PI3K and STAT3: A New Alliance

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ABSTRACT

Recent proteomic data have uncovered an interdependence of PI3K (phosphatidylinositol 3-kinase) and STAT3. In PI3K-transformed murine cells, STAT3 is phosphorylated on Y705 and activated in a PI3K-dependent manner, and dominant-negative STAT3 interferes with PI3K-induced oncogenic transformation. Phosphorylation of STAT3 in PI3K-transformed murine cells is mediated by the TEC (tyrosine kinase expressed in hepatocellular carcinoma) kinase BMX (bone marrow tyrosine kinase gene in chromosome X) and observations in glioblastoma stem cells reveal similar critical roles for STAT3 and BMX. These new data document an important role of STAT3 in PI3K-driven oncogenic transformation and mark BMX as a promising therapeutic target that could enhance the effectiveness of PI3K inhibitors.

Significance: The PI3K–TOR and STAT3 signaling pathways represent two distinct regulatory networks. The discovery of a functional link between these pathways is significant for our understanding of PI3K- and STAT3-driven oncogenic mechanisms and identifies the TEC kinase BMX as a new cancer target. *Cancer Discovery; 1(6);* 481–86. *©2011 AACR.*

INTRODUCTION

In cellular signaling networks, diverse inputs trigger a fixed sequence of events including branch points, cross-talk with other signaling networks, and feedback loops, which combine to induce characteristic effects on cellular functions. For such systems, it is possible to draw a map of interactions and connections that depicts the core pathway as well as ancillary branches and feedback loops. Although such maps commonly include a fixed set of proteins, additions and modifications are frequently made based on new data. In this review, we focus on a modification of an established signaling system in cancer, the PI3K–TOR (phosphatidylinositol 3-kinase/target of rapamycin) pathway, to incorporate the essential contribution of STAT3.

We first summarize the consensus features of the PI3K–TOR network and of STAT3 activation and signaling. Against this background, we consider highlights from new proteomic data obtained with PI3K-transformed cancer cells. Some of these data are not explicable by the known effects of upregulated PI3K and suggest STAT3 involvement. We examine evidence supporting this suggestion and explore the relevance of these findings for human cancer and for cancer therapy.

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CANONICAL PI3K AND STAT3 SIGNALING

PI3K is a lipid kinase that controls a core cellular signaling and regulatory network. This network responds to multiple inputs including growth signals as well as metabolic and nutritional cues (1–3). PI3K controls cell growth, proliferation and survival, anabolic and autophagic activities, and cytoskeletal organization. The oncogenic signal originating from hyperactive PI3K proceeds through AKT (cellular homolog of murine thymoma virus akt8 oncoprotein) and via TSC (tuberous sclerosis complex) and RHEB (Ras homolog enriched in brain) to activate TOR in a multiprotein complex referred to as TORC1. TORC1 stimulates protein synthesis and inhibits autophagy. Activation of AKT and TORC1 is necessary but probably not sufficient for oncogenic cellular transformation. Other essential elements of the PI3K-generated oncogenic signal, possibly involving the TORC2 complex, remain to be identified (4).

STAT3 belongs to a family of transcriptional regulators, which are mobilized in response to interferon and initiate the transcription of interferon-induced genes (5, 6). STAT3 is activated by phosphorylation on residue Y705, which induces homodimerization or heterodimerization with other STAT proteins and results in nuclear translocation and activation of the STAT3 transcriptional regulatory function. Activating phosphorylation of STAT3 can be triggered by cytokines such as interleukin-6 (IL-6) and also by receptor and nonreceptor tyrosine kinases such as epidermal growth factor receptor (EGFR) (7, 8) and SRC (cellular homolog of the Src oncoprotein of Rous sarcoma virus) (9). Activation by IL-6 is mediated by members of the JAK kinase (Janus-activated kinase) family; the tyrosine kinases EGFR and SRC can directly phosphorylate STAT3.

