Leaders

Apoptosis in haematological malignancies

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In recent years, there has been a considerable effort to understand the mechanisms by which cells cause their own demise. This process, known as programmed cell death or apoptosis, is an important regulatory mechanism in normal tissue development and homeostasis. Abnormalities in apoptotic pathways contribute to a diverse array of pathological processes including cancer, immune disorders, and neurodegenerative diseases.¹ The haematopoietic system provides many examples of the significance of apoptosis, both as a regulatory mechanism under normal conditions, and as a pathogenetic element in neoplasia.²⁻⁶ Specifically, acquired resistance to apoptosis can both facilitate the development of haematological malignancies and render these tumours resistant to therapy. This review highlights several examples illustrating the significance of apoptosis in haematological malignancies.

bcl-2 and the paradigm of apoptosis in haematological malignancies

The t(14;18)(q32;q21) translocation characteristic of follicular lymphoma places the bcl-2 oncogene under the regulatory control of the immunoglobulin heavy chain gene and leads to enhanced expression of bcl-2 in B cells. In contrast to the growth promoting effects of other oncogenes described at the time of its discovery, bcl-2 was shown instead to inhibit cell death.⁷ Initially, bcl-2 overexpression was shown to promote the survival of cytokine dependent cells after cytokine deprivation.⁸ Transgenic mice in which bcl-2 expression was targeted to B cells demonstrated follicular hyperplasia, and many eventually developed malignant lymphoma accompanied by translocation of c-myc.⁹⁻¹⁰ In contrast, bcl-2 knockout mice displayed massive loss of mature B and T lymphocytes via apoptosis, implying a requirement for bcl-2 in the survival of these lymphocytes.¹¹ The mechanisms by which bcl-2 inhibits apoptosis have not been defined precisely. However, interactions among bcl-2 family members, including bax, which promotes cell death, and bcl_{xL}, which inhibits cell death, appear to modulate the propensity of the cell to undergo apoptosis.¹²

The ability of bcl-2 to inhibit apoptosis is not limited to that induced by growth factor deprivation, nor is follicular lymphoma the only haematological malignancy influenced by bcl-2 expression. Indeed, bcl-2 inhibits apoptosis triggered by a multitude of otherwise lethal

stimuli.¹² Principal among these from a therapeutic standpoint is apoptosis induced by cytotoxic chemotherapy and radiation.¹³ In keeping with this property, elevated bcl-2 expression has been correlated with chemoresistance and poor prognosis in a number of haematological malignancies. For example, elevated bcl-2 expression correlated with an inability to attain complete remission and poor overall survival in patients with acute myeloid leukaemia (AML).¹⁴ In childhood acute lymphoblastic leukaemia (ALL), bcl-2 overexpression correlated with resistance to serum deprivation induced apoptosis, although an effect on response to chemotherapy has not been demonstrated.¹⁵ Nonetheless, the propensity of primary B precursor ALL cells to undergo spontaneous apoptosis in vitro has been shown to predict long term survival.¹⁶ A relative increase in the ratio of bcl-2:bax correlated with in vitro chemoresistance in primary chronic lymphocytic leukaemia (CLL) cells,¹⁷⁻¹⁸ while bcl-2 overexpression correlated with reduced overall survival.¹⁹ Finally, in diffuse large B cell lymphoma, increased bcl-2 expression independently predicted advanced stage and reduced survival in multivariate analysis.²⁰

bcr-abl and the role of apoptosis in chronic myeloid leukaemia

Chronic myeloid leukaemia (CML) is a myeloproliferative disorder characterised clinically by an accumulation of myeloid cells at all stages of maturation, and cytogenetically by the presence of the Philadelphia chromosome. The reciprocal translocation that produces the Philadelphia chromosome, t(9;22)(q34;q11), encodes a chimeric fusion protein, bcr-abl, which is thought to be pathogenetic in this disease.²¹ Paradoxically, CML precursors display normal proliferative indices, despite the massive accumulation of myeloid elements characteristic of the disease.²² This conundrum has recently been resolved with the recognition that the bcr-abl fusion protein inhibits apoptosis.²²⁻²³ Thus, by analogy with bcl-2 and follicular lymphoma, the enhanced survival of CML cells yields a net expansion of myeloid cells without the requirement of a proliferative increase. As with bcl-2, the mechanisms by which bcr-abl inhibits apoptosis are not precisely known. However, the available data suggest that the intracellular signalling pathways through which bcr-abl modulates cell survival are likely to be complex.²⁴

