Research article

SGOL1-AS1 enhances cell survival in acute myeloid leukemia by maintaining pro-inflammatory signaling

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ABSTRACT

Epigenetic dysregulation is a key feature of most acute myeloid leukemia (AML). Recently, it has become clear that long noncoding RNAs (lncRNAs) can play a key role in epigenetic regulation, and consequently also dysregulation. Currently, our understanding of the requirements and roles of lncRNAs in AML is still limited. Here, using CRISPRi screening, we identified the lncRNA SGOL1-AS1 as an essential regulator of survival in THP-1 AML cells. We demonstrated that SGOL1-AS1 interacts with chromatin-modifying proteins involved in gene repression and that SGOL1-AS1 knockdown is associated with increased heterochromatin formation. We also observed that loss of SGOL1-AS1 results in increased apoptosis and the downregulation of pro-inflammatory genes. In AML patients, high expression of SGOL1-AS1 correlates with both pro-inflammatory gene expression and poor survival. Altogether, our data reveal that SGOL1-AS1 is an essential regulator of cell survival in AML cell lines and a possible regulator of pro-inflammatory signaling in AML patients.

1. Introduction

Acute Myeloid Leukemia (AML) represents a group of hematological malignancies induced by recurrent genetic mutations or translocations. These mutations impair myeloid cell differentiation and induce uncontrolled clonal proliferation, leading to the rapid accumulation of abnormal myeloid progenitor cells, called myeloid blasts. AML is sustained by aberrant transcription, and accordingly, a high proportion of these recurrent mutations are related to transcription and epigenetic processes (Cancer Genome Atlas Research et al., 2013; Chen et al., 2013). Hence, the disruption of epigenetic regulation of transcription plays a significant role in AML development and disease progression (Figueroa et al., 2010).

Large-scale transcriptomic analyses have revealed that a significant proportion of the transcriptome is composed of noncoding RNAs (ncRNAs) (Carninci et al., 2005; Djebali et al., 2012). The largest class of ncRNAs are long ncRNAs (lncRNAs) (Iyer et al., 2015), defined as transcripts over 200 nucleotides with no coding potential (Kapranov et al., 2007; Mercer et al., 2009). Structurally similar to messenger RNA (mRNAs), they are commonly spliced and polyadenylated (Cabili et al., 2011). Interestingly, they generally show lower expression levels and greater cell-type specificity than mRNAs (Derrien et al., 2012; Djebali et al., 2012). Although lncRNAs were previously thought to be the by-product of background transcription (Huttenhofer et al., 2005), there is growing evidence that they have essential functions in several cellular processes, notably those related to transcriptional and epigenetic regulation. LncRNAs have been shown to interact with several chromatin-modifying enzymes and may act to regulate their recruitment (Khalil et al., 2009; Guttman et al., 2011). For example, HOTAIR and XIST are required to maintain H3K27me3 at specific loci (Penny et al., 1996; Rinn et al., 2007; Zhao et al., 2008; Gupta et al., 2010).

Given this emerging role of lncRNAs in epigenetics, it is not surprising that there is accumulating evidence of aberrant lncRNA expression in AML (Calin et al., 2007). However, the majority of studies have focused on using the deregulation of lncRNA expression as a diagnostic or prognostic tool and not many studies have focused on identifying lncRNAs that are functionally required for AML maintenance (Garzon et al., 2014; De Clara et al., 2017; Lei et al., 2018; Mer et al., 2018). One

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of the few studies in which large scale screening was used to define lncRNA functions in AML has been performed by Bester et al. who employed a CRISPR activation (CRISPRa) gain of function screen screening approach to identify lncRNA involved in the development of drug resistance in AML (Bester et al., 2018). Here we aimed to characterize new lncRNAs with functional roles in AML maintenance. Therefore, we performed a CRISPR interference (CRISPRi) loss of function lncRNA screen. Specifically, we utilized the dCas9-KRAB transcriptional repressor and a sgRNA library targeting hematopoietic lncRNA loci to identify lncRNAs that are required for the proliferation of THP-1 cells, a commonly used model of human MLL rearranged AML (Tsuchiya et al., 1980; Drexler et al., 2004). We identified the lncRNA, SGOL1-AS1, as essential for the expansion and survival of THP-1 cells. Previous publications have shown that expression of this lncRNA is upregulated in lung and colorectal cancer (Nasim et al., 2019; Asad Samani and Peymani, 2021). Furthermore, expression of SGOL1-AS1 in gastric carcinoma cells inhibits cell growth, migration and invasion (Huang et al., 2021), by sequestering these components away from their target sites. Finally, high SGOL1-AS1 expression correlated with poor survival in AML patients independently of subtype.

