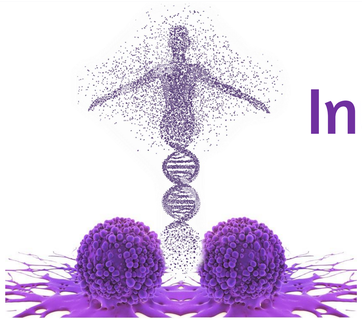


Investigation into the role of the long non-coding RNA MIAT in leukaemia

A. Ostini, M. Mourtada-Maarabouni

School of Life Sciences, Faculty of Natural Sciences, Keele University, Keele, UK

Correspondence: a.ostini@keele.ac.uk - m.m.maarabouni@keele.ac.uk

Keele UNIVERSITY

Introduction

Myocardial Infarction Associated Transcript (MIAT) also known as Gomafu, is a nuclear retained long non-coding RNA associated with higher risk of Myocardial Infarction (MI). The human MIAT gene contains 7 exons and is located on chromosome 22q12.1 (Fig.1). Four different MIAT transcript variants have been identified including NR_003491.3; NR_033319.2; NR_033320.2 and NR_033321.2 [1,4]. Mature MIAT is retained in the nucleus and it localises as punctuated pattern or spots that do not colocalise with known nuclear domains and their integrity remained unviolated even post-nuclear matrix preparation [2,4]. Extensive evidence attributed the role of oncogene to MIAT, this role appears to be the main regulatory machine of MIAT in the pathogenesis and progression of cancer [3]. In addition, data gathered so far, emphasize the regulatory role of MIAT in cellular growth, invasion, metastasis, and proliferation of cancer cells, asserting the valuable potential in both therapeutics and biomarker this transcript holds. MIAT is also reported to be over-expressed in leukaemia[1].

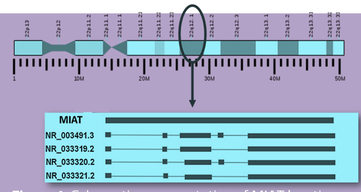


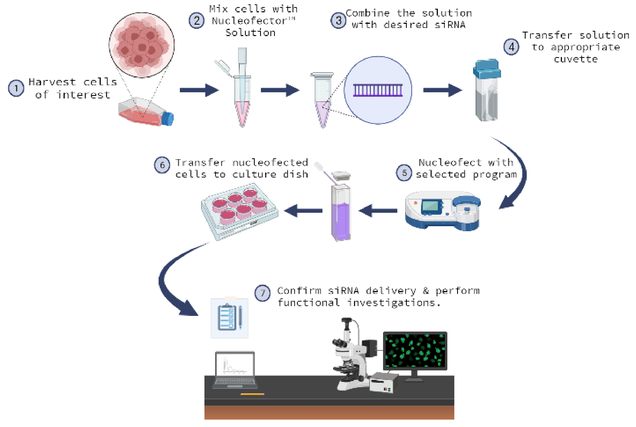
Figure 1. Schematic representation of MIAT location and its different variants. (Adapted from [3]).

Aims & Objectives

The current investigation aims to:

Elucidate the role of MIAT in the regulation of human leukemic cell fate decision. This will be achieved by investigating the effects of silencing MIAT on the short- and long- term survival of two leukemic cell lines Jurkat and CEM-C7.

Methodology



- Two types of leukemic cells lines were used in the study: human immortalized T lymphocyte cell line Jurkat, and human T lymphoblast cell line CEM-C7.
- MIAT silencing was achieved by transfecting cells with MIAT-specific siRNAs. The controls received scrambled siRNAs.
- Transfected cells were incubated for 24h. at 37°C and 5% CO_2 before being harvested and re-plated for 24h. and 48h. for further functional analysis.
- Culture viability, apoptosis and cell cycle were assessed for sort-term survival while clonogenic assay was performed to investigate long-term survival.
- Gene expression levels of MIAT were determined using TaqMan MIAT-FAM probes and eukaryotic 18S rRNA as endogenous control.

Results

MIAT silencing was confirmed through rt-PCR assessment.

