2008). These second messengers are positive regulators of mitogenic signaling—IP_3 induces calcium release from intracellular stores and DAG can initiate activation of Ras proteins via the RasGRP family of guanine nucleotide exchange factors. Calcium and DAG also activate protein kinase C, among other targets. The four members of the PLCβ subfamily are implicated as promoting cell growth downstream of activated G protein coupled receptors. Given this conventional view of PLC function, it is surprising that Xiao et al. found that mice deficient for PLC-β3 developed a neoplastic disease. Specifically, older mice frequently developed a myeloproliferative neoplasm (MPN), a differentiated hematopoietic malignancy that is frequently caused by mutations that activate signaling molecules such as BCR-ABL, N-Ras, SHP-2, and JAK-2 (Van Etten and Shannon, 2004). The authors pursued this initial observation in three ways.

First, they used state-of-the-art methods to evaluate the hematopoietic compartment in *PLC-β3*$^{-/-}$ mice. Stem and progenitor populations were expanded in the absence of *PLC-β3* and demonstrated excessive proliferation in vivo and in vitro. Importantly, myeloid hyperplasia developed in naive recipients that were transplanted with *PLC-β3*$^{-/-}$ bone marrow cells, which demonstrates that the abnormal accumulation and infiltration of myeloid lineage cells is intrinsic to the hematopoietic system. The observation that the leukemia initiating population is restricted to the c-kit+ lineage- Sca1+ (KLS) CD34^{-} fraction is consistent with other murine models of MPN in which hematopoietic stem cells (HSCs), but not their more differentiated progeny, can transfer disease in vivo (Sabnis et al., 2009; Santaguida et al., 2009). Adoptive transfer and competitive reconstitution experiments using cells sorted based on expression of more selective stem cell markers, such as those in the signaling lymphocytic activation molecule (SLAM) family, will provide more precise information on the phenotypic and biologic characteristics of the leukemia-initiating population in this model (Kiel et al., 2005).

The authors next sought a biochemical mechanism to explain the tumor suppressor activity of *PLC-β3*. A key observation was hyperphosphorylation of Stat5 in multipotent progenitor cells. Stat5 is a key regulator of proliferation and survival downstream of activated cytokine receptors that are aberrantly activated in many myeloid malignancies (Murray, 2007). To assess the functional relevance of Stat5 to myeloproliferation, the authors infected *PLC-β3*$^{-/-}$ bone marrow cells with a dominant-negative Stat5 construct and found that this attenuated the ability of these cells to cause MPN in vivo. Importantly, expressing not only wild-type PLC-β3, but also a PLC-β3 protein lacking catalytic activity reduced proliferation and Stat5 phosphorylation. The authors went on to identify a carboxyl-terminal fragment of PLC-β3 as essential for biologic functions. How then, does loss of this PLC-β3 adaptor activity deregulate Stat5 signaling? Xiao and colleagues performed a comprehensive biochemical analysis in

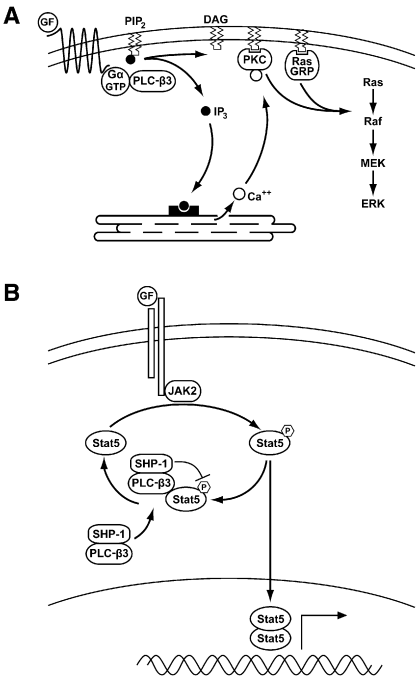

Figure 1. Distinct Roles of PLC-β3 in Growth Regulation

(A) PLC-β3 is a positive effector of mitogenic signals downstream of G-protein-coupled receptors. When a growth factor (GF) binds to its receptor, the associated Gα subunit becomes GTP-bound. This recruits PLC-β3, which cleaves PIP_2 into IP_3 and DAG. IP_3 induces calcium release from intracellular stores. These second messengers coordinately activate multiple signal transduction proteins, most notably protein kinase C (PKC) and RasGRP. These, in turn, initiate mitogenic signaling through Ras and the mitogen-activated protein kinase pathway.