Altogether our results indicate that the SGOL1-AS1 lncRNA can sequester negative regulators of transcription within the nucleus thereby supporting aberrant transcriptional activity in AML cells. Furthermore, the strong correlation between high SGOL1-AS1 expression and low survival in AML patients suggests a significant role for SGOL1-AS1 in AML progression in vivo, possibly through dysregulation of pro-inflammatory signaling.

2. Results

2.1. Identification of seventeen lncRNAs essential for THP-1 proliferation

To identify lncRNAs critical for AML proliferation, we opted for a large-scale CRISPRi screening strategy. THP-1 AML cells, originating from a one-year-old infant and carrying an MLL-AF9 translocation (Tsuchiya et al., 1980), were first transduced with a lentiviral vector expressing dCas9-KRAB linked to mCherry (pHR-SFFV-KRAB-dCas9–P2A-mCherry, Addgene Plasmid #60954). Subsequently, cells expressing high levels of mCherry were sorted to establish a polyclonal dCas9-KRAB P2A-mCherry, Addgene Plasmid #60954). Subsequently, cells expressing high levels of mCherry were sorted to establish a polyclonal dCas9-KRAB expressing cell line (dCas9-KRAB THP-1, Fig. S1a). A set of previously validated sgRNAs (Razdzieuska et al., 2016) confirmed that the dCas9-KRAB THP-1 line was functional. RT-qPCR results demonstrated efficient and specific knockdown of expression of individually targeted genes down to 30% or less of their original levels (Fig. S1b).

To identify lncRNAs that play a role in AML survival, we opted for a leukemia-focused sub-library from the CRISPRi Noncoding Library (CRINCL) published by Liu et al. (2017). This complete lentiviral guide library targets 16,401 lncRNA loci expressed across seven diverse human cell lines. For our screen, we selected the sub-library representing lncRNAs expressed in the human chronic myeloid leukemia K562 cell line and targeting 2831 lncRNAs with ten sgRNAs per lncRNA. Using the Cancer Cell Line Encyclopedia (CCLE) (Barretina et al., 2012), we found that 76% of lncRNAs targeted in the K562 library, representing 2150 lncRNAs, are expressed in the acute myeloid leukemia THP-1 cell line.

To initiate the screen, THP-1 cells were transduced with the lentiviral sgRNA library. The sgRNA lentiviral vectors constitutively express a BFP marker and a puromycin resistance gene, which were used to quantify the initial transduction efficiency (42%) (Figure 1a) and to select for sgRNA-expressing cells, respectively. The reference sample for the screen was taken after a 48h puromycin selection. Samples were sequentially harvested every seven days, up to an endpoint of 20 cell doublings (Figure 1a). SgRNAs were extracted from genomic DNA, and their representation was quantified by next-generation sequencing. The screen processing pipeline (https://github.com/mhoerbeck/ScreenProcessing) was used to compare the representation of guides in the starting and endpoint samples and identify lncRNAs playing a role in THP-1 proliferation or survival. In total, guide RNAs targeting 17 different lncRNAs (empirical FDR <0.01) displayed a significant change in representation (Figure 1b). Amongst them, we found the microRNA encoding lncRNA MIR17HG, previously implicated in maintaining MLL-rearranged AML (Mi et al., 2010; Wong et al., 2010), validating the efficacy of our CRISPRi screen.

