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The substitution pattern of anthocyanidins affects different cellular signaling cascades regulating cell proliferation

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The aglycons of the most abundant anthocyanins in food, cyanidin (cy) and delphinidin (del), represent potent inhibitors of the epidermal growth factor receptor (EGFR). Structure-activity studies show that the presence of vicinal hydroxy substituents at the phenyl ring at the 2-position (B-ring) is crucial for target interaction. The presence of a single hydroxy group or introduction of methoxy substituents at the B-ring results in a substantial loss of inhibitory properties. However, biological activity is not exclusively limited to compounds bearing vicinal hydroxy groups. A contradictory structure-activity relationship is observed for the inhibition of cAMP-specific phosphodiesterases (PDEs). Of the anthocyanidins tested, malvidin, bearing methoxy substituents in the 3′- and 5′-positions, most effectively inhibited cAMP hydrolysis. The absence of methoxy groups and/or replacement by hydroxy substituents was found to strongly diminish PDE-inhibitory properties. We found that either effective EGFR inhibition or effective PDE inhibition is required to achieve a shut-down of the central mitogen-activated protein kinase (MAPK) pathway, a signaling cascade crucial for the regulation of cell growth. This is consistent with the finding that efficient reduction of cell growth is limited to anthocyanidins that are potent EGFR- or PDE-inhibitors including cy and del or malvidin (mv), respectively. In summary, depending on the substitution pattern at the B-ring, anthocyanidins interfere with different signaling cascades involved in the regulation of cell growth.

Keywords: Anthocyanidins / Epidermal growth factor receptor / Mitogen-activated protein kinase / Phosphodiesterase

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1 Introduction

In the last decades the public interest in questions of health and life quality has steadily increased. Products promising wellness and longevity have developed into a huge, still growing market. One class of preparations available as food supplements are anthocyanin-rich fruit extracts. Anthocyanins are natural food colorants, widely-found in food of plant origin. Depending on pH and the presence of chelating metal ions, anthocyanins are intensely colored blue, violet or red, contributing substantially to the natural coloring of a multitude of foods, such as berries, grapes or cherries. Glycosides of the aglycons cyanidin (cy) and delphinidin (del) (Table 1) represent the most abundant anthocyanins in fruits [1]. The glycosides of malvidin (mv) are characteristic for grapes and grape products [2]. Anthocyanins and anthocyanidins have been associated with a broad spectrum of potentially positive health effects ranging from the treatment of diabetic retinopathy and various microcircular diseases to potential anti-inflammatory and chemoprotective properties [3 – 5]. The daily intake of anthocyanins in Germany was estimated to average 2.7 mg/person daily in 2002, and varied between 0 – 76 mg/person daily [6]. Especially the increasing popularity of food supplements and the developing market for functional foods might contribute substantially to a rise in daily intake levels among the respective consumer groups. However, despite the relatively high possible intake in humans, information on potential cellular mechanisms of these compounds is rather limited. Like the majority of flavonoids, anthocyanins and their aglycons have repeatedly been reported to possess antioxidative properties *in vitro* [7 – 11]. Recently, we showed that aglycons of the most abundant anthocyanins in food, cy and

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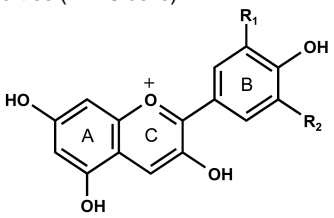
Abbreviations: cy, cyanidin; **del**, delphinidin; **EGFR**, epidermal growth factor receptor; **MAPK**, mitogen-activated protein kinase; **mv**, malvidin; **PDE**, phosphodiesterase; **pg**, pelargonidin; **PKA**, protein kinase A; **pn**, paeonidin

Table 1. Structure of anthocyanidins and *in vitro* growth inhibitory properties (HT29 cells)[a)]



