Clin Epigenet (2011) 2:401–404
DOI 10.1007/s13148-011-0046-2

REVIEW

Exposure and fetal growth-associated miRNA alterations in the human placenta

Matthew A. Maccani · Carmen J. Marsit

Received: 6 April 2011 / Accepted: 22 June 2011 / Published online: 9 July 2011

Abstract Researchers have begun to examine epigenetic alterations in the placenta, making key advances in understanding the epigenetic regulatory mechanisms of the placenta that define underlying processes of human development and disease. Examining changes in microRNA (miRNA) expression associated with environmental exposures and fetal growth is providing critical insights into the biology of development, response to in utero exposure, and future disease risk assessment. This review aims to highlight previous studies describing changes in miRNA expression in the human placenta associated with in utero exposure and fetal growth and seeks to assess the future directions in this exciting field of research.

Work in the developmental origins of health and disease has shifted to investigating the molecular mechanisms of fetal programming. Both in epidemiologic studies and model systems, the focus is on one of the most important tissues responsible for regulating in utero development—the placenta. The placenta can serve as a record of in utero exposure and pathology (Maccani and Marsit 2009). A number of studies have shown microRNA (miRNA) expressed in the human placenta (Barad et al. 2004; Lim et al. 2005), generating interest in further characterizing miRNA involvement in placental function and in the development of miRNA platforms with clinical utility as biomarkers of exposure or disease (Mouillet et al. 2011; Maccani and Marsit 2009).

One common yet potentially hazardous environmental exposure during pregnancy is cigarette smoke, and studies have linked cigarette smoke exposure during pregnancy to placental as well as fetal complications (Shiverick and Salafia 1999; Argente et al. 2010). Maternal cigarette smoking during pregnancy is associated with the downregulation of *miR-16*, *miR-21*, and *miR-146a* in term human placental tissue, and two components of cigarette smoke, nicotine and benzo(a)pyrene, result in the significant dose-dependent downregulation of *miR-146a* in an in vitro model (Maccani et al. 2010). Avissar-Whiting et al. showed that *miR-146a* is particularly sensitive to dysregulation by bisphenol A exposure in placental cell lines, further suggesting that *miR-146a* may be especially sensitive to modulation by exposure to these environmental toxicants (Avissar-Whiting et al. 2010; Maccani et al. 2010). These studies suggest that potentially hazardous environmental toxicants may modulate miRNA expression in the placenta, thereby underscoring the need for future work to better understand the possible downstream effects of toxicant-dysregulated miRNA expression in the placenta.

Future experiments aimed at further characterizing the modulation of miRNA in the placenta by cigarette smoke and other toxicants are needed. Experiments conducted using cigarette smoke condensate, as reported by Crane-Godreau et al. (2009), may prove especially useful in characterizing the effects of complex mixtures of cigarette smoke components on placental cells. Mouse models of chronic environmental tobacco smoke exposure as described previously (Xiong et

M. A. Maccani · C. J. Marsit (✉)
Departments of Pathology and Laboratory
Medicine and Epidemiology,
Brown University,
Box G-E5, Providence, RI 02912, USA
e-mail: Carmen_Marsit@brown.edu

M. A. Maccani
e-mail: Matthew_Maccani@brown.edu

C. J. Marsit
Center for Environmental Health and Technology,
Department of Epidemiology, Brown University,
Providence, RI, USA

al. 2011), utilizing various long-term gestational exposures and examinations at multiple timepoints in pregnancy, could elaborate on the normal and exposure-modulated roles of miRNA in placenta function. Additionally, such findings using an in vivo model system may provide key mechanistic data that can further strengthen current epidemiologic association studies.

Work to characterize associations of dysregulated miRNA expression in the placenta with fetal growth is also ongoing. Placental miRNA have been detected in maternal plasma, and circulating miRNA and other circulating nucleic acids have been detected in the maternal sera of pregnant and non-pregnant women (Chim et al. 2008; Gilad et al. 2008; Mouillet et al. 2010, 2011). Chim et al. (2008) suggested that several placental miRNAs (*miR-141*, *miR-149*, *miR-299-5p*, and *miR-135b*) were highly expressed in maternal plasma during pregnancy and noted that such expression patterns may serve as clinical biomarkers for pregnancy monitoring. Gilad et al. (2008) showed that sera from pregnant women contained placental miRNA levels higher than miRNA levels in sera from non-pregnant women; additionally, they showed that miRNA levels in the sera of pregnant women correlated with pregnancy stage. Working with porcine placentas from day 30 and day 90 of gestation, Su et al. (2010) showed differential expression of miRNA associated with pregnancy stage.

