Published in final edited form as:
Curr Opin Pediatr. 2014 April ; 26(2): 243–251. doi:10.1097/MOP.0000000000000073.

Environmental Influence of microRNA in Children's Health

Maya Kappil[1] and **Jia Chen**[1,2,3,4]

[1]Department of Preventive Medicine, Mount Sinai School of Medicine, New York, NY, USA

[2]Department of Pediatrics, Mount Sinai School of Medicine, New York, NY, USA

[3]Department of Oncological Sciences, Mount Sinai School of Medicine, New York, NY, USA

[4]Department of Medicine, Hematology and Medical Oncology, Mount Sinai School of Medicine, New York, NY, USA

Abstract

Purpose of review—Understanding the effects of *in utero* exposures to environmental agents is of great importance as the resulting deregulation of biological processes can affect both fetal development and health outcomes that manifest later in life. Due to the established role in developmental processes and inherent stability *ex vivo*, microRNAs (miRNAs) have emerged as attractive candidates to explore the impact of such exposures during this critical window of susceptibility. In this review, we summarize the findings of studies assessing miRNAs as markers of *in utero* environmental exposures and as candidates for the molecular basis through which these exposures exert their influence on children's health. Recent Findings: To date, miRNA expression profiles due to various *in utero* environmental exposures, including xenochemicals, endogenous factors and nutritional status, have been reported.

Summary—While the validity of the identified exposure-specific miRNA profiles remains to be established, the findings thus far do raise interesting questions worth addressing in future studies. Gaps that remain to be addressed include linking specific *in utero* exposures to subsequent health outcomes based on established miRNA expression profiles and experimentally validating putative downstream targets of the deregulated miRNAs.

Keywords

miRNA; *in utero* exposures; xenochemicals; endogenous exposures; nutrition

1. Introduction

The impact of environmental exposures on human health ranges from acute effects, such as skin irritation, to chronic long-term consequences, including cognitive and reproductive dysfunction. The life stage during which the exposure occurs has been deemed a critical determinant on the health consequences realized. The physiologic and metabolic characteristics of fetal development, including the immaturity of the blood-brain barrier and

Corresponding author: Jia Chen, Departments of Preventive Medicine, Pediatrics, Oncological Sciences, and Medicine, Hematology and Medical Oncology, Mount Sinai School of Medicine, 1468 Madison Avenue, New York, NY 10029, USA., Phone: 212-241-7592., jia.chen@mssm.edu.

the reduced detoxification and elimination capacity of the internal organs such as liver and kidneys, mark the intrauterine experience as a period of heightened susceptibility[1]. In addition to impacting fetal development, environmental exposures can also modify the genome to program health outcomes experienced later in life. The molecular mechanism underlying this fetal programming, also referred to as the "developmental origins of health and disease (DOHAD)"[2], has not been clearly delineated. However, mounting evidence suggests that epigenetics plays a vital role.

The epigenome refers to heritable, quasi-stable yet dynamic and environmentally responsive elements that regulate gene expression without changes in the DNA sequence itself[3]. Various epigenetic marks have been identified, including transcriptional regulation through DNA methylation and histone modification, as well as post-transcriptional regulation through microRNAs (miRNAs). MicroRNAs are small non-coding RNAs, typically 21 – 23 nucleotides in length, formed from transcripts that take on a characteristic hairpin structure[4]. The process to generate miRNAs is initiated through the transcription of primary miRNA (pri-miRNA) by RNA polymerase II[5]. Processing by DROSHA, an RNAse III endonuclease, generates a 60–70nt stem loop intermediate with a 3′ overhang[6,7]. This precursor miRNA (pre-mRNA) is actively transported from the nucleus to the cytoplasm via Ran-GTP and Exportin-5, where it is further processed by an additional RNAse III endonuclease, DICER, into a double-stranded miRNA:miRNA* complex[8,9,10]. The mature single-stranded miRNA is loaded into the RNA induced silencing complex (RISC), where it serves as a guide for targeting the RISC to mRNAs based on complementarity[11,12]. The degree of complementarity between a miRNA and its targets determines the mechanism of post-transcriptional regulation. Near perfect pairing results in cleavage of the target mRNA while partial pairing, a phenomenon more commonly observed in animals, results in either mRNA decay or translational repression[13].

To date, over 2000 unique mature human miRNAs have been annotated and catalogued in the publicly-accessible database miRBase (www.miRbase.org), and up to one-third of all mRNA transcripts are believed to be regulated by miRNAs[14]. As targeting only requires partial complementarity, a single miRNA can bind multiple mRNA transcripts and each mRNA can be bound be multiple miRNAs. In this way, miRNAs are able to modulate entire gene networks, including biological processes such as development, stem cell differentiation, hematopoiesis, cardiac/muscle development, neurogenesis, insulin secretion, cholesterol metabolism and immune response[15]. Significant modifications in the expression of miRNAs likely result in the deregulation of these processes, ultimately triggering disease. Children are a population of special interest in determining the impact of miRNA deregulation as the heightened susceptibility to early life environmental exposures may play a role in modulating miRNA expression levels. Additionally, the downstream effects due to the resulting deregulation of miRNAs are likely more pronounced since many of the miRNA-regulated physiologic processes are most active early in the developmental process. Hence, this review focuses on miRNAs as markers of *in utero* environmental exposures and as candidates for the molecular basis through which these exposures exert their influence on children's health.

