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Review Article

The Association of nm23-H1 Expression with a Poor Prognosis in Patients with Peripheral T-Cell Lymphoma, Not Otherwise Specified

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nm23-H1 was originally identified as a protein that is expressed at a lower than usual level in metastatic cancer cells. The nm23 genes play critical roles in cellular proliferation, differentiation, oncogenesis, and tumor metastasis. Peripheral T-cell lymphoma (PTCL) is relatively rare, accounting for only 10% to 15% of non-Hodgkin's lymphomas. We examined whether nm23-H1 is a prognostic factor of peripheral T-cell lymphoma, not otherwise specified (PTCL-NOS). PTCL is more aggressive and has a poorer prognosis than diffuse large B-cell lymphoma. nm23-H1 was positive in 44.1% of PTCL-NOS patients. nm23-H1 expression was not correlated with age, performance status (PS), lactate dehydrogenase (LDH) level, or stage. The nm23-H1-positive group had significantly shorter overall survival (OS). OS was significantly shorter in patients with the following clinicopathologic features: age > 60 years, PS of 2-4, LDH > normal, bone marrow involvement, or nm23-H1-positive lymphoma. The nm23-H1 protein may be an important prognostic factor in PTCL-NOS. Because our results suggest that nm23-HI is produced by lymphoma cells, we expect to see the development of new treatments targeting nm23 overexpression.
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Keywords: peripheral T-cell lymphoma, nm23-H1, prognostic factor

INTRODUCTION

Peripheral T-cell lymphoma (PTCL) is relatively rare, accounting for only 10% to 15% of non-Hodgkin's lymphomas.[1,2] T-cell lymphomas are subdivided into PTCL, not otherwise specified (PTCL-NOS), angioimmunoblastic T-cell lymphoma (AITL), and anaplastic large-cell lymphoma, with PTCL-NOS being the most common entity among these rare diseases.[2] The international prognostic index (IPI)[3] is generally used as a prognostic factor. It may be used in PTCL patients for risk stratification in order to identify patients for clinical trials. For example, clinical trials in which high-dose chemotherapy followed by autologous hematopoietic stem cell transplantation is performed in an up-front manner are conducted for cases at a high risk according to the IPI. Numerous reports about biological prognostic factors have been published, but there are few prognostic factors that can be practically used in a clinical context.

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nm23-H1 was originally identified as a protein that is expressed at a lower than usual level in metastatic cancer cells. The nm23 genes play critical roles in cellular proliferation, differentiation, oncogenesis, and tumor metastasis. We previously established an enzyme-linked immunosorbent assay technique for determination of the serum level of nm23-H1 protein,[4,5] and reported that this level in patients with aggressive lymphoma was significantly higher than that in healthy controls, and that a high nm23-H1 level was associated with poor prognosis in aggressive lymphoma.[5] In our previous immunohistochemical study on cytoplasmic nm23-H1 expression in diffuse large B-cell lymphoma, we found that patients with positive cytoplasmic staining had significantly poorer prognosis than patients with negative staining.[6] In addition, serum nm23-H1 showed a high level in non-Hodgkin's lymphoma in comparison with that in normal controls and we reported that it also showed a high level especially in PTCL and adult T-cell leukemia/lymphoma.[4,5]

NM23

The *nm23* gene was initially identified as a putative metastasis suppressor gene on the basis of its reduced expression in certain highly metastatic cell lines and tumors.[7] This gene was independently identified as a regulator of imaginal disc differentiation late in *Drosophila* development as Awd.[7,8]