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One phenotype associated with the ability of bcr-abl to inhibit DNA damage induced apoptosis is a relatively prolonged G2/M arrest following genotoxic injury.²⁵ Such a prolongation of arrest would potentially allow the cell to repair otherwise lethal damage, and thereby enjoy a survival advantage.²⁵ Significantly, bcr-abl is not a universal inhibitor of apoptosis. In contrast to the relative resistance against genotoxic agents displayed by CML cells in vitro and in vivo, these cells remain sensitive to apoptosis induced by cytotoxic T cells²⁶ and natural killer cells.²⁷ These observations provide a biological basis for the clinical observation that allogeneic bone marrow transplantation is the only therapy with curative potential in patients with CML. Clearly, dissection of apoptotic pathways may thus yield novel therapeutic windows of opportunity.

p53 abnormalities in haematological malignancies

Modulation of apoptosis appears to be one of the biological activities of the p53 tumour suppressor gene, and loss of wild-type p53 function is associated with chemoresistance in some systems.²⁸⁻²⁹ Interestingly, p53 mutations are less common in haematological malignancies than in solid tumours, in which p53 represents the most frequently mutated gene.³⁰ Nonetheless, p53 mutations are associated with disease progression and poor prognosis in a number of haematological malignancies, and these associations may be due, at least in part, to an impairment in p53 dependent apoptosis in response to therapy. For example, acquisition of p53 mutations is associated with progression of follicular lymphoma to diffuse large cell lymphoma,³¹⁻³² chronic phase CML to myeloid blast crisis,³³ myelodysplastic syndrome to AML,³⁴ and relapse of T-ALL.³⁵⁻³⁶ Similarly, although they are seen in only a minority of cases, p53 mutations are associated with chemoresistance and poor overall survival in AML,³⁷ childhood ALL,³⁸ CLL,³⁷ mantle cell lymphoma,³⁹⁻⁴⁰ and myelodysplastic syndromes.³⁷⁻⁴¹ Indeed, all cases of myelodysplastic syndromes with mutant p53 reported to date have been classified morphologically as high grade.³⁷⁻⁴¹ These clinical observations are consistent with the notion that loss of p53 function yields an attenuated apoptotic response to cytotoxic chemotherapy, and hence, a poor clinical outcome.

Fas and the paradigm of receptor mediated apoptosis in haematological malignancies

Fas (also known as APO-1 and CD95) is a member of the tumour necrosis factor (TNF) superfamily of membrane receptors,⁴² several of which modulate apoptosis.⁴³ Binding of Fas by the Fas ligand (FasL) induces apoptosis in susceptible cells. One might therefore predict that acquired resistance to Fas mediated apoptosis in a normally susceptible cell could produce clonal expansion and, ultimately, a haematological malignancy. In keeping with this prediction, patients with a rare lymphoproliferative disorder, which is sometimes associ-

ated with autoimmunity, have recently been shown to harbour mutations in Fas.⁴⁴⁻⁴⁵ In principle, Fas expression might also sensitise haematological malignancies to immunotherapy, as occurs with graft versus leukaemia and graft versus lymphoma effects in allogeneic bone marrow transplant recipients.⁴⁶

