MR IMAGING OF GENE EXPRESSION IN ZEBRAFISH

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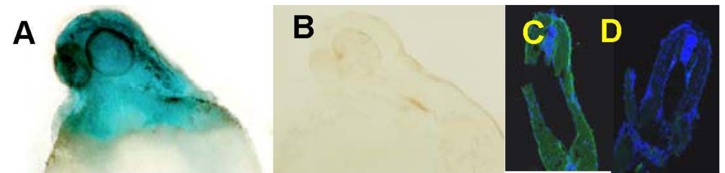
Background

The Green Fluorescent Protein reporter system in zebrafish revolutionized the study of localized gene expression involved in vertebrate development. This was in largely due to the transparent nature of the developing zebrafish embryo. As the fish develops and matures it becomes opaque and the light from the GFP is unable to penetrate the skin, resulting in loss of signal strength and spatial information. Interest in the use of adult zebrafish as a valuable vertebrate system for the study of genomics, development, human disease processes, and for drug discovery remains high for several reasons. They are inexpensive to acquire and maintain, and just as with mice, animal models of human disease can be created by genetic and environmental manipulation. In addition adult zebrafish are able to regenerate damaged tissues including cardiac muscle and spinal cord. For this reason zebrafish biology is an area currently of great interest in the field of stem cell and regenerative biology. Understanding of how this occurs in the adult fish may offer insight into new therapies for human tissue repair in conditions such as in spinal cord injury and myocardial infarction. The study of the genes involved in these processes would be greatly supported if there was an in vivo imaging system for zebrafish that could detect temporal gene expression in the adult fish. If in addition this technique was on an anatomical background, accurate spatial information would also be available. We are developing an MRI visible reporter system with ferritin as the reporter gene.

Methods and Results



We have developed an in vivo system to image zebrafish, and have previously imaged ferritin expression in bacteria and zebrafish cells. To create ferritin expressing zebrafish single cell embryos were microinjected with ferritin transcripts and were allowed to develop over time. They were assayed for Fe levels by Prussian blue histological stain (**A ZHF+LF, B control** at 50 hrs post fertilization) and ferritin heavy chain expression by immunhistochemistry (**C ZHF+LF, D control at 6 weeks**). High resolution MRI was performed using an in house built coil and a T2*W pulse sequence which is sensitive to small amounts of iron (such as is found in ferritin). The ferritin causes a local area of susceptibility around it resulting in a loss of signal and so darkening in the image The measured signal intensity in the body and tail of the transgenic embryo is only 60% that of the control at 50hrspf (**Fig.F**). The yolk sack of both is dark due to iron in the yolk. The embryos are viable, and at 6wpf (**Fig.G,H**) some ferritin is still detected in brain and muscle on MRI, though the difference is less than at the embryo stage.

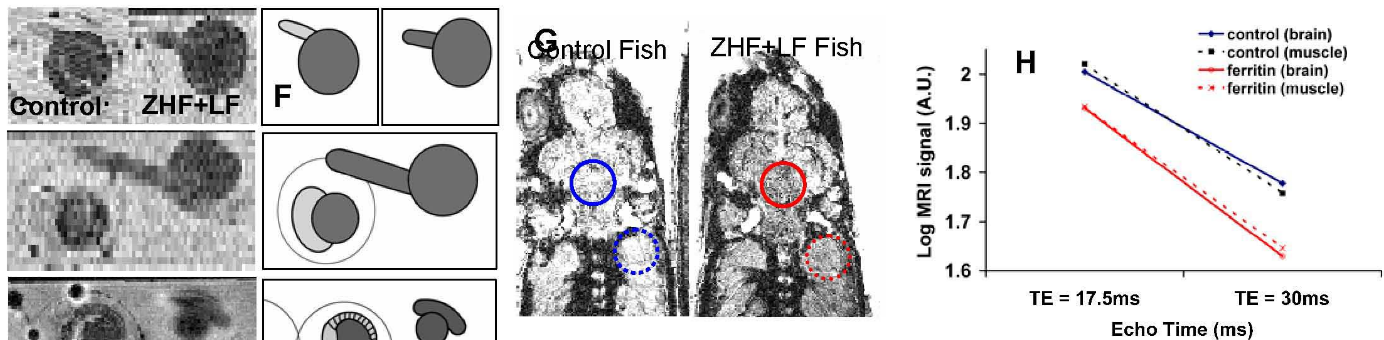


Fig F MRI of 50hpf embryos at different orientations:
Fig G,H MRI of 6wpf fish **imaged together** with a T2* weighted sequence.

Discussion and conclusion

Using this system we demonstrate the proof in principle steps required for generating a ferritin based MRI detectable reporter system in Zebrafish. Issues of micro and in vivo imaging of zebrafish have been resolved and we are in a position to develop the ferritin system as a reporter of individual gene expression. The difference between this system and an exogenous system is that the MR contrast is generated from within the cells themselves and not from the addition of exogenous contrast agents which may or may not get to its target.