The role of apoptosis in myelodysplastic syndromes

Apoptosis or programmed cell death is a cellular process characterized by morphologic and biochemical changes, including fragmentation of cellular proteins and nucleic acids, to maintain a homeostatic balance between cell growth and cell death.[1] In the field of hematology default apoptosis appears in true malignancies, such as leukemia and lymphoma, with consequent uncontrolled cell growth; in contrast, in the bone marrow of patients with myelodysplastic syndrome (MDS), which is a clonal disorder of the stem cell, characterized by dysplastic hematopoiesis and enhanced risk of leukemic transformation, higher percentages of apoptotic hematopoietic precursors were observed than in normal subjects. This phenomenon is important in early MDS, whereas in advanced MDS the percentages of bone marrow apoptotic cells are lower than in normal subjects and similar to those observed in acute leukemia. Thus, it has been hypothesized that a high level of apoptosis may be responsible for the ineffective hematopoiesis in MDS, with the apparent paradox of peripheral cytopenia associated with hypercellular bone marrow.[2-9] This phenomenon is also clearly evident in *in vitro* cell cultures in which cells cannot form colonies in spite of the use of hematopoietic growth factors.[10] On the other hand, during the transformation of MDS into acute leukemia, apoptosis – enhanced during the preleukemic state – appears to diminish so that an uncontrolled growth of the pathologic stem cell clone is permitted. The leukemic transformation could be due to the acquisition of genetic lesions that may either block programmed cell death or promote cell proliferation in excess of the rate of apoptotis. Thus, the neoplastic clone may acquire a sufficiently great proliferative advantage to overcome the pre-neoplastic clone and expand in an uncontrolled way. Although the real, complete pathogenesis of these hematologic disorders is not yet perfectly known, the apoptotic process could be one of the most important mechanisms.[11-13]

Apoptosis, triggered by the bone marrow microenvironment and/or intrinsic cellular defects, is regulated at different levels by numerous factors such as oncogenes and their protein products, hematopoietic growth factors, immunologic factors, cell-cell or cell-stromal interactions, and critical adhesion receptors.[14] Various oncoproteins are involved in the control of cell proliferation, differentiation and apoptosis. Overexpression or dysregulation of oncogenes such as bcl-2, c-myc, fas, ras, p53, which can occur through many mechanisms (translocations, mutations, deletion or gene amplification), may play a crucial role in oncogenesis by affecting intracellular growth control, stimulating cytokine production and promoting or suppressing apoptosis. For example, whereas p53 inhibits cell growth and suppresses tumor progression by the induction of programmed cell death, bcl-2 is an inhibitor of apoptosis; ras gene family members play important roles in the transduction of growth factor signals and protect cells against apoptosis. Many studies have shown an association between raised levels of apoptosis and an increased ratio of pro-apoptotic to anti-apoptotic oncoproteins in hematopoietic precursors of MDS patients.[5,7,15-19]

Apoptosis is mediated by the accumulation of free radicals inside the cells, with consequent oxidative damage of DNA.[20,21] Some cytokines, such as tumor necrosis factor (TNF)α, interleukin (IL)-1β, interferon (IFN)γ, transforming growth factor (TGF)β may be involved in the regulation of this phenomenon, by suppressing or inducing apoptosis, depending on the levels of the cytokines and the degree of hematopoietic differentiation. Altered levels of cytokines produced by marrow stromal cells have been observed in peripheral blood and bone marrow from MDS patients; in particular, high bone marrow levels of TNFα seem closely related to the increase of apoptotic cells.[22] Suboptimal production of stimulatory cytokines, as granulocyte or granulocytic colony-stimulating factor, IL3, and erythropoietin, might impair the survival and differentiation of the hematopoietic stem cells.

Recent studies have shown that caspases, a cysteine-protease family, are the final effectors of the cell death program. Caspases are activated by a cascade mechanism and cause the destruction of important cellular components. Targets of caspases are cytoskeleton proteins and enzymes repairing nucleic acids. In normal conditions caspases are not casually activated and, after activation, they are controlled by protein inhibitors of apoptosis.[1]

Although some of the molecular mechanisms of the apoptotic pathway are known, the precise role of this process in the pathogenesis of MDS is still controversial. In particular, which cellular compartment is most involved in apoptosis is unknown and it is uncertain whether apoptosis has essential physiopathologic importance or is only secondary to the condition of ineffective hematopoiesis that characterizes these syndromes. Thus, its clinical importance and possible therapeutic implications need to be clarified.

In the paper by Ramos *et al.*[23] in this issue of Haematologica the influence of marrow cell apoptosis and oncoprotein expression on prognosis in MDS patients was examined. Whereas the apoptotic rate was higher in MDS patients than in controls but did not correlate with peripheral cytopenia and had no prognostic influence on the overall survival, a relation was observed between p53 expression and clinical data. These findings further suggest that apoptosis has a role in ineffective hematopoiesis of MDS but mechanisms additional to increased apoptosis are involved in the pathogenesis of the disorder. On the other hand, they demonstrate the need of studies to incorporate additional biologic and molecular features into MDS classification in order to improve prognostic evaluation and treatment of this disease.