We subsequently confirmed the phenotypes of six hits (SGOL1-AS1, MYB-AS1, LINC00649, LINC00624, LH00626, LH17064) from our screen by performing competition between non-transduced cells and cells transduced with single sgRNAs and evaluating the proportional loss of sgRNA expressing cells, measured using BFP fluorescence. For both experiments, the phenotype of the sgRNA is calculated by log2 (relative proportion of sgRNA) and is determined by read counts or BFP fluorescence for the screen and competition experiments, respectively. When comparing results for days 7, 14, and 21, we observed a good correlation between the loss of sgRNAs within the screen and individual sgRNAs, showing our screen has performed well in estimating sgRNA phenotypes (Figure 1c). Overall, the sgRNAs targeting the lncRNA SGOL1-AS1 displayed the most pronounced phenotype on THP-1 maintenance, achieving the strongest phenotype score in both the screen and the competition experiments.

2.2. A5O mediated knockdown confirms a phenotypic role for SGOL1-AS1

We next wanted to exclude the possibility that the proliferative defects observed upon targeting SGOL1-AS1 were the result of dCas9-KRAB mediated silencing of the overlapping protein-coding gene, SGOL1. SGOL1 encodes the Shugoshin 1 protein, which is important for chromatid cohesion in mitosis, and as such the growth defect observed could be caused by SGOL1 dysregulation. Therefore, we used antisense oligonucleotides (ASOs) to directly knockdown the SGOL1-AS1 transcript without affecting transcription in the SGOL1 locus (Figure 2a). Transfection of the different SGOL1-AS1 ASOs in THP-1 cells resulted after three days in reduced lncRNA transcript levels down to 5% of the normal levels (Figure 2b). Loss of SGOL1-AS1 correlated with reduced proliferation of THP-1 cells (Figure 2c). Furthermore, transfection of MV4-11 cells, another model of MLL-rearranged AML, with ASOs targeting SGOL1-AS1 also led to reduced cell proliferation (Fig S2 e).

We thereafter used ASO transfection to further define the requirement for SGOL1-AS1 in THP-1 maintenance. Although this allows for strand-specific targeting of SGOL1-AS1, it also might result in possible under-estimation of the longer-term phenotype due to the use of transient transfections. Replating in semisolid media indicated that ASO-based knockdown of SGOL1-AS1 significantly reduced the colony-forming capacity of THP-1 after 10 days (Figure 2d). The cell cycle (Fig S2a-b) or differentiation (Fig S2 c-d, f) status of the cells was unaffected by ASO-based knockdown after 5 days. In contrast, we observed an increase in apoptotic annexin V positive THP-1 cells upon SGOL1-AS1 knockdown (Fig. 2e-f). Altogether these results indicate that the loss of SGOL1-AS1 leads to induction of apoptosis, resulting in reduced proliferation of cells.

2.3. The SGOL1-AS1 isoform expressed in THP-1 is a nuclear transcript polyadenylated transcript that localizes to nuclear bodies

Having confirmed a role for SGOL1-AS1 in THP-1 maintenance, we set out to further characterize the properties of this lncRNA in this cell line. Six possible isoforms of SGOL1-AS1, originating from four transcriptional start sites (TSS), are predicted by Gencode (Fig. S2g).
However, we only detected by PCR a single four exon isoform arising from the TSS closest to the 3' end of the SGO1 gene (Figure 2a, Fig. S2g). This isoform is predicted to have low coding potential using CPC2 and CPAT (Fig. S2h). Investigation of 3' end processing of SGOL1-AS1 showed enrichment in cDNA generated with oligo-dT primers indicating that SGOL1-AS1 is polyadenylated (Fig. S2i). To further characterize SGOL1-AS1, we investigated its cellular location. Subcellular fractionation demonstrated a strong enrichment for SGOL1-AS1 within the nucleus, above the levels of the nuclear marker U6 (Figure 2g). This nuclear localization was confirmed using RNA FISH (Figure 2h), detecting SGOL1-AS1 as discrete dots within the nucleus. There were on average two of these foci per nucleus, and no foci were observed in the absence of the probe or upon RNase treatment (Figure 2i).

