In Situ Self-assembly of Peptides for Cancer Therapy

Literature seminar #2 2024/6/6 M1 Mayo Yamazaki

Contents

Introduction

■ What is the significance of self-assembly of peptides?

ALP-instructed self-assembling peptides

• Design of self-assembling peptides selectively targeting cancer cells

- 1. D-peptides vs. L-peptides
- 2. The number of the enzyme recognition sites
- 3. Addition of sites that react with other enzymes
- Summary & Perspectives

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Summary & Perspectives

Advantage of self-assembly for therapy

Challenges in the therapeutic effects of drugs

- Weak drug-target interaction
 → Limitation of therapeutic effects
- Drug resistance of target proteins

Advantage of self-assembly of peptides for therapy

- Self-assembling in situ
- Multivalent interactions
- Extended duration
- Rapid response
 - \rightarrow Improvement for pharmacokinetics
- Mechanical stress



Controlling noncovalent local interactions by self-assembly → Development of supramolecular assemblies functioning in vivo 2024/6/6

What causes self-assembly in vivo environment



Introduction

- Specific pH
- Redox environment
- Presence of specific enzymes

Strategy

Introducing <u>biological reaction sites</u> in the design of <u>precursors</u> for self-assembling peptides

→ Peptides can self-assemble in a specific environment

While reactive oxygen species (ROS) are mainly generated in the mitochondria, as well as, to a lesser degree, by the membrane-bound NADPH oxidase (NOX), the cellular reducing agent glutathione is present in high concentration (10 mM) throughout the cytosol. During the endocytic pathway, the pH inside the vesicles decreases significantly from 6.3 in the early endosomes to 5.5 in the late endosomes to 4.7 in the lysosomes, whereas the cytosolic pH is typically neutral, at around 7.2.

Weil, T. et al. Nat. Rev. Chem. 2022, 6, 320-338

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EISA; Enzyme-instructed self-assembly



Biomimetic multi-step process

 <u>Enzymatic transformation</u> of non-self-assembling precursors to self-assembling molecules
 <u>Self-assembling</u> of molecules

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Introduction

Higher expression of ALP on cancer cells



Alkaline phosphatase (**ALP**, Protein tyrosine phosphatase)

- On the cell membrane
- Highly expressed in some cancer cells
 - \rightarrow bio-marker of cancer

Challenges in targeting ALPs for therapy
ALPs play a crucial role in the liver and brain!
Inhibitor of ALP will lead side effects
→ undruggable...

EISA strategy Using ALP as <u>a trigger</u> for self-assembly of peptides

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Introduction

Mechanism of self-assembly of peptides

Ulijn, R. V. et al. Chem. Soc. Rev. 2014, 43, 8150-8177



Design of ALP-instructed self-assembling peptides



Yang, Z., Xu, B. et al. Angew. Chem. Int. Ed. 2007, 46, 8216–8219

Design of self-assembling peptides selectively targeting cancer cells

How can we design self-assembling peptides that selectively target cancer cells?



- 1. D-peptides vs. L-peptides
- 2. The number of the enzyme recognition sites
- 3. Addition of sites that react with other enzymes

Li, J., Xu, B. *et al. J. Am. Chem. Soc.* **2013**, *135*, 9907–9914 Zhou, J., Xu, B. *et al. J. Am. Chem. Soc.* **2016**, *138*, 3813–3823 Feng, Z., Xu, B. *et al. J. Am. Chem. Soc.* **2017**, *139*, 15377–15384

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• Summary & Perspectives

D-peptides vs. L-peptides



Active sites of ALP

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Expect to be more

biostable precursor

Self-assembling ability of D-peptides



 Precursors L-peptides and D-peptides undergo dephosphorylation at similar rates upon being treated with ALP. \leftarrow 31P NMR shows the conversion of 1.0 wt % of (A) **1a** and (B) **1b** catalyzed by the phosphatase (0.02 U/mL) at pH 7.6 at 3 min and 4, 12, 24, and 48 h. Time-dependent rheology study of 1.0 wt % of (C) **1a** and (D) **1b** catalyzed by the phosphatase (0.02 U/mL) at pH 7.6.

