Increased expression of phosphorylated NBS1, a key molecule of the DNA damage response machinery, is an adverse prognostic factor in patients with de novo myelodysplastic syndromes

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ABSTRACT

The expression of activated forms of key proteins of the DNA damage response machinery (pNBS1, pATM and γH2AX) was assessed by means of immunohistochemistry in bone marrow biopsies of 74 patients with de novo myelodysplastic syndromes (MDS) and compared with 15 cases of de novo acute myeloid leukemia (AML) and 20 with reactive bone marrow histology. Expression levels were significantly increased in both MDS and AML compared to controls, being higher in high-risk than in low-risk MDS. Increased pNBS1 and γH2AX expression possessed a significant negative prognostic impact for overall survival in MDS patients, whereas pNBS1 was an independent marker of poor prognosis.

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1. Introduction

Myelodysplastic syndromes (MDS) are clonal hematopoietic stem cell disorders with great heterogeneity in terms of clinical, biological and prognostic features, which has been ascribed to the variety of genetic lesions contributing to MDS pathogenesis [1]. They are characterized by ineffective hematopoiesis, peripheral blood cytopenias (anemia, neutropenia and/or thrombocytopenia), dysplasia in one or more myeloid cell lines and an increased risk of progression to acute myeloid leukemia (AML). Prognostic classification of various types of MDS concerning survival and risk of evolution to AML is based on the percentage of bone marrow blasts, cytogenetic findings and number of cytopenias; based upon the above, the International Prognostic Scoring System (IPSS) [2] subdivides MDS into four categories, namely low risk, intermediate-1 risk (INT-1), intermediate-2 risk (INT-2) and high risk; these are grouped into low-risk MDS (including low risk and INT-1) and high-risk MDS (including INT-2 and high risk MDS).

About 50% of MDS cases display aneuploidy (the state of a cell containing an aberrant number of whole or parts of the chromosomes) [3,4] at the time of diagnosis, a finding indicative of chromosomal instability (CIN) at early stages of hematopoietic stem cell clonal evolution. Although molecular mechanisms underlying chromosomal instability remain largely unknown, formation of DNA double-strand breaks (DSBs) can lead to this type of genomic instability [5]. Besides, formation of DNA DSBs activates the DNA damage response (DDR) pathway which, according to the “oncogene-induced DNA damage model for cancer development and progression” [6], represents an antitumor barrier raised in precancerous lesions, by inducing p53-dependent cell cycle arrest, apoptosis and senescence, features that have been described in MDS [7,8].
An early event in response to DNA DSBs is the recruitment of MRN complex proteins (MRE11, RAD50 and NBS1), which serves as the sensor of DNA breakage and leads to the phosphorylation of NBS1 [9,10]. Phosphorylated NBS1 (pNBS1) activates through phosphorylation the key signal transducer of the DDR pathway, protein kinase ATM, which in turn phosphorylates many substrate proteins, including histone protein H2AX, whose rapid phosphorylation leads to $\gamma$H2AX foci formation at nascent DSB sites [9]. The aforementioned events finally induce the activation of tumor suppressor protein p53, which leads to cell cycle arrest, apoptosis or senescence, constituting an evolutionary pressure for the transition from precancerous lesion to full blown malignancy [6]. These processes can be monitored by means of either immunofluorescence or immunohistochemistry, using primary antibodies that detect the activated, phosphorylated forms of NBS1, ATM and H2AX and p53 [11,12].

Following publication of the aforementioned cancer development model, many studies performed mainly on solid tumors confirm the initial prediction, i.e. the activation of DDR in precancerous lesions [11–14]. Concerning neoplastic hematopoietic stem cell disorders, two studies performed on bone marrow specimens from MDS and AML patients detected activation of the DDR machinery in preleukemic lesions, i.e. MDS, in contrast to normal bone marrow samples; their results were contradictory concerning the expression of these molecules in AML samples [15,16]. Moreover, in a recent study we have shown that proteins involved in the Non Homologous End Joining (NHEJ) mechanism of DSBs repair are expressed in MDS cases [17].

