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Evidences for mutations in the histone modifying gene *SETD2* as critical drivers in leukemia development

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Cancer is thought to be largely caused by genetic mutations that alter nucleotide sequences of DNA, yet epigenetic factors are increasingly recognized to also play causative roles in cancer development. The idea of "epimutations" involves aberrations in chemical modification of the chromatin or DNA that do not change nucleotide sequence. While it is widely accepted that aberrations of DNA methylation contribute to carcinogenesis, the functional importance of histone modifications and other chromatin features in the development of human cancer remains to be elucidated. Recent efforts in large-scale sequencing of cancer genomes have revealed mutations in numerous chromatin regulatory genes, and thus demand further investigations on the epigenetic mechanisms as driving forces in cancer development.

SETD2 is a key histone modifying enzyme gene that is responsible for catalyzing trimethylation at the lysine 36 of histone H3 (H3K36me3). During transcriptional elongation in normal cells, H3K36me3 marks the zone on exons for accurate gene transcription and plays an important role in the regulation of alternative splicing. Recent studies also demonstrated that SETD2-mediated H3K36me3 is required for DNA mismatch repair (MMR). H3K36me3 facilitates MMR in cells by recruiting the MMR machinery to the chromatin during cell cycle. *SETD2* was previously known for its frequent mutations in kidney cancer. A recent comprehensive study evaluating 12 major types of cancer by an international study also showed that *SETD2* mutations are specific to certain types of solid tumors including kidney, lung and bladder cancers. In blood cancer, targeted sequencing of limited exons of *SETD2* did not find mutations in an acute leukemia patient cohort consisting of childhood B-cell acute lymphoblastic leukemia (ALL) and adult acute myeloid leukemia (AML) [1]. One recent report identified *SETD2* mutations in T-cell ALL, a specific type of acute leukemia originating from lymphoid lineage [2]. Despite the implications of *SETD2* mutations in the pathogenesis of a number of human cancers, the functional role of *SETD2* mutations has not been clearly defined.

Our new study [3] has demonstrated a definitive role of histone modifying gene *SETD2* in a specific type of human blood cancer, leukemia. Disruption of the SETD2 function resulted in global loss of H3K36me3. In the presence of a major chromosomal translocation, SETD2 disruption greatly accelerated both initiation and progression of the disease.

Acute leukemia characterized by chromosomal translocations usually requires additional molecular disruptions to develop the full blown malignancy. Through whole genome sequencing, we looked for cooperating events and identified mutations in *SETD2* in the genome of a monozygotic twin child who carried a major chromosomal translocation involving the MLL gene and had developed acute leukemia.

Intrigued by the *SETD2* mutations in the genome of the leukemic twin sister, we carried out a subsequent screening

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on more than 240 acute leukemia patients collected from several major medical centers in China and revealed a 6.2% frequency of *SETD2* mutations in the entire leukemia cohort and 22% in patients with MLL translocations. More importantly, *SETD2* mutations are associated with a broad spectrum of leukemia other than the MLL-rearranged leukemia subtype, which makes our finding more relevant in the clinical setting. *SETD2* mutations are accompanied by multiple major chromosomal translocations including *MLL-AF9*, *MLL*-PTD and *AML1-ETO*, in addition to *MLL-NRIP3* that was identified from the twin patient in this study.

By taking advantage of multiple cancer patient cohorts in public databases, we performed comprehensive and powerful genomic analyses, which uncovered a mutation spectrum of *SETD2* in human leukemia that is distinct from what has been observed in solid tumors. These analyses identified point mutation, rather than large deletion or epigenetic silencing, as a major mechanism in disrupting the function of SETD2 in acute leukemia.

To directly test the functional role of SETD2 disruption in leukemia development, we performed a series of *in vitro* and *in vivo* experiments. In conjunction with a major chromosomal abnormality, SETD2 disruption greatly increased the self-renewal potential and the number of leukemia stem cells (LSCs). LSCs are the "seed" cells that replicate themselves to promote and sustain leukemic cell growth. By examination of the expression profile of more than 20000 genes, the study was able to pinpoint the molecular pathways that may contribute to stemness of LSCs in *SETD2* mutated leukemia. Moreover, specific inhibition of the mTOR pathway significantly decreased the self-renewal potential of LSCs.

The series of experimentation in conjunction with extensive bioinformatic analyses provide novel insights into both cellular and molecular mechanisms upon the disruption of SETD2 in the heterogeneous leukemia cell population and the complex signaling landscape. In addition, functional disruption of SETD2 appears to be involved in both initiation and progression stages of leukemia development, thereby further justifying *SETD2* mutation as a critical driver mutation through the course of leukemia development.

