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Combinatorial Haplo-Deficient Tumor Suppression in 7q-Deficient Myelodysplastic Syndrome and Acute Myeloid Leukemia

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Heterozygous deletions of chromosome 7 are frequent in myelodysplastic syndrome (MDS) and acute myeloid leukemia (AML). In this issue of *Cancer Cell*, Chen and colleagues identify *MLL3* as a novel haploinsufficient tumor suppressor on 7q that, in combination with *NF1* suppression and *TP53* deficiency, mediates MDS and AML phenotypes in mouse and human systems.

Heterozygous deletions or losses of chromosome 7 (−7/7q-) are frequently observed in patients with myelodysplastic syndrome (MDS) and acute myeloid leukemia (AML) and are associated with chemoresistance and poor outcome. Previous studies have examined the genetics and functional genomics of patients with −7/7q-, including at the stem cell level, and identified genes involved in disease pathogenesis (Wong et al., 2010; Pellagatti et al., 2010; Barreyro et al., 2012; McNerney et al., 2013). However, no definitive single haploinsufficient tumor suppressor in this region has thus far been functionally validated in its capacity to induce overt MDS or AML in an experimental model. Experimental heterozygous deletion of one of the commonly deleted regions failed to result in overt myeloid disease in mice (Wong et al., 2010), and previous studies did not detect biallelic inactivation of any candidate tumor suppressors (Curtiss et al., 2005). Recent advances in our knowledge of the multi-step pathogenesis of myeloid malignancies and functional work on co-occurring disease alleles have highlighted limitations in the study of single candidate alleles and shown striking disease phenotypes when combinatorial allelic genotypes were examined (Jan et al., 2012; Abdel-Wahab et al., 2013). This leads to the hypothesis that haploinsufficiency of candidate genes in regions commonly deleted in cancers (e.g., here, on chromosome 7) may have detectable tumor suppressing activity only in the context of other cooperating genetic events.

In an approach to identify and experimentally interrogate such context-dependent combinatorial candidate tumor suppressors in −7/7q- AML, Chen et al. (2014; in this issue of *Cancer Cell*) analyzed their own and other published genomic data on AML patients and focused on one particular candidate 7q tumor suppressor, the mixed lineage leukemia 3 (*MLL3*) gene. Chen et al. (2014) noticed that haploinsufficiency of *MLL3* (by deletion of 7q, loss of chromosome 7, or *MLL3* nonsense mutation) frequently co-occurred with monoallelic deletion of the neurofibromin 1 (*NF1*) gene in combination with *TP53* inactivation. *MLL3* is a member of the *MLL* family (comprising other members that are frequently rearranged in human leukemias) and contains a SET domain that can transfer a methyl group to lysine 4 of histone H3. In addition, MLL3 can mediate histone H3 K27 demethylation through a physical interaction with the histone H3 demethylase UTX. NF1 is a potent suppressor of RAS oncogenic signaling, and its inactivation has been shown to induce a myeloproliferative phenotype (Le et al., 2004).

To functionally interrogate potential leukemogenic cooperativity among MLL3, NF1, and TP53, Chen et al. (2014) leveraged several elegant and complementary model systems. First, they used retroviral delivery of short hairpin RNAs (shRNAs), directed against *Mll3* or *Nf1* (sh*Mll3* or sh*Nf1*) and labeled with different fluorophores, into *p53*-deficient hematopoietic stem and progenitor cells (HSPCs), followed by syngeneic transplantation. They observed striking cooperativity in the induction and progression of an aggressive transplantable AML when *Mll3* and *Nf1* were simultaneously suppressed (Figure 1). Importantly, suppression of *Mll3* and *Nf1* in wild-type HSPCs was not leukemogenic, and sh*Mll3* alone in $p53^{-/-}$ HSPCs did not accelerate p53-induced thymic lymphomas, demonstrating that indeed the combinatorial suppression of all three tumor suppressors (i.e., Mll3, Nf1, and p53) is required for AML initiation. Analysis of gene expression in the formed AML cells showed residual expression levels of both *Mll3* and *Nf1* at about 50% of their levels in controls. As the initially transduced HSPC populations were polyclonal, this indeed suggested that cells with intermediate *Mll3* and *Nf1* knockdown have a selective advantage during leukemogenesis. To further support their hypothesis of *Mll3* haploinsufficiency in AML pathogenesis, Chen et al. (2014) utilized in vivo CRISPR/Cas9 genome editing technology to disrupt *Mll3*. Strikingly, and consistent with shRNA knockdown experiments, CRISPR/Cas9 targeting of *Mll3* in sh*Nf1;*$p53^{-/-}$ cells led to disease acceleration and AML development as opposed to T-ALL development in recipient mice transplanted with cells of the same background, but transduced

