In Vivo Ultra High Field Magnetic Resonance Microimaging to Track the Development of Malignant Melanoma in Zebrafish

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Introduction: Zebrafish have emerged as one of the most promising and cost-effective model systems to study cancer susceptibility and carcinogenesis. Recent studies have demonstrated that zebrafish cancer has genomic and histological similarities with human cancers, suggesting that experiments in zebrafish cancer models will be highly relevant for clinical studies (1). Most tumors in zebrafish develop late in life, when fish are no longer transparent, limiting *in vivo* optical imaging methods. Thus, non-invasive imaging to track tumors in adult zebrafish remains challenging. In this study, we applied magnetic resonance microimaging (μMRI) to track spontaneous melanomas in stable transgenic zebrafish models expressing a RAS oncoprotein and lacking P53 (mitf:Ras::mitf:GFP X p53-/-) (2). Tumors in live adult zebrafish were visualized and live imaging of tumors at ultra-high field (17.6T) was used to reveal tumor heterogeneity. To our knowledge, this is the first report demonstrating the application of μMRI to detect the locations, invasion status and characteristics of internal melanomas in zebrafish and suggests that non-invasive μMRI can be applied for longitudinal studies to track tumor development and real-time assessment of therapeutic effects in zebrafish tumor models.

Methods: For *in vivo* μMRI, anesthsized wild-type and transgenic fish (nacre:Ras::nacre:GFP X p53-/-fish) were imaged in a closed flow-through chamber, which was specially designed to support living zebrafish inside the magnet (Fig 1B) (3). MR images were acquired using a 9.4-T and 17.6T vertical wide-bore imaging systems equipped with a Bruker Avance console and 1000-mT/m gradients. A series of coronal and sagittal T_2-weighted images were acquired using the rapid acquisition with relaxation enhancement (RARE) sequence. The settings used were, TE = 10.5 (22.5 ms effective), TR =2000 ms, RARE factor (echo train length) = 4 and averages = 2. An in-plane resolution of 78 x 78 μm was achieved with slice thickness of 200 μm in an acquisition time as low as 4 minutes. T_2 relaxation was measured using a Multi-Slice Multi-Echo sequence, with TE = 8.5, 17, 25.5, 34, 42.5, 51, 59.5, 68 ms, TR = 1500 ms, averages = 2, with an in-plane resolution of 78 x 78 μm and a slice thickness of 1 mm.

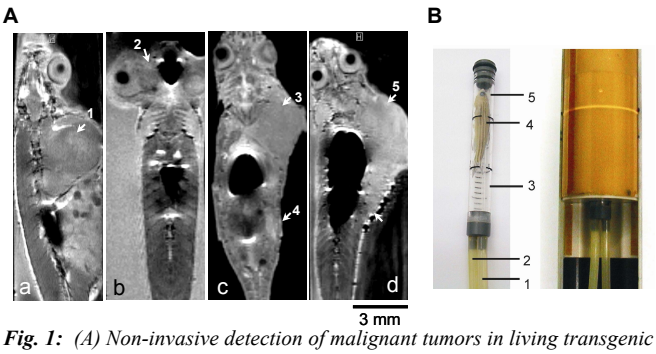

Fig. 1: *(A) Non-invasive detection of malignant tumors in living transgenic zebrafish using μMRI. (1) malignant tumor seen in: (1) trunk muscles and abdomen (2) near eye; (3) back muscles; (4) intestine; (5) back muscle (B) Flow through setup specially designed for imaging live zebrafish*

Results and Discussion:.
Fig. 1A show images of 4 live transgenic zebrafish (a-d) showing tumors at locations such as in trunk muscles and abdomen that are penetrating into the myoseptum and ovary, near the eye, intestine, and liver (Fig. 1A). An excellent 3 way correlation between tumor visualized by in vivo μMRI , ex vivo μMRI and in histological section was obtained (Fig. 2A). The tumor shown in Fig. 2 was heterogeneous as revealed by histological analysis of the same tumor (Fig. 2C). Live imaging of a tumor at 17.6 T revealed that the tumor is highly heterogeneous, which was also confirmed by histological analysis of the same tumor (Fig. 3). The heterogeneity of tumors was reflected by the significant differences in transverse relaxation time, T_2 measured in various regions of tumor. In conclusion, our results demonstrate the feasibility of μMRI technique to detect internal tumors in live adult zebrafish non-invasively and reveal that melanomas in stable transgenic zebrafish models expressing a RAS oncoprotein and lacking P53 are heterogeneous in nature.

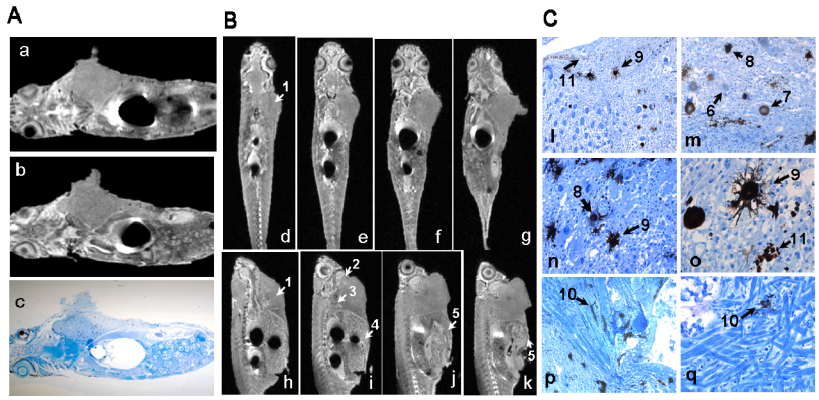

Fig. 2: *(A) Comparison of images of same transgenic zebrafish, with large abdominal tumor, obtained by (a) in vivo μMRI, (b) ex vivo μMRI, and (c) after histological sectioning. (B) Successive μMRI slices in coronal (d-g) and sagittal (h-k) planes showing abdominal tumor (C) High magnification view of tumor showing its heterogeneity and cell morphology.*

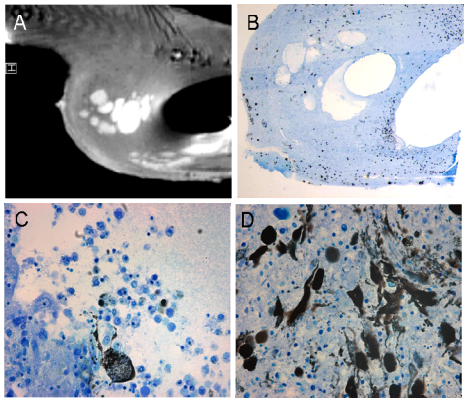

Fig. 3: *Heterogeneity of the malignant abdominal tumor in transgenic zebrafish visualized by (A) μMRI at 17.6T and (B-D) after histological sectioning.*

References: (1) Amatruda JF, Shepard JL, Stern HM, Zon LI. Cancer Cell 2002; 1:229-231. (2) Michailidou C, Jones M, Walker P, Kamarashev J, Kelly A, Hurlstone AFL. Disease Models & Mechanisms, 2009; DOI: 10.1242/dmm.001149 (in press); (3) Kabli S, Spaink HP, De Groot HJM, Alia A. J Magn Reson Imaging 2008; 29:275-281.

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