Anthocyanidin	R_1	R_2	Growth inhibition IC_{50} (μM)[a)]
Delphinidin (del)	OH	OH	35 ± 5
Cyanidin (cy)	OH	H	57 ± 3
Pelargonidin (pg)	H	H	213 ± 66
Paeonidin (pn)	OCH_3	H	90 ± 21
Malvidin (mv)	OCH_3	OCH_3	35 ± 6

a) Inhibition of cell growth was determined by the sulforhodamine B assay [30]. HT29 cells were treated for 72 h with the respective compound. The data presented are the mean ± SD of at least three independent experiments each performed in quadruplicate. The IC_{50} value (concentration inhibiting cell growth to 50% of the control) was calculated by linear regression.

del, as well as the grape-typical mv, in the micromolar range inhibit the growth of human tumor cells *in vitro* [12]. Investigating the potential mechanism of action, we focused on central signaling cascades crucial for the regulation of cell growth.

The extracellular-signal-regulated/mitogen-activated protein kinase (ERK/MAPK) pathway (Fig. 1) represents one of the major intracellular signaling cascades regulating cell proliferation [13, 14]. Activation of a respective cell surface receptor, such as the epidermal growth factor receptor (EGFR), initiates an exchange of GDP *versus* GTP at the G-protein Ras. GTP-loaded Ras recruits the serine/threonine kinase Raf-1 from the cytosol to the cell membrane, resulting in the activation of kinase activity. Raf-1, as an interface between cell surface receptors and nuclear transcription, is the entry point to the ERK/MAPK pathway. Effective inhibition of the upstream located EGFR results in a shutdown of the subsequent kinase cascade, leading to the inhibition of cell growth [12, 15 – 18].

Recently, we showed that the anthocyanidins cy and del are highly potent inhibitors of the tyrosine kinase activity of the EGFR that effectively down-regulate subsequent MAPK cascade activity [12]. mv, however, lacking substantial EGFR inhibitory properties, also affects MAPK activity [12], suggesting that other upstream signaling elements than EGFR are targeted.

The MAP kinase cascade is connected to several other signaling pathways in a complex network. One important regu-

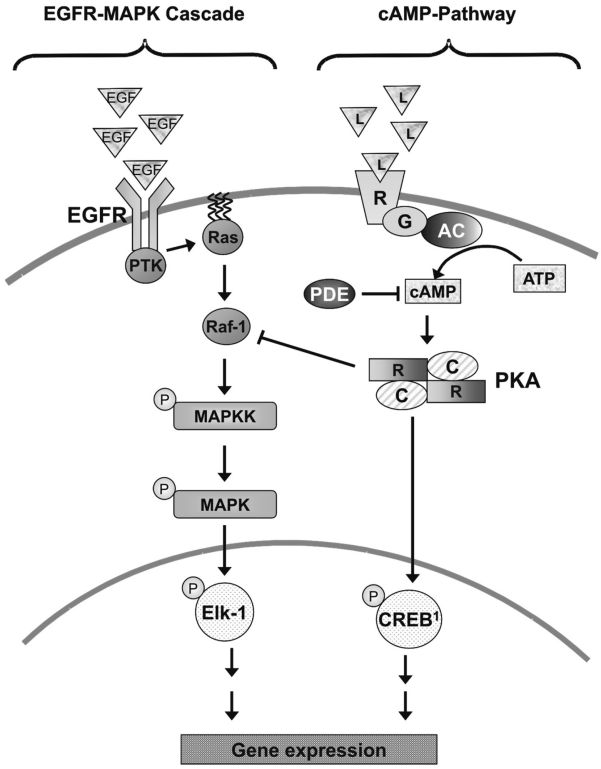


Figure 1. Simplified scheme of the crosstalk between the MAPK cascade and the cAMP pathway. EGF, epidermal growth factor; EGFR, epidermal growth factor receptor; PTK, protein tyrosine kinase; Ras, GTP-binding protein; Raf-1, serine/threonine kinase; MAPKK, mitogen-activated protein kinase kinase; Elk-1, Ets-like kinase (transcription factor); L, ligand; R, receptor; G, GTP-binding protein; AC, adenylyl cyclase; cAMP, cyclic adenosine monophosphate; PKA, protein kinase A (R = regulatory subunit, C= catalytic subunit); CREB, cAMP-responsive element binding protein; [1]transcription factors of the CREB/ATF-family.

