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H. 知的財産権の出願・登録状況

H-1 特許取得

1. 発明の名称：新規ペプチド
   発明者：山﨑基生、高橋憲行、南野直人、佐々木一樹、高尾敏文、里見佳典
   PCT/JP2006/314969 号
2. 発明の名称：新規ポリペプチド及びその用途
   発明者：中里雅光、高尾敏文
   PCT/JP2006/309192 号
3. ネフローゼ症候群の疾患関連たんぱく質およびその使用、出願日：平成１８年６月２１日
   出願番号：特願 2006-171584
4. ネフローゼ症候群の疾患関連たんぱく質およびその使用、出願日：平成１８年９月８日
   出願番号：特願 2006-244433

H-2 実用新案登録

なし

H-3 その他

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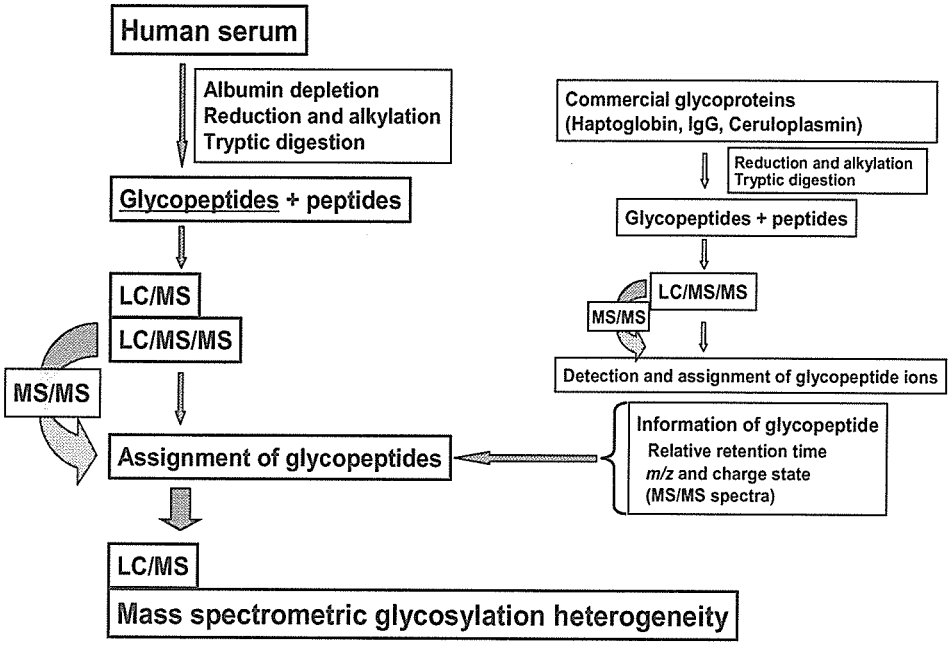


図 1 Strategy of human serum glycomics

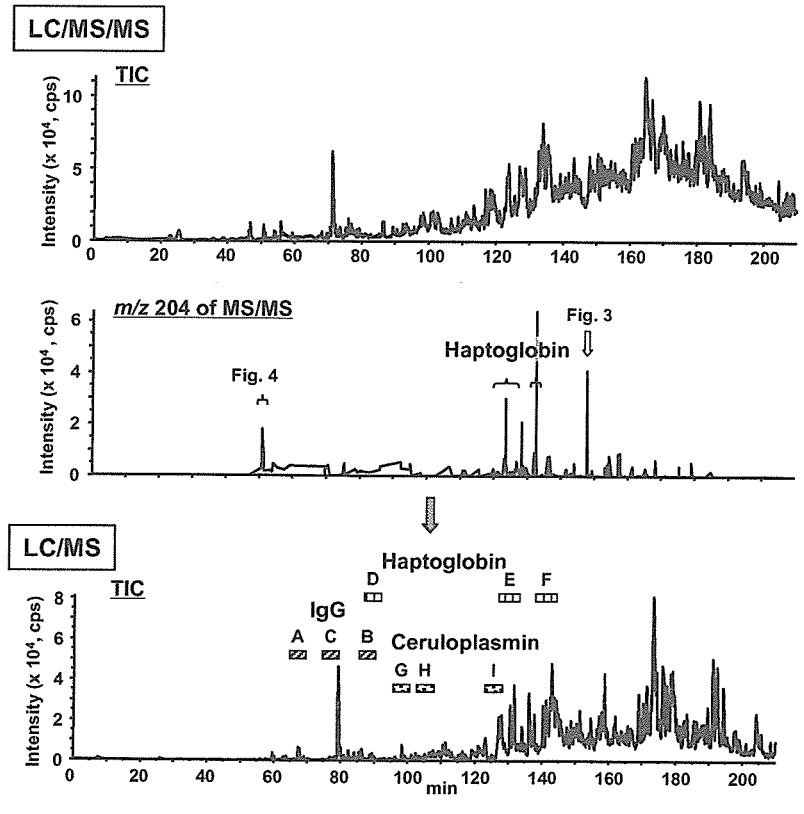


図 2 LC/MS/MS of tryptic digest of human serum

(A) Total ion chromatogram of LC/MS/MS. (B) Intensity at *m/z* 204 in data-dependent MS/MS. Some product ion spectra were shown in Figure 3 and 4. (C) Total ion chromatogram of LC/MS. Mass spectrometric glycosylation heterogeneity was calculated by mass spectra of glycopeptides.

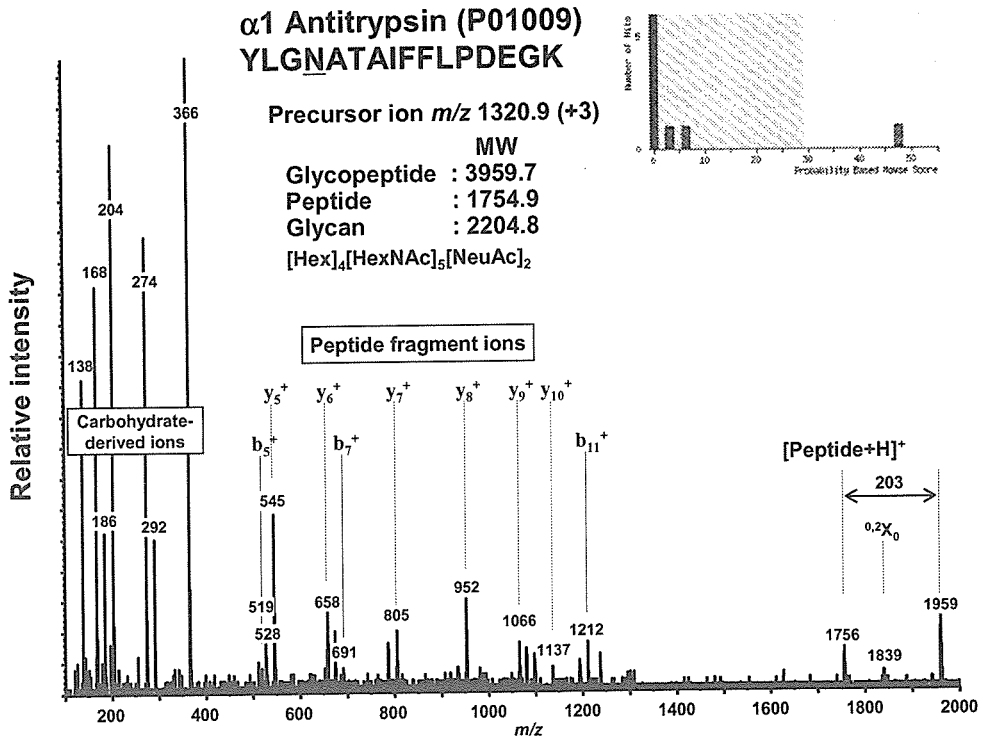


図 3 . Product ion spectrum of glycopeptide ion at *m/z* 1320.9 (+3)