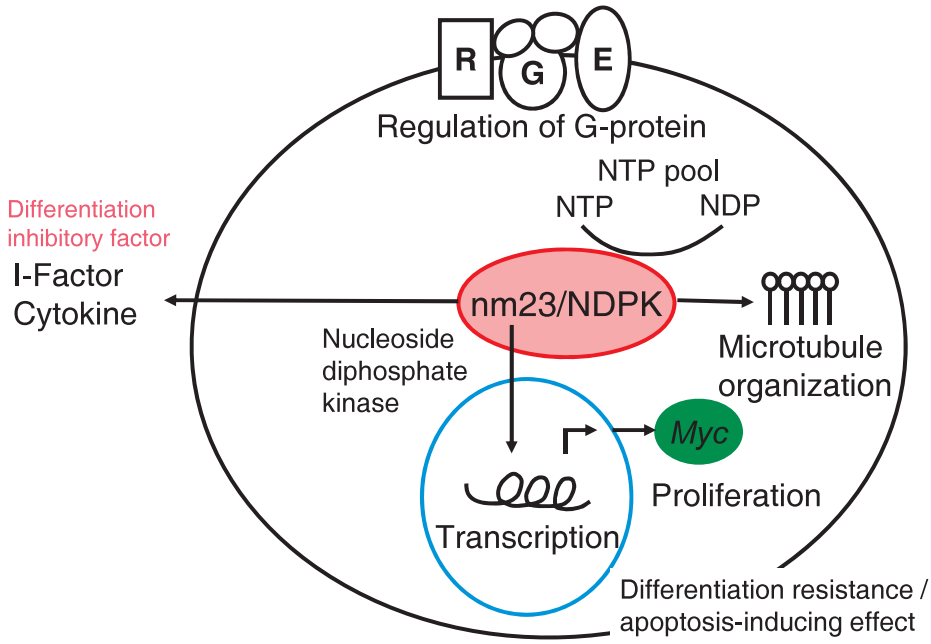

Fig. 1. nm23 is a multifunctional protein that is identical to nucleotide diphosphate kinase. This protein is involved in a variety of cellular regulatory functions.

Table 1. nm23 expression and metastasis in human cancers

Inverse correlation between nm23 expression and metastasis in
Breast cancer
Gastric cancer
Lung cancer other than small cell carcinoma
Hepatocellular carcinoma
Melanoma
Positive correlation between nm23 expression and metastasis in
Neuroblastoma
Thyroid carcinoma
Acute myelogenous leukemia
Non-Hodgkin lymphoma

Marino N, *et al*. Cancer Metastasis Rev (2012)

Thus far, ten human homologues (nm23-H1 to nm23-H10) have been described, with nm23-H1 and nm23-H2 being the most studied. They have been separated into two groups based on sequence homology. Group I (H1 to H4) includes proteins with nucleotide diphosphate kinase activity and 58-88% similarity; however, group II (H6 to H10) consists of more divergent proteins, with only 25-45% similarity.[8,9]

nm23 is a multifunctional protein that is identical to nucleotide diphosphate kinase. This protein is involved in a variety of cellular regulatory functions. For example, the protein functions as a factor inhibiting differentiation and suppresses tumor metastasis (Fig. 1). The level of nm23-H1 expression is inversely correlated with metastatic potential in human cancers, such as breast, gastric, and non-small cell lung cancers, hepatocellular carcinoma, and melanoma.[8,9] In contrast, the opposite trend has been reported in neuroblastoma, thyroid carcinoma, acute myelogenous leukemia (AML), and non-Hodgkin lymphoma, although the mechanisms behind this discrepancy are not known (Table 1).[9,10]

Chemoattractants derived from an insulin-like growth factor, a platelet-derived growth factor, and serum including lysophosphatidic acid disrupt the cellular motor ability of cancer cells by overexpression and, via these mechanisms, nm23-H1 is thought to reduce metastasis and invasion. In addition, the signaling pathway of the nm23-H1 family has been reported on, and various associations with the intracellular proteins 3-glyceraldehyde phosphate dehydrogenase, Hsp70, Hsp90, telomerase, ROR, Rad, and Ras-associated GTPase have also been reported.[10-12] Okabe-Kado *et al*. detected a differentiation instruction-inhibiting factor (inhibitory factor; I-factor) throughout the culture supernatant of murine differentiation-resistant myeloid leukemia cells (M1 cells).[13] They subsequently purified I-factor and characterized it as an important factor controlling the differentiation potency of M1 cells. They also revealed that it is identical to nm23 protein and reported that nm23-H1 is a poor prognostic factor of AML. We established a system of measurement of serum nm23-H1 by enzyme-linked immunosorbent assay, which enabled easy measurement using only a little serum in a lymphoma case, and we found it to be a prognostic factor of non-Hodgkin's lymphoma.[4,5]

