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Apoptosis and Apoptosis Modulators in Myeloid Leukemia

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1. Introduction

Acute myeloid leukemia (AML) is one of the most common types of leukemia in adults (American Cancer Society, 2010) however overall survival rate remain poor despite advancement in treatment modality.

Since the last 50 years, systemic chemotherapy has greatly improved outcome in many types of cancers. The use of continuous infusion Arabinosylcytosine (Ara-C) combined with another agent, usually an anthracycline or anthracenedione, the "3+7" regimen, has been the backbone of induction therapy for AML cases (Yates et al., 1973). An attempt to add other drugs (Preisler et al., 1987) and intensification of the Ara-C dose (Schiller et al., 1992; Weick et al., 1996) to this approach has achieved some degree of success. Currently more work is attempted at improving patient outcome by intensifying the doses of anthracyclines (Lowenberg et al., 2010a) or by adding targeted therapies like gemtuzumabozogamicin (Lowenberg et al., 2010b; Nabhan et al., 2005).

For consolidation therapy, the use of Ara-C with or without other agents has been employed to maintain remission and cure. Allogeneic hematopoietic cell transplantation (HCT) based on initial cytogenetic (Cornelissen et al., 2007; Koreth et al., 2009) and molecular studies (Castaigne et al., 2004) have been proposed as an alternative consolidation therapies.

Induction therapy aims to produce complete remission (CR) defined as a marrow with less than 5% blast, a neutrophil count greater than 1000/mm^3 and a platelet count greater than 100,000/mm^3 (Cheson et al., 2003). Majority of younger patients (65-75%) will achieve CR after receiving induction treatment while CR in elderly group is much lower (40-50%).

Patients who do not respond to induction treatment display chemotherapy resistance (Estey et al., 1996). In trials done by the Southwest Oncology Group (SWOG), resistant disease was found in about 33% (patients younger than 56) out of 404 patients' enrolled into the studies, 62% for patients in between 56-65 year old, 61% for patients between 66-75 years old and 57% for age more than 75 year old (Frederick et al., 2006).

Resistance is also common at relapse (Estey et al., 1996). Relapse itself could be due to resistance to treatment in a subgroup of leukaemic cells which survived induction therapy despite CR. Patients usually relapse within two to three years after achieving CR.

2. Multi-drug resistance protein as a mechanism of drug resistance

Development of drug resistance is a major problem in AML therapy. It will eventually occur in most haematological malignancies treated with chemotherapy. Classically, drug resistance is divided into extrinsic and intrinsic (Jean-Pierre et al., 2003). Extrinsic resistance (host factors) refers to the inability of the drug to reach the tumour cell. It occurs when the bioavailability of the oral form varies from patient to patient like poor absorption resulting in low serum levels.

Intrinsic (cellular) resistance is due to properties of the tumour cell. It can be classified as simple resistance, when cells are resistant to only one particular drug, or as multidrug resistance (MDR) when cross resistance is observed among chemotherapeutic drugs with different biochemical targets. Multidrug resistance is more common than simple resistance and it can be due to several mechanisms. The most common pharmacological mechanism involved is due to an active efflux of drugs from the tumour cells or enhanced drug metabolism which prevented the drug from reaching its target in the nucleus.

The most important protein described in MDR cells is P-glycoprotein (P-gp), a transmembrane energy-dependent drug efflux pump, which is most efficient at transporting naturally occurring substances. It is encoded by the MDR1/ABCB1 gene and belongs to a superfamily of ABC (ATP binding cassette) transporters. P-gp expression in AML at initial presentation has been reported to be 20% to 40% (Motoji T et al., 2000). Increase in P-gp expression in leukaemic cells causes reduced intracellular concentration of cytotoxic drugs. There are many drugs used in AML that are transported by P-gp including anthracyclines and anthracenediones like daunorubicin and mitoxantrone, the vinca alkaloids (vincristine and vinblastine) and the epipodophyllotoxins (etoposide and teniposide).

Other ABC transport proteins that have been implicated in MDR include the multi-drug resistance associated proteins (MRP1/ABCC1) and the breast cancer resistance protein (BCRP/ABCG2). All these proteins are not unique to drug resistance cells but expressed in tissue with excretory and secretory functions. However, many studies have found that overexpression of these proteins correlate with poor treatment response (Damiani et al., 2010; Bendarra et al., 2005).

A non-ABC protein, found widely expressed in P-gp negative multidrug resistant cancer cell termed initially as lung resistance related protein (LRP) and now known as major vault protein (MVP) also has been implicated in drug resistance mechanism (Izquierdo et al., 1996; Huh et al., 2006). This protein is involved in bidirectional transportation of a variety of substrates between nucleus and cytoplasm. It is present in many cells and seems to be upregulated in cancer cells and has been found to be an adverse prognostic factor in AML (Styczynski et al., 2007). The expression of P-gp (Leith et al., 1999), MRP and LRP in AML was also found to correlate with advanced age (>60 years) and high white cell count (van delHeuvel et al., 2007). It also correlates with high risk of relapse (Daniela et al., 2007).

