Diagnostic Value of R2* in identifying ALK mutations in transgenic murine models of neuroblastoma

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Introduction

Neuroblastoma (NB) is the most common extra-cranial solid tumour of childhood and accounts for 15% of mortality in paediatric cancer. Approximately 50% of patients with poor prognosis disease exhibit amplification of the proto-oncogene *MYCN* associated with frequent relapse, aggressive tumour biology and enhanced tumour angiogenesis (1, 2). The TH-*MYCN* genetically-engineered-mouse model (GEMM) spontaneously develops tumours that closely recapitulate most major features of high-risk human NB; including the anatomical distribution, radiology and biological features (3). Recently, mutations in the anaplastic lymphoma kinase (*ALK*) gene were described in virtually all cases of hereditary NB and in approximately 15% of somatic NB tumour tissues, making *ALK* mutations the second most common genomic alteration in NB (4, 5). We used a similar GEM modelling approach to construct a new model of NB, which contains an *ALK* mutation (*ALK-F1174L*), concomitantly with the TH-*MYCN* transgene, since the *ALK-F1174L* mutation alone is not sufficient to drive tumour formation. Here, we present a multi-parametric imaging study, which compares the tumours from the TH-*ALK-F1174L*/TH-*MYCN* mice to TH-*MYCN* mice.

Materials and Methods

Tumour-bearing mice were identified by palpation. ^{1}H MRI was performed on a 7T Bruker horizontal bore microimaging system using a 3cm birdcage coil. Anatomical T_2w RARE coronal images were acquired through the abdomen of the mice (FOV=4cm, matrix=128x128, 20 slices, 1mm thick, 4 averages, TE_{eff}=36ms, TR=5000ms, turbo factor=8). Baseline R_2* was measured using a multi gradient-echo (MGE) sequence (TE=6-28ms, TR=200ms, 8 echoes, 8 averages, FOV=3cm, matrix=128x128, 3 axial slices, 1mm thick, NEX=8) Native T_1 and T_2 were quantified using an inversion recovery (IR) True-FISP sequence (baseline scan, TI=25-1450ms, 50 inversion times, TE=1.2ms, TR=2.5ms, scan TR=10s, 8 segments, NEX=8). Data Analysis: MGE, IR-trueFISP data were fitted on a pixel-by-pixel basis using in-house software (ImageView), providing parametric maps of R_2*, T_1, T_2.

Results and Discussion

Tumours from TH-*ALK-F1174L*/TH-*MYCN* mice demonstrated a significantly slower R_2* than TH-*MYCN* mice (Table 1, Figure 1.A). This difference was already evident on T_2*-weighted images. Signal intensity from tumours in the TH-*ALK-F1174L*/TH-*MYCN* decays slower than the signal arising from the tumour in the TH-*MYCN* mice, evident at TE=21.9ms (Figure 1.B). R_2* is sensitive to the concentration of deoxygenated haemoglobin, thus suggesting a difference in the vascular phenotype between the two models. At excision, the very dark-red colours of tumours in TH-*MYCN* mice contrast with the pale appearance of tumours in the TH-*ALK-F1174L*/TH-*MYCN* mice (Figure 1.D). Tumours from TH-*MYCN* mice present a large amount of "blood lakes" (Figure 1.C), which could result in vascular stasis. These structures are absent from the tumours in the TH-*ALK-F1174L*/TH-*MYCN* mice. Overall this study suggests that tumours from the TH-*ALK-F1174L*/TH-*MYCN* mice have a lower blood volume than TH-*MYCN* and/or may present more functional vessels. Investigation with DCE-MRI and USPIO imaging are on-going to assess potential differences vascular perfusion/permeability, and the architecture between the two models.

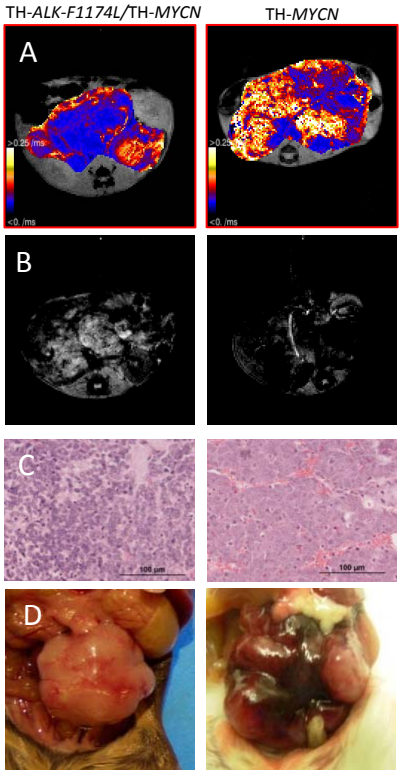

Figure 1. Characterisation of the tumour in the TH-*ALK-F1174L*/TH-*MYCN* and TH-*MYCN* mice. A, Representative parametric R_2* maps. B, T_2*-weighted images (TE= 21.9 ms). C, Representative H&E sections. D, Gross pathology.

Conclusions

We have demonstrated that the TH-*ALK-F1174L*/TH-*MYCN* model has a phenotypically different vasculature from the TH-*MYCN* model, identifying a role for the *ALK* gene in the regulation of tumour angiogenesis. If the TH-*ALK-F1174L*/TH-*MYCN* mice prove to recapitulate the clinical presentation of *ALK*-mutated childhood NB. T_2*-weighted imaging could be incorporated in diagnostic scanning, providing a method for identifying patients with mutations in *ALK*, and therefore helping with patient stratification and treatment decisions.

	TH-*ALK -F1174L*/TH-*MYCN*	TH-*MYCN*	p value
Volume	1941 ± 280	1495± 316	0.32
T_1	1888 ±18	1901 ± 31	0.70
T_2	73 ± 7	65 ± 6	0.45
R_2*	65 ± 4	118 ± 10	<0.0001

Table 1. Native tumour relaxation time values in the TH-*MYCN* and TH-*ALK* F1174/TH-*MYCN* mice. (Data = mean ± s.e.m, n=9, Student's 2-tailed unpaired t-test)

References

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