Cell Death Signalling Pathways in the Pathogenesis and Therapy of Haematologic Malignancies: Overview of Therapeutic Approaches

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Abstract. Malignant diseases, including haematologic malignancies, are associated with defects in the cell death mechanism. These defects are not only important for the growth advantage of the malignant clone, but when understood can be used for specific therapeutic targeting of malignant cells while sparing normal cells. The promising groups of agents that trigger, directly or indirectly, apoptosis of haematologic cancer cells are reviewed in this article. Some of the agents have recently been approved for therapy, some are under the clinical evaluation in various phases of clinical trials and some are tested under the experimental laboratory conditions.

Apoptosis and haematologic malignancies

It is a widely accepted paradigm that in transformed, unlike normal cells, even reversible damage can trigger and carry out programmed cell death. A huge body of evidence supports the fact that a great number of chemotherapeutic drugs act via inducing apoptosis in malignant cells. Drug resistance, then, is frequently associated with apoptosis programme deregulation. Deregulation of proteins that play important roles in controlling apoptotic cell death propagation is a hallmark of many haematologic malignancies (Bianco et al., 2006). Induction of strong and tumour-specific apoptotic response with minimal impact on normal tissues is the ultimate goal of emerging targeted therapy.

The extrinsic apoptotic pathway

TNF-related apoptosis inducing ligand (TRAIL) is a protein that mediates apoptosis in a variety of cell types. It is a member of the tumor necrosis factor (TNF) family and is expressed by a variety of cell types, including immune cells, tumor cells, and endothelial cells. TRAIL binds to its receptors, TRAIL-R1 and TRAIL-R2, which are expressed on the surface of cells. Upon activation, TRAIL-R1 and TRAIL-R2 undergo oligomerization and recruit death domain-containing proteins, such as FAS-associated death domain protein (FADD), which in turn recruit caspases, leading to cell death.

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FasL and anti-Fas antibodies

FasL, a 40 kD death ligand belongs to the major effector molecules of the cytotoxic T lymphocytes. Both FasL and its receptor Fas are expressed on activated T lymphocytes and NK cells. FasL can also bind to the inhibitory decoy receptor 3 (DcR3). Fas and FasL play crucial roles in immune regulations. Soluble FasL is only a weak agonist of Fas and can even antagonize the pro-apoptotic activity of membrane-bound FasL (Suda et al., 1997). As with TNF-α, in vivo murine studies using anti-Fas antibody showed severe toxicity side effects, especially haemorrhagic damage of the liver by apoptosis (Ogasawara et al., 1993). Fas-bearing malignant cells tend to be susceptible to FasL-induced apoptosis (Nguyen et al., 1996). Resistance to FasL-induced apoptosis is, however, not a rare finding.

Many haematologic malignancies have been shown to bear or shed FasL (Findley and Zhou 1999; Greil et al., 2003). It was demonstrated that such FasL-expressing transformed cells can kill Fas-expressing cells of host tissues, including cytotoxic T lymphocytes. This could contribute both to tumour invasiveness and toxic side effects associated with tumour progression (Zeytun et al., 2000). It has been reported that increased expression of FasL and Fas on haematologic progenitors might contribute to inefficient erythropoiesis in myelodysplastic syndromes (MDS) (Claessen et al., 2002). Data concerning the significance between the tumour expression of Fas/FasL, and biology of the tumour remain, however, controversial (Lepelley et al., 1998; Oshshima et al., 2000; Lee et al., 2001; Grullich et al., 2003; Hazar et al., 2005).

TNF-related apoptosis-inducing ligand (TRAIL)

Much expectation has been generated for TRAIL, a 32 kD protein, as a potential highly specific and efficient anti-cancer drug. TRAIL is not toxic for most normal tissues, with conflicting data concerning the liver and the brain (Jo et al., 2000; Hao et al., 2004). On the other side, TRAIL is a potent apoptosis inducer of a variety of transformed cell lines as well as primary tumour cells and is capable of inducing tumour regression of cancer xenotransplants in the immunodeficient mouse model, including various myelo- and lymphoproliferative diseases (Ashkenazi et al., 1999; Plasilova et al., 2002). TRAIL and its receptors are widely expressed in a variety of normal tissues. Hence, strong negative regulation of TRAIL-induced apoptosis must be anticipated in most tissues, which would favour TRAIL to both FasL and TNF-α for use in systemic therapy. Similarly to FasL, TRAIL appears to be essential natural killer (NK) /cytotoxic T cell weaponry against transformed cells (Takeda et al., 2002). Thus, tumour immune surveillance is speculated to be one of the main physiologic functions of TRAIL. TRAIL can interact with five receptors. Upon binding of TRAIL, death receptors (DR4, DR5) initiate the apoptotic process. Decoy receptors (DcR1, DcR2) are unable to transduce death signals, and their activities thus appear to be primarily anti-apoptotic. Osteoprotegerin (OPG), a soluble low-affinity (receptor) for TRAIL, neutralizes all activities elicited by TRAIL (Emery et al., 1998).

Many potential molecular mechanisms of resistance to TRAIL-induced apoptosis in cancer cells have been reported up to the present. Increased expression of decoy receptors has been correlated with resistance to TRAIL in acute and chronic myeloid leukemias (Bruserud, 2005; Riccioni et al., 2005). OPG might function as a paracrine survival factor against TRAIL-induced apoptosis in the bone marrow microenvironment in multiple myeloma cells (Shipman and Croucher, 2003). Overexpression of cFLIP, a dominant-negative inhibitor of caspase-8, has frequently been associated with TRAIL (and FasL)-resistant phenotype in haematologic malignancies. Targeting of cFLIP might represent a useful approach in restoring sensitivity to TRAIL-induced apoptosis. Deregulated expression of the inhibitor of apoptosis (IAP) family of caspase inhibitors contributes to the resistance to TRAIL-induced apoptosis in a wide range of acute as well as chronic leukemias.

