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Role of nm23 expression in nephroblastoma in determining prognosis and metastatic potential

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ABSTRACT

Nm23 gene family is associated with metastasis suppression and differentiation with known 4 types. Nm23 genes are expressed in different tumors where the levels are associated with reduced or increased metastatic potential. The aim of this study is to search for prognostic role and metastatic importance of nm23 protein expression in nephroblastomas. Monoclonal antibody specific for nm23 was applied immunohistochemically by LSAB method in formalin fixed paraffin embedded tissue sections of nephroblastoma in 30 children between 1-10 years of age in different stages of disease. Its prognostic role and relation with metastasis and other factors were searched. Seventeen of the cases were female (56.7%) while 13 were male (43.3%). The mean age was 4.3±2.4 (1-10). Thirteen cases were stage I (43.3%), one case was stage II (3.3%), 10 cases were stage III (33.3%), while 6 were stage IV (20%). Twenty-one cases were alive, while 9 cases were exitus. Mean survival time was 87.2±49.7 (8-154) months. Nm23 was positive in 20 cases (66.7%) and negative in 10 cases (33.3%). The mean survival time for nm23 negative cases was 50.90 months, while it was 105.35 months for positive cases. In Spearman correlation analysis, stage was positively related with nm23 status (p=0.0001) and Cox Regression analysis indicated nm23 protein expression as an independent factor for metastasis (p=0.010) and for prognosis (p=0.001). Log Rank Survival analysis showed a significant difference between nm23 positive and negative cases (p=0.019). Prognosis was better in nm23 positive cases. Our results revealed that negative nm23 protein expression might be a predictor for metastasis in nephroblastomas and it is an independent prognostic factor. It would worth studying the prognostic importance of nm23 in nephroblastoma and other tumors. [Turk J Cancer 2004;34(3):101-105]

KEY WORDS:

Nephroblastoma, nm23, prognosis

INTRODUCTION

Wilms tumor (nephroblastoma) is the most common malignant neoplasm of kidney in childhood. The patients enjoy a better prognosis and survival rates for favorable histology of Wilms tumor currently approach 90%. Histologically, the classic Wilms tumor has a triphasic appearance, being composed of variable admixtures of blastemal, epithelial and stromal elements (1).

Nm23 gene family is associated with metastasis suppression and differentiation. Nm23-1 and nm23-2 are well known to be putative metastasis suppressor genes. There are 4 types defined. Nm23-H1, nm23-H2, DR-nm23 and nm23-H4 genes encode nucleoside diphosphate (NDP) kinase (2).

These genes are expressed in different tumor types where their levels are alternatively associated with reduced

or increased metastatic progressive potential such as rectal cancer, nasopharyngeal carcinoma, serous ovarian carcinoma, thyroid carcinoma, lung cancer (8), breast cancer and retinoblastoma (3-10).

The prognostic role of nm23 protein expression and relationship with other prognostic factors such as age, sex, stage, metastasis, prognosis and survival have not been determined in nephroblastoma on tissue sections. The aim of this study is to examine the relationship of these parameters with immunohistochemical (IHC) expression of nm23 in our nephroblastoma series and to define new prognostic criteria for nephroblastomas determining metastatic potential.

MATERIALS AND METHODS

Nephroblastoma resection or tru-cut biopsy specimens from 30 cases diagnosed, and treated in Children's Education and Research Hospital by Oncology Study Group between 1991 and 1998 were included in this study. Patients had a mean of 96 months of follow-up. The cases were staged according to National Wilms Tumor Study Clinicopathologic Staging System (1). The staging was done clinically and confirmed by pathology. The patients were treated by one or more of surgery, chemotherapy (EPOC or NWTS) and radiotherapy according to individual features (11).

The histopathologic diagnosis was done by routine histopathologic slides. WT-1 staining was not necessary. Formalin-fixed and paraffin-embedded, well-preserved tissue blocks of tumors were used for IHC study. All tumor samples used for this study have been obtained at diagnosis prior to the treatment. IHC was performed by streptavidin-biotin peroxidase method. The 3μ sections were deparaffinised in xylene after keeping slides at 60°C overnight. Rehydration was done in decreasing alcohol concentrations. Endogenous peroxidase activity was blocked with hydrogen peroxide 3% for 15 minutes. The sections in citrate buffer (pH=6.0) were heated in microwave for 5 minutes 3 times. Nm23- NDP kinase Ab-1 (1/25 diluted, Neomarkers, USA) was applied as primary antibodies. It is a rabbit polyclonal antibody that recognizes the products of nm23-H1 and H2. Immunohistochemical expression of anti-nm23 polyclonal antibody recognises the product of cDNA. The epitope is aa 86- 102 and molecular weight of the antigen is 17kDa and 185kDa. Secondary antibodies (DAKO, Denmark) were applied and DAB (DAKO, Denmark) was used as chromogen. Invasive ductal carcinoma of breast was used as positive control and cytoplasmic staining was considered as positive. There was no nuclear staining. The nm23 expression was graded as negative and diffuse staining (Figure 1). Focal staining (staining lower than 1%) was considered as negative. The intensity of the staining was not considered while evaluating the expression. We used negative control for rabbit primary antibodies (DAKO, N1699, USA). The evaluation was blinded to any of the clinical features. Spearman Correlation Analysis, Cox Regression Analysis, Kaplan Meier method for the production of survival curves and Log-rank test for the comparison between groups were performed for statistical analysis. Chi-square test was performed for the relationship of sex and nm23 expression. P values less than 0.05 was considered to be statistically significant.

