Mutational Signatures in Osteoblast-Induced AML

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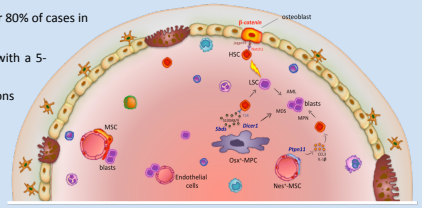
Abstract

Aberrant activation of β-catenin signaling in osteoblasts leads to MDS that progresses to AML in mice and humans. The disease can be transferred to healthy, bone marrow ablated mice following bone marrow transplantation from mice with activated β-catenin signaling in osteoblasts, suggesting that hematopoietic cells may have acquired permanent self-perpetuating genetic alterations that become independent of the initial mutation in osteoblasts leading to malignant transformation. Indeed, herein by screening for diagnostic mutations in myeloid, leukemic cells of mice with activated β-catenin signaling in osteoblasts using high throughput targeted DNA sequencing and subsequent validation by Sanger-based sequencing analysis, we identified genetic alterations in eight cancer related genes, six of which are commonly mutated in AML. These results support our initial hypothesis of secondary mutations induced by aberrant signaling in stromal cells that may lead to malignant transformation, provide mechanistic insights in the stroma-hematopoietic cell interaction and may provide additional therapeutic targets for disease treatment.

Introduction

- Acute myeloid leukemia (AML) is an aggressive malignancy of the bone marrow hematopoietic progenitors, the blood-forming cells, characterized by uncontrolled proliferation of immature myeloid cells that fail to develop resulting in impaired hematopoiesis and bone marrow failure
- AML is the most common acute leukemia in adults accounting for 80% of cases in this group
- Current therapies result in significant morbidity and mortality with a 5-year overall survival in less than 30% of patients [1,2]
- AML is associated with recurrent chromosomal structural variations and point mutations that act cooperatively rendering the disease highly heterogeneous and difficult amenable to single targeted therapies [1,2]
- In addition to mutations intrinsic to hematopoietic cells, genetic alterations in non-hematopoietic cells in the supporting stromal cells in the surrounding bone marrow microenvironment (niche), where the AML cells reside, are implicated in hematopoiesis and may lead to malignant transformation [3-5]



- Among them aberrant activation of β-catenin signaling in osteoblasts is sufficient to induce AML development in mice [6]. β-catenin signaling is activated in over 30% of patients with MDS/AML suggesting that this pathway may sustain dysplastic hematopoiesis and progression to MDS and AML in humans

Hypothesis: Osteoblasts and in particular osteoblasts with constitutively activated β-catenin have a pathogenetic role in the development of MDS and AML by inducing and acting synergistically with primary mutations in pre-malignant cells to lead to their oncogenic transformation

Materials & Methods

- Myeloid cells defined as CD11b^{+}Gr1^{+} were facs-purified from total bone marrow isolated from the tibia and femurs of four 4-week old male mice with constitutively activated β-catenin signaling in osteoblasts
- Genomic DNA was isolated and purified using AllPrep DNA/RNA kit from Qiagen and subjected to targeted sequencing by using IMPACT platform (Integrated Mutation Profiling of Actionable Cancer Targets) at Memorial Sloan Kettering Cancer Center
- Identified mutations were validated (present in myeloid cells, absent in tail) by conventional Sanger-based sequencing analysis:
  - 400-600bp genomic DNA encompassing the mutation was amplified using Q5 high-fidelity DNA polymerase
  - PCR products were purified from agarose gels using ZymoClean Gel DNA recovery kit
  - Purified PCR products were bi-directionally sequenced at Genewiz using primers spanning the mutation
  - Chromatogram analysis was conducted using SnapGene

References

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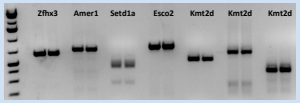
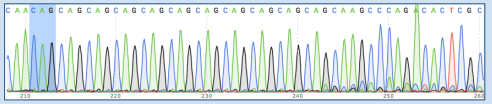
Results



