In vivo high field magnetic resonance imaging and spectroscopy of adult zebrafish

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5 General discussion and future outlook

The main focus of this thesis was to optimize and apply high resolution MR imaging and spectroscopic methods to obtain the anatomical and molecular information from a living adult zebrafish. The first results are promising and provide basis for applying these methods to monitor disease progression at anatomical and molecular levels using variety of available diseased zebrafish models.

5.1 Future perspectives of MR imaging of the adult zebrafish

One of the areas that stand to benefit from the zebrafish model is the live imaging of anatomical structures and molecular processes in adult zebrafish. Optical imaging studies in zebrafish are restricted to very early developmental stages due to opaqueness of the juvenile and the adult stages (1, 2). Magnetic resonance imaging is a non-invasive modality with exceptional soft tissue contrast. Its non-destructive nature allows a 3D analysis of different tissues in its original environment and the follow-up of the same animals, which is a clear advantage compared to classical histological studies (3, 4). MRI has not yet been applied to image live adult zebrafish. Because of the very small size compared to a mouse or a rat, MR imaging of adult zebrafish needs high resolution. In addition, being an aquatic animal, zebrafish requires special setup and several precautions for supporting *in vivo* imaging (5-7). As shown in chapter 2, we succeeded to

image live zebrafish using μMRI and obtained for the first time anatomical details from the living zebrafish. This was possible by using high magnetic field of 9.4T in combination with strong magnetic field gradients (1000mT/m) and specialized radio frequency coil (RF) coils. In addition, a 3D model of zebrafish was constructed from μMRI image slices using TDR-3D base software which allowed complete three-dimensional models of various structures such as brain, heart, liver, and swim bladder are constructed. While a three-dimensional atlas of zebrafish development is produced using the TDR-3D base from the histological sections is available (8, 9), at this time there is no atlas of the living adult zebrafish. We consider this work as a start that will pave the way for building a high-resolution anatomical atlas of adult zebrafish using both *ex vivo* and *in vivo* μMRI images. The results in chapter 2 demonstrate that high field μMRI provides sufficient resolution to get rapid anatomical details in adult zebrafish *ex vivo* as well as *in vivo*. In future high-resolution μMRI can be applied *in vivo* to study disease development, biological pathways, toxicologic mechanisms, and possible drug screening during various developmental stages in individual living zebrafish noninvasively.

It is well known that the signal-to-noise ratio of the MRI increases linearly with the field strength (10). Since an adult zebrafish is small compared to a mouse or a rat, it would be highly beneficial to further improve resolution by moving toward ultrahigh magnetic field. A first attempt to image zebrafish at ultra-high field (17.6T) is presented in Chapter 4. The comparison of the images of zebrafish between ultrahigh high field 9.4 T and 17.6T shows a clear difference in the resolution of the image. The images of 17.6 T were two times better in signal to noise and gave better

anatomical details especially in the brain and the small organs like the heart and the liver. In addition, signal to noise can be further improved by using the cryoprobe technology.