latory factor is the deactivating phosphorylation of the serine/threonine kinase Raf-1 by protein kinase A (PKA), a key enzyme in the cAMP-pathway (Fig. 1) [19 – 21]. The superfamily of cAMP-hydrolyzing phosphodiesterases (PDEs) comprise a central element regulating cAMP homeostasis. So far, 11 PDE isoenzyme families have been discovered and characterized by their gene homology, substrate specificity, kinetic characteristics, and susceptibility to selective modulators [22 – 24]. We showed previously that the cAMP-specific isoenzyme family PDE4 is responsible for the highest cAMP-hydrolyzing activity in many human tumor cells [25]. PDE4 belongs to the class of the so-called "low K_M-PDEs", contributing substantially to cAMP-homeostasis at physiological intracellular cAMP-levels. Inhibition of PDE4 in these cells results in a down-regulation of MAPK activity, presumably by PKA-mediated deactivating phosphorylation of the upstream located Raf-1 [25]. Treatment of these cells with a potent

PDE4 inhibitor leads to effective inhibition of tumor cell growth. The cells arrest in the G_1-phase of the cell cycle and subsequently undergo apoptosis [25 – 28].

In the present study, we investigated whether anthocyanidins not only affect the EGF-receptor activity, but whether they also interfere with the cAMP-signaling pathway. Furthermore, we determined the structural requirements of anthocyanidins that effectively inhibit the protein tyrosine kinase activity of the EGFR and/or that inhibit cAMP-hydrolysis.

2 Materials and methods

2.1 Chemicals

The anthocyanidins were obtained from Roth (Karlsruhe, Germany). For all assays freshly prepared solutions of the compounds were used. Purity and stability of the anthocyanidins were determined by HPLC with UV detection (540 nm), using a LiChrospher 100 RP18 column (250 mm/4 mm/5 µm; Merck Eurolab, Darmstadt, Germany) and A = H_2O/formic acid (90/10), B = MeOH + 0.1% HCl mixed A/B = 60/40 as mobile phase. The anthocyanidins were dissolved in DMSO for assays and diluted with B to an adequate concentration for HPLC analysis. Rolipram (4-[3-cyclopentyloxy-4-methoxy-4methoxyphenyl]-2-pyrrolidone) was kindly provided by Schering (Berlin, Germany).

2.2 Cell culture

The human vulva carcinoma cell line A431 [29] was cultured in MEM medium supplemented with 1% L-glutamine and the human colon carcinoma cell line HT29 was cultured in Dulbecco's modified Eagle's medium (DMEM) containing high glucose, both with 10% fetal calf serum (FCS) and 1% penicillin/streptomycin in humidified incubators (37°C, 5% CO_2). Both cell lines were obtained from the German Collection of Microorganisms and Cell Cultures (DSMZ, Braunschweig, Germany). Cell culture medium and supplements were obtained from Invitrogen (Karlsruhe, Germany). The cells were used for testing in passages between 10 to 30 after recultivation from −80°C stocks. Cells were routinely tested and found to be negative for mycoplasm contamination.

2.3 Sulforhodamine B assay

Effects on cell growth were determined according to the method of Skehan *et al.* [30] with slight modifications. Briefly, HT29 cells (4000 cells per well) were spread into 24-well plates and allowed to grow for 24 h before treatment. Thereafter, cells were incubated with the respective drug for 3 days in serum-containing medium, with a maximal solvent concentration of 1% dimethylsulfoxide. Incubation was stopped by addition of trichloroacetic acid (50% solution). After 1 h at 4°C, plates were washed four times with water. The plates were dried at room temperature over night or for 1 h at 37°C and were stained with a 0.4% solution of sulforhodamine B for 30 min in the dark. The excess of staining solution was washed out with 1% acetic acid. The dye was eluted with Tris-buffer (10 mM, pH 10.5) and quantified photometrically at 570 nm. Cytotoxicity was determined as percent survival, determined by the number of treated (T) over control (C) cells × 100 (% T/C).