In certain types of primary lymphomas and leukemias, the expression of TRAIL or/and its death receptors can be induced by radiation, cytostatics, pro tease inhibitors, antimetabolites and many other agents (Unnithan and Macklis, 2004). Conversely, resistant lymphomas and leukemias were sensitized to irradiation and/or chemotherapy-induced apoptosis after incubation with TRAIL (Belka et al., 2001). A synergistic effect of combining anti-tumour agents that act on different apoptotic pathways might prove optimal for maximal therapeutic efficacy in haematologic malignancies (Ballestero et al., 2004; Rosato et al., 2004).

\( \text{TRAIL death receptors (DR) and antibodies to DR4 and DR5} \)

Dozens of monoclonal antibodies (MoAbs) against specific surface antigens have been developed up to now for the treatment of various haematological malig-
nancies. Some of the antibodies have been in clinical use for years (e.g. anti-CD20/rituximab, anti-CD33/gemtuzumab-ozogamicin, anti-CD52/alemtuzumab), others only recently reached clinical trials. Antibodies against TRAIL death receptors induce the extrinsic apoptotic pathway in many cancer cell types. In addition to that, antibodies can consolidate elimination of target tumour cells by triggering other mechanisms, such as activation of complement or antibody-mediated cytotoxicity. Phase I and II clinical trials are under way testing the effects of anti-DR4 (HGS-ETR1/mapatumomab) and anti-DR5 (HGS-ETR2), respectively, in a variety of malignancies, including lymphoproliferative diseases (http://www.hgsi.com/products/index.html, http://www.cambridgeantibody.com/html/products).

**Cellular FLICE-like inhibitory protein (cFLIP): the inhibitor of the extrinsic apoptotic pathway**

The ratio of caspase-8 to cFLIP, its non-functional homologue/inhibitor, is critical for the assembly of DISC (death-inducing signalling complex) and subsequent extrinsic apoptotic cascade propagation. Upregulation of cFLIP has been associated with diverse haematologic cancer cell lines and primary malignancies, including Hodgkin lymphoma, B-cell non-Hodgkin lymphoma (NHL), B-cell chronic lymphocytic leukaemia (B-CLL) (Shain et al., 2002; Thomas et al., 2002; Dutton et al., 2004; Mathas et al., 2004). The sensitization of many cancer cells to death ligand-mediated apoptosis appeared to be mediated by cFLIP downregulation (Kim et al., 2002; Siegmund et al., 2002). Inhibition of cFLIP expression might be of particular importance for cancer therapies based on the induction of extrinsic apoptotic pathway.

Synthetic triterpenoids (e.g. CDDO, 2-cyano-3,12-dioxooleana-1,9-dien-28-oic acid) have recently been reported to activate apoptosis in acute myelogenous leukaemia (AML) cells mainly through downregulation of cFLIP with subsequent sensitization to TRAIL (Ito et al., 2000; Konopleva et al., 2002; Stadheim et al., 2002). Besides inhibition of cFLIP, triterpenoids block activation of NFkB and induce the intrinsic apoptotic pathway in diverse tumour cells (Chauhan et al., 2004; Ikeda et al., 2004; Inoue et al., 2004b).

**BAFF and APRIL: B-cell pro-survival signalling**

B-CLL cells, non-Hodgkin lymphoma or multiple myeloma cells rapidly undergo apoptosis when cultured in vitro. Exogenous administration of TNF superfamily members BAFF/BLyS (B-cell activating factor/B-lymphocyte stimulator) and APRIL (A proliferation antigen) could, however, significantly improve their in vitro survival. Non-malignant B cells up regulate BAFF/BLyS and APRIL upon stimulation by T-cell CD40 ligand. BAFF and APRIL thus represent upstream ligands of physiologic autocrine pro-survival pathways (Mackay et al., 2003; Schneider and Tschopp 2003). BAFF binds to three receptors: TACI (transmembrane activator and calcium modulator cyclophilin ligand interactor), BCMA (B-cell maturation antigen) and BAFF-R/BR3 (BAFF receptor). APRIL binds to TACI and BCMA. A splice variant of BAFF, designated δBAFF, can form heterotrimers with full-length BAFF, thereby neutralizing its effects (Gavin et al., 2003). Moreover, BAFF-APRIL heterotrimers have lately been reported to bind to TACI, which only underscores the complexity of the BAFF-APRIL ligand-receptor system. All three receptors recruit adaptor proteins of the TRAF family (TNF receptor-associated factors) and induce nuclear translocation of NFkB. Recent data suggest that many haematologic malignancies of B-cell origin can evade apoptosis by aberrant expression of the BAFF and APRIL cytokines (He et al., 2004; Kern et al., 2004; Moreaux et al., 2004; Nishio et al., 2005). BAFF levels have been reported elevated in the serum of patients with a variety of B-lymphoid malignancies. Moreover, the 13q32–34 locus encompassing the BAFF gene is often amplified in NHL (Rao et al., 1998). Neutralization of BAFF and/or APRIL by soluble TACI and BCMA receptors increased apoptosis of several B-cell lymphoma cell lines. Countering the pro-survival effects of aberrantly expressed BAFF and/or APRIL thus might represent a novel therapeutic approach in the treatment of various B-cell malignancies.

