Carcinogenesis, 2016, Vol. 37, No. 12, 1144–1151

doi:10.1093/carcin/bgw105
Advance Access publication October 7, 2016
Original Manuscript

ORIGINAL MANUSCRIPT

Exposure to polychlorinated biphenyls and prostate cancer: population-based prospective cohort and experimental studies

Imran Ali, Bettina Julin, Anders Glynn[1], Johan Högberg, Marika Berglund, Jan-Erik Johansson[2,3], Swen-Olof Andersson[2,3], Ove Andrén[2,3], Edward Giovannucci[4–6], Alicja Wolk, Ulla Stenius and Agneta Åkesson*

Institute of Environmental Medicine, Karolinska Institutet, Stockholm SE 171 77, Sweden, [1]The National Food Agency, Uppsala SE 751 26, Sweden, [2]School of Health and Medical Sciences, Örebro University, Örebro SE 701 82, Sweden, [3]Department of Urology, Örebro University Hospital, Örebro SE 701 85, Sweden, [4]Department of Nutrition and [5]Department of Epidemiology, Harvard T. H. Chan School of Public Health, Boston, MA 02115, USA and [6]Channing Division of Network Medicine, Department of Medicine, Brigham and Women's Hospital and Harvard Medical School, Boston, MA 02115, USA

*To whom correspondence should be addressed. Tel: +46 8 524 87542; Fax: +46 8 30 45 71; Email: agneta.akesson@ki.se
Correspondence may also be addressed to Imran Ali. Tel: +46 8 524 87521; Fax: +46 8 33 69 81; Email: imran.ali@ki.se

Abstract

Polychlorinated biphenyls (PCBs) are highly persistent environmental pollutants and are undesirable components of our daily food. PCBs are classified as human carcinogens, but the evidence for prostate cancer is limited and available data are inconsistent. We explored the link between non-dioxin-like PCB and grade of prostate cancer in a prospective cohort as well as in cell experiments. A population-based cohort of 32 496 Swedish men aged 45–79 years was followed prospectively through 1998–2011, to assess the association between validated estimates of dietary PCB exposure and incidence of prostate cancer by grade (2789 cases, whereof 1276 low grade, 756 intermediate grade, 450 high grade) and prostate cancer mortality (357 fatal cases). In addition, we investigated a non-dioxin-like PCB153-induced cell invasion and related markers in normal prostate stem cells (WPE-stem) and in three different prostate cancer cell lines (PC3, DU145 and 22RV1) at exposure levels relevant to humans. After multivariable-adjustment, dietary PCB exposure was positively associated with high-grade prostate cancer, relative risk (RR) 1.35 [95% confidence interval (CI): 1.03–1.76] and with fatal prostate cancer, RR 1.43 (95% CI: 1.05–1.95), comparing the highest tertile with the lowest. We observed no association with low or intermediate grade of prostate cancer. Cell invasion and related markers, including MMP9, MMP2, Slug and Snail, were significantly increased in human prostate cancer cells as well as in prostate stem cells after exposure to PCB153. Our findings both from the observational and experimental studies suggest a role of non-dioxin-like PCB153 in the development of high-grade and fatal prostate cancer.

Introduction

Persistent organic pollutants such as polychlorinated biphenyls (PCBs) have become ubiquitous environmental pollutants. Food generally constitutes the dominating source of exposure to these highly lipophilic and persistent chemicals that magnify in the food chain via adipose tissue accumulation (1–4). PCBs comprise of 209 different congeners with variable toxicity and are often divided into two subgroups: dioxin-like and non-dioxin-like PCBs. PCB153, a non-dioxin-like PCB, is the most abundant congener in food and in blood (5,6).

Received: July 1, 2016; **Revised:** September 5, 2016; **Accepted:** October 6, 2016

Abbreviations

ATCC	American Type Culture Collection
BMI	body mass index
CI	confidence interval
DMSO	dimethyl sulfoxide
FFQ	food frequency questionnaire
MET	metabolic equivalent
PCB	polychlorinated biphenyl
RR	relative risk
TGF-β1	transforming growth factor-β1

PCB mixtures and individual congeners may have tumor-initiating, tumor-promoting and tumor-progressing activities (7). Deregulation of cell-cycle control and cell proliferation, suppression of cell-to-cell communication and adhesion and increased invasiveness are examples of effects in various model systems (8). The International Agency for Research on Cancer (IARC) recently concluded the evidence for the carcinogenicity of PCBs in humans to be sufficient, with the highest level of certainty for melanoma and limited evidence for non-Hodgkin lymphoma and breast cancer. Data for cancers at other sites such as the prostate were too sparse for any conclusions to be drawn (8). Increased prostate cancer mortality has been observed in workers with high cumulative exposure to PCBs (9–11), whereas results from case–control studies utilizing biomarkers of PCB exposure are clearly inconsistent (7,12–16).

Prostate cancer is one of the leading cancer forms in men world wide with recorded cases exceeding 1.1 million in 2012. The link between diet and prostate cancer risk has so far been elusive (17), whereas any association with food contaminant exposure has not been explored. The aim of this study was to assess the association between validated estimates of dietary PCB exposure and tumor grade-specific prostate cancer incidence and prostate cancer death in a large population-based prospective cohort of middle-aged and elderly men. To explore our epidemiological observations in experimental settings, we assessed the markers of invasiveness, one factor that may affect aggressiveness of prostate cancer, in prostate cancer cell lines and prostate stem cells after exposure to PCB153 at a low concentration relevant to humans.

Materials and methods

Epidemiological data

Study population

The population-based Cohort of Swedish Men (COSM) was established in 1997, when all men aged 45–79 years and residing in two counties in central Sweden received an invitation along with a self-administrated questionnaire, including 350 items on diet and other lifestyle factors (response rate 49%; n = 48 850) (18). The cohort is representative of Swedish males aged 45–79 years, in terms of age distribution, educational level and prevalence of overweight (18). Ethical approval for the study was granted by the Regional Ethical Review Board in Stockholm, Sweden and return of the completed questionnaire was considered to imply informed consent.

