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REVIEW

Strategies targeting apoptosis proteins to improve therapy of chronic lymphocytic leukemia

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ABSTRACT

A typical feature of chronic lymphocytic leukemia (CLL) is the impaired ability of the leukemic cells to execute their apoptotic suicide program. Various strategies have been developed to restore apoptosis in CLL cells *ex vivo*. This article reviews the strategies targeting proteins that directly regulate the mitochondrial pathway of apoptosis and caspase activation: (i) inhibiting the expression or activity of prosurvival proteins of the Bcl-2 and IAP (inhibitor of apoptosis protein) families, which are overexpressed in CLL cells and (ii) upregulating proapoptotic BH3-only members of the Bcl-2 family (which are antagonists of the prosurvival members). Preclinical and clinical data have revealed that inhibiting the activity of prosurvival Bcl-2 proteins with BH3 mimetics (so-called because they mimic BH3-only proteins) is an attractive strategy for CLL therapy. Recent results suggest that the development of BH3 mimetics capable of directly activating the apoptosis effectors Bax and Bak may also be envisaged.

1. Introduction

Apoptosis is a cell death program occurring in either physiological or pathological conditions to eliminate useless and dangerous cells. The apoptotic program is executed by specific proteases belonging to the family of caspases, which are activated in response to various stress signals through two distinct pathways: the extrinsic or death receptor pathway and the intrinsic or mitochondrial pathway [1]. In the latter, the permeabilization of the mitochondrial outer membrane elicits the release in the cytoplasm of two critical molecules: cytochrome *c* that triggers the cascade of caspase activation and second mitochondria-derived activator of caspases (SMAC) that neutralizes the activity of caspase antagonists called inhibitors of apoptosis proteins (IAP). The mitochondrial outer membrane permeabilization (MOMP) is strictly regulated by proteins of the Bcl-2 family (sharing one to four domains of Bcl-2 homology, BH1 to BH4). This family is divided into three subfamilies: antiapoptotic members (Bcl-2 and the closely related Bcl-xL, Bcl-w, Mcl-1 and A1) and two proapoptotic subfamilies: first, BH3-only proteins (so-called because they have only the BH3 domain, such as Puma, Bim, Noxa, Bid), which are antagonists of the antiapoptotic members, and second, the MOMP executioners (mainly Bax and Bak). Specific interactions between proteins of the three subfamilies control Bax and Bak activation and thus MOMP: notably antiapoptotic members sequester Bax and Bak, and the interactions of BH3-only proteins with the antiapoptotic members induce Bax/Bak release and activation; some of the BH3-only proteins can also directly activate Bax and Bak [1].

Chronic lymphocytic leukemia (CLL) is characterized by the clonal expansion and accumulation of a CD5-positive subpopulation of B cells in the blood, bone marrow, lymph node and spleen. This condition is thought to derive from an imbalance between proliferation and apoptosis. Contrarily to normal B lymphocytes, the leukemic cells accumulating in the blood are mostly unable to trigger their suicide program. This typical feature of CLL results from both defective mechanisms in the leukemic cells and an excess of survival signals delivered by microenvironment cells [2–6]. Hence, the transcription factor nuclear factor-κB (NF-κB), the phosphatidylinositol-3-kinase (PI3K)/AKT pathway (that leads to the phosphorylation of many apoptosis-related proteins) and pathways involved in the B-cell receptor (BCR) signaling are constitutively activated, which stimulates the transcription of numerous antiapoptotic proteins and their overexpression. The main overexpressed antiapoptotic factors in CLL cells are members of the IAP family such as X-linked IAP (XIAP) and the Bcl-2 family proteins Bcl-2 and Mcl-1 [4–6]. The crucial role of Mcl-1 in drug resistance, disease progression and outcome in CLL patients is now well documented [7]. In some CLL cases, losses of micro-interfering RNAs miR-15a and miR-16 due to the common deletion 13q14 (resulting in Bcl-2 overexpression) [8] and inactivation of p53 (allowing the transcription of proapoptotic molecules, including the BH3-only proteins Puma and Noxa) also contribute to the apoptosis deficiency.

