Using different platform for miRNA expression profiling can affect experimental results

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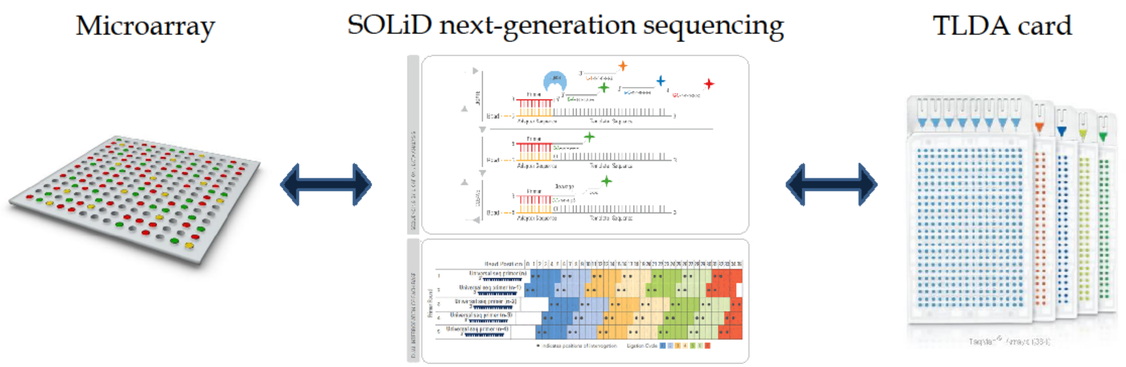
Introduction

There are 3 principal high-throughput methods that have been widely used to determine miRNAs expression levels: microarrays, qPCR based arrays and next generation sequencing. Our aim was to compare these platforms for the detection of miRNA profiles in normal and adenomatous pituitary samples.

Material and methods

Using 4 normal pituitary (NP) and 8 nonfunctioning pituitary adenoma (NFPA) samples we determined miRNA expression profile by GeneChip® microRNA Galaxy Array v1, SOLiD next generation sequencing (NGS) and TaqMan Low Density Array (TLDA). For biological validation we measured the expression of 22 miRNAs by individual TaqMan assay on additional samples as well (N_{NFA}:24, N_{NP}:10).

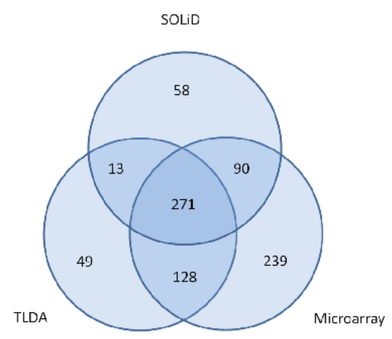


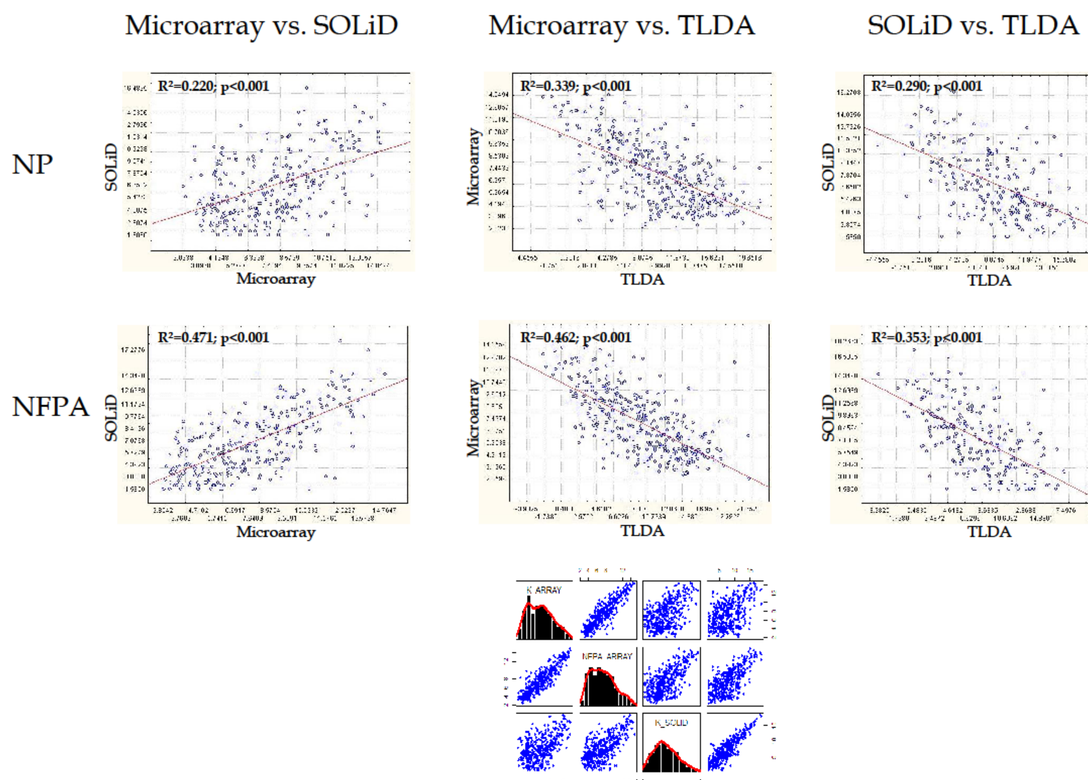
Results 1. Correlation of different platforms