We excluded from the baseline population, those men with incorrect national registration numbers and those who were diagnosed with cancer, based on linkage of the cohort to the National Cancer Registry, those who reported an implausible energy intake (±3 SD of mean log transformed energy, *n* = 567) and those diagnosed with diabetes prior to 1998 (*n* = 4250, based on self-reports and on National Hospital Discharge Registry data), as diabetes is both associated with decreased risk of prostate cancer (19), and the dietary advice given to diabetics may lead to increased exposure to PCB. Furthermore, since fish consumption is a major source to the dietary exposure to PCBs in Sweden, we excluded men with incomplete answers on the questions on fish consumption (*n* = 8593). Thus, the analytical cohort for the primary analysis consisted of 32 496 men.

Assessment of diet, PCB and covariates

Dietary intake was assessed at baseline using a 96-item food frequency questionnaire (FFQ). Participants reported their average frequency of consumption of each food item during the previous year according to eight predefined categories, ranging from never/seldom to more than three times/day. The consumption of, e.g. dairy products, was assessed using open-ended questions. The validity of the FFQ (correlation coefficient, *r*) was 0.65 for macronutrients, 0.77 for calcium and 0.72 for selenium from the diet alone, based on a random population-based sample of 248 men of similar age from the study area, who completed the FFQ and 14 repeated 24 h recalls during 1 year (20).

The average daily dietary PCB exposure was calculated by multiplying the consumption frequencies from the FFQ by age-specific portion sizes and the average content of PCB153 in each food item at the time of baseline, as described in detail elsewhere (1). Dietary PCB exposure values were adjusted to the mean energy intake in the cohort (2600 kcal/day) using the residual-regression method (21), and the long-term validity against biomarkers (six PCB congeners in serum) was $r = 0.30–0.58$ in women based on the same FFQ (1). The major dietary sources of PCB exposure were fish (63%), dairy products (16%) and meat products (11%).

We obtained questionnaire data on family history of prostate cancer, education, height, weight, smoking habits and physical activity. Body mass index (BMI) was calculated as weight (kg) divided by height^2 (m^2). The time spent per day at specific activities was multiplied by its typical energy expenditure requirements [expressed in metabolic equivalents (METs) and summarized in MET-h/day (22).

Ascertainment of prostate cancer cases

Incident cases of prostate cancer occurring from baseline (1 January 1998) through 31 December 2011 were identified by linkage of the cohort to the National Cancer Registry, close to 100% complete (23). Information on tumor grade was ascertained through medical records and the Swedish Prostate Cancer Quality Registry. We defined incident cases as low grade if Gleason score was <7, as intermediate grade if Gleason score was equal to 7 and as high grade if Gleason score was >7. Information on prostate cancer death was ascertained through linkage to the Swedish Cause of Death Register. Classification of deaths was based on International Classification of Diseases (ICD-10, code 61 for prostate cancer).

Statistical analysis

Follow-up was censored at date of invasive prostate cancer diagnosis, death or end of follow-up, whichever occurred first. Separate analyses were performed where fatal prostate cancer as the primary cause of death was the outcome. We used Cox proportional hazards regression models with age as the timescale to estimate hazard ratios (herein referred to as relative risks or RRs) with 95% confidence intervals (CIs) of prostate cancer by tertiles of dietary PCB exposure. In the multivariable analysis, we adjusted for age (years), family history of prostate cancer (yes, no, unknown), years of education (≥12, <12 years), BMI (18.5–<25, 25–<30, ≥30 kg/m^2), MET-h/day (tertiles), smoking status (ever, never), total energy intake (kcal continuous), alcohol consumption (<0.1, 0.1–<5, 5–<10, 10–<15, ≥15 g/day) and weight loss (>5 kg yes, no). Because dietary intake of selenium, lycopene and calcium has been evaluated as probable protective factors for the risk of prostate cancer (17), we also included tertiles of these variables in the multivariable analysis. In additional models, we adjusted for consumption of total fish (herring, salmon and cod; <1, 1–2, >2 servings/week). Finally, we performed analyses stratified by low (≤2 servings/week) and high (>2 servings/week) fish consumption (only for high-grade tumors). Missing values—treated as a separate 'missing category' in the models—were generally very few (<2%) with the exception of physical activity (~20%). The Schoenfeld's residual test indicated no violation of the proportional hazard assumption (24). Linear trends across categories were tested using the median PCB values within categories as a continuous variable. All reported P-values were two-sided and values <0.05 were considered statistically significant. Statistical analysis was performed with Stata, version 13 (StataCorp, College Station, TX).

Experimental data

Cell culture and exposure

Cell invasive growth in relation to PCB153 exposure was explored using three different types of human prostate epithelial cancer cells. PC3 (ATCC® CRL-1435™) and DU145 (ATCC® HTB-81™) are metastatic cell lines and 22RV1 (ATCC® CRL-2505™) is a castration-resistant cell line. The normal human prostate epithelium cells (WPE-stem) (ATCC-CRL-2887) were used in the epithelial-to-mesenchymal transition study. All cell lines were purchased from American Type Culture Collection (ATCC, Manassas, VA) and resuscitated from early-passage liquid nitrogen stocks. Cells were cultured for <2 months before re-initiating culture and routinely inspected microscopically for stable phenotype. ATCC uses morphology, karyotyping and PCR-based approaches to confirm the identity of human cell lines.

PC3 cells were cultured in RPMI 1640 supplemented with 10% inactivated fetal bovine serum, 1 mM sodium pyruvate, 2 mM L-glutamine and penicillin/streptomycin. DU145 cells were grown in Dulbecco's modified Eagle's medium with 10% inactivated fetal bovine serum, penicillin/streptomycin and 1 nM sodium pyruvate. 22RV1 cells were grown in RPMI 1640 supplemented with 10% inactivated fetal bovine serum and penicillin/streptomycin. WPE-stem cells were cultured, according to the procedure for ATCC CRL-2887, in keratinocyte serum-free medium (Invitrogen 17005) with bovine pituitary extract, epidermal growth factor human recombinant and antibiotic–antimycotic (Invitrogen 15240).

PCB153 (2,2′,4,4′,5,5′-hexachlorobiphenyl) was purchased from Neosyn with a highest purity (>99.2%). Purification was performed using fractionation on active carbon at the Department of Chemistry, Umeå University, Sweden (25), resulting in a highly purified PCB153 (> 99.9999%) with extremely low levels of polychlorinated dibenzo-*p*-dioxins/polychlorinated dibenzofurans and dioxin-like PCBs (< limit of detection—a few pg/g PCB).

