Fazi, F., Racanicchi, S., Zardo, G., Starnes, L.M., Mancini, M., Travaglini, L., Diverio, D., Ammatuna, E., Cimino, G., Lo-Coco, F., et al. (2007). Cancer Cell *12*, 457–466.

Jamieson, C.H., Ailles, L.E., Dylla, S.J., Muijtjens, M., Jones, C., Zehnder, J.L., Gotlib, J., Li, K., Manz, M.G., Keating, A., et al. (2004). N. Engl. J. Med. *351*, 657–667.

Lu, J., Getz, G., Miska, E.A., Alvarez-Saavedra, E., Lamb, J., Peck, D., Sweet-Cordero, A., Ebert, B.L., Mak, R.H., Ferrando, A.A., et al. (2005). Nature *435*, 834–838.

Mullighan, C.G., Miller, C.B., Radtke, I., Phillips, L.A., Dalton, J., Ma, J., White, D., Hughes, T.P., Le Beau, M.M., Pui, C.H., et al. (2008). Nature *453*, 110–114.

Ren, R. (2005). Nat. Rev. Cancer *5*, 172–183.

Sercan, H.O., Sercan, Z.Y., Kizildag, S., Undar, B., Soydan, S., and Sakizli, M. (2000). Leuk. Lymphoma *39*, 385–390.

Shah, N.P., and Sawyers, C.L. (2003). Oncogene *22*, 7389–7395.

Yi, R., Poy, M.N., Stoffel, M., and Fuchs, E. (2008). Nature *452*, 225–229.

Zhang, L., Huang, J., Yang, N., Greshock, J., Megraw, M.S., Giannakakis, A., Liang, S., Naylor, T.L., Barchetti, A., Ward, M.R., et al. (2006). Proc. Natl. Acad. Sci. USA *103*, 9136–9141.

The Ups and Downs of Src Regulation: Tumor Suppression by Cbp

Marilyn D. Resh[1,*]
[1]Cell Biology Program, Memorial Sloan-Kettering Cancer Center, 1275 York Avenue, New York, NY 10065, USA
*Correspondence: m-resh@ski.mskcc.org
DOI 10.1016/j.ccr.2008.05.011

Tight control of the tyrosine kinase activity of c-Src is critical for regulating its oncogenic potential. In a recent issue of *Molecular Cell*, Oneyama et al. (2008a) report that the membrane-bound adaptor protein Cbp (also known as PAG) can suppress c-Src-mediated cell transformation and tumorigenesis by binding and sequestering c-Src within lipid rafts. Cbp is also a raft-associated binding partner for Csk, a negative regulator of c-Src. However, the authors show that Cbp-mediated Src suppression is Csk independent. These findings suggest that Cbp is a tumor suppressor whose expression is downregulated during Src-driven cancer progression.

Src family kinases (SFKs) are membrane-bound tyrosine protein kinases that share a common domain structure. The founding member of the family, Src, was originally identified as a retroviral oncoprotein that induces transformation of avian cells and sarcoma formation in chickens. It is now well established that the cellular proto-oncoprotein c-Src plays an influential role in human cancers. Increased levels of c-Src protein and/or tyrosine kinase activity have been detected in multiple tumor types, including breast, colon, lung, head and neck, and pancreatic cancers (Ishizawar and Parsons, 2004). Src activation promotes tumor progression, metastasis, and angiogenesis, while blockade of Src kinase activity results in decreased tumor cell proliferation, migration, and invasion. These findings have served as an impetus for the development of Src kinase inhibitors, several of which are currently in clinical trials for therapeutic treatment of a variety of human cancers.

The kinase activity of SFKs is tightly regulated. A critical negative regulatory tyrosine resides in the C-terminal tail of all SFKs (Tyr527 in c-Src). When Tyr527 is phosphorylated, c-Src is inactive. Dephosphorylation of Tyr527, or mutation of Tyr527 to Phe, activates c-Src and induces cellular transformation. Crystallographic studies have revealed that the structural basis for c-Src regulation involves intramolecular interactions between phospho-Tyr527 and the SH2 domain, and between the SH3 domain and a polyproline-rich region. As a result, the kinase domain is maintained in a closed, inactive conformation in resting cells.

The kinase that phosphorylates Tyr527 is Csk, C-terminal Src kinase. Csk contains an SH3, SH2, and kinase domain but lacks the N-terminal membrane-binding motif (SH4) found in SFKs. Thus, a mechanism must exist to allow cytosolic Csk to gain access to its membrane-bound substrate. In 2000, two groups identified a transmembrane protein, Cbp (also called PAG), that binds Csk (Brdicka et al., 2000; Kawabuchi et al., 2000). Phosphorylation of Cbp by SFKs serves to recruit Csk to membrane-bound Cbp. Csk then phosphorylates and consequently inactivates membrane-bound SFKs.

