ROLE OF THE INTERFERON ALPHA INDUCIBLE PROTEIN 6 (IFI6) IN ESTABLISHING CHEMORESISTANCE IN ACUTE MYELOID LEUKEMIA

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INTRODUCTION

Acute Myeloid Leukemia (AML) presents a major therapeutic challenge. While initial response to standard 7+3 chemotherapy is observed in most patients, relapse with chemo-resistant disease is a frequent event, leading to a dismal overall survival rate of <40%. Emerging evidence points towards a population of rare AML cells, termed persistent blasts, that survive chemotherapy and fuel relapse. However, the mechanisms by which these blasts evade the cytotoxic effects of treatment remain unclear. The prevailing hypothesis implicates quiescent leukemia stem cells (LSCs), since are found within the persistent blast population and their dormant state inherently renders them resistant to chemotherapy. However, why chemoresistance persists in the progeny of LSCs as they emerge from quiescence to regenerate the leukemic population is unclear. Genomic analyses of paired primary/relapse AML samples showed persistence of multiple genetic clones at relapse (our unpublished results) suggesting that treatment resistance is not due to genetic mechanisms. Alternatively, persistent blasts may acquire resistance through phenotypic adaptation to chemotherapy. We developed novel mouse models of AML that faithfully recapitulate the features of chemo-resistant disease observed in patients: initial response to a treatment regimen adapted from the clinic (3+5) followed by inevitable relapse. Persistent and relapsing cells exhibit true chemoresistance, both in vitro and in vivo. Resistance is not associated with the emergence of new genetic mutations, suggesting a non-genetic mechanism at play. We observed that chemo-persistent blasts encompass both proliferating and quiescent populations, including quiescent LSCs. Single-cell transcriptomic analyses revealed unique transcriptional features in chemotherapy-persistent blasts. These blasts were characterized by activation of interferon (IFN) signaling in both quiescent and proliferating populations. Interestingly, the chemoresistance-associated IFN gene signature was able to stratify AML patients treated with a 3+7 chemotherapy regimen. Silencing the most highly upregulated IFN target gene, IFI6, did not affect growth of leukemia cells. However, it completely reversed the chemoresistant phenotype in both in vitro and in vivo experiments. Notably, IFI6 is known to negatively regulate innate immunity, suggesting that AML cells adapt to chemotherapy by preventing excessive activation of interferon signaling after treatment, thus protecting LSCs from cell death or senescence induced by IFN.

AIM

i) Generate AML PDX models of chemoresistance

ii) Investigate cell-cycle and clonal dynamics of AML during chemotherapy

iii) Characterize transcriptional phenotypic states of chemotherapy-resistant blasts and establish their role in relapse development

iv) Identify candidate genes responsible of chemo-resistance and relapse and validate their role in leukemogenesis and chemo-resistance

