MicroRNAs in maternal plasma for the non-invasive prenatal diagnosis of Down syndrome (Trisomy 21)

ABSTRACT

Most developmental processes are under the control of small regulatory RNAs called microRNAs (miRNAs). We hypothesise that different foetal developmental processes might be reflected by extracellular miRNAs in maternal plasma and may be utilised as biomarkers for the non-invasive prenatal diagnosis of chromosomal aneuploidies. In this proof-of-concept study, we report on the identification of extracellular miRNAs in maternal plasma of Down syndrome pregnancies. Using high throughput-quantitative PCR (HT-qPCR), 104 miRNAs were identified in maternal plasma via comparison of seven Down syndrome pregnancies with age and foetal sex matched controls. Six hundred and ninety-five miRNAs were identified. Thirty-six significantly differentially expressed mature miRNAs were identified as potential biomarkers. Hierarchical cluster analysis of these miRNAs resulted in the clear discrimination of Down syndrome from euploid pregnancies. Gene targets of the differentially expressed miRNAs were enriched in signalling pathways such as mucin type O-glycans, ECM-receptor interaction, Wnt-beta and endocytosis, which have been previously associated with Down syndrome. miRNAs are promising and stable biomarkers for a broad range of diseases and may allow a reliable, cost-efficient diagnostic tool for the non-invasive prenatal diagnosis of Down syndrome. Furthermore, they exhibit the potential of being the first prognostic marker to characterise the severity of Down syndrome disabilities and may allow for antagoMIr strategies to treat common Down syndrome features in the future.

RESULTS: Predictive miRNA targets and Down syndrome severity

These findings provide evidence that the differentially expressed miRNAs in this study are not randomly identified, but point towards its strong and unknown Down syndrome pathomechanisms. Thus, miRNA exhibit the potential to be the first prognostic factors to explore the severity of Down syndrome symptoms by NIPD.

DISCUSSION

A wide range of organ systems are affected in Down syndrome individuals, with some congenital whereas others are progressive, and include cardiac malformations, increased frequency of childhood leukaemia, varying degrees of intellectual disability and central nervous system abnormalities (7). MiRNAs are considered to play an active role in the regulation of developmental processes. With the assumption that ~50% of all genes are miRNA-controlled (8), we were able to identify Down syndrome-specific miRNA profiles in maternal plasma. These miRNA pattern have potential to be used for non-invasive diagnostic purposes. When using a subset of 10 or 20 miRNAs, a clear identification of Down syndrome is possible. The exact transport mechanisms of extracellular, cell-free miRNAs into the maternal circulation are currently unknown. Trisomy 21 may lead to a deregulation of gene expression including miRNAs. These miRNAs may then enhance the dysregulation of genes in a genome-wide fashion, resulting in mild or severe symptoms, depending on the dysregulation. It has been previously demonstrated that cells can select some miRNAs for cellular release while others are retained (9). Their exocytosis may represent a proteome mechanism in which harmful miRNAs are actively enveloped and secreted out of the cells via exosomes. The Down syndrome-specific miRNA profile may therefore reflect the severity of symptoms e.g., in respect to mental retardation and cardiomyopathy, thus classifying miRNAs as the first prognostic biomarkers in the NIPD of Down syndrome. Furthermore, miRNA may also allow for antagomiR strategies to cure or at least partially reduce serious Down syndrome symptoms during prenatal and postnatal development of Down syndrome patients in future.

Take-home messages:

- Down syndrome-specific miRNA profiles can be found in maternal plasma.
- miRNAs may be of foetal and not of placental origin.
- miRNAs may represent the first prognostic marker for the characterisation of the severity of Down syndrome symptoms.
- miRNA may allow for antagomiR strategies to ‘treat’ Down syndrome symptoms in future.

Hypothesised mechanisms on the entrance of foetal / placental miRNAs in the maternal circulation.

Although differentially expression patterns of miRNAs between Down syndrome pregnancies compared to euploid pregnancies can be observed, the exact transport mechanism of extracellular, cell-free miRNAs into the maternal circulation are currently unknown. Here, we present some hypothetical mechanisms:

A. Connection of the mother and foetus via the placenta. B. Chorionic villi are responsible for sustaining the placenta with nutrients and oxygen. The intervillus space is filled with maternal blood. C. Cellular release mechanisms and extracellular transportation systems of miRNAs (10). In the cytoplasm, miRNAs can be incorporated into small vesicles, exosomes, which stem from the endosome, and are released from cells when multivesicular bodies coalesce with the plasma membrane. MiRNAs are also found in circulation in a microparticle-free form (associated with Ago-2 or HDL).

REFERENCES

- A: "High throughput-quantitative PCR (HT-qPCR) was performed on individual samples using the SmartChip Human miRNA Panel V3.0 (WaverGen, CA, USA) in accordance with the manufacturer’s instructions. HT-qPCR analysis was performed using both qBase Software (Biogazelle, Belgium) and BioConductor (HT-qPCR Pack).
- B: "The WaferGen miPCR Software report generated which provides a short overview on raw data (replicates) 1C’s difference between sample and NTC, and normalized relative quantities (NRQ).
- C: "These data were also used as a template for downstream analysis using R and BioConductor. In order to identify predicted miRNA targets, the Diana miPath tool v2 was used."