2.4 Tyrosine kinase assay

The EGFR was isolated from A431 cells. Forty Petri-dishes (145 cm^2) with 3.6×10^6 cells per plate were cultivated for four days. The cell layer was washed thoroughly with PBS and the cells were harvested by trypsin/EDTA treatment. After centrifugation (2000 × *g*, 8 min) the cells were resuspended in 10 mL KMP-buffer (20 mM PIPES × NaOH, 1 mM $MgCl_2$, 5 mM KCl, 0.2 mM PMSF) and lysed by ultrasonic treatment (3 × 10 s) on ice. The soluble EGFR was cleared from crude membrane particles by centrifugation (4 × 18 min, 15 000 × *g*, 4°C) with each centrifugation step in 10 mL KMP-buffer. Intracellular membranes were aggregated by $CaCl_2$ treatment (10 mM, 15 min) and removed by centrifugation (30 min, 7000 × *g*, 4°C). The EGFR-containing membrane fraction was subsequently sedimented by centrifugation at 100 000 × *g* (60 min, 4°C) and stored at –80°C. Further purification was achieved by affinity chromatography. The receptor-containing membrane pellet was solubilized in 10 mL KMP-buffer including 100 µL Triton X-100. The suspension was stirred on ice for 1 h. After centrifugation (100 000 × *g*, 60 min, 4°C) to remove insoluble particles, the supernatant was applied to a wheat germ lectin agarose column (Pharmacia Biotech, Uppsala, Sweden). After thoroughly rinsing (*ca.* 200 mL) with washing buffer (40 mM HEPES × NaOH, 500 mM NaCl, 1 mM PMSF, 10% glycerol, 0.05% Triton X-100) elution of the EGFR protein was achieved by 0.3 M *N*-acetylglucosamine in washing buffer. Fractions of 4.5 mL were collected, mixed with dithiothreitol (final concentration 1 mM) and directly applied to the tyrosine kinase assay. The most active fractions were pooled and stored until further testing in aliquots at –80°C. 96-well plates with high capacity for protein binding (Greiner Bio-One, Frickenhausen, Germany), were coated by incubation overnight at 37°C with 100 µL per well 0.1 mg/mL of the tyrosine kinase substrate poly (Glu : Tyr) 4 : 1 sodium salt in PBS. Excess poly (Glu : Tyr) 4:1 was removed by aspiration, and the plates were washed with PBS containing 0.1% Tween-20. Prior to the kinase reaction, 40 µL of purified membrane fraction was incubated with 10 µL of the test compound (in 10% DMSO to give a final DMSO concentra-

tion of 1%) for 10 min. The kinase reaction was initiated by adding 50 µL of prewarmed (37°C) ATP-solution (50 mM HEPES, pH 7.2, 10 mM $MgCl_2$, 2 mM $MnCl_2$, 200 µM ATP) and proceeded at 37°C for 30 min. The kinase reaction was terminated by aspiration of the reaction mixture and the plate was washed with PBS containing 0.1% Tween 20. The phosphorylation of tyrosine residues was determined using an antiphosphotyrosine-peroxidase conjugated antibody (Roche, Mannheim, Germany), 0.6 U/mL in 1% BSA/PBS, 75 µL per well (at 37°C, 60 min). Excess antibody was removed by aspiration, the plate was washed again with PBS containing 0.1% Tween-20, and the peroxidase reaction was started by addition of 100 µL ABTS® (Roche, Mannheim, Germany) per well. The absorbance was measured at 405 nm 15 to 60 min after incubation at 37°C.