**The intrinsic apoptotic pathway**

**B-cell lymphoma 2 (Bcl-2) family**

The Bcl-2 family of proteins represents key regulators of the mitochondrial apoptotic pathway. The decreased sensitivity to mitochondrial apoptosis is associated with a drug-resistant phenotype in many tumours (Hockenbery et al., 1990; Letai, 2005). Overexpression of Bcl-2 anti-apoptotic regulator is a hallmark of follicular lymphoma. Furthermore, Bcl-2 deregulation is a common finding in chronic lymphocytic leukaemia (CLL), diffuse large B-cell lymphoma (DLBCL), multiple myeloma (MM), and acute leukemias. Interestingly, Epstein-Barr virus (EBV), implicated in the development of Burkitt lymphoma, HIV-related lymphomas, and posttransplantation lymphomas, encodes a Bcl-2 homologue BHRF1 (Henderson et al., 1993; Oudejans et al., 1995). Upregulation of Mcl-1, another Bcl-2 family antiapoptotic molecule, is a common finding in MM and CLL (Derenne et al., 2002). Based on the experimental data from mouse models, correction of an apoptotic defect itself can be detrimental for transformed cells (Letai et al., 2004). Strategies either to down regulate/block the expression of anti-apoptotic Bcl-2 members or to upregulate-supplement the pro-apoptotic members of the Bcl-2 family have been recently developed. The two
major approaches include antisense oligonucleotides to Bcl-2 anti-apoptotic members and small blocking peptides mimicking the BH3-only pro-apoptotic proteins. Antisense phosphorothioate 18-mer oligonucleotide oblimersen sodium (G3139, Genasense) has been designed to bind to the first six codons of the Bcl-2 mRNA (Cotter et al., 1994). Apart from having strong, dose-dependent anti-tumor effects in various in vitro and in vivo studies, oblimersen was also capable to potentiate the effects of a spectrum of cytostatic agents, monoclonal antibodies, steroids and radiation. Early clinical trials have shown only modest, if any, single-agent oblimersen benefits for the patients with MM and metastatic melanoma (Flaherty et al., 2001). Phase I and II studies testing oblimersen in combination with conventional chemotherapy, on the other hand, have brought encouraging results in patients with non-Hodgkin lymphoma, CLL, and AML (Waters et al., 2000; Marcucci et al., 2003, 2005; van de Donk et al., 2004; Badros et al., 2005; O’Brien et al., 2005). In a phase III clinical trial of CLL oblimersen in combination with chemotherapy improved major responses compared to chemotherapy alone. The results of phase III studies in patients with MM and CLL are awaited. Despite the initial disappointment with single-agent oblimersen, further evaluations of its anti-tumour activity in combination with diverse cytotoxic agents seem warranted. Other antisense strategies include antisense Bcl-xL, bispecific antisense Bcl-2/Bcl-xL, and antisense Mcl-1 oligonucleotides (Zangemeister-Wittke et al., 2000; Gautschi et al., 2001; Tortora et al., 2003).

Compounds mimicking BH3-only proapoptotic members of the Bcl-2 family represent promising anti-cancer agents. The BH3-only proteins include Bim, Bmf, Bik, Bad, Bid, Puma, Noxa, and Hrk. The BH3-only proteins mediate diverse death stimuli from the environment and from within the cell. Activated BH3-only proteins inactivate anti-apoptotic Bcl-2-like molecules (e.g., Bcl-2 or Bcl-xL), which results in the liberation of proapoptotic BH123 members Bax/Bak with subsequent induction of mitochondrial apoptosis. Binding of specifically designed molecules to the surface pocket of Bcl-2 or Bcl-xL would mimic the binding of BH3-only proteins, and trigger the mitochondrial apoptotic pathway by blocking its inhibitors (Wang et al., 2003b). To BH3 mimetic also belong derivatives of vitamin E succinate. Their anti-tumour action in addition involves enhanced production of reactive oxygen species (ROS) and synergism with TRAIL in inducing apoptosis of tumour cells (Weber et al., 2003).

Regulators of apoptosis

Inhibitor of apoptosis proteins (IAPs)

IAPs comprise a family of at least eight proteins that compromise the effector phase of apoptosis through blocking or inactivation of caspases (Shi, 2004). The overexpression of IAPs is associated with a drug-resistant phenotype of cancer cells, making IAPs promising molecules for targeted therapy of various cancers (de Graaf et al., 2004; Schimmer, 2004). To date, XIAP (X-linked IAP) and survivin have gained the most interest. Survivin is overexpressed in many tumours and transformed cells (Ambrosini et al., 1997). The exact mechanism of the anti-apoptotic function of survivin remains, however, elusive. Some studies have demonstrated a direct interaction with caspases-3 and -7. In addition, survivin is a regulator of cell cycle progression and mitosis (Ambrosini et al., 1998). XIAP, in addition to the inhibition of caspase-3 and -9 (Huang et al., 2004), is an activator of the NFκB pathway (Holecik et al., 2001). As with the Bcl-2 anti-apoptotic proteins, two approaches impacting IAP molecules have been designed: antisense oligonucleotides and small protein inhibitors (Wang et al., 2004). Phase I clinical trial of XIAP and Survivin antisense oligonucleotides in diverse malignancies are under way in the United Kingdom. In addition to that, small protein inhibitors are being developed that would mimic the blocking activity of the endogenous SMAC/Diablo protein, a potent natural inhibitor of IAPs. Polyphenylurea-based XIAP antagonists, for example, have recently been shown to induce apoptosis in several leukaemia cell lines and primary AML cell samples (Schimmer et al., 2004; Carter et al., 2005).

Heat-shock proteins (HSPs) and heat-shock protein inhibitors (HSPI)

HSPs act mainly as chaperone molecules that interact with a variety of cellular proteins to disaggregate, refold, and renature misfolded or aggregated proteins. HSPs include anti-apoptotic and pro-apoptotic proteins. Overexpression of Hsp27, Hsp70, and Hsp90 subfamilies have been implicated in the protection of various cancer cells against drug-induced apoptosis (Ray et al., 2004; Guo et al., 2005; Schepers et al., 2005; Thomas et al., 2005). Therapeutical inhibition of inducible HSP expression or activity using HSP inhibitors (HSPI) recently emerged as novel therapeutic strategies in anti-cancer protocols.