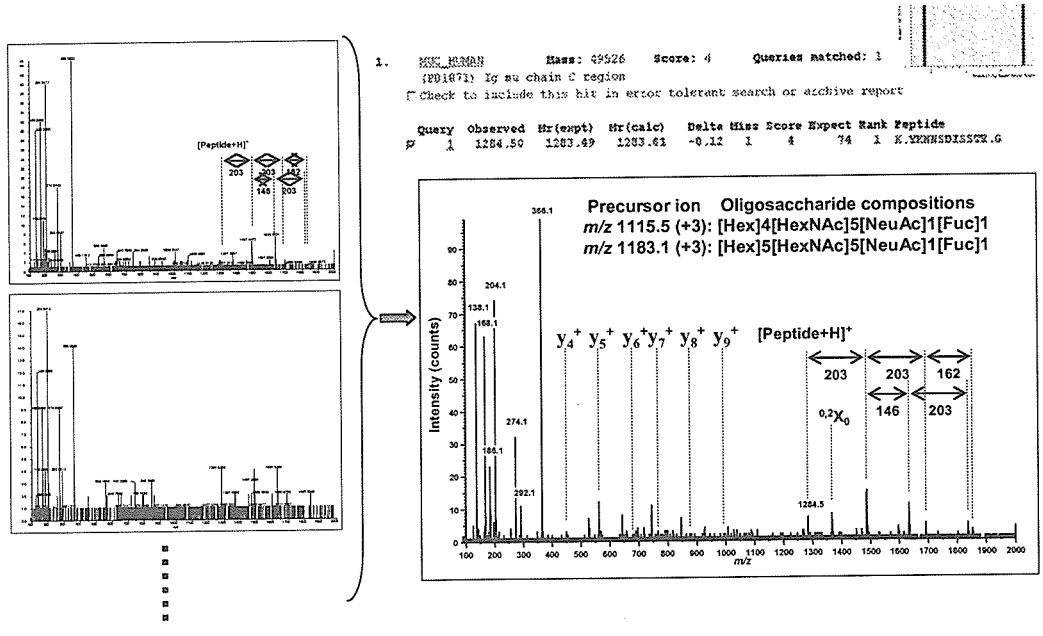


図 4 . Data-processing of product ion spectra of *m/z* 1115.5 and 1183.1 (+3)

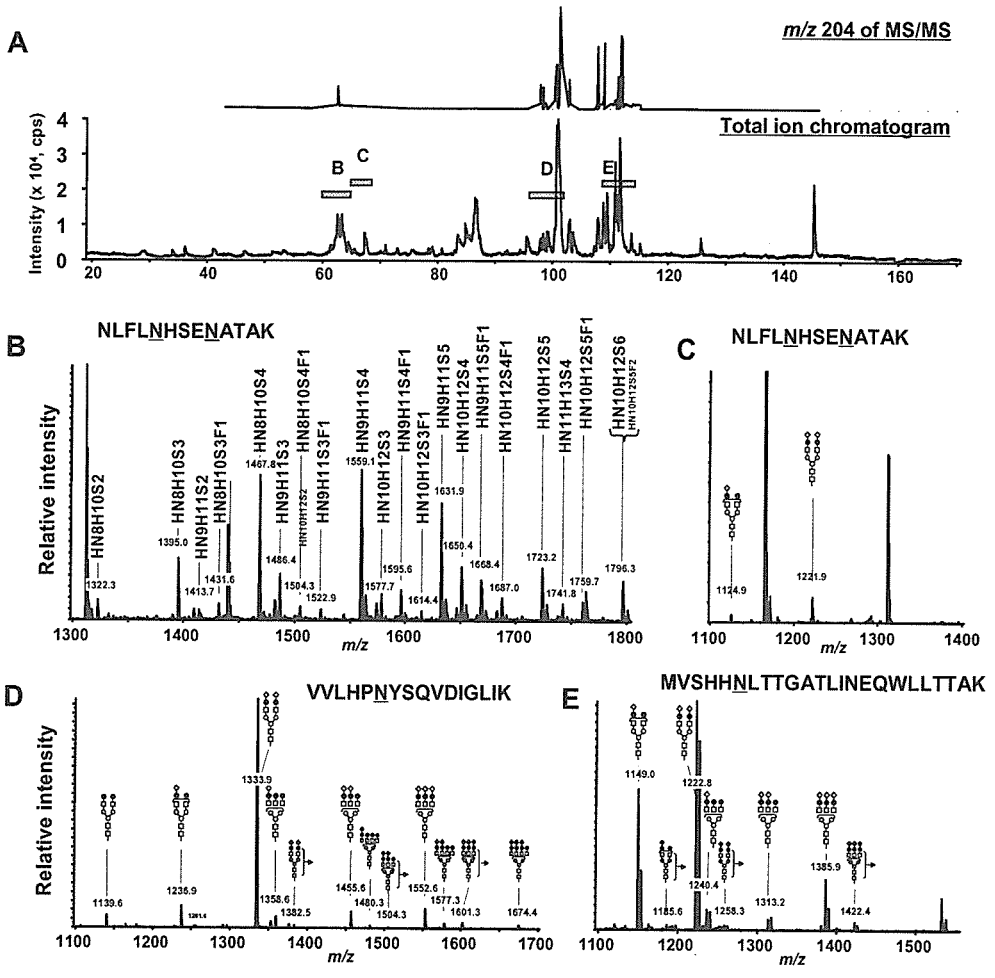


図 5 Site-specific glycosylation analysis of human haptoglobin. (A) Total ion chromatogram of LC/MS/MS, with an inset showing intensity at *m/z* 204 in data-dependent MS/MS. (B-E) Mass spectra of glycopeptides.

表 1

Summary of analysis of serum glycoproteome.

