MRI Phenotyping of Craniofacial Development in Transgenic Mice Embryos

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Objective

To evaluate the molecular etiology of cleft palate formation in Wnt1-cre:tgfbr2fl/fl mouse embryos using μMRI.

Introduction

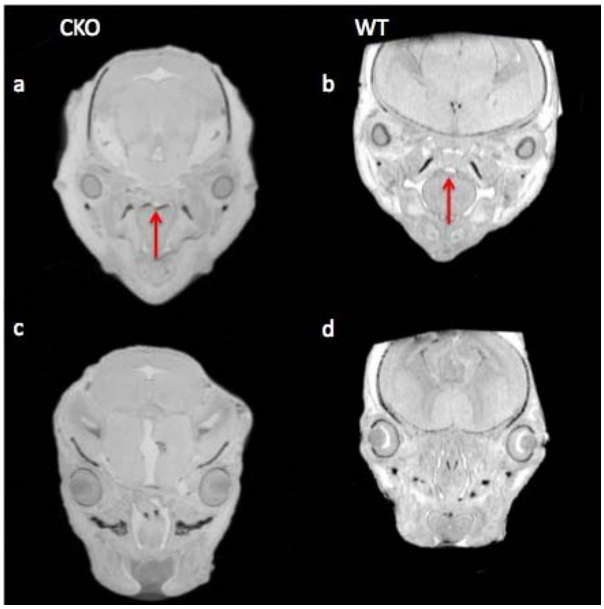
Craniofacial malformations are involved in three fourths of all congenital birth defects in humans, affecting the development of head, face or neck. TGF-β signaling plays a critical role in regulating palatal fusion. Animal model of inactivated tgfb2 in cranial neural crest (CNC) cells show TGF-β signaling is vital for regulating cell proliferation in the facial region [1]. Microscopic MRI (μMRI) is an emerging technique for high-throughput phenotyping of transgenic mouse embryos, and is capable of visualizing abnormalities in craniofacial development. μMRI methods rely on reduction of the tissue T1 relaxation time by penetration of a gadolinium chelate contrast agent [2]. Shortened relaxation time improves signal-to-noise ratio (SNR) of the images acquired with gradient echo sequences by reducing the repetition time (TR) requirements and thus enabling more averages.

Methods

E 14.5 *Tgfbr2$^{fl/fl}$; Wnt1-Cre* mouse embryos were fixed in 10% buffered formalin for 24 hours and placed into phosphate buffer with 0,01% azide. Prior to imaging, samples were soaked in 5mM Prohance solution for 8 hours resulting in a reduction of the intrinsic T1 relaxation time (final T1 ~ 100 ms)[3]. Following enhancement each sample was placed in a homemade holder and submerged in an MR invisible perfluropolyether (Galden) solution (fluorine based). Imaging was performed on a Bruker 11.7T system with a home built volume coil. T1 maps were generated from the MR images acquired using an inversion recovery sequence. All parameters were chosen to guarantee optimal contrast. 3D Volume imaging was performed using a gradient-echo sequence with FOV 8.960 x 8.960 x 8.960 mm3 FOV and matrix size 256 X 256 X 256 resulting in a isotropic spatial resolution of ~ 35 μm.

Results and Discussion

Shown is a representative image of a E15.5 wild type (WT) and knockout (CKO) embryo. The image comparison reveals significant morphological differences in the facial region. Arrows in both images (a,b) indicate where palate fusion should occur. In the case of the CKO embryo the fusion is clearly disrupted, also in the nasal cavity region one can notice the cleft formation in CKO compared to WT. Conclusion: μMRI is a potentially useful tool to assess morphological changes in transgenic mice with craniofacial malformations. Further studies are being conducted to optimize the imaging technique for tissue density differentiation within the facial region.



References

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