Plant Flavone Apigenin Binds to Nucleic Acid Bases and Reduces Oxidative DNA Damage in Prostate Epithelial Cells

Haripaul Sharma1,2,*, Rajnee Kanwal1,2, Natarajan Bhaskaran1,2, Sanjay Gupta1,2,3,4

1 Department of Urology, Case Western Reserve University, Cleveland, Ohio, United States of America, 2 Department of Urology, The Urology Institute, University Hospitals Case Medical Center, Cleveland, Ohio, United States of America, 3 Department of Nutrition, Case Western Reserve University, Cleveland, Ohio, United States of America, 4 Division of General Medical Sciences, Case Comprehensive Cancer Center, Cleveland, Ohio, United States of America

Abstract

Oxidative stress has been linked to prostate carcinogenesis as human prostate tissue is vulnerable to oxidative DNA damage. Apigenin, a dietary plant flavone, possesses anti-proliferative and anticancer effects; however, its antioxidant properties have not been fully elucidated. We investigated sub-cellular distribution of apigenin, its binding to DNA and protective effects against H2O2-induced DNA damage using transformed human prostate epithelial RWPE-1 cells and prostate cancer LNCaP, PC-3 and DU145 cells. Exposure of cells to apigenin exhibited higher accumulation in RWPE-1 and LNCaP cells, compared to PC-3 and DU145 cells. The kinetics of apigenin uptake in LNCaP cells was estimated with a Kₚ value of 5 μmole/L and Vₘₐₓ of 190 pmoles/million cells/h. Sub-cellular fractionation demonstrated that nuclear matrix retains the highest concentration of apigenin (45.3%), followed by cytosol (23.9%), nuclear membranes (17.9%) and microsomes (12.9%), respectively. Spectroscopic analysis of apigenin with calf-thymus DNA exhibited intercalation as the dominant binding mode to DNA duplex. Apigenin exposure resulted in significant genoprotective effects in H2O2-stressed RWPE-1 cells by reduction in reactive oxygen species levels. In addition, apigenin exposure suppressed the formation of 8-hydroxy-2′-deoxyguanosine and protected exposed cells from apoptosis. Our studies demonstrate that apigenin is readily taken up by normal prostatic epithelial cells and prostate cancer cells, and is incorporated into their nuclei, where its intercalation with nucleic acid bases may account for its antioxidant and chemopreventive activities.

Citation: Sharma H, Kanwal R, Bhaskaran N, Gupta S (2014) Plant Flavone Apigenin Binds to Nucleic Acid Bases and Reduces Oxidative DNA Damage in Prostate Epithelial Cells. PLoS ONE 9(3): e91588. doi:10.1371/journal.pone.0091588

Editor: Rana Pratap Singh, Jawaharlal Nehru University, India

Received January 13, 2014; Accepted February 12, 2014; Published March 10, 2014

Copyright: © 2014 Sharma et al. This is an open-access article distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.

Funding: The research work is supported by United States Public Health Service Grant RO1CA108512 and Endowment funds to SG. The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Competing Interests: The authors have declared that no competing interests exist.

* E-mail: sanjay.gupta@case.edu

† These authors contributed equally to this work.

Introduction

Prostate cancer has the highest incidence of any cancer in American men and is the second leading cause of cancer-related mortality [1]. The American Cancer Society estimates that in 2013, approximately, 238,590 new cases of prostate cancer were diagnosed and 29,720 men died of this disease [1]. Although the reasons for this high incidence are unknown, human prostate tissue may be particularly vulnerable to oxidative DNA damage by free radicals which are thought to play a critical role in the multi-step process of carcinogenesis [2–4]. Several etiological factors have been proposed in the genesis of prostate cancer, including increased cellular turnover, loss of DNA repair enzymes, impairment of antioxidant signaling network and persistent chronic inflammation in the prostate gland [5–9]. The resulting oxidative stress, characterized by the generation of reactive oxygen and nitrogen species in the local milieu, produces permanent genomic alterations and cellular DNA damage marked by accumulation of 8-hydroxy-2′-deoxyguanosine (8-OHdG). Studies demonstrate that 8-OHdG is the most prevalent DNA damage product and when incorporated into DNA leads to point mutation via an A→T substitution [3,10]. We have previously demonstrated that persistent chronic inflammation in the prostate gland, associated with increased accumulation of 8-OHdG in prostatic epithelium, promotes premalignant and malignant changes [9,11]. Conversely, reduced 8-OHdG levels, consistent with reduced oxidative stress, have been reported in subjects receiving plant-based diets rich in flavonoids and polyphenols [12–15]. These diets are characterized by conspicuous consumption of green tea and plant flavones rich in apigenin.

