miR-145 is associated with placental growth in mice

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Introduction

- Abnormal placental development and function can result in fetal growth restriction (FGR).
- FGR is associated with increased risk of infant morbidity and mortality and has life-long impacts on health.
- The insulin-like growth factor (IGF) axis is imperative for normal placental and hence fetal development .
- The actions of IGF-I and –II in regulating both human and murine placental development and function are mediated by IGF1R [1, 2].
- Modulating expression of IGF1R in the placenta may improve placental growth and improve outcomes of pregnancies complicated by FGR.
- In the human placenta, microRNAs (miRs) regulate expression of components of the IGF axis [3,4].
- One miR- miR-145- modulates human placental growth by targeting IGF1R [4], so it may be possible to utilise miR-145 based drugs to manipulate placenta growth in humans.
- Prior to testing in humans, miR-based drugs would need to be tested in mice, however it is unclear whether miRs and specifically miR-145, influence placental and fetal growth in mice.

Hypotheses and Aims

Hypotheses:

- **miR-145 is expressed in the mouse placenta**
- **miR-145 regulates murine placental and fetal growth by targeting IGF1R**

Aims:

- To ascertain whether miR-145 is expressed in the mouse placenta and fetus
- To determine if miR-145 is associated with placental growth in mice by examining both placenta growth and miR-145 expression across gestation
- To determine the relationship between miR-145 expression and IGF1R in the mouse placenta and in the fetus

Methods

Pregnant C57BL/6J mice

↓

Cull mice @ E12.5, E15.5 or E18.5

↓

Harvest placenta and fetus

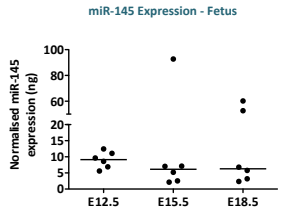
↙ Fix tissue and embed in paraffin wax → ↙ Immunohistochemistry (IHC) to assess levels of Ki67 / IHC for IGF1R expression

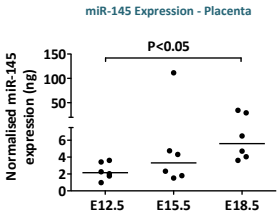
↘ Homogenise tissue, extract total RNA → ↙ RT-QPCR for miR-145 expression / RT-QPCR for IGF1R expression

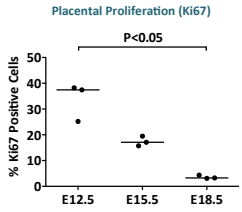
- miR-145 and IGF1R were quantified by RT-QPCR using specific primers and values were normalised to 5s and 18s rRNA, respectively.
- IGF1R expression was assessed using IHC.
- Levels of proliferation were analysed by performing IHC for Ki67 and expressing the number of Ki67+ cells as a percentage of total number of nuclei (haematoxylin stained).

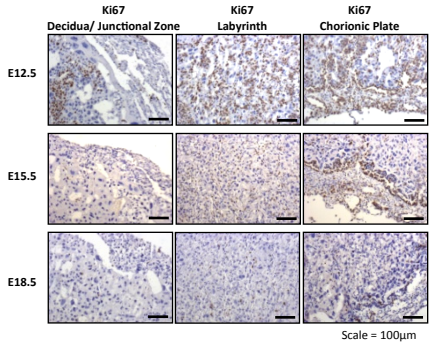
Results

1) miR-145 is expressed in murine placenta and inversely correlates with placental growth



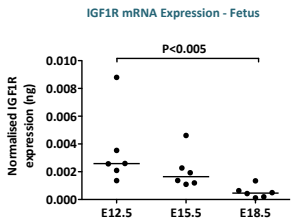


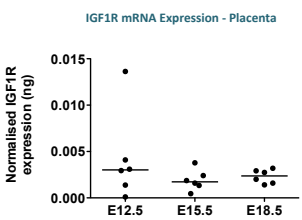


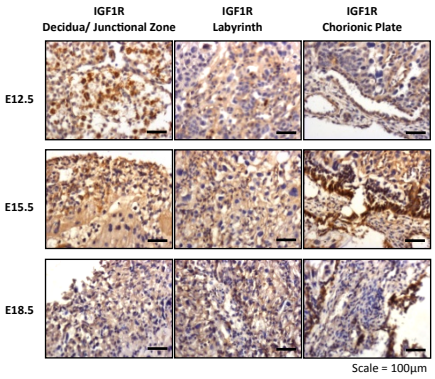


- QPCR demonstrated that miR-145 is expressed in the mouse fetus but does not alter with gestation.
- QPCR confirms that miR-145 is expressed in the mouse placenta, and levels significantly increase (P<0.05) over gestation.
- Analysis of cell proliferation (Ki67) reveals that proliferation significantly decreases (P<0.05) over gestation.
- These results are consistent with results found in studies on the human placenta [4] and suggest that miR-145 may negatively regulate placental growth in mice.

2) IGF1R is expressed in the fetus and placenta throughout gestation







- IHC demonstrates that IGF1R is expressed throughout the placenta (decidua, junctional and labyrinth zones) in mice and protein expression appears to reduce with gestation.
- However, QPCR confirms that IGF1R mRNA expression does not alter (P>0.05) in the mouse placenta over gestation.
- These results are consistent with the human placenta whereby studies have shown that miR-145 acts by reducing IGF1R protein and not mRNA expression [3].
- QPCR also demonstrated that IG1R mRNA is expressed in the mouse fetus and this significantly decreases (P<0.005) over pregnancy.

Conclusions

- miR-145 is expressed in the mouse placenta and is significantly increased over gestation, similar to data observed in the human placenta [4]
- Proliferation significantly decreases over gestation in the mouse placenta, and inversely correlates with placental growth; this is also consistent with studies in the human placenta.
- There was no change in placental IGF1R mRNA throughout pregnancy, however IHC appears to demonstrated that IGF1R protein expression decreases towards term; this discrepancy between mRNA and protein expression is consistent with the known actions of miRs in the human placenta: data from this lab group suggests that miR-145 targets IGF1R protein degradation as opposed to repression of mRNA translation [4].
- These results combined suggests that miR-145 is likely to have similar actions in regulating placental growth in mice as it does in humans, however the role of miR-145 in regulating IGF1R expression in mice remains to be established.
- Ongoing work will examine the potential therapeutic role of miR-145 based drugs to improve placental and fetal growth using mouse models of FGR.

References

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