Cells Designed to Deliver Anticancer Drugs by Apoptosis

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ABSTRACT

We describe a new drug delivery strategy that uses genetically engineered endothelial cells (ECs) to deliver drugs to tumor cells by apoptosis. Immortalized ECs were genetically engineered to express a flk-1:fas fusion protein. Exposure to the flk-1 ligand, vascular endothelial growth factor (VEGF), which is overexpressed by many tumors, these cells underwent extensive apoptosis. These apoptotic ECs, when loaded with drug, promote drug delivery by phagocytosis of drug-loaded apoptotic bodies by the tumor cells and by increased drug transport through the more permeable apoptotic membrane. In the current study, severe combined immunodeficient mice bearing solid tumors that expressed high levels of VEGF were treated either intratumorally or i.v. every 4 days for a total of five doses with saline control, free Taxol, and immortalized ECs expressing the flk-1:fas fusion protein (imEC/HFF) loaded with Taxol (imEC/HFF-T). Intratumoral treatments also included imEC/HFF and imECs loaded with Taxol (imEC-T). Tumor size was monitored for a minimum of 44 days. Whether administered intratumorally or i.v., imEC/HFF-T cells produced greater inhibition of tumor growth than all other treatments, including Taxol. It was noteworthy that 5 of 16 of the imEC/HFF-T-treated animals were tumor free at the termination of the studies, compared with 2 of 16 animals treated with Taxol. A cell distribution experiment showed that flk-1:fas fusion protein expression ECs as well as parental ECs accumulated in tumor and spleen with the highest level, followed by liver, lung, kidney, and brain. Significant apoptosis of flk-1:fas expression cells was observed in tumor, apparently driven by VEGF secreted from tumor cells. Apoptosis-induced drug delivery offers a new avenue for targeted drug delivery research that uses biological control mechanisms.

INTRODUCTION

Delivery of drugs to tumors continues to be a formidable challenge because of the inability to selectively and uniformly target the desired therapeutic agent to tumor cells. These pitfalls can in part be attributed to the inability of current drug delivery strategies to respond to tumor-specific events that would enhance drug delivery. For instance, liposomal and drug-conjugate systems deliver their payload through chemically controlled variables, such as polymer degradation or hydrolysis of drug-carrier bonds. A new drug delivery strategy is presented based on the use of apoptosis as a biological mechanism to control delivery of drugs from carrier to tumor cells.

Apoptosis of drug-loaded carrier cells could result in delivery of drugs to tumor cells by initiation of phagocytosis, by enhancement of drug transport through a permeable apoptotic membrane. In the current investigation, severe combined immunodeficient mice bearing solid tumors that expressed high levels of VEGF were treated either intratumorally or i.v. every 4 days for a total of five doses with saline control, free Taxol, and immortalized ECs expressing the flk-1:fas fusion protein (imEC/HFF) loaded with Taxol (imEC/HFF-T). Intratumoral treatments also included imEC/HFF and imECs loaded with Taxol (imEC-T). Tumor size was monitored for a minimum of 44 days. Whether administered intratumorally or i.v., imEC/HFF-T cells produced greater inhibition of tumor growth than all other treatments, including Taxol. It was noteworthy that 5 of 16 of the imEC/HFF-T-treated animals were tumor free at the termination of the studies, compared with 2 of 16 animals treated with Taxol. A cell distribution experiment showed that flk-1:fas fusion protein expression ECs as well as parental ECs accumulated in tumor and spleen with the highest level, followed by liver, lung, kidney, and brain. Significant apoptosis of flk-1:fas expression cells was observed in tumor, apparently driven by VEGF secreted from tumor cells. Apoptosis-induced drug delivery offers a new avenue for targeted drug delivery research that uses biological control mechanisms.

MATERIALS AND METHODS

Chemicals. Paclitaxel was obtained from Mead-Johnson (Taxol® Injection; Bristol-Myers Co., Princeton, NJ). Fas polyclonal antibody and SV40 monoclonal antibody were obtained from Santa Cruz Biotechnology, Inc. (X-20; Santa Cruz, CA). PARP monoclonal antibody was purchased from Oncogen (Oncogen Research, Cambridge, MA). ApopTag Plus used for the detection of apoptosis via the TUNEL method was from Oncor (Gaithersburg, MD). scid mice (12–18 g) used for the efficacy study were provided by the Laboratory Animal Facility of Fox Chase Cancer Center. Matrigel was obtained from Becton Dickinson (Medford, MA).

