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Aurora-A Expression Is Independently Associated with Chromosomal Instability in Colorectal Cancer[1]

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Abstract

AURKA (the official symbol for Aurora-A, STK15, or BTAK) regulates the function of centrosomes, spindles, and kinetochores for proper mitotic progression. AURKA overexpression is observed in various cancers including colon cancer, and a link between AURKA and chromosomal instability (CIN) has been proposed. However, no study has comprehensively examined AURKA expression in relation to CIN or prognosis using a large number of tumors. Using 517 colorectal cancers in two prospective cohort studies, we detected AURKA overexpression (by immunohistochemistry) in 98 tumors (19%). We assessed other molecular events including loss of heterozygosity (LOH) in 2p, 5q, 17q, and 18q, the CpG island methylation phenotype (CIMP), and microsatellite instability (MSI). Prognostic significance of AURKA was evaluated by Cox regression and Kaplan-Meier method. In both univariate and multivariate logistic regressions, AURKA overexpression was significantly associated with CIN (defined as the presence of LOH in any of the chromosomal segments; multivariate odds ratio, 2.97; 95% confidence interval, 1.40-6.29; $P = .0045$). In multivariate analysis, AURKA was associated with cyclin D1 expression ($P = .010$) and inversely with *PIK3CA* mutation ($P = .014$), fatty acid synthase expression ($P = .028$), and family history of colorectal cancer ($P = .050$), but not with sex, age, body mass index, tumor location, stage, CIMP, MSI, *KRAS*, *BRAF*, *BMI*, LINE-1 hypomethylation, p53, p21, β-catenin, or cyclooxygenase 2. AURKA was not significantly associated with clinical outcome or survival. In conclusion, AURKA overexpression is independently associated with CIN in colorectal cancer, supporting a potential role of Aurora kinase-A in colorectal carcinogenesis through genomic instability (rather than epigenomic instability).

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Abbreviations: AURKA, Aurora-A; BMI, body mass index; CI, confidence interval; CIN, chromosomal instability; CIMP, CpG island methylator phenotype; FASN, fatty acid synthase; HPFS, Health Professionals Follow-up Study; HR, hazard ratio; LOH, loss of heterozygosity; MSI, microsatellite instability; MSS, microsatellite stable; NHS, Nurses' Health Study; OR, odds ratio

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Introduction

Chromosomal instability (CIN) in cancer is characterized by frequent chromosomal abnormalities including translocations, gains, and losses of chromosomes or their segments. Chromosomal instability promotes carcinogenesis through loss of tumor suppressors and copy number gains of oncogenes [1]. Causes of CIN are still poorly understood but possibly include mitotic spindle checkpoint gene (e.g., *BUB1* and *BUB1B*) deregulation [2], DNA checkpoint gene (e.g., *TP53*) mutation [3], cell cycle regulator (e.g., *FBXW7*) inactivation [4], telomere dysfunction [5], and abnormal centrosome number and function [6–8]. Centrosome dysfunction causes abnormal centrosome segregation during mitosis, which may lead to CIN [6–8].

AURKA (the official symbol for Aurora-A, also known as STK15/BTAK) is a member of the Aurora family of cell cycle–regulating serine/threonine kinases and functions in centrosome regulation and mitotic spindle formation [9,10]. Activation of AURKA in experimental systems confers malignant phenotype by inducing centrosome amplification and genomic instability, indicating *AURKA* as an oncogene [11–13]. AURKA expression has been reported in various human cancers [14–20], including colorectal cancers [16]. *AURKA* amplification correlates with CIN in colorectal cancer [21]. However, to our knowledge, no study has comprehensively examined the relation between AURKA protein overexpression and CIN in colorectal cancer or whether the relation is independent of other related molecular features including microsatellite instability (MSI) and the CpG island methylator phenotype (CIMP).

