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SDF-1 Blockade Enhances Anti-VEGF Therapy of Glioblastoma and Can Be Monitored by MRI[1,2]

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Abstract

Despite the approval of antiangiogenic therapy for glioblastoma multiforme (GBM) patients, survival benefits are still limited. One of the resistance mechanisms for antiangiogenic therapy is the induction of hypoxia and subsequent recruitment of macrophages by stromal-derived factor (SDF)-1α (CXCL-12). In this study, we tested whether olaptesed pegol (OLA-PEG, NOX-A12), a novel SDF-1α inhibitor, could reverse the recruitment of macrophages and potentiate the antitumor effect of anti–vascular endothelial growth factor (VEGF) therapy. We also tested whether magnetic resonance imaging (MRI) with ferumoxytol as a contrast agent could provide early information on macrophage blockade. Orthotopic human G12 glioblastomas in nude mice and rat C6 glioblastomas were employed as the animal models. These were treated with bevacizumab or B-20, both anti-VEGF antibodies. Rats were MR imaged with ferumoxytol for macrophage detection. Tumor hypoxia and SDF-1α expression were elevated by VEGF blockade. Adding OLA-PEG to bevacizumab or B-20 significantly prolonged the survival of rodents bearing intracranial GBM compared with anti-VEGF therapy alone. Intratumoral CD68+ tumor associated macrophages (TAMs) were increased by VEGF blockade, but the combination of OLA-PEG + VEGF blockade markedly lowered TAM levels compared with VEGF blockade alone. MRI with ferumoxytol as a contrast agent noninvasively demonstrated macrophage reduction in OLA-PEG + anti-VEGF–treated rats compared with VEGF blockade alone. In conclusion, inhibition of SDF-1 with OLA-PEG inhibited the recruitment of TAMs by VEGF blockage and potentiated its antitumor efficacy in GBM. Noninvasive MRI with ferumoxytol as a contrast agent provides early information on the effect of OLA-PEG in reducing TAMs.

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Introduction

Despite the recent approval of bevacizumab for treatment of glioblastoma (GBM), the survival of such patients is still poor. GBMs treated with bevacizumab invariably progress, and first-line use of bevacizumab did not improve overall survival in two randomized trials [1,2]. Thus, therapies overcoming resistance or evasion of VEGF blockade are urgently needed [3].

Bevacizumab, an antibody blocking vascular endothelial growth factor (VEGF), exerts its function by inhibiting tumor angiogenesis [4]. Studies have shown that antiangiogenic therapy can induce tumor hypoxia and lead to an influx of bone marrow–derived cells, including macrophages, into the tumors and facilitate therapeutic resistance [5,6]. Tumor-associated macrophages (TAMs) are known to promote tumor growth by their proangiogenic action [7–9].

We have previously demonstrated that hypoxia is responsible for macrophage infiltration of tumors postirradiation by upregulating

Abbreviations: GBM, glioblastoma multiforme; OLA-PEG, olaptesed pegol; TAMs, tumor-associated macrophages; VEGF, vascular endothelial growth factor.

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stromal cell–derived factor 1-α (SDF-1α) (CXCL-12) and that tumor response can be enhanced by blocking the SDF-1 pathway [10]. Olaptesed pegol (OLA-PEG, previously known as NOX-A12) is a novel PEGylated mirror-image RNA oligonucleotide with high binding affinity to SDF-1. We previously showed the inhibitory effect of OLA-PEG on SDF-1α–dependent migration of monocyte *in vitro* and the prolonged survival of rats with autochthonous brain tumors treated with the drug combined with irradiation [11]. Because tumor irradiation blocks or severely limits local angiogenesis [12], we asked the question using two GBM models of whether blocking the SDF-1 pathway would increase the therapeutic efficacy of anti-VEGF therapy, which also targets angiogenesis,

Ferumoxytol, a product containing ultrasmall superparamagnetic iron oxide nanoparticles, is an FDA-approved iron supplement for anemic patients. As it can be phagocytosed by TAMs and imaged by magnetic resonance (MR) [13,14], we also investigated whether it could be used to noninvasively image by MR imaging (MRI) changes in TAM levels in tumors produced by anti-VEGF therapy combined with SDF-1 blockage.

In the present study, we found that SDF-1 blockade could potentiate the therapeutic effect of anti-VEGF therapy in GBM animal models by inhibiting macrophage recruitment and further reduces tumor vasculature. To provide a clinically relevant early therapeutic evaluation, we also found that reduction in macrophage influx by OLA-PEG could be noninvasively imaged by MRI with ferumoxytol as a contrast agent.

