interruptions in tyrosine kinase inhibitor treatment (resulting from either medication noncompliance or intolerance).

This study introduces AID and immune diversification mechanisms into the already complex biology of CML. Future investigations into the role of AID and BCR-ABL1 in causing tyrosine kinase inhibitor resistance, genomic instability, and progression to blast crisis will be important for understanding the pathogenesis of the disease as well as for developing therapeutic interventions to prevent these processes. Additional insight is likely to be gained through investigations into the multilineage dynamics of the CML HSC and factors that regulate expression of PAX5 and AID.

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Src Substrate Surprise

G. Steven Martin[1,*]
[1]Department of Molecular and Cell Biology and Cancer Research Laboratory, University of California, Berkeley, Berkeley, CA 94720, USA
*Correspondence: gsm@berkeley.edu
DOI 10.1016/j.ccr.2009.08.017

In this issue of *Cancer Cell*, Lowy and colleagues show that the SH2 domain of tensin-3 is regulated by phosphorylation by Src and that this phosphorylation promotes the oncogenic function of tensin-3. Phosphorylation of the SH2 domain represents a novel mechanism for the regulation of SH2 ligand binding.

Src is a nonreceptor tyrosine kinase that is activated by a variety of mechanisms in human cancer. Its biological effects are mediated by the phosphorylation of a plethora of protein substrates. A primary role of tyrosine phosphorylation is to generate docking sites for proteins containing SH2 or PTB domains, thereby promoting protein-protein interactions and the formation of macromolecular complexes that function in intracellular signal transduction (Pawson, 2004). The binding activity of SH2 domains is primarily regulated by the phosphorylation of the ligand, although some SH2 domain-target interactions are phosphorylation independent.

Many prominent Src substrates are found at focal adhesions, including the focal adhesion kinase FAK and the Crk-associated substrate Cas. Focal adhesions are sites of integrin-dependent substrate adhesion generated by Rho-ROCK-activated actomyosin-dependent contractility. They serve as sites for the exertion of force on the substratum. They also serve as sites of intracellular signal transduction, mediating adhesion-dependent signals that result in anchorage-dependent cell proliferation.

Tensins are another family of focal adhesion proteins that can serve as Src substrates. There are four members of the family in mammals (Lo, 2004). Tensins 1–3 contain three distinct regions (Figure 1A): the N-terminal domains bind to the side of F-actin and target the molecule to focal adhesions, and the central region of the tensins is nonconserved, whereas the C terminus contains an SH2 domain and a PTB domain. The PTB domain interacts, not with tyrosine phosphorylated proteins, but with NPXY motifs in the integrin tails of β1, 3, 5, and 7. Tensin-1 null fibroblasts show migration defects, and the focal adhesion localization and SH2 domains of tensin-1 are required for this promigratory function (Chen and Lo, 2003). The tensin SH2 domain binds to tyrosine-phosphorylated Cas and FAK, which are pro-oncogenic. However, it also binds to nonphosphorylated RhoGAP7, aka DLC1 (deleted in liver cancer 1), a focal adhesion-localized protein that is a known tumor suppressor, and to the related tumor suppressor DLC3 (Liao et al., 2007; Qian et al., 2007).

Perhaps consistent with the fact that tensins interact with both pro- and antioncogenic proteins, expression profiling and overexpression studies have supported both pro- and antioncogenic functions for tensins. In addition, the oncogenic functions of tensins may be isoform specific (Yam et al., 2006; Katz et al., 2007). Intrigued by this complexity, Qian et al. (2009) decided to examine the functions of different tensins in both Src-transformed fibroblasts and a panel of human cancer cell lines. Knockdown of tensin-3 inhibited transformation by Src and cell migration and growth of the human cancer lines. These effects were not observed upon knockdown of the other tensin family members. In addition,

