2008

Biphasic MLL takes helm at cell cycle control: Implications in human mixed lineage leukemia

Han Liu
Washington University School of Medicine in St. Louis

Shugaku Takeda
Washington University School of Medicine in St. Louis

Emily H.Y. Cheng
Washington University School of Medicine in St. Louis

James J.D. Hsieh
Washington University School of Medicine in St. Louis

Follow this and additional works at: http://digitalcommons.wustl.edu/open_access_pubs

Recommended Citation
http://digitalcommons.wustl.edu/open_access_pubs/3018

This Open Access Publication is brought to you for free and open access by Digital Commons@Becker. It has been accepted for inclusion in Open Access Publications by an authorized administrator of Digital Commons@Becker. For more information, please contact engeszer@wustl.edu.
Biphasic MLL takes helm at cell cycle control
Implications in human mixed lineage leukemia

Han Liu, Shugaku Takeda, Emily H.Y. Cheng and James J.D. Hsieh*

Molecular Oncology, Department of Medicine, Siteman Cancer Center, Washington University School of Medicine, St. Louis, Missouri, USA

Abbreviations: MLL, mixed lineage leukemia or myeloid lymphoid leukemia; HRX, human trithorax; ALL-1, acute lymphoblastic leukemia 1; trx, trithorax; Taspase1, threonine aspartase 1; K4, lysine 4; SET, su(var)3-9, enhancer of zeste, trithorax; Trx-G, trithorax group proteins; Pc-G, polycomb group proteins; HSC, hematopoietic stem cells; CMP, common myeloid progenitors; GMP, granulocytic/monocytic-restricted progenitors; MEP, megalakaryocytic/erythroid-restricted progenitors; aa, amino acid; TFIIA, basal transcription factor II A; ALF, TFIIA-like factor; HCF, host cell factor; CDK, cyclin dependent kinase; CDKI, CDK inhibitor; UPS, ubiquitin-proteasome system; SCF, Skp1-Cul1-F-box protein; APC, anaphase-promoting complex/cyclosome; FYRN, F (phenylalanine) Y (tyrosine) rich N (amino) terminus; FYRC, F (phenylalanine) Y (tyrosine) rich C (carboxy) terminus; PHD, plant homeodomain; DNMT, DNA methyltransferase homology domain; TA, transactivation domain; CS, cleavage site; Rb, retinoblastoma protein; AML, acute myeloid leukemia; ALL, acute lymphoblastic leukemia

Key words: MLL, taspase1, E2F, cyclin, CDK inhibitors, Skp2, Cdc20, Hox, cell cycle, leukemia

Discovered in 1992 from cloning the gene involved in human leukemias carrying chromosome band 11q23 translocations, the MLL/HRX/ALL-1 gene has since attracted scientists from various disciplines by its diverse functions in normal physiological and pathological processes. MLL is the human orthologue of Drosophila trithorax (trx)—the founding member of trithorax group proteins, Trx-G. Leukemogenic11q23 translocations fuse the common MLL N-terminal 1400aa in-frame with a wide variety of fusion partners that share no structural or functional homology. The 500 kD precursor MLL undergoes evolutionarily conserved site-specific cleavage mediated by Taspase1, generating the mature MLLN320/C180 heterodimer which methylates histone H3 at lysine 4 with its carboxy-terminal SET domain. Extensive biochemical and genetic studies on MLL/trx have established its critical role in maintaining the expression of Hox/hematopic genes. By contrast, the involvement of MLL in many other essential cellular processes remains unclear. Recent reports including ours began to elucidate the intricate interplay between MLL and the cell cycle machinery, which ensures proper cell cycle phase transitions. Thus, this review will focus on this novel activity of MLL and discuss the implications of its deregulation in MLL leukemias.

The MLL Leukemia

Although modern therapy for acute leukemia has improved greatly, there are genetic subsets of patients who respond very poorly to chemotherapy or relapse shortly post induction chemotherapy. Leukemias with chromosome band 11q23 translocations are associated with early relapse, which involves the human Mixed Lineage Leukemia (MLL/HRX/ALL-1) gene.1-12 MLL translocations are found in at least 10% of de novo ALL and AML in children and adults. Of note, 80% of acute lymphoblastic leukemia (ALL) and acute myeloid leukemia (AML) that arise in infants, and almost all cases of secondary AML that arise after treatment with topoisomerase II inhibitors are associated with MLL translocations. The leukemia blasts in infant ALL are usually CD19+, HLA-DR+, TdT+, CALLA- and cytoplasmic Ig-, consistent with an early precursor B-ALL. Furthermore, they also express the myelomonocytic marker, CD15.13-15 The mixed phenotype of these blasts has led to a proposal that 11q23 abnormalities transform a multipotent stem cell or interrupt a differentiating cell.

