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ARTICLE

Acute myeloid leukemia

CD8^{+} T cells expand stem and progenitor cells in favorable but not adverse risk acute myeloid leukemia

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Abstract

CD8^{+} T cell immunosurveillance is crucial in solid tumors and T cell dysfunction leads to tumor progression. In contrast, the role of CD8^{+} T cells in the control of leukemia is less clear. We characterized the molecular signature of leukemia stem/progenitor cells (LSPCs) and paired CD8^{+} T cells in patients with acute myeloid leukemia (AML). Epigenetic alterations via histone deacetylation reduced the expression of immune-related genes in bone marrow (BM)-infiltrating CD8^{+} T cells. Surprisingly, a silenced gene expression pattern in CD8^{+} T cells significantly correlated with an improved prognosis. To define interactions between CD8^{+} T cells and LSPCs, we performed comprehensive correlative network modeling. This analysis indicated that CD8^{+} T cells contribute to the maintenance/expansion of LSPCs, particularly in favorable risk AML. Functionally, CD8^{+} T cells in favorable AML induced the expansion of LSPCs by stimulating the autocrine production of important hematopoietic cytokines such as interleukin (IL)-3. In contrast, LSPCs in aggressive AML were characterized by a higher activation of stemness/proliferation-related pathways and develop independent of BM CD8^{+} T cells. Overall, our study indicates that CD8^{+} T cells support and expand LSPCs in favorable risk AML whereas intermediate and adverse risk AML possess the intrinsic molecular abnormalities to develop independently.

Introduction

Acute myeloid leukemia (AML) is a heterogeneous group of hematologic malignant diseases, characterized by maturation arrest and increased proliferation of myeloid blasts [1, 2]. Based on the (cyto)genetic profile of AML blasts, patients can be classified into three different risk groups: favorable risk (40–45%), intermediate risk (25–35%), and adverse risk (25–30%) [3].

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Leukemia stem cells (LSCs) represent a minor fraction of the bulk leukemia cell population that reconstitutes and propagates the disease. They are resistant to chemotherapy and irradiation and therefore are the main cause of relapse [4–8]. LSCs possess stem cell properties such as self-renewal and quiescence that are regulated by cell-intrinsic and cell-extrinsic mechanisms [9–11]. As cell-intrinsic drivers, LSCs display molecular abnormalities that lead to constitutive activation of the nuclear factor kB (NF-kB) [2], Wnt [12], and Notch signaling pathways [13]. In addition, similar to hematopoietic stem cells (HSCs), LSCs interact with cells of the bone marrow (BM) microenvironment, including endothelial cells, specialized BM stromal cells, and osteoblasts [14–16]. These cells constitute the HSC niche and regulate maintenance, self-renewal, and differentiation of HSCs and LSCs [17]. Immune cells are part of the BM microenvironment and interact with HSCs as well as leukemia stem and progenitor cells (LSPCs). For example, CD8^{+} T cells have the potential to support the maintenance of HSCs and facilitate their engraftment after allogeneic stem-cell transplantation [18, 19].

It is well documented that AML responds to immune-mediated therapies such as allogeneic hematopoietic stem

cell transplantation and donor lymphocyte infusions [14, 20]. Similarly, AML blasts can be lysed by adoptively transferred gene-modified T cells in xeno-transplantation experiments [21]. However, several studies indicate that LSCs escape elimination by CD8$^+$ T cell and natural killer (NK) cells by down-regulating important molecules/pathways for immune recognition or by the expression of immune-inhibitory molecules [22, 23]. Experimental evidence indicates that cytokines secreted by immune cells and defined cell–cell interactions such as the CD70/CD27 interaction expand HSCs and LSCs and contribute to disease progression [24, 25]. However, the role of the adaptive immune system in the control of human AML and especially in the regulation of LSCs is poorly understood.

We performed a comprehensive transcriptomic profiling of BM-derived LSCs and leukemia progenitor cells together with paired CD8$^+$ T cells of AML patients from different molecular risk groups. This analysis indicated that epigenetic mechanisms silence the gene expression of CD8$^+$ T cells in AML. Importantly, a silenced gene expression pattern correlated with improved prognosis. Correlation network modeling revealed that CD8$^+$ T cells regulate LSPC in favorable risk but not in adverse risk AML. Functionally, we show that CD8$^+$ T cells induce the autocrine production of the hematopoietic cytokines such as IL-3 in favorable risk AML that expands LSPCs. The interaction of CD8$^+$ T cells with LSPCs was gradually lost from favorable risk to intermediate and adverse risk AML. In contrast, LSPCs from patients with intermediate and adverse risk AML had a higher expression of genes related to stemness and cell proliferation. This study indicates that LSPCs in favorable risk AML are regulated by extrinsic signals such as BM-infiltrating CD8$^+$ T cells, whereas mainly cell-intrinsic mechanisms drive LSPC expansion in aggressive AML.

Materials and methods

Patients

Blood and BM aspirates from patients diagnosed with AML at the Department of Medical Oncology, University Hospital Bern were prospectively collected. Thirty patients were selected from this repository based on the FACS immune-phenotype of the AML cells and the risk category. The clinical and molecular characteristics of the AML patients and controls are listed in Supplementary Table 1. This study was approved by the local ethical committee (Kantonale Ethikkommission Bern, KEK122/14).

Molecular profiling and correlation network modeling

Transcriptomic analysis was performed on 111 different well-defined FACS-purified samples of hematopoietic stem/progenitors and paired CD8$^+$ T cells from 30 AML patients and 7 controls. Modeling of several tens of thousands of predictive correlation network was assessed to map potential links between genes expressed in stem/progenitor cells and paired CD8$^+$ T cells in all AML patients of different risk groups and controls. Selection of investigated genes, biological signaling, and correlations were further functionally validated. For details, see Supplementary Methods.

Data availability

All transcriptomic data compiled for this study have been deposited in NCBI GEO under the accession code GSE117090. In addition, expression data from GEO public repository was assessed as a validation cohort (GSE6891).