Construction of flk-1:fas cDNA and Transfection. Two flk-1:fas plasmids were constructed, pEGFP-N3/flk-1:fas and pcDNA3- Hygro/flk-1:fas. The 3′ region of the extracellular domain (282–2571) of flk-1 (kindly provided by Dr. J. G. Flanagan Harvard University, Cambridge, MA) was fused to the 5′ end of the fas transmembrane and intracellular domain (515–1027), which was obtained by reverse transcription-PCR from S49 mouse lymphoma cells (ATCC, Rockville, MD). A linker nucleotide sequence (AGA TGG CGA) that codes for three amino acids was inserted between these two fragments. The flk-1:fas cDNA was cloned into either pEGFP-N3 (Clontech, Palo Alto, CA) or pcDNA3-Hygro (provided by Dr. A. Godwin, Fox Chase Cancer Center) vectors. An end-cod signal was added to the fragment in the pcDNA3-Hygro/flk-1:fas plasmid. The pEGFP-N3/flk-1:fas plasmid was transfected into bovine pulmonary arterial ECs (CCL209; ATCC) using a calcium phosphate protocol (3), and stable colonies were selected by selection in G418 (400 μg/ml). The pcDNA3-Hygro/flk-1:fas and pEGFP-N3/flk-1:fas plasmids were transiently transfected into imECs (see below) using the calcium phosphate protocol. The flk-1:fas fusion protein was detected by Western blot analysis using a fas antibody.

Cell Culture. ECs were cultured in DMEM containing 10% fetal bovine serum. ECs transfected with the pEGFP-N3/flk-1:fas plasmid and imECs were cultured in DMEM containing 10% FBS and 400 μg/ml G418. The human glioma cell line, SF188/V+, which overexpresses VEGF (4), was maintained in DMEM containing 10% FBS and 400 μg/ml G418. All cell lines were maintained at 37°C in a humidified atmosphere of 5% CO2.

TUNEL Assay. VEGF-induced apoptosis was detected by a standard method (ApopTag Plus; Oncor) using the TUNEL method. Briefly, ECs that stably expressed the flk-1:fas fusion protein were treated with either 50 ng/ml of recombinant VEGF (Sigma Chemical Co., St. Louis, MO) or 3% SF188/V+ conditioned medium for 0, 8, and 24 h. Cells were collected by trypsinization and combined with the dead cells floating in the medium and then fixed by 7% formalin solution. Slides were prepared by cytoospin and then processed according to the product instructions. The slides were analyzed by fluorescent oxynuclease I-mediated dUTP nick-end-labeling (scid, severe combined immunodeficient; ATCC, American Type Culture Collection; EC, endothelial cell; imEC, immortalized EC; HFLC, high-performance liquid chromatography; SC, saline control; SRR, sulfathalidine B.)
microscopy (Zeiss III RS Germany), with the bright blue fluorescent cells counted and expressed as the percentage of apoptotic cells.

**Cell Growth Inhibition Assay.** A standard SRB assay (5) was used to evaluate VEGF-induced cell growth inhibition. Parental ECs, EC/pEGFP-N3 (vector only), and EC/N3FF (stable flk-1:fas expressing) cells (i.e., 500) in 200 µl of DMEM were plated in 96-well microplates and incubated overnight. After the attachment period, 100 µl of various dilutions (i.e., 0.001–11.11% v/v) of SF188/V+ conditioned medium were added to each well and then incubated for 6 days at 37°C in 5% CO₂,95% air. The cells were then fixed in 10% trichloroacetic acid and stained by SRB (4% SRB in 1% acetic acid). After removal of the unbound SRB by washing, bound SRB was dissolved in 10 molar Tris buffer, and the absorption at 570 nm was measured in a microplate reader (model 3550; Bio-Rad, Hercules, CA). Cell survival was calculated as an absorption ratio compared with a blank.