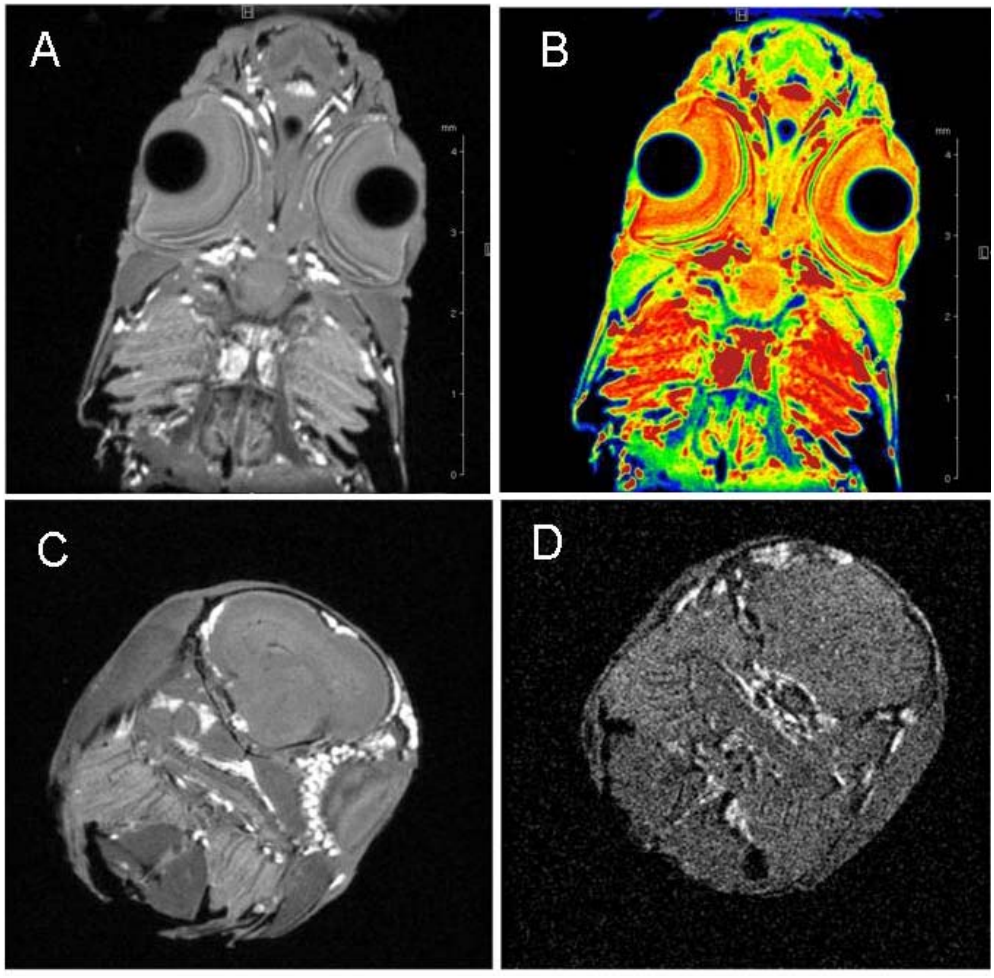

Figure 5.1 *Images from the head of adult zebrafish obtained by using micro-imaging cryoprobe (A-C) and conventional Micro2.5 probe (D) at 9.4T with exactly the same experimental data acquisition and processing parameters. Slice in coronal and axial plane were obtained using 3D MSME pulse sequence (TE= 5.4 ms; TR= 1800 ms; ns=4). The image resolution is 43 µm.*

