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REVIEW ARTICLE

# Effects of anthocyanins on the prevention and treatment of cancer

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Anthocyanins are a class of water-soluble flavonoids, which show a range of pharmacological effects, such as prevention of cardiovascular disease, obesity control and antitumour activity. Their potential antitumour effects are reported to be based on a wide variety of biological activities including antioxidant; anti-inflammation; anti-mutagenesis; induction of differentiation; inhibiting proliferation by modulating signal transduction pathways, inducing cell cycle arrest and stimulating apoptosis or autophagy of cancer cells; anti-invasion; anti-metastasis; reversing drug resistance of cancer cells and increasing their sensitivity to chemotherapy. In this review, the latest progress on the anticancer activities of anthocyanins and the underlying molecular mechanisms is summarized using data from basic research *in vitro* and *in vivo*, from clinical trials and taking into account theory and practice.

## LINKED ARTICLES

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## Abbreviations

ABC, ATP-binding cassette; AP-1, activator protein-1; APC, adenomatous polyposis coli; ARE, antioxidant response element; Atg5, autophagy-related gene 5; BCRP, breast cancer resistance protein; cAMP, cyclic AMP; C-3-G, cyanidin-3-glucoside; Cy-g, cyanidin-3-O-β-glucopyranoside; FDGP, freeze-dried grape powder; HCC, hepatocellular carcinoma cells; iNOS, inducible NO synthase; 3-MA, 3-methyladenine; MEK, MAPK/ERK kinase; 8-OHdG, 8-hydroxydeoxyguanosine; P-gp, P-glycoprotein; PMBE, *Pinus massoniana* bark extract; RTK, receptor TK; siRNA, small interfering RNA; TG, Thapsigargin; TPA, 12-O-tetradecanoylphorbol-13-acetate; YGM-3, 3-(6,6′-caffeylferulylsophoroside)-5-glucoside of cyanidin; YGM-6, 3-(6,6′-caffeylferulylsophoroside)-5-glucoside of peonidin

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## Tables of Links

| TARGETS | | |
|---|---|---|
| **Other protein targets**[a] | **Enzymes**[e] | MMP2 |
| Bcl-2 | Akt (PKB) | MMP9 |
| TNF-α | Caspase family | MPO |
| **Catalytic receptors**[b] | CDK1 subfamily | PI3K |
| PDGFR | COX-2 | PKA |
| VEGFR | EGFR | p38 MAPK |
| **Nuclear hormone receptors**[c] | ERK1 | Raf |
| AP-1 | ERK2 | uPA |
| **Transporters**[d] | iNOS | |
| ABCB1, P-gp | JNK1 | |
| ABCG2 | MEK1 | |

| LIGANDS | |
|---|---|
| cAMP | Nitric oxide (NO) |
| EGF | $PGE_2$ |
| IFN-γ | Thapsigargin |
| Keap1 | TPA |
| LPS | |

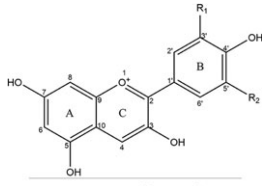
These Tables list key protein targets and ligands in this article that are hyperlinked to corresponding entries in http://www.guidetopharmacology.org, the common portal for data from the IUPHAR/BPS Guide to PHARMACOLOGY (Southan *et al.*, 2016), and are permanently archived in the Concise Guide to PHARMACOLOGY 2015/16 ([a,b,c,d,e]Alexander *et al.*, 2015a,b,c,d,e).

## Introduction

Anthocyanins exist widely in plants and are the most abundant flavonoids. Anthocyanins are responsible for the varied colours of flowers and fruit that change with the change of seasons and are cell vacuole components. For example, strawberries, grapes, apples, purple cauliflower and corn can present a red, blue or purple colour respectively (Cooper-Driver, 2001). To date, the number of reported types of anthocyanins exceeds 500, and these different anthocyanins can be detected in 27 families and 72 genera of plants (Sarma *et al.*, 1997). Research since the 1980s has mainly focused on their extraction (Pace *et al.*, 2014; Carbonaro *et al.*, 2015), isolation (Correa-Betanzo *et al.*, 2014), purification and absorption (McGhie and Walton, 2007), metabolism (Jian and Giusti, 2010), bioavailability (Fernandes *et al.*, 2014), pharmacokinetics (Kay, 2006; Semaming *et al.*, 2015) and their analytical techniques (Welch *et al.*, 2008). On the basis of studies in cell lines from the gastrointestinal tract, such as the intestines (Li *et al.*, 2011), oesophageal, stomach, colorectal, liver, breast, cervical and prostate cancers (Bowen-Forbes *et al.*, 2010; Rugina *et al.*, 2012; Hafidh *et al.*, 2013; Jin *et al.*, 2013; Li *et al.*, 2014; Bishayee *et al.*, 2016), animal models (Jiang *et al.*, 2014) and human clinical trials (https://clinicaltrials.gov), anthocyanins have antioxidant (He and Giusti, 2010), bacteriostatic, anti-inflammation, anti-ageing and anticancer functions (Bagchi *et al.*, 2004; Cui and Li, 2014; Chen *et al.*, 2016b). They can be applied for the prevention of cardiovascular disease (Alvarez-Suarez *et al.*, 2014; Cerletti *et al.*, 2016), obesity control (Wu *et al.*, 2013), the alleviation of diabetes (Li *et al.*, 2015a) and cancer therapy (Bobe *et al.*, 2006). Here, we summarize briefly the possible antitumour or anticancer roles of anthocyanins in the different stages of tumourigenesis and carcinogenesis, highlighting their sources, structural characteristics and health effects, and focusing on their pharmacological aspects in the prevention and treatment of cancer *in vitro* and *in vivo*.

## The chemical structure and characteristics of anthocyanins

Anthocyanin is the common name for a class of flavonoids that are easily dissolved in water. Their basic structural unit is 2-phenylchromenylium (flavylium) (Figure 1) (Hou *et al.*, 2004; Jing and Giusti, 2010). They exist in natural products, mainly in a form combined with glucose, galactose and rhamnose (Liu *et al.*, 2010), and can be divided into at least six common types, such as pelargonidin, cyanidin, delphinidin, peonidin, petunidin and malvidin, according to the different substituent groups on the flavylium B-ring (Holton and Cornish, 1995). The research has indicated that the *ortho*-dihydroxyphenyl structure on the B-ring is the active site that inhibits tumour growth and metastasis (Hou *et al.*, 2004; Xu *et al.*, 2010). In recent years, because of increased health consciousness (Pascual-Teresa, 2014), people have paid close attention to anthocyanins' roles in




| | $R_1$ | $R_2$ |
|---|---|---|
| Cyanidin | OH | H |
| Delphinidin | OH | OH |
| Malvidin | $OCH_3$ | $OCH_3$ |
| Pelargonidin | H | H |
| Peonidin | $OCH_3$ | H |
| Petunidin | $OCH_3$ | OH |

**Figure 1**
Chemical structure of the anthocyanidins most commonly found in foods.

tumour prevention and cancer therapy because of their extensive sources, low cytotoxicity and safe consumption. This review summarizes the related experimental and clinical data at the cellular and molecular levels *in vitro* and in clinic trails *in vivo*, analyses the existing critical questions and provides directions for further research.

