A MENage à Trois in Leukemia

Liya Roudaia[1] and Nancy A. Speck[1,*]
[1]Department of Biochemistry, Dartmouth Medical School, Hanover, NH 03755, USA
*Correspondence: nancy.speck@dartmouth.edu
DOI 10.1016/j.ccr.2008.06.009

Menin binds to the N terminus of the chromatin-remodeling histone methyltransferase MLL and is essential for the transforming activity of at least several oncogenic MLL fusion proteins. In this issue, Yokoyama and Cleary (2008) show that menin's essential, and perhaps only, contribution to leukemia is to tether a third protein, LEDGF—a chromatin-associated protein implicated in leukemia and several other disease states—to MLL. Thus, this study identifies a new, critical player in leukemias caused by MLL fusion proteins and defines the biochemical function of menin in the MLL complex.

The myeloid/lymphoid or mixed-lineage leukemia (*MLL*) gene is targeted by at least 50 different chromosomal translocations in leukemias with a particularly poor prognosis (Krivtsov and Armstrong, 2007). The translocations result in the production of chimeric proteins, all of which contain the N-terminal portion of MLL fused with a large assortment of partners that seem to contribute either a transactivation or oligomerization domain to the oncogenic fusion protein. MLL is a very large nuclear protein that is homologous to *Drosophila* trithorax and functions to maintain gene expression during development. It contains at least five conserved domains, including AT hooks that can mediate DNA binding and a SET domain that can methylate lysine 4 on histone H3 tails (a positive transcriptional epigenetic mark).

MLL interacts with a large number of proteins. Yokoyama, Cleary, and colleagues previously purified an MLL complex containing eight polypeptides including several members of a Set1 methyltransferase complex as well as a ubiquitously expressed protein called menin (Yokoyama et al., 2004). In a reciprocal experiment, the MLL homolog MLL2 was immunoprecipitated from 293T cells using antibodies against menin (Hughes et al., 2004). Menin is a nuclear protein whose loss or inactivation causes multiple endocrine neoplasia type 1 (MEN1, OMIM 121100), a syndrome characterized by a triad of tumors of parathyroid, enteropancreatic endocrine, and anterior pituitary gland origin (Chandrasekharappa and Teh, 2003). Somatic MEN1 mutations and loss of heterozygosity have also been found in a variety of sporadic tumors of endocrine and nonendocrine origin. Menin binds with high affinity to a conserved sequence located at the extreme N terminus of MLL that is retained in all oncogenic MLL fusion proteins (Caslini et al., 2007; Yokoyama et al., 2005). A small deletion removing the menin binding site in the MLL-ENL, MLL-GAS7, MLL-AF10, or MLL-AF9 oncogenic fusion proteins destroys their transforming activity (Caslini et al., 2007; Yokoyama et al., 2005). Similarly, loss of menin obliterates the ability of multiple MLL fusion proteins to sustain transformation of hematopoietic cells, establishing the MLL/menin duo as an essential contributor to MLL oncoprotein-mediated leukemogenesis (Yokoyama et al., 2005). However, menin does not contain any known protein domains, and it has been shown to interact with multiple nuclear proteins including histone methyltransferases, histone deacetylases, and various transcription factors. Thus, the exact molecular contribution of menin to endogenous MLL and MLL fusion protein activity is poorly understood.

In a study in this issue of *Cancer Cell*, Yokoyama and Cleary (2008) purified MLL-interacting proteins from cells overexpressing tagged versions of both MLL and menin, with the notion that more weakly interacting proteins might be identified by this strategy. One of the proteins they copurified was LEDGF (lens epithelium-derived growth factor), a protein previously implicated in cancer, autoimmunity, and AIDS (Engelman and Cherepanov, 2008). LEDGF is also directly involved in leukemia through translocation-induced fusion to the NUP98 protein (Ahuja et al., 2000). LEDGF is a member of the hepatoma-derived growth factor-related family of proteins. It appears to be involved in a number of transcriptional processes—it was found associated with RNA polymerase II (RNA pol II), and it regulates stress-induced genes (Shinohara et al., 2002). LEDGF also binds tightly to the HIV-1 integrase and is thought to play a major role in tethering the HIV-1 preintegration complex to active chromatin through a PWWP domain and AT hooks located in the N-terminal half of the protein (Engelman and Cherepanov, 2008). The PWWP domain is conserved in other chromatin-binding proteins involved in DNA repair, methylation, and transcription and has been shown to target DNA methyltransferases to chromatin (Chen et al., 2004; Ge et al., 2004).