**Western Blot Analyses.** Western blot analysis was used to detect the protein expression of flk-1:fas, SV40, and PARP. Total protein was extracted from cells by lysis buffer (50 mM HEPES, 150 mM NaCl, 1.5 mM MgCl₂, 100 mM NaF, 1 mM EGTA, 10% glycerol, 1% Triton X-100, 0.2 mM sodium orthovanadate, 10 mM sodium pyrophosphate, 1% phenylmethylsulfonyl fluoride, and 1% aprotinin). Protein concentrations were quantitated using a standard method (6), and then equal amounts of protein were subjected to SDS-PAGE on 4–15% Tris-glycine gel (Bio-Rad). Separated proteins were transferred to nitrocellulose membranes, blotted with their corresponding antibodies, and detected by ECL (Amersham, Arlington, IL).

**PARP Cleavage in flk-1:fas-expressing ECs.** PARP (a substrate of caspase 3) cleavage was used to confirm fas-mediated apoptosis of EC/N3FF or imEC/HFF cells after treatment with VEGF. Briefly, either parental or transfected cells were incubated with or without 3% SF188/V+ conditioned medium for 0, 2, 6, and 24 h. Cellular protein (including the floating cells in the medium) were extracted by lysis buffer as noted above. PARP proteins were blotted with a PARP monoclonal antibody and detected with enhanced chemiluminescence. The PARP antibody recognized both the intact M₅ 116,000 PARP molecule and a M₅ 85,000 degradation fragment.

**Immortalized ECs.** ECs were immortalized by transfection of SV40 large T antigen using pSV3-neo plasmid (ATCC) using a calcium phosphate protocol (3). Forty-eight hours after transfection, the medium was replaced by G418-containing medium (400 µg/ml), and different colonies were selected and expanded in culture. Western blot analysis was used to confirm SV40 protein expression in the stable clones. imECs were further characterized by measuring their growth rate, response to VEGF stimulation, and tumorigenicity in SCID mice. imECs were transiently transfected with the pcDNA3/A TPRT antigen using pSV3-neo plasmid (ATCC) using a calcium phosphate protocol. Cells (1 × 10⁶/ml) were incubated with Taxol at a concentration of 110 µg/ml for 2.5 h at 37°C. Taxol-loaded imECs and imEC/HFF cells are referred to as imEC-T and imEC/HFF-T, respectively. After incubation, the cells were collected by centrifugation, followed by two cold PBS washes, and a final centrifugation. Each cell pellet was resuspended in 800-1000 µl of saline, and 50 µl of the cell suspension were analyzed for total cellular protein (6) and intracellular Taxol concentration. The quantity of Taxol loaded into the cells was determined by a HPLC method. To prepare samples for HPLC analysis, the cell suspension was deproteinized by adding three volumes of methanol, and a supernatant was obtained by centrifugation. Aliquots (50 µl) of the supernatant were injected onto an HPLC system that consisted of an ODS C₁₈ column (Hypersil, 5 µm, 150 mm × 4.6 mm; Alttech, Deerfield, IL) and a mobile phase of 45% acetonitrile in water. Taxol was detected at 230 nm and quantitated based on peak areas.

**Animal Efficacy Studies.** SF188/V+ glioma cells (2 × 10⁶ cells/mouse) were implanted s.c. in the right back flank region of scid mice. All treatments were repeated every 4 days for a total of five doses. Tumor volume and body weight were monitored every 4 days until the tumor reached ~10% of the body weight in the SC treatment group (i.e., 44 days intratumoral group; 56-day i.v. group). Tumor volume was calculated as: tumor volume = width² × length/2. Statistical comparisons of tumor volume were made for each treatment at each monitoring time using a nonparametric Wilcoxon test with P < 0.05 indicative of statistical significance.

**Tissue Distribution and Apoptosis in Vivo.** Parental ECs and EC/N3FF (stable flk-1:fas expressing) cells were labeled with PKH26 as described previously (1). Briefly, either 10 million parental ECs or EC/N3FF cells were collected by trypsinization, washed with serum free medium, pelleted by centrifugation, and then resuspended to 2 ml of serum-free medium. The cells were labeled with PKH26 according to the manufacturers’ specifications. The labeling time was 2 min. After labeling, the labeled cells were diluted with an equal volume of serum and washed (five times) with complete medium and transferred to a new tube after each wash. The final cell pellet was resuspended in complete medium and used for the in vivo tissue distribution study.