The consensus interaction networks of PI3K–TOR and STAT3 do not currently include established or default lines of communication (Fig. 1), but increasing evidence from

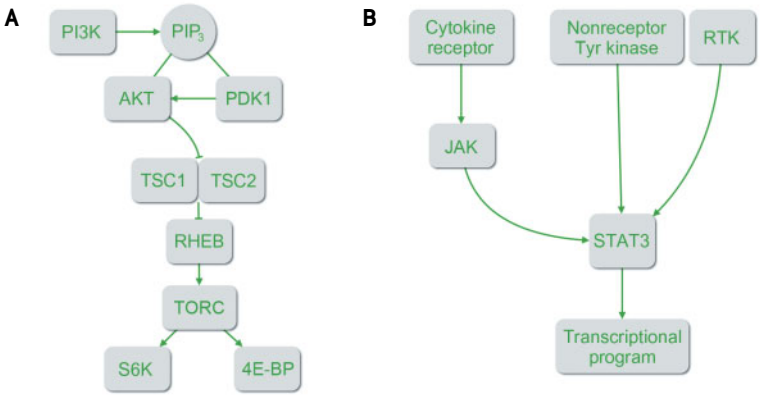

Figure 1. Core elements of two distinct signaling pathways. **A,** PI3K–TOR pathway. The product of PI3K, PIP_3 (phosphatidylinositol 3,4,5-trisphosphate), recruits the two serine-threonine kinases AKT and PDK1 (3-phosphoinositide–dependent protein kinase-1). PDK1 phosphorylates and thereby activates AKT. AKT has numerous targets. By phosphorylating TSC2, it inactivates the GTPase activity of the TSC complex. As a result, the GTP-bound form of the RAS-like protein RHEB increases, and RHEB activates TORC1. Important targets of TORC1 are S6K and 4E-BP. Phosphorylation of these targets increases the rate of protein synthesis. **B,** activation of STAT3. Two principal routes lead to the phosphorylation and activation of STAT3. Binding of a cytokine to its cognate receptor leads to receptor dimerization and transphosphorylation of the associated JAKs. These kinases then phosphorylate STAT3. The phosphorylation results in dimerization, nuclear translocation, and transcriptional regulator activity. Alternatively, STAT3 can be directly phosphorylated by receptor tyrosine kinases (RTK) and by nonreceptor tyrosine kinases.

basic and clinical studies suggests that PI3K and STAT3 signaling in cancer is interdependent.

ACTIVATION OF STAT3 IN MURINE CELLS TRANSFORMED BY PI3K

Methods for analysis of the global proteome are now available and are increasingly applied to cancer cells to identify changes that are specific to oncogenic transformation (10). Such studies require comparison with a normal progenitor cell that, in its genetic and epigenetic makeup, is identical to the cancer cell except for the differences that are the cause of the oncogenic transformation. Stable transfection with an activated oncogene can generate such an isogenic pair; a more stringent method is to knock in the activated oncogene into the genome of the normal progenitor to effect oncogenic transformation (11, 12).

A recent study has used SILAC (stable isotope labeling with amino acids in cell culture) in conjunction with tandem mass spectroscopy to identify and analyze proteomic differences between the murine embryonic fibroblast line C3H 10T1/2 and its isogenic, PI3K-transformed descendant (13). The transformed cells carry the H1047R mutation of *PIK3CA* as an actively expressed and stably integrated transgene. *PIK3CA* encodes the PI3K catalytic subunit p110α, and H1047R is a highly oncogenic mutant of this gene (14, 15). The transformed C3H 10T1/2 cells are derived from a single clone; unlike their progenitors, they are capable of anchorage-independent proliferation and display characteristic features of cancer cells: rounded cell shape, reduced adhesion, and multilayered growth. They show elevated expression of p110α and constitutive phosphorylation of the downstream targets AKT and ribosomal protein S6. In this isogenic pair of cell lines, SILAC identified about 50,000 peptides, revealing the relative abundance of more than 4,000 proteins. In the transformed cells, about 100 proteins are upregulated, and nearly 50 proteins are downregulated by a factor of >2 ($p < 0.05$). Notable among the upregulated proteins are several whose expression is controlled by STAT transcription factors. The corresponding genes contain interferon-stimulated response elements or interferon γ activation sites. These sites bind transcriptional regulatory complexes that include STAT proteins. The SILAC data therefore suggest elevated STAT activity, which is confirmed by Western blots for phosphorylated STAT proteins (16). Both STAT3 and STAT6 show enhanced tyrosine phosphorylation in the transformed cells. Because of the prominent role of STAT3 in cancer, initial investigations have focused on the connection between this member of the STAT family and PI3K-induced oncogenic transformation.

