

$p16^{INK4A}, p15^{INK4B}$

$p14^{ARF}$

p16^{INK4A}, p15^{INK4B}

p14^{ARF}



가

가

가

가

27

(,)

)

(

)

, 가

	1
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	<i>p16^{INK4A}</i> , <i>p15^{INK4B}</i>	<i>p14^{ARF}</i>19

p16^{INK4A}, p15^{INK4B} p14^{ARF}

10-40%가

p16^{INK4A}

p16

p15^{INK4B}

p14^{ARF}

p16^{INK4A}

p14^{ARF}

p16^{INK4A}, p15^{INK4B}

p14^{ARF}

, 79

p16^{INK4A}, p15^{INK4B}

p14^{ARF}

p16^{INK4A},

p15^{INK4B}

p14^{ARF}

33%, 15%, 30%

, 33%,

4%, 22%

가

p16^{INK4A}

p14^{ARF}

p15^{INK4B}

73%

가

,

70%

가 .

- $p16^{INK4A}$ $p14^{ARF}$

.

: $p16^{INK4A}$, $p15^{INK4B}$, $p14^{ARF}$, ,

p16^{INK4A}, p15^{INK4B} p14^{ARF}

< >

I.

1/4

가

,

가

(oncogene)

가

가

가 가

1

K-Ras

² *APC, DCC, p53*

가

3-5

3

가

가

p53, APC, DCC

E-Cadherin, p16

hMLH1 DNA

6-10

11-15

가

p16^{INK4A}

9

21 (

9p21)

cyclin-dependent

kinase inhibitor

CDK4/6

가

(late G1 phase)

(S phase)

DNA

16

9p21

p16^{INK4A}

$p15^{INK4B}$ $p14^{ARF}$ 가 $p14^{ARF}$ p16
 alternative splicing encode ,¹⁷⁻²⁰ MDM2-p53 complex
 p53 ²¹⁻²⁴ $p14^{ARF}$
 $p15^{INK4B}$
^{25,26}
 $p16^{INK4A}$
 0-10% ,²⁷⁻³⁰
 20-55% ³¹⁻³⁴ 가
 (microsatellite instability) $p16^{INK4A}$
³⁵ $p14^{ARF}$
 30% 10%
 가 , 61 22 (36%)
 가 ³⁶
 p16
 $p15, p14^{ARF}$
 $p16^{INK4A}$
 가 - , $p16^{INK4A}$
 $p16^{INK4A}$ $p15^{INK4B}, p14^{ARF}$

$p16^{INK4A}$, $p15^{INK4B}$ $p14^{ARF}$

II.

1.

6 (SNU-1, SNU-16, SNU-601, SNU-638,
 SNU-668, SNU-719)^{37,38} 1995 1 1999 12
 79

(1).

1.

가	10	4 ^a	8	10
(Adenoma-Carcinoma in same lesion)				
가	69	52	17	0
(Single Adenoma)				
	79	56	25	10

^a, 가 2

가 .

2.

가.

DNA

microtome 4 μm hematoxylin eosin

4~5 10 μm 가

hematoxylin eosin

. DNA

10 μm

xylene

1.5 ml

tube 500 μl lysis buffer (100 mM Tris, pH 8.0, 50

mM EDTA, 150 mM NaCl, 0.5% SDS, 200 $\mu\text{g/ml}$ proteinase K) 가 , 50°C

phenol:chloroform:isoamylalcohol

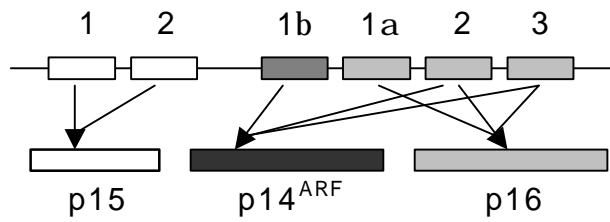
(25:24:1) 가 , 13,000 rpm 4

chloroform 500 μ l
 , isopropanol 500 μ l 10M Ammonium
 acetate 100 μ l 가 30 . 14,000 rpm 10
 DNA , DNA TE buffer (pH 8.0)
 , -20°C .