(B) PLC-β3 attenuates JAK/Stat signaling. Receptors for cytokines and related ligands lack intrinsic kinase activity and instead initiate signaling by activating the protein tyrosine kinase JAK2. This results in phosphorylation of the receptor and other JAK2 substrates, such as Stat5. Phosphorylated Stat5 translocates to the nucleus and acts as a transcription factor. In this issue of *Cancer Cell*, Xiao et al. demonstrate that a carboxyl terminal domain of PLC-β3 can recruit the protein tyrosine phosphatase SHP-1 to Stat5, resulting in its dephosphorylation. This novel, growth inhibitory activity of PLC-β3 is required to prevent neoplasia in the hematopoietic system.

the Ba/F3 lymphoid cell line to elucidate the underlying mechanism. These cells are an ideal choice, as they depend upon interleukin 3, a cytokine that activates Stat5, for growth and survival. The authors demonstrate a tripartite "SPS" complex that includes PLC-β3, Stat5, and the protein tyrosine phosphatase SHP-1 and implicated phosphorylated Stat5 as a SHP-1 substrate. Genetic and functional analysis of motheaten viable *(Me^v/Me^v)* mice, which have a hypomorphic allele of the gene encoding SHP-1, affirmed the functional importance of SHP-1 phosphatase activity in the SPS complex. Together, these studies strongly support the idea that the carboxyl terminus of PLC-β3 restrains hematopoietic growth by colocalizing activated Stat5 and SHP-1, thereby promoting Stat5 dephosphorylation (Figure 1B). While the current studies do not directly address if specific cytokines are essential coconspirators in the development of MPN, there are intriguing candidates. The thrombopoietin receptor is expressed on HSC and signals, in part, through Stat5. PLC-β3 mutant myeloid progenitors are also hypersensitive to both IL-3 and granulocyte-macrophage colony stimulating factor (GM-CSF). Interestingly, aberrant Stat5 activation in response to low concentrations of GM-CSF is a biochemical feature of the juvenile myelomonocytic leukemia and chronic myelomonocytic leukemia subtypes of human MPN (Kotecha et al., 2008).

A final aspect of this ambitious and far-ranging paper is studies addressing the role of PLC-β3 in lymphoma and lymphoid leukemia. The authors find that loss of PLC-β3 cooperates with deregulated *Myc* expression in a classic model of B lineage leukemia. In this system, mice that are heterozygous for the mutant allele show markedly reduced PLC-β3 protein expression in the absence of "second hit" mutations. Consistent with this, two of six human Burkitt's lymphoma cell lines showed low levels of PLC-β3 and elevated Stat5 phosphorylation. Overexpressing PLC-β3 inhibited the growth of these cells, but not of a line that showed high endogenous levels of the protein. The authors also identified a subset of human chronic lymphocytic leukemia samples that showed concomitant elevated levels of phosphorylated Stat5 and low PLC-β3 expression. These intriguing correlative data set the stage for future experiments to understand the mechanisms underlying reduced PLC-β3 expression in some human cancers, to determine if there is a cause-and-effect relationship with deregulated Stat5 signaling, and to investigate if a hematopoietic malignancies with this biochemical profile are uniquely sensitive to small molecule inhibitors that interfere with Stat5 activation or output.

A provocative aspect of this work is that an important physiologic role of PLC-β3 is encoded in a noncatalytic domain. Furthermore, this unexpected new tumor suppressor activity of PLC-β3 may oppose (or at least counterbalance) other growth-promoting properties of the protein that are mediated by the production of IP_3 and DAG. Taken together, the results of Xiao and colleagues demonstrate that it may be impossible to functionally categorize signal transduction proteins using simple binary concepts. Indeed, in cellular contexts where Stat5 is not expressed, PLC-β3 may have predominately pro-growth properties. Alternatively, the absence of PLC-β3 mutations in human cancers may reflect the fact that complete loss of protein function may negatively impact cell fitness. New analytical tools may be required to represent the complicated interactions of multidomain signaling proteins. In the meantime, the rigorous studies of Xiao et al. provide an excellent example of how interrogating primary cells with a defined genetic alteration can uncover novel and unanticipated biochemical interactions that are physiologically important in growth regulation and oncogenesis.

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