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Short Communication

Blind confirmation of pathological alterations detected in small animals by Magnetic Resonance Imaging

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ABSTRACT

Among different imaging techniques, MRI is rarely employed in small animals because it is cost- and time-consuming and demands substantial expertise. However, MRI has a high spatial resolution and the possibility to simultaneously extract physiological and anatomical information are its great advantages when the number of animals that can be injected with cells is limited. Here we evaluate the performance of MRI in detecting pathological alterations in a mouse injected with potential metastatic cells from a human specimen. MRI was carried out with a clinical 1.5T system, every 2 weeks, starting from 30 days post injection till 90 days post-injection when the mouse was terminated. At the second week of MRI monitoring, a bilateral increase in signal intensity in the kidneys was observed. In addition, T2-weighted images showed enlargement of the spleen. Blind histopathological analysis performed on the terminated mouse indeed confirmed metaplasia of the myeloid component of spleen and glomerulonephritis in the kidneys. Thus, MRI with its properties that allow to reduce the number of mice to obtain relevant information, may be the method of choice to *in vivo* monitor the effects of cells present in limited number in human specimens, whose *in vitro* manipulation or expansion is not recommended.

KEYWORDS: neuroblastoma, imaging, human cells, small animals

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INTRODUCTION

Small animals are frequently used as models of human pathology. In cancer research, mice are injected with neoplastic cells and tumor growth is monitored till autopsy, when pathological analysis of organs and tissues confirms absence/presence of tumor growth [1]. In recent years, intravenous or orthotopic injection of neoplastic cells has been preferred to subcutaneous injection because of the higher correspondence with the clinical conditions [2]. The use of these models, however, increased the challenge of correctly monitoring tumor growth till animal termination.

Among different imaging techniques, fluorescent and bioluminescent imaging are increasingly used because of their relative simplicity and limited cost, but bioluminescent or fluorescent tumor cells need to be generated by transfection of recombinant plasmids, thus requiring long *in vitro* selection and expansion of transfected cells [3]. Recently, fluorescent probes able to passively enter into the cells have been developed [4], but their imaging properties are still limited. Magnetic Resonance Imaging (MRI) is rarely employed because it is cost- and time-consuming and demands substantial expertise. However, MRI has the highest spatial

resolution and physiological/molecular and anatomical information can be extracted simultaneously, thus limiting the number of animals to obtain relevant information [5].

Since the development of the cancer stem cell theory (see [6] for review), the evaluation of the tumorigenic potential of different subsets of neoplastic cells from human specimens has further increased the need to correctly monitor tumor growth by non invasive techniques. The number of cells, in fact, is limited and their *in vitro* manipulation or expansion may alter their properties.

Herein, we tested whether MRI could correctly monitor the *in vivo* fate of cells selected from a human specimen and show that the pathologic alterations observed were fully confirmed by a blind pathological analysis performed on the terminated mouse.

MATERIALS AND METHODS

Mice and cells

NOD/scid mice (NOD.CB17-Prkdcscid/J, Jackson Laboratories, Bar Harbor, ME) were housed at the Animal Facility of the IRCCS San Martino-Istituto Nazionale per la Ricerca sul Cancro, Genoa, in specific pathogen-free colony and were fed sterile food and water.

The injected human cells were GD2-positive neuroblastoma cells immunomagnetically selected from the umbilical cord blood of a child that developed a neuroblastoma tumor [7]. To resemble the clinical situation the potential metastatic cells were injected orthotopically into the adrenal gland after laparotomy performed under general anesthesia. The mouse was anesthetized by ip administration of a 1:1 mixture of Xylazine chloridate (Xilor®, Bio98 srl, Italy) and Ketamine chloridate (Imalgene 1000®, Merial Italia SpA, Italy) at 5-10 mg/kg and 80-100 mg/kg, respectively.

After orthotopic injection and recover from anaesthesia, the mouse was monitored twice a week for weight loss (>15%), presence of tumour masses, or any other sign of disease. The experiment was approved by the Institution Animal Care and Use Committee and was conducted according to Institutional guidelines.

Magnetic Resonance Imaging

MRI was performed with a clinical 1.5T MR system (Philips, Gyroscan NT-Intera), every 2 weeks, starting from 30 days post injection, under general anaesthesia as described above. A naive mouse was used as control. The mice were placed in prone position on a surface coil placed on a support filled with water at 37 °C to preserve their body temperature. The temperature during the experiment was 28 °C and the mean acquisition time was 30 minutes for each experiment.

