MOLECULAR ONCOLOGY 7 (2013) 1069–1082

available at www.sciencedirect.com

ScienceDirect

www.elsevier.com/locate/molonc

p27^{kip1} maintains a subset of leukemia stem cells in the quiescent state in murine MLL-leukemia

Jun Zhang[a,b,1], *Christopher S. Seet*[b,1], *Clare Sun*[b], *Jing Li*[a], *Dewen You*[b], *Andrew Volk*[b,c], *Peter Breslin*[b,d,e], *Xingyu Li*[a], *Wei Wei*[b], *Zhijian Qian*[f], *Nancy J. Zeleznik-Le*[b], *Zhou Zhang*[a,g,**], *Jiwang Zhang*[b,h,*]

[a]*Department of Biology, College of Life and Environment Science, Shanghai Normal University, 100 Guilin Road, Shanghai 200234, PR China*
[b]*Oncology Institute, Cardinal Bernardin Cancer Center and Department of Pathology, Loyola University Chicago, Maywood, IL 60153, United States*
[c]*Molecular Biology Program, Loyola University Medical Center, United States*
[d]*Department of Biology, Loyola University Chicago, United States*
[e]*Department of Molecular and Cellular Physiology, Loyola University Medical Center, United States*
[f]*Department of Medicine, University of Illinois at Chicago, United States*
[g]*Department of Pharmacology, Shenyang Pharmaceutical University, 110016, PR China*
[h]*Department of Pathology, Loyola University Medical Center, Maywood, IL 60153, USA*

ARTICLE INFO

Article history:
Received 7 June 2013
Received in revised form
22 July 2013
Accepted 31 July 2013
Available online 20 August 2013

Keywords:
MLL-leukemia
p27^{kip1}
Leukemia stem cell
Quiescence

ABSTRACT

MLL (mixed-lineage leukemia)-fusion genes induce the development of leukemia through deregulation of normal MLL target genes, such as *HOXA9 and MEIS1*. Both *HOXA9* and *MEIS1* are required for MLL-fusion gene-induced leukemogenesis. Co-expression of *HOXA9* and *MEIS1* induces acute myeloid leukemia (AML) similar to that seen in mice in which MLL-fusion genes are over-expressed. p27^{kip1} (p27 hereafter), a negative regulator of the cell cycle, has also been defined as an MLL target, the expression of which is up-regulated in MLL leukemic cells (LCs). To investigate whether p27 plays a role in the pathogenesis of MLL-leukemia, we examined the effects of *p27* deletion ($p27^{-/-}$) on MLL-AF9 (MA9)-induced murine AML development. HOXA9/MEIS1 (H/M)-induced, *p27* wild-type ($p27^{+/+}$) and $p27^{-/-}$ AML were studied in parallel as controls. We found that LCs from both MA9-AML and H/M-AML can be separated into three fractions, a CD117^{-}CD11b^{hi} differentiated fraction as well as CD117^{+}CD11b^{hi} and CD117^{+}CD11b^{lo}, two less differentiated fractions. The CD117^{+}CD11b^{lo} fraction, comprising only 1–3% of total LCs, expresses higher levels of early hematopoietic progenitor markers but lower levels of mature myeloid cell markers compared to other populations of LCs. p27 is expressed and is required for maintaining the quiescent and drug-resistant states of the CD117^{+}CD11b^{lo} fraction of MA9-LCs but not of H/M-LCs. *p27* deletion significantly compromises the leukemogenic capacity of CD117^{+}CD11b^{lo} MA9-LCs by reducing the frequency of leukemic stem cells (LSCs) but does not do so in H/M-LCs. In addition, we found that p27 is highly expressed and required for cell cycle arrest in the CD117^{-}CD11b^{hi} fraction in both types of LCs. Furthermore, we

* *Corresponding author*. Oncology Institute, Cardinal Bernardin Cancer Center and Department of Pathology, Loyola University Chicago, Maywood, IL 60153, United States. Tel.: +1 708 327 3343.
** *Corresponding author*. Department of Biology, College of Life and Environment Science, Shanghai Normal University, Shanghai 200234, China.
E-mail address: jzhang@lumc.edu (J. Zhang).
[1] These authors contributed equally to the research.
1574-7891/$ – see front matter Published by Elsevier B.V. on behalf of Federation of European Biochemical Societies.
http://dx.doi.org/10.1016/j.molonc.2013.07.011

found that *c-Myc* expression is required for maintaining LCs in an undifferentiated state independently of proliferation. We concluded that p27 represses the proliferation of LCs, which is specifically required for maintaining the quiescent and drug-resistant states of a small subset of MA9-LSCs in collaboration with the differentiation blockage function of c-Myc.

Published by Elsevier B.V. on behalf of Federation of European Biochemical Societies.

1. Introduction

MLL protein, a large histone methyltransferase, maintains the expression of a subset of genes by specifically methylating histone H3 at lysine 4 (H3K4) in the promoter regions of its target genes (Milne et al., 2002). Most MLL-target genes, such as those within the *Hox* loci and many other genes, are master regulators of development and are also key regulators of hematopoiesis (Abramovich and Humphries, 2005). Gene knockout studies suggested that Mll is dispensable for the initiation of stage-specific expression of its target *Hox* genes but is absolutely required for the maintenance of expression of such genes during early development (Yu et al., 1995). Mice with homozygous *Mll* mutation suffer early embryonic lethality with significant defects in both yolk sac primary and AGM definitive hematopoiesis (Yagi et al., 1998; Hess et al., 1997; Ernst et al., 2004a,b). The self-renewal and proliferation of hematopoietic stem cells (HSCs) are significantly compromised by Mll inactivation (McMahon et al., 2007; Jude et al., 2007). The hematopoietic defects in *Mll*-knockout mice ($Mll^{-/-}$) might be mainly due to a reduction in the expression of *Hox* genes because the proliferative defects of hematopoietic stem and progenitor cells (HSPCs) derived from $Mll^{-/-}$ embryonic stem cells can be largely rescued by the overexpression of *Hox*-cluster genes (Ernst et al., 2004a,b).

