Molecular and cellular aspects of extramedullary manifestations of acute myeloid leukemia

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ABSTRACT

The myeloid extramedullary tumor is a solid tumor formed by infiltration of immature myeloid cells in various tissues of the body. This tumor is also identified as chloroma or myeloid sarcoma (MS). MS is a manifestation of acute myeloid leukemia (AML) occurring at presentation or during treatment or relapse. MS is associated with multiple chromosomal abnormalities and molecular mutations since patients with these disorders bear a high potential for MS manifestation. There is a high incidence of extramedullary infiltration (EMI) in AML. AML patients with EMI have a worse prognosis than patients without it. Hematopoietic stem cells and leukemic stem cells reside in a special bone marrow microenvironment called niche, which is essential for their normal functions. Cancers are exploited dysfunctional cell-cell and matrix-cell interactions, which convert a normal niche into a neoplastic niche. This study summarizes the current knowledge on the molecular and cellular characteristics of AML with EMI and extramedullary niches in AML patients.

Key words: Acute myeloid leukemia; extramedullary infiltration; niche

INTRODUCTION

Acute myeloid leukemia (AML) is an aggressive myeloid neoplasm characterized by maturation arrest of myelopoiesis leading to an accumulation of myeloblasts in the blood and bone marrow (BM).[1] AML is a complex and heterogeneous disease strongly associated with genetic and epigenetic changes in the hematopoietic progenitors.[2] These changes lead to disruption of several signaling pathways that result in increased proliferation, survival and accumulation of leukemic cells.[3]

Normal hematopoietic stem cells (HSCs) reside in a specialized area of the BM microenvironment known as niche, which regulates their survival and function. Two distinct niches exist in the BM: Vascular and endosteal/osteoblastic niche. The vascular niche is localized in close proximity to the osteoblastic niche, at the inner surface of bone cavity with abundant bone-forming osteoblasts. The vascular niche is composed of sinusoidal endothelial cells lining blood vessels, and it promotes the proliferation and differentiation of short-term HSCs. The endosteal niche includes osteoblasts, osteoclasts, glial non-myelinating Schwann cells and regulatory T-cells, and it is located in the endosteme. The vascular niche contains CXCL12-abundant reticular cells, nestin-positive mesenchymal stem cells and leptin receptor-positive cells.[4] HSC niches are present in different tissues during development, first in the aorta-gonad-mesonephros (AGM) region and yolk sac, then in the placenta, fetal liver, spleen and BM. After birth, the BM is the primary site of HSC maintenance and hematopoiesis, but the niche can shift to extramedullary sites in response to hematopoietic stress.[5]

AML may present with extramedullary-AML at initial diagnosis or in relapse. Myeloid sarcoma (MS) is defined as an extramedullary mass composed of myeloid blasts occurring in anatomic sites other than BM.[6] Extramedullary...
infiltration (EMI) is fairly common in AML patients. In addition, MS has been observed in all age groups, and may occur anywhere in the body. The most common tissues include soft tissues, bone, peritoneum, lymph nodes and gastrointestinal tract. Other occasional sites include male and female urogenital system and central nervous system (CNS).[7] Moreover, several studies have found a worse prognosis in cases of acute leukemia with EMI, which may be explained by a poor response to chemotherapy and disease relapse.[8] Evaluation of the cellular and molecular structures of extramedullary niches, as well as the migration and homing of leukemic cells, may help in designing diagnostic and therapeutic techniques and preventing relapse. However, there is still little information in this regard. The aim of this study was to investigate the characteristics of leukemic cells and the changes in their microenvironment that promote to EMI.

**GENETIC AND MOLECULAR FEATURES OF EXTRAMEDULLARY INFILTRATION IN AML**

Extramedullary leukemia (EML) is also called MS, granulocyte sarcoma and chloroma. In the WHO classification, MS is an important subgroup of myeloid neoplasias and acute leukemia. MS may occur simultaneously with, before or after the diagnosis of AML.[9] Genetic mutations and molecular aberrations are an important tool for the evaluation of acute leukemia and assessment of prognosis. However, there is very limited information on the role of genetic mutations in MS.[10] Although the overall incidence of MS in AML has been reported at 1.4-9%, it is particularly high in some subtypes of AML, reaching 18-24% in AML patients with t(8:21) and 25% in pediatric AML.[11] Other genetic abnormalities diagnosed in EML patients include t(15:17), t(9:11), t(1:11), t(8:17), del(16q), del(5q), del(20q), monosomy 7, trisomy 4 and inv(16).[12] According to studies, the involvement in this type of cytogenetic abnormalities has been limited to chest and uterus.[19,20] Furthermore, another study suggests a link between MLLr and lymph node involvement.[21] More studies are needed to confirm these observations.