Cells were treated separately with PCB153 (10 nM) dissolved in dimethyl sulfoxide (DMSO) and with transforming growth factor-β1 (TGF-β1) (0.4 and 4 ng/ml) dissolved in phosphate-buffered saline, as well as with the combination of both PCB153 and TGF-β1. Controls were treated with DMSO. The final concentration of DMSO did not exceed 0.2% (vol/vol) in the medium and DMSO alone had no effect on the cells. The chosen PCB153 concentration (10 nM = 3608 ng/l) is close to the plasma PCB153 concentrations observed in our validation study among women in ~2004 (n = 201; median: 940 ng/l; min–max: 227–2722 ng/l = <1–7.5 nM) but below the highest sum of three non-dioxin-like PCBs (CB 153, 138 and 180) observed in plasma (5660 ng/l) (1). Noteworthy, men generally have higher plasma PCB concentrations than women (26).

Western blot analysis of markers of invasiveness

Markers of invasiveness including MMP9, MMP2, Slug and Snail were analyzed by western blot. Cells were lysed in IPB-7 buffer (triethanolamin-HCl, 1M, pH 7.8; NaCl, 5M; sodium deoxycholate, 4%; Igepal CA-63 or Nonidet P-40, 10%) with protease inhibitors (1 mg/ml phenylmethylsulfonyl fluoride, 0.1 mg/ml trypsin inhibitor, 1 mg/ml aprotinin, 1 mg/ml leupeptin, 1 mg/ml pepstatin, 1 mM Na_3VO_4 and 1 mM NaF). The protein was quantified by using Coomassie Plus, Bradford Assay kit (Pierce/Thermo scientific, Rockford, MD). The samples were subjected to sodium dodecyl sulfate–polyacrylamide gel electrophoresis and blotted onto a polyvinylidene difluoride membrane (Bio-Rad, Hercules, CA) and protein bands were subsequently probed with primary antibodies against the matrix metallopeptidases MMP9, MMP2 and Slug from Cell Signaling (Beverly, MA), Snail and β-actin from Santa Cruz Biotechnology (Santa Cruz, CA). Depending on the primary antibody, protein bands were probed with secondary antibodies, goat anti-rabbit IgG-horseradish peroxidase sc-2004 and goat anti-mouse IgG-horseradish peroxidase sc-2005 from Santa Cruz Biotechnology. Protein bands were visualized with the ECL detection kit (AmershamTM GE Healthcare Bio-sciences, AB, Uppsala, Sweden). β-Actin was used as loading control. The densitometry analysis was made with Image J version 1.34s software (National Institute of Health, Bethesda, MD; http://rsbweb.nih.gov/ij/index.html).

Cell invasion assay and epithelial-to-mesenchymal transition

Cell invasion assay was performed by using 8 μM pore size transwell Biocoat Control inserts (BD Biosciences, San José, CA) according to the manufacture's protocol. The cells were fixed with methanol and stained with tolubidine blue from Merck (Darmstadt, Germany). Images were taken and the number of transmembrane cells was counted with Image J version 1.34s software (National Institute of Health, Bethesda, MD; http://rsbweb.nih.gov/ij/index.html). Epithelial-to-mesenchymal transition is a cellular process included in invasiveness (27), and we also tested parameters indicating this process. Briefly, WPE-stem cells were treated repeatedly every third day with PCB153 (10 nM) and observations on the morphology of the cells were made on day 1, day 3, day 6 and day 9. TGF-β1 was used as positive control (28).

Statistical analysis

Unpaired *t*-test was applied to compare the treatment with control. Differences were considered significant at $P \leq 0.05$ for two-tailed tests and performed using GraphPad Prism version 5.04 for Windows, GraphPad Software (San Diego, CA; www.graphpad.com).

Results

Dietary PCB exposure and prostate cancer incidence and mortality

During a mean follow-up of 12 years (406 127 person-years), we ascertained 2789 incident cases of prostate cancer of which 1276 were low grade (Gleason score < 7), 756 intermediate grade (Gleason score = 7), 450 high grade (Gleason score > 7) and 307 undefined. During the same follow-up period, 357 fatal prostate cancer cases were ascertained. The mean dietary PCB exposure at baseline in the 32 496 men was 278 ng/day (5–95 percentiles 100–540 ng/day) corresponding to 3.5 ng/kg body weight and day. The mean consumption of fish was 1.9 servings/week. Men in the highest tertile of dietary exposure to PCB were older, more likely to have a higher education (>12 years) and had a higher consumption of fish (all types) and a higher intake of selenium and lycopene compared with men in the lowest tertile (Table 1).

We observed statistically significant higher risks for both high-grade (1.35; 95% CI: 1.03–1.76) and fatal tumors (RR: 1.43; 95% CI: 1.05–1.95), but not for overall, low- or intermediate-grade prostate cancer, when we compared the highest tertile of dietary PCB with the lowest (*P* for trend = 0.01 for high-grade and 0.03 for fatal tumors) (Table 2).

The major dietary sources contributing to the total PCB exposure were fish (63%) followed by dairy products (16%) and meat products (11%). Because fish has been observed to have beneficial effects on prostate cancer, we also adjusted the models for total fish consumption. This resulted in an increase in risk for high-grade tumors (RR: 1.51; 95% CI: 1.03–2.22). For fatal prostate cancer, additional adjustment for total fish consumption slightly attenuated the result in RR 1.27 (95% CI: 0.83–1.95) comparing the highest PCB tertile with the lowest. Fish consumption was, however, not associated with prostate cancer risk in these models adjusted for dietary PCB exposure. In additional analyses, we also assessed the association between fish consumption alone and high-grade and fatal tumors. Compared with the consumption of <1 serving of fish/week, 1–2 and >2 servings/week were associated with RRs: 0.86 (95% CI: 0.67–1.12) and 1.11 (95% CI: 0.82–1.50), respectively, for the high-grade tumors. The corresponding results for fatal prostate cancer were RR: 1.19 (95% CI: 0.87–1.62) for 1–2 servings/week and RR: 1.47 (95% CI: 1.03–2.08) for >2 servings/week.

