Vascular changes in tumors resistant to a vascular disrupting nanoparticle treatment

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ARTICLE INFO

ABSTRACT

Anti-angiogenic and vascular disrupting therapies rely on the dependence of tumors on new blood vessels to sustain tumor growth. We previously reported a potent vascular disrupting agent, a theranostic nanosystem consisting of a tumor vasculature-homing peptide (CGKRK) fused to a pro-apoptotic peptide [$_D$(KLAKLAK)$_2$] coated on iron oxide nanoparticles. This nanosystem showed promising therapeutic efficacy in glioblastoma (GBM) and breast cancer models. However, complete control of the tumors was not achieved, and some tumors became non-responsive to the treatment. Here we examined the non-responder phenomenon in an aggressive MCF10-CA1a breast tumor model. In the treatment-resistant tumors we noted the emergence of CD31-negative patent neovessels and a concomitant loss of tumor homing of the nanosystem. In vivo phage library screening in mice bearing non-responder tumors showed that compared to untreated and treatment-sensitive tumors, treatment sensitive tumors yield a distinct landscape of vascular homing peptides characterized by over-representation of peptides that target αv integrins. Our approach may be generally applicable to the development of targeted therapies for tumors that have failed treatment.

1. Introduction

All vertebrate tissues depend on blood circulation, and that includes tumors, which require angiogenesis as an essential step in tumor initiation and progression [1,2]. In the past decades, several therapies have been developed that specifically block tumor angiogenesis [3–6]. Some of the anti-angiogenic drugs have already been approved for the treatment of solid tumors, such as breast cancer [7–9]. The blood vessel-directed therapies can act synergistically with conventional chemotherapies and tend to have non-overlapping toxicities. However, like all anticancer therapies, anti-angiogenic agents and vascular disrupting agents (VDAs) trigger compensatory changes that undermine the efficacy. For example, current anti-angiogenic and VDA therapies preferentially eliminate immature tumor blood vessels, leaving mature vessels intact and even improving their function [10–12]. Some of the molecular markers that distinguish tumor vessels from normal vessels disappear as a result of the therapy, making it difficult to target the remaining vessels for destruction [13]. However, development of affinity probes to target tumors that have failed treatment and become

resistant has received little attention.

Recently we reported a novel tumor vessel-disrupting nanosystem consisting of three elements: (i) a tumor-homing 5-amino acid peptide (CGKRK) that specifically delivers its payload to the mitochondria of tumor endothelial cells and tumor cells, (ii) a pro-apoptotic peptide [$_D$(KLAKLAK)$_2$] that disrupts mitochondria, and (iii) multivalent presentation of the peptides on iron oxide nanoparticles, which greatly enhances the activities of the peptides [14,15]. The nanoparticles, dubbed nanoworms (NWs) because of their elongated shape [14], showed therapeutic efficacy against lentiviral-induced glioblastoma models and a MCF10-CA1 breast cancer model [15,16]. Nearly complete control was achieved in one glioblastoma model, but in a more aggressive glioma and in the breast cancer model, some tumors escaped the control and become resistant to the therapy.

In vivo phage display of peptides is used to discover for agnostic discovery of homing peptides specific for different pathologies including tumors, atherosclerotic plaques, wounds, and severe brain injuries [17–21].

Here, we compare the vasculature of MCF10-CA1a breast cancers
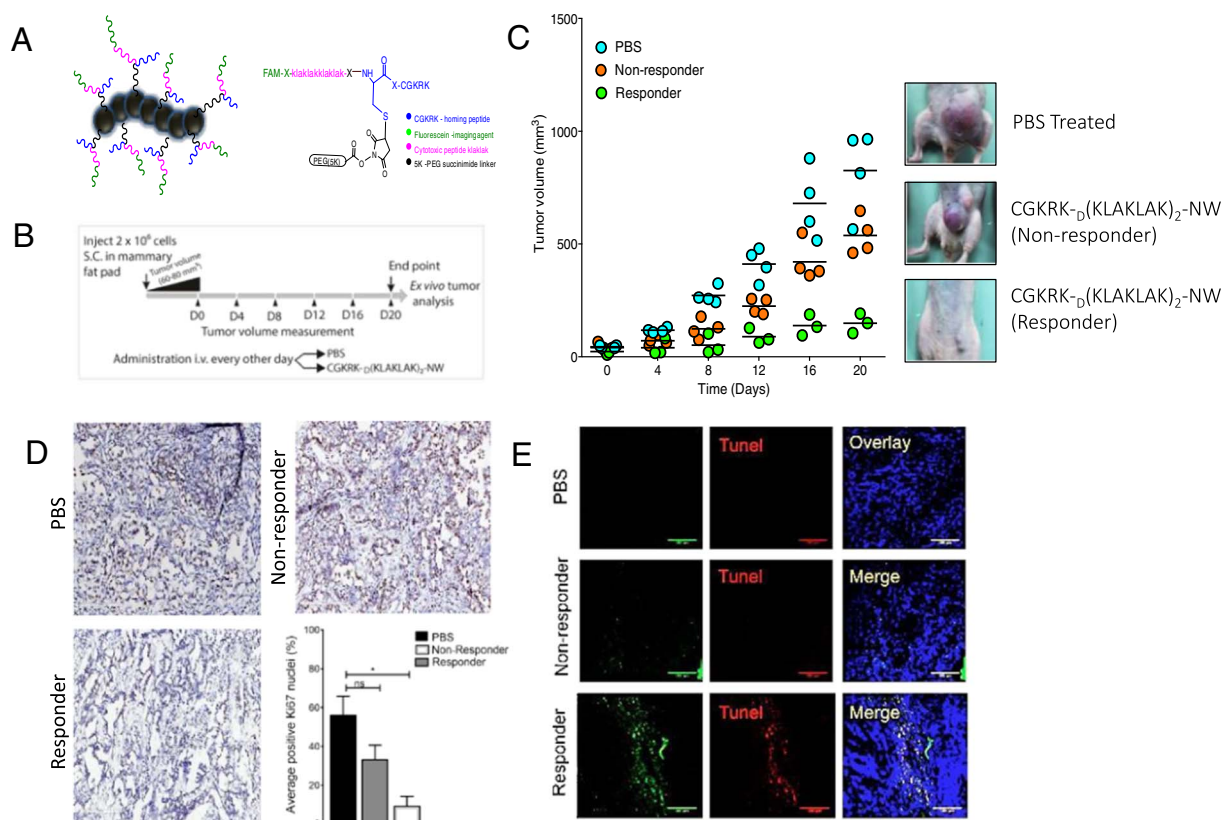