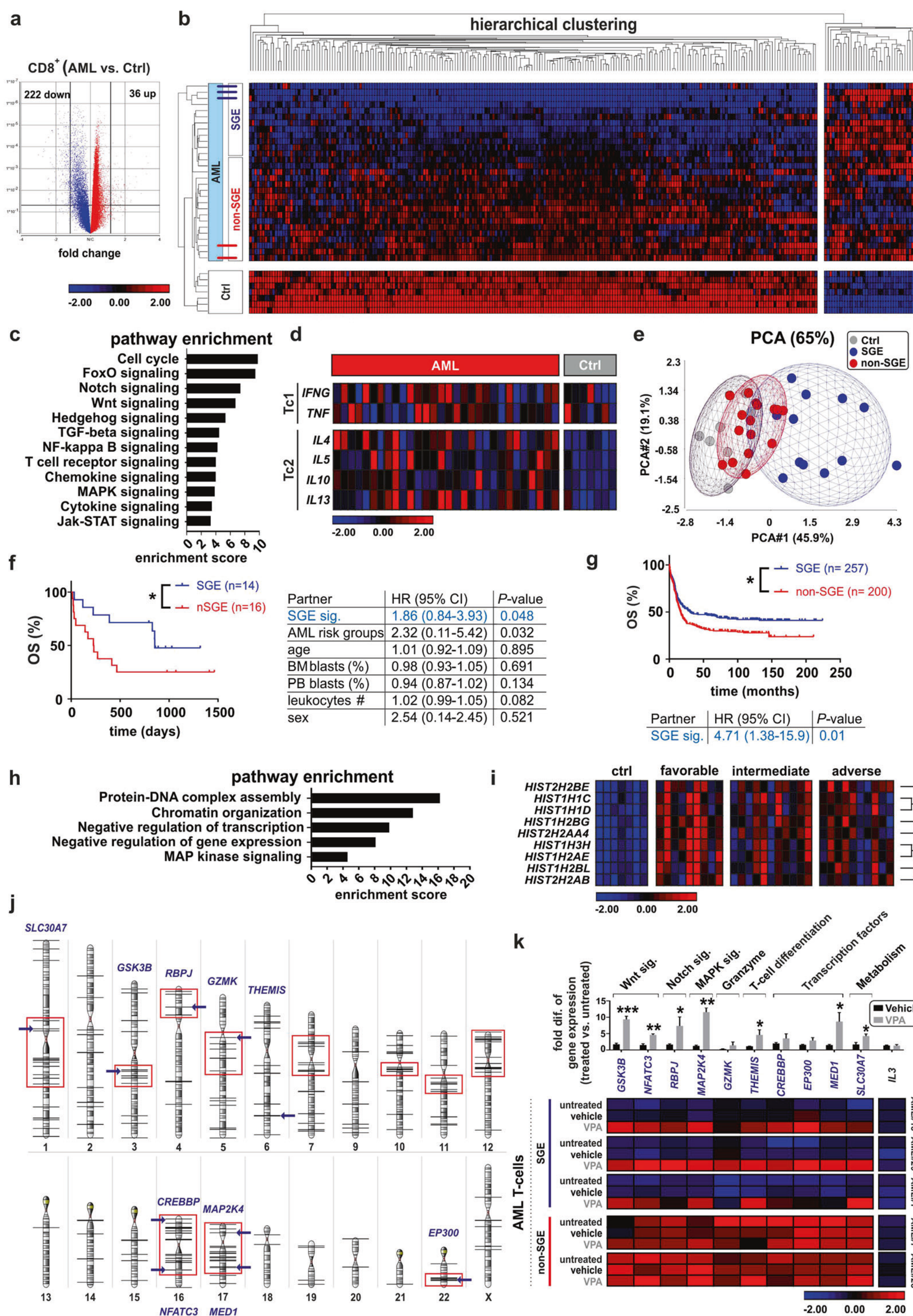
Complete methods are included in the Supplementary Appendix.

Results

A silenced gene expression pattern in CD8$^+$ T cells correlates with improved prognosis in AML

We first characterized the complete gene expression signature of FACS-purified CD34$^+$CD38$^-$ AML LSCs, CD34$^+$CD38$^+$ AML progenitors and paired CD8$^+$ T cells in the BM of AML patients at the time point of diagnosis (Supplementary Fig. 1, Supplementary Table 1). As expected, patients in the favorable risk group had a better overall survival in comparison to the other AML risk groups (Supplementary Fig. 2).

The expression of 258 genes was significantly changed in BM-infiltrating CD8$^+$ T cells from AML patients compared to healthy donors (Supplemental Dataset 1). Interestingly, 222 genes were down-regulated and only 36 genes were up-regulated (Fig. 1a, b). The down-regulated genes are involved in signaling pathways related to T cell activation, differentiation, and function, such as NFkB, Wnt, FoxO, T cell receptor (TCR), and cytokine/chemokine signaling (Fig. 1c). The expression profile of key genes for T cell polarization in CD8$^+$ T cells of AML patients revealed a TC1 phenotype in controls but a skewing towards a TC2 phenotype in AML patients (Fig. 1d). Out of the 222 down-regulated genes, we defined a 40-gene panel of genes that belong to the NFkB, Wnt, FoxO and Notch pathway, TCR and cytokine/chemokine signaling according to the results of the gene ontology enrichment



Partner	HR (95% CI)	P-value
SGE sig.	1.86 (0.84-3.93)	0.048
AML risk groups	2.32 (0.11-5.42)	0.032
age	1.01 (0.92-1.09)	0.895
BM blasts (%)	0.98 (0.93-1.05)	0.691
PB blasts (%)	0.94 (0.87-1.02)	0.134
leukocytes #	1.02 (0.99-1.05)	0.082
sex	2.54 (0.14-2.45)	0.521

Partner	HR (95% CI)	P-value
SGE sig.	4.71 (1.38-15.9)	0.01

and pathway analysis (Fig. 1c, Supplementary Table 2). Based on the calculated mean gene expression level of these 40 pre-defined genes, outcome-based cut-points were defined using X-Tile software [26]. AML patients with a mean gene expression below or above the defined cut-point were classified as patients with a "silenced gene expression (SGE) signature" or "non-silenced gene expression" (non-SGE) signature, respectively. Principal component analysis (PCA) revealed a closer similarity between the non-SGE group and healthy controls while patients with the SGE signature exhibited more distinct gene expression patterns (Fig. 1e). Importantly, patients with a SGE signature in BM CD8$^+$ T cells had a significantly better overall survival compared to patients with a

◀ **Fig. 1** A silenced gene expression pattern in $CD8^+$ T cells correlates with improved prognosis. **a** Volcano plot showing differentially expressed genes of leukemia $CD8^+$ T cells (AML vs. Ctrl); *y*-axis: negative log of *P*-value; *x*-axis: log2-fold change; red dots: up-regulated genes; blue dots: down-regulated genes. **b** Heatmap illustrating differentially expressed genes in $CD8^+$ T cells (AML vs. Ctrl). **c** Pathway enrichment analysis of 222 down-regulated genes. **d** Heatmap illustrating the expression profile of key genes for $CD8^+$ T cell phenotype polarization. Silenced gene expression (SGE); non-silenced gene expression (non-SGE). **e** PCA indicating the similarities between $CD8^+$ T cells with SGE or non-SGE signature and controls according to their gene expression profile. **f** Kaplan–Meier plots of overall survival (OS) for AML patients according to the 40-gene panel of $CD8^+$ T cells. Multivariate analysis for SGE signature adjusted for AML risk groups, age, percentage of blasts in BM and blood, leukocyte counts, and sex. **g** Kaplan–Meier plots of overall survival (OS) for AML patients in the validation cohort according to the 40-gene signature of $CD8^+$ T cells. Cox-regression analysis for SGE signature in the validation cohort. **h** Pathway enrichment analysis of 36 up-regulated genes. **i** Heatmap illustrating the expression profile of up-regulated histone organizer/regulator genes. **j** Karyogram panel shows significant enrichment of down-regulated genes to particular regions in the genome. Important selected genes are highlighted in blue. **k** Expression profile of selected genes from three AML patients with SGE signature (AML #15, favorable group; AML #29, intermediate group; AML #1, adverse group) and T cells from two AML patients with non-SGE signature (AML #7, favorable group and AML #28, adverse group). Analysis was performed immediately after FACS-purifying (untreated) and after 24 h treatment with VPA or vehicle. The fold differences of gene expression were calculated as the ratio of treated (VPA or vehicle) vs. untreated conditions. Statistics: **f**, **g** log-rank test; and multiple Cox-regression, **k** Student's *t*-test (2-tailed). $^*P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$. CI confidence interval, OS overall survival. (See also Supplementary Fig. 2–3; Supplementary Table 2; Supplementary Dataset 1)

non-SGE signature (Fig. 1f). Although the analysis in the different risk groups is limited by a rather small sample size, the expression of a SGE signature correlated with longer survival only in the favorable risk but not in the intermediate or adverse risk group (Supplementary Fig. 3). Multivariate analysis for the SGE signature adjusted for AML risk group, patient age, percentage of blasts in BM and blood, leukocyte counts, and sex, confirmed the SGE signature as an independent prognostic marker for overall survival in our analyzed AML patient cohort (Fig. 1f).

