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Haemopoietic growth factors and leukaemogenesis
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Haemopoietic stem cells give rise to all the different mature circulating blood cell types by a process of differentiation, proliferation and development. The pluripotent haemopoietic stem cells are required throughout the lifespan of an animal because mature cells, such as erythrocytes and neutrophils, are short lived. The defining characteristic of the stem cell population is therefore its continued pluripotentiality: it can both generate lineage-restricted progenitor cells which proliferate and develop into mature cells and self-renew to maintain the population of stem cells throughout the life of the organism. The structure and regulatory mechanisms that govern blood cell production have been the subject of intense research. It is hoped that, by studying the processes that balance survival, proliferation, differentiation and development, an understanding will be gained of how these events are disrupted in haemopoietic malignancies. Furthermore understanding the factors that regulate the self-renewal or differentiation of the stem cell are of clinical importance in that the stem cell is the key target for bone marrow transplantation and some gene therapy strategies.

Haemopoiesis occurs in the adult bone marrow. Within this microenvironment the proliferation and differentiation of the stem cells occurs in close contact with stromal cells and their associated extracellular matrix. These cell–cell and cell–extracellular matrix interactions are mediated in part by haemopoietic growth factors (HGFs) many of which are synthesized by stromal cells [1, 2]. HGFs can be present as integral membrane proteins of specific stromal cells or tightly associated with components of the extracellular matrix [3–5]. In consequence, HGFs are often compartmentalized within the stromal layer where their local concentrations are increased. Evidence for the importance of HGFs comes

Abbreviations used: AML, acute myeloid leukaemia; CML, chronic myeloid leukaemia; GM-CSF, granulocyte–macrophage colony-stimulating factor; HGF, haemopoietic growth factor; IL, interleukin; M-CSF, macrophage colony-stimulating factor; NF1, neurofibromatosis; SCF, stem cell factor; TGF, transforming growth factor; TNF, tumour necrosis factor.
from the biological effects observed in naturally occurring mutant mice [6]. For example, W mice cannot produce mature haemopoietic cells because of an intrinsic stem cell defect. The converse occurs in Steel mutant mice, which have a defective stromal microenvironment incapable of supporting haemopoiesis. The Steel and W mice lack the cytokine stem cell factor (SCF) and its receptor, c-Kit, respectively. This illustrates the intrinsic importance of one specific cytokine. The molecular cloning of the HGFs (and their receptors) has allowed an investigation of their role in haemopoiesis using genetically engineered mice with inactivated genes for specific HGFs. Such studies, in contrast with those with SCF and its receptor, indicate that the effects of some HGFs are not essential for blood cell production. For example, mice that are unable to respond to interleukin (IL)-3, granulocyte–macrophage colony-stimulating factor (GM-CSF) and IL-5 exhibit normal haemopoiesis [7]. However, knockout mice have also shown HGFs to have specific important functions, for example macrophage colony-stimulating factor (M-CSF) is essential for normal osteoclast function [8,9].

HGFs often act synergistically when added in combination to primitive cells. Initially this was discovered for IL-1 and M-CSF using the high proliferative potential colony-forming cell assay [10]. Alone, neither cytokine gave a growth stimulus to these cells, whereas their combination led to a massive proliferation and development of primitive cells to form macroscopic colonies in soft gel assays. Since then a wide range of cytokine combinations have been shown to induce a far greater than additive effect [11,12] on either the number of cells that proliferate in in vitro assays (synergistic recruitment) or the number of cell divisions observed (synergistic proliferation) from a single cell. It is possible that, in vivo, the primitive cell is receiving input from so many sources that the loss of one cytokine is of little importance in most instances. Thus redundancy is built into the haemopoietic system where there are known to be a large number of cytokines active on specific progenitor cell populations.

HGFs mediate their effects on cells by binding to specific receptor proteins, which activates intracellular signal-transduction pathways [13]. These signal-transduction pathways in turn activate a range of events, including transcription, which control cellular development as well as proliferation. The mechanism by which the specificity of the transcriptional response is generated is undoubtedly complex, but certain transcription factors are known to be associated with specific cellular events and appear to be regulated by lineage commitment. Recently the SCL/TAL-1 gene has been shown to be essential for the development of haemopoietic stem cells [14,15]. However, what regulates its expression remains unclear. The role of transcription factors in haemopoiesis is discussed in detail by Shivdasani and Orkin [16].