2.5 Inhibition of phosphodiesterase activity

HT29 cells (3×10^6 per plate) were spread in 10-cm Petri dishes (about 16 dishes per test) and harvested at a cell density of 60 – 70%. Before harvesting, medium was removed and cells were washed with 5 mL PBS. Harvesting and lysate preparation was performed at 4°C. Cells were harvested by scraping in buffer A (50 mM Tris-HCl, pH 7.4, 10 mM $MgCl_2$, 0.1 mM EDTA, 0.1 mM EGTA, 4 mM benzamidine hydrochloride, 0.5 µM trypsin inhibitor from soy beans, 0.1 mM phenylmethylsulfonylfluoride, 1 mM β-mercaptoethanol, 0.1 mM *N*-α-*p*-tosyl-L-lysine chloromethyl ketone, 1 µM pepstatin, 1 µM leupeptin) and homogenized with 40 strokes in a Wheaton homogenizer (tight pestle). After centrifugation (100 000 × *g*, 50 min), the supernatant (cytosol) was carefully removed and directly subjected to the PDE assay. PDE activity was determined according to the method of Pöch [31] with slight modifications. PDE-containing samples were incubated at 37°C in the presence or absence of test compounds with a mixture of cAMP and [^{3}H]-cAMP in a buffer containing 50 mM Tris-HCl, pH 7.4, 10 mM $MgCl_2$, and 1 mM AMP, resulting in a final cAMP concentration in the assay of 1 µM. Reaction was stopped at a maximal cAMP turnover of about 20% by adding $ZnSO_4$. [^{3}H]-5′-AMP was precipitated by addition of $Ba(OH)_2$ and separated by centrifugation at 10 000 × *g* for 5 min. Nonhydrolyzed [^{3}H]-cAMP was determined by liquid scintillation counting of the supernatant. PDE activity of each sample was determined in triplicate. The entire experiment was performed three times.

3 Results

3.1 Effect on cell growth

Growth inhibitory properties of anthocyanidins were determined by using the sulforhodamine B (SRB) assay. HT29 colon carcinoma cells were treated for 72 h with the respective compound in the presence of serum. Del and mv exhibited the highest potency for growth inhibition of HT29 cells, followed by cy (Table 1, Fig. 2). Pelargonidin (pg) exhibited the lowest growth inhibitory potential, one order of magnitude less than that for del and mv. The growth inhibition potency of paeonidin (pn) was intermediate to those of pg and cy (Fig. 2).

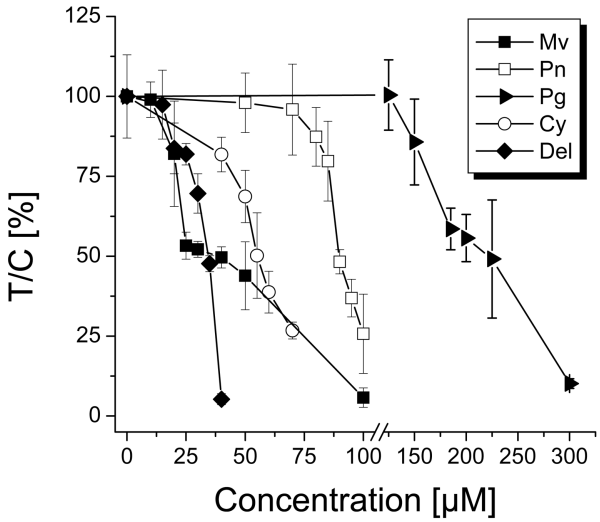


Figure 2. Inhibition of *in vitro* tumor cell growth by anthocyanidins. Growth inhibition was determined using the sulforhodamine B assay [30]. HT29 cells were incubated for 72 h with the respective compound. Growth inhibition was calculated as survival of treated cells over control cells (treated with the vehicle 0.1% DMSO) × 100 [T/C%]. The values given are the mean ± SD of at least three independent experiments, each performed in quadruplicate.

