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Ayanin diacetate-induced cell death is amplified by TRAIL in human leukemia cells

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ABSTRACT

Here we demonstrate that the semi-synthetic flavonoid ayanin diacetate induces cell death selectively in leukemia cells without affecting the proliferation of normal lymphocytes. Incubation of human leukemia cells with ayanin diacetate induced G_2-M phase cell cycle arrest and apoptosis which was prevented by the non-specific caspase inhibitor z-VAD-fmk and reduced by the overexpression of Bcl-x_L. Ayanin diacetate-induced cell death was found to be associated with: (i) loss of inner mitochondrial membrane potential, (ii) the release of cytochrome *c*, (iii) the activation of multiple caspases, (iv) cleavage of poly(ADP-ribose) polymerase and (v) the up-regulation of death receptors for TRAIL, DR4 and DR5. Moreover, the combined treatment with ayanin diacetate and TRAIL amplified cell death, compared to single treatments. These results provide a basis for further exploring the potential applications of this combination for the treatment of cancer.

1. Introduction

There are at least two fundamental apoptotic pathways, referred to as the extrinsic pathway and the intrinsic pathway, depending on the cell type [1,2]. In the extrinsic (or death receptor) pathway, apoptosis is mediated by death receptors and involves caspase-8 activation, while in the intrinsic (or mitochondrial) pathway diverse proapoptotic signals lead to the release of cytochrome *c* from mitochondria to cytoplasm that promotes the assembly of apoptosome and caspase-9 activation.

Tumor necrosis factor (TNF)-related apoptosis-inducing ligand (TRAIL) is a member of the TNF superfamily which is considered a promising anticancer agent. TRAIL selectively induces apoptosis in various types of cancer cells *in vitro* and *in vivo*, but has little or no toxicity toward normal cells [2,3]. TRAIL interacts with two specific pro-apoptotic receptors on the cell surface: death receptor 4 (DR4, also named TRAIL-R1) and death receptor 5 (DR5, also named TRAILR2) to activate the extrinsic apoptotic pathway [4]. After TRAIL binding to DR4 and DR5, the apical pro-caspase-8 zymogen is recruited to the receptor complex and activated, resulting in its autocatalytic processing [5]. Active caspase-8 initiates the proteolytic activation of downstream effector caspases-3, -6 and -7, which cleave cellular substrates to mediate apoptotic cell death. Although the predominant signaling event of TRAIL is apoptosis induction in susceptible cells, most hematologic malignancies, especially primary leukemia, are resistant to TRAIL [6,7]. However, previous studies have shown that TRAIL resistance can be overcome by the combination of chemotherapeutic drugs and irradiation [8].

Flavonoids are naturally occurring polyphenolic compounds which display a remarkable spectrum of biological activities and are of great current interest due to their anticancer activities [9]. Some flavonoids have been shown to induce cell cycle arrest and apoptosis, a kind of cell death which is thought to be an important response to most chemotherapeutic agents in leukemia cells. We have previously reported the preparation of the flavonoid derivative ayanin diacetate and evaluated its effect on the growth of five human tumor cell lines and found that it displays cytotoxic properties [10]. Here we have studied the effect of this compound on cell viability in human leukemia cells as well as in cells which over-express the anti-apoptotic protein Bcl-x_L. We have also evaluated whether caspase activation and death receptors are involved in the mechanism of action.

