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Night-shift work duration and risk of colorectal cancer according to *IRS1* and *IRS2* expression

Yan Shi^{#1,2}, Li Liu^{#2,3}, Tsuyoshi Hamada², Jonathan A Nowak⁴, Marios Giannakis^{5,6}, Yanan Ma^{7,8}, Mingyang Song^{9,10}, Daniel Nevo^{11,12}, Keisuke Kosumi², Mancang Gu², Sun A. Kim¹³, Teppei Morikawa¹⁴, Kana Wu¹⁵, Jing Sui^{7,16}, Kyriaki Papantoniou¹⁷, Molin Wang^{11,12}, Andrew T. Chan^{5,7,9,10}, Charles S. Fuchs^{7,18,19,20}, Jeffrey A. Meyerhardt⁶, Edward Giovannucci^{7,12,15}, Shuji Ogino^{2,4,5,12}, Eva S. Schernhammer^{#7,12,17}, Reiko Nishihara^{#2,4,5,11,12}, Xuehong Zhang^{#7}

¹Department of Medical Oncology, Chinese PLA General Hospital, Beijing, P.R. China

²Department of Oncologic Pathology, Dana-Farber Cancer Institute and Harvard Medical School, Boston, MA, USA

³Department of Epidemiology and Biostatistics, and the Ministry of Education Key Lab of Environment and Health, School of Public Health, Huazhong University of Science and Technology, Wuhan, P.R. China

⁴Program in MPE Molecular Pathological Epidemiology, Department of Pathology, Brigham and Women's Hospital and Harvard Medical School Boston, MA, USA

⁵Broad Institute of Massachusetts Institute of Technology (MIT) and Harvard, Cambridge, Massachusetts, USA

⁶Department of Medical Oncology, Dana-Farber Cancer Institute and Harvard Medical School, Boston, MA, USA

⁷Channing Division of Network Medicine, Department of Medicine, Brigham and Women's Hospital and Harvard Medical School, Boston, MA, USA

⁸Department of Biostatistics and Epidemiology, School of Public Health, China Medical University, Shenyang, Liaoning, PR China

Co-corresponding authors: Eva S. Schernhammer, MD, DrPH, Center for Public Health, Department of Epidemiology, Medical University of Vienna, Kinderspitalgasse 15, 1090 Vienna, Austria, Telephone: +43-40160-34704, eva.schernhammer@meduniwien.ac.at; Reiko Nishihara, MD, PhD, Program in MPE Molecular Pathological Epidemiology, Department of Pathology, Brigham and Women's Hospital, 60 Fenwood Road, BTM8002S, Boston, MA 02115, USA, Telephone: +1-857-307-4138; Fax: +1-617-582-8558, rnishiha@hsph.harvard.edu; Xuehong Zhang, MD, ScD, Brigham and Women's Hospital, Harvard Medical School, 181 Longwood Avenue, Room 453, Boston, MA 02115, USA, Telephone: +1-617-525-0342; Fax: +1-617-525-2008, xuehong.zhang@channing.harvard.edu.

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Use of standardized official symbols: We use HUGO (Human Genome Organisation)-approved official symbols (or root symbols) for genes and gene products, including *AKT*, *IGF1R*, *INSR*, *IRS1*, *IRS2*, and *PIK3CA*; all of which are described at www.genenames.org. The official symbols are italicized to differentiate from non-italicized colloquial names that are used along with the official symbols. This format enables readers to familiarize themselves with the official symbols for genes and gene products together with common colloquial names.

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⁹Clinical and Translational Epidemiology Unit, Massachusetts General Hospital and Harvard Medical School, Boston, MA, USA

¹⁰Division of Gastroenterology, Massachusetts General Hospital and Harvard Medical School, Boston, MA, USA

¹¹Department of Biostatistics, Harvard T.H. Chan School of Public Health, Boston, MA, USA

¹²Department of Epidemiology, Harvard T.H. Chan School of Public Health, Boston, MA, USA

¹³Laboratory of Human Carcinogenesis, National Cancer Institute, National Institutes of Health, Bethesda, Maryland, USA

¹⁴Department of Pathology, Graduate School of Medicine, The University of Tokyo, Tokyo, Japan

¹⁵Department of Nutrition, Harvard T.H. Chan School of Public Health, Boston, MA, USA

¹⁶Key Laboratory of Environmental Medicine Engineering, Ministry of Education, School of public Health, Southeast University, Nanjing, Jiangsu, P.R. China

¹⁷Department of Epidemiology, Center for Public Health, Medical University of Vienna, Vienna, Austria

¹⁸Yale Cancer Center, New Haven, CT, USA

¹⁹Department of Medicine, Yale School of Medicine, New Haven, CT, USA

²⁰Smilow Cancer Hospital, New Haven, CT, USA

These authors contributed equally to this work.

Abstract

Objective: We hypothesized that the risk of CRC in night-shift workers might be different according to insulin receptor substrates status.

