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Tumor-targeted delivery of liposome-encapsulated doxorubicin by use of a peptide that selectively binds to irradiated tumors

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Abstract

Tumor-targeted drug delivery improves anti-tumor efficacy and reduces systemic toxicity by limiting bioavailability of cytotoxic drugs to within tumors. Targeting reagents, such as peptides or antibodies recognizing molecular targets over-expressed within tumors, have been used to improve liposome-encapsulated drug accumulation within tumors and resulted in enhanced tumor growth control. In this report, we expand the scope of targeting reagents by showing that one peptide, HVGGSSV which was isolated from an *in vivo* screening of phage-displayed peptide library due to its selective binding within irradiated tumors, enabled highly selective tumor-targeted delivery of liposome-encapsulated doxorubicin and resulted in enhanced cytotoxicity within tumors. Targeting liposomes (TL) and non-targeting liposomes (nTL) were labeled with Alexa Fluor 750. Biodistribution of the liposomes within tumor-bearing mice was studied with near infrared (NIR) imaging. In the single dose pharmacokinetic study, the liposomal doxorubicin has an extended circulation half life as compared to the free doxorubicin. Targeting liposomes partitioned to the irradiated tumors and improved drug deposition and retention within tumors. The tumor-targeted delivery of doxorubicin improved tumor growth control as indicated with reduced tumor growth rate and tumor cell proliferation, enhanced tumor blood vessel destruction, and increased treatment-associated apoptosis and necrosis of tumor cells. Collectively, the results demonstrated the remarkable capability of the HVGGSSV peptide in radiation-guided drug delivery to tumors.

Keywords

radiation-guided drug delivery; phage-displayed peptide; liposomes; doxorubicin; lung cancer; xenograft model

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INTRODUCTION

Radiation plus chemotherapy enable a synergistic effects and lead to significant decreases in tumor growth rates, increases in cancer cell apoptosis, and improved clinical outcomes as demonstrated in breast, ovarian, and lung cancers [1]. However, side effects and systemic toxicity limit the tolerable dose of many chemoagents, including doxorubicin and cisplatin [2,3]. Nanocarriers and tumor-targeting ligands have increased drug deposition and retention within tumors, thus improving therapeutic efficacy while reducing toxic off-target effects [4,5]. Here, we demonstrate a new targeting peptide with very specific delivery and its use for targeted adjuvant therapy following radiation.

Targeting tumor-associated antigens for the delivery of nanocarrier-encapsulated drugs leads to higher drug accumulations within tumors and improved therapeutic efficacy [6]. Specific antibodies or peptide ligands can be conjugated to a vehicle loaded with cytotoxic drugs and promote tumor-specific drug uptake. Currently, most of the targeting strategies focus on the molecules that are overexpressed on the cancer cell surface such as prostate-specific antigen (PSA) [7], carcinoembryonic antigen (CEA) [8], epidermal growth factor receptor [9], and folate receptor [10]. However, application of this strategy in clinical treatment of cancer patients is limited due to the heterogeneity of tumors and the accessibility of tumor cell surface targets to the drug delivery vehicle. An alternative strategy is to deliver drugs by targeting the tumor endothelium. The tumor endothelium is morphologically and physiologically different from normal endothelium. For example, angiogenic markers such as VEGFR is expressed at levels 2-5 times higher in tumors than in normal tissue [11]. Targeting the tumor endothelium shows promise in the battle against cancer because it is readily available for binding from the blood stream [12-15]. In addition, destruction of the endothelial cells has been shown to arrest tumor growth and lead to tumor cell death [16,17]. Although targeting the tumor vasculature has generated encouraging results, exploration of this strategy is limited by the small number of validated tumor-specific endothelial biomarkers.

Phage display technology has been extensively applied to uncover new biomolecular targets [18]. A library of diversified peptides displayed on the surface of phage particles can be biopanned over a desired target regardless of whether the target is a purified molecule, molecular complex, cell or whole organ [19-22]. The physical linkage between the displayed entity (phenotype) and its genetic code (genotype) permits for fast decoding of the primary sequence of the isolated peptides. Phage display showed promises in identification of peptide probes that distinguish physiologically or pathological altered tissues from the normal tissues. Radiation induces new protein expression and improves accessibility for molecular-targeting [23,24]. By applying the *in vivo* screening of phage-displayed peptides against irradiated tumors, we isolated a panel of peptides that selectively bind to the irradiated tumors. Among those peptides, HVGGSSV distinguishes irradiated tumors from untreated tumors and normal tissues within multiple tumor models [25]. The selective binding of the HVGGSSV peptide suggests that this peptide possesses great potential in radiation-guided drug delivery to tumors. This work utilizes the HVGGSSV peptide to lead liposome-encapsulated doxorubicin to irradiated tumors, elevate the drug deposition and retention within the irradiated tumors, and improve tumor growth control.

