Ⅲ. 研究成果の刊行に関する一覧

書籍

著者氏名	論文タイトル名	書籍全体の編集者名	書籍名	出版者名	出版地	出版年	ページ
S. Hashiguchi, M. Yamamoto, S. Kitamoto, T. Nakashima, H. Yamanaka, D. Ishibashi, S. Sakaguchi, S. Katamine, Y. Ito, K. Sugimura	Prion-conformation-specific human antibodies established from phage display library. Prions	T. Kitamoto	Prions: Food and Drug Safety	Springer	Japan	2005	191-192

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Human antibodies specific to beta-sheet-rich isoform of human prion protein .

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The pathogenesis of prion disease involves a structural change of prion protein (PrP). A series of antibodies recognizing a distinct conformational change of prion is useful for not only understanding the mechanism of molecular conversion but also for diagnostic and therapeutic reagents. We have previously prepared two kinds of refolded recombinant human prions according to the Collinge's protocol (Science 283: 1935-1937, 1999). Alpha-helical confomation (α-PrP) of PrP23-231 was prepared under neutral pH condition whereas the beta-sheet-rich confomation (β-PrP) was prepared by refolding the PrP23-231 under reductive and pH4.0 condition that likely endosome-like condition. The soluble β form (β-PrP) exhibited partial resistance to proteinase K digestion, and composed of small spherical particles (diameter 3 nm). Direct panning was done against β-PrP using a large scale of the human scFv-displaying phage liberary. After two rounds of panning, we successfully isolated two clones (β-PrP7, β-PrP30) specifict to β-PrP. We also tested the binding activity of these clones using β-PrP samples incubated at 4°C by ELISA. The binding activity of β-PrP7 was dramatically increased dependent on the incubation time, indicating that β-PrP consists of various β-conformers and the content of ones of conformers change dynamically in the solution at 4°C. In addition to immnochemical examinations including epitope and affinity analysis, we are going to test the immunohistological ability of these antibodies using brain with prion disease.

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Antibody-phage library clones panned on ELISA plate coated with PAGE gels

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[Objective] Animal-based hybridoma technology requires a quantity of purified antigen for optimal antibody generation. In contrast, phage library provide a revolutionary means to produce monoclonal antibodies since this tech. is independent of immunization and requires an antigen at ng order. We have proposed a method (ultra high speed method: UHSM) which permits direct selection of scFv phage to a protein fractionated by Native-PAGE of habu-snake toxin[1)]. We here determined the sensitivity of UHSM, i.e., the quantity of target antigen, and the frequency of specific clone in phage library using a model system.

[Method] A minute amount of rhIL-18 mixed with N2a cell extracts were run on a Native-PAGE. The gel was fractionated into 8 fragments. Each gel fragment was incubated with PBS in well. After blocking with skim milk, each well was incubated with IL-18-specific scFv phage solution, followed by washing. Bound phage were eluted with 0.1M glycine-HCl(pH2.1). The titer of bound phage was determined by infection of *E. coli.* TG-1.

[Results] Anti-IL-18-scFv phage was recovered from fractions containing IL-18, but not from other fraction. The required amount of IL-18 was as little as 2 ng/lane of PAGE.

[Reference] 1) H. Kokuryo et al., 76th Annual Meeting of the Japanese Biochemical Society, 2003

プリオン研究会

2006年9月

岩手

全長リコンビナントプリオン分子から作製された繊維型及びオリゴマープリオンに対するヒト抗体ファージクローンの反応性について

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プリオン病の病態解析には、そのコンフォメーションを認識する抗体が有用となる。この作製のためには、1. リコンビナントプリオン蛋白から作製する方法あるいは、2. プリオン感染脳組織を用いて直接作製する方法がある。1.のアプローチは、*in vitro* で自在にコンフォメーションを制御でき、かつ大量に調整できる。私たちは、これまでにヒト一本鎖抗体(scFv)を提示したファージライブラリーを反応させ、そのコンフォメーションに特異的な抗体単離を試みてきた。

昨年度の本研究会において、Collinge らのプロトコールにもとづいて作製したβ型プリオン(β-PrP)に特異的に結合する抗体ファージクローン(β-PrP7, β-PrP30)を単離したことを報告した。

Collinge らは、プリオン 90-231 を用いて、プリオン fiber の作製に成功しているが、Hashiguchi らは、プリオン 23-231 を用いて fiber 形成を観察することは出来なかった。一方、最近 Baskakov らは、プリオン 23-231 を用いてβ型の fiber 及び oligomer を作製したことを報告した。そこで今回は、Hashiguchi らが単離したβ-PrP7, β-PrP30 ファージ抗体が、Baskakov らのプロトコールにより作製したプリオンに結合するかどうかを検討した。

表 1. リコンビナントプリオン蛋白のフォールディング結果のまとめ

Researcher	Seq.	CD	PK[4]	Conformation	Cytotoxicity
Collinge ら [1]	90 - 231	β-form	+	β-oligomer → fiber	?
Hashiguchi ら [2]	23 - 231	β-form	+	β-oligomer → -	?
Baskakov ら [3]	23 - 231	β-form	+	β-oligomer → fiber	+
Kubota ら	23 - 231	β-form	+	β-oligomer → fiber	?

1. Collinge J. et al., Science 283: 1935-37, 1999

2. プリオン研究会，仙台（2005）

3. Baskakov, I.V. et al., *J Mol Biol* 346, 645-59, 2005

4. PK = Proteinase K-resistance