Apigenin (4,5,7-trihydroxyflavone), a flavone subclass of flavonoid widely distributed in many herbs, fruits, and vegetables is a substantial component of the human diet and has been shown to possess a variety of biological characteristics, including chemopreventive activity and tumor growth inhibition [16]. Recent studies in several biological systems have shown that apigenin possesses anti-proliferative properties, and induces cell cycle arrest and apoptosis in various human and animal-derived cancer cell lines [17–20]. In transformed mouse liver cells, apigenin has been reported to reduce the toxicological effects of dioxin by suppressing the dioxin-induced activation of the aryl hydrocarbon receptor [21]. After dietary intake, apigenin becomes widely distributed in various tissues and is known to exert beneficial
effects [22]. Apigenin has been shown to protect endothelium-dependent relaxation of rat aorta against oxidative stress [23]. Furthermore, apigenin intake results in reduced levels of lipid peroxidation products and increased antioxidant enzymes, preventing hepatocarcinogenesis in rats exposed to N-nitrosodiethylnitrosamine and phenobarbital [24]. In addition, the bioavailability of apigenin has also been investigated in animals and human subjects. Short-term intake of apigenin-rich parsley by healthy human subjects increased the level of antioxidant enzymes erythrocyte glutathione reductase and superoxide dismutase [25]. However, the cellular distribution of ingested apigenin, its uptake in sub-cellular compartment and its anti-oxidative activity has not been fully elucidated.

In this study, we determined the sub-cellular distribution of apigenin in prostate cancer and normal prostate epithelial cells. We also studied the protective role of apigenin against oxidative stress caused by hydrogen peroxide. Our results demonstrate that apigenin preferentially accumulates in the nuclear matrix, particularly binds to nucleic acid bases and has the ability to reduce oxidative DNA damage in prostate epithelial cells.

Materials and Methods

Chemicals and Reagents

All chemicals and reagents were purchased from Sigma Chemical Co. (St. Louis, MO) unless otherwise specified. Tissue culture supplies were procured from Falcon (Becton-Dickinson Labware, Franklin Lakes, NJ). All tissue culture reagents and 2', 7'-dichlorofluorescein diacetate (DCF-DA) was purchased from Invitrogen (Grand Island, NY) whereas fetal bovine serum was purchased from Tissue Culture Biologicals (Tulare, CA).

Cell Culture

Human prostate cancer LNCaP, PC-3 and DU145 cells and transformed human prostate epithelial RWPE-1 cells were obtained from American Type Culture Collection (Manassas, VA). LNCaP, PC-3 and DU145 cells were cultured in RPMI 1640 medium containing 10% fetal bovine serum supplemented with 1% penicillin-streptomycin at 37°C.

Apigenin Stability

To measure the stability of apigenin during incubation was performed by incubating PC-3 cells with 20 μM apigenin in RPMI 1640 medium containing 10% FBS, and same concentration of apigenin incubated in culture medium without cells under similar culture conditions. Apigenin levels in the medium were detected at set intervals using HPLC.

Cellular Uptake of Apigenin

Human prostate cancer LNCaP, PC-3 and DU145 cells and human prostate epithelial RWPE-1 cells were seeded at a density of 1 x 10^5/mL in 100 mm culture plates with three replicates for each incubation time point. After 24 h of seeding the culture medium was replaced with fresh medium containing 20 μM apigenin. The time course of apigenin uptake by each cell line was determined by incubation with medium containing 20 μM apigenin for up to 16 h.

Kinetics of Apigenin Uptake

Because of higher uptake of apigenin by LNCaP cells, these cells were further studied for uptake absorption kinetics and cellular distribution of this compound. Cells were grown as triplicate cultures in RPMI 1640 medium supplemented with 10% fetal bovine serum and at various concentration of apigenin range from 1.25 μM to 20 μM up to 6 h. At each time point, cells were counted using a hemocytometer, and cellular apigenin was extracted and then measured using HPLC as described previously [26]. Finally, the kinetics of apigenin uptake by LNCaP cells was evaluated using the Michaelis-Menten kinetics model.

