CXCR4 Is a Novel Target of Cancer Chemopreventative Isothiocyanates in Prostate Cancer Cells

Kozue Sako1, Avani R. Vyas2, Sreenivasa R. Chinni3, Ali I. Amjad4, Rahul Parikh4, and Shivendra V. Singh2

Abstract

Isothiocyanates (ITCs) derived from cruciferous vegetables, including phenethyl isothiocyanate (PEITC) and sulforaphane (SFN), exhibit in vivo activity against prostate cancer in a xenograft and transgenic mouse model, and thus are appealing for chemoprevention of this disease. Watercress constituent PEITC and SFN-rich broccoli sprout extract are under clinical investigations but the molecular mechanisms underlying their cancer chemopreventive effects are not fully understood. The present study demonstrates that chemokine receptor CXCR4 is a novel target of ITCs in prostate cancer cells. Exposure of prostate cancer cells (LNCaP, 22Rv1, C4-2, and PC-3) to pharmacologically applicable concentrations of PEITC, benzyl isothiocyanate (BITC), and SFN (2.5 and 5 μM/L) resulted in downregulation of CXCR4 expression. None of the ITCs affected secretion of CXCR4 ligand (stromal-derived factor-1). In vivo inhibition of PC-3 xenograft growth upon PEITC treatment was associated with a significant decrease in CXCR4 protein level. A similar trend was discernible in the tumors from SFN-treated TRAMP mice compared with those of control mice, but the difference was not significant. Stable overexpression of CXCR4 in PC-3 cells conferred significant protection against wound healing, cell migration, and cell viability inhibition by ITCs. Inhibition of cell migration resulting from PEITC and BITC exposure was significantly augmented by RNAi of CXCR4. This study demonstrates, for the first time, that cancer chemopreventive ITCs suppress CXCR4 expression in prostate cancer cells in vitro as well as in vivo. These results suggest that CXCR4 downregulation may be an important pharmacodynamic biomarker of cancer chemopreventive ITCs in prostate adenocarcinoma. Cancer Prev Res; 8(5); 365–74. ©2015 AACR.

Introduction

Cancer chemoprevention with edible plants and/or their bioactive constituents is appealing because of their safety, epidemiologic evidence of risk reduction, preclinical indication of preventive efficacy, and cost-effectiveness. Cruciferous vegetables are a rich source of cancer chemopreventive phytochemicals collectively known as isothiocyanates (ITCs; refs. 1, 2). Cancer chemoprevention by ITCs, which occur naturally as thioglucoside conjugates in widely consumed vegetables such as watercress, garden cress, mustard, and broccoli, was first documented by Wattenberg more than three decades ago (3). Phenethyl isothiocyanate (PEITC) abundant in watercress and the garden cress constituent benzyl isothiocyanate (BITC) were shown to inhibit breast cancer induced by 7,12-dimethylbenz[a]anthracene in rats when administered 4 hour before the carcinogen treatment (3). Since then, the chemopreventive activity of ITCs was extended against other chemical carcinogens (1, 2). Epidemiologic association of cancer risk reduction with increasing intake of cruciferous vegetables provides additional support for their chemopreventive effect (4–6). PEITC and BITC have been studied extensively for their anticancer preventive efficacy in preclinical models as well as mechanistic characterization (1, 2). The ClinicalTrials.gov lists three completed or ongoing clinical trials on PEITC or watercress juice. On the other hand, preclinical efficacy and mechanistic studies on SFN have primarily focused on the synthetic racemic (D,L-SFN) analogue of naturally occurring L-isomer (2). Majority of the ongoing or completed clinical trials on SFN in healthy volunteers or cancer patients have used standardized broccoli sprout extract.