There have been extensive trials conducted on AML therapy to circumvent drug resistance like reversion of P-gp, targeted agents against DNA replication and repair, cell cycling and apoptosis.

With the extensive knowledge on P-gp efflux mechanism and its contribution to drug resistance in AML, quinine and cyclosporine were tested to reverse the P-gp action. However, these substances did not significantly improve the response rate in AML (Eric et al., 2003; Solary et al., 1996; Liu et al., 1998; Tallman et al., 1995). Combination of tetrandrine, a potent inhibitor of the MDR-1 efflux pump, with induction therapy also showed no

significant difference in response between P-gp positive and P-gp negative patients (Wen et al., 2006). Nevertheless, an early study revealed by using P-gp reversal modulators, the emergence of drug resistance could be prevented (Futscher et al., 1996). However, a recent randomized phase III trial involving 302 newly diagnosed AML patients, evaluated the effect of P-gp inhibitor valspodar (PSC-833) showed no difference in overall disease survival (Jonathan et al., 2010). Similar result was obtained in another phase III randomized trial involving poor risk AML patients when valspodar was added in the induction therapy (Peter et al., 2004)

3. Molecular 'signatures' in AML

AML is characterized by a high degree of heterogeneity with respect to chromosome abnormalities, gene mutations and expression of multiple genes. The heterogeneous nature of AML has significant clinical impact as there are marked differences in survival following intensive chemotherapy (explained in detail elsewhere in this book). The World Health Organization (WHO) classifies AML by cytogenetics, morphology, immunophenotype and clinical features (Swerdlow et al., 2008). Diagnostic karyotype emerges as the most significant prognostic factor as determined in multivariable analyses that take into account age, type of AML (*de novo* or secondary) and presenting white blood cell count (WBC), and accordingly provides the framework for current risk stratified treatment approaches (Grimwade, 2007). Nevertheless as cytogenetic and molecular genetic aberrations are not mutually exclusive the expression of downstream target genes that encode proteins involved in complex biologic networks are affected (Mrozek et al., 2009) and may alter predictability of standard prognostic markers. Microarray genome-wide gene-expression profiling (GEP) and microRNA-expression profiling assays have revealed AML signatures and may be readily applicable for diagnosis and outcome class prediction in AML (Mrozek et al., 2009). Many of the molecules involved are known mediators of signal transduction pathways and apoptosis.

4. Apoptotic molecules in AML

Apoptosis occurs principally via two separate yet interlinked signaling mechanisms: the extrinsic pathway, activated by proapoptotic receptor signals at the cellular surface (members of tumor necrosis factor, TNF, family), and the intrinsic pathway (members of Bcl-2 family), activated by mitochondrial signals from within the cell. These pathways converge through "effector" caspases, which orchestrate the apoptotic program. Nevertheless, each requires different initiation caspases to begin the process. The *extrinsic* pathway is activated by engagement of death receptors on the cell membrane. The death receptors involved in the extrinsic apoptotic pathway belong to the TNF receptor superfamily that include Fas (CD95 or Apo1), TNFR1 (TNF receptor 1), death receptor 3 (DR3/Wsl-1/APO-3/TRAMP/LARD), death receptor 4 (DR4/TRAIL−R1), death receptor 5 (DR5/TRAIL−R2) and DR6. These receptors are characterized by an intracellular death domain. There are also decoy receptors (i.e. DcR1 and DcR2) that contain no death domain or a truncated death domain and can bind ligand but cannot signal. Therefore, these decoy receptors function as antagonists to inhibit death ligand/death receptor−induced apoptosis. Binding of ligands, such as FasL, tumor necrosis factor-alpha (TNF-alpha) and TNF-related apoptosis-inducing ligand (TRAIL) to their respective membrane receptors Fas, TNF-R and

TRAIL-R induces trimerization of the receptors and recruitment of adaptor proteins such as the Fas-associated death domain (FADD) to the death domain. This then recruits procaspase-8 which then leads to the formation of the oligomeric death-induced signaling complex (DISC). DISC in turn promotes activation of caspase-8 and a cascade of other caspase enzymes that culminates with cell death (reviewed in Elrod and Sun, 2008).