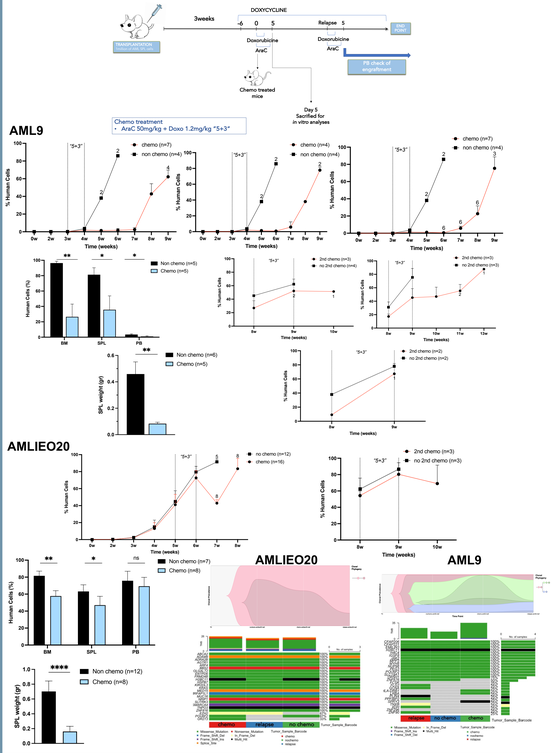
METHODS

- The first available AML PDX models of chemoresistance
- Incorporation of a label-retaining system to monitor and isolate quiescent cells (Tet-Off H2B-GFP)
- Treatment schedule as in patients (AraC + Doxorubicin, "5+3")
- Whole Exome Sequencing (WES)
- Single Cell RNA sequencing
- Lineage Tracing
- IFI6 shRNA for in vitro and in vivo validation

RESULTS

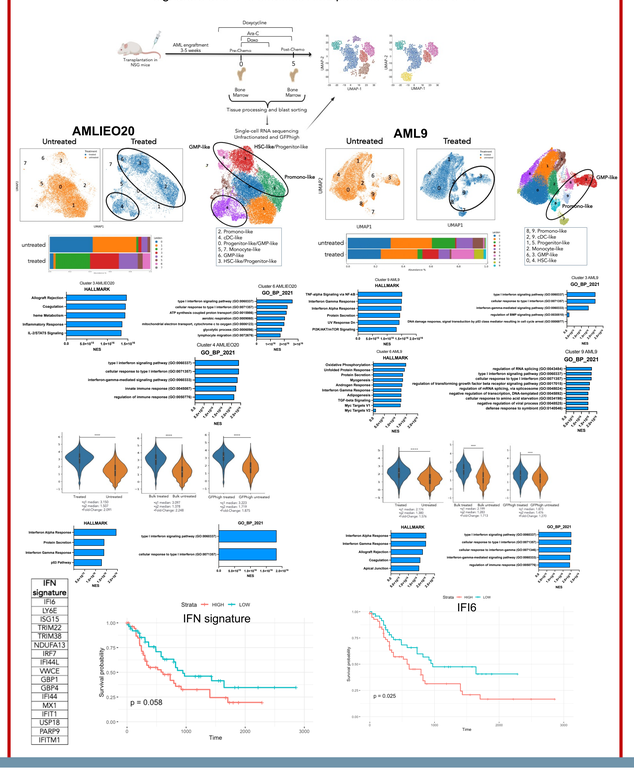
GENERATION OF AML PDX MODELS OF CHEMORESISTANCE

We established the first two AML PDX models of **non-genetic chemoresistance**. We demonstrated how the "5+3" chemotherapy regimen induces complete (**AML9**) partial (**AMLIEO20**) disease remission. In both models chemotherapy persistent cells and relapsing leukemias are chemo-resistant and chemoresistance does not select specific DNA mutations.