2. Materials and methods

2.1. Reagents

Ayanin (5,3′-dihydroxy-3,7,4′-trimethoxyflavone) was isolated from the aerial parts of *Pulicaria canariensis* ssp. *lanata* and ayanin diacetate was obtained by acetylation of the natural product as

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described previously [10]. Antibodies for poly(ADP-ribose) polymerase (PARP), for cytochrome *c*, caspases-7, -8, Bax and Bid were purchased from BD PharMingen (San Diego, CA, USA). Antibody for caspase-3 was from Assay Designs (Ann Arbor, MI, USA). Anti-caspase-6 and -9 monoclonal antibodies were from Medical & Biological Laboratories (Nagoya, Japan). Anti-Bcl-2 monoclonal antibody was from Santa Cruz Biotechnology (Santa Cruz, CA, USA). Monoclonal antibodies for α-tubulin and β-actin were purchased from Sigma (Saint Louis, MO, USA). Antibodies for TRAIL, DR4 and DR5 were from Abcam (Cambridge, UK). Anti-$p21^{Cip1}$ and PVDF membranes were purchased from Millipore (Billerica, MA, USA). Secondary antibodies were from GE Healthcare Bio-Sciences AB (Little Chalfont, UK).

2.2. Cell culture and cytotoxicity assays

The human leukemia HL-60, U937 and Molt-3 cells were from DSMZ (German Collection of Microorganisms and Cell Cultures, Braunschweig, Germany) and grown as previously described [11]. HL-60 cells transfected with the pSFFV-neo plasmid (HL-60/neo) or pSFFV-bcl-x_L plasmid (HL-60/Bcl-x_L) (donated by Dr. Angelika Vollmar and which were established by Dr. K.N. Bhalla) were cultured as described [11]. Human peripheral blood mononuclear cells (PBMC) were isolated as described [11]. Cytotoxicity assays on human tumor and human PBMC cells was analyzed by colorimetric MTT assay [11].

2.3. Quantification of apoptosis and analysis of cell cycle by flow cytometry

The flow cytometric evaluation of the cell cycle status and apoptosis was performed by staining DNA with propidium iodide as described [11]. Apoptosis was also determined by translocation of phosphatidylserine to the cell surface using an Annexin V–FITC apoptosis detection kit (BD PharMingen, San Diego, CA, USA) according to the manufacturer's protocol.

2.4. Western blot analysis

Immunoblot analysis of caspases, cytochrome *c*, Bcl-2 family members and PARP was performed as previously described [11].

2.5. Analysis of mitochondrial membrane potential ($\Delta\Psi_m$)

The membrane potential was measured by flow cytometry using the fluorochrome 5,5′,6,6′-tetrachloro-1,1′,3,3′-tetraethylbenzimidazolylcarbocyanine iodide (JC-1, 10 μM) [11].

2.6. Statistical analysis

Statistical significance of differences between control and treated samples were calculated using Student's *t*-test. *P* values of <0.05 were considered significant.

3. Results

3.1. Ayanin diacetate inhibits the growth and cell viability and induces apoptotic cell death in human leukemia cell lines

We have previously shown that ayanin diacetate (Fig. 1A) inhibits the cellular growth of HL-60, Molt-3 and Raji cells in a time- and concentration-dependent manner [10]. Here, we examined its effect on the growth of human leukemia U937 cell line and found that it displays an IC_{50} value of 22 ± 1 μM, similar to the reported value for HL-60 cells (IC_{50} = 18 ± 7 μM), and that over-expression of the anti-apoptotic protein Bcl-x_L confers protection to the HL-60 cells (Fig. 1B). Control experiments with normal lymphocytes show no appreciable toxicity at 30 μM ayanin diacetate. As a positive control, HL-60 cells were also included in the experiment and, as expected, there was an important reduction in the proliferation of these cells (Fig. 1C). When HL-60 cells were incubated with this flavonoid the percentage of apoptotic cells increased fourfold and similar results were obtained in U937 cells and the lymphoid Molt-3 cell line (Fig. 1D). Estimates of apoptosis obtained from Annexin V–FITC staining were similar (data not shown).