To validate the prognostic value of this 40-genes panel in a larger cohort of AML patients, we analyzed the expression of these genes in a publically available dataset comprising gene expression of non-fractionated BM cells of AML patients [27, 28]. Our defined 40-gene panel showed significantly better overall survival for patients with a SGE signature than for patients with a non-SGE signature. Cox-regression analysis further confirmed the SGE signature as a prognostic marker for overall survival (Fig. 1g).

In our analysis, we observed a systemic down-regulation of genes in AML $CD8^+$ T cells compared to controls. Chromatin modification mainly via histone deacetylation is one of the key mechanisms for gene silencing [29]. Only 36 genes were up-regulated in AML $CD8^+$ T cells (Fig. 1b). Pathway enrichment analysis revealed that these 36 up-regulated genes are primarily involved in the control of chromatin organization or negative regulation of transcription and gene expression (Fig. 1h). Nine of these 36 up-regulated genes are controlling chromatin organization/regulation (Fig. 1i). Chromosomal position-based gene-mapping analysis (karyogram) showed that down-regulated genes were not randomly distributed all over the genome but significantly enriched in some particular regions. These data suggest altered histone organization in particular chromosomal regions in leukemic CD8 T cells (Fig. 1j).

Histone deacetylation catalyzed by the histone deacetylase (HDAC) is a central switch of permissive to repressive chromatin domains leading to transcriptional silencing [30]. To functionally test the effect of histone deacetylation on the observed SGE signature, we treated FACS-purified $CD8^+$ T cells isolated from three AML patients with SGE signature and from two AML patients with non-SGE signature with the HDAC inhibitor, valproic acid (VPA). VPA treatment significantly reversed the SGE phenotype and increased the expression of 10 selected key genes involved in the regulation of Wnt-, Notch-, MAPK-signaling, T cell differentiation, and in metabolism. In contrast, VPA did not change the expression of these genes in T cells with non-SGE signature (Fig. 1k). In addition, VPA treatment did not change the expression of *IL3* in $CD8^+$ T cells from different AML patients, indicating that *IL3* gene expression in T cells is not regulated by histone deacetylation (Fig. 1k).

Overall, these data indicate that important genes involved in $CD8^+$ T cell activation, differentiation, and function are down-regulated in AML due to pathologic epigenetic alterations mainly mediated via histone deacetylation. Down-regulation of these genes in $CD8^+$ T cells correlates with an improved prognosis of AML patients.

LSPCs from AML patients display a dysregulated expression of genes involved in proliferation, stemness, and immune-recognition

We next investigated the gene expression signature of $CD34^+CD38^-$ AML LSCs and $CD34^+CD38^+$ AML progenitors. HSPCs from healthy donors (Ctrl) had a similar gene expression profile and therefore clustered together in the PCA. In contrast, the gene expression of AML cells was more diverse and differed from healthy controls (Fig. 2a). In AML LSCs and progenitors, 403 and 1309 genes were differentially expressed compared to controls, respectively (Fig. 2a, Supplementary Dataset 1). A comprehensive pathway analysis of the differentially expressed genes revealed that mainly pathways related to stemness, cell proliferation, cell cycle, or immune cell signaling were changed in AML LSCs and progenitor cells (Fig. 2b). The