Although progress has been made in understanding cytokine-stimulated survival and proliferation at the cellular and intracellular level, little is still known of the molecular mechanisms that influence developmental 'decisions' such as whether the stem cell self-renews or differentiates. At present there is conflicting evidence on the stochastic versus deterministic nature of haemopoietic cell development. Committed progenitor cells can be isolated that respond to HGF stimulation by undergoing lineage determination. Alternatively, recent work has shown that erythroid development of primitive progenitor cells does not depend on expression of the erythropoietin (EPO) or the erythropoietin receptor (EPO R) genes [17]. These data come from experiments on EPO/EPO R null mice in which early and late erythroid progenitor cells were unaffected (BUF-e and CFU-e respectively) although CFU-e differentiation was badly affected. These and further data from IL-5/5 receptor transgenic mice [18], in which there is no observed eosinophilia (IL-5 acting very much like a growth factor for all lineages in these mice), argue for a stochastic commitment process not led by the HGF stimulation of specific differentiation signal-transduction pathways. However, it is possible that more mature cells, such as the bipotent granulocyte–macrophage colony-forming cells, do respond to specific HGFs by undergoing lineage commitment [19], whereas more primitive cells are governed in their differentiation by other unknown processes. This awaits further investigation.

Myeloid leukaemias are characterized by the disruption of the normally tightly regulated processes of proliferation, self-renewal and development. The majority of blast cells from patients with acute (AML) or chronic (CML) myeloid leukaemia still require haemopoietic growth factors for their survival and proliferation, although in some patients the blast cells do
exhibit growth-factor-independent proliferation (this factor independence has been shown to be an indicator of a poor prognosis) [20]. AML blast cells cannot be induced to undergo maturation to mature cells by cytokines; there is, however, a shift in the immunophenotype of AML blast cells in response to cytokines in some cases [21]. The requirement of AML cells for a proliferative stimulus can often be provided in an autocrine fashion. AML cells have been shown to produce M-CSF, GM-CSF, IL-1β, IL-6 and tumour necrosis factor (TNF). IL-1 and TNF do not act directly to stimulate proliferation of AML cells but instead promote the production of cytokines, such as GM-CSF, that can promote growth [22–24]. Furthermore the growth potentiator transforming growth factor β (TGFβ) (see below) can, in some cases of AML, increase the production of IL-1 and TNF and increase cell proliferation [25]. On the other hand, TNF and TGFβ interfere with the proliferation of AML blast cells in response to SCF by down-regulating the SCF receptor [26,27], demonstrating the complex autocrine and paracrine interactions that may occur to support the growth of leukaemic cells. Recently point mutations in the cytoplasmic domain of the granulocyte colony-stimulating factor (which promotes the development of myeloid cells to neutrophils and acts in synergy with other factors to stimulate the proliferation of stem cells) receptor have been linked to the development of AML in patients with severe congenital neutropenia [28]. The point mutations appear not to be the cause of the congenital neutropenia but increase the risk of developing AML [29].

There is now evidence of differential responses of normal and leukaemic cells to both HGF stimulation and inhibition. For example, in a long-term marrow culture system, primitive CML progenitor cells are resistant to the inhibitory effects of macrophage inhibitory protein 1α whereas normal progenitor cell growth is inhibited [30]. Thus one aspect of the process of leukaemogenesis may not only be a reduced requirement for a growth factor but a lack of response to a growth inhibitor. This aberrant response of leukaemic cells to HGFs not only arises because of abnormal HGF production or receptor interaction but also by disruption of downstream signalling pathways and DNA-transcription factors. Signalling proteins, such as Ras, are mutated in some cases of AML leading to their constitutive activation [31]. In the case of mutated ras this may not be the primary leukaemogenic lesion occurring in AML cells but it is certainly a marker for the progression of myelodysplastic disease (a clonal disorder where 25–45% of patients progress to acute leukaemias) to AML. In the myelodysplastic syndromes, a large proportion of patients have mutated N- and K-ras proto-oncogenes [32,33]. Some studies have suggested that there is a linkage between the ras mutations and poor prognosis [32]. Other proteins that interact with Ras or potentiate its activity may therefore also be associated with increased risk of AML. The neurofibromatosis (NF1) disease is associated with increased incidence of leukaemias, and the NF1 gene product can potentiate Ras activity but there is no evidence for gross abnormalities in the NF1 gene in the myelodysplastic syndromes [34].

