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Acute Leukemias

INTEGRATION OF MULTIPLE RNA-SEQ DATASETS ENHANCES DEEP PROFILING OF NPM1-MUTATED FLT3-ITD NEGATIVE AML

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Introduction: Acute Myeloid Leukemia (AML) is an aggressive, molecularly heterogeneous malignancy with limited survival despite advances in its characterization and treatment. Nucleophosmin (NPM1) mutations without FLT3-ITD (NPM1-mut, FLT3-ITDneg) define a subgroup with favorable outcomes using chemotherapy alone. However, up to 40% of these patients relapse. Attempts to refine prognosis through additional gene mutations have failed. We therefore deeply analyzed the biological landscape of this clinically relevant subgroup using an integrated RNA-seq dataset, aiming to identify novel prognostic and biological biomarkers, including through machine-learning (ML) approaches.
Methods: Three public and one unpublished RNA-seq datasets were included in the study. We created the 'Gu' algorithm (EHA 2025 - PS1463) to generate an n = 894 unified RNA-seq dataset with strong biological coherence. Synthetic data for data augmentation purposes were generated via the "synthpop" R package, performing a 300× augmentation that yielded n = 2,682 synthetic samples. Several bioinformatic and ML analyses were performed to investigate the clinically relevant NPM1mut FLT3-ITDneg AML subgroup.
Results: The unified dataset demonstrated correct data integration and biological coherence (Figure 1a), also reproducing the ELN 2022 risk stratification (Fig. 1b). Within NPM1-mutated AML (n = 308, 187 FLT3-wt), the transcriptomic profile was heterogeneous, contrasting with homogeneous entities such as APL or t(8;21) AML. The NPM1-mut signature was confirmed, with HOX genes upregulated and CD34/CD133 downregulated (Figure 1c); new genes related to NPM1mut were identified. Focusing on the NPM1mut FLT3-ITDneg AML, a distinct transcriptomic signature based on FLT3-ITD mutational status was identified (Figure 1d) beside a group of genes that was stably upregulated in NPM1-mut independently of FLT3, allowing definition of an NPM1-mut "core" (mainly HOX genes). Transcriptomic dissection revealed two subgroups (Figure 1e): a "FLT3-like" cluster resembling FLT3-ITD AML, and an "NPM1-pure" cluster enriched in inflammatory/immune genes. RAS mutations prevailed in FLT3-like and IDH2 in NPM1-pure cases. No survival difference emerged under standard therapy. Finally, genes with prognostic value were identified, including genes related to immune regulation. A scoring system based on the expression of a 4-gene-panel could effectively stratify prognosis (Fig 1f). Validations are ongoing.
Conclusions: Our work opened an innovative window into the biology of NPM1-mutated FLT3-wild-type AML, revealing a distinctive transcriptomic signature that had never emerged from previous analyses, as limited sample size had so far precluded deep investigations on this subgroup. Several potential biomarkers for further prognostic stratification and new targets for therapy have emerged. Biological validations are ongoing in our lab.