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Despite recent progress of chemo-immunotherapy [9], CLL is still incurable. Designing novel apoptosis-based therapeutic strategies thus appeared important for improving the treatment of CLL patients. Two main types of strategies have been investigated: (i) targeting proteins that directly regulate the MOMP and caspase activation in leukemic cells and (ii) indirect strategies of interfering with survival signals arising from microenvironment cells. As regards the latter, the inactivation of the PI3K/AKT, NF-κB and BCR signaling pathways turned out to be promising in clinical trials with some agents such as idelalisib (a PI3Kδ isoform inhibitor) [10] and ibrutinib (a Bruton tyrosine kinase inhibitor) [11]. The inactivation of other types of microenvironment signals with chemokine receptor inhibitors or lenalidomide for example as well as indirect activation of the p53 pathway by various agents (e.g., nutlins) also appeared of interest for CLL therapy. These indirect strategies have been extensively documented [2–6,12–17].

The present article reviews direct strategies targeting molecules that control the initial events in triggering mitochondrial apoptosis (i.e., MOMP and caspase activation): first, inhibiting the expression or activity of prosurvival Bcl-2 and IAP family proteins and second, upregulating proapoptotic BH3-only proteins. Preclinical studies showing that these strategies can reactivate apoptosis in CLL cells and available clinical data are recapitulated and perspectives for the development of novel CLL therapeutics are discussed.

2. Inhibition of prosurvival protein expression

2.1. Specific inhibition of Bcl-2 and XIAP protein expression

Attempts to specifically inhibit the expression of prosurvival proteins were performed by using antisense oligonucleotides that target mRNA for degradation. Several antisense oligonucleotides targeting Bcl-2 (e.g., oblimersen) were designed and extensively studied. However clinical trials with oblimersen in CLL were unsuccessful probably because of off-target effects [18–20]. Antisense oligonucleotides targeting caspase inhibitors of the IAP family were also generated, including an XIAP inhibitor (AEG35156), which was found to induce apoptosis in CLL cells, but no significant therapeutic effects were recorded in CLL patients with this type of compounds [21–23]. Another possibility to selectively inhibit antiapoptotic protein expression is silencing with small interfering RNA (siRNA) or short hairpin RNA (shRNA) that mimics endogenous micro-interfering RNA (miR). Actually, Mcl-1 siRNA can induce CLL cell death *in vitro* [24], and both miR-15a1 and miR-16-1 can downregulate Bcl-2 and provoke apoptosis in a leukemic cell model [8]. However, the treatment of patients with siRNA or shRNA comes up against difficulties [25,26].

2.2. Non-specific transcriptional inhibition of Bcl-2 and IAP family proteins

This strategy turned out to be of great interest by using notably flavopiridol, which is a well known and potent inducer of apoptosis in CLL patients' cells *ex vivo* [27]. Indeed, this plant-derived flavonoid can reduce the transcriptional activity of RNA Polymerase II via its property of cyclin-dependent kinase (CDK) inhibitor [28]. This effect of flavopiridol on transcription is not specific and affects mainly short-lived proteins, including many antiapoptotic proteins such as Mcl-1 and Bcl-xL (but not Bcl-2), several IAP (XIAP, c-IAP-2, survivin), c-Myc (a transcriptional activator of Mcl-1) and Mdm-2 (a p53 antagonist) [28,29]. The transcriptional inhibition of Mcl-1 and XIAP is the major mechanism by which flavopiridol exerts its proapoptotic effects in CLL cells [27–29]. Although the first clinical trials of flavopiridol in CLL patients did not prove convincing [30], a phase II study using a novel pharmacologically based schedule has provided significant results with treatment responses in 53% of the patients and increases in progression-free survival time [31]. While predictive markers for toxicity of flavopiridol remain to be defined, its combination with chemo-immunotherapy has been recently proposed to eradicate residual CLL disease [30]. Other CDK inhibitors such as SNS-032 and dinaciclib are also apoptosis inducers in CLL cells by downregulating mainly Mcl-1 and XIAP expression [32,33], and their clinical evaluation is ongoing. Furthermore, translation inhibition that preferably targets labile proteins is another approach to downregulate antiapoptotic proteins such as Mcl-1. Homoharringtonine and silvestrol are two translational inhibitors capable of inducing CLL cell apoptosis via Mcl-1 downregulation [34,35]. While silvestrol exerts *in vivo* antileukemic activity in the TCL-1 transgenic mouse model of CLL [35], it is not known whether translation inhibitors have therapeutic activity in CLL patients.

