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Delphinidin-3-O-glucoside and delphinidin-3-O-rutinoside mediate the redox-sensitive caspase 3-related pro-apoptotic effect of blackcurrant juice on leukaemia Jurkat cells

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ABSTRACT

Blackcurrant, a rich source of anthocyanins, has been reported to exhibit an antiproliferative effect on several types of solid tumour cancer cells, but its active molecules and the precise mechanism of their action remain unclear. The aim of the present study was to investigate the anticancer effect of blackcurrant-derived products (juice (BCJ), extract (BCE) and anthocyanins, namely cyanidin-3-O-glucoside, cyanidin-3-O-rutinoside, delphinidin-3-O-glucoside and delphinidin-3-O-rutinoside) in Jurkat cells and to characterize the underlying mechanism. Cell cycle and apoptosis were assessed by flow cytometry, the formation of reactive oxygen species (ROS) by dihydroethidine and protein expression by Western blotting. BCJ and BCE inhibited the proliferation and induced G_2/M phase cell cycle arrest and apoptosis in Jurkat cells associated with an increased expression of p73 and caspase 3, dephosphorylation of Akt and Bad, and down-regulation of UHRF1 and Bcl-2. A pro-apoptotic response was also observed in response to two major blackcurrant anthocyanins, delphinidin-3-O-rutinoside and delphinidin-3-O-glucoside. The BCJ- and BCE-induced activation of caspase 3 was markedly inhibited by pretreatment with N-acetylcysteine. BCE and the two active anthocyanins induced the formation of reactive oxygen species. BCJ and its major anthocyanins induced a redox-sensitive caspase 3-dependent apoptosis in Jurkat cells, involving a dysregulation of the Akt/Bad/Bcl-2 pathway.

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1. Introduction

There is now more and more evidence suggesting that the onset of cancer is affected by food habits and life style. Both experimental and population based studies have shown an inverse relationship between consumption of fruits and vegetables and the risk of cancer and cardiovascular diseases (Liu, 2013; Schini-Kerth, Auger, Kim, Etienne-Selloum, & Chataigneau, 2010). Polyphenol-rich fruits such as berries have received much attention as potential chemopreventive and chemotherapeutic agents, as indicated by preclinical studies and clinical trials (Seeram, 2008, 2013; Wang et al., 2013; Woode et al., 2012).

Acute lymphoblastic leukaemia (ALL) is the most common haematological cancer occurring in childhood. The overall cure rate in children is near 90%, but prognosis still remains poor in adults, with cure rates of only 30–40% (Inaba, Greaves, & Mullighan, 2013). Dysregulation of apoptosis is a hallmark of this malignancy, characterized by high levels of the anti-apoptotic proteins Bcl-2 (B-cell lymphoma/leukaemia 2) and UHRF1 (Ubiquitin-like PHD Ring Finger 1) (Alhosin et al., 2011; Cimmino et al., 2005; Hanada, Delia, Aiello, Stadtmauer, & Reed, 1993). The induction of apoptosis by targeting Bcl-2 and UHRF1 proteins and/or Bcl-2- and UHRF1-regulating proteins such as Bad (Bcl-2-associated death promoter), and tumour suppressor gene p73, which is known to function as a p53 analogue in the p53-deficient Jurkat cells, could be a promising therapeutic approach in ALL (Abusnina et al., 2011; Alhosin et al., 2010, 2011; Bronner et al., 2007).

Blackcurrant represents a considerable source of dietary polyphenols, mainly anthocyanins (250 mg/100 g of fresh fruit), which contribute about 80% to the total phenolic compounds (Gavrilova, Kajdzanoska, Gjamovski, & Stefova, 2011; Slimestad & Solheim, 2002). Anthocyanins have been shown to have a broad spectrum of biological activities, including antioxidant, anti-inflammatory and protection of the vascular function (Kuntz et al., 2014; Speciale, Cimino, Saija, Canali, & Virgili, 2014; Zhu et al., 2013). Anthocyanin-rich extracts derived from blackcurrants have previously been shown to exert chemopreventive actions against diethylnitrosamine-initiated hepatocarcinogenesis in rats by attenuating oxidative stress and cytotoxic activity against HT29 human colon cancer cells and HepG2 human liver cancer cells (Bishayee et al., 2010; Thoppil et al., 2012; Wu, Koponen, Mykkanen, & Torronen, 2007) but the precise mechanism of action still remains unclear, and its activity against haematological malignancies has been poorly investigated. The main objective of this work was to study the anticancer effect of blackcurrant-derived products (juice (BCJ), extract (BCE) and major anthocyanins) in acute lymphoblastic leukaemia Jurkat cells, and to characterize the underlying mechanism. In particular, since previous studies have indicated that a pro-oxidant event is involved in the pro-apoptotic effect of *Corema album* leaves chalcones and *Aronia melanocarpa* juice polyphenols (Leon-Gonzalez, Lopez-Lazaro, Espartero, & Martin-Cordero, 2013; Sharif et al., 2012), experiments were performed to evaluate the role of reactive oxygen species (ROS).

2. Materials and methods

2.1. Preparation of blackcurrant juice (BCJ) and extract (BCE)

Blackcurrant (*Ribes nigrum* L., Grossulariaceae) juice concentrate (66.2° Bx) was provided by Eckes-Granini (Nieder-Olm, Germany) and diluted to 11.6° Bx in distilled water. BCJ extract (BCE) was prepared by using size exclusion chromatography: 2.5 mL of BCJ concentrate were added to a Sephadex® LH-20 (GE Healthcare, Uppsala, Sweden) column and washed with 250 mL H_2O to remove sugars and organic acids; elution was performed with 0.1% trifluoroacetic acid on methanol, followed by acetone/water (60:40, v/v) to remove the tannins. The column was then washed with 500 mL of H_2O before a new run.