## The anti-carcinogenic activities of anthocyanins in the initial stage of tumourigenesis

### *Antioxidant*

Anthocyanins can act on the antioxidant system (Bowen-Forbes *et al.,* 2010; Li *et al.,* 2015a), where they scavenge free radicals, thereby reducing damage to the genome of normal cells by oxidative stress and the subsequent malignant transformation by gene mutation, thus preventing the occurrence of tumours (Shih *et al.,* 2007; Yi *et al.,* 2010). Yi *et al.* (2010) found that the antioxidant effect of anthocyanins is determined by the 3′, 4′, 5′ hydroxyl on the B-ring and the 3′ hydroxyl on the C-ring. Shih *et al.* (2007) and Thoppil *et al.* (2012) found that anthocyanins (cyanidin, delphinidin and malvidin) could act on antioxidant response element (ARE) through the Keap1-Nrf2 pathway and inhibit the activity of cysteinyl aspartate specific proteinase-3 (caspase-3) by regulating the expression of phase II antioxidases (glutathione reductase, glutathione peroxidase, glutathione transferase and quinone oxidoreductase), thus playing a role in antioxidant protection. In short, it is the promotion of the expressions of ARE-regulated phase II enzymes by anthocyanins that defend normal cells against oxidative stress.

### *Anti-inflammation*

Chronic inflammation is often a harbinger of a tumour. The abnormal overexpression and secretion of inflammatory factors are critical to tumourigenesis. It is reported that anthocyanins can control the expression and secretion of inflammatory factors by inhibiting the transcription factor NF-κB, through multiple pathways to exert their anti-inflammatory function (Esposito *et al.,* 2014; Peiffer *et al.,* 2014). For example, cyanidin-3-glucoside (C-3-G), delphinidin-3-glucoside and petunidin-3-glucoside inhibit the activation of NF-κB induced by external stimuli (e.g. LPS or IFN-γ) by acting on the PI3K/PKB and MAPK pathways (Afaq *et al.,* 2005; Limtrakul *et al.,* 2015) and can inhibit the expression of COX-2 and inducible NO synthase (iNOS), as well as the production of their products $PGE_2$ and NO (Haseeb *et al.,* 2013; Jeong *et al.,* 2013; Peiffer *et al.,* 2014). Miyake *et al.* (2012) and Burton *et al.* (2015) found that anthocyanins could also block the activation of STAT3 and inhibit the expression of NF-κB.

### *Anti-mutagenesis*

During the transformation of normal cells towards cancer cells, somatic cell hypermutation can lead to instability of the genome and cause cancer (Martincorena and Campbell, 2015). Yoshimoto *et al.* (1999) used four different kinds of sweet potato root as experimental materials to investigate their anti-mutation effect and found that *Salmonella typhimurium* TA98 presented reverse mutation under the action of a heterocyclic mutagen, while adding four different kinds of sweet potato root, whose main ingredients are 3-(6,6′-caffeylferulylsophoroside)-5-glucoside of cyanidin (YGM-3) and 3-(6,6′-caffeylferulylsophoroside)-5-glucoside of peonidin (YGM-6), could inhibit the reverse mutation of TA98 in a dose-dependent manner. Thus, it was concluded that YGM-3 and YGM-6 could inhibit the reverse mutation of normal cells induced by a mutagen. Oxidative stress from free radical abnormalities can lead to DNA injury and mutation of related genes – oncogenes and anti-oncogenes – resulting in carcinogenesis and finally causing cancer. Therefore, anthocyanins with antioxidant properties may protect human cells from malignant mutation from extreme levels of ROS and free radicals by inhibiting point mutations, thereby exerting their anti-mutagenesis effects in human somatic cells.

## The anti-carcinogenic activities of anthocyanins in the cancer formation stage

### *Differentiation induction*

Differentiation induction is a phenomenon whereby malignant cells differentiate towards normal and mature cells under the effect of differentiation inducers. A large number of malignant cells undergo mitosis, and these cells are poorly differentiated (Charepalli *et al.,* 2015; Thwe *et al.,* 2016). Anthocyanins can induce terminal differentiation of tumour cells and block tumourigenesis. Through detecting the markers and kinase inhibitors in the cell differentiation process, Fimognari *et al.* (2004) found that cyanidin-3-O-β-glucopyranoside (Cy-g) could induce the differentiation of human acute promyelocytic leukaemia cell line HL-60 in a dose-dependent way by activating PI3K and PKC. Under treatment by Cy-g (200 $mg \cdot mL^{-1}$), HL-60 cells presented differentiation characteristics, such as increased adhesion and enhanced activity of esterase, and the expression of oncogene c-Myc was decreased. However, following treatment by PI3K and PKC inhibitors, the effect of Cy-g to induce the differentiation of HL-60 was significantly reduced. Serafino *et al.* (2004) found that Cy-g could induce the differentiation of melanoma cell line TVM-A12 by up-regulating cAMP levels, and the expressions of tyrosinase and the differentiation marker MART-1. Liu-Smith and Meyskens recently validated Cy-g's effects on the induction of melanoma cell differentiation (Liu-Smith and Meyskens, 2016). To some extent, the degree of differentiation determines the degree of tumour malignancy, and anthocyanins might play roles in the cancer formation stage by inducing differentiation, further determining the size of final tumour and its malignancy.

### *Inhibiting cellular transformation*

Cellular transformation is one of the mechanisms underlying tumourigenesis. Some carcinogens, such as 12-O-tetradecanoylphorbol-13-acetate (TPA) and EGF, can induce the transformation of various cell lines through the transcription factors AP-1 and NF-κB in the Raf-MEK-ERK and PI3K/Akt pathways (Burton *et al.,* 2015). Limtrakul *et al.* (2015)

