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Is miR-376c a biomarker or mediator of pre-eclampsia?

miR-376c: a marker or mediator of pre-eclampsia?

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Text

The remodelling of uteroplacental arteries is a key process in the first half of human pregnancy. The transformation from low-flow/high-resistance to high-flow/low-resistance arteries that ensures sufficient blood supply to meet the requirements of the fetus, is caused by a loss of elasticity and vasomotor control. This remodelling is facilitated by perivascular invasion of extravillous trophoblast cells and infiltration of vessel walls by endovascular trophoblast cells. Impaired trophoblast invasion of the myometrial segments is therefore associated with placental hypoperfusion and may play a role in the pathogenesis of some cases of intrauterine growth restriction and preeclampsia. Preeclampsia is a multisystem disorder of pregnancy triggered by placental ischemia and release of placental factors causing widespread endothelial dysfunction [1]. The reasons for altered trophoblast invasion in preeclampsia are incompletely understood, but similarities between development of normal placenta and development of cancer point toward a key role of growth factors in normal and abnormal placentation [2].

MicroRNAs (miRNA) are becoming increasingly recognised as critical regulators of cellular function. These short non-coding RNA species act via repression of gene targets mediated through binding to miRNA “seed sequences” usually, but not exclusively, located in the 3′ untranslated region. This interaction leads to a block in translation of the gene to protein or induction of messenger RNA degradation. As such, the expression pattern and potential dysregulation of individual miRNA species, or “miRNA signatures” in individual cells, tissues and organs are critical in understanding the role of miRNAs in health and disease. Although the discovery of miRNA was initially made in 1993 [3], it is only in recent years that full

recognition of their broad importance in cellular homeostasis as well as the potential of miRNAs as therapeutic entities and as biomarkers of disease has been established.

In contrast to diseases such as those of the heart and cancer, relatively little is known about miRNA during pregnancy, both at the level of normal placental growth and function and in the pathophysiology of complications in pregnancy, including preeclampsia. This lack of data is a consequence of the relative lack of studies in this area, and the complications of addressing an exclusively human disease. In addition, there are challenges in addressing the discrepancy between the key pathophysiological changes that are initiated well before 20 weeks' gestation and the clinical symptoms that are not manifest until after 20 weeks gestation. With preeclampsia being an exclusively human disease, clinical studies on miRNA expression are often restricted to placenta tissue at the time of delivery. Nevertheless, the available studies confirm dysregulation miRNAs that play a role in the regulation of trophoblast cell invasion such as miR-195 [4], miR-29b [5], and mitochondrial function such as miR-210 [6]. In this issue of *Hypertension* [7], Fu and colleagues assess, in detail, one particular miRNA, miR-376c and its expression and function in normal pregnancy and in preeclampsia using a range of cellular assays, molecular manipulations and patient materials. MiR-376c is expressed within a region of imprinted DNA on human chromosome 14, a region rich in non-coding RNA species including an abundance of distinct miRNAs (http://www.ensembl.org/Homo_sapiens/Location/View?db=core;r=14:100505828-102472517;region=AL132709.5). Indeed, miR-376c itself is transcribed from the miR376 cluster pri-miRNA complex that encodes 5 separate mature miRNAs, a region recently linked to the development of glioma [8]. The experiments presented in this study can clearly be

divided into evaluation of miR-376c in clinical material, assessment of function *in vitro* by manipulation of miR-376c in relevant systems and the mechanism of action.

The clinical sample dataset presented essentially leads to the hypothesis that downregulation of this miRNA is important in preeclampsia. The data demonstrate that miR-376c is modestly upregulated during the time course of normal pregnancy through the three trimesters, but is downregulated in the placental tissue RNA samples of preeclamptic cases that have term births (but intriguingly not in those with pre-term births) compared to age-matched controls. Importantly, plasma levels of miR-376c are lower in both pre-term and term samples vs controls. Cases were recruited from sites in China and Canada and, although not large patients numbers, the sample set shows significant differences in levels of miR-376c. . In Fig. 1A-D the authors present their data in box plots representing median values, 25% and 75% percentiles, and minimum and maximum values. Although they used an appropriate data presentation, it appears (Fig 1D-F) that the values are not normally distributed; yet, parametric statistics have been used. Further rigorous analysis using larger patient populations will be important, both for clarity, validation and assessment of the influence of geographical variation. The authors have reported relative miR-376c levels with U6 as an internal control in these samples. It is well known for plasma/serum-derived samples that stringent controlling and normalisation is difficult. A number of strategies have therefore evolved, including spiking controls or normalisation using entire miRNA expression datasets that would be invaluable for inclusion in future studies for confirmation of this important finding. This is especially important in defining the utility of miR-376c, or indeed any other miRNA, as a biomarker of the preeclamptic condition. Nevertheless, the

data presented certainly suggest that miR-376c is downregulated and that this may be important in the pathophysiology of preeclampsia.

Next, the authors present a thorough and compelling series of experiments that define the effect of miR-376c manipulation using an immortalised cell line, stable transfectants and placental villous tip explants. They show that simple overexpression of miR-376c in any of these model systems has, in general, the overall effect of enhancing trophoblast cell growth and migration and conversely, downregulation of miR-376c reduces these effects on trophoblast cells. Clearly, caution is required when artificially manipulating miRNA by overexpression or inhibition, since the resulting levels achieved can be dramatically affected and beyond the levels altered in the disease under study. Although lacking details such as Cy3-labelled visualisation of transfection percentages and locales in the explant model, these studies support the concept that downregulation of miR-376c hinder trophoblast proliferation and migration, supporting the hypothesis that originated from assessment of clinical material.

