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The RNA-binding protein Quaking regulates mesenchyme survival during lung development

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Background:

Every 10 minutes a baby is born with congenital diaphragmatic hernia (CDH) worldwide. These babies have a hole in their diaphragm and abdominal organs in their chest, compressing the lungs and causing breathing problems and mortality at birth. The microRNA miR-200b promotes normal lung development and counters a fibrotic-like phenotype in a rat model of CDH (Nitrofen model) and represses the RNA-binding protein Quaking.

Objective:

We aim to develop a new prenatal therapy to promote lung growth, hypothesizing that Quaking plays a key role in mediating lung development.

Methods:

Quaking protein expression in normal prenatal rat lungs was assessed at gestational days (E) 13, 15, 18, and 21 with immunostaining. Pregnant rats were administered Nitrofen to induce CDH in the fetuses. E21 Nitrofen lungs were compared to control using immunostaining (quantified with ImageJ), RT-qPCR, and RNAscope (*in situ* hybridization) for Quaking. Normal E13 rat lungs were cultured *ex vivo* at an air-liquid interface. Quaking translation was blocked using a vivo-morpholino, and knockdown was verified with immunostaining. Lung branching was checked daily over four days of culture and later immunostained.

Results:

Immunostaining showed Quaking protein expression in every lung cell across the developmental stages studied. The Nitrofen group had 24% more Quaking-positive cells surrounding the airways (mesenchyme) ($P < 0.05$), a 2.1-fold increase in mRNA (RT-qPCR, $P < 0.05$), and stronger staining via RNAscope (qualitative data). Immunostaining showed 50% diminished Quaking signal intensity after knockdown according to ImageJ ($P < 0.05$) and the lungs showed a 58% reduction in small airway numbers ($P < 0.05$). This stunted growth was likely from increased apoptosis according to active caspase-3 staining (preliminary data).

Conclusion:

Quaking is widely expressed during lung development and is likely important for cell survival. The Quaking-positive mesenchyme could maintain this population of cells further into lung development, leading to fibrotic-like lungs in CDH.