2.2. Chemical analysis

The total polyphenol concentration of BCJ and BCE was measured by the Folin–Ciocalteu method and expressed as gallic acid equivalents (GAE). BCJ contained 2.7 g GAE/L and BCE contained 310.4 ± 0.1 mg GAE/g. The Ribereau-Gayon method was performed for the determination of total anthocyanins (Gonzalez-Rodriguez, Perez-Juan, & Luque de Castro, 2002). BCE contained 81.0 ± 0.1 mg/g.

HPLC-DAD analysis was performed in a liquid Elite Lachrom chromatograph (Merck Hitachi, Krefeld, Germany) equipped with an L2450 photodiode array detector. Separation of anthocyanins was carried out according to Tabart, Kevers, Pincemail, Defraigne, and Dommes (2010). Absorbance was recorded at 518 nm. Standards of cyanidin-3-*O*-glucoside, cyanidin-3-*O*-rutinoside, delphinidin-3-*O*-glucoside and delphinidin-3-*O*-rutinoside were purchased from Extrasynthese (Genay Cedex, France).

2.3. Cell culture and treatments

Jurkat and Molt-4 cell lines were obtained from the American Type Culture Collection (Manassas, VA, USA). Peripheral blood mononuclear cells (PBMCs) from healthy donors were isolated by Ficoll density-gradient centrifugation (Lymphocyte Separation Medium, MP Biomedicals). Cells were cultured in RPMI 1640 medium containing 10% foetal bovine serum (Lonza, Verviers, Belgium), 2 mM L-glutamine, 100 U/mL penicillin and 100 U/mL streptomycin (Sigma-Aldrich, St Louis, MO, USA) in a humidified incubator with 5% CO_2 at 37 °C, as previously described (Achour et al., 2008). Cells were grown for 24 h and then they were exposed to BCJ, BCE or an anthocyanin for specified times. In some experiments, the antioxidant N-acetylcysteine (NAC, Sigma-Aldrich) was added to the culture medium 30 min before treatment.

2.4. Cell viability analysis

Jurkat cells were seeded in 96-well plates at a density of 2×10^4 cells/well, grown for 24 h and exposed to BCJ at different concentrations for 24 h. Then 20 µL of MTS [3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulphophenyl)-2H-tetrazolium] reagent (CellTiter 96® Aqueous One Solution,

Promega, Charbonnières-Les-Bains, France) were added to each well and incubated for 2 h. Absorbance was then measured at 490 nm using a multiwell plate reader. The percentage of viable cells was calculated as a ratio of the optical density (OD) value of each treated cell sample to the OD value of the corresponding control.

2.5. Cell cycle phase distribution analysis

Jurkat cells were seeded in 80 cm^2 culture flasks at a density of 2×10^5 cells/mL, grown for 24 h and then exposed to BCJ at different concentrations for 24 h. Thereafter, cells were collected, washed and fixed in 70% ethanol. After incubation for at least 2 h at 4 °C, cells were washed, treated with an RNase solution (Sigma-Aldrich) and stained with the DNA fluorochrome propidium iodide (PI, 50 μg/mL, Sigma-Aldrich) for 30 min at room temperature. Propidium iodide fluorescence was then measured by flow cytometry (FACScan, BD Biosciences, San Jose, CA, USA). A minimum of 20,000 cells were acquired per sample, and the data were analysed using the Modfit LT® software (Verity Software House, Topsham, ME, USA). The percentage of cells in G_0/G_1, S and G_2/M phases was determined from DNA content histograms (Sharif et al., 2010).

2.6. Apoptosis analysis

The annexin V-FITC/PI apoptosis assay (BD Biosciences Pharmingen, San Diego, CA, USA) was used to detect early and late apoptosis. Apoptosis rates were assessed by flow cytometry on Jurkat and Molt-4 leukaemia cell lines or on PBMCs, after exposure to either BCJ, BCE or an anthocyanin at different concentrations. At least 10,000 events were recorded and represented as dot plots (Sharif et al., 2011).

2.7. Determination of ROS formation

The level of cellular ROS formation was determined as previously described (Alhosin et al., 2010). Briefly, Jurkat cells exposed for 24 h to 75 μg/mL BCE or 100 μM of an anthocyanin were stained with dihydroethidium (DHE), which is a redox-sensitive fluorescent probe. DHE is rapidly oxidized to ethidium (a red fluorescent compound) by superoxide anions, which is then trapped in the nucleus by intercalating into DNA, leading to an increase of ethidium fluorescence intensity. After staining with DHE, cells were subjected to flow cytometric examination (BD FACSCalibur, Becton Dickinson, Franklin Lakes, NJ, USA). Data were acquired and analysed using the CellQuest software (BD Biosciences, San Jose, CA, USA).

2.8. Western blot analysis

Proteins from whole cell lysates were extracted, separated on 10 or 12% SDS-polyacrylamide gels and transferred onto nitrocellulose membranes as previously described (Alhosin et al., 2010). Membranes were then probed overnight at 4 °C with an appropriate primary antibody (mouse monoclonal anti-p73 antibody, BD Biosciences; rabbit polyclonal anti-cleaved caspase 3 antibody; mouse monoclonal anti-UHRF1 antibody, mouse polyclonal anti-p-Bad, rabbit polyclonal anti p-Akt Ser473, Cell Signaling Technology, Danvers, MA, USA; or mouse monoclonal anti-Bcl-2, Millipore, Darmstadt, Germany). Membranes were thereafter incubated for 1 h with the corresponding horseradish peroxidase-linked secondary antibody. Immunoreactive bands were detected using ECL chemiluminescence substrate solution (GE Healthcare Europe GmbH, Saclay, France). Membranes were stripped subsequently and reprobed with a mouse monoclonal anti-β-tubulin antibody (Abcam, Paris, France).