An important component of this study is a detailed account of the proposed mechanism of action of miR376c in the setting of trophoblast function. The authors focus on the transforming growth factor- β and Nodal pathway. Using reporter assays, they show that both activin-like kinase (ALK)5 and ALK7 are direct, validated targets of miR-376c. They further show that manipulation of miR-376c leads to the reciprocal alteration in ALK5 and ALK7 at the protein level. Moreover, siRNA to ALK5 (but not ALK7) mimics the effect of miR376c overexpression and the effects of miR-376c can be overridden by plasmid-mediated overexpression of ALK5 or ALK7. Combining siRNAs or plasmid systems in a single experiment to manipulate both ALK5 and ALK7 simultaneously may be a useful strategy to

further refine these experiments. Furthermore, miR-376c has many other putative gene targets and whilst it is beyond the scope of the current paper, the impact of miR-376c on other targets expressed in trophoblasts would be important to assess globally the transcriptional influence of this miRNA in trophoblasts. Mechanistically, the authors conduct the necessary experiments to show that miR-376c overexpression acts to repress both TGF- β - and Nodal-induced signalling (blocked pSMAD2/3 activation) and expression of pathway responsive genes (e.g. p21). Thus, the study provides compelling data to suggest that miR-376c plays an important role in normal pregnancy through regulation of ALK5 and ALK7 activation mediated by TGF- β and Nodal, respectively and that downregulation in preeclampsia combined with elevated TGF- β and Nodal levels leads to enhanced activation of ALK5/ALK7, in turn resulting in reduced trophoblast growth and invasion and ultimately, preeclampsia.

In any complex system, interplay between different cell types and their environmental cues impact on disease progression. Within any given cell type, many miRNA are expressed and their levels are governed by processes including transcriptional regulation, miRNA processing and activity. In addition, the secretion of miRNA from cells has the capacity to impact the function of other cells locally should the miRNA be taken up. As suggested, this may certainly impact upon similar signalling systems in alternate cell types, such as endothelium or smooth muscle. It is very clear that the effect of miR-376c on trophoblast cell function is potent, and is focused on signalling mediated by TGF- β and Nodal. What about other miRNAs? It has been established that miRNAs can work alone or in concert with other miRNAs to affect the transcriptome in healthy cells and in the progression of disease [9]. With respect to the latter, this may be via multiple mechanisms, including different

miRNAs acting upon different biological pathways relevant to the pathogenesis of a particular disease, or different miRNAs acting together upon a single biological pathway or system. In support of the latter, the same group that published this study also very recently reported the role of miR-378a-5p using a very similar series of samples and experiments [10]. Essentially, they showed that miR-378-5p was also downregulated in preeclamptic placental tissue compared to controls and that the function and mechanism was to target the 3'UTR of Nodal to repress Nodal-induced repression of trophoblast growth and invasion. Also, Zhu *et al.* [11] found dysregulation of 34 miRNAs in placentas from women with preeclampsia; one of the differentially regulated miRNAs was miR195. More recently, Bai *et al.* [4] confirmed downregulation of miR-195 and demonstrated that ActRIIA, the type II receptor for ActivinA and Nodal, is a target of miR-195/mir195. Thus, there is likely to be complex interplay and regulation of different miRNAs species that impact on pathways relevant to trophoblast function, and/or endothelial function and the pathogenesis of preeclampsia. A systems approach to address this would clearly be warranted.

Preeclampsia affects between 2 and 7 percent of pregnant women, and despite years of ongoing research the etiology of the disorder remains incompletely understood. One of the most consistently described features of the condition are reduced levels of angiogenic factors such as VEGF compared to normal pregnancy, in part due to presence of a soluble form of the VEGF receptor (sFlt-1), and possibly triggered by placental hypoxia. Another important factor associated with the pathogenesis of preeclampsia is soluble endoglin, which acts as a TGF-beta antagonist. The finding by Fu and colleagues [7] that TGF-beta signalling can also be altered through miR-376c and its actions on Nodal is in line with the

overall concept of preeclampsia being a disorder of impaired vasculogenesis and provides an explanation at the level of gene transcription.

In order to establish miR-376c as a predictive or diagnostic biomarker of preeclampsia, the study by Fu and colleagues [7] requires confirmation in independent and larger cohorts. Already at this stage, however, it provides a better understanding of the processes leading to impaired trophoblast invasion in preeclampsia. Even if the study by Fu *et al.* elegantly demonstrated dysregulation of miR-376c in plasma samples from early pregnancy it remains unclear if miR-376c expression was downregulated prior to pregnancy and if it remains downregulated in vascular tissues after pregnancy. In the light of the key role of TGF- β signalling in a wide range of conditions including cardiovascular and renal diseases [12] and the increased cardiovascular risk of women with a history of preeclampsia [13] it will be important to study if dysregulation of TGF- β targeting miRNAs provides a link between preeclampsia and other vascular diseases.

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