Analyses of Mature microRNA Molecules and Sequences by MALDI Mass Spectrometry.

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Abstract

Rationale: Matrix-assisted laser desorption/ionization (MALDI) is a soft ionization technique used in mass spectrometry, allowing the analysis of biomolecules and large organic molecules. Nucleic acids can be directly observed without amplification by this method but are harder to detect than peptides, especially in longer sequences, due to their tendency to fragment when laser is irradiated. Previous publications demonstrated microRNA (miRNA) let-7d and miR-21 as molecules that differentiate disease to normal in pulmonary disease such as idiopathic pulmonary fibrosis. Although these findings suggest the role of miRNA molecules as potential biomarkers, current PCR and chip hybridization based assays require biological control samples to be processed in parallel, making it complicated for diagnostic use. Because mature miRNA molecules are smaller in size (21 – 23 nt) by nature, the effect of nucleotide chain fragmentation can potentially be minimal. We hypothesized MALDI mass spectrometry can serve as an alternative method for miRNA expression and sequence analyses.

Materials and Methods: Mature sequence molecules for hsa-miR-16a, hsa-let-7d, hsa-miR-21 were prepared (Sigma-Aldrich Japan, Ishikari, Japan). 0.1 ug of oligonucleotides were eluted in nuclease-free water, and their yields were titrated by serial dilution. Oligonucleotides were then mixed with 3-hydropicolinic acid matrix and subjected to AXIMA Performance MALDI mass spectrometry (Shimadzu Corporation, Kyoto, Japan) for detection and sequence analysis of miRNA molecules.

Results: MALDI mass spectrometry revealed the spectra of the whole mature molecules of miR-16a, let-7d, miR-21, as well as their sequence from 5' end up to 10 nucleotides. The spetra were detectable as low as 3.75 picomoles without amplification processes, and the yields were measurable by HPLC through ultra-violet laser adsorption ratio of 260/280.

Conclusions: MALDI mass spectrometry and liquid chromatography demonstrated potential as alternative methods for qualitative and quantitative analysis of mature microRNA. This novel approach has advantage over conventional methods in terms of its capability to directly observe the microRNA molecules without amplification.

Background

- New demands arise for the analysis and quantitation of microRNA.
- Chromatography methods are possible alternatives.
  - UV
  - Fluorescence
  - MS/MALDI
- microRNA (miRNA) biomarker discovery and RNomics
  - more non-coding RNAs are inevitably discovered.

Aims

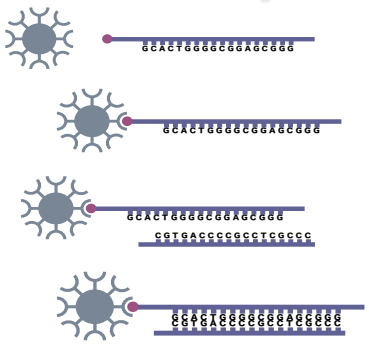
- Detect and observe microRNA molecules directly without amplification.
- Application of "fishing process" in biological samples.

Materials and Methods

- Mature sequence molecules for hsa-miR-16a, hsa-let-7d, hsa-miR-21 were prepared (Sigma-Aldrich Japan, Ishikari, Japan).
- 0.1 ug of oligonucleotides were either eluted in nuclease-free water or spiked to RNA pool extracted from biological samples, and their yields were titrated by serial dilution.
- Oligonucleotides were then mixed with 3-hydropicolinic acid matrix and subjected to AXIMA Performance MALDI mass spectrometry (Shimadzu Corporation, Kyoto, Japan) for detection and sequence analysis of miRNA molecules.
- Hybridized targets were verified by miRNA qRT-PCR.

Experimental Scheme

microRNA 'Fishing'



miR-16-1

5'-UAGCAGCACGUAAAUAUUGGCG-3'
3'-TTATCGTCGTGCATTTATAACCGC-5'

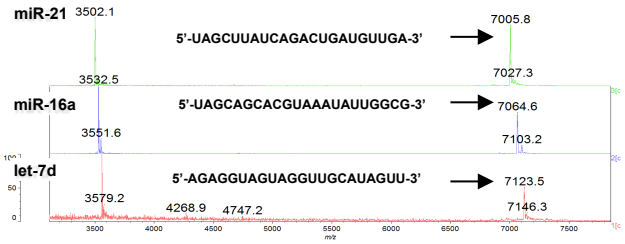
Let-7d

5'-AGAGGUAGUAGGUUGCAUAGUU-3'
3'-TTTCTCCATCATCCAACGTATCAA-5'

- Biotinylated antisense DNA probes are attached to avidin-coated magnetic beads.
- Target microRNA chain is attached to the probe-beads complex.
- microRNA molecules are detached from the magnetic beads, then visualized with MALDI mass spectrometry.

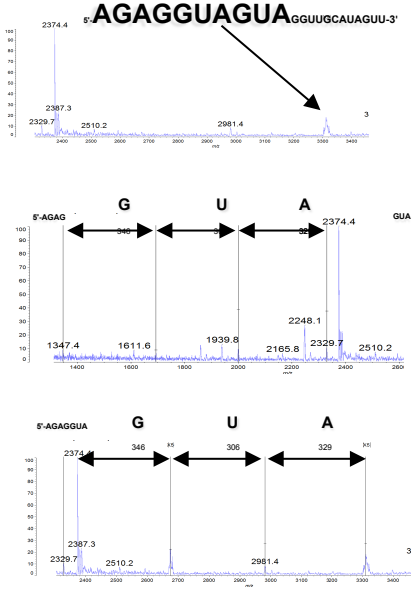
Results

microRNA molecules can be distinguished by MALDI mass spectrometry.

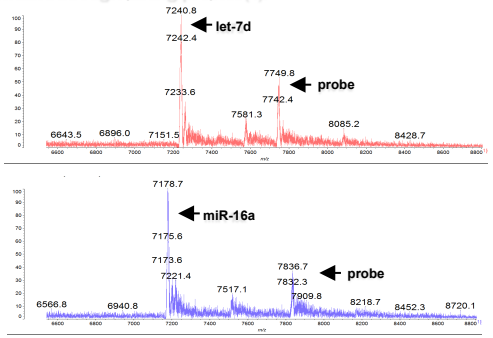


Results

In source decay (ISD) enables identification of 5'end conserved MOTIF and sequence analysis of mature miRNA let-7d.

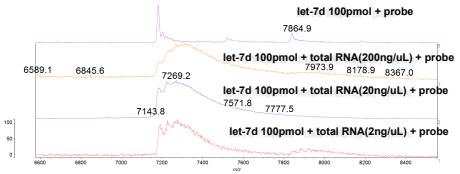


Mature microRNA molecules are collected and identified through 'fishing' process (1).

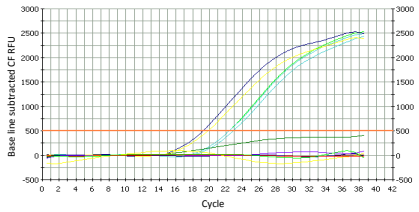


Results

Mature microRNA molecules are collected and identified through 'fishing' process (2).



Validation of target microRNA by qRT-PCR.



Conclusions

- Mature microRNA molecules can be distinguished by MALDI mass spectrometry.
- 'Fishing' for the target microRNA molecules is possible by applying antisense oligo-probe and biotinylated beads.
- Verified the "fished' molecules as target by miR qRT-PCR.
- Direct observation of oligonucleotides (e.g. microRNA, siRNA) opens a new field in order to apply these molecules as biomarkers, as well as studying these compounds in forms of pharmaceutical agents.

References

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