MATERIALS AND METHODS

Preparation of liposomes

Preparation and drug-loading of the liposomes were carried out as described [26-28]. Briefly, liposomes were made with cholesterol and 1,2-Distearoyl-sn-Glycero-3-Phosphocholine (DSPC) at a molar ratio of cholesterol:DSPC=45:55. Maleimide-PEG2000-

DSPE (1,2-Distearoyl-sn-Glycero-3-Phosphoethanolamine-N-[Maleimide(Polyethylene Glycol)2000]) or Amine-PEG2000-DSPE (all from Aventi Polar Lipids, Inc. Alabaster, AL) were included in liposomes (2% of total phospholipids for each) for conjugating a cysteine-containing peptide (Genemed Synthesis Inc., San Antonio, TX) or N-(Succinyl)-Alexa Fluor 750 (Invitrogen, Carlsbad, CA), respectively. Lipids were dissolved in chloroform (10 mg/ml) and mixed in a round bottom flask attached to a rotary evaporator. A thin lipid film formed after the chloroform was evaporated under vacuum. The lipid film was rehydrated in 500 mM of ammonium sulfate with 2 mM of desferrioxamine mesylate (pH 5.5) at 45 °C. Liposomes were subsequently extruded repeatedly through polycarbonate membrane filters with a pore size of 100 nm. Conjugating the liposomes with peptides or Alexa Fluor 750 was conducted as instructed from the manufacture. One targeting peptide [25] (NH₂-GCNHVGGSSV-COOH) and a control peptide with a scrambled amino acid sequence (NH₂-GCSGVSGHGN-COOH) were used to prepare targeting liposomes (TL) and non-targeting liposomes (nTL), respectively. Desalting columns were utilized to change buffers and remove the unconjugated free peptide or dye from the liposomes. The final concentration of liposomes was adjusted to 1 mg/ml. Loading of doxorubicin was driven by the pH gradient generated from the ammonium sulfate within the liposomes. Briefly, doxorubicin (from Sigma, 2 mg/ml in PBS) was mixed with the liposome suspension and maintained at room temperature for 2 hours. Free drug was removed by passing the liposome suspension through a desalting column. The drug concentration was determined with a fluorophotometer (excitation/emission at 480/550 nm) [29].

Cell culture and tumor xenograft models

Murine Lewis lung carcinoma (LLC) and human lung cancer H460 cells were purchased from American Type Culture Collection (ATCC, Manassas, VA) and were maintained in Dulbecco's Modified Eagle Medium (DMEM) cell culture medium supplemented with 10% fetal bovine serum, 1 mM Na pyruvate, and 50 ug/ml penicillin and streptomycin. Tumor cells were subcutaneously implanted (5×10^5 cells per injection) in both hind limbs of eight-week old C57/BL6 Foxn1 null/null nude mice (Harlan Laboratories, Prattville, AL). The tumor models were used for pharmacokinetics and tumor growth studies when the tumor size reached 0.5 cm in diameter. Irradiation of the tumors was conducted with a Therapax DXT 300 X-ray machine (Pantak Inc., East Haven, CT) while other parts of body were shielded. All of the animal works were conducted according to protocols approved by Vanderbilt University Institutional Animal Care and Use Committee (IACUC).

Near infrared imaging

Four hours after irradiation, 100 μ l (1 mg/ml) of the TL or nTL labeled with Alexa Fluor 750 were injected into the circulation *via* the tail vein. Biodistribution of the liposomes within the tumor-bearing mice was monitored with near infrared (NIR) imaging. NIR images were taken with an IVIS imaging system (Xenogen Corp., Hopkinton, MA) at various time points (5, 12, 24, 50, and 72 hours) after the liposomes administration. Radiance (photons/sec/cm²) was measured within the tumor region (region of interest, ROI) using the program provided by Xenogen.

