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Hematopoietic modulators as potential agents for the treatment of leukemia

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1. ABSTRACT

Leukemias are the most common malignancy of childhood and have the highest mortality among aging people. Leukemias are a group of blood disorders characterized by an accumulation of leukemic cells in the peripheral blood of patients as a result of disturbances in proliferation and differentiation. Refractory leukemia remains the most common therapeutic challenge. In recent years, the presence of a cancer stem cell population in leukemias has been proposed as a cause for the refractory phenomenon. Insights into the cellular and molecular features of leukemia led to a new point of view in the choice of novel therapeutic agents. New agents for the treatment of this disease should selectively target leukemia stem cells or exhibit higher cytotoxic effects in cancer cells than in normal cells. A special interest is focused on anticancer agents from biological and natural sources that can be used in the treatment of leukemia. This review discusses the characteristics of some of these potential new agents.

2. INTRODUCTION

Conventional therapies efficiently reduce the high proliferative population of tumor cells, but may fail to completely eliminate all tumor cells. The inefficiency of most treatments can be associated with several factors, such as increased efflux or decreased uptake of drugs, resistance to apoptosis, drug metabolism, interaction with a tumor microenvironment, repair of DNA damages, and ultimately the presence of cancer stem cells (1). Leukemia is a heterogeneous disease characterized by a mutation in hematopoietic progenitor cells, which promotes accumulation of immature cells by the abnormal proliferation and differentiation of a hematopoietic clonal population. However, similar to other types of cancers, leukemias also display cells in distinct phases of differentiation (2-6). Among these different populations, a rare subpopulation exhibits stem cell features, such as indefinite proliferation, self-renewal, and low proliferation rate; this population is generally called leukemia stem cells (1, 5, 7).

Leukemias represent approximately 3% of all cancers; however, around 10% of deaths from cancer result from leukemias and lymphomas (8). Conventional therapies against leukemia target different characteristics of the disease. Therefore, antileukemic drugs may act against receptors or other molecular targets in deregulated signaling pathways affecting proliferation and differentiation (such as the kinase inhibitor Dasatinib), affect proliferation (such as Daunorubicin), or may induce differentiation and cell death (such as Tretinoin and ATRA) (9). Although several anti-leukemia agents exist, the mortality rate currently remains high. Thus, the identification of new treatments against leukemic cancer remains an important area of investigation.

The concept of leukemia stem cells is changing the strategies for cancer treatment. Current knowledge of stem cell biology can help in the search for new drugs against cancer, especially for a drug that can selectively affect the leukemia stem cell population. This review focuses on signaling molecules and new agents that can regulate hematopoiesis and could be used as modulators of primitive clonal populations in leukemia.

3. FREE RADICALS AND THE HEMATOPOIETIC SYSTEM

Although the occurrence of free radicals in the body has been related to both the aging process and the development of several disorders, biological functions exerted by the presence of free radicals are extremely important for organism homeostasis (10). Over the last several years, studies have been performed to understand the participation of free radicals in the biology of stem cells, especially the hematopoietic stem cell. Reports have shown the influence of free radicals in controlling self-renewal, proliferation and differentiation processes in hematopoietic stem cells (11-13). Studies have described that the hematopoietic stem cells are predominantly located in the osteoblastic niche, where there is a lower supply of oxygen than in other regions of the bone marrow (14-16). A relationship between the quiescent state of the hematopoietic stem cell and low oxygen presence has been hypothesized. The hypoxic state in trabecular areas has been hypothesized to potentially prevent the differentiation of hematopoietic stem cells, whereas in areas with higher levels of reactive oxygen species (ROS), such as in bone marrow vascular areas, the presence of free radicals seems to be responsible for the maturation of the cells (12, 17, 18).

Several studies have been performed to elucidate the participation of free radicals in hematopoiesis. For example, hematopoietic stem cells have been shown to be sensitive to H_2O_2, which is released into the medium by the growing cells (11). Furthermore, ROS elevation has been shown to promote alterations in the hematopoietic stem cell (HSC) population; for example, an increase in ROS levels induces p38 mitogen-activated protein kinase (p38 MAPK) activation, and this alteration limits the lifespan of the HSCs *in vivo*, leading to the exhaustion of this cell population (19). Conditional deletion of FoxO transcription factor family members leads to a decrease in the primitive hematopoietic population by an increase in ROS levels (20). Moreover, a decrease in Mdm2 expression, a negative suppressor of p53, results in an increase in ROS levels, cell cycle arrest, senescence and cell death in the hematopoietic stem/progenitor cell compartment (21). All of these effects could be reversed by the use of antioxidants (11, 19-21).

The importance of the regulation of oxidative stress has also been described in the process of differentiation. In erythropoiesis, ROS participation is particularly important, because its accumulation results in hemolysis and shortened red blood cell lifespan (22). In response to this effect, a compensatory balance occurs in which the erythroid cell precursors give rise to the mature erythroid cells by increasing their rate of proliferation and differentiation (22). In addition, levels of Nrf2 protein (also known as nuclear factor (erythroid-derived 2)-like 2, NFE2L2), which induces the activation of cytoprotective genes in response to increased ROS levels, decreases during megakaryocytic maturation, suggesting that ROS participates in the maturation process of these cells (23).

The ability of several drugs in clinical use to cause leukemia cell death is dependent on an increase in ROS levels. The clinically used synthetic retinoid 4-HPR (Fenretinide) promotes cell death in the B-precursor lymphoblastic leukemia cell line YCUB-2 by inducing an increase in ROS levels (24). Diallyl disulfide is a chemopreventive agent that induces apoptosis mediated by ROS-activated c-Jun N-terminal kinases (JNK) in human myeloid leukemia HL60 cells (25). Baicalin, a compound obtained from *Scutellaria baicalensis*, induces apoptosis through the activation of caspase-3 and an increase in ROS levels in HL60 cells (26). In K562, a human erythroleukemia cell line, a primitive $CD34^+$ subpopulation of cells possesses greater resistance to imatinib than the bulk cells; this population is sensitive to the combination of simvastatin and imatinib through elevated ROS levels (27).

Taken together, these findings reveal important aspects of ROS as a regulator of the differentiation, maintenance and survival of hematopoietic cells. This new field of study recognizes free radicals as second messenger molecules controlling hematopoiesis, which is consistent with the effects of ROS-inducing agents in leukemia cell survival.

4. P2 RECEPTORS AS HEMATOPOIETIC REGULATORS

The members of the P2 receptor family, ionic channels (P2X) and metabotropic receptors (P2Y), have emerged as important regulators of several physiological processes in the hematopoietic system (28-35). The presence of P2 receptors in hematopoietic cells was first described in mast cells, in which stimulation with ATP induces the release of histamine (36). Subsequent studies have demonstrated the presence of the $P2X_7$ receptor in monocytes and macrophages (32, 33, 37-39). Other

differentiated cells, such as lymphocyte B and T cells (40, 41), erythroblasts and erythrocytes (29, 42) and granulocytic cells (34, 43-45) also express P2 receptors.