2. MicroRNA and Children's Health

A number of studies have implicated miRNAs in various pediatric health outcomes. As summarized in Table 1, these studies showcase the wide-ranging utility of miRNAs as markers of disease, in terms of diagnosis, prognosis, disease subtype classification, treatment monitoring, and agents of intervention. However, greater understanding is also required in identifying the upstream elements capable of disrupting the expression profile of miRNAs, including environmental factors, ultimately resulting in disease.

3. MicroRNA and the *in utero* Environment

Studies that have investigated the responsiveness of miRNAs to early life environmental exposures are summarized in Table 2. The assessed exposures fall into three general categories: xenochemicals, endogenous agents and nutrition. The group of xenochemicals includes ethanol and tobacco smoke, established teratogens known to cross the placenta and induce fetal malformation. The remaining chemicals in this group, including the endocrine disruptor BPA, have suspected but not yet verified detrimental effects on normal human fetal development. Studies revolving around endogenous factors, including parental stress and hypoxia, indicate that miRNAs are responsive not only to exogenously introduced chemical exposures, but also to the dysregulation of the internal environment. Finally, nutrition is highlighted as its own distinct category since the importance of the nutritional state in fetal development and programming has been previously established, the most well-documented of which links fetal malnutrition with later onset of metabolic diseases in studies conducted on individuals exposed to the Dutch famine[54]. The following sections highlight findings from representative exposures in each category.

A. Tobacco Smoke

Two human observational studies have been carried out to date assessing the role of miRNAs as potential mediators of the impact of tobacco smoke on *in utero* development. Maccani et al[46] compared the expression level of candidate miRNAs in 25 placentas obtained at the time of delivery from women with and without a history of smoking during pregnancy. Out of the 4 selected targets, a significant downregulation of miR-16, miR-21 and miR-146a was observed due to maternal cigarette smoking. Furthermore, miR-146a was significantly downregulated following exposure to both nicotine and benzopyrene in TCL-1, a cell line derived from 3rd trimester extravillous cells, while no impact due to either agent was observed on the remaining miRNAs.

The impact on miRNA expression levels due to tobacco smoke exposure during the gestational period was also addressed in a study conducted by Herberth et al[47]. As this study was particularly concerned with the effect of tobacco smoke on immune-related responses, two candidate miRNAs, miR-155 and miR-223, previously implicated in Treg cell formation and function, were selected for analysis. In this prospective study of mother-child pairs, increasing levels of miR-223 were observed in both maternal and cord blood with increasing levels of cotinine in maternal urine.

B. Parental Stress

In a study by Morgan and Bale[52] the impact of prenatal stress on brain development was assessed. For this purpose, the offspring of male mice who were prenatally exposed to maternal stressors were analyzed. Microarray analysis of brain-derived miRNAs revealed that the profile of F2 male offspring stemming from a stress-exposed lineage was more similar to the profile of non-exposed F2 female offspring than non-exposed F2 male offspring, suggesting dysmasculinazation of the brain among the F2 stress-exposed males. Specifically, three miRNA species, miR-322, miR-574-3p and miR-873, were observed to be downregulated among the F2 stress-exposed offspring, while the expression level of β-glycan, a predicted target common to all three miRNAs, was determined to be upregulated compared to F2 control offspring. Finally, following treatment with the aromatase inhibitor formestane, the brain miRNA profile of formestane-treated male mice clustered more closely to control female mice than control male mice, further suggesting that the miRNA environment is sensitive to hormonal influence dysregulation. The overall findings from this study, therefore, suggest that the changes incurred during *in utero* development can be transmitted trans-generationally.

C. Nutrition

The effects of a maternal high fat diet on the miRNA profile of the resulting offspring was assessed in a murine model by Zhang et al[53]. Microarray analysis of liver-derived miRNAs from female offspring identified 10 miRNAs that were upregulated and 23 miRNAs that were downregulated among offspring exposed to a high-fat diet. While all targets were not successfully validated, the downregulation of miR-483* was consistently observed as the greatest fold-change among high-fat diet exposed offspring. The authors determined that the genetic location of miR-483* lies within the intron of *Igf2*, a gene known to be critical in regulating fetal growth, indicating that the transcription of both is likely regulated by the same promoter. Paradoxically, among high fat exposed mice, the downregulation of miR-483* was observed alongside an upregulation of *Igf2*.

4. Future Perspective

Findings relaying the impact of *in utero* exposures on miRNA expression levels thus far have raised several interesting questions that will need to be further addressed. Principal among these is determining whether the observed changes in miRNA expression levels reflect a causal pathway between exposure and outcome, identifying the downstream gene targets impacted by deregulated miRNAs, and delineating variability introduced due to methodological and biospecimen specifications.