Our interest in ITCs was initially sparked by epidemiologic studies suggesting an inverse relationship between intake of cruciferous vegetables and the risk of prostate cancer (7, 8). Prostate adenocarcinoma remains a leading cause of cancer mortality among American men despite a comprehensive understanding of the underlying biology, genomic landscape, and the risk factors (9–12). Population-based evidence prompted us to test the efficacy of PEITC and SFN for prevention of prostate cancer using a transgenic mouse model (Transgenic Adenocarcinoma of Mouse Prostate; TRAMP; refs. 13–15). Dietary administration of
PEITC (3 μmol/g diet) for 19 weeks to male TRAMP mice resulted in a statistically significant decrease in the incidence of poorly differentiated prostate cancer when compared with mice fed with basal diet (13). Oral SFN administration (6 μmol/mouse three times per week) inhibited the incidence of prostatic intraepithelial neoplasia and well-differentiated cancer, but not poorly differentiated cancer in TRAMP mice (14). However, inhibition of poorly differentiated prostate cancer by oral SFN was achievable with coadministration of an autophagy inhibitor (chloroquine) as SFN is known to induce cytoprotective autophagy (15, 16). SFN was also effective in preventing pulmonary metastasis in the TRAMP mouse model (14). Feeding of 240 mg of broccoli sprout/day to TRAMP mice exhibited retardation of prostate tumor growth (17). The growth of PC-3 and LNCaP human prostate cancer cells implanted in athymic mice was retarded significantly by PEITC or SFN administration (18–20). PEITC also exhibited in vivo growth-inhibitory activity against a cell line (TRAMP-C1) derived from a TRAMP tumor (21).

PEITC and SFN have been the focus of intense mechanistic studies to gain insights into the biology of prostate cancer chemoprevention by these agents (2, 18, 19, 21–24). Mechanisms of prostate adenocarcinoma chemoprevention by PEITC and SFN include apoptotic or autophagic cell death induction (autophagy is cytoprotective for SFN), suppression of oncogenic pathways (e.g., NF-κB), and inhibition of cell proliferation (2, 18, 19, 21–24). The present study explores the role of chemokine receptor CXCR4, which is implicated in prostate cancer progression and metastasis (25) in anticancer effects of PEITC, BITC, and SFN using human prostate cancer cells.

Materials and Methods

Ethics statement

The use of mice for the in vivo studies was approved by the University of Pittsburgh (Pittsburgh, PA) Institutional Animal Care and Use Committee. Prostate tumor tissues from our previously published studies (15, 26) were used to determine the in vivo effect of PEITC and SFN administration on CXCR4 protein expression. For the in vivo xenograft experiment with PEITC, PC-3 cells stably expressing luciferase were injected immediately before use. LNCaP, C4-2, 22Rv1, and PC-3 human prostate cancer cells were acquired from the ATCC or UroCor and last authenticated in 2011 or 2012. Each cell line was found to be of human origin and free of pathogen contamination. PC-3 cells stably transfected with CXCR4 plasmid (hereafter abbreviated as CXCR4-PC-3) or empty vector (Neo_PC-3) have been described previously (27).

Western blotting

After treatment, cells were collected and lysed as described by us previously (28). TRAMP tumor tissues were processed as previously described (13). Proteins were separated by SDS-PAGE and transferred onto a membrane. Western blotting was performed as described previously (28) except that dilution of CXCR4 antibody and membrane exposure time were optimized. Enhanced chemiluminescence reagent was used for immunodetection of the band.

Immunofluorescence microscopy

LNCaP or PC-3 cells were plated on coverslips in 12-well plates, allowed to attach overnight, and then exposed to PEITC, BITC, SFN, or DMSO (control) for 24 hours. After washing with BD Perm/Wash buffer, cells were fixed with 4% formaldehyde at room temperature for 1 hour. After washing with BD Perm/Wash buffer, Alexa Fluor 488-conjugated secondary antibody was added (1 hour at room temperature). Subsequently, cells were washed and treated with DAPI (10 ng/ml) for 5 minutes at room temperature to stain nuclear DNA. Cells were washed with PBS and examined under a fluorescence microscope at ×100 objective magnification.

RT-PCR and quantitative real-time PCR

Total RNA from DMSO-treated control cells or those treated with the desired ITCs was isolated using RNeasy kit. The cDNA was synthesized with the use of SuperScript III reverse transcriptase and oligo(dT)20 primer. PCR was performed using specific primers: CXCR4 forward: 5′-GGAGCTGTTGGCTGAAGG-3′ and CXCR4 reverse: 5′-GAGCTGATGGCAGTCCAAAT-3′ (PCR product size, 345 bp); with the following amplification conditions: 94°C for 5 minutes, 28 cycles at 94°C for 15 seconds, 54°C for 30 seconds, 72°C for 1 minute, and extension at 72°C for 10 minutes. qPCR was done using SYBR Green master mix (Applied Biosystems-Life Technologies) under same conditions, with the number of cycles changed to 40. Relative gene expression was calculated using the method described by Livak and Schmittgen (29).