In addition, recent reports have demonstrated the participation of P2 receptors in the regulation of proliferation and differentiation of a primitive hematopoietic subset. Early investigations have shown that P2 receptors are modulated during cellular differentiation of HL60 cells into granulocytes or monocytes (35, 46, 47). Recently, has shown that ATP can also act synergistically with cytokines to induce an increase in the human hematopoietic progenitor/stem cells (48). Moreover, ATP and analogs are able to induce myeloid differentiation and decrease the primitive hematopoietic population in murine bone marrow cultures (28). The ability of ATP to induce low proliferation and myeloid differentiation differs from other hematopoietic modulators, such as cytokines. ATP prevents the self-renewal of primitive hematopoietic cells, which are exhausted after some days of stimulus (28, 49). Recently, we have confirmed that ATP is able to induce hematopoietic stem cell differentiation by the direct activation of P2 receptors (49).

Trabecular and vascular areas in bone marrow are two important regulatory niches for hematopoietic stem cells (14-16). Recently, the expression of P2 receptors in primitive hematopoietic cells has been shown (28, 31, 48-50), suggesting that P2 receptors participate in the regulation of these cells. In addition, ATP is known to be released by osteoblasts and endothelial cells under physiological conditions or mechanical stimulation (51-55). Thus, osteoblasts and endothelial cells, which are present in the hematopoietic stem cell microenvironment, may control hematopoiesis through their interactions with hematopoietic stem cells and the release of signaling molecules, such as ATP.

In the hematopoietic stem cell niche, the extracellular ATP concentration can be regulated by its secretion and degradation. Because ATP can induce hematopoietic stem cell differentiation (28), specialized osteoblasts and stromal cells, which regulate the quiescence of the hematopoietic stem cell, could express high levels of enzymes that catalyze the hydrolysis of a nucleotide, such as CD39 and CD73.

In addition, a particular P2 receptor expressed in bone marrow cells may be related to the process of elimination of injured cells. The $P2X_7$ receptor is an ion channel receptor activated by ATP, but also induces the opening of a large pore to allow the permeation of molecules of approximately 1 kDa. This receptor is associated with cellular processes including the release of molecules (56), proliferation (57) and cell death (58). The association between the expression and function of $P2X_7$ receptor with some disorders has been recognized, such as neurodegenerative diseases (59, 60) and cancers (61, 62). In hematological cancers, high level of $P2X_7$ receptor expression has been observed in several leukemias and leukemia cell lines (30, 63, 64). In contrast, downregulation of the $P2X_7$ receptor has been observed in aging myeloid cells (65). Thus, a decrease in the expression of $P2X_7$ receptor, which regulates cell death in myeloid cells, may be related to hematological disorders that are more common with advancing age, such as acute myeloid leukemia. However, the functional role of this receptor in the development of cancer is unclear, and the relationships among age, $P2X_7$ receptor expression and leukemia are still far from been completely understood. $P2X_7$-mediated cell death is likely to be a physiological defense against cancer cells, and the downregulation or nonfunctional expression of this receptor may be related to cancer development.

In contrast, some reports have described a direct effect of ATP on cell death in leukemia cells. High concentrations of extracellular ATP (> 1 mM) have shown cytotoxic activity in the K562 cell line and its multidrug resistant counterpart, Lucena-1, by a still non-identified receptor (66). In addition, ATP also induces apoptosis in leukemic HL60 and F-36P cells (67).

5. NATURAL COMPOUNDS WITH ANTILEUKEMIC ACTIVITIES

Natural products have long been a major source of unique chemicals with biological properties and have a long history of use for the treatment of several diseases. This broad diversity of molecules arises from millions of years of biological selection and is unmatched by any synthetic combinatorial chemistry library. Indeed, even with the rapid development and diversification of drug discovery technologies, natural products still provide the most dramatic impact in the area of cancer. The recent approval of ixabepilone, trabectedin, temsirolimus and romidepsin for cancer treatment (68, 69) highlight the important contribution of natural products, mainly from microbial sources, in anticancer therapy, even with the increasing use of molecular target-based therapy. In leukemia therapies, the value of natural products is demonstrated by agents such as the anthracyclines, the vinca alkaloids and the podophyllotoxins.

Natural products are produced by a wide range of different organisms, mainly plants and microorganisms. The later has been considered the most valuable source of secondary metabolites with biological and potential medical applications. Several factors contribute to this premise: 1) the large scale growing of microorganisms in culture media provides an unlimited supply of the material; 2) with the advent of metagenomics, potential producing gene clusters of new active compounds can be identified and expressed using appropriate fermentation conditions; 3) improvement of cultivation methods together with metagenomic analysis may unravel novel chemical structures from otherwise uncultivable microorganisms; 4) despite the microbial diversity and abundance most microorganisms remain unexploited as yet, especially in the marine environment, thus novel potential agents can be expected in the future; 5) it has been demonstrated that the major classes of plant-derived compounds, camptothecins, taxanes, podophyllotoxins, and the vinca alkaloids, are rather produced by endophytic fungi isolated from the original plant tissues, supporting microorganisms as a

major source of secondary metabolites with exquisite activities to the arsenal of anticancer drugs (68, 70).

A large number of medically useful secondary metabolites have been isolated from environmental microorganisms, such as actinomycetes, bacilli and filamentous fungi. The most popular anticancer chemotherapeutics used in leukemia treatment refers to the antibiotics of the class of anthracyclines, such as daunorubicin and doxorubicin. However, newly microbial compounds have shown interesting biological activities and unique mechanisms of action.

Pericosine A, a carbasugar isolated from the fungus *Periconiabyssoides* OUPS-N133, is able to inhibit the epidermal growth factor receptor and topoisomerase II, presenting *in vitro* and *in vivo* antitumor activities against P388 lymphocytic leukemia (71). The tubulin-binding cytotoxic maytansinoids have been isolated from various species, including the actinomycete *Actinosynnema pretiosum*. Their potent *in vitro* activities against lymphocytic leukemia have stimulated studies of structure-activity relationship, leading to analogs development and synthesis (72).

In recent years, a novel important signaling pathway has been gained attention, the phosphatidylinositol 3-kinase (PI3K)/Akt/mammalian target of rapamycin (mTOR) signaling pathway, which plays a significant role in the regulation of many cellular functions, such as metabolism, growth, proliferation, differentiation, autophagy and survival. Rapamycin is a macrolide ester produced by the bacterial strain *Streptomyces hygroscopicus*, which was first isolated from a soil sample on Easter Island. This compound was initially identified as an antifungal agent and studies of its effects in yeast led to the discovery of the mechanism of action and genes encoding the TOR proteins (73). Rapamycin, in turn, helped to decipher the physiological and pathological role of TOR in different cell types and led to the development of analogs, such as temsirolimus, everolimus and deforolimus. Deregulation of the mTOR signaling or its activation by chemotherapeutic agents is demonstrated in several tumors, including malignant hematopoietic disorders (74). Thus, targeting the mTOR signaling has attracted scientific and clinical interest. However, the complex regulation of the mTOR signaling and its role in normal hematopoiesis may lead to variable responses to inhibitors and difficult interpretation of results. Studies with primary AML blasts in short and long term cultures, as well as in clonogenic assays, suggested that rapamycin might target only the proliferating clone of cells (75). However, rapamycin in combination to other chemotherapeutics, such as etoposide, all-trans retinoic acid (ATRA) and histone deacetlylase (HDAC) inhibitors, has been considered promising therapeutic strategies in AML. For instance, co-treatment with etoposide decreased engraftment of acute myeloid leukemia cells in xenograft model (75). Another study reveals that temsirolimus increased ATRA-induced differentiation of acute pro-myelocytic leukemia in a synergistic fashion (76). Despite the initial disappointing results of rapamycin and its analogs in clinical trials, the ongoing studies aim to evaluate the potential of rapamycin combined with chemotherapeutics for leukemia treatment.

