

# Urokinase- type Plasminogen Activator(uPA), Plasminogen Activator Inhibitor- 1(PAI- 1) nm23

=Abstract=

Expression of Urokinase- type Plasminogen Activator (uPA),  
Plasminogen Activator Inhibitor- 1 (PAI- 1) and nm23 protein,  
as Prognostic Factors in Epithelial Ovarian Cancer

Kyung Tai Kim, M.D., Ho Sang Seo, M.D., Ki Heon Lee, M.D.,\*  
Young Jin Moon, M.D., Sam Hyun Cho, M.D., Hyung Moon, M.D.,  
Wan Sub Kim, M.D.,\*\* Moon Hyang Park, M.D.,\*\* Youn Yeoung Hwang, M.D.  
*Department of Obstetrics and Gynecology, Department of Pathology,\* College of Medicine, Hanyang University.  
Department of Obstetrics and Gynecology, College of Medicine, Sungkyunkwan University  
Samsung Cheil Hospital and Women's Healthcare Center, Seoul, Korea.\**

The prognosis of ovarian cancer remains poor, and there is a need to identify patients who are less likely to respond to treatment, in the hope that the identification of these patients with a poorer prognosis may allow the administration of more intensive or different treatment. But, most clinical and pathological factors were considered to lack satisfactory predictive power.

Recently, essential role of protease in tumor cell invasion and metastasis have been elucidated in tumor biology. Urokinase-type plasminogen activator (uPA) and its inhibitor, plasminogen activator inhibitor-1 (PAI-1), play a key role in tumor-associated proteolysis. Thus, the presence of both uPA and PAI-1 modulates the invasive and metastatic phenotype of cancer cells. Genetically, nm23 protein from chromosome 17q may act independently as a metastasis suppressor.

The purpose of this study was to determine the relative predictive power of some of those prognostic variables such as uPA, PAI-1 and nm23 protein in a selected group of patients of ovarian cancer.

Immunohistochemical staining was used to determine the overexpression of uPA, PAI-1 and nm23 protein. Specimens were rated positive and negative. Then, scored '1' in case of positive for uPA, PAI-1, and negative for nm23, and '0' in case of negative for uPA, PAI-1, and positive for nm23, respectively. The sum of scores were divided into three groups ( , , and

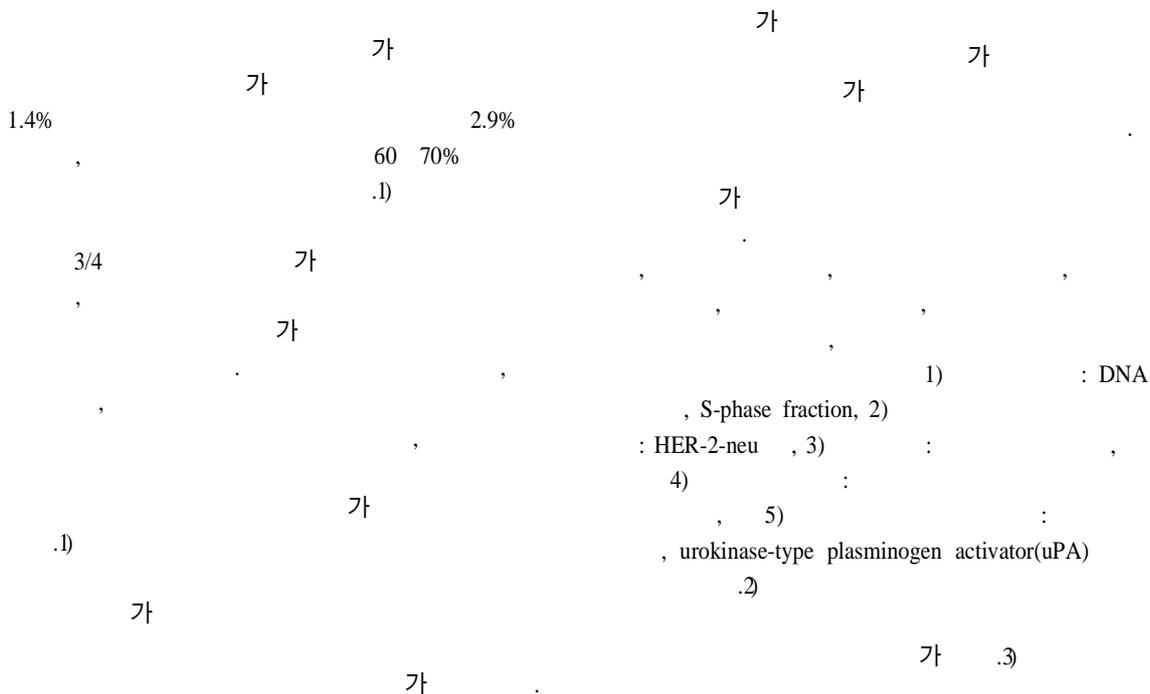
groups), and compared with clinico-pathologic parameters, clinical response, lymph node metastasis, recurrence and 5-year survival rate, retrospectively.