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References

1. Johnson DE. Programmed cell death regulation: basic mechanisms and therapeutic opportunities. Leukemia 2000; 14:1340-4.
2. Raza A, Mundle S, Iftikhar A, Gregory S, Marcus B, Khan Z, et al. Simultaneous assessment of cell kinetics and programmed cell death in bone marrow biopsies of myelodysplastics reveals extensive apoptosis as the probable basis for ineffective hematopoiesis. Am J Hematol 1995; 48:143-54.
3. Bogdanovic AD, Trpinac DP, Jankovic GM, Bumbasirevic VZ, Obradovic M, Çolovic MD. Incidence and role of apoptosis in myelodysplastic syndrome: morphological and ultrastructural assessment. Leukemia 1997; 11:656-9.
4. Aul C, Bowen DT, Yoshida Y. Pathogenesis, etiology and epidemiology of myelodysplastic syndromes. Haematologica 1998; 83:71-86.
5. Kornblau SM. The role of apoptosis in the pathogenesis, prognosis and therapy of hematologic malignances. Leukemia 1998; 12 Suppl 1:S41-S6.
6. Parker JE, Mufti GJ. Ineffective haemopoiesis and apoptosis in myelodysplastic syndromes. Br J Haematol 1998; 101:220-30.
7. Parker JE, Fishlock KL, Mijovic A, Czepulkowski B, Pagliuca A, Mufti GJ. "Low-risk" myelodysplastic syndrome is associated with excessive apoptosis and an increased ratio of pro- versus anti-apoptotic bcl-2-related proteins. Br J Haematol 1998; 103:1075-82.
8. Shimazaki K, Ohshima K, Suzumiya J, Kawasaki C, Kikuchi M. Evaluation of apoptosis as a prognostic factor in myelodysplastic syndromes. Br J Haematol 2000; 110: 584-90.
9. Alessandrino EP, Amadori S, Cazzola M, Locatelli F, Mecucci C, Morra E et al. Myelodysplastic syndromes: recent advances. Haematologica 2001; 86:1124-57.
10. Holm M, Hoyer M, Jensen I, Thomsen M, Hokland P. Dynamic cell cycle kinetics in vitro and in vivo in myelodysplastic syndromes with special reference to the influence of hematopoietic growth factors. Leuk Res 2000; 24: 999-1008.
11. Greenberg PL. Apoptosis and its role in the myelodysplastic syndromes: implications for disease natural history and treatment. Leuk res 1998; 22:1123-36.
12. Yoshida Y, Mufti GJ. Apoptosis and its significance in MDS: controversies revisited. Leuk Res 1999; 23:777-85.
13. Rosenfeld C, List A. A hypothesis for the pathogenesis of myelodysplastic syndromes: implications for new therapies. Leukemia 2000; 14:2-8.
14. Preisler HD, Bi S, Venugopal P, Raza A. Cytokines, molecular biological abnormalities, and acute myelogenous leukemia. Leuk Res 1997; 21:299-312.
15. Rajapaksa R, Ginzton N, Rott LS, Greenberg PL. Altered oncoprotein expression and apoptosis in myelodysplastic syndrome marrow cells. Blood 1996; 88:4275-87.
16. Davis RE, Greenberg PL. Bcl-2 expression by myeloid precursors in myelodysplastic syndromes: relation to disease progression. Leuk Res 1998; 22:767-77.
17. Padua RA, Guinn BA, Al-Sabah A, Smith M, Taylor C, Pettersson T et al. RAS, FMS and p53 mutations and poor clinical outcome in myelodysplasias: a 10-year follow-up. Leukemia 1998; 12:887-92.
18. Parker JE, Mufti GJ, Rasool F, Mijovic A, Devereux S, Pagliuca A. The role of apoptosis, proliferation, and the Bcl-2-related proteins in the myelodysplastic syndromes and acute myeloid leukemia secondary to MDS. Blood 2000; 96:3932-8.
19. Invernizzi R, Pecci A, Bellotti L, Ascari E. Expression of p53, bcl-2 and ras oncoproteins and apoptosis levels in acute leukaemias and myelodysplastic syndromes. Leuk Lymphoma 2001; 42:481-9.
20. Peddie CM, Wolf CR, Mc Lellan LI, Collins AR, Bowen DT. Oxidative DNA damage in CD34+ myelodysplastic cells is associated with intracellular redox changes and elevated plasma tumor necrosis factor-alpha concentration. Br J Haematol 1997; 99:625-31.
21. Moroni M, Porta C, Invernizzi R, Inzoli A, Bobbio-Pallavicini F, Bobbio-Pallavicini E. "TUNEL" evidence of reduced bone marrow cells apoptosis in a refractory anaemia patient treated with amifostine. Br J Haematol

1999; 104:420-6.