Romidepsin (FK288), a selective inhibitor of HDAC recently approved for the treatment of cutaneous T-cell lymphoma (69), is a bicyclic depsipeptide isolated from the Japanese strain of the soil Gram-negative bacterium *Chromobacterium violaceum* (77). Romidepsin has also demonstrated cytotoxic activities against acute and chronic leukemias. Although its mechanism of cell death is not completely studied, ROS induction, caspase activation and apoptosis induction are involved (78). Studies have also shown that romidepsin sensitizes chronic lymphocytic leukemia (CLL) to tumor necrosis factor-related apoptosis-inducing ligand (TRAIL)-mediated cell death through increased FADD recruitment (79). Moreover, preclinical studies have demonstrated romidepsin-induced apoptosis in CLL and acute myeloid leukemia (AML) at concentrations that effectively inhibit HDAC, suggesting a promising activity in combination therapy (80, 81).

Violacein is another potential anticancer agent isolated from the Amazonian strain of *C. violaceum* (82). This purplish indole derivative exhibits potent anticancer activity *in vitro* against a variety of human tumor cell lines, with major effects in leukemia, non-small-cell lung cancer and colon cancer cells, as well as *in vivo* in Ehrlich ascites tumor-bearing mice (83, 84). *In vitro* assays using HL60 myeloid leukemia cells resulted in 50% growth inhibition with 0.8 μM violacein, while citotoxicity to normal human peripheral monocytes and lymphocytes, and normal fibroblasts (V79 cell line) occurred at concentrations 10-fold and 6-fold higher, respectively (84-88). Furthermore, violacein was found to be safe when administered intraperitoneally to mice in a 35-day toxicity study, as demonstrated by a lack of mortality, and absence of hematotoxicity, renal and hepatotoxicity at doses up to 1 mg/kg (84). Of interest, violacein was first demonstrated to increase HL60 leukemia cell death with signs of apoptosis and to reduce cellular proliferation (85). Further studies evaluating its molecular mechanism of action indicated that cell death was preceded by activation of caspase 8, transcription of nuclear factor κB (NFκB) target genes, and p38 MAPK activation. Moreover, violacein directly activated tumor necrosis factor (TNF) receptor 1 signaling as demonstrated by its co-immunoprecipitation with TNF receptor-associated factor 2 (TRAF2). Additionally, pre-treatment of cells with infliximab, an antibody that antagonizes TNF-α–induced signaling, abolished violacein citotoxicity and the activation of the executor caspase 3, thus providing evidence of the role played by TNFα signaling in violacein-mediated HL60 cell death (87). It is interesting to note that the antitumor potential of violacein is not restricted to the acute myeloblastic subtype of leukemia, as recent results from our group have demonstrated its beneficial effects against BCR-ABL-positive chronic myeloid leukemia cells (K562 cell line), including its multidrug resistant variant, and studies of the molecular mechanisms of action suggested activation of specific signaling cascades depending on the cell type. This observation is corroborated by studies performed in

prostate and colon cancer cell lines and in Ehrlich ascites tumor (84, 89, 90).

In addition to the cytotoxic armamentarium of anticancer drugs, some natural compounds may also indirectly contribute in cancer therapy, such as the case of the immunomodulatory agents. In this respect, we have isolated a proteic aggregated polymer of magnesium ammonium phospholinolate-palmitoleate anhydride (P-MAPA) from *Aspergillus oryzae*, which present marked inhibition of tumor growth and concomitant lengthening of the host's life span of animals bearing transplantable lymphosarcoma-180, Ehrlich solid and ascites tumor, plasmacytoma, Walker 256 tumor and spontaneous mammary carcinoma (91). This unique natural product has been shown to be non-cytotoxic or genotoxic in cultured normal V79 fibroblasts and to human lymphocytes, and no cell growth-inhibiting activity were found against 53 tumor cell lines [91]. Interestingly, studies carried on in Ehrlich ascites tumor-bearing mice revealed that the protective effects of P-MAPA, administered intraperitoneally, is associated with its action on the hematopoietic system, stimulating myelopoiesis, as demonstrated by the increased number of bone marrow granulocyte-macrophage colonies, which counteracted the myelosupressive effect of the tumor. Concurrently, P-MAPA increased the life span of tumor-bearing mice and markedly reduced the number of tumor cells in the peritoneal cavity (91). In addition, increased lymphocyte proliferation, IL-2 production and NK cell activity were found regardless of tumor outgrowth. Likewise, P-MAPA stimulated IFN-γ production, which, in turn, may potentiate NK cell reactivity and activate macrophage functions (92). Taken together, these data clearly indicate that P-MAPA may function to reverse tumor-induced immunosuppression and may delay tumor outgrowth through immunotherapeutic mechanisms, which include modulation of the hematopoietic system, thus offering a valuable alternative strategy to overcome tumor growth.

6. ANTICANCER ACTION OF ANTIMICROBIAL PEPTIDES

Antimicrobial peptides or defense peptides are produced by most organisms. These peptides display innate immune system activity in mammalian and lower complex organisms (93). Most antimicrobial peptides are cationic, and usually amphiphilic or hydrophobic, exhibiting diverse structures. The majority of these peptides present a low molecular weight of less than 10 kDa (94). This class of peptides was initially studied to understand its role in protecting against microorganisms, such as bacteria, fungi and other pathogenic agents. For these reasons, the ability to produce cell death in bacteria and fungus has been extensively investigated. However, a newly identified property of these peptides under investigation is their cytotoxic effect in cancer cells.

In leukemia cell lines, such as HL60 cells, antimicrobial peptides have shown cell death potential. Treatment of HL60 cells with magainin, a cationic and amphipatic α-helix peptide that exhibits low toxicity in normal cells (95), induces tumor cell death by apoptosis (96). Cecropin A, a cationic antimicrobial peptide, also displays cytotoxic activity in HL60 and K562 cells (97). In another study, which was performed with K562 and HL60 cells, the peptide polybia-MPI was shown to induce leukemic cell death by necrosis without affecting NIH/3T3 normal fibroblasts. In addition, tachyplesin, a peptide with a disulfide bridge that forms a stabilized amphipatic β-sheet structure and exhibits antitumoral activity (98), also induces apoptosis in HL60 cells by the efflux of potassium ions (99).

Although several studies have reported that the antitumoral activity of cationic antimicrobial peptides occurs primarily through apoptosis and necrosis. However, the specific mechanism by which these peptides act in eukaryotic cells remains unclear. Investigation of their mechanisms of action revealed huge differences among the peptides, such as the type of cell death and the ability to differentially affect cancer or normal cells. For example, gomesin, a peptide with a disulfide bridge that forms a stabilized amphipatic β-sheet structure, presents high cytotoxic activity against cancer and normal cells (100). Nevertheless, the mode of action of this peptide in eukaryotic cells is not understood. Because gomesin induces the release of LDH, it has been suggested that cell death is a consequence of the opening of pores in cellular membrane or due to a detergent-like effect that results in membrane permeabilization in bacteria and eukaryotic cells (101-105). However, the study of Soletti and collaborators (78) in human neuroblastoma SH-SY5Y and rat pheochromocytoma PC12 cells suggested a mechanism other than a detergent-like action, which involves a complex intracellular calcium-mediated pathway is triggered prior to membrane permeabilization (105). In addition, recent results from our group have demonstrated that gomesin-induced cell death is related to both endocytosis of the peptide and calcium accumulation in mitochondria until its disruption, which happens prior to membrane permeabilization (unpublished results). In hepatocellular carcinoma, peptaiboils, a family of antibiotic peptides from fungi, have been demonstrated to suppress tumor growth by calcium influx, which in turn leads to the activation of μ-calpain and promotes the translocation of Bax to the mitochondria, triggering apoptosis and autophagy, with less evidence of cell permeabilization (106). Considering that the structural differences displayed by these peptides may play a role in their mechanisms of action, further studies are necessary to further clarify the mechanisms of antimicrobial peptides.

