

## Lewis

## CA19-9

### Factors Related to Increased CA19-9 & Lewis Antigen in Pancreatic Cancer Cell Lines

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**Purpose:** The 8 pancreatic cancer cell lines (BxPC-3, Capan-2, CFPAC-1, HPAC, Capan-1, AsPC-1, MIA PaCa-2, and PANC-1) were investigated to identify the factors which would increase CA19-9 related to the Lewis antigen. CA19-9 in serum is a well-known tumor marker, and is frequently used for the clinical diagnosis of pancreatic cancer. The oligosaccharide on the CA19-9 epitope is a sialylated Lewis A blood group antigen.

**Methods:**  $\beta$ Gal-T was detected by reverse transcriptase polymerase chain reaction (RT-PCR). The phenotypes and genotypes of Lewis antigen were determined by flow cytometry analysis and restriction fragment length polymorphism (RFLP), respectively. The phenotypes of sLe<sup>a</sup> were assessed by flow cytometry analysis and the sLe<sup>a</sup> on supernatants was detected by sodium dodecyl sulfate - polyacrylamide gel electrophoresis (SDS-PAGE). CA19-9 and DUPAN-2 on supernatants were measured by enzyme immunoassay.

**Results:** CA19-9 productions were possible from all cell lines since they all had  $\beta$ Gal-T and there were no genotypical Lewis negative (*le/le*). The elevation of CA19-9 was noted on Capan-2 and CFPAC-1, which were phenotypically Lewis positive (Le(a + b +)), as expected. Interestingly, it was also elevated in BxPC-3 even though the line was known to be phenotypically Lewis negative

(Le(a - b -)). Sialyl Le<sup>a</sup> appeared to play an important role in this phenomenon. Although CA 19-9 was not detected in the phenotypically Lewis negative pancreatic cell line without sialyl Le<sup>a</sup>, the levels of DUPAN-2 were variable.

**Conclusion:** It was revealed that an elevated CA19-9 was related with increased expression of Lewis gene, not merely the existence of the gene. Further investigations on the role of ST3Gal are warranted to explain the mechanisms of the variable levels of DUPAN-2 in Le(a - b -) cell lines. (J Korean Surg Soc 2002;63:317-325)

**Key Words:** Sialylation, N-acetyl glucosamine 1,3-galactosyl transferase ( $\beta$ Gal-T), 1,3/4 fucosyl transferase (Fuc-TIII, FUT3, Lewis antigen), CA19-9, DUPAN-2  
: Sialylation, N-acetyl glucosamine 1,3-galactosyl transferase ( $\beta$ Gal-T), Lewis antigen, CA19-9, DUPAN-2

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Cancer Antigen (CA) 19-9  
가 가 가  
(1,2) CA 19-9 sialyl Lewis<sup>a</sup> (sLe<sup>a</sup>)  
가 Lewis system  
(3)  
Lewis system  
(4) blood group  
Lewis system (5)  
CA 19-9 (Fig. 1) 가  
glycosyl transferase가 , N-acetyl gluco-