3.2. Ayanin diacetate induced G_2-M phase arrest in human myeloid leukemia cells

To determine whether ayanin diacetate-induced cell growth inhibition is mediated via alterations in cell cycle progression, we evaluated the effect of this compound on cell cycle phase distribution by flow cytometric studies. As shown in Fig. 1D, ayanin

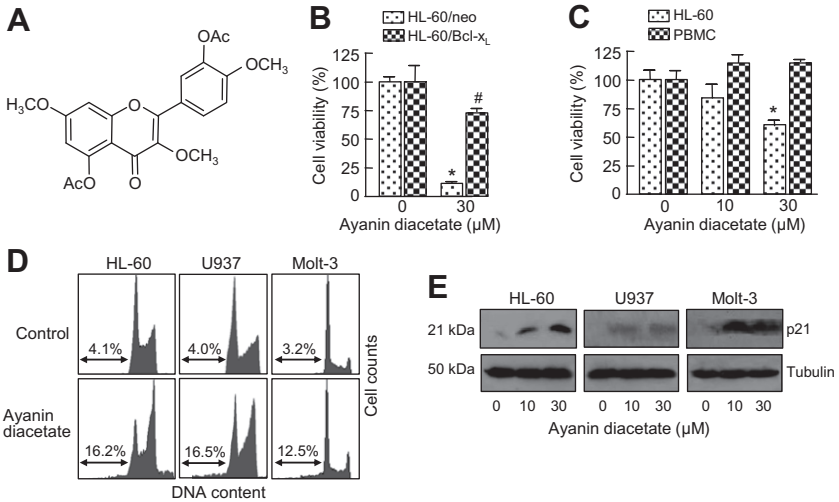

Fig. 1. Chemical structure of ayanin diacetate (A). Cells were cultured with the indicated concentrations of ayanin diacetate for 72 h (B) or for 24 h (C), and thereafter cell viability was determined by the MTT assay. $^{*}P < 0.05$, significantly different from untreated control. $^{\#}P < 0.05$, significantly different from ayanin diacetate treated HL-60/neo. (D) Cells were incubated with 30 μM ayanin diacetate for 24 h and the cell cycle phase distribution was determined by flow cytometry. Hypodiploid cells are shown in region marked with an arrow. (E) Effect of ayanin diacetate on $p21^{Cip1}$ expression. Cells were treated with the indicated concentrations of the flavonoid for 24 h and whole cell lysates were assayed by immunoblotting. Tubulin was used as a loading control.