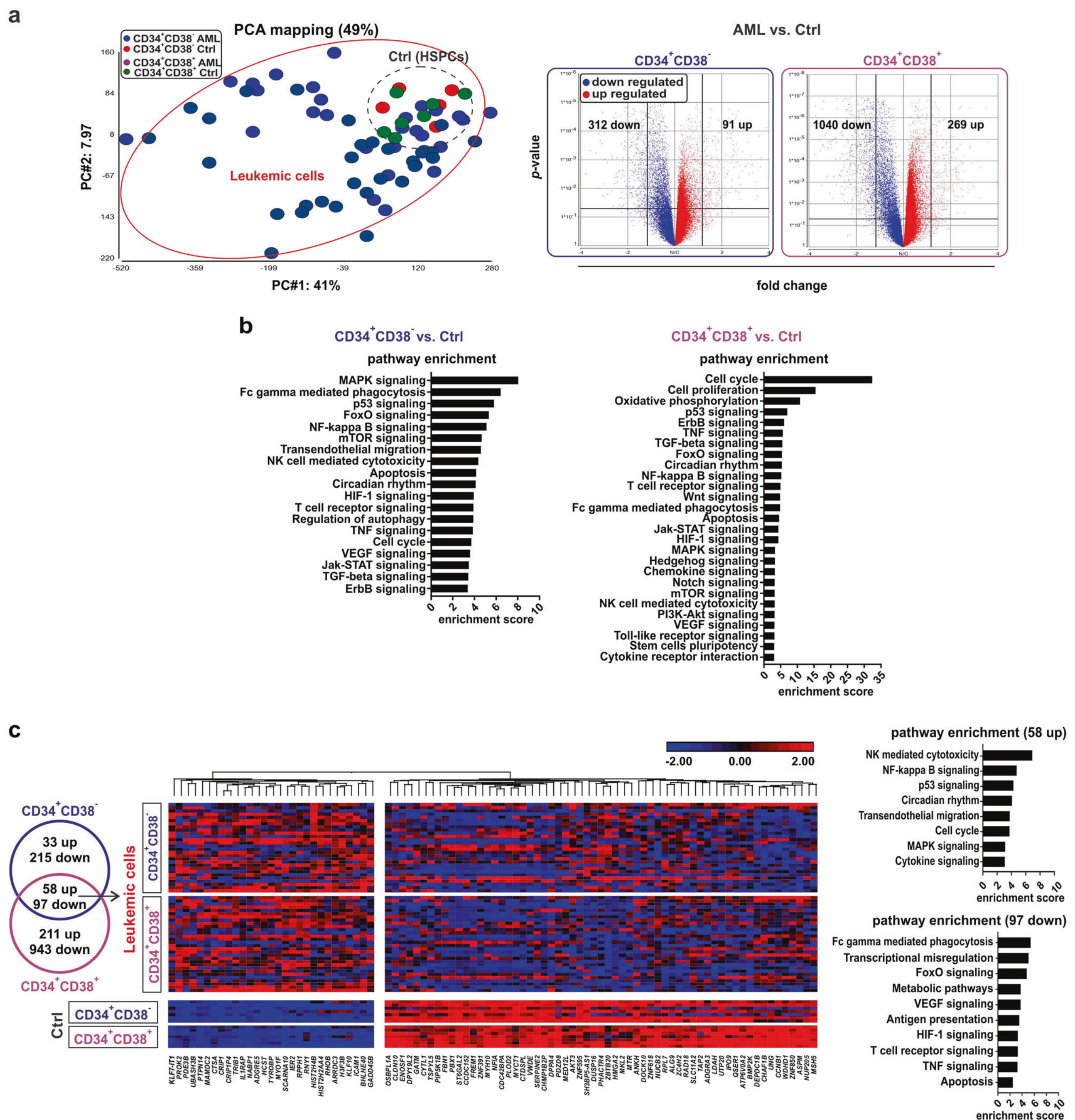


Fig. 2 Gene expression analysis of LSPCs from AML patients. **a** PCA of AML LSPCs and control HSPCs. Volcano plots representing differentially expressed genes of $CD34^+CD38^-$ and $CD34^+CD38^+$ AML and control cells; *y*-axis: negative log of *P*-value; *x*-axis: log2-fold change; red dots: up-regulated genes; blue dots: down-regulated genes. **b** Pathway enrichment analysis of differentially expressed genes in each cell population. **c** Venn-diagram of significant up/down-regulated genes within leukemia stem/progenitor cells (AML vs. Ctrl). Heatmap indicating the profile of 155 differentially expressed intersection genes (58 up-regulated and 97 down-regulated genes). Pathway enrichment analysis of 58 up-regulated and 97 down-regulated intersection genes. (See also Supplementary Dataset 1)

number of 155 genes (58 up-regulated and 97 down-regulated genes) were similarly dysregulated in AML LSCs and progenitors when compared to controls (Fig. 2c). Pathway enrichment analysis revealed that up-regulated genes are mainly involved in cell proliferation, cell cycle, or immune-related signaling while down-regulated genes are primarily involved in signaling pathways mediating antigen-presentation and interaction with immune cells (Fig. 2c). Taken together, AML LSCs and progenitors had a higher expression of genes involved in stemness and cell proliferation compared to HSPCs whereas the down-regulated genes and pathways facilitate immune-escape.

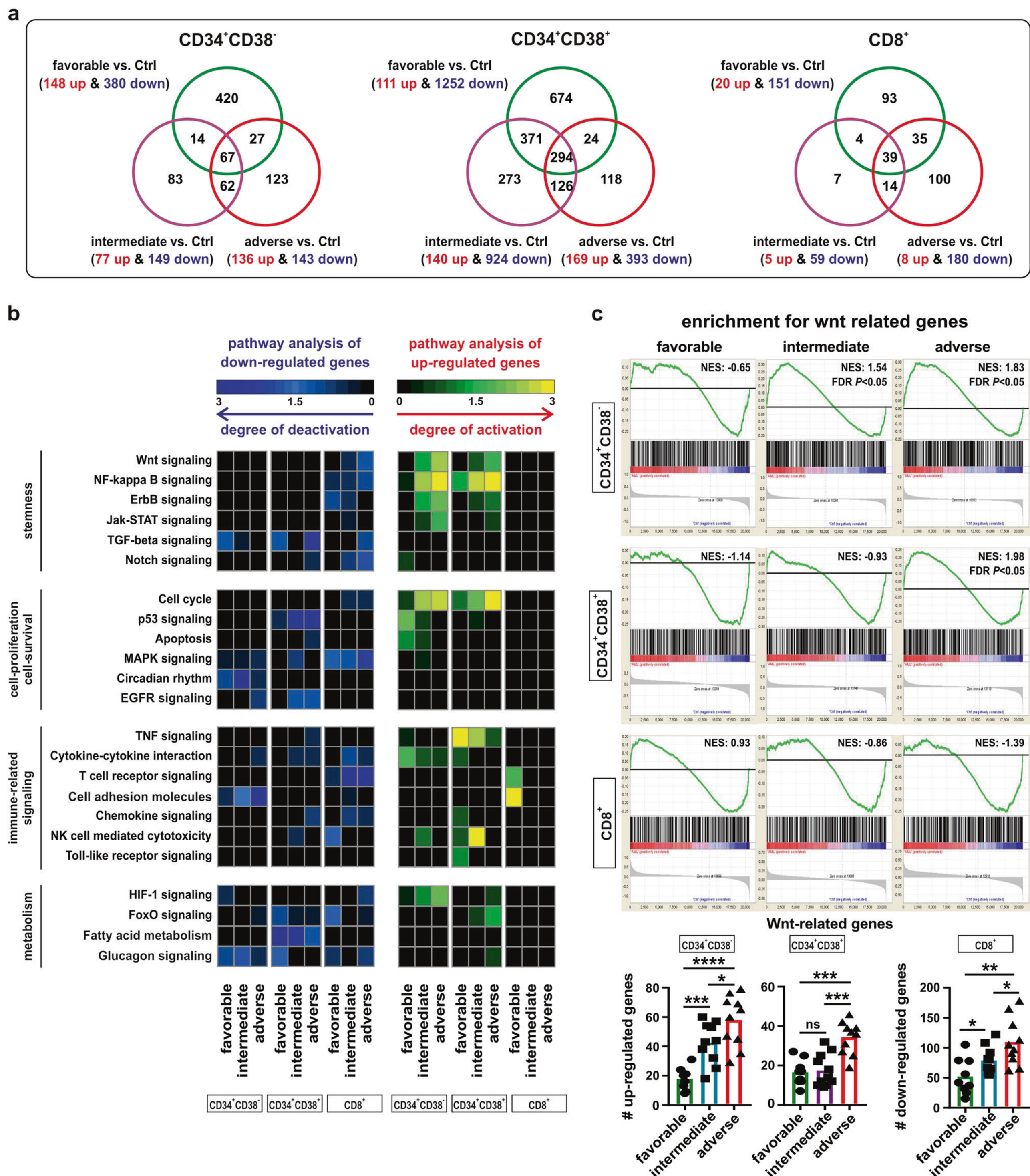


Fig. 3 Gene expression signature of leukemia stem/progenitor cells and paired CD8$^+$ T cells across AML risk groups. **a** Venn-diagrams showing differentially expressed genes in CD34$^+$CD38$^-$ LSCs, CD34$^+$CD38$^+$ progenitors and CD8$^+$ T cells across AML risk groups (favorable, intermediate, or adverse vs. Ctrl). **b** Pathway analysis of differentially expressed genes in LSPCs and CD8$^+$ T cells across AML risk groups. **c** Gene set enrichment analysis (GSEA) representing the normalized enrichment score (NES) of gene sets linked to Wnt signaling for LSPCs and CD8$^+$ T cells (favorable, intermediate, or adverse vs. Ctrl). Bar charts showing the number of up-regulated Wnt-related/target genes in AML patients from different risk categories vs. controls. Statistics: Student's *t*-test (2-tailed). $^*P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$, $^{****}P < 0.0001$. (See also Supplementary Dataset 1)

Genes involved in immunosurveillance are down-regulated whereas stemness-related genes are up-regulated in adverse risk AML

We next compared the gene expression profiles of AML LSCs, progenitors, and paired CD8$^+$ T cells between the three different risk categories (Fig. 3a). AML progenitors shared 294 dysregulated intersection genes across all AML risk categories. In contrast, only 67 intersection genes were found in AML LSCs. The gene expression profile of BM CD8$^+$ T cells differed significantly between the different risk groups with only 39 intersection genes that were similarly altered in all three-risk categories (Fig. 3a). AML progenitors, which comprise the highly proliferative AML cells, showed the highest expression of genes related to cell cycling. In contrast, genes involved in stemness- and leukemogenesis-related pathways were expressed at higher levels in AML LSCs (e.g., Wnt, ErbB, and NF-kB). These stemness-related pathways were expressed at highest levels in LSCs from adverse risk AML and at lowest levels in LSCs from the favorable risk group. This finding is in line with previous studies indicating that a LSC-related gene signature is a negative prognostic marker in AML [31, 32]. Furthermore, genes involved in immune-related pathways such as cytokine–cytokine receptor interactions and cytokine/chemokine signaling were more expressed in LSCs from favorable risk AML but were not expressed in adverse risk AML (Fig. 3b).