In this study using a large number (N = 517) of colorectal cancers, we examined AURKA overexpression in relation to CIN and patient survival. Because our tumor database included other related molecular events, we were able to assess whether there was an independent relation between AURKA and CIN. Our current data support AURKA as one of potential contributing factors for CIN in colorectal cancer.

Materials and Methods

Study Group

We used the databases of two large prospective cohort studies; the Nurses' Health Study (NHS; N = 121,700 women followed since 1976) [22,23], and the Health Professionals Follow-up Study (HPFS; N = 51,500 men followed since 1986) [23]. Data on height and weight were obtained by biennial questionnaire. A subset of the cohort participants developed colorectal cancers during prospective follow-up. Previous studies on the NHS and HPFS have described baseline characteristics of cohort participants and incident colorectal cancer cases and confirmed that our colorectal cancers were good representatives of a population-based sample [22,23]. Data on tumor location and stage were obtained through medical record review. We collected paraffin-embedded tissue blocks from hospitals where patients had undergone resections of colorectal cancers. On the basis of availability of adequate tissue specimens and results, a total of 517 colorectal cancers were included. Written informed consent was obtained from all study subjects. Among our cohort studies, there was no significant difference in demographic features between cases with tissue available and those without available tissue [23]. This current analysis represents a new analysis of AURKA on the existing colorectal cancer database that have been previously characterized for CIMP, MSI, p53, *KRAS*, *BRAF*, *PIK3CA*, long interspersed nucleotide element 1 (LINE-1), cyclooxygenase 2 (COX-2), and clinical outcome [23–26], which is analogous to novel studies using the well-described cell lines or animal models. In any of our previous studies, we have not examined AURKA expression or the relations of AURKA with clinical outcome and other molecular events. This study represents a unique novel study in term of 1) a large sample size analyzed for AURKA; 2) the comprehensive clinical and tissue molecular database used, including the long-term follow-up outcome data, CIMP, MSI, *KRAS*, *BRAF*, *PIK3CA*, p53, β-catenin, LINE-1 methylation, and COX-2; and 3) a number of molecular correlates that have been analyzed. Tissue collection and analyses were approved by the Harvard School of Public Health and Brigham and Women's Hospital Institutional Review Boards.

Histopathologic Evaluations

Hematoxylin and eosin–stained tissue sections were examined by a pathologist (S.O.) unaware of other data. The tumor grade was categorized as low (≥50% gland formation) *versus* high (<50% gland formation). The presence and extent of extracellular mucin were categorized as 0% (no mucin), 1% to 49%, or ≥50% of the tumor volume [27]. The presence and extent of signet ring cells were categorized as absent (0%) or present (≥1%) [27].

Sequencing of KRAS, BRAF, *and* PIK3CA

Genomic DNA was extracted from tumor and polymerase chain reaction (PCR) and Pyrosequencing targeted for *KRAS* (codons 12 and 13) [28], *BRAF* (codon 600) [29], and *PIK3CA* (exons 9 and 20) [30] were performed as previously described.

Microsatellite Instability Analysis

Microsatellite instability analysis was performed, using 10 microsatellite markers (D2S123, D5S346, D17S250, BAT25, BAT26, BAT40, D18S55, D18S56, D18S67, and D18S487) [27]. MSI-high was defined as the presence of instability in ≥30% of the markers. MSI-low was defined as instability in <30% of the markers, and microsatellite stable (MSS) tumors were defined as tumors without an unstable marker [27].

Loss of Heterozygosity Analysis

For loss of heterozygosity (LOH) analysis using microsatellite markers (D2S123, D5S346, D17S250, D18S55, D18S56, D18S67, and D18S487), we duplicated PCR in each sample to exclude allele dropouts of one of two alleles [27,31]. Loss of heterozygosity at each locus was defined as ≥40% reduction of one of two allele peaks in tumor DNA relative to normal DNA. Chromosomal instability (CIN) positivity was defined as the presence of LOH in any of the chromosomal segments among 2p, 5q, 17q, and 18q. CIN negativity was defined as the absence of LOH in any of the chromosomal segments with the presence of at least two informative segments.