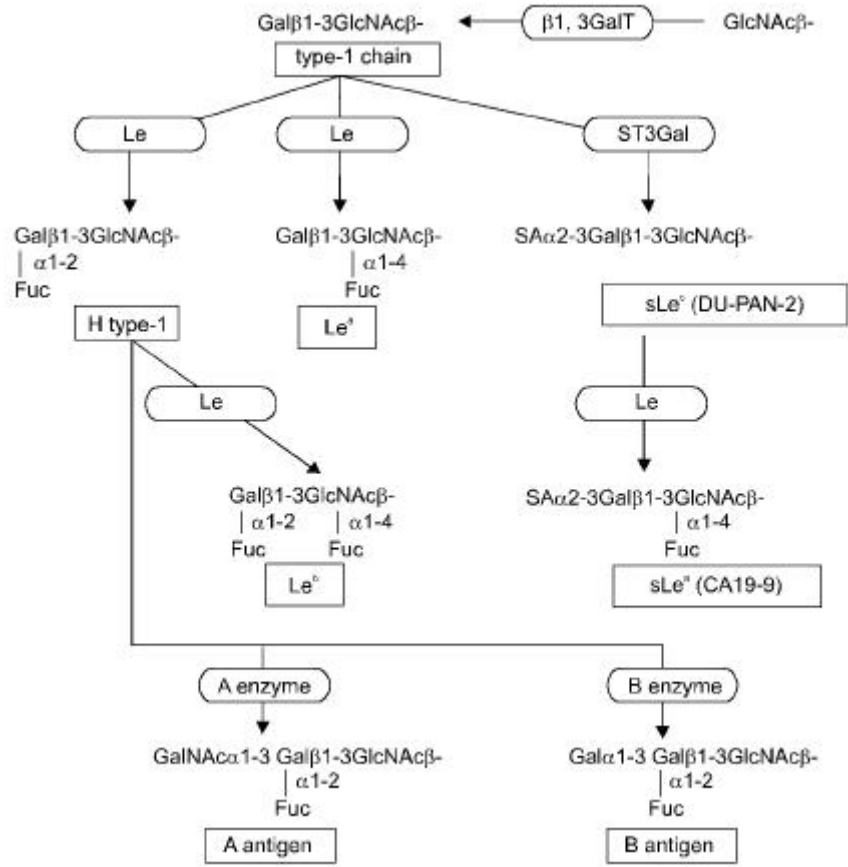


Fig. 1. Biosynthetic pathways for Le antigens with the type I chain in correlation with the synthesis of ABH antigen.

samine galactose type I chain Lewis  
 N-acetyl glucosamine 1, 3-galactosyl transferase ( 3Gal- Lewis  
 T) , , sialyl type 1 chain sialic acid  
 type I chain Gal residual galactose- 2, 3-sialyl  
 transferase (ST3Gal), , fucose sialyl-type 1 chain  
 GlcNAc residual 1, 3/4 fucosyl traseferase (Fuc- Lewis<sup>x</sup> Lewis<sup>a</sup>  
 TIII, FUT3, Lewis antigen) .(6) Lewis<sup>a</sup> (Le<sup>a</sup>)  
 CA 19-9 sialyl- (cross reactivity)가 (14)  
 Le<sup>c</sup> (DUPAN-2) 3Gal-T가 Lewis<sup>x</sup> s-Lewis<sup>x</sup>  
 type I chain CA 19-9 (15) CA 19-9  
 DUPAN-2 .(7)  
 가 3Gal-T 1994 genuine Lewis CA 19-9  
 cloning Human Genome Project 가 CA 19-9  
 4 가 (6,8) FUT3 Lewis  
 .(9-11) CA 19-9 Lewis  
 Lewis Lewis  
 CA 19-9 Lewis  
 .(12)  
 Lewis CA 19-9 가

BxPC-3, Capan-2, CFPAC-1, HPAC, Capan-1, AsPC-1, MIA PaCa-2, PANC-1

### 1) Cancer cell line

(BxPC-3, Capan-2, PANC-1, MIA PaCa-2, CFPAC-1, HPAC, Capan-1, AsPC-1) ATCC (American Type Culture Collection, Manassas, VA, USA)

ATCC (adenoCarcinoma)

ATCC

MIA PaCa-2 100 µg/ml penicillin-streptomycin (Gibco BRL, Grand Island, New York, USA), 4 mM L-glutamine, 10% DMEM (Gibco BRL), PANC-1 4 mM L-glutamine, 10% DMEM, Capan-1 4 mM L-glutamine, 20% IMDM (Gibco BRL), AsPC-1 20% RPMI-1640 (Gibco BRL), BxPC-3 CFPAC-1 10%

RPMI-1640 (Gibco BRL), Capan-2 10%

McCoy's 5a (Gibco BRL),

HPAC 5% DME+Ham's F12 (1 : 1) 75 cm<sup>2</sup>

80% 3

ml 0.25% trypsin, 0.03% EDTA

2 ml trypsin-EDTA 가

60%

3 5

가

### 2) -N-Acetylglucosamine 1, 3-Galactosyltransferase (3Gal-T5)

RNA DNase I

superscript preamplification system complementary DNA

cDNA 10 µl, competitor DNA 10 µl, beta 3 Gal T5 beta actin target primer set (Table 1) 0.2 um 50 µl reaction mixture AmpliTaq GoldTM (Perkin Elmer) (Competitive Reverse Transcriptase Polymerase Chain Reaction, RT-PCR) (16)