3.2 EGFR

The EGFR was isolated from A431 cells (human vulva carcinoma cells) by affinity chromatography. Effects of test compounds on EFGR protein tyrosine kinase activity were determined using an enzyme-linked immunosorbent assay (ELISA) adopted to 96-well plate format. As previously reported, cy and del are potent inhibitors of EGFR activity [12]. Pg, pn, and mv were found to be less potent EGFR inhibitors by several orders of magnitude compared to cy and del (Fig. 3). The structure-activity relationship characterizing the relative strength of inhibitory effect of anthocyanidins on EGFR protein tyrosine kinase activity can be summarized as cy ≈ del ≫ pg > pn > mv.

3.3 Cyclic AMP-specific PDEs

The cytosolic PDEs of HT29 cells were found to hydrolyze 134.4 ± 18.8 pmol cAMP/(min × mg protein). Addition of

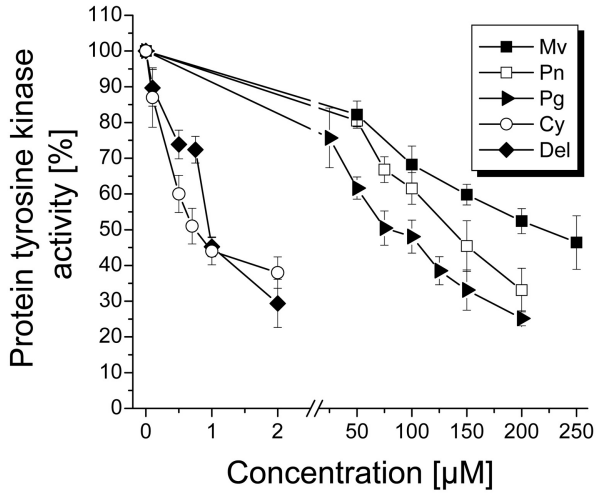


Figure 3. Inhibition of the tyrosine kinase activity of the EGF-receptor. The phosphorylation of tyrosine residues of a peptide poly (Glu/Tyr) was determined by ELISA using an anti-phosphotyrosine antibody linked to a peroxidase. The data presented are the mean ± SD of three independent experiments, each performed in quadruplicate.

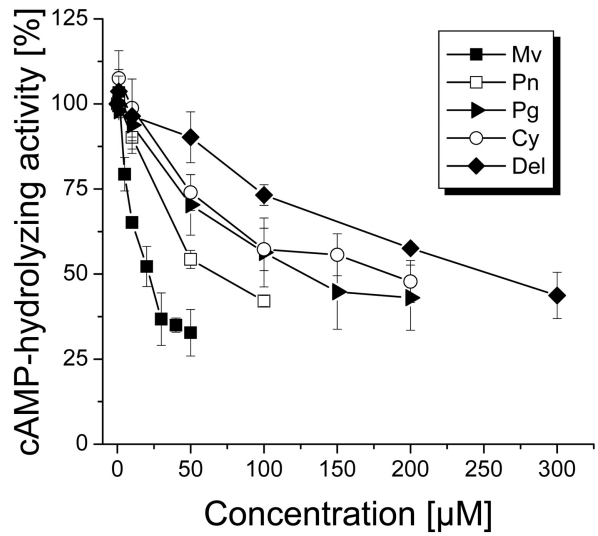


Figure 4. Inhibition of PDE activity. The inhibition of cAMP hydrolysis of cytosolic PDE from HT29 cells was determined according to the method of Poech [31]. The data presented are the mean ± SD of at least three independent experiments, each performed in triplicate.

rolipram (10 µM), a selective inhibitor of the cAMP-specific isoenzyme family PDE4, to the cytosol of HT29 cells in the PDE assay diminished cAMP-hydrolysis by 74 ± 4%, showing that PDE4 is the predominantly expressed PDE isoenzyme family in these cells. Mv was found to effectively inhibit the cytosolic cAMP-hydrolyzing activity of HT29 cells with an IC_{50}-value of 23 ± 5 µM, thus exhibiting the highest PDE-inhibitory activity among the anthocyanidins tested (Fig. 4). The structure-activity on the inhibition of cytosolic PDE-activity of HT29 cells by anthocyanidins can be summarized as mv > pn > pg ≈ cy > del.