found that black rice whole grain extracts might suppress LPS-induced inflammation via inhibition of the MAPK signalling pathway, leading to decreased NF-κB and AP-1 translocation. In addition, inflammation also has an important relationship with cellular transformation, and high expression of COX-2 and $PGE_2$ can enhance the tumorigenic effect (Hou *et al.,* 2004). Anthocyanins can act on Ras-ERK and PI3K/Akt pathways by decreasing the expression of AP-1 and thus inhibit the cellular transformation. Hou *et al.* (2004, 2005) found that delphinidin, cyanidin and petunidin could inhibit the transformation of mouse skin cell line $JB6P^+$ induced by TPA. Kang *et al.* (2008) found that delphinidin could bind with Raf1 or MEK1 in an ATP-non-competitive way to inhibit the expression of AP-1 and NF-κB in $JB6P^+$ cells treated with TPA and further inhibit the expression of COX-2 and the production of $PGE_2$. In addition, delphinidin could also weaken the TPA-induced cellular transformation through the Ras/Raf/MEK/ERK pathway by regulating the phosphorylation level of MEK, ERK, ribosomal protein S6 kinase and mitogen stress activator protein kinase. Early experiments demonstrated that this effect is associated with the antioxidant ability of anthocyanins to scavenge superoxide radicals (Hou *et al.,* 2004). Recently, Song *et al.* (2012) found that the 1,1-diphenyl-2-picrylhydrazyl radical 2,2-diphenyl-1-(2,4,6-trinitrophenyl) hydrazyl and 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt of cyanidin and C-3-G showed no significant difference in their free radical scavenging abilities; however, their abilities to inhibit cellular transformation are significantly different: cyanidin has a strong ability to inhibit transformation, while C-3-G has hardly any ability to inhibit transformation, which suggested that the effect of cyanidin to inhibit transformation is not associated with its antioxidant ability. Cyanidin can directly combine with PI3K in an ATP-competitive way to inhibit the expression of AP-1 and NF-κB through the PI3K/Akt/p70S6 pathway and inhibits the cellular transformation of $JB6P^+$ cells by treatment with EGF.

### *Inhibiting cell proliferation*

A significant characteristic of cancer cells is their uncontrolled cell cycle, which leads to continuous division and proliferation (Lee *et al.,* 2010; Li *et al.,* 2016a). Anthocyanins can selectively inhibit the proliferation of cancer cells but have little influence on the proliferation of normal cells (Malik *et al.,* 2003). The main manifestations of anthocyanins' inhibition of the growth and proliferation of cancer cells are summarized below from three aspects.

### *Inhibition of signalling pathways to block signal transduction*

Syed *et al.* (2008) found that delphinidin could inhibit the hepatocyte growth factor-induced phosphorylation and activation of hepatocyte growth factor receptors on human normal mammary cell line MCF-10 A and could block the Ras–ERK MAPK and PI3K/Akt pathways. Teller *et al.* (2009b) found that anthocyanins could also inhibit the autophosphorylation of receptor TKs (RTKs) extensively in cancer cells, and the inhibitory effect on oncogene ErbB3 was the most effective. Anthocyanins, especially malvidin, could inhibit the activity of PDE and the hydrolysis of cAMP effectively in human colon cancer HT29 cells and thus inhibit the MAPK signalling pathway (Marko *et al.,* 2004). In short, anthocyanins can inhibit the growth and proliferation of cancer cells by inhibiting different kinase signalling pathways *in vitro*.

### *Regulating the expression of anti-oncogenes and relevant proteins*

Malik *et al.* (2003) and Ha *et al.* (2015) found that in addition to up-regulating p53 in colon and prostate cancer cells to activate the DNA repair system, anthocyanins could also initiate the transcription of p21 and p27. p21, a broad-spectrum inhibitor of cyclin-dependent kinases (CDKs), can combine with CDKs and inhibit their activity to induce the cell cycle arrest of cancer cells. Anwar *et al.* (2016) found that a standardized berry anthocyanin rich extract inhibited the proliferation of Caco-2 cells by up-regulating the expression of p21Waf/Cif1, arresting their cell cycle, and further inducing them to undergo apoptosis by caspase-3 activation. Meanwhile, anthocyanins can also down-regulate the expressions of CDK-1 and CDK-2, inhibit the expressions of cyclin-B, cyclin-A and cyclin-E, promote the expressions of CDK inhibitors (CDKIs) and induce cancer cells to arrest at the G0/G1 and G2/M stages (Chen *et al.,* 2005). Thus, anthocyanins can inhibit cancer cell proliferation mainly by arresting the cell cycle at different division phases via up-regulating the expressions of anti-oncogenes and down-regulating the expressions of oncogenes, accompanied by the expressions of different cyclins and their partners CDKs and/or CDKIs.

### *Other signalling pathways*

Kausar *et al.* (2012) found that berry anthocyanidins could act on the β-catenin, Wnt and Notch pathways, as well as their downstream target proteins, to inhibit the growth and proliferation of human non-small-cell lung cancer cells synergistically.

Overall, tumours and cancer are referred to as signal pathway diseases, and this concept helps researchers to broaden their research and develop safer, targeted chemopreventive and/or chemotherapeutic treatments, including anthocyanins and other natural products.

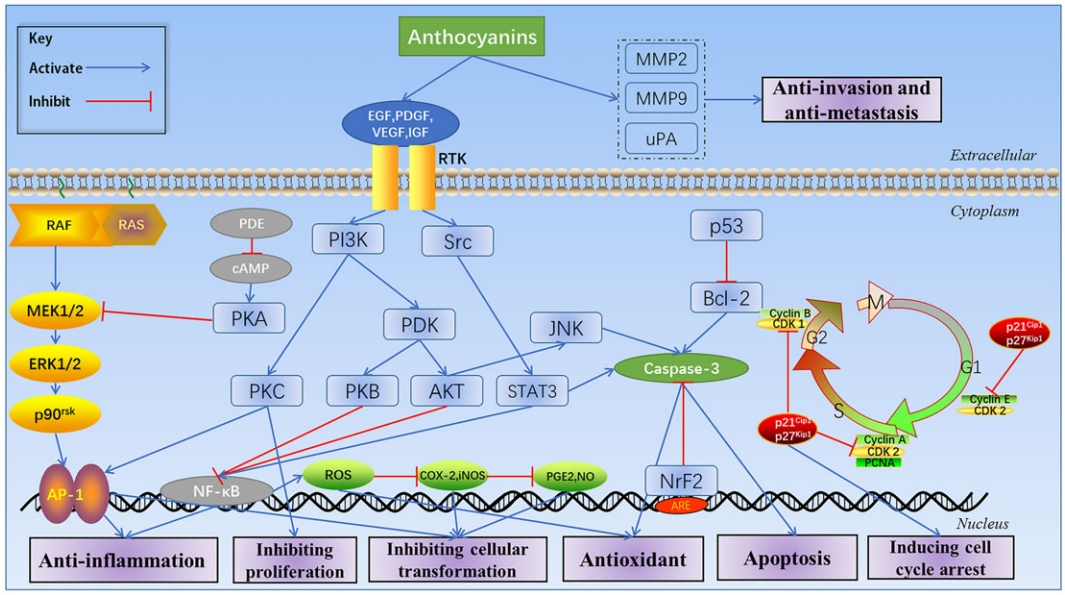
## The anti-carcinogenic activities of anthocyanins in the cancer development stage

### *Inducing apoptosis of tumour cells*

Malignantly transformed cells display uncontrollable growth, and their excessive proliferation leads to the formation of a tumour (Četojević-Simin *et al.,* 2015). Apoptosis of tumour cells is inhibited; therefore, dying cells cannot be eliminated normally (Naomi *et al.,* 2003). Anthocyanins can induce the apoptosis of cancer cells through the internal mitochondrial pathway and the external death receptor pathway (Figure 2).

