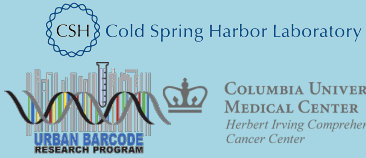


Genomic alterations driving activated b-catenin signaling in osteoblast-induced AML

Funded by:

The Pinkerton Foundation



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Abstract

Overactivation of b-catenin signaling in osteoblasts affects the maturation of hematopoietic stem cells resulting in accumulation of myeloid lineage precursors cells and the development of acute myeloid leukemia. This pathway is active in 38% of AML patients. However, the cause of its activation is not yet known. Here, we hypothesized that mutations within the osteoblasts drive activated b-catenin signaling as this pathway remains active in isolated osteoblasts grown in culture for several days, suggesting that factors intrinsic to the cells are responsible for overactivation of b-catenin. To test this hypothesis, we screened for genomic alterations in key genes affecting b-catenin activity by Sanger-based sequence analysis in osteoblasts collected from AML patients as well as healthy controls.

Introduction

- Acute Myeloid Leukemia (AML) is characterized by uncontrolled proliferation of myeloid precursors that have failed to differentiate and accumulate resulting in impaired hematopoiesis and bone marrow failure.
- AML is the most common acute leukemia present in adults affecting over 20,000 cases per year in the US
- Current treatments for AML include high doses of chemotherapy and allogeneic hematopoietic cell transplantation. Both of these treatments result in high morbidity and mortality with a 5 year survival period post-treatment in less than 30% of patients.
- Besides genetic alterations in hematopoietic cells, alterations in the supporting stromal cells in the bone marrow microenvironment affect hematopoiesis and can lead to malignancies. Osteoblasts, the bone forming cells and their progenitors in the marrow, have been implicated in leukemogenesis
- Constitutive activation of b-catenin signaling in osteoblasts has been shown to induce AML in mice while this pathway remains activated in the osteoblasts of 38% of AML patients
- B-catenin is a ubiquitous transcriptional activator in the canonical Wnt signaling pathway involved in many cellular processes.
- Its function as a transcriptional activator mainly depends on its nuclear availability which is tightly controlled through its interaction with two subcellular compartments: its interaction with cadherins that sequesters b-catenin to the plasma membrane and its interaction with a complex of proteins in the cytoplasm (destruction complex) that leads to its degradation
- Hypothesis: The constitutive activation of b-catenin signaling observed in AML patient's osteoblasts could be due to mutations in b-catenin itself, cadherins or proteins consisting the destruction complex that would fail to tightly regulate its presence in the nucleus.

Methods

- Osteoblast samples were harvested from an explant culture of bone chip (fragments) collected from AML patients during a biopsy. Samples were used from 5 AML patients with activated b-catenin signaling, 4 AML patients without activated b-catenin to check for specificity and 4 healthy controls.
- DNA was isolated by using the Qiagen DNA isolation kit
- Genes encoding for b-catenin and APC were amplified by PCR using Q5 high-fidelity DNA polymerase and primers spanning all the exons of b-catenin gene and nucleotides 3000-5000 of the APC gene that is commonly mutated in cancer
- PCR products were purified from agarose gels using ZymoClean Gel DNA recovery kit
- Purified PCR products were bi-directionally sequenced at Genewiz