SF188/V+ glioma cells (2 × 10⁶ cells/mouse) were implanted s.c. in the right back flank region of scid mice as described above. Fifteen days after implantation, the tumor grew to ~250 mm³ (10 mm × 10 mm) in size. Either labeled ECs or EC/N3FF were administered through a tail vein (one million cells/mouse) over 1 min to groups of four mice each. Two mice each were sacrificed at 10 min and 4 h after cell administration in each group. The liver, brain, lung, kidney, spleen, and tumor were removed immediately; one-half of each organ was freshly frozen in liquid nitrogen and stored at ~80°C until sectioned at 5-µm thick and analyzed by fluorescence microscopy for cell localization. The other half of each tissue was fixed in 10% neutral buffered formalin, and 5-µm-thick paraffin-embedded sections were made. Apoptosis was detected with the TACS TdT in situ Apoptosis Detection kit (R&D Systems, Inc., Minneapolis, MN). The apoptotic cells were labeled blue.

**RESULTS**

**VEGF-induced Apoptosis of ECs Expressing flk-1:fas Protein.** Our interest in developing ECs into AIDD systems was based on their potential ability to maintain high transgene expression and their ability to localize to active sites of tumor neovascularization (8, 9). The goal of these studies was to develop genetically engineered ECs that would apoptose in response to VEGF. A pEGFP-N3FF plasmid was constructed that consisted of the extracellular domain of flk-1, a VEGF receptor (10, 11), and the transmembrane and intracellular domains of fas (12). After transfection using a calcium phosphate protocol and selection in G418, stable transfecteds were obtained that expressed the flk-1:fas:EGFP fusion protein (Fig. 1, cell line referred to as EC/N3FF) at M₅ 250,000. EC/N3FF cells underwent appreciable apoptosis in the presence of VEGF (Fig. 2), with ~80% of the cells apoptotic at 24 h. Similar extent of apoptosis was achieved in the presence of either recombinant VEGF or conditioned medium obtained from human SF188/V+ glioma cells. VEGF-mediated cytotoxicity of EC/N3FF cells is shown in Fig. 3. A pronounced concentration effect is observed with as little as 0.5% (v/v) VEGF-conditioned medium causing 50% of the cells to die. VEGF had a converse and expected growth-stimulating action on parental ECs and vector-alone transfected ECs. PARP cleavage is an indicator of the fas apoptotic signal transduction pathway that uses caspase 3 (13, 14). It was found that EC/N3FF cells demonstrated VEGF-induced PARP cleavage (Fig. 4) after exposure to VEGF. Even in the absence of VEGF, there is a small amount of PARP degradation (Fig. 4, Lane 5), yet this is rapidly and significantly increased in the presence of VEGF at 2 and 6 h (Fig. 4, Lanes 6 and 7). Both parental and vector-transfected ECs (Fig. 4, Lanes 1–4) are virtually absent of PARP cleavage in the presence of VEGF, even at long incubation times. These data support a fas-mediated cell death pathway in EC/
N3FF cells. In summary, ECs expressing a novel flk-1:fas fusion protein (EC/N3FF) undergo apoptosis in response to VEGF, a growth factor secreted by tumors.

The senescent nature of ECs and the constitutive expression of the flk-1:fas fusion protein made working with EC/N3FF cells difficult and prompted the development of imECs. The rapid proliferation of imECs (i.e., doubling time of 34 h versus 140 h for parental ECs at the same passage) allowed large numbers of cells to be obtained and facilitated conduction of animal efficacy studies. ECs were transfected with a pSV3-neo plasmid containing SV40 large T antigen cDNA, and stable transfectants were selected with G418. Western blot analysis confirmed high expression of SV40 in numerous clones (data not shown). These cells were not transformed after 40 passages, had not lost contact inhibition, and were nontumorigenic in scid mice. Thus, it was feasible to use imECs in animal efficacy studies without the potential to compromise interpretation of tumor size as an end point.

