Blueberry flavonoids inhibit matrix metalloproteinase activity in DU145 human prostate cancer cells

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Abstract: Regulation of the matrix metalloproteinases (MMPs), the major mediators of extracellular matrix (ECM) degradation, is crucial to regulate ECM proteolysis, which is important in metastasis. This study examined the effects of 3 flavonoid-enriched fractions (a crude fraction, an anthocyanin-enriched fraction, and a proanthocyanidin-enriched fraction), which were prepared from lowbush blueberries (*Vaccinium angustifolium*), on MMP activity in DU145 human prostate cancer cells in vitro. Using gelatin gel electrophoresis, MMP activity was evaluated from cells after 24-hr exposure to blueberry fractions. All fractions elicited an ability to decrease the activity of MMP-2 and MMP-9. Of the fractions tested, the proanthocyanidin-enriched fraction was found to be the most effective at inhibiting MMP activity in these cells. No induction of either necrotic or apoptotic cell death was noted in these cells in response to treatment with the blueberry fractions. These findings indicate that flavonoids from blueberry possess the ability to effectively decrease MMP activity, which may decrease overall ECM degradation. This ability may be important in controlling tumor metastasis formation.

Key words: blueberry flavonoids, MMP activity, prostate cancer cells.

Résumé : La régulation de l'activité des métalloprotéases de la matrice (MMP), les médiateurs principaux de la dégradation de la matrice extracellulaire (MEC), est cruciale afin de contrôler la protéolyse qui est importante dans le processus métastatique. Cette étude a examiné les effets de trois fractions enrichies en flavonoïdes, soit un extrait brut, une fraction enrichie en anthocyanine, ainsi qu'une fraction enrichie en proanthocyanidine, préparées à partir de bleuet nain (ou airelle à feuille étroite) (*Vaccinium angustifolium*), sur l'activité des MMP des cellules cancéreuses de la prostate humaines DU145, après un traitement de 24 heures avec les extraits de bleuets. Toutes les fractions ont démontré une capacité à diminuer l'action de la MMP-2 et de la MMP-9. Des fractions testées, la fraction enrichie en proanthocyanidine s'est révélée la plus efficace quant à l'inhibition de l'activité MMP dans ces cellules. Aucune induction de mort cellulaire par apoptose ou par nécrose n'a été notée en réponse au traitement avec les fractions isolées du bleuet. Ces résultats indiquent que les flavonoïdes du bleuet ont la capacité de diminuer de façon efficace l'activité MMP, résultant en une diminution de la dégradation de la MEC, ce qui pourrait être important dans le contrôle de la formation des métastases tumorales.

Mots clés : flavonoïdes du bleuet, activités des métalloprotéases de la matrice (MMP), cellules cancéreuses de la prostate.

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Introduction

The role of extracellular matrix (ECM) integrity and ECM-degrading enzymes, such as the matrix metalloproteinases (MMPs or matrixins), in the process of cancer metastasis has been shown to be substantive, because ECM degradation is essential for tumor metastasis to occur (Stetler-Stevenson and Yu 2001; Pupa et al. 2002; Lynch and Matrisian 2002; Freije et al. 2003). MMP expression and activity are tightly regulated processes, indicating their importance in regular cellular dynamics and interactions (Pupa et al. 2002; Visse and Nagase 2003). Although not the only contributing factor to tumor metastasis, it has been clearly shown that many cancer cells demonstrate a pronounced increase in MMP

activity, and that overexpression of MMPs may be an important factor in both tumor invasion and tumor angiogenesis into foreign tissues (Stetler-Stevenson and Yu 2001; Pupa et al. 2002). Because of the pivotal role MMPs play in tumorigenesis and metastasis formation, MMP expression and regulation may prove to be a strategic target for the development of methods to combat and treat cancer (Pupa et al. 2002). Recent studies have shown that lowbush blueberries (*Vaccinium angustifolium*) have beneficial effects against certain chronic diseases (Bomser et al. 1996; Knekt et al. 2002). Blueberries contain relatively high concentrations of polyphenolic compounds, such as flavonoids and phenolic acids (Smith et al. 2000; Kalt et al. 2000). Flavonoids have been shown to have antioxidant effects in many different models, ranging from free-radical quenching to protection during hypoxia–ischemia insults (Kandaswami and Middleton 1995; Sweeney et al. 2002; Kahkonen and Heinonen 2003). Furthermore, flavonoids from *Vaccinium* species (lingonberry, bilberry, cranberry, and lowbush blueberry) have anticarcinogenic properties; they regulate ornithine decarboxylase, the activity or expression of which is altered in many tumor types (Bomser et al. 1996). In in vitro studies, green tea (*Camellia sinensis*) flavonoids, such as epigallocatechin, have also been shown to modify MMP activity and to modulate ornithine decarboxylase activity (Gupta et al. 1999; Garbisa et al. 2001).