The observation that the D-isoform is as cytotoxic as the native peptide suggested that the primary mode of action of these peptides against cancer cells involves their interaction with the cellular membrane through a non-receptor mediated mechanism (100). Another theory proposed that the preferential action against cancer cells is related to the cationic nature of most of these peptides. Important differences between the plasma membranes of normal and malignant cells and of extracellular matrix components are known (98, 107-110). In this regard, electrostatic interactions between cationic

antimicrobial peptides and anionic cell membrane components are believed to be a significant feature in the selective killing of cancer cells. A study with lipid bilayer giant unilamellar vesicles, composed of mixtures of the neutral lipid palmitoyloleoyl phosphatidylcholine with the negatively charged lipid palmitoyloleoyl phosphatidylglycerol or cholesterol, has shown that gomesin interacts preferentially with negative bilayers than with bilayers containing cholesterol (111). The preferential interaction of cationic peptides with cancer cells has been demonstrated for the amphipathic α-helix peptides BMAP-27 and NK-lysin (109, 110). However, a study with CHO-K1 and its defective mutant in the biosynthesis of glycosaminoglycans CHO-745 has shown that the presence of heparan sulfate in wild type cells is important to protect cells from the cytotoxic effects of the amphipathic α-helix KW5 and the amphipathic β-sheet lactoferricin peptides (107).

In a comparative study of AMPs with β-hairpin AMPs (tachyplesin, gomesin, polyphemusin II and protegrin and their linear analogs) different actions in cell death were observed by these peptides in K562 cell lineage. Depending on the concentration of AMPs it was possible to distinguish between two biological effects. At concentrations below the EC50, AMPs promoted cell death, although different intracellular mechanisms could be observed depending on the AMP used. However, at concentrations above the EC50, AMPs induced diverse types of membrane disruptions. Gomesin and protegrin displayed cytotoxic properties, whereas their linear (L) counterparts did not. Similarly, the analogs of tachyplesin and polyphemusin lost the ability to induce cell death when compared to their parent compounds. Lower concentrations of AMPs induced controlled cell death mechanisms such as apoptosis, secondary necrosis and necrosis/necroptosis. Each AMP tested promoted controlled cell death by a different intracellular mechanism. Gomesin, tachyplesin and L-tachyplesin promoted apoptosis, which was characterized by annexin labeling, sensitivity to Z-VAD, and caspase-3 activation. Gomesin and protegrin-induced cell death were also dependent on intracellular calcium mechanisms. N-acetyl cysteine, an antioxidant, was able to reduce protegrin cell death and necrostatin-1 inhibited gomesin, tachyplesin and L-polyphemusin II cytotoxicity (unpublished results).

In contrast, cationic antimicrobial peptides can also increase the cytotoxity of conventional chemotherapeutic drugs in cancer cells (112). For example, cecropin A, an agent that induces cell death in human lymphoblastic leukemia with little toxic effect on normal cells, enhances the effect of chemotherapeutic drugs, such as 5-fluorouracil and cytarabine (113). Furthermore, these peptides may also possess immunological properties because they are stored in the granules of neutrophils, mast cells and NK cells, although their immunomodulatory activities have not been fully investigated (93, 114). Antimicrobial peptides are likely to be able to induce effects either directly or indirectly on the immune system, such as inducing chemokine or cytokine release (115, 116).

7. CONCLUSIONS

In recent years, new mechanisms and features of hematopoietic stem cells and their environment have been discovered, providing evidence and new concepts that may be explored in the rational development of new therapies against blood cancers. The emergence of physiological molecules that can regulate the most primitive hematopoietic compartment and leukemia stem cells, such as ATP and reactive oxygen species, provides candidates for the development of new strategies against cancer cells. The interesting ability of ATP to induce the differentiation of hematopoietic stem cells and reduce the total percentage of this cell population by yet unknown mechanisms may be advantageous in cancer therapy. This observation may lead to the use of specific pharmacologic tools, such as specific analogs of ATP or antagonists directed against a particular receptor. In addition, the ability of ROS to modulate bone marrow development and hematopoietic stem cell biology suggests that compounds that affect the redox state may represent potential drugs that can be used in combination with conventional therapy. Alternatively, promising novel compounds isolated from natural sources, such as violacein, depsipeptide (FR901228), and the antimicrobial peptides are under investigation. Furthermore, these compounds offer the possibility for the development of new classes of therapeutic agents for cancer treatment, including leukemias, due to their cytotoxic and immunomodulatory abilities, thus opening new avenues for the treatment of the disease.

8. REFERENCES

1. Reya, T., S. J. Morrison, M. F. Clarke & I. L. Weissman: Stem cells, cancer, and cancer stem cells. *Nature*, 414, 105-11 (2001)

2. Huntly, B. J. & D. G. Gilliland: Cancer biology: summing up cancer stem cells. *Nature*, 435, 1169-70 (2005)

3. Steffen, B., C. Muller-Tidow, J. Schwable, W. E. Berdel & H. Serve: The molecular pathogenesis of acute myeloid leukemia. *Crit Rev Oncol Hematol*, 56, 195-221 (2005)

4. Lapidot, T., Y. Fajerman & O. Kollet: Immune-deficient SCID and NOD/SCID mice models as functional assays for studying normal and malignant human hematopoiesis. *J Mol Med*, 75, 664-73 (1997)

5. Bonnet, D. & J. E. Dick: Human acute myeloid leukemia is organized as a hierarchy that originates from a primitive hematopoietic cell. *Nat Med*, 3, 730-7 (1997)

6. Chan, W. I. & B. J. Huntly: Leukemia stem cells in acute myeloid leukemia. *Semin Oncol*, 35, 326-35 (2008)

7. Passegue, E. & I. L. Weisman: Leukemic stem cells: where do they come from? *Stem Cell Rev*, 1, 181-8 (2005)

8. McCubrey, J. A., L. S. Steelman, S. L. Abrams, F. E. Bertrand, D. E. Ludwig, J. Basecke, M. Libra, F. Stivala, M. Milella, A. Tafuri, P. Lunghi, A. Bonati & A. M. Martelli: Targeting survival cascades induced by activation of Ras/Raf/MEK/ERK, PI3K/PTEN/Akt/mTOR and Jak/STAT pathways for effective leukemia therapy. *Leukemia*, 22, 708-22 (2008)

9. Corey, S. J.: New agents in the treatment of childhood leukemias and myelodysplastic syndromes. *Curr Oncol Rep*, 7, 399-405 (2005)

10. Droge, W.: Free radicals in the physiological control of cell function. *Physiol Rev*, 82, 47-95 (2002)

11. Gupta, R., S. Karpatkin & R. S. Basch: Hematopoiesis and stem cell renewal in long-term bone marrow cultures containing catalase. *Blood*, 107, 1837-46 (2006)