Methods: Among 77,470 eligible women having night work assessed in the Nurses' Health Study, we documented a total of 1,397 CRC cases, of which 304 or 308 had available data on *IRS1* and *IRS2*, respectively. We used duplication method Cox proportional hazards regression analysis for competing risks to calculate HRs and 95% CIs for each CRC subtype. We measured tumor *IRS1* or *IRS2* expression by immunohistochemistry.

Results: Compared with women who never worked night-shifts, those working 15 years night-shifts had a marginal trend of increased overall risk of CRC ($P_{\text{trend}} = 0.06$, multivariable HR = 1.20, 95% CI, 0.99 to 1.45). Longer duration of night-shift work was associated with a higher risk of *IRS2*-positive tumors (multivariable HR = 2.69, 95% CI 1.48 to 4.89, $P_{\text{trend}} = 0.001$, 15 years night-shifts vs. never) but not with *IRS2*-negative tumors (multivariable HR = 0.90, 95% CI 0.54 to 1.51, $P_{\text{trend}} = 0.72$, $P_{\text{heterogeneity for IRS2}} = 0.008$). Similarly, the corresponding multivariable HRs were 1.81 for *IRS1*-positive tumors (95% CI 0.94 to 3.48, $P_{\text{trend}} = 0.06$) and 1.13 for *IRS1*-negative tumors (95% CI 0.71 to 1.80, $P_{\text{trend}} = 0.56$, $P_{\text{heterogeneity for IRS1}} = 0.02$).

Conclusion: Our molecular pathological epidemiology data suggest a potential role of *IRS* in mediating carcinogenesis induced by night-shift work.

Impact: Although these findings need validation, rotating night-shift might increase CRC risk in women with abnormal insulin receptor pathway.

Keywords

Cancer prevention; circadian disruption; cohort study; colon cancer; etiologic heterogeneity; insulin receptor substrate; metabolism; molecular pathological epidemiology; rectal cancer; rotating night-shift work

INTRODUCTION

Shift work is considered as a “probable” (class 2A) carcinogen to human by the International Agency for Research on Cancer.(1,2) Accumulating evidence suggests that shift work involving circadian rhythm disruption is associated with increased risk of some types of cancers such as breast and colorectal cancer (CRC).(3,4) Schernhammer et al previously reported an increased CRC risk with longer duration of night-shift work in the Nurses’ Health study (NHS) in 2003.(4) Subsequent studies (3,5,6) including a recent meta-analysis (7) reported similar findings. However, other studies (8,9) including a most recent one from Papatoniou et al failed to replicate these findings.(9) It is conceivable that dealing with inherently heterogeneous CRC as a single entity might have diluted any risk association with shift work that might exist for a specific molecular subtype of CRC. Further exploring the underlying biological mechanisms would be helpful to better understand these inconsistent associations. Given that CRC is a highly heterogeneous disease, night-shift work might have different effects on the development of different subgroups of CRC defined by tumor molecular characteristics. Thus, molecular pathological epidemiology (MPE) approach that integrates molecular pathology into epidemiological research (10) can link a certain exposure (such as shift work) to specific pathological signatures, thereby better elucidating the possible pathogenic effect of night-shift work on CRC development.

Recent experimental studies suggested that circadian rhythm disruption may be associated with β -cell dysfunction, glucose intolerance, and improper insulin secretion.(11,12) Similarly, population studies showed that night shift worker tended to have lower insulin sensitivity, hyperinsulinemia, insulin resistance, and develop metabolic syndrome, accordingly.(13–17) Insulin resistance is an adaptive process in insulin-sensitive tissues characterized by reduced insulin receptor substrate 1 (IRS1), and increased *IRS1* serine phosphorylation and attenuated downstream signaling. However, there is some evidence demonstrating that the presence of high insulin levels may not necessarily cause insulin resistance, but instead was associated with *IRS1* or *IRS2* expression and / or tyrosine phosphorylation, which could activate downstream the PI3K / MTOR pathway and subsequently promote mitogenesis and cell proliferation, as shown in colon cancer cells and mouse skeletal muscle cells.(18,19) In addition, human evidence reported positive association between high levels of insulin and risk of colon cancer.(20,21) Since *IRS1* and *IRS2* are two primary mediators of insulin-dependent mitogenesis and regulation of glucose metabolism in most cell types,(22) and abundantly expressed in CRC,(23) it appears plausible that *IRS1* and *IRS2* play a key role in colorectal carcinogenesis as part of the chronic metabolic disorder observed in night-shift workers.(24)

In light of this evidence, we hypothesized that longer duration of night-shift work might be associated with an increased risk of colorectal cancer overexpressing IRSs. To test our hypothesis, we prospectively investigated the association of duration of night-shift work with CRC risk according to tumor *IRS1* or *IRS2* expression in the Nurses' Health Study (NHS).

In this cohort, we have previously found that women who worked rotating night-shifts for at least 15 years were at an increased risk of CRC.(4) Integrating host factors (such as night-shift work) and tumor molecular features (such as IRSs expression) may enhance our understanding of the mechanisms through which night-shift work may act on colorectal carcinogenesis.