Glycopeptide					Oligosaccharide	Protein (Protein ID)	Theoretical MW
Retention time (min)	m/z[a]	charge	Observed MW	Relative peak intensity[b]	Observed MW	Glycopeptide Peptide sequence Deduced oligosaccharide composition[c]	Peptide Oligosaccharide
						IgG1(P01857)	
						EEQYNSTYR	1188.50
67.3	1479.6	+2	2957.1	13.1	1768.6	[HexNAc]4[Hex]5[Fuc]1	1768.64
67.3	1297.0	+2	2592.0	3.0	1403.5	[HexNAc]3[Hex]4[Fuc]1	1403.51
67.4, 67.6	1398.5	+2	2795.1	33.1	1606.5	[HexNAc]4[Hex]4[Fuc]1	1606.59
67.4, 67.7	1216.0	+2	2429.9	4.3	1241.4	[HexNAc]3[Hex]3[Fuc]1	1241.45
67.7	1317.5	+2	2633.0	27.8	1444.5	[HexNAc]4[Hex]3[Fuc]1	1444.53
67.9	1500.1	+2	2998.1	2.3	1809.6	[HexNAc]5[Hex]4[Fuc]1	1809.67
	1000.4	+3	2998.0				
67.9	1581.1	+2	3160.2	0.2	1971.7	[HexNAc]5[Hex]5[Fuc]1	1971.72
68.0	1406.6	+2	2811.1	0.9	1622.6	[HexNAc]4[Hex]5	1622.58
68.2, 68.4	1325.5	+2	2649.0	2.8	1460.5	[HexNAc]4[Hex]4	1460.53
68.2	1419.1	+2	2836.1	2.9	1647.6	[HexNAc]5[Hex]3[Fuc]1	1647.61
68.5	1244.5	+2	2486.9	1.4	1298.4	[HexNAc]4[Hex]3	1298.48
69.1	1625.1	+2	3248.1	2.2	2059.6	[HexNAc]4[Hex]5[NeuAc]1[Fuc]1	2059.73
	1083.7	+3	3248.1				
69.6	1544.1	+2	3086.2	0.5	1897.6	[HexNAc]4[Hex]4[NeuAc]1[Fuc]1	1897.68
						EEQYNSTYRVVSVLTVLHQDWLNGK*	2977.49
161.9, 192.2	1147.0	+4	4584.1		1606.6	[HexNAc]4[Hex]4[Fuc]1	1606.59
162.4	1106.5	+4	4422.1		1444.6	[HexNAc]4[Hex]3[Fuc]1	1444.53
						EEQYNSTYRVVSVLTVLHQDWLNGKEYK*	3397.69
156.4	1034.3	+5	5166.4		1768.7	[HexNAc]4[Hex]5[Fuc]1	1768.64
156.6, 157.1	1001.8	+5	5004.0		1606.3	[HexNAc]4[Hex]4[Fuc]1	1606.59
						IgG2 (P01859)	
						EEQFNSTFR	1156.51
85.4	1463.6	+2	2925.1	6.3	1768.6	[HexNAc]4[Hex]5[Fuc]1	1768.64
85.5	1281.0	+2	2560.0	1.4	1403.5	[HexNAc]3[Hex]4[Fuc]1	1403.51
85.7, 86.3	1382.5	+2	2763.1	19.4	1606.5	[HexNAc]4[Hex]4[Fuc]1	1606.59
85.7, 86.4	1200.0	+2	2397.9	2.8	1241.4	[HexNAc]3[Hex]3[Fuc]1	1241.45
85.7	1565.1	+2	3128.2	0.0	1971.7	[HexNAc]5[Hex]5[Fuc]1	1971.72
86.0	1484.1	+2	2966.2	1.0	1809.6	[HexNAc]5[Hex]4[Fuc]1	1809.67
86.5	1301.5	+2	2601.0	21.8	1444.5	[HexNAc]4[Hex]3[Fuc]1	1444.53
86.5	1390.5	+2	2779.0	0.0	1622.5	[HexNAc]4[Hex]5	1622.58
86.9	1403.0	+2	2804.1	1.8	1647.5	[HexNAc]5[Hex]3[Fuc]1	1647.61
87.0, 87.5	1309.5	+2	2617.0	0.1	1460.5	[HexNAc]4[Hex]4	1460.53
87.6	1228.5	+2	2454.9	0.2	1298.4	[HexNAc]4[Hex]3	1298.48
89.4	1609.1	+2	3216.2	1.6	2059.7	[HexNAc]4[Hex]5[NeuAc]1[Fuc]1	2059.73
	1073.1	+3	3216.2				
90.0	1528.1	+2	3054.2	1.5	1897.6	[HexNAc]4[Hex]4[NeuAc]1[Fuc]1	1897.68
	1019.1	+3	3054.1				
						IgG4 (P01861)	
						EEQFNSTYR	1172.51
76.4	1471.6	+2	2941.1	1.0	1768.6	[HexNAc]4[Hex]5[Fuc]1	1768.64
76.5	1289.0	+2	2576.0	0.2 [b]	1403.5	[HexNAc]3[Hex]4[Fuc]1	1403.51
76.6, 76.8	1390.6	+2	2779.1	3.4	1606.6	[HexNAc]4[Hex]4[Fuc]1	1606.59
76.5, 76.8	1208.0	+2	2413.9	0.4	1241.4	[HexNAc]3[Hex]3[Fuc]1	1241.45
76.7	1492.1	+2	2982.1	0.2	1809.6	[HexNAc]5[Hex]4[Fuc]1	1809.67
76.9	1309.5	+2	2617.0	3.6	1444.5	[HexNAc]4[Hex]3[Fuc]1	1444.53
77.0	1398.6	+2	2795.1	0.1	1622.6	[HexNAc]4[Hex]5	1622.58
76.9	1317.5	+2	2633.0	0.1	1460.5	[HexNAc]4[Hex]4	1460.53
77.0	1411.1	+2	2820.1	0.3	1647.6	[HexNAc]5[Hex]3[Fuc]1	1647.61
77.4	1236.4	+2	2470.8	0.1	1298.3	[HexNAc]4[Hex]3	1298.48
78.5	1617.1	+2	3232.1	0.3	2059.6	[HexNAc]4[Hex]5[NeuAc]1[Fuc]1	2059.73
79.1	1536.1	+2	3070.1	0.1	1897.6	[HexNAc]4[Hex]4[NeuAc]1[Fuc]1	1897.68
						Haptoglobin (P00738)	
						NLFLNHSENATAK	1457.73
86.3	1395.0	+4	5576.0	0.1	4118.3	[HexNAc]8[Hex]10[NeuAc]3	4118.45
87.0	1650.3	+4	6597.3	0.3	5139.6	[HexNAc]10[Hex]12[NeuAc]4	5139.81
87.6	1595.6	+4	6378.3	0.9	4920.6	[HexNAc]9[Hex]11[NeuAc]4[Fuc]1	4920.73
87.9	1559.1	+4	6232.3	2.7	4774.5	[HexNAc]9[Hex]11[NeuAc]4	4774.68
88.6	1504.3	+4	6013.0	0.1	4555.3	[HexNAc]8[Hex]10[NeuAc]4[Fuc]1	4555.60
88.9	1467.8	+4	5867.1	4.1	4409.4	[HexNAc]8[Hex]10[NeuAc]4	4409.54
90.4	1759.6	+4	7034.5	0.2	5576.8	[HexNAc]10[Hex]12[NeuAc]5[Fuc]1	5576.96

表 1 (continue)

90.7	1723.1	+4	6888.5	0.5	5430.8	[HexNAc]10[Hex]12[NeuAc]5	5430.90
91.5	1668.3	+4	6669.4	0.3	5211.6	[HexNAc]9[Hex]11[NeuAc]5[Fuc]1	5211.83
91.7	1631.8	+4	6523.3	0.4	5065.6	[HexNAc]9[Hex]11[NeuAc]5	5065.77
87.8	1124.7	+3	3371.2	–	1913.5	[HexNAc]4[Hex]5[NeuAc]1	1913.68
91.6, 92.1	1221.7	+3	3662.2	–	2204.5	[HexNAc]4[Hex]5[NeuAc]2	2204.77
						MVSHHNLTTGATLINEQWLLTTAK	2678.39
137.9	1531.7	+3	4592.1		1913.7	[HexNAc]4[Hex]5[NeuAc]1	1913.68
	1149.0	+4	4592.0	30.1	1913.6		
141.4	1221.8	+4	4883.1	88.7	2204.7	[HexNAc]4[Hex]5[NeuAc]2	2204.77
						M(O)VSHHNLTTGATLINEQWLLTTAK	2694.38
137.3	1153.0	+4	4608.1	28.9	1913.7	[HexNAc]4[Hex]5[NeuAc]1	1913.68
140.5, 141.1	1225.8	+4	4899.1	64.3	2204.7	[HexNAc]4[Hex]5[NeuAc]2	2204.77
	1634.1	+3	4899.1		2204.8		
						VVLHPNYSQVDIGLIK	1794.00
127	1358.6	+3	4072.8	2.2	2278.8	[HexNAc]5[Hex]6[NeuAc]1	2278.81
128.2	1236.9	+3	3707.7	3.1	1913.7	[HexNAc]4[Hex]5[NeuAc]1	1913.68
131.4	1455.6	+3	4363.8	4.8	2569.8	[HexNAc]5[Hex]6[NeuAc]2	2569.90
	1092.0	+4	4363.8				
131.8	1333.9	+3	3998.7	89.2	2204.7	[HexNAc]4[Hex]5[NeuAc]2	2204.77
	1000.7	+4	3998.7		2204.7		
134.1	1552.7	+3	4655.0	7.5	2860.9	[HexNAc]5[Hex]6[NeuAc]3	2861.00
134.1	1164.7	+4	4654.9		2860.9		
						Ceruloplasmin (P00450)	
						EHEGAIYPDNTTDFQR	1891.83
95.6	1415.2	+3	4242.7	0.1	2350.8	[HexNAc]4[Hex]5[NeuAc]2[Fuc]1	2350.83
	1061.7	+4	4242.6		2350.8		
96.3	1366.5	+3	4096.6	6.0	2204.8	[HexNAc]4[Hex]5[NeuAc]2	2204.77
	1025.2	+4	4096.7		2204.9		
98.1, 98.5	1633.9	+3	4898.7	0.4	3006.9	[HexNAc]5[Hex]6[NeuAc]3[Fuc]1	3007.06
	1226.0	+4	4899.9		3008.0		
98.8	1585.2	+3	4752.7	0.4	2860.9	[HexNAc]5[Hex]6[NeuAc]3	2861.00
	1189.2	+4	4752.6		2860.8		
						ENLTAPGSDSAVFFEQGTTR	2125.99
127.0	1493.2	+3	4476.6	0.0	2350.6	[HexNAc]4[Hex]5[NeuAc]2[Fuc]1	2350.83
127.4	1444.5	+3	4330.6	2.8	2204.6	[HexNAc]4[Hex]5[NeuAc]2	2204.77
129.3	1663.3	+3	4987.0	0.2	2861.0	[HexNAc]5[Hex]6[NeuAc]3	2861.00
						ELHHLQEQNVSNAFLDK	2021.00
104.3	1093.9	+4	4371.7	1.4	2350.7	[HexNAc]4[Hex]5[NeuAc]2[Fuc]1	2350.83
	1458.3	+3	4371.8	0.2	2350.8		
105.2	1057.4	+4	4225.7	4.5	2204.7	[HexNAc]4[Hex]5[NeuAc]2	2204.77
	1409.6	+3	4225.6	1.2	2204.6		
106.6	1294.6	+4	5174.2	1.2	3153.2	[HexNAc]5[Hex]6[NeuAc]3[Fuc]2	3153.12
106.8, 107.4	1258.0	+4	5027.9	2.0	3006.9	[HexNAc]5[Hex]6[NeuAc]3[Fuc]1	3007.06
107.7	1221.5	+4	4881.8	1.9	2860.8	[HexNAc]5[Hex]6[NeuAc]3	2861.00
						α1 Antitrypsin (P01009)	
						YLGNATAIFFLPDEGK	1754.89
154.6	1369.6	+3	4105.7	2.2	2350.8	[HexNAc]4[Hex]5[NeuAc]2[Fuc]1	2350.83
154.8	1320.9	+3	3959.7	140.6	2204.8	[HexNAc]4[Hex]5[NeuAc]2	2204.77
						α2 HSGP (P02765)	
						VCQDCPLLAPLNDTR	1772.81
136.9	1326.9	+3	3977.7		2204.8	[HexNAc]4[Hex]5[NeuAc]2	2204.77
						α2 Macroglobulin (P01023)	
						VSNQTLSLFFTVLQDVPVR	2162.17
187.9, 188.8	1505.3	+3	4512.7	5.1	2350.6	[HexNAc]4[Hex]5[NeuAc]2[Fuc]1	2350.83
188.3	1456.7	+3	4367.0	22.5	2204.8	[HexNAc]4[Hex]5[NeuAc]2	2204.77
						β2 Glycoprotein (P02749)	
						VYKPSAGNNSLYR	1467.75
83.5	1273.8	+3	3818.5	1.5	2350.8	[HexNAc]4[Hex]5[NeuAc]2[Fuc]1	2350.83
83.6	1225.2	+3	3672.5	6.9	2204.8	[HexNAc]4[Hex]5[NeuAc]2	2204.77
85.2	1492.6	+3	4474.6	0.3	3006.9	[HexNAc]5[Hex]6[NeuAc]3[Fuc]1	3007.06
85.4	1443.9	+3	4328.6	0.5	2860.8	[HexNAc]5[Hex]6[NeuAc]3	2861.00
						LGNWSAMPSCK	1250.54
109.7	1152.8	+3	3455.3		2204.7	[HexNAc]4[Hex]5[NeuAc]2	2204.77

