Institute of Experimental Morphology and Anthropology with Museum
Bulgarian Anatomical Society

Acta morphologica et anthropologica, 14
Sofia • 2009

(mini-review)

Ineffective Hematopoiesis in Cases of Myelodysplastic Syndromes — the Role of CD34+ Stem and Progenitor Cells

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Myelodysplastic syndromes (MDS) are a heterogenous group of disorders characterized by ineffective hematopoiesis as a result of bone marrow cell dysplasia. MDS may result due to changes in the hematopoietic stem cells (HSCs), inequilibrium between programmed cell death (apoptosis) and cell proliferation and often develops after chemo- and/or X-ray therapy. It's determined that ineffective erythropoiesis (the most common disorder of MDS) is primarily due to increased intramedullar apoptosis of differentiated erythroid cells and not as much to changes in the immature CD34+ hematopoietic progenitor cells. There are promising results showing improved erythropoiesis when HSCs or erythroid progenitors are cultured in vitro in the presence of different growth factors.

Key words: Myelodysplastic syndromes (MDS), bone marrow, hematopoiesis/erythropoiesis, growth and transcription factors, cell cultures, apoptosis.

The *aim* of the present work is to make a brief summary of the up-to-date scientific literature on the myelodysplastic syndromes and more specifically on the role of the CD34+ stem and progenitor cells for the ineffective hematopoiesis, in the development of anemia and pancytopenias. The article focuses on the role of the immature progenitors for the disturbed erythropoiesis — one of the most common features of MDS. Some transcription factors affecting hematopoiesis and stem cell development are also discussed. Special attention is paid to the effects of some growth factors on the behavior of cultured *in vitro* immature hematopoietic progenitors isolated from patients with MDS.

Myelodysplastic syndromes (MDS) are a heterogenous group of disorders characterized by ineffective hematopoiesis as a result of bone marrow cell dysplasia. Dysplasia can be observed in all three hematopoietic lineages — erythroid, myeloid and megakaryocytic. MDS can be characterized in the peripheral blood smears by the different degrees of pancytopenia (erythro-, leuko- and thrombocytopenia) [3]. MDS is found primarily in adults at the age between 60 and 75 years but it can also occur in younger indivi-duals and

children [7]. MDS patients are susceptible to anemia, bacterial infections and haemorrhages. There is also an increased risk of MDS progression in acute myeloid leukemia (AML). It's clinically determined that in cases of MDS erythropoiesis and megakaryocytopoiesis are more difficult to stimulate [6].

Anemia is the most often observed disorder in MDS, where more than 80% of the patients have hemoglobin concentrations less than 10 g/dl. MDS anemia is usually normocytic, but often it is macrocytic. Some of the morphological erythrocyte changes include poikilocytes, anisocytes, eliptocytes, mackrovalocytes and sometimes stomatocytes. Approximately 10% of the erythrocytes are nucleated [2]. In most cases the bone marrow is normo- or hypercellular, but in 10-15% of the cases it can be hypocellular [7, 15]. MDS develops as a result of genetic changes in the hematopoietic stem cells (HSCs), disequilibrium between programmed cell death (apoptosis) and cell proliferation and often after chemo- and/or X-ray therapy. It's determined that ineffectine erythropoiesis is primarily due to increased intramedullary apoptosis of differentiated erythroid cells and not as much to changes in the immature CD34+ hematopoietic progenitor cells [16]. Other reasons for disturbed cell differentiation may be disorders in signal transduction — decreased number of growth factor receptors, altered cell sensitivity to different cytokines, synthesis of pro- and-antiapoptogenic proteins, production of inhibitory cytokines such as interferon-gamma (IFN-γ), tumor necrosis factor-α (TNF-α), Fas-ligand (Fas-L), cell-mediated autoimmune suppression, etc.

According to the criteria of the French-American-British morphological classification (FAB) MDS is divided into the following subgroups: Refractory anemia (RA), Refractory anemia with ring sideroblasts (RARS), Refractory anemia with excess of blasts (RAEB) and Refractory anemia with excess of blasts in transformation (RAEB-T), the latter often referred as pre-leukemic state. The new classification of hematopoietic and lymphoid tissue tumors, accepted in 2001 by the World Health Organization makes minor changes in the diagnostic subclassification dividing the heterogeneous MDS group into RA, refractory cytopenia with multilineage dysplasia (RC-MLD) and RAEB, 5q syndrome. RAEB-T cases are now related to the group of acute myeloid leukemias.