### *The death receptor pathway*

Huang *et al.* (2012) determined the phosphorylation levels of p38, p53, protein Fas and FasL after treatment with the p38 inhibitor SB203580 and demonstrated that mulberry




## Figure 2

The main potential molecular mechanism of the antitumour effect of anthocyanins *in vitro*. Cancer cell growth might be inhibited by anthocyanidins through targeting RTKs (e.g. EGFR, PDGFR and VEGF/VEGFR) and acting on the Ras-MAPK and PI3K/Akt signal cascade pathway. Inflammation might also be inhibited by anthocyanins through acting on the PI3K/Akt and NF-κB pathway to suppress the expression of COX-2 and iNOS and prevent cancer by regulating the expression of phase II antioxidant enzymes to achieve antioxidation through the Nrf2/ARE signalling system. During cancer initiation, anthocyanins might prevent malignant transformation by targeting the MAPK pathway and AP-1 factor and by inhibiting RTK activity. Anthocyanins can initiate the expression of p21 and p27, whose products can combine with multiple cyclin-CDKs to down-regulate the expression of CDK-1 and CDK-2, further inhibiting the expression of cyclin-B, cyclin−A and cyclin–E, which promote the expression of CDK inhibitors and induce cancer cells to arrest at the G0/G1 and G2/M stages. During cancer development, anthocyanins can induce apoptosis of cancer cells by activating caspases, mediated by ROS and JNK/p38-MAPK. In addition, anthocyanins might exert their anti-metastatic activities by targeting the VEGF signalling pathway and extracellular matrix degradation (via MMP2, MMP9, uPA).

anthocyanin could induce the apoptosis of gastric cancer cells through the external receptor p38/Fas/FasL/Caspase-8 pathway. Chang *et al.* (2005) found that delphinidin could activate p38-FasL and the pro-apoptosis protein Bid pathway, thereby inducing the apoptosis of HL-60 cells in a time-dependent and dose-dependent manner.

### *The mitochondrial signalling pathway*

Mitochondria-mediated apoptosis include the caspase-dependent and caspase-independent pathways.

### *Caspase-dependent pathway*

Anthocyanins can act on proteins of the Bcl family and the inhibitor of apoptosis protein family to activate the apoptosis response through the caspase-dependent cascade (Shih *et al.*, 2007; Lee *et al.*, 2009; Charepalli *et al.*, 2016).

### *Caspase-independent pathway*

Anthocyanins extracted from potato could induce mitochondria to release endonuclease G and apoptosis-inducing factor through the JNK pathway to trigger caspase-independent apoptosis of prostate cancer LNCaP and PC-3 cell lines (Reddivari *et al.*, 2007; Liu *et al.*, 2016).

### *The endoplasmic reticulum signalling pathway*

Yu *et al.* (2011) investigated the mechanism underlying the anti-apoptotic effects of freeze-dried grape powder (FDGP) on human hepatocarcinoma Huh7 cells. Pretreatment with FDGP could inhibit the thapsigargin (TG)-induced expression of binding immunoglobulin protein/glucose-regulated protein 78 and cytochrome c and increased the TG-reduced expression of PCNA and caspase-12. Caspase-7 can be transported to the endoplasmic reticulum (ER) to react with caspase-12 to induce the apoptosis of cancer cells. By contrast, the protein Bax is also abundant in the ER, and research has confirmed that anthocyanins can cause down-regulation or up-regulation of pro-apoptotic proteins, such as Bax and Bcl-2 homologous antagonist/killer (Bak) (Pal *et al.*, 2013; Liu *et al.*, 2016). Therefore, anthocyanins might achieve their functions via the ER signalling pathway.

### *The lysosome signalling pathway – cooperation with an autophagy inhibitor*

Autophagy is a process whereby intracellular substances are degraded by the lysosome, which has a dual function in carcinogenesis: on the one hand, autophagy deficiency can promote malignant transformation and carcinogenesis; on the other hand, autophagy can restrict the necrosis and inflammation of a tumour, thereby relieving chromosome injury and the metabolic stress response in tumour cells. Anthocyanins can induce autophagy of cells in an autophagy-related protein 5 (encoded by Atg5)-dependent manner (Tsuyuki *et al.*, 2012; Schiavano *et al.*, 2015). Longo *et al.* (2008) found that after human hepatocellular

carcinoma (HCC) cell line PLC/PRF/5 was treated with anthocyanins, the expressions of downstream Bcl-2 family and rapamycin target proteins were down-regulated, while the expressions of eukaryotic initiation factor 2α and autophagy related gene LC3-II were up-regulated, which all suggested that anthocyanins can induce autophagy of human HCC cells. Using small interfering RNA (siRNA) to interfere to silence the Atg5 gene or using autophagy inhibitor 3-methyladenine (3-MA) to act on HCC cells treated with anthocyanins causes transfer of apoptin Bax into mitochondria, the release of cytochrome c and cleavage of caspase-3, leading to the apoptosis cascade response. Feng *et al.* (2010) found that cyanidin-3-rutinoside and delphinidin could not induce the apoptosis of HCC cells and could only cause growth retardation of cells via vacuolisation, which could be inhibited by the type III PI3K inhibitor 3-MA and proton pump inhibitors that blocked lysosome degradation. After using 3-MA and siRNA to interfere with autophagy marker LC3 and using delphinidin to treat human HCC SMMC7721 cells, delphinidin could induce autophagy of SMMC7721 cells. In addition, 3-MA and delphinidin acting together on SMMC7721 cells could lead to significant cell death.

### *Inhibiting angiogenesis of tumours*

Tumour angiogenesis is a restrictive condition for the growth and metastasis of malignant tumours. The process of angiogenesis is controlled by many cytokines, of which the most important positive regulatory factor is VEGF. Therefore, inhibiting the receptor of angiogenesis VEGF receptor (VEGFR) could inhibit the metastasis of tumours effectively (Chen *et al.,* 2015). Anthocyanins can inhibit RTK extensively, and the inhibitory effect on VEGFR-3 is especially significant (Teller *et al.,* 2009a). Delphinidin and cyanidin could strongly inhibit the expression of VEGF in vascular smooth muscle cells induced by PDGF by blocking the p38-MAPK and JNK pathways (Oak *et al.,* 2006). The inhibitory effect of delphinidin on angiogenesis is associated with not only VEGF inhibition but also inhibition of the PDGFR-β (Lamy *et al.,* 2008). Delphinidin could inhibit PDGF induced phosphorylation of PDGFR-β in pulmonary artery smooth muscle cells in a time-dependent and dose-dependent manner, thus inhibiting VEGF-induced microvessel formation in HUVECs. Hypoxia is a general pathophysiological characteristic of solid tumours. It is likely to induce angiogenesis of the tumour, and this process is mainly completed via the VEGF signalling pathway, mediated by hypoxia inducible factor-1α (HIF-1α). Inhibiting the protein level of HIF-1α could lead to decreased transcription activity of HIF-1α target genes, including VEGF (Huang *et al.,* 2011). Freeze-dried blackberry anthocyanins could reduce the expression of HIF-1α and VEGF in oesophagus tumour cells of F344 rats induced by N-nitrosomemethylbenzylamine and thus inhibit angiogenesis of oesophageal tumours (Wang *et al.,* 2009).