2.9. Statistical analysis

All values are expressed as means ± S.E.M. of at least three independent experiments. Statistical evaluation was performed using one-way ANOVA test, followed by a Bonferroni's post-hoc test, using GraphPad Prism software (version 5.04 for Windows, GraphPad Software Inc., La Jolla, CA, USA), or paired *t* test, as appropriate. $P < 0.05$ was considered as significant.

3. Results

3.1. Blackcurrant-derived products inhibit cell proliferation and induce G_2/M cell cycle arrest and apoptosis in Jurkat cells

BCJ treatment significantly inhibited the proliferation of lymphoblastic leukaemia Jurkat cells. As shown in Fig. 1A, 0.3 and 0.5% (v/v) treatment of Jurkat cells with BCJ significantly decreased the proliferation. Since cell growth is associated with an impaired ability to progress through the different phases of mitosis, the effect of BCJ treatment on the cell cycle phase distribution was examined. As shown in Fig. 1B, BCJ, at concentrations from 0.1% to 0.5% (v/v), significantly increased the percentage of cells in the G_2/M phase and decreased the percentage in S phase. Thus, BCJ inhibited the growth of Jurkat cells by promoting cell cycle arrest in the G_2/M phase.

Thereafter, apoptosis rates were assessed in Jurkat cells after treatment with 0.5% BCJ for different times (Fig. 1C) or with 0.1, 0.3 and 0.5% BCJ for 24 h (Fig. 1D). The annexin V-FITC/PI analysis by flow cytometry indicated that BCJ induced apoptosis in a time- and concentration-dependent manner. A significant increase of the percentage of apoptotic cells was observed after incubating Jurkat cells with 0.5% BCJ for 6 h (Fig. 1C) or with concentrations of or greater than 0.3% BCJ for 24 h (Fig. 1D). A pro-apoptotic activity of BCJ was also observed in the human acute lymphoblastic leukaemia cell line, Molt-4 (Fig. 1E).

In order to better evaluate the role of polyphenols in the pro-apoptotic effect of BCJ, a polyphenol-rich extract of BCJ (BCE) was prepared, and the cytotoxic effect was evaluated. As shown in Fig. 1E, more than 20% of the Jurkat cells were undergoing apoptosis after a 6-h treatment period with BCE (100 μg/mL) (Fig. 1F), and a significant apoptotic effect was observed with concentrations of or greater than 25 μg/mL BCE at 24 h (Fig. 1G).

The HPLC analysis of BCE showed the presence of four major anthocyanins: cyanidin-3-O-glucoside (2.2 ± 0.1 mg/g), cyanidin-3-O-rutinoside (22.1 ± 1.5 mg/g), delphinidin-3-O-glucoside (11.5 ± 0.6 mg/g) and delphinidin-3-O-rutinoside (41.0 ± 0.6 mg/g). Therefore, these four major anthocyanins of blackcurrant

