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Anthocyanin-rich bilberry extract induces apoptosis in acute lymphoblastic leukemia cells *via* redox-sensitive epigenetic modifications

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ABSTRACT

Polycomb group (PcG) proteins are epigenetic regulators that reduce tumor suppressor gene expression and promote cancer cell survival. The aim of the present study was to determine whether a bilberry extract (Antho 50) inhibits the expression of PcG proteins in Jurkat cells and, if so, to determine the underlying mechanism. Apoptotic rates and the formation of reactive oxygen species (ROS) were assessed by flow cytometry, and protein expression by Western blotting. Antho 50 induced apoptosis, augmented ROS formation, increased p73, p21 and cleaved caspase-3 expression levels, and decreased those of p-Akt, survivin, PcG proteins, HDACs, DNMT1 and UHRF1. The antioxidant enzyme PEG-catalase prevented the formation of ROS and apoptosis induced by Antho 50 and also by H_2O_2. These findings indicate that Antho 50 promotes apoptosis in Jurkat cells, in part, by decreasing the expression levels of PcG proteins, and, hence, the subsequent PcG proteins-dependent pro-survival events *via* a redox-dependent mechanism.

1. Introduction

Epidemiological studies have reported that a high intake of fruits and vegetables is associated with a reduced rate of cancer mortality (Turati, Rossi, Pelucchi, Levi, & La Vecchia, 2015). A recent meta-analysis of observational studies showed that a high adherence to the Mediterranean diet, which is rich in fruits and vegetables, reduced the overall cancer mortality risk by 10% (Schwingshackl & Hoffmann, 2014). Polyphenol-rich fruits, including edible berries such as cranberry, blackcurrant, blueberry and bilberry, have been shown to have strong chemopreventive and chemotherapeutic properties in different types of cancer cells (Alhosin et al., 2015; Diaconeasa, Leopold, Rugina, Ayvaz, & Socaciu, 2015; Katsargyris, Tampaki, Giaginis, & Theocharis, 2012). Moreover, polyphenols isolated from natural products, such as (-)-epigallocatechin-3-gallate (EGCG), resveratrol, and curcumin, have been shown to exert preventive and therapeutic anticancer effects through different mechanisms, including inhibition of cell proliferation, induction of cell cycle arrest, apoptosis, and anti-metastatic and anti-angiogenic responses (Li et al., 2010; Talero, Avila-Roman, & Motilva, 2012). Although the induction of reactive oxygen species (ROS) formation has been described as a main initiator event of these effects (Alhosin et al., 2010; Feng et al., 2007; Rigas & Sun, 2008; Sanchez-Duffhues et al., 2009; Sharif et al., 2010), the effect of dietary polyphenols on polycomb group (PcG) proteins and associated epigenetic regulators remains unclear.

PcG proteins play a main role in the regulation of gene expression by modifying chromatin structure including effects on histone acetylation and methylation (Simon & Kingston, 2009). The PcG is constituted by two complexes: the polycomb repressive complex 1 (PRC1) and the polycomb repressive complex 2 (PRC2), which act in a hierarchical mode. The PRC2 includes four core proteins: EZH2 (enhancer of zeste homolog 2), SUZ12 (suppressor of zeste 12 homolog), EED (embryonic ectoderm development) and RBBP4 (retinoblastoma-binding protein 4) (Simon & Kingston, 2009). As a first step of the regulatory circuitry, the PRC2 recruits to chromatin HDAC1 and HDAC2 in order to catalyze local histone deacetylations (Eckert, Adhikary, Rorke, Chew, & Balasubramanian, 2011). In a second step, the methyltransferase EZH2, associated with SUZ12 and EED, trimethylates the lysine 27 of histone H3 (H3K27-3M) (Eckert et al., 2011; Mills, 2010; Simon & Kingston, 2009). These events, along with the DNA methylation by DNA methyltransferases (DNMTs) allow the full repression of the target genes (Sparmann & van Lohuizen, 2006).