2.4. SGOL1-AS1 supports transcription of inflammatory genes and associates with repressive chromatin-modifying proteins

To define the mechanisms by which loss of SGOL1-AS1 induces apoptosis, we first evaluated the transcriptomic consequences of SGOL1-AS1 loss. Changes in gene expression in THP-1 five days post-ASO transfection were evaluated by RNA-seq (Figure 3a, Table S1). We detected 408 genes significantly downregulated upon SGOL1-AS1 knockdown, whereas only 57 were upregulated (adjusted p-value < 0.05). SGO1 expression was not affected by transfection with the ASO targeting SGOL1-AS1. To concentrate on the most significant changes, we selected the high confidence genes (absolute log2 fold change >0.5, adjusted p-value < 0.05). Gene Ontology (GO) analysis of the downregulated genes showed...
Figure 1: sgol1-AS1 targeting.

(a) Schematic representation of sgol1-AS1 and ASOs targeting.

(b) Bar graph showing expression relative to non-targeting control.

(c) Graph displaying cumulative cell count over days post-transfection.

(d) Bar graph comparing number of colonies relative to non-targeting control.

(e) Flow cytometry histograms for Annexin V and Hoechst staining.

(f) Bar graph illustrating % cells Annexin V+.

(g) Proportion of expression in nucleus and cytoplasm.

(h) Fluorescence images showing DAPI, sgol1-AS1 probe, and merge.

(i) Violin plot displaying FOCI per nucleus.
enrichment for genes implicated in defense response and response to cytokines (FDR < 0.05) (Figure 3b, Table S2, Table S3). Furthermore, Gene Set Enrichment Analysis (GSEA) indicated that genes implicated in similar pathways, including chemokine signaling and inflammatory response, were overrepresented in cells transfected with a non-targeting ASO in comparison to cells transfected with ASO targeting SGOL1-AS1 (FDR < 0.05) (Figure 3c). RT-qPCR confirmed the downregulation of cytokines signaling genes (IG20, CXL10, EGR1) upon ASO treatment (Figure 3d).

Next, to explore the mechanisms by which SGOL1-AS1 can influence gene expression we performed RNA affinity purification experiments (Panda et al., 2016). A biotinylated RNA bait was used to pulldown SGOL1-AS1 interacting proteins, which were subsequently identified by mass spectrometry (Figure 3e). In total, we identified 174 proteins significantly associated with SGOL1-AS1, either exclusively found (Fig. S3a), or enriched at log2FC > 1 and adjusted p < 0.05, in the SGOL1-AS1 pulldown (Fig. S3b, Table S4). Gene Ontology analysis of the SGOL1-AS1 interactors showed a strong enrichment for chromatin-binding proteins (Figure 3f). These chromatin-binding proteins, such as CBX1, JMJD1C, BMI1 and CBX4, are mainly involved in the regulation of gene expression and chromosome organization. As loss of SGOL1-AS1 leads to reduced transcription of several genes (Figure 3a), we hypothesized that SGOL1-AS1 acts to sequester these negative regulatory proteins and chromosomal organization proteins away from their target genes. We, therefore, investigated if SGOL1-AS1 loss led to increased heterochromatin formation. A global increase in H3K9me3, a heterochromatin marker, was observed by Western blot after the knockdown of SGOL1-AS1 (Fig. S3c-d). Altogether these results suggest that SGOL1-AS1 sequesters proteins involved in the negative regulation of gene expression and chromosome organization to prevent heterochromatin formation. Loss of SGOL1-AS1 leads to the release of these proteins, resulting in heterochromatin formation and an associated downregulation of gene expression, particularly of the genes involved in cytokine signaling.

2.5. High SGOL1-AS1 levels in AML patients correlate with poorer overall survival

Having identified SGOL1-AS1 as an epigenetic regulator that sustains the survival of human THP-1 AML cells, we next explored its expression patterns in patient samples. Using the RNA-seq data available from TCGA (151 AML patients) we compared SGOL1-AS1 expression to healthy bone marrow samples from Genotype Tissue Expression (GTEX). We found SGOL1-AS1 expression to be significantly upregulated in AML patient samples (Figure 4a). Furthermore, we also observed that higher SGOL1-AS1 expression correlates with poorer overall survival in the TCGA AML cohort (Figure 4b). Interestingly, this was not associated with either specific driver mutations or a specific AML subtype (Figure 4c-d). Altogether these results led us to analyze the expression of SGOL1-AS1 in normal cells. In line with the TCGA data we observed upregulation of SGOL1-AS1 in AML patients compared to Common Myeloid Progenitor and Megakaryocyte Erythrocyte Progenitor populations (Figure 4c).