表 1 (continue)

					Complement C3 (P01024)		
					TVLTPATNHMGNVTFTIPANR		2254.15
121.0	1265.9	+3	3794.6	5.4	1540.4	[HexNAc]2[Hex]7	1540.53
121.2	1211.8	+3	3632.5	47.8	1378.4	[HexNAc]2[Hex]6	1378.48
121.6	1157.8	+3	3470.4	10.2	1216.3	[HexNAc]2[Hex]5	1216.42
					Hemopexin (P02790)		
					SWPAVGNCSSALR		1404.65
115.3	1252.8	+3	3755.5	0.7	2350.8	[HexNAc]4[Hex]5[NeuAc]2[Fuc]1	2350.83
115.8	1204.1	+3	3609.4	10.3	2204.7	[HexNAc]4[Hex]5[NeuAc]2	2204.77
					ALPQPQNVTSLLGCTH		1735.86
115.8	1314.5	+3	3940.4	10.3	2204.5	[HexNAc]4[Hex]5[NeuAc]2	2204.77
					IgA1 (P01876), IgA2 (P01877)		
					LSLHRPALEDLLLGSEANLTCTLTGLR		2963.58
165.2, 165.7	1157.8	+4	4627.2	9.8 [d]	1663.6	[HexNAc]5[Hex]4	1663.61
165.8	1117.3	+4	4465.0	14.1 [d]	1501.4	[HexNAc]5[Hex]3	1501.56
165.9	1046.0	+4	4180.0	4.9	1216.4	[HexNAc]2[Hex]5	1216.42
169.2	1220.3	+4	4877.2	59.6	1913.6	[HexNAc]4[Hex]5[NeuAc]1	1913.68
168.8, 169.4	1256.9	+4	5023.5	1.5	2059.9	[HexNAc]4[Hex]5[NeuAc]1[Fuc]1	2059.73
169.9	1179.8	+4	4715.3	4.6	1751.7	[HexNAc]4[Hex]4[NeuAc]1	1751.62
170.0	1169.6	+4	4674.2	5.1 [d]	1710.6	[HexNAc]3[Hex]5[NeuAc]1	1710.60
	1271.2	+4	5080.8		2117.2	[HexNAc]5[Hex]5[NeuAc]1	2116.76
	1017.2	+5	5081.0		2117.4		
	1230.4	+4	4917.6		1954.0	[HexNAc]5[Hex]4[NeuAc]1	1954.70
	1343.9	+4	5371.6		2408.0	[HexNAc]5[Hex]5[NeuAc]2	2407.85
	1293.2	+4	5168.8		2205.2	[HexNAc]4[Hex]5[NeuAc]2	2204.77
					PALEDLLLGSEANLTCTLTGLR*		2357.21
174.4	1287.2	+3	3858.6	12.8	1501.4	[HexNAc]5[Hex]3	1501.56
176.7	1424.6	+3	4270.9	59.4	1913.7	[HexNAc]4[Hex]5[NeuAc]1	1913.68
	1492.5	+3	4474.5		2117.3		2116.76
					IgA2 (P01877)		
					TPLTANITK		957.55
84.1	1006.8	+3	3017.2	3.7	2059.7	[HexNAc]4[Hex]5[NeuAc]1[Fuc]1	2059.73
	1509.6	+2	3017.2	1.5	2059.6		
	1055.4	+3	3163.2	???	2205.6	[HexNAc]4[Hex]5[NeuAc]2	2204.77
84.1	1074.4	+3	3220.3	4.8	2262.8	[HexNAc]5[Hex]5[NeuAc]1[Fuc]1	2262.81
	1611.2	+2	3220.3	0.3	2262.8		
87.2	1103.8	+3	3308.3	1.3	2350.8	[HexNAc]4[Hex]5[NeuAc]2[Fuc]1	2350.83
					Ig mu (P01871)		
					YKNNSDISSTR*		1283.61
59.7	1115.5	+3	3343.4	40.1	2059.7	[HexNAc]4[Hex]5[NeuAc]1[Fuc]1	2059.73
60.3	1183.1	+3	3546.4	16.4	2262.8	[HexNAc]5[Hex]5[NeuAc]1[Fuc]1	2262.81
					Transferrin (P02787)		
					CGLVPVLAENYNK		1476.73
126.1	1252.8	+3	3755.5	0.9	2278.8	[HexNAc]5[Hex]6[NeuAc]1	2278.81
127.0	1131.1	+3	3390.4	1.7	1913.7	[HexNAc]4[Hex]5[NeuAc]1	1913.68
129.8	1349.9	+3	4046.7	1.6	2569.9	[HexNAc]5[Hex]6[NeuAc]2	2569.90
130.6	1228.2	+3	3681.5	46.8	2204.7	[HexNAc]4[Hex]5[NeuAc]2	2204.77
133.1	1446.9	+3	4337.8	0.8	2861.0	[HexNAc]5[Hex]6[NeuAc]3	2861.00
					QQQHLFGSNVTDCSGNFCLFR		2516.08
142.8, 143.8	1623.3	+3	4866.9	4.1	2350.8	[HexNAc]4[Hex]5[NeuAc]2[Fuc]1	2350.83
143.9	1181.2	+4	4720.9		2204.8	[HexNAc]4[Hex]5[NeuAc]2	2204.77
	1574.6	+3	4720.9	50.5	2204.8		
146.0	1842.0	+3	5523.0	0.9	3007.0	[HexNAc]5[Hex]6[NeuAc]3[Fuc]1	3007.06
146.2	1793.4	+3	5377.3	1.6	2861.2	[HexNAc]5[Hex]6[NeuAc]3	2861.00

[a], Underlines indicated that these ions were assigned by elucidating data-dependent MS/MS of LC/ESI MS/MS of human serum dig
Other ions were assigned by mass differences of glycan units or utilizing data of commercial glycoproteins (IgG, haptoglobin, and ce

[b], Centroid peak intensity in integrated MS spectra during glycopeptide eluting period.