In line with these findings, genes involved in pathways mediating immune cell function and target cell recognition such as TCR and chemokine signaling as well as cytokine–cytokine receptor interaction were more expressed in paired CD8$^+$ T cells from favorable risk AML but were down-regulated in the adverse risk group. Similarly, signaling pathways involved in T cell effector function, such as NF-kB and Notch signaling, were significantly down-regulated in CD8$^+$ T cells isolated from intermediate and adverse risk AML patients [33]. The Wnt pathway, which is crucial for the differentiation into memory T cells [33, 34], was inactivated in CD8$^+$ T cells from patients with adverse risk AML (Fig. 3b). These results suggest that T cells in intermediate and high risk AML are less functional than in favorable risk AML and might be exhausted. However, the analysis of markers defining exhausted CD8$^+$ T cells on gene level did not reveal differences between the AML risk groups (Supplementary Fig. 4). Importantly, the frequency of BM-infiltrating CD8$^+$ T cells did not differ between the AML risk groups or healthy controls (Supplementary Fig. 5). This indicates that functional but not numerical differences lead to the CD8$^+$ T cell-mediated expansion of LSPCs in favorable risk AML.

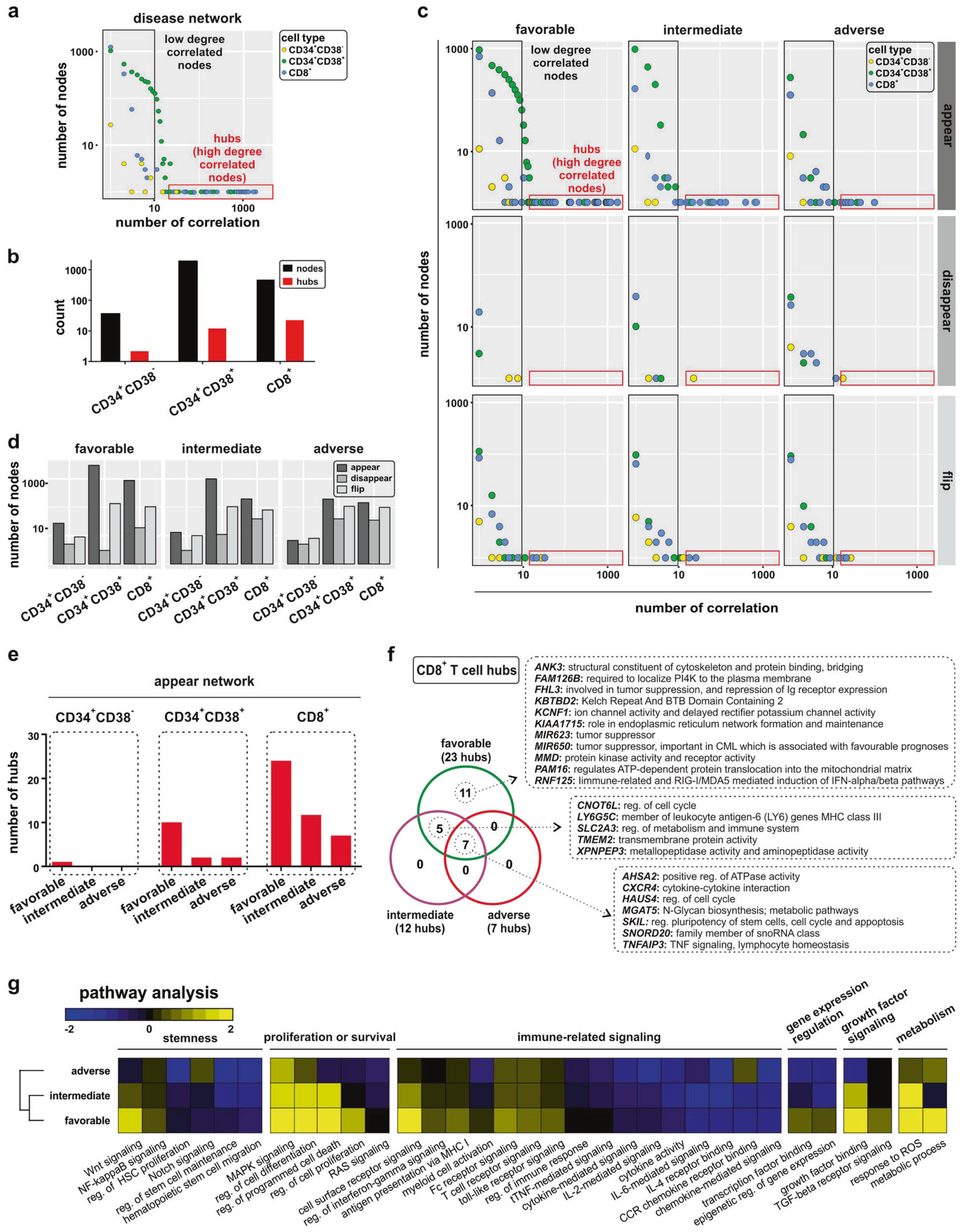
Wnt signaling is fundamental for stemness and effector function of LSCs and CD8$^+$ T cells, respectively [12]. Gene set enrichment analysis (GSEA) in our AML cohort revealed a significant enrichment for genes involved in Wnt pathway activation in LSPCs from intermediate and adverse risk group AML patients, but not in LSPCs from the favorable risk group. In contrast, Wnt-related genes were down-regulated in paired CD8$^+$ T cells from AML patients of the intermediate and adverse risk group (Fig. 3c). These results suggest that LSPCs of adverse risk AML patients develop and expand largely independently of CD8$^+$ T cells, whereas CD8$^+$ T cells are mainly active in favorable risk AML patients.

CD8$^+$ T cells regulate LSPCs in favorable but not adverse risk AML

To define possible interactions of genes/pathways in CD8$^+$ T cells with genes/pathways in AML LSPCs, comprehensive correlation networks were constructed. In each network, a node was defined as a gene expressed either in CD8$^+$ T cells or in LSPCs. A node (gene) in one cell that correlates significantly with more than 15 nodes in the other cell type was considered as a hub (high-degree correlated node) (Fig. 4a). The assumption was that a gene expressed in CD8$^+$ T cells communicates or coordinates other genes in LSPCs or vice versa. Three types of correlations were detected: (1) “appear”, a correlation present in AML but not in controls, (2) “disappear”, a correlation present in the controls and absent in AML, and (3) “flip”, where the direction of the correlation changes.