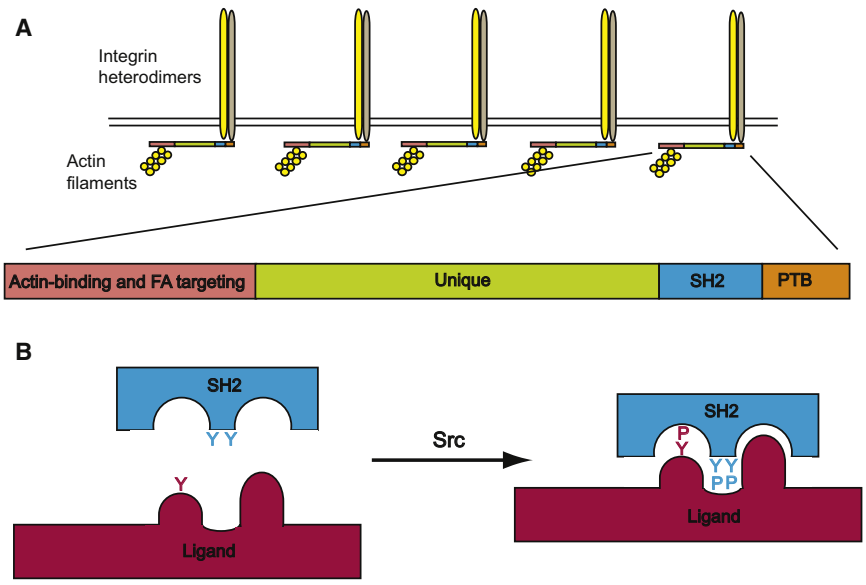

Figure 1. Tyrosine Phosphorylation of the SH2 Domain of Tensin-3

(A) Domain structure of tensin-3. In focal adhesions, tensins link integrin heterodimers to the actin cytoskeleton. The N terminus of tensin-3 contains domains that bind to F-actin and a focal adhesion targeting activity. The C terminus contains an SH2 domain and a PTB domain. The PTB domain interacts with NPXY motifs in the integrin tails of certain β integrins.

(B) The model of Qian et al. (2009). The authors propose that binding of ligands to the tensin-3 SH2 domain is promoted not only by Src-dependent tyrosine phosphorylation of the ligand but also by tyrosine phosphorylation of the SH2 domain itself.

knockdown of tensin-3 reduced the tumorigenicity of mammary cells derived from transgenic mice expressing polyoma middle-T (MMTV-PyMT).

Tensin-1 is known to be tyrosine phosphorylated in Src-transformed chicken embryo fibroblasts (Davis et al., 1991). Qian et al. observed that in their panel of human cancer cell lines, the level of phosphotyrosyl-tensin-3 correlated roughly both with malignancy and with the level of Src kinase activity. Furthermore, the level of phosphotyrosyl-tensin-3 was strongly reduced by pharmacological inhibition of Src or by Src siRNA knockdown. Tensin-3 was also phosphorylated at tyrosine in the MMTV-PyMT mouse model, in which endogenous Src is activated, and this phosphorylation was reversed by the Src inhibitor PP2. In addition, recombinant Src could phosphorylate tensin-3 in vitro. All these and other observations presented in the paper argue that tensin-3 is an authentic Src substrate in mammalian tumor cells.

If tyrosine phosphorylation of tensin-3 is biologically significant, it might be expected to influence its association with functionally important partners. Indeed, inhibition of Src not only decreased the phosphorylation of two other known Src substrates, Cas and the RNA-binding protein Sam68, but also decreased the level of these two proteins that associated with tensin-3 in coimmunoprecipitation assays. This decrease in association must be due at least in part to decreased tyrosine phosphorylation of the tensin-3 ligands. In addition, mutational analysis indicated that three tyrosine residues in the SH2 domain (Y1173, Y1206, and Y1256) were, unexpectedly, Src phosphorylation sites. Mutation of two of these residues (2F) or all three (3F) in resulted in reduced coprecipitation of a GST-SH2 fusion protein with Src, Cas, FAK, and Sam68. In contrast, however, interaction of the tensin-3 SH2 domain with two other ligands, DLC-1 and the integrin-linked kinase ILK, was unaffected by these mutations, indicating that the role of these three tyrosine residues is ligand specific.

Thus, three tyrosine residues in the tensin-3 SH2 domain are phosphorylated by Src, and the same residues appear to promote binding to ligands. Putting two and two together, the most obvious interpretation of these findings is that phosphorylation of these residues promotes binding. Indeed, in both coprecipitation and far-western assays, tyrosine phosphorylation of the SH2 domain was found to promote interaction with Src, Cas, and FAK, as well as with unidentified phosphotyrosyl-proteins in lysates of Src-transformed cells. The authors therefore proposed that phosphorylation of these residues directly affect ligand binding, as shown in Figure 1B. This interpretation is based on homology modeling of the tensin-3 SH2 domain, which suggests that the two tyrosine residues most significantly involved in this regulation are located close to the ligand-binding site of the SH2 domain. The binding sites within the ligands were not defined in this study, so that it is formally possible that phosphorylated and nonphosphorylated SH2 domains might bind different phosphotyrosyl sites in their ligands. In addition, the interaction of tensin-3 with Src may be mediated in part by the Src SH2 domain, with the phosphotyrosyl residues in tensin-3 acting as docking sites. More detailed biophysical and structural studies with defined peptide ligands will be needed to sort this out. Nevertheless it is clear that the tyrosine phosphorylation of the tensin-3 SH2 domain is somehow affecting its function, an observation for which there is no precedent.