Real-time PCR for Quantitative DNA Methylation Analysis

Sodium bisulfite treatment on genomic DNA and subsequent real-time PCR (MethyLight) were validated and performed as previously described [32]. We quantified DNA methylation in eight CIMP-specific promoters [*CACNA1G*, *CDKN2A* (p16), *CRABP1*, *IGF2*, *MLH1*, *NEUROG1*, *RUNX3*, and *SOCS1*] [24,33,34]. CIMP-high was defined as the presence of six or more of eight methylated promoters, CIMP-low as the presence of one to five of eight methylated promoters,

and CIMP-0 as the absence of methylated promoters, according to the previously established criteria [24,35].

Pyrosequencing to Measure LINE-1 Methylation

To accurately quantify relatively high methylation levels in LINE-1 repetitive elements, we used Pyrosequencing as previously described [25,36].

Immunohistochemistry for AURKA, p53, p21, Cyclin D1, β-Catenin, COX-2, and Fatty Acid Synthase

Tissue microarrays were constructed as previously described [23,37]. Methods of immunohistochemical procedures and examples of staining patterns can be found in the previous reports as follows: p53 [38], p21 (*CDKN1A*) [39,40], cyclin D1 [41], β-catenin [42], COX-2 [27,43], and fatty acid synthase (FASN) [27,44]. For AURKA, antigen retrieval was performed, and deparaffinized tissue sections in Antigen Retrieval Citra Solution (Biogenex Laboratories, San Ramon, CA) were treated with microwave in a pressure cooker for 25 minutes. Tissue sections were incubated with 3% H_2O_2 (30 minutes) to block endogenous peroxidase (Dako Cytomation, Carpinteria, CA). A primary antibody [mouse monoclonal to AURKA (ab13824), 1:100 dilution; Abcam Inc, Cambridge, MA] was applied, and the slides were maintained at 4°C overnight, followed by mouse secondary antibody (Vector Laboratories, Burlingame, CA) for 60 minutes, an avidin-biotin complex conjugate (Vector Laboratories) for 60 minutes, diaminobenzidine (5 minutes) and methyl-green counterstain. Nuclear AURKA expression was recorded as no expression, weak expression, moderate expression, or strong expression (Figure 1). AURKA overexpression was defined as moderate to strong expression in any portion of tumor cells or at least 50% of tumor cells with weak staining. Appropriate positive and negative controls were included in each run of immunohistochemistry. Each immunohistochemical maker was interpreted by one of the investigators (AURKA by Y.B.; cyclin D1 and β-catenin by K.N.; p53, p21, COX-2, and FASN by S.O.) unaware of other data. A random selection of 117 cases was examined for AURKA by a second observer (K.S.) unaware of other data, and concordance between the two observers was 0.85 (κ = 0.62, P < .0001), indicating substantial agreement. For each of the other immunohistochemical markers, a second observer (S.O. for β-catenin; K.S. for cyclin D1 and p21; K.N. for p53, COX-2, and FASN) examined a random selection of more than 100 tumors unaware of other data, and the concordance rate between the two observers was always greater than 0.82 [all κ > 0.61 (except for FASN, κ = 0.57), all P < .0001], indicating generally substantial agreement.

