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IN FOCUS

Src turns FHL1 to the dark side

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Phosphorylation by Src causes FHL1 to move into the nucleus and converts it from a tumor suppressor to a tumor promoter.

FHL1 belongs to a family of proteins that contain four and a half LIM domains, protein modules that mediate interactions with the actin cytoskeleton and transcriptional machinery. FHL1 itself localizes to both focal adhesions and the cell nucleus, and acts as a tumor suppressor by inhibiting cell growth and migration. However, in this issue, Wang et al. reveal that FHL1 can also promote tumor growth when it is phosphorylated by the tyrosine kinase Src (1).

In accordance with its function as a tumor suppressor, FHL1 is down-regulated in a variety of cancers, including lung, breast, and colon cancer. But, by binding and inhibiting the cell regulator CDC25C, FHL1 promotes resistance to radiation-induced DNA damage, and increased FHL1 expression therefore correlates with poorer survival rates in breast cancer patients who received radiotherapy (2). "This indicates that the role and mechanism of FHL1 in cancer progression is more complex and diverse than previously thought," explains Hongquan Zhang, from Peking University Health Science Center in China. "Therefore, we set out to investigate whether posttranslational modification of FHL1 plays an important role in cancer progression."

"Zhang and colleagues, led by Xiang Wang and Xiaofan Wei, focused on protein kinases that mediate integrin-based signaling at focal adhesions and found that the tyrosine kinase Src can bind to FHL1 and phosphorylate the protein on two tyrosine residues, Y149 and Y272 (1).

Src's activity at focal adhesions is regulated by its interaction with the integrin-binding protein kindlin-2 (3). The researchers found that kindlin-2 also binds and recruits FHL1, forming a tripartite complex with Src at focal adhesions. This complex is likely to be transient, however, because kindlin-2 and Src compete for binding to FHL1's fourth LIM domain. Overexpressing kindlin-2 inhibited FHL1's association with Src and suppressed its phosphorylation. In contrast, knocking down kindlin-2 enhanced FHL1 phosphorylation.

Focal Point (Left to right) Xiang Wang, Xiaofan Wei, Hongquan Zhang, and colleagues determine that the tumor suppressor FHL1 can be converted to a tumor promoter by the tyrosine kinase Src, which phosphorylates FHL1 and induces its translocation from focal adhesions to the cell nucleus, where it interacts with the transcription factor BCLAF1. The focal adhesion protein kindlin-2 opposes this process by competing with Src for binding to FHL1. Tissue sections from a lung adenocarcinoma patient show that, compared with surrounding, normal tissue (N), phospho-FHL1 levels (top right) are elevated in the tumor (T), even though total levels of FHL1 (bottom right) are reduced. PHOTOS COURTESY OF THE AUTHORS.

The researchers found that Src-dependent phosphorylation stimulates FHL1's translocation into the nucleus, where it binds to the transcription factor BCLAF1. To investigate whether this alters FHL1's tumor suppressor function, Wang et al. transfected wild-type and mutant versions of FHL1 into lung carcinoma cells. As expected, wild-type FHL1 suppressed the cells' growth and migration in vitro and a nonphosphorylatable version had an even stronger suppressive effect. In contrast, an FHL1 mutant in which Y149 and Y272 were replaced with phosphomimetic aspartate residues stimulated proliferation and migration.

The researchers saw a similar effect in vivo when they injected these cancer cells into mice. Cells expressing phosphomimetic FHL1 grew faster and formed much larger tumors than cells expressing wild-type or nonphosphorylatable FHL1. But knocking down BCLAF1 prevented phospho-FHL1 from promoting tumor growth, indicating that the interaction between these two proteins in the nucleus is crucial for FHL1's ability to stimulate cell proliferation.

Wang et al. then investigated whether phosphorylated FHL1 could have a similar effect in human tumors. The researchers examined a variety of patient samples and found that FHL1 phosphorylation was elevated in lung, liver, gastric, rectal, and esophageal cancers, even though the total levels of FHL1 expression were reduced in these tumors. "From our analysis of human cancer samples and databases, we've learned that it is insufficient for a study to only analyze the total level of a protein when evaluating its role in cancer," Zhang says. "It is important to examine the protein's posttranslational modifications and understand the roles of posttranslational modifications in cancer progression."

FHL1's role in tumor progression is likely to depend on the relative levels of kindlin-2 and Src activity, determining whether FHL1 remains unphosphorylated at focal adhesions, where it suppresses tumorigenesis, or is phosphorylated and translocated into the nucleus, where it can stimulate tumor growth. Zhang and colleagues now want to investigate the role of other adhesion-associated proteins in the occurrence and development of cancer.

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3. Liu, Z., et al. 2015. *FEBS Lett.* 589:2001–2010.

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