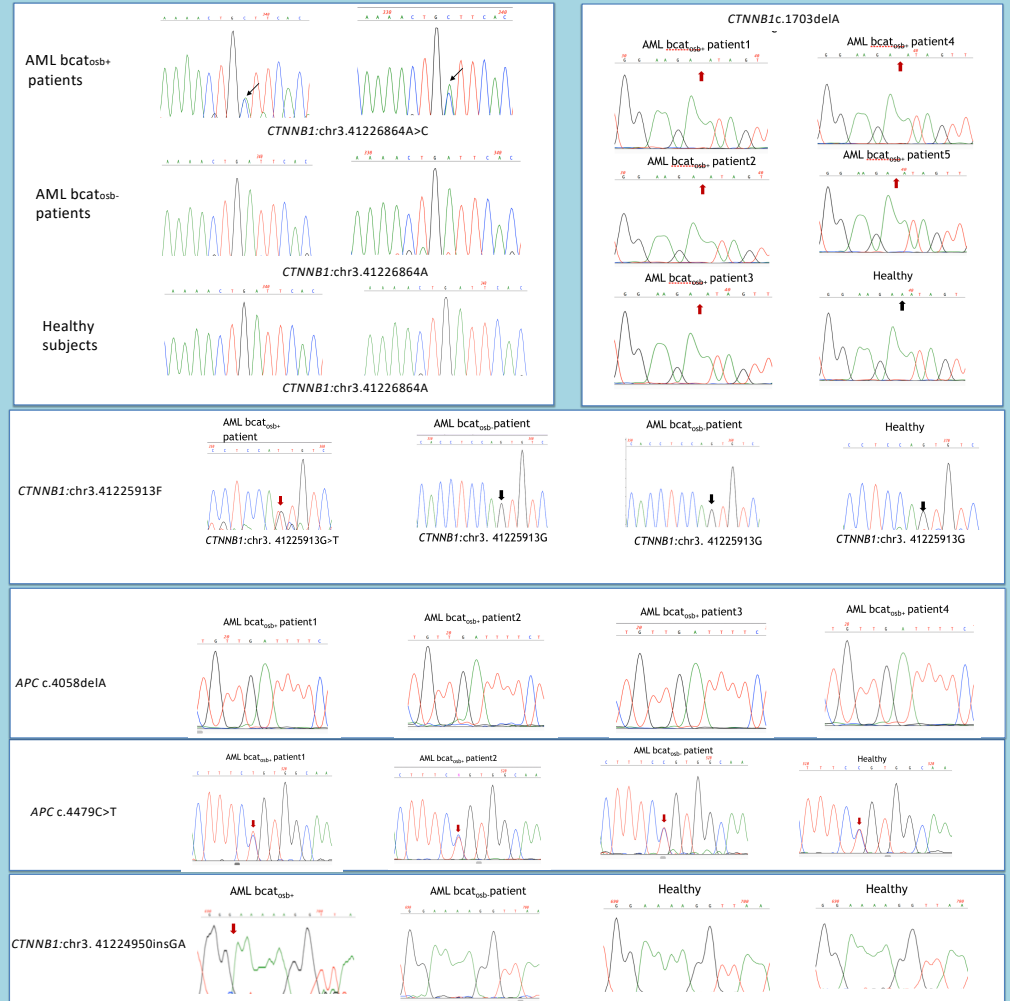
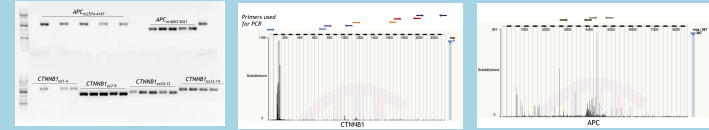
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Acknowledgments

We would like to express our gratitude to the Pinkerton Foundation, Science Sandbox – Simons Foundation, Harlem DNA Lab, Cold Spring Harbor Laboratory, and Columbia University Medical Center for making this research project possible. Special thanks to our mentors Dr. Ioanna Mosialou and Christine Marizzi, for guiding us throughout this lengthy yet fruitful experience, especially during the experimental process.

Results



Conclusions

- Preliminary analysis of 5 AML patients identified genomic alterations in both b-catenin and APC gene. The functional significance of these mutations is yet unknown and will be tested.
- Identifying genetic drivers of activated b-catenin signaling in osteoblasts may provide additional therapeutic targets for a subgroup of AML patients with activated b-catenin signaling in osteoblasts to improve their treatment options.