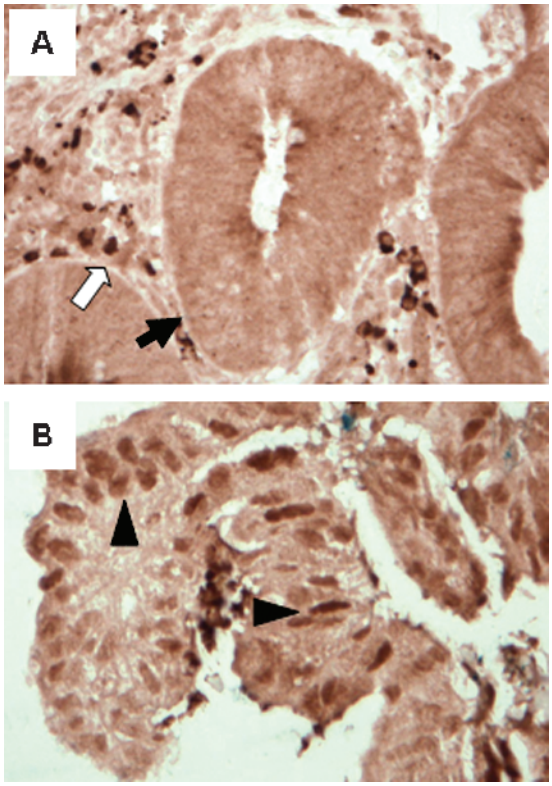

Figure 1. AURKA (Aurora-A) expression in colorectal cancer cells. (A) Negative for nuclear AURKA overexpression in colorectal cancer cells (arrow). Inflammatory cells serve as an internal positive control for AURKA positivity (white arrow). (B) Positive for nuclear AURKA overexpression in colorectal cancer cells (arrowheads).

Statistical Analysis

All statistical analyses used SAS program (Version 9.1; SAS Institute, Cary, NC). All P values were two-sided, and statistical significance was set at $P \leq .05$. For categorical data, the χ^2 test was performed, and odds ratio (OR) with 95% confidence interval (CI) was computed. To compare mean LINE-1 methylation levels, the t test assuming unequal variances was performed. The κ coefficient was calculated to assess an agreement between the two interpreters in immunohistochemical analyses. To assess independent relations of AURKA overexpression with CIN, a multivariate logistic regression analysis was performed. Odds ratio was adjusted for sex, age (continuous), body mass index (BMI, <30 *vs* ≥30 kg/m^2), family history of colorectal cancer (present *vs* absent), tumor location (right colon *vs* left colon *vs* rectum), tumor stage (I-II *vs* III-IV), tumor grade (low *vs* high), mucinous component (0 *vs* ≥1%), signet ring cell component (0 *vs* ≥1%), CIMP status (high *vs* CIMP-low/0), MSI status (high *vs* low/MSS), LINE-1 methylation (continuous), *BRAF*, *KRAS*, *PIK3CA*, p53, p21, cyclin D1, β-catenin, COX-2, and FASN.

For survival analysis, Kaplan-Meier method was used to assess survival time distribution according to AURKA status, and log-rank test was used to test significance of a deviation from the null hypothesis. For the analyses of colorectal cancer–specific mortality, death as a result of colorectal cancer was the primary end point and deaths as a result of other causes were censored. To assess independent effect of AURKA on mortality, we constructed a multivariate, stage-matched conditional Cox proportional hazard model to compute a hazard ratio (HR) according to AURKA status, adjusted for sex, age, year of diagnosis (continuous), BMI, family history of colorectal cancer, tumor location, stage, grade, CIMP, MSI, CIN, *BRAF*, *KRAS*, *PIK3CA*, LINE-1 methylation, p53, p21, cyclin D1, β-catenin, COX-2, and FASN.

Results

AURKA Overexpression in Colorectal Cancer

Among the 517 colorectal cancers in this study, 98 (19%) showed nuclear AURKA overexpression (i.e., AURKA-positive; Figure 1). Table 1 shows the frequencies of AURKA overexpression according to

Table 1. Frequency of AURKA Overexpression in Colorectal Cancer.