(buffer) 10 mM Tri-HCl (pH 8.3), 50 mM KCl, 1.5 mM MgCl<sub>2</sub>, 0.2 µM of each dNTP 0.0001% gelatin

95°C 11 pre-Heat step

95°C 1, 3 gal T5 65°C,

actin 60°C (annealing) 1, 72°C 2 10 µl

(aliquot) 1% agarose gel

ethidium bromide (band) 가

### 3) Lewis

(1) Lewis :

Le<sup>a</sup>

BG-5 (anti-Le<sup>a</sup>, Signet Laboratories, Inc., Dedham, MA, USA) 50, Le<sup>b</sup>

BG-6 (anti-Le<sup>b</sup>, Signet Laboratories, Inc., Dedham, MA, USA) 40

1, 2 anti-mouse IgM (FITC, Becton Dickinson, San Jose, CA) 1 : 100

15 (FACS: Becton Dickinson)

isotype (IgG1)

WinMDI (version 2.8; Scripps Research Institute, La Jolla, CA, USA)

(2) Lewis :

(DNA WizardTM, Promega, Madison, WI, USA)

DNA Le T59G

59A 59AS (17)

Table 2

Table 1. The primer sets used for competitive RT-PCR analysis

Target gene	Primer sets (Forward primer)	Size of PCR products target (base pairs)	Restriction enzyme for competitor DNA	Annealing temperature
3Gal-T5	5'-ACCACCAGCAGTGCAGCGGAAAC-3'	554	Eco8 II-XcmI	65°C
-Actin	5'-GATATCGCCGCGCTCGTCGTCGAC-3'	789	EcoO 109I-BstEII	60°C

**Table 2.** The primer sets used for amplification of the Le genes

Gene	Primer name	Primer sequences	Annealing temperature	Fragment size (bp)	Restriction enzyme
Le	59S	5'-ATGGCGCCGCTGTCTGGCCGCC-3'	65°C	120 (97+23)	Msp I
	59AS	5'-GAGGACCCACTGGGAGCCCT-3'			

가 Taq DNA polymerase 1 U, dNTP 250 μM, Tris-HCl (pH 9.0) 10 mM, KCl 40 mM, MgCl<sub>2</sub> 1.5 mM AccuPower™ PCR PreMix (Bioneer, , ) GeneAmp PCR system 9600 (Perkin Elmer Cetus, CT, USA) 94°C 1 , 65°C 30 , 72°C 30 25 . A59T PCR Msp I (Takara biotechnology Co., LTD, Shiga, Japan) 12 가 37°C 2 4% metaphor (MetaPhor<sup>®</sup> agarose, FMC<sup>®</sup>, ME, USA) . Le A59G 가 97 bp 23 bp .

**4) sialyl Le<sup>a</sup>**

(1) sialyl Le<sup>a</sup> : (anti-sialyl Le<sup>a</sup>, Dr. Narimatsu H , Soka University, Tokyo, Japan) 150 Lewis

**(2) SDS-PAGE (Sodium Dodecyl Sulfate - Polyacrylamide Gel Electrophoresis) Sialyl-Le<sup>a</sup>**

: EBC (40 mM Tris-Cl, 120 mM NaCl, 0.5% NP-40) protease inhibitor mixture ( Leupeptin 1 μg/ml, aprotin 1 μg/ml PMSF (100 μg/ml) 가 lysis buffer 12,000 rpm 20 Bio-rad Protein assay 10% acrylamide gel well 20 μg gel transfer chamber (Hoefer Pharmacia Biotech, San Francisco, CA, USA) NitroCellulose membrane (Amesharm-Pharmacia Biotech, Little Chalfont, England) membrane 5% skim milk 16 4°C bloCking PBST (phosphate saline, 0.5% Tween 20) 3 1 (anti-sialyl Le<sup>a</sup>, Dr. Narimatsu H , Soka University, Soga University, Tokyo, Japan) phosphate saline Tween 20 1 : 2,000 2

phosphate saline Tween 20 3 , 5 (anti-mouse Ig G HRP conjugated, Dako, Copenhagen, Denmark) 1 : 2,000 1 , phosphate saline Tween 20 3 , 5 ECL western blotting (Amersham-Pharmacia Biotech, Little Chalfont, England) X-ray film proCessor (FujiFPM 100A, Tokyo, Japan) 5) (1) CA19-9 : CA 19-9 EIA II (RoChe Diagnostics AG, Switzerland) 37

U/mL (2) DUPAN-2 : DUPAN-2 solid phase enzyme immunoassay (EIA;- Kyowa Medex, Tokyo, Japan) 150 U/mL .

