Dextran-CpG oligonucleotide conjugates for enhanced immune-stimulation for cancer therapy

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Introduction

- CpG oligonucleotide (CpG ODN) is a Toll-like receptor (TLR) 9 agonist that activates antigen-presenting cells (APCs), which in turn activates innate immune cells required for effectively inducing antitumor immune response.
- Intratumoral injection of CpG ODN has shown promise for reduced systemic toxicity and tumor regression through focusing the immune stimulation in tumors and draining lymph nodes.
- The fundamental limitation of injecting CpG intratumorally is its poor retention at the injection site.

Hypothesis: conjugating CpG ODN to a high molecular-weight dextran polymer followed by intratumoral injection enhances its tumor retention and transports it to the lymph node.

Methods

CpG-1668 (5'-TCCATGACGTTCCTGATGCT-3') modified with a 3' amine (3' CpG-NH_2) is conjugated to the amino-dextrans with different molecular weights, i.e. 20 kDa, 40 kDa, 70 kDa, and 110 kDa, using bis-arylhydrazone linking strategy **(Fig. 1)**.

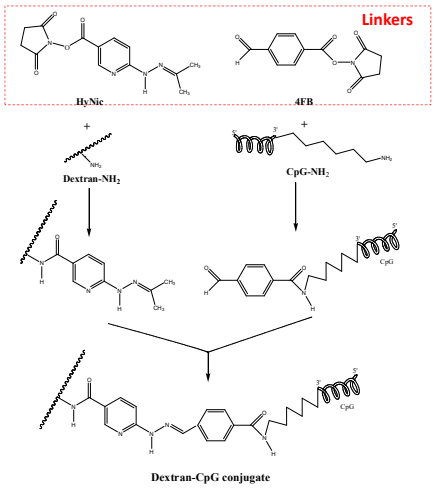

Fig. 1. Synthesis of CpG-conjugated dextran using bis-arylhydrazone linking strategy

Physico-chemical characterisations:

(1) Purity of CpG-conjugated dextrans was assessed by size exclusion chromatography (SEC) at two wavelengths, i.e. 260 nm and 354 nm
(2) Molar substitution ratio (MSR) of CpG in CpG-conjugated dextrans was determined by the formation of bis-aryl hydrazine bond measured at 354 nm
(3) CpG concentration in conjugates was quantified using Quant-iT™ Oligreen® ssDNA assay kit (Thermo Fisher Scientific, Pittburg, PA).

***In vitro* dextran uptake studies on splenocytes:**

For tracking purpose, dextran was fluorescently labelled with Dylight 633® NHS ester. The degree of Dylight substitution was determined by fluorescence spectroscopy (Em = 584 nm, Ex = 620 nm).

The uptake of fluorescently-labelled amino-dextrans on splenocytes was conducted at 37 °C for 24 h and was tested on four cell sub-populations (i.e., dendritic cells (DCs), macrophages, B cells, and T cells). To identify different cell types, cells were stained with antibodies against DCs (FITC-CD11c), macrophages (PE-F4/80), B cells (APC/H7-CD19), and T cells (FITC-CD3). Fluorescence were measured by flow cytometer (Gallios, Beckman Coulter, Franklin Lakes, NJ).

Results

Table 1. Physico-chemical properties of CpG-conjugated dextrans

Conjugates	CpG concentration (mg/mL)	CpG molar substitution ratio (MSR)
CpG-Dextran 20 kDa	0.356	3.58
CpG-Dextran 40 kDa	0.792	3.90
CpG-Dextran 70 kDa	2.493	4.02
CpG-Dextran 110 kDa	2.716	4.02

CpG-conjugated dextrans were successfully prepared, as evidenced by size exclusion chromatography. The molar substitution ratio of CpG in dextran conjugates was ranged from 3.58 to 4.02, indicating that three to four CpG molecules were linked to one dextran molecule **(Table 1)**.

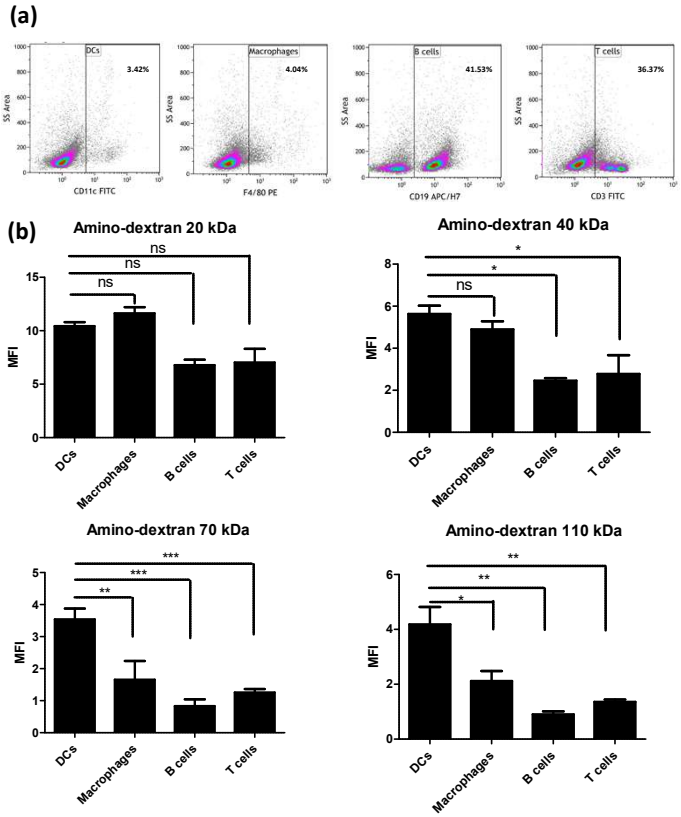

Fig. 2. (a) Representative gating strategy for dextran uptake experiment on DCs (CD11c-positive), macrophages (F4/80-positive), B cells (CD19-positive), and T cells (CD3-positive); (b) Cell uptake profiles of amino-dextrans (20 kDa, 40 kDa, 70 kDa, and 110 kDa) on four different sub-populations (DCs, macrophages, B cells, and T cells) of splenocytes. The bar graphs depict the mean MFI from three independent experiments ± S.E.M. Statistical significance was determined by one-way ANOVA with Bonferroni's post-hoc test; *** $p<0.001$; ** $p<0.01$; * $p<0.05$; ns, not significant.

Dextrans were fluorescently labelled with Dylight 633 with the degree of labelling being 1.5, 2.6, 3.1, 4.6 for dextran 20 kDa, dextran 40 kDa, dextran 70 kDa, and dextran 110 kDa, respectively. The uptake profiles of dextrans with the molecular weight ranging from 20 kDa to 110 kDa at 37 °C **(Fig. 2)** demonstrated that high molecular weight dextrans (i.e., greater than 70 kDa) were found to target DCs. This would be beneficial for promoting vaccine antigen presentation, leading to the initiation of robust antigen-specific T cell responses.

Conclusions

- The CpG-dextran conjugates were synthesized and their physico-chemical properties were evaluated.
- Further studies involving *in vivo* biodistribution of the conjugates and their immune efficacy following intratumoral injection will be tested.

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