A. Role of miRNAs in linking exposure to outcome

To address this question, it will first have to be established whether the observed changes in miRNA expression levels due to environmental exposures are the result of a direct effect or a surrogate indication of a different mechanism. This issue was highlighted in the study by Herberth et al, where the expression level of miR-223 in blood was found to vary among different blood cell-types, leading the authors to question whether the observed tobacco-related increase in miR-223 levels is indeed a direct response to the exposure or a by-stander

effect of smoke-induced changes in blood cell-type composition[43]. While the utility as a marker of exposure is not diminished in either case, the former would more directly associate miRNAs to the causal pathway linking exposures to putative deregulated physiological processes.

Given a substantiated impact of environmental exposures on miRNA expression levels and related physiological processes, more efforts into how these changes in expression profiles translate into health outcomes will also be warranted. To date, studies relating changes in miRNAs to various health outcomes and studies relating exposures to changes in miRNAs are often conducted separately. Few studies have linked the observed changes in miRNAs due to environmental exposures to known health effects. One such example includes the finding by Herberth et al[47] that higher levels of miRNA-223 in cord blood was also correlated with decreasing levels of Treg cells in newborns, which in turn was shown to be associated with a significantly higher risk of developing atopic dermatitis by the age of 3. Such findings pave the way to further understand the etiologic processes underlying the exposure-outcome relationship and offer possibilities for remediation and intervention.

B. Prediction of miRNA targets

In order to identify potential physiological processes that are affected by the exposure-incurred changes in miRNA profiles, studies often employ various existing web-based programs (e.g. miRBase, Targetscan, PicTar) to identify putative mRNA targets of the aberrantly expressed miRNAs. Using slightly varying algorithms, these programs scan a library of mRNA 3′UTR regions to identify potential binding sites for the miRNAs of interest. However, as binding of miRNAs to their targets requires only partial complementarity, prediction of targets typically yields up to 20% false-positives[55]. The substantial rate of false positives and the observed discrepancy in predicted targets across the various algorithms highlight the need to experimentally validate reported putative targets.

C. Methodological differences: NGS vs. microarray

Several high-throughput methodologies are currently in use to determine miRNA expression levels. Utilization of microarrays relies on nucleic-acid hybridization of labeled miRNA-derived cDNAs to complementary oligonucleotide probes immobilized on the array, with fluorescence intensity indicating the level of expression. This methodology is currently the most widely applied means of high-throughput determination of miRNA expression levels. However innate properties of miRNAs limit the sensitivity and specificity achieved with this method. For example, miRNAs of low abundance likely fall below the limit of detection on the array. There is also considerable sequence homology among miRNAs, often differing in only one nucleotide, which probes on the array may not be able to distinguish. Next generation sequencing is a methodology that is able to address several of these limitations. Additionally, unlike array-based methods, profiles generated using sequencing are not limited to previously identified miRNAs. However, the multiple steps involved in setting up the sequencing reaction offer various opportunities to introduce bias[56]. Furthermore, although the price is continuously dropping, the cost of sequencing at present is still higher than array-based methods.

D. Specimen issues: Placenta, cord blood, maternal plasma

As with any biomarker-related study, the biospecimen source of miRNAs greatly influences the interpretation of meaningful results. Placental tissue, umbilical cord blood and maternal sera are common sources for biomarkers reflecting the *in utero* experience in human observational studies. All three serve as easily obtainable, non-invasive sources of miRNAs. However, there are factors driving distinctions in the expression profile generated from these biospecimens that need to be considered. Both placenta and blood are composite tissues consisting of heterogeneous cell-types. Therefore, minimizing variability in cell-type composition across samples needs to be accounted for prior to analysis to reduce the likelihood of introducing bias into the study. Cord blood does provide access to specific cell-lineages that make this particular biospecimen an attractive source for studies focusing on the impact of environmental exposures on the differentiation potential of stem cell populations, such as neuronal precursors, and on the immune response of cytokines.

While the expression profile generated from placental tissue and cord blood reflects the exposure experience towards the end of pregnancy, maternal plasma offers a means to monitor dynamic changes in expression level throughout pregnancy, enabling the focus on specific gestational periods. However, the proportion of placental miRNA in circulation likely fails to account for the comprehensive expression profile of the placenta. Therefore, maternal plasma is best suited to develop screening markers, while cord blood and placental levels can also be analyzed to further etiologic understanding.

5. Conclusion

The implementation of miRNAs as indicators of environmental exposures relevant to children's health is still a nascent field, and the promise of their utility is just beginning to be realized. While mRNA transcript levels, the ultimate targets of miRNAs, can also be utilized for this purpose, one of the major attractions over their labile transcript counterparts is the inherent stability and robustness of miRNAs in bodily fluids under various conditions[57,58]. Furthermore, technological advances have made high-throughput assessment affordable, providing a means of feasible and sensitive detection. The relevance of this marker in these types of studies is also established by the fact that miRNAs are known to be involved in processes with heightened activity during early development. Hence, beyond reflecting extent of exposure to an environmental agent, an observed deregulation of miRNA levels can also point to the etiologic mechanism, ultimately linking a given exposure to an outcome. Already various exposure and outcome-related signatures have been identified. However, the lack of comparability in experimental conditions prevents deriving meaningful conclusions from the findings reported thus far. Even in studies focusing on the same exposure of interest, variability exists in the form of dosage, study population, biospecimen analyzed, detection methods utilized, and definitions for fold cut-offs. Hence, while technological advances yet to come will further facilitate the ability to assay and analyze expression profiles under various conditions resulting in novel contributions to the literature, future studies should also focus on replicating existing expression profiles. Only in establishing reproducible expression profiles and validating putative gene targets can the

ultimate goal of building a cohesive narrative tying environmental exposures to children's health outcomes be realized.

