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Could microRNAs be biomarkers for neural tube defects?

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Over the last decade there has been an explosion of interest in the function of non-coding RNAs in post-transcriptional regulation of gene expression (Okamura 2012). MicroRNAs (miRNAs) are small RNAs, typically 21-22 nucleotides in length. They are generally produced by the sequential action of the endonucleases Drosha and Dicer on miRNA primary transcripts, although Drosha-independent processing can occur in certain instances (Okamura 2012). Mature miRNAs bind to target mRNAs and facilitate assembly of an RNA-induced silencing complex, enhancing mRNA turnover and suppressing translation. There is also evidence for miRNA-mediated up-regulation of translation in some contexts (Mendell and Olson 2012; Vasudevan 2012). miRNAs have been found to influence a range of signalling pathways, both positively and negatively, and are implicated in various disease states, particularly cancer, cardiovascular and immune disorders (Mendell and Olson 2012).

In addition to studies of the cellular functions of miRNAs, attention has also focussed on their potential utility as biomarkers, exploiting the fact that extracellular miRNAs are also found in body fluids, including serum. In particular, abundance of circulating miRNAs has been correlated with a range of cardiovascular pathologies and several types of cancer (Mendell and Olson 2012). During pregnancy, cell-free fetal nucleic acids including miRNAs, as well as DNA and mRNA, are detected in amniotic fluid and maternal plasma and may form the basis of non-invasive diagnostic tests, potentially as biomarkers for fetal abnormalities (Miura *et al.* 2010; Edlow and Bianchi 2012). In the current issue, Gu *et al.* (2012) investigate whether miRNAs may provide biomarkers for neural tube defects (NTDs): one of the commonest group of congenital malformations (birth defects). NTDs arise when the neural tube, the embryonic precursor of the brain and spinal cord, fails to close correctly during development (Greene and Copp 2009; Copp and Greene 2010). Failure of closure in the spinal region causes spina bifida and in the cranial region results in anencephaly. NTDs are clinically severe: anencephaly being lethal at birth and spina bifida causing perinatal lethality or severe handicap that often requires lifelong healthcare interventions. Despite the successful establishment of folic acid fortification and supplementation programmes in many countries (Obican *et al.* 2010), NTDs continue to affect around 0.5-2 per 1,000 pregnancies, worldwide.

The causes of NTDs appear to be complex and multifactorial involving both genetic and environmental factors (Greene *et al.* 2009). Few single genes have yet been found to be

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major contributors to NTD susceptibility and, while several environmental risk factors are known (e.g. maternal diabetes, valproic acid), the causes of NTDs are not known or predictable in the majority of cases. Prenatal diagnosis of NTDs provides options for management of affected pregnancies, including elective termination, preparation for post-natal care or, in a few centres, *in utero* surgery. Screening was initially based on biochemical assay of maternal blood and/or amniotic fluid to detect elevated levels of alpha-fetoprotein (AFP), which is released from the fetal serum via open NTD lesions. Drawbacks of AFP screening include the possible failure to detect some NTDs, especially spina bifida, and the fact that elevated AFP is also associated with fetal abnormalities other than NTDs (Cameron and Moran 2009). Biochemical tests have largely been superseded by ultrasonography.

The identification of additional biomarkers for NTDs could offer an further avenue for prenatal screening, particularly where ultrasonography is not available, or might enable diagnosis earlier in gestation. In the current issue Gu et al., (2012) provide evidence that miRNAs, present in maternal serum during pregnancy, may provide such biomarkers. Abundance of miRNAs was compared by microarray between mid-gestation NTD and apparently normal pregnancies and differentially abundant miRNAs were further analysed by qRT-PCR in a larger set of samples. A subset of individual miRNAs was found to differ in abundance between control and NTD samples and combinatorial analysis was highly predictive of the presence of NTDs. Intriguingly, most of the apparently dysregulated miRNAs were associated with pregnancy, declining in abundance after caesarean or normal delivery. Potential limitations of the study include the relatively small sample size and the lack of correlation between microarray and qRT-PCR for some miRNAs in the direction of mis-regulation (increased or decreased abundance in NTDs). Nevertheless, the significant association of altered miRNA abundance and NTDs is striking and warrants further investigation in larger-scale studies.

