The influence of linker length on the properties of cathepsin S cleavable ^{177}Lu-labeled HPMA copolymers for pancreatic cancer imaging

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ABSTRACT

N-(2-hydroxypropyl)-methacrylamide (HPMA) copolymers have shown promise for application in the detection and staging of cancer. However, non-target accumulation, particularly in the liver and spleen, hinders the detection of resident or nearby metastatic lesions thereby decreasing diagnostic effectiveness. Our laboratory has pursued the development of cathepsin S susceptible linkers (CSLs) to reduce the non-target accumulation of diagnostic/radiotherapeutic HPMA copolymers. In this study, we ascertain if the length of the linking group impacts the cleavage and clearance kinetics, relative to each other and a non-cleavable control, due to a reduction in steric inhibition. Three different CSLs with linking groups of various lengths (0, 6 and 13 atoms) were conjugated to HPMA copolymers. In vitro cleavage studies revealed that the longest linking group (13 atoms) led to more rapid cleavage when challenged with cathepsin S. The CSL incorporated HPMA copolymers demonstrated significantly higher levels of excretion and a significant decrease in long-term hepatic and splenic retention relative to the non-cleavable control. Contrary to in vitro observations, the length of the linking group did not substantially impact the non-target in vivo clearance. In the case of HPAC tumor retention, the CSL with the null (0 atom) linker demonstrated significantly higher levels of retention relative to the other CSLs. Given these results, we find that the length of the linking group of the CSLs did not substantially impact non-target clearance, but did influence tumor retention. Overall, these results demonstrate that the CSLs can substantially improve the non-target clearance of HPMA copolymers thereby enhancing clinical potential.

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1. Introduction

Pancreatic ductal adenocarcinoma (PDAC) is the fourth leading cause of cancer-related deaths in the United States with a 5-year survival rate of only 6% and a median survival of approximately 6 months [1]. The poor prognosis for pancreatic cancer patients is mainly due to the asymptomatic nature of the early disease state with symptoms typically presenting only in advanced stages, where effective treatment options are severely limited [2,3]. For the small portion of the pancreatic cancer population (<10%) diagnosed with localized disease, surgical resection has been shown to be an effective treatment and is the current standard of care. Pre-operative staging is crucial in determining whether a patient is a viable candidate for surgical resection [4]. Inaccurate identification of patients with unresectable tumors leads to unnecessary surgeries that can result in significant increases in patient morbidity and mortality [4]. To date, accurate staging of pancreatic cancer represents a major challenge in patient treatment.

Several noninvasive imaging techniques are currently used for the diagnosis and staging of pancreatic cancer. These modalities include contrast-enhanced computed tomography (CT), abdominal ultrasound (US), magnetic resonance imaging (MRI) and MR cholangiopancreatography (MRCP) [4]. While these techniques have superb anatomical resolution, these imaging modalities do not have the sensitivity and specificity associated with nuclear imaging instrumentation, namely Single Photon Emission Computed Tomography (SPECT) and Positron Emission Tomography (PET) [5,6]. Recently, the fusion of nuclear imaging with the CT modality has resulted in hybrid imaging systems, SPECT/CT and PET/CT, which have been demonstrated to more accurately detect and stage a variety of cancers [6]. Unfortunately, the potential of these hybrid imaging systems for improving pancreatic cancer staging has not been fully realized due to the lack of an effective radiotracer to target the disease [4].

The development of polymer-based diagnostics and therapeutics for human disease has been an area of intense research and has yielded several drugs that have progressed to the clinic [7]. N-(2-hydroxypropyl)-methacrylamide (HPMA) copolymer is a polymeric platform that has been extensively investigated for a variety of biomedical applications including the development of SPECT and PET tracers [8–18]. HPMA copolymers are capable of targeting solid tumors either passively, through the enhanced permeability and retention (EPR) effect, and/or actively by inclusion of targeting vectors into the polymeric construct [13,14,19,20]. However, one major challenge for the development of diagnostic and/or therapeutic HPMA based drugs, as well as other polymer and nanomedicine systems, is opsonization and sequestration by the mononuclear phagocyte system (MPS) [21,22]. In many cases, the sequestration of these polymeric drugs leads to substantial accumulation in the liver and spleen. From a diagnostic perspective, this MPS accumulation in non-target organs can hinder identification of resident or nearby metastatic lesions thereby decreasing the diagnostic effectiveness. For therapeutic applications, the non-target accumulation of these polymeric drugs can lead to significant toxicities which may be dose-limiting.

Our laboratory is interested in developing synthetic approaches in which HPMA copolymers can be modified to significantly reduce the MPS accumulation thereby enhancing the diagnostic and/or radiotherapeutic efficacy of the agent. Recently, we have described the development of cathepsin S susceptible linkers (CSLs) [23], which degrade in the presence of cathepsin S, a lysosomal protease that is selectively and highly expressed in MPS tissues [24–26]. From our initial investigation, we found that CSLs significantly reduced long-term retention of ^{177}Lu-labeled HPMA copolymers in tissues associated with the MPS (i.e., liver and spleen). However, the high molecular weight HPMA copolymer (343 kDa) utilized in the study gave blood circulation times that were not ideal for diagnostic or therapeutic use. Herein, we report our continued investigation of the CSL design by evaluating the structure–activity impact of linking groups of varying size, Fig. 1, on the in vitro and in vivo efficacy of HPMA copolymer based radiopharmaceuticals. In conjunction with these studies, we utilize a lower molecular weight HPMA (109 kDa) copolymer with a blood circulation time that is more suitable for diagnostic and/or radiotherapeutic applications.