A possible link between PI3K and the activation of STAT6 remains to be investigated. STAT6 is primarily active in the transcriptional regulation of lymphocytes (17) and is involved in hematopoietic malignancies (18). STAT6 is strongly activated by IL-4 and IL-13 via JAK kinases but can also be activated by the receptor tyrosine kinase platelet-derived growth factor receptor. STAT6 is involved in the regulation of allergic responses and inflammation in asthma and ulcerative colitis, playing a key role in immune cells and in the epithelium. In solid tumors, STAT6 can have prosurvival functions but can also inhibit the growth of tumor cells (19–22). The experiments described in this review for the characterization of the PI3K–STAT3 connection could also be informative for STAT6, particularly in leukemias and lymphomas.

Upon phosphorylation, STAT proteins homodimerize but can also heterodimerize with other members of the STAT family. Because the transcriptional target spectra of different STAT proteins are similar but not identical (23), STAT heterodimers can act in complex ways. An example of this complexity is the interaction between STAT1 and STAT3 (24), which are mutually antagonistic. When they are incorporated into heterodimers, activated STAT1 downregulates STAT3 targets and STAT3 downregulates STAT1 targets. The balance of these effects depends on the levels of STAT expression and phosphorylation.

INTERDEPENDENCE OF PI3K AND STAT3

Activation of STAT3 in H1047R-transformed cells could reflect a generic property of the oncogenic phenotype without specific relevance to the PI3K-generated transforming signal, or it could play a specific role in the oncogenicity of PI3K. Expression of STAT3DB (dominant-negative STAT3 DNA-binding mutant) in chicken embryo fibroblasts induces resistance to transformation by the oncogenic p110α mutants H1047R and E545K, by constitutively active myristylated p110α, or by overexpression of the wild-type versions of p110β, p110γ, and p110δ. Activation of STAT3 is therefore not a dispensable feature of PI3K-transformed cells but plays an important role in the oncogenic process. Conversely, inhibition of PI3K by the small-molecule inhibitor GDC-0941 reduces the phosphorylation of STAT3, further suggesting that in this system, PI3K and STAT3 are linked by a mutual dependence.

CONNECTION OF PI3K TO STAT3 VIA TEC KINASES

PI3K signaling from p110 to TOR does not involve a tyrosine kinase. The PI3K-initiated activation of STAT3 by phosphorylation on Y705 must therefore be carried out by a PI3K-activatable kinase that operates outside the core PI3K pathway. The TEC kinase family meets this criterion (25, 26). Its members are TEC, tyrosine kinase expressed in hepatocellular carcinoma; BMX, bone marrow tyrosine kinase of chromosome X; BTK, Bruton agammaglobulinemia tyrosine kinase; ITK, IL-2–inducible T-cell kinase; and TXK, TXK tyrosine kinase. These are nonreceptor tyrosine kinases with diverse roles in the transmission of signals initiated by G-protein–coupled receptors, antigen and integrin receptors, and receptors of growth factors and cytokines (27–30). They contain a PH (pleckstrin homology) domain that mediates specific binding to the PI3K product PIP_3 (phosphatidylinositol 3,4,5-trisphosphate), which is followed by kinase activation by nearby receptor tyrosine kinases (RTK), nonreceptor tyrosine kinases, or autophosphorylation (31, 32). Most TEC family members are preferentially expressed in hematopoietic cells, but BMX (also known as ETK) and TEC kinase itself occur in several nonhematopoietic tissues. Of these, only BMX is phosphorylated and therefore activated in H1047R-transformed cells. BMX has previously been identified as an activator of STAT3, and BMX can phosphorylate STAT3 *in vitro* (33, 34).