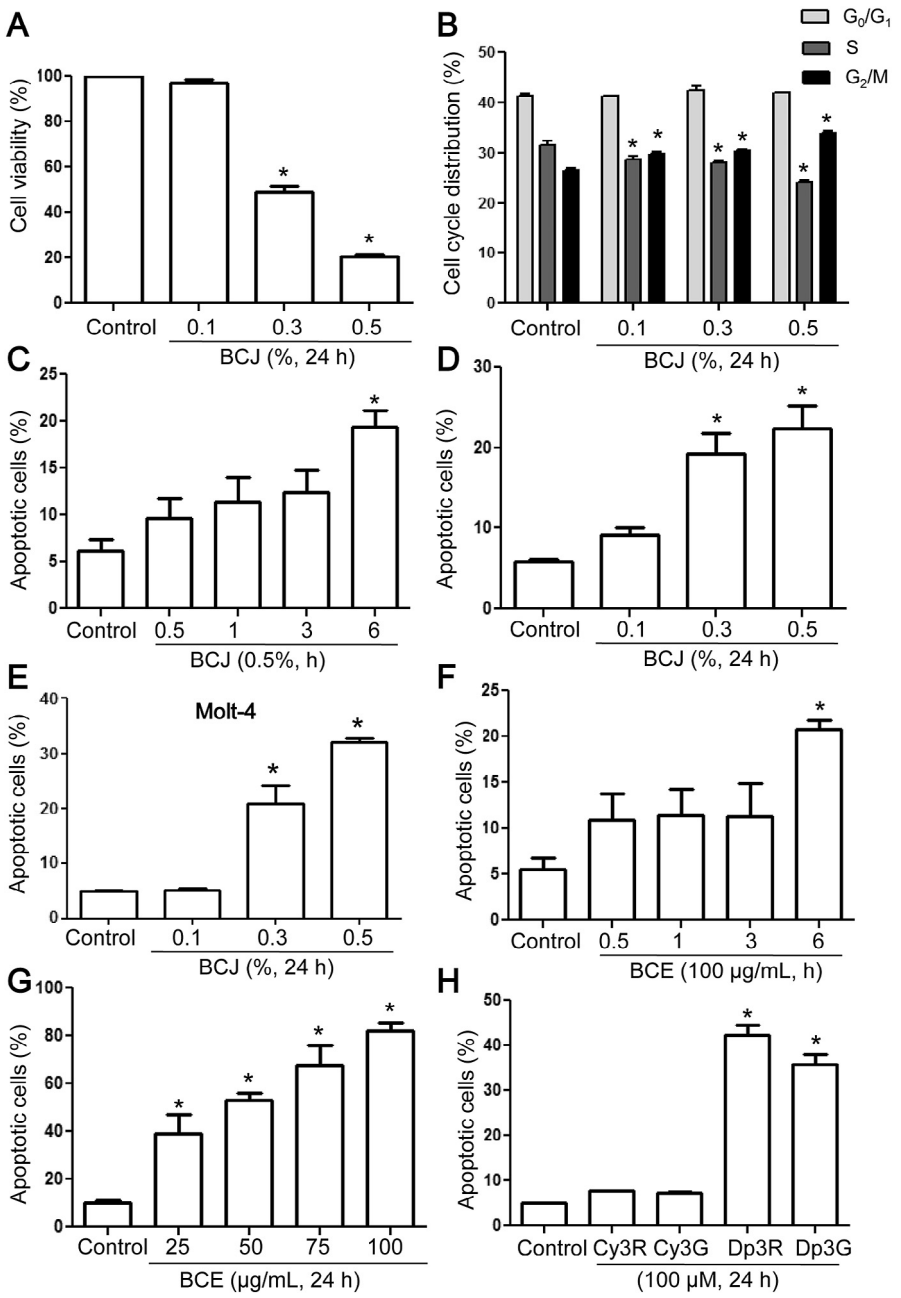

Fig. 1 – Blackcurrant-derived products decrease viability, cause cell cycle arrest and induce apoptosis in acute lymphoblastic leukaemia cells. Cells were exposed to increasing concentrations of blackcurrant juice (BCJ) for 24 h and cell viability was determined using the MTS assay (A). Jurkat cell cycle distribution (B). Apoptosis rates in Jurkat (C, D, F–H) and Molt-4 (E) cells were assessed by flow cytometry using the annexin V-FITC/PI apoptosis assay after different treatments. Values are shown as means ± S.E.M. (n = 3–6). *$P < 0.05$ versus respective control. Cyanidin-3-O-rutinoside (Cy3R), cyanidin-3-O-gluoside (Cy3G), delphinidin-3-O-rutinoside (Dp3R) and delphinidin-3-O-gluoside (Dp3G).

were evaluated for their pro-apoptotic effect. Treatment with delphinidin-3-O-glucoside and delphinidin-3-O-rutinoside significantly increased the percentage of apoptotic cells, whereas cyanidin-3-O-glucoside and cyanidin-3-O-rutinoside were inactive at 100 µM (Fig. 1H).

In order to determine the effect of BCJ on non-malignant cells, PBMCs from three healthy adult donors were incubated with increasing concentrations of BCJ, for 24 h. Although 0.3% BCJ promoted apoptosis in Jurkat cells by about 20% (Fig. 1D), no such effect was observed in PBMCs exposed to BCJ (1.5 ± 0.3% apoptosis at 3% BCJ).

3.2. *BCJ modulates the expression of proteins related to cell cycle and apoptosis in Jurkat cells*

To characterize the molecular events implicated in the BCJ induced cell cycle arrest and apoptosis, the expression level of various proteins was assessed using Western blot analysis.

Exposure of Jurkat cells to BCJ induced a time- and concentration-dependent activation of caspase 3 in Jurkat cells (Fig. 2A and B). An increase of the cleaved caspase 3 level appeared within 6 h of treatment, suggesting that the Jurkat cell viability is rapidly reduced after BCJ treatment (Fig. 2A). Next, we examined the expression level of apoptosis-related proteins after a 24-h treatment period with increasing concentrations of BCJ. As shown in Fig. 2B, p73 expression level was increased in a concentration-dependent manner by the BCJ, reaching about a two-fold increase at 0.5% BCJ. The BCJ-induced up-regulation of p73 expression was accompanied with a reduced expression of UHRF1 (Fig. 2B).

Next, the role of the p-Akt/p-Bad/Bcl-2 pathway, a major regulator of apoptosis, on BCJ-induced apoptosis was examined. As shown in Fig. 3, BCJ induced a decrease in Bcl-2 expression level in a concentration-dependent manner. Since the inactivation of p-Bad via its dephosphorylation induces Bcl-2 down-regulation leading to apoptosis (Yang, 2010), the state of p-Bad in response to BCJ was examined. The BCJ treatment of Jurkat cells induced a dephosphorylation of Bad (Fig. 3). Finally, as p-Akt can phosphorylate Bad at Ser112 and Ser136 promoting survival (Hu et al., 2009), the potential of BCJ to inhibit the constitutive phosphorylation of Akt in Jurkat cells was examined. As indicated in Fig. 3, BCJ caused the dephosphorylation of Akt at Ser473.

Similarly to BCJ, the anthocyanin-rich BCE also induced caspase 3 activation associated with an increased expression of p73 and the down-regulation of UHRF1 (Fig. 4).

3.3. *Blackcurrant-derived product-induced apoptosis is a redox-sensitive event*

The pro-apoptotic effect of various natural products such as *C. album* chalcones, red wine polyphenols and several alkaloids and terpenoids, has been shown to be a redox-sensitive event (Leon-Gonzalez et al., 2013; Martin-Cordero, Leon-Gonzalez, Calderon-Montano, Burgos-Moron, & Lopez-Lazaro, 2012; Rigas & Sun, 2008; Sharif et al., 2010). Therefore, experiments were performed to determine the role of ROS formation in the blackcurrant-derived product induced apoptosis in Jurkat cells. As indicated in Fig. 5A, BCE, delphinidin-3-*O*-glucoside and delphinidin-3-*O*-rutinoside induced a marked increase in the formation of ROS in Jurkat cells whereas cyanidin-3-*O*-glucoside and cyanidin-3-*O*-rutinoside were inactive. Treatment of Jurkat cells with the ROS inhibitor NAC significantly reduced the up-regulation of active caspase 3 induced by both BCJ and BCE (Fig. 5B). Altogether, these results indicate that blackcurrant anthocyanins-induced apoptosis is critically dependent on a redox-sensitive event.