In this study, 3 fractions from lowbush blueberry were tested to examine their effects on MMP activity in DU145 human prostate cancer cells. These fractions represent the major groups of flavonoids in these fruits: a crude fraction, which contains all flavonoids; an anthocyanin-enriched fraction (AN); and a proanthocyanidin-enriched fraction (PAC). The anthocyanins and the proanthocyanidins are groups of flavonoids that are believed to be the major active agents responsible for the anticarcinogenic properties of many flavonoid-containing fruits and vegetables (Bomser et al. 1996; Sartor et al. 2002; Sato et al. 2002). Therefore, in this study, we hypothesized that the gelatinolytic activity of MMPs in DU145 cells would be inhibited after exposure to the various flavonoid-containing fractions, which would indicate that these flavonoid-enriched fractions isolated from lowbush blueberry can affect and regulate the activity of MMPs.

Materials and methods

Preparation of blueberry fractions

The pressed juice from organic lowbush blueberries (*V. angustifolium*) was loaded onto a preconditioned C18 column, which was subsequently washed with water to remove sugars. The C18 column was then eluted with 100% methanol, containing 1% formic acid, until no more color was eluting off the column. The resultant fraction, referred to as the crude blueberry (CB) extract, was loaded onto an LH-20 column that had been equilibrated in 50% methanol in water. Elution with 60% methanol, 35% water, and 5% formic acid yielded coloured fraction no. 1; further elution of the column with 70% acetone and 30% water yielded coloured fraction no. 2.

HPLC analysis of fractions

Coloured fractions no. 1 and no. 2 were chromatographed on an Agilent 110 Series HPLC (Agilent Technologies, Kirkland, Que.), which was equipped with a quaternary pumping system, a temperature controlled autoinjector, a column heater, a diode array detector (DAD), a fluorescence detector, and an HP ChemStation for data storage and manipulation. Mass spectral analysis was carried out on an API-300 triple quadrupole, equipped with a TurboIonSpray source (MDS SCIEX, Concord, Ont.) with flow from the HPLC system split (1:1).

Analysis of coloured fraction no. 1 was performed on a Zorbax SB C18 column (Agilent Technologies), which was subjected to a gradient elution with H_2O (0.2% trifluoroacetic acid (TFA)) and CH_3CN (0.2% TFA). Absorbance was monitored at 280, 320, 360, and 520 nm. Conditions for mass spectral analysis in the positive mode included an IonSpray voltage of 5500 V and a turboprobe temperature of 450 °C. Liquid chromatography/mass spectrometry (LC/MS)-selective ion monitoring (SIM) was performed for the expected molecular ions and for respective fragments so that observed anthocyanins (Prior et al. 2001) and flavonol glycosides (Kader et al. 1996) could be identified. A chlorogenic-acid standard (ICN Nutritional Biochemicals, Costa Mesa, Calif.) and an anthocyanin standard (Extrasynthese, France), containing cyanidin, delphinidin, malvidin, peonidin, and petunidin-3-glucosides, aided in the identification of peaks on the LC chromatograms.

Coloured fraction no. 2 was chromatographed on a Luna Si (2) column (Phenomenex, Torrrance, Calif.). The ternary solvent system consisted of methanol, dichloromethane, and acetic acid – water (1:1). Absorbance was monitored at 280, 320, 360, and 520 nm; on the fluorescence detector, the excitation was set at 276 nm and the emission at 316 nm. Conditions for mass spectral analysis in the negative mode included an IonSpray voltage of –5000 V and a turboprobe temperature of 300 °C. Before the source was introduced, 0.75 mol ammonium hydroxide/L was introduced to the column effluent through a tee, at a flow rate of 0.04 mL/min. Assignments were accomplished by LC/MS SIM of the expected molecular ions or prominent fragments (Lazarus et al. 1999). Both epicatechin and catechin standards were obtained using Fluka (Sigma–Aldrich Corp., St. Louis, Mo.).