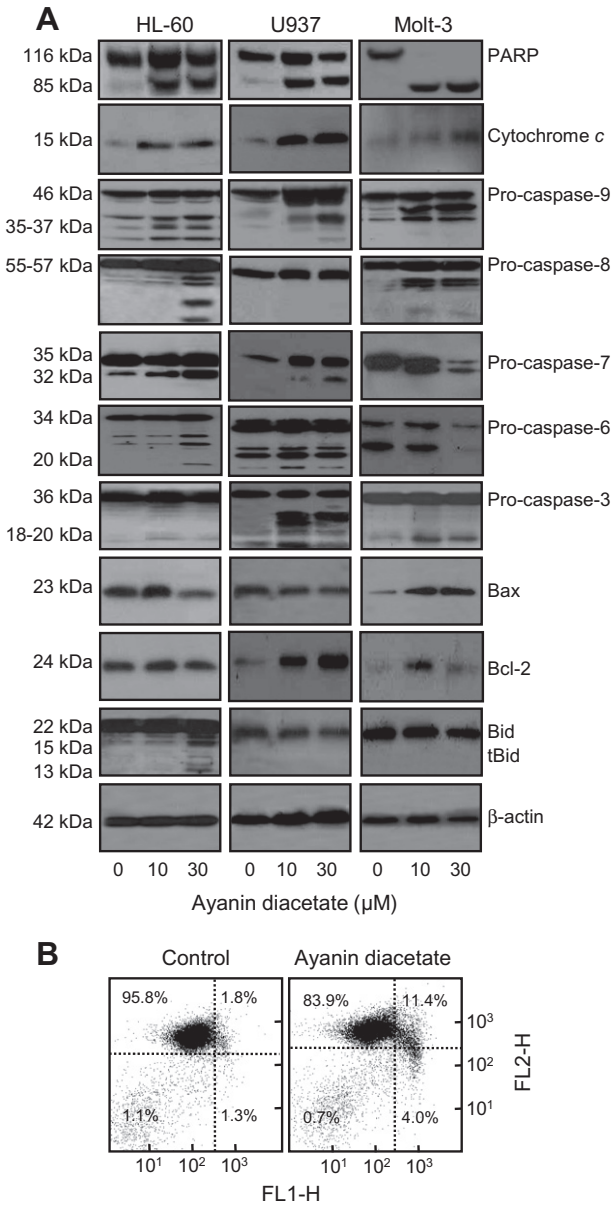

Fig. 2. (A) The cells were incubated in the presence of the indicated concentrations of ayanin diacetate, harvested at 24 h and cell lysates (or cytosolic extracts in the case of cytochrome *c*) were assayed by immunoblotting. (B) Effect of ayanin diacetate on the mitochondrial membrane potential ($\Delta\Psi_m$). HL-60 cells were treated with ayanin diacetate (30 μM) for 24 h and the intensity of JC-1 fluorescence was analyzed by flow cytometry. Similar results were obtained in two separate experiments each performed in triplicate.

diacetate-treated cells significantly accumulated in the G_2-M phase of the cell cycle with a parallel decrease of cells in the G_0/G_1 phase and a significant increase in the sub-G_1 population at 24 h. To characterize the molecular mechanism of G_2-M cell cycle arrest, we examined the expression of the cyclin-dependent kinases inhibitor $p21^{Cip1}$, which may take part in a G_2-M arrest through its interaction with cyclin/cyclin-dependent kinases complexes. Our results show that $p21^{Cip1}$ became detectable after 24 h of treatment with ayanin diacetate in HL-60, U937 and Molt-3 cells (Fig. 1E).

3.3. Ayanin diacetate-induced cell death is mediated by a caspase-dependent pathway

To determine whether caspases were involved, we examined whether ayanin diacetate induces PARP [poly(ADP-ribose) polymerase] cleavage, a hallmark of apoptosis that indicates activation of caspase. As shown in Fig. 2A, hydrolysis of the 116 kDa PARP protein to the 85 kDa fragment was detected in all cell lines analyzed after 24 h exposure at a concentration as low as 10 μM.

To assess whether ayanin diacetate-induced apoptosis involves the release of cytochrome *c* from mitochondria to cytosol, dose-response experiments were performed and cytosolic preparations were analyzed by immunoblotting. As demonstrated (Fig. 2A), a significant increase in the amount of cytochrome *c* in the cytosol was detected after 24 h of treatment in HL-60, U937 and Molt-3 cells. To evaluate whether this flavonoid directly induces the release of cytochrome *c* from mitochondria, these organelles were isolated from HL-60 and U937 cells and incubated during different time periods with the compound. Release of cytochrome *c* was observed in both cell lines, suggesting a direct effect of ayanin diacetate on mitochondria (results not shown). To explore whether the release of cytochrome *c* is associated with mitochondrial membrane potential dissipation, cells were incubated for 24 h with the flavonoid and analyzed using the probe JC-1. Our results show a significant loss of $\Delta\Psi_m$ (Fig. 2B), which suggests that the disruption of the mitochondrial membrane potential might be involved in cell death.

We also examined the effect of this compound on proteolytic processing of caspases. To this end, cells were treated with increasing concentrations of ayanin diacetate and the cell lysates were then subjected to immunoblot analysis (Fig. 2A). The results indicate that ayanin diacetate stimulates the cleavage of inactive pro-caspase-9 to the active 35–37 kDa fragment in all leukemia cells assayed which suggests a central role of the intrinsic pathway. Moreover, ayanin diacetate significantly promotes hydrolysis of procaspases -7, -6 and -3 at 24 h of treatment in HL-60, U937 and Molt-3 cells. We also investigated the expression of the Bcl-2 family members-which control the intrinsic pathway of apoptosis. As shown in Fig. 2A, ayanin diacetate decreased (HL-60 and U937) or increased (Molt-3) the Bax levels in a concentration dependent manner, while there was an increase of the anti-apoptotic protein Bcl-2 (U937 and Molt-3). Since it is known that Bid is a substrate for caspase-8, we also subjected cell lysates to Western blot analysis with an anti-Bid antibody. The results demonstrate that ayanin diacetate promotes Bid cleavage in HL-60 cells and a clear reduction of Bid in U937 and Molt-3 cells.