MLL-rearrangements are found in >70% of infant acute lymphoblastic leukemias (ALL), approximately 10% adult acute myeloid leukemias (AMLs) and >30% of therapy-related leukemias (Liu et al., 2009). MLL-fusion proteins, the products of *MLL* rearrangements, induce the development of leukemia through deregulating MLL target genes, such as *HOXA9*, *HOXA7*, *MEIS1* and *PBX1*. (Marschalek, 2011; Muntean and Hess, 2011; Faber et al., 2009; Dou and Hess, 2008; Zeisig et al., 2004; Kroon et al., 1998; Li et al., 2009; Andreeff et al., 2008). Although they lack histone methyltransferase activity, MLL-fusion proteins contain Menin/LEDGF binding sites, a nuclear localization sequence and a CxxC motif, all of which are features essential for the correct targeting of the MLL complex to the promoter regions of its target genes (Milne et al., 2010; Milne et al., 2005; Zeleznik-Le et al., 1994; Ayton et al., 2004). Interrupting the DNA binding ability of MLL-fusion proteins by disrupting these DNA binding motifs will significantly compromise their leukemogenic capacity due to their consequent failure to up-regulate MLL target genes (Slany et al., 1998; Cierpicki et al., 2010; Yokoyama et al., 2005; Caslini et al., 2007; Yokoyama and Cleary, 2008; Bach et al., 2009). The requirement for the wild-type (*WT*) allele of MLL for MLL-fusion protein-induced leukemic transformation suggested that both MLL-fusion protein and WT-MLL might be recruited to the regulatory regions of target genes. An MLL-fusion protein might be dependent upon WT-MLL's methyltransferase activity and hijack the normal transcription/elongation machinery to induce target gene expression (Milne et al., 2010; Muntean et al., 2010; Bernt et al., 2011; Chang et al., 2010; Thiel et al., 2011). Many MLL target genes are consistently up-regulated in human MLL cells (Slany, 2009; Imamura et al., 2002; Lawrence et al., 1999; Armstrong et al., 2002). Several of these genes, including *HOXA9*, *HOXA7*, *MEIS1* and *PBX1/2*, have been shown to be required for MLL-fusion protein-induced leukemia development as shown by genetic studies (Faber et al., 2009; Wong et al., 2007; Kumar et al., 2009; Ayton and Cleary, 2003). In addition, co-expression of HOXA9 and MEIS1 induces leukemic transformation in HSCs by promoting self-renewal/proliferation and blocking differentiation, which mimic most of the functions of MLL-fusion proteins (Zeisig et al., 2004; Kroon et al., 1998). These studies demonstrated the key role of *Hox*-cluster genes in the pathogenesis of MLL-fusion protein-induced leukemia.

$p27^{kip1}$ (p27 hereafter), a negative regulator of the cell cycle, has been identified as a direct target of MLL (Milne et al., 2005; Xia et al., 2005). Menin, the product of the tumor suppressor gene *Multiple endocrine neoplasia*, represses cell growth by inducing p27 expression (Horiguchi et al., 2009). It was found that Menin functions as an adaptor for the interface of MLL and LEDGF (lens epithelium-derived growth factor), which mediates the specific binding of MLL to chromatin and DNA (Milne et al., 2005; Caslini et al., 2007; Yokoyama and Cleary, 2008). Both Menin and LEDGF are required for the correct localization of MLL to its target genes and inducing target gene expression. Genetic disruption of the MLL/Menin/LEDGF interaction leads to down-regulation of MLL target gene expression in MLL-fusion gene-transduced cells and blocks the development of leukemia (Yokoyama et al., 2005; Caslini et al., 2007; Yokoyama and Cleary, 2008). In MLL-fusion gene-transduced LCs, Menin recruits both WT MLL and MLL-fusion proteins to the p27 promoter, which might cooperatively up-regulate p27 expression (Milne et al., 2005; Xia et al., 2005). In contrast to other leukemogenic fusion protein-related LCs, Flt3-L (Flt3-Ligand) specifically induces p27 expression in MLL-fusion-related LCs and represses their proliferation (Furuichi et al., 2007). This specific up-regulation of p27 in MLL LCs suggested that it might play a distinct role in the pathogenesis of MLL-fusion gene-induced leukemia.

To investigate the role of p27 in the development of MLL-fusion gene-induced leukemia, we evaluated the effects of deletion of the *p27* gene on the pathogenesis of MLL leukemia by comparative study of the *in vitro* growth behaviors and *in vivo* leukemogenic activity of $p27^{+/+}$ (p27 WT) and $p27^{-/-}$ (p27-knockout), MA9 (MLL-AF9) murine LCs, as well as $p27^{+/+}$ and $p27^{-/-}$ H/M (HOXA9/MEIS1) murine LCs. We found that in