The imECs were transfected with a pcDNA3-hygro/FF plasmid containing the flk-1:fas cDNA (i.e., designated imEC/HFF), and transient expression of the flk-1:fas fusion protein was assessed by Western blot analysis. Expression of flk-1:fas was time and VEGF dependent in imEC/HFF cells (Fig. 5). Reductions in flk-1:fas protein expression in the presence of VEGF likely reflects the ability of these cells to undergo apoptosis and, accordingly, lose protein expression. Similar to their nonimmortalized precursors, the imEC/HFF cells also exhibited PARP cleavage after exposure to VEGF, consistent with an intact fas signal transduction cascade (Fig. 5).

Animal Efficacy Studies Demonstrate Superior Activity of Taxol-loaded imEC/HFF Cells. Two separate efficacy studies, one using intratumoral administrations and the other using i.v., were con-
ducted in scid mice bearing s.c. human SF188/V+ tumors. Intratumoral administrations bypass rapid reticuloendothelial cell system clearance and indicate the potential usefulness of using AIDD systems systemically. The human SF188/V+ cell line overexpresses VEGF and was derived and characterized by us (4), based on our in vitro results (Figs. 2, 4, and 5) in which conditioned medium from SF188/V+ cells was shown to trigger apoptosis in flk-1-fas-expressing ECs. In the intratumoral study, multiple-dose treatments were administered on days 5, 9, 13, 17, and 21 and consisted of either a SC, imEC/HFF cells (cells that express the flk-1-fas fusion protein), imEC cells containing Taxol (imEC-T), imEC/HFF containing Taxol (imEC/HFF-T), or Taxol. The mean Taxol dose was essentially equivalent with Taxol doses of 0.40 ± 0.15 mg/kg/dose for the imEC/HFF-T and Taxol groups and 0.38 ± 0.15 mg/kg/dose for the imEC-T group. Because the dose was based on the measured intracellular Taxol concentration determined each day of treatment, there were small variations in the Taxol dose. The mean cell number/dose was 2.2 ± 0.7 million in the intratumoral study. It can be seen (Fig. 6A and Table 1) that the imEC/HFF-T treatments produced the greatest reduction in tumor size. These reductions were statistically significant (P < 0.05) compared with all other treatments in the intratumoral trial beginning on day 8, except when compared with the imEC-T group. Treatment with imEC/HFF-T always produced a greater reduction in tumor size compared with imEC-T, yet only differences on days 8, 12, and 16 were statistically significant, whereas from day 20 until day 44, Ps ranged from 0.06 to 0.14. In the imEC/HFF-T group, 6 of 10 animals had no measurable tumor after three doses, and 4 of 10 remained so throughout the 44-day monitoring period. For all other intratumoral treatments, there was only 1 animal tumor free in the Taxol group, again after three doses. It is of interest that both intratumoral Taxol-loaded cell treatments (i.e. imEC-T and imEC/HFF-T) resulted in greater reductions in tumor volume than Taxol alone. This could be attributed to the cells prolonging the residence time of Taxol in the tumor because of a lower clearance or elimination rate from the tumor compared with Taxol. The greater effect of imEC/HFF-T compared with imEC-T on tumor growth can be attributed to the desired apoptotic induction of drug delivery by enhanced drug release and stimulation of phagocytosis by tumor cells (1). This benefit should be enhanced by using a clonal population of flk-1-fas-expressing cells.

The same multiple-dose treatment schedule was used in the i.v. study as in the intratumoral study; however, only the SC, imEC/HFF-T, and Taxol groups were included in the i.v. study. The mean Taxol dose was 0.44 ± 0.22 mg/kg/dose for both the imEC/HFF-T (mean cell number/dose, 2.1 ± 0.6 million) and Taxol treatment groups. Similar to the intratumoral administration trial, i.v. dosing of imEC/HFF-T produced the greatest inhibition of tumor growth (Fig. 6B and Table 2). However, because of greater variability in tumor size, the differences between the imEC/HFF-T and Taxol groups did not reach statistical significance. There was 1 of 6 animals tumor free in each of the imEC/HFF-T and Taxol treatment groups after administration of the fifth dose. Thus, imEC/HFF-T treatment, either intratumorally or i.v., produced the greatest antitumor response in a xenograft tumor model that overexpressed VEGF.