[c], Oligosaccharide compositions were deduced from molecular weights.

[d], Other ions with same *m/z* were overlaped.

*, Missed cleavage or unexpected digestions.

All masses are monoisotopic. Cysteine residue was carboxymethylated. Potential N-glycosylation sites were underlined.
M(O), oxidized methionine; HexNAc, N-acetyl hexosamine; Hex, Hexose; NeuAc, N-acetyl neuraminic acid; Fuc, Fucose

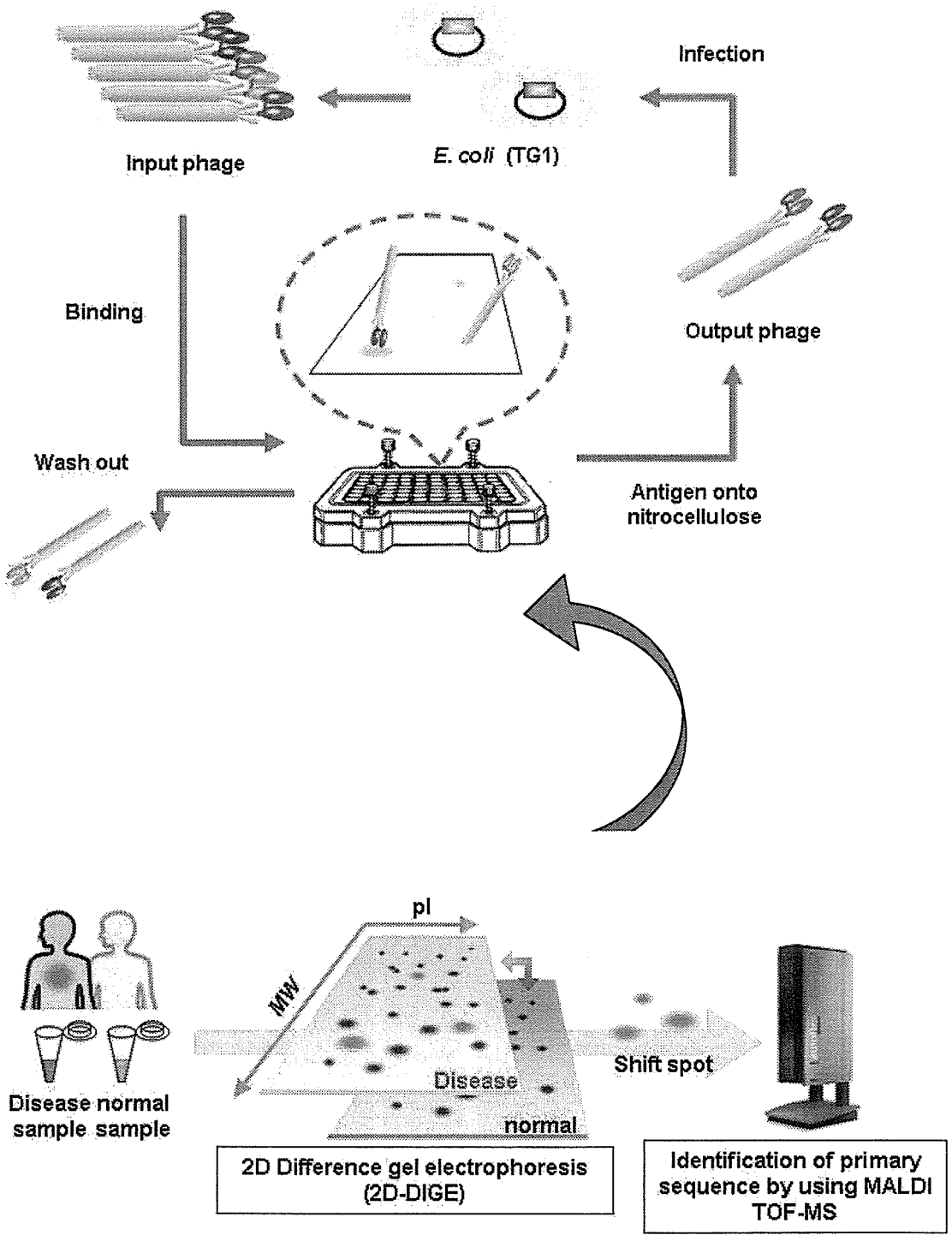
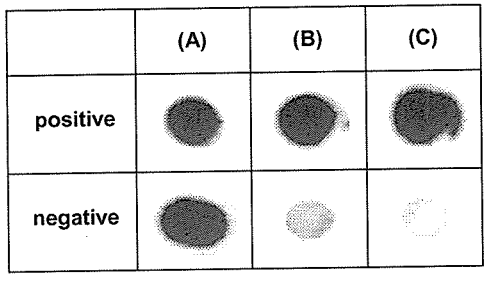


図 6 . membrane-based affinity panning system linked with 2D-DIGE analysis

< PVDF >



< nitrocellulose membrane >

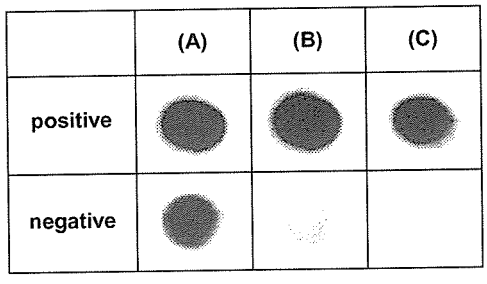


図 7 **Optimization of both membrane and blocking conditions for panning**

KDR were immobilized on PVDF or nitrocellulose membranes using the Bio-Dot Microfiltration Apparatus and then were blocked with blocking buffer ((A) 4% skim milk, (B) 10% skim milk, and (C) 10% skim milk & 25% glycerol). Anti-KDR scFv phage (positive) and anti-luciferase scFv phage (negative) were pre-incubated with 90%

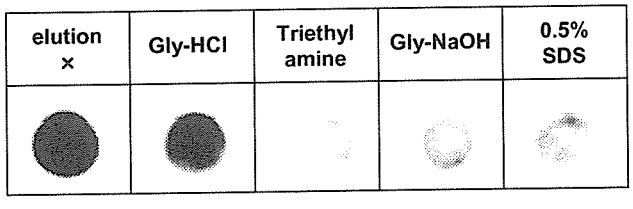


図 8 **Optimization of elution condition for panning**

Anti-KDR scFv antibody was reacted to KDR immobilized on the nitrocellulose membrane and then was washed five times with TBST. The apparatus incubated with elution buffer (Gly-HCl, triethylamine, Gly-NaOH and 0.5% SDS). Experimental protocol was described in the section of materials and methods.

< *panning input* >

< *panning in nitrocellulose output* >

(A)

(B)

(C)

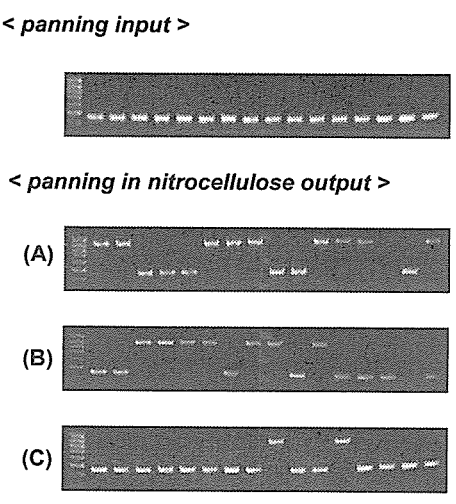


図 9 **Examination of panning conditions by using nitrocellulose membrane**

Model panning was performed by using the nitrocellulose membrane. The model library (anti-KDR scFv phage : negative phage = 1 : 100) were reacted to KDR ((A)5000 ng, (B) 50 ng, (C) 0.5 ng) immobilized on the membrane. After the binding step, the membrane was washed ten times with TBST. The bound phage was eluted with triethylamine. The eluted phage was then incubated in log phase TG1 cells and individual TG1 clones were picked at random. Inserts of 16 phage clones were amplified by PCR. Inserts of PCR products were analyzed on agarose gels.