The *intrinsic* pathway is triggered by various extracellular and intracellular stresses, including growth factor deprivation, DNA damage, oncogene induction, hypoxia and cytotoxic drugs. Cellular signals originated by various mechanisms by these different stresses converge on a cellular target represented by mitochondria. Mitochondrial membrane permeability is controlled by pro-apoptotic (Bax, Bak, Bad, Bid, Bim, Bmf, NOXA, PUMA, Bok, Bcl-G, Bfk) and anti-apoptotic (Bcl-2, Bcl-L, Mcl-1, Bcl-w, A1) members of the Bcl-2 family, inducing or preventing heterodimerization of pro-apoptotic members. A series of biochemical events is induced that lead to damage of the outer mitochondrial membrane, with the consequent release of cytochrome c and other pro-apoptotic molecules, such as Smac/DIABLO, from the inner membrane into the cytosol enabling the formation of the apoptosome, a large molecular complex formed by cytochrome c, apoptotic protease activating factor 1 (APAF-1) and caspase-9, and massive activation of caspases. These proteins all play crucial roles for cell survival and the loss of any of these proteins causes major deregulation of survival of some cell types (reviewed in Ashkenazi and Herbst, 2008).

Dysregulation of apoptosis plays an important role in the development of a variety of human pathologies, including cancer and particularly leukemia. The evasion of programmed cell death has been regarded as one of the six essential alterations in cellular physiology that dictate the growth of cancer cells and is a hallmark of virtually all cancers. Moreover, tumors that have alterations in proteins involved in cell death signaling are very frequently resistant to chemotherapy and are difficult to treat with chemotherapeutic agents that primarily act by inducing apoptosis (Testa et al., 2007).

Fas, DR4 and DR5 are generally expressed in both normal and malignant cells. An examination of patients with de novo AML revealed Fas was expressed on eight of nine (89%) patients tested (Tourneur et al., 2004). Another study showed expression of Fas on 62% of 29 AML patients (Min et al., 2004). Fas mutation was observed in 4/28 CML cases and none of the six AML cases tested (Rozenfeld-Granot et al., 2001). DR4 and DR5 mutations detected in cancers including chronic myelogenous leukemia were very low (0–10.6%) (Liu et al., 2005). On the other hand, DR4 and DR5 receptors were positive in 20 (69%) and 29 (100%) patients, respectively. This study also showed, relapse−free survival was significantly prolonged in patients with CD95−positive AML cells compared with patients with CD95−negative AML cells (73% versus 38% at 3 years; $p = 0.047$) using univariate analysis (Min et al., 2004). This was however not supported by another study on 99 AML patients where multivariate analysis showed no correlation with overall survival and disease free survival (Brouwer et al., 2001).

Three ligands (TNF-α, FasL and TRAIL) of the TNF-family and their respective four receptors (TNF-R1, Fas, TRAIL-R1 and TRAIL-R2) are potentially important as anti-cancer therapeutics. The demonstration that TNF-α selectively kills tumor cells but not normal cells, set it up for the first molecules to be studied. Unfortunately, marked pro-inflammatory effects precluded its systemic administration (Buzzoni and Butler, 1996). Fas was also excluded as agonistic antibodies triggering Fas activation was highly hepatotoxic causing death in mouse models (Ogasarawa et al., 1993). In contrast, TRAIL and agonistic anti-

TRAIL-R1/TRAIL-R2 antibodies appear to be well tolerated *in vivo*. TRAIL/Apo-2L exhibited potent anti-tumor activity and induces little cytotoxic effects in immunodeficient mice xenograft models implanted with several human tumor cell lines (Ashkenazi et al., 1999). However, the *in vivo* half-life of the TRAIL-ligand is very short (<4 minutes) (Kelley et al., 2001). Agonistic TRAIL-R1 and TRAIL-R2 antibodies do not bind to TRAIL decoy receptors, TRAIL-R3 and TRAIL-R4, which are frequently expressed on the membrane of tumor cells.

