

Global miRNA and pre-miRNA expression profiling of the NCI-60 cancer cell lines using novel LNATM enhanced microarrays

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Introduction

MicroRNAs (miRNAs) play a role in the initiation and progression of cancer, and may comprise a novel class of diagnostic and prognostic signatures.

The aim of this study is:

To discover the miRNAs that are differentially expressed within the NCI-60 cell line panel (a set of 59 human cancer cell lines) and to identify miRNAs that correlate to relevant cancer features.

Methods

Cell lines

The 59 cell lines (=NCI-60) were kindly provided by Dr. Susan Holbeck at NCI and represent cancers derived from the following tissues: breast, colon, kidney, lung, ovary, prostate, CNS, leukemia and melanoma.

RNA isolation

The cells were grown to near confluence before total RNA was isolated by guanidinium isothiocyanate/phenol: chloroform extraction (Trizol).

MicroRNA profiling

Figure 1

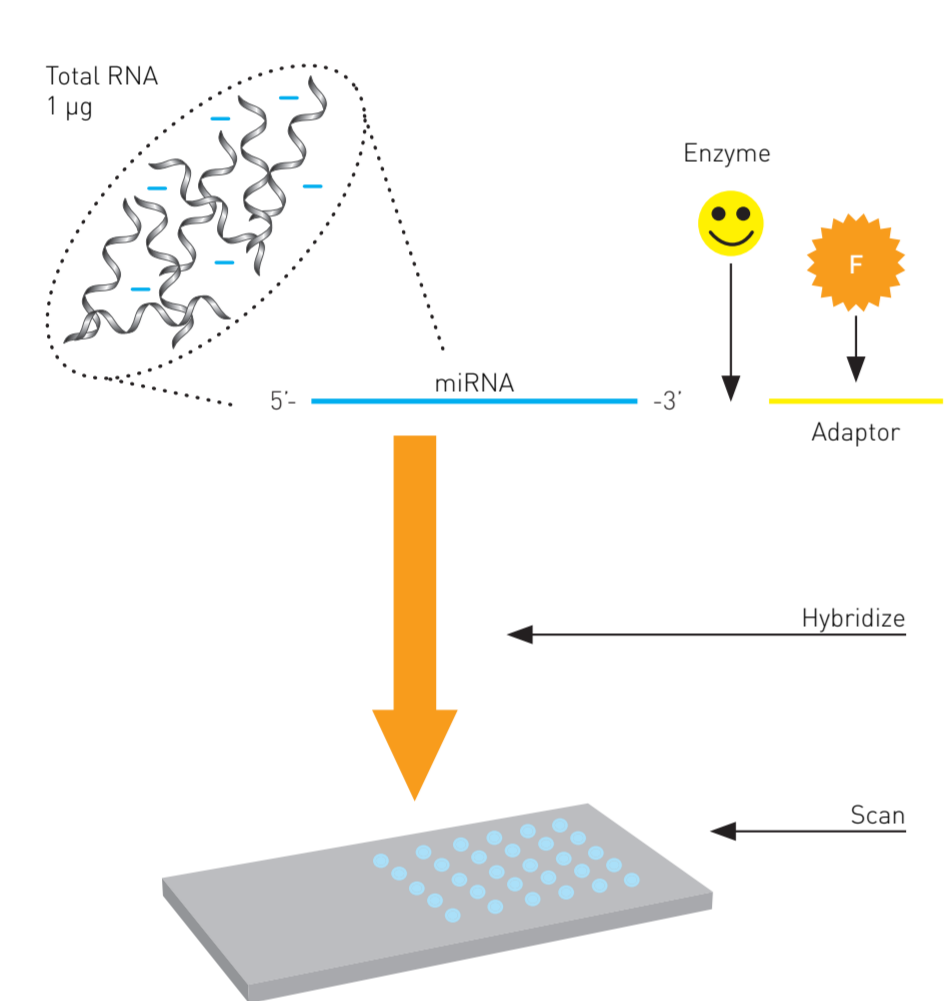


Figure 1. One µg total RNA was analyzed for miRNA expression on miRCURY LNATM microRNA Arrays (Ref. 1) containing 1m normalized capture probes for 2090 miRNAs, including 110 human miRPlusTM sequences not yet annotated in miRBase, plus 200 miRNAs discovered by 454 high throughput sequencing as well as the corresponding pre-miRNAs. All hybridizations were made against a common reference pool.