The involvement of TEC kinases in the activation of STAT3 is documented by the effect of the TEC kinase inhibitor LFM-A13. At concentrations that affect only TEC kinases, this inhibitor greatly reduces the phosphorylation of STAT3 at Y705. The JAK kinase inhibitor AG490, the SRC inhibitor Src1, and rapamycin have no such effect on the phosphorylation of STAT3 in H1047R-transformed cells. The new small-molecule, ATP-competitive inhibitors of TOR still require testing. Interestingly, treatment of H1047R-transformed cells with LFM-A13 leads to elevated phosphorylation of AKT, suggesting that TEC kinases can function as negative regulators of PI3K. In assays for oncogenic transformation conducted with chicken embryo fibroblasts, LFM-A13 strongly interferes with focus formation by a retroviral H1047R expression vector but does not affect transformation induced by SRC (16).

STAT3 can also be phosphorylated by TOR at the S727 target residue, and this phosphorylation enhances STAT3 activity (35–37). However, the level of S727 phosphorylation is high regardless of PI3K-induced transformation. This observation, together with the failure of rapamycin to affect STAT3 phosphorylation at Y705, suggests that TOR does not play an immediate role in the PI3K–STAT3 connection.

RELEASE OF A STAT3-ACTIVATING FACTOR BY H1047R-TRANSFORMED CELLS

Because activation of STAT3 by phosphorylation is part of the cellular response to interferon, it is important to examine a possible role of interferon in the PI3K–STAT3 connection. Transfection of nontransformed C3H 10T1/2 cells with polyinosinic:polycytidylic acid induces the production and release of interferon. Conditioned medium from these cells, placed on fresh C3H 10T1/2 cells, triggers an interferon response in these recipients including a large increase in ISG15 (interferon-induced protein 15), but it does not change the phosphorylation of STAT3. By contrast, conditioned medium from untreated H1047R-transformed C3H 10T1/2 cells strongly enhances the phosphorylation of STAT3 but has no effect on the abundance of ISG15. These data suggest that interferon is not involved in the link between PI3K and STAT3 but that H1047R-transformed cells release a factor capable of activating STAT3 in recipient cells. The identity of this factor and its mechanism of operation remain to be determined. IL-6 is a potential candidate for such a factor, because it is known to activate STAT3 (38). Although preliminary tests show that an IL-6 antibody had no detectable effect on the activity of the factor released by H1047R-transformed cells, the possibility that this factor is IL-6 remains open. It also remains an unanswered question whether this factor functions in an autocrine fashion and is involved in the activation of STAT3 in transformed cells. In other systems, IL-6 can readily form a self-stimulatory autocrine loop (39–41). Its paracrine activity, documented on normal C3H 10T1/2 cells, could also be an important factor in the remodeling of the tumor stroma by cancer cells (42–44).

THE PI3K–STAT3 CONNECTION IN HUMAN CANCER CELL LINES

For any given cell line, there is a simple test that can reveal an active link between PI3K and STAT3: the phosphorylation of STAT3 should be sensitive to the inhibition of PI3K and to the inhibition of TEC kinases. Many human cancer cells show phosphorylation of STAT3, and the level of this phosphorylation varies within wide boundaries (45, 46). In a small sample of such cell lines tested, about half show the diagnostic sensitivity of STAT3 phosphorylation to PI3K and to TEC kinase inhibition (16). However, the level of STAT3 phosphorylation is not correlated with a gain of function in PI3K. Cells carrying oncogenic mutations in p110α may or may not show elevated STAT3 phosphorylation, and the link between PI3K and STAT3 can be demonstrable in cells with low levels of STAT3 phosphorylation. Much larger numbers of cancer cell lines and of primary tumors will have to be examined to allow generalizations on the prevalence and relevance of the PI3K–STAT3 connection in human cancer.