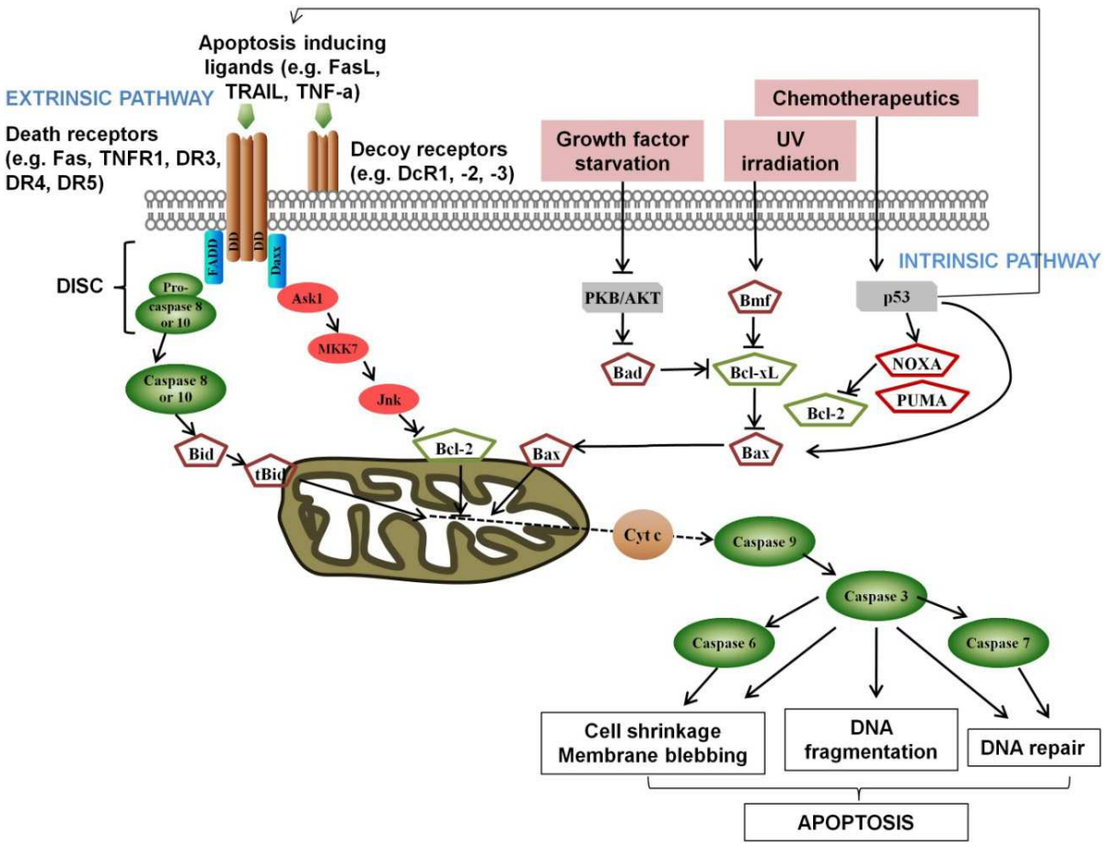

Fig. 1. Extrinsic and intrinsic pathways in apoptosis (see text for further details).

Antisense therapies involved the use of sequences of single-stranded DNA to complement and bind specific coding regions on mRNA hence forming DNA–mRNA which is then degraded by a ribonuclease, therefore gene expression and translation are prevented. Most widely studied were with XIAP (X-linked inhibitor of apoptosis) and antiapoptotic proteins Bcl-2.

Sufficient evidence exists to show that Bcl-2 was overexpressed in AML patients and predictive of worst outcome (Campos et al., 1993; Andreef and Konopleva, 2002). It seemed conceivable that Bcl-2 downregulation might lower the apoptotic threshold of leukemic cells and, through this mechanism, favor response to chemotherapy. Much success has been achieved. A phase I study using oblimersen, an antisense to Bcl-2, added during induction and then consolidation therapy, in elderly AML patients, induced remission in 14/27 patients, of which seven relapsed within 12.6 months (Marcucci et al., 2005); In a multicenter phase II trial, 12/39 relapsed AML patients treated with oblimersen and gentuzamab (anti-

CD33) achieved complete remission of which 10/12 survived for more than 6 months (Moore et al., 2004).

XIAP binds and inhibits caspases 3, 7, and 9, mediators of the apoptotic cascade. Downregulation of XIAP using multiple approaches (e.g., antisense, RNAi, knock-out animals and cell lines, immuno-depletion) in vitro and in vivo conditions resulted in increased caspase activation and/or cell death. Antitumor activity was also observed with the use of second generation anti-sense compound, AEG35156, in xenograft models of cancer (Lacasse et al., 2005) Results from clinical trials however, have been variable. While one study on five phase 1 (12–350mg/m2 AEG35156) and eight phase 2 (350 mg/m2 AEG35156) patients showed increased apoptotic cells and increase response (Bing et al., 2011) another study on 27 patients randomized to receive high dose Ara-C and idarubicin with or without AEG35156 (650 mg) found a lower overall response rate in the group which received the anti-XIAP drug (Schimmer et al., 2011).

The analysis of Mcl-1 protein expression in AML showed great heterogeneity, but the levels of the protein do not seem to correlate with response to standard chemotherapy (Kaufmann et al., 1998). Bad and Bcl-xL have been shown to be expressed in normal and leukemic hematopoietic precursor cells. Immature hematopoietic cells do not express Bcl-2 but do express Bcl-xL. CD34 positive cells express Bcl-2, Bcl-xL and Bad. Bcl-2 expression is higher on CD34 positive cells than on AML cells. Phosphorylated Bad was expressed in AML (Andreef et al., 1999).