A number of groups have also investigated associations of miRNA expression specifically with fetal growth and fetal growth restriction. Maccani et al. (2011) analyzed 107 human placenta samples for the expression of six candidate miRNA previously shown to be expressed in the placenta and suggested to regulate key cell and developmental processes. They found that placental *miR-16* and *miR-21* expression was reduced in infants with the lowest birthweights and that low *miR-16* expression in the placenta predicted an increased odds for small for gestational age (SGA) status using logistic regression models and controlling for other potential confounders (Maccani et al. 2011). Moreover, low placental expression of both *miR-16* and *miR-21* predicted a greater increased odds for SGA using logistic regression models controlling for potential confounders than did only low *miR-16* or low *miR-21* alone (Maccani et al. 2011). Mouillet et al. (2010) showed that plasma levels of miRNA are regulated in pregnancy and that fetal growth restriction may be associated with an increase in circulating miRNA levels. Taken collectively, these studies suggest that miRNA levels in the placenta as well as placental miRNA detectable in maternal serum may serve as important clinical biomarkers of pregnancy, pregnancy stage, and other pregnancy-related outcomes (Maccani and Marsit 2009; Prieto and Markert 2011). Furthermore, work by Pineles et al. (2007) showed differential expression of miRNA in placenta from patients with preeclampsia, suggesting that differential placental miRNA expression may have the clinical utility of being a biomarker of preeclampsia. A further study by Patel et al. (2011) demonstrated the modulation of specific miRNA levels by placenta growth factor in endothelial cells, a result which is quite interesting given the suggested role of placenta growth factor in ensuring normal pregnancy (Aubuchon et al. 2011). While many of these clinical conditions of pregnancy can be measured by a suite of current technologies, including ultrasound, miRNA biomarkers of risk for a number of diseases of pregnancy, such as preeclampsia, or even fetal risk for developing a childhood disease may prove useful in giving clinicians further tools to assess risk for disease in both pregnant mother as well as fetus.

Future work further characterizing associations between dysregulated miRNA expression in the placenta and assessments of fetal growth will be key in better understanding how miRNA may be involved in regulating pathways of fetal development and growth. A number of animal models have been generated to further study low birthweight in a controlled, experimental system (Desai et al. 2007; Tamashiro and Moran 2010), and it will be important to consider the role of miRNA in these animal models. Pernaute et al. (2011) have made important steps in characterizing the roles of miRNA in controlling cell cycle processes in the developing mouse embryo as well as in extra-embryonic tissues. Several studies have suggested that the insulin-like growth factor 2 (IGF-2) gene may be a major regulator of placental development and function (Coan et al. 2008, 2010; Constancia et al. 2002). As a result of this observation, it may be of critical importance to identify miRNA that reside within the IGF-2 gene, as well as miRNA that may target the IGF-2 gene. Since IGF-2 signals through the Akt and ERK pathways, alterations to either the expression of miRNA or the potential targeting of IGF-2 by miRNA may have major consequences both for the placenta and for the developing fetus.

Future work may also include studying the effects of viral or bacterial infections, such as sub-lethal doses of influenza A (Mackenzie et al. 1977) or LPS (Clark et al. 2004) at different stages during pregnancy on placental miRNA expression in model systems. One might hypothesize that miRNA particularly sensitive to infections and miRNA reported to be involved in regulating inflammatory pathways, such as *miR-146a* (Lu et al. 2010; Balasubramanyam et al. 2011; Aronica et al. 2010), might be especially sensitive to such infections. Mice generated with placenta-specific knockdown of specific miRNA using previously reported techniques for placental gene manipulation (Georgiades et al. 2007) may also prove essential in better characterizing the effect of dysregulated miRNA expression on placental function and fetal growth and development.

Conclusion

Knowledge of the molecular mechanisms underlying placental gene regulation may be enhanced by identifying miRNA biomarkers for exposure, burden, or risk for disease. Additionally, these aberrant patterns of miRNA expression may identify previously unknown pathways targeted for alteration, which can serve as targets for novel drug treatment or prevention strategies, thereby bringing miRNA diagnostics and therapeutics from benchtop to bedside. Important advances in characterizing placental miRNA expression associated with exposure and fetal growth continue to elucidate a better understanding of the epigenetic regulatory mechanisms of in the placenta. Future work investigating the molecular mechanisms underlying how cigarette smoke and other potentially hazardous in utero exposures may alter the expression of placental miRNA, as well as how such aberrant miRNA expression patterns may lead to dysregulation of their target gene protein levels, will be essential in further understanding the effects of environmental exposures on placental function and potentially downstream fetal programming.