Single-dose pharmacokinetic study

Four hours post irradiation (right tumor, 3 Gy), equal amounts of doxorubicin (1 mg/kg) in the form of the targeting liposomes-encapsulated doxorubicin (TL-Dox) or free doxorubicin (free Dox) were injected into the tail vein of tumor-bearing mice. Mice were sacrificed at various time points (5 minutes, 2, 5, 20, 30, 50 and 72 hours) after the drug administration to collect serum and tumor tissues. Doxorubicin within the plasma and tumors was extracted and quantified by fluorophotometer measurements. Each measurement was normalized with the weight of the tissue [30]. The half life of doxorubicin within the serum was calculated by

plotting the concentration of the drug within the plasma against the time post drug administration. A ratio of doxorubicin distribution within the tumors and serum was calculated to determine the efficiency of the drug delivery.

Tumor growth study

Tumor growth delay study was conducted in murine LLC and human H460 lung cancers implanted within hind limbs of C57/BL6 Foxn1 null/null nude mice. The tumors were treated with radiation alone, radiation plus free Dox, radiation plus nTL-Dox, TL-Dox, or radiation plus TL-Dox. Untreated tumors were used as controls. Each mouse group (n=6) received fractionated radiation at 3 Gy on day 1, 3, 5, 8, and 10 (15 Gy in total) on the right tumor. 100 μ l of PBS, free Dox, nTL-Dox or TL-Dox (1 mg Dox/kg) were administered through tail vein injection on days 2, 4, 6, 9, and 11. Tumor volumes were measured with calipers before irradiation and every other day over the treatment course. The fold change in tumor volume was calculated to determine the therapeutic efficacy of treatments.

Immunohistochemistry

In the treatment course, tumor tissues were recovered from sacrificed mice on day 5, prior to the third radiation fraction. Tissues were paraffin-embedded and sectioned for immunohistochemistry examination. Apoptotic cells were stained with TUNEL (Terminal deoxynucleotidyl transferase dUTP nick end labeling) reagents (DeadEnd Colorimetric TUNEL System, Promega, Madison, WI) and counted from more than five independent fields. Proliferating cells were identified by Bromodeoxyuridine (BrdU) incorporation. Briefly, 150 μ l of BrdU (10 mg/ml in PBS, from BD Biosciences, San Jose, California) were injected into blood vessels of tumor-bearing mice 4 hours before sacrifice. Following sectioning, BrdU-labeled cells were detected with a specific antibody against BrdU (Santa Cruz Biotechnology, Inc., Santa Cruz, CA) and a rhodamine-conjugated secondary antibody (Invitrogen, Carlsbad, CA). Nuclei were counterstained with 4',6-diamidino-2-phenylindole (DAPI). The percentage of proliferating cells among the counted cells was used to determine tumor cell proliferation rates within tumors. To detect the vascular function and density within the tumors, 100 μ l of biotinylated tomato lectin (10 μ g/ml in PBS, from Vector Laboratories, Burlingame, CA) [31] were injected intravenously 1 hour prior the animal sacrifice. After heart-perfusion of 30 ml of PBS and 10 ml of 4% paraformaldehyde, the tumor tissues were retrieved, embedded with paraffin, and sectioned for antibody staining. Blood vessels were detected with rhodamine-labeled anti-CD34 antibody (Vector laboratories), while the functional vessels that had been stained with the biotinylated lectin were detected with a streptavidin-conjugated Alexa Fluor 488 (Invitrogen). The tissue sections were counterstained with DAPI. Cell necrosis was detected with standard hematoxylin and eosin (H&E) staining. The functional blood vessels and necrosis within tumors were quantified upon the collected images as described [32,33] for statistic analysis.

Data analysis and Statistics

Group comparisons were analyzed by ANOVA.

RESULTS

Radiation-guided liposomes delivery to tumors

Upon the observed preferential binding of HVGGSSV to irradiated Lewis Lung Carcinoma (LLC) [25], targeting liposomes (TL) and non-targeting liposomes were prepared with the HVGGSSV peptide and a control peptide of scrambled amino acid sequence, respectively. A schematic illustration of the liposomes with peptide and NIR probe conjugation locations is shown in Fig. 1. Nude mice bearing LLC tumors were used as a model to study

biodistribution of the TL and nTL. NIR imaging of the nude mice showed that the dye-labeled lipids were mainly metabolized through the kidney and bladder. Upon the fluorescence signal from whole body of the tumor-bearing mice, it is indistinguishable between the TL and nTL in term of the clearance of the liposomes (data not shown). Compared to the nTL that dispersed throughout the whole body, TL had selectively increased accumulation within the irradiated tumors at 5 and 24 hours post intravenous (i.v.) administration (Fig. 2A). The decrease in fluorescence by 50 hours post injection suggests the retention time of the TL within the irradiated tumor is less than 2 days.