As processing not always correlates with activity, enzymatic activities of caspase-8, -9 and -3-like proteases (caspase-3/7) were also investigated in HL-60, U937 and Molt-3 cells. Cell lysates were obtained and assayed for cleavage of the tetrapeptides IETD-*p*NA, LEHD-*p*NA and DEVD-*p*NA, as specific substrates for caspases-8, -9 and -3/7, respectively. Induction of caspase-8, -9 and -3 activities was shown to increase in these cell lines (Fig. 3A). To confirm that ayanin diacetate-triggered apoptosis requires the activation of caspases, HL-60 cells were pretreated with the broad-spectrum caspase inhibitor z-VAD-fmk. The complete inhibition by z-VAD-fmk of apoptosis induced by ayanin diacetate shows that this apoptosis is caspase dependent (Fig. 3B). To know the contribution of caspase-3 the impact of the irreversible caspase-3 inhibitor z-DEVD-fmk was also evaluated. As shown (Fig. 3B) the percentage of hypodiploid cells was significantly decreased in presence of the inhibitor which suggests that caspase-3 is involved in ayanin diacetate-induced cell death.

3.4. Ayanin diacetate up-regulates death receptors DR4 and DR5 and the combination of ayanin diacetate and TRAIL enhances apoptosis

Previous reports have shown that the over-expression of death receptors leads to the apoptotic death of cancer cells [12,13]. For this reason we investigated whether this compound induces changes in the expression of TRAIL receptors in leukemia cells.

