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Night-Shift Work Duration and Risk of Colorectal Cancer According to *IRS1* and *IRS2* Expression

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ABSTRACT

Background: We hypothesized that the risk of colorectal cancer in night-shift workers might be different according to insulin receptor substrate status.

Methods: Among 77,470 eligible women having night work assessed in the Nurses' Health Study, we documented a total of 1,397 colorectal cancer 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% confidence intervals (CI) for each colorectal cancer subtype. We measured tumor *IRS1* or *IRS2* expression by immunohistochemistry (IHC).

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

Conclusions: Our molecular pathologic 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 colorectal cancer risk in women with abnormal insulin receptor pathways.

Introduction

Shift work is considered as a "probable" (class 2A) carcinogen to humans 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 (3, 4). Schernhammer and colleagues previously reported an increased colorectal cancer 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 Papantoniou and colleagues failed to replicate these findings (9). It is conceivable that dealing with inherently heterogeneous colorectal cancer as a single entity might have diluted any risk association with shift work that might exist for a specific molecular subtype of colorectal cancer. Further exploring the

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underlying biological mechanisms would be helpful to better understand these inconsistent associations. Given that colorectal cancer is a highly heterogeneous disease, night-shift work might have different effects on the development of different subgroups of colorectal cancer defined by tumor–molecular characteristics. Thus, a molecular pathologic epidemiology (MPE) approach that integrates molecular pathology into epidemiologic research (10), can link certain exposures (such as shift work) to specific pathologic signatures, thereby better elucidating the possible pathogenic effect of night-shift work on colorectal cancer 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 workers tended to have lower insulin sensitivity, hyperinsulinemia, and insulin resistance, and were more likely to 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 associations between high levels of insulin and risk of colon cancer (20, 21). Because *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 colorectal cancer (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 IRS. To test our hypothesis, we prospectively investigated the association of duration of night-shift work with colorectal cancer risk according to tumor *IRS1* or *IRS2* expression in the Nurses' Health Study (NHS).

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

Materials and Methods

Study population and assessment of night-shift work duration

Participants were identified from the NHS. Details of 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 United States. 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 Board at the Brigham and Women's Hospital (Boston, MA).

As described previously (4, 28), NHS participants were asked "how many years have you worked rotating night-shifts defined as 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 prespecified categories, which are never, 1–2, 3–5, 6–9, 10–14, 15–19, 20–29, and $\geq$30 years. We excluded women with a history of any cancer (other than nonmelanoma skin cancer), polyposis syndrome, ulcerative colitis, or Crohn 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. (**Fig. 1**).

Assessment of covariates

We collected information on potential colorectal cancer 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 II 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 on the basis of reported height and weight. In addition, we collected information on dietary factors including consumption of alcohol, vitamin D, folate, calcium, red meat, and 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 $r = 0.79$; $P < 0.0001$; ref. 31).

Ascertainment of incident colorectal cancer cases

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

IHC for *IRS1* and *IRS2* expression

We collected formalin-fixed paraffin-embedded archival tissue specimens of colorectal carcinoma resections from hospitals and laboratories and constructed tissue microarrays (TMA) from colorectal cancer blocks as described previously (32). Methods for tumor *IRS1* and *IRS2* IHC have been described previously (33). TMA sections were deparaffinized, rehydrated, and heated in a pressure cooker for 30 minutes at 95°C in Antigen Retrieval Citra Solution, pH 6 (BioGenex Laboratories). Sections were incubated with Dual Endogenous Enzyme Block (Dako) for 30 minutes, followed by the treatment with 10% FBS (Life Technologies) in Tris-buffered saline (TBS) for 30 minutes. Samples were then incubated at 4°C for 16 hours with *IRS1* antibody (rabbit 06–248, Millipore; 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) for 30 minutes, then treated with streptavidin-peroxidase (ABC Kit, Vector Laboratories) according to the manufacturer's instructions.