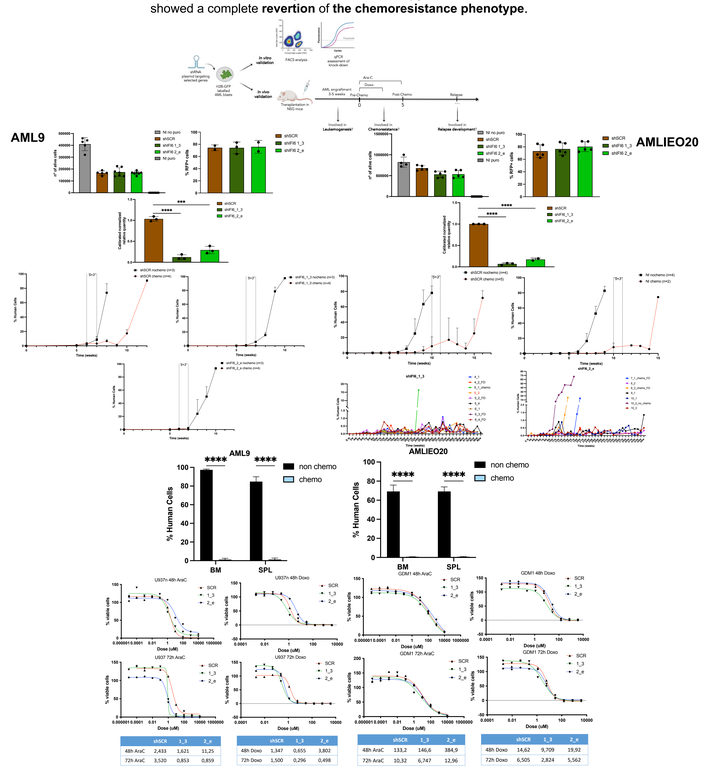
TRANSCRIPTIONAL CHARACTERIZATION OF PERSISTENT BLASTS

Chemo-persistent blasts are characterized by specific transcriptional features: **undifferentiated phenotype** and **up-regulation of interferon signalling pathway**. This chemoresistance-associated phenotype is shared by both persistent-proliferating and persistent-quiescent blasts, thus suggesting that quiescence is not a specific characteristic of resistance. The chemoresistance interferon-gene signature and IFI6 stratifies AML patients treated with 3+7.

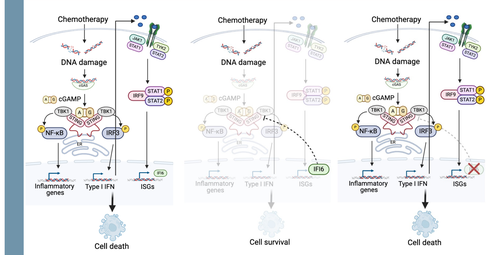


VALIDATION OF IFI6 GENE AS CHEMORESISTANCE GENETIC DETERMINANT

The shSCR does not modify the growth and the chemoresistance phenotyoe of AMLIEO20 blasts. In vivo shIFI6 increased the letency but with the same kinetics of leukemia growth (AMLIEO20 model). In vivo shIFI6 do not have any effect on latency or kinetic of leukemia growth (AML9 model). shIFI6-expressing blasts in both models maintain IFI6 silencing regardless of treatment. Therefore IFI6 is dispensable for leukemia growth. **Chemotherapy eradicates AML** in all **IFI6-silenced mice**, we showed a complete **revertion** of **the chemoresistance phenotype**.



CONCLUSIONS



IFI6 is activated by chemotherapy in our model systems, as part of the signalling pathway that connects DNA damage to interferon activation (cGAS-STING pathway)

- DNA damage in the nucleus results in the accumulation of cytoplasmic DNA (micronuclei and ds/ssDNA)
- Cytoplasmic DNA binds to and activates cGAS, that converts guanosine triphosphate (GTP) and ATP into the second messenger cyclic GMP-AMP (cGAMP)
- cGAMP binds and activates STING at the ER
- STING then activates transcription factors IRF3 and NF-κB (IKK) via TBK1 kinase
- IRF3 and NF-κB translocate into the nucleus to elicit the expression of IFNs and other cytokines/inflammatory genes.

IFI6 might prevent excessive activation of interferon/cytokine upon chemotherapy, which is detrimental for stem and differentiated cells

- DDR-Induced IFN-beta promotes cell senescence/apoptosis of differentiated cells
- IFN contributes to stem cell decline and premature aging
- We hypothesize that IFI6 antagonizes IFN signalling by direct interaction and inhibition of cGAS or STING at the ER

shIFI6 might "restore" excessive activation of interferon/cytokine upon chemotherapy, preventing blasts adaptation to chemotherapy and restoring chemosensitivity

- In shIFI6 AMLs, chemotherapy might induce hyper-activation of cGAS/STING, NF-κB, and IFN/cytokine in blasts upon chemotherapy, promoting cell senescence/apoptosis of differentiated and stem cells

Therapeutic activation of interferon signalling (IFN, cGAMP) might prevent occurrence of chemoresistance upon standard treatments in AMLs

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