The findings of the study raise a number of questions. Are the miRNAs identified in this study specific to NTDs or also altered in the presence of other 'open' defects, such as gastroschisis? How early in pregnancy do the characteristic changes in miRNA profile become informative? As the miRNAs associated with NTDs are also characteristic of pregnancy it will be also be important to evaluate levels over normal pregnancy to generate reference data for comparison. For example, a complicating factor in biochemical screens is the variation in maternal serum levels of AFP that occurs during pregnancy (Cameron and Moran 2009).

Do miRNA alterations reflect purely secondary manifestations of the presence of an open neural tube in the developing fetus or could there be a functional link with the primary defect in closure? In order for dysregulated miRNAs to influence risk of NTDs they should be present in the developing embryo prior to and/or at the stage of neural tube closure, days 21-28 of gestation in humans. Fetal DNA and mRNA detected in maternal circulation are principally of placental origin whereas those present in amniotic fluid derive from the fetus itself (Edlow and Bianchi 2012). Pregnancy-specific placental miRNAs have previously been identified in maternal serum (Miura *et al.* 2010) but there is little information on whether fetal miRNAs can cross the placenta. While they differ from those identified by Miura *et al.*, it appears likely that the pregnancy-specific miRNAs highlighted by Gu et al.

(2012) are of placental origin, while a possible effect on embryonic development would be supported by their concomitant alteration in amniotic fluid.

The potential for miRNA-mediated gene regulation in neural tube closure has so far received relatively little attention in genetic analysis of human NTDs. However, miRNA target sequences are predicted by bioinformatic analysis in genes that could be functionally relevant to NTDs in humans and mice, such as those encoding components of folate one-carbon metabolism (Stone *et al.* 2011). Moreover, genes that have been experimentally validated as regulated by specific miRNAs do, in some cases, produce NTDs in loss of function models. One example is *Mlin41* (*Trim71*) which is a regulatory target of let-7 and mi-125 (Maller Schulman *et al.* 2008). Given the large number of genes that have been implicated in the NTDs of mouse models, the possibility of aberrant regulation by miRNAs will be an important question to address.

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Abbreviations

NTDs	neural tube defects
miRNA	micro RNA

Reference List

- Cameron M, Moran P. Prenatal screening and diagnosis of neural tube defects. *Prenatal Diag.* 2009; 29:402–411. [PubMed: 19301349]
- Copp AJ, Greene NDE. Genetics and development of neural tube defects. *J Pathol.* 2010; 220:217–230. [PubMed: 19918803]
- Edlow AG, Bianchi DW. Tracking fetal development through molecular analysis of maternal biofluids. *Biochim Biophys Acta.* 2012
- Greene ND, Copp AJ. Development of the vertebrate central nervous system: formation of the neural tube. *Prenatal Diag.* 2009; 29:303–311. [PubMed: 19206138]
- Greene NDE, Stanier P, Copp AJ. Genetics of human neural tube defects. *Hum Mol Genet.* 2009; 18:R113–R129. [PubMed: 19808787]
- Maller Schulman BR, Liang X, Stahlhut C, Delconte C, Stefani G, Slack FJ. The let-7 microRNA target gene, *Mlin41/Trim71* is required for mouse embryonic survival and neural tube closure. *Cell Cycle.* 2008; 7:3935–3942. [PubMed: 19098426]
- Mendell JT, Olson EN. MicroRNAs in stress signaling and human disease. *Cell.* 2012; 148:1172–1187. [PubMed: 22424228]
- Miura K, Miura S, Yamasaki K, Higashijima A, Kinoshita A, Yoshiura K, Masuzaki H. Identification of pregnancy-associated microRNAs in maternal plasma. *Clin Chem.* 2010; 56:1767–1771. [PubMed: 20729298]
- Obican SG, Finnell RH, Mills JL, Shaw GM, Scialli AR. Folic acid in early pregnancy: a public health success story. *FASEB J.* 2010; 24:4167–4174. [PubMed: 20631328]
- Okamura K. Diversity of animal small RNA pathways and their biological utility. *Wiley Interdiscip Rev RNA.* 2012; 3:351–368. [PubMed: 22086843]

- Stone N, Pangilinan F, Molloy AM, Shane B, Scott JM, Ueland PM, Mills JL, Kirke PN, Sethupathy P, Brody LC. Bioinformatic and genetic association analysis of microRNA target sites in one-carbon metabolism genes. *PLoS One*. 2011; 6:e21851. [PubMed: 21765920]
- Vasudevan S. Posttranscriptional Upregulation by MicroRNAs. *Wiley Interdiscip Rev RNA*. 2012; 3:311–330. [PubMed: 22072587]