Sub-cellular Distribution of Apigenin

LNCaP cells were cultured in triplicates in RPMI 1640 medium supplemented with 10% FBS and apigenin at final concentration of 20 μM. After 48 h of incubation, cells were harvested and washed with cold PBS. Cells were separated by centrifugation at 600×g for 10 min at 4°C. Sub-cellular fractionation was carried out as previously described with some modifications [26]. Cell pellet was resuspended in hypotonic buffer containing 20 mM Tris-HCl, pH 7.4, 5 mM MgCl₂, 5 mM CaCl₂, 1 mM DTT and 1 mM EDTA and protease inhibitor cocktail for 45 min. Cytosolic fraction having microsomes was separated by centrifugation at 2000×g for 30 min at 4°C. The supernatant was removed and then ultra-centrifuged for 3 h at 100,000×g at 4°C to separate the microsomal fraction. The crude nuclear pellet from the low-speed centrifugation was resuspended in ice-cold low salt buffer containing 20 mM Tris-HCl, 5 mM MgCl₂, 2 mM KCl, 1 mM DTT, and 1 mM EDTA with protease inhibitors for 30 min. Then high salt concentration buffer containing 20 mM Tris-HCl, pH 7.4, 5 mM MgCl₂, 1.2 M KCl, 1 mM DTT, 1 mM EDTA and protease inhibitors was added drop wise at 4°C with constant stirring for 30 min and centrifuged for 30 min at 25000×g at 4°C to separate the nuclear matrix from nuclear membranes. All four fractions were concentrated to dryness by vacuum evaporation and reconstituted in 50% ethanol prepared in PBS to deproteinize the sample. After centrifugation at 10000×g for 10 min, supernatants were subjected to HPLC analysis to analyze apigenin content.

Apigenin Binding with DNA

Calf thymus (CT) DNA was prepared in double distilled water adjusted to pH 7.2, sonicated and filtered through a 0.45 μM filter. It was kept stirring for overnight at 4°C to obtain a homogeneous solution of polymerized DNA. Aqueous solution of apigenin was freshly prepared. Experiments were performed in 0.1 M phosphate buffer solution, with pH 7.4. 0.25 μM DNA solutions were prepared having varying concentrations of apigenin ranging from 0.06 mM to 0.4 mM. Absorption spectra of all solution were recorded from 230 nm to 500 nm using NanoDrop 1000.

Spectrum of Calf Thymus DNA Treated with Hydrogen Peroxide and Apigenin

CT-DNA was incubated with hydrogen peroxide (H₂O₂) at physiological pH 7.4. The reaction mixture consisted of 0.25 mM CT-DNA, and various concentration of H₂O₂. In another reaction 0.25 mM CT-DNA, mix with various concentration of apigenin then treated with H₂O₂. The incubation was carried out for 2 h at 37°C. Spectra were recorded with UV-visible spectrophotometer.

Reactive Oxygen Species Measurement

RWPE-1 cells were plated at 1x10⁶ cells per well in 96-well plates in appropriate culture medium. As cells reached to 75–80% confluence subsequently treated with different concentration of
apigenin for 16 h and 200 μM H₂O₂ for 6 h. The treatment medium was removed and cells were washed with phosphate-buffered saline (PBS) and then exposed to PBS containing 10 μM 2′, 7′-dichlorodihydrofluorescein diacetate (DCF-DA), a dye that fluoresces when ROS are generated. The cells were incubated with DCF-DA for 20 min, after which fluorescence intensity was determined using Fluostar Omega Spectrophotometer (BMG Labtech) at 480 nm excitation and 560 nm emission as previously described [27]. The values, expressed in percentage arbitrary fluorescence units, were compared across treatment groups.

8-OHdG Measurement

Measurement of 8-OHdG in cultured cells was performed with OxiSelect Oxidative DNA damage ELISA kit, Cell Biolabs, Inc. (San Diego, CA) as per vendor’s protocol. Briefly, DNA was converted to single stranded DNA and 8-OHdG was quantified by quantitative ELISA assay. The quantity of 8-OHdG in the specimens was determined by comparing its absorbance with known 8-OHdG standard curve as previously described [11].

Flow Cytometry–annexin V Assay

To distinguish the proportion of viable cells from cells undergoing apoptotic death, propidium iodide (PI) and annexin V staining assays were employed. RWPE-1 cells were treated with 10 μM and 20 μM of apigenin for 16 h alone or further incubated for 6 h with 200 μM H₂O₂. Later, the cells were harvested, washed twice with PBS, stained with PI and annexin V and analyzed using FACS cyrometer as described previously [27].