Acknowledgments

This work was supported by grants from the National Institutes of Health (R01 CA172460, R01 HD067611/R01ES022223-01A1, U01 ES019451) and Mount Sinai Children's Environmental Health Center Pilot Fund.

References

References of special interest (*)

1. Chance GW, Harmsen E. Children are different: environmental contaminants and children's health. Can J Public Health. 1998; 89(Suppl 1):S9–13. S10–5. [PubMed: 9654786]
2. Barker DJP. The origins of the developmental origins theory. J Intern Med. 2007; 261:412–7. [PubMed: 17444880]
3. Bollati V, Baccarelli A. Environmental epigenetics. Heredity (Edinb). 2010; 105:105–12. [PubMed: 20179736]
4. Lagos-Quintana M, Rauhut R, Lendeckel W, Tuschl T. Identification of novel genes coding for small expressed RNAs. Science. 2001; 294:853–8. [PubMed: 11679670]
5. Lee Y, Kim M, Han J, Yeom K-H, Lee S, Baek SH, Kim VN. MicroRNA genes are transcribed by RNA polymerase II. EMBO J. 2004; 23:4051–60. [PubMed: 15372072]
6. Lee Y, Ahn C, Han J, Choi H, Kim J, Yim J, Lee J, Provost P, Radmark O, Kim S, Kim VN. The nuclear RNase III Drosha initiates microRNA processing. Nature. 2003; 425:415–9. [PubMed: 14508493]
7. Lee Y, Jeon K, Lee JT, Kim S, Kim VN. MicroRNA maturation: stepwise processing and subcellular localization. EMBO J. 2002; 21:4663–70. [PubMed: 12198168]
8. Bohnsack MT, Czaplinski K, Görlich D. Exportin 5 is a RanGTP-dependent dsRNA-binding protein that mediates nuclear export of pre-miRNAs Exportin 5 is a RanGTP-dependent dsRNA-binding protein that mediates nuclear export of pre-miRNAs. 2004:185–191.10.1261/rna.5167604.Most
9. Hutvágner G, McLachlan J, Pasquinelli AE, Bálint E, Tuschl T, Zamore PD. A cellular function for the RNA-interference enzyme Dicer in the maturation of the let-7 small temporal RNA. Science. 2001; 293:834–8. [PubMed: 11452083]
10. Ketting RF, Fischer SE, Bernstein E, Sijen T, Hannon GJ, Plasterk RH. Dicer functions in RNA interference and in synthesis of small RNA involved in developmental timing in C. elegans. Genes Dev. 2001; 15:2654–9. [PubMed: 11641272]
11. Hammond SM, Bernstein E, Beach D, Hannon GJ. An RNA-directed nuclease mediates post-transcriptional gene silencing in Drosophila cells. Nature. 2000; 404:293–6. [PubMed: 10749213]
12. Gregory RI, Chendrimada TP, Cooch N, Shiekhattar R. Human RISC couples microRNA biogenesis and posttranscriptional gene silencing. Cell. 2005; 123:631–40. [PubMed: 16271387]
13. Zeng Y, Yi R, Cullen BR. MicroRNAs and small interfering RNAs can inhibit mRNA expression by similar mechanisms. Proc Natl Acad Sci U S A. 2003; 100:9779–84. [PubMed: 12902540]
14. Griffiths-Jones S. miRBase: microRNA sequences and annotation. Curr Protoc Bioinformatics. 2010; Chapter 12(Unit 12.9):1–10.
15. Williams AE. Functional aspects of animal microRNAs. Cell Mol Life Sci. 2008; 65:545–62. [PubMed: 17965831]
16. Schotte D, Chau JC, Sylvester G, Liu G, Chen C, van der Velden VH, Broekhuis MJ, Peters TC, Pieters R, den Boer ML. Identification of new microRNA genes and aberrant microRNA profiles in childhood acute lymphoblastic leukemia. Leukemia. 2009; 23:313–22. [PubMed: 18923441]
17. Zhang H, Luo XQ, Zhang P, Huang LB, Zheng YS, Wu J, Zhou H, Qu LH, Xu L, Chen YQ. MicroRNA patterns associated with clinical prognostic parameters and CNS relapse prediction in pediatric acute leukemia. PLoS One. 2009; 4:e7826. [PubMed: 19915715]

