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# POSTER ABSTRACTS

## 602.MYELOID ONCOGENESIS: BASIC

### Phase Separation of PML/RARα Microspeckles Governs Transcriptional Dysregulation through Genomic Rewiring of BRD4 in Acute Promyelocytic Leukemia

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**Introduction:** Abnormal nuclear morphologies are frequently observed in cancer cells, which have been implicated in biological reactions such as gene regulation and DNA damage repair. However, the nature of altered nuclear structures, particularly molecular components determining the specificity, regulatory functions and oncogenic activity of nuclear compartments, has remained largely enigmatic and awaits characterization. In acute promyelocytic leukemia (APL), the PML/RARα fusion oncoprotein, which is produced by t(15;17), has been long observed under the microscope for its ability in destroying PML nuclear bodies (NBs) and subsequently forming microspeckles. Restoration of PML NBs has been reported during the treatment with retinoic acid (RA) or arsenic trioxide (ATO). Despite being long observed, how PML/RARα-mediated microspeckles are formed and linked to APL leukemogenesis remains a mystery. To address this knowledge gap, we present a comprehensive analysis of the driving forces and constituent components of PML/RARα-assembled microspeckles and their functional implications in transcriptional dysregulation and leukemogenesis.
**Methods:** High-resolution imaging techniques, multi-omics strategies, integrative bioinformatics analysis, and in-depth functional validation are used in this study. We employ Immunofluorescence, Fluorescence recovery after photobleaching and in *vitro* droplet formation assays to determine the chemical and physical properties and constituent components of PML/RARα microspeckles. Co-IP and mass spectrometry are performed to identify interactome of PML/RARα. ChIP-seq is utilized to determine the exact genomic regions where PML/RARα microspeckles occur and to evaluate the impact on chromatin occupancy of PML/RARα and its co-factors upon genetic or pharmacological perturbation. RNA-seq is performed to evaluate the transcriptional output of repression of condensates mediated co-factor activity. Functional experiments, including CCK-8 assay and Flow cytometry, are used to assess the pathological functions of PML/RARα assembled and BRD4 recruited microspeckles.
**Results:** We uncover the biophysical mechanism of liquid-liquid phase separation (LLPS) underlying the assembly of PML/RARα microspeckles and elucidate their role in APL leukemogenesis. Our findings reveal that PML/RARα co-assembles with BRD4 to form *de novo* nuclear phase-separated condensates, which distinguish them from PML nuclear bodies. PML/RARα and BRD4 co-assembled condensates exhibit preferential occupancy on super-enhancers and broad-promoters, targeting genes essential for APL leukemogenesis. Mechanically, PML/RARα incorporates BRD4 into nuclear condensates, thereby facilitating its chromatin binding and redistribution. Importantly, blockage of the condensate-mediated co-activator BRD4 activity suppresses APL cell proliferation and induces apoptosis, thereby impairing PML/RARα-driven leukemogenesis.

Finally, perturbation of LLPS depletes the chromatin co-occupancy of PML/RARα and BRD4 and attenuates their target gene activation, reinforcing the importance of LLPS in transcriptional dysregulation.

**Conclusions:** In this study, we have provided a comprehensive analysis of PML/RARα-assembled microspeckles, shedding light on their driving forces, constituent components, and regulatory function. Our findings have resolved the long-standing cognitive stagnation regarding the morphological characteristics of PML/RARα microspeckles. We have demonstrated that PML/RARα forms *de novo* phase-separated condensates, which selectively recruit the co-activator BRD4 to gene loci with both super enhancers and broad promoters. This aberrant recruitment leads to dysregulated transcriptional programs and the development of malignant phenotypes. Importantly, our present study is the first to reveal the biophysical nature of PML/RARα-mediated microspeckles and elucidate the fundamental regulatory mechanism underlying their formation. We have also highlighted the role of PML/RARα phase separation in the pathogenesis of APL leukemogenesis. Therefore, our study provides valuable biophysical insights into the molecular basis for PML/RARα to exert its oncogenic activity.

**Disclosures** No relevant conflicts of interest to declare.

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