Indeed, there are reported examples of Fas induced apoptosis in primary haematological malignancies. For example, freshly isolated adult T cell leukaemia cells express high levels of Fas, and receptor binding triggered apoptosis in all nine cases evaluated in one series.⁴⁷ Many Burkitt's lymphoma cell lines are susceptible to Fas mediated apoptosis, and in one clinically refractory Fas negative case of primary Burkitt's lymphoma, induction of Fas in vitro rendered these cells sensitive to Fas mediated apoptosis.⁴⁸ Interferon α is used in the treatment of chronic phase CML and its antitumour activity appears to result from upregulation of Fas on CML progenitors.⁴⁹ In primary AML, in which expression of Fas is variable,⁴⁶⁻⁵⁰ binding of Fas induced apoptosis in about half of the cases tested.⁵⁰ Interestingly, susceptibility to Fas mediated apoptosis did not correlate with intensity of Fas expression,⁵⁰ implying modulation of Fas mediated apoptosis by other factors. Along these lines, bcl-2 has been shown to inhibit Fas mediated apoptosis.⁵¹ Therefore, susceptibility to Fas mediated apoptosis in AML may be regulated in part by bcl-2 that, like Fas, is also variably expressed in primary AML.⁴⁶

Like other members of the TNF receptor superfamily,⁵² Fas is expressed in many cases of Hodgkin's disease and non-Hodgkin's lymphomas.⁵³⁻⁵⁴ However, the clinical significance of Fas expression in these tumours remains to be determined. Likewise, the molecular basis for the variability in Fas expression observed among haematological malignancies has not been examined, although deletions and rearrangements of the gene encoding Fas appear to be uncommon in Hodgkin's disease and non-Hodgkin's lymphoma.⁵⁵

Haematopoietic growth factors and apoptosis in AML

Apoptosis plays a regulatory role in normal myelopoiesis, as myeloid precursors deprived of haematopoietic growth factors die an apoptotic death.²⁻³ Likewise, blasts from most patients with AML retain this property and cannot be sustained in vitro without exogenous haematopoietic growth factors.⁴⁻⁵ This distinction between haematopoietic growth factor dependent and independent AML has clinical significance; patients with AML whose blasts are independent of such trophic support fare poorly.⁵⁶⁻⁵⁷ Haematopoietic growth factors have also been shown to protect primary AML cells from chemotherapy induced apoptosis.⁵⁸ This observation implicates constitutive activation of haematopoietic growth factor mediated survival signals as a basis for the poor chemotherapeutic response of haematopoietic growth factor independent AML.⁵⁶⁻⁵⁷ Moreover, the observation that haematopoietic growth factors

inhibit drug induced apoptosis in AML may also account for the increase in persistent leukaemia that has been reported in patients treated with haematopoietic growth factors plus cytotoxic chemotherapy compared with those receiving chemotherapy alone.^{59 60}

Apoptosis and myelodysplasia

The myelodysplastic syndromes comprise a heterogeneous group of clonal diseases characterised by peripheral cytopenias and morphological evidence of dysplasia with or without an increase in myeloblasts. In most cases of myelodysplastic syndrome, the bone marrow is noticeably hypercellular, despite the evidence of bone marrow failure manifested clinically and in the peripheral blood. Recent work implicates accelerated intramedullary apoptosis as the pathogenetic basis for this paradox.

Using bone marrow biopsy samples and an in situ end-labelling method to identify DNA strand breaks generated during apoptosis, Raza and colleagues^{61 62} found a relative increase in the fraction of marrow cells undergoing apoptosis in patients with myelodysplastic syndrome compared with both normal marrows and those from patients with AML. Further, using a double labelling technique, they found that many of the S phase cells were also undergoing apoptosis, a phenomenon that was not observed in the controls. These authors suggested that increased apoptosis accounted for the ineffective haematopoiesis characteristic of myelodysplastic syndromes. Subsequent work has revealed that myelodysplastic syndrome marrow cells undergo apoptosis more rapidly than controls during incubation in vitro, further supporting an enhanced susceptibility to apoptosis in this disorder.⁶³

These observations have been confirmed and extended by a second group, who demonstrated that the increased apoptosis in myelodysplastic syndrome marrow cells compared with normals is restricted to the CD34+ subset.⁶⁴ Significantly, several patients in this study were treated with haematopoietic growth factors, and all of these treated patients demonstrated an increase in granulocytes, coupled with a decrease in the proportion of apoptotic CD34+ marrow cells.⁶⁴ This finding strengthens the proposed relation between enhanced susceptibility to apoptosis in marrow precursors, and peripheral cytopenias in patients with myelodysplastic syndromes.