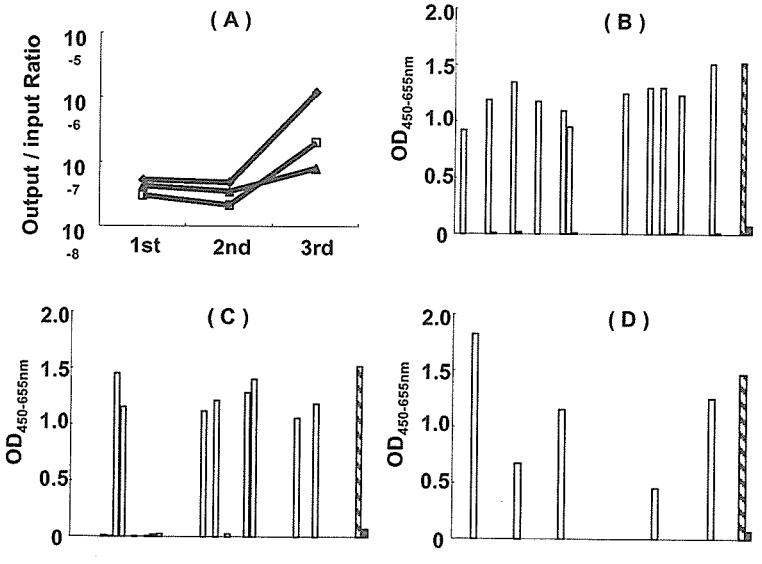


図 10 **Enrichment of antibodies to KDR from non-immune libraries for a small amount of protein by panning in nitrocellulose membrane**

Affinity panning was performed by using the nitrocellulose membrane. The scFv phage library were reacted to KDR () immobilized on the nitrocellulose membrane. (A) The ratio of phage titer at each panning round was plotted. (◆)5000 ng, (□) 50 ng, (▲) 0.5 ng (B) After panning, the binding properties of the selected phage clones were analyzed by ELISA. (B) 5000 ng, (C) 50 ng, (D) 0.5 ng. (◪) positive control (anti-KDR scFv phage) (■) negative control (anti-luciferase scFv phage)

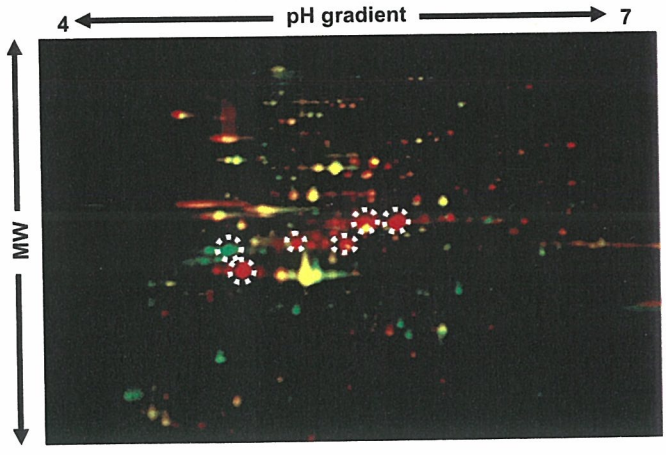


図11 **2D-DIGE image of fluorescently labeled proteins from mammary gland cell and mammary cancer cell**

Mammary gland cell and mammary cancer cell were labeled using cy3 and cy5, and then performed 2D electrophiresis. Red color shows spots elevated expression lebel in mammary cancer cell. Green color shows spots elevated expression lebel in mammary gland cell. Yellow color shows no expression difference spots.

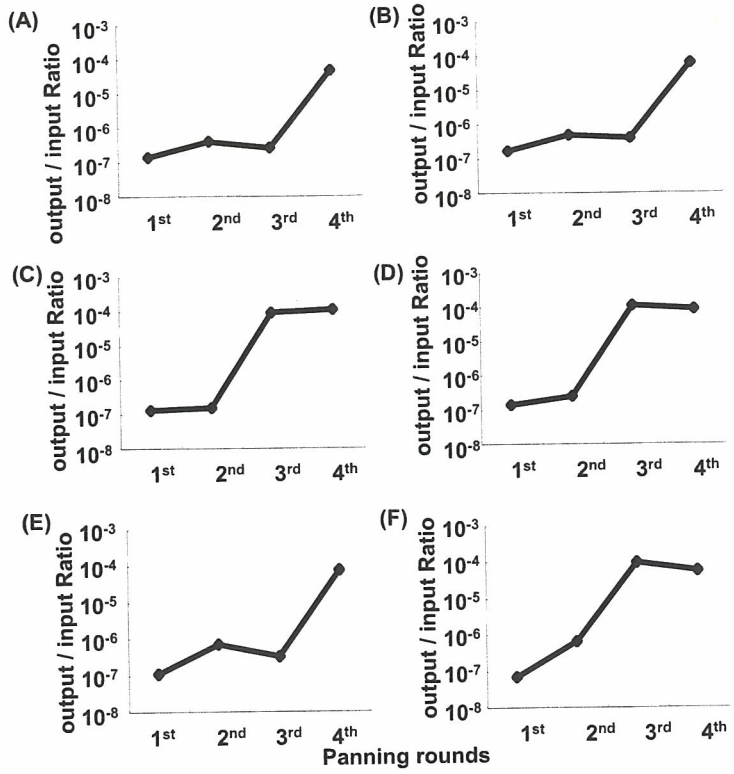


図12 **Enrichment of antibodies from 2D-DIGE spots directly by panning in nitrocellulose membrane**

Affinity panning was performed by using the nitrocellulose membrane. The scFv phage library were reacted to the spots ((A) spot 1, (B) spot 2, (C) spot 3, (D) spot 4, (E) spot 5, (F) spot 6) immobilized on the nitrocellulose membrane. Experimental protocol was described in the section of materials and methods.

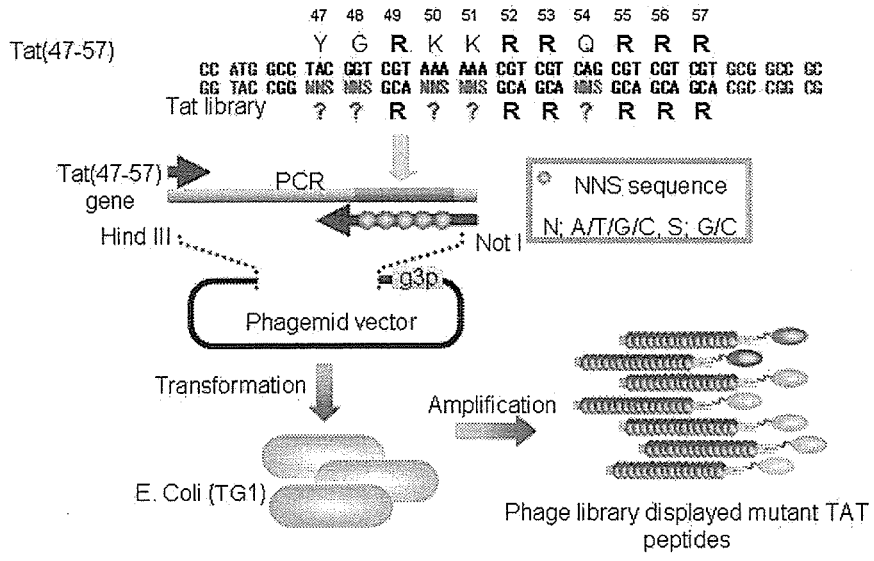


図 13. Construction of mutant TAT libraries

表 2 Nucleotide and amino acid sequences

clone	position					clone	position				
	47	48	50	51	54		47	48	50	51	54
1	T (ACC)	L (CTC)	T (ACC)	R (AGG)	H (AAC)	6	K (AAG)	E (GAG)	H (CAC)	L (CTC)	H (CAC)
2	N (AAC)	Y (TAC)	T (ACC)	G (GGC)	K (AAG)	7	D (GAC)	R (CGG)	N (AAC)	S (TCC)	N (AAC)
3	L (CTG)	T (ACG)	W (TGG)	T (ACC)	M (ATG)	8	H (CAC)	R (CGG)	P (CCC)	V (GTC)	F (TTC)
4	Y (TAC)	P (CCG)	I (ATC)	D (GAC)	P (CCC)	9	N (AAC)	K (AAG)	R (CGC)	Q (TAG)	K (AAG)
5	S (TCC)	K (AAG)	T (ACC)	W (TGG)	H (AAC)	10	A (GCC)	P (CCC)	D (GAC)	W (TGG)	A (GCC)

