

- HCV ,
(host factor)
가 가 .
- C (HCV) flaviviridae
9.5 kb 가 (positive strand) RNA
non-A, non-B ¹⁾ HCV
70%가
3 ligand
(binding pocket)
(discontinuous determinants)
(linear structure) peptide
ligand- (mimicking)
- 7 HCV
³⁾ .
HCV 1989 ⁴⁾ in
vitro HCV
life cycle
가 . HCV가 B
, T , monocyte
¹⁾ tropism
- .
HCV 31-35 kDa E1 58-74 kDa
E2가 . E2
⁵⁾ . E2
- E2
가 HCV
E2 peptide library biopanning
E2 (gD-E2)
. gD-E2 herpes simplex
gD HCV E2 CHO
anti-gD affinity column .
. BSA, gD, gD-E2 가
5 µg/ml coating buffer (0.1 M NaHCO₃, pH
8.6) microtiter plate (Maxisorb, Nunc, Denmark)
well 4 16 .
well 3% BSA/PBS
blocking . 7-mer 12-mer random peptide가
pIII peptide library (Ph.D.7
Ph.D.12, NEB) 10 µl 40 µl 3% BSA/TBS [50 mM
Tris-HCl (pH 7.5), 150 mM NaCl] 가 .
BSA gD phage peptide
- ²⁾ . 1
³⁾ .
⁴⁾ in
⁵⁾ .
⁶⁾ Pileri
A2R
cDNA library HCV tropism
transfection E2
, tetraspanin superfamily CD81
가 E2 T .
B , monocyte, HCV
B (HBV) asialoglycoprotein receptor (ASGPR), endonexin II, hepatitis B virus binding factor, transferrin receptor, preS1-BP35
⁷⁾ .

Random peptide library				C	E2	peptide mimotope			
library 50 μ l	BSA가	well				phage peptide	50 μ l		
	1					well	3		
	gD가	well				PBST	4		
			gD-E2가	HRP-conjugated anti-M 13	(Pharmacia, USA)				
well	3			1:5,000	1				
0.1% Tween 20 가				PBST	ABTS (Pierce, USA)	100 μ l			
TBS(TBST) buffer	50 μ l	elution			50 μ l	2% SDS			
buffer [0.1 M glycine-HCl (pH 2.2), 0.1% BSA]				가		405 nm			
gD-E2	phage peptide			BSA	gD	gD-E2	가 가		
1 M Tris-HCl (pH 9.1)	phage peptide								
panning	panning			Peptide sequencing					
phage (input pahge)	phage (output phage)					phage peptide			
(ratio, O/I ratio)						500 μ l			
E2- binder				200 μ l	5X PEG/NaCl	가	10		
OD ₆₀₀ nm=0.5	ER2537	20 ml			phage peptide		10,000 X		
40 μ l phage peptide	37			g	20				
1	100 ml	SB			10 μ l	iodide buffer (4 M Sodium iodide in TE			
[30 g Bacto-tryptone, 20 g Yeast extract, 10 g MOPS				buffer)	가	pellet	phage DNA		
(pH 7.0), per 1 liter]	가	37	16			250 μ l			
	10,000 X g, 20	4		10		10,000 X g	15		
	30 ml	5X PEG/NaCl [20%		80%		30 μ l	TE buffer		
PEG (w/v), 15% NaCl (w/v)]	가	4	30	phage DNA					
	PEG			DNA		dideoxynucleotide chain termination			
1 ml 3% BSA/TBS	phage peptide pellet					(ABI, USA)			
2 biopanning	2 panning					sequencing primer	vector sequence		
gD-E2	panning	2,		5'-GCC CTC ATA GTT AGC GTA ACG-3'					
1, 0.5 μ g/ml		5, 10, 20							
가									
E2	phage peptide			free form HCV					
O/I ratio가 가	biopanning			C		HCV가 free			
phage peptide	ER2537	plaque가		form	anti-HCV				
plate 100-200	plating			(immune complex)		10 ³	400 μ l		
plaque	1 ml	ER2537		HCV RNA (+)	1:4	1:43	PBS		
37	5			10,000 X g	5				
SB	900 μ l	100 μ l	37		goat anti-human IgG	(1:1,000)			
16		10,000 X g		g	15	free form HCV가	10,000 X		
	phage peptide			pellet					
BSA, gD, gD-E2가	1 μ g/ml			RT - nested PCR HCV					
microtiter plate	well	3% BSA/PBS	blocking	pellet		HCV			

