p53, bcl- 2   bax

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.   .

=Abstract=

Immunohistochemical Study for Expression of p53, bcl- 2 and Bax in Uterine Sarcoma
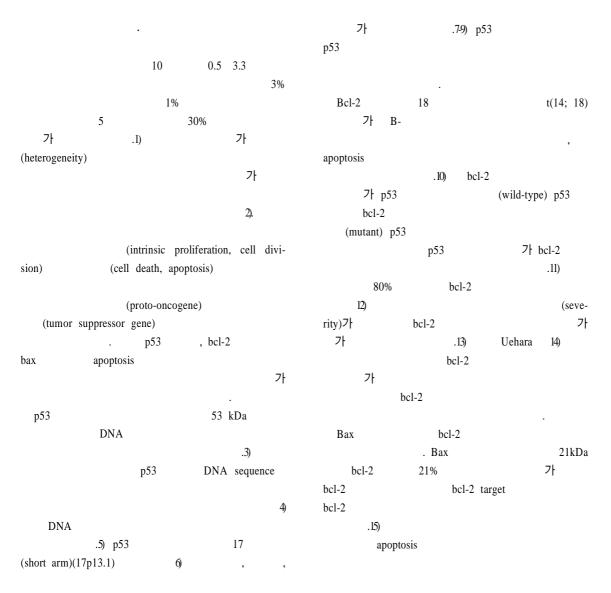
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Uterine sarcomas are rare tumors with unpredictable prognosis, comprising about 3% of uterine cancers. Little is known of epidemiologic risk factors and similarly, little work has been performed assessing molecular alterations in sarcomas. Proteins encoded by p53, bcl-2 and bax genes are important regulators of programed cell death, hence apoptosis. Alterations in the expression of these apoptosis-related genes can contribute to the development of most of human cancers, as well as possibly influence the prognosis of the cancer patients. Using antibodies specific for the p53, bcl-2 and bax proteins in combination with immunohistochemical methods, we examined for the first time the expression of these genes in 19 cases of uterine sarcoma, managed at Asan Medical Center between June, 1989 and December, 1996, including 13 leiomyosarcomas, 4 endodermal stromal sarcomas and 2 malignant mixed mullerian tumors. Twelve patients had stage I disease and 7 stage   , and 9 patients had tumors with mitoses less than 10 per 10 HPF, and the others had those with mitoses equal to or more than 10 per 10 HPF. The results were evaluated by semiquantitative analysis as non-(0%), low(1 25%), moderate(26 75%) and high expressors(76%), and the latter two were defined as tumors with overexpression. The immunoreactivity of bcl-2 and bax appeared in the cytoplasm, while that of p53 was localized solely in the nuclei. p53 immunostaining revealed 4 non-expressors, 7 low, 3 moderate and 5 high expressors, showing 42.1% rate of overexpression.

Immunostaining of bcl-2 showed 13 non-expressors, 2 low, 1 moderate and 3 high expressors, resulting 21.1% rate of overexpression and that of bax showed 1 non-expressors, 4 low, 6 moderate and 8 high expressors, resulting 73.7% rate of overexpression. We could not find any significant correlation among the degrees of the expressions of these three proteins. The overexpression of these three proteins did not show any significant association with stage of disease or mitotic count of tumor. In conclusion, although apoptosis-related factors such as p53, bcl-2 and bax are strongly suggested to play a certain role in tumorigenesis of uterine sarcoma, the correlation among them and prognostic implications need further investigation.

Keywords : Uterine sarcoma, Immunohistochemical staining, p53, bcl-2, bax

.   .7,9) p53

p53

10   0.5   3.3

3%   .

