Centrosome Aberrations in Acute Myeloid Leukemia are Correlated to Cytogenetic Risk Profile

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ABSTRACT

Genetic instability is a common feature in acute myeloid leukemia (AML). Recently, centrosome aberrations have been described as a possible cause of aneuploidy in many human tumors. To investigate whether centrosome aberrations correlate with cytogenetic findings in AML, we examined a set of 51 AML patients using a centrosome-specific antibody to pericentrin. All 51 AML analyzed displayed numerical and structural centrosome aberrations (36.0 ± 16.6%) as compared to peripheral blood mononuclear cells from 21 healthy volunteers (5.2 ± 2.0%; p<0.0001). In comparison to AML patients with normal chromosome count, the extent of numerical and structural centrosome aberrations was higher in patients with numerical chromosome changes (50.5 ± 14.2% vs 34.3 ± 12.2%; p<0.0001). When the frequency of centrosome aberrations was analyzed within cytogenetical defined risk groups, we found a correlation of the extent of centrosome abnormalities to all three risk groups (p=0.0015), defined as favorable (22.5 ± 7.3%), intermediate (35.3 ± 13.1%) and adverse (50.3 ± 15.6%). These results indicate that centrosome defects may contribute to the acquisition of chromosome aberrations and thereby to the prognosis in AML.
INTRODUCTION

Genetic instability is a common feature in acute myeloid leukemia (AML). Balanced chromosomal translocations such as t(15;17), t(8;21) and inv(16)/t(16;16) lead to leukemia-specific fusion-transcripts without gain or loss of genetic material whereas unbalanced chromosome abnormalities result in gains and losses of whole chromosomes or parts thereof (1, 2). In AML, both numerical and structural chromosome aberrations have been shown to provide information about the clinical course. In large AML clinical trials, especially numerical chromosome aberrations like losses of chromosomes 5 and 7 as well as complex aberrations were identified as adverse prognostic factors for survival. Defects in chromosome number are thought to occur through missegregation of chromosomes (3), but the mechanism by which this occurs has not been elucidated. There are many potential mitotic targets, which could cause unequal segregation of chromosomes, among those chromosomal, spindle microtubule, and centrosomal defects (4). Recently, centrosome aberrations have been described as a possible cause of numerical chromosome abnormalities in many human tumors (5-13). As the primary microtubule organizing center of most eukaryotic cells, the centrosome assures symmetry and bipolarity of the cell division process, a function that is essential for accurate chromosome segregation (4).

To investigate whether centrosome aberrations do occur in AML and correlate with cytogenetically defined subgroups, we examined a set of 51 patients with AML using a centrosome-specific antibody to pericentrin (14).
PATIENTS AND METHODS

Specimen selection. We examined a set of 51 patients with AML according to the French-American-British (FAB) classification (15). All patients were diagnosed and treated in our institution between July 2000 and February 2002. Cytogenetic studies were performed after short term cultures using standard protocols for G- or R-banding techniques. Karyotype changes were interpreted according to the 1995 ISCN nomenclature (16).

Centrosome staining. For enrichment of mononuclear cells, heparin was added to leukemia samples and centrifuged with Biocoll separating solution (Biochrom, Berlin, Germany). For centrosome immunostaining, cytospins were prepared. The cells were mounted on coated slides and fixed in -20°C acetone for 10 min, permeabilized in 0.2% Tween 20 for 5 min, blocked in PBS containing 1% BSA and 1% human immunoglobulin for 1 h, followed by standard indirect immunohistochemistry. Briefly, cytospins were stained using a polyclonal antibody to pericentrin (Convance, Richmond, CA). The slides were washed in PBS 3 times for 5 min each. The antibody-antigen complexes were detected by incubation for 1 hr at room temperature with a FITC-conjugated secondary goat anti-mouse IgG antibody (Convance, Richmond, CA). The slides were washed in PBS 3 times for 5 min each again. Slides were mounted with phosphate buffered glycerol (Euroimmun, Lübeck, Germany) and visualized under a fluorescence microscope (Axioskop, Zeiss, Jena, Germany) using a 100 x objective.