RT-nested PCR

pellet 3 200 μ l

4 RNAzol B solution

(TEL-Test. Inc, USA) 가 4 5

chloroform : isoamylalcohol (24:1) 200 μ l

4 , 12,000 rpm 14

isopropanol 500 μ l tRNA (50 μ g/ml) 2 μ l

가 HCV RNA

75% pellet 30 μ l diethyl

pyrocarbonate (DEPC)가 RNA

17 μ l HCV 5'-UTR primer 1

2 RT-PCR kit (Bioneer, Korea) 가 57

10 , 42 60 , 94 5

cDNA 94 30 ,

55 30 , 72 1 cycle 35

72 7

PCR 2 μ l nested primer 3 4

PCR kit (Bioneer, Korea) 가

DNA 2% agarose gel

primer

Primer 1 : 5'-CTGTGAGGAAGTACTGTCTT-3',

Primer 2 : 5'-GTCTCGTAGACCGTGACCATG-3'

Primer 3 : 5'-TTCACGCAGAAAGCGTCTAG-3'

Primer 4 : 5'-GCCTGATAGGGTGCTTGCGAGTG-3'

HCV - capture ELISA

HCV solid phase

phage peptide

capture ELISA . Capturing

antibody HCV E2

H25 10

μ g/ml coating buffer microtiter

plate 100 μ l 4 16

. DW 0.4% BSA/PBS blocking

. free form HCV 1:64

well 1

. PBST 3 , 1×10^{12} pfu

phage peptide 2

E2 phage

peptide .

(Neutralization of binding (NOB) assay)

gD-E2

E2 가 E2

T-

MOLT-4

10%

RPMI 1640 5% CO₂가

37 . 1 μ g gD-E2

2 X 10⁹, 6 X 10⁹, 1.8 X 10¹⁰, 5.4 X 10¹⁰

phage peptide 40 μ l 4 30

. 5 X 10⁵ MOLT-4 가

E-tube

PBST 3 pellet 1X

sample buffer [60 mM Tris-HCl (pH 6.8), 2% SDS, 25% glycerol, 14.4 mM 2-mercaptoethanol, 0.1% bromophenol blue]

10% polyacrylamide gel

gel nitrocellulose

membrane E2 H52

HRP-conjugated anti-mouse IgG

western blot . ECL

(Amersham, USA) , X-Omat film (Kodak, USA)

E2 biopanning

7 mer-, 12 mer peptide library gD-E2

6 biopanning panning

phage peptide output phage/input phage

(ratio) . 7-mer library 5

panning 가 . 12-mer

library , panning 가 O/I ratio가

가 가 ratio (

1A).

E2 - binder

library panning O/I ratio가 가 3

(7-mer library) 5 (12-mer library)