Based on gene expression profiles, several groups had reported that ALL possessing a rearranged MLL exhibits a highly uniform and distinct pattern that clearly distinguishes them from conventional ALL or AML.16-18 Their gene expression profiles are consistent with an early hematopoietic progenitor stage, expressing select multi-lineage markers and individual HOX genes. Studies using retrovirus-mediated gene transduction of MLL-Fusions in purified hematopoietic progenitors established that MLL leukemias can arise from hematopoietic stem cells (HSC), common myeloid progenitors (CMP), and granulocytic/monocytic-restricted progenitors (GMP), but not megalakaryocytic/erythroid-restricted progenitors (MEP).19-21

Functions of the MLL Gene

Located at the human chromosome band 11q23 breakpoints, MLL is the mammalian counterpart of Drosophila trithorax (Trx), the founder of trithorax group (Trx-G) genes.22-27 It is well characterized that trithorax group (Trx-G) proteins antagonize with polycomb group (Pc-G) proteins for proper Hox/hematopic gene expression.
Mll in the cell cycle driver’s seat

As a transcription coactivator, MLL/T rx is required for the maintenance of spatial patterns of Hox and HOM-C (homeotic complex) genes in vertebrates and invertebrates, respectively. Homozygous deficiency for MLL results in early embryonic lethality at embryonic day 10.5 (E10.5), exhibiting multiple patterning defects, and heterozygous deletion of MLL incurs homeotic transformation, indicating altered Hox gene expression. Recent studies also indicated an essential role of MLL in hematopoiesis. The MLL gene encodes a 3,969 amino acid, 500 kD protein consisting of multiple conserved domains with distinct biochemical properties (Fig. 1). (1) Three AT hooks, found near the N-terminus of MLL, mediate its binding to the minor groove of AT-rich DNA region. (2) A transcription repression domain consists of a cysteine-rich CXXC DNMT (DNA methyltransferase) homology region. (3) Four PHD fingers mediate protein-protein interactions. (4) A transactivation (TA) domain interacts with p300/CBP. (5) A C-terminal SET domain functions as a histone methyl transferase (HMT) that methylates Histone H3 at K4, marking transcriptionally active genes. Over the years, a plethora of MLL interaction partners have been identified, providing additional mechanistic insights regarding how MLL regulates complex gene expression. MLL complexes with Menin (a tumor suppressor), cell cycle regulators (E2Fs and HCF), Pc-G proteins (Bmi-1 and HPC2), HDACs (Histone Deacetylases), Cyp33 (a nuclear cyclophilin), CBP/P300 (Histone acetylase), INI1/SNF5 (chromatin remodeling factors) and core components of the H3K4 histone methyl transferase (WDR5 and Rbbp5).

In addition to the aforementioned complexity of MLL gene regulation, we and others showed that the 500 kD full length MLL precursor (MLLFL) undergoes evolutionarily conserved site-specific proteolysis to generate a mature MLLN320/C180 heterodimer, consisting of the processed N-terminal 320 kD and C-terminal 180 kD fragments.

Taspase1 Cleaves MLL: An Essential Step in MLL Activation

Proteolysis of MLL is an evolutionarily conserved process in that canonical cleavage sites (QV(L)D/GXXD) exist among all MLL orthologues from insects to vertebrates. To understand the biological significance of MLL cleavage, we purified the responsible protease. Biochemical purification of the MLL processing enzyme identified a previously uncharacterized orf13 (open reading frame) on human chromosome 20 (c20orf13). Detailed studies of c20orf13 confirmed that it encodes the bona fide protease mediating MLL proteolysis both in vitro and in vivo. We named it Taspase1 (threonine aspartase 1) and the discovery of Taspase1 founded a new class of endopeptidases that utilize the N-terminal threonine of its mature β subunit as the active site nucleophile to cleave polypeptide substrates after an aspartate.