Given this neatly intertwined set of reactions, one might assume that the ability of Cbp to regulate SFKs is dependent on Csk. However, a recent paper in *Molecular Cell* (Oneyama et al., 2008a) provides several new twists to this scenario. First, Oneyama et al. show that Cbp can function independently of Csk. The authors used mouse embryonic fibroblasts derived from $Csk^{-/-}$ mice. When c-Src is expressed in these cells, it is activated and promotes transformation (Oneyama et al., 2008b). The authors first noted that levels of endogenous Cbp mRNA and protein were reduced when activated c-Src was expressed. They then made the seminal observation that overexpression of exogenous Cbp reversed the

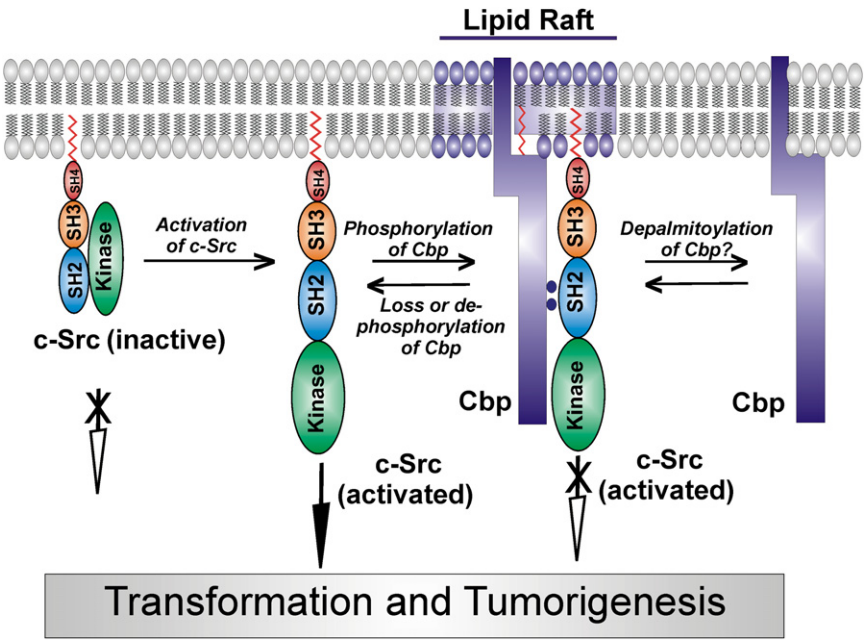

Figure 1. Csk-Independent Inactivation of c-Src by Raft-Associated Cbp
Membrane-bound c-Src can be activated by growth factor receptors, dephosphorylation of Tyr527, and/or binding of SH2 or SH3 ligands. Activated c-Src phosphorylates Cbp and is then recruited to lipid rafts. When associated with raft-bound Cbp, c-Src cannot access its substrates and is incapable of inducing cellular transformation and tumorigenesis. Cbp inactivation by downregulation, dephosphorylation, or depalmitoylation of Cbp could reactivate c-Src.

oncogenic effects of c-Src. Transformed cell morphology was reverted and growth in soft agar was reduced in vitro, and a striking suppression of tumor formation in nude mice occurred in vivo.

A second surprise came when the authors examined the mechanism of Cbp-mediated suppression. Cbp had no effect on intrinsic c-Src tyrosine kinase activity. Instead, Cbp expression altered c-Src localization. Cbp is a palmitoylated protein that concentrates in lipid rafts, membrane microdomains that are enriched in cholesterol and have different biophysical properties from the bulk plasma membrane. Oneyama et al. demonstrated that the SH2 domain of c-Src binds to tyrosine-phosphorylated Cbp. This resulted in recruitment of nearly all of the c-Src to the raft fraction, where it was associated with Cbp (Figure 1).

How does a change in subcellular localization regulate SFK function? The authors found that neither raft-associated c-Src nor raft-associated Fyn, another SFK, was able to transform cells. Moreover, redistribution of Fyn to non-raft fractions, by mutating a Fyn palmitoylation site, increased transforming activity. The association of c-Src and Fyn with rafts and/or raft-associated Cbp apparently blocks their ability to phosphorylate key substrates required for transformation.

If Cbp efficiently sequesters and functionally inactivates Src, one would predict that Cbp could act as a tumor suppressor in cells whose oncogenic potential is driven by activated c-Src. Indeed, loss of Cbp expression was noted in breast and colon cancer cell lines that overexpress c-Src. Conversely, overexpression of Cbp in a colon cancer cell line resulted in association of Cbp and c-Src, c-Src inactivation, and strong inhibition of tumor formation in nude mice.