## Inhibiting the invasion and metastasis of tumours

Invasion and metastasis are the two main aspects of cancer cells that threaten patients' health and life. Cyanidin could inhibit effectively the invasion and metastasis of breast cancer cells (BT474, MDA-MB231 and MCF-7) with high expression of ErbB2 by blocking the ErbB2/cSrc/FAK pathway (Xu *et al.,* 2010; Li *et al.,* 2016a). Syed *et al.* (2008) found that delphinidin could reduce the membrane translocation of PKCα and the phosphorylation of STAT3 in MCF-10 A cell lines mediated by hepatocyte growth factor, inhibit the nuclear translocation of NF-κB/p65 and thus inhibit the invasion of cells. Invasion and metastasis involve three main processes: adhesion, degradation and movement. Anthocyanins can act on some adhesion molecules and proteolytic enzymes to inhibit the adhesion and degradation of cells (Zhu *et al.,* 2008; Mauray *et al.,* 2012).

### *Adhesion*

Chen *et al.* (2011) found that delphinidin could reduce the expression of cell adhesion molecule-1 and P-selectin induced by oxidized LDL through the ROS/p38-MAPK/NF-κB pathway in a dose-dependent manner, thus inhibiting the adhesion of monocytes to endothelial cells.

### *Degradation*

Uridylyl phosphate adenosine (uPA) and MMPs are two key components for extracellular matrix degradation, which can promote the invasion of tumour cells and increase their migration ability. Anthocyanins can target uPA and MMPs and thus play a role in inhibiting tumour metastasis. Delphinidin could influence the expression of the uPA receptor and LDL receptor related protein, as well as the production of plasmin, by interfering with the clearance of uPA-plasmin activator inhibitor complex in malignant glioma U-87 cells, thus inhibiting the invasion of U-87 cells (Lamy *et al.,* 2007). In MDA-MB-453 cells, the capacity for migration, adhesion, motility and invasion was inhibited by black rice anthocyanins in a concentration-dependent manner, accompanied by decreased activity of a transfer promoting factor, urokinase-type plasminogen activator (Luo *et al.,* 2014). Ho *et al.* (2010) found that peonidin could reduce the expression of uPA in lung cancer cells by inhibiting the phosphorylation of ERK1/2 and thus inhibited the invasion of cancer cells. Delphinidin could target reduced nicotinamide adenine dinucleotide phosphate oxidase to inhibit the phosphorylation of MAPK-JNK1/2, MKK3/6-p38 and MEK-ERK1/2 and thus inhibit the expression of MMP-1 in human epidermal fibroblasts induced by UV-B radiation (Lim *et al.,* 2013b). In addition, cyanidin and pelargonidin could inhibit the expression of MMP-2 and MMP-9 by inhibiting the PI3K/Akt pathway in cancer cells and thus reducing the invasion of cancer cells (Shin *et al.,* 2011; Kuntz *et al.,* 2015). A study also found that *Vitis coignetiae* anthocyanins could inhibit the expression of MMP-2 and MMP-9 through inhibiting the activation of NF-κB in cancer cells (Yun *et al.,* 2010). In addition, it is reported that anthocyanins (including delphinidin-3-glucoside, cyaniding-3-glucoside and malvidin-3-glucoside) could inhibit the expression of the tight junction proteins, claudin-1 and claudin-3, by activating the p38-MAPK pathway in human colon cancer HCT-116 cells, thus inhibiting their invasion (Shin *et al.,* 2011).

## Reversal of multidrug resistance

Chemotherapy can inhibit proliferation or induce apoptosis of tumour cells by interfering with the DNA replication of tumour cells; however, multidrug resistance of tumour cells is a common reason for chemotherapy failure. A classical pathway for multidrug resistance of tumour cells is mediated by an ATP-binding cassette (ABC) transmembrane protein superfamily, which mainly includes P-glycoprotein (P-gp), multidrug resistance associated protein and breast cancer resistance protein (BCRP). The ABC superfamily is highly expressed in the pharmacological barrier. Therefore, targeting these proteins to reverse multidrug resistance could be used to assist chemotherapy to treat cancer. The anthocyanins have MWs low enough to penetrate the blood–brain barrier (Andres-Lacueva *et al.,* 2005). A further study found that cyanidin could inhibit the expression of P-gp in human epidermal carcinoma KB-C2 cells that overexpressed P-gp (Kitagawa, 2006). Anthocyanins have high affinity for BCRP, and among the anthocyanins determined, seven kinds (malvidin, petunidin, malvidin-3-galactoside, malvidin, cyaniding-3-galactoside, peonidin-3-glucoside and C-3-G) were potential substrates of BCRP, 12 types (cyanidin, peonidin, cyaniding-3,5-diglucoside, malvidin, pelargonidin, delphinidin, petunidin, delphinidin-3-glucoside, cyanidin-3-rutinoside, malvidin-3-glucoside, pelargonidin-3,5-diglucoside and malvidin-3-galactoside) were BCRP inhibitors and three kinds (malvidin, malvidin-3-galactoside and petunidin) demonstrated dual biological activity (Dreiseitel *et al.,* 2009). *In vitro*, black rice anthocyanin could inhibit the activity of topoisomerase and the formation of topoisomerase-DNA complex in colon cancer HT29 cells (Esselen *et al.,* 2011). All these results suggested that anthocyanins might function to change pharmacokinetics and reverse multidrug resistance.