The aim of the present study was to examine the expression of key proteins of the DDR machinery, i.e. pNBS1, pATM and $\gamma$H2AX, in bone marrow cells of adult de novo MDS and to determine their association with clinical and biological parameters, as well as with prognosis.

2. Materials and methods

2.1. Study population

The current study included 74 cases of newly diagnosed adult de novo MDS. Samples were obtained by bone marrow biopsy before the onset of any treatment. Diagnosis was issued according to the updated (2008) WHO classification [1]. Their clinical and biological characteristics are summarized in Table 1. Patients with low and INT-1 score according to the IPSS (47/70 patients) who were asymptomatic did not receive any treatment or received only growth factors (Epo and GM-CSF) and transfusions of red blood cells (RBC) and/or platelets (PLT) as needed. The vast majority of patients with INT-2 and high risk IPSS scores (19/23 patients) were treated with 5-azacytidine (Vidaza). Four patients were treated with AML chemotherapy (Daunorubicin and Idarubicin; 7+3), while one of them received additional allogeneic hematopoietic stem cell transplantation. In addition, 20 cases of bone marrow biopsies from age-matched patients (11 men, 9 women) in the process of non-Hodgkin lymphoma staging (7 cases of low-grade B-cell lymphomas, 10 of diffuse large B-cell lymphomas and 3 of T-cell lymphomas), without any evidences of lymphoma infiltration, served as controls, as well as 15 cases of de novo AML were added for comparison purposes; hence, the total study population consisted of 109 cases. All patients represented in this study underwent trephine biopsy in the Hematology Unit of the 2nd Department of Internal Medicine in “Attikon” University Hospital between December 2003 and September 2010. All of them had a follow-up period of minimum one year (median 24, range 1–148). All specimens were fixed in acetone-zinc formalin solution for 24 h followed by decalcification in Decal® for 3 h and were then embedded into paraffin. Hematoxylin-and-Eosin-stained slides, as well as appropriate immunostains were used for diagnostic purposes. The study was approved by the “Attikon” University Hospital Institutional Ethics Review Board; all patients had given informed consent for participation in the study.

2.2. Cytogenetic analysis

Cytogenetic analysis was performed by direct culture of bone marrow cells and G-bandning technique. Chromosomal aberrations were described according to the International System of Human Cytogenetic Nomenclature (ISCN 1995) recommendations [18].

<table>
<thead>
<tr>
<th>Table 1</th>
<th>Clinical and biological characteristics of MDS patients.</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. patients</td>
<td>74</td>
</tr>
<tr>
<td>Sex (male/female)</td>
<td>46/28</td>
</tr>
<tr>
<td>Median (range)</td>
<td>Age (years)</td>
</tr>
<tr>
<td>75 (41–87)</td>
<td></td>
</tr>
<tr>
<td>Hemoglobin (g/dL)</td>
<td>9.8 (5.7–14.1)</td>
</tr>
<tr>
<td>WBC ($\times 10^3$/mL)</td>
<td>4195 (1100–30.360)</td>
</tr>
<tr>
<td>ANC ($\times 10^3$/mL)</td>
<td>1715 (80–7425)</td>
</tr>
<tr>
<td>Platelets ($\times 10^3$/mL)</td>
<td>131,500 (7000–440,000)</td>
</tr>
<tr>
<td>Platelet count (%)</td>
<td>6.0 (20)</td>
</tr>
<tr>
<td>Overall survival (months)</td>
<td>24 (1–148)</td>
</tr>
<tr>
<td>Karyotype (67/74 patients)</td>
<td></td>
</tr>
<tr>
<td>Normal</td>
<td>41 (61%)</td>
</tr>
<tr>
<td>Abnormal</td>
<td>26 (39%)</td>
</tr>
<tr>
<td>Cyrogentic risk (67/74 patients)</td>
<td></td>
</tr>
<tr>
<td>Low</td>
<td>47 (70%)</td>
</tr>
<tr>
<td>Intermediate</td>
<td>11 (16.5%)</td>
</tr>
<tr>
<td>High</td>
<td>9 (13.5%)</td>
</tr>
<tr>
<td>WHO classification (74/74 patients)</td>
<td></td>
</tr>
<tr>
<td>RA</td>
<td>9 (12.2%)</td>
</tr>
<tr>
<td>RARS</td>
<td>3 (4.1%)</td>
</tr>
<tr>
<td>RCMD</td>
<td>23 (31.1%)</td>
</tr>
<tr>
<td>RAEB-1</td>
<td>23 (31.1%)</td>
</tr>
<tr>
<td>RAEB-2</td>
<td>16 (21.6%)</td>
</tr>
<tr>
<td>IPSS risk (70/74 patients)</td>
<td></td>
</tr>
<tr>
<td>Low</td>
<td>20 (28.6%)</td>
</tr>
<tr>
<td>Intermediate-1</td>
<td>27 (38.6%)</td>
</tr>
<tr>
<td>Intermediate-2</td>
<td>16 (22.9%)</td>
</tr>
<tr>
<td>High</td>
<td>7 (10.0%)</td>
</tr>
</tbody>
</table>

