MRI Characterisation of a Novel Transgenic Mouse Model of Neuroblastoma

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Introduction

Neuroblastoma is the most common extracranial childhood solid tumour, accounting for between 7-10% of paediatric cancers, and originates in peripheral nerve tissues (1). The proto-oncogene *MYCN* is amplified in 25% of high-risk neuroblastoma and is associated with an aggressive tumour phenotype, enhanced tumour angiogenesis and poor clinical prognosis (2). The Mycn oncoprotein is highly expressed in tumour but not in normal tissue, making it an attractive candidate for targeted therapeutics. To assess the relevance of *MYCN* overexpression in neuroblastoma, and to test Mycn-targeted therapeutics, a novel murine transgenic model that faithfully replicates the disease biology of high-risk neuroblastoma by targeting overexpression of *MYCN* to the neural crest has been constructed (3, 4). In this model, tumour origin is spontaneous and occurs in the correct tissue of origin. As a prelude to the assessment of novel therapeutics for the treatment of neuroblastoma, and given their posterior and abdominal localisation, the TH-*MYCN* model has been investigated by MRI. Specifically, the anatomical presentation and longitudinal development of the tumour *in situ*, as well as its established response to the chemotherapeutic agent cyclophosphamide *in vivo*, have been characterised. In addition, quantitative MRI parameters, and interrogation of the tumour vasculature by DCE-MRI, are also reported.

Materials and Methods

Mice with abdominal tumours identified by palpation were imaged on a 7T Bruker MicroImaging system (n=7) using the following protocol: **1- Anatomical images:** T_2-weighted coronal images were acquired through the mice (RARE, FOV=4cm, matrix=128x128, 20 slices, 1mm thick, NEX=4, TE_{eff} =36ms, TR=5000ms, turbo factor=8). Following the imaging session, mice (n=3) were treated with two i.p. doses of 100mg/kg cyclophosphamide (CP) (48 hours apart) and imaged 48 hours later. **2- Quantitative measurement of native relaxation times**. 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) was then used to quantify R_2*. 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) **3-DCE-MRI** data were acquired using IR True-FISP sequence with 60 dynamic scans (TI=130-1037ms, 8 inversion times, TR=4ms, TE=2ms, scan TR=10s, temporal resolution=20s, NEX=2) prior to and following i.v. injection of 0.1mmol/kg Gd-DTPA (Magnevist, Schering) **Data Analysis:** MGE, IR-trueFISP data were fitted on a pixel-by-pixel basis using in-house software (ImageView), providing maps of tumour spatial heterogeneity.

Results and Discussion

Anatomical presentation: All the TH-*MYCN* mice had developed abdominal tumours which originated from the adrenal glands and were located between the two kidneys (Figures 1 and 2). Their sizes were variable at the time of the colony weekly screening (by palpation), and the multi-slice T_2-weighted images typically revealed tumours filling the whole abdominal cavity resulting in compression of the gut, kidneys (often against the abdominal wall) and the spine. Displacement of the characteristically enlarged abdominal aorta and vena cava was also commonly evident.

Response to treatment with CP: Following treatment with CP, tumours were difficult to identify/undetectable (n=3), consistent with previous studies showing remission in this model, and which mirrors the sensitivity of childhood neuroblastoma to CP in the clinic (4). Further screening of these mice is ongoing to interrogate any potential relapse associated with mutations of the tumour suppressor gene p53.

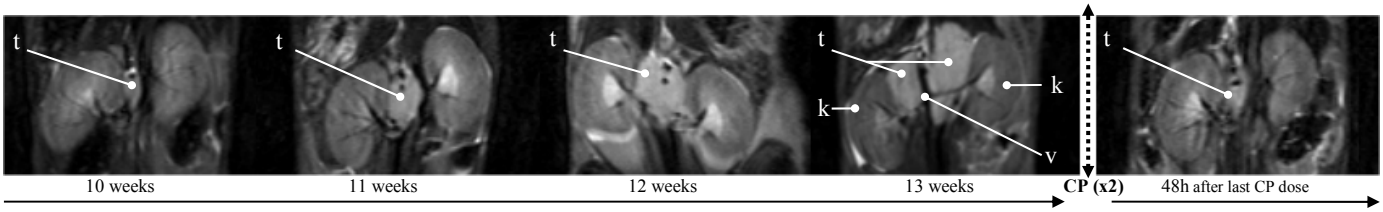
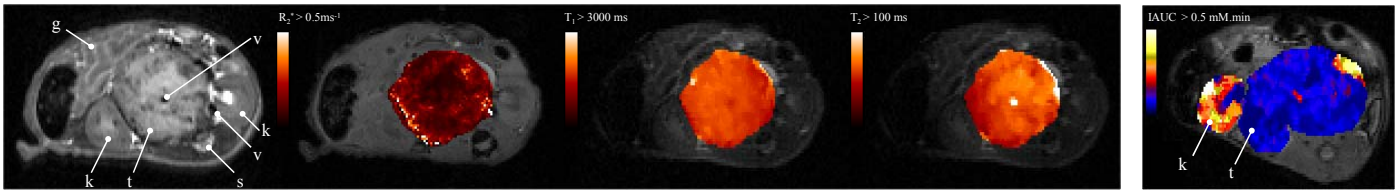


Figure 1. T_2-weighted coronal slice through the kidneys showing the progression of a TH-*MYCN* neuroblastoma over 3 weeks and its subsequent response to CP.

Quantitative MR characterisation: The tumours presented very heterogeneous spatial distribution of T_1, T_2 and R_2^* (Figure 2), consistent with the heterogeneity observed on the H&E stained sections (Figure 3). The relatively fast baseline R_2* is indicative of a large blood volume, confirmed by the intense red coloration of these tumours at excision, and the presence of a large proportion of blood lakes (5). The $IAUC_{60}$ following Gd injection indicates that these tumours are relatively well-vascularised.



R_2^*= 115 ± 3s^{-1} T_1= 1748 ± 14ms T_2= 55 ± 1 ms $IAUC_{60}$= 0.13 ± 0.01ms

Figure 2. Quantitative MRI characterisation of TH-*MYCN* neuroblastoma tumour model. From left, T_2-weighted axial images trough the mouse abdomen showing **g**ut, **k**idney, **s**pine, **t**umour and abdominal blood **v**essels. Calculated R_2*, T_1, T_2 maps and values (mean ± 1 s.e.m., n=7), and integrated area under the curve 60s ($IAUC_{60}$) map and average determined from DCE MRI (mean ± 1 s.e.m., n=3).

Conclusion

This study reinforces the TH-*MYCN* murine model as a faithful representation of human neuroblastoma with regards to its anatomical and radiological appearance, as well as its high sensitivity to CP treatment. It also demonstrates that the implementation of MRI screening would be an asset in the development of novel therapeutics for neuroblastoma and accelerate their clinical development by allowing simultaneous evaluation of MRI biomarkers of treatment response.

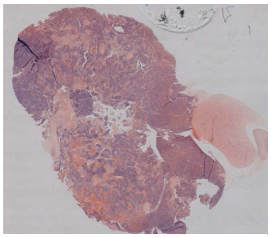


Figure 3. H&E section from a TH-*MYCN* neuroblastoma.

References

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