Measurement of CXCL12 secretion

Analysis of CXCL12 secretion was performed using a commercially available kit. PC-3 cells were plated into 6-well plates, allowed to attach, and then exposed to PEITC, BITC, SFN, or DMSO (control) for 12 or 24 hours. Conditioned medium was collected and spun down to remove debris and stored at −80°C. CXCL12 was measured in the medium by an immunoassay kit from R&D Systems. The concentration in the medium was calculated using a standard curve from serially diluted CXCL12 provided with the kit.
**RNAi**

PC-3 cells were seeded in 6-well plates and transfected at 70% confluence with a nonspecific (control) siRNA or CXCR4-targeted siRNA (100 or 200 nmol) using Oligofectamine. Twenty-four hours posttransfection, cells were treated with DMSO or the test agent for an additional 24 hours. Cells were collected and processed for immunoblotting, cell migration, or wound-healing assay. Knockdown of CXCR4 was confirmed by western blotting.

**Wound-healing assay**

PC-3 cells stably transfected with either CXCR4 plasmid or empty vector were seeded in 6-well plates. For PC-3 cells transiently transfected with CXCR4 siRNA, 1 × 10⁵ cells were placed in 48-well plates, and allowed to attach overnight. Cells were transfected with 200 nmol/L control or CXCR4 siRNA for 24 hours. A wound in the confluent monolayer culture was created by scratching with a pipette tip. The wounded cells were washed with PBS and incubated with F12K medium containing 1% FBS, puromycin (1 µg/mL), 1 mmol/L thymidine, and desired concentration of PEITC, BITC, or SFN. Cells were allowed to migrate for 10 hours, fixed with methanol, and stained with Giemsa staining solution. Migration of cells was quantified by measuring distances between the borders of cells using Image J software. At least three non-overlapping areas per well were examined for wound healing.

**Cell migration assay**

PC-3 cells transfected with a control (nonspecific) siRNA or CXCR4-targeted siRNA or Neo_PC-3 or CXCR4_PC-3 cells were suspended in serum-free medium containing DMSO or the test agent (PEITC or BITC) and placed in the upper compartment of the Transwell chamber. After 24 hours of incubation, nonmotile cells from the upper surface of the filter were removed using a cotton swab. The motile cells from the bottom face of the filter were fixed with methanol and stained with hematoxylin and eosin. At least five randomly selected areas were scored for cell migration.

**Cell viability assay**

Cell viability was determined by Trypan blue dye exclusion assay as described by us previously (30).

### Figure 1.

ITCs downregulated CXCR4 protein level in prostate cancer cells. A, structures of PEITC, BITC, and SFN. B, Western blot analyses showing effect of ITC treatment on CXCR4 protein level in LNCaP, 22Rv1, and C4-2 cells. GAPDH was probed as a loading control. C, immunofluorescence microscopy for effect of ITC treatment (5 µmol/L, 24 hours) on CXCR4 protein level in LNCaP cells. Western blotting was performed two to five times using independently prepared lysates. Data on effect of SFN in 22Rv1 cell were inconsistent.
Statistical analysis

One way ANOVA with Dunnett adjustment was used to determine statistical significance of difference for dose–response studies, whereas Bonferroni test was used for multiple comparisons (e.g., between Neo_PC-3 and CXCR4_PC-3 cells). Unpaired Student t test was used for binary comparisons.

Results

ITCs downregulated CXCR4 expression in prostate cancer cells

We used androgen-sensitive (LNCaP) and androgen-independent human prostate cancer cells (C4-2 and PC-3) and pharmacologically relevant concentrations of PEITC, BITC, and SFN (Fig. 1A) to determine their effect on CXCR4 protein. Level of CXCR4 protein was decreased after treatment with all three compounds in LNCaP cells (Fig. 1B). In LNCaP cells, this effect was generally more pronounced at the 5 μmol/L ITC dose. Suppression of CXCR4 protein after treatment with PEITC, BITC, and SFN was also evident in 22Rv1 and C4-2 cells (Fig. 1B). However, cell linespecific differences were also observed in the extent and kinetics of downregulation with each ITC compound. Data for 22Rv1 with SFN were also variable in five different experiments. Immunofluorescence microscopy confirmed ITC-mediated downregulation of the CXCR4 protein expression in LNCaP cells (Fig. 1C).