|a – White blood cells. |
|b – Absolute neutrophil count. |
c – World Health Organization classification (RA, refractory anemia; RARS, refractory anemia with ringed sideroblasts; RCMD, refractory cytopenia with multilineage dysplasia; RAEB, refractory anemia with excess blasts). |
d – International prognostic scoring system.

2.3. Immunohistochemistry

Immunostaining was performed on 3-µm deparaffinized sections. Primary antibodies against p53-NBS1 (phospho S1432) (rabbit monoclonal, clone EPR2470Y [abcam], 1:100 dilution), ATM (phospho S1981) (rabbit monoclonal, clone EP1890Y [abcam], 1:100 dilution); $\gamma$H2AX (phospho S139) (mouse monoclonal, clone 9F3 [abcam], 1:500 dilution) and p53 (mouse monoclonal, clone DO-7 [DAKO], 1:50 dilution) were used. For pATM, $\gamma$H2AX and p53, slides were immersed in a high pH target retrieval solution (K8004 [DAKO]), boiled in a microwave at 650 W for 20 min, and subsequently cooled at room temperature for 20 min. Endogenous peroxidase activity was blocked by means of the peroxidase-blocking reagent S2001. Immunohistochemistry was performed using the DAKO Autostainer Plus device with Envision™ detection and visualization kit (K5007 [DAKO]). For pNBS1 immunostaining, BondTM Automated Immunohistochemistry System was used. In sixteen randomly chosen cases (5 reactive, 4 RA, 1 RARS, 2 RAEB-1, 1 RAEB-2 and 3 AML) double immunostaining was performed using primary antibodies against CD34 (mouse monoclonal, clone QBEnd-10 [DAKO], 1:50 dilution) and $\gamma$H2AX. Double staining was performed with Envision™ G2 Doublestain System, Rabbit/Mouse (DAB+/Permanent Red), Code K3561. All sections were lightly counterstained with Hematoxylin for 25 sec prior to mounting.

2.4. Scoring of immunohistochemical staining

Cell counting was done blindly with an 60x objective [Nikon eclipse E400 microscope] by a trained (MK) and a qualified (PF) pathologist. At least 1000 marrow cells per case were assessed and the percentage of positive nuclei calculated. Double staining was scored by counting the percentage of $\gamma$H2AX positive cells in at least 100 CD34 positive cells, with blastic morphol-

2.5. Statistical analysis

MDS patients were divided into two subgroups (low/INT-1 risk and INT-2/high risk), according to their IPSS score. For categorization of pNBS1, $\gamma$H2AX, pATM, and p53 percentage of immunopositive cells, the X-tile algorithm [19] was used to generate optimal cutpoints: 35.8% (46th percentile) for pNBS1, 66.3% (72nd percentile) for pATM, 66.1% (75th percentile) for $\gamma$H2AX and 5.0% (76th percentile) for p53. Finally, extensive statistical analysis was performed (Online Supplementary Design and Methods).
Table 2
Frequency of the detected numerical and structural abnormalities in MDS patients.

