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Analysis of *nm23* Expression as a Prognostic Parameter in Renal Cell Carcinoma

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To identify the clinicopathological events including nm23 expression that underlies progression in renal cell carcinoma, a retrospective analysis of patients with renal cell carcinoma was performed. Ninety-eight cases of radical nephrectomies with extensive regional or para-aortic lymph node dissection were assessed for clinicopathological variables, and eighty-five cases underwent nm23/NDPK-A protein immunohistochemical staining. Significant parameters in survival were tumor size, histologic pattern, Fuhrman's nuclear grade, pathologic T(pT) stage, pathologic N stage, M stage, tumor thrombi, location of metastasis, and nm23 staining intensity. To assess the relationship with survival, the tumors with low and high nm23 expressions were compared. The fifty-nine patients with a high staining intensity had a significantly worse survival than did the twenty-six with a low staining intensity (p=0.0015). Additionally nm23 staining intensity was correlated with tumor size, Fuhrman's nuclear grade, pT, and distant metastasis. Therefore, the immunostaining intensity of nm23 protein could be used as a prognostic parameter with an inverse correlation.

Key Words : *Renal cell carcinoma, nm23, Immunohistochemistry, Prognostic parameter*

INTRODUCTION

The prognosis of renal cell carcinoma is influenced by nuclear differentiation, cell type, histologic pattern, tumor size, status of nodal involvement, and tumor stage, although the overwhelming determining factor in survival is surgical removal (Skinner et al., 1971 ; Fuhrman et al., 1982 ; Selli et al ., 1983 ; Golimbu et al., 1986 ; Medeiros et al., 1988 ; Giuliani et al., 1990). A major research goal is to identify the clinicopathological events including *nm23* expression that underlies progression in renal cell carcinoma.

nm23 was identified as a gene associated with tumor metastatic process in two types of murine melanoma cell lines which have different metastatic potentials (Steeg et al., 1988). In human breast carcinomas, low *nm23* RNA or protein expression levels were correlated with the presence of lymph node metastasis at surgery, and significantly reduced disease free and overall survival (Bevilacqua et al., 1989 ; Barnes et al., 1991 ; Hennessy et al., 1991).

On the contrary, *nm23*-H1 allelic deletion was observed in 20%(2/10) of metastatic renal carcinoma (Leone et al., 1991). Also, the level of *nm23* mRNA expression was increased in metastatic renal cancer cells (Radinsky et al., 1992).

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Although the RNA data may indicate the prognostic significance of *nm23* expression, its clinical usefulness would probably be precluded by technical requirements. We therefore attempted to evaluate *nm23* protein content in renal cell carcinoma tissues, determined by immunoperoxidase staining using anti-*nm23*/nucleoside diphosphate kinase A (NDPK-A) antibody as a prognostic parameter in renal cell carcinoma.

MATERIALS AND METHODS

Between 1985 and 1993, 181 patients underwent radical nephrectomy for renal cell carcinoma at Severance Hospital, Seoul, Korea. Of the 181 patients 98 fulfilled the following criteria; extensive regional or paraaortic lymph node dissection, no adjuvant chemotherapy or irradiation, and more than 6 months postoperative followup. There were 68 men and 30 women (ratio 2.27 : 1, mean age 53.1 years, range 27 to 77 years). Possible clinicopathological parameters were established from medical records including operative notes and pathological reports. Tumor slides were reviewed by a single pathologist in all cases. Followup periods ranged from 6 to 111 months (mean 37.87) and 3-year and 5-year followup was available on 47 and 23 of 98 patients, respectively.

