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Construction of peptide-vehicles, bioconjugates having modules for cancer cell surface capture and cell-penetrating peptide with anticancer agents

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ABSTRACT

Recently molecular targeting therapy has been applied to cancer chemotherapy, although in some cases side-effects are not negligible. Based on our bio-detection concept, that is, protein-protein interaction can be mimicked by using peptides, a novel cell-targeting concept designated peptide-vehicle has been proposed, which has conjugates consisting of the cancer cell recognition and cell penetrating peptides with anticancer drugs. The cancer cell surface protein can be captured by a cyclotide, containing protease resistant D-cystine. A library of cell penetrating peptides has been prepared and conjugated to the cyclotide. Anticancer molecules were recovered after clinical use, which were pooled, purified and derivatized for loading into the vehicle. The present paper describes construction of peptide-vehicles, bioconjugates focusing on more efficiency and cancer cell selective delivery for anticancer drugs.

1. Introduction

In the last decade pharmaceutical industries have focused on molecular targeting therapy and several drugs based on this concept have been successfully used as anticancer therapy, although in some cases side-effects are not negligible. Hence, we have proposed the construction of a cancer cell targeting delivery system based on our concept of bio detection with peptide arrays.[1] We have designated a peptide-vehicle, a novel bioconjugate focusing on more efficient drug delivery. The peptide-vehicle was constructed of recognition modules and cell penetration peptides carrying anticancer drugs. As the first target we have chosen lung cancer, which has the highest incidence rate of tumors in the world. The prognosis of this cancer is particularly poor and 95% patients do not survive. The recognition modules were fundamentally small cyclic peptides that mimicked proteins and captured cancer cell-surface proteins. Prior to the discovery of capturing peptides from patient's cells, we have applied a cyclotide, designated RecP, cyclic [cNGRGEQc], which had been discovered by the "one peptide on one bead" technology.[2] Several cell penetrating peptides (CPPs) reviewed by Koren, E. and Torchilin V. P.[3] have been selected and prepared. In a preliminary study we have used cancer cell-lines and the commercially available anticancer agent, methotrexate, and found efficacy of 200% improvements. Additionally, cell-selectivity was improved ca 1.5 times using the constructed vehicle.[4] The anticancer molecules used as cargos were obtained from the residuals after therapeutic use. Since these drugs contain several additives which interfere with derivatization, the collected and pooled drugs were purified. The present paper describes construction of peptide vehicle focusing on cell-targeted delivery, of which the concept is illustrated in Figure 1.

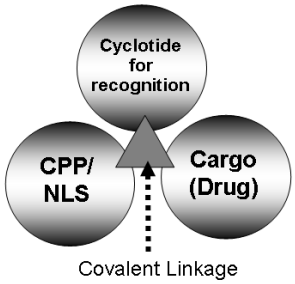

Figure 1. The concept of the peptide-vehicle consisting of three modules for effective cell-targeting delivery. Cell penetration peptide (CPP) or peptide for the nuclear localization signal (NLS), a cyclotide for capturing and anticancer agent (cargo) have been bound together with covalent linkages. Hence, the cargos have been loaded through their hydroxyl groups.

Materials used in the present study and characterization.

The major reagents used for peptide syntheses, amino acid derivatives, solid supports (Wang resin, TentaGel) (2-(1*H*-benzotriazole-1-yl)-1,1,3,3-tetramethylaminium hexafluoro-phosphate (HBTU), 1-hydroxybenzotriazol (HOBt) and *N*-ε-maleimidocaproic acid (EMCA) were from HiPep Laboratories (Kyoto, Japan). Camptothecin (CPT), tetrakis(triphenyl-phosphine)palladium(0), 4-dimethylaminopyridine (DMAP), *N*,*N*'-diisopropylcarbodiimide (DIPCDI), *N*,*N*-