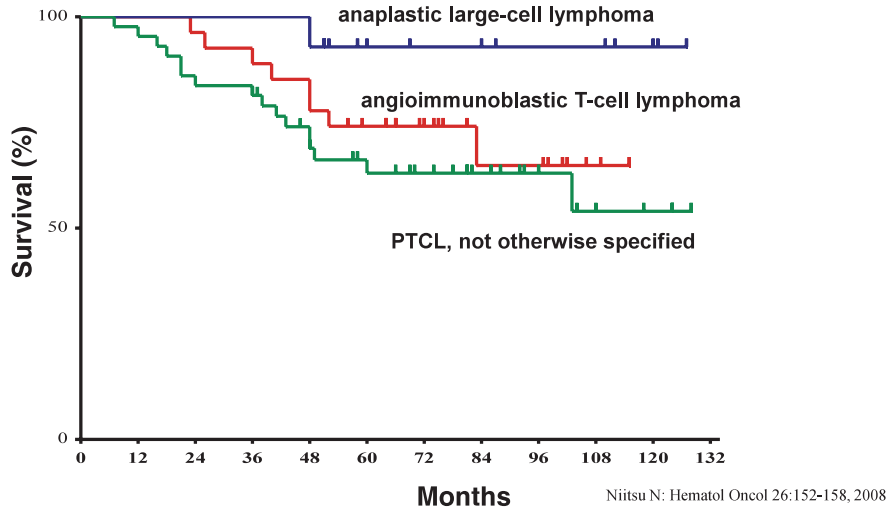

Fig. 2. Overall survival curves of patients with three types of peripheral T-cell lymphoma (peripheral T-cell lymphoma, not otherwise specified, angioimmunoblastic T-cell lymphoma, and anaplastic large-cell lymphoma) who received the CyclOBEAP regimen.

PERIPHERAL T-CELL LYMPHOMA, NOT OTHERWISE SPECIFIED (PTCL-NOS)

In the WHO classification, a subclass of PTCL was identified and termed PTCL-NOS.[1] However, this is a blanket term used to describe a heterogeneous array of lymphomas with differing histological characteristics, clinical features, responses to treatment, and associated prognosis. PTCL is more aggressive and has a poorer prognosis than diffuse large B-cell lymphoma.[1,2,14-16] Patients with PTCL-NOS usually present with systemic symptoms and generalized lymphadenopathy, as well as frequent involvement of bone marrow, skin, and spleen. In particular, PTCL-NOS is not responsive to conventional chemotherapies. The usefulness of high-dose chemotherapy followed by autologous hematopoietic stem cell transplantation has been reported, although a recent study did not confirm this.

IPI is generally used as a prognostic factor.[3] Recently, Gallamini *et al*.[17] analyzed patients with PTCL-NOS and proposed a new prognostic index for this condition including age, performance status (PS), lactate dehydrogenase (LDH) level, and bone marrow involvement. This PIT model was able to identify 4 groups of patients with different outcomes, and had overall superior predictive capacity compared with IPI.

