Personalized Single-Cell Proteogenomics Distinguish Leukemic and Nonmalignant Clones

- CITE-seq with WGS-guided personalized DNA probes is performed on three relapsed AML cases.
- Concurrent detection of genome lesions with immunophenotypic markers definitively resolves clonal relationships between CH and AML.

Oncogenic driver mutations are present in otherwise normal cells (clonal hematopoiesis, CH) coexisting with malignant cells in patients with cancer, including leukemia. Distinguishing CH from leukemia is essential to accurately estimate the disease burden and presents a challenge for therapies guided by minimal residual disease (MRD). In this work, Dillon et al. characterize three relapsed acute myeloid leukemia (AML) patient samples by whole genomic sequencing (WGS) and design patient-specific DNA probes to key oncogenic lesions, including translocations. These probes are then used for single-cell DNA-seq combined with antibody–oligonucleotide conjugates for multiparameter flow cytometry. This approach enables distinguishing nonmalignant hematopoietic clonal populations from AML clones at a resolution unattainable with previous methods. The findings reveal that genetic and morphologic clinical estimates of MRD are missing a considerably sized pool of AML cells. It also uncovers a layer of clinically relevant phenotypic diversity uncoupled from genetic and transcriptional level.

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Genome Subtyping with MRD-Directed Therapy in Pediatric ALL

- Among 598 pediatric ALL cases, 16 B-ALL and 8 T-ALL subtypes are identified by NGS.
- MRD-based risk assessment has improved in 6 novel subtypes.
- Combining MRD with genomic analysis of disease subtype improves the accuracy of risk-directed treatment.

While a number of new acute lymphoblastic leukemia (ALL) subtypes have been identified by next-generation sequencing (NGS)–based cancer genome analyses, their utility to guide clinical decisions remains to be explored. In this work, Jeha et al. have combined the powers of genomic subtyping and minimal residual disease (MRD) detection. They have performed next-generation genomic sequencing of 598 children treated for ALL within the Total Therapy Study 16 trial, which identified 16 B-ALL and 8 T-ALL subtypes. MRD <0.01% is associated with durable complete remission if achieved on day 8 for most subtypes; in contrast, when reached by day 42 MRD, it does not rule out a relapse for certain high-risk subtypes. In higher MRD groups, outcomes are associated with genotypes. For patients with MRD exceeding 1% at day 15, therapy intensification improved outcomes in three novel subtypes. The authors conclude that genomic analyses and MRD should be used together for risk-directed treatment. Six recently described subtypes—DUX4-rearranged, PAX5alt, BCR–ABL1-like, ETV6–RUNX1-like, MEF2D-rearranged, and ZNF384-rearranged ALL—have shown prognostic and therapeutic value with contemporary risk-directed treatment. These results demonstrate how novel genomic subtypes can improve precision of MRD-guided risk assessment in pediatric ALL.

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**ROBO1 Promotes Myeloma Dissemination and Proliferation**

- High ROBO1 expression is found in myeloma cell lines, but not in normal B lymphocytes or plasma cells.
- Engraftment of myeloma cells in mice is reduced in the absence of ROBO1.
- Ligand-independent cleavage of the cytoplasmic domain of ROBO1 underlies its MM-promoting activity.

In this study, Bianchi et al. have expanded the insight of the role of the ROBO1 transmembrane receptor and its natural ligands, extracellular matrix proteins of the SLIT family, in the development and biological properties of multiple myeloma (MM). shRNA-mediated ROBO1 knockdown is cytotoxic in MM, but not other hematologic malignancies or ROBO1-expressing cell lines. ROBO1 is rate limiting for myeloma growth in culture and in an extramedullary plasmacytoma mouse model. Using paired MM-derived cell lines with biallelic ROBO1 deletion and reintroduction of full-length ROBO1, this work demonstrates ROBO1 requirement for adhesion to stromal and endothelial cells and for homing and engraftment of myeloma to the bone marrow. These functions are mediated by the ROBO1 cytosolic domain, which the authors propose may be regulated by proteolysis and translocation to the nucleus. Proteome and transcriptome assays identify ROBO1-interacting proteins and ROBO1-dependent RNA processing, offering further insights into possible molecular mechanisms mediating ROBO1 effects on homing and adhesion.

