

## **Trophoblast-Specific Knockdown of the System A Amino Acid Transporter, *Slc38a2*/SNAT2, Causes Fetal Growth Restriction in Mice.**

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### **Introduction**

System A transporters/SNATs mediate sodium-dependent accumulation of small neutral amino acids in the trophoblast. Their activity in the human placenta is associated with birth weight. Placental System A activity is reduced in pregnancies complicated by fetal growth restriction but the mechanistic importance of individual placental SNAT isoforms for intrauterine growth is unknown. We hypothesized that trophoblast-specific SNAT2/*Slc38a2* knockdown, using lentiviral small-hairpin (sh) RNA delivery to the blastocyst trophectoderm, impairs fetoplacental growth in late gestation mice.

### **Methods**

Embryonic day (E) 3.5 mouse blastocysts were transduced with  $5 \times 10^5$  transforming units of a lentiviral vector bearing either a U6 promoter-driven, *Slc38a2* targeting shRNA (*Slc38a2*KD), or a non-targeting control shRNA (SCR), both with green fluorescent protein (GFP). After 4hr, SCR and *Slc38a2*KD blastocysts were transferred to contralateral uterine horns of pseudopregnant CD-1 female recipients (n=29). On E18.5, recipients were euthanized and fetuses and placentae collected and weighed. *Slc38a2* expression was determined by qPCR. Trophoblast plasma membrane SNAT2 abundance and *in vivo* System A capacity were determined by western blot and maternal-placental <sup>14</sup>C-methylaminoisobutyric acid clearance per gram placenta, respectively. Litter mean outcome measures were calculated for SCR and *Slc38a2*KD conceptuses and differences determined by paired Student's t-test. Results are mean  $\pm$  SEM.

### **Results**

Both SCR and *Slc38a2*KD transduced conceptuses exhibited trophoblast-specific GFP fluorescence. Placental, but not fetal, *Slc38a2* expression was 59% lower in *Slc38a2*KD compared to SCR conceptuses (P<0.001, n=6 litters). Placenta-specific *Slc38a2* knockdown reduced both fetal weight (-11%) and placental weight (-18%) compared to SCR controls (P<0.01, n=29 litters). Trophoblast plasma membrane SNAT2 abundance and *in vivo* System A amino acid transport capacity were also lower in *Slc38a2*KD than SCR placentas (P<0.05, n=9-13 litters). *Slc38a2* knockdown did not alter embryo implantation rate but diminished fetal viability, as measured by the percentage of transferred blastocysts surviving to term.

### **Discussion**

This study demonstrates, for the first time, a cause-and-effect relationship between reduced placental expression of the System A amino acid transporter *Slc38a2*/SNAT2 and fetal growth restriction in mice. We speculate that *Slc38a2*/SNAT2 deficiency mechanistically contributes to placental insufficiency in human pregnancies complicated by fetal growth restriction.