In this new report, whole-genome and targeted sequencing in a Chinese leukemia patient cohort revealed recurrent loss-of-function *SETD2* mutations. Importantly, extensive functional and genomic analyses demonstrated that disruption of SETD2 cooperates with multiple major chromosomal abnormalities to promote leukemogenesis via enhancing the self-renewal potential of LSCs and activating multiple signaling pathways (Figure 1). As the cancer genome field is shifting its trend from massive sequencing and cataloguing of genomic changes in patients to studies on clinical significance and functional importance of identified mutations, this study represents an important step toward that direction.

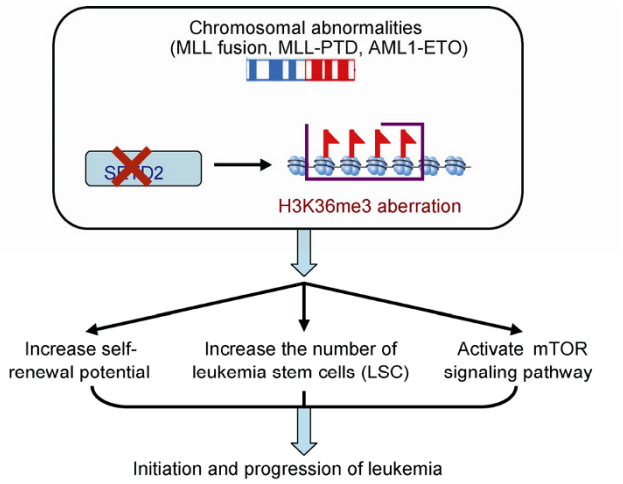

Figure 1 Histone modification aberration caused by SETD2 inactivation cooperating with chromosomal abnormalities promotes initiation and progression of leukemia.

Moreover, the distinct epigenetic mechanism shown by disruption of SETD2 in driving human leukemia offers a new opportunity for the development of cancer diagnostics and therapeutics [4].

A new study from Armstrong group further strongly suggested a role of SETD2 in chemotherapy resistance [5]. Consistent with the data obtained from the Chinese cohort, the frequencies of *SETD2* mutations in two different B-ALL populations were 5% and 12%, respectively. A higher prevalence of *SETD2* mutations was observed in patients with *MLL*-rearranged (22%) and *ETV6-RUNX1* (13%) *de novo* leukemias. To test the hypothesis that mutations in epigenetic regulators may play an important role in relapse, the authors carried out targeted sequencing on 472 epigenetic regulators and frequently mutated genes including *SETD2*. At diagnosis, 22% of patients had mutations in epigenetic regulators. Strikingly, epigenetic mutations were significantly enriched in the matched relapse samples (57%, P=0.0039). Eleven out of 30 patients gained somatic variants in the epigenetic regulators at the time of relapse. In sharp comparison, the mutation spectra in signaling genes such as *KRAS*, *NRAS* and *FLT3* were similar between the patients at diagnosis and relapse. This well-designed clinical investigation indicates the potential functional involvement of *SETD2* mutations in relapsed ALL patients that are often chemoresistant with a poor long-term survival.

Simon et al. had shown that global loss of H3K36me3 is associated with altered chromatin accessibility, and aberrant RNA processing and splicing in kidney cancer. Despite the functional and clinical significance of SETD2 in human leukemia, the underlying molecular mechanisms remain unclear as to how loss-of-function of SETD2 contributes to accelerated leukemia development and chemoresistance, and how global reduction of an active histone mark leads to activation of observed cancer stem cell signatures and on-

cogenic signaling pathways [3]. Interestingly, a high prevalence of *SETD2* mutation (22%) in *MLL* associated leukemia was reported in two independent cohorts [3,5]. Several major MLL fusion genes such as *MLL-AF9*, *MLL-AF4* and *MLL-ENL* are known to induce aberrant H3K79 methylation via recruitment of histone modifying enzyme DOT1L. An intrinsic link may exist between the SETD2-H3K36 and the MLL fusion-H3K79 pathways. Exploring this potential link will not only provide new biological insights into the pathogenesis of MLL leukemia, but also offer novel therapeutic strategies in dealing with this deadly disease. Finally, the association of *SETD2* mutations with multiple major chromosomal translocations implies a common mechanism in various subtypes of leukemia with *SETD2* mutations. Identification of such molecular commonality may provide an important basis for the development of novel therapeutic agents for a broader spectrum of human acute leukemias.

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