(homozygous deletions)

p16^{INK4A}, *p15^{INK4B}* *p14^{ARF}*
p16^{INK4A}, *p15^{INK4B}* *p14^{ARF}* exon (1)
 primer *hMSH6* ³⁹ primer
 Multiplex PCR (2).⁴⁰
 PCR 50 ng DNA , 0.2 mM dNTP, 1.5 mM MgCl₂, 1
 unit *Taq* polymerase (GIBCO-BRL, Grand Island, NY, USA), 10X PCR buffer, 1 μ Ci
 [α -P³²]dCTP (3000 Ci/mmol; NEN DuPont, Boston, MA, USA) ,
p16^{INK4A}, *p15^{INK4B}* *p14^{ARF}* exon primer 20 pmol *hMSH6*
 primer 5 pmol 20 μ l . PCR
 machine (Perkin-Elmer, Foster, CA, USA) 95°C 5
 (denaturation) , 95°C 15 , 58°C 15 , 72°C 30 33 cycles
 (amplification) . PCR 6% polyacrylamide gel 60W

1 , Gel Dryer (BIO-RAD, Hercules, CA, USA) gel , X-Ray Film (Kodak, Rochester, NY, USA)



1. $p16^{INK4A}$, $p15^{INK4B}$ $p14^{ARF}$ exon . 9p21

$p16^{INK4A}$ $p15^{INK4B}$ $p14^{ARF}$ 7†

$p14^{ARF}$ p16

alternative splicing encode .

$p16^{INK4A}$, $p15^{INK4B}$ $p14^{ARF}$

DNA

50%

hMSH6

2.

primer	sense	antisense	product size
<i>p15^{INK4B}</i> exon 1	atgcgcgaggagaacaag	ctccgaaacggttgactc	143
<i>p15^{INK4B}</i> exon 2	ctctgctctctctggcaggt	ggtgagagtggcaggtct	105
<i>p14^{ARF}</i> exon 1β	tgggtcccagtctgcagtta	gggatgtgaaccacgaaaac	186
<i>p16^{INK4A}</i> exon 1α	caacgcaccgaatagttacg	gcgctacctgattccaatc	123
<i>p16^{INK4A}</i> exon 2	cattctgttctctctggcagg	caccagcgtgtccaggaa	154
<i>p16^{INK4A}</i> exon 3	aacgcctgtttctttctgc	cctgtaggaccttcggtgac	109
<i>hMSH6</i>	ccctcagccaccaacaagca	ctgccaccacttctcatccc	288

. Sodium Bisulfite Modification DNA

Methylation-specific PCR (MSP) ²⁶ DNA ,
 DNA sodium bisulfite
 modification , ²⁶ Sodium bisulfite modification
 DNA 1 μg 가 50 μl 가 , 5.6 μl 5 N
 NaOH 가 , 37°C 15 . 30
 μl 10 mM hydroquinone, 520 μl 4 M Sodium bisulfite (pH 5.0) 가
 . Mineral oil , 55°C

16~20 . Sodium bisulfite DNA Wizard DNA
 purification resin (Promega, Madison, WI, USA) .
 50 μ l DNA , 5.6 μ l 5 N NaOH
 가 37°C 15 . , 5.5 μ l 10 M
 ammonium acetate 125 μ l ethanol 가 -20°C 30
 . 4°C 14,000 rpm 15
 DNA . 20 μ l
 , -20°C .