phage peptide plaque . plaque

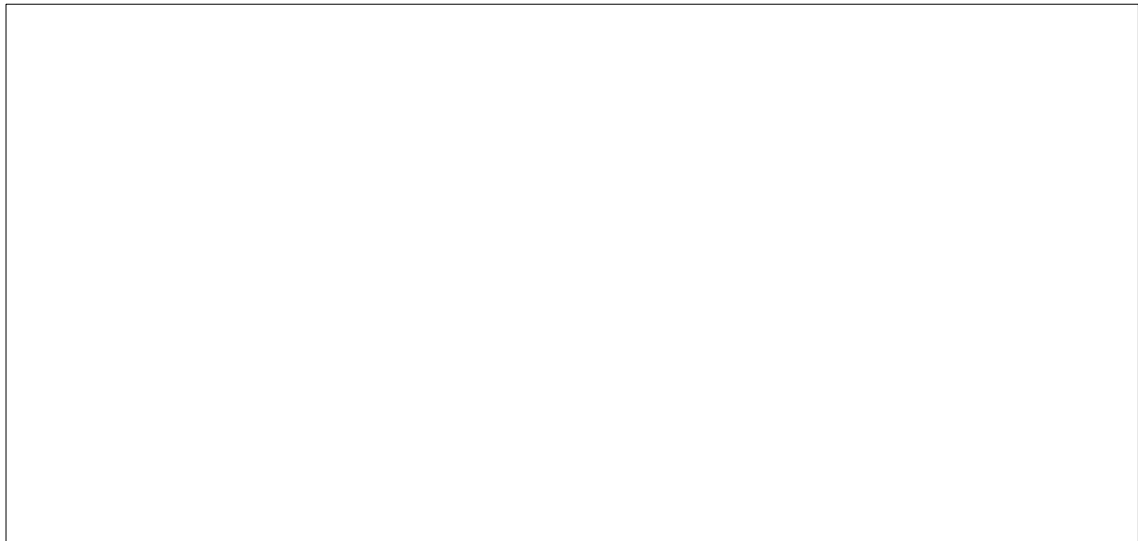


Fig. 1. Biopanning of phage peptide. (A) Output phage / Input phage (O/I) ratio. From each biopanning step, E2-binding phage peptides were eluted with 0.1 M glycine-HCl (pH 2.2), amplified and concentrated for subsequent panning. Enrichment of specific phages from either 7-mer or 12-mer is described as a ratio of eluted phages (output phage) over phages applied into reaction (input phage). (B) ELISA of phage peptides selected from either 7-mer or 12-mer phage peptide library. Phage peptides from each plaques were infected and amplified into *E.coli* ER2537. ELISA against E2 protein was performed using phages concentrated with PEG. Bound phages were detected using HRP-conjugated anti-M13 antibody and OPD as a color reagent.

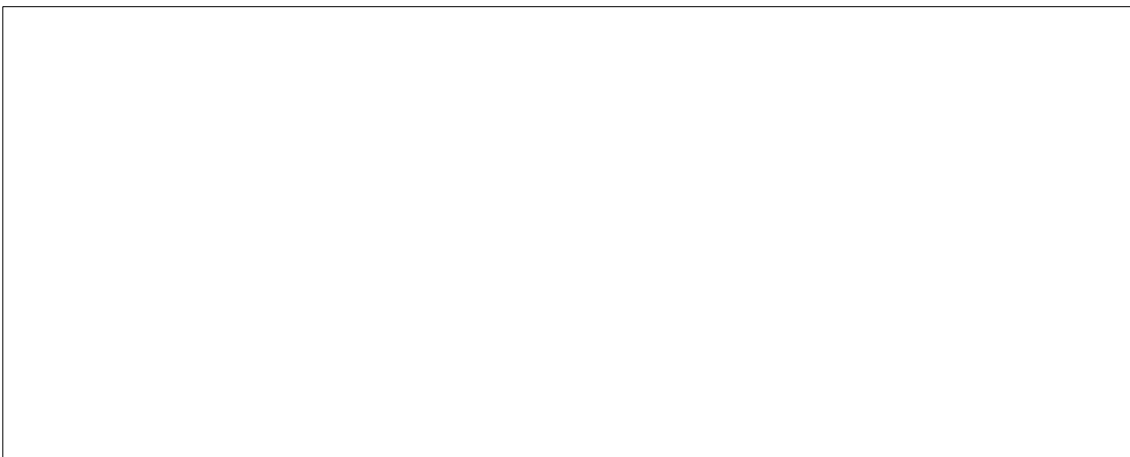


Fig. 2. Specificity of phage peptides. (A) Phage peptide sequences are determined. Conserved sequences are indicated as bold letters. (B) Phage peptides of indicated number were added and incubated into E2-coated wells of microtiter plate. Bound phages were detected using HRP-conjugated anti-M13 antibody and OPD as a color reagent.

phage	BSA, gD, gD-E2	1B),	12-mer library	phage
ELISA	. 7-mer library	phage	. phage peptide	
E2	0.025	BSA gD	gD-E2	6
12-mer library	phage	phage	. E2	
7-mer library	6 가	(0.15-0.27	.	