Figure 1. A diagram depicts the regulatory cascades initiated by the Taspase1-mediated cleavage of MLL. Taspase1 proenzyme undergoes autotwoproteolysis to form the mature α28/β22 protease which cleaves precursor MLL at conserved cleavage site (CS) to generate N-terminal 320 kD and C-terminal 180 kD fragments which heterodimerize as a fully functional histone H3 K4 methyl transferase. MLL actively participates in cell cycle progression (Left) and embryonic development (Right) through directly controlling cell cycle genes and HOX clusters, respectively. The four human HOX clusters are outlined. The activity of Cdc20 is kept in check by the cell cycle UPS, thus facilitating the accurate execution of the cell progression cycle. T denotes threonine.
The Intricate Interplay Between MLL and Cell Cycle Machinery

Eukaryotic cell cycle consists of a tightly orchestrated circular progression of a sequence of distinct phases—namely G1, S, G2, and M—designed for the execution of inherent genetic programs.57 Key regulatory components of the mammalian cell cycle machinery include E2Fs, Rbs, Cyclins, Cyclin dependent kinases (CDKs), and CDK inhibitors (CDKIs), which form complex positive and negative epistatic circuits to ensure accurate cell cycle progression.58 The molecular blueprint of a normal cell cycle details the central controls held by a series of CDKs which positively influence proliferation through phosphorylation of target proteins such as Rbs.59 This releases the repressive activities of Rbs on E2Fs—executors of the cell cycle genetic program.60–64 Individual CDKs need to complex with specialized Cyclins to form catalytically active CDK/Cyclins. Therefore, their activities depend on the availability of involved Cyclins. Additional regulation of CDK/Cyclins comes from negative factors, the CDKIs.65 Levels of Cyclins and CDKIs undulate during the cell division cycle, which in part is due to defined windows of degradation by the ubiquitin-proteasome system (UPS).66 UPS commences at substrate recognition and the subsequent covalent conjugation of ubiquitin by responsible E3 ligases, followed by degradation executed by the 26S proteasome.67 The two major E3 complexes involved in proteolyzing core components of the cell cycle machinery are SCF (Skp1-Cul1-F-box protein) and APC (anaphase-promoting complex/cyclosome) complexes, of which the substrate recognition is conducted by the variable components—F box proteins for SCF, and Cdc20 or Cdh1 for APC.68–70 Thus, two types of post-translational modifications—phosphorylation and ubiquitination—constitute the two main controls of cell cycle.

Our studies on cells deficient for Taspase1 or bearing non-cleavable alleles of MLL, MLL<sup>nc/nc</sup>, recognize the participation of MLL through E2Fs in regulating cell proliferation, linking MLL proteolysis to the heart of cell cycle gene programming.44 The essential role of MLL in cell cycle progression through the MLL-E2F axis is further supported by a recent report indicating that HCF-1, MLL and E2F form tertiary complex on E2F-responsive promoters during the G<sub>1</sub>/S phase transition, thereby inducing histone methylation and transcriptional activation.45 The active involvement of MLL/Trx in the cell cycle is conserved through evolution in that genetic studies in Drosophila also highlight a critical role of Trx in cell proliferation.71 Additionally, several recent reports also identified MLL as a direct activator of Cyclins and CDKIs.44,45,72,73

To further investigate how MLL regulates the cell cycle, we examined protein level of MLL through the cell cycle and observed a tightly controlled biphasic expression of MLL in all cell types.74 This unique expression is conferred by defined windows of degradation mediated by specialized cell cycle E3 ligases, SCFSkp2 and APC<sub>Cdc20</sub> (Fig. 1).74 Importantly, two peak expressions of MLL ensure corresponding G<sub>1</sub>/S transition and M phase progression. Disruption of this unique expression pattern by shRNA-mediated knockdown of MLL causes defects in G<sub>1</sub>/S entry and M phase progression.74 Furthermore, overexpression of MLL incurs specific S phase defects, indicating the importance of downregulating its activity in the S phase. However, whether this is resulted from sustained expression of downstream key cell cycle regulators, and/or overexpressed MLL invoked yet identified insults remains to be determined.

