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Pro-apoptotic activity against cancer stem cells differs between different parts of sweet sorghum

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ABSTRACT

The extracts from sweet sorghum stalk were previously shown to eliminate human colon cancer stem cells (CCSC) in a partial p53-dependent fashion. However, the underlying mechanisms remain unknown. In this study, we transfected CCSC with shRNA against p53 and treated with sweet sorghum phenolics extracted from different plant components. While all sweet sorghum components demonstrated anti-proliferative and pro-apoptotic effects in CCSC, phenolics extracted from the dermal layer and seed head were more potent in eliminating CCSC by elevating caspase 3/7 activity, poly ADP-ribose polymerase cleavage, and DNA fragmentation in a p53-dependent and partial p53-dependent manner, respectively. These effects were associated with decreases in β-catenin, cyclin D1, cMyc, and survivin protein levels. These results suggest that the anti-proliferative and pro-apoptotic effects of sweet sorghum extracts against CCSC are potentially via suppression of Wnt/β-catenin signalling in a p53-dependent (dermal layer) and partial p53-dependent (seed head) manner.

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1. Introduction

Colorectal cancer is the third most common cancer in men and the second most common in women (Jemal et al., 2011). Up to 80% of colon cancer cases are thought to be caused by environmental factors including diet (Reddy, Odhav, & Bhoola, 2003). Recent evidence implicates that bioactive compounds (e.g. phenolic acids, flavonoids, stilbenoids, and carotenoids) are partly responsible for imparting protective and

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Abbreviations: CCSC, Human colon cancer stem cells; PARP, Poly ADP ribose polymerase; BrdU, Bromodeoxyuridine; TUNEL, Terminal deoxynucleotidyl transferase (TdT) dUTP nick-end labelling; Wnt, Wingless/integrated; 5FU, 5-Fluorouracil; GSK-3β, Glycogen synthase kinase isoform 3β; ABTS, 2,2′-Azino-bis(3-ethylbenzothiazoline-6-sulfonic acid); TPC, Total phenolic content; AOA, Antioxidant activity; DAP, Days after planting; TE, Trolox equivalents

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preventive properties against chronic diseases (Liu, 2012; Reddy et al., 2003; Vanamala, Radhakrishnan, Reddivari, & Massey, 2012).

In colorectal cancers, aberrant Wnt/β-catenin signalling occurs in almost all cases and drives carcinogenesis (Bienz & Clevers, 2000). However, further genetic events must occur to fully develop disease malignancy (Firestein et al., 2008). The traditional pathway of colorectal cancer accounts for 70 to 85% of all colon cancers (Bienz & Clevers, 2000). This pathway starts with loss or mutation of adenomatous polyposis coli (APC) gene resulting in β-catenin accumulation, followed by mutation in K-ras, loss of 18q, and finally loss of p53 via loss of 17p (Worthley, Whitehall, Spring, & Leggett, 2007). Furthermore, it is estimated that p53 is abnormal in 50% to 75% of colorectal cancer cases, and this marks the transition from noninvasive to invasive disease (Worthley et al., 2007). Disruption of p53 is known to alter tumour responses to 5-fluorouracil (5FU), making it more difficult to effectively eliminate colorectal tumours by conventional chemotherapies (Damalas et al., 1999; Longley, Harkin, & Johnston, 2003). Excess β-catenin accumulation promotes transcriptionally active p53, whereas an active p53 results in the degradation of β-catenin (Oren, 2003; Sadot, Geiger, Oren, & Ben-Ze'ev, 2001). β-Catenin is also regulated by glycogen synthase kinase-3β (GSK-3β), which phosphorylates β-catenin at the N-terminus (Liu et al., 2002). Binding of APC and axin to GSK-3β and β-catenin facilitates this event and results in the ubiquitination and degradation of β-catenin (Liu et al., 2002). In the absence of such regulation, β-catenin accumulates in the cytosol and translocates in to the nucleus, where it interacts with members of the T-cell factor/lymphoid enhancer factor (TCF/LEF) family of transcription factors resulting in transactivation of pro-survival target proteins, such as cMyc, cyclin D1, and survivin (Fodde, Smits, & Clevers, 2001; Tarapore, Siddiqui, & Mukhtar, 2012).

