CELL SURFACE RECEPTOR TARGETED LIPOSOMES INHIBIT TUMOR GROWTH *IN VIVO*

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ABSTRACT SUMMARY

We report the inhibition of tumor growth by a synthetic $\alpha_v\beta_3$ integrin antagonist (IA) that is covalently attached to the surface of a dextran-coated liposome (DCL). *In vitro*, these novel IA-DCL conjugates bind to purified integrin with IC_{50} values that are similar to the IA, and IA-DCLs inhibit cell proliferation relative to controls including DCLs lacking IA. In the M21 human melanoma model, IA-DCL conjugates and IA-liposome conjugates were compared to controls including saline, DCLs lacking IA, and cyclo(RGDfV), a cyclic peptide that is similar to Cilengitide, which is in Phase II clinical trials. Intravenous injection of IA-DCLs derived from DPPC resulted in statistically significant differences in tumor volume quadrupling time *in vivo* relative to controls including buffer, nanoparticle lacking IA, and cyclo(RGDfV) ($P<0.05$). Non-statistically significant inhibition of tumor growth was observed for IA-DCLs prepared from DSPC and IA-liposome conjugates relative to controls.

Keywords: angiogenesis, drug targeting, tumor drug delivery

INTRODUCTION

Targeted nanoparticles are an emerging class of therapeutic agents. These agents may overcome current drug delivery limitations, including rapid drug clearance and poor intracellular delivery to target cells. The potential impact of such delivery is increased efficacy due to efficient inhibition of deleterious receptor-ligand interactions, and increased intracellular delivery of a therapeutic payload through receptor-mediated endocytosis.

Critical attributes of targeting nanoparticles are the specificity of the target receptor for diseased tissues and cells. The $\alpha_v\beta_3$ integrin is a promising therapeutic target because it is up-regulated on tumor cells and endothelial cells that comprise tumor vasculature, and less so on normal vasculature.[1-3] Additionally, this integrin is up-regulated during angiogenesis and has proven to be a promising therapeutic target for antibody agonists,[4,5] cyclic RGD peptides,[6] and RGD mimetics attached to liposomes.[7]

Drug delivery by nanoparticles targeted to disease-specific receptors up-regulated on endothelial cells may improve the therapeutic index of new or existing drugs. Mechanisms for improving efficacy may include multivalent display of the targeting agent, and increased pharmacokinetic half-life of the therapeutic agent. Targeting tumor endothelial cells is attractive because they are accessible and genetically more stable than tumor cells.[8] The delivery of anti-angiogenic agents with nanoparticles such as DCLs that are targeted to the $\alpha_v\beta_3$ integrin is therefore a promising anti-cancer strategy.

EXPERIMENTAL METHODS

Preparation of Liposomes All liposomes were prepared by homogenization. Compositions include DPPE-succinate, cholesterol, and either DSPC or DPPC at molar ratios of 5/50/55. Aminodextran was attached to the surface of the liposomes using EDAC coupling chemistry. After purification using tangential flow filtration (TFF), the amino groups were succinylated and the IA was attached to the nanoparticles to generate 100 nm IA-DCL conjugates.

Potency assays Binding to the $\alpha_v\beta_3$ integrin on 96-well plates was demonstrated in a competition assay with a biotin-vitronectin conjugate.[9] IA-DCLs and biotin-vitronectin conjugate were incubated with $\alpha_v\beta_3$ integrin-coated plates blocked with BSA. The plates were washed with buffer, and streptavidin-HRP conjugate was added. The plates were again washed with buffer, LumiGlo substrate (KPL) was added, and chemiluminescence was monitored using a Wallac plate reader.

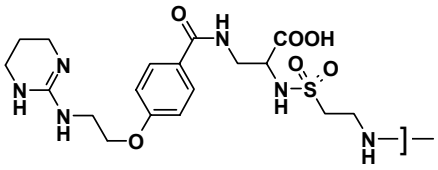
***In vitro* cell proliferation assays** Cell proliferation was assessed *in vitro* by incubating vesicles with human umbilical vein endothelial cells (HUVECs) at 80% confluence and by monitoring cell density over time with MTT.