**Tissue Distribution and Apoptosis of ECs after Systemic Administration.** There were EC/N3FF apoptotic cells in tumor at 10 min and 4 h, yet it was more pronounced at 4 h after systemic administration (Fig. 7A). In the EC group, apoptosis was much lower than in the EC/N3FF group, and only a few apoptotic cells could be seen. Apoptosis of either ECs or EC/N3FF was not observed in other tissues, except for a few apoptotic cells in spleen.

The pattern of tissue distribution of ECs and EC/N3FF in scid mice bearing s.c. tumors was analogous with the greatest accumulation in tumor, followed by the spleen, liver, lung, kidney, and brain. There appeared to be more cell debris or small broken cells in tumor after EC/N3FFF administrations compared with ECs (Fig. 7B).

**DISCUSSION**

AIDD is a new drug delivery strategy that uses apoptosis as a biological mechanism to promote drug delivery from genetically engineered carrier cells to tumor cells. This investigation demon-

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**Table 1 Summary of animal efficacy study after intratumoral treatments**

<table>
<thead>
<tr>
<th>Treatment group</th>
<th>No. of mice</th>
<th>Tumor size (mm³)</th>
<th>No. of tumor-free mice</th>
</tr>
</thead>
<tbody>
<tr>
<td>SC</td>
<td>10</td>
<td>1800 ± 181.5 (837.7–2658.8)</td>
<td>0/0</td>
</tr>
<tr>
<td>imEC/HFF</td>
<td>6</td>
<td>1904.3 ± 244.4 (1622–3106.8)</td>
<td>0/0</td>
</tr>
<tr>
<td>imEC/HFF-T</td>
<td>10</td>
<td>60.5 ± 40.2 (4.0–433.4)</td>
<td>6/4</td>
</tr>
<tr>
<td>Taxol</td>
<td>10</td>
<td>160.5 ± 273.4 (23.9–2380.8)</td>
<td>1/1</td>
</tr>
<tr>
<td>imEC-T</td>
<td>5</td>
<td>272.7 ± 190 (16.4–1098.1)</td>
<td>0/0</td>
</tr>
</tbody>
</table>

*Mean ± SE (range) for all measurable tumors.

*Number of mice without measurable tumors at the end of five doses/number of mice without measurable tumors at the end of the 44-day observation period.
strated three important aspects of AIDD that will provide a basis for future research:

(a) It was shown that ECs expressing a growth factor receptor:cell death domain fusion protein, flk-1:fas, underwent apoptosis upon activation by the growth factor, VEGF. This attribute supports the development of other engineered cells that apoptose in response to other growth factors that may be dominant in tumor progression. On the basis of our interest in brain tumors and the significant correlation between brain tumor growth and expression of VEGF (15, 16, 17), the current cell system was designed to use VEGF as the apoptotic trigger. The fusion protein receptors not only function to mediate apoptosis but may also serve as decoy receptors by interference of growth factor binding to native receptors located on ECs. In this manner, the decoy receptors may limit unabated neovascularization attributable to the mitogenic action of growth factors on normal ECs. Distinguishing a unique decoy receptor action for an AIDD system, such as imEC/HFF-T, is difficult because of the confounding effect of the cytotoxic agent on tumor growth. On the basis of the positive tumor growth observed after imEC/HFF (Fig. 6), there does not seem to be a decoy receptor function; however, only about 35–40% of the cells expressed the flk-1:fas fusion protein. A decoy receptor function for AIDD systems may be possible in stable expression systems planned for the future.