In univariate analysis, the positive rate of uPA was 36% (29/80), that of PAI-1 was 35% (28/80), and the negative rate of nm23 was 43% (34/80). The overexpression of uPA was higher in the low-grade tumor (p=0.0053), the overexpression of PAI-1 was positively correlated with the advanced stage of tumor (p=0.0001), more malignant histologic type (serous) of tumor (p=0.0013) and larger residual tumor mass (> 2 cm)(p=0.0480). The overexpression of nm23 protein was negatively correlated with advanced stage of tumor (p=0.0068) and low-grade tumor (p=0.011).

In scoring system, the number of patients with first group (I: score 0) was 24, group (score: 1 2) was 49, and group (score: 3) was 7. The mean age of patients was 46.4 years and mean follow-up time was 59 months. The rate of lymph node metastasis were 16.7%, 37%, and 75% respectively(p=0.0632). With increasing score in each group, the less clinical response rate was found (75% vs 71% vs 29%, p=0.0532). The 5-year survival rate of each group were 70% in I group, 65% in group, and 14% in group(p=0.0096).

In conclusion, the scoring system using immunohistochemical staining with rating of overexpression uPA, PAI-1 and nm23 protein may be useful as an important and powerful predictive prognostic indicator in patients with epithelial ovarian cancer.

**Keywords:** Urokinase-type plasminogen activator (uPA), Plasminogen activator inhibitor-1 (PAI-1), nm23 protein, Epithelial ovarian cancer



가 WHO 46.4 (20 79) ,  
 , 가 WHO 40 (50%), 26 (33%),  
 45) 6 (5%), 4 (5%) .  
 6) , FIGO  
 (fibrin) urokinase-type plasminogen ( ) 38 (48%) ( )  
 activator(uPA) 가 .7) , ( ) 42 (52%) .  
 (thrombin) 1 26 (33%), 2 3 21 (26%), 4가 12  
 uPA가 (15%) . 가 43  
 (54%), 2 cm 20 (25%), 2 cm 17  
 (21%) . cis-platinum  
 6  
 nm23 35 (44%)  
 Jacobs 8) 17 12 (34%) ,  
 allele 23 (66%) .  
 23 5 22%  
 가 .. 57 , 80 26  
 가 가  
 가 가  
 uPA, uPA,  
 plasminogen activator inhibitor-1  
 nm23  
 48가  
 uPA PAI-1, nm23  
 가 , ,  
 , .  
 1. 1985 1 1994 12  
 가 , 80  
 (1)

(Table 1).

Table 1. Profiles of Epithelial Ovarian Cancer(n=80)

Age	46.4(20 79)	Stage	37
Histo-type Serous	40		1
Mucinous	26		32
Endometriod	6		10
Undifferentiated	4	Residual tumor	
MMMT	2	None	43
Clear cell	1	<2 cm	20
Unclassified	1	2 cm	17
Grade 1	26		
2	21		
3	21		
4	12		
Second-look laparotomy(n=36)			
Negative	22		
Positive	14		
Microscopic	8		
Gross	6		
Recurrence	6/22(27%)		
Death	26		
Mean follow-up(months)	57.1		

Urokinase-type Plasminogen Activator(uPA), Plasminogen Activator Inhibitor-1(PAI-1) nm23

Barbara, CA. USA) 20

Buffer saline  
Meyer's hematoxylin

1)

0.5 μm

L-lysine

(2)

xylene 10 2 7+

2) Rehydration and Blocking

100% ethanol 95%, 75%, 50%

( ), + ( ), ++ ( )

- ( ), + ( ), ++ ( )