We could detect total 848 miRNAs of which only 271 were detected by all three approaches.

We found that microarray profile correlates with SOLiD with R^2: 0.471 and 0.220 ($p<0.01$) in NFPA and NP samples,
microarray and TLDA with R^2: 0.462 and 0.339 ($p<0.01$),
and SOLiD and TLDA with R^2: 0.353 and 0.290 ($p<0.01$), respectively.

Number of miRNAs detected





Results 2. Comparing different NGS bioinformatics approaches

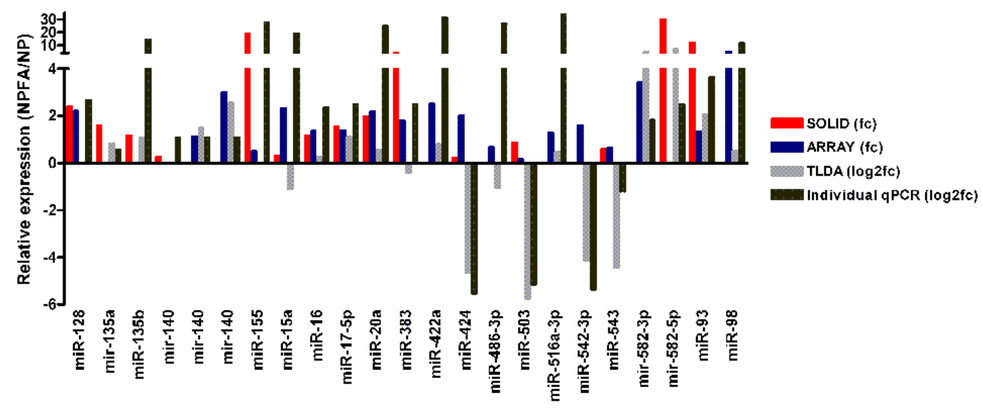
Next, we investigated if bioinformatics setting regarding NGS data analysis could affect the correlation.

1. Different minimum read number (3, 5 or 10 nt)
2. Different miRNA lengths (19-20-21-22-23 nt)
3. Different alignments (0 or 1 mismatches)
4. Different algorithms (CLCBio, Bowtie1, SureMir)

There was a small possibility to improve R^2 values depending on usage CLCBio, Bowtie1 or SureMir (our own algorithm); however this was not a major factor in determining the associations.

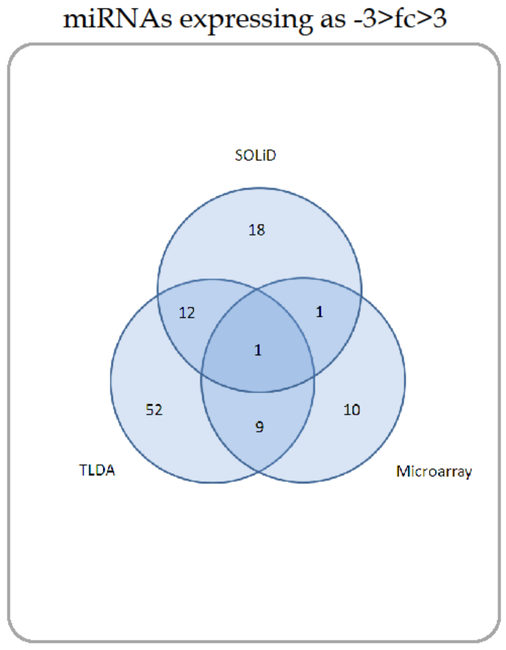
Results 3. Biological validation

Biological validation was also performed by comparing expression fold changes in NFPA compared to NP using individual TaqMan assays. Thereby we could poorly approach the results gained by comparing platforms by samples.
However the direction of expression changes could be validated as 81% of TLDA results,
72% of microarray and
72% of NGS results.