Molecular abnormalities associated with EML have not been systematically defined; however, a well-documented molecular abnormality is a mutation in the nucleophosmin (NPM-1) gene.[12] Nucleolar phosphoprotein or NPM-1 is localized in nuclear foci and is a multifunctional protein expressed in various cells.[22] NPM-1 gene mutation is the most common molecular genetic abnormality in AML, particularly AML with normal karyotype.[23] NPM-1 is mutated in almost 15% of cases of MS.[24] In a survey conducted on 89 AML patients, 15 patients (18%) had extramedullary manifestation at diagnosis, and 13 of them (87%) had mutated NPM-1.[25]

FMS-related tyrosine kinase 3-internal tandem duplication (FLT3-ITD) mutation is observed in 28-34% of cases of AML with normal cytogenetics. It plays an important role in cell proliferation, survival and differentiation of hematopoietic progenitor cells.[26] Some studies have found an association between FLT3-ITD mutation and EML, so that in one study, 15% of MS patients have this mutation.[27,28]

CD56, a neural cell-adhesion molecule, is expressed in normal, natural killer cells. Abrassent expression of CD56 in AML blasts, particularly AML with translocation t(8:21) correlates with a worse prognosis than CD56-negative cases.[29] An association has been described between the expression of CD56 and EMI, especially in lymph nodes (lymphadenopathy).[21,30,31] CD56 gene is in the 11q23.1 locus.[22] Due to this fact and to the connection between 11q23 mutation and EMI, MLLr is likely associated with aberrant expression of CD56 in EML. Some case reports and studies support this hypothesis.[26,21,33]

Minimal residual disease (MRD) assessment is an important feature of therapy management, especially in cases whose recurrence risk is high. There is not much information on MRD in MS patients, and only one study has evaluated the correlation between continuous detection of AML1-MTG8 chimeric transcripts in BM and peripheral blood, and extramedullary relapse in t(8:21) AML.[34]
Available information indicates that the prognosis of EML is poor with short overall survival. In an evaluation, the 5-year survival rate for patients with MS was 21%. Patients treated with chemotherapy showed longer survival than untreated patients. Although the mortality rate of acute leukemia patients has been reduced with the emergence of new therapies, many patients still suffer from refractory disease or relapse, and EMI is one of the main causes of poor prognosis in these patients.

**EXTRAMEDULLARY NICHE IN AML**

During development, HSCs are initially present in AGM and then migrate into the fetal liver and embryonic bone, which remains the only active site of hematopoiesis in adult life. Movement and homing of HSCs in the BM is associated with CXCL12 chemokine and its receptor CXCR4. Cancer subverts cell-cell and matrix-cell interactions and converts the normal niche to a neoplastic one.

ITD-FLT3 mutation, which is common in AML and MS patients, leads to deregulation of CXCR4 in AML leukemic cells since CXCR4 signaling is markedly decreased in patients with ITD-FLT3 compared with patients without it. It is thought that this mutation facilitates the infiltration of leukemic cells into visceral organs by reducing the homing of leukemic cells. Infiltration of leukemic cells in other organs is likely associated with chemokine receptor expression and different adhesion molecules. For example, NCAM1 or CD56, which is associated with a high incidence of MS, is highly expressed in the breast, testicular tissue, ovary and gut. This molecule is responsible for homing of leukemic cells in these tissues. Moreover, AML blasts isolated from skin show a group of specific chemokine receptors including CCR5, CXCR4, CXCR7, and CX3CR1 compared with AML blasts isolated from blood and BM. These cytokine-chemokine receptor interactions enable homing and survival of AML blasts in skin [Figure 1].

Expression of matrix metalloproteases may contribute to the increased incidence of EMI in some subtypes of AML. For example, in SHI-1 cells, a highly invasive human acute monocytic leukemia cell line, there is a high expression level of matrix metalloproteinase 2 (MMP-2), membrane-type 1 MMP and tissue inhibitor of metalloproteinase, which facilitate cell invasion. Moreover, it has been suggested that the specific binding of MMP-9 via its procatalytic domain to leukocyte surface I domains of beta-2 integrins is essential for precellular proteolysis and migration of AML-derived cells [Table 1].