Thirteen of these 98 patients were excluded for *nm23*/NDPK-A protein immunohistochemical staining ; eleven cases were excluded due to poorly preserved paraffin blocks, and two cases due to preoperative angioinfarction. Sections four micrometer thick from paraffin embedded renal cell carcinomas were mounted on poly-L-lysine coated slides, air dried, and heated at 60℃ for 20 minutes. Endogenous peroxidase activity was quenched with 0.3% H_2O_2 in methanol for 30 minutes. *nm23* staining was performed with the standard ABC immunoperoxidase technique (Vectastain® ABC kit, Vector Lab., Inc., Burlingame, CA, USA). The sections were incubated for 30 minutes at 25℃ with antiserum of anti-*nm23*/NDPK-A mouse monoclonal antibody, clone 37.6 (NCL-*nm23*, Novocastra Lab. Ltd., UK) (working dilution=1 : 100). The reaction product was developed by incubation for 6 minutes in 0.1M Tris-HCl buffer (pH 7.2), containing 1 mg/ml diaminobenzidine (Sigma Chemical Co., St. Louis, Mo,. USA) and 0.02% H_2O_2. Sections were lightly counterstained in hematoxylin. Portions of normal renal parenchyme, specimens from two cases of chronic pyelonephritis, two cases of renal tuberculosis, and two cases of well differentiated breast carcinoma were used as controls.

Each section for *nm23* staining was examined under low power (×4) to identify regions containing low and high staining tumor cells. The proportion of low- and high- staining tumor cells in each selected field was determined by manual counting at high power (×100) using an eyepiece graticule. At least 100 tumor cells were counted from areas designated by the pathologist as being representative of assigned grade, and away from areas of hemorrhage, necrosis and artifactual staining, thus controlling these factors. Slides were examined by two scoring systems of intensity (0, unstained tumor : equivalent to background staining of acellular stroma; +1, weakly stained : light brown stain slightly darker than background; +2, moderately stained; +3, strongly positive : intense brown stained) and relative abundance (0=<5%, 1=5-25%, 2=26-50%, 3=51-75%, 4=76-100%). Abundance of staining less than 5% was regarded as unstained tumor.

The predictive ability of the prognostic variables including *nm23* staining intensity was determined using Cox's proportional hazards linear regression model and verified with log-rank test. Also, the relationships between *nm23* staining intensity were classified as two groups (low : 0 and +1; high : +2 and +3) and prognostic variables were analysed with t (two-tail) and chi-square tests. All analyses were performed using SAS software (SAS institute, Cary, NC, USA).

RESULTS

Significant parameters in survival were tumor size (<5cm vs ≥5cm : p=0.032), histologic pattern (tubular, solid, papillary, and sarcomatoid : p=0.0455), Fuhrman's nuclear grade (I-IV : p=0.0001), pathologic T stage (1-4 : p=0.0001), pathologic N stage (0-3 : p=0.0001), M stage (0,1 : p=0.001), tumor thrombi (none ; V0, renal vein ; V1, and IVC ; V2 : p=0.0042), location of metastasis (none, lung, brain, bone, and others : p=0.0001), and *nm23* staining intensity (0-+3 : p=0.0043) (Table 1).

Staining intensity was determined as the highest staining intensity minimum of five percent when there were mixtures of several different staining intensities. Staining intensity was predominantly cytoplasmic (Fig. 1) and was correlated to the nuclear grade of tumor

Table 1. Three and five year survival function according to the parameters.

	3 year-survival function	5 year-survival function
Fuhrman's nuclear grade 1/2/3/4	1.0/0.66/0.4/0.16	—/0.56/0.33/0.0
pT1/T2/T3/T4	1.0/0.78/0.32/0.0	—/0.74/0.17/0.0
pN0/N1/N2/N3	0.72/0.34/0.12/0.0	0.53/0.34/0.0/0.0
Tumor thrombi V0/V1/V2	0.73/0.54/0.14	0.48/—/0.14
M0/M1	0.79/0.06	—/0.06
Tumor size <5cm/≥5cm	0.8/0.54	0.76/0.22
Histologic pattern Tubular/Solid/Papillary/Sarcomatoid	0.92/0.62/0.72/0.12	0.76/0.5/—/0.0
Metastatic location None/Lung/Brain/Bone/Others	0.94/0.41/0.0/0.0/0.5	—/0.0/0.0/0.0/0.0
nm23 intensity 0/+1/+2/+3	0.93/—/0.7/0.38	0.93/—/0.7/—

—; unestimated survival function.

