Biomaterials 32 (2011) 8518–8528

Contents lists available at SciVerse ScienceDirect

Biomaterials

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

Identification of a peptide that interacts with Nestin protein expressed in brain cancer stem cells

Samuel Beck [a], Xun Jin [b], Jinlong Yin [a], Sung-Hak Kim [b], Nam-Kyung Lee [a], Se-Yeong Oh [b], Xiong Jin [b], Min-Kook Kim [a], Eun-Bae Kim [a], Jee-Soo Son [a], Sung-Chan Kim [c], Do-Hyun Nam [d], Se-Hyuk Kim [e], Sang-Kee Kang [a], Hyunggee Kim [b,*], Yun-Jaie Choi [a,**]

[a] School of Agricultural Biotechnology, Seoul National University, Seoul 152-742, Republic of Korea
[b] School of Life Sciences and Biotechnology, Korea University, Seoul 136-713, Republic of Korea
[c] Department of Biochemistry, College of Medicine, Hallym University, Chuncheon, Republic of Korea
[d] Department of Neurosurgery, Samsung Medical Center and Samsung Biomedical Research Institute, Sungkyunkwan University School of Medicine, Seoul, Republic of Korea
[e] Department of Neurosurgery, Ajou University School of Medicine, Suwon, Republic of Korea

ARTICLE INFO

Article history:
Received 2 June 2011
Accepted 16 July 2011
Available online 30 August 2011

Keywords:
Peptide
Adhesion molecule
Phage display
Glioma stem cells
Brain tumors

ABSTRACT

Glioma stem cells (GSCs) are presumably major culprits for brain tumor initiation, progression, and recurrence after conventional therapies. Thus, selective targeting and eradication of GSCs may provide a promising and effective therapeutic approach. Here, we isolated a GSC-targeting (GSCT) peptide that demonstrated selective binding affinity for many undifferentiated GSCs using *in vitro* phage display technology. This GSCT peptide binds to isotypes of Nestin proteins specifically expressed in GSCs, enabling it to target Nestin-positive cells in human glioblastoma tissues. In human glioblastoma tissue specimens, the fluorescence-conjugated GSCT peptide could visualize putative GSC populations, showing its possible use as a diagnostic agent. GSCT peptide is also internalized into undifferentiated GSCs specifically *in vitro*, and moreover, intravenously injected GSCT peptide effectively penetrated into tissues, specifically accumulated in gliomas that arise from subcutaneous and orthotopic implantation, and predominantly targeted Nestin-positive cells in these tumors. Thus, our GSCT peptide may be useful for the development of more promising therapeutic and diagnostic modalities that target GSCs in brain tumors.

1. Introduction

Glioblastoma multiforme (GBM) is the most aggressive type of central nervous system malignancy with a median survival of ~14 months in newly diagnosed patients [1]. Despite intensive biological and clinical investigations spanning several decades, poor prognosis with frequent recurrence is mainly attributable to the failure of conventional treatments such as surgical resection, chemotherapy, and radiotherapy [1].

The recent identification of a subpopulation of glioma cells, known as glioma stem cells (GSCs), revealed that they possess several unique cellular traits: asymmetrical self-renewal, tumor-repopulating potential, and multi-lineage differentiation [2–5]. Thus, these data provide evidence that GSCs are a major culprit driving an intense heterogeneous nature of GBM. Additionally, when compared with their differentiated progenies and numerous established glioma cell lines, GSCs exhibit remarkably resistant behavior against irradiation and chemotherapy through the active DNA repair regulatory systems and high level expression of various ATP-binding cassette (ABC) transporters that allow antitumor drugs to be pumped out [6–8]. Clinically, such cellular behavior implicates GSCs as the principle drivers of tumor recurrence, inevitably occurring after various conventional treatments [9]. Thus, to overcome therapeutic limitations that current conventional therapies targeting bulk tumors fail to completely eradicate GSCs, new therapies that target and eliminate GSCs are necessary to prevent GSC-driven tumor progression and recurrence.

Phage display is a specialized tool for screening peptide ligands or antibody fragments that have binding affinities for target cells, organs, or proteins [10]. Until recently, numerous studies have been performed to screen and validate peptide ligands that target tumor tissues or the tumor vasculature [11]. Tissue-specific, peptide-mediated delivery of conventional drugs would promise effective

* Corresponding author. Tel.: +82 2 3290 3472.
** Corresponding author. Tel.: +82 2 880 4816.
E-mail addresses: hg-kim@korea.ac.kr (H. Kim), cyjcow@snu.ac.kr (Y.-J. Choi).

0142-9612/$ – see front matter Crown Copyright © 2011 Published by Elsevier Ltd. All rights reserved.
doi:10.1016/j.biomaterials.2011.07.048

and cost-saving control of disease while attenuating undesirable side effects [12,13]. Several peptides have been shown to not only target tumor tissues, but also induce cellular apoptosis, even in the absence of conjugates that induce this process [14]. Furthermore, tissue-specific peptides have been confirmed as probes for molecular imaging by delivering contrast molecules [15].

In this study, we used phage display technology to identify peptides that specifically bind to GSCs, but not to their differentiated counterpart cells, to provide reliable and effective GSC-targeting strategies.

2. Materials and methods

2.1. Cell culture

For *in vitro* biopanning assays, protein isolation for individual phage screening, competition assays, and peptide pull-down assays, GSCs (GSC1, GSC2, GSC3, Ajou13, and Ajou14) [16] were established and maintained as suspended neurospheres in neurobasal medium (NBE; Invitrogen) in the presence of B27, N2, bFGF, and EGF (R&D) [3]. For cell-based phage ELISA assays and peptide penetration assays, cells were maintained on laminin-coated plates as previously described [17]. To differentiate GSCs, cells were maintained in Dulbecco's Modified Eagle Medium (DMEM; Hyclone) containing 10% fetal bovine serum (FBS; Hyclone) for at least 2 weeks [3].