1) Fig. 2 BxPC-3 mucin Capan-2 mucin PANC-1 Soft agar fibroblast monolayer 가 MIA PaCa-2 (aneuploidy) CFPAC-1 cytokeratine (oncofetal antigen) HPAC Capan-1 AsPC-1

**2) 3Gal-T5**

8 554 base

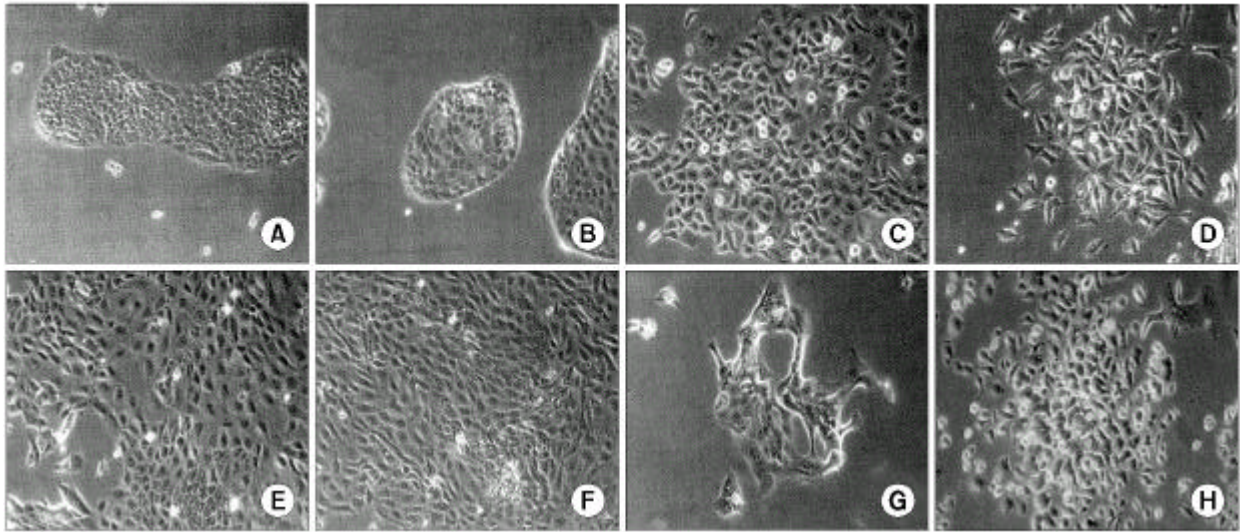


Fig. 2. Cellular appearance in 8 human pancreatic cancer cell lines.

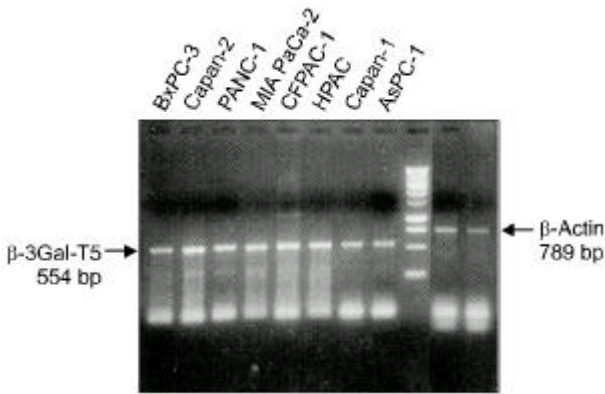


Fig. 3. 3Gal-T5 analysis in human pancreatic cancer cell lines by RT-PCR.

pairs  
 (Fig. 3). 3Gal-T5 type 1 chain  
 3Gal-T5 $\uparrow$  sLe<sup>a</sup> (CA19-9)

3) Lewis

(1) Lewis : 8  
 Capan-2 CFPAC-1 Lewis  
 Le (a+b+) Le (a-b-) (Fig. 4).  
 (2) Lewis : Le A59G  
 59A 59AS  
 가 97 bp 23 bp  
 MIA PaCa-2 Le/le 7  
 Le/Le (Fig. 5).