Hsp90 binds and stabilizes a variety of client proteins, including mutated p53, chimeric Bcr-Abl, aberrant Raf1, deregulated Akt, ErbB2, or ZAP70 (Blagosklonny, 2002). The treatment of CML cells with Hsp90 inhibitors herbimycin A (HMA), geldanamycin (GA), or its derivative 17-AAG has led to rapid destruction of the Bcr-Abl-positive clones. Furthermore, CML cells that have developed resistance to imatinib mesylate still remain susceptible to HSPI (Gorre et al., 2002). Besides CML, HSPI might be of therapeutic value in patients with CLL, AML, Hodgkin’s lymphoma, NHL, or MM (Castro et al., 2005; Thomas et al., 2005; Ma et al., 2006; Mitsiades et al., 2006).
Aberrantly activated pro-survival signalling pathways

The fate of each cell is determined by a variety of anti-apoptotic/mitogenic/pro-survival and pro-apoptotic/stress/death signals that are transduced via a complex interdependent net of signalling molecule cascades. Cytokines, hormones, and growth factors control all aspects of cell development, differentiation, growth, proliferation, and survival of haematopoietic cells. Cytokine/growth factor signalling is initiated by binding of a ligand to the corresponding receptor on the cell surface. Cytokine receptors can be divided into two distinct groups: receptor tyrosine kinases (RTKs) with intrinsic tyrosine kinase activity, such as PDGF, EGF, VEGF, and insulin receptors and cytokine receptors that signal through JAK-STAT (Janus kinase, signal transducers and activators of transcription) with receptor-associated tyrosine kinase proteins, e.g. Epo, Tpo, GM-CSF, and most of the interleukin receptors.

Kinases, such as protein-tyrosine kinases (PTKs), protein serine-threonine kinases, lipid kinases, protein kinases B and C, are important regulators of intracellular signal-transduction pathways. Perturbation of kinase signalling may result in constitutive activation of transcription factors, such as NFκB, and malignant transformation (Blume-Jensen and Hunter, 2001).

Janus kinase (JAK) – signal transducers and activators of transcription (STAT) cascade

The Janus kinase protein-tyrosine kinase members (JAK1, 2, 3, and Tyk2) are crucial components of
diverse signal transduction pathways that govern cellular survival, proliferation, differentiation and apoptosis. Binding of cytokines to cell surface receptors results in the receptor oligomerization with subsequent activation of Janus kinase molecules (JAKs). Activated JAKs phosphorylate cytoplasmic domains of the aggregated receptors, thereby creating docking sites for proteins called signal transducers and activators of transcription (STATs: STAT1, 2, 3, 4, 5a, 5b, and 6). Once bound to the cytokine receptors STATs become phosphorylated by JAKs, form homo- and hetero-dimers, and readily translocate to the nucleus, where they initiate transcription of diverse target genes. The JAK-STAT signalling pathways are controlled by at least three classes of negative regulators. These include suppressors of cytokine signalling (SOCS), protein tyrosine phosphatases (e. g. Src-homology 2-containing phosphatase-1, SHP-1), and protein inhibitors of activated STATs (PIAS). A number of diseases including haematologic malignancies are associated with deregulation of JAK-STAT signalling pathways (Benekli et al., 2003; Valentino and Pierre 2006).

Constitutive activation of JAK2 and STATs are believed to mediate neoplastic transformation and promote abnormal cell proliferation in various malignancies. STATs have been found constitutively activated in a variety of myeloid and lymphoid haematologic malignancies. Constitutively activated STAT1, 3, 5 and/or 6 have been detected in acute and chronic leukaemias, B-cell NHL, Burkitt lymphoma, several types of cutaneous lymphoma, in Reed-Sternberg cells from patients with Hodgkin’s disease, multiple myeloma, and others (Epling-Burnette et al., 2001; Spiekermann et al., 2001; Skinnider et al., 2002; Aoki et al., 2003; Benekli et al., 2003). Furthermore, STATs contribute to resistance of malignant cells to apoptosis and to cell-mediated cytotoxicity by interfering with the transcription/function of key apoptosis and cell cycle regulators, including Bcl-2, Mcl-1, Bcl-XL, c-Myc, cyclin D1, p21WAF1, and others. IL-6 and IL-10 autocrine/paracrine loops that belong to the major survival pathways identified in malignant B-cells are mediated primarily by the STAT3 signalling pathway (Alas et al., 2001). Mutation in JAK2 (V617F) has been associated with myeloproliferative disorders, most specifically with polycythemia vera (James et al., 2005; Kralovics et al., 2005; De Keersmaecker and Cools, 2006).

A number of strategies are being developed to target aberrantly activated JAK-STAT pathways at different levels of the signalling cascade. Monoclonal antibodies against the cytokine receptors (e. g. Sant7, IL-6 super-antagonist) represent the most upstream approach, while specific inhibitors of JAK and STAT proteins (SOCS, PIAS, SHP1) belong to downstream strategies (Campo et al., 2005; Tassone et al., 2005; Valentino and Pierre 2006). Inhibition of STAT expression by antisense oligonucleotides constitutes another possible way for therapeutic blockage of upregulated JAK-STAT signalling in tumours. Tyrophostin (AG490, Jak2 inhibitor) and Piceatannol (STAT3 inhibitor), respectively, have recently been reported to sensitize NHL and MM cell lines to a battery of chemotherapeutic drugs (Epling-Burnette et al., 2001; Alas and Bonavida, 2003).