The standard therapeutic option for patients with advanced-stage PTCL is CHOP (cyclophosphamide, doxorubicin, vincristine, prednisone) chemotherapy. For this treatment, an overall response rate of more than 60% has been reported; however, relapses are common and the 5-year overall survival (OS) rate is approximately 20-30%.[16] A few large-scale controlled trials with CHOP therapy for PTCL have been performed. The German non-Hodgkin's lymphoma group (DSHNHL) evaluated the outcome of all T-cell lymphomas based on treatment regimens received in 7 German high-grade studies. In the NHL-B1 trial, young good-prognosis patients with T-cell lymphoma had an improved 3-year event-free survival rate (71% vs. 50%) when etoposide was added to CHOP-14 or CHOP-21 (P = 0.01).[18,19] The GELA performed a trial that compared the ACVBP (doxorubicin, cyclophosphamide, vindesine, bleomycin, prednisone) regimen with the CHOP regimen for patients with high-risk PTCL. They reported that the ACVBP regimen was superior to standard CHOP with regard to both event-free survival and OS.[20] In addition, we report the results of a phase II study of the CyclOBEAP (cyclophosphamide, vincristine, bleomycin, etoposide, doxorubicin, prednisolone) regimen in patients with PTCL. The 5-year OS rate was 72% and the 5-year progression-free survival (PFS) rate was 61% among the 84 patients. The 5-year OS was 93% among the anaplastic large-cell lymphoma patients, 74% among the AITL patients, and 63% among the PTCL-NOS patients (Fig. 2). Among the PTCL-NOS and AITL patients, the 5-year OS rate was 67% and the 5-year PFS rate was 58%.[21]

NM23-H1 EXPRESSION IN PTCL-NOS[14]

We studied 102 consecutive, untreated PTCL-NOS patients, in whom the expression of nm23-H1 was studied by immunohistochemistry. Forty-five of these 102 patients with PTCL-NOS were positive for nm23-H1.

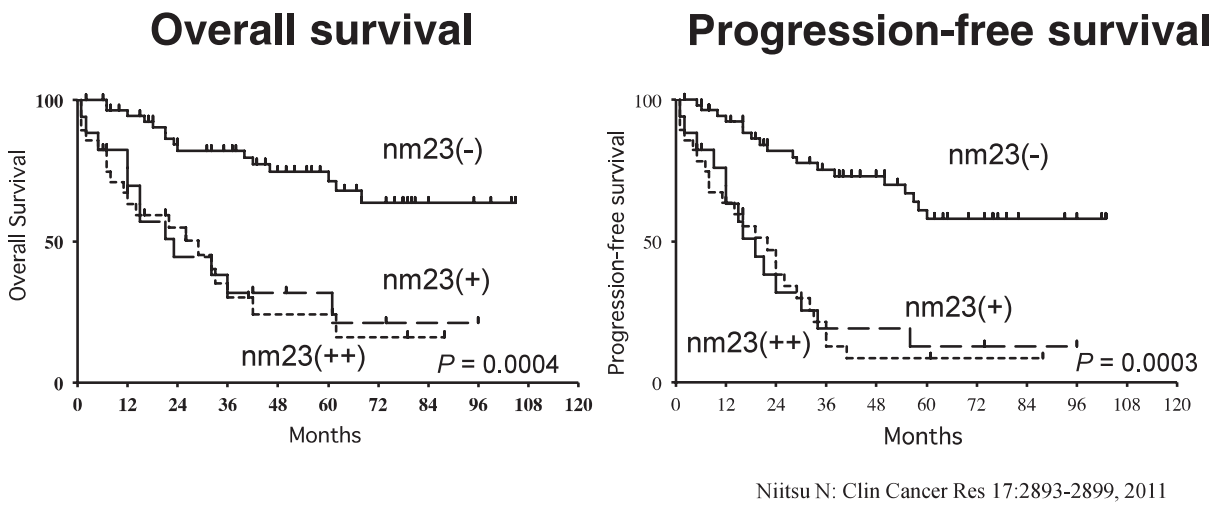

Fig. 3. Overall survival curve and progression-free survival curve of patients with peripheral T-cell lymphoma, not otherwise specified, according to the expression of cytoplasmic nm23-H1.