The authors demonstrate in multiple ways that MLL, menin, and LEDGF are a threesome. Both LEDGF and menin bind MLL via nonoverlapping sites, and the interaction of LEDGF with MLL requires menin. All three proteins occupy target genes (*HOXA*, *MEIS1*, and *CDKI*) that have been implicated in MLL oncoprotein-mediated disease and presumably work together to upregulate the expression of those genes.

Yokoyama and Cleary (2008) speculated that menin's role in this triad is to tether MLL to LEDGF and that LEDGF in turn directs the complex to chromatin via its PWWP domain (Yokoyama and Cleary, 2008). In an elegant series of experiments, they provide compelling evidence for this model by showing that MLL's dependence on menin and LEDGF can be entirely circumvented by simply substituting the menin binding site in an MLL oncoprotein (MLL-ENL) with the PWWP domain from LEDGF (Figure 1). This modified

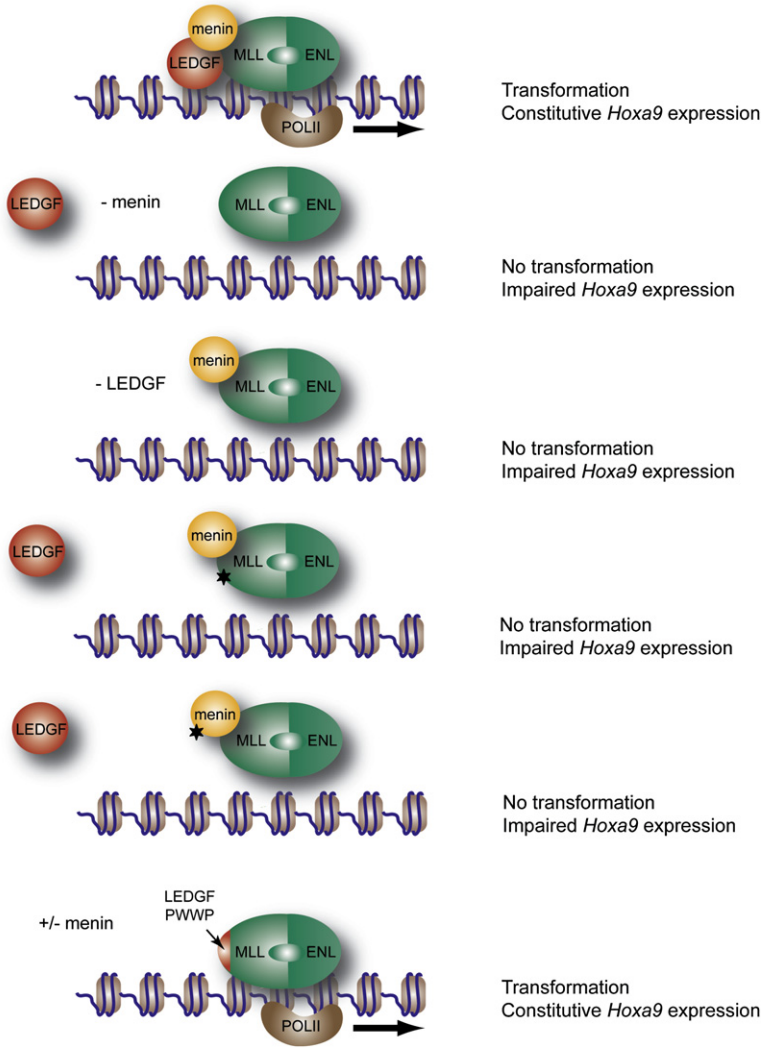

Figure 1. Multiple Lines of Evidence Demonstrate that Menin's Role Is to Tether LEDGF to MLL

Conditional deletion of menin, knockdown of LEDGF, and mutations that disrupt binding of LEDGF to either MLL or menin all impair the MLL-ENL fusion protein's transforming activity and constitutive expression of the MLL target *Hoxa9*. Fusion of LEDGF's chromatin-binding PWWP domain to MLL supplants menin activity.