Finally, we performed analyses stratified for low and high fish consumption. For high-grade tumors, the highest compared with lowest tertile of dietary PCB exposure was associated with RR: 1.20 (95% CI: 0.81–1.79) among those who consumed ≤2 servings of fish/week (P_{trend} = 0.45). The corresponding results among those consuming >2 servings of fish/week was 1.95 (95% CI: 0.46–8.20) (P_{trend} = 0.06).

Table 1. Age-standardized baseline characteristics by categories of PCB exposure in 32 496 men aged 45–79 years, Cohort of Swedish Men

	Tertiles of dietary PCB153 exposure, ng/day		
Characteristics[a]	<211	211–277	>277
Median PCB (ng/day)	168	242	376
Age (years)	55	56	59
Mean PCB153 (ng/day)[b]	157	243	432
Family history of prostate cancer (%)	9	9	9
≥12 years of education (%)	16	20	21
BMI (mean, kg/m^2)	26	26	26
Weight loss >5 kg within a year (%)	40	40	44
MET (h/day)	42	41	41
Ever smokers (%)	61	61	64
Mean alcohol intake (g/day)	14	15	16
Fish consumption (mean, g/day)	18.0	28.6	50.8
Fatty fish, e.g. herring (servings/week)	0.26	0.46	1.18
Medium fatty fish, e.g. salmon (servings/week)	0.16	0.41	0.75
Lean fish, e.g. cod (servings/week)	0.62	0.83	1.15
Selenium (mean, µg/day)[b]	33	38	46
Lycopene (mean, µg/day)[b]	2276	2474	2694
Calcium (mean, mg/day)[b]	1446	1466	1414
Total energy intake (mean, kcal)	2889	2713	2601

[a]All factors, except age were directly standardized to the age distribution of the study population.
[b]Energy-adjusted to 2600 kcal.

Table 2. RRs and 95% CIs of prostate cancer by tertiles of dietary PCB exposure, Cohort of Swedish Men 1998–2011

	Tertiles of dietary PCB153 exposure			
	1	2	3	P_{trend}
All invasive tumors				
Person-years	136 561	137 131	132 435	
No. of cases	854	915	1020	
Age-adjusted RR (95% CI)	1.00	1.02 (0.93–1.12)	1.02 (0.93–1.12)	0.65
Multivariable adjusted RR (95% CI)[a]	1.00	0.97 (0.88–1.07)	1.01 (0.90–1.12)	0.95
Low grade (Gleason score < 7)				
No. of cases	395	439	442	
Age-adjusted RR (95% CI)	1.00	1.04 (0.91–1.19)	0.98 (0.85–1.12)	0.64
Multivariable-adjusted RR (95% CI)[a]	1.00	0.99 (0.86–1.15)	0.97 (0.83–1.14)	0.71
Intermediate grade (Gleason score = 7)				
No. of cases	254	245	257	
Age-adjusted RR (95% CI)	1.00	0.91 (0.77–1.09)	0.85 (0.72–1.02)	0.09
Multivariable adjusted RR (95% CI)[a]	1.00	0.84 (0.70–1.02)	0.83 (0.67–1.01)	0.11
High grade (Gleason score > 7)				
No. of cases	129	125	196	
Age-adjusted RR (95% CI)	1.00	0.94 (0.73–1.20)	1.28 (1.03–1.60)	0.009
Multivariable adjusted RR (95% CI)[a]	1.00	0.96 (0.74–1.24)	1.35 (1.03–1.76)	0.01
Fatal prostate cancer				
Person-years	136 561	137 131	132 435	
No. of cases	91	117	149	
Age-adjusted RR (95% CI)	1.00	1.24 (0.95–1.64)	1.38 (1.06–1.79)	0.02
Multivariable adjusted RR (95% CI)[a]	1.00	1.28 (0.96–1.70)	1.43 (1.05–1.95)	0.03

[a]Adjusted for age (years), family history of prostate cancer (yes, no, unknown), years of education (≥12, <12 years), BMI (18.5–<25, 25–<30, ≥30 kg/m^2), MET-h/day (tertiles), smoking status (ever, never), total energy intake (kcal continuous), alcohol consumption (<0.1, 0.1–<5, 5–<10, 10–<15, ≥15 g/day), weight loss (<5 kg yes, no) and intake of selenium, lycopene and calcium (mg/day, tertiles).

PCB153 stimulated cell invasion and cell migration in prostate cancer cells and prostate stem cells

In three different human prostate cancer cells (PC3, DU145, 22RV1), derived from advanced prostate tumors (29), we explored whether cell invasive growth, one factor that might affect aggressiveness of prostate cancer, was increased by PCB153. The non-dioxin-like PCB153 was chosen because it is the most abundant PCB congener in food (30,31) and human blood (5,6) and has the highest abundance in blood in men from the study area (32) and in our validation study (1). Moreover, dioxin may have anti-carcinogenic effects in the prostate (33), whereas non-dioxin-like PCBs have been shown to stimulate aggressive growth in mammary cancer cells (34).

Our results in prostatic cancer cells show that PCB153 at 10 nM, a concentration 500 times lower than the concentration that kills cells *in vitro* (35), induced indicators of cell invasion (29,36) including MMP9, MMP2, Slug and Snail (Figure 1A and B) by 50–150%. In line with these results, PCB153 treatment (10 nM) significantly stimulated the invasion of PC3 and DU145 cells (Figure 1C and D); PC3 cells showed almost 4-fold and DU145 2.5-fold induction of cell invasion as compared with DMSO-treated controls ($P \leq 0.001$ and 0.01, respectively).

In WPE prostate stem cells, PCB153 (10 nM) induced the cell invasion markers MMP9, MMP2, Slug and Snail by 20–150% (Figure 2A). This was complemented by increase in the number of transmembrane invading cells (Figure 2B). We used TGF-β1 as positive control and in consistency with our previous findings (28), TGF-β1 changed WPE-stem cell morphology from epithelial-like to mesenchymal-like appearance (spindle shape, forming clusters; Figure 2C) and increased their levels of MMP9, Slug and Snail (Figure 2D). PCB153 treatment alone induced similar marked changes in the morphology (Figure 2C). Combined treatment with PCB153 and TGF-β1 had a more pronounced effect than TGF-β1 alone on some of the tested proteins (Figure 2D). Morphologically there was no pronounced effect of the combination as compared with the effect of TGF-β1 alone (Figure 2C). These experimental data support a role for PCB153 in stimulating invasive growth.