Potential abnormalities of the various initiator caspases in AML have been explored. Levels of caspase-8, caspase-2 and caspase-3 are heterogeneous in AML. AML with an immature phenotype (i.e., M0 and M1 AML) predominantly express caspase-8L (Mohr et al., 2005).The significance of caspases as prognostic indicators in AML are unclear as current reports are still controversial may be due to the different format of molecules examined (Svingen et al., 2000; Estrov et al., 1998; Holleman et al., 2005).

Expression of pro- and anti-apoptotic molecules continues to be studied in AML to correlate its mutated state, expression, activity or methylated state with treatment outcome (Testa et al., 2007). At present, the prognostic utility of measurements of pro- and antiapoptotic molecules for predicting clinical outcome and response to chemotherapy is uncertain.

5. Drug modulation of signaling, differentiation and apoptotic pathways

The study of cancer cell biology in predicting treatment outcome cannot stop at the presentation stage as cells continue to be modified by the microenvironment and are ultimately subjected to chemotherapy. While remarkable progress have been achieved in targeted therapies, for most tumors chemo- or radiotherapy is likely to remain in the near future. Both chemo- and radiotherapy are designed to kill cancer cells by damaging nuclear DNA. DNA damage triggers the DNA damage response (DDR) which have three critical goals: (i) halting cell cycle progression and division to prevent transfer of DNA damage to progeny cells; (ii) increasing accessibility of the damage sites to- and engagement of- the DNA damage repair machinery, and (iii) triggering apoptosis to exterminate cells whose damaged DNA cannot successfully be repaired (reviewed in Darzynkiewicz et al., 2009).

Chemotherapeutic drugs such as cisplatin, mitomycin, methotrexate, mitoxantrone, adriamycin, and bleomycin induce Fas expression in human cancer cells, primarily through a p53−dependent mechanism (Muller et al., 1998). Adriamycin, etoposide, Ara−C, cisplatin and camptosar were shown to induce the expression of DR4 and DR5 or only DR5

expression, through either p53−dependent, or p53−independent mechanisms (Wu et al., 1997; Guan et al., 2001; Sheikh et al., 1998). Etoposide was shown to induce DR5 expression in human acute leukemia cells (Wen et al., 2000).

To complete induction of cell death, chemotherapeutic drugs have to suppress survival mediators in activated signaling pathways. Paclitaxel treatment of transfected MDA MB-435 human breast carcinoma cell line was observed to downregulate phosphorylated Akt (Klos et al., 2003). Nevertheless, chemotherapy induction of cell death is not equal in all cells. Adriamycin produced differential responses in Akt phosphorylation and kinase activity in a panel of breast cancer cell lines. While MCF7, MDA468 and T47D cells showed a dose dependent increase in p-Akt levels; in contrast, SKBR3 and MDA231 cells showed a dose-dependent decrease and no or minimal change was detected in MDA361, MDA157 and BT474 cells (Li et al., 2005). The diversity in response may also be predictive of a heterogeneity in treatment outcome.

Other signaling molecules are activated by chemotherapeutic drugs leading to cell death. Ara-C induced apoptosis in HL-60 cell lines through the activation of p38 (Stadheim et al., 2000). Adriamycin was shown to activate Jnk in a T cell leukemia cell line (Yu et al., 1999). Leukemia cell lines (TF-1 and K562) primed for apoptosis were also revealed to stimulate Jnk and p38 phosphorylation (Tucker et al., 2004)

Certain cytokines have apoptotic activity. TNF-alpha and IFN-gamma induced the expression of DR5 in a number of cancer cell lines (Meng and El-Deiry, 2000). IFN-gamma had differential effect on induction of death receptors in colon carcinoma cell lines. While it raised the levels of CD95 membrane 6 – 8-fold, it had no effect on the TRAIL-receptors (DR4, DR5, DcR1 and DcR2) (van Geelan et al., 2003). Interferon−alpha was also reported to increase DR5 expression in human hepatoma (Shigeno et al., 2003).

