Global microRNA profiling using novel miRCURY LNA™ microarrays enables identification of tumors of unknown primary origin

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Introduction

MicroRNA profiling can constitute a recently discovered class of tissue specific, small, non-coding RNAs, which regulate the expression of genes involved in many biological processes, including development, differentiation, apoptosis and carcinogenesis.

Around 5% of all newly diagnosed metastatic cancers are cancers of unknown primary (CUP), Figure 1, where the site of the primary tumor cannot be determined, despite the use of advanced immunohistochemical and radiological techniques. Because effective cancer treatment depends on early identification of the primary tumor, CUP patients have poor prognoses with a median survival of 2–4 months and a 5-year survival rate of only 3%.

By applying a novel microarray platform based on locked nucleic acid (LNA) modified detection probes, which enable high sensitivity and sequence specific detection of miRNAs, we are able to identify tumor-specific markers in CUP patients, without delay, in order to offer the most relevant treatment. As part of this effort we are developing a classification test for diagnostic of CUP, enabling individualized treatment of this otherwise intractable group of cancers.

Methods

More than 500 tumor and normal adjacent tissue samples were collected from both histological and functional level, paraffin-embedded specimens. Table 1 lists 31 tissues that are included in our analysis, and which represent the most common tissues of origin for CUP.

Results

1) Microarray profiling

One microarray based profiling was performed on an miRCURY LNA™ Discovery arrays containing 1,186 human miRNAs, including 673 human miRNAs, 457 human miRNAs discovered by 3′A high throughput sequencing as well as the sequencing of primed miRNAs (Figure 2). All hybridizations were made against a common reference pool.

Data analysis

A miRNA expression database was established for identification of miRNAs with high discriminatory potential. In this database, a machine learning (random forest) clustering and supervised analysis was performed to generate a multiclass classifier.

Properties of the LNA™ capture probes and microarrays

Table 2

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Number of DNA capture probes</th>
<th>Number of LNA™ capture probes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lung</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>Breast</td>
<td>10</td>
<td>10</td>
</tr>
<tr>
<td>Colon</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>Kidney</td>
<td>10</td>
<td>10</td>
</tr>
<tr>
<td>Liver</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>Ovary</td>
<td>10</td>
<td>10</td>
</tr>
<tr>
<td>Bladder</td>
<td>10</td>
<td>10</td>
</tr>
</tbody>
</table>

Validation and Localization of miRNAs

With LNA™ technology, tissue specific markers can be validated with in situ hybridization (ISH). This can potentially reveal the strength of CUP classification. Below is shown an ISH experiment, which allows localization of specific miRNAs.

Conclusions

- We have generated a miRNA based classifier, which can identify the origin of the primary tumor in CUP patients with metastatic disease.
- The classifier is based on a limited number of microRNAs and may be improved by including spatial informations in the expression.
- In addition to these biological analyses, we are currently sampling Exiqon’s further bank of 150,000 specimens.