**MICRONRNAS’ SIGNIFICANCE IN EXTRAMEDULLARY AML**

MicroRNAs (miRNAs) are small, 18-25 nucleotide non-coding RNA molecules, which regulate gene expression by hybridizing to their complementary messenger RNA. Each miRNA has the potential to regulate several different transcripts through partially complementary target sequences. miRNAs participate in cell differentiation, proliferation and carcinogenesis. Several studies have shown that miRNAs play key roles in normal hematopoiesis and various hematological malignancies. Different miRNAs are also known in myelopoiesis and myeloid neoplasias like AML. Functional studies have shown that miRNAs play an important role in the pathogenesis of AML as either oncogenes or tumor suppressors. It has also been shown that distinct miRNA expression signatures are associated with response to chemotherapy and clinical outcomes. Based on our literature and database searches, there has been no study describing miRNA signatures in MS. However, some studies show a link between miRNAs and mutation-induced MS. MiR-100 is aberrantly expressed in a number of cancer cells, including AML cells. Increased expression of miR-100 in AML is associated with maturation block. In vitro studies indicate that increased expression of miR-100 in AML cells inhibits retinoblastoma 1 serine phosphates from human chromosome 3 and causes the release of E2F in addition to increased levels of phosphorylated retinoblastoma. These events induced proliferation and inhibited the differentiation of granulocyte/monocyte cells. In a study performed on 106 pediatric AML patients, it showed that this miRNA was associated with AML with extramedullary manifestation.

High expression level of miR-10 family is associated with AML with mutated NPM-1. Furthermore, miR-424 in AML patients with NPM-1 mutation was down-modulated. In AML patients with the FLT3-ITD mutation, miR-451 and miR-144 were down-regulated while miR-155 was overexpressed. As previously mentioned, NPM-1 and FLT3-ITD mutations, as well as some other cytogenetic abnormalities, are associated with increased risk of EMI. In summary, deregulated miRNAs in these disorders can be considered as candidate markers for future studies in MS patients [Table 2].
**Table 1: Evaluation of AML patients with extramedullary infiltration**

<table>
<thead>
<tr>
<th>Age (years), sex</th>
<th>Cytogenetics</th>
<th>Molecular test</th>
<th>CD markers</th>
<th>Extramedullary site</th>
<th>Subtype</th>
<th>Prognosis</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>51, female</td>
<td>46XX (50%)/45XX del (5)(q13q33), -7, add (15)(q22), -18</td>
<td>WT1+</td>
<td>MPO+, CD3+, CD20-, TdT-</td>
<td>Genital area</td>
<td>AML with multilineage dysplasia</td>
<td>Poor</td>
<td>[42]</td>
</tr>
<tr>
<td>16, female</td>
<td>PML-RAR at (15;17)</td>
<td>CD13+(85%), CD15+, CD33+, CD117+, CD34+, HLA-DR+, HLA-DR+, CD4+, CD11c+, CD13+, CD15+, CD33+, CD117+, CD34+, CD56+, CD45+</td>
<td>Right humerus, right proximal femur and distal tibia</td>
<td>Pulmonary</td>
<td>M5</td>
<td>[43]</td>
<td></td>
</tr>
<tr>
<td>19, male</td>
<td>11q23 (MLL-AF10) rearrangement, low-level</td>
<td>CD117+, CD33+, CD15+, CD4+, CD64+, CD4+, CD56+, CD64+</td>
<td>Left breast</td>
<td>M4</td>
<td>Poor</td>
<td>[20]</td>
<td></td>
</tr>
<tr>
<td>42, female</td>
<td>t(8;21)-RUNX1-RUNXIT1</td>
<td>FLT3 inhibition FLT3-ITD</td>
<td>MPO+, CD34+, Ki67 (60-70%)</td>
<td>Auditory canal</td>
<td>M2</td>
<td>M2</td>
<td>[45]</td>
</tr>
<tr>
<td>29, female</td>
<td>47, XX, +8, t(9;11) (p22; q23)</td>
<td>MLL-AF9 fusion gene</td>
<td>CD117+, CD33+, CD38+, CD15+, CD64+, CD4+, CD56+</td>
<td>Left and right breast</td>
<td>M4</td>
<td>CR</td>
<td>[20]</td>
</tr>
<tr>
<td>28, female</td>
<td>No overt cytogenetic aberration was shown</td>
<td>MLL-AF9 fusion gene</td>
<td>CD117+, CD33+, CD34+, CD38+, MPO+, HLA-DR+</td>
<td>Gastric</td>
<td>M5</td>
<td>CR</td>
<td>[46]</td>
</tr>
<tr>
<td>12, female</td>
<td>t(9;11)(p22;q23)</td>
<td>MLL gene rearrangement</td>
<td>CD45+, CD33+, CD4+, CD8, alpha-1-antitrypsin+, muramidase+, HLA-DR+</td>
<td>Abdomen</td>
<td>M5a</td>
<td>Poor</td>
<td>[47]</td>
</tr>
<tr>
<td>15, female</td>
<td>normal female karyotype - t(9;11)(p22;q23)</td>
<td>MLL gene rearrangement</td>
<td>CD45+, CD33+, CD117+, CD4+, CD1a+, CD13+, CD34+, CD33+, MPO+, HLA-DR+</td>
<td>Skin</td>
<td>M5</td>
<td>CR</td>
<td>[47]</td>
</tr>
<tr>
<td>10, male</td>
<td>45, X,-Y, del (2)(p21), t(8;21), q22, q22</td>
<td>MLL gene rearrangement</td>
<td>CD13+, CD33+, CD34+, CD117+, CD64+, CD4+, CD56+, MPO+</td>
<td>Appendix</td>
<td>M2</td>
<td>CR</td>
<td>[48]</td>
</tr>
<tr>
<td>38, female</td>
<td>t(6;21)</td>
<td>MLL gene rearrangement</td>
<td>CD13+, CD33+, CD34+, CD117+, CD64+, CD4+, CD56+, MPO+</td>
<td>Gastric</td>
<td>M4</td>
<td>Poor</td>
<td>[33]</td>
</tr>
<tr>
<td>57, male</td>
<td>47, XY, +8, t(9;11) (p22; q23)</td>
<td>11q23, tetrasomy 8</td>
<td>CD4+, CD13+, CD16+, CD33+, CD56+ and</td>
<td>Forearm and thigh</td>
<td>M5a</td>
<td>No relapse during chemotherapy</td>
<td>[33]</td>
</tr>
<tr>
<td>69, female</td>
<td>t(8;17), t(17;17)</td>
<td>HLA-DR+, MPO+, CD43+, CD33+, CD13+, CD65+, CD4+, CD34+, CD117+, CD34+, CD33+, CD56+ and MPO+</td>
<td>Eye, Pancreas</td>
<td>M1</td>
<td>Poor</td>
<td>[49]</td>
<td></td>
</tr>
<tr>
<td>47, male</td>
<td>t(15;17)</td>
<td>PML-RARA</td>
<td>MPO+</td>
<td>M2</td>
<td>Poor</td>
<td>[50]</td>
<td></td>
</tr>
<tr>
<td>1, male</td>
<td>t(15;17)</td>
<td>PML-RARA</td>
<td>MPO+</td>
<td>M2</td>
<td>Poor</td>
<td>[50]</td>
<td></td>
</tr>
<tr>
<td>13, female</td>
<td>47, XX, +21 and 46, del (x)(q22)</td>
<td>CD45+, CD117+, CD34+, CD43+, CD68+</td>
<td>Cardiac</td>
<td>M5</td>
<td>Poor</td>
<td>[52]</td>
<td></td>
</tr>
</tbody>
</table>