Cell culture

Human DU145 prostate adenocarcinoma cells (ATCC, Rockville, Md.) were cultured on 100-mm Falcon plastic tissue-culture dishes (Becton Dickinson Labware, Franidin Lakes, N.J.) in alpha minimal essential medium (Invitrogen, Burlington, Ont.), supplemented with 10% (*v/v*) fetal bovine serum (Hyclone/VWR Canlab, Mississauga, Ont.), at 37 °C in 5% CO_2. Defined medium (DM), which is a serum-free medium, was also used. DM consisted of alpha minimal essential medium supplemented with transferrin (Sigma, Oakville, Ont.) and insulin (Sigma). Blueberry-enriched DM was prepared by dissolving each of the fractions from lowbush blueberry (CB, coloured fraction no. 1, and coloured fraction no. 2) in DM, to a final concentration of 0.1, 0.5, and 1.0 mg/mL, respectively. Subconfluent cells were exposed

Fig. 1. Liquid chromatography/mass spectrometry (LC/MS)-selective ion monitoring (SIM) analysis of colored fraction no. 1 showed the presence of the following 21 anthocyanins and 3 flavonol glycosides: (1) delphinidin-3-galactoside, (2) delphinidin-3-glucoside, (3) cyanidin-3-galactoside, (4) delphinidin-3-arabinoside, (5) cyanidin-3-glucoside, (6) petunidin-3-galactoside, (7) cyanidin-3-arabinoside, (8) petunidin-3-glucoside, (9) peonidin-3-galactoside, (10) petunidin-3-arabinoside, (11) quercetin-3-glucoside, (12) malvidin-3-galactoside, (13) peonidin-3-glucoside, (14) quercetin-3-galactoside, (15) peonidin-3-arabinoside, (16) malvidin-3-glucoside, (17) malvidin-3-arabinoside, (18) quercetin-3-rhamnoside, (19) delphinidin-6-acetyl-3-galactoside, (20) cyanidin-6-acetyl-3-glucoside, (21) petunidin-6-acetyl-3-glucoside, (22) malvidin-6-acetyl-3-galactoside, (23) peonidin-6-acetyl-3-glucoside, (24) malvidin-6-acetyl-3-glucoside.
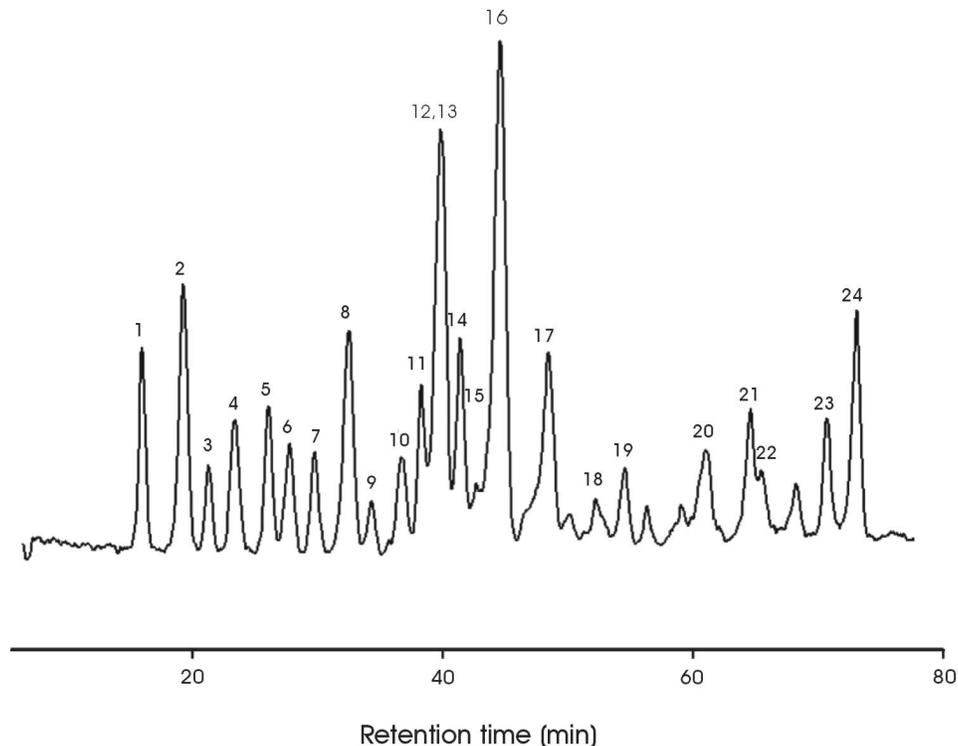
to blueberry-enriched DM for 24 h, and the resulting conditioned medium was analyzed for MMP activity.