MIAT Taq-Man probes were used to determine gene expression levels of MIAT and 18s rRNA was utilised as endogenous control (Fig. 2). Jurkat and CEM-C7 cells were transfected with negative siRNA or one of the three MIAT-specific siRNAs using nucleofection, cells were incubated for 24h., re-plated and incubated for further 24h. prior RNA isolation and rt-PCR assessment.

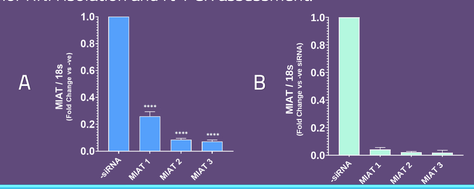


Figure 2. MIAT gene expression level in Jurkat cell line.
Relative expression of MIAT was measured by Real-Time PCR 24h. post-transfection, it was significantly lower for all MIAT-specific siRNAs in both Jurkat (A) and CEM-C7 (B) cells; **** indicate a p-value<0.001, as measured by One-way ANOVA tests with multiple comparisons (MCT). Data are represented as mean +/- SEM, n=3 experiments for Jurkat, n=2 experiments for CEM-C7.

MIAT-specific down-regulation and its effects on the short-term cell survival of Jurkat cells.

MIAT-specific down-regulation does not significantly affect the short-term cell survival of Jurkat cells. MIAT down-regulation does not lead to a statistically significant change in the number of viable cells as assessed with trypan blue exclusion assay (A & B), although a significant change is observed for MIAT 1/2 compared to the negative control as assessed with flow cytometry (C & D) after 24 and 48 h., respectively.

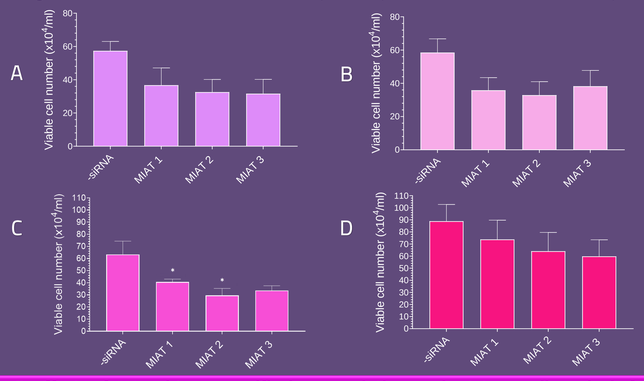


Figure 3. Effects of MIAT siRNAs-specific downregulation on short-term cell survival of Jurkat cells.
* indicate a p-value<0.05, as measured by One-way ANOVA tests with multiple comparisons (MCT). Data are represented as mean +/- SEM, n=4 experiments for Trypan Blue Exclusion Assay 24 h. (A) and 48 h. (B), n=3 experiments for Flow Cytometry MUSE® 24 h. (C) and 48 h. (D) respectively.

MIAT siRNA-specific downregulation increases the level of apoptosis.

MIAT-specific down-regulation increases the levels of apoptosis of both Jurkat (A) and CEM-C7 (B) cells. Cells were transfected using nucleofection and apoptosis was assessed 24h. post-nucleofection using Acridine Orange staining. The levels of apoptosis were significantly elevated for both cell lines and for all three different MIAT-specific siRNAs.

