平成19年　2月　9日

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２１世紀ＣＯＥプログラム

拠点：大学院工学系研究科
応用化学専攻、化学システム工学専攻、
化学生命工学専攻、マテリアル工学専攻

“化学を基盤とするヒューマンマテリアル創成”

平成１８年度リサーチ・アシスタント報告書

ふりがな 氏名	リュウ　ブンカイ 劉　文海	生　年　月　日
所属機関名	東京大学大学院工学系研究科化学生命工学専攻	
所在地	〒113-8656 東京都文京区本郷 7-3-1 東京大学大学院工学系研究科化学生命工学専攻　工学部５号館	
申請時点での学年	D1	
研究題目	Construction of a fluorescein-responsive chimeric receptor with strict ligand dependency	
指導教員の所属・氏名	東京大学大学院工学系研究科化学生命工学専攻 長棟　輝行	

I　研究の成果　（1000字程度）

[***Introduction***] In our previous study, we designed anti-fluorescein (FL) antibody/receptor chimeras in response to FL-conjugated BSA (BSA-FL). However, considerable background cell proliferation was observed without antigen. Therefore, we tried to redesign chimeric receptor constructs with different combinations of the domains containing anti-FL single chain Fv (ScFv), extracellular D1/D2 domains, transmembrane/intracellular domains of erythropoietin receptor (EpoR) or glycoprotein 130 (gp130), for construction of a strictly fluorescein-dependent chimeric receptor. We also tried to analyze the role of erythropoietin receptor domains in signal transduction.

[***Methods and Results***] We designed a series of chimeras. Firstly an anti-FL ScFv was fused to full-length EpoR. Next we tried to delete extracellular D1 or D2 domain of EpoR, to mutate transmembrane (TM) domain, to exchange the intracellular domain (ID) into that of gp130, and/or to insert several Ala residues into juxtamembrane domain to modulate the conformation of intracellular domain(Fig.1). Chimeric receptors were expressed in IL-3-dependent Ba/F3 cells to compare their growth characteristics. We found that BSA-FL acted as an inverse agonist at some chimeric receptors, whereas it also acted as an agonist at other chimeric receptors. We also found the effect on cell growth induced by the TM domain mutation and the insertion of Ala residues between TM and intracellular domains of chimeric receptors. Notably, one chimeric receptor, ScFv-EpoRTM-gp130ID (Fig.2), transduced a strict BSA-FL dependent growth signal without any background cell growth. Therefore, this chimera might be promising as a basis for cell growth-based screening of high-affinity ScFvs derived from a randomized antibody library, where simple culture in antigen-containing medium results in growing cells with a high-affinity antibody gene, leading to antibody selection.

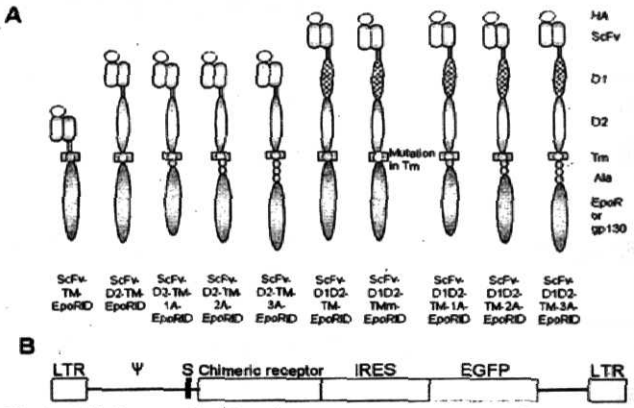

Fig.1 (A)Scheme of chimeric receptor constructs and vectors. (B) Schematic diagram of vectors. Retroviral vectors with long-terminal repeats (LTRs) and a packaging signal (ψ) are used. An immunoglobulin heavy chain secretion signal sequence (S) is located upstream of the chimeric receptor genes to enable their cell surface expression.

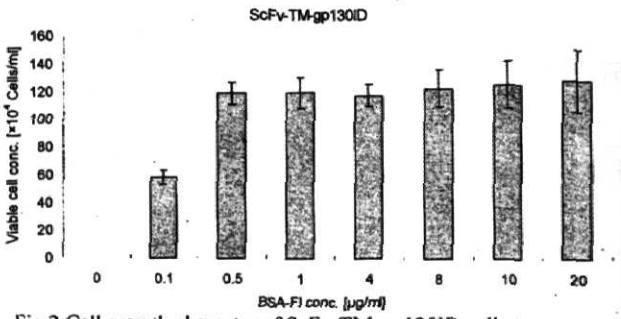

Fig.2 Cell growth character of ScFv-TM-gp130ID cells to BSA-Fl. Cells (10^4 cells/ml) were inoculated into 96-well plates on day 0 and cultured in the presence of BSA-Fl concentrations: 0, 0.1, 0.5, 1, 4, 8, 10, 20μg/ml . Viable cell concentration on day 6 is plotted with average.

氏　　名

Ⅱ（1）　学術雑誌等に発表した論文A（掲載を決定されたものを含む.）

共著の場合、申請者の役割を記載すること.

（著者、題名、掲載誌名、年月、巻号、頁を記入）

氏　　名　　劉　文海

Ⅱ（2）学会において申請者が口頭発表もしくはポスター発表した論文
（共同研究者（全員の氏名）、題名、発表した学会名、場所、年月を記載）

①Title:Construction of a fluorescein-responsive chimeric receptor with strict ligand dependency
Author:Wenhai Liu, Masahiro Kawahara, Hiroshi Ueda Teruyuki Nagamune
Proceeding: China/USA/Japan Joint Chemical Engineering Conference, Bejing, China, 2005, P06-18 (2005)(English)

②Title:A fluorescein-responsive chimeric receptor with strict ligand dependency
Author:Wenhai Liu, Masahiro Kawahara, Hiroshi Ueda Teruyuki Nagamune
Proceeding:The 78th Annual Meeting of the Japanese Biochemical Society, Kobe, Japan, 2005, 4P-552 (2005)(English)

③1．タイトル Construction of a fluorescein-responsive chimeric receptor with strict ligand dependency and analysis of the role of erythropoietin receptor domains in signal transduction
2．氏名(著者名)．Liu, M. Kawahara, H. Ueda and T. Nagamune
3．会議録名,開催地,開催年,頁-頁(発行年)(言語)
The 19th International and Annual Meeting of Japanese Association for Animal Cell Technology
Kyoto Japan, 2006, 100-101(2006)(English)

④1．タイトル Construction of a fluorescein-responsive chimeric receptor with strict ligand dependency—— For establishing a novel method to select high-affinity antibody against any antigen
2．氏名(著者名)．Liu, M. Kawahara, H. Ueda and T. Nagamune
3．会議録名,開催地,開催年,頁-頁(発行年)(言語)
創薬工学シンポジウム 東京, 2006, (日本語)

⑤1．タイトル Construction of a fluorescein-responsive chimeric receptor with strict ligand dependency and analyse the role of erythropoietin receptor domains in signal transduction
2．氏名(著者名)．Liu, M. Kawahara, H. Ueda and T. Nagamune
3．会議録名,開催地,開催年,頁-頁(発行年)(言語)

4th COE 21 International Symposium
東京, 112(2006)(English)

⑥1. タイトル 厳密にリガンド依存性を持つフルオセレイン応答性キメラ受容体の構築
2. 氏名(著者名). Liu, M. Kawahara, H. Ueda and T. Nagamune
3. 会議録名,開催地,開催年,頁-頁(発行年)(言語)
東京大学生命科学研究ネットワークシンポジウム
東京, 83(2006)(日本語)