Gelatin gel electrophoresis

Gelatinolytic activity was analyzed as described elsewhere (Samuel et al. 1992). Briefly, an aliquot of conditioned medium was mixed (4:1) with sample buffer, which consisted of 10% SDS and 0.1% bromophenol blue in 0.3 mol Tris–HCl/L (pH 6.8), and then incubated at 37 °C for 5 min. Aliquots of each sample were loaded into wells of a 5% stacking gel and resolved at a constant current at ambient temperature. The 10% resolving gel contained Type A gelatin (Sigma) to a final concentration of 1 mg/mL. After electrophoresis, gels were washed in 0.05 mol Tris–HCl/L (pH 7.4) and 2% Triton X-100 for 1 h at room temperature, followed by a 30-min wash in 0.05 mol Tris–HCl/L (pH 7.4) at room temperature. Gels were then incubated at 37 °C for 24 h in a substrate buffer containing 0.05 mol Tris–HCl/L (pH 7.4), 1% Triton X-100, and 0.005 mol $CaCl_2$/L. Following this incubation period, gels were stained with 0.1% Coomassie Brilliant Blue R-250 in a solution of acetic acid, methanol, and water (5:10:85 by volume). Prestained molecular-weight markers (Bio-Rad, Hercules, Calif.) were also resolved on the same gel. Gelatinase activity appeared as zones of clearing (due to gelatin degradation) against a blue background. As a loading control, identically loaded complementary Coomassie Brilliant Blue –

stained polyacrylamide gels without gelatin were used (Oetken et al. 1992). Staining of these gels produces a number of protein bands for each lane. The intensity of these stained bands is used to ensure that an equal amount of protein was added per lane for each individual zymogram (data not shown).

Necrotic and apoptotic cell-death assays

Cell cultures were analyzed for the presence of markers for either necrotic or apoptotic cell death. Lactate dehydrogenase (LDH) activity and caspase-3 activity are routinely used indicators of necrotic and apoptotic cell death (Gay et al. 1968; Cohen 1997). Commercially available assay kits were used to detect the activity of LDH (Diagnostic Chemicals Ltd., Charlottetown, P.E.I.) and caspase-3 (Sigma).

Results

Analysis of fractions

One gram of CB produced 0.75 g of coloured fraction no. 1 and 0.085 g of coloured fraction no. 2. Through HPLC analysis, coloured fraction no. 1 was found to contain mostly anthocyanins and flavonol glycosides, with less than 2% chlorogenic acid. Using LC/MS SIM, 21 anthocyanins were identified in the profile when monitored at 520 nm (Fig. 1). Using LC/DAD and a standard that contained cyanidin, delphinidin, malvidin, peonidin, and petunidin-3-glucosides,

the retention times of these 5 anthocyanins were confirmed. Malvidin-3-glucoside, petunidin-3-glucoside, and delphinidin-3-glucoside made up 37% of the anthocyanins. Glycosides of cyanidin and, in particular, peonidin were minor contributors to the total anthocyanin content of coloured fraction no. 1. Three flavonol glycosides, quercetin-3-glucoside, quercetin-3-galactoside, and quercetin-3-rhamnoside, were identified using LC/DAD monitoring at 360 nm and LC/MS SIM. The retention time of quercetin-3-glucoside was confirmed with a standard. Kaempferol glycosides were not detected with LC/DAD or LC/MS techniques. Chlorogenic acid was identified at a level of 13.5 µg/mg of coloured fraction no. 1 by monitoring the HPLC/DAD chromatogram at 320 nm and with the use of a standard. Thus, coloured fraction no. 1 is an AN.

Using LC/MS SIM, coloured fraction no. 2 was found to contain a range of proanthocyanidins, from monomers to decamers. Levels of the monomers epicatechin and catechin were determined to be 0.125 and 0.187 µg/mg, respectively, using the respective standards and LC/fluorescence detection analysis. When the chromatographic profile was monitored at 320 nm and 360 nm, no peaks were observed that corresponded to chlorogenic acid or flavonol glycosides, respectively. Thus, coloured fraction no. 2 is a PAC.