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CONCLUSION

EMI is a relatively common manifestation of AML, with increased incidence in specific subtypes.[7] Despite advances in the diagnosis and treatment of myeloid leukemias, there is insufficient information on the diagnosis, treatment and pathogenesis of EML.[38] Molecular and cellular studies of EML cases, as well as evaluation of the differences between AML patients with and without EMI, have revealed some features of EML. Elucidating the relationship between genetic abnormalities and sites prone to infiltration may contribute to the prevention and early detection of EML in target tissues. In many cases, MS is misdiagnosed at first, with the most common alternative diagnoses being lymphoma, melanoma, extramedullary hematopoiesis and inflammation. Given the aggressive nature of MS, early diagnosis with sensitive and specific tests is vital to these patients.[9] Available information suggests that ITD-FLT3 mutations, which are prevalent in patients with EML, may play an important role in the pathogenesis of disease. Therefore, ITD-FLT3 mutation scan should be evaluated as a diagnostic and prognostic factor in patients. Moreover, NPM-1 mutation, which also has a high prevalence in EML, should be evaluated as a prognostic test.

According to case report studies, common CD markers in EML include CD13, CD33, CD34, CD117, myeloperoxidase (MPO), CD56 and CD68; these should be considered in immunophenotype assessment of the disease [Table 1]. In a study conducted on MS patients, similar results were indicated, and CD68/KP1 was the most common positive marker in 100% of patients. Other markers, in order of positive frequency, were: MPO (83.6%), CD117 (80.4%), CD99 (54.3%), CD68/PG-M1 (51%), CD34 (43.4%), terminal deoxynucleotidyl transferase (31.5%), CD56 (13%), CD61 (2.2%), CD30 (2.2%) and CD4 (1.1%).[70] These data can be useful to develop a diagnostic immunophenotyping panel for MS patients.

Allogeneic hematopoietic stem cell transplantation (HSCT) is increasingly used as treatment procedure for AML patients, but there are no standard procedures for EML therapy. Furthermore, HSCT not only is not an effective procedure for EML, but it can also increase the risk of EML relapse in AML patients.[71] Studies reviewed in this article suggest that cases of AML that have blasts with relatively specific characteristics have a high-risk for non-hematopoietic tissue infiltration. These features may be very helpful in distinguishing patients susceptible to EMI. Further studies are needed to develop diagnostic and therapeutic standards for patients with EMI as well as sensitive and specific prognostic biomarkers.

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Conflicts of interest

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