Statistical Analysis

The experiments on cell culture and CT-DNA were repeated at least three times. Results were expressed as mean values ± SD. Statistical comparisons were made by ANOVA followed by a Dunnett’s multiple comparison test. p values <0.05 were considered significant.

Results

Cellular Uptake and Stability of Apigenin

The structure of apigenin is provided in figure 1A. To measure the stability of apigenin during the incubations, apigenin was dissolved in dimethyl sulfoxide, added in the culture medium to attain 20 μM concentrations and incubated with or without PC-3 cells for up to 96 h. More than 70% of apigenin remained in cell culture medium without PC-3 cells after 96 h whereas 30% loss of apigenin may be due to degradation. The concentration of apigenin in the medium of incubation containing PC-3 cells was lower than that of the corresponding incubation without cells which decreased to 76% at 96 h post-incubation (Figure 1B). This difference was probably due to cellular uptake of apigenin. Overall, these studies demonstrate that there is a significant uptake of apigenin by the cells.

Comparison of Apigenin Uptake by Non-tumorigenic and Prostate Cancer Cells

In the next experiment, LNCaP, PC-3 and DU145 prostate cancer cells as well as transformed human prostate epithelial RWPE-1 cells were incubated up to 16 h in the culture medium containing apigenin. At each time point the cells were harvested and extracted, and apigenin levels were measured. The time courses of apigenin uptake by RWPE-1, LNCaP, PC-3 and DU145 cells are shown in figure 2A. The initial uptake of apigenin was rapid up to 2 h, followed by a slower but sustained uptake that reached a plateau after 16 h post-incubation. Furthermore, incubation of 20 μM apigenin for 16 h exhibited an accumulation of 1.48 nmoles/million cells which was higher than in PC-3 and DU145 cells by a factor of 1.89 and 2.71, respectively. Almost similar uptake of apigenin was noted in androgen-responsive LNCaP cells as in RWPE-1 cells (Figure 2B). These results indicate a preferential uptake of apigenin by RWPE-1 and LNCaP cells was higher compared to PC-3 and DU145 cells.

Uptake Kinetics of Apigenin in Human Prostate Cancer LNCaP Cells

Because of higher uptake of apigenin by LNCaP cells, these cells were further studied for absorption kinetics and the results are shown in figure 3. The 6 h time point was selected because it is before the plateau level and within the linear range of apigenin intake in the previous experiment. The kinetics of apigenin uptake by LNCaP cells was saturable and concentration dependent. Apigenin absorption kinetics showed a Kₘ value of approximately 5 μmol/litre and a V₅₀ value of 190 pmoles/h/million cells (Figure 3A). A reciprocal plot between hours of incubation and absorption rate is linear which showed proportionality between these parameters (R² = 0.9061) (Figure 3B).

Sub-cellular Distribution of Apigenin in LNCaP Cells

Next we determined intracellular localization of apigenin within LNCaP cells. The cells were separated into four sub-cellular fractionations by lysis in hypotonic buffer and then differential centrifugation followed by quantitative measurement of apigenin by HPLC. As shown in figure 4, sub-cellular fractionation results exhibit that nuclear matrix retains the highest concentration of apigenin (45.3%), followed by cytosol (23.9%), nuclear membrane (17.9%) and microsomal fraction (12.9%), respectively. This preferential accumulation of apigenin in the nuclear matrix suggests its interaction with the nucleic acids.

Interaction of Apigenin with Calf Thymus DNA

In the next experiment, we determined the interaction of apigenin with calf thymus (CT) DNA. As shown in figure 5A, the absorption spectra of solution containing apigenin, CT-DNA and apigenin+CT-DNA were recorded from 230 nm to 500 nm. The absorbance value of DNA increased at 260 nm upon addition of apigenin and CT-DNA in accordance with the Beer’s Law (Figure 5B & C). This indicates that apigenin might have intercalated between the strands of DNA thereby increasing the absorption of DNA due to the unwinding of DNA double helical structure, which has been previously reported with other plant flavonoids [28–30].

Protection of Oxidative DNA Damage by Apigenin

To determine the antioxidant potential of apigenin, CT-DNA was incubated with increasing concentration of H₂O₂. As shown in figure 6A, incubation with H₂O₂ increased the peak absorbance in a dose-dependent manner. However, the presence of apigenin from 0.2 mM to 0.8 mM prevented H₂O₂-mediated damage to DNA as shown by the restoration of the peak absorbance of DNA (Figure 6B).