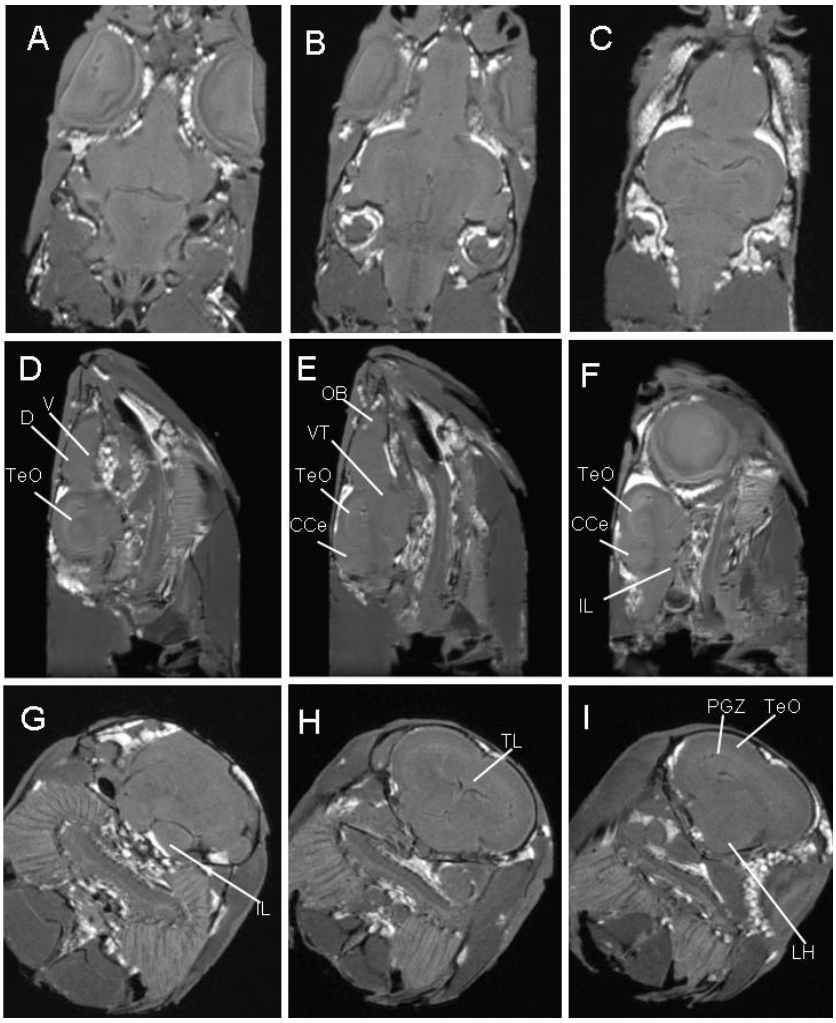

Figure 5.2 *Images from the head of adult zebrafish showing anatomical details in the brain obtained by using micro-imaging cryoprobe at 9.4T. Slice in axial plane (A-C), sagittal plane (D-F) and coronal plane (G-I) were obtained using 3D MSME pulse sequence (TE= 5.4 ms; TR= 1800 ms; ns=4; T; Scantime 31 min). The image resolution is 43 µm. V, ventral telencephalic area; D, dorsal telencephalic area; opticum tectum; OB, olfactory bulb; VT, ventral thalamus; CCe, cerebellar corpus; IL, inferior hypothalamus; TL, longitudinal torus; LH, lateral hypothalamic nucleus*

Cryoprobe technology improves signal/noise (S/N) ratios by reducing the operating temperature of the coil and the pre-amplifier. As a result, the efficiency of the coil is improved and the noise of the coil and the pre-amplifier are reduced (11, 12). In a pilot study, we used the cryoprobe in combination with 9.4T to get access to zebrafish anatomy with great details. The cryoprobe was equipped with a ^{1}H channel for 5 mm diameter samples, an RF coil operated at a temperature of 25 K and an integrated cryogenic preamplifier operated at 77 K. The temperature of the cryogenic probe was fully controlled by the Bruker CryoPlatform. The cooling of the CryoProbes is accomplished with a closed-looped helium gas flow via a flexible transfer line. Using 2D and 3D Multi slice multi echo (MSME) sequence, we observed an increase in the S/N ratio by a factor of 3-4, as compared to images obtained by conventional probe (Fig. 5.1). This improvement in S/N leads to a reduction in experimental time of upto 16. Due to the very small size of zebrafish brain, inadequate S/N ratio can be a major factor limiting the application of μMRI to get anatomical details from the zebrafish brain. As can be seen in Fig. 5.2, several structures within the brain are identifiable including the optic tectum, toris semicircularis, optic ventricle and cerebellum. Improved S/N ratio and possible reduction in experimental time with microimaging cryoprobe will pave the way in the future to follow the zebrafish development from embryo phase till the adulthood non-invasively.

5.2 High resolution localized MR spectroscopy of adult zebrafish brain and future perspective

Due to a similar basic organization of brain components as that of human, zebrafish is increasingly used for understanding brain diseases including neurodegenerative disorders (13, 14). However, there is an apparent lack of

information on the neurochemical composition of adult zebrafish brain *in vivo*.