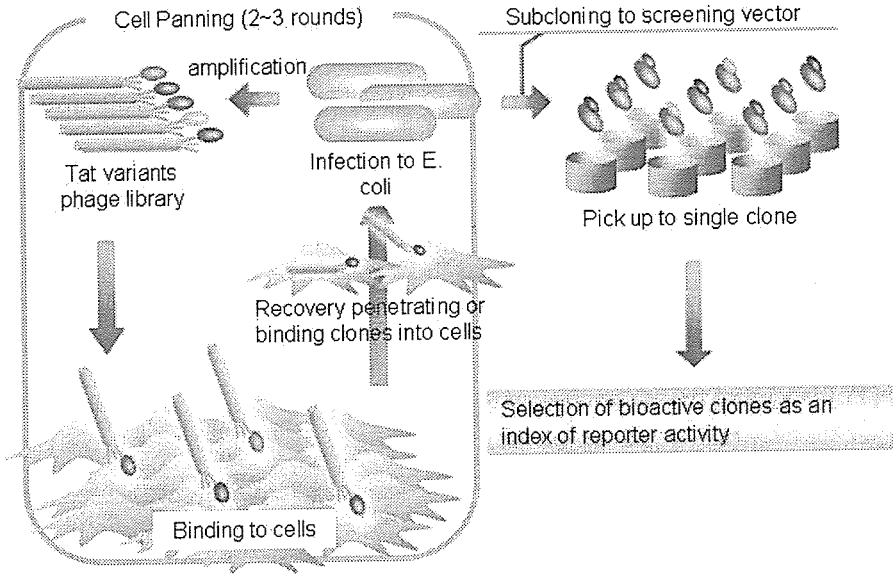


図14 Screening scheme for cell membrane permeable mutant TAT libraries

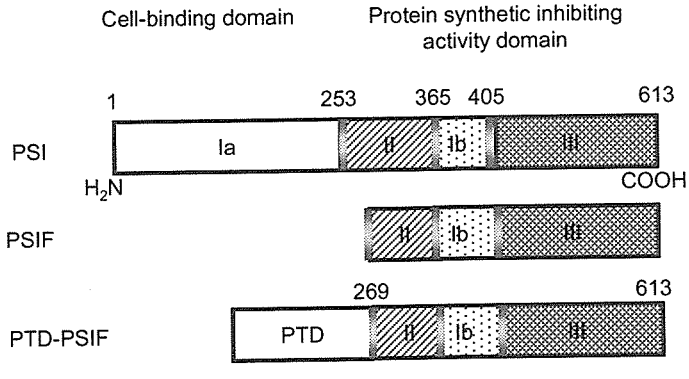


図 15 Schematic structures of PSI and PSIF.
Domain Ia is a binding domain, domain II is a translocating domain and domain III contains a ADP ribosylating enzyme which inactivates elongation factor (EF-2) in the cytosol and results in cell death.

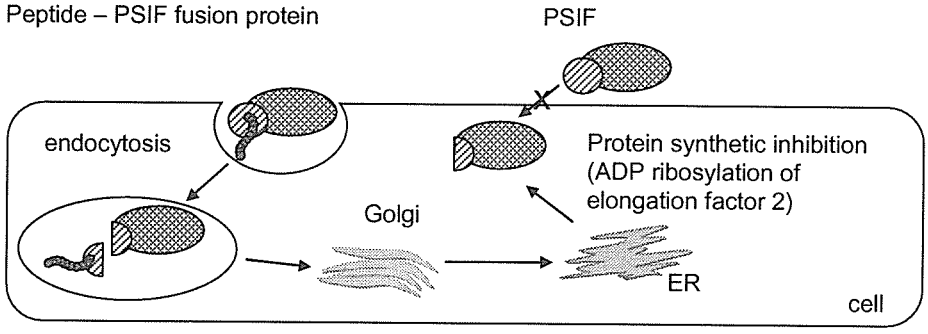


図16. Intoxication of cell by PSIF

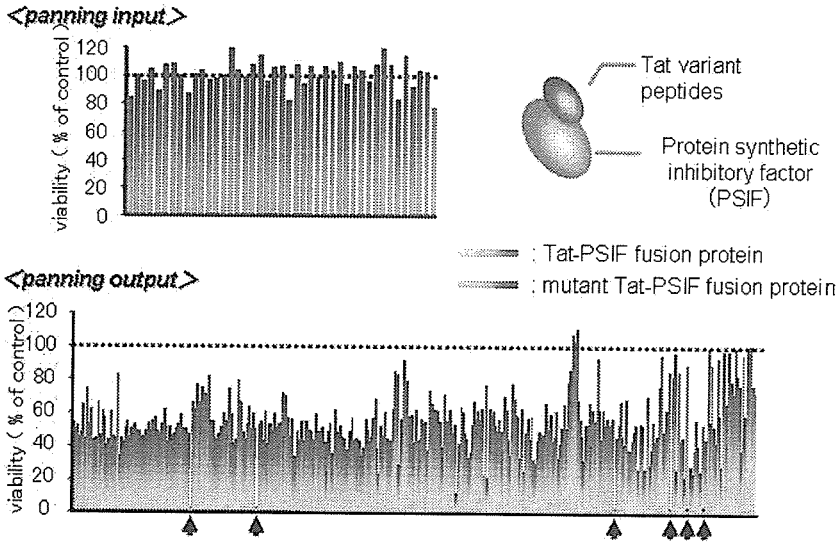


図 17 Selection of clones posses membrane permeability.
The intracellular translocation of selected clones were measured by cytotoxicity derived from PSIF in presence of 50 mg/ml cycloheximide. Tat peptide ( ) used as a control.

表3. Nucleotide and amino acid sequences of mutant TAT peptides

clone	sequence										
Tat	Y TAC	G GGT	R CGT	K AAA	K AAA	R CGT	R CGT	Q CAG	R CGT	R CGT	R CGT
2-62	W TGG	A GCC	R CGT	N AAC	R ACG	R CGT	R CGT	Q CAG	R CGT	R CGT	R CGT
2-83	E GAG	R AGG	R CGT	R CGG	T ACC	R CGT	R CGT	S AGC	R CGT	R CGT	R CGT
2-91	P CCC	Y TAC	R CGT	H CAC	Q CAG	R CGT	R CGT	S CAG	R CGT	R CGT	R CGT
2-2-6	R AGG	N AAC	R CGT	A GCC	R CGC	R CGT	R CGT	Q CGG	R CGT	R CGT	R CGT
2-8	P CCC	V GTG	R CGT	R CGC	P CCC	R CGT	R CGT	R CGG	R CGT	R CGT	R CGT
2-19	T ACC	H CAC	R CGT	L TTG	P CCC	R CGT	R CGT	R CGC	R CGT	R CGT	R CGT

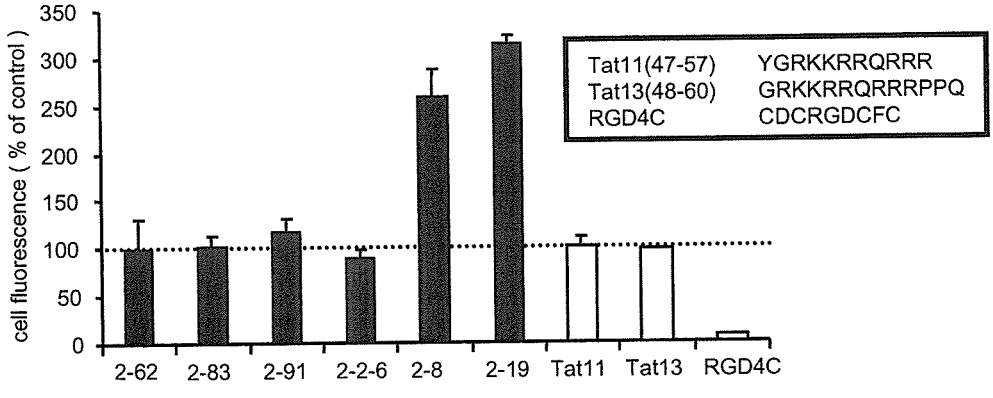


図 18 cellular uptake of selected clones in HaCat cells.
HaCat cells were incubated with fresh medium containing FITC-labeled peptide (10 mM). Quantification of the intracellular translocated peptides was measured using a FACScan flow cytometer. Samples were treated with 0.25% trypsin before FACS analysis. Each point represents the mean ±SD. Of three samples.