Effects of lowbush-blueberry fractions on MMP activity

Gelatinolytic activity of conditioned medium from DU145 cells after 24 h of treatment with CB fraction was determined. DU145 cells were exposed to 0.1, 0.5, and 1.0 mg/mL CB fraction. As shown in Fig. 2, in control cells not exposed to the CB fraction, discrete bands of gelatinolytic activity were noted at about 92 kDa and ~62–72 kDa. These gelatinolytic activities correspond to MMP-9, activated forms of MMP-2 and pro-MMP-2. Activity of MMP-9 was unaffected by 0.1 mg/mL CB fraction, but was progressively inhibited by 0.5 and 1.0 mg/mL (Fig. 2). The proteolytic activity of both the proenzyme forms of MMP-2 and activated isoforms of MMP-2 were apparently unaffected by 0.1 mg/mL CB fraction, but were progressively inhibited by 0.5 and 1.0 mg/mL. In this regard, 1.0 mg/mL CB fraction results in a pronounced decrease in gelatinolytic activity of the activated isoforms of MMP-2, and a complete inhibition of the activity of pro-MMP-2 (Fig. 2).

Gelatinolytic activity of conditioned medium from DU145 cells after 24 h of treatment with 0.1, 0.5, and 1.0 mg/mL AN fraction is shown in Fig. 3. Figure 3 also shows that MMP activity is differentially modulated after 24 h of exposure to the AN fraction from lowbush blueberry. MMP-9 activity was unaffected after exposure to either 0.1 or 0.5 mg/mL AN fraction, but was inhibited in the presence of 1.0 mg/mL. MMP-2 activity is unaffected by 0.1 mg/mL AN fraction, but is affected by 0.5 and 1.0 mg/mL. In this regard, pro-MMP-2 activity is virtually abolished in the presence of 1.0 mg/mL AN fraction.

Gelatinolytic activity of conditioned medium from DU145 cells after 24 h of exposure to 0.1, 0.5, and 1.0 mg/mL PAC fraction from lowbush blueberry is shown in Fig. 4. Figure 4 also shows that all MMP activity is dramatically reduced in the presence of the PAC fraction. Activity of MMP-9 is markedly reduced in the presence of 0.1 mg/mL of PAC, and is completely abolished in response to 0.5 and 1.0 mg/mL.

Fig. 2. Effects of crude fraction from lowbush blueberry (CB) on MMP activity in DU145 cells. Gelatin gel electrophoresis was performed on aliquots of conditioned medium from DU145 cells after 24-h exposure to CB fraction. Gelatinolytic activity in cells cultured in the absence of CB fraction is shown in lane 1, and gelatinolytic activity in cells after 24-h exposure to 0.1 mg/mL CB fraction is shown in lane 2, to 0.5 mg/mL is shown in lane 3, and to 1.0 mg/mL is shown in lane 4.
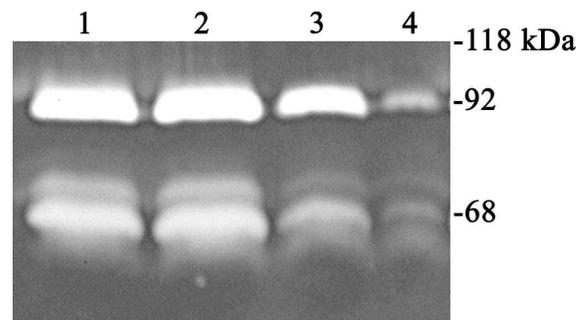

Fig. 3. Effects of anthocyanin-enriched fraction from lowbush blueberry (AN) on MMP activity in DU145 cells. Gelatin gel electrophoresis was performed on aliquots of conditioned medium from DU145 cells following 24 h exposure to AN fraction. Control cells (lane 1), and cells exposed to 0.1 mg/mL AN (lane 2), 0.5 mg/mL AN (lane 3), and 1.0 mg/mL AN fraction (lane 4).
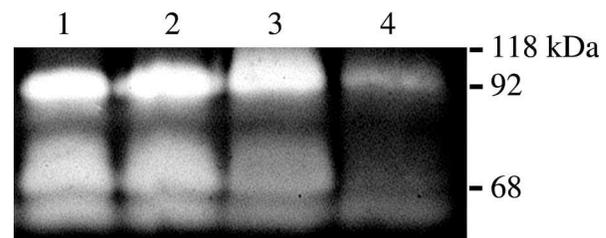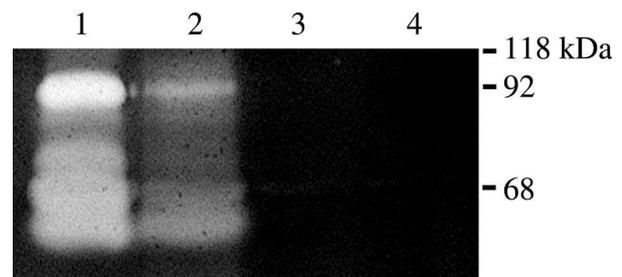