METHODS

Study population and assessment of night-shift work duration

Participants were identified from the Nurses' Health Study (NHS). Details for the study design and the population have been reported elsewhere.(25–27) A total of 121,700 female registered nurses aged 30 to 55 years were enrolled at baseline in 1976 in the U.S. A biennial questionnaire has been sent to all the participants since 1976 to collect updated information regarding demographics, lifestyle factors, and medical history. Returning the questionnaires was considered to imply informed consent. All procedures of the study were in accordance with the Declaration of Helsinki. The study protocol was approved by the institutional review boards at the Brigham and Women's Hospital, Boston, Massachusetts.

As described previously,(4,28) NHS participants were asked "how many years have you worked rotating night-shifts including at least 3 nights per month, in addition to days or evenings in that month" in 1988. Information on lifetime years of rotating night-shift work was collected in 8 pre-specified categories, which are never, 1 - 2, 3 - 5, 6 - 9, 10 - 14, 15 - 19, 20 - 29, and 30 years. We excluded women with a history of any cancer other than non-melanoma skin cancer, polyposis syndrome, ulcerative colitis, or Crohn's disease in or before 1988, or who did not report their night-shift work duration. A total of 77,470 women were included in this analysis. (Figure 1)

Assessment of covariates

We collected information on potential CRC risk factors including height, body weight, physical activity (METS-hours/week), cigarette smoking, history of sigmoidoscopy or colonoscopy screening, family history of colorectal cancer, history of type 2 diabetes, aspirin use, and menopausal status and use of menopausal hormones at baseline and updated in biennial follow-up questionnaires. Body mass index (BMI) was calculated based on reported height and weight. In addition, we collected information on dietary factors including consumption of alcohol, vitamin D, folate, calcium, red meat, processed meat using a validated food frequency questionnaire, with updates almost every 4 years.(29,30) Furthermore, in 1986, 2000, 2002 and 2008, we asked how many hours a woman slept, on average, in a 24-hour period (5 hours or less, 6 hours, 7 hours, 8 hours, 9 hours, 10 hours, or

11 hours or more). Self-reported sleep duration correlated well with sleep duration assessed by sleep diaries in this cohort (Spearman $r = 0.79$; $P < 0.0001$).⁽³¹⁾

Ascertainment of incident colorectal cancer cases

Participants or next-of-kin were asked for written permission to obtain medical records and pathological reports if they reported cancer on biennial questionnaires. Study researchers blinded to exposure status further reviewed medical and pathological records to confirm all possible CRC cancer cases, and extracted the information on anatomic location, stage and histological type of the cancer. CRC cases were defined as primary tumors with International Classification of Diseases-9 (ICD-9) codes 153 and 154 and with the histological subtypes, adenocarcinoma, signet-ring cell cancer, adenosquamous cancer, as well as undifferentiated cancer (excluding carcinoid, squamous cell cancer, and non-epithelial malignancies, such as sarcoma and lymphoma). We identified unreported fatal CRC and death from state vital statistics records and the National Death Index.

Immunohistochemistry for *IRS1* and *IRS2* expression

We collected formalin-fixed paraffin-embedded (FFPE) archival tissue specimens of colorectal carcinoma resections from hospitals and laboratories, and constructed tissue microarrays (TMA) from CRC blocks as previously described.⁽³²⁾ Methods for tumor *IRS1* and *IRS2* immunohistochemistry have been previously described.⁽³³⁾ TMA sections were deparaffinized, rehydrated, and heated in a pressure cooker for 30 min at 95°C in Antigen Retrieval Citra Solution, pH 6 (BioGenex Laboratories, Fremont, CA, USA). Sections were incubated with Dual Endogenous Enzyme Block (Dako, Carpinteria, CA, USA) for 30 min, followed by the treatment with 10% fetal bovine serum (Life Technologies, Carlsbad, CA, USA) in Tris-buffered saline (TBS) for 30 min. Samples were then incubated at 4°C for 16 h with *IRS1* antibody (rabbit 06-248, Millipore, Billerica, MA, USA; 1:200 dilution) or *IRS2* antibody (rabbit 06-506, Millipore; 1:500). After washing thoroughly in TBS, sections were incubated with anti-rabbit IgG (Vector Laboratories, Burlingame, CA, USA) for 30 min, then treated with streptavidin-peroxidase (ABC kit, Vector Laboratories) according to the manufacturer's instructions. Specimens were visualized using diaminobenzidine (Dako) and counterstained with hematoxylin. Sections processed with the replacement of primary antibody by TBS were used as a negative control.

Immunohistochemical assessment for *IRS1* and *IRS2* in all cases were interpreted by a pathologist (T.M.), and a random group of 76 cases was independently reviewed by a second pathologist (S.A.K.). Both pathologists were blinded to any information concerning the CRC cases. Concordance between the two pathologists indicated substantial agreement for both *IRS1* status (four levels) with a weighted κ of 0.74 (95% CI 0.61 to 0.86) and *IRS2* status (four levels) with a weighted κ of 0.77 (95% CI 0.65 to 0.89). Tumor cytoplasmic *IRS1* and *IRS2* expression status were scored as 1 (no or minimal staining), 2 (weak staining), 3 (moderately intense staining), and 4 (intense staining) based on the staining intensity in colorectal carcinoma cells.