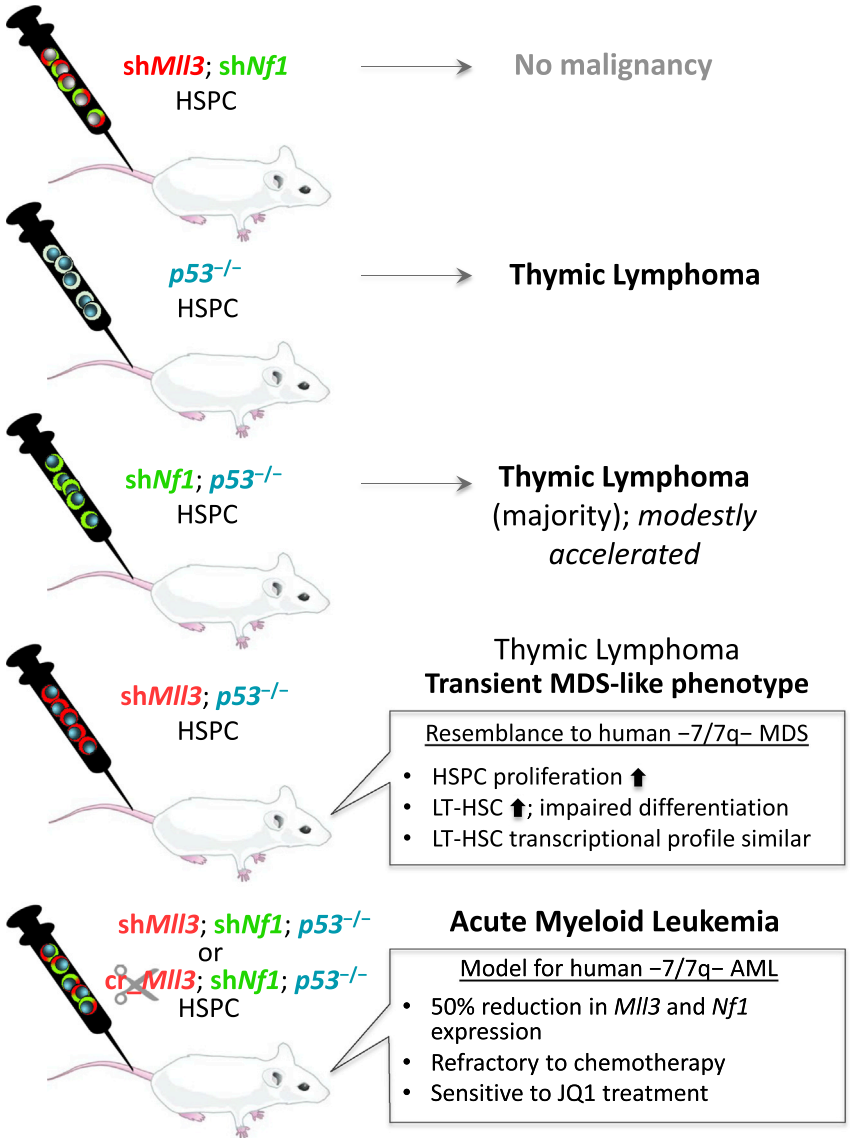

Figure 1. Leukemogenic Cooperation of *Mll3* and *Nf1* Haploinsufficiency in a p53-Deficient Background

Adoptive transfer of lentivirally transduced hematopoietic stem and progenitor cells (HSPCs) into syngeneic recipient animals leads to an AML recapitulating hallmarks of human −7/7q- AML, in contrast to thymic lymphoma induced by p53 deficiency only.

by control CRISPR/Cas9 targeting a noncoding region. Importantly, while CRISPR/Cas9-directed mutagenesis can lead to mutations in one or both target alleles, sequencing of the induced AML clones revealed that they were indeed heterozygous for wild-type *Mll3*, suggesting that *Mll3* haploinsufficiency rather than complete loss leads to AML formation.

Correlative analyses of Mll3 suppression-induced transcriptional changes with gene expression data from MDS patients' CD34$^+$ cells showed high similarity and a significant enrichment of gene expression differences found in −7/7q-MDS versus normal karyotype MDS, suggesting that MLL3 repression is, at least in part, causing the molecular phenotype observed in MDS patients' cells. At the mechanistic level, Chen et al. (2014) found reduced trimethylation of H3K4 and increased trimethylation of H3K27 at the loci of genes downregulated upon Mll3 suppression. While this is consistent with an Mll3-dependent epigenetic mechanism, further studies will be required to determine whether this is indeed a direct effect on histone modifications by Mll3 or Mll3-associated complexes or an alternative mechanism of transcriptional regulation.

To study the cellular phenotypes mediated by *Mll3*, Chen et al. (2014) examined sh*Mll3*;*p53*$^{-/-}$ HSPCs in comparison to sh*Renilla*;*p53*$^{-/-}$ controls. They discovered features consistent with human MDS (Will et al., 2012) transiently present in sh*Mll3*;*p53*$^{-/-}$ mice: (1) aberrant morphological features within the mature myeloid cell compartment, (2) impaired differentiation at the stem cell level concomitant with an increase in long-term HSCs, and (3) increased proliferative activity of immature cKit$^+$ cells. These findings indicate that *MLL3* plays an important role in HSC differentiation, but requires cooperating events for sustained MDS disease and progression to overt AML.