As can be seen in Fig. 2A, CXCR4 protein level was markedly decreased upon treatment with PEITC, BITC, and SFN in PC-3 cells. For PEITC, a 6-hour time point was also included but the results were inconsistent (results not shown). Immunofluorescence microscopy confirmed ITC-mediated downregulation of the CXCR4 protein expression in PC-3 cells (Fig. 2B).
suppression of CXCR4 protein was attributed to the transcriptional inhibition as revealed by RT-PCR (Fig. 2C) and quantitative real-time PCR (Supplementary Fig. S1).

**ITCs had no effect on CXCL12 expression or secretion**

We next determined the effect of ITCs on expression and secretion of CXCR4 ligand CXCL12. Expression of CXCL12 was not affected by PEITC, BITC, or SFN at least in PC-3 cells as determined by immunofluorescence microscopy (data not shown). As shown in Fig. 2D, secretion of CXCL12 was not affected either except for a modest decrease by BITC at the 24-hour time point. Similarly, SFN treatment failed to alter CXCL12 secretion in PC-3 cells (data not shown). Collectively, these results indicated transcriptional suppression of CXCR4 by aromatic ITCs (PEITC and BITC) and a thioalkyl ITC (SFN).

**Effects on PEITC and SFN administration on tumor CXCR4 protein level in vivo**

In a PC-3 xenograft study with five times/week oral administration of 9 μmol PEITC, the average tumor volume (mean ± SD) in control mice (879.2 ± 284.5) was about 1.9-fold higher in control mice (879.2 ± 284.5) compared with PEITC-treated mice (P = 0.068; ref. 26). Fresh-frozen tumor tissues from a second experiment of this study with 6 μmol PEITC/mouse (26) were used to determine the effect of PEITC administration on CXCR4 protein level in vivo. As shown in Fig. 3A, CXCR4 protein was detectable in most of the control PC-3 xenografts. The level of CXCR4 protein was lower by about 74% in the PC-3 xenografts from PEITC-treated mice when compared with controls with a P = 0.03 by unpaired Student t test (n = 7 for control and n = 8 for PEITC treatment group; Fig. 3B). We have shown previously that the incidence of well-differentiated cancer in the dorsolateral prostate of TRAMP mice is about three times/week (15). Western blotting for CXCR4 protein also showed its suppression in the tumors from SFN-treated TRAMP mice relative to control (Fig. 3C) but the difference was inconclusive (data not shown). We also determined the effect of CXCR4 knockdown in PC-3 cells using a siRNA (Fig. 4A) on wound-healing inhibition by PEITC and BITC (Fig. 4D). The wound-healing inhibition by PEITC, but not BITC was significantly augmented by CXCR4 knockdown. Difference between PEITC and BITC is not surprising as these compounds are known to exhibit mechanistic differences despite close structural similarity (1, 2).

**RNAi of CXCR4 augmented PEITC- and BITC-mediated inhibition of PC-3 cell migration**

Figure 5A depicts PC-3 cell migration that was decreased by about 28% (P < 0.05) by CXCR4 knockdown alone in the absence of PEITC treatment. Exposure to PEITC also resulted in a significant inhibition of migration capacity of PC-3 cells (Fig. 5B). PEITC-mediated inhibition of PC-3 cell migration was modestly but statistically significantly augmented by RNAi of CXCR4 but this effect was relatively more pronounced for BITC (Fig. 5B).

