Biochemical and Biophysical Research Communications xxx (2018) 1–7

Contents lists available at ScienceDirect

Biochemical and Biophysical Research Communications

journal homepage: www.elsevier.com/locate/ybbrc

Engineering of a tumor cell–specific, cytosol-penetrating antibody with high endosomal escape efficacy

Ji-sun Kim [a], Jae-Yeong Park [a], Seung-Min Shin [a], Seong-Wook Park [a], Sei-Yong Jun [a], Jin-Sun Hong [a], Dong-Ki Choi [b], Yong-Sung Kim [a, c, *]

[a] Department of Molecular Science and Technology, Ajou University, Suwon, 16499, Republic of Korea
[b] ORUM Therapeutics Inc., Daejeon, 34050, Republic of Korea
[c] Department of Allergy and Clinical Immunology, Ajou University Medical Center, Suwon, 16499, Republic of Korea

ARTICLE INFO

Article history:
Received 25 June 2018
Accepted 2 July 2018
Available online xxx

Keywords:
Cytosol-penetrating antibody
Cytotransmab
Tumor specificity
Endosomal escape
Cytosolic delivery

ABSTRACT

The main obstacles for practical uses of cytosol-penetrating peptides and proteins include their lack of cell- or tissue-specific targeting and limited cytosolic access owing to the poor endosomal escape ability. We have previously reported a cytosol-penetrating, human IgG1 antibody TMab4-WYW, generally referred to as a cytotransmab (CT), which reaches the cytosol of living cells but nonspecifically because it is endocytosed via a ubiquitously expressed receptor called heparan sulfate proteoglycan (HSPG). Here, our aim was to construct a next-generation CT with tumor cell specificity and improved endosomal escape efficiency. We first substantially reduced the HSPG-binding activity of TMab4-WYW and then fused a cyclic peptide specifically recognizing tumor-associated epithelial cell adhesion molecule (EpCAM) to the N terminus of the light chain for EpCAM-mediated endocytosis, while maintaining the endosomal escape ability in the light chain variable domain (VL), thus generating epCT05. Then, we separately engineered another CT, dubbed epCT65-AAA, with an endosomal escape ability only in the heavy chain variable domain (VH) but not in VL, by functional grafting of the endosomal escape motif of epCT05 VL to the VH. We finally combined the heavy chain of epCT65-AAA and the light chain of epCT05 to create epCT65 with endosomal escape capacity in both the VH and VL. epCT65 effectively localized to the cytosol of only EpCAM-expressing tumor cells and showed approximately twofold improved endosomal escape efficiency, as compared with CTs with endosomal escape motifs in either VH or VL. The full–IgG format CT, epCT65, with a tumor cell–specific cytosol-penetrating activity, has a great potential for practical medical applications, e.g., as a carrier for cytosolic delivery of payloads.

1. Introduction

Antibodies normally cannot cross intact cellular or subcellular membranes in living cells due to their large size and hydrophilicity [1]. Instead, antibodies can be internalized into cells by receptor-mediated endocytosis, but are usually degraded in lysosomes due to their inability to escape from intermediate early and/or late endosomes [2]. Our group recently reported cytosol-penetrating antibodies, referred to as cytotransmabs (CTs), which in the intact human IgG1 format can reach the cytosol of living cells after endocytotic internalization [3–5]. The previous best CT called TMab4-WYW localizes to the cytosol of cells after receptor-mediated endocytosis via cell surface–expressed heparan sulfate proteoglycan (HSPG) with a subsequent endosomal release into the cytosol. CTs including TMab4-WYW are not routed to degradative lysosomes but instead are dissociated from HSPG in acidified endosomes (pH 5.5–6.5) and next escape into the cytosol from the lumen of endosomes [3,4]. We have determined that the endosomal escape motif of TMab4-WYW resides in the light chain variable domain (VL) and is composed of a pH-sensing pair [AspL1 (where L = light chain with Kabat numbering [3]) in the framework region (FR) and MetL95 in the third complementarity-determining region of the VL (VL-CDR3)] and a membrane-binding motif of ^{92}WYW^{94} (TrpL92, TyrL93, and TrpL94) in VL-CDR3 (Fig. 1A) [4]. At the mildly acidified pH of endosomes, the carboxyl group of the

Abbreviations: GFP, green fluorescence protein; IgG, immunoglobulin G; CT, cytotransmab; SA, streptavidin.

* Corresponding author. Dept. of Molecular Science and Technology, Ajou University, 206 Worldcup-ro, Yeongtong-gu, Suwon, 16499, Republic of Korea.
E-mail address: kimys@ajou.ac.kr (Y.-S. Kim).