<table>
<thead>
<tr>
<th>Karyotype</th>
<th>n (%)</th>
<th>MDS subtype</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>41 (61.0)</td>
<td>6RA, 3 RARS, 15 RCMD, 11 RAEB-1, 6 RAEB-2</td>
</tr>
<tr>
<td>Abnormal</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Del (5q)</td>
<td>26 (39.0)</td>
<td>1 RA, 2 RAEB-1, 2 RAEB-2</td>
</tr>
<tr>
<td>Del (20q)</td>
<td>5 (19.0)</td>
<td>1 RA, 2 RAEB-1, 2 RAEB-2</td>
</tr>
<tr>
<td>Del Y</td>
<td>3 (11.5)</td>
<td>1 RA, 1 RCMD, 1 RAEB-1, 1 RAEB-2</td>
</tr>
<tr>
<td>Trisomy 8</td>
<td>3 (11.5)</td>
<td>1 RA, 1 RCMD, 1 RAEB-1, 1 RAEB-2</td>
</tr>
<tr>
<td>Del 11q</td>
<td>2 (8.0)</td>
<td>1 RCMD, 1 RAEB-2</td>
</tr>
<tr>
<td>Inv (9)</td>
<td>2 (8.0)</td>
<td>1 RAEB-1</td>
</tr>
<tr>
<td>Inv (3)</td>
<td>1 (4.0)</td>
<td>1 RCMD</td>
</tr>
<tr>
<td>Abnormalities of chromosome 7</td>
<td>7 (27.0)</td>
<td>1 RCMD, 5 RAEB-1, 1 RAEB-2</td>
</tr>
<tr>
<td>Complex karyotype</td>
<td>3 (11.5)</td>
<td>3 RAEB-2</td>
</tr>
</tbody>
</table>

MDS, myelodysplastic syndromes; RA, refractory anemia; RARS, refractory anemia with ringed sideroblasts; RCMD, refractory cytopenia with multilineage dysplasia; RAEB, refractory anemia with excess blasts.

3. Results

3.1. Cytogenetic analysis

Cytogenetic analysis was successful in 67 out of 74 patients. As shown in Table 2, structural or numerical abnormalities were found in 26 patients (39%).

3.2. Expression of proteins involved in DDR machinery

In the present study, we examined the expression of protein components of the DDR machinery i.e. pNBS1, γH2AX and pATM in 74 MDS patients. Expression of all molecules was localized in the nucleus. Any cytoplasmic staining was not further considered in this analysis. The percentage of immunopositive cells for the three DDR-involved proteins was significantly increased in MDS and AML compared to controls [for pNBS1, 38.7 (range: 0.0–85.8) in MDS patients, 80.7 (range: 65.5–95) in AML patients and 8.8 (range: 1.2–36.8) in normal controls (P < 0.001), for γH2AX, 32.2 (range: 1.2–93.4) in MDS patients, 86.8 (range: 35.1–96.8) in AML patients and 16.1 (range: 0.0–70.3) in controls (P < 0.001) and for pATM, 38.3 (range: 4.9–85) in MDS, 79.7 (range: 0.0–100) in AML and 19.3 (range: 8.4–34.7) in controls (P < 0.001)] (Online Supplementary Table S1). In addition, we found that the expression of the proteins that participate in the DDR machinery differed significantly among the subtypes of MDS and was higher in high risk MDS (RAEB-1 and RAEB-2) compared to low risk MDS (RA, RARS, and RCMD), categorized according to WHO classification (2008), and even more elevated in AML patients (P < 0.001 for all examined DDR-involved proteins) (Fig. 1: Online Supplementary Table S2).

Finally, in MDS patients the expression level of any one of pNBS1, γH2AX and pATM proteins was positively correlated to the expression levels of the rest two, while the expression of γH2AX and pATM also correlated with p53 expression (Online Supplementary Table S3).