Statistical analysis of microarray data

Background correction and normalization: The pre-processing of data was conducted in R applying the Limma package (Ref. 2). Background correction was performed using the "normexp + offset = 50" method (Ref. 3), which provides variance stabilization and guarantees no negative values. Intra-slide loess normalization was used to reduce systematic technical variation, such as dye bias.

Identification of differentially expressed miRNAs: The NCI-60 cell lines were grouped according to tissue of origin. Tissue specific miRNAs were selected by fitting a linear model to the normalized microarray data and calculating empirical Bayes, moderated, t-statistics (Ref. 4). The comparisons of interest were extracted by means of contrast fitting. The miRNAs with highest log-odd ratios (B-statistics) were selected for the unsupervised hierarchical clustering.

Results

Figure 2

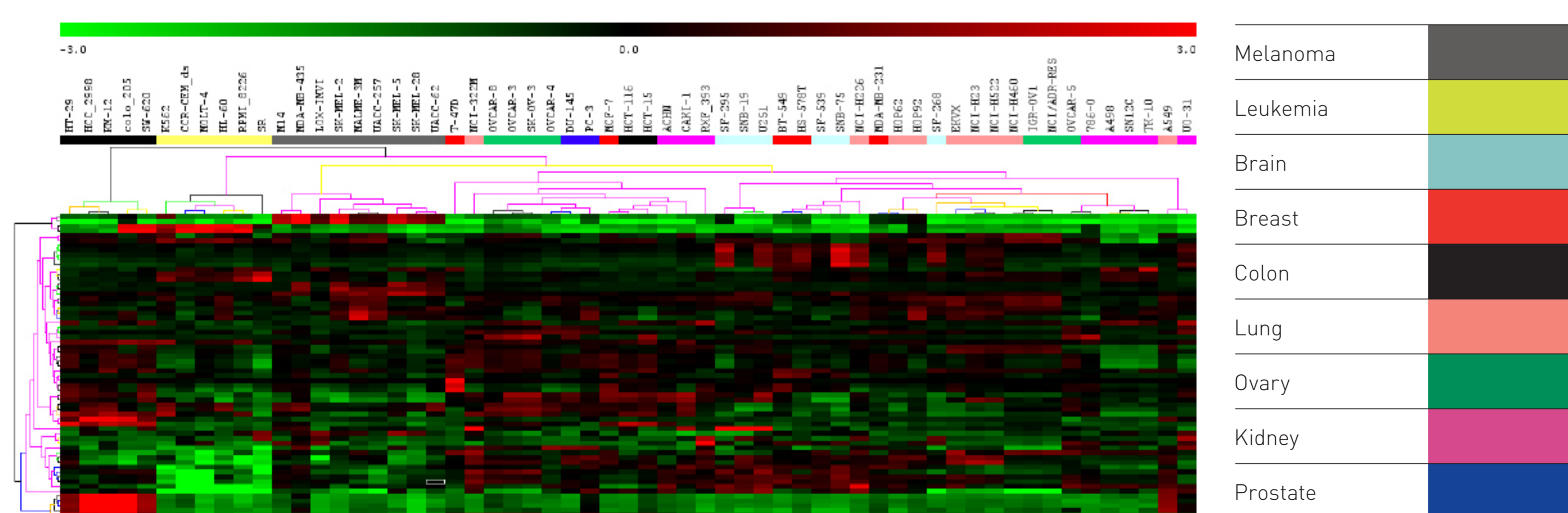


Figure 2. The heat map shows clustering of NCI-60 cell lines, based on expression of miRNAs that were specific for the tissue of origin. The miRNAs specific for each tissue of origin (selected based on B-statistic log-odds) were combined and used in unsupervised hierarchical clustering. The clustering shows clear separation of cell lines from melanoma (grey) and leukemia (yellow) origin. Both colon (black) and ovary (green) cell lines are split into two distinctive groups. Brain, lung and kidney cell lines are divided into smaller groups. The breast cancer cell lines do not cluster together.

