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IMMUNOPHENOTYPIC FEATURES OF LEUKEMIC STEM CELLS AND BULK OF BLASTS IN ACUTE MYELOID LEUKEMIA

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According to the modern concept, leukemic stem cells (LSC) in acute myeloid leukemia (AML) are distinct from the bulk of leukemic cells in bone marrow and peripheral blood of AML patients. Nevertheless, LSC are responsible for managing all the hierarchy of the bulk of leukemic blast populations. This mini-review provides brief information on the distinctive features of LSC and blast cells in cytologically recognized types of AML. The study of different phenotypes of LSC and blast cells in AML with the aid of up-to-date flow cytometric techniques is important both for the deep insight into the mechanisms of leukemogenesis and development of novel strategies of target therapy. The urgent need for extending the diagnostic panel of monoclonal antibodies used for diagnosing AML is beyond doubt.

Key Words: **acute myeloid leukemia, leukemic stem cells, bulk of blast cells, hematopoietic stem cells, targeted therapy, hematopoiesis.**

The study of antigen expression patterns in leukemic stem cells (LSC) and blast cells in different types of acute myeloid leukemia (AML) has a great significance for understanding the mechanisms of leukemogenesis and developing novel strategies of target therapy.

Human LSC were the first cancer stem cells discovered with the aid of xenogeneic transplantation into severe combined immunodeficient (SCID) mice and characterized with the panel of monoclonal antibodies to the differentiation antigens of human leukocytes by flow cytometry technique [1–5]. When the leukemic cells from the bone marrow and the peripheral blood of the patients with different types of AML diagnosed in accordance with French-American-British (FAB) classification were transplanted to SCID mice, it was convincingly demonstrated that the bulk of leukemic cells were not capable to proliferate and to develop the leukemic infiltrates in recipient animals. In fact, only few cells in the fraction of $CD34^+CD38^-$ representing not more than 0.2–1.3% of the total leukemic cells in AML patients possess clonogenicity [2, 3, 6–9]. Later on, it was proved that the origin of different types of AML is associated with the mutations accumulated in the normal hematopoietic stem cells (HSC), multipotent progenitor cells or committed progenitor cells such as cells of GM-CFU type and megakaryocyte-erythroid progenitor cells [1, 4].

LSC being in the origin of the clonal growth in different types of AML are the cells responsible for the propagation of the bulk of leukemic blasts. The latter represent more committed progeny or derivatives of LSC. LSC constitute a very small proportion of heterogeneous leukemic population in bone marrow and peripheral blood in AML patients. Nevertheless, it is these cells that are responsible for the relapse of the disease and resistant to the treatments available now [10, 11].

Similarly to HSC in normal hematopoiesis, LSC in AML have a limitless self-renewal capacity and high proliferation potential. Besides, LSC differentiate into more committed blast cells that ultimately sustain the entire mass of leukemic cells in bone marrow and blood of AML patients [4].

The comparison of HSC and LSC is essential to understand the precise disease driving mechanisms in AML. The data on the marker antigens for identifying HSC and myeloid progenitor cells in normal hematopoiesis are summarized in Table 1.

Table 1. Marker antigens for identifying HSC and myeloid progenitor cells in normal hematopoiesis [12–15]

HSC	CD34, CD90, CD117, CD123, CD164, CD166, HLA-DR, CD172, CD173, CD174, CD175, CD176, CD224, CD227, CD239
Multipotent progenitor cells (CFU-GMEMeg)	CD34, CD38, HLA-DR, CD45RA, CD33, CD117, CD123, CD213, CD230
Cell progenitors of granulocytes-monocytes (CFU-GM)	CD34, CD38, HLA-DR, CD45RA, CD33, CD13, CD15, CD64, CD115, CD116, CD123, CD131, CD183, CD213, CD230
Cell progenitors of granulocytes (CFU-G)	CD13, CD15, CD32, CD33, CD35, CD66, CD89, CD116, CD123
Cell progenitors of monocytes (CFU-M)	CD13, CD15, CD33, CD115, CD116, CD123, CD213, CD230
Early erythroid progenitors (BFU-E)	CD34, CD38, HLA-DR, CD45RA, CD33, CD71, CD36, CD238
Early megakaryocyte progenitors (CFU-Meg)	CD34, $CD38^{low}$, HLA-DR, CD33, CD41, CD61

Among the antigens given in Table 1, CD34, CD38, and HLA-DR are in focus of attention of oncohematologists. At present, CD34 is recognized as one of the most important markers of HSC that is widely used for isolation of stem cells and hematopoietic progenitor cells from bone marrow and peripheral blood with the aim of auto- and allogeneic transplantation.