25% 5 pH 7.4 Buffer

Saline 5

3) peroxidase

3% H<sub>2</sub>O<sub>2</sub> 1:4

( )

( )

7+ 20

endogenous peroxidase

, 5, PBS(Phosphate

Buffer Saline) 5

4)

Antisera 10%

(normal goat serum)

30

5)

2

Urokinase-type plasminogen activator, Plasminogen activator inhibitor Nm23 protein

(monoclonal antibody)

urokinase-type plasminogen activator(uPA) #3689 American Diagnostica Inc., Greenwich, Conn.(Human Urokinase Murine Mo Ab against β-chain(33 kD) fragment, affinity purified), plasminogen activator inhibitor-1 # 3785 American Diagnostica Inc., Greenwich, Conn.(Murine Mo Ab against PAI-1)

, nm23 NCL-nm23, Novocastra Lab.(Mouse Mo Ab against nm23/ NDPK-1 protein)

PBS 5 Link Antibody

5

6)

Buffer saline 5

Streptavidin-biotin(LSAB kit; DAKO, Santa

(3) (Scoring)

uPA, PAI-1 nm23

7+

uPA, PAI-1

(-, +)

0

(+)

Fig. 1. Positive uPA immunostaining of serous cystadenocarcinoma (×114).

Fig. 2. Positive PAI-1 immunostaining of mucinous cystadenocarcinoma (×114).

Fig. 3. Positive PAI-1 immunostaining of endometrioid adenocarcinoma (× 114).

1. UPA, PAI-1, nm23

80 uPA 36%(29/80)  
 , PAI-1 35%(28/80) , nm23  
 43%(34/80) (Table 2).

Table 2. Overexpression of uPA, PAI-1, and nm23 in Epithelial Ovarian Cancer(n=80)

uPA		
uPA(-)	51	64%
uPA(+)	29	36%
PAI-1		
PAI-1(-)	52	65%
PAI-1(+)	28	35%
nm23		
PAI(-)	34	43%
PAI(+)	46	57%

Fig. 4. nm23 protein was diffusely stained in endometrioid adenocarcinoma cells(× 114).

2. uPA

uPA  
 (univariate analysis) uPA

+, +++ 1  
 nm23 (-, +) 1 ,  
 (++, +++) 0  
 가  
 가 0  
 , 가 1 2 , 가 3  
 가 uPA(+), PAI-1(-), nm23(+)  
 1  
 (4)  
 , ( , , )  
 , (CA-125),  
 5  
 (5)  
 Student-t-test Fisher exact chi test  
 SAS

· , ,  
 ,  
 · ,  
 , 5  
 (Table 3).

Table 3. Overexpression uPA and Clinical Parameters

Parameters	uPA(-) (n=51)	uPA(+) (n=29)	p-value
Age	44	49	NS
Stage	and	AND	p=0.2468
Predominant			
Histologic type	Mucinous	Serous	p=0.2816
Grade	1 and 2	3 and 4	p=0.0053
Residual tumor	< 2 cm	> 2 cm	p=0.2460
CA 125	-	+	NS
LN metastasis	-	+	p=0.7388
Clinical response	CR	P	p=0.5481
Second look findings	-	+	p=0.4672
Recurrence	-	+	p=0.3089
Median survival(M)	38	30	p=0.1423
5-year survival	69%	50%	p=0.1319

3. PAI-1

PAI-1

PAI-1

가

가

가

, 5

nm23

(Table 5).

PAI-1

, 5

(Table 4).

Table 4. Overexpression PAI-1 and Clinical Parameters

Parameters	PAI-1( - ) (n=52)	PAI-1( + ) (n=28)	p-value
Age	43	52	NS
Stage	and	AND	p=0.0001
Predominant			
Histologic type	Mucinous	Serous	p=0.0013
Grade	1 and 2	3 and 4	p=0.1526
Residual tumor	< 2 cm	> 2 cm	p=0.0480
CA 125	-	+	NS
LN metastasis	-	+	p=0.0933
Clinical response	CR	P	p=0.1155
Second look findings	-	+	p=0.7956
Recurrence	-	+	NS
Median survival(M)	38	30	p=0.0746
5-year survival	69%	47%	p=0.0779