Calculation of centrosome aberrations. Immunostaining of centrosomes was judged satisfactory when the characteristic single or paired centrosome pattern was detected in negative controls. Centrosomes were considered structurally abnormal if they had a diameter at least twice that of centrosomes in nonmalignant control cells and numerically abnormal if they were present in numbers > 2 as described previously (11). At least 200 consecutive cells per sample were carefully examined.
Statistical analysis. Differences in the number of cells with centrosome aberrations among cytogenetically defined subgroups were analyzed by the application of the *t-test* for independent samples. All statistical analyses were performed by the statistical software SPSS for Windows, release 6.1.3 (SPSS Inc., Chicago, IL).
RESULTS AND DISCUSSION

To investigate whether centrosome aberrations do occur in AML and correlate with cytogenetically defined subgroups, we examined a set of 51 patients with AML using indirect immunofluorescence with an antibody to pericentrin. Our data set consisted of 30 peripheral blood and 21 bone marrow samples obtained from AML patients at the time of diagnosis (n=41) or at relapse (n=10). In particular, there were 27 males and 24 females with a median age of 60 years (range, 18 - 80). A total number of 33 patients were registered as having de novo AML, while 18 patients with secondary AML were analyzed. Cytogenetic information was available for 48 of 51 patients. The karyotype classification was similar to that used in other series (1, 2). Analogous to the MRC AML 10 trial, all AML patients with a specific abnormality were considered, irrespective of the presence of additional or secondary cytogenetic changes (1).

To determine whether blasts from AML patients harbor abnormal centrosomes, we analyzed 51 AML patients and compared the centrosome patterns with peripheral blood mononuclear cells from 21 healthy volunteers, including 8 males and 13 females with a median age of 24 years (range, 22 - 58). All 51 AML samples analyzed displayed numerical and structural centrosome aberrations as compared to the controls (Figure 1). Centrosome abnormalities were detectable in 36.0 ± 16.6% of the blasts in AML but in only 5.2 ± 2.0% of the controls (p<0.0001). These findings indicate that centrosome defects are a common feature of AML. Consistently, centrosome aberrations have been previously reported in solid tumors of different origin including brain, breast, lung, colon, prostate, pancreas, bile duct and head and neck (5-11). Hematological malignancies like non-Hodgkin’s lymphomas and myelodysplastic syndromes also display centrosome aberrations at high frequencies (12-13).
To test for the hypothesis that centrosome abnormalities are associated with chromosome aberrations in AML, we compared the centrosome aberration patterns of 48 AML patients with available karyotype information. In comparison to 34 AML patients with normal chromosome count, the extent of numerical and structural centrosome aberrations was higher in 14 AML patients with numerical chromosome changes (50.5 ± 14.2% vs 34.3 ± 12.2%; p<0.0001). In line with this finding, recent studies have provided evidence that centrosome aberrations result in chromosome missegregation and may lead to malignant transformation (17-19). Specifically, centrosome hyperamplification, induced by p53 mutations or Mdm2 overexpression, has been shown to induce aneuploidy (17). In another study, overexpression of tumor amplified kinase STK15/BTAK induced centrosome amplification, aneuploidy, and malignant transformation (18). In addition, it has been demonstrated that centrosome duplication in somatic cells is controlled by the phosphorylation status of the retinoblastoma (Rb) protein, release of the transcription factor E2F from Rb binding, and subsequent activation of cyclin-dependent kinase (cdk) 2 in late G1 phase of the cell cycle (20-22).
Consequently, the commonly observed abrogation of the p53 and Rb pathways in human malignancies including AML will not only facilitate progression towards DNA replication, but may also deregulate the centrosome duplication cycle (4, 23, 24).
Table 1. Centrosome aberrations in acute myeloid leukemia are correlated to cytogenetic risk profile