Specificity of the TL to irradiated tumors was further studied in an expanded single dose pharmacokinetic study, in which mice bearing both of irradiated and untreated tumors were used to determine whether the selective accumulation of the TL within irradiated tumors was due to passive dosing or active targeting mediated by the HVGGSSV peptide. LLC tumors were implanted in both sides of the hind limbs of C57/BL6 mice, only the tumors in the right limbs were irradiated while the left tumor within the same animal was used as control. Quantitative fluorescence imaging was conducted at 24 hours post liposome administration (Fig. 2B). The TL with the HVGGSSV peptide had significantly ($p<0.05$) increased accumulation within the irradiated tumors as compared to the non-irradiated tumors. There was no significant difference in term of the nTL accumulation within the irradiated or untreated tumors (Fig. 2C). All these data collectively suggested that accumulation of the TL within the irradiated tumors was a result of the selective targeting effect of the HVGGSSV peptide.

Tumor-targeted liposome delivery resulted in enhanced drug delivery

To further study whether the TL can selectively deliver chemotherapeutic agents to the irradiated tumors, TL-Dox and free Dox were compared in a single dose pharmacokinetic study by use of mice bearing LLC tumors. The half life of the free Dox within plasma was less than two hours, compared to more than 14 hours observed on the TL-Dox (Fig. 3A). This observation is consistent to the previous reports that showed that liposome encapsulated drugs had increased half life within circulation that facilitated tumor-targeted drug delivery [27,34]. Although free Dox reached its highest drug concentration within tumors in the first few hours post administration, the concentration of the drug within the tumors remained similar to that in plasma at most of the time points. Doxorubicin delivered by the TL had improved accumulation and retention within tumors, as indicated by the increasing ratio of doxorubicin within the tumor to that in the plasma (Fig. 3B). The TL-Dox levels within the non-irradiated tumors remained similar to those in the plasma at most of the time points in this study suggesting the enhanced drug delivery and retention was a result of the specific binding of the TL within irradiated tumors, not due to the passive accumulation because of the increased circulation time. Accumulation of doxorubicin within tumors was also directly visualized by the autofluorescence of doxorubicin within tumor sections (Fig. 3C). Doxorubicin fluorescence was more pronounced within irradiated tumors that had been treated with TL-Dox as opposed to the other conditions.

Tumor-targeted drug delivery resulted in more cell death and decreased cell proliferation within tumor

Doxorubicin prevents cell proliferation and induces apoptosis. Therefore, in a therapeutic study with mice bearing LLC tumors, tumor tissues were retrieved after two doses of Dox and IR for cell proliferation and apoptosis evaluation. Compared to the other treatment groups, radiation combined with TL-Dox resulted in significantly decreased tumor cell proliferation (Fig. 4A&B). Tumors treated with radiation and the TL-Dox had more cell apoptosis than the tumors receiving other treatments (Fig. 4C&D). Apoptotic cells are indicated by arrows in the representative images of the TUNEL staining (Fig. 4C).

Tumor-targeted drug delivery resulted in improved tumor growth delays and blood vessel destruction within tumor

Therapeutic efficacy of the tumor-targeted delivery of doxorubicin was determined by the change in tumor volume over the treatment course. In both of the LLC (Fig. 5A) and H460 (Fig. 5B) tumor models, similar tumor growth control was observed. The TL-Dox alone had no significant therapeutic benefit on the tumor growth control. Although radiation alone significantly suppressed the tumor growth, its effect on the tumor growth control was farther enhanced by the combination with the TL-Dox. Combination of the free Dox or nTL-Dox with radiation did not further enhance the therapeutic efficacy of the radiation.

In addition to the tumor growth study, LLC tumors were retrieved after the treatment course to determine the functionality of tumor-associated blood vessels and tumor cell necrosis. IR and drug treatment resulted in dysfunctional blood vessels (stained as green in Fig. 5C) among the tumor-associated blood vessels (stained as red in Fig. 5C). Shutdown of the blood vessels within the TL-Dox and IR-treated tumors lead to extensive necrosis (marked with dashed lines in Fig. 5E) of tumor cells. Compared to the tumor treated with free Dox and IR, or the nTL/Dox and IR, the tumors treated with TL-Dox and IR had few functional blood vessels (Fig. 5D) and more necrotic tumor cells (Fig. 5F)