12. Jang, Y. Y. & S. J. Sharkis: A low level of reactive oxygen species selects for primitive hematopoietic stem cells that may reside in the low-oxygenic niche. *Blood*, 110, 3056-63 (2007)

13. Juntilla, M. M., V. D. Patil, M. Calamito, R. P. Joshi, M. J. Birnbaum & G. A. Koretzky: AKT1 and AKT2 maintain hematopoietic stem cell function by regulating reactive oxygen species. *Blood*, 115, 4030-8 (2010)

14. Li, Z. & L. Li: Understanding hematopoietic stem-cell microenvironments. *Trends Biochem Sci*, 31, 589-95 (2006)

15. Zhang, J., C. Niu, L. Ye, H. Huang, X. He, W. G. Tong, J. Ross, J. Haug, T. Johnson, J. Q. Feng, S. Harris, L. M. Wiedemann, Y. Mishina & L. Li: Identification of the haematopoietic stem cell niche and control of the niche size. *Nature*, 425, 836-41 (2003)

16. Calvi, L. M.: Osteoblastic activation in the hematopoietic stem cell niche. *Ann N Y Acad Sci*, 1068, 477-88 (2006)

17. Suda, T., F. Arai & A. Hirao: Hematopoietic stem cells and their niche. *Trends Immunol*, 26, 426-33 (2005)

18. Parmar, K., P. Mauch, J. A. Vergilio, R. Sackstein & J. D. Down: Distribution of hematopoietic stem cells in the bone marrow according to regional hypoxia. *Proc Natl Acad Sci U S A*, 104, 5431-6 (2007)

19. Ito, K., A. Hirao, F. Arai, K. Takubo, S. Matsuoka, K. Miyamoto, M. Ohmura, K. Naka, K. Hosokawa, Y. Ikeda & T. Suda: Reactive oxygen species act through p38 MAPK to limit the lifespan of hematopoietic stem cells. *Nat Med*, 12, 446-51 (2006)

20. Tothova, Z., R. Kollipara, B. J. Huntly, B. H. Lee, D. H. Castrillon, D. E. Cullen, E. P. McDowell, S. Lazo-Kallanian, I. R. Williams, C. Sears, S. A. Armstrong, E. Passegue, R. A. DePinho & D. G. Gilliland: FoxOs are critical mediators of hematopoietic stem cell resistance to physiologic oxidative stress. *Cell*, 128, 325-39 (2007)

21. Abbas, H. A., D. R. Maccio, S. Coskun, J. G. Jackson, A. L. Hazen, T. M. Sills, M. J. You, K. K. Hirschi & G. Lozano: Mdm2 is required for survival of hematopoietic stem cells/progenitors via dampening of ROS-induced p53 activity. *Cell Stem Cell*, 7, 606-17 (2010)

22. Ghaffari, S.: Oxidative stress in the regulation of normal and neoplastic hematopoiesis. *Antioxid Redox Signal*, 10, 1923-40 (2008)

23. Motohashi, H., M. Kimura, R. Fujita, A. Inoue, X. Pan, M. Takayama, F. Katsuoka, H. Aburatani, E. H. Bresnick & M. Yamamoto: NF-E2 domination over Nrf2 promotes ROS accumulation and megakaryocytic maturation. *Blood*, 115, 677-86 (2010)

24. Goto, H., H. Takahashi, H. Fujii, K. Ikuta & S. Yokota: N-(4-Hydroxyphenyl)retinamide (4-HPR) induces leukemia cell death via generation of reactive oxygen species. *Int J Hematol*, 78, 219-25 (2003)

25. Yi, L., X. X. Ji, M. Lin, H. Tan, Y. Tang, L. Wen, Y. H. Ma & Q. Su: Diallyl disulfide induces apoptosis in human leukemia HL-60 cells through activation of JNK mediated by reactive oxygen. *Pharmazie*, 65, 693-8 (2010)

26. Lu, H. F., S. C. Hsueh, Y. T. Ho, M. C. Kao, J. S. Yang, T. H. Chiu, S. Y. Huamg, C. C. Lin & J. G. Chung: ROS mediates baicalin-induced apoptosis in human promyelocytic leukemia HL-60 cells through the expression of the Gadd153 and mitochondrial-dependent pathway. *Anticancer Res*, 27, 117-25 (2007)

27. Chen, R., W. Xiao, D. Li & S. Mu: Combination of simvastatin and imatinib sensitizes the CD34+ cells in K562 to cell death. *Med Oncol* (2010)

28. Paredes-Gamero, E. J., C. M. M. P. Leon, R. Borojevic, M. E. M. Oshiro & A. T. Ferreira: Changes in Intracellular Ca2+ Levels Induced by Cytokines and P2 Agonists Differentially Modulate Proliferation or Commitment with Macrophage Differentiation in Murine Hematopoietic Cells. *Journal of Biological Chemistry*, 283, 31909-31919 (2008)

29. Paredes-Gamero, E. J., R. B. Craveiro, J. B. Pesquero, J. P. Franca, M. E. Oshiro & A. T. Ferreira: Activation of P2Y1 receptor triggers two calcium signaling pathways in bone marrow erythroblasts. *Eur J Pharmacol*, 534, 30-8 (2006)

30. Zhang, X. J., G. G. Zheng, X. T. Ma, Y. H. Yang, G. Li, Q. Rao, K. Nie & K. F. Wu: Expression of P2X7 in human hematopoietic cell lines and leukemia patients. *Leuk Res*, 28, 1313-22 (2004)

31. Wang, L., S. E. Jacobsen, A. Bengtsson & D. Erlinge: P2 receptor mRNA expression profiles in human lymphocytes, monocytes and CD34+ stem and progenitor cells. *BMC Immunol*, 5, 16 (2004)

32. Steinberg, T. H., A. S. Newman, J. A. Swanson & S. C. Silverstein: ATP4- permeabilizes the plasma membrane of mouse macrophages to fluorescent dyes. *J Biol Chem*, 262, 8884-8 (1987)

33. Coutinho-Silva, R. & P. M. Persechini: P2Z purinoceptor-associated pores induced by extracellular ATP in macrophages and J774 cells. *Am J Physiol*, 273, C1793-800 (1997)

34. Suh, B. C., J. S. Kim, U. Namgung, H. Ha & K. T. Kim: P2X7 nucleotide receptor mediation of membrane pore formation and superoxide generation in human promyelocytes and neutrophils. *J Immunol*, 166, 6754-63 (2001)

35. Adrian, K., M. K. Bernhard, H. G. Breitinger & A. Ogilvie: Expression of purinergic receptors (ionotropic P2X1-7 and metabotropic P2Y1-11) during myeloid differentiation of HL60 cells. *Biochim Biophys Acta*, 1492, 127-38 (2000)

36. Cockcroft, S. & B. D. Gomperts: The ATP4- receptor of rat mast cells. *Biochem J*, 188, 789-98 (1980)

37. Naumov, A. P., Y. A. Kuryshev, E. V. Kaznacheyeva & G. N. Mozhayeva: ATP-activated Ca(2+)-permeable channels in rat peritoneal macrophages. *FEBS Lett*, 313, 285-7 (1992)

38. Nuttle, L. C. & G. R. Dubyak: Differential activation of cation channels and non-selective pores by macrophage P2z purinergic receptors expressed in Xenopus oocytes. *J Biol Chem*, 269, 13988-96 (1994)