3.3. Relationships between pNBS1, γH2AX and pATM expression, and MDS patients’ clinicopathological variables

Positive correlations were observed between pNBS1, γH2AX and pATM expression status and blast count (r₁ = 0.462, P < 0.001, r₂ = 0.274, P = 0.018 and r₃ = 0.342, P = 0.003 respectively). For γH2AX, this finding was also confirmed in double-stained slides, whereby the median percentage value of CD34+ blasts immunostained for γH2AX was 5-fold higher in high risk MDS and more than 9-fold higher in AML cases compared to controls. No difference was detected between controls and low risk MDS. Additionally, a significant negative correlation was found between pNBS1 expression status and hemoglobin as well as white blood count (WBC), suggesting higher pNBS1 expression in patients with

Fig. 1. Immunohistochemistry of reactive bone marrow, low-risk MDS, high-risk MDS and AML representative cases for pNBS1, γH2AX and pATM. Box-and-whiskers diagrams show differences in mean values of percentage of immunopositive cells. Error bars represent standard errors of the mean.
anemia ($r_s = -0.357, P = 0.002$) and leucopenia ($r_s = -0.245, P = 0.035$). Finally, a negative correlation was observed between γH2AX expression and platelet count, implying higher γH2AX expression in patients with thrombocytopenia ($r_s = -0.241, P = 0.038$) (Online Supplementary Table S4). In addition, expression status of each protein under study was categorized into two groups (positive vs. negative), as described in the “Design and Methods” section. Thus, among the 74 MDS cases examined, 40 (54.1%) were classified as pNBS1-positive and 34 (45.9%) as pNBS1-negative, 18 (24.3%) as γH2AX-positive and 56 (75.7%) as γH2AX-negative and 20 (27.0%) as pATM-positive and 54 (73.0%) as pATM-negative. pNBS1-positive patients had more frequently abnormal karyotype ($P = 0.011$), intermediate or high cytogenetic risk profile ($P = 0.041$) and belonged to a more advanced IPSS category ($P = 0.006$) (Online Supplementary Table S5).

Regarding pATM expression, pATM-positive patients had more often abnormal karyotype compared to the pATM-negative MDS population ($P = 0.046$), while γH2AX-positive patients belonged to an advanced IPSS category compared to the negative subgroup of patients, ($P = 0.007$). When the percentage of each protein expression was analyzed as a continuous variable, it was shown that the expression of all three analyzed DDR machinery molecules increased gradually among, normal controls, patients with low/INT-1 and INT-2/high MDS, and AML patients (Fig. 1).

3.4. p53 Expression and its correlations with DDR-involved proteins and clinicopathological characteristics of MDS patients

We also examined the expression status of p53 protein and its association with DDR-involved proteins, as well as with clinicopathological characteristics of MDS patients. The percentage of p53-immunopositive cells was significantly increased in MDS and AML compared to normal controls [median % positivity: 1.6 (range: 0.0–72.8) in MDS patients vs. 45 (range: 9.3–95.0) in AML patients vs. 0.0 (range: 0.0–1.6) in normal controls, $P < 0.001$]. In addition, we found that the expression of p53 differed significantly among the different subtypes of MDS, being higher in high risk MDS (RAEB-1 and RAEB-2) compared to low-risk MDS (RA, RARS, and RCMD), according to WHO (2008) classification, and even more elevated in AML ($P < 0.001$) (Online Supplementary Tables S1 and S2). Moreover, p53 expression was positively correlated with the expression of γH2AX ($P = 0.016$) and pATM ($P = 0.007$) (Online Supplementary Table S3). A significant association was also observed between the p53% positivity and the karyotype category, as MDS patients with normal karyotype were less frequently p53-positive compared to MDS patients with abnormal karyotype ($P = 0.021$) (Online Supplementary Table S6).