**Receptor tyrosine kinase (RTK) and Bcr-Abl signalling pathways**

Approximately 20 different RTK subfamilies have been identified, all of which share similar structure that includes a ligand-binding extracellular domain, a single transmembrane domain and an intracellular tyrosine kinase domain. Signaling by RTKs requires ligand-induced receptor oligomerization, which results in tyrosine autophosphorylation of the receptor subunits. This both activates catalytic activity and generates phosphorylated tyrosine residues that mediate the specific binding of cytoplasmic signalling proteins containing Src homology-2 (SH2) and protein tyrosine-binding (PTB) domains (Blume-Jensen and Hunter 2001). Gain-of-function mutations of RTKs are germane to the leukaemic transformation. The aberrant activation of RTKs result in enhanced signalling of several important pro-survival cascades, including Ras-Raf (MAPKKK)-MEK (MAPKK)-Erk (MAPK) or PI3K-Akt-mTOR pathways, which both contribute to increased proliferation, accelerated growth, and weakened susceptibility to apoptotic stimuli of leukaemic cells (Skorski et al., 1997). Fit-3 receptor, RTK class III, is the most commonly mutated gene in acute myeloid leukaemia, while c-kit (stem cell factor receptor) mutations are strongly linked to the development of mast cell malignancy. Constitutive activation of RTKs causes aberrant activation of Ras/Rho (Ras homologue) GTPase oncoproteins. Aberrant activation of Ras/Rho proto-oncogenes by point mutation, overexpression or amplification represents the most frequent molecular mechanisms of enhanced Ras-signalling in solid cancers. The frequency of gain-of-function ras mutations in haematologic malignancies varies from 5% to 15% in acute lymphocytic leukaemia (ALL) up to 65% in chronic myelomonocytic leukaemia (CMML). Ras mutations are a common finding in multiple myeloma as well as in myelodysplastic syndrome (MDS) patients. Posttranslational prenylation (farnesylation, geranylgeranylation) is a crucial step in attachment of Ras/Rho to the inner leaflet of the plasmatic membrane and is indispensible for proper signal transduction. The inhibition of Ras/Rho prenylation by farnesyltransferase inhibitors (FTI) or geranylgeranyltransferase inhibitors (GGTI) thus represents a powerful approach to inhibit aberrant Ras/Rho signalling in cancer cells (Lobell et al., 2001). The anti-tumour effects of FTI members tipifarnib (Zarnestra) and lonafarnib (Sarasar) have already been evaluated in clinical trials. For the treatment of haematologic malignancies, combinations of FTIs with standard chemotherapy agents emerge as the most promising strat-
ogy (Cortes et al., 2003; Lancet and Karp 2003; Mesa et al., 2003; Kurzrock et al., 2004; Yanamandra et al., 2006).

Signalling pathways aberrantly activated by the chimeric non-receptor PTK Bcr-Abl is very similar to the signalling pathways activated by RTKs, including the Ras-Raf (MAPKKK)-MEK (MAPKK)-Erk (MAPK), JAK-STAT and PI3K-Akt-mTOR pathways (Deininger et al., 2000). The Bcr-Abl translocation (Philadelphia (Ph) chromosome) is an example of consistent chromosomal abnormality associated with a specific type of leukaemia. The reciprocal translocation t(9; 22) involves the non-receptor PTK c-Abl on chromosome 9 and a breakpoint cluster region (BCR) on chromosome 22 (Shitovelman et al., 1985). A majority of patients with CML, and a significant fraction of Ph-positive patients with ALL have one of three different versions of this translocation (Kurzrock et al., 2003). The first therapeutic agent of the novel targeted therapy molecules ever introduced to clinical use was PTK inhibitor imatinib mesylate (Gleevec, Glivec), formerly known as signal transducer inhibitor-571 (STI-571) or CGP57148B. Imatinib mesylate acts mainly via blocking the chimeric PTK Bcr-Abl (Goldman and Melo, 2003; Pardanani and Tefferi 2004). Since imatinib targets also other kinases in addition to Bcr-Abl (e. g. c-kit or PDGFR), its activity was explored for myeloproliferative disorders harbouring an $ETV6-PDGFR-\alpha$ fusion gene, hypereosinophilic syndrome with a $FIP1-PDGFR-\alpha$ fusion gene and solid tumours with gain-of-function mutations of c-kit and PDGFR RTKs (Apperley et al., 2002; Cools et al., 2003a; Arora and Scholer, 2005). In addition to imatinib, other small molecule inhibitors of receptor and non-receptor PTKs desatinib (BMS-354825, Sprycel) and nilotinib (AMN-107), have generated much promise in clinical trials in imatinib mesylate-resistant CML patients (Schittenhelm et al., 2006). PTKI N-benzoylstaurosporine (PKC412), gefitinib (Iressa), and erlotinib (Tarceva) are under clinical investigation for the treatment of refractory leukaemia, multiple myeloma and MDS patients are under way (Lee and Dominguez, 2005; Milella et al., 2005).

Disruption of MAPK signalling plays a pivotal role in the pathogenesis of cancer, including haematologic malignancies (Platanias, 2003). Constitutive activation of the Raf-Mek-Erk cascade abrogates the requirement of normal haematopoietic cells for growth factors, which results in cytokine independence. Erk kinase has been detected either constitutively activated or overexpressed in the majority of acute leukaemias. In addition to that, the very central role for Erk signalling is suggested in the pathogenesis of a rare form of leukaemia, the natural killer large granular lymphocyte leukaemia. In CML, the Erk pathway seems to be activated as a result of Bcr-Abl transformation. Moreover, the Raf-Mek-Erk cascade is essential for the multiple myeloma IL-6-dependent plasma cells. Clinical trials with MAPK inhibitors, alone or in combination either with standard chemotherapy or other experimental agents (e. g. Bcl-2 inhibitors, cell-cycle inhibitors, etc.) for the treatment of refractory leukaemia, multiple myeloma and MDS patients are under way (Lee and Dominguez, 2005; Milella et al., 2005).