18. De Oliveira JC, Scrideli CA, Brassesco MS, Morales AG, Pezuk JA, de Queiroz RP, Yunes JA, Brandalise SR, Tone LG. Differential miRNA expression in childhood acute lymphoblastic leukemia and association with clinical and biological features. Leuk Res. 2012; 36:293–8. [PubMed: 22099053]
19. Fulci V, Colombo T, Chiaretti S, Messina M, Citarella F, Tavolaro S, Guarini A, Foa R, Macino G. Characterization of B- and T -Lineage Acute Lymphoblastic Leukemia by Integrated Analysis of MicroRNA and mRNA Expression Profiles. 2009; 1082:1069–1082.
20. Kaddar T, Chien WW, Bertrand Y, Pages MP, Rouault JP, Salles G, Ffrench M, Magaud JP. Prognostic value of miR-16 expression in childhood acute lymphoblastic leukemia relationships to normal and malignant lymphocyte proliferation. Leuk Res. 2009; 33:1217–23. [PubMed: 19195700]
21. Tan Z, Randall G, Fan J, Camoretti-Mercado B, Brockman-Schneider R, Pan L, Solway J, Gern JE, Lemanske RF, Nicolae D, Ober C. Allele-specific targeting of microRNAs to HLA-G and risk of asthma. Am J Hum Genet. 2007; 81:829–34. [PubMed: 17847008]
22. Su XW, Yang Y, Lv ML, Li LJ, Dong W, Miao-Liao Gao LB, Luo HB, Yun-Liu Cong RJ, Liang WB, Li YB. Association between single-nucleotide polymorphisms in pre-miRNAs and the risk of asthma in a Chinese population. DNA Cell Biol. 2011; 30:919–23. [PubMed: 21663520]
23. Collison A, Mattes J, Plank M, Foster PS. Inhibition of house dust mite-induced allergic airways disease by antagonism of microRNA-145 is comparable to glucocorticoid treatment. J Allergy Clin Immunol. 2011; 128:160–167.e4. [PubMed: 21571357]
24. Abu-Elneel K, Liu T, Gazzaniga FS, Nishimura Y, Wall DP, Geschwind DH, Lao K, Kosik KS. Heterogeneous dysregulation of microRNAs across the autism spectrum. Neurogenetics. 2008; 9:153–61. [PubMed: 18563458]
25. Sarachana T, Zhou R, Chen G, Manji HK, Hu VW. Investigation of post-transcriptional gene regulatory networks associated with autism spectrum disorders by microRNA expression profiling of lymphoblastoid cell lines. Genome Med. 2010; 2:23. [PubMed: 20374639]
26. Vaishnavi V, Manikandan M, Tiwary BK, Munirajan AK. Insights on the functional impact of microRNAs present in autism-associated copy number variants. PLoS One. 2013; 8:e56781. [PubMed: 23451085]
27. Xu J, Hu Z, Gu H, Yi L, Cao H, Chen J, Tian T, Liang J, Lin Y, Qiu W, Ma H, Shen H, Chen Y. Functional variant in microRNA-196a2 contributes to the susceptibility of congenital heart disease in a Chinese population. Hum Mutat. 2009; 30:1231–6. [PubMed: 19514064]
28. Xing HJ, Li YJ, Ma QM, Wang AM, Wang JL, Sun M, Jian Q, Hu JH, Li D, Wang L. Identification of microRNAs present in congenital heart disease associated copy number variants. Eur Rev Med Pharmacol Sci. 2013:2114–2120. [PubMed: 23884835]
29. Zhu S, Cao L, Zhu J, Kong L, Jin J, Qian L, Zhu C, Hu X, Li M, Guo X, Han S, Yu Z. Identification of maternal serum microRNAs as novel non-invasive biomarkers for prenatal detection of fetal congenital heart defects. Clin Chim Acta. 2013; 424:66–72. [PubMed: 23707860]
30. Oglesby IK, Bray IM, Chotirmall SH, Stallings RL, O'Neill SJ, McElvaney NG, Greene CM. miR-126 is downregulated in cystic fibrosis airway epithelial cells and regulates TOM1 expression. J Immunol. 2010; 184:1702–9. [PubMed: 20083669]
31. Megiorni F, Cialfi S, Dominici C, Quattrucci S, Pizzuti A. Synergistic post-transcriptional regulation of the Cystic Fibrosis Transmembrane conductance Regulator (CFTR) by miR-101 and miR-494 specific binding. PLoS One. 2011; 6:e26601. [PubMed: 22028919]
32. Ramachandran S, Karp PH, Jiang P, Ostedgaard LS, Walz AE, Fisher JT, Keshavjee S, Lennox KA, Jacobi AM, Rose SD, Behlke MA, Welsh MJ, Xing Y, McCray PB Jr. A microRNA network regulates expression and biosynthesis of wild-type and DeltaF508 mutant cystic fibrosis transmembrane conductance regulator. Proc Natl Acad Sci U S A. 2012; 109:13362–7. [PubMed: 22853952]
33. Oglesby IK, Chotirmall SH, McElvaney NG, Greene CM. Regulation of cystic fibrosis transmembrane conductance regulator by microRNA-145, -223, and -494 is altered in ΔF508 cystic fibrosis airway epithelium. J Immunol. 2013; 190:3354–62. [PubMed: 23436935]

