CD44 receptor targeting with Hyaluronic acid-conjugated PLGA nanoparticles loaded with SN 38

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ABSTRACT SUMMARY

For the first time we have decorated PLGA nanoparticles with HA using EDC coupling reaction and used PLGA-HA nanoparticles for the specific delivery of SN38 to ovarian cancer. The specificity of PLGA-HA NPs and cytotoxicity of SN38 loaded nanoparticles was evaluated in ovarian cancer cell lines.

PLGA-HA nanoparticles showed high specificity towards CD 44 receptor +ve ovarian cancer cells and internalized by receptor mediated endocytosis. Drug loaded targeted nanoparticles showed significantly higher cytotoxicity on ovarian cancer cell lines.

INTRODUCTION

Ovarian cancer is the fifth leading cause of cancer deaths in American women. Primary therapy for ovarian cancer is surgery followed by chemotherapy. Conventional chemotherapy cannot cure recurrent and resistant tumors and dose related systemic toxic effects limit chemotherapy. Delivering high amounts of drug to especially to tumor microenvironment could be better idea to circumvent these problems. In this study we have used hyaluronic acid (HA), which can bind with high affinity to CD44 receptors over expressed on many cancer types, to decorate PLGA nanoparticles. Nanoparticles were loaded with SN38, a water insoluble, poorly stable, highly toxic drug. The targetability and toxicity of SN38 loaded of PLGA-HA nanoparticles was tested in ovarian cancer cell lines.

EXPERIMENTAL METHODS

HA decorated nanoparticles (PLGA-HA NPs) were prepared in two steps. Firstly, PLGA-PEG-NH$_2$ polymer conjugate was prepared as reported earlier [1]. Nanoparticles were prepared by o/w single emulsion solvent evaporation method. HA was conjugated with free –NH$_2$ groups protruding on the surface of PLGA-PEG-NH$_2$ nanoparticles by EDC coupling reaction. Presence of hyaluronic acid on the surface of nanoparticles was confirmed and quantified a reported CTAB turbidimetric assay [2].

CD44 receptor expression on various cell lines was determined by flow cytometry. To evaluate the specificity of the nanoparticles, FITC was loaded into nanoparticles. CD44 +ve and CD44 –ve cell lines were incubated for 30min with FITC loaded PLGA-HA NPs and non-targeted PLGA NPs and the percent cellular uptake was determined by flow cytometry.

RESULTS AND DISCUSSION

CTAB turbidimetric assay confirmed the conjugation of HA on to nanoparticles and it was quantified as 0.116±0.031 mg (n=3) per 1mg of PLGA-HA nanoparticles.

Based on the results from receptor expression analysis, SKOV-3 & OVCAR-8 are
used as CD44 +ve cells (with high CD44 expression levels) and CHO cells (with negligible CD44 expression levels) are used as the negative control.

As shown in Figure 1 the uptake PLGA-HA nanoparticles was significantly higher in CD44 +ve cell lines in comparison to CD44 –ve cell line at both concentrations 5µg/ml (** P< 0.001) and 25µg/ml (*P< 0.05).

In mechanistic studies, the cellular internalization of PLGA-HA nanoparticles in the presence of excess HA or colchicine significantly reduced and the cellular internalization at 4°C is very negligible. There mechanistic studies clearly show that PLGA-HA nanoparticles were internalized by CD44 mediated receptor endocytosis.

SN38 loaded nanoparticles were of size 230-270 nm and an average zeta potential of -30 to -35mV. SN38 encapsulation efficiency in PLGA-HA and PLGA nanoparticles were 75.83±4.12 and 81.85±5.33, respectively.

IC50 values of SN38 loaded nanoparticles were shown in the table 1. PLGA-HA nanoparticles showed increased cytotoxicity in CD44 +ve cells. Blank PLGA-HA nanoparticles were showed no signs of cytotoxicity even at the concentration of 100µg/ml in CHO cells.

### Table 1: IC50 values of Sn38 loaded nanoparticles in OVCAR-8 and SKOV-3 cells.

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<thead>
<tr>
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<th>OVCAR-8</th>
<th>SKOV-3</th>
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<tbody>
<tr>
<td>SN38</td>
<td>514.90 ± 13.36</td>
<td>10.0 ± 0.14</td>
</tr>
<tr>
<td>SN38-NP</td>
<td>372.87 ± 7.92</td>
<td>8.14 ± 0.27</td>
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<tr>
<td>SN38-HA</td>
<td>229.90 ± 7.65</td>
<td>6.21 ± 0.14</td>
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### CONCLUSION

The present study demonstrated that tumorspecific, biocompatible hyaluronic acid decorated PLGA nanoparticles could be better choice for targeted SN38 delivery.

### REFERENCES