Figure 3

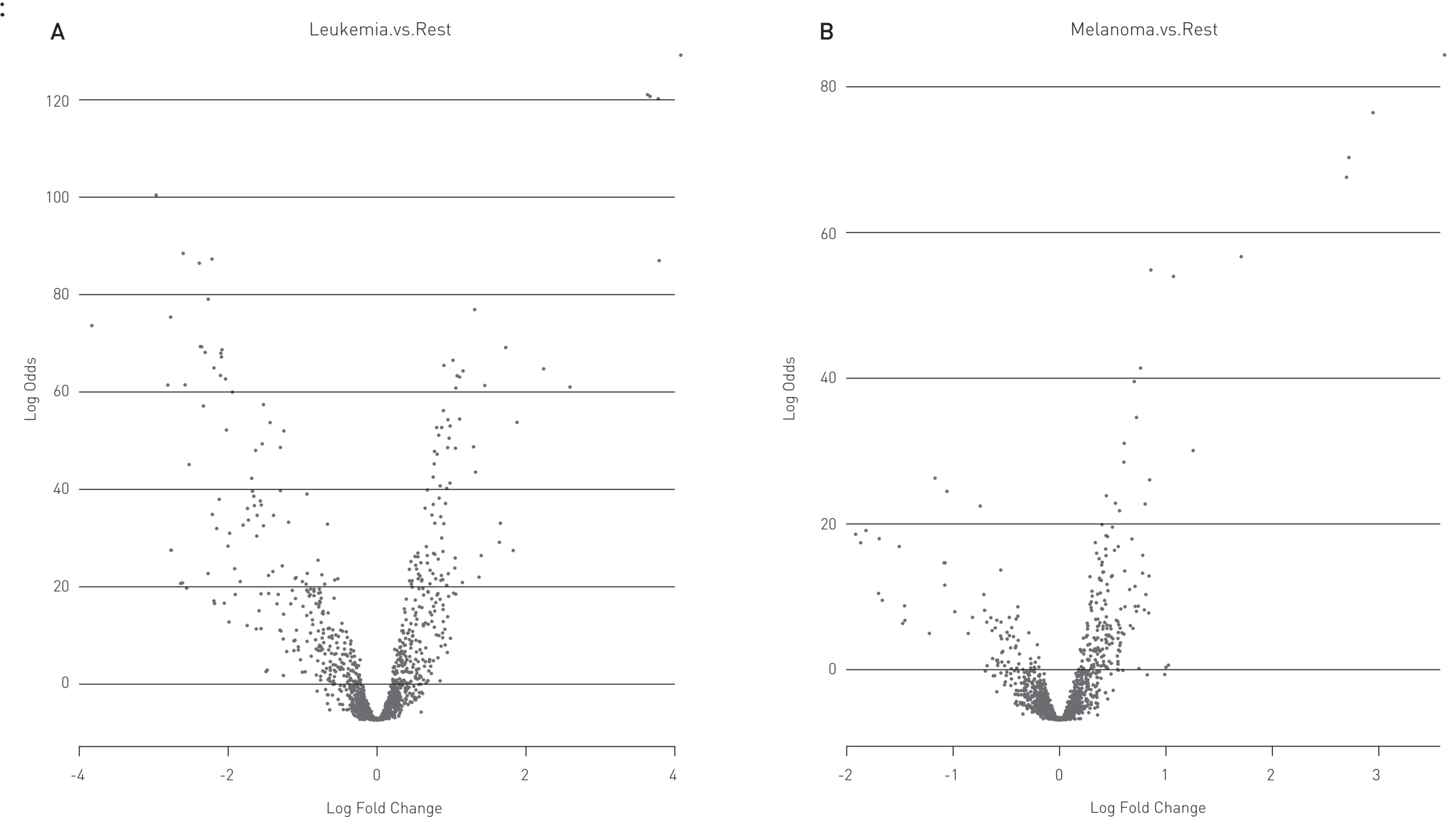


Figure 3. Volcano plots showing the miRNAs, which have significantly different expression in melanoma (a) and leukemia (b) cell lines when compared to the rest of the groups. The x-axis represents changes in expression (log fold change), the y-axis shows statistical significance of the change in log-odds (B-statistics).

Conclusion

- NCI-60 cell lines, except breast, grouped according to the tissue of origin
- microRNAs with expression profiles characteristic for each group were discovered with varying significance
- Support vector machine (SVM) analysis showed that it is possible to classify the leukemia and melanoma cell lines according to expression of selected miRNAs (data not shown).
- These findings will be validated on patient tissue samples.

Perspectives

The next part of the study will focus on the relationship between miRNAs expression profiles and drug resistance.

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