Materials and Methods

Tumors and Animals

GBM12 (G12), a serially passaged human glioblastoma, was a generous gift from Dr. Jann Sarkaria (Mayo Clinic, MN) and was passaged as previously described [15]. A total of 300,000 G12 cells were implanted intracranially into nude mice (NCI Frederick, MD). Rat C6 cells were obtained from ATCC and were authenticated by them. A total of 500,000 cells were injected intracranially into Sprague-Dawley rats purchased from Charles River. Tumor cells were injected into the brain as previously described [10]. All animal procedures were approved by Stanford University's Administrative Panel on Laboratory Animal Care. For survival analysis, animal numbers are as follows: for G12 (bevacizumab) experiment: G12 control (n = 5), OLA-PEG (n = 5), Beva (n = 6), Beva + OLA-PEG (n = 6); for C6 (B-20) experiment: C6 control (n = 8), treated with OLA-PEG (n = 10), B-20 (n = 14), or B-20 + OLA-PEG (n = 14)

Drug Treatment

Because of the different tumor models and different anti-VEGF antibodies, treatment regimens differed in dose and time points in the two models. OLA-PEG (a generous gift from NOXXON Pharma AG, Germany) was prepared as previously described [11], and the solution was injected subcutaneously every other day at a dose of 10 mg/kg until animal sacrifice. Bevacizumab (Genentech, CA) was injected intraperitoneally at a dose of 5 mg/kg, twice a week, based on a previous report [16]. B-20 (Genentech, CA) was given once, at a dose of 5 mg/kg. B-20 is a cross-species VEGF-blocking antibody [17,18]. For mice with G12 tumors, treatment was started 14 days after cell implantation; for rats with C6 tumors, OLA-PEG was started 6 days and B-20 7 days after implantation. Necrotic tumors were not harvested when collecting samples.

MRI Assessment of Intracerebral Macrophage Influx

MRI of C6 tumors was performed 7 days after treatment initiation with a 1-T animal MR scanner (Bruker ICON) located in the Stanford Small Animal Imaging Center. MRI scans were taken prior to and 24 hours after intravenous injection of 27.92 mg/kg (0.5 mmol [Fe]/kg) ferumoxytol. Ferumoxytol was a generous gift from Dr. Heike E. Daldrup-Link (Stanford University, CA).

Coronal T2-weighted RARE pulse sequences were obtained with a 180° flip angle, a 3300-millisecond repetition time (TR), and a 120-millisecond echo time (TE). T1 relaxation time was obtained with a T1-FLASH-T1mapIR sequence with a 218.7-millisecond TR, a 6.0-millisecond TE, and multiple increasing inversion time (TI) of 25 to 200 milliseconds. T2 relaxation time was obtained with T2map-MSME sequence with a 1500-millisecond TR and multiple echo of 17 to 119 milliseconds. T2* relaxation time was obtained with Flash-bas sequence with 325.7-millisecond TR, an increasing echo of 9 to 54 milliseconds, and a 30° flip angle. All sequences (2-10 acquisitions) were acquired with a field of view of 27 × 27 mm, a matrix of 160 × 160 pixels, and a slice thickness of 1.0 mm.

The acquired MR images were transferred as DICOM images and processed by the ParaVision 5 software. Average signal intensities of the whole tumor, tumor rim, and tumor center as well as background noise were measured by operator-defined regions of interest. T2-relaxation times of the tumor were calculated based on T2map-MSME sequences and converted to R2-relaxation rates (R2. 1/T2), which is proportional to contrast agent concentration. The relative change in R2 (ΔR2) between pre- and postcontrast MRI was determined as a quantitative measure of tumor contrast enhancement. In addition, T1 and T2* relaxation rates of the tumors were calculated from the multi-TI and multiecho pulse sequences using nonlinear function least square curve fitting on a pixel-by-pixel basis. T_1 relaxation times were calculated using inversion recovery sequences with a fixed TR of 218.7 milliseconds, a TE of 6.0 milliseconds, and increasing TI of 25 to 200 milliseconds. T_2^* relaxation times were calculated with a fixed TR of 325.7 milliseconds and increasing TEs of 9 to 54 milliseconds. The relaxation times of the tumors were derived by region of interest measurements of the tumors on the resultant T1, T2, and T2* maps, and the results were converted to R1, R2, and R2* relaxation rates (s − 1).

Immunofluorescent Staining and Quantification

Endothelial cells were stained by rat anti-mouse CD31 for G12 tumors at a 1:200 dilution (BD Biosciences). Macrophages were stained with a dilution of 1:400 rabbit anti-mouse/rat CD68 (Abcam). SDF-1α antibody (Santa Cruz Biotechnology) was used at a concentration of 1:100. Tumor hypoxia was evaluated by staining with rabbit anti-human hypoxia inducible factor (HIF)-1α (Abcam, 1:100 dilution). Frozen sections were stained with primary antibodies overnight in a cold room, and Alexa Fluor 488 anti-rat or Alexa Fluor 555 anti-rabbit antibodies were used for secondary antibodies where appropriate (Life Technologies, 1:500 dilution) for 1 hour at room temperature. Immunofluorescent images were acquired as previously described [10]. Only intratumoral area images were acquired by identifying the nuclei density difference. Signal pixels were measured by Image-Pro Plus (MediaCybernetics) using a ×20 objective with ×10 eyepieces of the fluorescence microscope.

Statistical Analysis

Statistical analysis was performed as previously described [10]. Student's t test was used when appropriate. $P < .05$ was considered