4) Sialyl-Le<sup>a</sup>

(1) Sialyl-Le<sup>a</sup> :  
 sialyl-Le<sup>a</sup> Capan-2 CFPAC-1  
 BxPC-3  
 (Fig. 6).

(2) SDS-PAGE Sialyl-Le<sup>a</sup>  
 anti-sialyl-Le<sup>a</sup> Western blot analysis  
 CFPAC-1, BxPC-3, Capan-2 Sialyl-Le<sup>a</sup>  
 (Fig. 7).

5)

CA 19-9 sialyl-Le<sup>a</sup>  
 BxPC-3, Capan-2, CFPAC-1  
 51.6, 979.9, 424 (U/ml)  
 DUPAN-2 CFPAC-1, Capan-1, AsPC-1  
 301, 20,600, 1,760 (U/ml)  
 (Table 3).

CA 19-9 sialyl Lewis<sup>a</sup> (sLe<sup>a</sup>) 가  
 Lewis system CA 19-9  
 N-acetyl glucosamine 1,3-galactosyl  
 transferase (3Gal-T), galactose- 2,3-sialyl transferase (ST3-  
 Gal), fucosyl transferase (Fuc-TIII, FUT3, Lewis enzyme)  
 가 glycosyl transferase가 .(6)

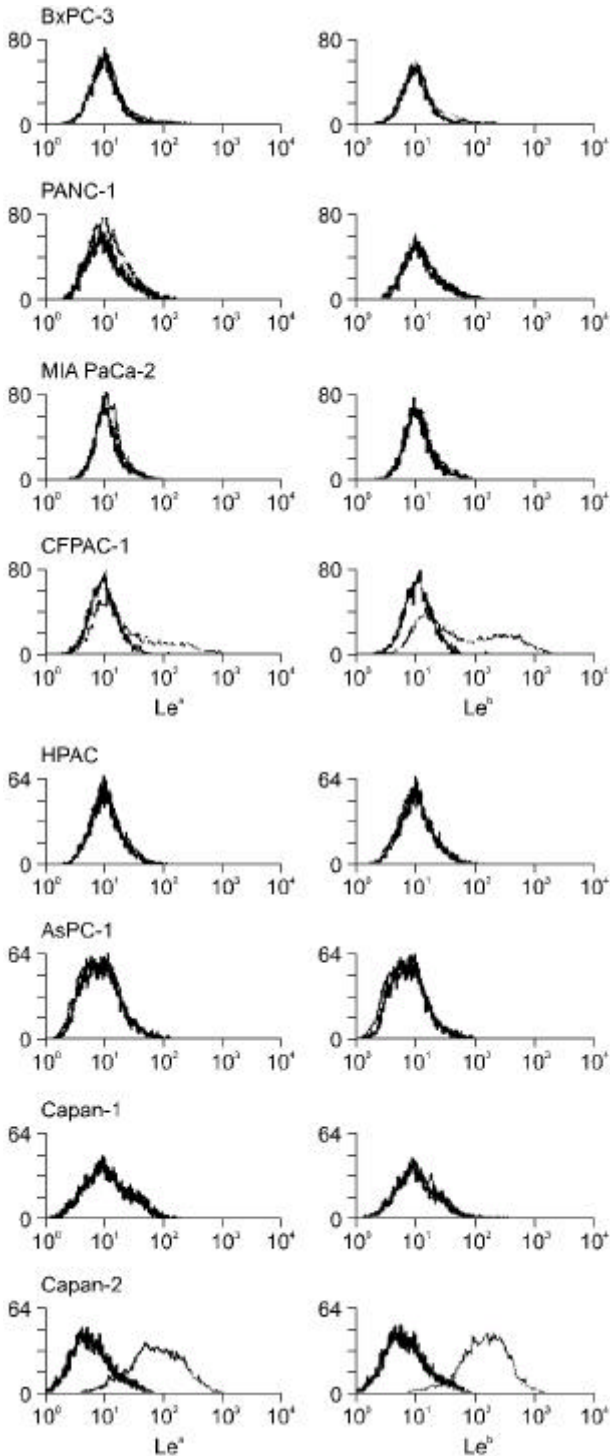


Fig. 4. Lewis phenotyping in human pancreatic cancer cell lines by flow cytometry analysis.