3.4 Effects on cell signaling and growth inhibition

Comparing the IC_{50}-values for the different endpoints (inhibition of cAMP hydrolysis, EGFR activity and cell growth) clearly shows an inverse structure-activity relationship for inhibition of PDE activity compared to inhibition of the EGF receptor (Fig. 5). Effective inhibition of tumor cell growth was found to be either associated with potent inhibition of the EGFR activity (del and cy) or PDE-inhibition (pn and mv). The monohydroxylated pg only marginally affected both potential targets, showing the smallest effect on tumor cell growth in HT29 cells (Fig. 5).

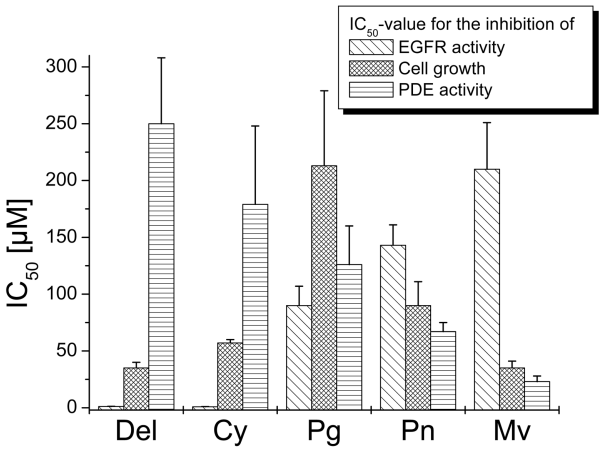


Figure 5. Comparison of the structure-activity relationship for the effect of anthocyanidins on different biological endpoints by the respective IC_{50}-values. Growth inhibitory effects on HT29 cells were determined using the sulforhodamine B assay (Fig. 2). The inhibition of tyrosine kinase activity of the EGFR was assessed by ELISA (Fig. 3). Effects on the PDE activity were determined as inhibition of the cAMP hydrolysis by cytosolic PDE originating from HT29 cells (Fig. 4). From the data presented in Figs. 2–4 the IC_{50}-values were calculated by linear regression.

4 Discussion

In the present study we show that the anthocyanidins del, cy, and mv potently inhibit the growth of the human colon carcinoma cell line HT29 (Table 1, Fig. 2). Flavonoids of different structural classes are known to possess antiproliferative properties and have been reported to inhibit the proliferation of colon carcinoma cells [35–37]. For del and cy, the data presented in this study are comparable to the results of earlier studies on the inhibition of the proliferation of a

human large cell lung carcinoma (LXFL529L), a vulva carcinoma cell line (A431) [12], and the human colon carcinoma cell line HCT115 [37]. From the cell lines tested so far, HT29 showed the highest sensitivity to the growth inhibitory properties of mv. In HT29 cells mv was found to be equipotent to del (Table 1, Fig. 2), whereas in LXFL529L cells, a substantial difference in effectiveness was observed [12]. For LXFL529L cells, del is a potent inhibitor of tumor cell growth; mv, however, induces only marginal growth inhibitory effects with an IC_{50}-value above 100 μM [12]. These data indicate cell line specificity for the growth inhibitory properties of the different anthocyanidins.

Considering the growth inhibitory effect of del, cy, and mv on HT29 cells (Fig. 2), we focused further studies on the underlying mechanism of action and structural requirements for effecting growth inhibition. The MAP kinase pathway represents one of the major signaling cascades regulating cell proliferation. Effective inhibition of the upstream located EGFR leads to inactivation of the downstream kinase cascade, thus inhibiting cell growth. Many tumor cell lines have been shown to overexpress the EGFR as compared with nonmalignant cell lines, thus suggesting a promising target for anticarcinogenic compounds. Growth of HT29 cells is potently inhibited by the specific synthetic EGFR inhibitor tyrphostin AG1478 (data not shown), indicating substantial expression of the receptor in these cells.