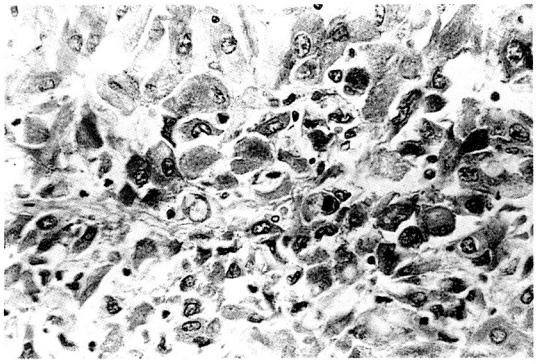

Fig. 1. Immunostaining for *nm23* protein showing positive reaction predominantly in cytoplasm of tumor cells (ABC method with DAB chromogen, ×400).

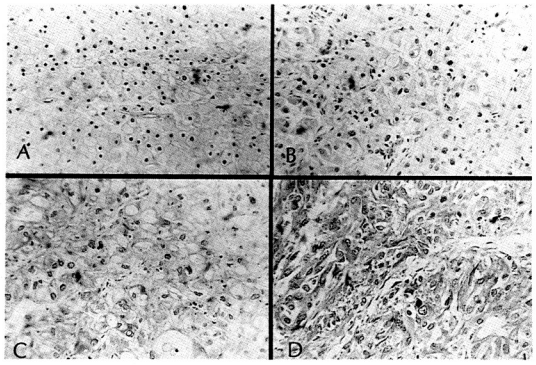

Fig. 2. Expression of *nm23* immunoreactivity in renal cell carcinoma. Tumor cells show positive reaction in various degrees for *nm23*. A, *nm23* immunostaining was negative in grade 1 renal cell carcinoma (×250). B, Focal weak positivity for *nm23* in grade 2 renal cell carcinoma (×250). C, Moderate degree of *nm23* immunostaining in grade 3 renal cell carcinoma (×250). D, Sarcomatoid renal cell carcinoma showed diffuse, intense positive reaction for *nm23* (×) (ABC method with DAB chromogen).

cell (Fig. 2).

The fifty-nine patients with high staining intensity (+2 and +3 : 21 and 38 patients each) exhibited significantly worse survival than did the twenty-six with low staining intensity (0 and +1 : 14 and 12 patients each) (p=0.0015) (Fig. 3).

The relative abundance of *nm23* staining was 1 in 37 patients, 2 in 42 patients, and 3 in 6 patients. A relative abundance of 4 was not seen in any patients.

Nonsignificant parameters in survival were laterality, location of tumor, cell type (clear, granular, or mixed), adrenal gland invasion, multiplicity of tumor (10/98 : 10.2%), and sex. Additionally *nm23* staining intensity was correlated with tumor size. The mean tumor size of low staining intensity group was 5.36 ± 2.53 cm and high staining intensity group, 7.65 ±3.52 cm, respectively. The difference in mean tumor size between the two staining intensity group was statistically significant (Table 2). And the differences in Fuhrman's nuclear grade (I-IV), pathologic T stage (1-4), and M

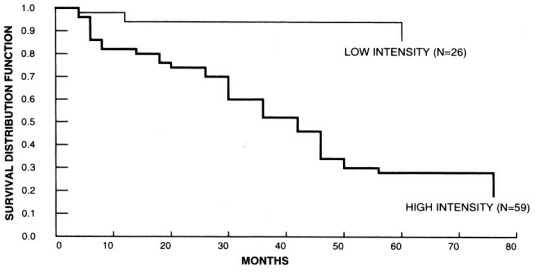

Fig. 3. Survival of patients with low and high *nm23* protein staining in 85 patients. The survival curve shows difference in survival between low intensity group and high intensity group(p = 0.0015).