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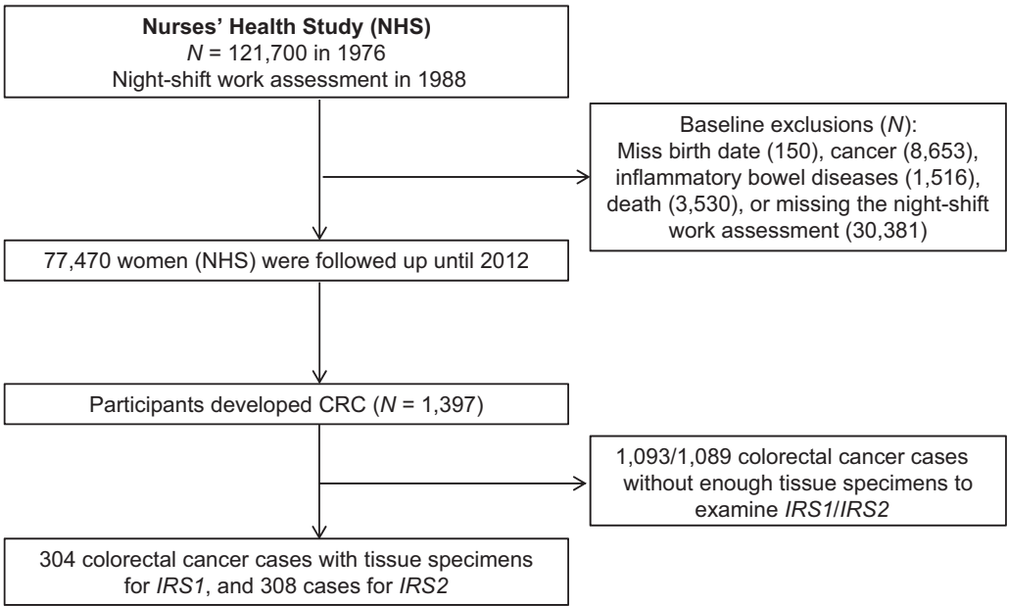

Figure 1.
Flowchart of the study population in the NHS.

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.

IHC assessment for *IRS1* and *IRS2* in all cases were interpreted by a pathologist (T. Morikawa), and a random group of 76 cases was independently reviewed by a second pathologist (S.A. Kim). Both pathologists were blinded to any information concerning the colorectal cancer cases. Concordance between the two pathologists indicated substantial agreement for both *IRS1* status (four levels) with a weighted κ of 0.74 (95% CI, 0.61–0.86) and *IRS2* status (four levels) with a weighted κ of 0.77 (95% CI, 0.65–0.89). 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, colorectal cancer diagnosis, or the end of follow-up (June 1, 2012), whichever came first. We used duplication-method Cox proportional hazards regression for competing risks data to calculate age-adjusted and multivariable-adjusted HRs and 95% confidence intervals (CI) for each colorectal cancer subtype (34). Multivariable HRs 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 II diabetes. For covariates, when appropriate, we have calculated the cumulative averages by averaging all prior intakes up to each questionnaire cycle. All models were stratified by age (in months) and year of questionnaire return (every 2 years since baseline questionnaire return). To retain sufficient statistical power in the analyses, we divided duration of rotating shift work into three categories with never, 1–14, and $\geq$15 years as main exposure. When appropriate, we calculated cumulative averages for covariates including consumption 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 analyses, tumors were classified as *IRS1*- or *IRS2*-positive (moderate/intense) and *IRS1*- or *IRS2*-negative (negative/weak) with scores 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_{trend} was calculated using a Wald test. We also conducted a sensitivity analysis using inverse probability weighting method as described previously to reduce the potential bias due to the availability of tumor samples (34, 35). The outcome of interest was the incidence of a specific subtype of colorectal cancer, but not colorectal cancer, and therefore, cases without the specific biomarker data were treated as censored at 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 noncases 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).

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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 nonitalicized 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.

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 (**Fig. 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 II diabetes (**Table 1**). In addition, demographic or clinical features were similar according to availability of tumor *IRS1* or *IRS2* status (Supplementary Table S1). Among the colorectal cancer 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.

Table 1. Age-adjusted baseline characteristics of participants by night-shift work duration in the NHS (women, at 1988).