The relationship between nm23-H1 expression and clinicopathologic factors

There was no correlation between nm23-H1 expression and age, PS, serum LDH level, stage, or bone marrow involvement, but a significantly higher percentage of patients with a high PIT score showed nm23-H1 expression. Furthermore, the expression at the time of diagnosis was significantly lower in patients who subsequently achieved a complete response than in those who failed to do so. Thus, a close relationship between nm23-H1 expression and therapeutic responsiveness was found.

Survival of patients with PTCL-NOS according to the expression of nm23-H1

Fig. 3 shows the OS and PFS of patients with PTCL-NOS according to the expression of nm23-H1. The OS rates of patients in the nm23-H1 "weakly positive" and "strongly positive" expression groups were significantly lower than for those in the nm23-H1 "negative" group, and the same was true for PFS. We also evaluated the significance of nm23-H1 expression among patients who were grouped according to the PIT by incorporating the nm23-H1 weakly positive and strongly positive groups into a single nm23-H1 "positive" group. In PIT groups 1 and 2, OS and PFS were worse among patients who were positive for nm23-H1. The same was true for PIT groups 3 and 4 (Fig. 4). Therefore, we were able to predict the therapeutic outcome in each PIT risk group of PTCL-NOS using nm23-H1 expression at diagnosis.

Univariate and multivariate analyses of OS and PFS in patients with PTCL-NOS

OS was significantly shorter in patients with the following clinical features: older age, poor PS, elevated serum LDH level, bone marrow involvement, and nm23-H1 positivity. The same was true for PFS, except for PS.

A multivariate analysis was conducted using the prognostic factors that were shown to be statistically significant in PTCL-NOS. It is evident that nm23-H1 expression is an important and independent prognostic factor for patients with this type of lymphoma.

The CyclOBEAP regimen for patients with PTCL with nm23-H1

As for nm23-H1 expression in the PTCL-NOS and AITL patients in the present study, the 5-year OS of the nm23-H1-positive group was 46.7% and that of the nm23-H1-negative group was 83.3% (Fig. 5A), while the 5-year PFS of the nm23-H1-positive group was 45.3% and that of the nm23-H1-negative group was 81.4% ($P = 0.0007$) (Fig. 5B). The nm23-H1-positive group showed significantly poorer prognosis.

EXTRACELLULAR NM23 PROTEIN AS A THERAPEUTIC TARGET FOR ACUTE LEUKEMIA[22]

Fig. 6 shows the extracellular functions of nm23-H1 protein derived from AML cells on normal peripheral blood mononuclear cells (PBMNC). Extracellular nm23-H1 protein promotes the growth and survival of AML cells, which is mediated by MAP kinase activation, STAT activation, and