ACTIVATION OF STAT3 IN GLIOBLASTOMA STEM CELLS BY BMX

Recent investigations on glioblastoma complement the studies in cell culture and document the activation of STAT3 by BMX (33). Glioblastomas contain a minority population of cells, referred to as glioblastoma stem cells, that self-renew and generate identical tumors on transplantation (47). They are characterized by specific progenitor cell markers, including CD133 (cluster of differentiation 133, prominin 1), OLIG2 (oligodendrocyte transcription factor 2), and SOX2 (sex-determining region Y-box 2). These cells are also distinguished from the rest of the cell population by the expression of BMX. The expression of BMX is also an important differentiator between normal neural progenitor cells and glioblastoma stem cells. In glioblastoma stem cells, BMX mediates the activation of STAT3. Active STAT3 is essential for the maintenance of self-renewal and of tumorigenicity (33, 48). These properties of cancer stem cells depend on IL-6 signaling (49). IL-6 can activate PI3K, and this in turn mediates the autophosphorylation of BMX (27). Additionally, glioblastomas frequently carry inactivating mutations of PTEN (phosphatase and tensin homolog) or activating mutations of the PI3K regulatory subunit p85 that result in enhanced PI3K activity (50–52). Therefore, it seems likely that the PI3K–STAT3 connection is active in glioblastoma stem cells and forms the backbone of a regulatory pathway that determines and sustains stemness. The IL-6–STAT3 axis is not confined to glioblastoma; it is also well established in pancreatic and colon cancer and may be of universal significance in oncogenesis (53–55).

UNIVERSALITY OF THE ALLIANCE BETWEEN PI3K AND STAT3

It is possible that the full oncogenic activity of PI3K always depends on the phosphorylation of STAT3. This requirement would mark the proteins of the PI3K–STAT3 signaling chain as potential therapeutic targets in PI3K-driven tumors. However, currently available data seem not to support such a view. The human cancer cell lines HCC-1954 and T-47D both carry the H1047R mutation of p110α, but STAT3 phosphorylation in these cells is not affected by a PI3K inhibitor or by a TEC kinase inhibitor. In these instances, the H1047R mutation could function independently of STAT3 or it could be irrelevant for the oncogenic phenotype. Alternatively, STAT3 phosphorylation may be independent of the PI3K pathway and mediated by other activation mechanisms such as JAK- or RTK-induced phosphorylation. However, there is an alternative interpretation of these observations, suggested by the work on glioblastoma. As in glioblastoma and other cancers, the tumor-initiating cells in the cell lines probably constitute only a small fraction of the cell population (56), but Western blots of culture lysates used to determine the sensitivity of STAT3 phosphorylation to PI3K and TEC kinase inhibitors reflect the average in protein levels of the entire cell population and are therefore not suitable for characterization of minority cell types. The tumor-initiating cells have to be isolated from the heterogeneous population, and only with these cells would it be possible to decide the question of the universality of the PI3K–STAT3 alliance. In the meantime, it is possible to explore the PI3K–STAT3 signaling chain for "druggability." In preliminary tests, the TEC kinase inhibitor LFM-A13 has greatly enhanced the cytostatic and cell-killing effectiveness of PI3K inhibitors. This observation suggests that targeting TEC kinases, and specifically BMX, in conjunction with inhibition of PI3K could result in therapeutic benefit. The combination with PI3K inhibitors would be important because of the possibility that TEC kinase inhibitors activate AKT. BMX is an attractive drug target for two additional reasons (33): It is not expressed in neural progenitor cells that share several properties with glioblastoma stem cells. On a general scale, mice in which BMX is genetically inactivated are largely normal (57). Therefore, BMX does not have essential functions in development, and a specific BMX inhibitor is unlikely to cause significant side effects.