3Gal-T가 type I chain  
 CA19-9 DUPAN-2 Lewis  
 CA19-9  
 sialyl-Le<sup>c</sup> (DUPAN-2) .(7)

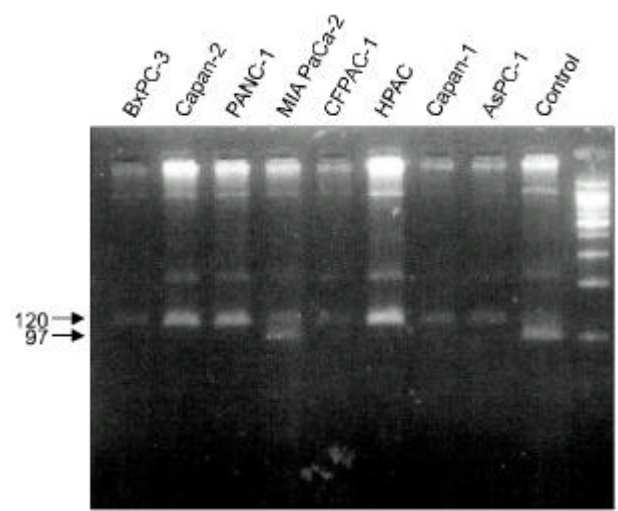


Fig. 5. Lewis genotyping in human pancreatic cancer cell lines by PELP.

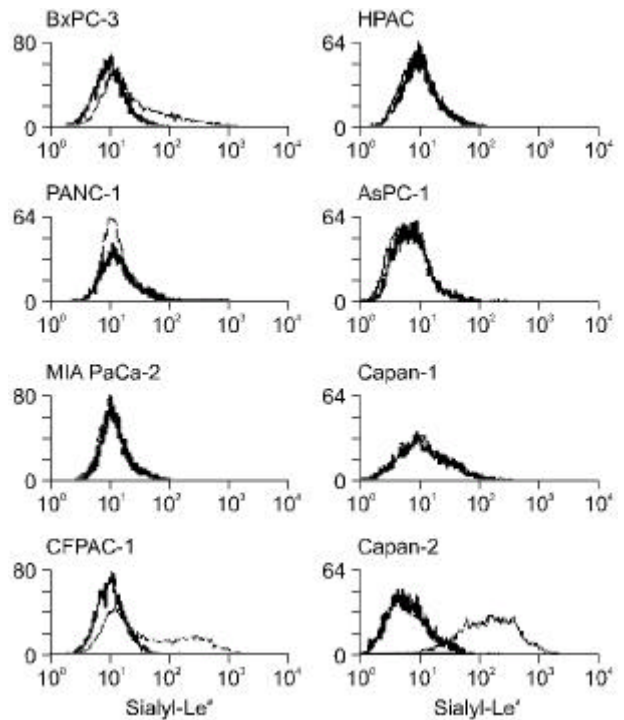
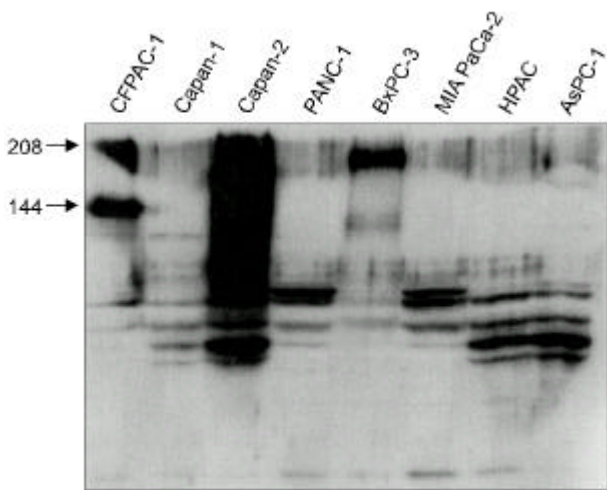


Fig. 6. Sialyl-Le<sup>a</sup> phenotyping in human pancreatic cancer cell lines by flow cytometry analysis.