. Methylation-Specific PCR (MSP)

DNA Sodium bisulfite DNA
p16^{INK4A} , *p15^{INK4B}* *p14^{ARF}*
 (3)^{25,26} PCR ,
 (2).
 PCR 가 20 μ l 가 100 ng DNA, 0.2 mM dNTP, 1.5 mM
 MgCl₂, 1 pmol/ μ l sense antisense primer, 1 unit *Taq* polymerase (GIBCO-BRL,
 Grand Island, NY, USA), 10X PCR buffer . PCR machine
 (Perkin-Elmer, Foster, CA, USA) 95°C 45 , 55°C 45 , 72°C
 1 35 cycles , 6X dye 4 μ l 2% agarose gel 15 μ l

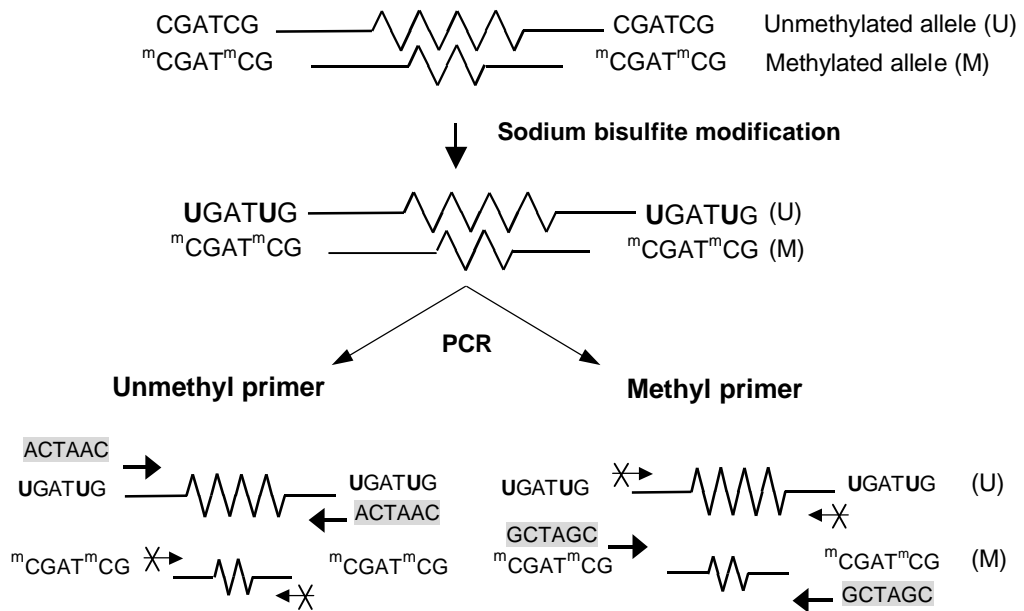
loading PCR . DNA 100bp
 ladder size marker (Promega, Madison, WI, USA) .

3. MSP primer sequence

gene		primer set	annealing temp (°C)	genomic position
<i>p15^{INK4B}</i>	U	F TGTGATGTGTTTGTATTTTGTGGTT	54	+34 ~ +187
		R CCATACAATAACCAAACAACCAA		
	M	F GCGTTCGTATTTTGCGGTT	55	+40 ~ +187
		R CGTACAATAACCGAACGACCGA		
<i>p14^{ARF}</i>	U	F TTTTTGGTGTTAAAGGGTGGTGTAGT	52	+195 ~ +326
		R CACAAAAACCCTCACTCACAACAA		
	M	F GTGTAAAGGGCGGCGTAGC	58	+201 ~ +322
		R AAAACCCTCACTCGCGACGA		
<i>p16^{INK4A}</i>	U	F TTATTAGAGGGTGGGGTGGATTGT	58	+167 ~ +316
		R CAACCCCAAACCACAACCATAA		
	M	F TTATTAGAGGGTGGGGCGGATCGC	65	+167 ~ +317
		R GACCCCGAACCGCGACCGTAA		

U : unmethylated-specific primer, M : methylated-specific primer,

F : forward primer, R : reverse primer

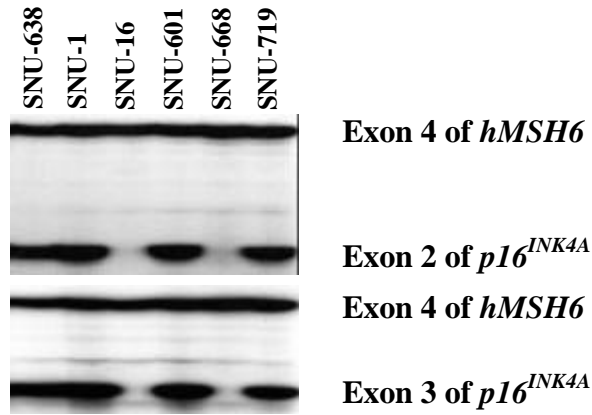


2. MSP . DNA sodium bisulfite ,
 cytosine uracil , cytosine
 primer set PCR

III.