4. Discussion

Anthocyanins represent a flavonoid subclass that has been shown to have a broad range of biological effects and, in particular, antimutagenesis and anticarcinogenesis properties (Kahkonen et al., 1999; Schumacher et al., 2011; Sorrenti et al., 2012). Blackcurrant juice is a rich source of polyphenols which contains predominantly anthocyanins, about 250 mg/100 g of

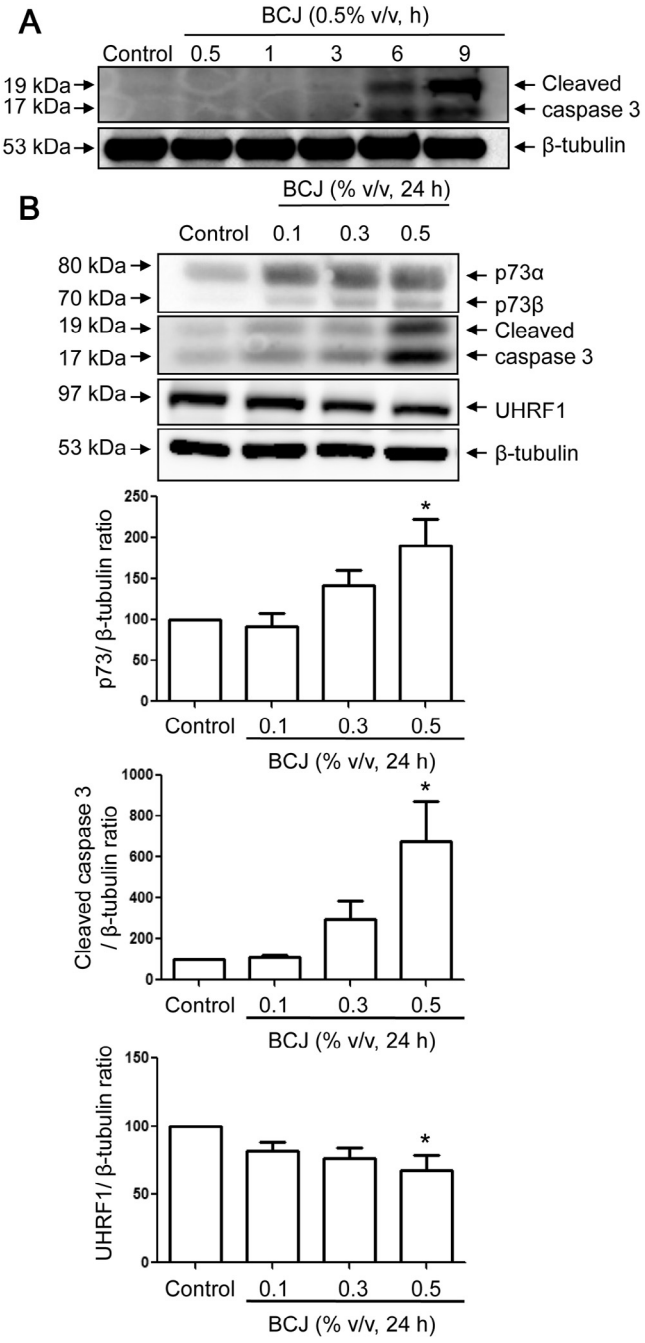

Fig. 2 – Blackcurrant juice (BCJ) stimulates the expression of pro-apoptotic markers in Jurkat cells. (A) Cells were exposed to 0.5% BCJ for different times. The expression level of cleaved caspase 3 was assessed by Western blot analysis. Similar findings were observed in three independent experiments. (B) Cells were exposed to increasing concentrations of BCJ for 24 h. The expression levels of p73, cleaved caspase 3 and UHRF1 were assessed by Western blot analysis. Values are shown as means ± S.E.M. (n = 3–5). *P < 0.05 versus respective control.