Statistical analysis

We calculated person-years for each participant from 1988 when the shift work questionnaire was returned, to the date of death, CRC diagnosis, or the end of follow-up (June 1, 2012), whichever came first. We used the duplication-method Cox proportional hazards regression for competing risks data to calculate age-adjusted and multivariable-adjusted hazard ratios (HRs) and 95% confidence intervals (CIs) for each CRC subtype.(34) Multivariable hazard ratios were adjusted for age, BMI, smoking, history of colorectal cancer in a parent or sibling, history of sigmoidoscopy / colonoscopy, postmenopausal status and hormone use, physical activity, regular aspirin use, alcohol consumption, total intake of vitamin D, folate, calcium, red meat and processed meat, sleep duration, and history of type 2 diabetes. For covariates, when appropriate, we have calculated the cumulative averages by averaging all the prior intakes up to each questionnaire cycle. All the models were stratified by age (in months) and year of questionnaire return (every two years since baseline questionnaire return). To retain sufficient statistical power in the analysis, we divided the duration of rotating shift work into three categories as never, 1 – 14, and 15 years as main exposure. When appropriate, we calculated cumulative averages for covariates including consumptions of alcohol, vitamin D, folate, calcium, red meat and processed meat. For each covariate with missing data (generally 2 – 3%), we assigned a separate “missing” indicator to include those participants in the multivariable Cox models. We found no violation of the proportional hazard assumption.

To retain statistical power in subgroup analysis, tumors were classified as *IRS1*- or *IRS2*-positive (moderate / intense) and *IRS1*- or *IRS2*-negative (negative / weak) with the score ranging from 3 to 4 and 1 to 2, respectively. We examined the statistical significance of the difference in associations according to cancer subtypes using the likelihood ratio test that compared the model fit that allowed separate associations by different tumor *IRS1* or *IRS2* expression status with the model fit that assumed a common effect. Linear trend tests were conducted using the median of each category of night-shift work duration as a continuous variable, and the *P* value for trend was calculated using a Wald test. We also conducted a sensitivity analysis using inverse probability weighting method as previously described to reduce the potential bias due to the availability of tumor samples.(34,35) We used duplication-method Cox regression cause-specific hazards regression for competing risk data (34) to assess the association between night-shift work and risks of colorectal cancer subtypes by *IRS1* or *IRS2*. In this competing risk model, the outcome of interest is the incidence of a specific subtype of CRC, but not CRC, and therefore, cases without the specific biomarker data were treated as censored as the diagnosis of colorectal cancer. The weight was set as the reciprocal of the predictive probability for each case with the corresponding *IRS1* or *IRS2* marker, whereas, it was set as 1 for non-cases or cases without the corresponding *IRS1* or *IRS2* marker in the weighted Cox regression models.

We did a secondary data analysis stratified by primary tumor location (colon vs. rectum). All analyses were performed using the SAS software (SAS Institute, Version 9.2, Cary, NC), and a two-sided *P* value less than 0.05 was considered statistically significant for the overall risk testing. For subtype analysis, the primary hypothesis test was the heterogeneity in the association with various colorectal cancer subtypes. To account for multiple testing for two

biomarkers (*IRS1* and *IRS2*), we adjusted for the statistical significance level to 0.025 (0.05/2).

RESULTS

Among 77,470 eligible participants reporting their night-shift work history in 1988 with 1,708,790 person-years of follow up, we documented a total of 1,397 incident colorectal cancer cases, of which 304 or 308 had available *IRS1* or *IRS2* expression data respectively (Figure 1). Compared with women who never worked rotating night-shifts, women with longer duration of rotating night-shift work were more likely to be a smoker, overweight, sleepless, and developing type 2 diabetes (Table 1). Additionally, demographic or clinical features were similar according to availability of tumor *IRS1* or *IRS2* status (Supplementary Table S1). Among the CRC cases with available tissue for *IRS1* or *IRS2* expression analysis, 86 (28.2%) and 102 (33.1%) had moderate or intense *IRS1* and *IRS2* expression, respectively.

Consistent with our previous report,(4) we observed a trend of increased overall risk of CRC with at least 15 years of night-shift work ($P_{\text{trend}} = 0.06$). Additionally, this positive association appeared to persist when we restricted our analyses to women with available *IRS1* or *IRS2* expression data (Table 2). We also examined the association between night-shift work duration and CRC risk by primary tumor sites. We found a similar significant trend of increasing risk of rectal cancer (15+years vs. never: multivariable HR=1.54; 95%CI, 1.03 to 2.29) as in Papantoniou et al (same comparison, multivariable HR=1.60; 95%CI, 1.09 to 2.34).