**Inhibition of cell migration and cell viability by ITCs in PC-3 cells was attenuated by ectopic expression of CXCR4**

Cell migration was more intense for CXCR4-overexpressing cells compared with Neo_PC-3 cells (Fig. 5C). PEITC treatment decreased cell migration in Neo_PC-3 cells but this effect was ablated in CXCR4-overexpressing cells (Fig. 5D). CXCR4 overexpression also conferred partial but significant protection against BITC-mediated inhibition of PC-3 cell migration (Fig. 5D). Western blotting for CXCR4 was performed using lysates from Neo_PC-3 and CXCR4_PC-3 cells after 24-hour treatment with
DMSO (control) or 2.5 and 5 μmol/L PEITC. Generally consistent with the results in un-transfected PC-3 cells (Fig. 2A), expression of CXCR4 protein was decreased by 50% to 70% after PEITC treatment in Neo_PC-3 cells (Fig. 6A). PEITC treatment was relatively less effective in downregulating CXCR4 protein in CXCR4_PC-3 cells in comparison with Neo_PC-3 cells (Fig. 6A). Viability of Neo_PC-3 cells was significantly decreased after 24-hour treatment with PEITC, BITC, and SFN; albeit in the order of PEITC > BITC > SFN (Fig. 6B). Similar to cell migration assay, CXCR4 overexpression conferred significant protection against cell viability inhibition by each compound (Fig. 6B). Previous studies have shown that CXCR4/CXCL12 signaling activates AKT, which is a prosurvival signal (31). In agreement with these results, S473 phosphorylation of AKT was >2-fold higher in CXCR4_PC-3 cells when compared with empty vector transfected control cells (Fig. 6C). Phosphorylation of AKT was decreased to varying extent upon treatment of CXCR4_PC-3 and Neo_PC-3 cells with each compound. A modest attenuation of pAKT suppression upon CXCR4 overexpression in the presence of 2.5 μmol/L PEITC or BITC was not consistent in different experiments. Previous studies have also shown ERK activation in CXCR4 signaling (25). Level of phospho-ERK2 was modestly higher in CXCR4_PC-3 cells than in Neo_PC-3 (Fig. 6D), but ERK activation was not consistently affected by ITCs in either cell type (Fig. 6D). These results indicated that CXCR4-mediated

Figure 4. Stable overexpression of CXCR4 conferred protection against wound-healing inhibition by PEITC and BITC. A, Western blot analyses show overexpression (left) or knockdown (right) of CXCR4 protein in PC-3 cells. B, results of scratch assay showing the effect of PEITC and BITC treatments (10-hour treatment) on wound healing in PC-3 cells transfected with CXCR4 plasmid or empty vector. C, quantitation of wound healing. The results shown (mean ± SD) are representative of two independent experiments (n = 3). Significantly different compared with *corresponding control, and †between Neo_PC-3 and CXCR4_PC-3 cells by one-way ANOVA followed by Bonferroni test. D, bar graphs showing effect of CXCR4 knockdown using a siRNA on wound-healing inhibition by PEITC or BITC. The results shown are mean ± SD (n = 2). Significantly different compared with *corresponding control and †between control siRNA and CXCR4 siRNA by one-way ANOVA followed by Bonferroni test.
protection against cell proliferation inhibition by ITCs was independent of AKT or ERK activation.

Discussion

The present study reveals that CXCR4 is a novel target of widely-studied cancer chemopreventive ITCs at least in prostate cancer cells. Despite a close structural similarity between PEITC and BITC (Fig. 1A), marked differences have been documented in their cancer protective effect as well as associated mechanisms (2, 3). However, CXCR4 seems to be a common target of both these compounds as well as for a commonly studied thioalkyl-type ITC. We also show that CXCR4 downregulation by ITCs is not a cell line-specific effect as both androgen-sensitive and androgen-independent cells are susceptible to CXCR4 downregulation by ITCs. It is important to point out that downregulation of CXCR4 protein upon 48-hour treatment of PC-3 and DU145 cells with 10 μmol/L SFN was documented previously but functional significance of its suppression was not studied (32). Since submission of this manuscript, BITC-mediated downregulation of CXCR4 was shown in a human glioma cell line but the functional significance of this observation was not studied (33). Even though expression of CXCR4 mRNA is decreased after treatment with ITCs (Fig. 2C), the upstream mediator(s) of transcriptional repression remain unknown. It is plausible that CXCR4 downregulation by ITCs is mediated by NF-κB as this transcription factor, which is a known regulator of CXCR4 expression, is inhibited by ITC treatment in prostate cancer cells (22, 32). Recent studies have also implicated ERG (ETS-related gene) in transcriptional regulation of CXCR4 (34, 35), and the possibility of ERG suppression by ITCs needs to be explored in future studies. In this context, it is interesting to note that the androgen-sensitive LNCaP cell line is relatively more sensitive to CXCR4 downregulation by ITCs compared with PC-3 cells especially at the...
may be required for intense SFN dosing regimen (e.g., daily treatment or higher dose) treated TRAMP mice, but the difference did not reach statistical significance. A similar trend is observed from prostate tumors of SFN-treated mice clearly exhibit statistically significant downregulation of CXCR4 protein following treatment with PEITC (&lt;3.5 μmol/L) and SFN is also observed in prostate cancer cells after PEITC and/or SFN treatment have also been documented (36, 37). Other possible molecular mediators include hypoxia-inducible factor 1α, Ets1, CREB3, Krüppel-like factor 2, and miRNA 494-3p (38–42). However, more work is needed to determine which of these mechanism(s) accounts for ITC-mediated downregulation of CXCR4.