PWWP-domain-containing MLL-ENL fusion protein, now incapable of binding either menin (because its binding site is missing) or LEDGF (whose binding to MLL requires menin), can perform functions that normally require all three proteins. For example, PWWP-MLL-ENL can occupy the *Hoxa9* locus (a well-known MLL target gene), upregulate the expression of Hoxa9, and, most importantly, transform myeloid lineage cells and induce leukemia in mice with the same latency as the unmodified MLL-ENL protein. To further drive this point home, the authors show that PWWP-MLL-ENL (but not MLL-ENL) is no longer dependent on menin to maintain the transformation of myeloid progenitors. They also show that knocking down LEDGF reduces both the clonogenic potential of MLL-ENL transformed cells and Hoxa9 expression. Finally, the authors examined the ability of mutant menin proteins found in MEN1 patients to bind MLL and LEDGF and to partner with MLL fusion proteins in leukemia. More than 300 different mutations, including missense and in-frame deletions, have been found throughout the length of the *MEN1* gene in various tumors (Chandrasekharappa and Teh, 2003). Yokoyama and Cleary found that some mutated menin proteins retain their association with MLL but cannot bind LEDGF and cannot cooperate with MLL fusion proteins in leukemia. Therefore, menin's liaison with LEDGF is essential for the oncogenic activity of MLL fusion proteins, and potentially for its tumor suppressor activity in MEN1.

In summary, Yokoyama and Cleary (2008) demonstrate a key role for LEDGF in MLL's leukemic activity and in addition show that menin's only job is to tether MLL to LEDGF. These results raise several interesting and important questions. For example, do all MLL fusion proteins require LEDGF? The requirement for LEDGF was demonstrated for MLL-ENL and MLL-AF10, both of which contain MLL fused to the transactivation domains of putative DNA-binding proteins. But MLL can also be fused to proteins with oligomerization domains, to septins, and to histone acetyltransferases (Krivtsov and Armstrong, 2007). Whether these other types of MLL fusion proteins transform by the same mechanism as MLL-ENL and MLL-AF10, and whether they also require LEDGF, is unknown. Global chromatin immunoprecipitation studies showed that MLL occupies up to 90% of the sites occupied by RNA pol II. Is LEDGF co-occupying all of these sites? If not, what is the functional outcome of LEDGF occupancy? LEDGF is variously thought to influence the efficiency of HIV-1 integration and target site selection. Does LEDGF affect the efficiency of MLL binding or activity? Does it bind chromatin cooperatively with MLL (or for that matter, can MLL occupy chromatin in the absence of LEDGF)? And is the MLL/menin/LEDGF threesome the final story, or is there a poly family of MLL-associated proteins, all of which are essential for MLL's function in development and leukemia? The answers to these and other questions will undoubtedly shed light on the epigenetic regulation of gene expression by MLL and its oncogenic derivatives and will hopefully yield much-needed targets for treating the difficult MLL fusion protein leukemias.

REFERENCES

Ahuja, H.G., Hong, J., Aplan, P.D., Tcheurekdjian, L., Forman, S.J., and Slovak, M.L. (2000). Cancer Res. *60*, 6227–6229.

Caslini, C., Yang, Z., El-Osta, M., Milne, T.A., Slany, R.K., and Hess, J.L. (2007). Cancer Res. *67*, 7275–7283.

Chandrasekharappa, S.C., and Teh, B.T. (2003). J. Intern. Med. *253*, 606–615.

Chen, T., Tsujimoto, N., and Li, E. (2004). Mol. Cell. Biol. *24*, 9048–9058.

Engelman, A., and Cherepanov, P. (2008). PLoS Pathog. *4*, e1000046.

Ge, Y.Z., Pu, M.T., Gowher, H., Wu, H.P., Ding, J.P., Jeltsch, A., and Xu, G.L. (2004). J. Biol. Chem. *279*, 25447–25454.

Hughes, C.M., Rozenblatt-Rosen, O., Milne, T.A., Copeland, T.D., Levine, S.S., Lee, J.C., Hayes, D.N., Shanmugam, K.S., Bhattacharjee, A., Biondi, C.A., et al. (2004). Mol. Cell *13*, 587–597.

