

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_{x_L}, 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.

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