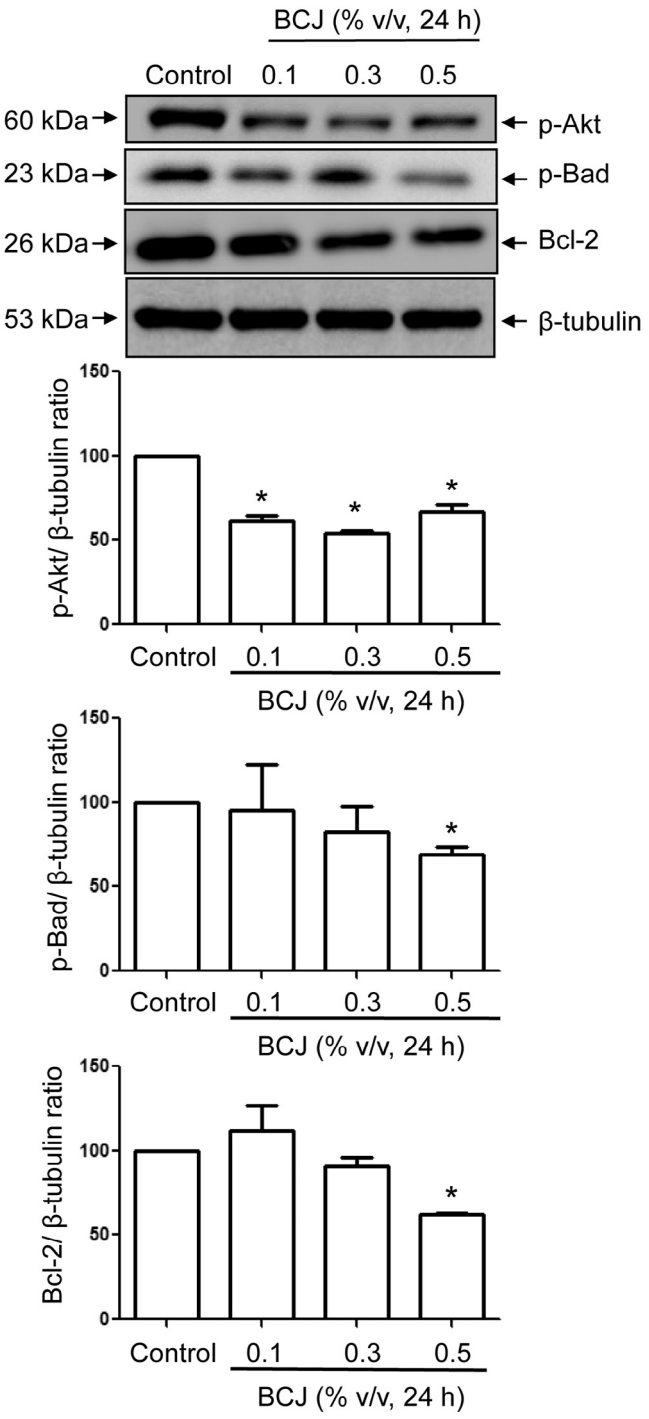

Fig. 3 – Blackcurrant juice (BCJ) reduces the expression of anti-apoptotic proteins in Jurkat cells. Cells were exposed to increasing concentrations of BCJ for 24 h. The expression levels of p-Akt, p-Bad and Bcl-2 were assessed by Western blot analysis. Values are shown as means ± S.E.M. (n = 3–6). *P < 0.05 versus respective control.

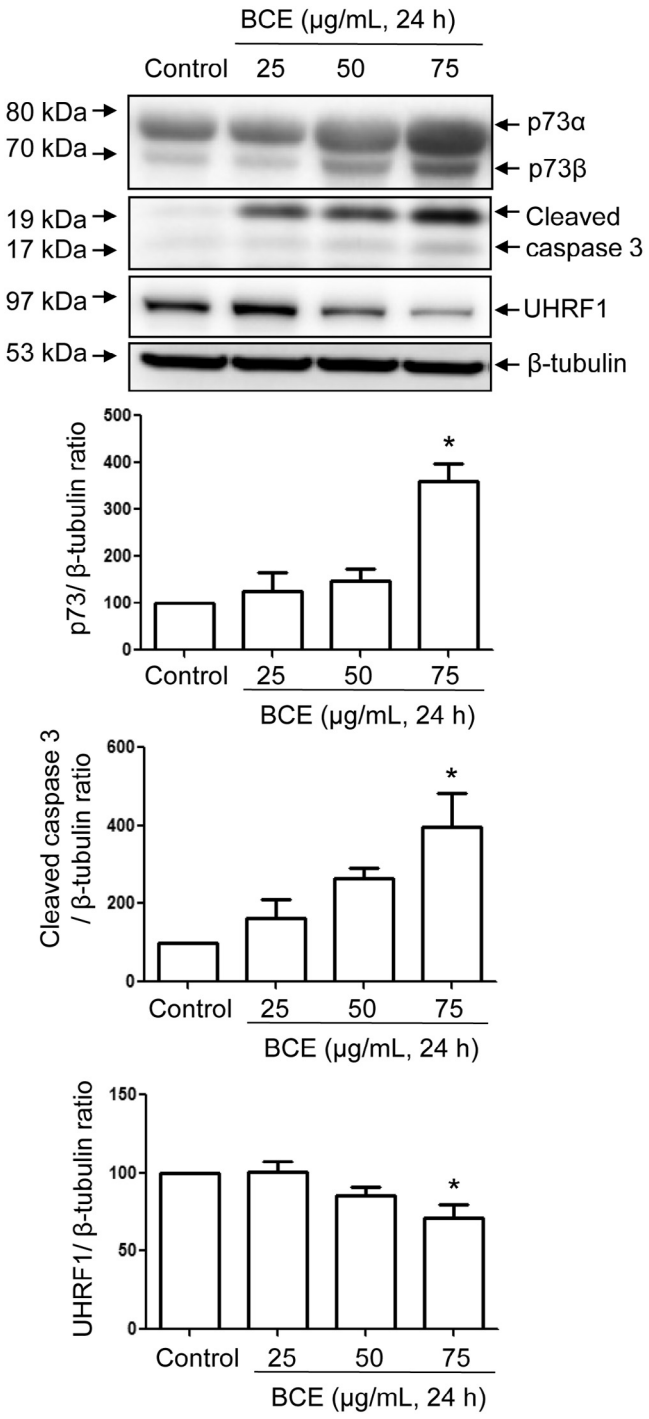

Fig. 4 – Blackcurrant extract (BCE) induces a concentration-dependent pro-apoptotic effect in Jurkat cells. Cells were exposed to increasing concentrations of BCE for 24 h. The expression levels of p73, cleaved caspase 3 and UHRF1 were assessed by Western blot analysis. Values are shown as means ± S.E.M. (n = 3–5). *P < 0.05 versus respective control.

fresh fruit (Gavrilova et al., 2011). Previous studies have indicated that a blackcurrant berry extract has chemopreventive activities against the diethyl nitrosamine-induced hepatocellular carcinogenesis in rats (Bishayee et al., 2011), and antiproliferative activity on gastric cancer cells via the induction of apoptosis (Jia, Xiong, Kong, Liu, & Xia, 2012). The precise mechanism of action of blackcurrant anthocyanins remains unclear, and its potential against other types of cancers, such as leukaemia, has been poorly investigated. The present