We then tested our primary hypothesis that the association between duration of rotating night-shift work and CRC risk might differ according to *IRS1* or *IRS2* expression. We found that the positive association of longer duration of rotating night-shift work appeared to differ by tumoral *IRS1* or *IRS2* status. Compared with women who never worked rotating night-shifts, women with at least 15 years of rotating night-shift work had a trend of an increased risk for *IRS1*-positive tumors (multivariable HR = 1.81, 95%CI 0.94 to 3.48, $P_{\text{trend}} = 0.06$), but not for *IRS1*-negative tumors (multivariable HR = 1.13, 95%CI 0.71 to 1.80, $P_{\text{trend}} = 0.56$, $P_{\text{heterogeneity}}$ for *IRS1* subtypes = 0.02). Likewise, a stronger association was observed for the *IRS2*-positive tumors (multivariable HR = 2.69, 95% CI 1.48 to 4.89, $P_{\text{trend}} = 0.001$) but not for the *IRS2*-negative tumors (multivariable HR = 0.90, 95% CI 0.54 to 1.51, $P_{\text{trend}} = 0.72$, $P_{\text{heterogeneity}}$ for *IRS2* subtypes = 0.008) (Table 2).

In order to reduce possible bias due to the availability of tumor specimens after diagnosis of CRC, we conducted a sensitivity analysis using the inverse probability weighting (IPW) method as previously described. We observed similar differential associations by both *IRS1* ($P_{\text{heterogeneity}} = 0.001$) and *IRS2* status ($P_{\text{heterogeneity}} = 0.001$) (Supplementary Table S2). In addition, the similar pattern was observed regardless of tumor locations in either colon or rectum, although the heterogeneity test did not reach statistical significance (Supplementary Table S3).

DISCUSSION

As the third most commonly diagnosed cancer both in women and men in US and worldwide,(36,37) CRC comprises a group of heterogeneous diseases in which each tumor arises and behaves in a unique fashion due to its distinctive genetic and epigenetic background. The potential pro-tumorigenic effects of night-shift work on CRC may thus differ by specific tumor molecular subtypes. In this large U.S. prospective cohort of nurses, we found that working a rotating night-shift for at least 15 years was associated with higher risk of *IRS2*-positive and had a trend of higher risk of *IRS1*-positive CRCs, but not negative tumors, compared with women who never worked rotating night-shifts.

Consistent with previous studies including Schernhammer's in 2003,(3–5) we observed positive associations between rotating night-shift work and CRC risk. However, Papantoniou et al published an updated analysis of Schernhammer et al of night shift work and CRC in NHS, and newly adding data from the NHS2 cohort,(9) which we did not include in the current paper due to lack of tumor marker data in NHS2. Our findings regarding to overall association of night-shift work duration (i.e., 15+ years night shift work vs. never) and CRC risk in the NHS cohort (multivariable HR=1.20, 95%CI: 0.99 to 1.45) are consistent with these results (multivariable HR=1.15, 95%CI: 0.95 to 1.39). There were also some differences in the inclusion criteria and covariate adjustment between these two studies. Specifically, Papantoniou included 130 additional CRC cases (N=1,527) compared to ours (N=1,397), as these 130 CRC cases were histologic subtypes of malignancies (carcinoid, leiomyosarcoma, and squamous cell cancer), which may have different pathogenesis and were therefore not suitable for MPE analysis. Lastly, we adjusted for additional covariates in our paper that were not included in Papantoniou's paper, all of which likely contributed to the slight difference in magnitude of the aforementioned associations.

Working at night and rotating shifts could lead to a series of unfavorable alterations of the sleep cycle and cell cycle,(38) lipid and carbohydrate metabolism, and insulin resistance. (39,40) Because these alterations play a role in regulation of cell proliferation, the observed positive association is biological plausible.(1,41) The insulin resistance system involving the insulin receptor (*INSR*) and *IGF1R* pathways is the primary system responsible for many manifestations of metabolic disorders. Insulin is also considered as a growth factor for tumor formation by stimulating proliferation, inhibiting apoptosis or activating the *INSR* and *IGF1R* pathway.(42) Recent population studies showed that circulation *IGF1* and *IGF2*, and some of genetic variants in the *INSR* or *IGF1R* pathway (such as single-nucleotide polymorphisms in *IGF1*, *IGFBP3*, *INSR* and *IRS*), were associated with CRC risk. (43–48) Therefore, the influence of night-shift working on colorectal cancer might partially act through the *INSR* and *IGF1R* pathway. The positive associations of CRC risk and night-shift work observed for *IRS2*-positive tumors, and the marginally significant association for *IRS1*-positive tumors support this possibility.