These data not only highlight the significance of biphasic MLL expression in orchestrating cell division cycle but also uncover a novel mechanism by which the cell cycle machinery regulates MLL.74 This establishes another irreversible post-translational regulation of MLL through degradation in addition to Taspase1-mediated site-specific
proteolysis. As MLL directly activates transcription of Cyclins that exhibit periodic expression during cell proliferation, it is imperative for a cell to incorporate MLL expression into the intricately assembled cell cycle circuitry, ensuring a correct transition of progressive phases.

It seems counterintuitive at the first glance that MLL/Trx activates both Cyclins 44,45 and CDKIs 72,73 positive and negative regulators of the cell cycle, respectively. It is plausible that MLL/Trx targets downstream genes in a cell cycle phase-dependent manner. Indeed, a cell cycle specific recruitment of MLL/Trx to its target genes such as CyclinA has been reported.45 Biphasic expression of MLL assures target genes to be activated and inactivated following a tightly choreographed temporal sequence. Based on available reports, we propose the following model detailing how MLL coordinates the G1/S transition (Fig. 2).44,45,73-76 In the mid-G1 phase, the rising MLL level activates both p27 and CyclinE that assemble into an inactive CDK2/CyclinE/p27 complex which prepares the cell for G1/S entry. In the late G1 phase, MLL further activates CyclinE leading to an excess of active CDK2/CyclinE complex that marks p27 for degradation through phosphorylation. Downregulated p27 along with the concurrent increase of CyclinE eventually marshals cells through the G1/S transition. Once entering the G1/S transition, MLL is recruited to the promoter of CyclinA.45 Increased CyclinA replaces CyclinE, thereby facilitating the assembly of CDK2/CyclinA complex for S phase progression. Upon a successful entry into the S phase, MLL is no longer needed and thus degraded by the SCF5Skp2 E3 ligase (Fig. 2).

**Models of the MLL Leukemia**

Human chromosome 11q23 aberrations disrupting MLL lead to infant and therapy-related leukemias. These balanced translocations fuse the N-terminal ~1400 aa of MLL in-frame with a wide spectrum of fusion partners, generating pathognomonic leukemogenic MLL-Fusions. Mysteriously, among these (>50) fusion partners, no shared characteristics were recognized based on sequence or structure homology.1-11 Genetic studies on mice carrying individual MLL-Fusions reveal several fundamental aspects concerning the MLL leukemia. First, MLL-Fusions from 11q23 translocations are responsible for the leukemogenesis.77-81 Second, fusion partners are indispensable.82 Third, partners can be as non-specific as bacterial galactosidase (LacZ) in that mice bearing MLL-LacZ developed myeloid leukemias after a prolonged latency.82 Fourth, individual partners determine the phenotypes of resulting leukemias. For example, mice carrying MLL-AF4 or MLL-AF9 develop lymphoid versus myeloid malignancies, mimicking their respective human counterparts.78,80,83 Detailed analyses of individual MLL-Fusions using retrovirus-mediated gene transduction of hematopoietic stem/progenitor cells provide insightful mechanistic explanations regarding the MLL leukemia.84-89 These studies established important models such as transactivation and dimerization—emphasizing the gain of Hox gene expression, thus interfering blood lineage commitment (Fig. 3).85,90-92 However, these two models are only suitable for subsets of MLL leukemias since not all fusion partners contain transactivation or dimerization domains.

As mice bearing MLL-LacZ developed leukemias, it has been postulated that LacZ induces leukemias through either oligomerizing or stabilizing the MLL N-terminus—two non-mutually exclusive mechanisms.82 Although stabilization may contribute to MLL leukemogenesis, it has not been further examined. Our initial studies on the site-specific proteolysis of MLL indicated that proteolytic cleavages of MLL not only regulates its activation, but also stability.50 Based on our prior studies on the unstable nature of MLL N-terminus, we had proposed a model in which fusion partners stabilize the common MLL N-terminus for downstream gene regulation—a mechanism compatible with the prevailing activation and dimerization models.50 In a recent report, we further investigated this mechanism and discovered that degradation of MLL initiates at its N-terminal ~1400 aa that is universally retained in all documented MLL leukemia fusions.74 Importantly, the prevalent MLL-Fusions, including MLL-AF4, MLL-AF9, MLL-ENL and MLL-ELL, and leukemogenic MLL-LacZ all exhibit resistance to degradation mediated by respective cell cycle UPS. This is in part due to the diminished interaction between N-terminal MLL and Skp2 or Cdc20, which results in an uncontrolled constant presence of MLL-Fusions through the cell cycle.74