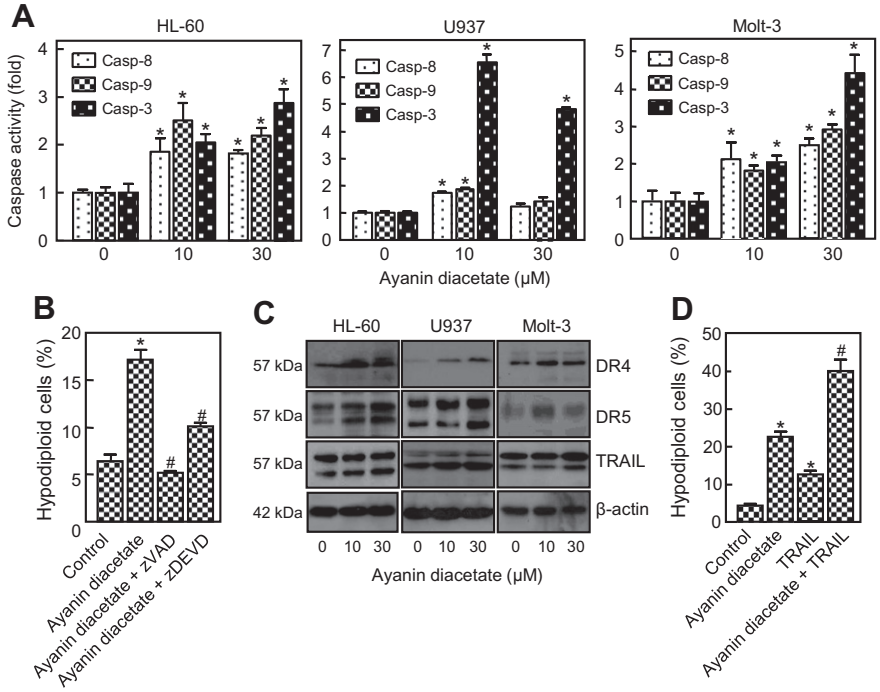

Fig. 3. (A) Caspase activation in response to ayanin diacetate. Results are expressed as fold-increase in caspase activity compared with control. (B) Effect of cell-permeable caspase inhibitors on ayanin diactetate-stimulated apoptosis. (C) Ayanin diacetate up-regulates the expression of the TRAIL receptors DR4 and DR5 in human leukemia cells. Cells were treated with various concentrations of the flavonoid for 24 h and cell lysates were assayed by immunoblotting. (D) HL-60 cells were treated with 30 μM ayanin diacetate, 40 ng/ml TRAIL and the combination of ayanin diacetate and TRAIL for 24 h and the sub-G_1 population was analyzed by flow cytometry. Values represent means ± SE of three independent experiments each performed in triplicate. $^{*}P < 0.05$, significantly different from untreated control. $^{\#}P < 0.05$, significantly different from ayanin diacetate treatment alone.

To this end, HL60, U937 and Molt-3 cells were treated during 24 h and cell lysates were subjected to immunoblot analysis with antibodies which recognize DR4, DR5 and its ligand (TRAIL). As shown in Fig. 3C, ayanin diacetate up-regulates expression of DR4 and DR5 in all the leukemia cell lines assayed. To investigate whether this flavonoid can sensitize HL-60 leukemia cells to TRAIL-induced apoptosis, we carried out experiments with a combination of ayanin diacetate and TRAIL. Treatment with 40 ng/ml TRAIL alone or 30 μM ayanin diacetate alone resulted in ~12% and ~22% apoptotic cell death, respectively, while the combination treatment enhanced apoptosis to ~40% (Fig. 3D).

4. Discussion

Although blood cancer cells are highly sensitive to cytotoxic drugs, they often become resistant after initial therapy. Therefore, there is a need for new drugs that induce specific cell death pathways in leukemia cells. Here, we show that ayanin diacetate effectively induces cell death selectively in leukemic cells but not in normal lymphocytes. This flavonoid causes cell cycle arrest at the G_2-M phase which was associated with induction of the Cdk inhibitor $p21^{Cip1}$. Up-regulation of $p21^{Cip1}$ appears to be an important event in ayanin diacetate-induced antiproliferative effect and might be the consequence of tubulin disorganization.

The experiments shown here demonstrate that antiproliferative properties of ayanin diacetate are dependent on caspases, since initiator and executioner caspases were activated and cell death was inhibited by the general caspase inhibitor z-VAD-fmk. The release of cytochrome *c* may be the initiating event in apoptosis, or it may be downstream of caspase activation, such as occurs in the death receptor pathway which depends on the activation of caspase-8 [14]. Ayanin diacetate initiated redistribution of cytochrome *c* into the cytosol which was correlated with the dissipation of $\Delta\Psi_m$ and caspase-3 activation. Upon treatment of the mitochondrial fraction from HL-60 and U937 cells resulted in a time-dependent release of cytochrome *c*. This suggests that cytochrome *c* release could be the result of a direct action of ayanin diacetate on the mitochondria.

We also observed a concentration-dependent activation of caspases-9 and -3, in accordance with the cytochrome *c* release, emphasizing that the intrinsic pathway plays an important role in the cell death. Moreover, our results indicate that HL-60/Bcl-x_L cells were partially resistant compared with the parental cell line, which confirms the significant role of the mitochondria in ayanin diacetate-induced cell death.