IRS proteins are a family of cytoplasmic proteins composed of six members (*IRS1* to *6*) that regulate numerous processes such as growth, metabolism, survival and proliferation.(49) *IRS1* and *IRS2* were identified as the first two dominant members of the *IRS* family, which act as the mediators of the *INSR* and *IGF1R* pathway and play a central role in maintaining

diverse cellular functions, such as metabolism and proliferation.(24,50) In normal metabolic regulation, these proteins contribute to the insulin-regulated glucose homeostasis through promoting glucose uptake and utilization, and regulating the biosynthesis of macromolecules that are required for cell growth and proliferation.(50) When human circadian rhythms are disrupted, such as in night-shift workers, glucose homeostasis is dysregulated, leading to hyperinsulinemia and insulin insensitivity, as well as potentially insulin resistance. In vitro, high levels of insulin may stimulate *IRS1* tyrosine phosphorylation, which is associated with activation of *PI3K / AKT* and *MAPK* pathway, and mitogenesis in mouse skeletal muscle cells.(19) Similarly, chronic insulin exposure may be associated with *IRS1* and *IRS2* expression, *AKT* activation and chemo-resistance in some colon cancer cells.(18) Phosphorylation of *IRS1* tyrosine sites could activate downstream pathways including *PI3K / AKT*, *MAPK*, and *PAK1*, which increase proliferation and cell survival in cancer cell.(51,52) Many studies have focused on the increased expression level or activity of IRSs in different human cancers including colorectal cancer, and correlated these with poor prognosis, potentially defining them as oncogenic proteins.(23,53) In light of this evidence, night-shift workers may experience different degrees of metabolic disorders such as insulin oscillations or hyperinsulinemia, which can stimulate IRSs and its downstream signaling. This disruption can eventually result in tumor occurrence as the duration of exposure (i.e., night-shift work) increases. Hence, it is plausible that the higher risk of longer duration of shift work appeared in IRSs-positive CRC but not IRSs-negative tumors.

We also observed slightly stronger positive associations with *IRS2*-positive than *IRS1*-positive tumors, suggesting a possible different role of *IRS1* and *IRS2* in tumorigenesis. To date, most such research has focused on breast cancer. Using the PyV-MT mouse model of mammary tumor progression, it was reported that tumor onset and growth were equivalent in the absence of either *IRS1* or *IRS2*.(54,55) However, the absence of *IRS2* was associated with the regression of mammary tumor metastasis but *IRS1* cannot compensate for this loss. (55) And in *irs1*-/- tumors, *IRS2* activation was enhanced and associated with a higher frequency of metastasis.(54) Moreover, *IRS1* was expressed predominantly in estrogen receptor positive, well-differentiated breast cancer cell lines, whereas *IRS2* was expressed in estrogen receptor negative, poorly-differentiated metastatic breast cancer cells.(56,57) Taken together, these studies suggest that *IRS2* might play a different role than *IRS1* in tumor initiation, aggressiveness, and progression. Further functional studies in colon cancer pre-clinical models are warranted to further clarify these potential biological mechanisms.

Our study has several strengths, including the prospective design with a large sample size, long-term follow-up with high follow-up rate, and validated CRC outcomes. The repeated assessments of a variety of dietary and lifestyle risk factors allowed better confounding control. Furthermore, the availability of tumor *IRS1* and *IRS2* data in these cohorts enabled us to identify tumor subtypes that are more susceptible to night-shift work, which provide potential mechanistic insights.

Our study has some potential limitations. First, information on lifetime shift work exposure was self-reported, and only inquire once with no further updates beyond 1988. We are unable to evaluate the impact of changes or different intensities or patterns of night-shift work. However, it is likely that these self-reported data among these nurses were reliable

because other self-reported measures by these nurses have been reasonably accurate.(4) Second, not all colorectal cancer cases in the NHS cohort have tumor specimen data from which we can assess their *IRS* status. However, patients with or without *IRS* data were highly comparable. Additionally, to address possible bias due to the availability of tumor specimens, we used IPW in sensitivity analyses and results remained essentially unchanged. Nonetheless, the number of cases with *IRS* data in our study was limited and chance can therefore not be ruled out. Finally, due to sparse data in certain tumor subtype analyses especially in the long-term shift worker group, we could not have enough power to analyze these association by stratify or adjust for other potential confounding molecular features. So, these results should be interpreted cautiously.

In conclusion, our prospective cohort study showed that working at least 15 years of rotating night-shift was associated with higher risk of CRC, particularly for *IRS2*-positive tumors, and with a trend for higher risk of *IRS1*-positive CRCs with increasing duration of night shift work. Our findings suggest a role of *IRS*s, especially for *IRS2*, in mediating pro-tumorigenic effects of night-shift work on CRC. Future studies with more available tumor specimens and functional experiments are needed to confirm these findings and better clarify the underlying mechanisms.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Abbreviations:

CI	confidence interval
CRC	colorectal cancer
FFPE	formalin-fixed paraffin-embedded
FFQ	food frequency questionnaire

HR	hazard ratio
IGF1R	insulin like growth factor 1 receptor
INSR	insulin receptor
IRS	insulin receptor substrate
METS	metabolic equivalent task score
MPE	molecular pathological epidemiology
NHS	Nurses' Health Study
TBS	Tris-buffered saline
TMA	tissue microarrays