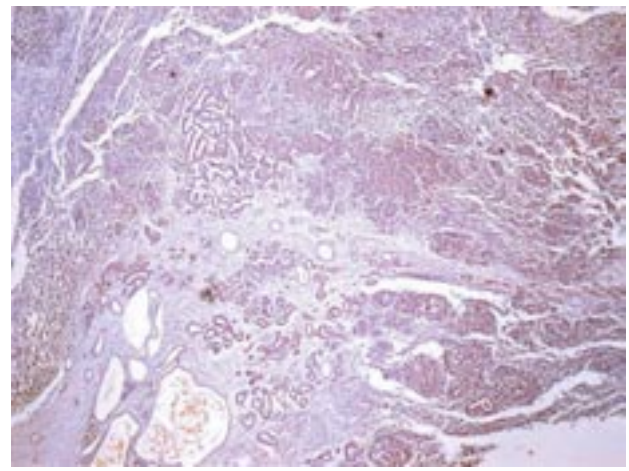

Fig 1. Diffuse nm23 expression in nephroblastoma (DAB, x40)

RESULTS

Seventeen of the cases were female (56.7%) while 13 were male (43.3%). The mean age was 4.3±2.4 (1-10). The distribution of the cases according to nm23 status is shown in table 1. Thirteen cases were stage I (43.3%), one case was stage II (3.3%), 10 cases were stage III (33.3%), while 6 were stage IV (20%).

Table 1
The summary of the variables according to nm23 expression

	n=30	nm23(+) n:20	nm23(-) n:10	p
Sex (M/F)	13/17	10/10	3/7	0.259
Prognosis (Alive/ex)	21/9	18/2	3/7	0.001
Metastasis (+/-)	9/21	3/17	6/4	0.010
Mean Age	4.3±2.4	4.5±2.6	4.00±1.8	0.595
Stage	I:13, II:1, III:10, IV:6	I:13, III:6, IV:1	II:1, III:4, IV:5	0.0001
Mean Survival (month)	87.2	105.4	50.9	0.019
Tumor diameter (cm)	13.8±4.8	14.7±5.1	12.2±3.9	0.192

Twenty-one cases were alive, while 9 cases were exitus. Mean survival time was 87.2±49.7 (8-154) months. Nm23 was positive in 20 cases (66.7%) and negative in 10 cases (33.3%). The mean survival time for nm23 negative cases was 50.9 months, while it was 105.4 months for positive cases.

There were 9 cases with metastasis and 3 of them showed nm23 expression, in the primary tumor. Twenty three of the cases were triphasic, while 7 were biphasic. None of the cases included anaplasia or sarcomatoid areas, all were in favorable histology group. The positive cases showed all diffuse or more than 80% cytoplasmic staining. There was no nuclear staining. The expression differs in epithelial, blastemal or mesenchymal component of the tumor for some cases. In 5 cases stromal component was negative. Expression in any component was evaluated. In three cases there was a focal expression in the epithelial (tubular) component, and these cases were considered as negative because the expression rate was less than 1%. The tubular epithelial cells showed cytoplasmic staining in nonneoplastic renal parenchyma in the cases in which peripheral nonneoplastic tissue was available. This was considered as an intrinsic control although we studied positive and negative control slides.

Nm23 negative expression is correlated with metastasis (p=0.010), stage of the disease (p=0.0001) and prognosis (p=0.001), and not correlated with tumor diameter (p=0.192), or age (p=0.595) of the patients in Spearman correlation analysis. Chi-square test did not show statistical relationship between sex and nm23 expression (p=0.259). Cox Regression analysis indicated nm23 as an independent factor for metastasis (p=0.010) and for prognosis (p= 0.017). Log Rank analysis showed a significant difference for overall survival between nm23 positive and negative cases (p=0.019) (Figure 2).