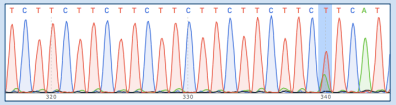
Setd1aA>G

Setd1aSer447Gly

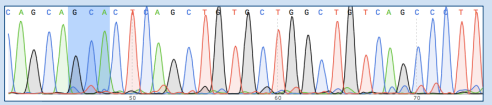
Zfhx3Gln1746del



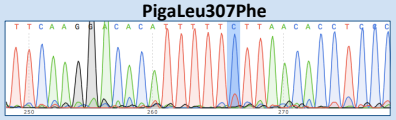
Amer1Glu382Asp



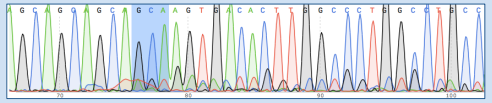
Kmt2dGln3610del



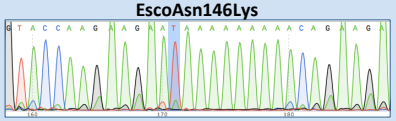
PigaLeu307Phe



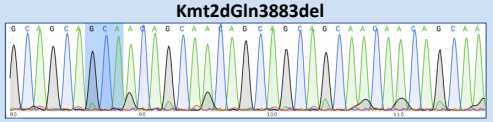
Kmt2dGlnd4049del



EscoAsn146Lys



Kmt2dGln3883del



Gene	Chromosome	Variant class	Reference_Allele	Tumor_Seq_Allele2	HGVSc	Aminoacids
Setd1a	chr7:127785314-127785314	SNV	G	A	c.1336G>A	p.Gly446Ser
Setd1a	chr7:127785317-127785317	SNV	A	G	c.1339A>G	p.Ser447Gly
Setd1a	chr7:127785318-127785320	DEL	GTG	–	c.1340_1342delNNN	p.Gly460del
Setd1a	chr7:127785320-127785320	SNV	G	A	c.1342G>A	p.Gly448Ser
Whsc1l1	chr8:25641028-25641030	DEL	TCC	–	c.408_410delNNN	p.Pro147del
Zfhx3	chr8:108947522-108947524	DEL	CAG	–	c.5203_5205delNNN	p.Gln1746del
Fanca	chr8:123288136-123288136	SNV	C	T	c.2426G>A	p.Gly809Asp
Esco2	chr14:65831422-65831422	SNV	A	T	c.438T>A	p.Asn146Lys
Kmt2d	chr15:98845132_98845134	DEL	TGC	–	c.12144_12146delNNN	p.Gln4049del
Kmt2d	chr15:98845669_98845671	DEL	TGC	–	c.11607_11609delNNN	p.Gln3883del
Kmt2d	chr15:98846446_98846448	DEL	TGC	–	c.10830_10832delNNN	p.Gln3610del
Amer1	chrX:95427365-95427365	SNV	T	A	c.1146A>T	p.Glu382Asp
Piga	chrX:164428607-164428607	SNV	C	T	c.919C>T	p.Leu307Phe

Discussion

- In agreement with the development of cell autonomous AML in mice with constitutively activated β-catenin signaling in osteoblasts, by screening for diagnostic mutations in myeloid, leukemic cells of mice with activated β-catenin signaling in osteoblasts we identified genetic alterations in eight cancer related genes.
- Six of these genes have been reported to be commonly mutated in MDS and AML such as *Setd1a, Kmt2d, Whsc1l1, Zfhx3, Piga, and Fanca*; the pathogenetic role of which will be tested in subsequent functional studies.
- The results suggest that in addition to the evolution of cooperative mutations in hematopoietic stem cells, the stromal niche may play a distinct role by providing cooperative signals leading to the transformation of pre-leukemic mutant states to AML.
- Targeting and interrupting these cooperating signals in the niche may provide new therapeutic options that could possible be used alone or in combination with current treatments to improve disease outcome in AML patients.