High Resolution Pre-clinical MRI in Murine Braf-induced Thyroid Tumor Targeted Therapy

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Introduction: Mutations of *BRAF* are found in ~ 45% of papillary thyroid cancers (PTC), and are enriched in tumors with more aggressive properties (1-3). We developed mice with a thyroid-specific knock-in of oncogenic *BRAF* (*LSL-BRAF^{V600E}/TPO-Cre)* to explore the role of pharmacological treatments in a physiologically relevant mouse model of PTC. Thyroid specific BRAF activation led to the development of classic infiltrative PTC with complete penetrance by 3 weeks. Murine Braf-induced PTCs were treated with the specific allosteric MEK1/2 inhibitor PD325901 or rapamycin for three weeks, beginning at 3 weeks. Conventional tumor volume measurement through dissection is both invasive and inaccurate due to the small size of thyroid. With the increase of scanner field strength in MRI, particularly pre-clinical MRI, MRI has become more sensitive and suitable for small animal studies. In this work we chose non-invasively measured thyroid volume by high resolution MRI as the tumor biomarker.

Methods:LSL-BRAF^{V600E} mice harbor a latent oncogenic Braf knock-in allele, which following Cre-mediated recombination results in endogenous expression of the oncoprotein (4). TPO-Cre mice express Cre recombinase under the control of the thyroid peroxidase gene promoter, which is active only in thyroid follicular cells beginning at E14.5 (5). PD352901 and rapamycin were administered to mice once daily by oral gavage at doses of 25mg/kg and 7.5mg/kg respectively. High resolution MR imaging of the transgenic mouse thyroid tumors was done on a 200 MHz Bruker Biospec scanner (Bruker Biospin MRI GmbH, Ettlingen, Germany) with a custom-built 26 mm ID birdcage resonator. Mice were anaesthetized with 1% isoflurane gas during scanning, and physiologically monitored (SA Instruments, Inc., Stony Brook, New York). Mouse thyroid tumor images were acquired using T2-weighted RARE sequence with a coronal slice of 0.4 mm thick, with a spatial resolution of 82 x 104 µm. We used the following acquisition parameters: TR = 2 s, TE= 45 ms, RARE factor 8, with an average of 40 scans and 32 minutes of acquisition time. H&E staining confirmed that the tumors occupied the entire thyroid gland, therefore the volume of the entire thyroid gland was determined pre- and post-treatment.

Results and Conclusions: A high resolution mouse thyroid tumor MRI image is shown in Figure 1. Thyroid tumor volume, as determined by MRI before and after treatment, was decreased in PD 325901 mice(n=7) (p=0.0002, Figure 2B), compared with vehicle (n=5, Figure 2A), however no effect was observed in mice treated with rapamycin(n=4) (Fig 2C). The reduction in thyroid volume in PD325901 animals was associated with a significant reduction in the proliferative index of the tumors (p=0.01, Fig 2D). However, there was no difference in the apoptotic index, or in the histopathological appearance of the lesions. Levels of phosphorylated ERK, a downstream target of MEK, were profoundly inhibited 6h after the last dose indicating PD325901 successfully inhibited ERK (Figure 3). These results demonstrate the murine Braf-induced thyroid tumors are sensitive to Mek inhibition, but not rapamycin, and that MRI is a valid non-invasive mechanism to measure thyroid tumor burden in this genetically engineered mouse model of thyroid cancer.

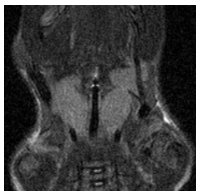

Figure 1

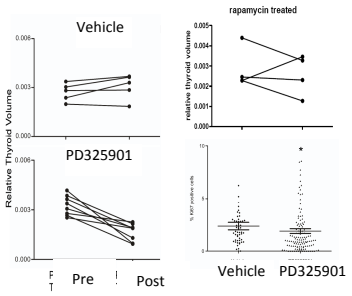

Figure 2

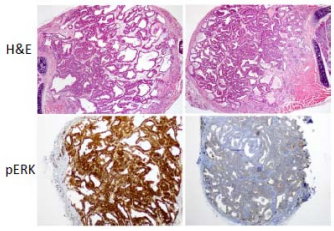

Figure 3

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