EGFR-targeted stearoyl gemcitabine nanoparticles show enhanced anti-tumor activity

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INTRODUCTION
Previously, we reported a novel stearoyl gemcitabine nanoparticle formulation and demonstrated that the nanoparticles were more effective than gemcitabine hydrochloride (HC) in controlling the growth of model mouse or human tumor cell lines, clearly indicating the in vivo efficacy of gemcitabine can be improved using nanoparticles.

Among the earliest growth factors characterized and sequenced, epidermal growth factor (EGF, a 75 kDa androgenic cell surface glycoprotein) has been intensely tied with normal cellular function including cell proliferation, survival, adhesion, migration, and differentiation. More than ten ligands, including EGF, have been distinguished to exhibit strong receptor-ligand affinity towards epidermal growth factor receptor (EGFR). Although EGFR is expressed in a variety of normal cell types, there are numerous studies reporting the over-expression of EGFR in various tumor cells.

In the present study, the feasibility of further improving the anti-tumor activity of the stearoyl gemcitabine nanoparticles by actively targeting them into tumors was evaluated. It was hypothesized that conjugation of EGF on the surface of the stearoyl gemcitabine nanoparticles would increase the delivery of the nanoparticles into tumor cells, and thus, improve the resultant anti-tumor activity.

MATERIALS & METHODS
Stearoyl-gemcitabine-loaded nanoparticles were engineered from lecithin:glycerylmonooleate:in-water emulsion. Considering the nanoparticles have a solid lipid core, the hydrophilic gemcitabine molecule was made lipophilic by synthesizing a previously reported stearic acid amide derivative of gemcitabine, 4-(N-stearoylamino-gemcitabine) and incorporated it into the nanoparticles.

Recombinant murine EGF was conjugated onto nanoparticles by forming a stable thioether group, and preparing the GemC18-NPs with 1,2-distearyloxy-3-(phosphatidyl)-serine (Makalide/cholesterol) (PEG-Makalide). This mixture was then incubated overnight in a nitrogen-enriched atmosphere. Un-conjugated EGF was removed using a Sepharose® 4B column. The purified EGF-conjugated GemC18-NPs are named EGF-GemC18-NPs (Scheme 1). A control to EGF, ovariulin (OW) was conjugated onto the GemC18-NPs to form OVA-GemC18-NPs.

Many cancer cells over-express EGFR. Therefore, anti-cancer activity of the EGF-targeted GemC18-NPs were characterized using human breast adenocarcinoma models, MDA-MB-468, MDA-MB-231, and MCF-7, which express different levels of EGFR (1 x 10^6, 2 x 10^6, and 1 x 10^5 cells, respectively).

EXPERIMENTAL RESULTS
Table 1: Physical characterization of nanoparticles

<table>
<thead>
<tr>
<th>Formulation</th>
<th>Size (nm)</th>
<th>Polydispersity index</th>
<th>Zeta potential (mV)</th>
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<tbody>
<tr>
<td>OVA-GemC18-NPs</td>
<td>215 ± 8</td>
<td>0.32 ± 0.08</td>
<td>-28.4 ± 0.5</td>
</tr>
<tr>
<td>GemC18 (mg/ml)</td>
<td>4.4 ± 1.2</td>
<td>4.3 ± 1.1</td>
<td></td>
</tr>
<tr>
<td>EGF or OVA (mg/ml)</td>
<td>462 ± 39</td>
<td>330 ± 42</td>
<td></td>
</tr>
</tbody>
</table>

Figure 1: Flow cytometric analysis of MDA-MB-468 and MDA-MB-231 cells treated with various concentrations of EGF-GemC18-NPs and EGF (5 nM, 10 nM, 20 nM, 40 nM) for 4 h. (A) Typical flow cytometric graphs of cells after 6 h of incubation with fluorescence-labeled (EGF-GemC18-NPs (green) or EGF (grey)). (B) Flow cytometric graphs of cells after 6 h of incubation with EGF-GemC18-NPs or EGF (grey). Cell nucleus was stained with DAPI (blue). Nanoparticles were labeled with fluorescence and shown in green.

Figure 2: Flow cytometric and fluorescent microscopic analyses of tumor tissue sections treated with EGF-GemC18-NPs by tumor cells expressing different levels of EGFR.

Figure 3: Typical flow cytometric graphs of cells after 6 h of incubation with fluorescence-labeled (EGF-GemC18-NPs (green) or EGF (grey)). Cell nucleus was stained with DAPI (blue). Nanoparticles were labeled with fluorescence and shown in green.

Figure 4: Anti-tumor activity of EGF-GemC18-NPs in nude mice with pre-established MDA-MB-468 tumors. (A) Tumor growth curves. (B) Mean tumor volumes (p = 0.05). EGF-GemC18-NPs vs. OVA-GemC18-NPs. (C) Tumor growth curves in mice used in immunohistochemistry analysis. In A and C, * p < 0.05, EGF-GemC18-NPs vs. OVA-GemC18-NPs.

CONCLUSIONS
It was previously shown that formulating a stearoyl gemcitabine derivative into nanoparticles significantly improved the resultant anti-tumor activity. Data in the present study showed that the anti-tumor activity of stearoyl gemcitabine nanoparticles can be further improved by actively targeting them into tumors.

ACKNOWLEDGEMENTS
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