Morphological Analyses of wildtype and HMGN-manipulated Xenopus laevis Embryos using High Resolution Magnetic Resonance Imaging at 17.6 Tesla

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Introduction

High resolution magnetic resonance microscopy is a rapidly evolving technique for reconstruction of the three-dimensional embryonic structure in developmental biology [1]. The absence of perturbations due to histological sectioning provides a better representation of the geometry and spatial relationship between embryonic organ systems, which is particularly important in phenotyping transgenic models. In this study, magnetic resonance microscopy was used to study the influence of high mobility group N (HMGN) proteins on wildtype Xenopus laevis embryonic development.

Methods

In vitro fertilization and culture of embryos as well as manipulation of HMGN protein levels were performed as described previously [2,3]. Manipulated and wild type sibling embryos were grown until the wild type controls reached Nieuwkoop and Faber stage 35, upon which they were fixed for 2h at room temperature in MEMFA and stored in 100% methanol at -20°C. Since formaldehyde-based fixation is known to lead to very short tissue T_2 values [4,5], prior to MRI experiments, embryos were rehydrated in a descending methanol series, placed in phosphate-buffered saline and immersed in a perfluorinated solution (Fluorinert FC-43; 3M Corp., Minneapolis, MN), to reduce magnetic susceptibility effects and to maximize the dynamic range of the signal from the embryo. MRI was performed on a 17.6 T (750 MHz) widebore magnet using an AVANCE console and Micro2.5 microimaging gradients capable of 1 T/m maximum gradient strength (Bruker Analytic, Rheinstetten, Germany). The embryo was placed in a 2.5 mm o.d. glass capillary and inserted into a 2.5 mm i.d. four-turn solenoid radiofrequency coil. Three-dimensional data sets of each embryo were acquired using a 3D spin echo sequence. Data acquisition parameters were empirically optimized to give best contrast, leading to a T_R of 600 ms, a T_E of 5.4 ms, number of averages 16 (wild type), 18 (HMGN overexpressed) and 10 (knock down), spatial resolution 33x14x14 µm (wild type), 14x14x14 µm (HMGN injected and knock down). Total data acquisition times were ~ 14 h for each sample: the field-of-view for the wild type in the long-direction was twice that in the other two embryos due to its physically larger size. The MR data sets were zero-filled to 256x128x128 before inverse Fourier transformation. Images were analyzed using the software package Amira™ 3.0. 3D digital reconstruction of the embryos was performed using the Voltex tool and the segmentation editor tool was used to simultaneously slice the embryos in all three axial planes in parallel windows.

Results

The images in Figure 1 showed that deviation of HMGN protein levels leads to imperfect closure of the blastopore, results in microcephalic embryos and causes a distorted and shortened body axis. Furthermore, the data revealed that myotome differentiation was impaired following HMGN injection. Compared to the wild type data set, horizontal and sagittal sections of HMGN1-injected embryos showed that the myotome organization was more loose. Moreover, malformed or missing structures in HMGN manipulated embryos were identified, i.e. reduced development of brain, eyes, and spinal cord, the reduced development and organization of the somites and the absence of a notochord and a spinal cord in the posterior region of the HMGN1-injected or HMGN2 knock-down tadpoles. Differences in anterior malformations caused after increasing or reducing HMGN protein levels are also evident.

Discussion

The data presented here show that high field magnetic resonance microscopy, at a spatial resolution of ~15x15x15 µm, is very useful in assessing morphological changes in embryonic development of *Xenopus* laevis, a classic biological developmental model, which is readily amenable to genetic modification.

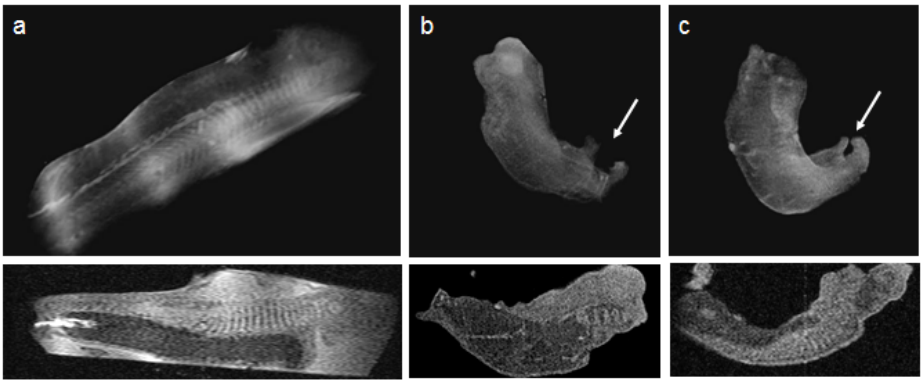

Figure 1: (top) 3D-Maximum Intensity Projectons, (bottom) individual slices from datasets, (a) wild type, (b) knock-down and (c) overexpressed embryos; the arrows indicate imperfect blastopore closure

References

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