The stem cell theory of cancer states that aberrant stem cell populations are the cause of colorectal cancers, as stem cells are more prone to acquire mutations necessary to manifest the disease because of their long life span as well as self-renewal property (Bitarte et al., 2011; Kasdagly, Radhakrishnan, Reddivari, Veeramachaneni, & Vanamala, 2014). Aberrant Wnt/β-catenin signalling defines CCSC in the tumour situation (Vermeulen et al., 2010). CCSC are chemotherapy- and radiation-resistant, making it difficult to eradicate the disease (Li, Wicha, Schwartz, & Sun, 2011). It is imperative, therefore, that secondary prevention strategies target aberrant β-catenin signalling, p53 inactivation, and cancer stem cell populations. There is evidence for this already as curcumin and a curcumin analogue successfully targeted stem cells derived from colon cancer cell lines (Lin et al., 2011). Resveratrol, a bioactive compound in red grapes, was also shown to inhibit prostate cancer stem-like cells (Pandey et al., 2011). We have shown that combination of grape seed extract, rich in proanthocyanidins, with resveratrol resulted in p53-dependent and p53-independent apoptosis in colon cancer HCT116 cells (Radhakrishnan, Reddivari, Sclafani, Das, & Vanamala, 2011). These evidences warrant further investigation into the development of novel bioactive compound treatments for colorectal cancer.

Sorghum is an important food source worldwide, placing fifth in cereal crop cultivation (Reddy, Ashok Kumar, & Ramesh, 2007). Sweet sorghums are varieties of *Sorghum bicolor*, which concentrate fermentable sugars in the stalks. There is a growing interest in utilizing these sugars for biofuel conversion. Sweet sorghum and other crops can be made more attractive for biofuel conversion if the biofuel waste can be utilized for production of value-added byproducts (Cherubini, 2010; Yu, Zhang, Zhong, Zhang, & Tan, 2012). Value-added byproducts could include bioactive extracts prepared for the purposes of human health. However, plant maturity can impact the content and composition of bioactive compound including phenolics. Maturation of *S. bicolor* has been shown to alter the levels of total phenolic compounds in the stalk, leaves and seed head (Ring, Waniska, & Rooney, 1988). The effects of these changes in maturation on the health-benefiting properties associated with the bioactives in *S. bicolor* need to be explored.

Our previous work on the bioactivity of sweet sorghum stalk components showed that dermal layer extracts were more potent *in vitro* against CCSC, as compared to pith (Massey, Reddivari, & Vanamala, 2014). However, as the whole above ground portion of the plant is used for the biorefinery approach, it is important to evaluate the bioactivity of extracts from different parts of the sweet sorghum plant in direct comparison with one another. There is a lack of such information with respect to sweet sorghums. In this study, we compared the *in vitro* anti-cancer activities of pith, dermal layer, leaf, and seed head from Dale and M81E sweet sorghum varieties, harvested at four different times, to understand the effects of time of harvest (plant maturity). We also investigated the extent to which sweet sorghum extract bioactivity is dependent on the p53 status of the cells, and its capacity to suppress β-catenin and β-catenin's downstream target proteins, cMyc, survivin, and cyclin D1, involved in proliferation.

2. Materials and methods

2.1. *Reagents*

Sodium carbonate, monobasic sodium phosphate, dibasic sodium phosphate, and sodium chloride were purchased from Fisher Scientific (Pittsburgh, PA, USA). Potassium persulfate was purchased from Mallinckrodt Chemicals (Hazelwood, MO, USA). 2,2′-Azino-bis(3-ethylbenzothiazoline-6-sulphonic acid) (ABTS), Folin–Ciocalteu reagent, Trolox, and gallic acid were purchased from Sigma (St. Louis, MO, USA). Apigeninidin and luteolinidin were purchased from Santa Cruz Biotechnology (Santa Cruz, CA, USA) and Indofine Chemical Co. (Hillsborough, NJ, USA), respectively. Standards for polyphenols were procured from Sigma (St. Louis, MO, USA), and CCSC and colon cancer stem cell media were obtained from Celprogen (San Pedro, CA, USA).

2.2. *Sweet sorghum extraction*

The sweet sorghum used in this study was generously provided by Great Valley Energy LLC (Bakersfield, CA, USA). Dale and M81E, two varieties of sweet sorghum, were grown in Bakersfield, CA, and harvested at 117, 125, 138 and 152 days for the dermal layer and 131, 145, 155, and 160 days for the seed head. Dale and M81E were selected as representative

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