34. Bhattacharyya S, Kumar P, Tsuchiya M, Bhattacharyya A, Biswas R. Regulation of miR-155 biogenesis in cystic fibrosis lung epithelial cells: Antagonistic role of two mRNA-destabilizing proteins, KSRP and TTP. Biochem Biophys Res Commun. 2013; 433:484–488. [PubMed: 23524258]
35. Sebastiani G, Di U, Onlus M. Increased expression of microRNA miR-326 in type 1 diabetic patients with ongoing islet autoimmunity. 2011:862–866.10.1002/dmrr
36. Nielsen LB, Wang C, Sorensen K, Bang-Berthelsen CH, Hansen L, Andersen ML, Hougaard P, Juul A, Zhang CY, Pociot F, Mortensen HB. Circulating levels of microRNA from children with newly diagnosed type 1 diabetes and healthy controls: evidence that miR-25 associates to residual beta-cell function and glycaemic control during disease progression. Exp Diabetes Res. 2012; 2012:896362. [PubMed: 22829805]
37. Salas-Pérez F, Codner E, Valencia E, Pizarro C, Carrasco E, Perez-Bravo F. MicroRNAs miR-21a and miR-93 are down regulated in peripheral blood mononuclear cells (PBMCs) from patients with type 1 diabetes. Immunobiology. 2013; 218:733–7. [PubMed: 22999472]
38. Chen Y, Stallings RL. Differential patterns of microRNA expression in neuroblastoma are correlated with prognosis, differentiation, and apoptosis. Cancer Res. 2007; 67:976–83. [PubMed: 17283129]
39. Bray I, Bryan K, Prenter S, Buckley PG, Foley NH, Murphy DM, Alcock L, Mestdagh P, Vandesompele J, Speleman F, London WB, McGrady PW, Higgins DG, O'Meara A, O'Sullivan M, Stallings RL. Widespread dysregulation of MiRNAs by MYCN amplification and chromosomal imbalances in neuroblastoma: association of miRNA expression with survival. PLoS One. 2009; 4:e7850. [PubMed: 19924232]
40. Prats-Puig A, Ortega FJ, Mercader JM, Moreno-Navarrete JM, Moreno M, Bonet N, Ricart W, Lopez-Bermejo A, Fernandez-Real JM. Changes in Circulating MicroRNAs Are Associated With Childhood Obesity. J Clin Endocrinol Metab. 2013; 98:E1655–60. [PubMed: 23928666]
41. Avissar-Whiting M, Veiga KR, Uhl KM, Maccani MA, Gagne LA, Moen EL, Marsit CJ. Reprod Toxicol. 2010; 29:401–406. [PubMed: 20417706]
42. Sathyan P, Golden HB, Miranda RC. Competing interactions between micro-RNAs determine neural progenitor survival and proliferation after ethanol exposure: evidence from an ex vivo model of the fetal cerebral cortical neuroepithelium. J Neurosci. 2007; 27:8546–57. [PubMed: 17687032]
43. Wang LL, Zhang Z, Li Q, Yang R, Pei X, Xu Y, Wang J, Zhou SF, Li Y. Ethanol exposure induces differential microRNA and target gene expression and teratogenic effects which can be suppressed by folic acid supplementation. Hum Reprod. 2009; 24:562–79. [PubMed: 19091803]
44. Soares AR, Pereira PM, Ferreira V, Reverendo M, Simões J, Bezerra AR, Moura GR, Santos MA. Ethanol exposure induces upregulation of specific microRNAs in zebrafish embryos. Toxicol Sci. 2012; 127:18–28. [PubMed: 22298809]
45. Balansky R, Longobardi M, Ganchev G, Iltcheva M, Nedyalkov N, Atanasov P, Toshokova R, De Flora S, Izzotti A. Transplacental clastogenic and epigenetic effects of gold nanoparticles in mice. Mutat Res. 201310.1016/j.mrfmmm.2013.08.006
46. Maccani MA, Avissar-Whiting M, Banister CE, McGonnigal B, Padbury JF, Marsit CJ. Maternal cigarette smoking during pregnancy is associated with downregulation of miR-16, miR-21, and miR-146a in the placenta. Epigenetics. 2010; 5:583–589. [PubMed: 20647767]
47*. Herberth G, Bauer M, Gasch M, Hinz D, Roeder S, Olek S, Kohajda T, Rolle-Kampczyk U, von Bergen M, Sack U, Borte M, Lehmann I. Maternal and cord blood miR-223 expression associates with prenatal tobacco smoke exposure and low regulatory T-cell numbers. J Allergy Clin Immunol. 2013 In this study, maternal cotinine levels was positively associated with miR-223 expression levels in cord blood. Additionally, miR-223 expression levels was negatively associated with Treg cell numbers, while low Treg cell counts was associated with an increased risk of atopic dermatitis. These findings are of interest as they suggest a potential etiologic mechanism linking an exposure to an outcome through the deregulation of miRNAs. 10.1016/j.jaci.2013.06.036
48. Zhu C, Yu ZB, Zhu JG, Hu XS, Chen YL, Qiu YF, Xu ZF, Qian LM, Han SP. Differential Expression Profile of MicroRNAs during Differentiation of Cardiomyocytes Exposed to Polychlorinated Biphenyls. Int J Mol Sci. 2012; 13:15955–66. [PubMed: 23443104]

49. Wang F, Liu W, Ma J, Yu M, Jin Y, Dai J. Prenatal and neonatal exposure to perfluorooctane sulfonic acid results in changes in miRNA expression profiles and synapse associated proteins in developing rat brains. Environ Sci Technol. 2012; 46:6822–9. [PubMed: 22594572]