Implications and Complications

The findings of Oneyama et al. illustrate the multifunctional ability of Cbp to suppress Src function in Csk-dependent and -independent modes. Csk activity is decreased in some colorectal cancers, implying that Csk-independent regulation of Src by Cbp may be operative in human tumors. These findings also predict that downregulation of Cbp should occur in order for Src-driven tumor formation to progress. c-Src induces Cbp loss at the transcriptional level, but the mechanisms responsible are unknown.

Cbp is likely to have a more general role in regulating normal as well as oncogenic signaling by receptor protein tyrosine kinases. EGFR, PDGFR, and FGFR phosphorylate Cbp in response to ligand. For EGFR, this occurs via c-Src (Jiang et al., 2006). Overexpression of Cbp blocks EGFR-mediated c-Src activation, signaling, and cell transformation, while loss of Cbp function has the reverse effect. Thus, Cbp may regulate the synergistic interaction between c-Src and EGFR in breast cancer.

Cbp contains multiple potential sites for protein:protein interaction that enable it to serve as a scaffold for signaling proteins. The cytoplasmic domain has two proline-rich SH3-binding motifs and ten tyrosines, nine of which are SFK target phosphorylation sites. Phosphorylated Cbp can recruit the SH2 domain-containing proteins Csk, SFKs, and SOCS1 (Ingley, 2008). In addition, Cbp binds to EBP50, an ezrin-binding protein, thereby linking Cbp-containing rafts to the actin cytoskeleton.

How can Cbp function be turned off (Figure 1)? Dephosphorylation of Cbp would result in dissociation of the bound SFK and other signaling proteins. The phosphatase (or phosphatases) that dephosphorylates Cbp has not been definitely identified, although SHP2 and CD45 may play a role. Alternatively, Cbp could be removed from lipid rafts by depalmitoylation. Many palmitoylated proteins undergo dynamic cycles of palmitoylation/depalmitoylation (Resh, 2006). Depalmitoylation would promote redistribution of Cbp, and Cbp-associated proteins, from raft domains into the bulk plasma membrane, where signaling properties may differ.

The findings of Oneyama et al. complicate our understanding of lipid rafts: do they play a positive or negative regulatory role in signal transduction? There is ample evidence in the literature that rafts are positive hubs for signaling by activated receptors and their associated SFKs. For example, SFKs need to be localized in rafts to mediate downstream signaling by immune cell receptors (Resh, 2006). Moreover, two recent studies have shown that SFKs are active (Solheim et al., 2008) and can drive cancer cell growth (Tauzin et al., 2008) even when bound to raft-associated Cbp.

The findings of Oneyama et al. stand in stark contrast—raft-associated c-Src was inactive. These discrepancies might be due to differences in the SFK, fatty acylation status, cell type, extent of Csk-Cbp interaction, and/or association with different subsets of rafts. Perhaps the best way to reconcile these disparate findings is to propose that Cbp can function as both a positive and negative regulator of SFKs. In this regard, the work of Oneyama et al. broadens our appreciation of the multiple levels at which SFK activity and tumorigenesis can be regulated.

REFERENCES

Brdicka, T., Pavlistova, D., Leo, A., Bruyns, E., Korinek, V., Angelisova, P., Scherer, J., Shevchenko, A., Hilgert, I., Cerny, J., et al. (2000). J. Exp. Med. *191*, 1591–1604.

Ingley, E. (2008). Biochim. Biophys. Acta *1784*, 56–65.

Ishizawar, R., and Parsons, S.J. (2004). Cancer Cell *6*, 209–214.

Jiang, L.Q., Feng, X., Zhou, W., Knyazev, P.G., Ullrich, A., and Chen, Z. (2006). Oncogene *25*, 5495–5506.

Kawabuchi, M., Satomi, Y., Takao, T., Shimonishi, Y., Nada, S., Nagai, K., Tarakhovsky, A., and Okada, M. (2000). Nature *404*, 999–1003.

Oneyama, C., Hikita, T., Enya, K., Dobenecker, M.-W., Saito, K., Nada, S., Tarakhovsky, A., and Okada, M. (2008a). Mol. Cell *30*, 426–436.

Oneyama, C., Hikita, T., Nada, S., and Okada, M. (2008b). Genes Cells *13*, 1–12.

Resh, M.D. (2006). Nat. Chem. Biol. *2*, 584–590.

Solheim, S.A., Torgersen, K.M., Tasken, K., and Berge, T. (2008). J. Biol. Chem. *283*, 2773–2783.

Tauzin, S., Ding, H., Khatib, K., Ahmad, I., Burdevet, D., van Echten-Deckert, G., Lindquist, J.A., Schraven, B., Din, N.U., Borisch, B., et al. (2008). Blood *111*, 2310–2320.