Images were obtained on coronal and sagittal planes. The following sequences were applied: T1-weighted turbo-spin-echo-sequences (repetition time in ms/echo time in ms/flip angle/number of acquisitions/450/20/90°), T2-weighted turbo-spin-echo sequences (repetition time in ms/echo time in ms/flip angle/number of acquisitions/3111/130/90°) performed with a field of view of 100 x 100 mm, a matrix of 256 x 256 pixels and a slice thickness of 2 mm, T2-weighted turbo-spin-echo fat saturation sequences (repetition time in ms/echo time in ms/flip angle/number of acquisitions/2616/130/90°) performed with a field of view of 100 x 100 mm, a matrix of 256 x 256 pixels.

Histopathological analysis

Ninety days post-injection, the mouse injected with the human cells and a naive mouse were terminated by CO asphyxiation and autopsies were performed. All the organs, including decalcified spine and posterior hind bones, were fixed in formalin, paraffin-embedded and then sectioned at 5 μm. After staining with Mayer's Ematossilin, sections were analyzed blind to the MRI data. Kidneys' sections were also stained with PAS, Jones and Trichrome Green Stain (Vetana Medical Systems, Tucson, AZ) according to the manufacturer's procedures.

RESULTS AND DISCUSSION

At the second week of MRI monitoring (day +45 after injection), a bilateral increase of signal intensity, better appreciated on T2 and T2-fat suppression weighted images, appeared in the kidney cortex and medulla of the injected mouse, as compared to the naive mouse (Figure 1). The injected mouse also showed a significant enlargement of the spleen (Figure 2). No other

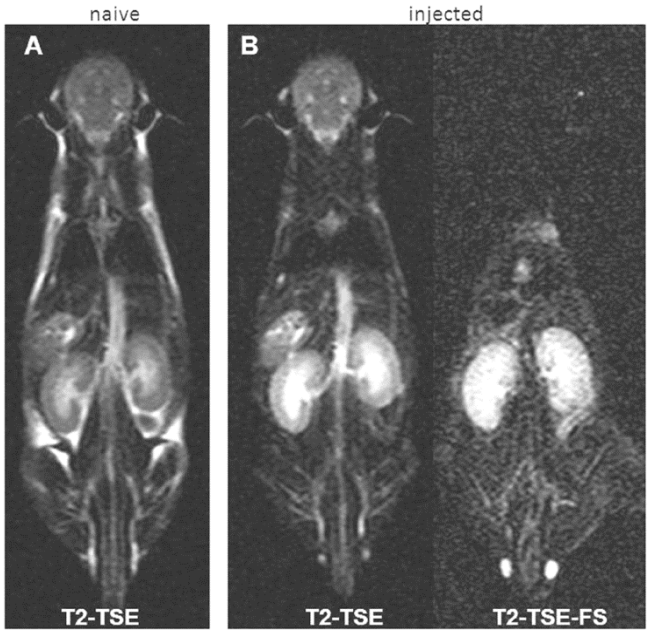

Figure 1. Magnetic Resonance of a naive (A) and human cells-injected mouse (B) on coronal planes. T2- and T2-fat suppression weighted images showed a bilateral signal intensity increase in the kidney cortex and medulla.