(b) A second important aspect of the investigation was that therapeutic amounts of a cytotoxic drug were delivered to solid tumors by a cell carrier. The quantity of drug that can be delivered to the tumor by the cell system is dependent on many variables. Of primary importance will be the initial amount of drug loaded into the cells, the ability to limit drug leakage from the cells outside the tumor and nonspecific apoptosis (see below), and the ability of the drug-loaded apoptotic cells to promote their uptake by tumor cells. Initial drug loading was achieved by a simple incubation of Taxol with the imECs that will likely be applicable to other lipophilic drugs. Alternate procedures, such as electroporation, may be applied to hydrophilic compounds. A phenomenon referred to as nonspecific apoptosis may have limited the potential advantage of AIDD after i.v. administration. Nonspecific apoptosis is apoptosis of drug-carrier cells induced by the loaded cytotoxic drug rather than specific apoptosis induced by the desired growth factor and could cause drug release outside the tumor.

Table 2. Summary of animal efficacy study after i.v. treatments

<table>
<thead>
<tr>
<th>Treatment group</th>
<th>No. of mice</th>
<th>Tumor size (mm³)</th>
<th>No. of tumor-free mice</th>
</tr>
</thead>
<tbody>
<tr>
<td>SC</td>
<td>6</td>
<td>1188 ± 194.7</td>
<td>0/0</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(664.8–1929.2)</td>
<td></td>
</tr>
<tr>
<td>Taxol</td>
<td>6</td>
<td>994.2 ± 385.7</td>
<td>0/1</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(697.6–2553.2)</td>
<td></td>
</tr>
<tr>
<td>imEC/HFF-T</td>
<td>6</td>
<td>603.3 ± 303.8</td>
<td>0/1</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(168.8–1656.2)</td>
<td></td>
</tr>
</tbody>
</table>

* Mean ± SE (range) for all measurable tumors.

* Number of mice without measurable tumors at the end of five doses/number of mice without measurable tumors at the end of the 56-day observation period.

Fig. 7. Apoptosis (A) and tissue distribution (B) of PKH26-labeled flk-1:fas fusion protein expression cells (EC/FF) and parental CCL209 ECs after i.v. administration to scid mice bearing s.c. SF188/VEGF tumors. In A, apoptosis is indicated by blue-labeled apoptotic cells in tumor at 10 min and 4 h after administration. In B, fluorescent PKH26-labeled ECs and EC/N3FF are observed in tumor and spleen at 10 min and 4 h after administration.
limiting the quantity available to the tumor. Nonspecific apoptosis may be addressed by the use of prodrugs that require metabolic activation within the tumor cell yet remain inactive in the drug-carrier cell whose enzymatic profile is genetically altered. Thus, inert prodrugs within the carrier cells would not initiate nonspecific apoptosis, permitting greater quantities of drugs to be available to tumor cells.

Of the two mechanisms ascribed to AIDD, enhanced drug release and phagocytosis, phagocytosis has the most potential to increase the selectivity of drug delivery to the tumor because a carrier cell–tumor cell conduit prevents release of the drug into the interstitial fluid and its availability to noncancerous cells. Macrophage recognition of apoptotic cells is a burgeoning research area that will increase the identification of both macrophage receptors and apoptotic cell ligands, such as phosphatidylserine (18). This area of research could provide important input into how AIDD systems could be genetically modified to enhance their recognition and phagocytosis by tumor cells.

The final important aspect of the investigation was the demonstration of favorable antitumor activity of imEC/HFF-T after i.v. administration. The ability of a drug delivery system to target a tumor after systemic administration offers a definitive advantage to systems that require regional administration in terms of ease of use and broad application to tumor cells. AIDD offers three fundamentally unique features that may increase the fraction of the dose available to the tumor after systemic administration.

AIDD is a new drug delivery strategy that uses apoptosis as a biological control mechanism to deliver drugs from carrier cells to tumors. AIDD offers three fundamentally unique features that may result in selective drug delivery to tumors and produce antitumor response: (a) the apoptotic triggering mechanism can promote and direct drug uptake by tumor cells from apoptotic carrier cells; (b) AIDD systems could interfere with tumor angiogenesis if fusion proteins expressed on the carrier cells serve as decoy receptors for growth factors secreted by the tumors; and (c) genetic manipulation of the carrier cells offers many opportunities to optimize selective drug delivery to tumors, such as through the use of enzyme-activated prodrug systems. Clearly, many challenges remain before optimally designed AIDD systems reach the clinic, yet the opportunities afforded through the use of genetic engineering support the future development of AIDD systems.

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