## Experiments performed in different models

### *Animal models*

In a BALB/c nude mice model that received a transplant of human breast cancer cell line MDA-MB-453, growth of cancer and the formation of its blood vessels were clearly inhibited by oral delivery of an anthocyanin-rich extract from black rice (100 mg·kg$^{-1}$ in the diet) (Chang *et al.,* 2010). The study observed lower expressions of COX-2, iNOS and c-Jun, and angiogenic factors such as MMP-9, MMP-2 and uPA in tumour tissue (Chang *et al.,* 2010). In a mouse model of adenomatous polyposis coli (APC) intestinal cancer, the number of mice that developed caecum cancer was reduced significantly ($P < 0.05$) (Lim *et al.,* 2013a), compared with the control group, when they were fed with purple sweet potatoes rich in anthocyanins. Another mouse model of APC showed that mucosal expression of COX-2 and cPLA2 was significantly decreased in the 0.5% anthocyanin-rich extract group, by 32 and 62%, respectively, compared with the control group (Park *et al.,* 2015). In a rat model of F344 colon cancer, the number of all tumours, which included glandular tumours and cancers, was reduced by 42, 45 and 71% compared with the control group, after a diet with 2.5, 5 and 10% freeze-dried powders from black raspberries, and the 8-hydroxydeoxyguanosine (8-OHdG) level in urine was reduced by 73, 81 and 83% respectively (Kocic *et al.,* 2011). Treatment with *Hibiscus* anthocyanins (HAs) caused reduction in the levels of aspartate transaminase, alanine transaminase, uric acid and myeloperoxidase. In addition, the results showed that oral administration of HAs (0.2%) × inhibited the progression of N-nitrosomethylurea-induced leukaemia by approximately 33.3% in rats (Tsai *et al.,* 2014). Bishayee *et al.* (2011) tested the effects of black currant extract rich in anthocyanins on the prevention of liver cancer. Mice were fed with preventive anthocyanin for 4 weeks and fed for another 22 weeks after injecting diethylnitrosamine into the abdominal cavity to induce liver cancer. The result indicated that black currant anthocyanins could reduce the risk of abnormal proliferation before cancers formation, reduce the number of hyperplastic nodules and decrease in the sizes any nodules that did form. The effect was dose-dependent, which was confirmed by later pathological examinations.

### *Human research*

Epidemiological studies have demonstrated that consumption of fruit and vegetables rich in polyphenols is associated with reduced risk of colorectal cancer, while a recent study conducted by Nimptsch *et al.* (2016) did not support the hypothesis that a higher habitual intake of any flavonoid subclass decreases the risk of colorectal cancer. Although epidemiological studies do not show a lower risk of most human cancers after taking anthocyanins, anthocyanin intake might reduce oxidative damage (Wang and Stoner, 2008). DNA oxidative damage decreased in human subjects who had drunk a juice rich in anthocyanins and showed significant increases in reduced glutathione, compared with the control group (Weisel *et al.,* 2006). Recently, Cerletti *et al.* (2016) summarized the results of two EU-funded projects, which indicated that in breast cancer patients, moderate wine consumption may have a protective effect on skin toxicity induced by radiotherapy, attributing this effect to the possible antioxidant effect of the polyphenols contained in wine. In another study, the treatment group drank freeze-dried powders from black raspberries every day, and after 6 months, 8-iso-PGF$_2$ and 8-OHdG levels were both decreased in their urine (Kresty *et al.,* 2006). Urinary 8-OHdG is considered a marker of total DNA damage in humans, while 8-Iso-PGF2, a prostaglandin-like compound produced via COX-independent enzymes, is used as a marker of lipid peroxidation. Both 8-OHdG and 8-Iso-PGF2 are indicators of oxidative status *in vivo* (Kresty *et al.,* 2006; Cocate *et al.,* 2014). A randomized, placebo-controlled, double blind, monocentric evaluation, including 300 patients, is reported in Table S1 of Cerletti *et al.*'s report in 2016 (ClinicalTrials.gov ID: NCT02195960). This trial is ongoing and will be completed in the coming months (Cerletti *et al.,* 2016).

## Pharmacokinetics

Yang *et al.* (2011) summarized the bioavailability, pharmacokinetics and metabolism of anthocyanins in their published review. De Ferrars *et al.* (2014) identified 17 $^{13}$C-labelled compounds in serum, which are metabolized to a structurally diverse range of metabolites that exhibit dynamic kinetic profiles. Research on the metabolites suggested that

## Table 1

Summary of the experimental data on the health effects of anthocyanins from cancer cell lines *in vitro* and from animal models *in vivo*

| Anthocyanins | Source | Properties | Effects | Laboratory model | Reference |
|---|---|---|---|---|---|
| Cyanidin | Seeds of corn | Antioxidant, anti-carcinogenesis | 2-amino-1-methyl-6-phenylimidazo [4,5-b]pyridine (PhIP) clearly exerts promoting effects on 1,2-dimethylhydrazine-induced colorectal carcinogenesis; these can be reduced by 5.0% potential of purple corn colour in the diet, under the present experimental conditions | F344 rats treated with PhIP | (Hagiwara *et al.*, 2001) |
| | Sweetpotato | Antioxidative, anti-mutagenic, and anti-proliferative | The multiplication medium extract, which exhibited the highest radical scavenging activities and anti-proliferation activities, contained the highest level of anthocyanins | Human leukaemia HL-60 cells | (Konczak-Islam *et al.*, 2003) |
| | Blackberry | Synergistically or additively in producing anticancer effects | Anthocyanins and non-anthocyanin phenolics in anthocyanin-containing blackberry extracts act synergistically or additively in producing anticancer effects | Human colon cancer HT-29, breast cancer MCF-7 and leukaemia HL-60 cells | (Dai *et al.*, 2009) |
| | Black currant | Up-regulation of Bax and down-regulation of Bcl-2 expression | Mechanistic studies revealed that the anthocyanin-rich black currant skin extract-mediated proapototic signal during experimental hepatocarcinogenesis might be propagated via the up-regulation of Bax and down-regulation of Bcl-2 expression at the translational level | Rats treated with diethylnitrosamine and human liver cancer HepG2 cells | (Bishayee *et al.*, 2011) |
| | Chokeberry | Antioxidant, anti-proliferative | Cyanidin glycosides inhibited HeLa human cervical tumour cell proliferation and increased the generation of reactive oxygen species after 48 h of treatment, suggesting that they could be responsible for the anti-proliferative activity | Human colon cancer HT-29 cells | (Rugina *et al.*, 2012) |
| | Tart cherry | Anti-inflammatory, anti-carcinogenesis | Mice that were fed anthocyanin-rich extract (at any dose) in combination | APC (Min) mice | (Bobe *et al.*, 2006) |