1. (homozygous deletions)

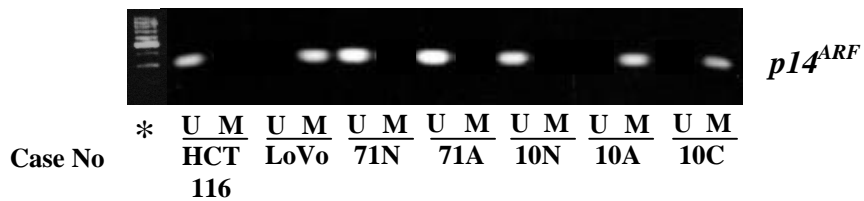
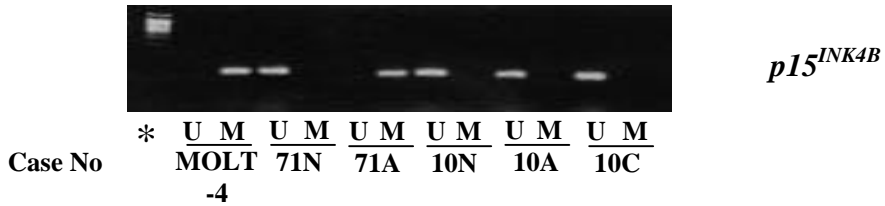
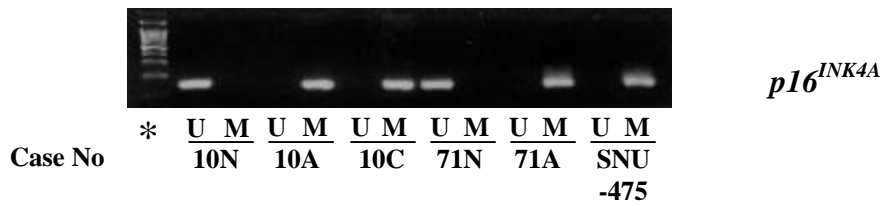
6 $p16^{INK4A}$, $p15^{INK4B}$ $p14^{ARF}$ exon
 , 33% (2/6)
 (3).



3.

genomic DNA , $p16^{INK4A}$, $p15^{INK4B}$ $p14^{ARF}$
 exon $hMSH6$
 PCR , PCR 6% polyacrylamide gel
 . $p16^{INK4A}$ exon 2 exon 3 SNU-16 SNU-668

79 $p16^{INK4A}$, $p15^{INK4B}$ $p14^{ARF}$ exon
, $p15^{INK4B}$ exon 1 13% (10/79), $p15^{INK4B}$ exon 2
4% (3/79), $p14^{ARF}$ exon 1 β 8% (6/79), $p16^{INK4A}$ exon 1 α 5% (4/79), $p16^{INK4A}$
exon 2 25% (20/79), $p16^{INK4A}$ exon 3 4% (3/79) , $p16^{INK4A}$ exon 2
가 . $p16^{INK4A}$, $p15^{INK4B}$ $p14^{ARF}$
34% (27/79), 15% (12/79) 32% (25/79) .
 $p16^{INK4A}$ 50% (5/10), $p15^{INK4B}$ 10% (1/10), $p14^{ARF}$ 50%
(5/10) . exon $p15^{INK4B}$ exon
2, $p14^{ARF}$ exon 1 β , $p15^{INK4B}$ exon 1, $p16^{INK4A}$ exon 1 α ,
 $p16^{INK4A}$ exon 3 10% (1/10) , $p16^{INK4A}$ exon 2 40%
(4/10) 가 . ,
 $p16^{INK4A}$ $p14^{ARF}$ $p15^{INK4B}$
.
. $p16^{INK4A}$, $p15^{INK4B}$ $p14^{ARF}$
 $p16^{INK4A}$, $p15^{INK4B}$ $p14^{ARF}$
MSP (4).