The highest number of nodes was detected in AML progenitor cells and in CD8$^+$ T cells, whereas few nodes were detected in AML LSCs (Fig. 4a, b). Interestingly, the highest number of hubs were detected in CD8$^+$ T cells, whereas fewer hubs were found in LSPCs (Fig. 4a, b, Supplementary Dataset 2). We next analyzed these networks in the different AML risk categories (Fig. 4c, d). The number of nodes in the “appear network” in AML LSPCs and CD8$^+$ T cells gradually decreased from the favorable risk to the adverse risk group (Fig. 4d). In contrast, the number of nodes in “disappear” and “flip networks” did not change across AML risk groups (Fig. 4c, d). Importantly, the majority of hubs in CD8$^+$ T cells were present in the favorable risk group and their number gradually decreased in the intermediate and adverse risk groups (Fig. 4e). No CD8$^+$ T cells hubs were detected in intermediate and adverse risk groups that were not present in the favorable risk group (Fig. 4e, f). Only few hubs were identified in CD34$^+$CD38$^+$ progenitors. However, similar to CD8$^+$ T cells, reduced number of hubs were identified with increased risk category (Fig. 4e, Supplementary Fig. 6).

The majority of nodes within AML LSPCs, which were connected to CD8$^+$ hubs, were up-regulated compared to controls (Supplementary Fig. 7). The number of nodes in



LSPCs connected to given hubs in CD8$^+$ T cells was gradually decreased from favorable to intermediate and adverse risk AML (Supplementary Table 3).

In order to identify the signaling pathways in AML LSPCs, which are modulated by the identified CD8$^+$ T cell hubs, we performed a pathway analysis. The significantly

◀ **Fig. 4** Correlation network modeling between leukemia stem/progenitor cells and paired $CD8^+$ T cells. **a** Schematic view of correlation networks for AML samples ("disease"). The red box represents hubs with a high degree of correlations and black box indicates low degree correlated nodes. **b** Quantification of hub and node genes in LSPCs and $CD8^+$ T cells. **c** Schematic view of correlation networks that only appear in one or more of the AML groups but not in the healthy controls ("appear"); networks which are only detectable in control samples but not in any of the AML risk groups ("disappear"); networks that are significant in both the control group and patient groups but have opposite signs ("flip"). **d** Bar graph representing the number of node gene in the appear, disappear, and flip networks, in different AML risk categories. **e** Quantification of hub genes in the appear network in AML risk categories. **f** Venn-diagram of $CD8^+$ T cells' hubs genes in different AML risk categories. **g** Pathway analysis of node genes within LSPCs that show significant correlation to $CD8^+$ T cell hubs. (See also Supplementary Fig. 6–7; Supplementary Table 3; Supplementary Dataset 2)

altered pathways in LSPCs included stemness, cell proliferation and survival, immune-related signaling, gene expression regulation, growth factor signaling, and metabolism which were most activated in favorable risk AML (Fig. 4g, Supplementary Fig. 7).

Taken together, our correlation network modeling indicated that $CD8^+$ T cells regulate LSPCs in favorable risk AML. This interaction is reduced or absent in intermediate and adverse risk AML.

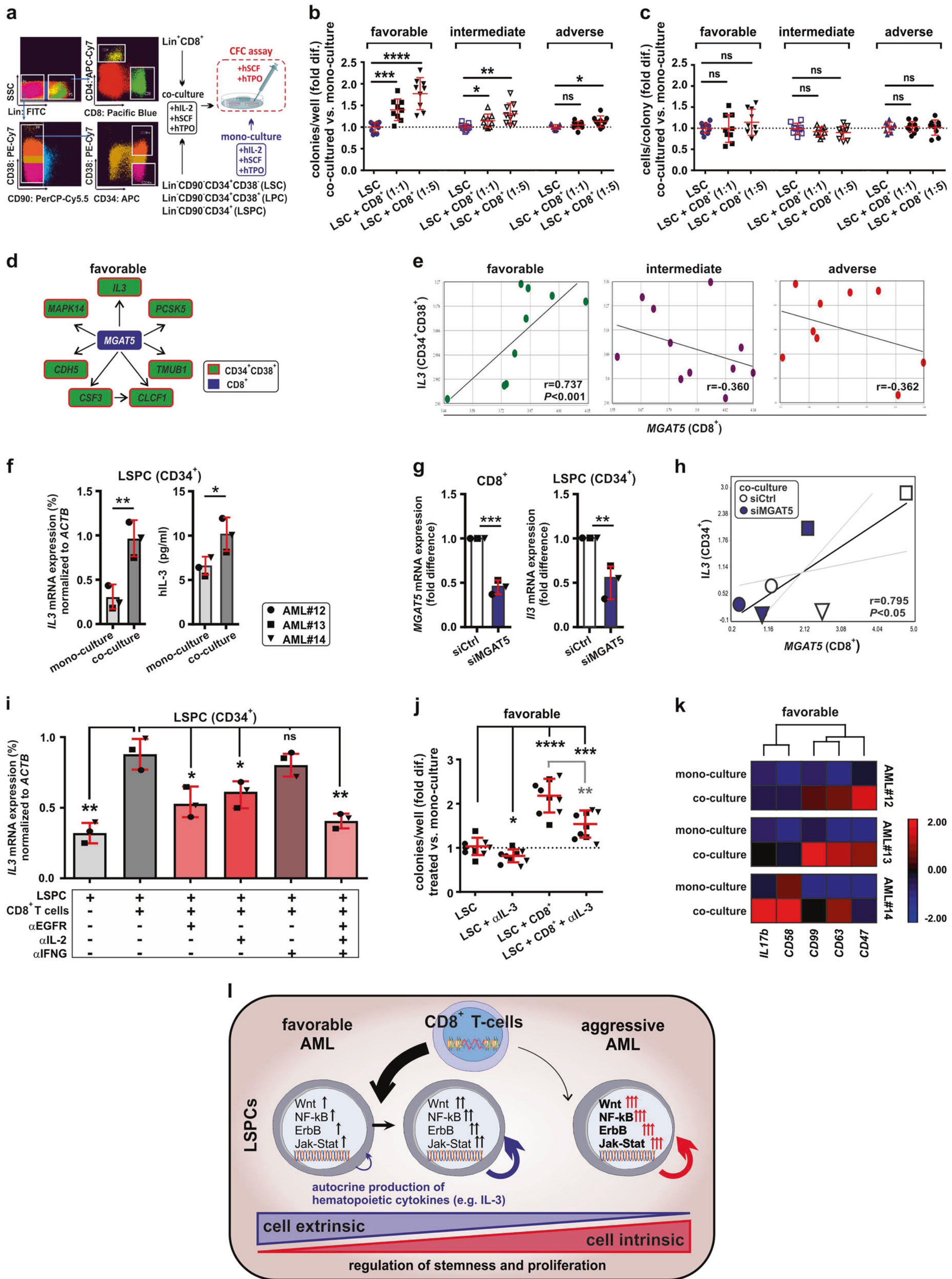
$CD8^+$ T cells induce the expansion and maintenance of LSPCs in favorable risk AML

To functionally analyze the potential influence of $CD8^+$ T cells on LSPCs, we FACS-purified both LSCs or AML progenitor cells together with paired $CD8^+$ T cells from BM of nine different AML patients (Fig. 5a, Supplementary Table 1). The co-culture of $CD8^+$ T cells with LSCs resulted in an up to 2-fold increase in colony-formation compared to LSC mono-culture in the favorable risk group. In contrast, the increase in colony formation in intermediate and adverse risk was less pronounced and only observed at higher T cell:LSC ratios (Fig. 5b). In addition, the number of cells per colonies was not significantly changed, suggesting that differentiation was not affected by co-culture of LSPCs with $CD8^+$ T cells (Fig. 5c). Further re-plating of cells derived from primary colony assays showed a significant difference in the colony-formation capacity of co-cultured LSCs with a higher ratio of $CD8^+$ T cells compared to LSC mono-culture. This indicates that LSCs with unimpaired self-renewal capacity are expanded by $CD8^+$ T cells (Supplementary Fig. 8).