THE FUSION OF TWO NETWORKS

Figure 2 schematically presents the oncogenic signals originating in PI3K. The product of PI3K, PIP_3, serves as a joint platform and as the branching point for two pathways that both contribute to the oncogenic cellular phenotype. PIP_3 recruits AKT and its activating kinase PDK1 (3-phosphoinositide–dependent protein kinase-1) (58) and also recruits BMX (31, 59). The recruitment of these kinases is mediated by PH domains. The binding of different PH domains to PIP_3 is characterized by dissociation constants that depend on the amino acid sequence of the PH domain. It is therefore conceivable that such differences in affinity mediate a compartmentalization, facilitating the AKT-PDK1 and the BMX-BMX interactions. The signaling pathway connecting AKT to TOR has been studied in great detail. The BMX to STAT3 pathway has not been analyzed to the same extent. Signaling components that lie between PI3K and BMX and STAT3 have not been identified. Alternative pathways

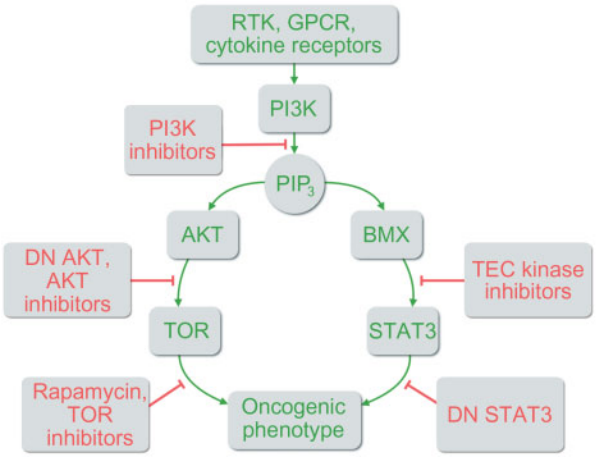

Figure 2. Two branches of oncogenic signal initiated by PI3K. PIP_3 functions as a starting platform and a branching point of two pathways, the AKT–TOR pathway and the BMX–STAT3 pathway. Both are required for full oncogenic transformation. Individual steps in these pathways can be repressed by specific inhibitors or dominant-negative constructs. The AKT–TOR pathway stimulates cell growth and survival; the BMX–STAT3 pathway includes inflammation-related targets. DN, dominant negative; GPCR, G-protein–coupled receptor.

of PI3K-dependent activation of STAT3 also deserve exploration. These pathways include possible noncatalytic functions of PI3K that could lead to activation of a tyrosine kinase and to phosphorylation of STAT3 (60, 61).

The relevant target proteins that induce the oncogenic phenotype downstream of TOR and of STAT3 are not completely known. The TORC1 complex activates protein translation, but this activity is probably not the complete spectrum of oncogenic signals that originate in TOR. Similarly, the transcriptional targets of STAT3 that are essential for oncogenic transformation remain to be identified. STAT3 activates the antiapoptotic proteins BCL-X_L (B-cell lymphoma/leukemia-x, long), MCL1 (induced myeloid leukemia cell differentiation protein Mcl-1), and Survivin (53) as well as Cyclin D1 (62) and NF-κB (nuclear factor κ-light-chain-enhancer of activated B cells) (63). STAT3 also collaborates with MYC and NF-κB in transcriptional activation (64, 65) and is connected to IL-6 in an autocrine loop that can function as an important determinant of oncogenicity (66, 67).

At PIP_3, the PI3K signal bisects. The branch proceeding through AKT carries signals that primarily stimulate cell growth and survival. The branch that is transmitted by BMX and STAT3 involves signals related to inflammation, including IL-6 and NF-κB. Recent studies have documented the importance of the link between promotion of cell growth and inflammation in oncogenesis (65, 68). With the connection to STAT3, the PI3K signal integrates growth-promoting and inflammatory functions.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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