L-peptides





Optical images and TEM images of the hydrogels formed by using ALP (1.0 U/mL) to treat 0.4 wt % of (A) 1a and (B) 1b at pH 7.6.

✓ Hydrogelators L-peptides and D-peptides self-assemble to form long, flexible, and uniform nanofibers.

Chirality of these two hydrogelators → little difference in the properties of the resulting hydrogels

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D-peptides

Li, J., Xu, B. et al. J. Am. Chem. Soc. 2013, 135, 9907–9914

D-peptides can resist proteinase



Figure S12. The molecular structures of **1a** and **1b**, and their time-dependent course of the digestions by proteinase K.

✓ D-peptides resists proteinases ↔ L-peptides undergoes proteolytic hydrolysis

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D-peptides

Short Summary of D-peptides



D-peptides

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- ✓ D-peptides can act as a substrate of phosphatase.
- ✓ **D-peptides** can self-assemble by enzyme-instruction.
- ✓ D-peptides are intrinsically resistant to proteolytic hydrolysis.

Li, J., Xu, B. et al. J. Am. Chem. Soc. 2013, 135, 9907–9914

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The number of phospho-Tyr in one peptide

Enzyme recognition sites



Molecular structures of the precursors (a-2p, b-2p, c-2p, a-1p, b-1p, and c-1p) that have one or two phosphotyrosine residues and the corresponding self-assembling d-peptides (i.e., hydrogelators a, b, and c).

Zhou, J., Xu, B. et al. J. Am. Chem. Soc. 2016, 138, 3813-3823

Self-assembling ability with ALP



in the solutions of different precursors (a-2p, a-1p, b-2p, b-1p, c-2p, and c-1p) or nanofibers in the hydrogels formed by treating the solutions of the precursors with alkaline phosphatase (ALP). C = 0.5 wt %, pH = 7.4, [ALP] = 1 U/mL. Insets are optical images of the solutions of the precursors and the hydrogels formed after enzymatic dephosphorylation. The scale bar is 100 nm. (B) Time-dependent rheometry to

TEM images of aggregates/nanofibers

show the gelation points (that is, at the gel state, where storage modulus (G') dominates loss modulus (G'') of different precursors treated with ALP (0.05 U/ml). C = 0.5 wt %.

- ✓ ^DF^DY^DF^DY exhibits **higher** ability to self-assemble (due to the orientation of ^DFs)
- ✓ **One phosphorylated precursors** form hydrogels much faster than their corresponding two phosphorylated precursors

Zhou, J., Xu, B. et al. J. Am. Chem. Soc. 2016, 138, 3813–3823

- solubility of the precursors. ✓ +ALP
 - \rightarrow All can self-assemble in water to form nanofibers.

 \checkmark Two phospho-tyr residues enhance the aqueous

Selectivity for cancer cells



✓ The precursors relatively selectively inhibited the survival of cancer cells over normal cells.

✓ HeLa, SK-OV-3, A2780cis (higher or a little higher)
 IC50 value for the monophosphorylated precursor was smaller compared to the diphosphorylated precursor.

Enzyme recognition sites

(B) IC50 of different precursors/hydrogelators against different cell lines after 48-h incubation.

Cell line	Cell origin	ALP expression levels	
HeLa	cervical cancer cell	Higher	
SK-OV-3	drug-resistant	A little higher	
A2780cis	cell	Higher	
T98G	glioblas-toma multiforma tumor cell	A little higher	
Saos-2	bone osteosarcoma cell	Much higher	
HS-5	immortalized normal stromal cell	(Standard)	

Short Summary of the number of enzyme recognition sites



Enzyme recognition sites

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 ✓ By increasing the number of enzyme recognition sites, peptides can avoid organizing in cells with moderately high ALP expression
 → improving selectivity for targeting cells with very high ALP expression

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Lower selectivity of monophosphorylated peptides



How can we improve selectivity for cancer cells over normal cells with highly ALP expression?