Table 2. Correlation* between *nm23* staining intensity and tumor size.

Intensity	Number of cases	Mean (cm)	Standard Deviation	Standard Error	2-Tail probability
Low	26	5.3600	2.539	0.508	
					0.004
High	59	7.6467	3.527	0.455	

* t-test(2-tail)

stage (0 and 1) between the two staining intensity group were statistically significant (Table 3-5). Other clinicopathological parameters such as tumor laterality, location, histologic pattern, cell type, pN stage, tumor thrombi, adrenal gland invasion, and multiplicity of tumor were not correlated with *nm23* staining intensity.

nm23 stainings were negative in all control specimens ; portions of normal renal parenchyme, two cases of chronic pyelonephritis, and two cases of renal tuberculosis. Meanwhile, *nm23* stainings of breast carcinomas were positive which were predominantly cytoplasmic and perinuclear.

DISCUSSION

Surgical extirpation is the only effective means at present of curing renal cell carcinoma. Thus the anatomic extent and pathologic features of the tumor have been premier variables determining survival (Bassil et al., 1985 ; Golimbu et al., 1986 ; Giuliani et al., 1990). Similarly to other reports we have observed the significance of parameters such as pathologic stage and nuclear grade. According to our results, the patients with relatively small tumors less than 5cm in diameter, low pathologic T stage, tubular pattern, low nuclear grade, and no regional lymph node metastasis might have a good prognosis. Additionally *nm23* staining intensity for tumor tissue reflected the malignant degree of renal cell carcinoma and may be used as one of the prognostic parameters.

Two types of *nm23* gene have been identified, designated as *nm23*-H1 and *nm23*-H2. *nm23*-H1 is very similar to Dictyostelium nucleoside diphosphate (NDP) kinase (Wallet et al., 1990) and abnormal wing disc (*awd*) gene product (Biggs et al., 1990). Also it is identical to the NDP Kinase A in humans. Eighty-eight percent of *nm23*-H2 is identical to the *nm23*-H1 in amino acid sequence. After the initial report of *nm23* gene in melanoma cell lines with different metastatic potential had been described (Steeg et al., 1988), several investigations on *nm23* in other types of malignancies regarding its prognostic implications were carried out. For instance the patients with high *nm23* expressions in breast carcinoma exhibited better survival than patients with low expressions (Bevilaqua et al., 1989 ; Barnes et al., 1991 ; Hennessy et al., 1991). However in renal cell carcinoma, restriction fragment length polymorphism showing somatic allelic deletion of *nm23*-H1 was observed only in patients with metastasis (Leone et al., 1991). Meanwhile, *nm23* mRNA expression levels were increased in highly metastatic renal cell carcinoma cell line compared to nonmetastatic cell line. Therefore, in renal cell carcino-

Table 3. Correlation * between *nm23* staining intensity and Fuhrman's nuclear grade.

Grade \ Intensity	Low	High	Total
1	8	0	8
2	16	21	37
3	2	29	31
4	0	9	9
Total	26	59	85

*Chi-Square test : Pearson's Coefficient=0.00000

Table 4. Correlation * between *nm23* staining and pathologic T stage.

T stage \ Intensity	Low	High	Total
1	7	1	8
2	16	28	44
3	2	27	29
4	1	3	4
Total	26	59	85

* Chi-Square test : Pearson's Coefficient=0.00106

Table 5. Correlation* between *nm23* staining and M stage.

M stage \ Intensity	Low	High	Total
0	23	37	60
1	3	22	25
Total	26	59	85

*Chi-Square test : Pearson's Coefficient=0.00516

ma, *nm23* expression might lack antimetastatic function or reflect tumor proliferation, as seen in neuroblastoma (Hailat et al., 1991).

Our study demonstrated an inverse correlation between *nm23* expression and disease-free survival of the patients. In conclusion, it was not clear from these results whether the increased *nm23* expression in renal cell carcinoma suppresses metastasis consistently, or is associated with disease progression.