In contrast some cytokines exert protective effect from chemotherapeutic drug induced cell death, decreasing the effectiveness of cancer radiotherapy and chemotherapy. Normal hematopoietic cells, like other normal cell types, die by the process of apoptosis when deprived of viability inducing cytokines that include colony stimulating factors (CSFs) and various other cytokines. Induction of apoptosis by cancer chemotherapy such as vincristine, adriamycin, methotrexate and Ara-C was suppressed by IL (interleukin)-6, IL-3, granulocyte-CSF (G-CSF), granulocyte-monocyte CSF (GM-CSF) and IFN-gamma in myeloid leukemia cells (reviewed in Lotem and Sachs, 2002). These cytokines upregulate pro-survival molecules such as Bcl-2 [IL-2, IL-3, stem cell factor (SCF), IFN-gamma], Bcl-xL [IL-3, IL-6, IL-7, IL15, GM-CSF, IFN-gamma and erythropoietin (EPO)] and other apoptosis suppressing genes such as Survivin (Carter et al., 2001), X-linked inhibitor of apoptosis protein (XIAP) and cellular inhibitor of apoptosis 2 (cIAP2) (Digicylioglu and Lipton, 2001) that are caspase inhibitors and FLICE-like inhibitory protein (FLIP), that may disrupt the ability of cell surface molecules such as Fas to activate apoptosis (Kovalovich et al., 2001) Some myeloid leukemic cells are autonomous and do not require an exogenous source of cytokines for viability (Griffin and Lowenberg,1986), while others do. Thus, it is possible to suppress leukemia not only by cytotoxic agents or by induction of terminal differentiation, but also by decreasing the in vivo supply of apoptosis suppressing cytokines or the response of leukemic cells to these cytokines (reviewed in Sachs, 1996).

A characteristic abnormality of leukemia cells is that they are blocked at an early stage of their development. Myeloid leukemic cells however can be induced to differentiate to non dividing mature granulocytes and macrophages by different cytokines, including cytokine

independent myeloid leukemic cells that were induced to differentiate with IL-6. Different myeloid leukemic clones however have different blocks and ability to undergo differentiation by cytokines. Our own work on in vitro cultured AML blasts exhibited different degrees of spontaneous apoptosis. Univariate analysis of 13 AML patients revealed blasts with lower levels of cell viability after 72h culture was significantly correlated with a longer disease free survival . Within a smaller number of samples (n=7) we observed blasts with lower levels of cell viability were associated with reduced levels CD34 and higher levels of CD16, indicating an increased level of cell differentiation (Maha et al., 2008). The observations may indicate an abnormal developmental program in leukemic cells which may be reprogrammed epigenetically by appropriate differentiation inducing cytokines. Constitutive expression of transcription factors such as c-myc, c-myb and E2F1 (Gonda and Metcalf, 1984; Blatt et al., 1992; Melamed et al., 1993) as well as others such as the homeobox gene Hox B8 (Hox 2.4) (Blatt et al., 1992) or GATA-1 (Tanaka et al., 2000), disrupted the ability of cells to undergo cytokine induced differentiation (reviewed in Lotem and Sachs, 2002)

Cytokines as a differentiation treatment against leukemia however has been disappointing. Hematopoietic leukemia cell lines of myeloid origin such as K562, U937, HL-60, CS-1, KG-1, MUTZ-3, or ex vivo AML or chronic myeloid leukemia (CML) blasts were modestly permissive to induction of in vitro differentiation by EPO, G-CSF, GM-CSF, IL-4, IL-6, SCF, or synergistic combinations of several cytokines (Leung et al., 2005; Koss et al., 1996; Goliaei et al. 1998; Kamano et al., 1994; Kamijo et al., 1990). A niche for hematopoietic cytokines in differentiation therapy exists in the treatment of congenital neutropenia disorder. The administration of G-CSF to patients has overcome a block of myeloid differentiation leading to a substantial prolongation of their survival (Berliner, 2008).

Clinically, differentiation therapy has been most successful in acute promyelocytic leukemia (APL) using all-trans-retinoic acid (ATRA) as the inducer. This targeted APL cells carrying the chromosomal translocation between chromosomes 15 and 17 [t(15;17)(q22;q21)]. Subsequently, APL therapy was improved with the combination regimen of ATRA with cytotoxic chemotherapy. Currently, complete remission rates of up to 90% to 95% are achievable using ATRA/ATO (arsenic trioxide) and anthracycline-based chemotherapy (Niu et al., 1999; Soignet et al., 2001; Raffoux et al., 2003; Ghavamzadeh et al., 2006; Mathews et al., 2006; Estey et al., 2006; Sanz et al., 2008).

Another targeted treatment with tyrosine kinase inhibitor (TKI) imatinib for the treatment of CMLalso achieved better success. Gefinitib and erlotinib which inhibit the intracellular tyrosine kinase activity of epidermal growth factor receptor (EGFR), induce a differentiation program in myeloid leukemia cells that corresponds to neutrophil maturation (Stegmaier et al., 2005; Boehrer et al., 2008a; Boehrer et al., 2008b).

These results together emphasize further not only the heterogeneity of leukemias but also complexity of host-cancer interaction and its influence on outcome in survival and also during induction of cell death.