4. nm23

nm23

nm23

Table 5. Overexpression nm23 and Clinical Parameters

Parameters	nm23( + ) (n=46)	nm23( - ) (n=34)	p-value
Age	48.3	49.9	NS
Stage	and	AND	p=0.0068
Predominant			
Histologic type	Mucinous	Serous	NS
Grade	1 and 2	3 and 4	p=0.0110
Residual tumor	< 2 cm	> 2 cm	NS
CA 125	-	+	NS
LN metastasis	-	+	p=0.0642
Clinical response	CR	P	p=0.6205
Second look findings	-	+	NS
Recurrence	-	+	NS
Median survival(M)	37.6	32.7	NS
5-year survival	72%	58%	p=0.2266

5. uPA, PAI- 1

nm23

(

, )

1)

80

24 ,

49 ,

7

2)

(Table 6)

Table 6. Scoring System and Clinico-pathological Parameters

	0(n=24)	1 2(n=49)	3(n=7)	p value
Age(Mean: years)	42	47.5	57.2	p=NS
Stage and	16(67)	22(45)	0(0)	p=0.0051
and	8(33)	27(55)	7(100)	
Histologic type				p=0.0388
Serous	10(42)	25(51)	5(100)	
Mucinous	13(54)	13(27)	0(0)	
Endometrioid	0	5	1	
Others	1	6	1	
Grade 1 and 2	20(83)	27(55)	0(0)	p=0.0001
3 and 4	4(17)	22(45)	7(100)	
Residual tumor				p=0.3990
< 2 cm	22(92)	37(76)	4(47)	
2 cm	2(8)	12(24)	3(53)	

( ): %

(1) 42 , 47.5 ,  
57.2 가 .  
(2) (0 )  
16 (67%), 8 (33%)  
(3 ) 7 (100%) 가  
가 ( , ) 가  
( , ) ,  
가 (p=0.0051).  
(3) 가  
가  
가  
가  
(p=0.038).  
(4) 가 1 2,  
가 3 4  
20 (83%), 3 4가 4 (17%) ,  
7 (100%) 3, 4  
가 (3 )  
가 , 가 (0 )  
가 (p=0.0001).  
(5) 2 cm  
, 2 cm  
2 (8%), 12 (24%), 3 (53%)  
(p=0.3990).  
3) CA-125  
CA-125가 35 U/ml  
, 71%,  
79%, 86%  
CA-125  
173.1, 209, 289.3 가

(Table 7).

4) 80  
43 15  
35% .  
12.5%(2/12), 32%(10/27),  
75%(3/4) , 가

(p=0.0632)

(Table 8).

Table 7. Scoring System and Serum CA 125 Antigen

	0(n=21)	1 2(n=47)	3(n=7)
CA 125			
35 U/ml	15(71%)	37(79%)	6(86%)
<35 U/ml	6(29%)	12(21%)	1(14%)
Median(U/ml)	173.1	209.0	289.3
p=0.7183			

Table 8. Scoring System and Retroper

	0(n=12)	1 2(n=27)	3(n=4)
LN metastasis			
LN(+)	2(25%)	10(32%)	3(75%)
LN(-)	10	17	1

Total LN(+): 15/43=35%

p=0.0632

5) 가  
67%(16/24), 71%(35/49),  
29%(2/7) 가  
, 5 (21%),  
10 (21%), 3 (43%) 가  
가 가 (p=0.0532)

(Table 9).

Table 9. Scoring System and Clinical Response

Response	0(n=24)	1 2(n=49)	3(n=7)
CR	16(67%)	35(71%)	2(29%)
PR	2(8%)	9(0%)	0(0%)
No change	1(4%)	4(8%)	2(29%)
PD	5(21%)	10(21%)	3(43%)

p=0.0532

6) 80  
36 .  
50%(5/10), 67%(16/24), 50%  
(1/2)  
가 . 27%(6/22)

Urokinase- type Plasminogen Activator(uPA), Plasminogen Activator  
Inhibitor- 1(PAI- 1) nm23  
40%(2/5), 19%(3/16), 100%(1/1)  
가 (Table 10).

Table 10. Scoring System and the results of 2nd-look laparotomy(n=36)

2nd-look op.	0(n=10)	1 2(n=24)	3(n=2)
Negative	5(50%)	16(67%)	1(50%)
Positive	5(50%)*	8(33%)	1(50%)*
Microscopic( + )	2(20%)	5(20%)	1(100%)
Gross( + )	3(30%)	3(13%)	0(0%)
Recurrence	2/5(40%)*	3/16(19%)*	1/1(100%)*

\*p=0.7314; Recurrence rate: 6/22(27%)

7) 5  
5 70%, 65%,  
14% 가 5 가  
(p=0.0096)(Fig. 5).