Phosphatidylinositol 3 kinase (PI3K)
– Akt/protein kinase B (PKB) – mammalian target of the rapamycin (mTOR) pathway

PI3Ks are a family of related enzymes that are capable of phosphorylating the 3 position hydroxyl group of the inositol ring of phosphatidylinositol (Foster et al., 2003). The PI3K-Akt/PKB signalling pathway plays a pivotal role in conducting survival signals in a wide range of cells (Song et al., 2005). Several upstream activators of PI3K-Akt/PKB have been described up to the present, among them RTKs and Ras/Rho proteins. The most important negative regulator of PI3K cascade is phosphatase and tensin homologue (PTEN) tumour suppressor, frequently mutated in diverse tumours (Kennedy et al., 1999; Suhara et al., 2002). Conditional deletion of the PTEN tumour suppressor gene in mouse adult haematopoietic cells led to the development of leukaemias within weeks (Yilmaz et al., 2006). Constitutively activated Akt is a frequent finding in haematologic malignancies, especially in AML and mantle cell lymphoma patients. It has been shown that the growth of MM cells depends on the PI3K-Akt cascade. Activated Akt kinase inhibits function and/or transcription of many pro-apoptotic Bcl-2 members, including Bad and Bim, which leads to decreased susceptibility of...
the malignant cells to apoptosis. The therapeutic blockage of the aberrantly activated PI3K-Akt pathway is therefore of eminent interest for experimental treatment of many haematologic malignancies.

The mammalian target of rapamycin (mTOR), a large serine-threonine kinase, is a direct downstream effector of the PI3K-Akt pathway that controls the activation of ribosomal protein translation. Translation control is effectuated through two major cascades: the activation of the 40S ribosomal protein S6 kinase 1 (p70S6K1), and the inhibition of 4E-binding protein 1 (4E-BP1), which consequently dissociates from the eukaryotic initiation factor 4E (eIF-4E) (Janus et al., 2005). Through the 4E-BP1 and p70S6K1 pathways, mTOR controls the translation of mRNAs that accelerates the cell cycle progression from G1 to S phase. In addition to that, mTOR is a principal regulator of cyclin-dependent kinase (CDK) inhibitor p27kip1, retinoblastoma (Rb) protein, cyclin D1, c-Myc, or STAT3 (Recher et al., 2005a). Rapamycin, a mTOR inhibitor, is a natural product with antimicrobial, immunosuppressive, and anti-tumour qualities. Rapamycin analogues sirolimus (Rapamune), everolimus (RAD001), and temsirolimus (CCI-779) have been shown to exert antiproliferative effects in many leukaemia, lymphoma and myeloma cell lines (Pene et al., 2002; Giles and Albitar 2005). Phase II and III clinical trials of mTOR inhibitors in patients with acute and chronic leukaemias, and relapsed mantle cell lymphoma are under way, and further studies combining mTOR inhibitors with standard chemotherapy are warranted (Giles and Albitar 2005; Recher et al., 2005b; Witzig et al., 2005; Teachey et al., 2006).

Protein kinase C (PKC) pathway

The protein kinase C (PKC) is a family of phospholipid-dependent serine-threonine kinases that act as key components of phospholipase C (PLC)-coupled growth factor receptor signaling pathways. Conventional PKCs (isoforms α, β, βII, and γ) require Ca2+, diacylglycerol (DAG), and a phospholipid for activation. Novel PKCs (isoforms δ, ε, η, and θ) require DAG, but not Ca2+ for activation (Steinberg, 2004). Intensive cross-talk exists between PKC and many important pro-survival signalling pathways. PKCs can directly activate the Ras-Raf-Mek-MAPK pathway by phosphorylating Raf (Ueda et al., 1996). PKC is activated by 3-phosphoinositide-dependent protein kinase-1 (PKD1) a downstream target/effector of PI3K, which underscores the complexity and interdependence of several pivotal pro-survival cascades. PKC is thought to be an important player in carcinogenesis and cancer progression. PKC-α and PKC-β have been linked to increased invasion, proliferation, drug resistance and genetic instability, while PKC-δ is thought to increase apoptosis (Koivunen et al., 2006).

ISIS 3521, an antisense phosphorothioate oligonucleotide to PKC-α has demonstrated anti-tumour activity in patients with relapsed low-grade NHL (Nemunaitis et al., 1999; Rao et al., 2004). UCN-01 (7-hydroxystaurosporine) was originally developed as a protein kinase C (PKC) inhibitor but was subsequently shown to inhibit several other kinases, including DNA damage kinase Chk1, PDK1, and PKB (also known as Akt). UCN-01 has triggered apoptosis in a set of leukaemia cell lines (Rahmani and Grant 2002; Yamauchi et al., 2002). In addition to that, strong synergetic effects between UCN-01 and agents that inhibit the PI3K, RTK or MAPK pathway, respectively, have been recently reported in a variety of preclinical studies (Dai et al., 2001, 2005; Jia et al., 2003; Cory et al., 2005; Hahn et al., 2005). More potent and specific inhibitors of the PI3K-Akt pathway are currently in development, and clinical trials testing their anti-tumour effects are warranted.

The staurosporine derivative PKC412 (N-benzoylstaurosporine) is a more selective inhibitor of the conventional isoforms of PKC. In addition to that, PKC412 reverses the efflux function of the multidrug resistance 1 (MDR1) gene product, P-glycoprotein (P-gp), and most importantly has potent inhibitory effects against a variety of class III RTKs (e.g. VEGF-R, PDGFR, Flt-3) (Levis et al., 2002; Weisberg et al., 2002; Stone et al., 2005). Consequently, PKC412 was found to potentially inhibit the growth of B-CLL and acute leukaemia cell lines. Inhibition of PKC thus offers a novel approach to the chemotherapy of acute leukemias and B-cell malignancies (Propper et al., 2001; Cools et al., 2003b; Chen et al., 2004; Stone et al., 2005). Bryostatin 1, which belongs to PKC modulators, can induce differentiation of CLL cells and exerts anti-leukaemic activity in vivo (Drexler et al., 1989; Battle and Frank 2003). Phase I and II clinical trials have demonstrated potential for bryostatin 1 as a therapeutic agent for refractory leukemias and indolent haematologic malignancies. The exact mechanism of action of bryostatin 1 remains, however, elusive. Recently, bryostatin 1 and UCN-01 have both been reported capable of eliciting apoptosis in human myeloid leukaemia cells, yet through disparate mechanisms (Wang et al., 2003a).