*(Continues)*

**Table 1** (Continued)

| Anthocyanins | Source | Properties | Effects | Laboratory model | Reference |
|---|---|---|---|---|---|
| | | | with sulindac had fewer tumours and a smaller total tumour burden (total tumour area per mouse) in the small intestine compared with mice fed with sulindac alone | | |
| | Wild-grown berries | Induction of autophagy | Anthocyanin-induced autophagy switched to apoptosis, as shown by the activation of Bax, cytochrome c and caspase 3, terminal deoxynucleotide transferase-mediated dUTP nick-end labelling-positive fragmented nuclei and cells with sub-G(1) DNA content, which were prevented by z-VAD | Human liver cancer PLC/PRF/5and HepG2 cells | (Longo *et al.*, 2008) |
| | Black rice | Suppress metastasis | Black rice anthocyanins suppress metastasis in breast cancer cells by targeting the RAS/RAF/MAPK pathway | Human breast cancer cell lines MCF-10A and MCF-7 | (Chen *et al.*, 2015) |
| | Black rice whole grain | Anti-inflammation | Polar fraction of black rice whole grain extracts might suppress LPS-induced inflammation via the inhibition of the MAPK signalling pathway, leading to decrease of NF-kB and AP-1 translocation | RAW 264.7 macrophage cells | (Limtrakul *et al.*, 2015) |
| | Black currant | Antioxidant | Blackcurrant bioactive anthocyanins exert chemo-preventive actions against diethylnitrosamine-inflicted hepatocarcinogenesis by attenuating oxidative stress through activation of the Nrf2 signalling pathway | Rats | (Thoppil *et al.*, 2012) |
| Delphinidin | Grape | Block carcinogen-DNA adduct formation | Concord grape extract and a component grape anthocyanin have breast cancer chemo-preventive potential caused in part | Block carcinogen-DNA human breast epithelial MCF-10F cells treated with benzo[a]pyrene adduct formation | (Singletary *et al.*, 2007) |

*(Continues)*

**Table 1** (Continued)

| Anthocyanins | Source | Properties | Effects | Laboratory model | Reference |
|---|---|---|---|---|---|
| | | | to their capacity to block carcinogen-DNA adduct formation, modulate the activities of carcinogen-metabolizing enzymes and suppress ROS in these noncancerous human breast cells. | | |
| | Blueberry | Inhibit MMP-2 and MMP-9 proteolytic activity | Gallic acid inhibited MMP-2 and MMP-9 proteolytic activity with very similar potency. NMR and molecular modelling experiments confirmed the interaction of Gallic acid with MMP-2 and suggested that it takes place within the catalytic centre | Human fibrosarcoma HT1080 cells | (Filipiak *et al.*, 2014) |
| | Roselle | Antitumour | Treatment with HAs caused reduced levels of AST, ALT, uric acid and MPO. The results showed that oral administration of HAs (0.2%) remarkably inhibited progression of NMU-induced leukaemia by approximately 33.3% in rats | N-nitrosomethylurea-induced leukaemia in rats | (Tsai *et al.*, 2014) |
| | Muscadine grape skin | Mediated invasion, migration and osteoclastogenesis | Snail regulation of CatL might occur via STAT3 signalling and can be antagonized by Muscadine grape skin extract, leading to decreased cell invasion, migration and bone turnover | LNCaP prostate and MCF-7 breast human cancer cells | (Burton *et al.*, 2015) |
| Malvidin | Blueberry | Antioxidant, anti-proliferative, stimulate apoptosis | The ARF-T was shown to have the highest anthocyanin content and antioxidant activity and inhibited B16-F10 melanoma murine cells proliferation at concentrations higher than 500 $\mu g \cdot mL^{-1}$. In addition, ARF-T stimulated apoptosis and increased | Mouse melanoma B16-F10 cells | (Bunea *et al.*, 2013) |

*(Continues)*

**Table 1** (Continued)

| Anthocyanins | Source | Properties | Effects | Laboratory model | Reference |
|---|---|---|---|---|---|
| | | | total LDH activity in metastatic B16-F10 melanoma murine cells | | |
| | Blueberry | Increase apoptosis through DNA fragmentation and caspase-3 activity | Apoptosis was confirmed in HT-29 cells when treated with anthocyanins from blueberry cultivars at 50–150 $\mu g \cdot mL^{-1}$ concentrations; however, these same concentrations decreased quinone reductase and glutathione-S-transferase activities rather than induced them | Human colon cancer HT-29 cells | (Srivastava *et al.*, 2007) |
| | Blackberry | Antioxidant, anti-proliferative and anti-inflammatory activities | The hydrolysed pulp and seed extracts showed significant antiproliferative activity. However, non-hydrolysed extracts showed much less activity | Human lung cancer A549 cells | (Aqil *et al.*, 2012) |
| | Bilberries and blueberries | Antioxidant | Anthocyanins had intracellular antioxidant activity if applied at very low concentrations ($<1 \mu g \cdot L^{-1}$; nM range), thereby providing a long-sought rationale for their health protecting effects in spite of their unfavourable pharmacokinetic properties | Human colon cancer (Caco-2), human hepatocarcinoma (HepG2), human endothelial (EA.hy926) and rat vascular smooth muscle (A7r5) | (Bornsek *et al.*, 2012) |
| Pelargonidin | Pomegranate | Antioxidant, antitumour and anti-inflammatory | Pomegranate fruit extract possesses anti-skin tumour-promoting effects in CD-1 mouse. Pomegranate fruit extract is capable of inhibiting conventional, as well as novel, biomarkers of TPA-induced tumour promotion; therefore, it may possess chemopreventive activity in a wide range of tumour models | TPA-induced skin tumour in CD-1mice | (Afaq *et al.*, 2005) |
| Peonidin | Sweet potato P40 | Antioxidant, anti-proliferative, induce cell-cycle arrest, apoptosis | Dietary P40 at 10–30% significantly suppressed azoxymethane-induced formation of aberrant crypt foci in the colons of CF-1 mice in conjunction | Human colonic SW480 cancer cells | (Lim *et al.*, 2013a) |

*(Continues)*

**Table 1** (Continued)

| Anthocyanins | Source | Properties | Effects | Laboratory model | Reference |
|---|---|---|---|---|---|
| | | | with, at least in part, a lesser proliferative PCNA and a greater apoptotic caspase-3 expression in the colon mucosal epithelial cells | | |
| | *Eugenia jambolana* | Anti-proliferative, pro-apoptotic | Java plum fruit extract anthocyanins suppressed ($P < 0.05$) proliferation in HCT-116 cells and elevated ($P < 0.05$) apoptosis in both HCT-116 cells and colon CSCs | HCT-116 colon cancer cell line and colon CSCs | (Charepalli *et al.*, 2016) |
| Petunidin | Jamun, Blackberry | Antioxidant and anti-proliferative | The hydrolysed pulp and seed extracts showed significant anti-proliferative activity. However, non-hydrolysed extracts showed much less activity | Human lung cancer A549 cells. | (Aqil *et al.*, 2012) |
| | Blueberry | Antiproliferative, antioxidant, metastasis control | The ARF-T had the highest anthocyanin content and antioxidant activity and inhibited B16-F10 melanoma murine cells proliferation at concentrations higher than 500 $\mu g \cdot mL^{-1}$. In addition, ARF-T stimulated apoptosis and increased total LDH activity in metastatic B16-F10 melanoma murine cells | B16-F10 metastatic melanoma murine cells | (Bunea *et al.*, 2013) |
| | Black soybean | Suppress oxidative stresses, decreasing inflammatory responses | Mucosal expression of COX-2 and cPLA2 were decreased significantly in the 0.5% AE group, by 32 and 62%, respectively, compared with the control group | APC (Min/+) mice | (Park *et al.*, 2015) |