Addition of CES-reacted sites

Dual enzyme-instructed



<u>Strategy</u>

Carboxy esterase in some cancer cells are downregulated

OMe (Increasing hydrophobicity and neutralizing negative charge of amino acid residues, substrate of CES)

vs. NHMe (Not react with CES)

Inhibitory activities against different cell lines



 Peptides with much lower selfassembling ability were generated by hydrolysis of the methyl ester bond of 1p with CES.

Cell line	Cell origin	ALP expression levels	CES expression levels
Saos-2	bone osteosarcoma cell	Much higher	Down regulated
MCF-7	Breast adenocarcinom a cell		Higher
T98G	glioblas-toma multiforma tumor cell	A little higher	Higher
HS-5	immortalized normal stromal cell	(Standard)	(Standard)

Dual enzyme-instructed

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Lower toxicity by introducing CES-reacted moiety



Feng, Z., Xu, B. et al. J. Am. Chem. Soc. 2017, 139, 15377–15384 Feng, Z., Xu, B. et al. J. Am. Chem. Soc. 2017, 139, 3950–3953

Molecular transformation in cell

Dual enzyme-instructed



ALP activities: Saos-2 > HepG2 > HS-5

↔ HepG2: high expression level of CES results in hydrolyzing 26% of carboxyl methyl ester

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Short Summary of addition of other enzymes-reacted sites

Dual enzyme-instructed



ET = Enzymatic Transformation; SA = Self-Assembly; DA = Disassembly

✓ Dual enzyme-instructed self-assembling peptides can selectively inhibit the osteosarcoma cells without harming liver cells.

<u>Next</u> : Activity of dual enzyme-instructed self-assembling peptides in vivo

Feng, Z., Xu, B. et al. J. Am. Chem. Soc. 2017, 139, 15377–15384 Feng, Z., Xu, B. et al. J. Am. Chem. Soc. 2017, 139, 3950–3953

Activity of self-assembling peptides in vivo

1P

1P

Saline Saos2-luc

1P Saos2-luc Saline Saos2-lung

1P Saos2-lung

Luciferase Intensity (pl/s/cm²/sr) (10⁶)

×10⁶

G 120-

vivir

80-

60.

0.4

_ 0.2

Saline

Saline

Е

1w

2w



Orthotopic mouse model of osteosarcoma

- Iuciferase-labeled Saos-2 cells (Saos2-luc)
- highly metastatic subtype (Saos2-lung) cells



Dual enzyme-instructed



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Feng, Z., Xu, B. et al. Chem. 2019, 5, 2442-2449

Effect of CES-reacted sites in vivo



✓ 2P and 3P which did not have CES-reacted moiety inhibited the growth of tumor less effectively than 1P.



Figure S9. (A) Images of osteosarcoma tumor growth using in vivo imaging system (IVIS) for orthotopic osteosarcoma model established by Saos2-luc and Saos2-lung cells at week 1-4 after **2P**, **3P** or saline treatment. (B) Quantification of luciferase intensity. Data are presented as mean ± standard deviation (S.D.). ***P < 0.001, **P < 0.01.



Feng, Z., Xu, B. et al. Chem. 2019, 5, 2442-2449

Figure S10. Kaplan-Meier survival curves for orthotopic osteosarcoma nude mice (n = 5) treated with 2P or 3P.

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Summary

How can we design self-assembling peptides that selectively target cancer cells?



- 1. To maintain bio-stability, D-peptides were utilized.
- 2. Adjusting the number of enzyme recognition sites increased target cell selectivity.
- 3. Adding sites recognized by other downregulated enzymes improved cancer cell selectivity.

Li, J., Xu, B. *et al. J. Am. Chem. Soc.* **2013**, *135*, 9907–9914 Zhou, J., Xu, B. *et al. J. Am. Chem. Soc.* **2016**, *138*, 3813–3823 Feng, Z., Xu, B. *et al. J. Am. Chem. Soc.* **2017**, *139*, 15377–15384

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Perspectives

- Multiple enzymes can control the EISA process and can precise regulate of the formation of the assemblies in different cellular environments.
- ← Need to design peptides with information of expression level of enzymes
- EISA has possibility to inhibit the multiple pathways of cells simultaneously in space and time.
- ← Not rely on ligand-receptor binding



Thank you for your kind attention!

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