MGAT5 was identified as a hub in $CD8^+$ T cells of all risk groups. However, while *MGAT5* positively correlated with the expression of *IL3* and other important genes in $CD34^+CD38^+$ progenitors in favorable risk AML patients, this correlation is lost in intermediate and adverse risk AML (Fig. 5d, e, Supplementary Fig. 9, Supplementary Dataset 2).

Our results suggest that $CD8^+$ T cells regulate LSPCs in favorable AML by inducing the production of important hematopoietic cytokines such as IL-3. To address this possibility, we co-cultured FACS-purified $CD8^+$ T cells with paired LSPCs derived from the same patients of favorable risk AML and analyzed the expression of *IL3* mRNA and protein. LSPCs of all risk groups expressed the IL-3 receptor alpha (IL-3Ra). While, $CD8^+$ T cells did not express IL3Ra on mRNA and protein levels (Supplementary Fig. 10). Co-culture with $CD8^+$ T cells resulted in an up to 3-fold increase in *IL3* mRNA expression in LSPCs and an up to 2-fold increase in IL-3 protein expression in culture supernatants compared to LSPCs mono-culture from favorable risk AML (Fig. 5f). In healthy controls, the expression of *MGAT5* in $CD8^+$ T cells did not correlate with *IL3* expression in progenitors (Supplementary Fig. 11a). In addition, co-culture of $CD8^+$ T cells with $CD34^+$ HSPCs derived from healthy donors did not increase the level of *IL3* mRNA expression (Supplementary Fig. 11b). However, co-culture of pooled $CD8^+$ T cells derived from three good risk AML patients significantly increased the colony formation capacity of both, AML LSCs and control HSCs (Supplementary Fig. 11c). Interestingly, colony formation in LSCs was increased significantly more than in HSCs. This suggests that AML LSCs have a higher proliferative capacity in response to stimulation by $CD8^+$ T cells than normal HSCs. Therefore, favorable risk AML LSPCs respond to similar interactions with $CD8^+$ T cells as normal HSPCs. However, only BM-infiltrating $CD8^+$ T cells of AML patients but not of healthy donors induce IL-3 production in LSCs and HSCs.

Knockdown of *MGAT5* gene in $CD8^+$ T cells using a siRNA before initiation of the co-culture revealed an up to 2-fold decrease in *IL3* mRNA expression in LSPCs (Fig. 5g). The level of *MGAT5* gene expression after siRNA treatment of CD8 T cells significantly correlated with the *IL3* expression in co-cultured LSPCs (Fig. 5h). In silico pathway analysis predicted that *MGAT5* expressed in $CD8^+$ T cells triggers the expression of *IL3* in LSPCs via EGF/EGFR, IL-2 or IFNγ signaling (Supplementary Fig. 9a). Correlation analysis confirmed a significant positive correlation between *IL3* expression in $CD34^+CD38^+$ progenitors and *EGF*/*EGFR* or *IL2* expression in $CD8^+$ T cells in favorable risk AML but not in intermediate or adverse risk AML (Supplementary Fig. 9b–c). We tested this concept experimentally by co-culturing FACS-purified $CD34^+$ LSPCs with paired $CD8^+$ T cells in the presence of neutralizing antibodies to these specific cytokines and growth factor receptor. Blocking EGFR and IL-2, but not IFNγ reduced *IL3* mRNA expression in FACS-purified $CD34^+$



LSPCs (Fig. 5i). This finding indicates that CD8^{+} T cells induce IL-3 production in LSPCs mainly by IL-2 and EGF/EGFR signaling.

Neutralization of IL-3 in the co-culture of CD8^{+} T cells with LSCs from favorable risk AML patients significantly decreased the colony-formation capacity (Fig. 5j). In

◀ **Fig. 5** $CD8^+$ T cells regulate LSPCs in favorable risk AML. **a** Experimental setup. **b**, **c** Fold differences in the numbers of colonies or cells from co-cultured conditions (LSCs with $CD8^+$ T cells) vs. monocultured (LSCs only) condition (three patients per each AML subgroup; #3, #22, and #28 from adverse risk group; #5, #17, and #21 from intermediate risk group; #14, #15, and #26 from favorable risk group). **d** An example of a correlation network in favorable group: *MGAT5* hub gene in $CD8^+$ T cells is predicted as a coordinator for different genes in LSPCs. **e** Correlation analysis of *MGAT5* hub in $CD8^+$ T cells vs. *IL3* in $CD34^+CD38^+$ cells in different AML risk categories. **f** *IL3* gene expression in re-purified LSPCs from monoculture or co-culture with $CD8^+$ T cells; (three AML patients from favorable risk category). IL-3 protein in culture supernatants of LSPCs monocultures and co-cultures with $CD8^+$ T cells. **g** *MGAT5* and *IL3* gene expression quantification upon siRNA-mediated gene silencing of *MGAT5*. The fold differences of gene expression were calculated as the ratio of siMGAT5 treated condition vs. siCtrl in re-purified cell populations (three AML patients from favorable risk category). **h** Correlation analysis of *MGAT5* expression in $CD8^+$ T cells vs. *IL3* in LSPCs after *MGAT5* gene knockdown. **i** *IL3* gene expression in FACS-purified favorable risk AML $CD34^+$ LSPCs from mono-culture or co-culture with $CD8^+$ T cells in the presence of neutralizing antibodies to EGFR, IL-2, or IFNγ ($n = 3$; mean ± SD). **j** Fold differences in the numbers of colonies from LSC monocultures- and co-cultures with $CD8^+$ T cells upon neutralization with αIL-3 antibody (three patients from favorable risk AML). **k** Heatmap illustrating expression profile of five selected genes in re-purified LSPCs after mono-culture or co-culture with $CD8^+$ T cells (three AML patients from favorable risk category). **l** Graphical scheme of the interaction between $CD8^+$ T cells and LSPCs. Statistics: Student's *t*-test (2-tailed). $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$, $^{****}P < 0.0001$. (See also Supplementary Fig. 8–11; Supplementary Dataset 2)

addition, co-culture of $CD34^+$ LSPCs with $CD8^+$ T cells resulted in the up-regulation of selected genes that are regulated by hubs with documented function in hematopoiesis, leukemia development, cell proliferation, or immune tolerance (*CD47*, *CD58*, *CD63*, *CD99*, and *IL17b*) (Fig. 5k, Supplementary Table 3) [35–39].

Taken together, these findings indicated that BM $CD8^+$ T cells induce pathways in favorable risk LSPCs that promote leukemia development, such as the autocrine production of hematopoietic cytokines. In contrast, LSPCs in more aggressive forms of AML develop largely independent of $CD8^+$ T cells (Fig. 5l).