39. Hickman, S. E., J. el Khoury, S. Greenberg, I. Schieren & S. C. Silverstein: P2Z adenosine triphosphate receptor activity in cultured human monocyte-derived macrophages. *Blood*, 84, 2452-6 (1994)

40. Padeh, S., A. Cohen & C. M. Roifman: ATP-induced activation of human B lymphocytes via P2-purinoceptors. *J Immunol*, 146, 1626-32 (1991)

41. Wiley, J. S., R. Chen & G. P. Jamieson: The ATP4-receptor-operated channel (P2Z class) of human lymphocytes allows Ba2+ and ethidium+ uptake: inhibition of fluxes by suramin. *Arch Biochem Biophys*, 305, 54-60 (1993)

42. Sluyter, R., A. N. Shemon, J. A. Barden & J. S. Wiley: Extracellular ATP increases cation fluxes in human erythrocytes by activation of the P2X7 receptor. *J Biol Chem*, 279, 44749-55 (2004)

43. Cockcroft, S. & J. Stutchfield: ATP stimulates secretion in human neutrophils and HL60 cells via a pertussis toxin-sensitive guanine nucleotide-binding protein coupled to phospholipase C. *FEBS Lett*, 245, 25-9 (1989)

44. Balazovich, K. J. & L. A. Boxer: Extracellular adenosine nucleotides stimulate protein kinase C activity and human neutrophil activation. *J Immunol*, 144, 631-7 (1990)

45. Idzko, M., E. Panther, H. C. Bremer, S. Sorichter, W. Luttmann, C. J. Virchow, Jr., F. Di Virgilio, Y. Herouy, J. Norgauer & D. Ferrari: Stimulation of P2 purinergic receptors induces the release of eosinophil cationic protein and interleukin-8 from human eosinophils. *Br J Pharmacol*, 138, 1244-50 (2003)

46. Martin, K. A., S. B. Kertesy & G. R. Dubyak: Down-regulation of P2U-purinergic nucleotide receptor messenger RNA expression during *in vitro* differentiation of human myeloid leukocytes by phorbol esters or inflammatory activators. *Mol Pharmacol*, 51, 97-108 (1997)

47. Buell, G., A. D. Michel, C. Lewis, G. Collo, P. P. Humphrey & A. Surprenant: P2X1 receptor activation in HL60 cells. *Blood*, 87, 2659-64 (1996)

48. Lemoli, R. M., D. Ferrari, M. Fogli, L. Rossi, C. Pizzirani, S. Forchap, P. Chiozzi, D. Vaselli, F. Bertolini, T. Foutz, M. Aluigi, M. Baccarani & F. Di Virgilio: Extracellular nucleotides are potent stimulators of human hematopoietic stem cells *in vitro* and *in vivo*. *Blood*, 104, 1662-70 (2004)

49. Barbosa, C. M., C. M. Leon, A. Nogueira-Pedro, F. Wasinsk, R. C. Araujo, A. Miranda, A. T. Ferreira & E. J. Paredes-Gamero: Differentiation of hematopoietic stem cell and myeloid populations by ATP is modulated by cytokines. *Cell Death Dis*, 2, e165 (2011)

50. Casati, A., M. Frascoli, E. Traggiai, M. Proietti, U. Schenk & F. Grassi: Cell-autonomous regulation of hematopoietic stem cell cycling activity by ATP. *Cell Death Differ* (2010)

51. Ostrom, R. S., C. Gregorian & P. A. Insel: Cellular release of and response to ATP as key determinants of the set-point of signal transduction pathways. *J Biol Chem*, 275, 11735-9 (2000)

52. Alvarenga, E. C., R. Rodrigues, A. Caricati-Neto, F. C. Silva, E. J. Paredes-Gamero & A. T. Ferreira: Low-intensity pulsed ultrasound-dependent osteoblast proliferation occurs by via activation of the P2Y receptor: Role of the P2Y(1) receptor. *Bone*, 46, 355-362 (2010)

53. Hayton, M. J., J. P. Dillon, D. Glynn, J. M. Curran, J. A. Gallagher & K. A. Buckley: Involvement of adenosine 5'-triphosphate in ultrasound-induced fracture repair. *Ultrasound Med Biol*, 31, 1131-8 (2005)

54. Qin, K. R., C. Xiang, Z. Xu, L. L. Cao, S. S. Ge & Z. L. Jiang: Dynamic modeling for shear stress induced ATP release from vascular endothelial cells. *Biomech Model Mechanobiol*, 7, 345-53 (2008)

55. Thuringer, D.: The vascular endothelial growth factor-induced disruption of gap junctions is relayed by an

autocrine communication via ATP release in coronary capillary endothelium. *Ann N Y Acad Sci*, 1030, 14-27 (2004)

56. Lopez-Castejon, G., J. Theaker, P. Pelegrin, A. D. Clifton, M. Braddock & A. Surprenant: P2X(7) receptor-mediated release of cathepsins from macrophages is a cytokine-independent mechanism potentially involved in joint diseases. *J Immunol*, 185, 2611-9 (2010)

57. Monif, M., C. A. Reid, K. L. Powell, M. L. Smart & D. A. Williams: The P2X7 receptor drives microglial activation and proliferation: a trophic role for P2X7R pore. *J Neurosci*, 29, 3781-91 (2009)

58. Kawamura, H., F. Aswad, M. Minagawa, K. Malone, H. Kaslow, F. Koch-Nolte, W. H. Schott, E. H. Leiter & G. Dennert: P2X7 receptor-dependent and -independent T cell death is induced by nicotinamide adenine dinucleotide. *J Immunol*, 174, 1971-9 (2005)

59. Diaz-Hernandez, M., M. Diez-Zaera, J. Sanchez-Nogueiro, R. Gomez-Villafuertes, J. M. Canals, J. Alberch, M. T. Miras-Portugal & J. J. Lucas: Altered P2X7-receptor level and function in mouse models of Huntington's disease and therapeutic efficacy of antagonist administration. *FASEB J*, 23, 1893-906 (2009)

60. Takenouchi, T., K. Sekiyama, A. Sekigawa, M. Fujita, M. Waragai, S. Sugama, Y. Iwamaru, H. Kitani & M. Hashimoto: P2X7 receptor signaling pathway as a therapeutic target for neurodegenerative diseases. *Arch Immunol Ther Exp (Warsz)*, 58, 91-6 (2010)

61. Li, X., L. Zhou, Y. H. Feng, F. W. Abdul-Karim & G. I. Gorodeski: The P2X7 receptor: a novel biomarker of uterine epithelial cancers. *Cancer Epidemiol Biomarkers Prev*, 15, 1906-13 (2006)

62. Slater, M., S. Danieletto, A. Gidley-Baird, L. C. Teh & J. A. Barden: Early prostate cancer detected using expression of non-functional cytolytic P2X7 receptors. *Histopathology*, 44, 206-15 (2004)

63. Adinolfi, E., L. Melchiorri, S. Falzoni, P. Chiozzi, A. Morelli, A. Tieghi, A. Cuneo, G. Castoldi, F. Di Virgilio & O. R. Baricordi: P2X7 receptor expression in evolutive and indolent forms of chronic B lymphocytic leukemia. *Blood*, 99, 706-8 (2002)

64. Chong, J. H., G. G. Zheng, X. F. Zhu, Y. Guo, L. Wang, C. H. Ma, S. Y. Liu, L. L. Xu, Y. M. Lin & K. F. Wu: Abnormal expression of P2X family receptors in Chinese pediatric acute leukemias. *Biochem Biophys Res Commun*, 391, 498-504 (2010)