Peptide sequence		phage peptide		dose		E2
		1C 1, 1H 1, 1B 2	phage	dose		
E2	6	phage peptide	7†	2B 1	phage	dose
DNA		E2	(2B).		
Ser-His-Phe-Try-Arg-Ala-Pro (SHFWRAP) 7		HCV - capture ELISA				
conserved	(2A).				
E2	(binding specificity)	HCV RNA titer	7†	10 ⁶		C

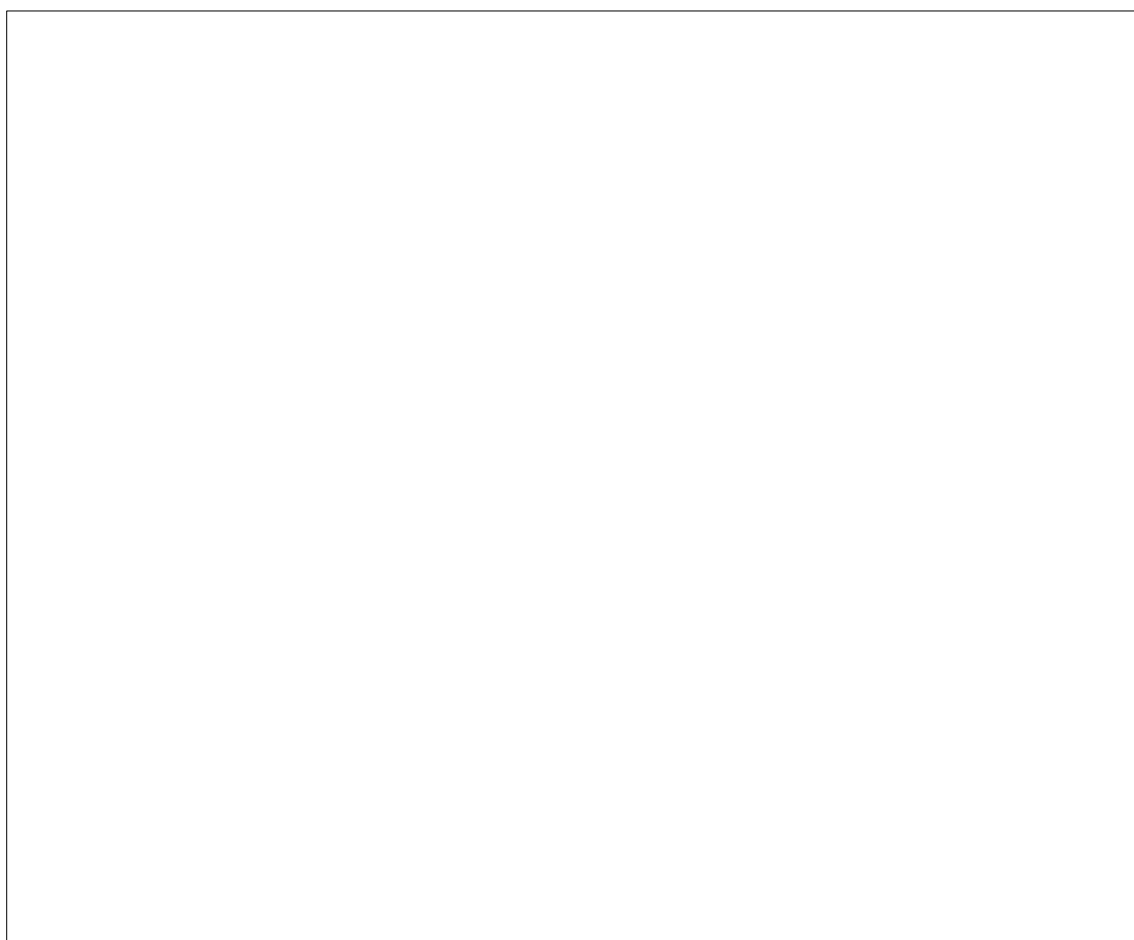


Fig. 3. Detection of HCV particles using phage peptides. (A) Diagram of strategy for amplification of 5'UTR of HCV RNA genome using nested primers. (B) The sera from patients with hepatitis C diluted to 1:4 or 1:4³ were incubated with anti-human IgG antibody, centrifuged to restore pellets and supernatant fractions. Each fraction was treated with RNAzol B solution for purification of RNA, and subjected to RT-nested PCR for amplification of RNA genome (P: pellet, S: supernatant). (C) The sera of patients with hepatitis C were incubated in wells of microtiter plate coated with anti-E2 rabbit sera (Rbt) and anti-E2 monoclonal antibody (H25), respectively. The captured viral particles with antibodies as indicated above were detected with anti-E2 monoclonal antibody and HRP-conjugated anti-mouse IgG antibody, or anti-E2 rabbit antibody and HRP-conjugated anti-rabbit IgG antibody, respectively. (D) Phage peptides of indicated numbers from each clone were added into the wells coated with H25 monoclonal antibody of microtiter plate where the patient's serum were preincubated. After washing with PBST, bound phage peptides were detected using HRP-conjugated anti-M13 antibody and OPD as a color reagent.