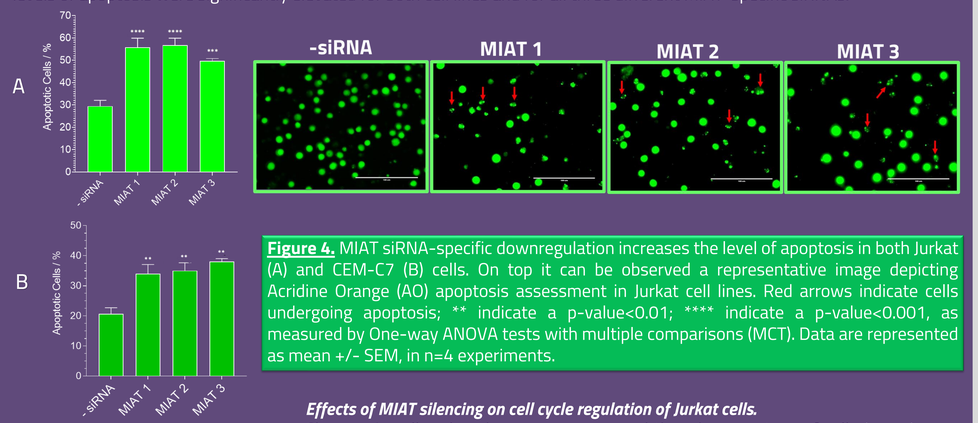


Figure 4. MIAT siRNA-specific downregulation increases the level of apoptosis in both Jurkat (A) and CEM-C7 (B) cells. On top it can be observed a representative image depicting Acridine Orange (AO) apoptosis assessment in Jurkat cell lines. Red arrows indicate cells undergoing apoptosis; ** indicate a p-value<0.01; **** indicate a p-value<0.001, as measured by One-way ANOVA tests with multiple comparisons (MCT). Data are represented as mean +/- SEM, in n=4 experiments.

Effects of MIAT silencing on cell cycle regulation of Jurkat cells.

Changes in cell cycles phases were assessed though percentage of cells (mean). MIAT silencing did not significantly perturbed the cell cycle of Jurkat cells.

Figure 5. Effects of MIAT siRNAs-specific downregulation on cell cycle of Jurkat cells.
Cells were transfected with negative control siRNA and MIAT-specific siRNAs (MIAT 1/2/3); cells were thereafter assessed 48h. post-replating. Statistically significant changes were detected performing One-Way ANOVA tests with multiple comparisons (MCT). Data are represented as mean +/- SEM, n= 4 experiments.

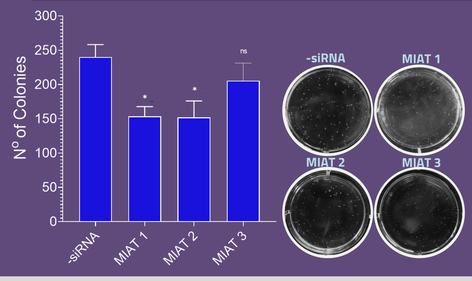


Figure 6. Effects of MIAT silencing on long-term survival of Jurkat cells.
Cells were seeded after transfection and incubated (37°C, 5% CO_2) for 2-3 weeks, colonies formed were thereafter counted. MIAT knockdown induced by MIAT-specific siRNAs led to a statistically significant reduction of the number of colonies formed. * represent p value < 0.05 as measured with One-Way ANOVA (MCT). Data are represented as mean +/- SEM, n=4 experiments.

Summary & Conclusion

- MIAT silencing through siRNAs-mediated transfection was confirmed with Real-Time PCR.
- MIAT silencing did inhibit short-term cell survival, however the effects were not statistically significant.
- MIAT silencing significantly promotes cell death in both Jurkat and CEM-C7 cell lines.
- MIAT silencing did not significantly affect cell cycle profile.
- MIAT silencing significantly reduced long-term survival of Jurkat cells.

References

1. Ghafouri-Fard S., Azimi T., Taheri M. (2020). "Myocardial Infarction Associated Transcript (MIAT): Review of its impact in the tumorigenesis". Biomedicine & Pharmacotherapy. 133, pp. 111040.
2. Sone M., Hayashi T., Tarui H., Agata K., Takeichi M., Nakagawa S. (2007) "The mRNA-like noncoding RNA Gomafu constitutes a novel nuclear domain in a subset of neurons." J. Cell Sci., 120 (15), pp. 2498–2506.
3. Bountali A., Tonge D., Mourtada-Maarabouni M. (2019). "RNA sequencing reveals a key role for the long non-coding RNA MIAT in regulating neuroblastoma and glioblastoma cell fate". International Journal of Biological Macromolecules, 130, pp.878-891.
4. Da C., Gong C., Nan W., Zhou K., Wu Z. and Zhang H. (2020). "The role of long non-coding RNA MIAT in cancers". Biomedicine & Pharmacotherapy, 129, p.110359.