As activation and dimerization models are only applicable to certain MLL-Fusions, a unifying model could exist to explain the wide variety of fusion partners. As MLL-LacZ is resistant to cell cycle phase-specific degradation, it is possible that partner proteins with oligomerizing capacity stabilize the respective MLL-Fusions (Fig. 3). Consequently, MLL target genes become constantly activated due...
The Contributions of Cell Cycle Deregulation to MLL Leukemias

HOX gene clusters encode highly conserved transcription factors with critical roles in embryonic development and hematopoiesis. As HOX genes are the best characterized MLL targets, significance of their deregulation in MLL leukemias has been extensively studied. Hox deregulation is required for certain but not all MLL-Fusions induced leukemias. For instance, HoxA7 and HoxA9 are prerequisite for MLL-ENL initiated leukemia but are dispensable for leukemias induced by MLL-AF9 and MLL-GAS7. Therefore, it is likely that deregulation of critical pathways other than Hox genes plays an instrumental role in MLL leukemias. A few reports including ours have begun to explore the roles of MLL/MLL-Fusions in cell cycle, genome integrity, etc.,—an uncharted area demanding further efforts. Initial emphasis on Hox gene deregulation provides an important yet oversimplified view concerning the MLL leukemia. Hence, future elucidation and validation of additional signaling pathways controlled by MLL and their respective deregulation incurred by MLL-Fusions would likely yield novel targets for future treatment of the MLL leukemia.

Our observed cell cycle defects from perturbations of MLL expression led to hypothesize that MLL-Fusions interfere with the cell cycle, which is supported by prior overexpression studies using either MLL-AF4 or MLL-AF9. The resistance of MLL-Fusion to cell cycle phase-specific degradation results in its non-oscillating expression during the cell division cycle. Although the consequences of such deregulated expression remain to be determined, the loss of temporal control of MLL targets likely contributes to the ultimate MLL leukemia phenotype.

The Trx-G and Pc-G in Oncogenesis

Genetic evidences obtained from studying flies and mammals establish the fundamental roles of Pc-G and Trx-G proteins in maintaining Hox/homeotic gene expression, thus ensuring the correct installment of complex segmental body plans in higher organisms. Recent studies revealed an even more perplexed relationship between these two groups of proteins in regulating non-Hox/homeotic pathways. For example, they target similar cell cycle genes such as CyclinA and p16. Furthermore, deregulation of factors belonging to either groups affects hematopoiesis and contributes to various oncogenic processes. 

Conclusions and Future Directions

Although our recent studies elucidated the intimate relationship between MLL and the cell cycle control, the in vivo consequences incurred by non-oscillating expression of MLL-Fusions remain to be elucidated. Studies by us and others clearly indicate the importance to avoid expressing MLL/MLL-Fusions at a nonphysiological level—expression of MLL/MLL-Fusions beyond or below the physiological range incurs undesirable cell cycle consequences that may easily lead to an erroneous conclusion. Therefore, to critically examine cell cycle defects associated with MLL-Fusions, one has to employ cells bearing knockin allele of MLL-Fusion of which its expression is directly controlled by the endogenous MLL promoter.

The most characterized biochemical property of MLL is its activity to methylate Histone H3 at K4 position with its C-terminal SET domain, thus marking the active epigenome. However, this highly conserved SET domain only represents less than 5% of MLL which is made of 3,969 amino acid residues. Furthermore, the ubiquitous expression of MLL implicates its participation in essential cellular processes other than cell fate decisions. Recent positioning of MLL in the cell division cycle inaugurated the search for non-canonical functions of MLL. Although it has been a decade since its
Figure 4. A diagram highlights the intricate relationship and draws the analogy between MLL, a Trx-G protein and Bmi-1, a Pc-G protein, in embryogenesis and oncogenesis.