Tumor growth inhibition *in vivo* The M21 melanoma model was prepared by subcutaneous injection of tumor cells into nude mice. When tumors reached 47-225 cm^3, the mice received four doses of placebo or therapeutic agent by intravenous injection every other day and tumor volume was measured until the tumors had quadrupled in size. Cyclo(RGDfV) was administered by interperitoneal injection. P-values were obtained using Tukey's W procedure with normalized tumor volumes at 13 days post treatment and for tumor volume quadrupling times. IA doses for IA-DCLs containing DPPC (IA-DCL(P)), IA-liposomes (IA-Ls), and cyclo(RGDfV) were 60 mg/kg total IA or cyclic peptide. The dose for IA-DCLs containing DSPC (IA-DCL(S)) was 36 mg/kg total IA. Total DCL or liposome doses were 317, 300, 317, and 350 mg/kg, respectively.

RESULTS AND DISCUSSION

Dextran-coated liposomes are colloidally stable carriers for the covalent attachment of drugs that target cell-surface receptors. The $\alpha_v\beta_3$ integrin antagonist (IA)[10] shown in Fig. 1 was attached at loadings of up to 5% (w/w) and the potency of the IA-DCL conjugate was similar to that observed for unmodified IA. IA-DCLs inhibit the proliferation of HUVECs *in vitro* where the DCL carrier has no effect. A representation of the IA-DCL conjugate is shown in Figure 1B.

A



IA

B

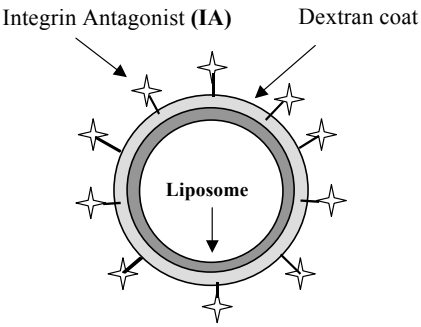


Fig. 1. A. Structure of the $\alpha_v\beta_3$ integrin antagonist. **B.** IA-dextran-coated liposome conjugate.

Statistically significant differences in normalized tumor volumes were observed for IA-DCLs prepared with DPPC in the M21 human melanoma model at total IA doses of 60 mg/kg and 300 mg/kg DCL when compared to buffer and DCL lacking IA (ANOVA, N=8, Tukey's W procedure, $P<0.05$). Comparisons of tumor volume quadrupling times (TVQTs) show statistically significant differences for the same IA-DCL conjugate relative to buffer, DCL, and cyclo(RGDfV), which is similar to Cilengitide, a drug in Phase II clinical trials. TVQTs were 23±8.8, 11.7±2.2, 11.9±2.6, and 14.1±5.0, respectively. Non-statistically significant inhibition of tumor growth was observed for IA-DCLs prepared from DSPC and IA-liposome conjugates relative to the controls. The data are shown in Fig. 2.

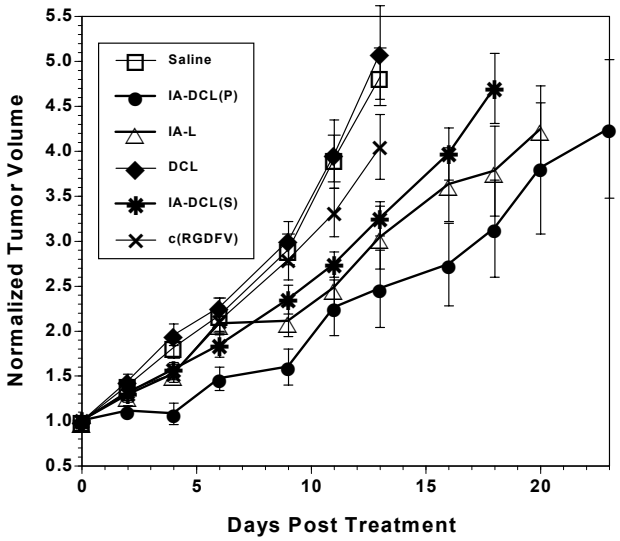


Fig. 2. Inhibition of tumor growth in the M21 human melanoma model in nude mice with IA-DCLs and IA-liposome conjugates. Abbreviations: c(RGDFV), cyclo(Arg-Gly-Asp-D-Phe-Val); DCL, dextran-coated liposome; IA, integrin antagonist; L, liposome; (P), DPPC; (S), DSPC.

CONCLUSION

$\alpha_v\beta_3$ integrin-targeted, dextran-coated liposomes are effective anti-tumor agents. These agents inhibit tumor growth in a human xenograft melanoma model *in vivo*, and inhibit cell proliferation of endothelial cells *in vitro*. These studies demonstrate the ability to enhance the pharmacological properties of small molecules through attachment to DCLs. The unique targeting aspects of IA-DCLs make them a promising new class of anti-angiogenesis agents that warrant further *in vivo* evaluation.

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