50. Singh NP, Singh UP, Guan H, Nagarkatti P, Nagarkatti M. Prenatal exposure to TCDD triggers significant modulation of microRNA expression profile in the thymus that affects consequent gene expression. PLoS One. 2012; 7:e45054. [PubMed: 23024791]

51*. Donker RB, Mouillet JF, Chu T, Hubel CA, Stolz DB, Morelli AE, Sadovsky Y. The expression profile of C19MC microRNAs in primary human trophoblast cells and exosomes. Mol Hum Reprod. 2012; 18:417–24. This study attempted to characterize the impact of hypoxia on expression levels in exosomal miRNA in addition to unfractionated miRNA. While no differential exosomal packaging due to hypoxia was observed, the findings are still noteworthy as they address the potential impact of environmental exposures on the role of miRNAs in intercellular communication, in addition to their influence on intracellular activities. [PubMed: 22383544]

52*. Morgan CP, Bale TL. Early prenatal stress epigenetically programs dysmasculinization in second-generation offspring via the paternal lineage. J Neurosci. 2011; 31:11748–55. The authors of this study report an altered brain miRNA expression profile in the F2 generation of mice exposed to stress during pregnancy compared to the F2 generation of unexposed mice. These findings indicate that changes incurred *in utero* can be transmitted transgenerationally. [PubMed: 21849535]

53. Zhang J, Zhang F, Didelot X, Bruce KD, Cagampang FR, Vatish M, Hanson M, Lehnert H, Ceriello A, Byrne CD. Maternal high fat diet during pregnancy and lactation alters hepatic expression of insulin like growth factor-2 and key microRNAs in the adult offspring. BMC Genomics. 2009; 10:478. [PubMed: 19835573]

54. Painter RC, Roseboom TJ, Bleker OP. Prenatal exposure to the Dutch famine and disease in later life: an overview. Reprod Toxicol. 20:345–52. [PubMed: 15893910]

55. Gusev Y, Schmittgen TD, Lerner M, Postier R, Brackett D. Computational analysis of biological functions and pathways collectively targeted by co-expressed microRNAs in cancer. BMC Bioinformatics. 2007; 8 (Suppl 7):S16. [PubMed: 18047715]

56. De Planell-Saguer M, Rodicio MC. Analytical aspects of microRNA in diagnostics: a review. Anal Chim Acta. 2011; 699:134–52. [PubMed: 21704768]

57. Chen X, Ba Y, Ma L, Cai X, Yin Y, Wang K, Guo J, Zhang Y, Chen J, Guo X, Li Q, Li X, Wang W, Zhang Y, Wang J, Jiang X, Xiang Y, Xu C, Zheng P, Zhang J, Li R, Zhang H, Shang X, Gong T, Ning G, Wang J, Zen K, Zhang J, Zhang CY. Characterization of microRNAs in serum: a novel class of biomarkers for diagnosis of cancer and other diseases. Cell Res. 2008; 18:997–1006. [PubMed: 18766170]

58. Weber JA, Baxter DH, Zhang S, Huang DY, Huang KH, Lee MJ, Galas DJ, Wang K. The microRNA spectrum in 12 body fluids. Clin Chem. 2010; 56:1733–41. [PubMed: 20847327]

Key Points

- MicroRNA expression profiles reflecting *in utero* environmental exposures are continually emerging.
- Published expression profiles will need to be validated to establish exposure-specific signatures.
- Additional gaps that remain to be addressed in future studies include experimentally validating putative downstream gene targets and linking miRNAs deregulated due to *in utero* exposures to later health outcomes.