MDS possess variable potential for progression into AML, due to the heterogenity within the group. Many methods for evaluation of the clinic and the risk of transformation into AML are proposed in search of prognostic markers. At presence, the International Prognostic Scoring System (IPSS) is the main method for risk evaluation of MDS patients, including the risk of transformation into AML, considering the presence of cytogenetic aberrations, the degree of cytopenia and the number of blasts, but not the directly inderlying pathogenetic mechanisms [4, 8].

P a r k e r et al. [12] determine increased apoptosis in patients with RA and RARS with more than 50% apoptotic CD34+ cells. The ratio apoptosis/proliferation is equalized in cases of RAEB, and in patients with RAEB-T, apoptosis is reduced. Similar results were obtained [1] showing that along with the increase of the number of bone marrow blast cells and the degree of dysplasia a parallel increase in the intracellular concentrations of pro-apoptotic proteins (caspase-3) and anti-apoptotic proteins (bcl-2) is observed. Studies show that apoptosis affects both CD34+ and CD34- hematopoeitc progenitor cells [12]. M o n r e a l et al. [10] observe a significant increase in the number of immature hematopoietic cell population in the high risk MDS groups indicating a blockade in stem cell differentiation. The authors show that the increased number of CD34+ cell is significantly associated with disease progression [10].

When CD34+ and CD34+CD38- are cultured *in vitro* in serum-free medium at optimal concentrations of hematopoietic growth factors such as stem cell factor (SCF), thrombopoietin, interleukin-3 (IL-3), granulocyte colony-stimulating factor (G-CSF), granulocyte-macrophage colony-stimulating factor (GM-CSF) and erythropoietin (Epo), dis-

turbed cell proliferation is observed [11]. Results of other authors [5] show, that purified erythroid progenitors differentiate into mature enucleate erythrocytes in the presence of Epo and insulin. A stimulatory synergistic effect of SCF and Epo on the formation of BFU-E colonies in patients with RA and RARS is observed, but not in RAEB and RAEB-T. The total number of the erythroid colonies formed in cases of MDS is lower than that in healthy donors [19]. When myeloid growth factors are used, myelopoiesis is stimulated (mainly neutrophils) while the erythroid response is weaker. Reduced erythropoiesis is observed in mice (when high doses of G-CSF are used) as a result of erythroid progenitor cell migration from bone marrow to the peripheral blood and spleen [6].

In cell cultures *in vitro* is also determined the role of the apoptotic factor Fas, which is considered to play a key role in the activation of apoptosis in cases of MDS. The increased Fas production in cases of MDS may be due to elevated TNF-α or IFN-γ levels [15]. The same authors observe increased number of circulating (IFN-γ-producing) CD4+ T-lymphocytes, which they consider as evidence for the lymphocyte-mediated suppression of hematopoiesis, causing anemia and increased apoptosis of the hematopoetic progenitor cells. A possible source of TNF-α are the macrophages present in the bone marrow which number also increases in MDS patients [12]. In liquid cultures supplemented with Epo, Fas causes apoptosis of differentiating erythroid progenitors [12]. Claessens et al. [5] suggest that overexpression of Fas and FasL could induce apoptosis in *in vitro* semi-solid cultures as a result of direct cell-to-cell contacts between immature- and mature Fas–L-expressing cells. The level of anti-apoptotic proteins such as $FLIP_L$ is reduced in MDS thus making progenitor cells more susceptible to apoptotic stimuli [12]. It's suggested that the increased number of apoptotic cells and disturbed differentiation are a result of insufficient amount of anti-apoptotic proteins as Bcl-2, Bcl-X_L, and excess of pro-apoptotic proteins like Bad, Bax, Bim, or both [5].