Krivtsov, A.V., and Armstrong, S.A. (2007). Nat. Rev. Cancer *7*, 823–833.

Shinohara, T., Singh, D.P., and Fatma, N. (2002). Prog. Retin. Eye Res. *21*, 341–358.

Yokoyama, A., and Cleary, M.L. (2008). Cancer Cell *14*, this issue, 36–46.

Yokoyama, A., Wang, Z., Wysocka, J., Sanyal, M., Aufiero, D.J., Kitabayashi, I., Herr, W., and Cleary, M.L. (2004). Mol. Cell. Biol. *24*, 5639–5649.

Yokoyama, A., Somervaille, T.C., Smith, K.S., Rozenblatt-Rosen, O., Meyerson, M., and Cleary, M.L. (2005). Cell *123*, 207–218.

Deregulating EMT and Senescence: Double Impact by a Single Twist

Marjon A. Smit[1] and Daniel S. Peeper[1,*]
[1]Division of Molecular Genetics, The Netherlands Cancer Institute, Plesmanlaan 121, 1066 CX Amsterdam, The Netherlands
*Correspondence: d.peeper@nki.nl
DOI 10.1016/j.ccr.2008.06.012

The acquisition of a fully malignant phenotype is limited by several barriers, including cellular senescence and the requirement to undergo an epithelial-mesenchymal transition (EMT). Deregulation of these processes is believed to occur by largely independent events. In this issue of *Cancer Cell*, Ansieau et al. (2008) challenge this view.

Tissues often contain several small and inconspicuous neoplastic lesions, which rarely progress to full malignancy. It is believed that multiple factors underlie this phenomenon. These include apoptosis, the necessity to acquire invasive competence, and the activation of a cellular senescence program. The last of these is receiving increased attention, moving from being a phenomenon seen exclusively in cultured cells to one constituting a critical tumor suppression function in vivo. It can be triggered by telomere malfunction or unscheduled activation of oncogenic signaling (Prieur and Peeper, 2008). Cellular (oncogene-induced) senescence is characterized by a largely irreversible cell-cycle arrest, often a typical flattened cell morphology, and induction of a tumor suppressor network.

Another factor proposed to limit progression of epithelial tumors is the requirement to undergo epithelial-mesenchymal transition (EMT). While important for embryonic development, EMT is often adopted by cancer cells, endowing them with a migratory and/or invasive phenotype. It is characterized by decreased cell adhesion, which is usually accompanied by the downregulation of E-cadherin. This important epithelial adhesion protein is regulated by several transcription factors, including Twist, Snail, and Zeb1.

Although these tumor-restricting processes are generally seen as largely independent traits, Ansieau et al. (2008) report that, in fact, they may not be distinctly autonomous as previously thought. The authors asked whether Twist1 (overexpression of which is linked to breast cancer infiltration; Yang et al. [2004]) and its close cousin Twist2 are activated in cancer. To address this, they analyzed MMTV-*ErbB2*-driven murine mammary tumors, where *TWIST2* levels were increased in the majority of these lesions. When extending this study to human breast carcinomas, the authors observed instead that *TWIST1* was upregulated in half of the cases.

The notion that Twist1 may play a relatively more important role in human breast cancer was supported when Twist proteins were depleted from T47D human breast cancer cells harboring multiple copies of *ERBB2*. The authors observed that only *TWIST1* knockdown resulted in a moderate increase in senescence-associated β-galactosidase activity (SA-β-gal, a commonly used marker of senescence). This was accompanied by a modest increase in the number of cells that actually underwent cell-cycle arrest, which may be explained by assuming that the genetic wiring implementing senescence-associated proliferative arrest in these cancer cells is disrupted. In human RPMI 7951 melanoma cells harboring a mutant $BRAF^{E600}$ oncoprotein, codepletion of the two Twist proteins resulted in a similar response. An interesting question prompted by these observations is whether in the same setting, Twist is required for the invasive and metastatic capacities of these cells. Previous work has shown this to be the case within the context of murine breast tumor cells (Yang et al., 2004).

A connection between Twist, senescence, and the cell-cycle machinery was initially suggested almost a decade ago (Maestro et al., 1999). Twist1 and Twist2 (also called Dermo1) were identified in a screen for genes antagonizing Myc-induced apoptosis. While Twist1 reversed