To gain further insights into the role of SGOL1-AS1 in patients, we adopted a ‘guilt-by-association’ approach. The top 200 correlated genes were selected based on their Pearson Correlation Coefficient. GO analysis revealed enrichment for 43 processes, with a significant overlap with the terms identified in the human THP-1 cell line, including the inflammatory response (Figure 4d). Among the most enriched terms was response to molecule of bacterial origin, which encompassed response to cytokines and response to biotic stimulus (Fig. 4e-f). These results indicate that SGOL1-AS1 is upregulated in AML patients and that its expression in patients correlates with the activation of similar pathways as the ones down regulated upon loss of SGOL1-AS1 in THP-1, suggesting a similar function of SGOL1-AS1 in THP-1 and patients.

3. Discussion

In this study, we performed a CRISPRi screen to identify lncRNAs critical for the proliferation of THP-1 AML cells. In addition to the previously identified lncRNA MIR17HG which harbors the mir-17-92 cluster (Mi et al., 2010; Wong et al., 2010), we identified 17 lncRNAs that are important for AML cell proliferation and survival. The most pronounced effect on survival in our CRISPRi screen was observed for lncRNA SGOL1-AS1 prompting us to characterize this lncRNA further. We confirmed the strength of this phenotype using internally controlled growth assays, which highlighted the phenotype of SGOL1-AS1 further.

We identified several interaction partners of SGOL1-AS1. A number of these proteins are associated with repressive heterochromatin and, therefore, reduction in transcription. We also observed that loss of SGOL1-AS1 leads to an increase in heterochromatin and a reduction in gene expression, particularly of those genes involved in inflammatory pathways. Furthermore, we found that SGOL1-AS1 transcripts have distinct localization within the nucleus of AML cells. Therefore, we propose that SGOL1-AS1 might act similarly to several other lncRNAs, such as lncPRESS1 and TSIX (Zhao et al., 2008; Jain et al., 2016; Morris and Cooper, 2017), as a decoy for these repressive chromatin factors. In this model, SGOL1-AS1 sequesters these repressive proteins away from target genes, maintaining an open chromatin state and active transcription at target regions. Loss of SGOL1-AS1 induces the release of repressive chromatin complexes and downregulation of the expression of target genes.