Discussion

It is well documented that the immune system contributes to the control of solid tumors, a process called "tumor immunosurveillance". However, cancer cells evade immune recognition and elimination by cytotoxic $CD8^+$ lymphocytes via various mechanisms, including loss of antigen and the expression of immune inhibitory molecules [40, 41]. Thus, tumor-infiltrating lymphocytes (TILs) are often dysfunctional or "exhausted" due to the interaction with immune inhibitory ligands expressed by tumor cells [40]. The presence of dysfunctional $CD8^+$ T cells in TILs is a negative prognostic and predictive factor for the response to treatment with immune-checkpoint inhibitors [42, 43].

In the present study, we analyzed the gene expression signature of BM $CD8^+$ T cells together with paired AML LSCs and progenitor cells. The majority of differentially expressed genes were down-regulated in $CD8^+$ T cells derived from AML patients. The down-regulated genes are involved in key functions of T cell activation and differentiation including NFkB, Notch, Wnt, FoxO, TCR, and cytokine/chemokine signaling. This indicates that similar to TILs in solid tumors, $CD8^+$ T cells in the AML BM are dysfunctional. Surprisingly, patients with dysfunctional $CD8^+$ T cells as indicated by a SGE signature had a significantly better survival compared to patients with non-SGE signature. This difference may be explained by the fact that TILs comprise a population of activated tumor-specific T cells whereas the BM is a secondary lymphoid organ that contains mainly memory T cells with different specificities [44].

The up-regulated genes in $CD8^+$ T cells of AML patients included main drivers of histone deacetylation and epigenetic regulation, which correlated with the SGE signature in our study population. HDAC enzymes regulate key functions in T cells, such as maturation, migration, and TCR signaling [45]. Recently, it was reported that the transcription factors Tcf1 and Lef1 favor $CD8^+$ T cell development by HDAC mediated suppression of lineage-inappropriate genes [46]. In addition, HDAC inhibitors enhance antitumor activity of antigen-specific cytotoxic T cells against solid tumors and multiple myeloma [47]. Similarly, treatment of AML and myelodysplasia patients with azacitidine and VPA induces a $CD8^+$ T-cell response to the MAGE cancer testis antigen [48]. This suggests that a SGE signature of T cells might be reversed by treatment with demethylating agents.

To define possible interactions of $CD8^+$ T cells with human AML LSPCs, we performed a comprehensive correlation network analysis. The goal of this analysis was to identify genes in $CD8^+$ T cells that regulate pathways in LSPCs and vice versa (hubs). Interestingly, most hub genes were identified in $CD8^+$ T cells whereas only few hubs were present in LSPCs. The number of $CD8^+$ T cell hubs was the highest in favorable risk AML and then gradually lower from intermediate to adverse risk AML. In addition, a given $CD8^+$ T cell hub correlated with the highest number of genes (nodes) in LSPCs from favorable risk group and this number was gradually reduced in intermediate to adverse risk AML. In addition, AML progenitors expressed more node genes than AML LSCs, indicating that $CD8^+$ T cells predominantly interact with progenitor cells. Our finding suggests that $CD8^+$ T cells regulate LSPCs in favorable risk AML while more aggressive forms of AML develop independently of BM-infiltrating $CD8^+$ T cells.

According to our comprehensive network modeling, hubs in $CD8^+$ T cells modulate stemness, cell-proliferation and cell-survival, immune-related signaling, and growth factor signaling mainly in favorable risk AML. $CD8^+$ T cells' hub genes in favorable risk AML, correlate with an increased gene expression of cytokine/chemokines such as *IL3* and *IL17B*, and cell surface molecules (e.g., *CD47*, *CD58*, *CD63*, and *CD99*) that have been associated with hematopoiesis, leukemia development, cell proliferation, or immune tolerance [35–39, 49].

Our results indicated that $CD8^+$ T cells induce IL3 production in AML LSPCs via IL2 and EGF signaling leading to their expansion. This is in agreement with reports documenting that IL-3 increases proliferation and expansion of CML stem cells and AML blasts [50, 51]. Interestingly, the level of IL-3Ra expression on AML blasts is associated with increased cellularity, enhanced proliferation, and with poor prognosis [52].

$CD8^+$ T cells regulate LSPCs mainly in favorable risk AML, while more aggressive forms of AML develop independently of BM-infiltrating $CD8^+$ T cells. The frequency of AML-specific T cells is rather low [53, 54]. Therefore, the majority of the analyzed $CD8^+$ T cells in the BM may be BM-resident memory $CD8^+$ T cells that are not leukemia-specific. We document that in adverse risk AML, the positive correlation between *IL2* or *EGF* expressed in $CD8^+$ T cells to *IL3* in AML progenitors was lost (Supplementary Fig. 9c). We did not detect differences in the level of gene silencing in $CD8^+$ T cells in different risk categories (Fig. 1, Supplementary Dataset 1). In addition, we also tested the expression of markers used to characterize exhausted $CD8^+$ T cells (*CD244* (2B4), *CD160*, *TIGIT*, *HAVCR2*, *LAG3*, *PDCD1* (PD-1)). This analysis did not reveal a preferential expression of exhaustion markers in T cells of intermediate or adverse risk AML (Supplementary Fig. 4). In addition, genes involved in immune-related pathways such as cytokine–cytokine receptor interactions and cytokine/chemokine signaling were expressed in LSCs from favorable risk AML but were not expressed in adverse risk AML (Fig. 3b). Therefore, we suggest that the lack of interaction between $CD8^+$ T cells and LSPCs in intermediate and adverse risk AML is not due to alterations in T cells but due to molecular changes in LSPCs in intermediate and high risk AML that render them independent in regard of proliferation and expansion. AML LSCs and progenitors in aggressive AML had a higher expression of genes involved in stemness and cell proliferation (as an intrinsic driver of leukemia) compared to LSPCs in favorable risk AML, whereas the down-regulated genes and pathways facilitated immune-escape and immune-tolerance. Stemness signatures in blasts are an important negative prognostic marker [12, 24]. The canonical Wnt pathway, which is central for HSC maintenance and development, is constitutively active in myeloid leukemia and of crucial importance for LSCs [24, 55, 56]. Fusion proteins such as AML1-ETO, MLL-ENL, PLZF-RARα, and PML-RARα induce Wnt signaling via activation of γ-catenin [57–59]. In addition, activating mutations in FLT3 are associated with high β-catenin levels and correlate with poor overall survival in AML patients [59, 60]. We document that stemness-related pathways, especially the Wnt pathway, were more active in adverse risk than in intermediate or favorable risk AML.

AML is a very heterogeneous disease. It is therefore not surprising that the interaction of $CD8^+$ T cells with LSPCs also varies in different molecular subtypes of AML. Interestingly, although the prognostic risk groups still comprise a molecular very heterogeneous group of diseases, we found a clear difference in the regulation of LSPCs. Favorable risk AML have less intrinsic molecular abnormalities that drive proliferation and stemness. The disease development depends on external cues from the niche, e.g., from $CD8^+$ T cells. In contrast, more aggressive AML is propagated mainly by cell-intrinsic mechanisms and develop independent of immune cells.

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Author contributions RR designed and performed experiments, analyzed and interpreted data, and wrote the manuscript. CR designed experiments and interpreted data. CS and RB analyzed the data. SH performed experiments. AFO designed experiments, wrote the manuscript, and supervised the project.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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