1%   Bcl-2   18   t(14; 18)

5   30%   B-

.1)   ,

(heterogeneity)   apoptosis

.10)   bcl-2

p53   (wild-type) p53

2).   bcl-2

(mutant) p53

(intrinsic proliferation, cell divi-   p53   bcl-2

sion)   (cell death, apoptosis)   .11)

80%   bcl-2

(proto-oncogene)   12)   (seve-

(tumor suppressor gene)   rity)   bcl-2

.   p53   , bcl-2   .13)   Uehara   14)

bax   apoptosis   bcl-2

.   bcl-2

p53   53 kDa   .

DNA   Bax   bcl-2

.3)   . Bax   21kDa

p53   DNA sequence   bcl-2   21%

bcl-2   bcl-2 target

4)   bcl-2

DNA   .15)

.5) p53   17   apoptosis

(short arm)(17p13.1)   6)   ,   ,

p53, bcl-2   bax

.

p53, bcl-2
bax

,

p53, bcl-2   bax

.

.

1.
1989   6   1996   12

(paraffin embedded block)
19   .

FIGO   .

2.
ABC
4㎛
poly-L-lysine
3%
5   3   PBS   .
peroxidase   0.3%
hydrogen peroxide methanol   30
PBS   . p53
1: 200   p53
(polyclonal rabbit anti-human p53 CM-1
antibody, Dako corporation, CA, USA) , bcl-2
1: 40   bcl-2   (monoclonal
anti-bcl-2 antibody(clone 124), Dako, Glostrup, De-
nmark) , bax   1: 1000   bax
(N-19 rabbit polyclonal antibody, Santa
Cruz Biotechnology, Santa Cruz, CA, USA)
4   .
PBS   (avidin-biotinylated

peroxidase complex)   30   .   0.2
mg/ml 3,3'-diaminobenzidine(DAKO, USA)   0.003%
hydrogen peroxide   Tris-HCL buffer
Mayer   hematoxylin   .

3.
2
. 400
5   (semiquantitative
analysis)
. , 25%
1 , 25%   75%   2 ,
75%   3
.   1   , 2
, 3   .

1
.   bcl-2
bax   (follicular hyper-
plasia)   p53
p53
.

4.

(2   or 3)
p53, bcl-2   bax
,   (FIGO stage)
(mitotic   count)
Fisher's two tailed exact test
0.05   .

.

1.
19   46.9 ± 13.1
(mean ± S.D.)   22   71   .
FIGO   12 (63.2%),   7 (36.8
%)   .
(leiomyosarcoma)   13
(68.4%)
(endometrial stromal sarcoma)   4 (21.1%),
(malignant mullerian tumor)   2

p53, bcl- 2 bax

(10.5%) . 10 high
power field (HPF) 10 11 (57.9%)
11 8 (42.1%) .

Table 1 .

Table 1. Clinical characteristics of patients with uterine sarcoma

Characteristics	No. of patients(%)
No. of patients studied	19
Age(mean ± S.D.)	46.9 ± 13.1
(range)	22 71
FIGO stage	
	12(63.2%)
	0(0%)
	7(36.8%)
	0(0%)
Histologic Type	
Leiomyosarcoma	13(68.4%)
Endometrial stromal sarcoma	4(21.1%)
Malignant mixed mullerian tumor	2(10.5%)
Mitotic count	
10/10HPF	11(57.9%)
10/10HPF	8(42.1%)

2. p53, bcl- 2 bax

p53 19 4
(21.1%), 7 (36.8%), 3 (15.8%)
5 (26.3%) 42.1%
. Bcl-2 13 (68.4%),
2 (10.5%), 1 (5.3%) 3
(15.8%) 21.1% bax
1 (5.3%), 4
(21.1%), 6 (31.6%) 8 (42.1
%) 73.7% .
(Table 2) (Fig. 1)

3. p53, bcl- 2 bax

p53 8 bcl-2
(0%) p53

11 7 bcl-2 (63.6%) 4
(36.4%)
(p 0.103). p53
8 7 bax
(87.5%) p53 11 7
bax (63.6%)
(p 0.263).
bcl-2 4 3 bax
(75.0%) bcl-2
15 11 bax
(73.3%) (p 1.000)(Table 3).