Acknowledgments This work has been supported by grants from the NIH-NCRR (P20RR018728), the NIEHS Superfund Basic Research Program (P42ES013660), and the NIEHS Training Program in Environmental Pathology (T32ES007272, MAM).

Conflict of interest statement The authors do not have any financial relationship with the organizations which sponsored the research. Furthermore, the authors have no conflict of interest to declare.

References

Argente J, Mehls O, Barrios V (2010) Growth and body composition in very young SGA children. Pediatr Nephrol 25(4):679–685. doi:10.1007/s00467-009-1432-2

Aronica E, Fluiter K, Iyer A, Zurolo E, Vreijling J, van Vliet EA, Baayen JC, Gorter JA (2010) Expression pattern of miR-146a, an inflammation-associated microRNA, in experimental and human temporal lobe epilepsy. Eur J Neurosci 31(6):1100–1107. doi:10.1111/j.1460-9568.2010.07122.x

Aubuchon M, Schulz LC, Schust DJ (2011) Preeclampsia: animal models for a human cure. Proc Natl Acad Sci U S A 108 (4):1197–1198. doi:10.1073/pnas.1018164108

Avissar-Whiting M, Veiga KR, Uhl KM, Maccani MA, Gagne LA, Moen EL, Marsit CJ (2010) Bisphenol A exposure leads to specific microRNA alterations in placental cells. Reprod Toxicol 29(4):401–406. doi:10.1016/j.reprotox.2010.04.004

Balasubramanyam M, Aravind S, Gokulakrishnan K, Prabu P, Sathishkumar C, Ranjani H, Mohan V (2011) Impaired miR-146a expression links subclinical inflammation and insulin resistance in Type 2 diabetes. Mol Cell Biochem 351(1–2):197–205. doi:10.1007/s11010-011-0727-3

Barad O, Meiri E, Avniel A, Aharonov R, Barzilai A, Bentwich I, Einav U, Gilad S, Hurban P, Karov Y, Lobenhofer EK, Sharon E, Shiboleth YM, Shtutman M, Bentwich Z, Einat P (2004) MicroRNA expression detected by oligonucleotide microarrays: system establishment and expression profiling in human tissues. Genome Res 14(12):2486–2494. doi:10.1101/gr.2845604

Chim SS, Shing TK, Hung EC, Leung TY, Lau TK, Chiu RW, Lo YM (2008) Detection and characterization of placental microRNAs in maternal plasma. Clin Chem 54(3):482–490. doi:10.1373/clinchem.2007.097972

Clark DA, Manuel J, Lee L, Chaouat G, Gorczynski RM, Levy GA (2004) Ecology of danger-dependent cytokine-boosted spontaneous abortion in the CBA x DBA/2 mouse model. I. Synergistic effect of LPS and (TNF-alpha+IFN-gamma) on pregnancy loss. Am J Reprod Immunol 52(6):370–378. doi:10.1111/j.1600-0897.2004.00237.x

Coan PM, Fowden AL, Constancia M, Ferguson-Smith AC, Burton GJ, Sibley CP (2008) Disproportional effects of Igf2 knockout on placental morphology and diffusional exchange characteristics in the mouse. J Physiol 586(Pt 20):5023–5032. doi:10.1113/jphysiol.2008.157313

Coan PM, Vaughan OR, Sekita Y, Finn SL, Burton GJ, Constancia M, Fowden AL (2010) Adaptations in placental phenotype support fetal growth during undernutrition of pregnant mice. J Physiol 588(Pt 3):527–538. doi:10.1113/jphysiol.2009.181214

Constancia M, Hemberger M, Hughes J, Dean W, Ferguson-Smith A, Fundele R, Stewart F, Kelsey G, Fowden A, Sibley C, Reik W (2002) Placental-specific IGF-II is a major modulator of placental and fetal growth. Nature 417(6892):945–948. doi:10.1038/nature00819

Crane-Godreau MA, Maccani MA, Eszterhas SK, Warner SL, Jukosky JA, Fiering S (2009) Exposure to cigarette smoke disrupts CCL20-mediated antimicrobial activity in respiratory epithelial cells. Open Immunol J 2:86–93

Desai M, Gayle D, Han G, Ross MG (2007) Programmed hyperphagia due to reduced anorexigenic mechanisms in intrauterine growth-restricted offspring. Reprod Sci 14(4):329–337. doi:10.1177/1933719107303983