6. In vivo drug induced molecular profiles: Potential predictor of drug resistance

The in vivo molecular changes in acute myeloid leukemia cells early after start of conventional genotoxic chemotherapy are incompletely understood, and it is not known if early molecular modulations reflect clinical response. As increasing evidence is proposing

tumor-host mechanisms as important for effective chemotherapy, there is an immediate need to investigate these issues in vivo in human cancer (Oyan et al., 2009)

For that purpose, blasts from patients undergoing chemotherapy were collected as a 'natural' and rich source to study response of these cells to the myriad of signals they were subjected to. Even though cells undergo cell death, as white blood cell counts may decline at early stages of chemotherapy, very low percentages of apoptotic cells were detected. Oyan et al. (2009) comparing treated ('3+7', idarubicin + Ara-C) with untreated AML cells from seven patients, observed upregulation of 113 genes (23 of unknown function) at early time points (2 – 4 hours) and 108 genes at late time points (18 – 24 hours). Among the 113 genes a substantial number (31 genes) were related to the tumor suppressor p53 (Oyan et al., 2009). p53 is implicated to affect a variety of cellular processes, the most undisputed roles of p53 are to induce growth arrest and to induce apoptosis (Bates and Vousden, 1996). p53 is the most commonly mutated gene in a variety of human cancers (Greenblatt et al., 1994). In AML however, mutations of p53 are rare, occurring in approximately 5% to 10% (Fenaux et al., 1992) but in these cases it correlates with worse outcome (Wattel et al., 1994). Wild-type p53 appears to change the balance in expression of apoptosis-inducing versus apoptosis-suppressing genes in favor of the former and thus induce apoptosis.

In tune with the above, a significant increase in gene expression of the apoptosis facilitators PUMA and Bax and a decrease in the Bcl-2 /Bax ratio as well as Bcl-2 /PUMA were observed for most of the AML samples. The mRNA profile of three other pro-apoptotic mediators Bad , Bak1 and Bim did not change significantly during the first hours, but the level of gene expression varied across patients. Altogether five tumor necrosis factor-related receptor genes were modulated 2–4 h after induction therapy (Oyan et al., 2009).

Induction of ligand to death receptor during chemotherapy was also supported by Devemy et al. (2001) who observed increased TNF transcripts in treated AML cells. We also studied molecular changes in paired AML samples at diagnosis and during chemotherapy (Ara-C + daunorubicin). We showed increased TNF-alpha was significantly higher in chemo-sensitive patients. Thus, expression of TNF-alpha early during chemotherapy may be a marker to predict good treatment outcome. In chemo-resistant cases, a higher, though not significant, percentage of cases expressed IL-1beta and IL-18 (Maha et al., 2009).

We observed a significantly higher percentage of chemo-responsive AML patients with blasts cells increased for the expression of IL-6. This was consistent with Devemy et al (2001) who reported that increase of IL-6 transcripts during remission induction therapy of AML patients was accompanied by a fall in blood count and bone marrow cellularity. The role of cytokines in the induction of cell differentiation is well established. Oyan et al (2009) also observed several receptors expressed on monocytic/macrophage lineage cells were upregulated, probably related to chemotherapy induced differentiation of the leukemic cells. Thus, induction of cytokines expression in drug responsive AML patients may be due to induction of cell differentiation.

Comparing blasts profiles before and during early chemotherapy also revealed upregulation of genes potentially involved in interaction between AML blasts and the host microenvironment. Chemokine receptors CXCR4 and CX3CR1 were upregulated in the late phase after start of chemotherapy indicating intention to home into a microenvironment that favours their growth and survival. This supports the hypothesis that the host response in chemotherapy is crucial for persistent remission (Oyan et al., 2009).

We further examined activation of signaling molecules in AML blasts. Chemotherapy increased the percentage of cases showing phosphorylation of the Akt molecules and Forkhead transcription factor (FKHR) but no significant differences were observed between chemo-resistant and chemo-sensitive cases. We however, observed a significantly higher percentage of chemo-resistant cases showing phosphorylation and inactivation of the pro-apoptotic Bad molecule. A higher percentage of chemo-sensitive cases were phosphorylated for p38, and Jnk (Maha et al., 2009). In summary, we were able to show in chemo-sensitive cases, chemotherapy stimulated IL-6, induced apoptosis by up-regulating TNF-alpha and downregulated phosphorylated Bad. In reverse, in chemo-resistant cases, cells survived by maintaining high levels of phosphorylated Bad maybe through protective role of IL-1b and IL-18 cytokines (Maha et al., 2009).

Most anticancer drugs exert their effects by the induction of apoptosis and/or interfering with cell cycle progression. Often these drugs give rise to specific patterns of cell death and cell cycle arrest that vary according to the drug used and the molecular status of the target cell. Simple in vitro methods may aid in this investigation. Drug cytotoxicity and sensitivity of individual tumor samples was demonstrated by combining cytochrome c and propidium iodide staining of DNA content and detected on flowcytometry. This method elucidated mitochondrial resistance mechanisms which may prove useful in identifying the apoptosis-sensitive cell cycle phase for a given tumor sample/anticancer drug combination. It offers the opportunity to design personalized drug regimens and to identify new combined treatment modalities (Mohr et al., 2004).