3Gal-T FUT3 CA 19-9  
 가 3Gal-T  
 5 가 3Gal-T5가  
 sLe<sup>a</sup> 가 가 .(6) 3Gal-  
 T5 554 base pairs PCR pro-  
 ducts target 5'-ACCACCAGCAGTGCAGCGG-

**Table 3.** CA 19-9 & DUPAN-2 values in the supernatant human pancreatic cancer cell lines with phenotype and genotype of Lewis antigen and presence of sialyl Le<sup>a</sup>

Cell lines	Lewis system		Sialyl Le <sup>a</sup>		Supernatant	
	Phenotyping	Genotyping	Flow cytometry	Western blot analysis	CA 19-9	DUPAN-2
BxPC-3	Le (a - b -)	Le/Le	weak +	+	51.6	<25
Capan-2	Le (a+b+)	Le/Le	+	+	979.9	<25
PANC-1	Le (a - b -)	Le/Le	-	-	0.6	<25
MIA PaCa-2	Le (a - b -)	Le/le	-	-	0.6	<25
CFPAC-1	Le (a+b+)	Le/Le	+	+	424	301
HPAC	Le (a - b -)	Le/Le	-	-	0.9	<25
Capan-1	Le (a - b -)	Le/Le	-	-	3.9	20600
AsPC-1	Le (a - b -)	Le/Le	-	-	1.9	1760



**Fig. 7.** CA19-9 to cancer cell lines lysate by SDS-PAGE and transfer to the blot.

Lewis

BxPC-3, MiaPaca-2    Panc-1

BxPC-3

Le<sup>a</sup> , sLe<sup>a</sup> , MIA PaCa-2, Panc-1

(22)

Lewis

PAC-1    CA 19-9    (23)

Le<sup>a</sup>, sLe<sup>a</sup>

SDS-PAGE    sLe<sup>a</sup>    mucin

(CFPAC-1, Capan-1, Capan-2, BxPC-3, AsPC-1)

(PANC-1, MIA PaCa-2, HPAC)

mucin    (24)    가

Capan-2, BxPC-3    Band가    CFPAC-1, anti-sLe<sup>a</sup>

CA 19-9    DUPAN-2    Lewis

가    MIA PaCa-2

CA 19-9    DUPAN-2    (BxPC-3, Capan-2)

CA 19-9    DUPAN-2가    (Capan-1, AsPC-1)

CA 19-9    Capan-2, PANC-1, HPAC

ST3Gal    가    (25,26)    sialylated carbohydrate

(27)

c-DNA microarray    sLe<sup>a</sup>

Capan-1    Capan-2    가

corrected ratio가    0.15, Capan-1    1.21, Capan-2    2.79

Capan-2    가

sialylated carbohydrate

AAAC-3' primer set    8

554 base pairs    sLe<sup>a</sup> type I chain

Fucosyltransferase    (determinant)

(1,2) fucosyltransferase (FUT1, FUCT2)

(1,3) fucosyltransferase (FUT3, FUCT4, FUT5, FUT6, FUT7)    가    (19,20)    FUT3가    Lewis type

fucosyltransferase    (21)    19

FUT3(9)    5가    가 cloning    (6,8)    (9-11)

T59G, G508A, T1067A    가

T59G, T202C, C314G, C445A, G508A, T1067A    가

T59G    T1067A    가

가

(12)    T59G



가가 CA19-9	가 ST3Gal	가 가
Lewis 8	CA 19-9	
type 1 chain		3Gal-T
RT PCR	Lewis RFLP	Flow Cytometry Analysis, sLe <sup>a</sup>
blot method	Flow Cytometry Analysis, sLe <sup>a</sup>	Western DUPAN-2
1) 8 galactosyl transferase가		1, 3-
2) Lewis Lewis (a-b) 가 Le/le	Le (a+b+) Lewis 7	Capan-2 CFPAC-1 Le MIA PaCa-2 Le/Le
3) sialyl-Le <sup>a</sup> BxPC-3 Western blot	Capan-2 BxPC-3, PANC-1, CFPAC--	CFPAC-1
1 Sialyl-Le <sup>a</sup>		
4) CA 19-9 BxPC-3, 51.6, 979.9, 424 (U/ml)	sialyl-Le <sup>a</sup> Capan-2, CFPAC-1	
DUPAN-2 301, 20600, 1760 (U/ml)	CFPAC-1, Capan-1, AsPC-1	
		CA 19-9 가가 CA 19-9
가	가	
(bloCking antibody)		가
Lewis		DUPAN-2 가
	ST3Gal	가

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