REFERENCES

Barnes R, Masood S, Barker E, Rosengard AM, Coggin DL, Crowell T, King CR, Porter-Jordan K, Wargotz ES, Liotta LA, Steeg PS. *Low nm23 protein expression in infiltrating ductal breast carcinomas correlates with reduced patient survival. Am J Pathol 1991; 139: 245-50.*

Bassil B, Dosoretz DE, Prout GR Jr. *Validation of the tumor, nodes and metastasis classification of renal cell carcinoma. J Urol 1985; 134: 450-4.*

Bevilacqua G, Sobel ME, Liotta LA, Steeg PS. *Association of low nm23 RNA levels in human primary infiltrating ductal breast carcinomas with lymp node involvement and other histopathological indicators of high metastatic potential. Cancer Res 1989; 49; 5185-90.*

Biggs J, Hersperger E, Steeg PS, Liotta LA, Shearn A. *A drosophila gene that is homologous to a mammalian gene associated with tumor metastasis codes for a nucleoside diphosphate kinase. Cell 1990; 63: 933-40.*

Fuhrman SA, Lasky LC, Limas C. *Prognostic significance of morphologic parameters in renal cell carcinoma. Am J Surg Pathol 1982; 6: 655-63.*

Giuliani L, Giberti C, Martorana G, Rovida S. *Radical extensive surgery for renal cell carcinoma: Long-term results and prognostic factors. J Urol 1990; 143: 468-74.*

Golimbu M, Tessler A, Joshi P, Al-Askari S, Sperber A, Morales P. *Renal cell carcinoma: survival and prognostic factors. Urology 1986; 27: 291-301.*

Hailat N, Keim DR, Melhem RF, Zhu X, Eckerskorn C, Brodeur GM, Reynolds CP, Seeger RC, Lottspeich F, Strahler JR, Hanash SM. *High level of p19/nm23 protein in neuroblastoma are associated with advanced stage disease and with N-myc gene amplification. J Clin Invest 1991; 88: 341-5.*

Hennessy C, Henry JA, May FEB, Westely BR, Angus B, Lennard TWJ. *Expression of the antimetastatic gene nm23 in human breast cancer: an association with good prognosis. J Natl Cancer Inst 1991; 83: 281-5.*

Leone A, McBride OW, Weston A, Wang MG, Anglard P, Cropp CS, Goepel JR, Lidereau R, Callahan R, Linehan M, Rees RC, Harris CC, Liotta LA, Steeg PC. *Somatic allelic deletion of nm23 in human cancer. Cancer Res 1991; 51: 2490-3.*

Medeiros LJ, Gelb AB, Weiss LM. *Renal cell carcinoma: prognostic significance of morphologic parameters in 121 cases. Cancer 1988; 61: 1639-51.*

Radinsky R, Weisberg HZ, Staroselsky AN, Fidler IJ. *Expression level of the nm23 gene in clonal populations of metastatic murine and human neoplasms. Cancer Res 1992; 52: 5808-14.*

Selli C, Hinshaw WM, Woodard BH, Paulson DF. *Stratification of risk factors in renal cell carcinoma. Cancer 1983; 52: 899-903.*

Skinner DG, Colvin RB, Vermillion CD, Pfister RC, Leadbetter WF. *Diagnosis and management of renal cell carcinoma: a clinical and pathologic study of 309 cases. Cancer 1971; 28: 1165-77.*

Steeg PS, Bevilacqua G, Kopper L, Thorgeirsson UP, Talmadge JE, Liotta LA, Sobel ME. *Evidence for a novel gene associated with low tumor metastatic potential. J Natl Cancer Inst 1988; 80: 200-4.*

Wallet V, Mutzel R, Troll H, Barzu O, Wurster B, Veron M, Lacombe ML. *Dictyostelium nucleoside diphosphate kinase highly homologous to nm23 and awd proteins involved in mammalian tumor metastasis and drosophila development. J Natl Cancer Inst 1990; 82: 1199-202.*