Discussion

In the present study, we combined an observational study among men with cell-based experiments. We observed in the large population-based prospective cohort that dietary PCB exposure was associated with increased risk of high-grade and fatal, but not low- or intermediate-grade, prostate cancer tumors. At similar low PCB concentrations as normally found in human plasma, the non-dioxin-like PCB153 induced cell invasion, supporting the potential aggravating effect on prostate cancer development observed in the male cohort.

Previous studies assessing the association between biomarkers of PCB exposure and prostate cancer risk in the general non-occupationally exposed population show inconsistent

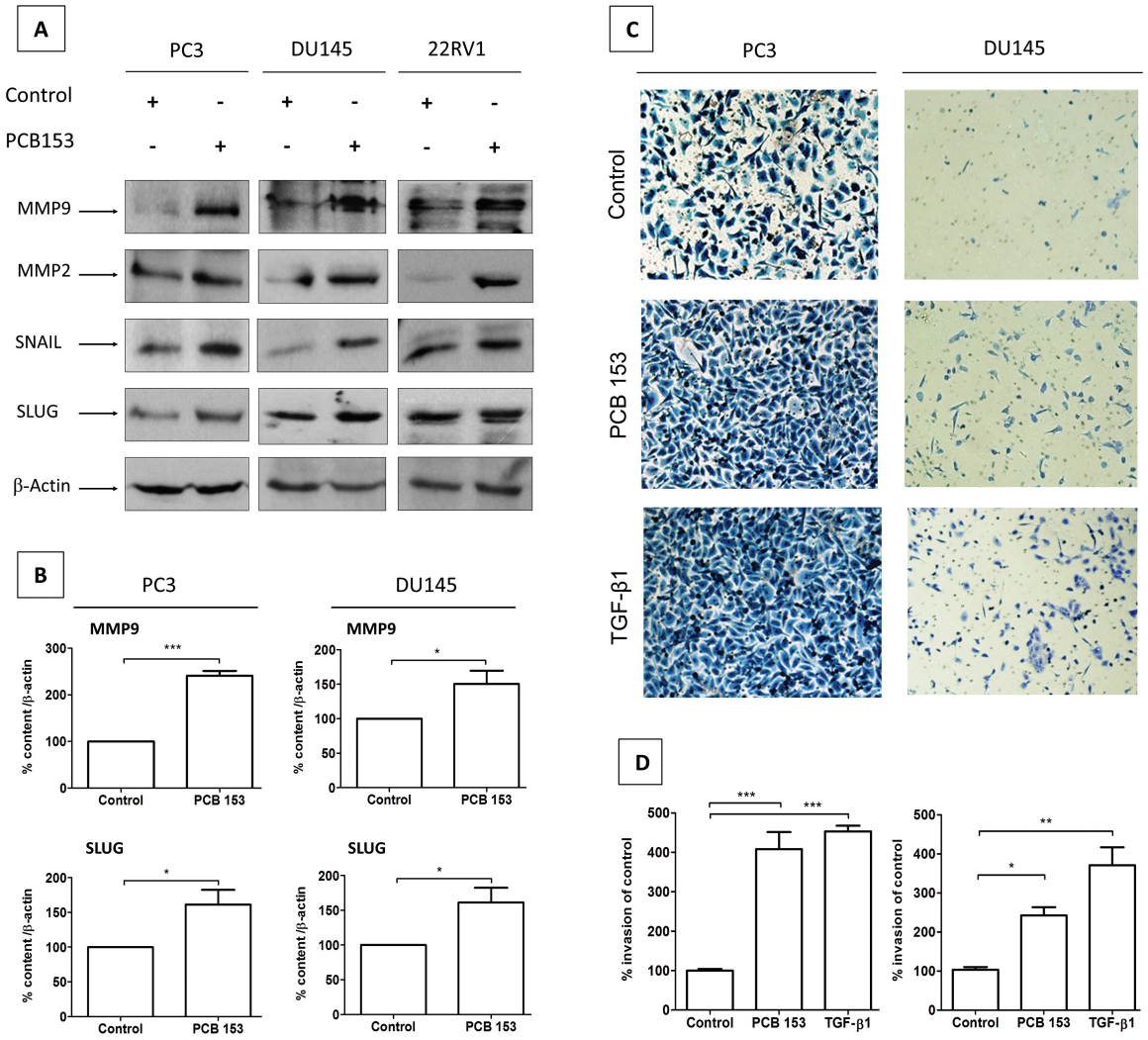

Figure 1. PCB153 stimulates cell invasion of prostate cancer cells. (**A**) Representative blots of MMP9, MMP2, Snail and Slug in PC3, DU145 and 22RV1 cells treated with PCB153 (10 nM) for 24 h. (**B**) Densitometry analysis of MMP9 and Slug in PC3 and DU145, respectively. (**C**) Representative images by light microscopy (original magnification ×10) of PC3 and DU145 cells in invasive assay employing PCB153 and positive control TGF-β1 (5 ng/ml). (**D**) Quantification of number of transmigrated PC3 or DU145 cells after exposure to PCB153 (10 nM) for 72 h. The data are reported as the percentage of the vehicle control (100%) and presented as the means ± SEM ($n = 4$). $^{*}P \leq 0.05$, $^{**}P \leq 0.01$, $^{***}P \leq 0.001$ significantly different.