Table 1

Studies assessing miRNAs in pediatric health outcomes

Outcome	miRNA-related assessment	Impact on outcome	Reference
Acute lymphocytic leukemia (ALL)	miRNA expression signature profile	Diagnostic: Case-control differences	Schotte et al, 2009[16]; Zhang et al, 2009[17]; Oliveira et al, 2011[18]
	miRNA expression signature profile	Disease subtype classification: T-ALL/MLL vs ALL	Schotte et al, 2009[16]
	miRNA expression signature profile	Disease sub-type classification: T-ALL vs B-ALL	Fulci et al, 2009[19]
	miRNA expression signature profile	Treatment monitoring: Response to prednisone	Zhang et al, 2009[17]
	miRNA expression signature profile	Clinical prognosis	Zhang et al, 2009[17]
	miRNA expression signature profile	Clinical prognosis	Kaddar et al, 2009[20]
Asthma	Presence of SNPs in HLA-G impacts miRNA binding to 3′UTR region	Etiology: Case-control difference	Tan et al, 2007[21]
	Presence of SNPs in miRNA genetic sequence	Etiology: Case-control differences	Su et al, 2011[22]
	Antagomir-targeted downregulation of miRNA	Intervention: Reduces eosinic inflammation, mucus hypersecretion, TH2 cytokine production, airway hyper- responsiveness	Collison et al, 2011[23]
Autism	miRNA expression signature profile	Diagnostic: Case-control differences	Abu-Elneel et al, 2008[24]
	miRNA expression signature profile	Diagnostic: Case-control differences	Sarachana et al, 2010[25]
	In silico analysis	Etiology: miRNAs known to be deregulated in autism present in CNV loci	Vaishnavi et al, 2013[26]
Congenital Heart Disease (CHD)	Presence of SNP in miRNA coding region	Etiology: Case-control differences	Xu et al, 2009[27]
	In silico analysis	Etiology: miRNAs present in CHD-related CNV loci	Xing et al, 2013[28]
	miRNA expression signature profile	Diagnostic: Case-control differences	Zhu et al, 2013[29]
Cystic Fibrosis	miRNA expression signature profile	Diagnostic: Case-control differences	Oglesby et al, 2010[30]
	In silico/in vitro analysis of CFTR 3′UTR binding targets	Etiology: miRNAs regulate CFTR gene expression	Megiorni et al, 2011[31]
	In silico/in vitro analysis of miRNAs and binding targets involved in CFTR regulation	Treatment: restore function of mutated CFTR protein	Ramachandran et al, 2012[32]
	miRNAs involved in CFTR regulation	Etiology: miRNAs regulate CFTR gene expression	Oglesby et al, 2013[33]
	In vitro analysis: biogenesis and maturation of candidate miRNA	Etiology: RNA binding proteins regulate maturation of miRNA upregulated in CF	Bhattacharya et al, 2013[34]
Diabetes, Type 1 (T1D)	Candidate miRNA expression analysis	Clinical Prognosis	Sebastiani et al, 2011[35]
	miRNA expression signature profile	Diagnostic: Case-control differences	Nielsen et al, 2012[36]
	Candidate miRNA expression analysis	Diagnostic: Case-control differences	Salas-Perez et al, 2013[37]
Neuroblastoma	miRNA expression signature profile	Disease sub-type classification	Chen and Stallings, 2007[38]
	miRNA expression signature profile	Clinical prognosis	Bray et al, 2009[39]

Outcome	miRNA-related assessment	Impact on outcome	Reference
Obesity	miRNA expression signature profile	Diagnostic: miRNA expression pattern associated with obesity markers	Prats-Puig et al 2013[40]

Table 2

Impact of *in utero* environmental exposures on miRNA expression levels

Environmental agent	miRNA	Target gene/pathway	Function	Species	Biospecimen/Celltype	Reference
Xenochemical						
BPA	miR-146a		TLR/cytokine signaling	Human	Placenta cell line	Avissar-Whiting et al, 2010[41]
Ethanol	miR-21	*Jag-1*	Notch ligand/proliferation of neuroepithelial cells	Mouse	Cerebral cortex-Derived neurospheres	Sathyan et al, 2007[42]
	miR-335	*ELAVL2*	Promotes neuronal maturation			
	miR-9					
	miR-153					
	miR-10a	*HOXa1*		Mouse	Brain	Wang et al, 2009[43]
	miR-10b					
	miR-9					
	miR-145					
	miR-153a	*Pou4fl*	Regulation of transcription	Zebrafish	Embryo	Soares et al, 2012[44]
	miR-30d	*Pou4fl*				
	miR-725					
	let7k					
	miR-100					
	miR-738					
	miR-732					
Gold nanoparticles	let-7a		Cell proliferation k-RAS activation Apoptosis	Mouse	Liver and Lung	Balansky et al, 2013[45]
	miR-183		Apoptosis Cell adhesion			
Maternal smoking	miR-146a	*BCL2L2*/*EDA*	Pro-survival NFκβ pathway	Human	Placenta	Maccani et al, 2010[46]
	miR-16	*PLAB1*/*SATB1*	Cell cycle/cell proliferation transcription factors			
	miR-21	*TRAF6*	NFκβ/TLR4 pathway			
	miR-223		Progenitor cell proliferation/granulocyte differention	Human	Cord blood	Herberth et al, 2013*[47]

Environmental agent	miRNA	Target gene/pathway	Function	Species	Biospecimen/Celltype	Reference
PCBs	miR-762	*Wnt1*	Cardiovascular differentiation	Mouse	P19 cells embryonal carcinoma	Zhu et al, 2012[48]
	miR-29a	*GSK3β*	Cardiovascular differentiation			
	miR-324-5p	*NKX2.5*	Heart development			
PFOS	miR-19b-c	*Cdk5* *Smad1* *Sox11b* *Pou5f1* *Bax* *Vsx1*	Brain and nervous system development	Rat	Brain	Wang et al, 2012[49]
	miR-19d					
	miR-181b-c					
	miR-735					
	miR-739					
TCDD	mir-122		Metabolism	Mouse	Thymus	Singh et al, 2012[50]
	miR-181a		T cell sensitivity and selection			
	miR-23a	*Fas*	Apoptosis			
	miR-18b	*FasL*	Apoptosis			
	miR-31	*CYP1A1*	Metabolism			
	miR-182	*AhR*	Metabolism			
Endogenous Factor						
Hypoxia	mR-520c-3p			Human	Placenta	Donker et al, 2012*[51]
Paternal stress	miR-322	*β-glycan*	TGF superfamily	Mouse	Brain	Morgan et al, 2011*[52]
	miR-574-3p					
	miR-873					
Nutrition						
High fat diet	miR-483*	*ZSWIM3*		Mouse	Liver	Zhang et al, 2009[53]