Reduction of Oxidative Stress and H₂O₂-mediated Oxidative DNA Damage by Apigenin

Next we determined the protective effect of apigenin from oxidative stress. Exposure of RWPE-1 cells with H₂O₂ caused a significant increase in reactive oxygen species (ROS) generation as...
measured by the addition of DCF-DA in the culture medium, which converts to highly fluorescent dichlorofluorescein in the presence of intracellular ROS. Pretreatment of cells with 10 μM and 20 μM apigenin caused significant decrease in ROS generation \( (p < 0.001) \), compared to \( \text{H}_2\text{O}_2 \)-treated cells \( \text{(Figure 7A)} \). We also determined the levels of 8-OHdG, a hallmark of oxidative stress DNA base damage. As shown in \textbf{figure 7B}, the levels of 8-OHdG in DNA were significantly higher in \( \text{H}_2\text{O}_2 \)-treated cells than in untreated cells or in cells treated with apigenin. Apigenin significantly decrease the levels of 8-OHdG induced by \( \text{H}_2\text{O}_2 \) treatment \( (p < 0.001) \). These results suggest that apigenin has the ability to protect the cells from oxidative-mediated cellular injury.

**Protection of Human Prostate Epithelial Cells from \( \text{H}_2\text{O}_2 \)-induced Cell Death by Apigenin**

Next we examined whether apigenin could decrease \( \text{H}_2\text{O}_2 \) -mediated cellular injury and death of RWPE-1 cells. The cells were treated with 200 μM \( \text{H}_2\text{O}_2 \) for 6 h. As shown in \textbf{figure 8A}, exposure of cells to \( \text{H}_2\text{O}_2 \) resulted in 71.4% increase in annexin V staining demonstrating increase oxidative stress-mediated apoptosis. To confirm the protective effect of apigenin, the cells were treated with 10 μM and 20 μM apigenin for 16 h and later exposed to 200 μM \( \text{H}_2\text{O}_2 \) for 6 h. Treatment of RWPE-1 cells with apigenin resulted in a marked decrease in \( \text{H}_2\text{O}_2 \)-mediated apoptotic cell death \( \text{(Figure 8B)} \). Treatment with apigenin alone did not induce substantial apoptosis in these cells. Overall, these results suggest that apigenin has the ability to protect prostate epithelial cells from \( \text{H}_2\text{O}_2 \)-mediated cellular injury and apoptosis.

**Discussion**

In this study we explored cellular uptake of apigenin in transformed human prostate epithelial cells and various prostate cancer cells. We evaluated its sub-cellular distribution, DNA binding activity, and quenching of \( \text{H}_2\text{O}_2 \)-induced ROS and oxidative stress in \textit{in vivo} cell cultures and \textit{in vitro} systems, using calf thymus DNA. Our results, for the first time, demonstrate that apigenin preferentially accumulates in the nuclear matrix, binds with the DNA to reduce oxidative DNA damage and apoptosis in prostate epithelial cells.

Reported studies to date indicate that frequent consumption of plant-based food products rich in flavones may be beneficial in
reducing the risk of prostate cancer [31]. Apigenin, a plant flavone, has received considerable attention due to its wide distribution in the plant kingdom and because of its health benefits and chemopreventive properties [16 and references therein]. Many studies have demonstrated that apigenin possesses a wide range of biological activities, including anticancer, antiviral, antibacterial, antioxidant and anti-inflammatory effects [16–20]. These biological activities are considered to be related to its intracellular distribution and interaction with several biological targets. In our studies, apigenin accumulation in human prostate cancer cells is in the following order of magnitude: LNCaP, PC-3, DU145 cells. The highest level of apigenin accumulation occurs in transformed human prostate epithelial RWPE-1 cells. These results indicate that apigenin preferentially accumulates in cells which possess functional androgen receptor (AR). Furthermore, studies demonstrate that androgens, via the androgen receptor, induce oxidative stress in normal and prostate cancer cells [32,33]. Androgens modulate the production of ROS via both the induction of fatty acid oxidation in the mitochondria and via the induction of NADPH oxidase activity [34]. Our previous studies demonstrate that high caloric intake increases oxidative stress in the mouse prostate via the NOX family of ROS-generating NADPH oxidases and sustained activation of NF-κB and STAT-3 transcription factors [35,36]. In the present study, apigenin accumulation in cells with functional AR may have the potential to interfere with AR signaling. Previous studies have demonstrated that apigenin interferes with AR signaling and inhibits androgen-responsive genes [37]. However, further studies are needed to clarify the interactions between AR and apigenin.