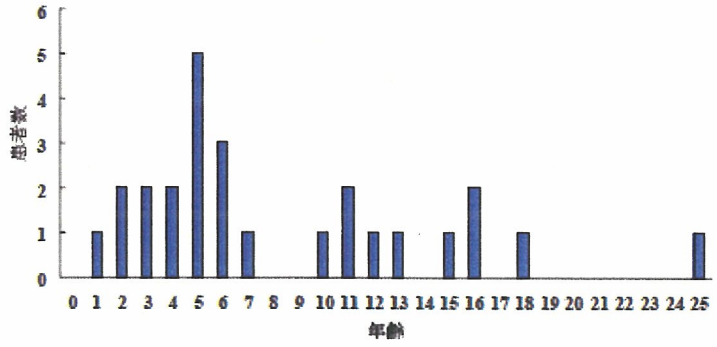


図 19　患者の年齢分布

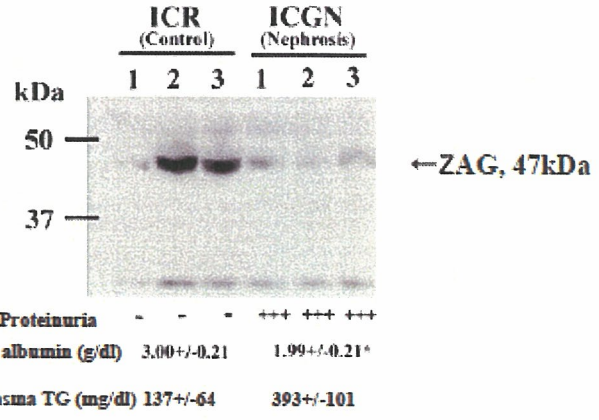


図 20　モデルマウスにおける血清 ZAG

	患者数計	微小変化	び慢性メサンギウム増殖	FSGS	慢性増殖性糸球体腎炎	その他	生理所見なし	不明
ネフローゼ症候群	21	6	2	2	0	3	4	4
ステロイド感受性	16	4	1			3	4	4
ステロイド抵抗性	4	1	1	2				
不明	1	1						
FSGS腎移植後	2			2				
IgA腎症	2		2					
その他	1				1			

表 4　疾患の内訳

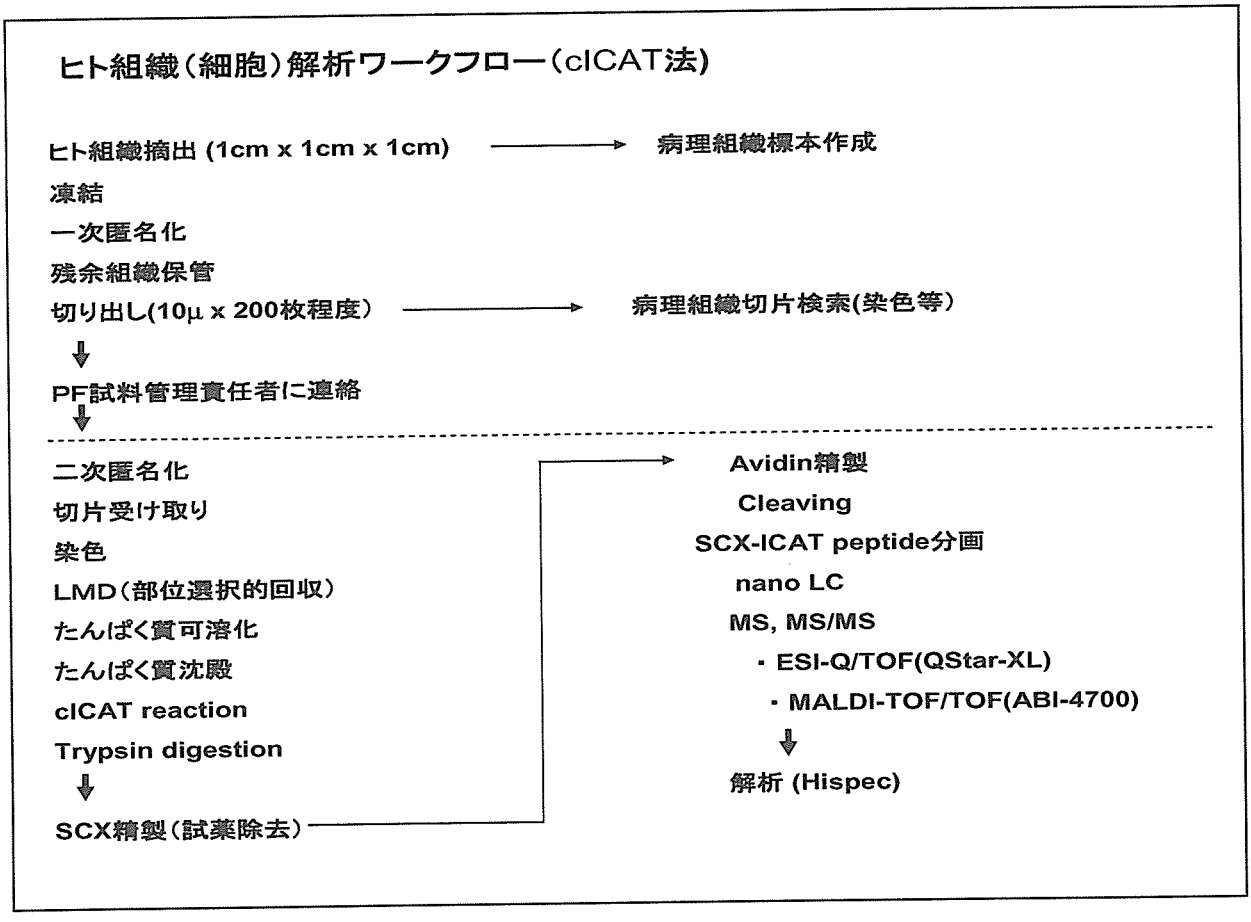


図 21. ヒト組織（細胞）cICAT 解析ワークフロー

表 5. 研究協力機関からのヒト試料の受け入れ状況一覧表

（平成 19 年 1 月 31 現在）

研究協力機関名称	ヒト試料種類	疾患名称	受け入れ状況 症例数	試料数
国立成育医療センター	血清	ネフローゼ	24	51
	血清	FSFG	2	7
	血清	IgA 腎症	2	2
	血清	膜性増殖性糸球体腎炎	1	1
国立精神・神経センター	血清	パーキンソン病	12	12
	血清	パーキンソン病症候群(多系統萎縮症)	5	5
国立循環器病センター	血清	脳動脈閉塞による急性期脳卒中	8	16
	血清/血漿	腎血管性高血圧	15	30
	血清	糖尿病治療前後の動脈硬化	6	12
	血清	冠動脈疾患を有する家族性ｺﾚｽﾃﾛｰﾙ血症	0	0
	血清	急性期心不全症	9	18
	血清	高ｺﾚｽﾃﾛｰﾙ血症	10	20
	血清/血漿	急性心筋梗塞及び類似疾患	22	44
	組織	心疾患	0	0
	血清	肺高血圧症	0	0
	血清	周産期心筋症	0	0
国立長寿医療センター	血清	骨粗鬆症	18	18
		認知症	14	14
国立国際医療センター	血清	糖尿病合併動脈硬化症	120	120
	血清	閉塞性肺疾患	10	10
	血清	早期糖尿病腎症	0	0
大阪府立成人病センター	血清	胃がん	15	26
	組織	胃がん（正常部位、病巣部位）	11	11
大阪大学医学部	血清	運動ニューロン病（ALS）	14	14
	組織	乳がん	8	8
	血清	COPD	21	21
	血清	間質性肺炎	5	5
	組織	間質性肺炎	0	0
合　計			352	465
	内訳：			
	血清		319	414
	血漿		16	32
	組織		19	19