<table>
<thead>
<tr>
<th>Abnormality</th>
<th>Total No.</th>
<th>Median number (range) of blasts with abnormal centrosomes in %</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>numerical</td>
</tr>
<tr>
<td>Normal controls*</td>
<td>21</td>
<td>0.5 (0.0 - 2.0)</td>
</tr>
<tr>
<td>AML patients (total)**</td>
<td>48</td>
<td>10.8 (5.0 - 24.5)</td>
</tr>
<tr>
<td>Favorable risk</td>
<td>5</td>
<td>8.5 (3.5 - 16.0)</td>
</tr>
<tr>
<td>t(15;17)</td>
<td>1</td>
<td>10.5</td>
</tr>
<tr>
<td>t(8;21)</td>
<td>2</td>
<td>5.3 (3.5 - 7.0)</td>
</tr>
<tr>
<td>inv(16)</td>
<td>2</td>
<td>12.3 (8.5 - 16.0)</td>
</tr>
<tr>
<td>Intermediate risk</td>
<td>32</td>
<td>9.5 (5.0 - 17.5)</td>
</tr>
<tr>
<td>No abnormality</td>
<td>21</td>
<td>11.0 (5.0 - 17.5)</td>
</tr>
<tr>
<td>+4</td>
<td>1</td>
<td>12.0</td>
</tr>
<tr>
<td>+8</td>
<td>2</td>
<td>11.3 (7.5 - 15.0)</td>
</tr>
<tr>
<td>+9</td>
<td>1</td>
<td>9.5</td>
</tr>
<tr>
<td>+13</td>
<td>1</td>
<td>6.5</td>
</tr>
<tr>
<td>Only structural</td>
<td>6</td>
<td>8.0 (7.5 - 16.0)</td>
</tr>
<tr>
<td>Adverse risk</td>
<td>11</td>
<td>14.5 (9.5 - 24.5)</td>
</tr>
<tr>
<td>Complex</td>
<td>6</td>
<td>15.5 (9.5 - 18.0)</td>
</tr>
<tr>
<td>-5</td>
<td>1</td>
<td>24.5</td>
</tr>
<tr>
<td>-7</td>
<td>3</td>
<td>14.0 (10.5 - 14.5)</td>
</tr>
<tr>
<td>del(5q)</td>
<td>1</td>
<td>16.0</td>
</tr>
</tbody>
</table>

* Peripheral blood mononuclear cells from healthy volunteers were analyzed as controls.
**Analogous to the MRC AML 10 trial, all AML patients with a specific abnormality were considered, irrespective of the presence of additional or secondary cytogenetic changes.
Since karyotype changes provide prognostic information in AML (1), we correlated centrosome abnormalities to cytogenetical defined risk groups according to the MRC AML 10 Trial as shown in Table 1. We found a statistically significant correlation of the extent of centrosome abnormalities to all three risk groups (p=0.0015), defined as favorable (22.5 ± 7.3%), intermediate (35.3 ± 13.1%) and adverse (50.3 ± 15.6%). This difference was mainly due to structural rather than numerical centrosome aberrations, fitting nicely to the ultrastructural observation that in human breast cancers anaplastic morphology and abnormal mitoses correlate to excess pericentriolar material rather than to an increase in centriole or centrosome numbers (25). The description of p53 and Rb pathway alterations in AML patients with an inferior prognosis and unfavorable cytogenetics further suggests a pathophysiological link to the induction of centrosome aberrations (23, 24).

In conclusion, our results indicate that centrosome defects are a common feature of AML and suggest that they may contribute to the acquisition of an increasing karyotypic instability. Since the extent of centrosome abnormalities correlates to cytogenetically defined risk groups in AML, the prognostic importance of centrosome patterns should be studied in prospective trials.
ACKNOWLEDGEMENTS

We would like to thank Mrs Brigitte Schreiter for excellent technical assistance.
REFERENCES


FIGURE LEGENDS

Figure 1. **Centrosome aberrations in acute myeloid leukemia.** Indirect immunofluorescence staining of normal bone marrow (A) and acute myeloid leukemia cells (B, C). Cells were immunostained with an antibody to pericentrin, followed by a FITC-conjugated secondary antibody.
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