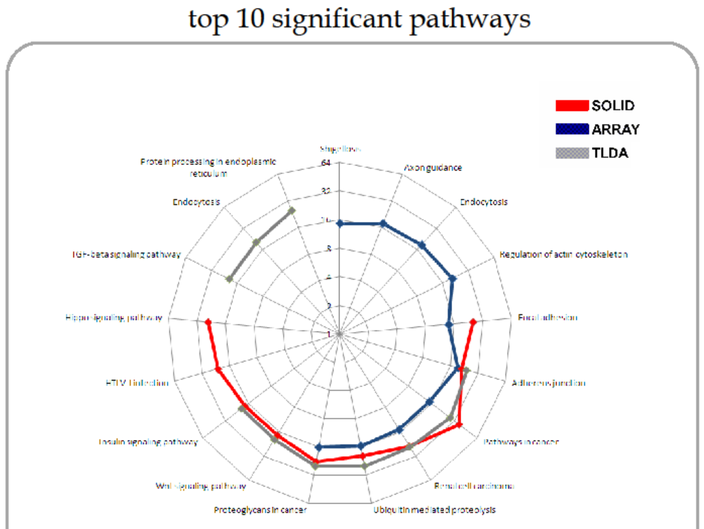


Results 4. Comparing pathway & network analyses

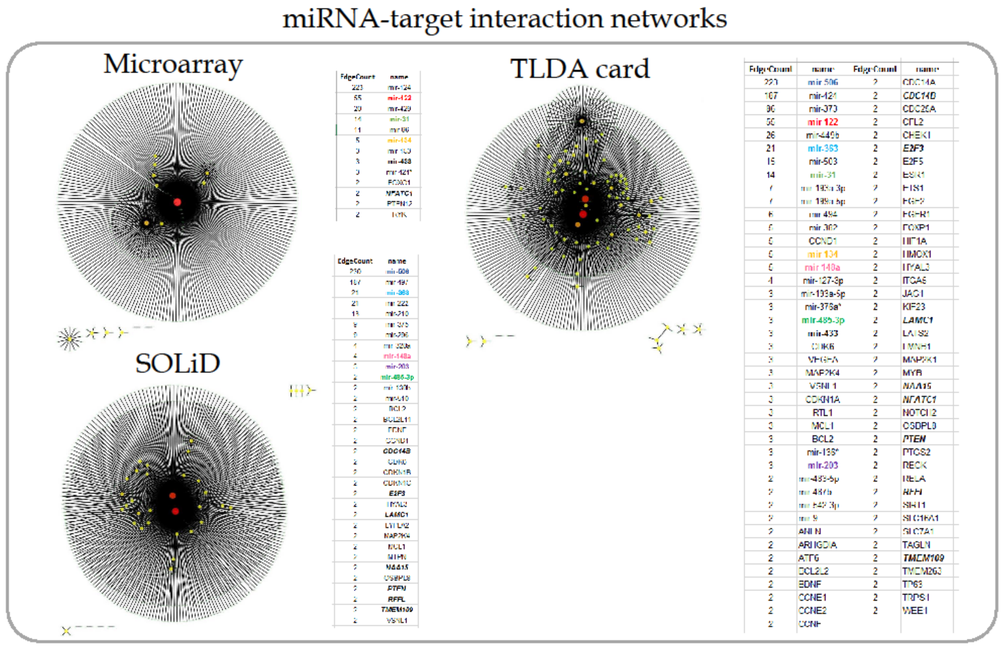
miRNAs expressing as -3>fc>3



top 10 significant pathways



miRNA-target interaction networks



Summary and Conclusion

1.) miRNA expression profiles measured by **different platforms showed poor correlation** and they were hardly comparable.

2.) Results correlated from **similar platform showed stronger association than similar sample groups**.

3.) However, individual miRNA expression from microarrays and NGS **results were replicable** in an acceptable percentage by **qPCR**.

4.) Selection of **screening method can influence experimental results** obtained by analyses using high-throughput data (e.g. pathway analysis).

5.) High-throughput miRNA **(pathway & network) analysis however showed better overlap** than significant miRNA lists among different platforms. This can be explained by the characteristics of miRNA's effect.

*Effect of miRNAs have "**divergent**" properties where the same miRNA targets multiple genes. It is also "**convergent**" in nature, where multiple miRNAs have augmented effect on the same target.*

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