The core of human PRC1 includes B-cell specific Moloney murine leukemia virus integration site 1 (BMI1), and ring finger protein 1B

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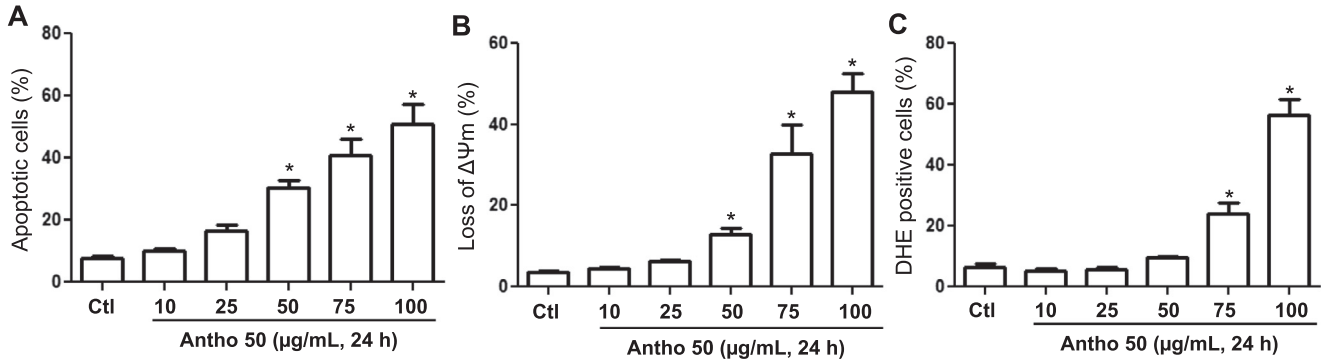

Fig. 1. Antho 50 induced apoptosis in Jurkat cells. Cells were exposed to increasing concentrations of Antho 50 for 24 h. (A) Cell apoptosis rates were assessed by flow cytometry using the annexin V-FITC/PI apoptosis assay. (B) The mitochondrial membrane potential and (C) the levels of reactive oxygen species (ROS) were assessed using flow cytometry. Values are shown as means ± S.E.M. (n = 3–4); *, $P < 0.05$ versus respective control (Ctl).