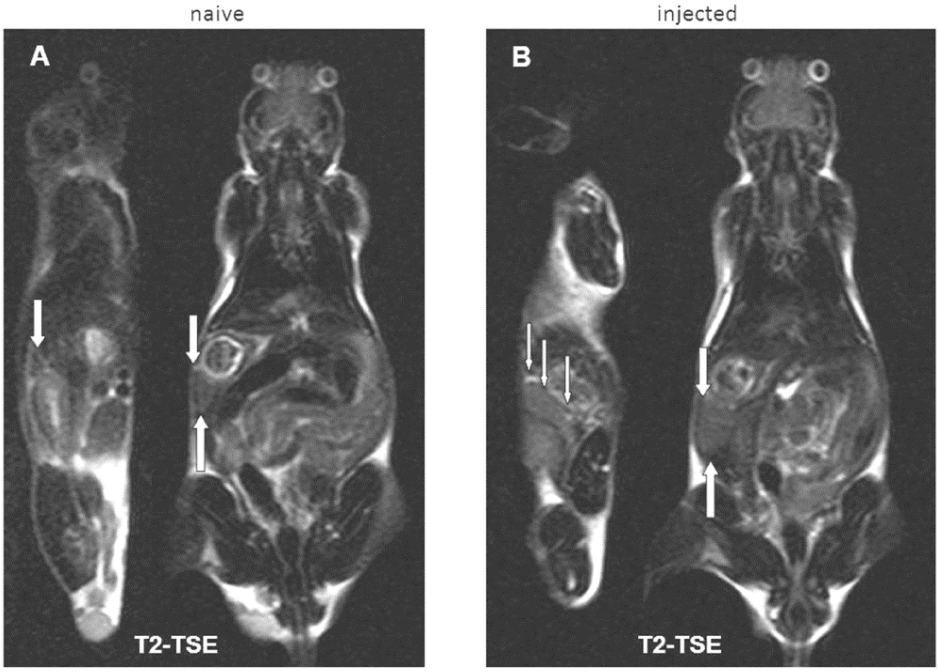

Figure 2. Magnetic Resonance of a naive (A) and human cells-injected mouse (B) on sagittal and coronal planes. T2-weighted images showed normal spleen (arrows) in the naive (A) and a visible enlargement (arrows) in the injected mouse (B).

pathological imaging, including tumor growth in the adrenal gland, was observed during the following 2 week interval of MR monitoring (Figure 3).

The absence of tumor growth revealed by MRI was confirmed by the blind histopathological analysis performed on the terminated mouse. No macro or micro metastasis was found in the

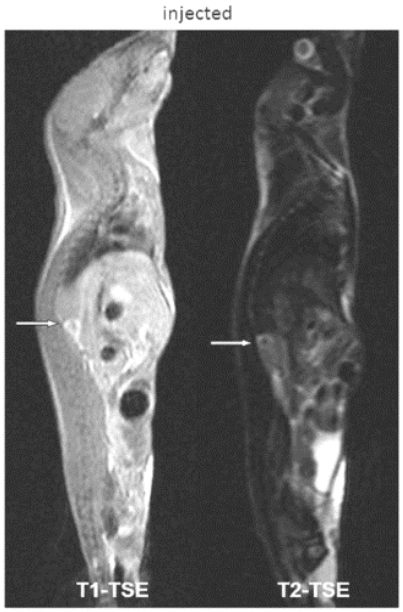

Figure 3. Magnetic Resonance of human cells-injected mouse on sagittal planes. No tumor growth was observed in the adrenal gland (arrow) on both T1-weighted (left) and T2-weighted (right) images.

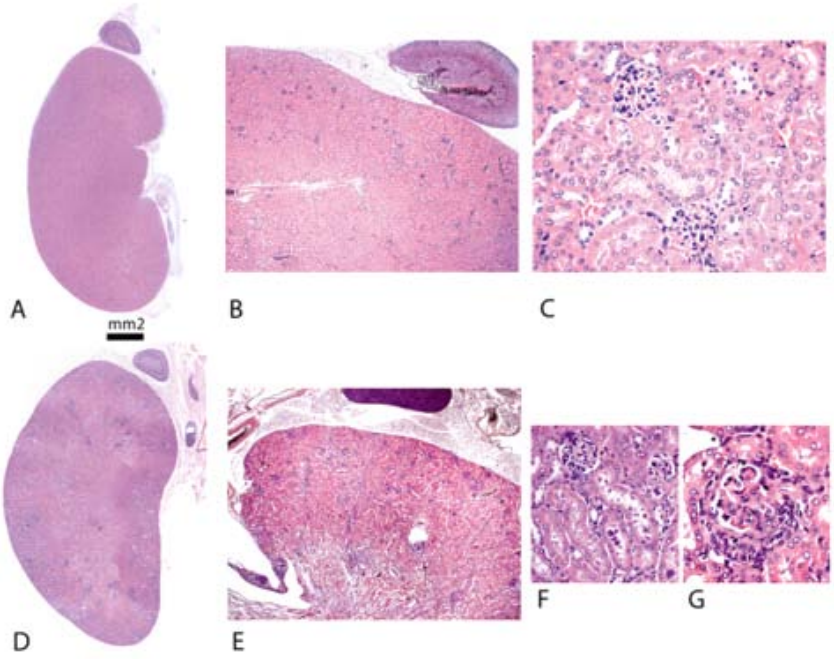

Figure 4. Kidney of a naive (panels A, B and C) and of an injected mouse (panels D, E, F and G). Sections, stained with Mayer's Ematossilin, at 1x (A and D) and 4x (B and E) magnification. Sections, stained with PAS (panels C, F and G) indicated membrane alterations in the tube (panel F) and in the glomerulo (panel G). Magnification was 40x (panels C and F) and 63x magnification (panel G).