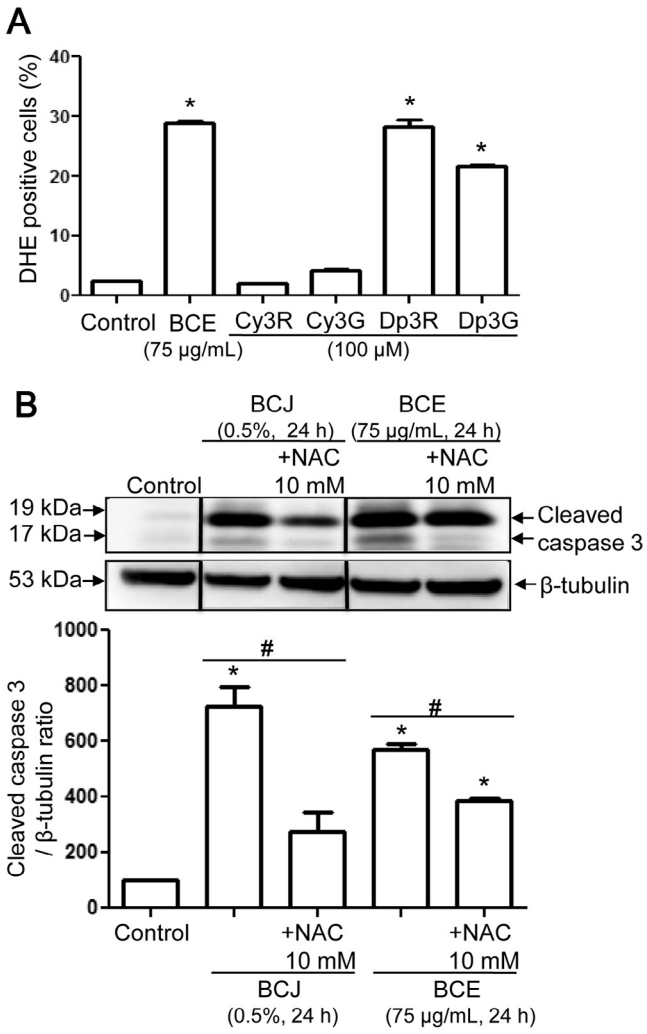

Fig. 5 – Blackcurrant derived products induce a redox-sensitive apoptosis in Jurkat cells. (A) Jurkat cells were exposed either to BCE or an anthocyanin [cyanidin-3-O-rutinoside (Cy3R), cyanidin-3-O-glucoside (Cy3G), delphinidin-3-O-rutinoside (Dp3R) or delphinidin-3-O-glucoside (Dp3G)] before addition of dihydroethidium (DHE), and determination of ROS formation by flow cytometry. Values are shown as means ± S.E.M. (n = 3); *P < 0.05 versus control. (B) Cells were exposed to the antioxidant N-acetylcysteine (10 mM) for 30 min prior to addition of either BCJ or BCE. The expression level of cleaved caspase 3 was assessed by Western blot analysis. Values are shown as means ± S.E.M. (n = 3). *P < 0.05 versus control, #P < 0.05 versus corresponding BCJ or BCE.

findings indicate that BCJ inhibited the growth of acute lymphoblastic leukaemia Jurkat cells by inducing a G_2/M phase cell cycle arrest associated with the induction of caspase 3-mediated apoptosis. In addition, BCJ also induced apoptosis in another human acute lymphoblastic leukaemia cell line, Molt-4, but not in PBMCs from healthy donors, indicating that BCJ possesses selective cytotoxicity towards leukaemia cells.

In order to characterize the mechanism involved in the pro-apoptotic signalling pathway activated by BCJ, the expression status of the tumour suppressor gene p73 was determined. p73, a structural and functional homologue of p53 acts as a guardian of DNA in p53-deficient cells such as the Jurkat cells and plays an important role by inducing cell cycle arrest (Alhosin et al., 2010; Boominathan, 2007; Jost, Marin, & Kaelin, 1997). The present findings indicate that BCJ induced a concentration-dependent up-regulation of p73 expression, suggesting that this cell cycle checkpoint regulator mediates the pro-apoptotic activity of BCJ in Jurkat cells. In addition, BCJ also caused a concomitant down-regulation of the epigenetic integrator UHRF1, which is a member of a subfamily of RING-finger type E3 ubiquitin ligases known to bind to methylated DNA and to recruit DNA methyltransferase-1 to regulate cell cycle progression and gene expression (Avvakumov et al., 2008; Bronner et al., 2007; Hopfner, Mousli, Oudet, & Bronner, 2002). Considering that UHRF1 is a downstream target of p73 (Alhosin et al., 2010) and of p53 (Arima et al., 2004), the present study suggests that BCJ triggers G_2/M cell cycle arrest and apoptosis of the p53-deficient lymphoblastic leukaemia Jurkat cell by targeting UHRF1 most likely through a p73-dependent pathway. The pro-apoptotic activity of BCJ is associated with a time- and concentration-dependent expression of active caspase 3, a major effector of the p73-related pro-apoptotic pathway (Alhosin et al., 2010). Furthermore, the present study indicates that BCJ-induced activation of caspase 3 is associated with dephosphorylation of Akt and Bad proteins. These findings are consistent with previous ones indicating that carnosic acid-induced inactivation of Akt causes dephosphorylation of Bad leading to apoptosis through the down-regulation of the anti-apoptotic protein Bcl-2 in HL-60 human myeloid leukaemia cells (Wang et al., 2012). They are also consistent with the fact that a polyphenol-rich extract of centipedegrass induced apoptosis in acute lymphoblastic leukaemia by decreasing the levels of p-Akt, p-Bad, and Bcl-2 together with the activation of caspase 3 (Bai et al., 2015). Altogether, these findings indicate that BCJ is able to kill leukaemic cells through a caspase 3-dependent pro-apoptotic pathway associated with the up-regulation of p73 expression, the activation of caspase 3, the down-regulation of UHRF1 and of Akt and Bad dephosphorylation leading to Bcl-2 down-regulation.