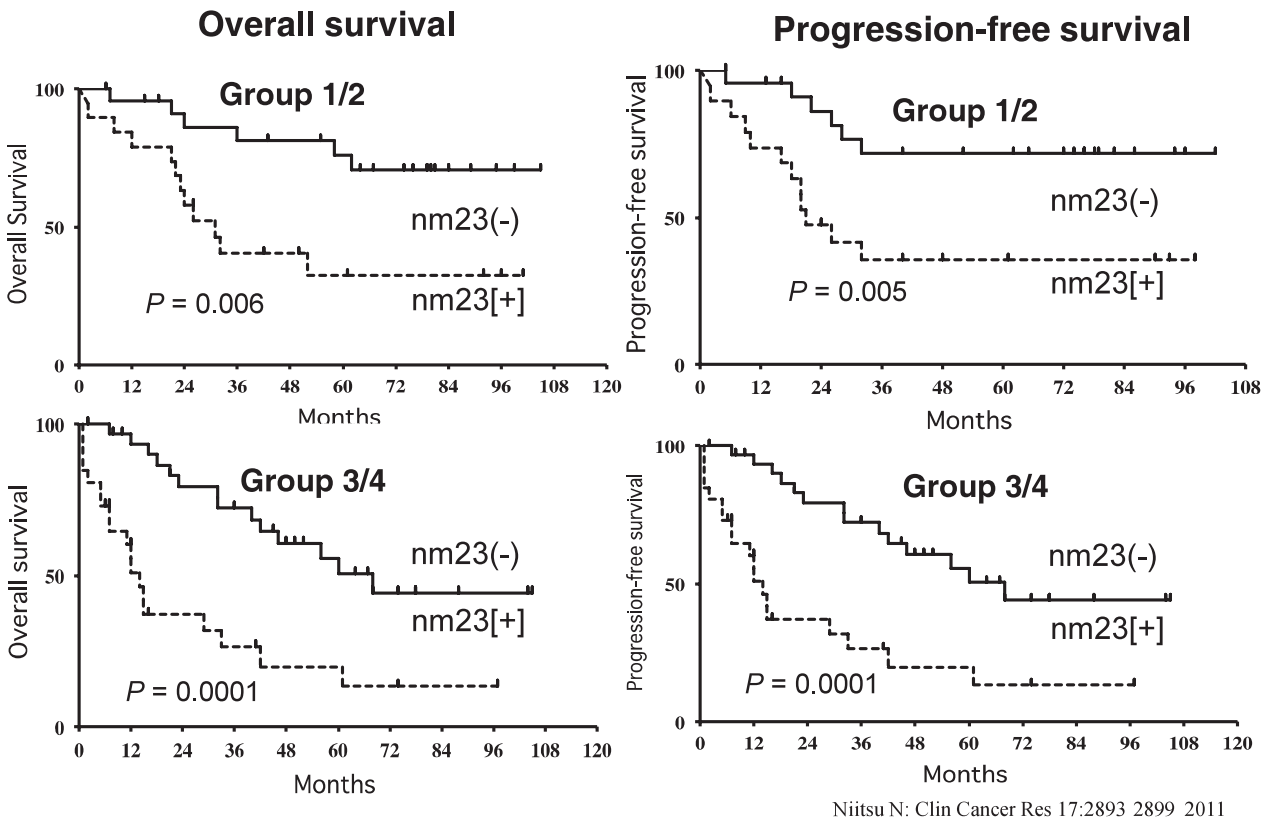

Fig. 4. Overall survival curve and progression-free survival curve of patients with peripheral T-cell lymphoma, not otherwise specified, according to the prognostic index for T-cell lymphoma.

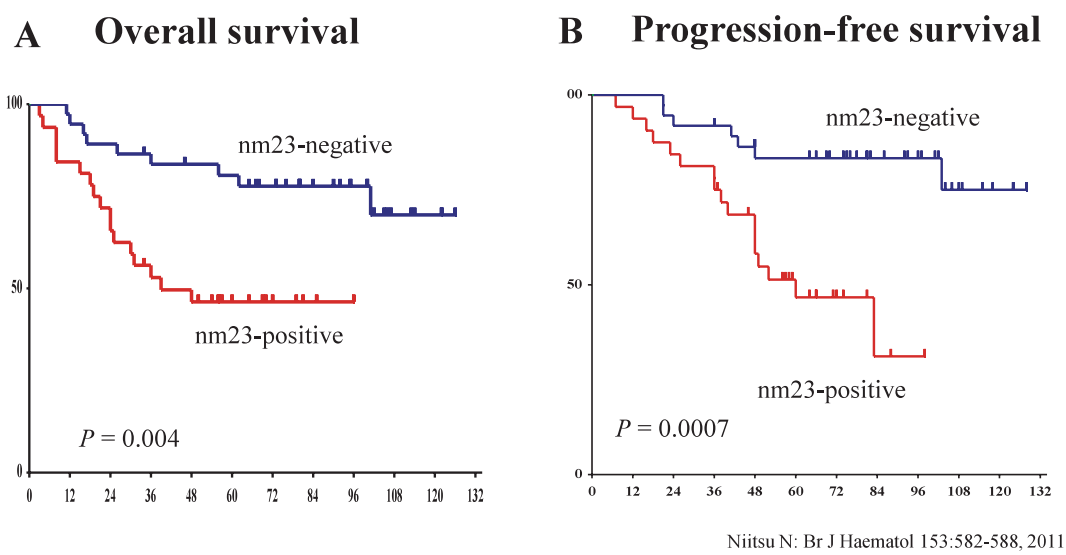

Fig. 5. Overall and progression-free survival curves of patients with peripheral T-cell lymphoma, not otherwise specified, +angioimmunoblastic T-cell lymphoma who received the CyclOBEAP regimen according to the presence or absence of cytoplasmic nm23-H1 expression.