Clinical or Pathologic Feature	Total *N*	AURKA (+)	Univariate OR (95% CI)	*P*
All cases	517	98 (19%)		
Gender				
Men	203	35 (17%)	1	
Women	314	63 (20%)	1.20 (0.76-1.90)	
Age (years)				
≤59	118	20 (17%)	1	
60-69	233	43 (18%)	1.10 (0.62-1.99)	
≥70	166	35 (21%)	1.31 (0.71-2.41)	
BMI (kg/m^2)				
<25	209	43 (21%)	1	
25-30	192	36 (19%)	0.89 (0.54-1.46)	
≥30	87	12 (14%)	0.62 (0.31-1.24)	
Family history of colorectal cancer				
(–)	396	84 (21%)	1	Referent
(+)	121	14 (12%)	0.49 (0.26-0.89)	.018
Tumor location				
Right colon (cecum to transverse colon)	243	53 (22%)	1	
Left colon (splenic flexure to sigmoid)	165	25 (15%)	0.64 (0.38-1.08)	
Rectum	89	14 (16%)	0.66 (0.35-1.28)	
Stage				
I	106	18 (17%)	1	
II	158	30 (19%)	1.14 (0.60-2.18)	
III	138	19 (14%)	0.78 (0.39-1.57)	
IV	71	15 (21%)	1.31 (0.61-2.81)	
Tumor grade				
Low	456	82 (18%)	1	
High	45	11 (24%)	1.48 (0.72-3.03)	
Mucinous component				
0%	267	43 (16%)	1	
1%-49%	116	27 (23%)	1.58 (0.92-2.71)	
≥50%	64	16 (25%)	1.74 (0.90-3.34)	
Signet ring cell component				
0%	387	72 (19%)	1	
≥1%	29	7 (24%)	1.39 (0.57-3.38)	

Only significant *P* values are described.

various clinical and pathologic features. AURKA overexpression was inversely associated with family history of colorectal cancer (OR, 0.49; 95% CI, 0.26-0.89; *P* = .018).

AURKA, CIN, and Other Molecular Features in Colorectal Cancer

Table 2 summarizes the frequencies of AURKA overexpression according to status of CIN and other molecular features in colorectal cancer. Chromosomal instability (CIN) was defined as the presence of LOH in any of the chromosomal segments among 2p, 5q, 17q, and 18q. AURKA overexpression was significantly associated with CIN (OR, 2.17; 95% CI, 1.09-4.32; *P* = .024).

AURKA overexpression was inversely associated with *KRAS* mutation (OR, 0.56; 95% CI, 0.35-0.92; *P* = .021), *PIK3CA* mutation (OR, 0.25; 95% CI, 0.09-0.71; *P* = .0050), and β-catenin activation (OR, 0.60; 95% CI, 0.36-0.99; *P* = .046; Table 2).

Multivariate Analysis to Assess Independent Relations with AURKA

We performed multivariate logistic regression analysis to examine whether AURKA was independently associated with CIN (Table 3). AURKA overexpression was significantly associated with CIN (multivariate OR 2.97; 95% CI, 1.40-6.29; *P* = .0045) independent of other variables. In addition, AURKA seemed to be associated with cyclin D1

Table 2. Frequency of AURKA Overexpression in Colorectal Cancer According to Various Molecular Features.