Normal HSC as well as the majority of LSC are CD38-negative. The lineage commitment of HSC is as-

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Abbreviations used: AML – acute myeloid leukemia; HSC – hematopoietic stem cells; LSC – leukemic stem cells.

sociated with the decreased expression of CD34 and increased level of CD38 detection. HSC lack expression of the histocompatibility antigens class II (HLA-DR).

The materials of ten International Workshops on Differentiation Antigens of Human Leukocytes (including the recent one held in 2014) may be useful for characterizing immunophenotype of LSC. According to [1–13], the marker antigens for identifying LSC in AML are such as follows: $CD34^+$, $CD38^-$, $HLA\text{-}DR^+$, $CD25^+$, $CD26^+$, $CD32^+$, $CD36^+$, $CD44^+$, $CD45RA^+$, $CD47^+$, $CD71^+$, $CD90^+$, $CD96^+$, $CD99^+$, $CD117^+$, $CD123^+$, $CD133^+$, $IL\text{-}1RAP^+$, $CD184^+$, $CD366^+$, $CD371^+$.

It is worth note that only limited set of markers characteristic for HSC and LSC are routinely used for immunophenotyping blast cells for diagnosing AML (both AML with recurrent genetic abnormalities and AML not otherwise specified) (Table 2).

Table 2. Panel of monoclonal antibodies for diagnosing AML (WHO, 2016) [14–17]

Markers of hematopoietic progenitor cells	CD34, CD38, HLA-DR, TdT, CD117, CD71
AML	CD34, CD38, HLA-DR, CD13, CD33, CD7, CD11b, CD11c, CD14, CD15, CD65, CD64, CD36, CD68, CD163
Acute erythroid leukemia	CD71, CD36, Gly-A
Acute megakaryoblastic leukemia	CD61, CD41, CD42

After the 5th International Workshop held in 1993 in Boston (USA), such panel was supplemented only with one antigen CD163 (Kobe, Japan, 1998). It would be highly desirable to extend the spectrum of the antigens used for acute leukemia diagnosis utilizing such novel LSC markers as CD366, CD371 and IL-1RAP. At the same time, it should be mentioned that even CD38 is not used routinely for diagnosing AML. Meanwhile, the percentage of $CD34^+CD38^-$ leukemic blasts in bone marrow and peripheral blood seems to correlate to some extent with LSC content that might be useful from practical point of view for prognosis of the disease, monitoring of the minimal residual disease and early detection of the relapses, especially in the setting of resource-poor countries [15, 16, 18, 19].

The said above may be exemplified by the data on immunophenotyping of blast cells in AML with recurrent cytogenetic abnormalities. In AML with t(8;21), the bulk of the blasts strongly expresses CD34 (the major antigen of HSC and LSC). In a subset of blasts, in some cases the maturation pathway towards $CD34^-$ $CD38^{++}$ monocytes is manifested. In AML with inv(16) or t(16;16), the blasts coexpress the immature markers CD34 and CD117. At the same time, monocytic compartment is also identified in some blasts with the expression of CD14 [14].

What is noticeable is that in different studies the relative frequencies of each separate type of AML delineated based on cytomorphological and cytochemical features of the bulk of blast cells in accordance with FAB classification (the group of AML not otherwise specified in WHO classification (2016)) are in fact very close (Table 3).

Table 3. Percentage of distribution of the separate cytological variants of AML diagnosed in different laboratories [15–19]

AML types	Percentage of distribution according to the data given in following references			
	[15]	[16]	[17]	[18, 19]
AML with minimal differentiation (M0)	–*	2–3	< 5	–
AML without maturation (M1)	15–20	15–20	5–10	15–20
AML with maturation (M2)	25–30	25–30	10	30
Acute promyelocytic leukemia (M3)	5–10	5–10	5–8	–
Acute myelomonocytic leukemia (M4)	20–25	15–20	5–10	15–20
Acute monoblastic/monocytic leukemia (M5)	15	10	5	15
Acute erythroid leukemia (M6)	–	3–4	–	–
Acute megakaryoblastic leukemia (M7)	1.2	1	–	–

Note: *Data not given.