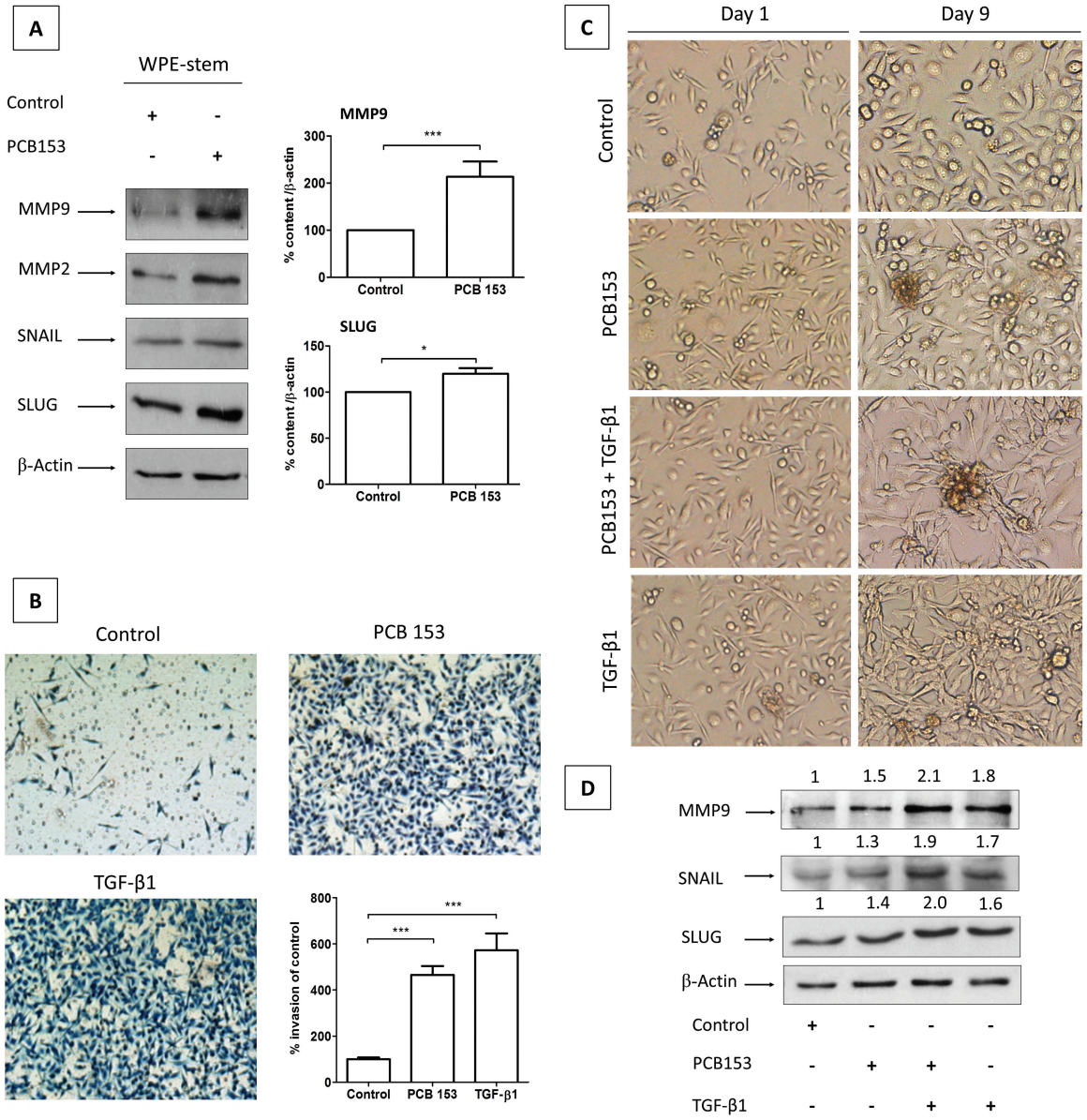

Figure 2. PCB153 stimulates cell invasion and affects epithelial-to-mesenchymal transition of prostate stem cells. (**A**) Representative blots of MMP9, MMP2, Snail, Slug and densitometry analysis of MMP9 and Slug in WPE-stem cells treated with PCB153 (10 nM) for 24 h. (**B**) Representative images by light microscopy (original magnification ×10) of WPE-stem cells and quantification of number of transmigrated cells after exposure to PCB153 (10 nM) and positive control TGF-β1 (4 ng/ml) for 72 h. The data are reported as the percentage of the vehicle control (considered as 100%) and presented as the means ± SEM ($n = 4$). $^{*}P \leq 0.05$, $^{***}P \leq 0.001$, significantly different from control. (**C**) Representative images by light microscopy (original magnification ×10) of WPE-stem cells during epithelial-to-mesenchymal transition process through days 1–9 and after repeated exposure to PCB153 (10 nM) and TGF-β1 (4 ng/ml). (**D**) Representative blots of MMP9, Snail and Slug on day 9 after repeated exposure to PCB153 (10 nM) and TGF-β1 (4 ng/ml). The values on the each blot are calculated through densitometry analysis and are based on single experiment as compared with control (considered as one).

results (7,12–16). Based on 58 cases and only 20 controls, the concentrations of the non-dioxin-like PCB153 in abdominal fat were associated with significantly increased odds of higher prostate-specific antigen concentrations (>16.5 ng/ml) at diagnosis of prostate cancer as compared with those with lower levels and controls (14). Similarly, higher serum levels of non-dioxin-like PCBs (including PCB153) were associated with higher odds of prostate cancer based on 58 cases with moderately or poorly differentiated tumors (7). In contrast, no increased risk was observed for advanced prostate cancer (extra prostatic or metastatic cancer or Gleason score 8–10) based on the sum or on individual serum PCBs in 201 prospective cases from Japan (16). Based on 576 cases of prostate cancer in men from the French West Indies, tertiles of serum PCB153 were inversely associated with odds of low-grade prostate cancer (Gleason <7 and 3+4), whereas no association was observed for the 101 cases with high-grade score (Gleason >7 and 4+3, $P = 0.04$ for heterogeneity) (13). The present study is the first to utilize data on dietary exposure of PCB in relation to prostate cancer incidence and mortality, enabling a large sample size and high study power. We used Gleason score 8–10 to identify high-grade prostate cancer with severe prognosis and observed similar increased risk for high grade as for fatal prostate cancer tumors. Higher prostate cancer mortality has been observed at high cumulative PCB exposure in occupationally exposed electrical capacitor manufacturing workers (9,10). Individual susceptibility, such as cytochrome

P450 polymorphism (37,38) affecting a potential PCB-driven carcinogenesis, may contribute to the inconsistent results observed across populations.