4.

13 p53
2 , 6
5 (38.5%) . Bcl-2
9 , 1 , 3 (23.1%)
bax 1 , 3
9 (69.2%) .
4 p53
2 , 1 1 (25.0%)
bcl-2 2 , 1
1 (25.0%) bax
1 , 3 (75.0%) .
p53 bax
2 (100%) bcl-2
2 (0%).

.

5. FIGO

I 12 p53
3 , 4 , 5 (41.7%)
bcl-2 7 , 2 ,
3 (25.0%) bax 1
, 3 , 8 (66.7%) .
7 p53 1
, 3 , 3 (42.9%) bcl-2
6 , 1
(14.3%) bax

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Table 2. Results of immunohistochemical staining of p53, bcl-2 and bax protein in patients with uterine sarcoma

Case No.	Age	Para	Stage	Histology	Mitotic count	Intensity of staining			F/U duration(Mo.)	Patient status
						p53	bcl-2	bax		
1	45	1		LMS	3	2	0	3	97	alive
2	49	3		LMS	4	1	0	1	81	alive
3	42	3		LMS	4	3	0	2	24	expire
4	46	2		LMS	4	0	0	2	33	expire
5	50	2		LMS	1	0	0	0	64	alive
6	70	3		LMS	1	2	0	2	3	loss
7	71	6		LMS	1	3	1	2	1	loss
8	34	2		ESS	1	0	0	1	54	alive
9	50			MMMT	4	3	0	3	1	expire
10	58	4		LMS	3	1	2	3	48	expire
11	37	2		LMS	1	1	3	1	24	alive
12	36	2		LMS	1	1	3	3	21	alive
13	27			MMMT	1	2	0	3	29	alive
14	43	1		LMS	1	1	0	3	12	loss
15	22	0		ESS	1	1	3	2	20	alive
16	44	2		LMS	2	3	0	1	18	alive
17	47	3		ESS	1	0	1	3	10	alive
18	64	4		ESS	4	3	0	3	10	alive
19	56	3		LMS	1	1	0	2	8	alive

*Histology: LMS; leiomyosarcoma, ESS; endometrial stromal sarcoma, MMMT; malignant mixed mullerian tumor
*Mitotic count: 0; none, 1; 1-10/10HPF, 2; 11-20/10HPF, 3; 21-30/10HPF, 4; 31-40/10HPF
*Intensiity of staining: 0; negative, 1; 25%, 2; 25-75%, 3; 75%

1 , 6 (85.7%) . .

p53 (41.7% vs 42.9%) bcl-2
(25.0% vs 14.3%) bax
(66.7% vs 85.7%)
(p 0.106, 1.000, 0.603) (Table 4).

6.

10 high power field
(HPF) 10 11 p53
3 , 5 , 3
(27.3%) bcl-2 6 , 2
, 3 (27.3%) bax
1 , 2 , 8 (72.7%)

10 high power
field (HPF) 11 8 p53
1 , 2 , 5
(62.5%) bcl-2 7 ,
1 (12.5%) bax
2 , 6
(75.0%) . p53
11/10HPF
(27.3% vs 62.5%) bcl-2
(27.3% vs 12.5%)
(p 0.181, 0.603) bax
(72.7% vs 75.0%) (p 1.000) (Table 5).

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Fig. 1. Staining of case 16. (A) This uterine leiomyosarcoma shows hypercellularity and occasionally nuclear pleomorphism with mitotic activity (H&E, × 200). (B) Diffuse nuclear immunoreactivity of p53 is noted in the leiomyosarcoma, grade 3+ (× 400). (C) Compared to infiltrated lymphocytes, no immunoreactivity to the cytoplasm of tumor cells is noted in bcl- 2 immunostaining (× 400). (D) A few tumor cells show positive reactivity for bax protein, grade 1+ (× 400).