Fig. 4. Effects of proanthocyanidin-enriched fraction from lowbush blueberry (PAC) on MMP activity in DU145 cells. Gelatin gel electrophoresis was performed on aliquots of conditioned medium from DU145 cells after 24-h exposure to PAC fraction. Control cells (lane 1), and cells exposed to 0.1 mg/mL PAC (lane 2), 0.5 mg/mL PAC (lane 3) and 1.0 mg/mL PAC fraction (lane 4).

MMP-2 activity is also virtually eliminated after exposure to 0.1 mg/mL PAC, and is completely abolished in response to 0.5 and 1.0 mg/mL. To further characterize the effect of the PAC fraction on MMP activity, the effects of the PAC fraction at lower concentrations was evaluated. Figure 5 shows the effect of 24-h exposure to 0.01 mg/mL and to 0.05 mg/mL

Fig. 5. Effects of lower concentrations of PAC fraction from lowbush blueberry on MMP activity in DU145 cells. Gelatin gel electrophoresis was performed on aliquots of conditioned medium from DU145 cells after 24-h exposure to various concentrations of PAC fraction. Gelatinolytic activity in control cells (lane 1), and gelatinolytic activity in cells exposed to 0.01 mg/mL (lane 2), 0.05 mg/mL (lane 3), 0.1 mg/mL (lane 4), 0.5 mg/mL (lane 5), and 1.0 mg/mL (lane 6) PAC fraction, respectively.
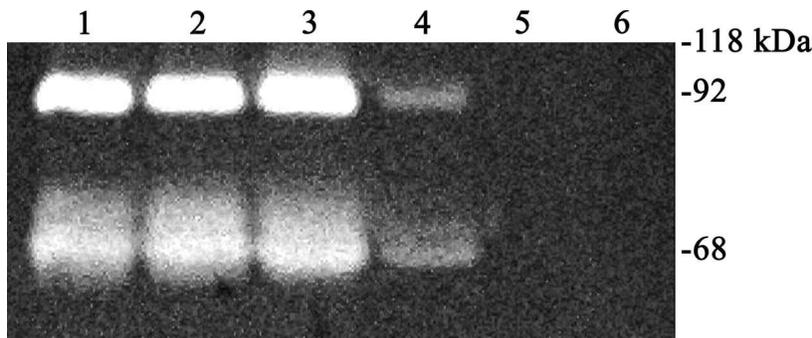

Fig. 6. Lactate dehydrogenase (LDH) activity as a measure of necrotic cell death. LDH activity was measured in DU145 cells after 24-h exposure to defined medium (control cells, lane 1), defined medium supplemented with CB fraction (1.0 mg/mL, lane 2), defined medium supplemented with AN fraction (1.0 mg/mL, lane 3), and defined medium supplemented with PAC fraction (1.0 mg/mL, lane 4). Results indicated are from duplicate experiments with 5 culture plates per condition tested.
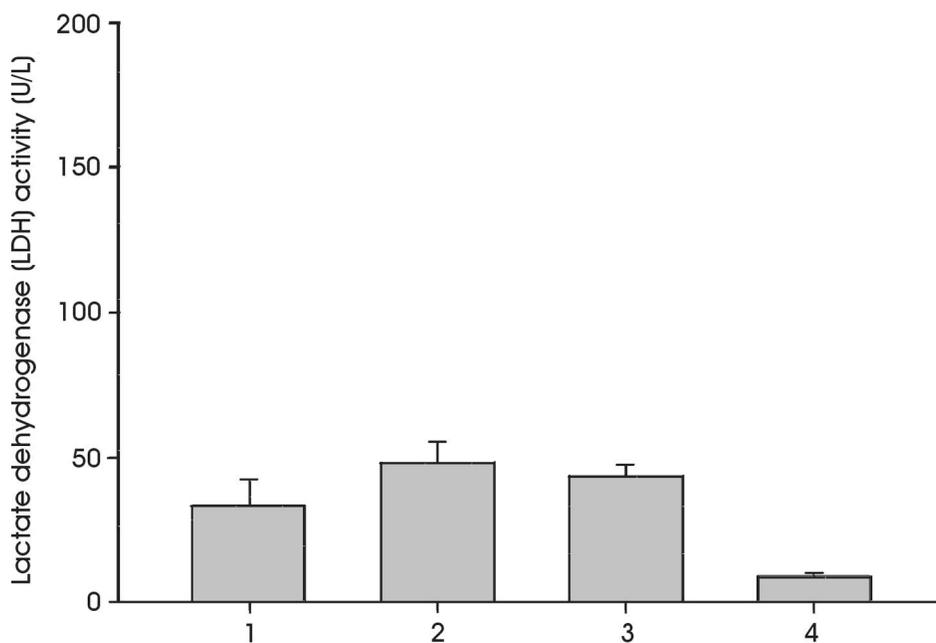