45. Tyagi S, Chabes AL, Wysocka J, Herr W. EZF activation of S phase promoters via asso-
ciation with HCF-1 and the MLL family of histone H3K4 methyltransferases. Mol Cell 2007;
27:87-96.
46. Rozenblatt-Rosen O, Rozovskaia T, Burakov D, Sedkov Y, Tillib S, Blechman J, Nakamura T, 
Crocce CM, Mao A, Canaani E. The C-terminal SET domains of all-1 and TRITHORAX 
interact with the IN11 and SNR1 proteins, components of the SWI/SNF complex. Proc 
47. Dou Y, Milne TA, Tackett AJ, Smith ER, Fukuda A, Wysocka J, Allis CD, Chair BT, 
Hess JL, Roeder RG. Physical Association and Coordinating Function of the H3 K4 
Regulation of MLL K4 methylation activity by its core components. Nat Struct Mol Biol 
49. Yokoyama A, Kitaibayashi I, Ayton PM, Cleary ML, Ohki M. Leukemia proto-oncprotein 
MLL is proteolytically processed into 2 fragments with opposite transcriptional properties. 
of MLL generates a complex of N- and C-terminal fragments that confers protein stability 
52. Khan JA, Dunn BM, Tong L. Crystal structure of human Tsaapae, a crucial protase 
54. Zhou H, Spicuglia S, Hsieh JJ, Mitsiou DJ, Hubsy T, Veena GST, Korsmeyer SJ, 
Sunnitnen HG. Undeaved TFL1A is a substrate for taspae 1 and active in transcription. 
55. Mazo AM, Huang DH, Meier BA, Dawid IB. The trithorax gene, a trans-acting regulator 
of the bithorax complex in Drosophila, encodes a protein with zinc-binding domains. Proc 
Natl Acad Sci USA 1990; 87:2112-6.
56. Hess JL, Yu BD, Li B, Hanson R, Korsmeyer SJ. Defects in yolk sac hematopoiesis in Mll-
57. Ayton PM, Cleary ML. Transformation of myeloid progenitors by MLL oncoproteins is 
59. Lavau C, Sivilassy SJ, Slany R, Cleary ML. Immunolocalization and leukemic transforma-
tion of a myelomonocytic precursor by retrovirally transduced HRX-ENL. J Mol Biol 1997; 
16:422-37.
60. Slany RK, Lavau C, Cleary ML. The oncogenic capacity of HRX-ENL requires the 
transcriptional transactivation activity of ENL and the DNA binding motifs of HRX. Mol Cell 
Biol 1998; 20:1655-64.
62. Lavau C, Du C, Thirman M, Zelenskii-le N. Chromatin-related properties of CBP fused to 
63. Lavau C, Luo RT, Du C, Thirman M. Retrversion-mediated gene transfer of MLL-ELL 
transforms primary myeloid progenitors and causes acute myeloid leukemias in mice. Proc 
64. DiMartino JP, Miller T, Ayton PM, Landewe T, Hess JL, Cleary ML, Shilatifard A. The 
AF1 carboxy-terminal domain of ELL is required and sufficient for immortalization of myeloid progenitors by MLL-ELL. Blood 2002; 99:5780-5.
65. So CW, Cleary ML. MLL-AFX requires the transcriptional effector domains of AFX to 
transform myeloid progenitors and transdominantly interfere with forkhead protein function. 
66. So CW, Lin M, Ayton PM, Chen EH, Cleary ML. Dimerization contributes to oncogenic 
Dimerization of MLL fusion proteins immortlalizes hematopoietic cells. Cancer Cell 2003; 
1:197-207.
69. Lawrence HJ, Sauvageau G, Humphries RK, Largman C. The role of HOX homeobox 
70. Ayton PM, Cleary ML. Transformation of myeloid progenitors by MLL oncoproteins is 
71. So CW, Karusky H, Woon P, Weissman IL, Cleary ML. Leukemic transformation of 
hematopoietic progenitors by MLL-GAS7 in the absence of Hox7 or Hox9. Blood 2004; 
103:3192-9.
72. Kumar AR, Hudson WA, Chen W, Nishiuchi R, Yao Q, Kersey JH. Hox9 influences the 
phenotype but not the incidence of MLL-AF9 fusion gene leukemia. Blood 2004; 
103:1823-8.
amino terminal sequences with menin is required for transformation. Cancer Res 2007; 
67:7275-83.
74. Casolini C, Serna A, Rossi V, Introna M, Biondi A. Modulation of cell cycle by graded 