Characteristics	Night-shift work duration		
	Never	1–14 years	≥15 years
N of participants	31,382	40,359	5,729
Age, years[a]	54.7 (7.2)	54.7 (7.1)	54.8 (7.1)
Race (White), %	97.9	97.5	96.5
BMI, 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	1,747 (519)	1,782 (525)	1,789 (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	1,093 (514)	1,088 (507)	1,056 (508)
Sleep duration, %			
Sleep <6 h, %	3.1	3.8	8.4
Sleep 6 h–<7 h, %	20.5	22.4	28.2
Sleep 7 h–<8 h, %	50.2	49.5	44.0
Sleep 8 h–<9 h, %	21.9	20.3	15.7
Sleep ≥9 h, %	4.3	3.9	3.6
History of type II diabetes, %	4.1	4.3	6.7

Note: Values were means ± SD or percentages and were standardized to the age distribution of the study population.
Abbreviation: METS, metabolic equivalent task score.
[a]Value was not age adjusted.

Consistent with our previous report (4), we observed a trend of increased overall risk of colorectal cancer with at least 15 years of night-shift work (P_{trend} = 0.06). In addition, 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 colorectal cancer 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, 1.03–2.29) as in Papantoniou and colleagues (same comparison, multivariable HR = 1.60; 95% CI, 1.09–2.34).

We then tested our primary hypothesis that the association between duration of rotating night-shift work and colorectal cancer 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, 0.94–3.48; P_{trend} = 0.06), but not for *IRS1*-negative tumors (multivariable HR = 1.13; 95% CI, 0.71–1.80; P_{trend} = 0.56; $P_{heterogeneity}$ for *IRS1* subtypes = 0.02). Likewise, a stronger association was observed for the *IRS2*-positive tumors (multivariable HR = 2.69; 95% CI, 1.48–4.89; P_{trend} = 0.001) but not for the *IRS2*-negative tumors (multivariable HR = 0.90; 95% CI, 0.54–1.51; P_{trend} = 0.72; $P_{heterogeneity}$ for *IRS2* subtypes = 0.008; **Table 2**).

To reduce possible bias due to the availability of tumor specimens after diagnosis of colorectal cancer, we conducted a sensitivity analysis using the inverse probability weighting (IPW) method as described previously. We observed similar differential associations by both *IRS1* ($P_{heterogeneity}$ = 0.001) and *IRS2* status ($P_{heterogeneity}$ = 0.001; Supplementary Table S2). This 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 the United States and worldwide (36, 37), colorectal cancer 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 protumorigenic effects of night-shift work on colorectal cancer 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 colorectal cancers and had a trend of higher risk of *IRS1*-positive colorectal cancers, 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 colorectal cancer risk. However, Papantoniou and colleagues published an updated analysis of Schernhammer and colleagues of night-shift work and colorectal cancer in NHS, and newly adding data from the NHS2 cohort (9), which we did not include in this study due to lack of tumor marker data in NHS2. Our findings with regard to the overall association of night-shift work duration (i.e.,

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Table 2. Night-shift work duration and colorectal cancer risk according to tumor *IRS1* and *IRS2* expression status in the NHS.

	Night-shift work duration				
	Never	**1–14 years**	**≥15 years**	P_{trend}[a]	$P_{heterogeneity}$[b]
Total colorectal cancer in the full cohorts					
No. cases (N = 1,397)	536	718	143		
Age-adjusted HR (95% CI)	1 (ref)	1.01 (0.90–1.13)	1.28 (1.06–1.54)	0.008	
Multivariable HR (95% CI)[c]	1 (ref)	1.01 (0.90–1.13)	1.20 (0.99–1.45)	0.06	
Total colorectal cancer among women with *IRS1* data					
No. cases (N = 304)	122	146	36		
Age-adjusted HR (95% CI)	1 (ref)	0.91 (0.71–1.16)	1.42 (0.97–2.06)	0.05	
Multivariable HR (95% CI)[c]	1 (ref)	0.90 (0.70–1.14)	1.31 (0.89–1.91)	0.13	
IRS1					
Negative/weak					
No. cases (N = 218)	90	105	23		
Age-adjusted HR (95% CI)	1 (ref)	0.88 (0.66–1.17)	1.23 (0.77–1.94)	0.36	0.02
Multivariable HR (95% CI)[c]	1 (ref)	0.87 (0.65–1.15)	1.13 (0.71–1.80)	0.56	0.02
Moderate/intense					
No. cases (N = 86)	32	41	13		
Age-adjusted HR (95% CI)	1 (ref)	0.98 (0.62–1.56)	1.96 (1.02–3.77)	0.03	
Multivariable HR (95% CI)[c]	1 (ref)	0.97 (0.61–1.54)	1.81 (0.94–3.48)	0.06	
Total colorectal cancer among women with *IRS2* data					
No. cases (N = 308)	119	153	36		
Age-adjusted HR (95% CI)	1 (ref)	0.98 (0.77–1.25)	1.46 (1.00–2.13)	0.04	
Multivariable HR (95% CI)[c]	1 (ref)	0.97 (0.76–1.23)	1.35 (0.92–1.97)	0.11	
IRS2					
Negative/weak					
No. cases (N = 206)	90	98	18		
Age-adjusted HR (95% CI)	1 (ref)	0.83 (0.62–1.11)	0.98 (0.59–1.63)	0.95	0.008
Multivariable HR (95% CI)[c]	1 (ref)	0.83 (0.62–1.10)	0.90 (0.54–1.51)	0.72	0.008
Moderate/intense					
No. cases (N = 102)	29	55	18		
Age-adjusted HR (95% CI)	1 (ref)	1.46 (0.93–2.29)	2.92 (1.61–5.30)	0.0004	
Multivariable HR (95% CI)[c]	1 (ref)	1.42 (0.90–2.22)	2.69 (1.48–4.89)	0.001	