AE, anthocyanin-rich extract; ALT, alanine transaminase; ARF-T, anthocyanin rich-fraction obtained from cultivar Torro; AST, aspartate transaminase; MPO, myeloperoxidase; NMU, N-nitroso-N-methylurea.

anthocyanins have longer enterohepatic recycling, which leads to prolonged residence time (Lila *et al.,* 2016). Cerletti *et al.* (2016) also found three main metabolites in urine, namely, delphinidin-3-glucoside, C-3-G and cyanidin-3-(6-malonylglucoside), reached a plateau level after the first week of ingestion of red orange juice. These compounds were considered markers of anthocyanin bioavailability in subsequent human studies. The peak plasma concentration of the four anthocyanins in black raspberries could be detected 2 h after taking oral black raspberries, whose elimination fits first-order kinetics (Stoner *et al.,* 2005). Four to eight hours after taking black raspberries, the anthocyanins are excreted through the urine in the form of complete anthocyanin or methyl derivatives. Experiments *in vitro* proved that anthocyanins in the range of $10^{-6}$ to $10^{-4}$ M could inhibit the growth of malignant tumours, regulate cell signal transduction and induce apoptosis (Cooke *et al.,* 2005; Wang and Stoner, 2008). When anthocyanins are taken, their concentration can reach a level of $10^{-8}$ M ~ $10^{-7}$ M or it can be far lower than that *in vitro* to have an anticancer effect (Yang *et al.,* 2011). The bioavailability of anthocyanins is very low (<1% in plasma); however, their presence in their native form has been described recently in colonic tissues of patients, suggesting they can directly interact with colonic tissues (Núñez-Sánchez *et al.,* 2015). It remains unclear whether anthocyanins in experimental concentrations can play a role against cancer in the human body and whether they function as the original molecules or as metabolites.

In short, we have summarized the chemical structure of anthocyanins, their potential molecular mechanisms in tumour prevention and anticancer therapy, and the current research status of their pharmacological effects on both cell lines *in vitro* and animal model *in vivo* and in human clinic trails. Notably, their precursors, proanthocyanidins, share many of same functions with anthocyanins. Proanthocyanidins are molecules polymerized with flavan-3-ols (Chen *et al.,* 2016a) and are extracted from flowers, fruit, leaves and barks of many plants. Proanthocyanidins, especially proanthocyanidin B from *Pinus massoniana* bark extract (PMBE), can scavenge free radicals and has antioxidant (Cui *et al.,* 2005b), anti-inflammation, antitumour and apoptosis induction and anti-invasion effects at the cellular level *in vitro* (Cui *et al.,* 2005a; Li *et al.,* 2015b, 2016b) and in a mouse model *in vivo* (Li *et al.,* 2007; Zhang *et al.,* 2012). Animal experiments by Li *et al.* (2007) showed that *Pinus koraiensis* bark procyanidins extract has antitumour activity against U12 cervical cancer mice. Treatment with 100, 200 and 300 mg·kg$^{-1}$ PMBE reduced the tumour weight and volume of S180-bearing NIH mice by 9–67% and 13–68%, respectively (Zhang *et al.,* 2012). In human trials, according to the No.NCT00100893 (https://clinicaltrials.gov), IH636 grape seed extract, which is rich in proanthocyanidins, has been used in a phase I clinical trial to study its side effects and the best dose to prevent breast cancer in postmenopausal women (40–75 years old) at risk of developing breast cancer.

## Conclusions

The anticancer effect of anthocyanins differs according to the different substituents on their B-rings. Anthocyanins with *ortho*-dihydroxyphenyl on their B-rings show the most obvious anticancer activity. Table 1 summarizes all the experimental data from cancer cell lines *in vitro* and animal models *in vivo*. Increasing evidence indicates that the main molecular mechanism of their antitumour effects lies in inhibiting the cancer cell growth and metastasis by targeting RTKs (EGFR, PDGFR and VEGF/VEGFR) by acting on Ras-MAPK and PI3K/Akt signal cascade pathways. At the initial stage, anthocyanin inhibits inflammation by acting on the PI3K/Akt and NF-κB pathway to suppress the expression of COX-2 and iNOS, thus preventing normal cells from transformation by regulating the expression of phase II antioxidant enzymes to achieve antioxidation through Nrf2/ARE signal system. During the formation phase, anthocyanins prevent carcinogenesis by targeting the MAPK pathway and AP-1 and by inhibiting RTK activity and its signal cascade pathway to regulate the expression of cancer-related genes, which leads to cell cycle arrest and DNA repair. At the development stage, anthocyanins induce apoptosis of cancer cells by activating caspase, mediated by ROS and JNK/p38-MAPK. In addition, anthocyanins inhibit cancer metastasis by targeting the VEGF signal pathway and extracellular matrix degradation. Furthermore, anthocyanins can reverse the multidrug resistance of cancer cells to improve their chemotherapy sensitivity. Figure 2 briefly summarizes the above related signal pathways.

Increasing *in vitro* experimental data have shown that anthocyanins can interfere with multiple signal pathways to exert their anticancer activities. However, it must be pointed out that most of these experiments were performed *in vitro*, and there is not necessarily an epidemiological connection between intake of anthocyanin and risk of carcinogenesis (Wang and Stoner, 2008). Data from animal models and *in vitro* and *in vivo* experiments indicated that anthocyanins have the potential to prevent and treat cancers; however, the metabolism of anthocyanins remains unclear. Considering that anthocyanins are rapidly metabolized into several active metabolites by the host and/or by gut microbiota and the evaluation of bioavailability of anthocyanins is complex (Núñez-Sánchez *et al.,* 2015), more sensitive instruments and efficient methods should be developed to further this research. More clinical trials are required to validate the potential anticancer activities of anthocyanins before their wide clinical use. In addition, experiments show that their availability in the human body is lower than that *in vitro* (Cooke *et al.,* 2005, Núñez-Sánchez *et al.,* 2015). Therefore, improving the availability and the stability of anthocyanin *in vivo* represents another challenge to researchers. The latest review of preclinical and clinical evidence for the potential benefits of edible berries for human aerodigestive and gastrointestinal tract cancers indicated that the exact mechanisms of actions of phytochemicals (including anthocyanins) remain largely unexplained. These mechanisms include chronic inflammation and cancer; modulation of carcinogenesis; phytochemical interaction with vital proteins; signal pathways; and regulation of cancer cell proliferation, immortality and metastasis, and have attracted significant research attention (Bishayee *et al.,* 2016). The mechanism of action of anthocyanins is summarized in Figure 2. Limited evidence from clinical data indicates that the use of anthocyanins can improve life quality (Bishayee *et al.,* 2016) and might provide

a survival advantage in terminal care for cancer patients, However, further rigorous scientific investigation and more clinical proof are required.

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## Conflict of interest

The authors declare no conflicts of interest.

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