65. Paredes-Gamero, E. J., J. L. Dreyfuss, H. B. Nader, M. E. M. Oshiro & A. T. Ferreira: P2X(7)-induced apoptosis decreases by aging in mice myeloblasts. *Experimental Gerontology*, 42, 320-326 (2007)

66. Bernardo, A. A., F. E. Pinto-Silva, P. M. Persechini, R. Coutinho-Silva, J. R. Meyer-Fernandes, A. L. de Souza & V. M. Rumjanek: Effect of extracellular ATP on the human leukaemic cell line K562 and its multidrug counterpart. *Mol Cell Biochem*, 289, 111-24 (2006)

67. Yoon, M. J., H. J. Lee, J. H. Kim & D. K. Kim: Extracellular ATP induces apoptotic signaling in human monocyte leukemic cells, HL-60 and F-36P. *Arch Pharm Res*, 29, 1032-41 (2006)

68. Bailly, C.: Ready for a comeback of natural products in oncology. *Biochem Pharmacol*, 77, 1447-57 (2009)

69. Vandermolen, K. M., W. McCulloch, C. J. Pearce & N. H. Oberlies: Romidepsin (Istodax, NSC 630176, FR901228, FK228, depsipeptide): a natural product recently approved for cutaneous T-cell lymphoma. *J Antibiot (Tokyo)* (2011)

70. Newman, D. J. & G. M. Cragg: Microbial antitumor drugs: natural products of microbial origin as anticancer agents. *Curr Opin Investig Drugs*, 10, 1280-96 (2009)

71. Yamada, T., M. Iritani, H. Ohishi, K. Tanaka, K. Minoura, M. Doi & A. Numata: Pericosines, antitumour metabolites from the sea hare-derived fungus Periconia byssoides. Structures and biological activities. *Org Biomol Chem*, 5, 3979-86 (2007)

72. Cassady, J. M., K. K. Chan, H. G. Floss & E. Leistner: Recent developments in the maytansinoid antitumor agents. *Chem Pharm Bull (Tokyo)*, 52, 1-26 (2004)

73. Wullschleger, S., R. Loewith & M. N. Hall: TOR signaling in growth and metabolism. *Cell*, 124, 471-84 (2006)

74. Martelli, A. M., C. Evangelisti, F. Chiarini & J. A. McCubrey: The phosphatidylinositol 3-kinase/Akt/mTOR signaling network as a therapeutic target in acute myelogenous leukemia patients. *Oncotarget*, 1, 89-103 (2010)

75. Xu, Q., J. E. Thompson & M. Carroll: mTOR regulates cell survival after etoposide treatment in primary AML cells. *Blood*, 106, 4261-8 (2005)

76. Nishioka, C., T. Ikezoe, J. Yang, S. Gery, H. P. Koeffler & A. Yokoyama: Inhibition of mammalian target of rapamycin signaling potentiates the effects of all-trans retinoic acid to induce growth arrest and differentiation of human acute myelogenous leukemia cells. *Int J Cancer*, 125, 1710-20 (2009)

77. Ueda, H., T. Manda, S. Matsumoto, S. Mukumoto, F. Nishigaki, I. Kawamura & K. Shimomura: FR901228, a novel antitumor bicyclic depsipeptide produced by Chromobacterium violaceum No. 968. III. Antitumor activities on experimental tumors in mice. *J Antibiot (Tokyo)*, 47, 315-23 (1994)

78. Mizutani, H., Y. Hiraku, S. Tada-Oikawa, M. Murata, K. Ikemura, T. Iwamoto, Y. Kagawa, M. Okuda & S. Kawanishi: Romidepsin (FK228), a potent histone deacetylase inhibitor, induces apoptosis through the generation of hydrogen peroxide. *Cancer Sci*, 101, 2214-9 (2010)

79. Inoue, S., N. Harper, R. Walewska, M. J. Dyer & G. M. Cohen: Enhanced Fas-associated death domain recruitment by histone deacetylase inhibitors is critical for the sensitization of chronic lymphocytic leukemia cells to TRAIL-induced apoptosis. *Mol Cancer Ther*, 8, 3088-97 (2009)

80. Byrd, J. C., G. Marcucci, M. R. Parthun, J. J. Xiao, R. B. Klisovic, M. Moran, T. S. Lin, S. Liu, A. R. Sklenar, M. E. Davis, D. M. Lucas, B. Fischer, R. Shank, S. L. Tejaswi, P. Binkley, J. Wright, K. K. Chan & M. R. Grever: A phase 1 and pharmacodynamic study of depsipeptide (FK228) in chronic lymphocytic leukemia and acute myeloid leukemia. *Blood*, 105, 959-67 (2005)

81. Odenike, O. M., S. Alkan, D. Sher, J. E. Godwin, D. Huo, S. J. Brandt, M. Green, J. Xie, Y. Zhang, D. H. Vesole, P. Stiff, J. Wright, R. A. Larson & W. Stock: Histone deacetylase inhibitor romidepsin has differential activity in core binding factor acute myeloid leukemia. *Clin Cancer Res*, 14, 7095-101 (2008)

82. Duran, N. & C. F. Menck: Chromobacterium violaceum: a review of pharmacological and industiral perspectives. *Crit Rev Microbiol*, 27, 201-22 (2001)

83. Duran, N., G. Z. Justo, C. V. Ferreira, P. S. Melo, L. Cordi & D. Martins: Violacein: properties and biological activities. *Biotechnol Appl Biochem*, 48, 127-33 (2007)

84. Bromberg, N., J. L. Dreyfuss, C. V. Regatieri, M. V. Palladino, N. Duran, H. B. Nader, M. Haun & G. Z. Justo: Growth inhibition and pro-apoptotic activity of violacein in Ehrlich ascites tumor. *Chem Biol Interact*, 186, 43-52 (2010)

85. Melo, P. S., S. S. Maria, B. C. Vidal, M. Haun & N. Duran: Violacein cytotoxicity and induction of apoptosis in V79 cells. *In vitro Cell Dev Biol Anim*, 36, 539-43 (2000)

86. Melo, P. S., G. Z. Justo, M. B. de Azevedo, N. Duran & M. Haun: Violacein and its beta-cyclodextrin complexes induce apoptosis and differentiation in HL60 cells. *Toxicology*, 186, 217-25 (2003)

87. Ferreira, C. V., C. L. Bos, H. H. Versteeg, G. Z. Justo, N. Duran & M. P. Peppelenbosch: Molecular mechanism of violacein-mediated human leukemia cell death. *Blood*, 104, 1459-64 (2004)

88. Bromberg, N., G. Z. Justo, M. Haun, N. Duran & C. V. Ferreira: Violacein cytotoxicity on human blood lymphocytes and effect on phosphatases. *J Enzyme Inhib Med Chem*, 20, 449-54 (2005)

89. de Carvalho, D. D., F. T. Costa, N. Duran & M. Haun: Cytotoxic activity of violacein in human colon cancer cells. *Toxicol In vitro*, 20, 1514-21 (2006)

90. Kodach, L. L., C. L. Bos, N. Duran, M. P. Peppelenbosch, C. V. Ferreira & J. C. Hardwick: Violacein synergistically increases 5-fluorouracil cytotoxicity, induces apoptosis and inhibits Akt-mediated signal transduction in human colorectal cancer cells. *Carcinogenesis*, 27, 508-16 (2006)