	Random peptide library	C	E2	peptide mimotope
	(immunoprecipitation)	RT-nested	H25	capture ELISA
PCR	free form	HCV		가
. High titer	4	1:4	HCV	(capturing)
	pellet	immune	form HCV가	, free
complex form	1:64	3	3C).	(
HCV RNA	pellet		HCV	H25
.	1:64		capture ELISA	phage peptide가
(3A,B).			1C 1, 1H 1, 1B2	. E2
<i>in vitro</i>		HCV		phage
capture antibody	solid phase	ELISA plate	dose	phage dose
	HCV		HCV	phage
			3D).	(



Fig. 4. Neutralization of binding of E2 protein to MOLT-4 cells. (A) gD-E2 of indicated amount was allowed to react with 0.5 μ g gD, then mixture was added into MOLT-4 cells. After washing with PBST, the cells were treated with sample buffer and subjected to SDS-PAGE and Western blot. E2 protein bound to MOLT-4 cells was detected using anti-E2 monoclonal antibody as a primary antibody and HRP-conjugated anti-mouse IgG as a secondary antibody and ECL as a color reagent. (B) E2 protein was mixed with phage peptides of indicated numbers from each clone, and incubated with 5×10^5 MOLT-4 cells. After washing in PBST, the cells were treated with sample buffer and subjected to SDS-PAGE and Western blot. E2 protein bound to MOLT-4 cells was detected as described elsewhere. (C) Graph describing a densitometric analysis based on the (A), in which the signal of each phage peptide was compared with that of control phage peptide and the results were indicated as percentage.

spike protein gp41¹³⁾ , /

E2 MOLT-4 () phage peptide ,

gD-E2 MOLT-4 E2

gD competition . 4A HCV

gD-E2 gD MOLT-4 ,

gD-E2 E2 ,

가 HCV

Phage peptide dose gD-E2 E1 E2 ,

MOLT-4 E2 ligand⁵⁾ .

1C1 1B2 phage dose

MOLT-4 E2 HCV E2

1C1 가 1H1 (E2

2B1 2G1 (peptide mimotope phage peptide library mimotope

4B,C). , random peptide filamentous phage phage peptide library

/ (enveloped virus) peptide ligand , high-throughput

(family) assay peptide

Rhabdoviridae rabies virus trimeric G protein (neuron) neural -

cell adhesion molecule (NCAM) endocytosis . Endosome pH peptide ,

가 active motif

endosomal membrane

가 . puumala hantavirus echovirus 22

influenza virus¹¹⁾ . 가 integrin gamma¹⁴⁾ .

carboxylase 7 12

. Herpes simplex virus type 1 gB, gC 7-/12-mer phage peptide library E2

human cytomegalovirus gB panning

가 heparan sulfate proteoglycan(HSPG) . 7-mer phage peptide library 12-mer

, gD library . 7

integrin

plasma membrane¹²⁾ , 5 panning output

Lentivirus human immunodeficiency virus type phage input phage (ratio) 7-mer

1 gp 120 helper T CD4 library가 12-mer library 4

chemokine CCR-5 .

	Random peptide library	C	E2	peptide mimotope
	12-mer library			
E2	phage peptide			
	3			peptide
mimotope	, Ser-His-Phe-Try-Arg-Ala-Pro (SHFWRAP)			가
	. 3 phage peptide E2			
	, C			
	HCV			
	E2 inhibition test			
	. IC 1 IB2			
MOLT-4	E2			
	, IH 1 E2			
	. IC 1 IB2가 E2			binding
motif	T			
	T			가
				peptide mimotope
				Swiss
Institute of Bioinformatics (SIB)				Fasta3
				α -helix β
-sheet	(linear structure)			
3	conformational motif			
peptide mimotope				
	, mimotope			
	3			
	가			peptide
mimotope	conformational motif			
가				
	peptide mimotope bovine serum			
albumin(BSA)	keyhole limpet hemocyanin(KLH)			
carrier protein				
	-peptide mimotope			
	cDNA library			
	가			
	.			
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. (KRF99-04 1-F00093)