Table 3. Correlation among overexpressions of p53, bcl- 2 and bax protein in uterine sarcoma

	bcl-2( )	bcl-2( )	p value*
p53( ) p53( )	7(63.65) 8(100%)	4(36.4%) 9(0%)	0.103
	bax( )	bax( )	p value*
p53( ) p53( )	4(36.4%) 1(12.5%)	7(63.6%) 7(87.5%)	0.263
	bax( )	bax( )	p value*
bcl-2( ) bcl-2( )	4(26.7%) 1(25.0%)	11(73.3%) 3(75.0%)	1.000

*Fisher's two-tailed exact test

Table 4. Association of overexpressions of p53, bcl- 2 and bax protein with FIGO stage in uterine sarcoma

	FIGO stage		p value*
p53( ) p53( )	7(58.3%) 5(41.7%)	4(57.1%) 3(42.9%)	0.106
bcl-2( ) bcl-2( )	9(75.0%) 3(25.0%)	6(85.7%) 1(14.3%)	1.000
bax( ) bax( )	4(33.3%) 8(66.7%)	1(14.3%) 6(85.7%)	0.603

*Fisher's two-tailed exact test

Table 5. Association of overexpressions of p53, bcl-2 and bax protein with mitotic count in uterine sarcoma

	mitotic count		P value*
	10/10HPF	10/10HPF	
p53( )	8(72.7%)	3(37.5%)	0.181
p53( )	3(27.3%)	5(62.5%)	
bcl-2( )	8(72.7%)	7(87.5%)	0.603
bcl-2( )	3(27.3%)	1(12.5%)	
bax( )	3(27.3%)	2(25.0%)	1.000
bax( )	8(72.7%)	6(75.0%)	

*Fisher's two-tailed exact test

7.

19   3   p53
        1   2   bcl-2
                                          bax
    2 ,   1   .
        4   p53   ,
            1   2   . bcl-2
                3 ,   1   bax
    4
                                                  4

                .

                .

        (soft tissue)   (leiomyosarcoma)
        p53

        .16-18)   Soini 16) 36
                        25
p53

                p53
                    .   p53

3   Bur 19) 7
1
    Liu 20)   (mixed mesodermal
tumor) 41 ,   4
    1   46
27 (59%)   Jeffers 21) 23
                    , 10
    18   (mi-
crowave antigen retrieval)   p53
        ,
                    ,   56.5%(13/23), 60.0%(6/10)   5.6
%(1/18)
                                      .
1%   78.9%   25%

        42.1%(8/19)
    Liu 20)   Jeffers 21)
                .   Liu 20)   , Jeffers
    21)
                                          ,   Liu
    20)
                                              Jeffers
    21)   ,
    (microscopic field)
                    "focal" ,
                                "diffuse"

                                          25%
                                              .

                                                  Liu 20)
        25%(1/4), Jeffers 21)   34.8
%(8/23)   38.5%(5/13)

                                  .

        p53   p53
                        . Liu 20)
46   33   SSCP(single

strand conformational polymorphism) DNA p53 (viru-

p53 lence)

17 p53 .

16 4 apoptosis

81.0%, 75.0% p53 25) p53

. Jeffers 21)

56.5%(13/23) SSCP

1 (7.7%, 1/13)

10 2 p53

26)

p53 p53 52 31

p53 (57%) p53

.

27). ,

, p53

. p53

p53 28,29)

.

, p53 bcl-2 p53

p53

p53 (wild-type) p53 bcl-2

(mutant) p53

. p53

Jeffers 21) p53 bcl-2

11). bcl-2

p53

30).

FIGO 41.7% Bcl-2 p53

42.9% S

10/10HPF p53 .