Conclusion

Acquired resistance to apoptosis in haematological malignancies may promote clonal expansion and enhance the likelihood that subsequent mutations do not lead to elimination of the neoplastic clone. Because the efficacy of cytotoxic chemotherapy relies on its ability to induce programmed cell death, resistance to apoptosis typically correlates with chemoresistance, a phenomenon that is best characterised in haematological malignancies. Measurement of apoptotic tendency in primary haematological malignancies may augment the prognostic precision of conventional clinical and pathological classification, and thereby identify

biologically distinct subsets of tumours requiring more aggressive, and perhaps even novel, therapeutic strategies. Finally, enhanced susceptibility to apoptosis may be pathogenetic in bone marrow failure syndromes such as myelodysplastic syndromes.

- 1 Thompson C. Apoptosis in the pathogenesis and treatment of disease. *Science* 1995;267:1456-62.
- 2 Williams G, Smith CA, Spooner E, Dexter TM, Taylor DR. Haematopoietic colony stimulating factors promote cell survival by suppressing apoptosis. *Nature* 1990;343:76-9.
- 3 Lotem J, Sachs L. Rescue from programmed cell death in leukemic and normal myeloid cells. *Blood* 1991;78:953-60.
- 4 Sachs L, Lotem J. Control of programmed cell death in normal and leukemic cells: new implications for therapy. *Blood* 1993;82:15-21.
- 5 Lotem J, Sachs L. Control of apoptosis in hematopoiesis and leukemia by cytokines, tumor suppressor and oncogenes. *Leukemia* 1996;10:925-31.
- 6 Sachs L. The control of hematopoiesis and leukemia: from basic biology to the clinic. *Proc Natl Acad Sci USA* 1996;93:4742-9.
- 7 Korsmeyer S. bcl-2 initiates a new category of oncogenes: Regulators of cell death. *Blood* 1992;80:879-86.
- 8 Vaux D, Cory S, Adams TM. Bcl-2 promotes the survival of hematopoietic cells and cooperates with c-myc to immortalize pre-B cells. *Nature* 1988;335:440-2.
- 9 McDonnell T, Deane N, Platt FM, Nunez G, Jaeger U, McKearn JP, Korsmeyer SJ. bcl-2 immunoglobulin transgenic mice demonstrate extended B cell survival and follicular lymphoproliferation. *Cell* 1989;57:79-88.
- 10 McDonnell T, Korsmeyer SJ. Progression from lymphoid hyperplasia to high-grade malignant lymphoma in mice transgenic for the t(14;18). *Nature* 1991;349:254-6.
- 11 Veis D, Sorenson CM, Shutter JR, Korsmeyer SJ. Bcl-2-deficient mice demonstrate fulminant lymphoid apoptosis, polycystic kidneys, and hypopigmented hair. *Cell* 1993;75:229-40.
- 12 Yang E, Korsmeyer SJ. Molecular thanatopsis: a discourse on the bcl2 family and cell death. *Blood* 1996;88:386-401.
- 13 Miyashita T, Reed JC. Bcl-2 oncoprotein blocks chemotherapy-induced apoptosis in a human leukemia cell line. *Blood* 1993;81:151-7.