DISCUSSION

This study demonstrated that the liposome-encapsulated doxorubicin was selectively delivered to the irradiated tumors by use of a phage display-derived peptide. This TL-Dox had a long circulation half life and enhanced doxorubicin deposition within the tumors such that it last for more than 20 hours post the intravenous administration of the drug when 2-fold or higher doxorubicin were detected within the irradiated tumors than those within the blood stream. The NIR imaging and single dose pharmacokinetic studies suggested that the selective accumulation of liposome-encapsulated doxorubicin was resulted from the peptide-targeted delivery instead of the passive dosing effect. The improved drug accumulation within tumors resulted in measurable increases in tumor cell apoptosis and necrosis, and reductions in cell proliferation, blood vessel functionality and tumor growth rates. The tumor-specific delivery was regulated by spatially and temporally delivered radiation. The untreated tumors did not accumulate the HVGGSSV-decorated liposomes. These data, in combination with previous works demonstrating the specificity of the HVGGSSV peptide in multiple tumor models [25], suggests specificity of the tumor-targeted drug delivery can be modulated with the phage display-derived peptide that selectively binds to the irradiated tumors.

Radiation plays important roles in the clinical treatment of most cancer patients, and the biological effects extend beyond direct cytotoxicity. Radiation increases vessel permeability by inducing expression of several inflammatory molecules. This phenomenon has been explored for targeting drug delivery and enhanced drug release [23,24,35-37]. Compared to traditional targeted drug delivery that utilizes molecular targets over-expressed within tumors [19,38,39], the radiation-guided drug delivery provides possibilities to concurrently improve targeting selectivity and intratumoral drug deposition efficiency with the spatially and temporally controlled delivery of radiation. The HVGGSSV peptide was isolated from *in vivo* screening of a phage-displayed peptide library against irradiated tumors, it demonstrated robust selectivity to the irradiated tumor across multiple cancer models [25]. The peptide did not bind to irradiated or LPS-inflamed normal tissues, and showed basal level accumulation within untreated tumors. Recently, Tax-interacting protein 1 (TIP-1) was identified as the molecular target that enables the peptide binding within irradiated tumors [40]. Radiation induces translocation of the predominantly intracellular TIP-1 protein onto the plasma membrane surface within cancer cells. Studies also showed that TIP-1

translocation on the cell surface is not associated with radiation-induced inflammation. These data suggested that radiation-guided drug delivery by use of the HVGGSSV peptide would have minimal off-target effects in the radiation tract unlike targeting the radiation-induced inflammatory molecules.

Most of the conventional chemotherapeutic agents lack selectivity to cancer cells, the poor bioavailability within tumor and non-specific distribution of the drug throughout the whole body introduced systemic toxicity and limited effective treatment of the patients [2,3]. Drugs encapsulated within nano-sized vehicles can be passively accumulated within tumor through the leaky tumor, by prolonged circulation time and the Enhanced Permeability and Retention effect [6]. However, tumor-limited drug deposition and retention is more efficient by active targeting with tumor-specific antibodies or peptides [4,5,34,41]. As evidenced with immunohistochemistry studies on the tumor tissues, the improved drug deposition within the irradiated tumors by the targeting liposomes resulted in increased cell apoptosis and decreased cell proliferation. Direct cytotoxic effects of doxorubicin cause cell apoptosis and decrease proliferation rate. Additionally, the dramatic reduction of the functional blood vessels within the IR plus TL-Dox treated tumors may also increase the number of apoptotic cells. Over the whole treatment course, the IR plus TL-Dox showed the best inhibitory effect on the tumor growth.

In contrast to the significantly elevated cell apoptosis, necrosis and reduced blood vessel functionality, TL-Dox and IR treatment only moderately affected tumor growth as compared to other treatment groups. The modest tumor growth control with the radiation-guided and tumor-targeted drug delivery may be attributed to the tumor models utilized in this study. The high sensitivity of the LLC and H460 tumor models to radiation alone hindered the ability to signify the full therapeutic potential of the radiation-guided tumor-targeted drug delivery. Also, the collapse of the tumor-associated blood vessels following the first drug dose could hinder subsequent drug delivery. We envision that more significant tumor growth control effects will be observed on radioresistant tumor models. Likewise, to better illustrate the therapeutic efficacy, a single administration of a high dose of the TL-Dox would be desirable to avoid effect of vessel collapse on the subsequent drug delivery [42]. Alternatively, lower IR doses can be delivered to induce the HVGGSSV peptide binding by concurrent treatment of the tumor with tyrosine kinase inhibitors (TKIs) [25]. The lower IR dose will highlight the therapeutic efficacy of the radiation-guided drug delivery. In fact, combination therapies with synergistic effects, such as the HVGGSSV drug delivery and TKIs, are desirable in delivering treatment to tumors. Therefore, in future studies we will investigate the effects of concurrent TKI therapy and alternative dosing schedules in a radioresistant model.