Given these findings, what is the biological significance of this novel mode of regulation? Does the tyrosine phosphorylation of the tensin-3 SH2 domain affect its oncogenic function? Consistent with this idea, GST fusions containing the tensin-3 SH2 domain functioned as dominant-negative mutants in cell migration and colony growth assays, whereas the 2F and 3F substitution mutants did not. Furthermore, overexpression of full-length tensin-3 promoted cell migration and colony growth, and this effect was again abolished by the 2F mutation. As noted earlier, knockdown of tensin-3 reduced tumorigenicity in the MMTV-PyMT mouse model. It would be very interesting to determine whether the effects of tensin-3 knockdown in this system could be reversed by wild-type tensin-3 but not by the 2F or 3F mutant, as would be predicted by the authors' model, but this rescue experiment was not performed.

In summary, although the precise molecular details remain to be clarified, Lowy and colleagues have uncovered

a novel mode of regulation of an SH2 domain that affects both the biochemical properties of the SH2 domain and the biological effects of the molecule in which it resides. It remains to be determined whether other SH2 domains are regulated in a similar manner. The ligand-specificity of this mode of regulation is intriguing, given that Src, Cas, and FAK, whose association with tensin-3 is tyrosine phosphorylation dependent, are all pro-oncogenic, whereas the association of tensin-3 with DLC1, a tumor suppressor, is not affected by tyrosine phosphorylation. Thus the biological complexity of tensin-3 function—oncogenic in some contexts, antioncogenic in others—may reflect not only the complexity of the targets to which it binds but also the complexity of its regulation.

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A Molecular Link between AKT Regulation and Chemotherapeutic Response

Christian C. Dibble[1] and Brendan D. Manning[1,*]
[1]Department of Genetics and Complex Diseases, Harvard School of Public Health, Boston, MA 02115, USA
*Correspondence: bmanning@hsph.harvard.edu
DOI 10.1016/j.ccr.2009.08.011

The protein kinase AKT is frequently activated in human cancers and has been implicated in resistance to chemotherapy. In this issue of *Cancer Cell*, Pei et al. show that FKBP51 negatively regulates AKT through the phosphatase PHLPP. This regulation appears to be a determinant of chemosensitivity in cancer cells.

Despite increased interest in targeted therapeutics, cytotoxic chemicals remain the front-line defense against malignant tumors. However, malignant tumors vary greatly in their response to chemotherapeutic agents. A compound might elicit a strong apoptotic response in one tumor but have little to no effect on another. Furthermore, tumors initially sensitive to treatment can develop resistance to specific classes of agents or, in some instances, develop a more generalized chemoresistance to a large variety of agents with distinct modes of action. Therefore, there has been much interest in defining molecular mechanisms underlying this differential response and identifying markers to predict response. A hallmark of cellular transformation is evasion from apoptotic stimuli, and oncogenic pathways promoting aberrant cell survival can also affect the general response of a tumor cell to chemotherapeutics.

AKT (also known as PKB) is a critical survival kinase that is normally activated in a growth-factor-dependent manner but is constitutively activated at a high frequency in tumors through oncogenic events affecting its upstream regulation (Engelman et al., 2006). The most important upstream activators of AKT are the class I phosphoinositide 3-kinases (PI3K), which generate the lipid second messenger phosphatidylinositol-3,4,5-trisphosphate (PIP3). PI3K lipid products bind directly to the pleckstrin homology (PH) domain of AKT, thereby recruiting AKT to the plasma membrane where it is subsequently activated (see below). Therefore, the activation status of PI3K and the levels of its lipid products are the key factors dictating the level of AKT activity in a cell. PTEN is a lipid phosphatase that removes the critical 3-phosphate from PIP3, thereby reversing PI3K signaling and blocking AKT activation. Importantly, PTEN is encoded by a tumor-suppressor gene that is frequently mutated in human cancers, and these mutations are the likely cause of aberrant AKT activation in many tumors. Activating mutations and amplifications affecting the gene encoding the p110α isoform of PI3K (*PIK3CA*) are also common in cancers. Furthermore, other common oncogene products, such as Ras, receptor tyrosine kinases (e.g., EGFR, HER2, c-Met), and fusion proteins (e.g., BCR-Abl), are all potent activators of PI3K that lead to increased AKT activation. The large number of oncogenes and tumor suppressors upstream of AKT results in aberrantly elevated levels of AKT activation in the majority of sporadic cancers.

Full activation of AKT requires phosphorylation on two highly conserved