- 14 Campos L, Rouault J-P, Sabido O, Oriol Pascale, Roubi N, Vasselon C, et al. High expression of bcl-2 protein in acute myeloid leukemia cells is associated with poor response to chemotherapy. *Blood* 1993;81:3091-6.
- 15 Coustan-Smith E, Kitanaka A, Pui C-H, McNinch L, Evans WE, Raimondi SC, et al. Clinical relevance of bcl-2 overexpression in childhood acute lymphoblastic leukemia. *Blood* 1996;87:1140-6.
- 16 Kumagi M-A, Manabe A, Pui C-H, Behm FG, Raimondi SC, Hancock ML, et al. Stroma-supported culture of childhood B-lineage acute lymphoblastic leukemia cells predicts treatment outcome. *J Clin Invest* 1996;97:755-60.
- 17 Thomas A, El Roubi S, Reed JC, Krajewski S, Silber R, Potmesil M, et al. Drug-induced apoptosis in B-cell chronic lymphocytic leukemia: relationship between p53 gene mutation and bcl-2/bax proteins in drug resistance. *Oncogene* 1996;12:1055-62.
- 18 McConkey D, Chandra J, Wright S, Plunkett W, McDonnell TJ, Reed JC, et al. Apoptosis sensitivity in chronic lymphocytic leukemia is determined by endogenous endonuclease content and relative expression of BCL-2 and BAX. *J Immunol* 1996;156:2624-30.
- 19 Robertson L, Plunkett W, McKonnell K, Keating MJ, McDonnell TJ. Bcl-2 expression in chronic lymphocytic leukemia and its correlation with the induction of apoptosis and clinical outcome. *Leukemia* 1996;10:456-9.
- 20 Hermine O, Haioun C, Lepage E, d'Agay M-F, Briere J, Lavignac C, et al. Prognostic significance of bcl-2 expression in aggressive non-Hodgkin's lymphoma. *Blood* 1996;87:265-72.
- 21 Daley G, Van Etten RA, Baltimore D. Induction of chronic myelogenous leukemia in mice by the P210^{bcr/abl} gene of the Philadelphia chromosome. *Science* 1990;247:824-30.
- 22 Bedi A, Zehnbauser BA, Barber JP, Sharkis SJ, Jones RJ. Inhibition of apoptosis by BCR-ABL in chronic myeloid leukemia. *Blood* 1994;83:2038-44.
- 23 McGahon A, Bissonnette R, Schmitt M, Cotter KM, Green DR, Cotter TG. BCR-ABL maintains resistance of chronic myelogenous leukemia cells to apoptotic cell death. *Blood* 1994;83:1179-87.
- 24 Cortez D, Kadlec L, Pendergast AM. Structural and signaling requirements for BCR-ABL-mediated transformation and inhibition of apoptosis. *Mol Cell Biol* 1995;15:5531-41.
- 25 Bedi A, Barber JP, Bedi GC, El-Deiry WS, Sidransky D, Vala MS, et al. BCR-ABL-mediated inhibition of apoptosis with delay of G2/M transition after DNA damage: a mechanism of resistance to multiple anticancer agents. *Blood* 1995;86:1148-58.
- 26 Fuchs E, Bedi A, Jones RJ, Hess AD. Cytotoxic T cells overcome BCR-ABL-mediated resistance to apoptosis. *Cancer Res* 1995;55:463-6.
- 27 Roger R, Issaad C, Pallardy M, Leglise M-C, Turhan AG, Bertoglio J, et al. BCR-ABL does not prevent apoptotic death induced by human natural killer or lymphokine-activated killer cells. *Blood* 1996;87:1113-22.

