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Research paper

Syntenin-targeted peptide blocker inhibits progression of cancer cells

Jiahui Liu [b], Jiale Qu [b], Wanding Zhou [a], Yujia Huang [a], Linyan Jia [a], Xiaojie Huang [a], Zhen Qian [a], Jiang Xia [b, **], Yongsheng Yu [a, c, *]

[a] Clinical and Translational Research Center, Shanghai First Maternity and Infant Hospital, Tongji University School of Medicine, Shanghai, China
[b] Department of Chemistry, The Chinese University of Hong Kong, Shatin, Hong Kong SAR, China
[c] Institute for Advanced Study, Tongji University, Shanghai, China

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ABSTRACT

The multidomain adaptor protein syntenin is known to mediate cancer cell metastasis and invasion through its tandem PDZ1 and PDZ2 domains, leading to the postulation that the PDZ tandem may serve as a potential drug target for cancer treatment. Here we report the development of high-affinity peptide blockers to target the syntenin tandem PDZ domain, and elucidate that blocking syntenin correlates with the inhibition of cell migration and spreading. Two strategies are employed to derive high-affinity blockers from the low-affinity natural binding peptides: first, dimerization of the C termini of natural syntenin-binding peptides confers dimer peptides with much higher affinity than the monomers; second, unnatural amino acid substitution at P-1 and P-2 positions of the PDZ-binding sequence increases the binding affinity. Through several rounds of optimization, we discovered a dimeric peptide that binds tightly to syntenin tandem PDZ domain, with a dissociation constant of 0.21 μM based on fluorescence polarization measurement. The peptide dimer inhibits the migration and invasion of syntenin high-expression human cancer cells through attenuating the ERK phosphorylation of the MAPK kinase pathway. This work showcases an effective strategy to derive high-affinity blocker of multidomain adaptor proteins, which resulted in a syntenin-targeted antagonist with potential pharmaceutical values for the treatment of syntenin over-expressing cancers.

1. Introduction

The cytosolic adaptor protein syntenin contains a unique tandem PDZ domain architecture (PDZ1 and PDZ2 spaced by a 4-amino acid linker), an N-terminal domain (NTD) that is structurally uncharacterized and a short C-terminal domain (Fig. 1) [1]. The tandem PDZ domains bind to a wide variety of PDZ ligands with low to moderate affinity [2,3]. Due to the degenerative specificity in ligand recognition, syntenin has been found to interact with a wide range of intracellular signal proteins, such as syndecan [4], IL-5 receptor α subunit (IL5Rα) [5], neuroglian [1], proTGF-α [6], glutamate receptors [7], neurofascin [1], syndecan-4 [4], ephrin B [8,9], ephrin A7 [8], PTP-η [10], neurexin I [11], and merlin [12]. This diversity implies that syntenin may be involved in cellular functions such as cell adhesion [13], protein trafficking [6,14], and activation of the ERK1/2 MAPK kinase pathway [15].

Several lines of evidence suggest that syntenin plays a key role in metastasis of tumor cells. For instance, the expression level of syntenin was found to be much higher in metastatic cell lines as compared with non-metastatic cancer-cell lines [15]. Also, up-regulation of syntenin was correlated with the migration of non-metastatic cancer cells [16], and genetic knockdown of syntenin inhibited cell migration and invasion [17,18]. It was therefore postulated that inhibiting the function of syntenin, in particular the ligand-binding property of the tandem PDZ domain, may be an effective way of preventing metastatic cancer spreading [19]. Kegelman and coworkers recently developed small-molecule syntenin inhibitors using innovative fragment-based drug design and NMR approaches, and found that syntenin inhibitors reduced invasion gains in glioblastoma multiforme cells following radiation [20]; syntenin-targeted inhibition therefore produced a similar effect as genetic knockdown. In this report, we use an alternative strategy: epitope-based inhibitor discovery to develop high-affinity

* Corresponding author. Clinical and Translational Research Center, Shanghai First Maternity and Infant Hospital, Tongji University School of Medicine, Shanghai, China.
** Corresponding author.
E-mail addresses: jiangxia@cuhk.edu.hk (J. Xia), yongshengyu@tongji.edu.cn (Y. Yu).

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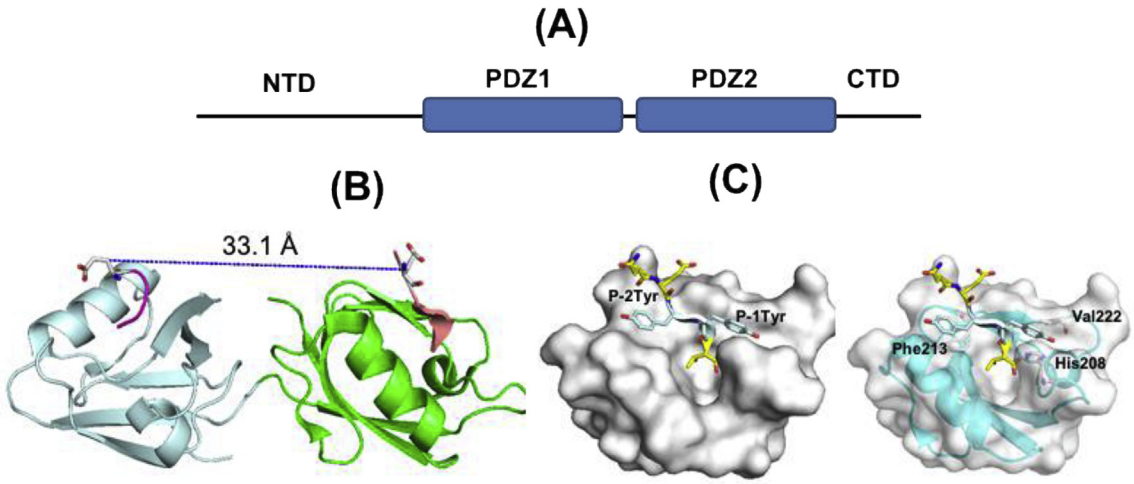