91. Justo, G. Z., N. Duran & M. L. Queiroz: Myelopoietic response in tumour-bearing mice by an aggregated polymer isolated from Aspergillus oryzae. *Eur J Pharmacol*, 388, 219-26 (2000)

92. Justo, G. Z., N. Duran & M. L. Queiroz: Natural killer cell activity, lymphocyte proliferation, and cytokine profile in tumor-bearing mice treated with MAPA, a magnesium aggregated polymer from Aspergillus oryzae. *Immunopharmacol Immunotoxicol*, 25, 305-19 (2003)

93. Easton, D. M., A. Nijnik, M. L. Mayer & R. E. Hancock: Potential of immunomodulatory host defense peptides as novel anti-infectives. *Trends Biotechnol*, 27, 582-90 (2009)

94. Mandard, N., P. Bulet, A. Caille, S. Daffre & F. Vovelle: The solution structure of gomesin, an antimicrobial cysteine-rich peptide from the spider. *Eur J Biochem*, 269, 1190-8 (2002)

95. Matsuzaki, K., K. Sugishita, M. Harada, N. Fujii & K. Miyajima: Interactions of an antimicrobial peptide, magainin 2, with outer and inner membranes of Gram-negative bacteria. *Biochim Biophys Acta*, 1327, 119-30 (1997)

96. Cruz-Chamorro, L., M. A. Puertollano, E. Puertollano, G. A. de Cienfuegos & M. A. de Pablo: *In vitro* biological activities of magainin alone or in combination with nisin. *Peptides*, 27, 1201-9 (2006)

97. Ceron, J. M., J. Contreras-Moreno, E. Puertollano, G. A. de Cienfuegos, M. A. Puertollano & M. A. de Pablo: The antimicrobial peptide cecropin A induces caspase-independent cell death in human promyelocytic leukemia cells. *Peptides*, 31, 1494-503 (2010)

98. Chen, J., X. M. Xu, C. B. Underhill, S. Yang, L. Wang, Y. Chen, S. Hong, K. Creswell & L. Zhang: Tachyplesin activates the classic complement pathway to kill tumor cells. *Cancer Res*, 65, 4614-22 (2005)

99. Zhang, H. T., J. Wu, H. F. Zhang & Q. F. Zhu: Efflux of potassium ion is an important reason of HL-60 cells apoptosis induced by tachyplesin. *Acta Pharmacol Sin*, 27, 1367-74 (2006)

100. Rodrigues, E. G., A. S. Dobroff, C. F. Cavarsan, T. Paschoalin, L. Nimrichter, R. A. Mortara, E. L. Santos, M. A. Fazio, A. Miranda, S. Daffre & L. R. Travassos: Effective topical treatment of subcutaneous murine B16F10-Nex2 melanoma by the antimicrobial peptide gomesin. *Neoplasia*, 10, 61-8 (2008)

101. Fazio, M. A., V. X. Oliveira, Jr., P. Bulet, M. T. Miranda, S. Daffre & A. Miranda: Structure-activity relationship studies of gomesin: importance of the disulfide bridges for conformation, bioactivities, and serum stability. *Biopolymers*, 84, 205-18 (2006)

102. Moraes, L. G., M. A. Fazio, R. F. Vieira, C. R. Nakaie, M. T. Miranda, S. Schreier, S. Daffre & A. Miranda: Conformational and functional studies of gomesin analogues by CD, EPR and fluorescence spectroscopies. *Biochim Biophys Acta*, 1768, 52-8 (2007)

103. Schaeffer, M., A. de Miranda, J. C. Mottram & G. H. Coombs: Differentiation of Leishmania major is impaired by over-expression of pyroglutamyl peptidase I. *Mol Biochem Parasitol*, 150, 318-29 (2006)

104. Moreira, C. K., F. G. Rodrigues, A. Ghosh, P. Varotti Fde, A. Miranda, S. Daffre, M. Jacobs-Lorena & L. A. Moreira: Effect of the antimicrobial peptide gomesin against different life stages of Plasmodium spp. *Exp Parasitol*, 116, 346-53 (2007)

105. Soletti, R. C., L. del Barrio, S. Daffre, A. Miranda, H. L. Borges, V. Moura-Neto, M. G. Lopez & N. H. Gabilan: Peptide gomesin triggers cell death through L-type channel calcium influx, MAPK/ERK, PKC and PI3K signaling and generation of reactive oxygen species. *Chem Biol Interact*, 186, 135-43 (2010)

106. Shi, M., H. N. Wang, S. T. Xie, Y. Luo, C. Y. Sun, X. L. Chen & Y. Z. Zhang: Antimicrobial peptaibols, novel suppressors of tumor cells, targeted calcium-mediated apoptosis and autophagy in human hepatocellular carcinoma cells. *Mol Cancer*, 9, 26 (2010)

107. Fadnes, B., O. Rekdal & L. Uhlin-Hansen: The anticancer activity of lytic peptides is inhibited by heparan sulfate on the surface of the tumor cells. *BMC Cancer*, 9, 183 (2009)

108. Wang, J. & D. L. Rabenstein: Interaction of heparin and heparin-derived oligosaccharides with synthetic peptide analogues of the heparin-binding domain of heparin/heparan sulfate-interacting protein. *Biochim Biophys Acta*, 1790, 1689-97 (2009)

109. Schroder-Borm, H., R. Bakalova & J. Andra: The NK-lysin derived peptide NK-2 preferentially kills cancer cells with increased surface levels of negatively charged phosphatidylserine. *FEBS Lett*, 579, 6128-34 (2005)

110. Risso, A., M. Zanetti & R. Gennaro: Cytotoxicity and apoptosis mediated by two peptides of innate immunity. *Cell Immunol*, 189, 107-15 (1998)

111. Domingues, T. M., K. A. Riske & A. Miranda: Revealing the lytic mechanism of the antimicrobial peptide gomesin by observing giant unilamellar vesicles. *Langmuir*, 26, 11077-84 (2010)

112. Hoskin, D. W. & A. Ramamoorthy: Studies on anticancer activities of antimicrobial peptides. *Biochim Biophys Acta*, 1778, 357-75 (2008)

113. Hui, L., K. Leung & H. M. Chen: The combined effects of antibacterial peptide cecropin A and anti-cancer agents on leukemia cells. *Anticancer Res*, 22, 2811-6 (2002)

114. Lai, Y. & R. L. Gallo: AMPed up immunity: how antimicrobial peptides have multiple roles in immune defense. *Trends Immunol*, 30, 131-41 (2009)

115. Juarez, J., K. F. Bradstock, D. J. Gottlieb & L. J. Bendall: Effects of inhibitors of the chemokine receptor CXCR4 on acute lymphoblastic leukemia cells *in vitro*. *Leukemia*, 17, 1294-300 (2003)

116. DeMarco, S. J., H. Henze, A. Lederer, K. Moehle, R. Mukherjee, B. Romagnoli, J. A. Robinson, F. Brianza, F. O. Gombert, S. Lociuro, C. Ludin, J. W. Vrijbloed, J. Zumbrunn, J. P. Obrecht, D. Obrecht, V. Brondani, F. Hamy & T. Klimkait: Discovery of novel, highly potent and selective beta-hairpin mimetic CXCR4 inhibitors with excellent anti-HIV activity and pharmacokinetic profiles. *Bioorg Med Chem*, 14, 8396-404 (2006)

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