Experiments shown in this paper demonstrate that both initiator caspases (caspases-8 and -9) are activated in HL-60, U937 and Molt-3 cells. Caspase-8 has two preferred substrates, caspase-3 which after activation may directly cause cell death, and Bid, a pro-apoptotic member of Bcl-2 family. Truncated Bid (tBid) decreases mitochondrial membrane potential and favors the release of apoptotic factors such as cytochrome *c*. Bid is the link in the cross-talk between the extrinsic and the intrinsic apoptotic pathways. Ayanin diacetate increased caspase-8 activity leading to the cleavage of Bid and the stimulation of caspase-3 activity in HL-60 cells. In U937 and Molt-3 cells a clear reduction of Bid levels was also observed, although tBid was not detected. Since caspase-8 is essential for the apoptotic response downstream of death receptors we also evaluated the expression of TRAIL and its pro-apoptotic death receptors. Our results show that ayanin diacetate induces TRAIL receptors DR4 and DR5. Although previous studies have shown that p53 has been implicated in the up-regulation of DR4 [15] and DR5 [13,16], we found that ayanin diacetate mediates DR4 and DR5 expression through a p53 independent mechanism since HL-60 and U937 cells lack functional p53 [17].

Resistance toward apoptosis is a key factor in the survival of malignant cells and induction of death receptors expression is

one potential strategy that may overcome this resistance [18]. Many tumors remain resistant to treatment with TRAIL, which has been related to the dominance of anti-apoptotic signals [19]. There is a great interest in the discovery of new drugs targeting the TRAIL-death receptors with the aim to either potentiate TRAIL effect, or to increase DR4 and DR5 receptor expressions [20,21]. At least one of the reasons for such interest is the fact that activation of DR4 or DR5 receptors has been shown to selectively induce apoptosis in a variety of tumour and transformed cells but not in most normal cells [22].

In summary, our results show that ayanin diacetate displays cytotoxic properties, induces G_2-M arrest and apoptosis in human leukemia cells. Ayanin diacetate-induced cell death is accompanied by the activation of multiple caspases, mitochondrial release of cytochrome *c*, PARP cleavage and reduced by the over-expression of Bcl-x_L. We also provide evidence that ayanin diacetate induces DR4 and DR5 expression and increases sensitivity to TRAIL. The findings of this study suggest that this flavonoid and/or flavonoid analogs might be useful in the development of new therapeutic agents against cancer.

