A dynamic process of macrophage infiltration during tumor progression

Y-H. Hsu[1], C-M. Shih[1], S-S. Lin[1], K-P. Chow[2], and C. Chang[1]

[1]Institute of Biomedical Sciences, Academia Sinica, Taipei, Taiwan, [2]Department of Microbiology and Immunology, Chang-Gung University, Taoyuan, Taiwan

Synopsis

The aim of the present study is to provide insights into the role of tumor-associated macrophages (TAMs) by employing superparamagnetic iron oxide (SPIO) nanoparticles-enhanced MRI to longitudinally trace the spatial and temporal evolution of TAM infiltration during tumor progression. Our data demonstrated that TAMs facilitated the tumor neovascularization by incorporating into and/or coordinating the new vessel formation and TAM infiltration was accompanied by *in situ* tumor mass expansion, implying sequential TAM recruitment resulting in stepwise tumor outgrowth.

Introduction

Solid tumors comprise not only malignant cells but also macrophages infiltrating from peripheral blood circulation. These TAMs may be up to 50% of the tumor mass [1]. Clinical and experimental evidence has suggested a contribution of TAMs to tumor cell proliferation and angiogenesis. Although it is postulated that TAMs may facilitate tumor neovascularisation in response to the stimuli in avascular tumor areas, the underlying mechanisms are still unclear [2]. A serial assessment of the distribution of TAMs in tumor mass can provide an invaluable insight of the *in vivo* role of TAMs during tumor progression. However, conventional histological analysis is limited to sampling from different individuals each at a particular fixed time point, providing insufficient information about the dynamic process of TAM infiltration. To elaborate the spatial and temporal distribution of TAM in the same neoplasm during tumor progression, the aims of the present MRI study are 1) to longitudinally trace the fate of TAMs after intravenous injection of SPIO particles into tumor-bearing mice [3]; 2) to histologically clarify if SPIO are internalized by TAMs; 3) to map the distributions of TAMs and blood vessels/proliferating tumor cells at different stages of tumor development.

Materials and Methods

Solid tumor was first generated by subcutaneous injection of malignant LMP1-transfected BALB/c3T3 cells into SCID mice [4], followed by transplantation of fragmented grown tumor into BALB/c mice. The transplantation of tumor fragments from the tumor-bearing BALB/c mice was routinely performed to other normal BALB/c mice for model maintenance [5]. The day of tumor transplantation was designated as Day 0. All MRI experiments were performed using a 7 Tesla PharmaScan 70/16 (Bruker, Germany). The tumor-bearing mice (n = 6) at Day 5 were first scanned for control images by T2WI using RARE sequence (TR/TE = 5000/60 ms, RARE factor = 8, and NEX = 4) and T2*WI using 2D FLASH sequence (TR/TE = 600/13 ms, NEX = 2, and flip angle = 15°). The SPIO particles (30 mg/kg; Feridex IV, Berlex Laboratories, USA) were administrated via ophthalmic vein. T2WI and T2*WI were then applied immediately post SPIO, and at Day 7, 9, 11, 14, and 21. All images were transverse and obtained using a FOV of 3.0 cm, a slice thickness of 1 mm, and a 256*128 matrix that was zero-filled to 256*256. The mice were perfused with 4 % paraformaldehyde after MRI, and the tumors were paraffinized and sectioned at 5 μm. H&E staining was used for correlating the tumor morphology to the images, while Prussian blue (PB) staining was applied to detect the SPIO in tumors. Antibodies against CD68 and CD31 were utilized to identify TAMs and examine the tumor vascular network, respectively.