We reported previously that the anthocyanidins del and cy represent highly potent inhibitors of EGFR tyrosine kinase activity [12]. The present structure-activity study clearly shows that the presence of vicinal hydroxy substituents on the B-ring is crucial for the interaction with the EGFR. Substantial inhibitory properties are limited to compounds bearing a catechol structure element, such as cy and del. The presence of a single hydroxy group, as for pg, is not sufficient for effective target interaction. The presence of methoxy substituents on pn and mv almost completely negates EGFR inhibitory properties (Fig. 3).

In intact cells the efficient EGFR inhibitors cy and del have been shown to affect the downstream signaling cascades. Cy and del inhibit MAPK activity, as measured by phosphorylation of the transcription factor Elk-1 in the concentration range where growth inhibition is observed [12]. Thus, the anthocyanidins cy and del act as potent inhibitors of the EGFR, down-regulating the activity of the downstream mitogen-activated signaling cascade. It is likely that this effect on cellular signaling contributes substantially to the growth-inhibitory potential of cy and del. However, mv, which lacks potent EGFR-inhibitory properties in HT29 cells (Fig. 3), exhibited growth inhibitory properties comparable to cy and del (Fig. 2). These results suggest that, at least in the case of mv, other cellular targets than the EGFR must be affected. As we previously showed, treatment of A431 cells with mv results in a similar reduction of MAPK activity as for the activity reduction induced by the potent EGFR inhibitor cy [12]. We conclude from these results that mv affects a signaling element upstream of Elk-1, but downstream of the EGFR.

The MAPK cascade interacts with several other signaling pathways in a complex network of crosstalks. One important regulatory factor is the deactivating phosphorylation of the serine/threonine kinase Raf-1 by PKA, a downstream element of the cAMP-pathway. One of the major factors affecting cAMP homeostasis is the expression and activity of cAMP-hydrolyzing PDEs. Several flavonoids of different classes have been reported to inhibit PDE activity [32 – 34].

We could show that anthocyanidins bearing methoxy residues at the B-ring (mv, pn) inhibit cAMP hydrolysis in HT29 cells (Fig. 4). Rolipram, a selective inhibitor of the cAMP-specific isoenzyme family PDE4, is commonly used to determine the presence and to quantify PDE4 activity. At a concentration of 10 μM rolipram, 74% of the cAMP-hydrolyzing activity in the cytosol of HT29 cells was inhibited, indicating that PDE4 is the predominantly active PDE isoenzyme family. The extent of PDE inhibition by mv or pn did not exceed 74% of the total cAMP hydrolysis. Thus, there is no indication that other PDE isoenzyme families than PDE4 are targeted by these compounds. PDE4 has been shown to be the predominantly expressed cAMP-hydrolyzing isoenzyme family in many human tumor cells [25]. Therefore, effective inhibition of PDE4 by food constituents might be of interest for chemoprevention.

Compared to the results obtained for the inhibition of EGFR activity, an opposite structure-activity relationship is observed for the inhibition of PDE4 activity by anthocyanidins (Fig. 5). The substitution pattern of mv, bearing methoxy residues in 3′- and 5′-positions on the B-ring, together with the hydroxy group in 4′-position, common among all anthocyanidins, appears to represent an effective motif for target-enzyme interaction. In contrast to EGFR inhibition, a loss of methoxy groups and/or the presence of vicinal hydroxy groups strongly decreases PDE4 inhibitory properties. The effective inhibition of PDE4 activity by mv might explain the effect of the compound on Elk-1 phosphorylation without requiring EGFR-inhibitory properties.

In summary, several anthocyanidins were shown to inhibit the growth of human tumor cells *in vitro*. However, depending on their specific structures, anthocyanidins affect different cellular signaling elements that are crucial for the regulation of cell proliferation. Considering the roles of the different cellular targets of anthocyanidins thus far identified as important to carcinogenesis and growth control,

these results imply that potential use of anthocyanidins might be of interest in terms of designing future chemoprevention strategies.

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