What stands out in the data above is rather high coincidence of the detection of expressed antigens on the blast cells in AML patients including those associated with LSC. In particular, the results of wide-scale research provided by S.A. Lugovskaya, M.E. Pochtar, and N.N. Tupitsyn [16] are fully consistent with the data of B. Bain [15] as well as the data on immunophenotypes of corresponding cytological AML variants summarized by WHO experts in [17].

In **AML with minimal differentiation (M0)**, the steady simultaneous expression of CD34, CD38, HLA-DR is evident. Sometimes CD13 and CD117 and less often CD33 are detectable [15]. Myeloperoxidase (MPO) activity is not revealed cytochemically while monoclonal Ab detects the presence of MPO protein. The analogous data on the expression of CD34, CD38, HLA-DR, CD13, and CD117 are given by the authors of the modernized WHO classification [17]. CD33 is expressed in blast cells in 60% of cases; TdT is present in the nuclei of the blast cells in half of the patients.

The phenotype of the blast cells in **AML without maturation (M1)** is $CD34^+HLA\text{-}DR^+CD33^+$. CD117 and sometimes CD116 could be detected. Meanwhile CD13, CD14 and CD15 are not found out. The same immunophenotype of the bulk of leukemic cells in bone marrow and blood of AML M1 patients is given in B. Bain's monograph [15].

In **AML with maturation (M2)**, the cells express frequently CD34, HLA-DR and CD117. In addition, the positive expression of the markers indicative of granulocyte maturation (CD11b, CD15, CD65) is detected. CD13 and CD33 may be found regularly, while CD14 is not present [15]. According to modernized WHO classification (2016), immunophenotype of blast cells is following: $CD34^{+/-}HLA\text{-}DR^+$ and/or $CD117^+$. Besides, one or several antigens associated with myelopoiesis are expressed: CD13, CD33, CD65, CD116, CD15.

The phenotype of the blast cells in **acute myelomonocytic leukemia (M4)** in most cases is $CD34^+CD117^+$, frequently HLA-DR is expressed, and in 30% of the patients the cells are $CD7^+$. In a fraction of the blasts, myeloid antigens CD13, CD33, CD65 and CD15 are expressed. The antigens inherent to the system of mononuclear phagocytes (CD14, CD4,

CD11b, CD11c, CD36, CD68, CD163) also could be detected. The same predominant phenotype of blast cells in bone marrow and blood of AML M4 patients is summarized in B. Bain's monograph [15].

In **acute monoblastic/monocytic leukemia (M5)** expression of CD34 is observed only in 30% of cases. CD117 is detected more frequently and HLA-DR expression is almost common. The distinctive feature is also the presence of at least two markers of those characteristic for monocytic lineage (CD14, CD4, CD11b, CD11c, CD64, CD68, CD36). The markers of myeloid differentiation such as CD13, CD33, CD15, CD65 are also detectable. According to B. Bain [15], the reaction for CD34 and CD117 in the blast cells in AML M5 may be either positive or negative. Also among frequently detected antigens are CD33, CD15, CD11b, CD65. CD14 is also rather frequent. More mature monocytoid cells express CD11c, CD14, CD64 and CD68. CD7 and CD56 are expressed rarely.

In **acute erythroid leukemia (M6)**, most blast cells express glycophorin A (Gly-A). The reaction for CD34, HLA-DR, CD33, CD11b in the blast cells might be either positive or negative. In addition, CD36, CD71, CD117, CD235a are expressed. CD14 is not detectable.

In **acute megakaryoblastic leukemia (M7)**, the blast cells predominantly express CD34 and HLA-DR. Also, CD61, CD41, CD42a, CD42b are expressed. As to CD34 and CD33, these antigens may be detectable only in utmost non-differentiated megakaryoblasts. In half of the cases, CD7 is detectable and in 23% of the cases — CD2. The positive reaction for CD117 is frequent while CD11b, CD14 as well as CD13 and CD15 are not detectable.

The bone marrow in AML patients contains both LSC and residual normal HSC as indicated by the recovery of normal hematopoiesis. The data that are far from comprehensive suggest that at present there is a reasonable opportunity for identifying LSC with the aid of up-to-date flow cytometric techniques. It is these cells that seem to have the highest therapy resistance [20].

The assessment of LSC frequency among bulk leukemic blasts both at time of diagnosis and after induction therapy [21, 22] using the broad panel of monoclonals should strongly improve prognostic impact as to the detection of minimal residual disease.

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