Androgen receptor signalling plays a pivotal role in the development and progression of prostate cancer (39). It has been shown that PCBs are able to activate or inhibit the androgen receptor transcriptional activity (40,41) but the detailed mechanism needs to be investigated further. For the non-dioxin-like PCBs, experimental animal studies do not provide clear evidence on the carcinogenicity. Nevertheless, non-dioxin-like PCBs have been suggested to induce their carcinogenic effects via several aryl hydrocarbon receptor-independent mechanisms, including activation of the constitutive androstane and pregnane xenobiotic receptors, and perturbation in cell–cell communication and cell adhesion (8). The extracellular matrix degradation enzymes MMP9 and MMP2 are well-established markers of cell invasion and tumor cell invasion and metastasis closely correlates with their activity (29,42). Our findings show that PCB153 induced MMP9, MMP2, Slug and Snail in three different prostate cancer cell lines as well as in prostate stem cells. Epithelial-to-mesenchymal transition is a process by which epithelial cells lose their cell–cell adhesion and gain migratory and invasive properties (27,43), and our experimental data on the transmembrane cell invasion and effects on cell morphology, indicating epithelial-to-mesenchymal transition, suggest that PCB153 at low concentrations can facilitate invasive growth in the later phases of prostate cancer development. Previous work shows that PCB153 at nanomolar concentrations induced the phosphorylation of MDM2 (25), and that MDM2 promoted the invasion and migration of breast cancer cells by inducing MMP9 expression (44), so although PCB153-induced effects on MDM2 in prostate remains to be shown, these data are in line with our results. We conclude that the present experimental *in vitro* data may explain the observational finding that PCB exposure was associated with increased risk of high-grade and fatal tumors, but not with low- and intermediate-grade tumors.

The established risk factors for prostate cancer are very few and the role of westernization, eating habits and exposure to environmental pollutants is unclear. Considerable research has been trying to elucidate the relationship between fish consumption and prostate cancer. Fish is proposed to be beneficial for prostate cancer, presumably due to the anti-inflammatory effects of marine long-chain fatty acids (45,46). Results have, however, been mixed as summarized in several recent systematic reviews (45,47), and even a higher risk of prostate cancer in relation to biomarkers of these marine fatty acids has been observed especially for high-grade cancers (48). The variable presence of contaminants such as PCBs in fish, depending on type and source of the fish, could explain these unexpected contradictive findings.

The major strengths of the epidemiological study include its population-based and prospective study design, which eliminates recall and selection bias. Although the response rate was 49%, the participants were considered representative (18). We were able to ascertain a relatively large number of cases according to tumor grade and the case ascertainment is very high, thanks to matching of the cohort with the nearly complete cancer registers. Moreover, the findings from the experimental study based on three different prostate cancer cell lines with different invasive potential, as well as prostate stem cells, exposed to PCB153 at relevant concentrations in humans, supported the results from observational study.

Most important limitation is whether the estimated intake of PCB provides a valid measure of exposure. Dietary assessments are subject to misclassification due to the difficulty of reporting diet correctly and the mean PCB concentrations in specific foods may not account for all the variability in its content in the reported food. The estimated dietary PCB exposure showed, however, reasonable validity against PCB biomarkers which support a ranking of subjects into low, medium and high dietary PCB exposure. Moreover, the prospective design of this study, most probably, makes the exposure misclassification non-differential. Although the *in vitro* study was based on several different cell lines and on stem cells, no primary prostate cancer cells were used. This should be considered while evaluating the strength of the data. Furthermore, *in vivo* experiments studying the effect of PCB on cancer invasion are needed to confirm the results.

In conclusion, both the prospective population-based and experimental data suggest the involvement of PCB153 in the development of high-grade and fatal prostate cancer, but future studies are needed to confirm or refute these findings.

Funding

This work was supported by the Swedish Cancer Society (120840 to A.Å., 150306 to A.W.); the Swedish Research Council (825-2008-5997 to A.W.) and the Swedish Research Council Formas (216-2009-1284 to AÅ).

Conflict of Interest Statement: None declared.

References

1. Bergkvist, C. et al. (2012) Validation of questionnaire-based long-term dietary exposure to polychlorinated biphenyls using biomarkers. Mol. Nutr. Food Res., 56, 1748–1754.
2. Liem, A.K. et al. (2000) Exposure of populations to dioxins and related compounds. Food Addit. Contam., 17, 241–259.
3. Meharg, A.A. et al. (1995) Dioxins released from chemical accidents. Nature, 375, 353–354.
4. Milbrath, M.O. et al. (2009) Apparent half-lives of dioxins, furans, and polychlorinated biphenyls as a function of age, body fat, smoking status, and breast-feeding. Environ. Health Perspect., 117, 417–425.
5. Glynn, A.W. et al. (2003) Organochlorines in Swedish women: determinants of serum concentrations. Environ. Health Perspect., 111, 349–355.
6. Covaci, A. et al. (2002) Persistent organochlorine pollutants in human serum of 50–65 years old women in the Flanders Environmental and Health Study (FLEHS). Part 2: correlations among PCBs, PCDD/PCDFs and the use of predictive markers. Chemosphere, 48, 827–832.
7. Ritchie, J.M. et al. (2005) Comparison of proposed frameworks for grouping polychlorinated biphenyl congener data applied to a case-control pilot study of prostate cancer. Environ. Res., 98, 104–113.
8. Lauby-Secretan, B. et al. (2013) Carcinogenicity of polychlorinated biphenyls and polybrominated biphenyls. Lancet Oncol., 14, 287–288.
9. Ruder, A.M. et al. (2014) Mortality among 24,865 workers exposed to polychlorinated biphenyls (PCBs) in three electrical capacitor manufacturing plants: a ten-year update. Int. J. Hyg. Environ. Health, 217, 176–187.
10. Prince, M.M. et al. (2006) Mortality and exposure response among 14,458 electrical capacitor manufacturing workers exposed to polychlorinated biphenyls (PCBs). Environ. Health Perspect., 114, 1508–1514.
11. Charles, L.E. et al. (2003) Electromagnetic fields, polychlorinated biphenyls, and prostate cancer mortality in electric utility workers. Am. J. Epidemiol., 157, 683–691.
12. Aronson, K.J. et al. (2010) Plasma organochlorine levels and prostate cancer risk. J. Expo. Sci. Environ. Epidemiol., 20, 434–445.
13. Emeville, E. et al. (2015) Associations of plasma concentrations of dichlorodiphenyldichloroethylene and polychlorinated biphenyls with prostate cancer: a case-control study in Guadeloupe (French West Indies). Environ. Health Perspect., 123, 317–323.
14. Hardell, L. et al. (2006) Adipose tissue concentrations of persistent organic pollutants and the risk of prostate cancer. J. Occup. Environ. Med., 48, 700–707.