Fig. 1. Design of the theranostic nanosystem and activity in MCF10Ca1a breast tumor model. (A) A chimeric peptide combining a tumor-homing peptide (CGKRK) and a pro-apoptotic peptide is covalently coupled to iron oxide NWs (length 40–50 nm; Park et al., 2008). An extra cysteine was added to the N-terminus of the CGKRK peptide for the NW coupling. The drug peptide and the fluorophor were attached to the free N-terminus of the same cysteine residue. (B) Schematic representation of the treatment regimen. (C) Mice bearing MCF10CA1a orthotopic tumor xenografts were intravenously injected with peptide-coated NWs or PBS every other day for 3 weeks at a dose of 5 mg/kg. PBS, n = 5; CGKRK$_D$[KLAKLAK]$_2$-NWs, n = 7. The tumors were grouped into responder and non-responder tumors. One of three independent experiments with similar results is shown. (D) Sections from the treated tumors were stained with an antibody against proliferating cell nuclear antigen (Ki67). The graph shows the percentage of Ki67 positive nuclei. The results are expressed as a mean $\pm$ SD (*$P < 0.01$, one way ANOVA, Kruskal-Wallis test n = 3 mice per group, scale bar = 200 μm). (E) TUNEL staining of treated tumors. Merged image: NWs, green; TUNEL-positive nuclei, red; DAPI-stained nuclei. Blue. Scale bars, 100 μm. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

that are either sensitive or resistant to the nanosystem. We then use *in vivo* phage display to identify peptides that selectively recognize the vasculature of the treatment-resistant tumors.

2. Results

2.1. Development of therapeutic resistance in breast tumors treated with CGKRK-$_D$[KLAKLAK]$_2$-NWs

The CGKRK-$_D$[KLAKLAK]$_2$-NW-based nanosystem used in this study, and the treatment schedule are schematically depicted in Fig. 1A and B. As has been reported [15,16], the nanosystem therapy resulted in significant overall reduction of tumor volume compared to control-treated mice. The treated mice appeared to fall into two categories, one with very small tumors (and occasional cures), termed the responders, and another with large tumors, the non-responders (Fig. 1C). The tumor volume in these sub-groups correlated with tumor cell proliferation and apoptosis; the responder group showed significantly reduced cell proliferation as measured by Ki67 staining (Fig. 1D), whereas the non-responder group was similar to the PBS-treated group. Conversely, the responder tumors displayed more TUNEL staining than the PBS and non-responder groups (Fig. 1E). This dichotomy in the tumor responses suggested that comparison of the two groups may provide clues to designing strategies that overcome the treatment resistance.

2.2. Vascular changes in therapy-resistant tumors

The nanosystem therapy used here was designed to target and disrupt the tumor vasculature based on the homing properties of the CGKRK peptide. Analysis of the vascular changes after completion of the treatment showed a striking 75% reduction of blood vessels detectable with CD31 staining in the responder tumors relative to the PBS control group (Fig. 2A). The non-responder vascular density was intermediate, and the vessels appeared larger than in the other groups. Perfusion with labeled tomato lectin to map patent vascular structures revealed a different picture. In the PBS- treated and responder groups, most of the CD31 + vessels were also positive for lectin staining (Fig. 2B). In contrast, over 20% of the non-responder vessels were positive for the lectin, but not for CD31 (Fig. 2B, bar graph). In agreement with the lectin results, ultrasound analysis of the non-responding tumors revealed efficient tumor circulation in the non-responder tumors (Fig. 2C).

The presence of lectin-positive, CD31-negative vessels in the non-responder tumors suggested the presence of an alternative circulatory system not based on endothelially-lined blood vessels of mouse origin. We next stained the sections with an anti-human CD31 antibody to examine the possibility that the alternative circulation was derived from the human tumor cells through trans-differentiation into endothelial cells. Such trans-differentiation has been observed in other tumor types [22], including glioblastomas [23,24]. We observed no staining for human CD31 (not shown), but some of the lectin-positive vessels in non-responder tumors stained with human-specific anti-