There is evidence that Raf, a component of the Ras/Raf mitogen-activated protein kinase pathway, may be dysregulated in some erythroleukaemias [35]. Furthermore factor-independent leukaemic cell lines exhibit hyperphosphorylated activated Raf-1 protein kinase, unlike non-leukaemic cell lines where cytokines are required for its activation. In the case of CML there is clear involvement of a protein tyrosine kinase in the molecular pathology of the disease. CML is a clonal haemopoietic stem cell disorder, characterized by a marked expansion in the myeloid cell population. A reciprocal translocation, t(9,22)(q34;q11), results in the expression of an abnormal gene, which encodes the BCR/ABL protein. This protein has a dysregulated constitutive tyrosine kinase activity and appears to promote a range of signalling events. The activation of Ras appears to be a key feature of BCR/ABL-mediated transformation [36]. BCR/ABL has been shown to bind GRB2 (an adaptor for the exchange factor mSOS) and also to co-immunoprecipitate with SHC, linking it to the activation of Ras and its associated signalling pathway [37]. Interestingly it has also been demonstrated that BCR/ABL inhibits the action of p120GAP [38]. Thus it is possible that BCR/ABL activates Ras not only by the GRB/SOS pathway but also by inhibiting its inactivation by GTPase-activating protein (GAP). It has also been reported that BCR/ABL activates the phosphatase SYP [39], which may be responsible for the deactivation of negative regulators of signal transduction leading to an increase in signalling from BCR/ABL. Signal transducers and
activators of transcription are also activated by BCR/ABL [40], this being an event normally associated with the addition of cytokines such as IL-3 to haemopoietic cells. The exact consequence of the expression of this constitutively active BCR/ABL tyrosine kinase during the chronic phase of this disease is unclear as the cells remain cytokine-dependent and are not differentiation-blocked. Recently, however, it has been suggested that BCR/ABL confers enhanced survival potential and drug resistance on the cells [41]. The mechanism whereby suppression of apoptosis occurs remains unclear but evidence from the viral ABL tyrosine kinase suggests that mitogen-activated protein kinase is not involved, but protein kinase C β2 activation may be a key event leading to survival. Progression of CML from the chronic phase to the acute phase is often accompanied by secondary mutations in other oncogenes, including bcl-2 and that for p53 (the tumour suppressor protein), further implicating BCR/ABL in the inhibition of apoptosis. Thus one of the major effects of BCR/ABL may be to impose a genetic instability on the primitive haemopoietic cell leading to the eventual blast crisis observed in this disease.

A number of other chromosomal translocations involving genes encoding transcription factors have also been implicated in leukaemogenesis. The impaired developmental response in one form of AML (acute promyelocytic leukaemia) has been ascribed to the expression of the oncogenic transcription factor PML–RARα [42,43]. The chromosomal translocation t(15;17) results in the translocation of the retinoic acid receptor α (RARα) locus to the PML gene. The PML gene encodes a transcription factor with the PML–RARα fusion protein being transcriptionally active. Acute promyelocytic leukaemia is characterized by an increase in promyelocyte progenitors that are maturation-blocked. Treatment for this disease has been dramatically improved with the use of all-trans-retinoic acid which may remove the apparent dominant negative effect of PML–RARα on transcriptional regulation of maturation. As mentioned above, the SCL transcription factor is an essential element in haemopoietic cell development. It is also associated with T-cell acute lymphoblastic leukaemia as a chimaeric fusion protein resulting from a T(1;14)(p33;q11) translocation. These examples demonstrate the importance of identifying the products of chromosomal translocations in the leukaemias and other malignant diseases, the differences in activity of the fusion proteins and their normal counterparts allowing rational drug design.

In the case of cell survival pathways, one particular protein has been characterized as a consequence of its role in a B-cell malignancy, follicular lymphoma. A chromosomal translocation, t(14;18)(q32;q31), results in the aberrant overproduction of BCL-2, which suppresses the onset of apoptosis but does not stimulate cell cycle progression [44]. The HGF-mediated expression of BCL-2 in myeloid progenitor cells may have a critical role in the regulation of haemopoietic cell survival. BCL-2 also suppresses cytotoxic drug-induced apoptosis in bone marrow cells [45], which may be of some significance in the response of leukaemic cells to chemotherapeutic agents. This and other evidence indicate that not only regulation of development but also survival can be compromised in the leukaemic cell.

Thus the understanding of the role of HGFs in the survival, proliferation and differentiation of haemopoietic cells is contributing to the understanding of leukaemogenesis and it is hoped, will lead to novel strategies to treat these diseases.

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