Molecular Feature	Total *N*	AURKA (+)	Univariate OR (95% CI)	*P*
CIN*				
(–)	105	11 (10%)	1	Referent
(+)	291	59 (20%)	2.17 (1.09-4.32)	.024
CIMP status (no. of methylated CIMP markers)				
CIMP-0 (0)	224	43 (19%)	1	
CIMP-low (1-5)	202	30 (15%)	0.73 (0.44-1.22)	
CIMP-high (6-8)	77	21 (27%)	1.58 (0.86-2.88)	
MSI status				
MSS	376	66 (18%)	1	
MSI-low	52	11 (21%)	1.26 (0.62-2.58)	
MSI-high	87	21 (24%)	1.49 (0.86-2.61)	
BRAF mutation				
(–)	437	80 (18%)	1	
(+)	67	17 (25%)	1.51 (0.83-2.77)	
KRAS mutation				
(–)	327	72 (22%)	1	Referent
(+)	189	26 (14%)	0.56 (0.35-0.92)	.021
PIK3CA mutation				
(–)	397	86 (22%)	1	Referent
(+)	62	4 (6%)	0.25 (0.09-0.71)	.0050
LINE-1 methylation				
≥70%	61	9 (15%)	1	
50%-70%	360	66 (18%)	1.30 (0.61-2.76)	
<50%	76	19 (25%)	1.93 (0.80-4.63)	
p53 expression				
(–)	311	67 (22%)	1	
(+)	202	31 (15%)	0.66 (0.41-1.05)	
p21				
Expressed	104	24 (23%)	1	
Lost	403	73 (18%)	0.73 (0.44-1.24)	
Cyclin D1 expression				
(–)	142	21 (15%)	1	
(+)	349	75 (21%)	1.57 (0.93-2.68)	
β-Catenin†				
Inactive (score 0-2)	294	62 (21%)	1	Referent
Active (score 3-5)	175	24 (14%)	0.60 (0.36-0.99)	.046
COX-2 expression				
(–)	90	23 (26%)	1	
(+)	426	74 (17%)	0.61 (0.36-1.05)	
FASN expression				
(–)	350	70 (20%)	1	
(+)	65	7 (11%)	0.48 (0.21-1.10)	

*Chromosomal instability was defined as the presence of LOH in any of the chromosomal segments among 2p, 5q, 17q, and 18q.
†β-Catenin activation score is based on the method previously described [72].

Table 3. Multivariate Logistic Regression to Show Independent Relationship between CIN and AURKA Overexpression in Colorectal Cancer.

Variable Independently Associated with AURKA	Multivariate OR (95% CI)	*P*
CIN*	2.97 (1.40-6.29)	.0045
Other significant variables		
PIK3CA mutation	0.24 (0.08-0.70)	.010
Cyclin D1 expression	1.93 (1.15-3.24)	.012
FASN expression	0.37 (0.15-0.92)	.032
Family history of colorectal cancer	0.50 (0.26-0.96)	.036

The multivariate logistic regression model included age, sex, BMI, tumor location, stage, grade, mucinous component, signet ring cells, CIMP, MSI, *KRAS*, *BRAF*, LINE-1 methylation, p53, p21, β-catenin, COX-2, FASN, and the variables listed in the table. Only significant variables are listed.
*Chromosomal instability was defined as the presence of LOH in any of the chromosomal segments among 2p, 5q, 17q, and 18q.

expression (P = .012) and inversely with *PIK3CA* mutation (P = .010), FASN expression (P = .032), and family history of colorectal cancer (P = .036; Table 3); however, considering multiple hypotheses testing and these P values between .05 and .01, any of these additional associations might simply be a chance event.

AURKA and Patient Survival

We assessed the influence of AURKA overexpression on clinical outcome of patients with stage I to IV colorectal cancer and adequate follow-up. We have previously shown that clinical outcome data in our two independent cohort studies are valid and reliable in detecting significant molecular predictors of patient survival [25,26,43,44]. There were a total of 216 deaths, including 124 colorectal cancer–specific deaths. In Kaplan-Meier analysis, AURKA was not significantly associated with colorectal cancer–specific (log rank, P = .67) or overall survival (log rank, P = .78). We also performed Cox regression analysis to assess patient mortality according to AURKA status (Table 4). For both cancer-specific and overall mortality, AURKA overexpression was not significantly related with patient outcome in univariate analysis, stage-matched analysis, or multivariate analysis. When we assessed patients with colon cancers, AURKA remained unrelated with patient mortality.

Discussion

We conducted this study to examine the relationship between AURKA overexpression and CIN in colorectal cancer. In addition, we assessed the relationship of AURKA with clinical, pathologic, and other molecular features and with patient survival. We found that AURKA was associated with CIN, independent of any of the clinical, pathologic, and molecular variables examined. Our rich tumor database allowed us to examine whether there is an independent relationship of AURKA with CIN as well as with clinical outcome. Our data support the hypothesis that AURKA overexpression is one of the contributing factors for CIN during colorectal cancer development.

