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Two novel miRNAs encoded by oncogene *MSI1*

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INTRODUCTION

The RNA-binding protein Musashi1 (*MSI1*) plays a critical role in normal cell proliferation, as well as in the differentiation and development of several organs. Aberrant expression of *MSI1* has been observed in numerous cancers. *MSI1* is considered as an activator in tumorigenesis and has been reported as a prognostic biomarker [1]. miRNAs, as highly stable circulating ncRNAs in body fluids, have emerged as more specific and sensitive diagnostic and prognostic biomarkers [2]. In this regard, we were interested in finding whether any novel miRNAs are encoded by the *MSI1* gene which could be considered as novel biomarkers in future studies and if there is a possible overlap between the expression pattern or function of the novel miRNAs and their host gene.

METHOD

The intronic regions of the *MSI1* gene were analyzed for stem-loop structures using SSCprofiler. miRNA precursor futures like MFE (Minimum Free Energy), presence of potential Drosha/Dicer cleavage sites and production of mature miRNAs were analyzed by MiPred, miRNA-dis, FomMIR, MiR-Find and CID-miRNA. For experimental analysis, miRNA precursors were transfected into HEK293T cells. Production of mature miRNAs from predicted precursors was confirmed by RT-qPCR and sequencing. Also, endogenous expression of novel miRNAs was detected in cancer cell lines and breast cancer samples. Clinical samples were collected during surgical excision of malignant tumors from breast cancer patients (Informed consent was obtained from all participants). For the prediction of target genes of novel miRNAs, DIANA and TargetScan websites were used and dual luciferase assay was used to validate the predicted genes.

Results and Discussion

Totally 40 stem-loop structures were detected in both strands of intronic regions of *MSI1* gene by SSCprofiler. Structures with unusual loops, many branches and extremely high or low MFEs were removed from the study. The optimal MFE range was assumed between -30 to -55 kcal/mol. Three websites, FomMIR, MiR-Find and CID-miRNA were used to determine if the selected precursors could produce potential mature miRNAs. Conservation status of predicted precursors was analyzed in UCSC. According to BLAST search programs of MiRBase, none of the candidate mature miRNAs were among the previously reported miRNAs in miRNA databases. The flowchart of bioinformatics studies is illustrated in Fig. 1.

Finally, two structures within intron 4 of *MSI1* gene, named MSM2 and MSM3, were selected for experimental studies. Structural features of MSM2 and MSM3 are shown in Fig. 2.

In order to confirm our bioinformatics predictions, two miRNA precursors were transfected into HEK293T cells and exogenous expression of the mature miRNAs was detected by RT-qPCR and sequencing analysis. Two mature miRNAs, MSM3-3p and MSM3-5p were generated by MSM3 precursor and one, MSM2-5p, was derived from MSM2. Also, significant expression of two miRNAs, MSM2-5p and MSM3-3p, was observed in MCF-7 and SH-SY5Y cells, while MSM3-5p expression was not detected in any of the cell lines (Fig. 3-4). Subsequently, we investigated the expression of the novel miRNAs in clinical samples. Given the high expression levels of both candidate miRNAs in MCF-7 cells, we specifically chose breast cancer samples. Expression of novel miRNAs was exclusively detected in clinical samples exhibited elevated *MSI1* levels (Fig. 5), implying a shared expression regulatory mechanism between novel miRNAs and their host gene *MSI1*. While the majority of clinical samples were ER/PR positive with high levels of *MSI1*, the novel miRNAs showed both decreased and increased expression patterns in these samples.

Based on bioinformatics predictions, *BNC2* and *NCAM1* were identified as the two most reliable target genes for MSM2-5p, while *PDE11A*, *ELAVL2*, and *PRKAA2* were selected as the target genes for MSM3-3p. Subsequent dual luciferase assay confirmed a direct interaction between MSM3-3p and the 3′UTR region of *PDE11A (Fig.* 6).

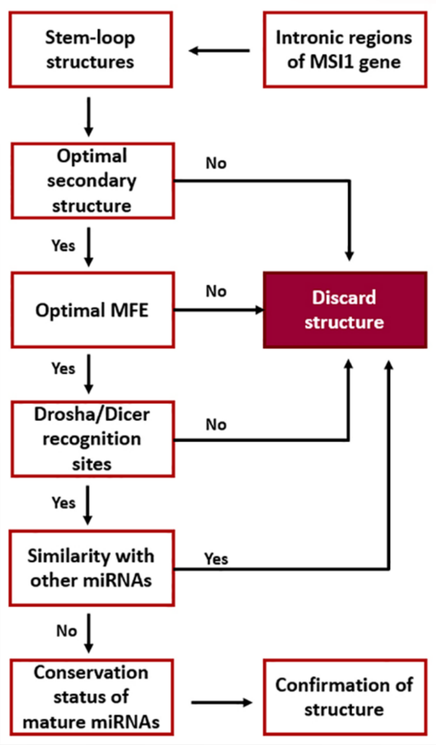

Figure 1: Flowchart of bioinformatics pipeline used for prediction of novel miRNAs.