Fig. 1. Syntenin and its peptide binding properties. (A) The domain organization of syntenin. (B) The structure of syntenin tandem PDZ bound with two separate PDZ ligands (PDZ ID: 1w9e). (C) The structure of syntenin PDZ2 bound with the peptide NEYYV (PDB ID: 1w9o). Both tyrosine residues at P-1 and P-2 reside at hydrophobic clefts of the binding site, and interact with His 208, Val 222, and Phe 213, respectively.

syntenin-targeted peptide inhibitor for the blockage of syntenin-mediated signals.

Because natural PDZ–peptide binding interactions are often weak and promiscuous, it is very challenging to develop epitope-based PDZ inhibitors. We utilized proximal reactivity to develop reactive peptides against a PDZ protein inside cells [21]. Its success notwithstanding, this strategy cannot be applied to syntenin as it does not have a reactive residue (for example cysteine) at the peptide-binding site. On the other hand, Strømgaard and co-workers developed a dimeric peptide inhibitor that binds to the tandem PDZ domain of PSD >1000 fold tighter than the natural PDZ epitope [22,23]. Therefore, we reason that a peptide dimer containing two binding epitopes appropriately spaced by a linker will bind the syntenin tandem PDZ domain (through simultaneous binding to both PDZ domains) with much higher affinity, and such a peptide dimer can effectively block syntenin-mediated cell migration, proliferation, and spreading (Fig. 1).

2. Results and discussions

2.1. PDZ binding peptides

Four peptides derived from the natural binding proteins of syntenin were chosen as the parental syntenin blockers: RVAFFEEL (an epitope from Merlin) [3], TNEFYA (an epitope from syndecan) [3], DKEYYV (an epitope from neurexin) [2], LEDSVF (an epitope from interleukin-5 receptor α) [3]. Peptides were synthesized by solid phase synthesis and fluorescently labeled at the N termini spaced by a flexible linker (the dye and the linker will not affect peptide binding as PDZ domains only recognize the C-terminal sequence) (Table 1, Supporting Table S1 and Fig. S1). We measured the binding affinity of these peptides with recombinantly expressed single-domain PDZ1 and PDZ2 proteins by fluorescence polarization (FP), and confirmed that they bind with only moderate affinities: the dissociation constants K_D with PDZ1 ranging from 282 μM for **p1**, 94 μM for **p2**, 81 μM for **p3**, to 58 μM for **p4** and the dissociation constants K_D with PDZ2 ranging from 531 μM for **p1**, 68 μM for **p2**, 33 μM for **p3,** to 60 μM for **p4** respectively (Table 1, Figs. S2 and S3 in the Supporting Information). This observation is consistent with the previous findings that syntenin PDZ domains bind to their natural peptide ligands with low to moderate affinity.

2.2. Peptide dimerization increases the affinity towards tandem PDZ domain

We next synthesized dimeric peptides by crosslinking peptide N termini through a linker PEG_3 by cysteine–maleimide bio-conjugation reaction. According to the crystal structures of syntenin tandem domain, the distance between the β-carbon of the P-3 amino acids of the two peptides is measured to be 33.1 Å, so the length of the linker will allow the dimeric peptide to bind to both domains (Fig. 1) (another linker PEG_2 gave similar affinity with no significant difference, see Fig. S4 in the Supporting Information). The combination of peptides **p2**, **p3**, and **p4** gave six dimeric peptides **2-2**, **2-3**, **2-4**, **3-3**, **3-4**, and **4-4** (Fig. 2A). The dissociation constants K_D between ligands and syntenin tandem PDZ domain were measured to be 24 μM for **2-2**, 3.1 μM for **2-3**, 24 μM for **2-4**, 0.80 μM for **3-3**, 3.4 μM for **3-4**, and 98 μM for **4-4** (Fig. 2B). Compared with monomeric peptides **p2**, **p3**, and **p4**, which showed dissociation constants of 43 μM for **p2**, 35 μM for **p3**, 65 μM for **p4** between ligands and syntenin tandem PDZ domain (Fig. S5 in the Supporting Information), most dimeric ligands have an obvious increase in the binding affinity. This is especially true for dimeric ligands containing **p3**: the affinity of **2-3** and **3-4** was about 3 μM, ten times higher than the original binding motifs **p2**, **p3** and **p4**. Furthermore, the homodimeric peptide **3-3** showed the highest

Table 1
Synthetic peptides derived from natural binding partners of syntenin and their binding affinities with syntenin PDZ1 and PDZ2 domains.

Peptides	Sequences		*Kd* (PDZ1) (μM)	*Kd* (PDZ2) (μM)
p1	FAM C G S Y	G S R V A F F E E L	282 ± 29	531 ± 114
p2	FAM	G C G S T N E F Y A	94 ± 6	68 ± 4
p3	FAM	G C G S D K E Y Y V	81 ± 7	33 ± 2
p4	FAM	G C G S L E D S V F	58 ± 4	60 ± 5