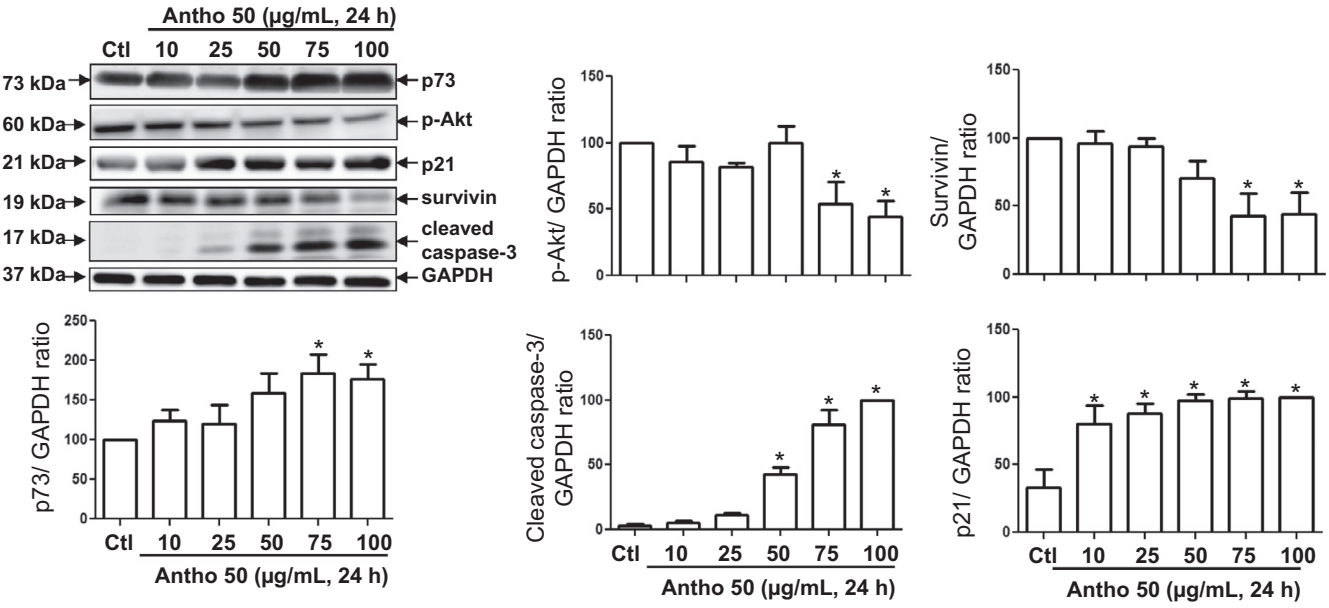

Fig. 2. Antho 50 induced upregulation of pro-apoptotic markers and downregulation of pro-survival proteins in Jurkat cells. Cells were exposed to increasing concentrations of Antho 50 for 24 h and the expression levels of p73, p21, caspase-3, p-Akt and survivin was assessed by Western blot analysis. Values are shown as means ± S.E.M. (n = 3–4); *, $P < 0.05$ versus respective control (Ctl).

(Ring 1B) (Qi et al., 2013). The trimethylation of H3K27 creates an epigenetic docking site for the chromo-domain of BMI1 (Eckert et al., 2011), which promotes the ubiquitination of histone H2A at lysine 119, leading to transcriptional repression (Li et al., 2006).

There is increasing evidence that up-regulated expression of PcG proteins contributes to cancer development (Sparmann & van Lohuizen, 2006). Indeed, PcG proteins are commonly over-expressed in cancer cells and tumors, and this event is linked to histone hyper-methylation and ubiquitination, leading to chromatin compaction and silencing of genes involved in the control of cell proliferation (Eckert et al., 2011; Sparmann & van Lohuizen, 2006). BMI1 levels are increased in primary myeloid leukemia and leukemic cell lines (Shen et al., 2012) and it has also been shown to play an important role in cell proliferation in various cancer types, including colon, lung, and breast, by repressing the expression of different tumor repressors (Mills, 2010). Similarly, increased levels of EZH2 expression have been linked with poor prognosis and aggressive tumor metastasis (Smits et al., 2010). EZH2 has been shown to stimulate cell proliferation, and down-regulation of EZH2 expression retarded cell proliferation and induced cell cycle arrest in cancer cells (Smits et al., 2010). Although EZH2 is presented as a promising therapeutic strategy for numerous cancer types, as mentioned before, inactivation of *Ezh2* gene expression has been detected in 17% of early T cell precursor acute lymphoblastic leukemia (ETP-ALL) patients, and it was associated with poor clinical outcomes (Zhang et al., 2012). The inactivation of the PRC2 is mechanistically linked with an increase of cell stemness and pro-survival pathways (Danis et al., 2016). However, lower rates (less than 5%) of *Ezh2* mutations has been detected in childhood T-ALL cohorts (Schäfer et al., 2016), and high EZH2 levels has been correlated with lower probability of disease-free survival (DFS) compared to T-ALL negative for EZH2 (D'Angelo et al., 2015).

Berry fruits represent a major source of anthocyanins, a subclass of polyphenols highly present in the human diet (180–215 mg/day in the United States of America) that have been shown to exert potent anticancer effects, by inducing a mitochondrial pro-apoptotic response *via* an intracellular ROS production in leukemic HL-60 cells (Feng et al., 2007). Anthocyanins are glycosides of anthocyanidins and sugary components such as glucose, galactose, or arabinose (Popović et al., 2016). Among anthocyanins, delphinidin-3-*O*-glucoside and delphinidin-3-*O*-rutinoside from blackcurrant juice have been shown to induce apoptosis in Jurkat human leukemia cells (Leon-Gonzalez et al., 2015), and a bilberry extract standardized to contain 50% anthocyanins (Antho 50) selectively induced apoptosis in chronic lymphocytic leukemia cells by targeting the Bcl-2/Bad pathway (Alhosin et al., 2015).