2. Materials and methods

2.1. Materials

All chemicals were used without further purification unless otherwise noted. Fluorescein isothiocyanate (FITC), N-hydroxysuccinimide (NHS), N,N'-dicyclohexylcarbodiimide (DCC), 4,4'-Azobis(4-cyanovaleric acid) (V-501), 4-cyano-4-(phenylcarbono-thioylthio)pentanoic acid (CTP), thioanisole, 3,6-dioxa-1,8-octanedithiol (DODT), triisopropylsilane (TIS), Tween-20, Tris-buffered saline, RIPA buffer, protease inhibitor cocktail, ethylenediaminetetraacetic acid (EDTA), ninhydrin, glycine, sodium acetate, insulin, transferrin, hydrocortisone and cysteine cathepsin S isolated from human spleens were obtained from Sigma–Aldrich (U.S.). Acetonitrile (HPLC Grade), formic acid (HPLC Grade), N,N-dimethylformamide (DMF, Peptide Synthesis Grade), dichloromethane (DCM, Peptide Synthesis Grade), methanol (MeOH), N-methylpyrrolidone (NMP, Peptide Synthesis Grade), N,N-Diisopropylethylamine(DIEA), trifluoroacetic acid (TFA, Peptide Synthesis Grade), L-ascorbic acid, ethylene glycol, tin(II) chloride, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES), bovine serum albumin (BSA), sodium hydroxide, sodium dodecyl sulfate (SDS), sodium bicarbonate, glutathione, epidermal growth factor (EGF), 2-mercaptoethanol, 0.9% sodium chloride (NaCl) solution (irrigation USP), Dulbecco's Modified Eagle Medium (DMEM), and phosphate buffered saline (PBS) were obtained from Fisher Scientific (U.S.). N-(2-Hydroxypropyl) methacrylamide (HPMA) and N-(3-aminopropyl) methacrylamide (APMA) hydrochloride were purchased from Polysciences (U.S.). 5-(3-(Methacryloylaminopropyl) thioureidyl) fluorescein (APMA-FITC) was synthesized in our lab as previously described [23]. 1-[1-(Cyano-2-ethoxy-2-oxoethylideneaminooxy) dimethylaminomorpholino)] uronium hexafluorophosphate (COMU), fluorenylmethyloxycarbonyl (Fmoc)-protected natural amino acids, Fmoc-NH-(PEG)$_2$-COOH (13 atoms), H-Pro-2-Trt resin and the 2-chlorotritylchloride (2-ClTrt-Cl) resin were purchased from NovaBiochem (U.S.). Fmoc-5-aminovaleric acid (Fmoc-5-Ava-OH) was obtained from Advanced ChemTech (U.S.). 1, 4, 7, 10-Tetraazacyclododecane-1, 4, 7, 10-tetraacetic acid (DOTA) was purchased from Macrocyclics (U.S.). Lutetium-177 trichloride was obtained from PerkinElmer (U.S.). The human pancreatic adenocarcinoma (HPAC) CRL-2119 cell line was purchased from American Type Culture Collection (ATCC) (U.S.). Matrigel™ was
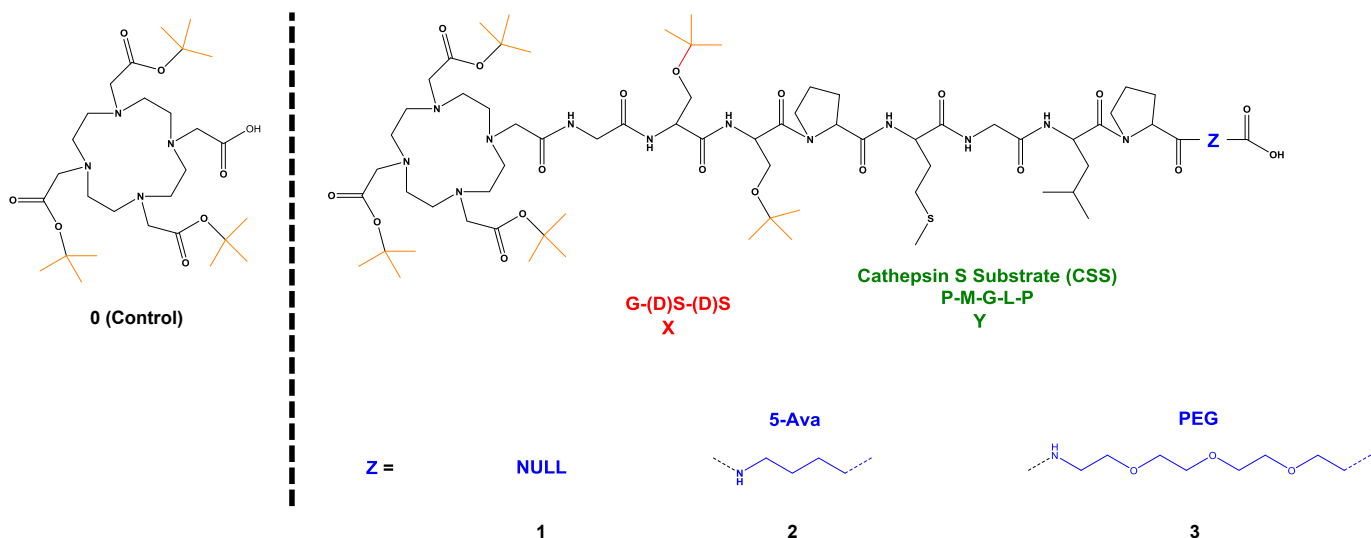

Fig. 1. Schematic design of CSLs with intact orthogonal protection.