The absorption and bioavailability of flavonoids remain critical issue in evaluating its cancer preventive effects. We determined how cellular uptake of apigenin changes in human prostate cancer LNCaP cells by exposing them to various concentration of apigenin up to 40 μM. The Michaels-Menton kinetics of cellular uptake was characterized by saturation at high apigenin concentration suggesting that the process of apigenin uptake by LNCaP cells might be through passive diffusion. The K_m value for the uptake of apigenin is high, relative to the concentration obtained in human and mouse plasma, which indicates that apigenin has low binding affinity with plasma proteins. Thus far, there are no reports of apigenin receptors or binding proteins that facilitate its cellular uptake.

Growing evidence suggests that chronic inflammation with low levels of reactive oxygen species (ROS) production plays an important role in causing DNA damage and development of cancer [8,9]. Reactive oxygen species oxidize DNA bases, leading to mutation and DNA hypermethylation [10,11]. Reactive oxygen species induce peroxide formation in membrane lipid molecules, altering the physiochemical properties of membranes and damage membrane-bound proteins and other macromolecules. In addition, reactive oxygen species exert deleterious chemical effects on proteins that can alter normal cellular functions. Our previous findings in a prospective 5-year follow-up study in needle biopsy specimens demonstrate a strong association between chronic prostatic inflammation, premalignant, and malignant changes in

Figure 3. Kinetics of apigenin uptake by human prostate cancer LNCaP cells. (A) Dose-dependent kinetics of apigenin uptake in LNCaP cells incubated for 6 h with apigenin at concentration ranging from 1.25 μM to 40 μM. The rate of apigenin uptake was measured as (cellular apigenin) million cells−1 h−1. Points±SD of experiments performed three times. (B) Data evaluated using Michaelis-Menten kinetics by constructing a reciprocal plot between 1/C and 1/V. Details are described in materials and methods section. doi:10.1371/journal.pone.0091588.g003

Figure 4. Sub-cellular distribution of apigenin in human prostate cancer LNCaP cells. The cells were incubated with 20 μM apigenin for 48 h with approximately 5×10^6 cells and processed for different fractions. Bars±SD of experiments performed three times. Distribution is represented as 100% apigenin in all the fractions. Details are described in materials and methods section. doi:10.1371/journal.pone.0091588.g004
the prostatic epithelium [9]. Mechanistically, inflammatory cells are drawn to the site of inflammation and consequently myeloperoxidase and phagocytic NADPH oxidase derived ROS are released. Parallel secretion of inflammatory cytokines acerbates inflammatory process via NF-κB and STAT-3 activation and favors cellular ROS formation [38]. These pro-oxidative changes in the prostate microenvironment in combination with genetic susceptibility such as defects in encoding for GSTP1 and DNA repair enzymes may contribute to initiation of prostate carcinogenesis [39]. In the present study we demonstrate that apigenin exposure significantly quenches ROS generation and protects prostate epithelial cells from oxidative DNA damage, and may
thereby inhibit carcinogenesis. Furthermore, we have shown that apigenin suppresses NF-κB activation. Additional studies are needed to precisely evaluate the effects of apigenin in decreasing inflammatory mediators and epigenetic modification.

Oxidative stress initiates DNA modification and mutagenic lesions that contribute to pathologic processes in various diseases, including cancer [40–42]. 8-hydroxy-2'-deoxyguanosine (8-OHdG) is a major base product that is formed after an oxidative insult to DNA [43]. Large amounts of 8-OHdG are produced in mammalian cells, either as a by-product of normal oxidative metabolism or as a result of exogenous sources of ROS. Studies have shown that 42% of men aged 55–80 years exhibit prostatic DNA damage, reflected by levels of 8-OHdG, which results from oxidative modification of guanine [2,44]. Oxidative damage to the DNA base leads to a point mutation by an A→T substitution when incorporated into DNA [45]. It has been demonstrated that hydroxyl radical (OH•), singlet oxygen (O2•), or peroxinitrite anion (ONOO•) is responsible for the formation of 8-OHdG. Levels of 8-OHdG in tissues may increase either because there is a strong DNA damaging stimulus or because one of the specific DNA repair mechanism is deficient [4–8]. Our studies demonstrate that apigenin protects against oxidative DNA damage.
Further studies are needed to clarify the mechanistic pathways responsible for this effect.