In order to identify the compounds involved in the pro-apoptotic effect of the BCJ, the polyphenolic content of the BCJ was extracted and the effect of the anthocyanin-rich extract (BCE) was studied in Jurkat cells. The present findings indicate that BCE caused a caspase 3-dependent apoptosis in Jurkat cells, accompanied by an up-regulation of p73 and down-regulation of UHRF1 expression. As anthocyanins have been described as mediators of apoptosis in different cancer cells, including non-small cell lung and colon cancer cells (Pal et al., 2013; Shin et al., 2009), the effect of the main anthocyanins identified in BCE was studied. Delphinidin glycosides, but not the cyanidin derivatives, induced a pro-apoptotic effect on Jurkat cells. Filipiak et al. have also observed that delphinidin-3-O-glucoside inhibited the cell viability of HT1080 (human fibrosarcoma cell line), whereas cyanidin-3-O-glucoside was inactive (Filipiak et al., 2014). Altogether, these findings indicate that the hydroxyl group at position 5′ of the B ring of delphinidin is of major importance for the induction of apoptosis on Jurkat cells (Fig. 6).

Although polyphenols are well-known to have antioxidant effects, a prooxidant effect has been linked with their

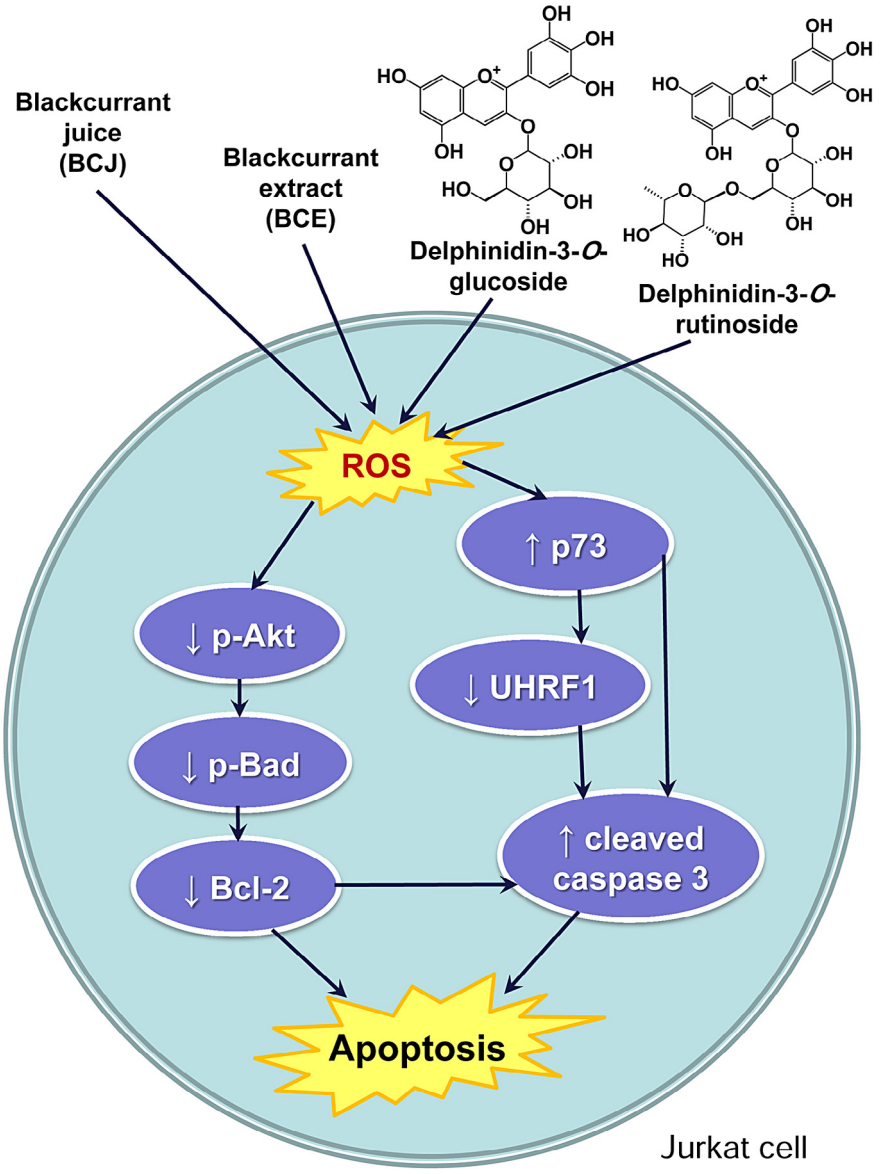

Fig. 6 – Schematic summarizing the pro-apoptotic signalling cascade induced by blackcurrant anthocyanins in Jurkat cells.

apoptotic effect in various types of tumour cells. Indeed, ROS have been shown to mediate the pro-apoptotic effect of *A. melanocarpa* and pomegranate polyphenols on leukaemia and oral squamous cell carcinoma cells (Sharif et al., 2012; Weisburg et al., 2010). The present findings indicate that incubation of Jurkat cells with either the BCE or the active anthocyanins delphinidin-3-O-glucoside and delphinidin-3-O-rutinoside induced apoptosis accompanied by an increased formation of ROS. The fact that the antioxidant NAC significantly reduced the BCJ- and the BCE-induced activation of caspase 3, further supports a role of ROS in the pro-apoptotic effect in Jurkat cells. These findings are consistent with previous ones indicating that anthocyanins act as prooxidant agents to compromise cellular protective mechanisms, such as by triggering the mitochondrial apoptotic pathway in colon cancer cells (Cvorovic et al., 2010), and that delphinidin is able to mobilize endogenous copper ions from human lymphocytes leading to oxidative degradation of cellular DNA (Hanif et al., 2008). Thus, the prooxidant response seems to be a key event in the blackcurrant polyphenols-mediated activation of the p73-related cytotoxic effect in Jurkat cells.

Altogether, blackcurrant-derived products and anthocyanins inhibit the proliferation of Jurkat cells in a redox-sensitive way by inducing apoptosis through the activation of p73 and caspase 3, and the down-regulation of UHRF1 and the p-Akt/p-Bad/Bcl-2 pathway. The pro-apoptotic activity is due, at least in part, to delphinidin-3-O-glucoside and delphinidin-3-O-rutinoside and is critically dependent on a prooxidant event.

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