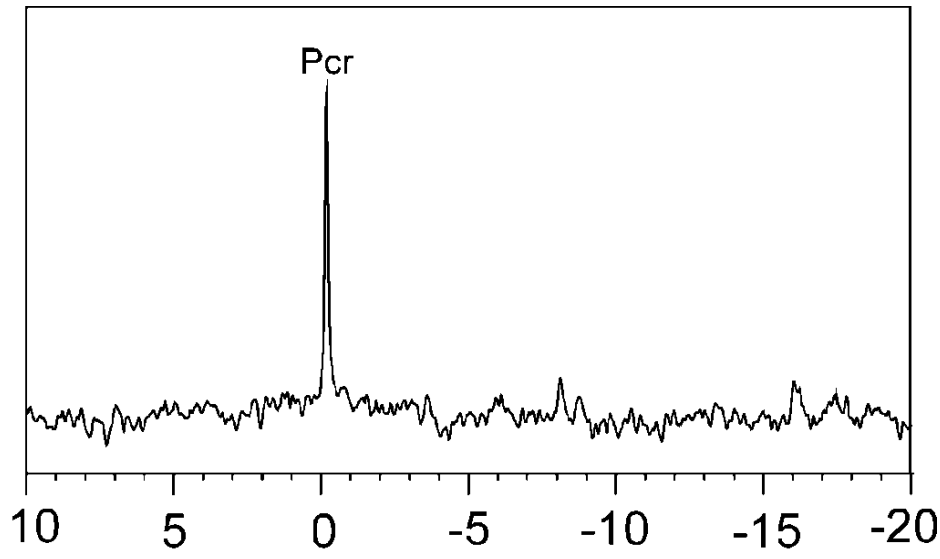

Figure 5.3 *In vivo high resolution localized ^{31}P MR spectrum from zebrafish brain obtained at 9.4T using ^{31}P_PRESS sequence with TE = 13.45 ms; TR = 1000 ms; Number of averages = 256; spectral width = 5000 hz; scantime = 15 min. ^{31}P chemical shifts in ppm.*

In chapter 3, we have successfully optimized a high resolution localized *in vivo* MRS technique to get access to the zebrafish brain and obtained for the first time the neurochemical composition of zebrafish brain. Our results showed that zebrafish brain contains the same basic neurochemical composition as that found in human, suggesting that zebrafish is a good model for studying human brain diseases. Due to the very small size of the zebrafish brain, the regional neurochemical information can not be obtained in this study. Future studies with more advance pulse sequences, better localization and use of high magnetic field such as 17.6T, or use of a

cryoprobe may provide access to localized regions in the zebrafish brain. Further development of *in vivo* MRS for zebrafish brain could also include localized ^{31}P and ^{13}C MR spectroscopy which can be important for more extensive analysis of brain changes at the molecular level (6, 16). In a preliminary experiment, we applied ^{31}P MR spectroscopy to obtain a spectrum from the zebrafish brain.

Due to a small size of the voxel (3μl) placed over entire brain, only resonances of phospho-creatine were clearly observed (Fig. 5.3). These results suggest that 9.4 T is not sensitive enough to measure resonances of ATP and P_i in the zebrafish brain. Future experiments at ultra high field 17.6 T may provide better resolution for ^{31}P MR spectroscopy. The use of *in vivo* localized MRS in combination with μMRI in zebrafish brain can be useful for longitudinal studies to monitor biochemical changes during disease progression and treatment using different available zebrafish models in the near future.

5.3 Monitoring spontaneous melanomas in transgenic zebrafish with μMRI and beyond

Zebrafish is emerging as a most promising model system in cancer research. The majority of the tumors in zebrafish develop late in life, when fish are no longer transparent, limiting *in vivo* optical imaging methods (17, 18). Thus *in vivo* imaging of tumor development remains demanding. In Chapter 4, we have successfully applied μMRI to visualize and characterize the tumors in transgenic zebrafish melanoma model at high (9.4T) and ultrahigh (17.6T) magnetic fields. Anatomical locations and invasion status of the tumors were clearly observed. In addition, we have shown that the T_2

relaxation time can provide a means to evaluate the heterogeneity of the malignant tumor (19). Such non-invasive μMRI studies can be applied in the future, for longitudinal studies to track tumor development or the effects of anti tumor drugs in various available zebrafish tumor models. In addition, if the tumors are homogeneous and solid, it will be easy to apply proton localized spectroscopy to detect the metabolic profile of the tumor and to specifically monitor the changes in the level of choline. It is already known from other studies that many tumors contain high amount of choline than normal tissue (20). In addition to anatomical imaging and localized spectroscopy, MR angiography can be applied to see vascular network in the tumor as well as in other parts of the body (21). It will however be very challenging to apply MR angiography in such a small zebrafish because the vessels are very thin and the water flow outside the fish might influence the signal of flowing blood. Finally, μMRI can be combined with targeted MRI contrast agents to follow specific processes. For example, *in vivo* visualization of gene expression has been visualized in living *X. laevis* embryos using MRI in combination with contrast agent that can indicate reporter gene expression (22, 23). Use of such contrast agents in combination with *in vivo* μMRI methods developed in this thesis will be a powerful tool to bridge the gap between the genome wide studies, the morphological, the physiological and the functional studies of the living adult zebrafish.

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