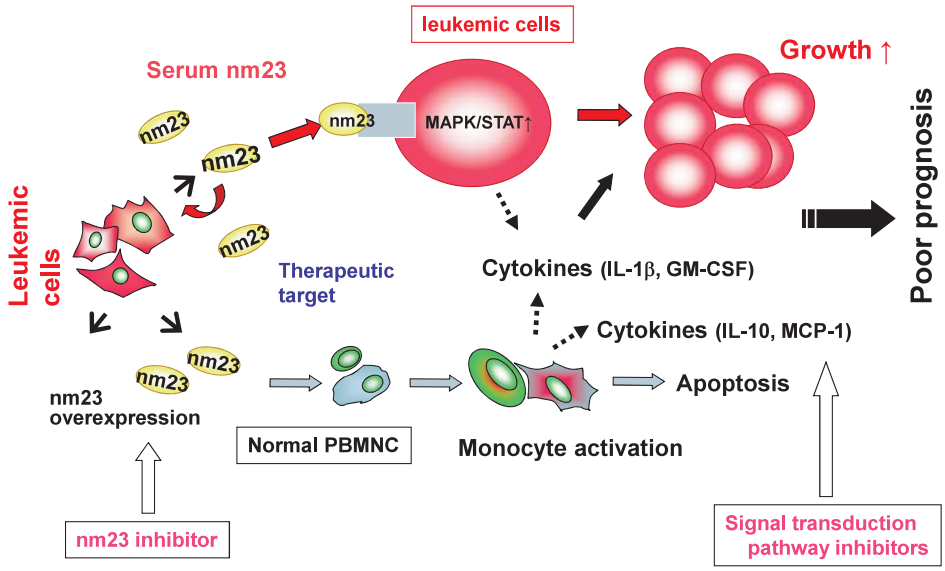

Fig. 6. Extracellular nm23 protein as a therapeutic target for acute myelogenous leukemia.

cytokine release. On the other hand, extracellular nm23-H1 protein also affects normal PBMNC survival, activates monocytes, and induces cytokine production. Some of these cytokines, especially GM-CSF and interleukin-1β, directly promote the survival and growth of primary cultured AML cells. Moreover, nm23-H1 induces immunosuppressive cytokines, such as interleukin-10. Therefore, the cytokine-inducing effect of extracellular nm23-H1 protein on normal PBMNC may be associated with a poor prognosis in AML. Taken together, these observations suggest that extracellular nm23-H1 may play an important role in the progression of leukemia, and that inhibitors of extracellular nm23-H1 protein or inhibitors of the extracellular functions of this protein should be evaluated as possible tools for treatment. Because it is possible that the same mechanisms operate in lymphoma, further study along the same lines is also recommended for this condition.

CONCLUSION

nm23-H1 protein appears to be an important prognostic factor in PTCL-NOS. Because our results indicate that nm23-H1 is produced by lymphoma cells, it is expected that new treatments targeting nm23-H1 overexpression will be developed in the future.

nm23 overexpression in non-Hodgkin's lymphoma is associated with a poor prognosis that is similar to that in the rituximab era. Therefore, prognostic improvement may be anticipated by reducing the expression of nm23.

It is suggested that we aim to develop, and use for diagnosis, a molecular target for the control of nm23-overexpressing lymphoma for the improvement of its treatment. We should also examine extracellular proteins as treatment targets including nm23, including in terms of its localization, and search for an aptamer for the target protein; it will also be necessary to promote development for translational research in the future.

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