PKC-δ, Ca2+-independent PKC isofrom, plays a central role in the genotoxic stress response leading to the induction of apoptosis in cells exposed to DNA-damaging agents. In addition to that, PKC-δ is a potent activator of NFκB and cyclin D1, two of the major targets for several growth stimulatory signalling pathways (Soh and Weinstein 2003), and phosphorylates p53 tumour suppressor, which leads to its ubiquitination and subsequent degradation of p53 by proteasome (Chernov et al., 1998). Blockade of constitutively activated PKC-δ by its specific inhibitor rottlerin has been shown effective in triggering apoptosis in a variety of CLL cells (Barragan et al., 2002; Ringshausen et al., 2002).
Nuclear factor κ B (NFκB) transcription factors and ubiquitin/proteasome system inhibitors

In mammalian cells the NFκB family consists of RelA (p65), RelB, c-Rel, p105/p50 (NFκB1), and p100/p52 (NFκB2). NFκB is kept quiescent in the cytoplasm as a dimer bound to its repressors, inhibitors of NFκB (IkB) family. The IkB family includes IkBo, IkBβ, IkBe, and Bcl-3. Phosphorylation of IkB by IkB-kinase (IKK) as a consequence of diverse upstream stimuli is followed by rapid ubiquitination and degradation of IkB in 26S proteasome, which results in the translocation of liberated NFκB to the nucleus with subsequent target gene transcription initiation or repression (Siebenlist et al., 1994). Loss of normal regulation of NFκB has been associated with accelerated growth, resistance to apoptosis, and propensity to form metastases. Constitutive nuclear NFκB activity has been detected in a plethora of haematologic and solid malignancies, including multiple myeloma, Hodgkin lymphoma, B-CLL and several non-Hodgkin lymphoma subtypes (Lu and Stark, 2004; Kim et al., 2006). NFκB2, c-Rel, and Bcl-3 proteins are encoded by the genes located in the regions frequently involved in rearrangements or amplifications of diverse B-cell malignancies and cutaneous lymphomas. Mutations in the IkBa gene have been detected in Hodgkin’s lymphoma and are suggested to render NFκB constitutively active in Hodgkin’s cells (Emmerich et al., 1999; Nishikori et al., 2003).

The ubiquitin/proteasome complex is responsible for the degradation of ubiquitin-tagged intracellular molecules (Zhang et al., 2004). Proteasome inhibitors have been shown to increase susceptibility of tumour cells to apoptosis in vitro, as well as to inhibit tumour growth, angiogenesis and metastases in vivo. Transformed and proliferating cells are more sensitive to proteasome inhibition than are normal and resting cells. Proteasome inhibitors fall into three categories: peptide aldehydes (e.g. MG-132, MG-115, ALLN, PSI), peptide boronates (e.g. PSI-341), and non-peptide inhibitors (e.g. lactacystin). Peptide aldehydes reversibly block the chymotrypsin-like activity and inhibit lysosomal cysteine and serine proteases and calpains. The peptide boronates, such as bortezomib, are reversible, more potent, and selective than peptide aldehydes. Bortezomib (Velcade, PSI-341), a dipeptide boronic acid analogue, reversibly inhibits, in a dose-dependent fashion, 26S proteasome subunit, which results in the inhibition of constitutively active NFκB (Kim et al., 2006). Bortezomib proved anti-tumor effects in many diverse clinical trials, and in 2003 was approved by US FDA for the treatment of relapsed multiple myeloma. Several preclinical and clinical studies testing the effects of single-agent bortezomib or/and combinations of bortezomib with other cytotoxic agents in various haematologic and solid malignancies are currently under way (Gatto et al., 2003; Pham et al., 2003; Yu et al., 2003; Satou et al., 2004; Chauhan et al., 2005). The inhibition of IKKB represents an alternate approach in targeting the constitutively active NFκB in multiple myeloma and other haematologic malignancies (Kim et al., 2006). Recently, synthetic triterpenoids have emerged as a novel group of experimental anti-tumour agents with distinct NFκB-inhibiting properties (Stadheim et al., 2002; Chauhan et al., 2004).

Cell-cycle progression and gene transcription machinery

Cyclin-dependent kinases (CDKs), cyclin dependent kinase inhibitors (CDKIs)

In mammalian cells, cyclin-dependent kinases (CDKs), cyclins, and CDK inhibitors (CDKI) represent core regulators of the cell cycle machinery. They function within several pathways, including the p16(INK4A)-cyclin D1-CDK4/6-pRb-E2F, p21(WAF1)-p27(KIP1)-cyclinE-CDK2, and p14(ARF)-MDM2-p53 pathways. The importance of the CDK-cyclin complexes in cell proliferation is underscored by the finding that deregulation of the CDK activity is found in most human tumours. The inhibition of CDK may lead to both cell cycle arrest and apoptosis (Shapiro, 2004).

Flavopiridol was the first potent CDKI to reach clinical trial. By competitive inhibition of ATP binding, synthetic flavone flavopiridol inhibits multiple CDKs, which results in cell cycle arrest or apoptosis, depending on the cell type and drug concentration (Chowdhury et al., 2005). Possible anti-tumour mechanisms of flavopiridol include decrease of cyclin D1, downregulation of anti-apoptotic regulators including Mcl-1, stabilization of p53 tumour suppressor, and inhibition of angiogenesis mainly through attenuation of VEGF signaling (Gojo et al., 2002; Chen et al., 2005). The anti-tumor effects of flavopiridol are mediated at least in part through disruption of STAT3 signalling (Lee et al., 2006). Flavopiridol has been shown effective in inducing apoptosis in a variety of haematopoietic neoplasms, including multiple myeloma, and leukemias (Gojo et al., 2002; Karp et al., 2005; Dispensieri et al., 2006). Seliciclib (CYC202, R-roscovitine), a more potent aminopurine synthetic analogue of olomoucine, represents another potent small molecule CDK inhibitor, with molecular mechanisms of anti-tumour activities similar to those of flavopiridol. Seliciclib is currently undergoing phase II clinical testing in diverse B-cell malignancies, including multiple myeloma and mantle cell lymphoma (Whittaker et al., 2004; Alvi et al., 2005; Lacrima et al., 2005; MacCallum et al., 2005; Raje et al., 2005).
Histone deacetylase (HDAC) inhibitors (HDACI)