3. Inhibition of prosurvival protein activity

3.1. Bcl-2 family proteins

The BH3 mimetic concept has proposed that small molecules capable of mimicking BH3-only proteins might be useful for anticancer therapy [36,37]. This concept is based on the fact that BH3-only proteins are the natural, specific antagonists of prosurvival Bcl-2 family members (whose activity is to sequester the MOMP effectors Bax and Bak). Such BH3 mimetic compounds should bind to the prosurvival Bcl-2 proteins and antagonize their activity, resulting in Bax/Bak release and activation, and thus apoptosis induction [37]. The various BH3 mimetics that have been designed are short peptides modeled on BH3 domains or small organic molecules (identified by screening either natural product libraries or computer-designed compounds for their capacity to interact with Bcl-2 proteins). There are two main criteria to define an authentic BH3 mimetic: binding to the targets with the same high affinity as the BH3-only proteins and induction of Bax/Bak-dependent apoptosis [37]. Some putative BH3 mimetics do not fully respond to these criteria and act via off-target effects (e.g., generation of reactive oxygen species (ROS), endoplasmic reticulum stress response, induction of the BH3-only Noxa, caspase-independent or autophagic cell death) [37,38].

The organic molecule ABT-737 is an authentic BH3 mimetic [37,38]. By binding to Bcl-2, Bcl-xL and Bcl-w (but not Mcl-1 or A1) [39,40], it induces mitochondrial apoptosis of CLL cells [41]. Its oral derivative ABT-263 (navitoclax), which has been designed for clinical application [42], has shown significant therapeutic activity in a phase I dose-escalation study in relapsed or refractory CLL patients, with a partial response rate of 35% and satisfactory progression-free survival of 25 months [43]. The ABT-199 derivative that binds only to Bcl-2 has been further developed in order to prevent the marked thrombocytopenia caused by navitoclax (resulting from Bcl-xL inhibition) [44]. ABT-199 can also trigger CLL cell apoptosis [45,46] and has proved promising in a clinical trial with reduced tumor burden in the first three CLL patients recruited [44]. The antileukemic activity of ABT-199 in CLL was further confirmed with 84% of overall response rate, including 20% of complete responses [47].

Two putative BH3 mimetics were found to promote mitochondrial apoptosis in CLL cells: obatoclax (synthesized from screening a natural product library), which binds to all prosurvival Bcl-2 proteins albeit with low affinity [48,49], and gossypol, a plant-derived polyphenol [50,51]; both were further shown to act at least partly through off-target mechanisms [37,38,52]. The gossypol's isomer AT-101 is more active and overcomes stroma-mediated Mcl-1 induction and apoptosis resistance *in vitro* [53]. However, neither of these agents has shown significant therapeutic effects in clinical trials in CLL; phase II studies of obatoclax in combination with other drugs are ongoing [54,55].

3.2. IAP family proteins

The strategy to mimic SMAC (the endogenous antagonist of IAP activities) has also been developed [21]. Although a monovalent small molecule designed to bind to the IAP BIR3 domain and capable of antagonizing XIAP displays proapoptotic properties in CLL cells [22], no