Results and Discussion

All tumor fragments exhibited gradual growth after implantation (Fig. 1). Both T2 and T2* images showed a hypointensity (red arrow) in one side of the implanted tumor after SPIO injection at Day 5. At Day 7, a budding of new tumor mass was observed nearby the hypointensity found at Day 5, along with a newly-detected hypointensity (white arrow). This pattern, early-detected hypointensity leads to new tumor mass generation and additional hypointensity in nearby area, appeared to be repeated from Day 7 to 21. Moreover, the regions with high tumor proliferation were found to be composed of multiple hotspots of TAM accumulation, developing from inner to outer area during tumor progression (Fig. 1). These results suggested sequential TAM recruitment from peripheral vessels resulting in stepwise tumor outgrowth. The tumors showing hypointensity post SPIO were collected to clarify if MR hypointensity was resulted from the SPIO (Fig. 2A). PB-stained sections confirmed a high spatial correlation between the MR hypointensity and the SPIO particles (blue spots) (left panel in Fig. 2B). The enlarged views clearly demonstrated the existence of cells internalized SPIO particles in tumor tissue. Interestingly, from Day 7, the SPIO-labeled cells were often found in proximity to hemorrhages (right panel in Fig. 2B), indicating a relationship between SPIO-labeled cells and the newly-formed vessels that have lost hierarchy. Immunostaining further proved that at Day 14, these SPIO-labeled cells were CD68-positive TAMs (Fig. 3A). The spatial relationship between TAMs and tumor neovascularisation was further examined by histology. Our data showed that the SPIO-labeled TAMs were frequently observed in the leading edge of CD31-positive vessel lumen (Fig. 3B), implying that TAMs may facilitate the tumor neovascularization by incorporating into and/or coordinating the new vessel formation [6].

Conclusion

Our results demonstrate that the *in vivo* dynamics of TAM infiltration during tumor development could be readily traced by SPIO-enhanced MRI. The images obtained over time showed that TAM infiltration is discontinuous in a sporadic fashion. The localization of these hotspots appears to progress repeatedly with stepwise tumor outgrowth. By histological examination, this may be mainly due to the infiltrated TAMs act as coordinators to facilitate neovascularization which promotes tumor cell proliferation.

References

1. Murdoch, et al., (2004) *Blood. 104: 2224-34.* **2.** Lewis, et al., (2006) Cancer Res. 66: 605-12. **3.** Rogers, et al., (2006) *Nat Clin Pract Cardio vasc Med. 3: 554-62.* **4.** Li, et al., (1996) *Oncogene. 12: 2129-35.* **5.** Singh, et al., (1992) *J Exp Med. 175: 139-46.* **6.** Anghelina et al., (2006) *Am J Pathol. 168: 529-41.*

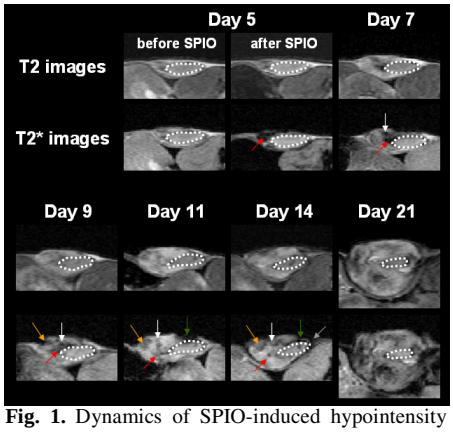

Fig. 1. Dynamics of SPIO-induced hypointensity during tumor development. Dot lines, the location of implanted tumor fragment. Colored arrows, the different waves of SPIO-induced hypointensity.

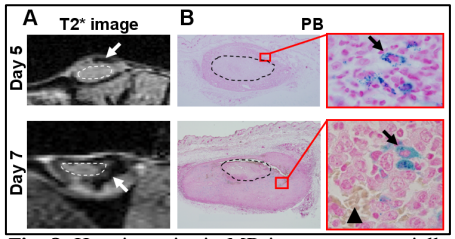

Fig. 2. Hypointensity in MR images was spatially correlated well with SPIO-labeled cells in tumor. **(A)** T2* images showed the hypointensity (arrows) in tumor post SPIO. **(B)** PB staining revealed the existence of SPIO (blue reactions) in tumors. The enlarged views (red squares) showed the cells with SPIO (arrows). Arrow heads, hemorrhages.

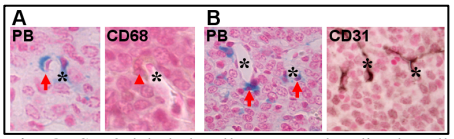

Fig. 3. SPIO-labeled cells were colocalized well with CD68-postive TAMs and CD31-positive blood vessels in tumor at Day 14. Two adjacent sections were used. Arrows, SPIO-labeled cells (blue). Arrow heads, CD68-positive cells (brown). Stars, CD31-positive vessels (brown).