Noninvasive monitoring of orthotopic glioblastoma therapy response using RGD-conjugated iron oxide nanoparticles

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Abstract
Noninvasive imaging techniques have been considered important strategies in the clinic to monitor tumor early response to therapy. In the present study, we applied RGD peptides conjugated to iron oxide nanoparticles (IONP-RGD) as contrast agents in magnetic resonance imaging (MRI) to noninvasively monitor the response of a vascular disrupting agent VEGF121/rGel in an orthotopic glioblastoma model. RGD peptides were firstly coupled to IONPs coated with a crosslinked PEGylated amphiphilic triblock copolymer. In vitro binding assays confirmed that cellular uptake of particles was mainly dependent on the interaction between RGD and integrin $\alpha_v\beta_3$ of human umbilical vein endothelial cells (HUVEC). The tumor targeting of IONP-RGD was observed in an orthotopic U87 glioblastoma model. Finally, noninvasive monitoring of the tumor response to VEGF121/rGel therapy at early stages of treatment was successfully accomplished using IONP-RGD as a contrast agent for MRI, a superior method over common anatomical approaches which are based on tumor size measurements. This preclinical study can accelerate anticancer drug development and promote clinical translation of nanoprobes.

1. Introduction
The detailed information of tumor progression in response to therapy is important to improve patient selection for specific treatment strategies and guides adaptation of treatment at an early stage [1]. Anatomical approaches based on measurements of tumor size are extensively applied for assessing therapy response so far, but significant limitations exist, such as the presence of tumors that cannot be measured, poor measurement reproducibility, and mass lesions that persist following therapy [2]. Noninvasive imaging techniques are emerging more and more as important tools to monitor response to therapies with novel mechanisms of action, often predicting the success of therapy before conventional measurements have changed [3,4]. The development of advanced imaging strategies, although still challenging, not only allow the detection and monitoring of tumor development, but also facilitate a broad understanding of the cellular and molecular events that propagate tumor angiogenesis, as well as those occurring in response to therapy.

A highly versatile device in monitoring tumor progression and therapy response is magnetic resonance imaging (MRI), since this technique provides a high spatial resolution and excellent contrast of opaque soft tissue [5]. However, the low sensitivity of MRI often reduces the success of imaging approaches. Recently, iron oxide nanoparticles (IONPs) have shown their suitability for use as sufficiently high tissue contrast agents for MRI in terms of their intrinsic properties and versatile surface functionality [6–9]. Among these applications, the detection of initial and further development of tumors using IONPs as contrast agents is of particular interest. Glioblastoma (GBM) is the most common form of primary brain tumor, and its aggressive nature and evasiveness...