There is now considerable published scientific data regarding the interaction of plant flavonoids with various proteins and lipids [28–30,46,47]. Our work on spectroscopic study of the interaction of plant flavones with various proteins and lipids in vitro suggests that classic intercalation is the dominant binding mode and may affect reactions associated with enzymes on DNA molecules. In particular, apigenin has been shown to inhibit the activities of various proteins attached to DNA, such as DNA polymerase, cAMP-response element binding proteins, DNA topoisomerase, and histone deacetylases [48-51]. Ours is the first study demonstrating the sub-cellular distribution of apigenin, documenting its interactions with DNA and elucidating its role in inhibiting oxidative stress within cells. Although several mechanisms by which apigenin might prevent prostate cancer have been demonstrated and/or are under investigation, our data are consistent with the concept that its antioxidant activity in the nucleus accounts for its documented capacity to serve in the chemoprevention of prostate cancer.

Author Contributions

Conceived and designed the experiments: HS RK SG. Performed the experiments: HS RK NB. Analyzed the data: HS RK SG. Wrote the paper: RK SG.

References

1. American Cancer Society. Cancer Facts and Figures 2012. Available: http://www.cancer.org/Cancer/ProstateCancer/index. Accessed 23 Dec 2013.

2. Malins DC, Johnson PM, Wheeler TM, Barker EA, Polissar NL, et al. (2001) Voinson MA. Age-related radical-induced DNA damage is linked to prostate cancer. Cancer Res 61: 6025–6028.

3. Miyake H, Hara I, Kamidono S, Eto H (2004) Oxidative damage in patients with prostate cancer and its response to treatment. J Urol 171: 1533–1536.

4. Bostwick DG, Alexander EE, Singh R, Shan A, Qian J, et al. (2000) Santella RM, Oberley LW, Yau T, Zhong W, Jiang X, Oberley TD. Antioxidant enzyme expression and antioxidant species damage in prostatic intraepithelial neoplasia and cancer. Cancer 89: 123–134.

5. Kuanse H, Rodrigues IS, Los-Gruenewaroski R, Reis MB, Fugani PE, et al. (2013) Baseline serum protein markers: a new diagnostic tool for prostate cancer. Int J Clin Pract 67: 1365–1371.

6. Yeh CC, Lee C, Daihya R (2001) DNA mismatch repair enzyme activity and gene expression in prostate cancer. Biochem Biophys Res Commun 285: 409–413.

7. Battini V, Maders LD, Bagatini MD, Oeztug GL, Chiesa J, et al. (2011) Oxidative stress and antioxidant status in prostate cancer patients: relation to Gleason score, treatment, and bone metastasis. Biomed Pharmacother 65: 516–524.

8. Arsov-Sarafinovska Z, Eken A, Matevski N, Ezermed O, Sayal A, et al. (2009) Increased oxidative/nitrosative stress and decreased antioxidant enzyme activities in prostate cancer. Clin Biochem 42: 1229–1235.

9. MacKinnon GT, Eisenberg R, Fleshman RL, Taylor JM, Fu P, et al. (2006) The influence of chronic inflammation in prostate carcinogenesis: a 5-year follow-up study. J Urol 176: 1012–1016.

10. Lim KS, Taghizadeh K, Wiedow JS, Babi R, Shafiee A, et al. (2012) Sequence-dependent variation in the reactivity of 8-OH-dG and 2-deoxyguanosine toward oxidation. Chem Res Toxicol 25: 366–373.

11. Kanwal R, Pandey M, Bhaskaran N, Mcleman GT, Fu P, et al. (2014) Protection against oxidative DNA damage and stress in human prostate by apigenin-rich parsley. Mol Cancer 13: 8–18.

12. Chen L, Stacey-Sawczuk M, Duncan C, Shariat R, Ghoos L, et al. (2001) Oxidative DNA damage in prostate cancer patients consuming tomato sauce-based entrees as a whole-food intervention. J Natl Cancer Inst 93: 1872–1879.

13. Jin BH, Qian LB, Chen S, Li J, Wang HP, et al. (2009) Protective role of apigenin on the status of lipid peroxidation and antioxidant defense against hepatocarcinogenesis in Wistar albino rats. Phytomedicine 16: 369–374.

14. Hege HL, Prashanth SN, Seetharamappa J (2012) Interaction of antioxidant flavonoids with calf thymus DNA analyzed by spectroscopic and electrochemical methods. J Pharm Biomed Anal 63: 40–46.

15. Ramoselos DF, Selmaj OW (2003) Flavourings and cancer prevention: a review of the evidence. J Nutr Gerontol Geriatr 31: 206–238.