22. Verhoef GE, De Shower P, Ceuppens JL, Van Damme J, Goossens W, Boogaerts MA. Measurement of serum cytokine levels in patients with myelodysplastic syndromes. Leukemia 1992; 6:1268-72.

23. Ramos F, Fuertes-Nunez M, Suarez-Vilela D, Fernandez-Lopez A. What does apoptosis have to do with clinical features in myelodysplastic syndrome? Haematologica 2002; 87:381-91.

Human herpesvirus 8-associated primary effusion lymphoma in human immunodeficiency virus-negative patients: a clinico-epidemiologic variant resembling classic Kaposi's sarcoma

The term primary effusion lymphoma (PEL) defines an extranodal non-Hodgkin's lymphoma, usually classified as a B-cell lymphoma, that grows in liquid-phase within body cavities. A mandatory requisite for the diagnosis is the demonstration of human herpesvirus 8 (HHV-8) genome within tumor cells. HHV8 was first identified in late 1994 within acquired immunodeficiency syndrome (AIDS)-Kaposi's sarcoma (KS) lesions,[1] subsequently in classic, iatrogenic, and endemic (African) KS variants[2,3] and finally, in 1995, within large-cell type, AIDS-related intracavitary lymphomas.[4] HHV-8 infection is also associated with multicentric Castleman's disease[5] and a range of post-transplantation hematologic conditions, such as bone marrow failure[6] and lymphoproliferative disorders.[7,8] In 1996, body cavity based lymphomas harboring HHV-8 were proposed as a new entity with the name of PEL, to be distinguished from other primary and secondary lymphomatous effusions.[9]

PEL typically presents with recurrent effusions but without a solid component. The most common sites of involvement are the pleural, peritoneal and pericardial cavities delimited by mesothelium. Tumor cells may be co-infected by Epstein-Barr virus (EBV) and show large-cell morphology, with plasmablastic or immunoblastic features. Immunophenotypic features include positive staining for CD45, CD45R0, CD138, and activation-associated antigens, and negative staining for B-/T-cell-associated antigens. PEL cases exhibiting aberrant expression of B-, T- and NK-markers have also been reported. The B-cell lineage derivation of PEL cells is established on the basis of clonal rearrangements of the heavy immunoglobulin (Ig) genes and recent polymerase chain reaction (PCR)-based findings of a preferential expression of certain lambda light chain genes, suggesting clonal proliferation by an antigen selection process.[10] In contrast to other non-Hodgkin's B-cell lymphoma types, neither c-MYC nor other proto-oncogene rearrangements are detected in PEL. Likewise, a wild type of the tumor suppressor gene p53 is expressed, while mutations of the BCL6 5' non-coding regions have been recently documented in most of the analyzed cases.[11] PEL cells show complex karyotypes, the most frequent chromosomal abnormalities being trisomy 7, 12 and aberrations of chromosomal bands 1q21-q25.[11] The postulated normal cell(s) counterpart is unknown, but the expression of CD138/syndecan-1 and CD45R0 antigens, together with frequent BCL6 mutations reflect a late stage of B-cell differentiation.[12] Recently, the expression status of MUM1/IRF4 (multiple myeloma 1/interferon regulatory factor 4) protein, which is involved in physiologic B-cell maturation, has been shown to cluster selectively with PEL among lymphomatous effusions, corroborating the notion that PEL originates from post-germinal center, preterminally differentiated B-cells.[13]

As to disease pathogenesis, the role of HHV-8 in PEL development is widely accepted, whereas more controversy exists on possible co-factors that may trigger the transformation of HHV-8-infected lymphoid cells and their tropism for body cavities.

Over the last few years, PEL has been mainly described in human immunodeficiency virus (HIV)-positive patients.[4,9,14] In the non-HIV setting, this entity remains almost unreported but it may be hypothesized that its epidemiology correlates with the distribution of HHV-8 infection, which is known to have a peculiar ethnic/geographic pattern, being higher in the ethnic groups at risk for classic KS, namely those of Jewish descent or those living in the Mediterranean basin (i.e. Israel, Greece, Spain and Italy)[15-17] and sub-Saharan Africa.[18] So far, two examples of PEL have been reported in HIV-negative transplant recipients, one from Haiti[19] and one from Italy,[20] but none from sub-Saharan Africa where KS accounts for a high proportion of all malignancies. However, one case of a black man from South Africa with KS and unexplained pleural effusions containing bizarre cells was reported prior to the discovery of HHV-8.[21] To date, there are 20 well-documented cases developing in elderly subjects[9,22,23,34] or even in centenarians[35] of Eastern European/Mediterranean or Jewish ancestry, supporting the existence of a distinct clinico-epidemiologic variant of PEL paralleling classic KS, i.e. *classic PEL*, as recently suggested also by Klepfish *et al.*[34]