3.5. Prognostic role of DDR-involved proteins in MDS

Follow-up information was available for 70 out of 74 patients; 26 deaths were recorded, all of which due to causes related to MDS. The median overall survival (OS) was 24 months (range: 1–148 months). Kaplan–Meier analysis revealed significantly reduced OS of pNBS1-positive compared to pNBS1-negative MDS patients ($P = 0.002$), of γH2AX-positive compared to γH2AX-negative MDS patients ($P = 0.005$) and reduced OS of p53-positive compared to p53-negative MDS patients ($P = 0.024$). On the other hand, Kaplan–Meier OS curves did not differ significantly between pATM-positive and pATM-negative MDS patients (Fig. 2).

To evaluate the prognostic effect of the positive expression of DDR machinery proteins on the OS in MDS patients, we performed Cox regression analysis. As demonstrated in Online Supplementary Table S7, univariate Cox regression analysis revealed that pNBS1 and γH2AX positivities carry a significant negative prognostic impact for OS in MDS patients [hazard ratio (HR)=3.65, 95% confidence interval (CI)=1.52–8.73, $P = 0.004$ and HR=2.98, 95% CI=1.32–6.75, $P = 0.009$, respectively]. Interestingly, the multivariate Cox regression models, adjusted for IPSS risk and patients’ age, revealed an independent negative prognostic significance of pNBS1 positivity for OS (HR=2.79, 95% CI=1.15–6.81, $P = 0.024$) (Online Supplementary Table S7).

4. Discussion

In this retrospective study we assessed the expression of pNBS1, pATM and γH2AX in a series of MDS as well as de novo AML patients. To the best of our knowledge, no studies dealing with the expression of all three key molecules of DNA-damage response in MDS patients have so far been published. Our data show that the phosphorylation events of these proteins correlate among each other, indicating the participation in a common activated pathway in MDS cases. Moreover, the expression of all molecules significantly correlates with blast count and is increased in MDS cases compared to controls, with a statistical significant further increase in AML cases compared to MDS cases. Accordingly, in MDS patients, the expression of all 3 molecules is positively correlated with the type of MDS, being higher as the prognostic IPSS category worsens. The baseline positive cells counted in tissue sections from cases served as controls may represent an overestimation, supposing that lymphomas induce DNA damage in distant proliferative tissues in humans, in analogy to what has been shown in a mouse tumor model [20].

DDR activation in MDS has also been suggested in recent studies, through the detection of pATM and/ or γH2AX [15,16,21]. Furthermore, although in one of them [16], pATM but not γH2AX expression was significantly increased in AML samples compared to MDS, correlating with the blast percentage, in the second [15], no further increase of pATM, pCHK2 and γH2AX was detected in AML cases. These discrepancies might be related to the AML population under investigation, this including de novo AML cases in the first study ($n = 5$) compared to MDS-related AML in the second ($n = 10$). It is well known that the activation of DNA damage response is an early event during neoplastic transformation raising a barrier to progression, through increased apoptosis or senescence of transformed cells [6]. Increased apoptosis (which may underlie ineffective hematopoiesis [7,22,23]), as well as senescence have been described in MDS patients [8]. Furthermore, even in low-risk MDS subtypes, DNA double-strand breaks have been detected by comet assay [24], suggesting a causative role for the activation of DDR in MDS. The fact that pNBS1, pATM and γH2AX are overexpressed in MDS, even in low grade, is an indicator of activation of DNA damage response pathway early in preleukemic stages.

Among precancerous disorders, MDS are unique in that in the usual diagnostic workflow, information concerning the degree of cell differentiation (assessed by means of complete blood count), as well as the presence of cytogenetic abnormalities (through classical karyotype analysis) is essential in the evaluation of every suspected case. MDS is, accordingly, an ideal human model to investigate possible correlations between DDR activation and degree of cellular differentiation or ploidy status. Interestingly, increased expression of pNBS1 was found in patients with lower levels of hemoglobin ($P = 0.002$) and WBC ($P = 0.035$), whereas increased expression of γH2AX was related to thrombocytopenia ($P = 0.038$). In previous studies, evidence of inhibition of differentiation after DNA damage has been observed in skeletal myogenesis [25,26] and pancreatic organogenesis [27]. It is considered that the differentiation checkpoint is a barrier that inhibits the expression of differentiation-specific genes in precursor cells after DNA damage, preventing the generation of differentiated cells bearing...
Fig. 2. Overall survival of MDS patients in relation to pNBS1 (A), γH2AX (B), pATM (C) and p53 (D) expression.