adrenal gland where the human cells were injected (Figure 4D), while the kidneys of the injected mouse, as compared to the naive mouse (Figure 4A, B and C), appeared slightly hydropic (Figure 4D) showing signs of focal glomerulonephritis (4E), involving the tube (4F) and the glomerulo (4G). In addition, the spleen of the injected mouse was greatly enlarged, as compared to a naive mouse

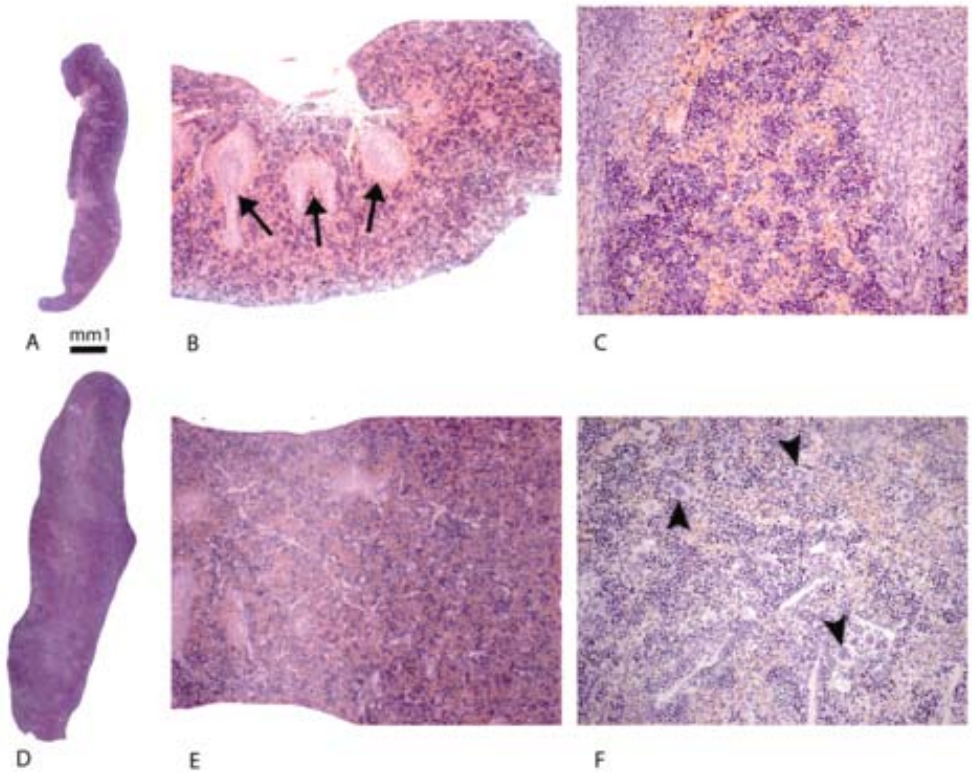

Figure 5. Spleen of a naive (panels A, B and C) and of an injected mouse (panels D, E and F). Sections, stained with Mayer's Ematossilin, at 1x (A and D), 4x (B and E), and 20x magnification (C and F). Arrows indicate white substance (panel B). Arrowheads indicate myeloid precursors (panel F).

(Figure 5D and 5A, respectively), and the white component present in normal spleen (Figure 5B) was almost absent (5E), because of an intense metaplasia (Figure 5F), involving the myeloid component (Figure 5C).

Thus, the anatomical modifications in the kidneys and spleen detected by MRI were fully confirmed by the histopathological analysis. Possibly these anatomical alterations were the result of cytokines/growth factors and albumin present in the cell suspension [8] that was not extensively washed with serum-free medium to limit loss of injectable cells.

CONCLUSIONS

The MRI monitoring, performed at 15 days interval, was safe and well tolerated and our results confirmed its great soft-tissue resolution. Among different imaging techniques, MRI is rarely employed because it is cost- and time-consuming and demands substantial expertise. However, MRI has two advantages over techniques that use radionuclides or optical probes. They are the higher spatial resolution (micrometers rather than several millimeters) and the fact that physiological and anatomical information can be extracted simultaneously, as demonstrated in the case of the renal alterations in our mouse. Thus, MRI may be the method of choice to *in vivo* monitor the effects of cells present in limited number in human specimens, whose *in vitro* manipulation or expansion is not recommended.

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