4. *p16^{INK4A}, p15^{INK4B} p14^{ARF}*

. Sodium bisulfite DNA (U)

(M)

PCR, PCR 2% agarose gel

SNU-475, MOLT-4, LoVo *p16^{INK4A}, p15^{INK4B} p14^{ARF}*

,^{25,41,42} HCT116 *p14^{ARF}* .²⁵ *

100bp ladder size marker, N, A, C

10A, 10C *p16^{INK4A} p14^{ARF}*

, 71A *p15^{INK4B}*

$p16^{INK4A}$, $p15^{INK4B}$ $p14^{ARF}$

33% (26/79), 4% (3/79), 22% (17/79) 가 ,

$p15^{INK4B}$ 가 , $p16^{INK4A}$ 40% (4/10),

$p14^{ARF}$ 40% (4/10) 가 ,

$p16^{INK4A}$ $p14^{ARF}$

(SNU-16, SNU-668)

4 (SNU-1, SNU-601, SNU-638, SNU-719) $p16^{INK4A}$

$p14^{ARF}$ promoter , $p15^{INK4B}$

(SNU-719)

, 가 10 22

(4 , 8

10) (4).

, $p16^{INK4A}$, $p15^{INK4B}$ $p14^{ARF}$

, 50% (2/4),

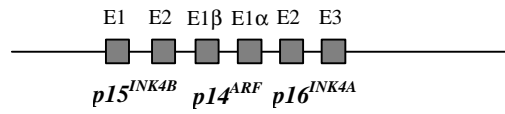
63% (5/8), 70% (7/10)

가 (

4).

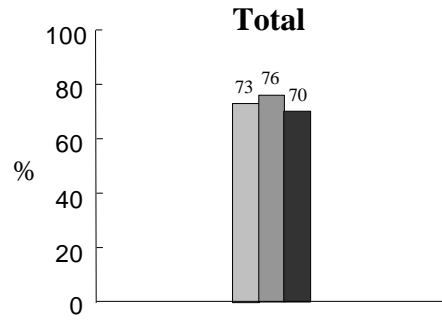
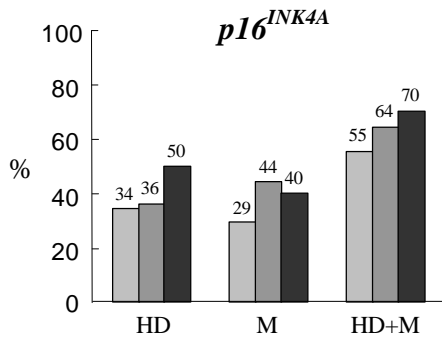
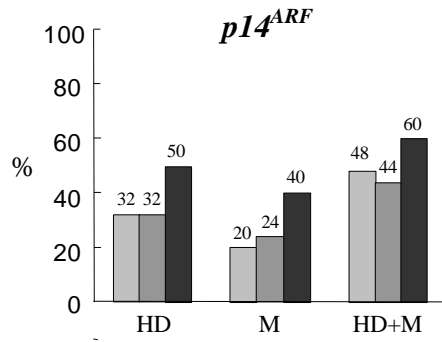
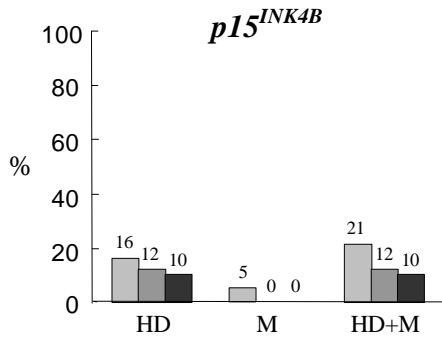
4. 10 - (adenoma-carcinoma in same lesion)