7. Conclusion

The heterogeneity in AML continues to elude the best methods to characterize them. Genome and proteome-wide analysis has further revealed complexity in the makeup of the leukemic cell. The rapid advancement in targeted therapies implied the urgent need for alternative therapy and the readiness of the community to embrace it. Nevertheless so far, combinatorial medicine still holds out as the best option for successful treatment. If targeted therapies remain the way forward it will eventually bank deeply on the ability to identify molecular signatures in the individual leading to the establishment of personalized medicine.

In the meantime, the mechanisms in leukemogenesis, drug resistance and relapse remain an area of much research. From cell biology to cytogenetics to molecular defects to signaling pathways, all have contributed to a better understanding of cancer biology. New knowledge in epigenetics and microRNA remain to be elucidated.

Current diagnostic and prognostication are based on the assumption that the phenotype of the leukemic cell is static and thus definitive. There is much evidence that suggest otherwise. Activation of oncogenes leads to constitutive expression of signal transduction pathways involved in cell survival and anti-apoptotic activities. These pathways are multiple and made up of a myriad of molecules that are receptive to the environment. The host-cancer microenvironment is a dynamic microcosm of interacting signals and cascading molecules that constantly respond to stimuli in the surrounding to find a balance that maintains survival. In the course of treatment, blast cells are exposed to DNA damaging cytotoxic agents which trigger a gamut of other signaling mediators to exert the opposite effect. It would appear that a struggle ensues in which the strength of the victor determines whether the blast cell would maintain life or be pushed off-balance and replaced with a new

profile signaling cell death. This new phenotype corresponded to a sensitive response to chemotherapy. On the other hand, cells may strengthen on pro-survival features which corresponded with resistance to chemotherapy. A few reports, including ours, are lending support to this hypothesis.

Unsurprisingly, chemotherapy-induce phenotype is not confined purely to either a survival or an apoptosis profile but a complex mix of conflicting signals to survive or die in addition to triggers to shut down cell proliferation, induce terminal differentiation or activate inflammatory responses. Thus, further elucidation of these profiles would involve assignment of each of the modulated molecule to its rightful pathway.

The immaturity feature in leukemias will undoubtedly be a factor that will further compound the heterogeneity in results obtained. An example is the striking correlations found between lower Bax/Bcl-2 ratio and higher progenitor marker expression, such as CD34, CD117 and CD133 antigens, confirming the link between this apoptotic index and the maturation pathways (Del Principe et al., 2005). Attempts to induce cell death by triggering death receptors has so far achieved mix results with the use of TNF-alpha, Fas ligands and the tumor necrosis factor-related apoptosis-inducing ligand (TRAIL) (i.e., DR4 and DR5). These molecules also selectively kill cancer cells while sparing normal cells (reviewed in Elrod and Sun, 2008). These results indicate a preferential expression of specific death receptors on different tissues.

Selection of lab methods for prognostication depends on the ability to identify lineage, maturation stages, genetic aberrations and activated signal transduction pathways. This feat may include the difficult task of combining surface markers, cytokines (secreted proteins) and phosphorylated proteins (unstable intracellular proteins) in the same tube on the same platform such as flowcytometry. Furthermore many of these proteins such as TNF-a, IL-6, p38 and Jnk have dual function of pro-survival and pro-apoptosis capabilities depending on the stimulating conditions cells are exposed to at that period of time. Precise markers will be required to differentiate these situations. Altogether, all of these add up to an interesting and exciting field of research for the immediate future.

8. References

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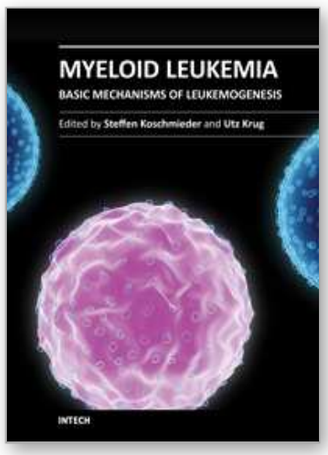

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The current book comprises a series of chapters from experts in the field of myeloid cell biology and myeloid leukemia pathogenesis. It is meant to provide reviews about current knowledge in the area of basic science of acute (AML) and chronic myeloid leukemia (CML) as well as original publications covering specific aspects of these important diseases. Covering the specifics of leukemia biology and pathogenesis by authors from different parts of the World, including America, Europe, Africa, and Asia, this book provides a colorful view on research activities in this field around the globe.

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