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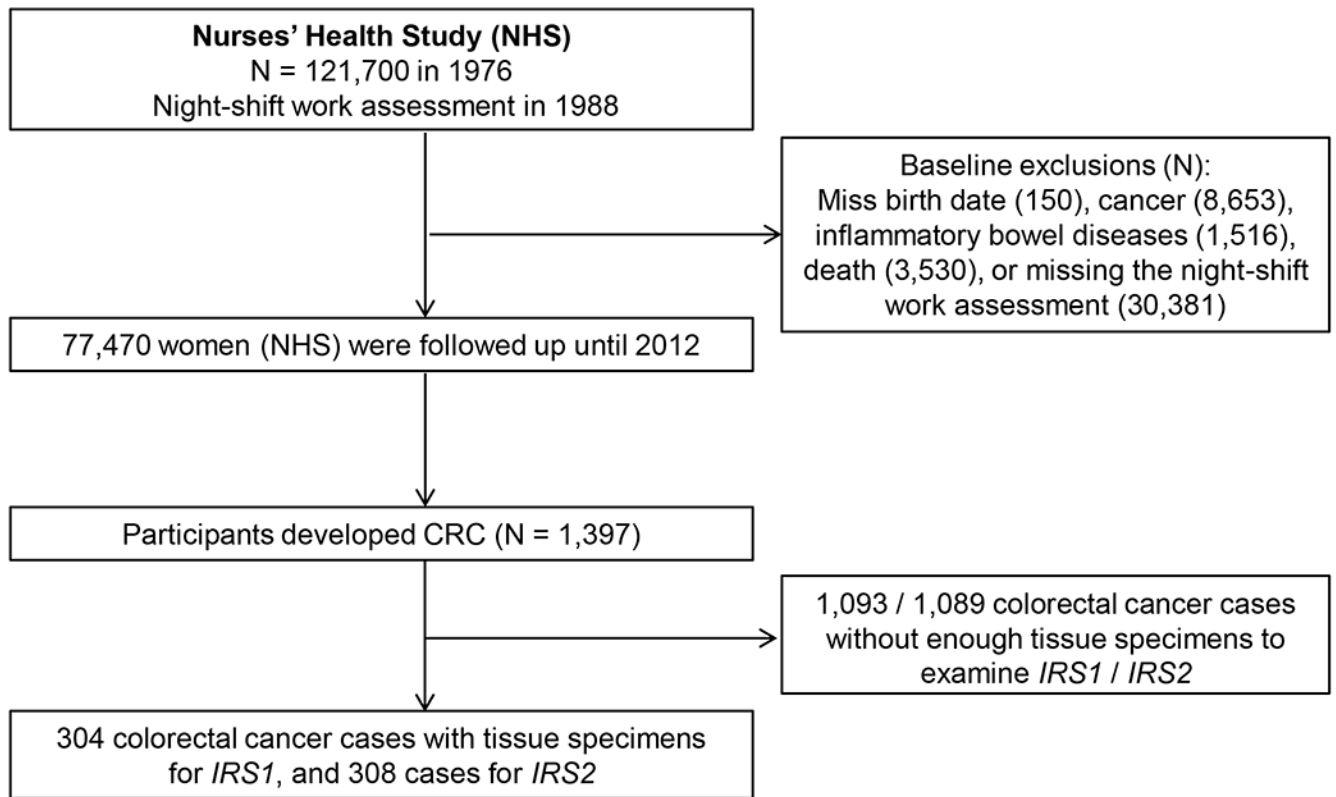


Figure 1.
Flow chart of the study population in the Nurses' Health Study.

Age-adjusted baseline characteristics of participants by night-shift work duration in the Nurses' Health Study (Women, at 1988)

Table 1.

Characteristic	Night-shift work duration		
	Never	1 - 14 years	15 years
N of participants	31,382	40,359	5,729
Age, years*	54.7 (7.2)	54.7 (7.1)	54.8 (7.1)
Race (White), %	97.9	97.5	96.5
Body mass index, kg/m ²	25.3 (4.8)	25.6 (4.9)	26.9 (5.5)
Family history of colorectal cancer, %	11.4	11.6	12.1
History of sigmoidoscopy / endoscopy, %	12.4	12.6	11.4
Postmenopausal status, %	72.1	72.5	76.0
Postmenopausal hormone use, %	37.5	38.1	35.5
Total activity, METS - hours/week [§]	14.6 (20.8)	16.0 (22.0)	16.6 (24.0)
Regular aspirin use (2 or more tablets/week), %	39.3	40.9	42.6
Smoking status, %			
Never, %	45.6	43.3	42.2
Smoking, pack years 0 - 10, %	16.8	17.4	14.4
Pack years > 10, %	36.3	37.9	41.7
Total alcohol intake, g/day	6.2 (10.6)	6.3 (10.7)	5.2 (10.3)
Total vitamin D, IU/day	341 (252)	343 (253)	337 (255)
Total folate intake, µg/day	403 (221)	407 (224)	395 (218)
Total energy intake, kcal/day	1747 (519)	1782 (525)	1789 (556)
Red meat, servings/week	2.2 (1.4)	2.2 (1.4)	2.2 (1.4)
Processed meat, servings/week	1.0 (1.3)	1.0 (1.3)	1.1 (1.3)
Total calcium intake, mg/day	1093 (514)	1088 (507)	1056 (508)
Sleep duration, %			
Sleep < 6h, %	3.1	3.8	8.4
Sleep 6h -< 7h, %	20.5	22.4	28.2
Sleep 7h -< 8h, %	50.2	49.5	44.0
Sleep 8h -< 9h, %	21.9	20.3	15.7
Sleep 9h, %	4.3	3.9	3.6

Characteristic	Night-shift work duration		
	Never	1 - 14 years	15 years
History of type 2 diabetes, %	4.1	4.3	6.7

Values were means \pm standard deviation (SD) or percentages and were standardized to the age distribution of the study population.

