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RAPID COMMUNICATION

Drug-induced amplification of nanoparticle targeting to tumors

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Abstract Nanomedicines have the potential to significantly impact cancer therapy by improving drug efficacy and decreasing off-target effects, yet our ability to efficiently home nanoparticles to disease sites remains limited. One frequently overlooked constraint of current active targeting schemes is the relative dearth of targetable antigens within tumors, which restricts the amount of cargo that can be delivered in a tumor-specific manner. To address this limitation, we exploit tumor-specific responses to drugs to construct a cooperative targeting system where a small molecule therapeutic modulates the disease microenvironment to amplify nanoparticle recruitment *in vivo*. We first administer a vascular disrupting agent, ombrabulin, which selectively affects tumors and leads to locally elevated presentation of the stress-related protein, p32. This increase in p32 levels provides more binding sites for circulating p32-targeted nanoparticles, enhancing their delivery of diagnostic or therapeutic cargos to tumors. We show

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that this cooperative targeting system recruits over five times higher doses of nanoparticles to tumors and decreases tumor burden when compared with non-cooperative controls. These results suggest that using nanomedicine in conjunction with drugs that enhance the presentation of target antigens in the tumor environment may be an effective strategy for improving the diagnosis and treatment of cancer.

Introduction

Nanotechnology has enabled numerous novel and improved approaches for cancer diagnosis and therapy. In particular, active targeting of nanoparticles, or the attachment of affinity ligands to the surface of particles to recognize and bind pathological markers, has arisen as an attractive strategy to precisely deliver cargos to disease sites while simultaneously reducing side effects [1]. Efforts to improve the targeting of nanomaterials have largely focused on engineering the properties of individual nanoparticles, including geometry, surface chemistry, ligand type, and ligand density [2—4]. However, one major factor that limits the effectiveness of active targeting is the paucity of targetable antigens available for nanoparticle binding within a tumor [5]. A promising approach for overcoming this limitation is to leverage disease responses to therapy to greatly enhance the number of existing binding sites, or induce the presentation of novel targets. Previously, localized treatments such as radiation [6] and hyperthermia [7,8] have been used to induce the expression of vascular antigens that serve as binding targets to recruit nanoparticles to tumors. Unfortunately, application of these methods is confined to clinical scenarios where disease sites are known and accessible, and thus preclude the treatment of disseminated disease, which is the primary cause of mortality in cancer [9].

Our strategy is to identify proteins that are selectively induced in the tumor microenvironment following treatment with drugs and leverage them as receptors for targeted nanoparticles. The arsenal of systemic therapies designed to treat metastatic cancer includes traditional cytotoxic drugs [10], molecularly targeted agents [11,12], immunotherapy [13,14], and vascular disrupting agents (VDAs) [15,16], which operate through distinct modes of action. These drugs are attractive inducing agents because many are clinically-approved or in trial stages and are frequently administered in combination with other therapeutics [11,12,17]. Drug-induced antigens have been utilized as biomarkers of therapeutic responses or as antibody targets [18—20], but to our knowledge, these changes have never been used to target nanoparticles to tumors. Here, we investigate the ability of systemically administered drugs to increase the prevalence of tumor-specific antigens to amplify nanoparticle targeting to tumors. In this report, we demonstrate that the small molecule VDA, ombrabulin, enhances the presentation of a stress protein called p32 in human tumor xenografts implanted in mice (Figure 1). p32 is specifically expressed in tumors and serves as the target receptor of the cyclic nonapeptide, LyP1, which was discovered through *in vivo* phage display [21,22]. We then use ombrabulin to induce greater levels of p32 in tumors and deliver two different LyP1-decorated nanoparticles to tumors: a prototypical imaging agent (magnetofluorescent iron oxide nanoworms) and a prototypical therapeutic agent (doxorubicin-loaded liposomes). We show that this cooperative targeting system amplifies the recruitment of targeted nanoparticles to tumors by three- to five-fold over non-cooperative controls, and improves the tumor burden and survival of mice in a preclinical therapeutic study.

Results

Characterization of ombrabulin-induced p32 presentation

Ombrabulin is a microtubule-binding agent that impacts tumor vasculature by effecting a rapid sequence of events shortly after administration, including morphological and functional changes in endothelial cells that increase vascular permeability, and culminate in extensive hemorrhagic necrosis within the tumor [15,23,24] (Figure 2A and B). Widespread extravasation of red blood cells into the tumor interstitium was observed within 1 h, and central necrosis was evident between 6 and 24 h following drug administration (Figure 2C). The selective vulnerability of tumor vessels to tubulin-binding compounds has been attributed to their immature development and defective pericyte coverage relative to normal vessels [15,16]. We hypothesized that the antitumor activity of ombrabulin might increase tumor presentation of p32 [p33/gC1q receptor/hyaluronan binding protein 1 (HABP1)], a mitochondrial protein that is found at elevated levels on the surface of stressed tumor and tumor-associated cells in a wide range of tumor types, particularly in hypoxic or nutrient-deprived regions [22]. To investigate the ability of ombrabulin to increase p32 presentation within tumors, we intravenously injected different doses of the drug (0, 30, 60 mg/kg) into nude mice ($n = 3$ mice per condition) bearing bilateral human MDA-MB-435 tumors. At 4 and 24 h, mice were euthanized and p32 presentation was assessed *via* immunofluorescent staining of tumor sections (Figure 2D). We observed that the percentage of p32-positive staining within the tumor trended upward with both time and ombrabulin dose, showing nearly a four-fold increase in p32-positive area at 24 h after a 60 mg/kg dose (Figure 2E). To assay for increased p32 presentation at the surface of surviving cancer cells, tumors were dissociated into single cell suspensions for quantification of p32 surface expression by live-cell staining and flow cytometry (Figure S1). Consistent with the trend observed in tumor sections, flow-based examination of the tumor population revealed that the percentage of cancer cells positive for surface p32 increased significantly by up to four-fold at 24 hrs after ombrabulin treatment ($^{***}P < 0.005$ by one-way ANOVA with