Chen et al. (2014) furthermore suggest that modest transcriptional changes in HSC, even if they lead to transient functional alterations, can determine transformation of a particular lineage at a later stage or lead to a switch of the lineage affected by transformation (a phenomenon also observed in human leukemias at relapse though infrequently). Further studies will be required to determine the step-wise temporal acquisition of aberrations and their precise cell type(s) of occurrence.

Lastly, Chen et al. (2014) studied the role of *Mll3* in resistance to chemotherapy. They observed that mice harboring sh*Mll3*;sh*Nf1*;p53$^{-/-}$ (MNP) AML were highly resistant to cytarabine plus doxorubicin therapy, which resembles the current standard in induction chemotherapy used for AML treatment. Furthermore, MLL3 suppression in a chemosensitive type of leukemia (*AML1-ETO*;*Nras*^{G12D}) led to a remarkably reduced sensitivity to chemotherapy, suggesting that MLL3 suppression plays a role in conferring chemotherapeutic resistance. They went on to show that MNP AML, as well as several AML cell lines carrying −7/7q-, are sensitive to treatment with the bromodomain and extra-terminal (BET) family inhibitor JQ1, demonstrating the potential utility of their model as a tool for the preclinical identification and study of novel agents effective

in these highly chemoresistant subtypes of AML with poor prognosis. Additional studies will be required to elucidate a possible mechanistic link between *Mll3* function and the JQ1-mediated, and potentially Myc-dependent, antiproliferative effect.

In summary, Chen et al. (2014) have shown that *Mll3* is a haploinsufficient tumor suppressor on 7q that can act in conjunction with heterozygous *Nf1* deletion (and possibly other mechanisms of RAS pathway activation) and *Tp53* deficiency to induce an aggressive transplantable AML in a murine model. *Mll3* suppression leads to a partial differentiation block at the level of HSC and MDS-like cellular and molecular features. Furthermore, Chen et al.'s mouse model of Mll3 suppression-induced AML represents an interesting new tool for the preclinical evaluation and mechanistic study of novel pathways and compounds that may be effective for the treatment of −7/7q- and possibly other types of chemotherapy-resistant AML.

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A New Lnc in Metastasis: Long Noncoding RNA Mediates the ProMetastatic Functions of TGF-β

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TGF-β signaling promotes metastasis by controlling the expression of downstream target genes. In this issue of *Cancer Cell*, Yuan and colleagues discover a novel TGF-β-induced lncRNA, lncRNA-ATB, which stimulates EMT through sequestering miR-200s and facilitates colonization by stabilizing *IL-11* mRNA, thus promoting both early and late steps of cancer metastasis.

The transforming growth factor-β (TGF-β) pathway plays crucial roles during development and homeostasis and exerts strong antiproliferative effects on normal and premalignant cells. However, advanced-stage cancers often become insensitive to the tumor-suppressive actions of TGF-β. Instead, advanced cancers benefit from TGF-β's profound metastasis-promoting effects, such as epithelial-to-mesenchymal transition (EMT) induction, angiogenesis promotion, altered extracellular matrix deposition, immune suppression, and increased metastatic colonization (Ikushima and Miyazono, 2010; Massagué, 2008). These prometastatic responses to TGF-β are mediated by a variety of downstream effector proteins, including transcription factors (e.g., AP-1, ID1, SNAIL, SLUG, TWIST, and ZEB1/2), cytokines, growth factors, and other ligands (e.g., ANGPTL4, PTHrP, IL-11, JAGGED1, PDGF-B, CTGF, and VEGF), matrix proteins and proteases (e.g., TNC, MMPs) (Ikushima and Miyazono, 2010; Massagué, 2008), and a growing number of microRNAs (miRNAs) (Butz et al., 2012).

In recent years, long noncoding RNAs (lncRNAs), a new class of noncoding RNAs longer than 200 nucleotides, were recognized to regulate a wide variety of physiological and pathological processes through diverse mechanisms. For example, lncRNAs ANRIL and HOTAIR promote tumor growth or metastasis by recruiting chromatin-remodeling complexes to alter gene transcription, while tumor-suppressing lncRNA GAS5 and tumor-promoting lncRNA HULC act as decoys for glucocorticoid receptor and miR-372, respectively (Gutschner and Diederichs, 2012). Although the diversity and abundance of lncRNAs seem to rival that of mRNAs in any given cell type, there is little understanding of crucial lncRNAs functioning downstream of the TGF-β pathway. In this issue of

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Due to a production error, the reference for Chen et al. (2014) was incorrect. The correct reference is listed below, and the online version of the article has been updated.

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