Investigating the role of SuFu in cerebellar development

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The mutation

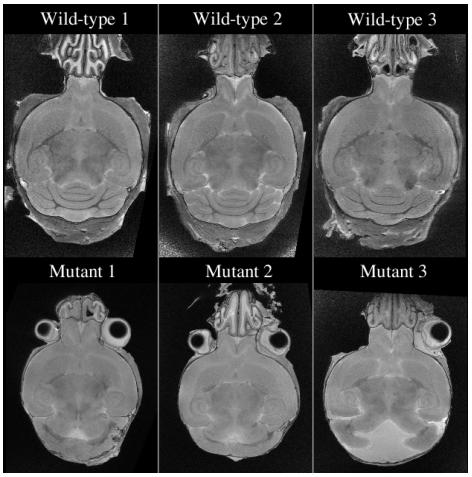
Isotropic 3D MR imaging is increasingly being found useful for characterizing anatomical differences arising from developmental mutations in mouse models of human diseases. The Hoxb7Cre; $SuFu^{-/loxP}$ transgenic mouse model utilizes Cre recombinase expression driven by the Hoxb7 promoter located upstream of Cre. Since the Hoxb7 promoter activity is selectively present in certain tissues such as the mid-hindbrain and the ureteric bud cells of the kidney in mice, conditional deletion of SuFu expression can be achieved. SuFu (suppressor of fused) is a negative regulator of the Sonic Hedgehog signaling pathway where it inhibits the transcriptional activation of Sonic Hedgehog target genes and promotes the proteolytic processing of the downstream effectors of the signaling pathway, the Gli proteins. Therefore, this mutant strain of mice allows one to investigate the role of SuFu in cerebellar and renal development by conditional knockout. To generate this mouse model $SuFu^{+/-}$ mice expressing Cre driven from a Hoxb7 promoter were crossed with mice having exons 4-8 flanked by loxP sites. It has been previously shown that Cre expression driven by the Hoxb7 promoter is present in the mid-hindbrain by embryonic day11.5. However, the time point and region from which SuFu expression is first deleted from the brains of Hoxb7Cre; $SuFu^{-/loxP}$ mice is yet to be determined.

Sample preparation

SuFu mutants (n=3) and their wild-type litter mates (n=3) at ages p21 and p22 were anesthetized with a combination of Ketamine (100 mg/kg) and Rompun (20 mg/kg) via intraperitoneal injection. A previously described sample preparation protocol for scanning was used with slight modifications (Tyszka et al., 2006). Thoracic cavities were opened and animals were perfused through the left ventricle with 20 mL of phosphate-buffered saline (PBS) (pH 7.4) at room temperature (25 °C). This was followed by infusion with 20 mL of iced 4% paraformaldehyde (PFA) in PBS. Following perfusion, the heads were removed along with the skin, lower jaw, ears and the cartilaginous nose tip. The remaining skull structures containing the brain were allowed to postfix in 4% PFA at 4 °C for 12 hours. Following a washout period of 5 days in PBS and 0.01% sodium azide at 15 °C, the skulls were transferred to a PBS and 2 mM ProHance® (Bracco Diagnostics Inc., Princeton, NJ) solution for at least 7 days at 15 C before MR imaging.

MR imaging

A multi-channel 7.0-T MRI scanner (Varian Inc., Palo Alto, CA) with a 6-cm inner bore diameter insert gradient set was used to acquire anatomical images of brains within skulls. Prior to imaging, the samples were removed from the contrast agent solution, blotted and placed into 13-mm-diameter plastic tubes filled with a proton-free susceptibility-matching fluid (Fluorinert FC-77, 3M Corp., St. Paul, MN). Three custom-built, 14-mm-diameter solenoid coils with a length of 18.3 cm and over wound ends were used to image three brains in parallel. Parameters used in the scans were optimized for grey/white matter contrast: a T2-weighted, 3D fast spin-echo sequence, with TR/TE = 325/32ms, four averages, field-of-view 12x12x25 mm and matrix size = 432x432x780 giving an image with 32 µm isotropic voxels. Total imaging time was 11.3 hours (Henkelman et al., 2006).



Data processing and analysis

In order to analyze the brain differences between the SuFu mutants and their wild-type litter mates, all scans were linearly (3 translations, 3 rotations) aligned. In the image on the right side, the top row shows a transverse slice of the wild-type brains, whereas the bottom row shows the corresponding slices of the SuFu mutants. It is visually clear that there are considerable differences between the two genotypes. Striking differences of variable degree can be observed in the cerebellum, which is heavily affected in the mutants. As well there are clear differences in brain size. In the picture below a volume rendering of a wild-type (top row) and a mutant (bottom row) brain are shown. Again we clearly see the malformation of the cerebellum in the mutant.

Conclusion

MRI techniques have been increasingly used for phenotyping mouse models (Nieman et al., 2005; Kovacevic et al., 2005). This study has provided the proof of principle of the power of MR imaging of SuFu mutants, resulting in striking visual malformations of the cerebellum. Longitudinal *in vivo* imaging of live SuFu mutants would allow a complete natural history of neuropathology and can deepen our knowledge about the phenotype of the SuFu mutant.

References

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