$p16^{INK4A}$, $p15^{INK4B}$ $p14^{ARF}$



Patient No.	Lesion	Exon 1	Exon 2	Exon 1b	Exon 1a	Exon 2	Exon 3
1	High-grade adenoma Carcinoma						
2	High-grade adenoma Carcinoma						
3	High-grade adenoma Carcinoma	■		■			■
4	Low-grade adenoma Carcinoma				▨		
5	Low-grade adenoma Carcinoma			▨		■	
6	Low-grade adenoma High-grade adenoma Carcinoma			▨		■	
7	High-grade adenoma Carcinoma			▨	▨		
8	Low-grade adenoma High-grade adenoma Carcinoma				▨	■	
9	High-grade adenoma Carcinoma						
10	High-grade adenoma Carcinoma			▨	▨	■	

■, ; ▨, ; □,



5. *p16^{INK4A}, p15^{INK4B}* *p14^{ARF}* *p16^{INK4A}, p15^{INK4B}* *p14^{ARF}* (HD)

(M) 56 ()

25 (), 10 ()

% Total

$p16^{INK4A}$, $p15^{INK4B}$ $p14^{ARF}$ (

 ,) (5).

 $p16^{INK4A}$ $p14^{ARF}$

 , $p15^{INK4B}$

 (21%, 12/56)

 (12%, 3/25), (10%, 1/10)

 .

 ,

 73% (41/56), 76% (19/25), 70% (7/10)

 가 (5).

IV.

가

1q, 5q, 7q, 9p, 11p, 11q,

13q, 16q, 17p 18q

가

⁴³⁻⁴⁶ APC, ⁶ p53 ⁷

DCC ⁸ 가

50%

E-Cadherin, hMLH1

가

DNA

⁹⁻¹⁰

TGFbRII, BAX DNA

가

,^{12,13} p53

(mutation)

¹¹

17p

,¹⁴

Kang

¹⁵ DAP-K, THBS1, TIMP-3

가

9p21

3

가

coding exon

reading

frame encode p16 p14,¹⁷⁻²⁰ homologue 가

p15 . $p16^{INK4A}$

, , , 60% .⁴⁷⁻

⁵¹ $p16^{INK4A}$ (homozygous deletions)

(point mutation), (methylation) , ⁴⁷⁻⁵¹ $p14^{ARF}$

$p15^{INK4B}$

.^{25,26}

9p21 $p16^{INK4A}$, $p15^{INK4B}$

$p14^{ARF}$

, .

$p16^{INK4A}$, $p15^{INK4B}$ $p14^{ARF}$ 가

50% (5/10), 10% (1/10), 50% (5/10) $p16^{INK4A}$

$p14^{ARF}$. 가

$p15^{INK4B}$ (15%, 12/79) $p16^{INK4A}$ (34%, 27/79) $p14^{ARF}$ (32%, 25/79)

. .

, $p16^{INK4A}$

0-10% .²⁷⁻³⁰

, Comparative multiplex PCR

가 .⁵²⁻⁵⁵

$p16^{INK4A}$, $p15^{INK4B}$ $p14^{ARF}$ exon primer

$hMSH6$ primer multiplex PCR

, densitometric analysis⁵² $hMSH6$

$p16^{INK4A}$, $p15^{INK4B}$ $p14^{ARF}$

50%

$p15^{INK4B}$ lymphoma, leukemia^{56,57},

가 . $p16^{INK4A}$, ,

, 40,58-60 가 , $p14^{ARF}$

가 .²⁵ $p16^{INK4A}$ 가

20-55% ,³¹⁻³⁵ $p14^{ARF}$ Iida³⁶ 36%

, $p16^{INK4A}$ $p14^{ARF}$ 가

. $p15^{INK4B}$ 4%

(3/79) , $p16^{INK4A}$ 40% (4/10),

33% (26/79) 가 . $p14^{ARF}$ 가

40% (4/10), 22% (17/79)