PAC fraction on MMP activity in DU145 cells. At these concentrations, the PAC fraction was unable to inhibit MMP activity in these cells, suggesting that a threshold value of the PAC fraction is required for the inhibitory effect to be expressed. For comparison, the remaining lanes in Fig. 5 are replicate lanes of 0.1, 0.5, and 1.0 mg/mL PAC fraction, respectively, shown in Fig. 4. Results presented in Figs. 2–5 are representative of observations noted from, at a minimum, 3 separate experiments.

Necrotic and apoptotic cell death in DU145 cells in response to blueberry fractions

To determine whether the decrease in MMP activity in response to treatment with the blueberry fractions was due to a toxic effect on the cells, the contribution of generalized cell death, resulting from cellular necrosis, and programmed cell death, resulting from cellular apoptosis, was evaluated.

LDH and caspase-3 activities were used as indicators of necrotic cell death and apoptotic cell death, respectively. Biochemical assays were performed to quantify the presence and the activity of LDH in DU145 cells exposed to the highest concentration of CB, AN, and PAC fractions from lowbush blueberry, namely 1.0 mg/mL. Figure 6 shows LDH activity in DU145 cells in the absence and presence of these fractions. Treatment of these cells with CB, AN, and PAC fractions (1.0 mg/mL) for 24 h did not result in any more LDH activity than in control cells, indicating that increased necrotic cell death was apparently not a contributing factor to the reduction in MMP activity noted in these cells in response to treatment with these blueberry fractions. Figure 7 shows caspase-3 activity in DU145 cells in the absence and presence of blueberry fractions. Treatment of DU145 cells with CB, AN, and PAC fractions (1.0 mg/mL) did not result in any more caspase-3 activity than in control cells, indicating that apoptotic

Fig. 7. Caspase-3 activity as a measure of apoptotic cell death. Caspase-3 activity was measured in DU145 cells after 24-h exposure to defined medium (control cells, lane 1), defined medium supplemented with CB fraction (1.0 mg/mL, lane 2), defined medium supplemented with AN fraction (1.0 mg/mL, lane 3), and defined medium supplemented with PAC fraction (1.0 mg/mL, lane 4). Results indicated are from duplicate experiments with 5 culture plates per condition tested.
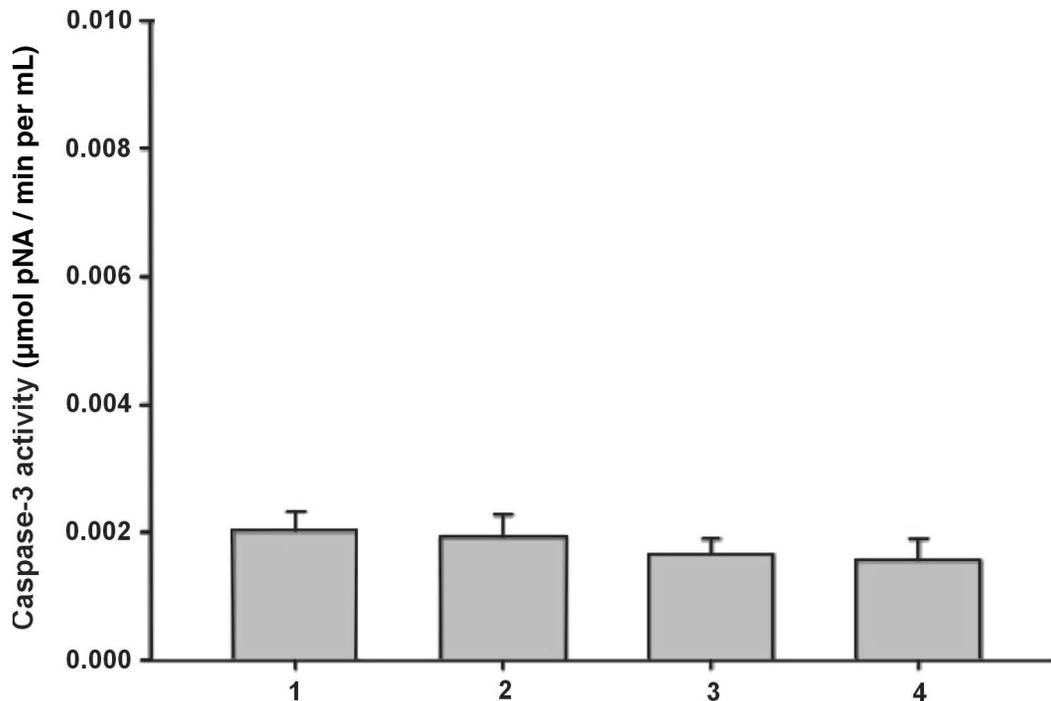
cell death was also not a contributing factor to the reduction in MMP activity noted in these cells in response to treatment with these blueberry fractions. These observations suggest that the decreased expression of MMPs observed in DU145 cells in response to treatment with blueberry fractions were target-directed and not the result of cellular death.