16. Ripple MO, Henry WF, Rago RP, Wilding G (1997) Prooxidant-antioxidant shift induced by androgen treatment of human prostate carcinoma cells. J Natl Cancer Inst 89: 40–48.

17. Whelan KF, Lu JP, Fridman E, Wolf A, Honig A, et al. (2009) Apigenin inhibits oxidative stress-induced macromolecular damage in N-Phosphoryl ribose 1 ADP-ribosyltransferase-1 deficient human colorectal carcinoma cell line SW480. Biochem Pharmacol 78: 2879–2885.

18. Nakanishi N, Shukla S, Kanwal R, Srivastava JK, Gupta S (2012) Increase of active oxygen-1 enzyme activity in the nucleus accounts for its documented capacity to serve in the chemoprevention of prostate cancer.

19. Janjua NK, Siddiqui A, Qasab R, Qureshi S, Qureshi R, et al. (2009) Spectrophotometric analysis of flavonoid-DNA binding interactions at physio- logical conditions. Spectrochim Acta A Mol Biomol Spectrosc 74: 1135–1137.

20. Huang L, Zhang J, Qiu X, Hu Q, Wang H, et al. (2009) Apigenin protects prostate cancer xenografts from tumor necrosis factor-alpha-induced apoptosis. Anticancer Res 29: 233–240.

21. Zhang S, Qin C, Safe SH (2003) Flavonoids as aryl hydrocarbon receptor agonists/antagonists: effects of structure and cell context. Environ Health Perspect 111: 1877–1882.

22. Gradelotto A, Bady JP, Berges R, Tressy L, Chagnon MC, et al. (2005) Pharmacokinetics and metabolism of apigenin in female and male rats after a single oral administration. Drug Metab Dispos 33: 49–54.

23. Jain BH, Qian L, Chen S, Wang HP, et al. (2009) Apigenin protects endothelium-dependent relaxation of rat aorta against oxidative stress. Eur J Pharmacol 616: 200–205.

24. Singh JP, Selvendiran K, Banu SM, Padmavathi R, Sakhthakesaran D (2009) Protective role of apigenin on the status of lipid peroxidation and antioxidant defense against hepatocarcinogenesis in Wistar albino rats. Phytomedicine 16: 369–374.

25. Meyer H, Boloriniw A, Wolfam G, Lemosien J (2006) Bioavailability of apigenin and luteolin from apiin-rich parsley in humans. Ann Nutr Metab 50: 167–172.

26. Lai A, Pajovic N, Pang Y, Zhu D, Calaminin B (2006) Absorption and subcellular localization of lycopene in human prostate cancer cells. Mol Cancer Ther 5: 2079–2085.

27. Bhaskaran N, Shukla S, Kanwal R, Srivastava JK, Gupta S (2012) Oxidative stress in the nucleus accounts for its documented capacity to serve in the chemoprevention of prostate cancer.

28. Li SF, Ma Y, Wang Y, Msida M, Lin J, et al. (2010) Apigenin reduces oxidative DNA damage. Mutagenesis 25: 814–819.

29. Jayasooriya RG, Kang SH, Kang CH, Choi YH, Moon DO, et al. (2012) Apigenin decreases cell viability and telomerase activity in human leukemia cell lines. Food Chem Toxicol 50: 2605–2611.

30. Zou Y, Gao Q, Wang X, Wu Q, Yang Q, et al. (2012) Antioxidant activity of apigenin in the nucleus accounts for its documented capacity to serve in the chemoprevention of prostate cancer.
Apigenin Reduces Oxidative DNA Damage

Kuzuhara T, Yagi Y, Yamaguchi K, Suganuma M, Fujiki H (2006) DNA and RNA as new binding targets of green tea catechins. J Biol Chem 281: 17446–17456.

Ohno S, Shinoda S, Toyoshima S, Nakazawa H, Makino T, et al. (2002) Effects of flavonoid phytochemicals on cortisol production and on activities of steroidogenic enzymes in human adrenocortical H295R cells. J Steroid Biochem Mol Biol 80: 355–363.

Constantinou A, Mehta R, Runyan C, Rao K, Vaughan A, et al. (1995) Flavonoids as DNA topoisomerase antagonists and poisons: structure-activity relationships. J Nat Prod 58: 217–223.

Pandey M, Kasr P, Shaila S, Abbas A, Fu P, et al. (2012) Plant flavone apigenin inhibits HDAC and remodels chromatin to induce growth arrest and apoptosis in human prostate cancer cells: in vitro and in vivo study. Mol Carcinog 51: 952–962.