$p16^{INK4A}$ $p14^{ARF}$

. Kang¹⁵

$p16^{INK4A}$ 61 7 (11%) $p16^{INK4A}$

$p16^{INK4A}$ 가 33% (26/79)

DNA MSP , Kang ¹⁵ modified 가

$p16^{INK4A}$, $p15^{INK4B}$ $p14^{ARF}$

2 (33%) (SNU-16, SNU-668) 4 (67%)

(SNU-638, SNU-1, SNU-601, SNU-719) $p16^{INK4A}$ promoter

methylation , 가 $p16^{INK4A}$

Lee ²⁹ Song ³¹

가 ,

가 (in vitro)

가 가 ⁶¹ 가

가

$p16^{INK4A}$ $p14^{ARF}$

, $p15^{INK4B}$

(21%, 12/56) (12%,

3/25) (10%, 1/10)

$p16^{INK4A}$ $p14^{ARF}$

$p16^{INK4A}$ $p14^{ARF}$

3

, $p16$ (germ-line) 가

$p16$ 가 .⁶² $p16$

pRB

cdk4 cdk6

, $p14^{ARF}$ MDM2 , p53

.⁶³ $p16$ $p14^{ARF}$ p53 Rb

Shahnavaz ⁶⁴

$p16^{INK4A}$

$p16^{INK4A}$

10

plasma serum DNA

65

saliva $p16^{INK4A}$, DAP-K, MGMT ,⁶⁶

3 sputum $p16^{INK4A}$.⁶⁷ $p16^{INK4A}$

marker 가

$p16^{INK4A}$

marker

가

$p16^{INK4A}$

$p16^{INK4A}$ $p14^{ARF}$ 가

가

V.

79

$p16^{INK4A}$, $p15^{INK4B}$ $p14^{ARF}$

1. $p16^{INK4A}$ exon 2 가 .

2. $p16^{INK4A}$, $p14^{ARF}$, $p15^{INK4B}$

3. (73%, 41/56)

(76%, 19/25), (70%, 7/10)

4.

$p16^{INK4A}$ $p14^{ARF}$

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Abstract

Alterations of p16^{INK4A}, p15^{INK4B} and p14^{ARF} genes
in gastric adenomas and carcinomas

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Gastric adenoma is a pre-cancerous lesion of the stomach and is associated with intestinal type carcinoma. The adenoma-carcinoma sequence in gastric carcinogenesis is believed to exist in a subset of gastric carcinomas and might develop through accumulative series of genetic alterations similar to that of colorectal cancer.

The *p16^{INK4A}* gene encodes two cell cycle regulator proteins, p16 and p14^{ARF}, by alternative splicing. This genetic locus also contains another cell cycle regulator gene, *p15^{INK4B}*, which encodes p15. The methylation of the *p16^{INK4A}* and *p14^{ARF}* gene has been demonstrated in some gastric carcinomas, however the inactivation mechanisms of this cell regulator proteins are not well characterized in gastric adenomas.

To characterize the role of the above 3 cell cycle regulator proteins in gastric adenomas, we examined the genomic status of the $p16^{INK4A}$, $p15^{INK4B}$ and $p14^{ARF}$ genes in 79 gastric adenoma tissues, 10 gastric carcinomas and 6 gastric cell lines.

In 79 gastric adenomas, the frequency of homozygous deletions and/or hypermethylation was 57% (45/79) in $p16^{INK4A}$, 19% (15/79) in $p15^{INK4B}$ and 47% (37/79) in $p14^{ARF}$. The overall frequency of $p16^{INK4A}$, $p15^{INK4B}$ and $p14^{ARF}$ alterations was 73% (59 of 79 cases) in gastric adenomas and was similar to gastric carcinomas (70%, 7/10). There is no significant genetic difference between low-grade and high-grade gastric adenoma.

Our results showed that the inactivation of p16 and p14^{ARF} may play an important role in early stage gastric carcinogenesis.

Key Words : $p16^{INK4A}$, $p15^{INK4B}$, $p14^{ARF}$, homozygous deletions, methylation