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References

[1] G. Kroemer, L. Galluzzi, C. Brenner, Mitochondrial membrane permeabilization in cell death, Physiol. Rev. 87 (2007) 99–163.
[2] A. Ashkenazi, Targeting the extrinsic apoptosis pathway in cancer, Cytokine Growth Factor Rev. 19 (2008) 325–331.
[3] H. Walczak, R.E. Miller, K. Ariail, B. Gliniak, T.S. Griffith, M. Kubin, W. Chin, J. Jones, A. Woodward, T. Le, C. Smith, P. Smolak, R.G. Goodwin, C.T. Rauch, J.C. Schuh, D.H. Lynch, Tumoricidal activity of tumor necrosis factor-related apoptosis-inducing ligand in vivo, Nat. Med. 5 (1999) 157–163.
[4] S.K. Kelley, A. Ashkenazi, Targeting death receptors in cancer with Apo2L/TRAIL, Curr. Opin. Pharmacol. 4 (2004) 333–339.
[5] C. Kantari, H. Walczak, Caspase-8 and Bid: caught in the act between death receptors and mitochondria, Biochim. Biophys. Acta 1813 (2011) 558–563.
[6] C.S. Mitsiades, S.P. Treon, N. Mitsiades, Y. Shima, P. Richardson, R. Schlossman, T. Hideshima, K.C. Anderson, TRAIL/Apo2L ligand selectively induces apoptosis and overcomes drug resistance in multiple myeloma: therapeutic applications, Blood 98 (2001) 795–804.
[7] A. Younes, B.B. Aggarwall, Clinical implications of the tumor necrosis factor family in benign and malignant hematologic disorders, Cancer 98 (2003) 458–467.
[8] S. Shankar, R.K. Srivastava, Enhancement of therapeutic potential of TRAIL by cancer chemotherapy and irradiation: mechanisms and clinical implications, Drug Resist. Updates 7 (2004) 139–156.
[9] B.H. Havsteen, The biochemistry and medical significance of flavonoids, Pharmacol. Ther. 96 (2002) 67–202.
[10] J. Triana, M. López, F.J. Pérez, F. León, J. Quintana, F. Estévez, J.C. Hernández, J. González-Platas, I. Brouard, J. Bermejo, Secondary metabolites from two species of *Pulicaria* and their cytotoxic activity, Chem. Biodivers. 11 (2011) 2080–2089.
[11] F. Torres, J. Quintana, F. Estévez, 5,7,3′-trihydroxy-3,4′-dimethoxyflavone-induced cell death in human leukemia cells is dependent on caspases and activates the MAPK pathway, Mol. Carcinog. 49 (2010) 464–475.
[12] J.P. Sheridan, S.A. Marsters, R.M. Pitti, A. Gurney, M. Skubatch, D. Baldwin, L. Ramakrishnan, C.L. Gray, K. Baker, W.I. Wood, A.D. Goddard, P. Godowski, A. Ashkenazi, Control of TRAIL-induced apoptosis by a family of signaling and decoy receptors, Science 277 (1997) 818–821.
[13] G.S. Wu, T.F. Burns, E.R. McDonald 3rd, W. Jiang, R. Meng, I.D. Krantz, G. Kao, D.D. Gan, J.Y. Zhou, R. Muschel, S.R. Hamilton, N.B. Spinner, S. Markowitz, G. Wu, W.S. El-Deiry, KILLER/DR5 is a DNA damage-inducible p53-regulated death receptor gene, Nat. Genet. 17 (1997) 141–143.
[14] T. Kuwana, J.J. Smith, M. Muzio, V. Dixit, D.D. Newmeyer, S. Kornbluth, Apoptosis induction by caspase-8 is amplified through the mitochondrial release of cytochrome *c*, J. Biol. Chem. 273 (1998) 16589–16594.
[15] X. Liu, P. Yue, F.R. Khuri, S.Y. Sun, p53 upregulates death receptor 4 expression through an intronic p53 binding site, Cancer Res. 64 (2004) 5078–5083.
[16] J.J. Chen, C.W. Chou, Y.F. Chang, C.C. Chen, Proteasome inhibitors enhance TRAIL-induced apoptosis through the intronic regulation of DR5: involvement of NF-κB and reactive oxygen species-mediated p53 activation, J. Immunol. 180 (2008) 8030–8039.
[17] G.S. Wu, W.S. El-Deiry, Apoptotic death of tumor cells correlates with chemosensitivity, independent of p53 or bcl-2, Clin. Cancer Res. 2 (1996) 623–633.
[18] A. Ashkenazi, Targeting death and decoy receptors of the tumour-necrosis factor superfamily, Nat. Rev. Cancer 2 (2002) 420–430.
[19] F.H. Igney, P.H. Krammer, Death and anti-death: tumour resistance to apoptosis, Nat. Rev. Cancer 2 (2002) 277–288.
[20] C. Vermot-Desroches, E. Sergent, B. Bonnin, J. Wijdenes, Characterization of monoclonal antibodies directed against TRAIL or TRAIL receptors, Cell. Immunol. 236 (2005) 86–91.
[21] M. Ishii, M. Iwai, Y. Harada, T. Kishida, H. Asada, M. Shin-Ya, Y. Itoh, J. Imanishi, T. Okanoue, O. Mazda, Soluble TRAIL gene and actinomycin D synergistically suppressed multiple metastasis of TRAIL-resistant colon cancer in the liver, Cancer Lett. 245 (2007) 134–143.
[22] D. Mérino, N. Lalaoui, A. Morizot, P. Schneider, E. Solary, O. Micheau, Differential inhibition of TRAIL-mediated DR5-DISC formation by decoy receptors 1 and 2, Mol. Cell. Biol. 26 (2006) 7046–7055.