Note: 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.

Abbreviation: No., number.

[a]Linear trend test using the median years of each category.

[b]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).

[c]Multivariable HRs were adjusted for age (in month), adult BMI (<25, 25–<27.5, 27.5–<30, or ≥30 kg/m^2), 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 (<6 h, 6–<7 h, 7–<8 h, 8–<9 h, or ≥9 h), and history of type II diabetes (yes or no).

15+ years night-shift work vs. never) and colorectal cancer risk in the NHS cohort (multivariable HR = 1.20; 95% CI, 0.99–1.45) are consistent with these results (multivariable HR = 1.15; 95% CI, 0.95–1.39). There were also some differences in the inclusion criteria and covariate adjustment between these two studies. Specifically, Papantoniou included 130 additional colorectal cancer cases (N = 1,527) compared with ours (N = 1,397), as these 130 colorectal cancer 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. Finally, we adjusted for additional covariates in our study that were not included in Papantoniou's study, 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 biologically 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 of *IGF1* and *IGF2* and some of the genetic variants in the *INSR* or *IGF1R* pathway (such as single-nucleotide polymorphisms in *IGF1, IGFBP3, INSR*, and *IRS*) was associated with colorectal cancer 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 colorectal cancer 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,

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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 chemoresistance 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 IRS 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 IRS and their 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 IRS-positive colorectal cancer but not IRS-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 ER^{+}, well-differentiated breast cancer cell lines, whereas *IRS2* was expressed in ER^{-}, 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 preclinical 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 colorectal cancer 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 inquired 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. In addition, 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 did not have enough power to analyze these associations by stratifying or adjusting for other potential confounding molecular features. Hence, 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 colorectal cancer, particularly for *IRS2*-positive tumors, and with a trend for higher risk of *IRS1*-positive colorectal cancers with increasing duration of night-shift work. Our findings suggest a role of IRS, especially for *IRS2*, in mediating protumorigenic effects of night-shift work on colorectal cancer. Future studies with more available tumor specimens and functional experiments are needed to confirm these findings and better clarify the underlying mechanisms.

Disclosure of Potential Conflicts of Interest

C.S. Fuchs is Director at CytomX Therapeutics, has ownership interest (including patents) in CytomX Therapeutics and Entrinsic Health, and is a consultant for Agios, Bain Capital, KEW, Merck, Merrimack Pharma, Pfizer, Sanofi, Taiho, Unum Therapeutics, CytomX Therapeutics, Celgene, Dicerna, Five Prime Therapeutics, Gilead Sciences, Eli Lilly, Entrinsic Health, and Genentech. J.A. Meyerhardt is an advisory board member for COTA Healthcare and Ignyta and is a member of a grant review committee through the National Comprehensive Cancer Network (NCCN) for Taiho Pharmaceutical. No potential conflicts of interest were disclosed by the other authors.

Disclaimer

The content is solely the responsibility of the authors and does not necessarily represent the official views of NIH. The funders had no role in design and conduct of the study; collection, management, analysis, and interpretation of the data; and preparation, review, or approval of the manuscript. The authors assume full responsibility for analyses and interpretation of these data.

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