Fig. 5. Scoring System and 5-Year Survival.

(tumor stroma: extracellular matrix)  
(fibrin), fibronectin,  
proteoglycan, laminin, (collagen)

(protease)  
(uPA, ca-  
thepsin, collagenase )  
.12-14)  
uPA  
Dano 12)  
uPA가  
1976 Astedt  
Holmberg9)  
uPA가  
.10,11)15-17)  
uPA  
16) .13-15)  
uPA  
(proenzyme) pro-uPA가  
uPA  
.18) , pro-uPA plasmin,  
kallikrein 19) cathepsin B20)  
uPA uPA  
plasminogen plasmin , plasmin  
uPA plasmin  
.19) uPA plasminogen activator  
inhibitor type-1(PAI-1)  
plasmin  
2-macroglobulin, 2-antiplasmin  
plasmin  
Plasmin  
, fibronectin, proteoglycan,  
laminin procolla-  
genase type collagenase type  
.21) Collagenase type  
collagen type

가 ,  
 (neo-vascularization) 가 ,  
 uPA (uPA-R) (domain) 가  
 18 32  
 (amino-terminal fragment; ATF) ,  
 (Growth Factor-like domain; GFD)  
 ATF -chain ,  
 uPA-R ATF GFD 가 uPA  
 pro-uPA  
 uPA-R .23)  
 uPA가  
 1994 Kuhn  
 .24) uPA가 (p=0.03),  
 (p=0.004) ,  
 3, 4  
 (p=0.059) .  
 (p=0.7388) (p=0.2460) ,  
 (p=0.0053).  
 가  
 2528)  
 uPA  
 uPA  
 uPA  
 , uPA 가 가  
 가  
 , Duffy 25)  
 (multivariate analysis) uPA가  
 가  
 PAI-1 , Kuhn 24)  
 , (p=0.0480)  
 (p=0.1526) 가 .  
 가 가  
 가 .7) 가  
 가 pro-coagulant initiating pathway  
 uPA initiated pathway  
 Gastl 29) 가 가  
 가

가 ,  
 (neo-vascularization) 가 ,  
 30)  
 가 가  
 IL-6 . IL-6가 ,  
 가 가  
 가 가  
 17  
 (17q) (tumor suppressor gene)가  
 nm23 .31) 17  
 (early onset  
 breast cancer) 가 (fa-  
 miliary breast and ovarian cancer syndrome) 32)  
 17q12-q23  
 . 337 17q allele  
 .34)  
 가  
 .35) nm23 가  
 .3436)  
 nm23 가  
 nm23 human ery-  
 throcyte nucleoside disphosphate (NDP) kinase  
 . nm23 nm23-  
 H1 nm23-H2  
 NDP kinase A B subunit .3637) NDP  
 kinase  
 (microtubule assembly), (signal  
 transduction), (transcription regulation),  
 (cellular adhesion)  
 .3740)  
 Alessandra 39) nm23-H1 allele  
 가  
 , nm23-H1  
 가 ,  
 .39)  
 nm23 가  
 ,  
 (p=0.0068), (p=0.0642)

uPA, PAI-1, nm23

5

가 , 가

가  
가

uPA, PAI-1 nm23 가

uPA, PAI-1, nm23

(0 ), (1-2 ), (3 )

가  
가

5  
가

(tumor-associated protease)

,4) uPA PAI-1 가

5

uPA Cathepsin D가

가

uPA, PAI-1, nm23

cathepsin D, B, D-dimer, pro-  
coagulant, 가

가가 (protease inhi-  
biting activity) 가 tranexamic acid, apro-  
tinin, warfarin, heparin  
4)

80

uPA, PAI-1, nm23

1. uPA uPA 35%(29/80) 가

2. PAI-1 PAI-1 35%(28/80) 가

3. nm23 nm23 43%(34/80)

4. uPA, PAI-1

nm23 1

(0 ), (1-2 ), (3 )

가

(p=0.0051), (p=0.038), (p=0)

5. 가 (p=0.0632), (p=0.0532)

6.

7. 5 70%, 65%, 14% 가 5 가 (p=0.0096).

uPA, PAI-1, nm23

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