15. Pavuk, M. et al. (2004) Environmental exposure to PCBs and cancer incidence in eastern Slovakia. Chemosphere, 54, 1509–1520.
16. Sawada, N. et al. (2010) Plasma organochlorines and subsequent risk of prostate cancer in Japanese men: a nested case-control study. Environ. Health Perspect., 118, 659–665.
17. World Cancer Research Fund (WCRF) (2007) Food, Nutrition and the Prevention of Cancer: A Global Perspective. World Cancer Research Fund/American Institute for Cancer Research, Washington, DC.
18. Harris, H.R. et al. (2013) The Swedish mammography cohort and the Cohort of Swedish Men: study design and characteristics of two population-based longitudinal cohorts. OA Epidemiol., 1, 16.
19. Giovannucci, E. et al. (2007) The role of obesity and related metabolic disturbances in cancers of the colon, prostate, and pancreas. Gastroenterology, 132, 2208–2225.
20. Messerer, M. et al. (2004) Sensitivity and specificity of self-reported use of dietary supplements. Eur. J. Clin. Nutr., 58, 1669–1671.
21. Willett, W. et al. (1986) Total energy intake: implications for epidemiologic analyses. Am. J. Epidemiol., 124, 17–27.
22. Norman, A. et al. (2001) Validity and reproducibility of self-reported total physical activity–differences by relative weight. Int. J. Obes. Relat. Metab. Disord., 25, 682–688.
23. Mattsson, B. et al. (1984) Completeness of the Swedish Cancer Register. Non-notified cancer cases recorded on death certificates in 1978. Acta Radiol. Oncol., 23, 305–313.
24. Grambsch, P.M. et al. (1994) Proportional hazards tests and diagnostics based on weighted residuals. Biometrika, 81, 515–526.
25. Al-Anati, L. et al. (2009) Non-dioxin-like-PCBs phosphorylate Mdm2 at Ser166 and attenuate the p53 response in HepG2 cells. Chem. Biol. Interact., 182, 191–198.
26. Bjermo, H. et al. (2013) Fish intake and breastfeeding time are associated with serum concentrations of organochlorines in a Swedish population. Environ. Int., 51, 88–96.
27. Thiery, J.P. et al. (2009) Epithelial-mesenchymal transitions in development and disease. Cell, 139, 871–890.
28. Ghalali, A. et al. (2014) Phosphatase and tensin homolog deleted on chromosome 10 (PTEN) and PH domain and leucine-rich repeat phosphatase cross-talk (PHLPP) in cancer cells and in transforming growth factor β-activated stem cells. J. Biol. Chem., 289, 11601–11615.
29. Ghalali, A. et al. (2014) Atorvastatin prevents ATP-driven invasiveness via P2X7 and EHBP1 signaling in PTEN-expressing prostate cancer cells. Carcinogenesis, 35, 1547–1555.
30. Atuma, S.S. et al. (1996) Survey of consumption fish from Swedish waters for chlorinated pesticides and polychlorinated biphenyls. Chemosphere, 33, 791–799.
31. Glynn, A.W. et al. (2000) PCB and chlorinated pesticide concentrations in swine and bovine adipose tissue in Sweden 1991–1997: spatial and temporal trends. Sci. Total Environ., 246, 195–206.
32. Glynn, A.W. et al. (2000) Serum concentrations of organochlorines in men: a search for markers of exposure. Sci. Total Environ., 263, 197–208.
33. Feng, S. et al. (2013) Role of aryl hydrocarbon receptor in cancer. Biochim. Biophys. Acta, 1836, 197–210.
34. Liu, S. et al. (2010) Polychlorinated biphenyls (PCBs) enhance metastatic properties of breast cancer cells by activating Rho-associated kinase (ROCK). PLoS One, 5, e11272.
35. Merritt, R.L. et al. (2007) Influence of persistent contaminants and steroid hormones on glioblastoma cell growth. J. Toxicol. Environ. Health. A, 70, 19–27.
36. Jadaan, D.Y. et al. (2015) Cellular plasticity in prostate cancer bone metastasis. Prostate Cancer, 2015, 651580.
37. Negri, E. et al. (2003) Environmental exposure to polychlorinated biphenyls (PCBs) and breast cancer: a systematic review of the epidemiological evidence. Eur. J. Cancer Prev., 12, 509–516.
38. Ghisari, M. et al. (2013) Genetic polymorphisms in CYP1A1, CYP1B1 and COMT genes in Greenlandic Inuit and Europeans. Int. J. Circumpolar Health, 72, 21113.
39. Lonergan, P.E. et al. (2011) Androgen receptor signaling in prostate cancer development and progression. J. Carcinog., 10, 20.
40. Casati, L. et al. (2013) Androgen receptor activation by polychlorinated biphenyls: epigenetic effects mediated by the histone demethylase Jarid1b. Epigenetics, 8, 1061–1068.
41. Hamers, T. et al. (2011) *In vitro* toxicity profiling of ultrapure non-dioxin-like polychlorinated biphenyl congeners and their relative toxic contribution to PCB mixtures in humans. Toxicol. Sci., 121, 88–100.
42. Barbieri, A. et al. (2015) The stress hormone norepinephrine increases migration of prostate cancer cells *in vitro* and *in vivo*. Int. J. Oncol., 47, 527–534.
43. Singh, A. et al. (2010) EMT, cancer stem cells and drug resistance: an emerging axis of evil in the war on cancer. Oncogene, 29, 4741–4751.
44. Chen, X. et al. (2013) MDM2 promotes invasion and metastasis in invasive ductal breast carcinoma by inducing matrix metalloproteinase-9. PLoS One, 8, e78794.
45. Lovegrove, C. et al. (2015) Systematic review of prostate cancer risk and association with consumption of fish and fish-oils: analysis of 495,321 participants. Int. J. Clin. Pract., 69, 87–105.
46. McCarty, M.F. et al. (2014) Omega-3 and prostate cancer: examining the pertinent evidence. Mayo Clin. Proc., 89, 444–450.
47. Aucoin, M., et al. (2016) Fish-derived omega-3 fatty acids and prostate cancer: a systematic review. Integr. Cancer Ther. doi:10.1177/1534735416656052.
48. Brasky, T.M. et al. (2013) Plasma phospholipid fatty acids and prostate cancer risk in the SELECT trial. J. Natl. Cancer Inst., 105, 1132–1141.