- 28 Lowe S, Ruley HE, Jacks T, Housman DE. p53-dependent apoptosis modulates the cytotoxicity of anticancer agents. *Cell* 1993;74:954-67.
- 29 Lotem J, Sachs L. Hematopoietic cells from mice deficient in wild-type p53 are more resistant to induction of apoptosis by some agents. *Blood* 1993;82:1092-6.
- 30 Imamura J, Miyoshi I, Koeffler HP. p53 in hematologic malignancies. *Blood* 1994;84:2412-21.
- 31 Lo Coco F, Gaidano G, Louie DC, Offit K, Chaganti RS. p53 mutations are associated with histologic transformation of follicular lymphoma. *Blood* 1993;82:2289-95.
- 32 Sander C, Yano T, Clark HM, Harris C, Longo DL, Jaffe ES. p53 mutation is associated with progression in follicular lymphomas. *Blood* 1993;82:1994-2004.
- 33 Nakai H MS, Toguchida J, Yandel DW, Ishikazi K. Frequent p53 mutations in blast crisis of chronic myelogenous leukemia, especially in myeloid crisis harboring loss of chromosome 17p. *Cancer Res* 1992;52:6588-93.
- 34 Kitagawa M, Yoshida S, Kuwata T, Tanizawa T, Kamiyama R. p53 expression in myeloid cells of myelodysplastic syndromes. Association with evolution to overt leukemia. *Am J Pathol* 1994;145:338-44.
- 35 Hsiao M, Yu AL, Yeargin J, Ku D, Haas M. Nonhereditary p53 mutations in T-cell acute lymphoblastic leukemia are associated with the relapse phase. *Blood* 1994;83:2922-30.
- 36 Diccianni M, Yu J, Hsiao M, Mukherjee S, Shao LE, Yu AL. Clinical significance of p53 mutations in relapsed T-cell acute lymphoblastic leukemia. *Blood* 1994;84:3105-12.
- 37 Wattel E, Preudhomme C, Hecquet B, Vanrumbeke M, Quesnel B, Dervite I, et al. p53 mutations are associated with resistance to chemotherapy and short survival in hematologic malignancies. *Blood* 1994;84:3148-57.
- 38 Marks D, Kurz BW, Link MP, Ng E, Shuster JJ, Lauer SJ, et al. High incidence of potential p53 inactivation in poor outcome childhood acute lymphoblastic leukemia at diagnosis. *Blood* 1996;87:1155-61.
- 39 Greiner TC, Moynihan MC, Chan WC, Lytle DM, Pederson A, Anderson JR, Weisenburger DD. p53 mutations in mantle cell lymphoma are associated with variant cytology and predict a poor prognosis. *Blood* 1996;87:4302-10.
- 40 Hernandez L, Fest T, Cazorla M, Teruya-Feldstein J, Bosch F, Peinado MA, et al. p53 gene mutations and protein overexpression are associated with aggressive variants of mantle cell lymphomas. *Blood* 1996;87:3351-9.
- 41 Sugimoto K, Hirano N, Toyoshima H, Chiba S, Mano H, Takaku F, et al. Mutations of the p53 gene in myelodysplastic syndrome (MDS) and MDS-derived leukemia. *Blood* 1993;81:3022-6.
- 42 Nagata S, Golstein P. The Fas death factor. *Science* 1995;267:1449-56.
- 43 Bazzoni F, Beutler B. The tumor necrosis factor ligand and receptor families. *N Engl J Med* 1996;334:1717-25.
- 44 Rieux-Laucat F, Le Deist F, Hivroz C, Roberts IA, Debatin KM, Fischer A, et al. Mutations in Fas associated with human lymphoproliferative syndrome and autoimmunity. *Science* 1995;268:1347-9.
- 45 Fisher G, Rosenberg FJ, Straus SE, Dale JK, Middleton LA, Lin AY, et al. Dominant interfering mutations impair apoptosis in a human autoimmune lymphoproliferative syndrome. *Cell* 1995;81:935-46.
- 46 DiGiuseppe J, LeBeau P, Augenbraun J, Borowitz MJ. Multiparameter flow-cytometric analysis of bcl-2 and Fas expression in normal and neoplastic hematopoiesis. *Am J Clin Pathol* 1996;106:345-51.
- 47 Debatin K-M, Goldman CK, Waldmann TA, Krammer PH. APO-1-induced apoptosis of leukemia cells from patients with adult T-cell leukemia. *Blood* 1994;81:2972-7.