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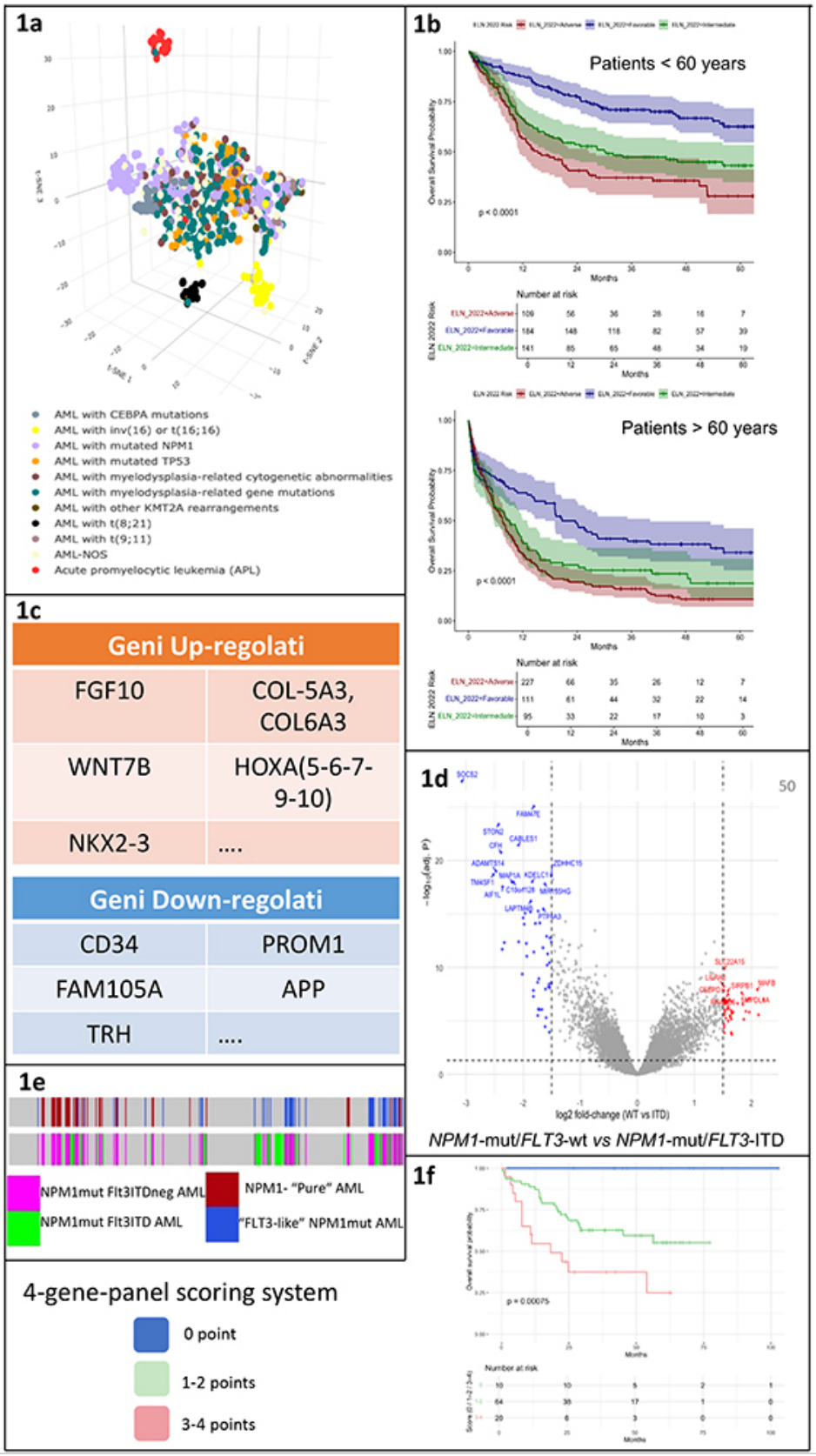
1a
AML with CEBPA mutations
AML with inv(16) or t(16;16)
AML with mutated NPM1
AML with mutated TP53
AML with myelodysplasia-related cytogenetic abnormalities
AML with myelodysplasia-related gene mutations
AML with other KMT2A rearrangements
AML with t(8;21)
AML with t(9;11)
AML-NOS
Acute promyelocytic leukemia (APL)
1b
Patients < 60 years
Patients > 60 years
Overall Survival Probability
Months
Number at risk
ELN 2022 Risk
1c
Geni Up-regolati
FGF10
COL-5A3, COL6A3
WNT7B
HOXA(5-6-7-9-10)
NKX2-3
....
Geni Down-regolati
CD34
PROM1
FAM105A
APP
TRH
....
1d
log2 fold-change (WT vs ITD)
NPM1-mut/FLT3-wt vs NPM1-mut/FLT3-ITD
1e
NPM1mut Flt3ITDneg AML
NPM1- "Pure" AML
NPM1mut Flt3ITD AML
"FLT3-like" NPM1mut AML
1f
4-gene-panel scoring system
0 point
1-2 points
3-4 points