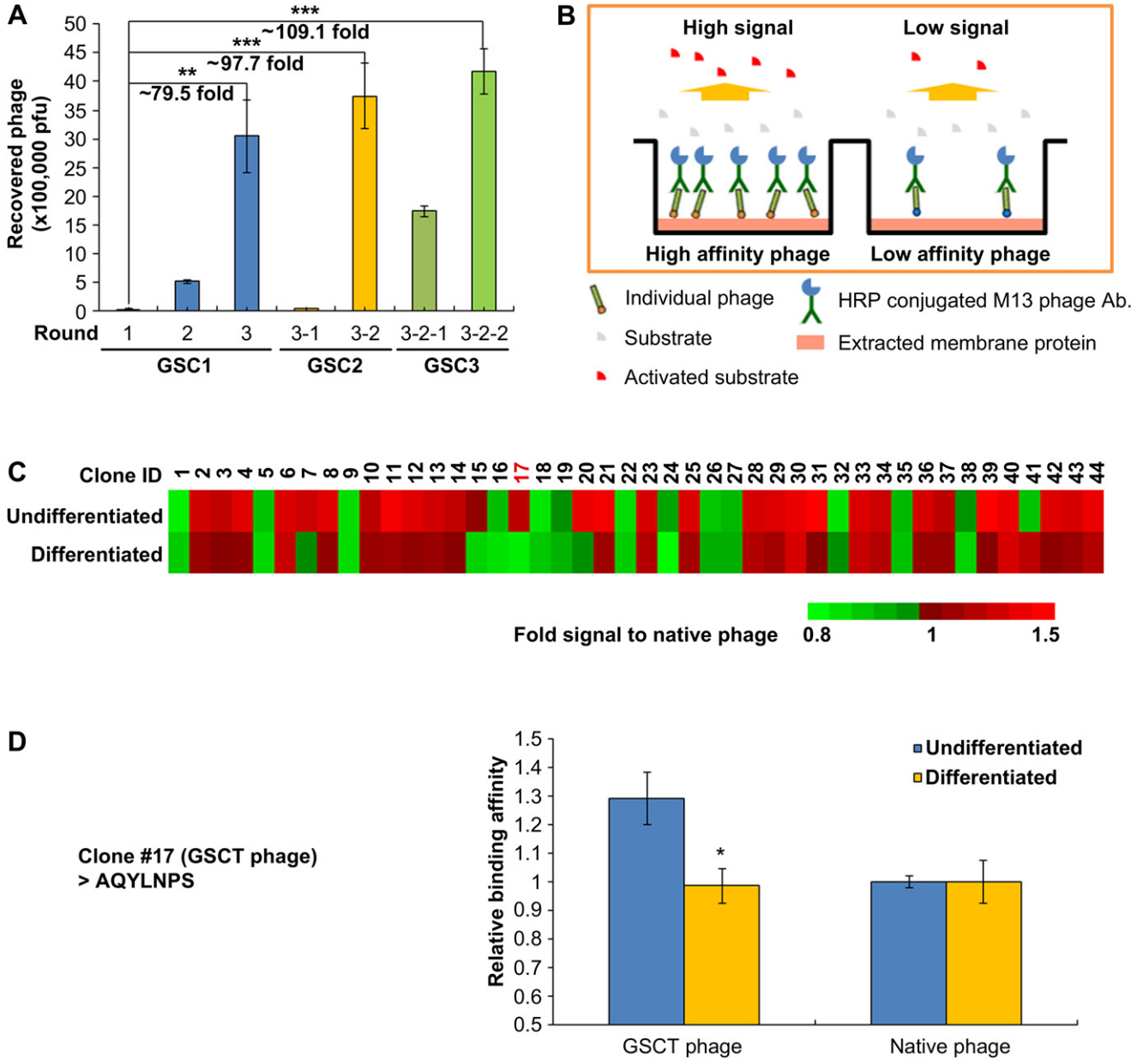

Fig. 1. Identification of glioma stem cell-targeting (GSCT) phage and peptide. (A) GSC-targeting phages were isolated by *in vitro* biopanning of a custom PhD.7. M13 phage library to three human GSC cell lines. In each round, 1×10^{11} plaque-forming units (PFUs) of phages were incubated with GSCs. After washing away unbound phages, the remaining phages (bound phages) were eluted with a low pH buffer, and phage titers were determined by plaque-forming assays. After three rounds of biopanning to GSC1, the resulting phages were subjected to two rounds of biopanning to GSC2, and then two additional rounds of biopanning to GSC3 using enriched phages from biopanning with GSC2. $^{**}P < 0.01$, $^{***}P < 0.001$. (B) Schematic diagram showing a rapid screening method (a protein extract-based phage ELISA) with individual phages. (C) Binding affinities of individual phages obtained from the phage pools that were converged from the biopanning described in (A). Using a protein extract-based phage ELISA method, individual phages were incubated with insoluble membrane proteins extracted from undifferentiated GSC3 grown in NBE medium (supplemented with epidermal growth factor (EGF) and basic fibroblast growth factor (bFGF)) and differentiated counterpart cells grown in DMEM (supplemented with 10% FBS). Signals subtracted with background binding affinities of native M13 phages were shown as a heatmap. (D) Deduced amino acid sequence of clone #17 (GSCT) phage, and its relative binding affinity to insoluble membrane proteins extracted from undifferentiated GSC3 and their differentiated counterpart cells. A native phage that contains no random peptide was used as a negative control.