- 48 Schattner E, Mascarenhas J, Bishop J, Yoo DH, Chadburn A, Crow MK, et al. CD4+ T-cell induction of Fas-mediated apoptosis in Burkitt's lymphoma B cells. *Blood* 1996;88:1375-82.
- 49 Selleri C, Sato T, Del Vecchio L, Luciano L, Barrett AJ, Rotoli B, et al. Involvement of Fas-mediated apoptosis in the inhibitory effects of interferon- α in chronic myelogenous leukemia. *Blood* 1997;89:957-64.
- 50 Komada Y, Zhou Y-W, Zhang X-L, Xue H-L, Sakai H, Tanaka S, et al. Fas receptor (CD95)-mediated apoptosis is induced in leukemic cells entering G1B compartment of the cell cycle. *Blood* 1995;86:3848-60.
- 51 Itoh N, Tsujimoto Y, Nagata S. Effect of bcl-2 on Fas antigen-mediated cell death. *J Immunol* 1993;151:621-7.
- 52 Gruss H-J, Dower SK. Tumor necrosis factor ligand superfamily: involvement in the pathology of malignant lymphomas. *Blood* 1995;85:3378-404.
- 53 Kondo E, Yoshino T, Yamadori I, Matsuo Y, Kawasaki N, Minowada J, Akagi T. Expression of bcl-2 protein and Fas antigen in non-Hodgkin's lymphomas. *Am J Pathol* 1994;145:330-7.
- 54 Xerri L, Carubaccia N, Parc P, Hassoun J, Birg F. Frequent expression of Fas/APO-1 in Hodgkin's disease and anaplastic large cell lymphomas. *Histopathology* 1995;27:235-41.
- 55 Xerri L, CN, Parc P, Birg F. Search for rearrangements and/or allelic loss of the fas/APO-1 gene in 101 human lymphomas. *Am J Clin Pathol* 1995;104:424-30.
- 56 Lowenberg B, van Putten WLJ, Touw IP, Delwel R, Santini V. Autonomously proliferating of leukemic cells in vitro as a determinant of prognosis in adult acute myeloid leukemia. *N Engl J Med* 1993;328:614-19.
- 57 Hunter A, Rogers SY, Roberts IAG, Barrett AJ, Russell N. Autonomously growth of blast cells is associated with reduced survival in acute myeloblastic leukemia. *Blood* 1993;82:899-903.
- 58 Kaplinsky C, Lotem J, Sachs L. Protection of human myeloid leukemic cells against doxorubicin-induced apoptosis by granulocyte-macrophage colony-stimulating factor and interleukin 3. *Leukemia* 1996;10:460-5.
- 59 Estey E, Thall PF, Kantarjian H, O'Brien S, Koller CA, Beran M, et al. Treatment of newly-diagnosed acute myelogenous leukemia with granulocyte-macrophage colony-stimulating factor (GM-CSF) before and during continuous-infusion high-dose ara-C + daunorubicin: comparison to patients treated without GM-CSF. *Blood* 1992;79:2246-55.
- 60 Zittoun R, Suci S, Mandelli F, de Witte T, Thaler J, Stryckmans P, et al. Granulocyte-macrophage colony-stimulating factor associated with induction treatment of acute myelogenous leukemia: A randomized trial by the European Organization for research and treatment of cancer leukemia cooperative group. *J Clin Oncol* 1996;14:2150-9.
- 61 Raza A, Gezer S, Mundle S, Gao X-Z, Alvi S, Borok R, et al. Apoptosis in bone marrow samples involving stromal and hematopoietic cells in 50 patients with myelodysplastic syndromes. *Blood* 1995;86:268-76.
- 62 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 myelodysplasias reveals extensive apoptosis as the probable basis for ineffective hematopoiesis. *Am J Hematol* 1995;48:143-54.
- 63 Mundle S, Venugopal P, Cartledge JD, Pandav DV, Broady-Robinson L, Gezer S, et al. Indication of an involvement of interleukin-1 β converting enzyme-like protease in intramedullary apoptotic cell death in the bone marrow of patients with myelodysplastic syndromes. *Blood* 1996;88:2640-7.
- 64 Rajapaksa R, Ginzton N, Rott LS, Greenberg PL. Altered oncoprotein expression and apoptosis in myelodysplastic syndrome marrow cells. *Blood* 1996;88:4275-87.