The role of inflammation and cytokine expression in patient prognosis is well appreciated in many hematological malignancies, including AML (Carey et al., 2017; Binder et al., 2018; Camacho et al., 2021). Our data indicate that SGOL1-AS1 can be an important player in maintaining the transcription of the genes involved in these pathways. Loss of SGOL1-AS1 led at the transcriptional level to the downregulation of a large number of genes with most notably genes involved in cytokine signaling. We observed in particular a requirement for SGOL1-AS1 for the expression of pro-inflammatory cytokines (CCL2 and CXL10). Whether these cytokines are direct targets of SGOL1-AS1 remains to be determined.
Figure 3. SGOL1-AS1 Expression maintains the expression of inflammatory genes. (a) Volcano plot of changes in gene expression after ASO treatment. Fold change was determined by mean expression in ASO treated divided by mean expression in non-Targeting. P value is the adjusted p-value of the differential expression, as determined by DeSeq2. Differentially expressed genes (padj < 0.05, absolute value of log2 fold change > 0.5) are colored in blue for downregulated genes, and red for upregulated genes. (b) Bar graphs showing the enrichment of Gene Ontology (GO) terms in significantly downregulated genes, as determined by ToppFun. (c) Gene Set Enrichment Analysis of genes related to Chemokine signaling (Top) and Inflammatory Response (Bottom). (d) Expression of inflammatory genes after ASO treatment, as determined by RT-qPCR (Student t-test with Holm-Sidak correction for multiple comparisons. n = 6 Biological replicates. Presented is the mean and SEM). (e) Scheme summarizing the RNA in vitro pulldown assay performed to identify SGOL1-AS1 associated proteins. (f) Bar graphs showing the enrichment of non-redundant Gene Ontology (GO) terms for Molecular function in proteins associated with SGOL1-AS1 (left) and Biological Processes in the chromatin binding proteins identified (right) as determined by ToppFun. Presented are the top 5 GO terms, after filtering of GO terms for similarity using REVIGO. For all figures shown (* = p < 0.05).
Figure 4. SGOL1-AS1 is upregulated in AML patients and correlates with poorer overall survival. (a) Boxplot showing the expression of SGOL1-AS1 in AML patients from the TCGA cohort (n = 173), compared to healthy bone marrow from GTEx (n = 70), produced by GEPIA (Colored Box represents the interquartile range, the middle line represents the median). (b) Kaplan-Meier plot, showing the separation of patients based on SGOL1-AS1 expression. SGOL1-AS1 high patients (red) are defined as the top 40% of patients ranked on SGOL1-AS1 expression, and SGOL1-AS1 low patients are the bottom 40% of patients ranked on SGOL1-AS1 expression (Mantel–Cox Test). (c) Violin plots showing SGOL1-AS1 expression in Blueprint Consortium data, including expression in AML, Hematopoietic stem Cells (HSC), Multipotent Progenitors (MPPs), Common Myeloid Progenitors (CMP), Common Lymphoid Progenitors (CLP), Granulocyte Monocyte Progenitors (GMP) and Megakaryocyte-erythroid Progenitors (MEP). P Value is the adjusted pValue, as determined by DEseq2. (d) Venn Diagram showing the overlap of the GO biological process terms enriched in the Top 200 correlating genes with SGOL1-AS1 in the TCGA dataset (Blue) or downregulated genes upon ASO treatment. (e) Bar graph showing the enrichment of the GO biological process terms in the top correlating genes from TCGA, after filtering with REVIGO (left) and the redundant terms filtered by REVIGO (right). For all figures shown (* = p < 0.05**** = p < 0.0001). See also Fig. S4.
Using a guilt-by-association approach revealed similar upregulation of
innate immune response pathways in AML patients with high levels of
SGOL1-AS1 expression. The involvement of SGOL1-AS1 in regulating cell
survival and cytokine expression suggests that targeting of SGOL1-AS1
expression, in combination with other treatments, may provide a new
therapeutic avenue for AML. However, further investigation would be
required to determine the exact therapeutic potential in AML patients.
SGOL1-AS1, has been previously studied in other cancer types, with
varied associations between healthy and cancerous tissue in colorectal
cancer, and gastric carcinoma (Asad Samani and Peymani, 2021; Huang
et al., 2021). Interestingly in the latter, SGOL1-AS1 has been character-
ized as a cytoplasmic lncRNA that is downregulated in gastric cancer cells.
The different cellular localization observed in gastric cancer and AML could possibly reflect distinct SGOL1-AS1 isoforms expressed
(Huang et al., 2021). This may also explain why in gastric carcinoma
SGOL1-AS1 is a marker of good prognosis (Huang et al., 2021), while we
observed that in AML patients high SGOL1-AS1 expression is associated
with poor survival across different subtypes of AML.

In conclusion, we have identified SGOL1-AS1 as a critical lncRNA for
the cell survival of THP-1 AML cells and demonstrated its role in the
regulation of cytokine signaling in these cells. Mining of patient datasets
suggests that SGOL1-AS1 plays a similarly important role in AML patients.

Declarations

Author contribution statement

Ewan Selkirk: Conceived and designed the experiments; Performed the experiments; Analyzed and interpreted the data; Contributed re-
agents, materials, analysis tools or data; Wrote the paper.

Rahima Patel; Duncan Smith: Performed the experiments;

Anna Hoyle; Michael Lie-a-Ling: Performed the experiments;

Georges Lacaud, Conceived and designed the experiments; Analyzed and interpreted the data; Contributed re-
agents, materials, analysis tools or data; Wrote the paper.

Declaration of interest’s statement

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Data availability statement

Data associated with this study has been deposited at Raw and
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