Antioxyen-rich extracts from black raspberries inhibit growth promoting and anti-apoptotic signaling pathways in human bladder carcinoma cell lines

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Abstract
Bladder cancer is the 4th most common cancer diagnosed among US males and females, respectively, accounting for over 60,000 cases per year. Epidemiologic and laboratory data suggests that diets rich in fruits and vegetables are associated with a reduced risk of bladder cancer. Evidences have been proposed that anthocyanin pigments, the most abundant phenolic compounds in fruits and vegetables, and particularly rich in many berries, have anti-cancer activity. We are currently evaluating the effects of black raspberry (BR) phenolics and their impact on cell growth and signaling of normal bladder epithelial cell lines and malignant bladder tumor cell lines that are representative of superficial and invasive bladder cancer. Anthocyanins were extracted from frozen black raspberries and the amounts and composition determined by HPLC/UV detection and high pressure liquid chromatography for flavonoids and isoflavonoids and mass spectrometry detection. Extraction with 80% acetonitrile followed by partitioning with chloroform yielded 120 mg cyanidin-3-glucoside equivalents/100 g BR powder. Whereas, higher extraction with warm deionized water (50 °C, 0.2 M formic acid) was 25% less. The anthocyanin profile was cyanidin-3-glucoside (50%), cyanidin-3-glucoside-cyanidin-3-sambubioside (30-35%) and cyanidin-3-glucoside-cyanidin-3-rutinoside (30-15%). Initial studies with poorly differentiated bladder cancer cell lines showed that exposure (10-24 h) to 15 µg anthocyanin equivalents/mL inhibited proliferation of J2 cells by 20% without significantly altering the HT1197 bladder cell line. AKT and ERK signaling pathways participate in bladder cell proliferation resistance to apoptosis, and regulation. Thus, J2 cells were exposed to the anthocyanin-rich extract and activated or phosphorylated AKT and ERK were assessed. Exposure of these cells to the extract was associated with reduced phosphorylation of AKT (80-90%) and ERK, with attenuation of total AKT and ERK levels. These data suggest that the BR anthocyanin-rich extract can modulate key signaling pathways associated with cellular proliferation of transformed bladder epithelial cells. Further studies of anti-bladder cancer activity of black raspberries are warranted.

Objectives
Quantify the effects of anthocyanin rich black raspberry extracts (BRE) on bladder cancer cell proliferation.
To determine if BRE act via inhibition of AKT and ERK phosphorylation status.

Methods
Cell Culture. Human bladder cancer cells (J2). T24 (RT) were purchased from ATCC, cells were cultured in RPMI 1640 supplemented with 10% fetal bovine serum, 1% penicillin/streptomycin, 1% FBS at 37°C with 5% CO2. All cells were passaged 2-3 times per week.
Black raspberry extraction. Fresh Black Raspberries were purchased from Dr. Laura Keatley’s laboratory and rinsed at 20°C. Upright extraction was performed for 20 min at 20°C with stirring and centrifugation with 12,000 rpm. Extracts were dried to estimate 80% total anthocyanin content.
High-Performance Liquid Chromatography/Analyzer Detection and Mass Spectrometry (HPLC/MS). The anthocyanins were analyzed by using HPLC with a Photodiode Array (PDA) detector and a Q-TOF MS (Agilent Technologies) operated in the electrospray ionization mode. Mass spectra were acquired in both full scan and selected reaction monitoring mode. The compounds were quantified using standard curves of cyanidin-3-glucoside.

Conclusions
The acetone/chloroform extraction methodology produces a consistent antioxidant-rich extract from black raspberries that can be employed for in vitro and in vivo studies.
Anthocyanin rich extracts of black raspberries inhibit proliferation of aggressive / muscle invasive (U2) and superficial / non-invasive (RT4) bladder cancer cells.
The phosphorylation of AKT and ERK, both involved in promoting anti-apoptotic pathways, is inhibited by black raspberry extract.

Future Directions
To compare the effects of anthocyanins on cell proliferation and signaling pathways of normal human bladder urothelial cells and bladder cancer cells.
To examine the ability of black raspberries and extracts to alter bladder carcinogenesis in murine models.

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References
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