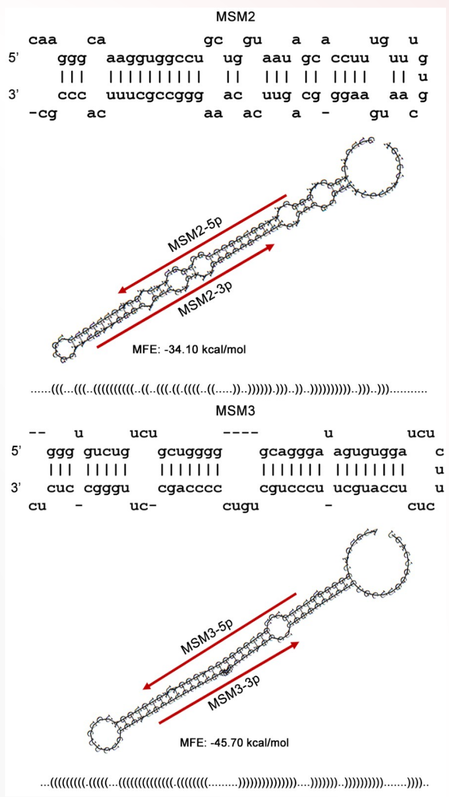

Figure 2: Structural features of predicted precursors. Secondary structure and minimal free energy of MSM2 and MSM3 miRNA precursors. Positions of predicted mature miRNAs are shown by red arrows.

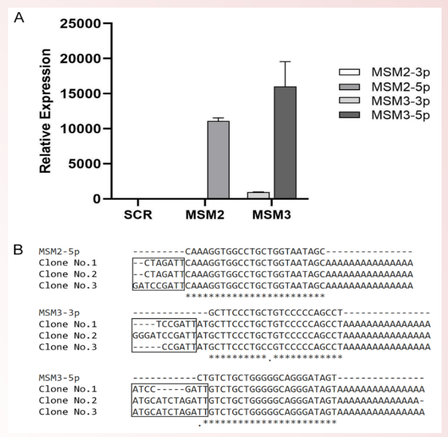

Figure 3: Exogenous expression of predicted miRNAs. (A) Relative expressions of mature miRNAs,*p-value < 0.05. (B) Sequencing results of RT-qPCR products. The first rows represent the sequences of predicted mature miRNAs.

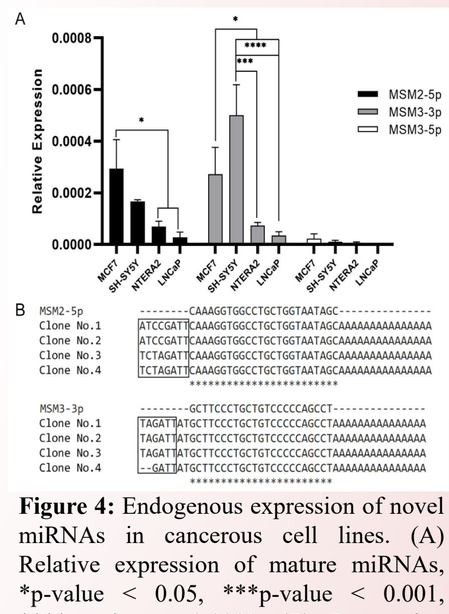

Figure 4: Endogenous expression of novel miRNAs in cancerous cell lines. (A) Relative expression of mature miRNAs, *p-value < 0.05, ***p-value < 0.001, ****p-value < 0.0001. (B) Sequencing results of RT-qPCR products. The first rows represent the sequences of predicted mature miRNAs.

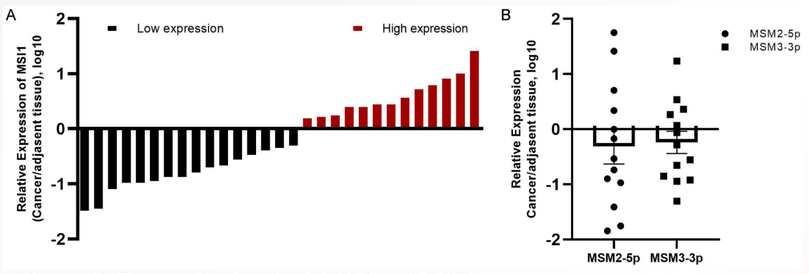

Figure 5: Expression level of *MSI1* and MSI1-encoded novel miRNAs in breast cancer samples. (A) The mRNA expression level of *MSI1* (n=29). (B) MSM2-5p and MSM3-3p expression in clinical samples with increased *MSI1* levels (n=13).

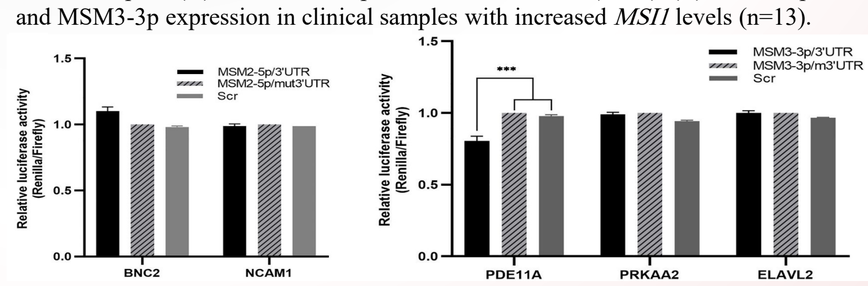

Figure 6: Dual luciferase activity assay was used to determine the direct interaction between the predicted miRNAs and putative target genes, *** p-value < 0.001.

Conclusion

Our RT-qPCR and sequencing results confirmed the presence of two novel miRNAs within the intronic region of the *MSI1* gene. Direct interaction between MSM3-3p and PDE11A, as a tumor suppressor gene, suggests an oncogenic role for this miRNA. However, additional studies are necessary to fully understand the precise roles of these novel miRNAs in diverse cancers and evaluate their potential as biomarkers.

References:
[1] A. E. Kudinov, J. Karanicolas, "Musashi RNA-Binding Proteins as Cancer Drivers and Novel Therapeutic Targets", Clin. Cancer Res. 23 (2017).
[2] A Chakrabortty, DJ Patton, "miRNAs: Potential as Biomarkers and Therapeutic Targets for Cancer", Genes. 14 (2023).

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