*See article, p. 338.*

**Cyclophosphamide Extends BiTE Efficacy against Advanced Myeloma by Preventing T-cell Exhaustion**

- T-cell exhaustion limits efficacy of BiTE therapy in mice with high myeloma burden.
- IMiD combination with BiTE boosts T-cell activation but does not prevent T-cell exhaustion.
- Cytotoxic therapy combined with BiTE improves T-cell persistence and confers long-lasting immune protection against tumor rechallenge.

Bispecific T-cell engager (BiTE) antibodies hold promise as off-the-shelf alternatives to CAR T cells. In multiple myeloma, responses to BCMA-targeting immunotherapies are robust but of limited duration. Here Meermeyer et al. explored immune mechanisms of BiTE response and resistance. They have engineered Vk*Myc mice to express human Cereblon, thus generating an immunocompetent murine model of myeloma sensitive to immune-modulatory drugs (IMiD). In this model, they find IMiDs synergize with BiTEs at low but not high tumor burden. In the latter setting, IMiDs still boost BiTE-induced T-cell activation. As more T-cell activation leads to more T-cell exhaustion, this initial benefit turns into a subsequent drawback, with no net gain in survival. In contrast, cyclophosphamide improves BiTE long-term therapeutic effects in mice with low and high tumor loads. This study identifies a roadblock and offers a mechanistic basis for overcoming it with rational combinations for maximizing beneficial responses to this promising line of therapy.

*See article, p. 354.*
KDM5A Is Required for MYC-Driven Transcription in Myeloma

Ohguchi et al. identify KDM5A as a crucial regulator of myeloma cell proliferation, Myc levels, and expression of Myc target genes, first by expression analysis in a large myeloma cohort and then by loss-of-function experiments using siRNA knockdown and a newly developed small-molecule inhibitor, JQKD82. By ChIP-seq, RNA-seq, and reporter assays, KDM5A is mapped to promoters of Myc-dependent genes, where it colocalizes with Myc and promotes transcription. This effect is specific to KDM5A and not seen with other KDM5 proteins, which are otherwise similar in structure, substrate activity, and cellular functions. The authors propose that KDM5A inhibition following hyperinduction of the H3K4me3-mark “anchors” the RNA polymerase II–phosphorylating complex away from its target, thereby preventing transcriptional elongation of Myc-driven genes. Taken together, the data suggest that targeting KDM5A represents a promising therapeutic strategy in KDM5A-dependent malignancies.

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Oncofusion Proteins Are Selectively Vulnerable to Heat Shock

Throughout evolution of species, proteins are optimized for robust performance in stress environments, including heat shock. Here Maimaitiyiming et al. postulated that as products of chance translocations of unrelated genes, oncofusion proteins may lack thermostability. Focusing on the acute promyelocytic leukemia (APL) driver oncofusion protein PML–RARα, they demonstrate its selective destabilization at 42°C in cell lines with ectopic expression and endogenous translocation, as well as in primary patient blasts. Most APL cases respond to ATRA and arsenic therapy, but approximately 1% are refractory or develop resistance. Importantly, hyperthermia potentiated the effect of drugs and induced destabilization of therapy-resistant PML–RARα mutants. Mechanistically, PML–RARα degradation requires NCoR–PML–RARα complex formation and ubiquitination by SIAH2, following autolysosomal proteolysis. Hyperthermia with arsenic reduced tumor burden in three patients with refractory APL. Collectively, these findings invite broader exploration of heat shock sensitivity as a possible Achilles heel of oncofusion-driven neoplasms.

See article, p. 388.

In This Issue is written by Blood Cancer Discovery editorial staff. Readers are encouraged to consult the original articles for full details.