Downregulation of CXCR4 protein following treatment with PEITC and SFN is also observed in vivo. The PC-3 xenografts from PEITC-treated mice clearly exhibit statistically significant decrease in CXCR4 protein expression in comparison with control. A similar trend is observed from prostate tumors of SFN-treated TRAMP mice, but the difference did not reach statistical significance due to small sample size. It is also possible that a more intense SFN dosing regimen (e.g., daily treatment or higher dose) may be required for in vivo suppression of CXCR4 expression.

Nevertheless, these results are encouraging and suggest that CXCR4 may be a predictive pharmacodynamic biomarker of ITCs in prostate adenocarcinoma.

Several studies have evaluated CXCR4 overexpression in prostate and other tumors (25, 43, 44). Analysis of CXCR4 expression in humans using high-density tissue microarrays from a cohort of over 600 patients revealed significantly elevated levels of this chemokine receptor in localized cancer and metastatic disease (43). High expression of CXCR4 in metastatic prostate cancers from patients on androgen deprivation therapy was associated with poor survival, but not predictive of clinical response to hormonal therapy (44). In a mouse model, a positive correlation was observed for CXCL12 levels in tissues with metastatic lesions (45). Several studies have suggested a role for CXCR4 in metastasis of prostate cancer (46, 47). Because treatment with PEITC and SFN results in inhibition of distant site metastasis (13–15), it is reasonable to consider that antimetastatic activity of ITCs is mediated, at least in part, via downregulation of CXCR4.

Figure 6.
Cell viability inhibition by ITCs was significantly attenuated by CXCR4 overexpression in PC-3 cells. A, Western blot analysis showing effect of PEITC treatment (24 hours) on CXCR4 protein levels in Neo_PC-3 and CXCR4_PC-3 cells. B, effects of PEITC, BITC, and SFN treatments (24 hours) on viability of Neo_PC-3 and CXCR4_PC-3 cells. The results shown are mean ± SD (n = 3). Significantly different compared with "corresponding control, and "between Neo_PC-3 and CXCR4_PC-3 cells by one-way ANOVA followed by Bonferroni test. C, Western blotting for S473 phosphorylated AKT using lysates from Neo_PC-3 or CXCR4_PC-3 after 24-hour treatment with DMSO or specified ITC compound. D, Western blotting for phospho-ERK and total ERK using lysates from Neo_PC-3 or CXCR4_PC-3 after 4-hour treatment with DMSO or specified ITC compound. The letters C, B, P, and S respectively signify corresponding control, and between Neo_PC-3 and CXCR4_PC-3 cells by one-way ANOVA followed by Bonferroni test.
CXCR4/CXCL12 signaling is mechanistically linked to AKT-1, ERK, and MMP-9 in prostate cancer cells (31, 48). CXCR4 is localized in lipid rafts of prostate cancer cells and initiates AKT phosphorylation (31). Loss of PTEN results in induction of both CXCR4 and CXCL12 expression and these effects are reversed by AKT inhibition (31). Expression of CXCR4 is also increased by overexpression of AKT-1 in DU145 cells (48). The present study reveals that CXCR4-mediated attenuation of cell proliferation inhibition by ITCs is independent of AKT or ERK activation status. Other possibilities need to be explored to explain these observations. For example, forced expression of zinc-finger transcription factor SLUG in prostate cancer cells causes induction of CXCR4 and CXCL12 leading to promotion of cell migration (49). The effect of PEITC or SFN on SLUG is not yet known in prostate or other cancers, but we previously reported downregulation of this transcription factor in BITC-treated breast cancer cells (50). Thus, it is reasonable to conclude that suppression of SLUG likely contributes to cell migration and proliferation inhibition by ITCs.

In conclusion, the present study shows that CXCR4, but not CXCL12, is transcriptionally downregulated after treatment with PEITC, SFN, and/or BITC in prostate tumor cells. We show further that CXCR4 downregulation is functionally important as inhibition of cell viability and migration by ITCs is attenuated by its overexpression.

References


CXCR4 Is a Novel Target of Cancer Chemopreventative Isothiocyanates in Prostate Cancer Cells

Kozue Sakao, Avani R. Vyas, Sreenivasa R. Chinni, et al.