Histone acetylation and deacetylation performed by diverse histone acetyltransferases (HATs) and histone deacetylases (HDACs) represent essential posttranslational modifications of the core nucleosomal histones that affect chromatin structure and gene expression. Acetylation of specific residues in histones H3 and H4 has been associated with an open chromatin configuration and a permissive gene transcription state. HDAC inhibitors (HDACI) represent a new class of targeted anti-cancer agents. HDACI can be divided into several groups, according to their chemical structure: short-chain fatty acids (e.g. sodium butyrate, valproic acid, VPA), hydroxamic acids (e.g. suberoylanilide hydroxamic acid, SAHA), and cyclic tetrapeptides (e.g. trapoxin). HDACI can induce programmed cell death and autophagy in cancer cells, although through yet incompletely understood molecular mechanisms (Peart et al., 2003; Shao et al., 2004). HDACI exert synergistic effects with various other cytotoxic drugs, including TRAIL, ATRA (all-trans retinoic acid), or bortezomib (Inoue et al., 2004a). Upregulation or constitutive activation of HDACs has been associated with various hematologic malignancies (Marks et al., 2001). Administration of VPA as a monotherapy or in combination with ATRA is of therapeutic benefit for patients with MDS and leukemias (Kuendgen et al., 2004, 2006; Bug et al., 2005; Raffoux et al., 2005). Phase I clinical trials have shown that SAHA has anti-tumour activity in solid and haematological tumours (Kelly et al., 2003, 2005). Depsipепtide (FK228), a natural HDACI that was isolated from Chromobacterium violaceum, and its derivatives induced apoptosis in a number of acute myeloid and chronic lymphocytic leukemia cells, especially when combined with proteasome inhibitor bortezomib (Arora et al., 2003; Klisovic et al., 2003; Pei et al., 2004; Byrd et al., 2005; Escobar-Diaz et al., 2005; Sutheesophon et al., 2006).

Iron chelators

Development of iron chelators has focused primarily on their use in the treatment of iron overload. Rapidly proliferating cells, including tumour cells, display greater sensitivity to iron deprivation than normal cells. The mechanisms involved in the anti-proliferative and apoptotic effect of iron chelation include the inhibition of ribonucleotide reductase (RR), hypophosphorylation of the retinoblastoma protein (pRb), decreased expression of cyclins A, B, D and Cdk2, and production of free radicals by iron-chelator complexes (Le and Richardson, 2002; Buss et al., 2003; Richardson, 2005). Several established chemotherapeutics used in the management of haematologic malignancies, such as hydroxyurea, fludarabine, doxorubicine, and cladribine, are able to bind iron, and their anti-tumour effect is at least partly attributed to inhibition of ribonucleotide reductase and to the production of free radicals (Kalinowski and Richardson, 2005; Xu et al., 2005). The anti-tumour activity of deferoxoxamide (DFO), chelator currently used to treat iron overload, is limited due to its poor ability to penetrate the cell membrane and chelate intracellular iron pools. Other, more potent iron chelators, developed specifically as anti-cancer drugs, are currently evaluated in preclinical and various stages of ongoing clinical trials (Lovejoy and Richardson 2003; Buss et al., 2004). Pyridoxal 4-isonicotinoyl hydrazone (PIH) analogues, desferrithiocin, O-trensox, and tachpyridine represent examples of iron chelators currently being explored for their anti-cancer activity in pre-clinical studies. Triapine (3-aminopyridine-2-carboxaldehyde thiosemicarbazone, 3-AP), synthetic iron chelator in the most advanced stage of evaluation, is evaluated for treatment of solid tumours and lymphomas in clinical trials. Apart from their use as single therapeutic agents, chelators can be potentially used in combination therapy of cancer as chemotherapy or radiotherapy sensitizing agents.

Conclusion

Deregulation of apoptosis contributes to both transformation and maintenance of the malignant haematopoietic phenotype. Detailed knowledge of the control of apoptotic pathways could aid in the rational design of effective therapeutics for a variety of human diseases including leukaemia (Klener Jr. et al., 2006). In conjunction with the growing understanding of the apoptosis mechanisms, multiple new potential opportunities for therapy of haematologic malignancies are being uncovered. These opportunities include discovery and development of new agents, the potential for more effective use of existing anticancer drugs through a better understanding of their effects on apoptosis pathways, and for attacking apoptosis resistance mechanisms through the control of apoptosis-regulatory gene expression (Reed and Pellecchia 2005). Many additional agents can indirectly modulate apoptosis pathways. HDAC inhibitors, for example, can reduce transcription of anti-apoptotic Bcl-2 family proteins in cancers, and are currently under investigation in clinical trials. Upstream of Akt, a compound that inhibits the kinase activity of Bcr/Abl (STI-571, Gleevec) has been approved for CML. Similarly, small-molecule drugs and monoclonal antibodies directed against various cell-surface growth factor receptors with PTK activity (e.g., HER-2; EGFR; IGFR) are expected to shut down the Akt pathway, and thus should render tumour cells more sensitive to apoptosis. Based on the detailed understanding of the cellular pathways that control expression of apoptosis-regulatory genes and from knowledge of the tumour-specific genetic lesions that affect sensitivity or resistance to apoptosis-inducing agents, the treatment strategies can be optimized for individual patients.
References