(27.3% vs 62.5%) bcl-2 G1

(p 0.181) p53

p53 . Bcl-2

. p53

(adenocarcinoma) bcl-2

p53 10 15% . Bcl-2 free

radical bcl-2

40 50% 22-24) .

bcl-2

40% p53

.　bcl-2

DNA　　　　　　　　　　　　　　　　　　　　.　　　　　　　(endometrioid)

32)　　　.　　　　　　　　bcl-2

bcl-2　　　　　p53　　　　　　　　　　　　　　　　　　　　　　　　　　　　　　　11) bcl-2

　　　　　　　　　　　　　　　　　　　　　　　　bcl-2

　　　　　　　　　p53　　　　　　　　　　　　　　　　　　　　　　　　　，　　　　，

, Liu　　20)　　　　　　　　　　　　　　, p53　　　　　　　　　　　　　　　　　33)

　　　8　　　　　bcl-2　　　　　　　　　　bcl-2

　　(mutant) p53　　　　bcl-2　　　　　　　　　　　　　　　　，

　　　　　　　p53　　　　　　　　　　　　　FIGO　　　　　bcl-2

bcl-2　　　　　　　　　　　　　　　　　　(25.0%　vs　14.3%)

　　　　　　bcl-2　　　　　　　4

　　p53　　　　　　　　bcl-2　　　(27.3%　vs　12.5%) bcl-2

　　　　　p53

Eliopoulos　30)　　　　　　　　　　　(　　p　1.000,　0.603).

.　　bcl-2

15　　　p53　　　　　　　7　　bcl-2

(46.7%)

　　　　　　　　　　　　　　.

p53　　　bcl-2　　　　　　　　　　　　　　　　　.

Bcl-2　　　platinum　　　　　　　bax

，　　　　　　　　　　　　　bax

　　　　　　bcl-2

.　　　　　　　.　　　bax　　bcl-2

21%　　　　　　　　　　　　　　　bcl-2

(severity)　　bcl-2　　　　　　　bcl-2　target　　　bcl-2

apoptosis　　　　　　　(Oltvai　et　al:　1993).　　bcl-2

apo-　bax

ptosis　　　　　　　　　　　.　　p53　　　　bax

(dysplastic)　　　　(transcription)

bcl-2　p53

.13)　　　　　.　　　　apoptosis

Uehara　14)　　　　　bcl-2　　　　　bcl-2　　　bcl-2

bax　　，　bcl-2

bcl-2　　　　(ratio)

(Oltavi　et　al:　1993).

Henriksen　31)　　bcl-2　bax

bcl-2

bcl-2　　　　　4　　3　bax

(75.0%) bcl-2　　　　　15

bcl-2　　　11　　bax　　(73.3%)

-       p53, bcl- 2   bax   -

(p   1.000)   ,

          .   bax   .

FIGO   1.   13   ,

(66.7%   vs   85.7%)   4   ,

     (p   0.603)   2   19   . FIGO

   bax   12   ,   7

    (72.7%   vs   75.0%) (p   1.000) bax   .   10

               Gazza-   high   power   field(HPF)   10   11   ,

niga   34)   bax   11   8   .

    bcl-2   2.

                 p53   38.5%(5/13),

              Brmbilla   35)   bcl-2   23.1%(3/13), bax   69.2%(9/13)

    bcl-2/bax   1   ,   bcl-2   .   p53   bcl-2

                 25%(1/4)   , bax   75%(3/4)

            .   p53   bax   100%(2/2)

          19   bcl-2   .

                        .

      p53,   bcl-2   bax   3.

       p53   41.7%(5/12),   42.9

                 %(3/7)   . bcl-2   25%

                 (3/12),   14.3%(1/7)   . bax

           .   66.7%(8/12),   85.7%(6/7)

                      .

                 4.

          p53   10 HPF

               10   27.3%(3/11),   11

                    62.5%(5/8)   .

          bcl-2   27.3%(3/11),   12.5%(1/8)

                 bax   72.7%(8/11),   75%(6/8)

          .   .

     1989   6   1996   12   5.   p53,   bcl-2   bax

             p53   8

             bcl-2   bax   87.5%

(paraffin-embedded   block)   (7/8)

      19   .

      p53,   bcl-2   bax   6.

19

p53, bcl-2

bax

.

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