Human CD34+ hematopoietic progenitor cells express receptors for IFN-γ [11] which suggests an important role of the cytokine in blood cell formation. The *in vitro* effects of IFN-γ on hematopoiesis are bipotent. Some authors [18, 20] suggest, that IFN-γ inhibits proliferation of hematopoietic stem- and progenitor cells, stimulating their differentiation. This cytokine is thought to be an important mediator in different acute and chronic bone marrow disorders such as aplastic anemia, MDS, etc. [20]. For this reason Selleri et al. [15] suggest the use of cyclosporin for suppression of IFN-γ production, as part of the medical treatment of patients with immune-mediated bone marrow suppression. It's determined [14], that in combination with Epo, IFN-γ reduces significantly erythroid progenitor cell apoptosis in healthy individuals.

Depending on the chromosome abnormalities in MDS it's established that IFN-γ stimulates expression of different genes. In cases of monosomy 7, the interferon stimulates the expression of apoptotic genes and in cases of trisomy 8, it enhances chemo- and cytokine synthesis by cultured *in vitro* CD34+ progenitor cells [21]. Parker et al.[12] report low ratio of pro-apoptotic versus anti-apoptotic proteins in the presence of chromosome 7 abnormalities.

The most frequent chromosome changes in MDS patients are deletions of chromosome 5, 7, 11, 12, 20 and trisomy of chromosome 8 [7].

Nuclear factor — kappa B (NF-κB) is an important transcription factor for CD34+ progenitor as well as erythroid cell development. It regulates the expression of specific genes such as c-myb, c-myc, genes for growth factors, immunoreceptors, adhesion molecules, viruses, etc. [22]. The antiapoptotic effects of NF-κB for different cell types is observed [17]. Its expression is stimulated by cytokines, affecting hematopoiesis such as Epo, GM-CSF, IL-3, IFN-γ, TNF-α, TGF-β1, etc., which suggest a key role of NF-κB as a secondary messenger in signal transduction and regulation of hematopoiesis/erythropoiesis [14]. This transcription factor is expressed in normal BFU-E and its quantity

decreases in the process of cell differentiation [22]. P y a t t et al. [13] show that human bone marrow CD34+ cells express NF-κB, which suggest its role in cell survival and differentiation. The transcription factor is also necessary for the activation of T-lymphocytes, monocytes/macrophages and neutrophils, for B-cell maturity, regulates the immune response reactions, etc.

There are data in the literature for NF-κB role in oncogenesis [17]. NF-κB expression is highly increased in patients with AML and it's suggested that this transcription factor may be used as a marker for distinguishing normal and leukemic stem cells.

M a r a t h e f t i s et al. [9] find elevated GATA-1 expression in bone marrow mononuclear cells, CD34+ progenitors and committed CD71+ erythroid cells. The authors also report a positive correlation between the transcription factor and apoptosis and suggest that up-regulation of GATA-1 transcription may be responsible for the cytopenias in the peripheral blood of MDS patients. The levels of GATA-1 expression also correlate with the progression of the disease [9].

It's interesting that the bone marrow stromal cells have a reduced capability for growth factor production. In long-term bone marrow cell cultures stromal cells are incapable of maintaining normal proliferation and differentiation of cultured CD34+ cells as a result of altered cell-to-cell interactions. In some cases, the stromal cells may even stimulate hematopoietic progenitor cell apoptosis as a result of defective cytokine and/or Fas- and other apoptotic protein production [12, 17]. S h e t t y et al. [16] find that when measuring apoptosis in bone marrow biopsies using the in situ end labeling (ISEL) technique stromal cells were apoptotic. The same authors show that apoptosis affected predominantly the erythroid and myeloid cells from all stages of maturation [16].

The use of cytokines for MDS treatment doesn't show exacerbations or disease progression. Although single cases are reported that after treatment with rhGM-CSF and Epo MDS progression to acute leukemia is registered [6], therapy with these recombinant growth factors shows promising results [7]. Combinations of hematopoietic growth factors can be beneficial for the treatment of MDS.

Although apoptosis is a typical sign in MDS its accurate evaluation is problematic and its significance remains unclear [16]. Whether it is a compensatory mechanism to the increased cell proliferation or a pathological state itself remains to be elucidated.

In the future the role of growth factors essential for the hematopoietic progenitor cell development needs to be elucidated with regard to their use in the treatment of MDS and to restore normal hematopoiesis. The quantitative changes of key erythroid transcriptional factors should also be clarified with a view to their possible contribution for the development and progression of MDS.

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