* Value was not age adjusted.

[§] METS, metabolic equivalent task score.

Night-shift work duration and colorectal cancer risk according to tumor *IRS1* and *IRS2* expression status in the Nurses' Health Study

Table 2.

	Night-shift work duration			<i>P</i> _{trend} [*]	<i>P</i> _{heterogeneity} [¶]
	Never	1 - 14 years	15 years		
Total colorectal cancer in the full cohorts					
No. cases (N = 1,397)	536	718	143		
Age-adjusted HR (95% CI)	1 (ref)	1.01 (0.90 to 1.13)	1.28 (1.06 to 1.54)	0.008	
Multivariable HR (95% CI) [§]	1 (ref)	1.01 (0.90 to 1.13)	1.20 (0.99 to 1.45)	0.06	
Total colorectal cancer among women with <i>IRS1</i> data					
No. cases (N = 304)	122	146	36		
Age-adjusted HR (95% CI)	1 (ref)	0.91 (0.71 to 1.16)	1.42 (0.97 to 2.06)	0.05	
Multivariable HR (95% CI) [§]	1 (ref)	0.90 (0.70 to 1.14)	1.31 (0.89 to 1.91)	0.13	
<i>IRS1</i>					
Negative / weak					
No. cases (N = 218)	90	105	23		
Age-adjusted HR (95% CI)	1 (ref)	0.88 (0.66 to 1.17)	1.23 (0.77 to 1.94)	0.36	0.02
Multivariable HR (95% CI) [§]	1 (ref)	0.87 (0.65 to 1.15)	1.13 (0.71 to 1.80)	0.56	0.02
Moderate / intense					
No. cases (N = 86)	32	41	13		
Age-adjusted HR (95% CI)	1 (ref)	0.98 (0.62 to 1.56)	1.96 (1.02 to 3.77)	0.03	
Multivariable HR (95% CI) [§]	1 (ref)	0.97 (0.61 to 1.54)	1.81 (0.94 to 3.48)	0.06	
Total colorectal cancer among women with <i>IRS2</i> data					
No. cases (N = 308)	119	153	36		
Age-adjusted HR (95% CI)	1 (ref)	0.98 (0.77 to 1.25)	1.46 (1.00 to 2.13)	0.04	
Multivariable HR (95% CI) [§]	1 (ref)	0.97 (0.76 to 1.23)	1.35 (0.92 to 1.97)	0.11	
<i>IRS2</i>					
Negative / weak					
No. cases (N = 206)	90	98	18		
Age-adjusted HR (95% CI)	1 (ref)	0.83 (0.62 to 1.11)	0.98 (0.59 to 1.63)	0.95	0.008
Multivariable HR (95% CI) [§]	1 (ref)	0.83 (0.62 to 1.10)	0.90 (0.54 to 1.51)	0.72	0.008

Moderate / intense	Night-shift work duration			P_{trend}^*	$P_{heterogeneity}^{\dagger}$
	Never	1 - 14 years	15 years		
No. cases (N = 102)	29	55	18		
Age-adjusted HR (95% CI)	1 (ref)	1.46 (0.93 to 2.29)	2.92 (1.61 to 5.30)	0.0004	
Multivariable HR (95% CI) [§]	1 (ref)	1.42 (0.90 to 2.22)	2.69 (1.48 to 4.89)	0.001	

CI, confidence interval; HR, hazard ratio.

Duplication-method Cox proportional cause-specific hazards regression for competing risks data was used to compute HRs and 95% CIs.

All analyses were stratified by age (in month) and year of questionnaire return.

* Linear trend test using the median years of each category.

[†] The likelihood ratio test was used to test for the heterogeneity of the associations between night-shift work duration (median) and colorectal cancer risk according to the expression of *IRS1* and *IRS2* (ordinal).

[§] Multivariable hazard ratios were adjusted for age (in month), adult BMI (< 25, 25 -< 27.5, 27.5 -< 30, or ≥ 30 kg/m²), smoking (0, 1-10, or > 10 pack-years), history of colorectal cancer in a parent or sibling (yes or no), history of sigmoidoscopy/colonoscopy (yes or no), postmenopausal status and hormone use (premenopause, postmenopause and never use hormone, postmenopause and current use hormone, postmenopause and past use hormone), physical activity (< 3, 3 -< 27, ≥ 27 METS - hours/week), regular aspirin use (yes or no), alcohol consumption (0 -< 5, 5 -< 15, or ≥ 15 g/day), total intake of vitamin D, folate, calcium, red meat and processed meat (all in tertiles), sleep duration (< 6h, 6 -< 7h, 7 -< 8h, 8 -< 9h, or ≥ 9h), and history of type 2 diabetes (yes or no).