Discussion

This study demonstrated that MMP activity from human prostate cancer cells, specifically DU145 cells, decreases after exposure to flavonoid-enriched fractions from lowbush blueberry. These results are in keeping with other studies investigating the effects of flavonoids in cancer models (Garbisa et al. 2001; Sartor et al. 2002; Sato et al. 2002). Although the gelatinolytic activity of the MMPs in DU145 cells decreases in response to treatment with blueberry fractions, the effects of each flavonoid-enriched fraction on MMP activity is varied. This is not unexpected; although the anthocyanins and proanthocyanidins are both flavonoids, they comprise distinct flavonoid subclasses and, as such, have different chemical structures. The CB fraction contains approximately 75% anthocyanins and 8.5% proanthocyanidins, which may have had synergistic or additive effects. To the best of our knowledge, this study is the first to demonstrate a link between bioactive-containing fractions isolated from lowbush blueberry and the inhibition of MMP expression. The PAC fraction contains a range of proanthocyanidins, from monomers to decamers (Fig. 1). The AN fraction contains a number of potentially bioactive compounds, including 4 main glucosides (malvidin, petunidin, delphinidin and quercetin), quercetin-3-galactoside, and quercetin-3-rhamnoside (Fig. 1). Antho-

cyanins and proanthocyanidins from other sources have been shown to affect MMP activity. Delphinidin has been shown to decrease the activity of MMP-2 (in human neuroblastoma cells) and MMP-9 (in HT-1080 human fibrosarcoma cells) (Sartor et al. 2002). Green tea catechins, which are very similar to proanthocyanidins, have been shown to downregulate MMP activity in cancer cells (Gupta et al. 1999; Garbisa et al. 2001). Epigallocatechin-3-gallate has been reported to inhibit MMP-2 and MMP-9 activity (Sartor et al. 2002). Myricetin, which is a proanthocyanidin, also effectively inhibits the activity of MMPs (Sartor et al. 2002). In addition, the proanthocyanidin nobiletin has been shown to decrease the activity of MMP-1 and MMP-9 in vitro (Sato et al. 2002). The results presented in this study are consistent with these observations. Studies are currently underway to define the exact nature of the bioactive compounds found in these fractions isolated from lowbush blueberries.

It is important to note that these blueberry fractions did not induce necrotic cell death in these cells. These findings indicate a specificity between the action of the bioactive components contained in these blueberry fractions and the inhibition of MMP (MMP-2 and MMP-9) activity in DU145 cells. InStudies looking at the mechanism(s) involved in this inhibitory process are ongoing.

MMP-2 and MMP-9 are the major mediators of basement membrane degradation and, as such, are possible targets for the development of novel anticancer treatments. Our findings demonstrate that flavonoid-enriched fractions from lowbush blueberries can downregulate the activities of specific MMPs in a target-directed manner, suggesting that further understanding of the complex properties of flavonoids, in particular those from lowbush blueberry, may allow for the further

development and refinement of the role of flavonoids in the prevention of carcinogenesis and metastasis.

In conclusion, our results are the first to describe a potential regulatory effect of flavonoids isolated from lowbush blueberry on human prostate cancer cells. This regulatory effect was directed at the activity of MMP-2 and MMP-9. These activities may be one of several targets of the potential antiproliferative effects associated with flavonoid-containing fractions isolated from lowbush blueberries.

Acknowledgements

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