https://doi.org/10.1016/j.bbrc.2018.07.008
0006-291X/

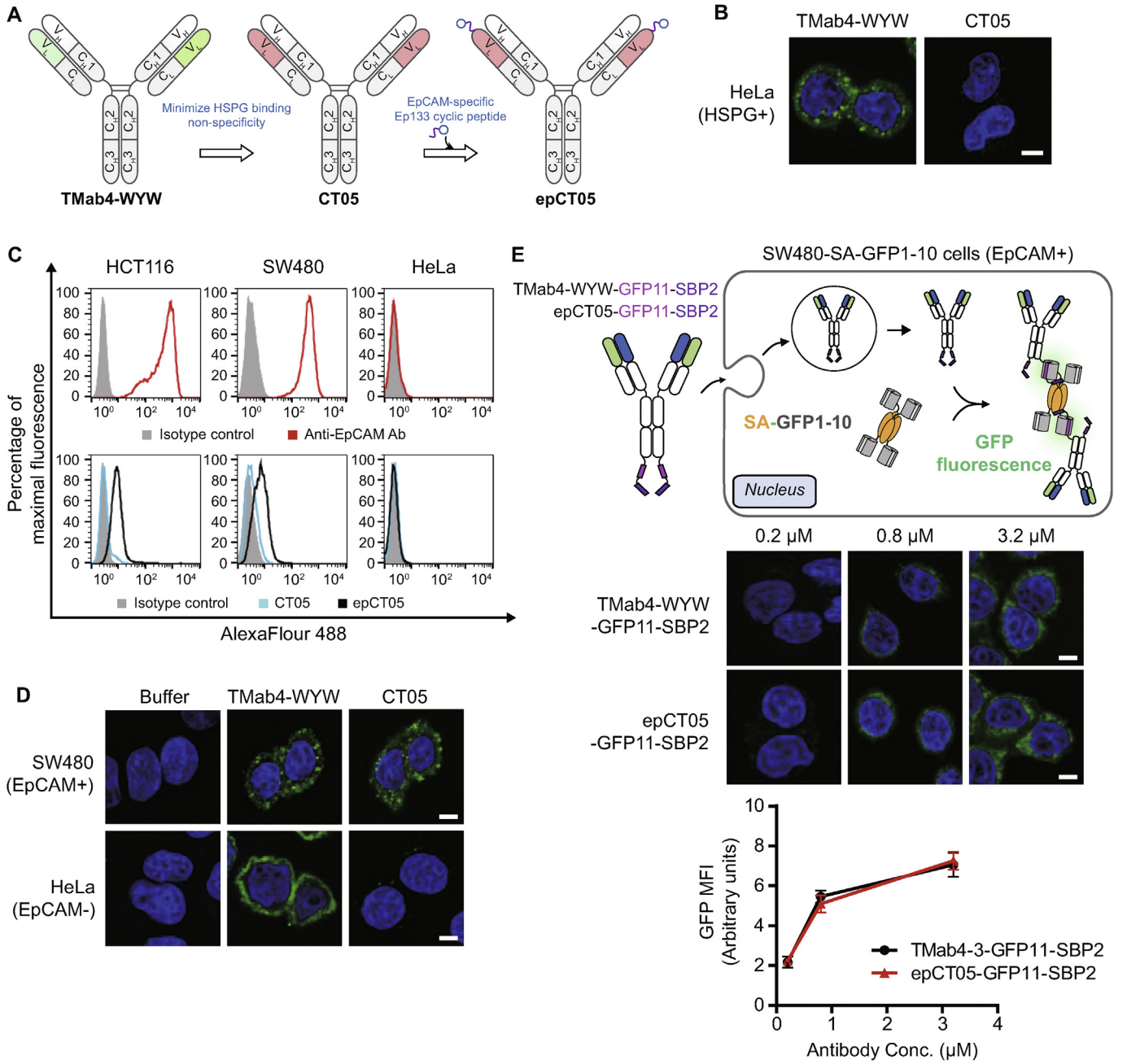

Fig. 1. Creation of antibody epCT05 penetrating into the cytosol of cells after EpCAM-mediated endocytosis. (**A**) A schematic diagram of CT engineering procedures. (**B**) Comparison of cellular internalization and localization between TMab4-WYW and CT05 in HeLa cells treated with the antibodies (1 μM) for 6 h at 37 °C. Internalized antibodies were visualized with an Alexa Fluor 488–conjugated anti-human IgG antibody (*green*). The blue color represents Hoechst 33342–stained nuclei. (**C**) Flow cytometric analysis of the cell surface expression levels of EpCAM on human tumor cells analyzed by means of an Alexa Fluor 488–labeled anti-EpCAM antibody (upper panels), and cell surface–binding levels of the indicated antibodies, coincubated at 100 nM with 300 IU/mL heparin for 1 h at 4 °C with the indicated cells before analysis (lower panels). (**D**) Cellular internalization and localization of the indicated antibodies (green) in EpCAM-positive SW480 cells and EpCAM-negative HeLa cells, treated with antibodies (1 μM) for 6 h at 37 °C prior to confocal fluorescence microscopy. The blue color represents Hoechst 33342–stained nuclei. (**E**) Cellular internalization and cytosolic localization of GFP11-SBP2–fused antibodies epCT05 and TMab4-WYW, as assessed by confocal microscopy measuring complemented GFP signals (*green*) in SW480-SA-GFP1-10 cells after treatment with the indicated concentration of the antibodies for 6 h at 37 °C. The blue color represents Hoechst 33342–stained nuclei. The bottom panel shows the MFI of GFP in cytoplasmic regions of cells compared with that in the PBS-treated control. Error bars denote SD (n = 20 cells per group). In panels B, D, and E, the scale bar is 10 μm. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

negatively charged Asp (or Glu) residues gets more protonated and thereby more hydrophobic, facilitating new hydrophobic interactions with MetL95, thus shortening the distance between AspL1 and MetL95. This AspL1–MetL95 interaction subsequently triggers local structural rearrangement of the MetL95-neighboring residues of ^{92}WYW^{94}, forcing the side chains to become upright in a favorable conformation to interact with the endosomal membrane. This interaction causes membrane lipid flip-flop, subsequently leading to the formation of membrane toroidal pores composed of the CT and a phospholipid for the endosomal escape of the CT into the cytosol. Consequently, the endocytosed CT escapes into the cytosol from endosomes.

Please cite this article in press as: J.-s. Kim, et al., Engineering of a tumor cell–specific, cytosol-penetrating antibody with high endosomal escape efficacy, Biochemical and Biophysical Research Communications (2018), https://doi.org/10.1016/j.bbrc.2018.07.008