unresolved lesions [28,29]. In accordance to these ex vivo studies, our results may suggest that the activation of DDR machinery in MDS cases might interfere with differentiation of hematopoietic lineages, providing a second potential mechanism beyond apoptosis for the observed peripheral cytopenias.

Another interesting finding of the present work was the positive correlation between the expression of molecules involved in the DDR machinery, namely pNBS1 and pATM and aneuploidy ($P = 0.011$ and 0.046, respectively). It is well known that aneuploidy, the numerical and structural alteration of chromosomes, is a common characteristic of tumor cells [30]. Overall, about 50% of MDS patients possess an abnormal karyotype, highest frequencies being found in patients who have refractory anemia with excess of blasts [3]. The role of aneuploidy in the process of carcinogenesis remains obscure, as both oncogenic as well as tumor suppressor properties have been attributed to cells with an aberrant number of chromosomes [31]. Higher expression of DDR molecules in aneuploid MDS cases raises the possibility that even in early stages of carcinogenesis, aneuploidy is proportional to genetic instability [32,33] probably through oncogene induced DNA DSBs [6]. On the other hand, higher expression of DDR molecules might be the result of differential gene expression and stress response in aneuploid cells, as has been recently described in yeast and mammalian cells [34] and this could explain the positive correlation of pNBS1 expression with intermediate and high cytogenetic risk cases, in which a wide variety of different karyotypes are included. Interestingly, in all three MDS cases with trisomy 8, high expression of pNBS1 was found, suggesting a correlation between MYC oncogene overexpression and its transcriptional target, NBS1 [35].

Our results suggest that overexpression of the activated form of key molecules of DDR machinery might have an adverse effect on prognosis in MDS patients. In fact, univariate analysis ($n = 70$) indicates that increased expression of pNBS1 and γH2AX is associated with poor prognosis ($P = 0.004$ and 0.009, respectively), whereas
multivariate analysis (n = 67) confirmed this association only for increased expression of pNB1 (P = 0.024). Moreover, pATM over-expression was associated in univariate analysis with a trend for reduced overall survival (P = 0.082). As suggested earlier for head and neck squamous cell carcinomas [36], NBS1 may act as an oncoprotein, participating in the MDS-related development of AML, possibly through the activation of PI3K/AKT, a pathway that is activated in high risk MDS [37]. In accordance, NBS1 overexpression has been associated with worse prognosis in uveal melanomas [38], whereas NBS1 defect is negatively related to survival in breast cancer patients, indicating a tumor suppressor function in the context of that disease [39].

In conclusion, our results show that key molecules of DNA damage response pathway are expressed in their activated form in MDS and AML. Further studies are needed to better elucidate the role of this pathway in MDS and AML pathogenesis. Activated NBS1 may be a potential independent prognostic factor by means of standard immunostaining during initial MDS diagnosis, thus identifying patients with poor prognosis; these high risk patients may further benefit from therapeutic targeting of phosphorylated NBS1, as has been shown ex vivo for BCR/ABL+ leukemic cells [40], an option that needs to be supported by additional studies.

Conflict of interest statement

Nothing to report.

Role of the funding source

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Contributions. MK, SGP and PGF provided the conception and design of the study, acquisition, analysis and interpretation of data; drafting the article and revising it critically for important intellectual content.

MK and PGF performed experiments.

CKK carried out the statistical analysis and drafted the manuscript.

PE and AT supplied the analysis of data.

VP, IGP, VGG, EP were responsible critically for important intellectual content of the article.

All authors have read and approved the submitted form of the manuscript.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.leukres.2013.08.018.

References