In summary, this study demonstrated that the phage display-derived hexapeptide HVGGSSV enabled radiation-guided selective drug delivery to tumors. Unique specificity of the peptide as illustrated within multiple tumor models suggests promising potentials of the peptide in tumor-targeted drug delivery.

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Figure 1.

Schematic of the liposome: 100 nm liposomes were prepared containing both maleimide and amine functionalized PEG chains. The maleimide group was used to attach the peptide through the thiol group on a cystine. The amine was used to conjugate a fluorescent AlexaFluor 750 to the liposome. Therapeutic liposomes were loaded with doxorubicin.

Figure 2.

Targeted liposomes (TL) localized in the irradiated tumors. Intensity of the AlexaFluor 750 correlates to the local concentration of liposomes. (A) Nude mice bearing LLC tumors on both hind limbs received 3Gy irradiation on the right tumor only (indicated by the arrow). Enhanced TL accumulation could be detected 5 hrs after the liposome injection and persisted through 24 hrs post injection. By 50 hrs post injection the differential accumulation was no longer evident. Non-targeting liposomes (nTL) did not accumulate in either the irradiated or the untreated tumors. (B) NIR images of the TL and nTL within C57BL/6 mice bearing LLC tumor on both hind limbs that received 3Gy irradiation on the right tumor only. The Radiance (photons/sec/cm²) from the tumor regions (circled) was quantified 24 hrs post liposome injection. (C) Quantification of the liposome accumulation within tumors. A significant accumulation difference was detected between the irradiated and non-irradiated tumors treated with the TL. The nTL controls did not accumulate in either tumor. * $p < 0.05$, $n = 5$, the Student t -test.

Figure 3.

TL loaded with doxorubicin increased the circulation life and intratumoral accumulation of doxorubicin. (A) By measuring the fluorescent signature of doxorubicin, the circulation half life is increased nearly 7-fold when encapsulated in a TL (TL-Dox) as opposed to administrated as a free drug (free Dox). (B) The ratio of drug within the tumor to those within the serum increases over time for TL-Dox within irradiated tumors. There are minimal concentration differences between the tumor and plasma compartment for TL-Dox in non-irradiated tumors or free Dox. (C) Direct imaging of the tumor illustrates the increased doxorubicin fluorescence in tumors receiving both irradiation and TL-Dox.

Figure 4.

Proliferation and apoptosis within tumor tissues. (A) Proliferation of cancer cells within tumor tissues was detected with *in vivo* incorporation of BrdU. The proliferating cells were stained as red and nuclei as blue with DAPI. (B) Quantification of the proliferating cells. More than 100 cells were counted in each of 10 independent fields to calculate ratio of the BrdU-positive cells. Average $\pm$ standard deviation were shown. (C) Apoptosis detected with TUNEL assay. The apoptotic cells were indicated with arrows. (D) Quantification of the apoptotic cells. Scatter plot showed the number of the apoptotic cells in 10 independent fields, median $\pm$ interquartile were presented. * $p < 0.05$, $n = 10$.

Figure 5.

Therapeutic effect of the radiation-guided tumor-targeted drug delivery. Fold change of the (A) LLC and (B) H460 tumors in the treatment course. The TL-Dox was compared to the nTL-Dox and Free Dox to show the TL-Dox was more efficient to improve tumor growth control efficacy of ionizing radiation. (C) Blood vessel function. TL-Dox plus IR resulted in more efficient blood vessel shutdown within H460 tumors. Functional blood vessels were detected by intravenously administrated lectin (green) while tumor-associated blood vessels (red) were stained with antibody against the Von Willebrand Factor (vWF). The cell nuclei (blue) were stained with DAPI. For each of the treatment groups, two representative images (C) and fractions of the functional blood vessels (D) from 10 independent fields were shown. (E) H&E staining to show the necrotic cells within the tumor tissues. The necrosis areas were marked with dashed lines in the representative images. Highlight of the necrotic areas are shown in the images of high magnification in the lower panel. Fractions of the necrotic area within the imaged fields (F) showed the significant effect of the combined treatment with TL-Dox and IR on tumor cell necrosis. ** $p < 0.01$; * $p < 0.05$, $n = 10$, the student's t -test.