Georgiades P, Cox B, Gertsenstein M, Chawengsaksophak K, Rossant J (2007) Trophoblast-specific gene manipulation using lentivirus-based vectors. Biotechniques 42(3):317–318, 320, 322–315

Gilad S, Meiri E, Yogev Y, Benjamin S, Lebanony D, Yerushalmi N, Benjamin H, Kushnir M, Cholakh H, Melamed N, Bentwich Z, Hod M, Goren Y, Chajut A (2008) Serum microRNAs are promising novel biomarkers. PLoS ONE 3(9):e3148. doi:10.1371/journal.pone.0003148

Lim LP, Lau NC, Garrett-Engele P, Grimson A, Schelter JM, Castle J, Bartel DP, Linsley PS, Johnson JM (2005) Microarray analysis shows that some microRNAs downregulate large numbers of target mRNAs. Nature 433(7027):769–773. doi:10.1038/nature03315

Lu LF, Boldin MP, Chaudhry A, Lin LL, Taganov KD, Hanada T, Yoshimura A, Baltimore D, Rudensky AY (2010) Function of miR-146a in controlling Treg cell-mediated regulation of Th1 responses. Cell 142(6):914–929. doi:10.1016/j.cell.2010.08.012

Maccani MA, Marsit CJ (2009) Epigenetics in the placenta. Am J Reprod Immunol 62(2):78–89. doi:10.1111/j.1600-0897.2009.00716.x

Maccani MA, Avissar-Whiting M, Banister CE, McGonnigal B, Padbury JF, Marsit CJ (2010) Maternal cigarette smoking during pregnancy is associated with downregulation of miR-16, miR-21 and miR-146a in the placenta. Epigenetics 5(7)

Maccani MA, Padbury JF, Marsit CJ (2011) miR-16 and miR-21 expression in the placenta is associated with fetal growth. PLoS ONE 6(6):e21210. doi:10.1371/journal.pone.0021210

Mackenzie JS, Williams K, Papadimitriou J (1977) Influenza A virus and its influence on the outcome of pregnancy in the mouse. Dev Biol Stand 39:489–496

Mouillet JF, Chu T, Hubel CA, Nelson DM, Parks WT, Sadovsky Y (2010) The levels of hypoxia-regulated microRNAs in plasma of pregnant women with fetal growth restriction. Placenta 31 (9):781–784. doi:10.1016/j.placenta.2010.07.001

Mouillet JF, Chu T, Sadovsky Y (2011) Expression patterns of placental microRNAs. Birth Defects Res A Clin Mol Teratol. doi:10.1002/bdra.20782

Patel N, Tahara SM, Malik P, Kalra VK (2011) Involvement of miR-30c and miR-301a in immediate induction of plasminogen activator inhibitor-1 by placental growth factor in human pulmonary endothelial cells. Biochem J 434(3):473–482. doi:10.1042/BJ20101585

Pernaute B, Spruce T, Rodriguez TA, Manzanares M (2011) miRNA-mediated regulation of cell signaling and homeostasis in the early mouse embryo. Cell Cycle 10(4):584–591

Pineles BL, Romero R, Montenegro D, Tarca AL, Han YM, Kim YM, Draghici S, Espinoza J, Kusanovic JP, Mittal P, Hassan SS, Kim CJ (2007) Distinct subsets of microRNAs are expressed differentially in the human placentas of patients with preeclampsia. Am J Obstet Gynecol 196(3):261.e1–261.e6. doi:10.1016/j.ajog.2007.01.008

Prieto DM, Markert UR (2011) MicroRNAs in pregnancy. J Reprod Immunol 88(2):106–111. doi:10.1016/j.jri.2011.01.004

Shiverick KT, Salafia C (1999) Cigarette smoking and pregnancy I: ovarian, uterine and placental effects. Placenta 20(4):265–272. doi:10.1053/plac.1998.0377

Su L, Zhao S, Zhu M, Yu M (2010) Differential expression of microRNAs in porcine placentas on days 30 and 90 of gestation. Reprod Fertil Dev 22(8):1175–1182. doi:10.1071/RD10046

Tamashiro KL, Moran TH (2010) Perinatal environment and its influences on metabolic programming of offspring. Physiol Behav 100(5):560–566. doi:10.1016/j.physbeh.2010.04.008

Xiong Z, Leme AS, Ray P, Shapiro SD, Lee JS (2011) CX3CR1+ lung mononuclear phagocytes spatially confined to the interstitium produce TNF-{alpha} and IL-6 and promote cigarette smoke-induced emphysema. J Immunol. doi:10.4049/jimmunol.1003221