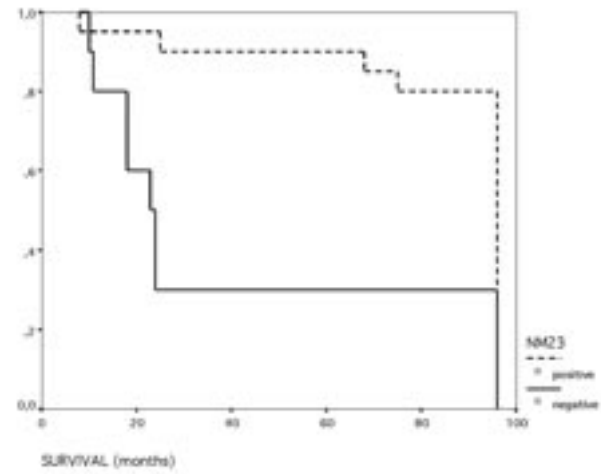

Fig 2. Survival curves of nm23 positive and negative cases in 8 year follow up (p=0.019)

DISCUSSION

The attempt to further identify the patients at high risk to develop metastases for tumors is very necessary to avoid

unnecessary therapeutic procedures. Although the survival rate of children and adolescents with nephroblastoma has increased, successful therapy has been associated with long-term toxicity. Strategies for prevention of treatment related toxicity includes developing new prognostic parameters.

Some recently studied prognostic factors for nephroblastoma include multidrug resistance-related protein 1 (MRP1), neurotrophin-receptor TrkB, heat shock protein 70 (HSP70), epidermal growth factor receptor (EGFR), transforming growth factor alpha and c-erb B-2 (12-15). Between these MRP1, TrkB and TGF-alpha were negatively correlated with prognosis and HSP70 expression were much expressed in cases who survived. Prognostic role of nm23 in nephroblastoma has not been studied yet.

It is suggested that biological significance of nm23 expression might be different in different tissues and neoplasms (7). Nm23-H1 is known as a differentiation inhibitory factor. Plasma levels of nm23-H1 can also be measured by ELISA method. In myelodysplastic syndrome, nm23-H1 level was low in patients with low international prognostic scoring system (16). This suggests that nm23-H1 may be useful as a prognostic marker for MDS, especially in low risk patients. IHC expression of nm23 was found more intensive in patients with nonrecurring disease and living patients among 50 ovarian cancer patients (6). In non-small cell lung cancer nm23 was found to be a suppressor of systemic but not lymphatic metastasis (17). In breast cancer, the expression of NDP kinase/nm23 has been reported to correlate with good prognosis and a lack of nodal metastasis (9). But in retinoblastoma nm23 staining was observed to indicate a tendency to metastases (10). In thyroid follicular carcinoma a significant inverse association was observed between metastatic disease and nm23-H1 expression (7). Nm23-H1 expression was related with tumor progression in nasopharyngeal carcinoma (4). In rectal cancer nm23 expression failed to correlate with distant metastasis in the series of Gunther et al (3). In vitro transfection experiments show that the nm23 gene suppresses metastasis, although the evidence from clinical studies is contradictory (17). In a series of human colorectal carcinoma reported by Ayhan et al. (18), reduced expression of nm23 protein detected by Western blotting technique was found to be associated with advanced tumor stage and distant metastasis.

It has been suggested that nm23 immunoreactivity might be a prognostic and differentiating factor in neuroblastoma (19,20). Overexpression of wild type nm23-1 proteins was defined to stimulate differentiation in neuroblastoma cell lines (21). Nm23 gene has been documented as metastasis-suppressor gene in normal development and differentiation (22). It has been identified in several aggressive neuroblastomas; mutated proteins might be related to the aggressiveness of neuroblastoma (23). Increased number of nm23-H1 copies was thought to be a predictor for poor prognosis independently in a series of 154 neuroblastoma cases (24).

Predicting the focus for investigating the metastatic potential of tumors is somewhat controversial. Should we examine the probable factors in the primary tumor or metastatic focus? The metastatic tumor cells might sometimes change some of their phenotypic properties. In this study we studied nm23 protein IHC expression in primary tumor tissues. We did not include specimens from metastases, recurrences or resections after treatment, because this study is designed to elucidate the usefulness of searching nm23 expression in primary tumors.

In our series statistical significance of metastasis, prognosis, stage and survival time all indicate prognostic significance of nm23 negativity. Absence of molecular genetic analysis is a disadvantage but it is not available in formalin-fixed paraffin embedded tissues for nm23. It is not possible yet to claim to decrease the amount of therapy by nm23 positivity alone. More series need to be studied both by genetic and IHC studies. Nm23 status must also be correlated by other prognostic factor.

We conclude that negative nm23 protein expression might be a predictor for metastasis in nephroblastomas and it is an independent prognostic factor. It would worth studying the prognostic importance of nm23 in nephroblastoma and other tumors.

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