Studying molecular alterations is important in cancer research [45–56]. Our resource of a large number (N = 517) of colorectal cancers derived from the two independent prospective cohort studies has enabled us to precisely estimate the frequency of colorectal cancers with a specific molecular feature (such as AURKA overexpression, MSI, etc.). The large number of cases has also provided us with a sufficient power in the multivariate logistic regression analysis and survival analysis. Thus, in survival analysis, we can conclude that AURKA expression is not significantly associated with patient survival.

Accumulating evidence suggests that AURKA activation is related to cancer development through CIN [11–13,57]. AURKA is mainly localized at spindle poles and the mitotic spindle during mitosis, where it regulates the function of centrosomes, spindles, and kinetochores, all of which are required for proper mitosis progression [9,10]. AURKA protein expression has also been related with TERT (telomerase) activity, thus possibly related with telomere function [16]. *AURKA* gene amplification [58,59] and *AURKA* gene overexpression [58] have been reported in colorectal cancer. Another study using colon cancer cell lines and a small number (N = 48) of human colorectal cancer tissues has shown that high copy number of *AURKA* is associated with CIN and AURKA protein overexpression [21]. However, the discrepancy in the frequencies of *AURKA* amplification and AURKA protein overexpression has been reported in several other cancers [13,19,60]. To the best of our knowledge, a significant relationship between AURKA protein overexpression and CIN has not been shown using a large number of colorectal cancer tissues. Molecular correlates with AURKA protein overexpression are important for the better understanding of genetic and epigenetic alterations during the colorectal carcinogenic process, especially in relation to CIN.

It remains controversial whether AURKA expression level correlates with malignant phenotype or patient prognosis in human cancers. Studies on several types of human cancers have indicated that AURKA overexpression may be related with poor prognosis [15,17,18,61] or with higher recurrence rate [62]. However, other studies have shown that AURKA expression is not associated with patient prognosis [20,63]. Another study has shown that activation of AURKA is associated with an early stage disease in ovarian cancer [19]. In a previous study on colorectal cancer (N = 200) [16], AURKA overexpression was associated with high-grade tumor, but the relationship between AURKA and clinical outcome was unclear. In our current study (N = 517), AURKA overexpression was not associated with tumor grade. This discrepancy might be due to a difference in the patient cohorts or the methods to assess AURKA overexpression or simply due to a chance variation between different studies. In addition, our analysis found that AURKA overexpression was not associated with prognosis of patients with stage I to IV colorectal cancer. Our findings suggest that AURKA overexpression may not mark an aggressive type of colorectal cancer.

Interestingly, we did observe inverse relations between AURKA and *PIK3CA* mutation as well as FASN expression in colorectal cancer, independent of clinical, pathologic, and other molecular variables. In various cancers including colorectal cancer, mutant *PIK3CA* stimulates the PI3K-AKT signaling pathway [64]. Fatty acid synthase overexpression has been associated with the PI3K-AKT pathway

Table 4. AURKA Expression and Patient Mortality in Colorectal Cancer.

	Total N	Cancer-Specific Mortality				Overall Mortality			
		Deaths/Person-Years	Univariate HR (95% CI)	Stage-matched HR (95% CI)	Multivariate HR (95% CI)	Deaths/Person-Years	Univariate HR (95% CI)	Stage-matched HR (95% CI)	Multivariate HR (95% CI)
Colon and rectal cancers									
AURKA (–)	398	103/3180	1 (referent)	1 (referent)	1 (referent)	179/3180	1 (referent)	1 (referent)	1 (referent)
AURKA (+)	89	21/688	0.90 (0.57-1.45)	0.98 (0.60-1.60)	0.97 (0.57-1.65)	37/688	0.95 (0.67-1.36)	1.00 (0.69-1.45)	1.03 (0.69-1.53)
Colon cancers									
AURKA (–)	324	78/2648	1 (referent)	1 (referent)	1 (referent)	144/2648	1 (referent)	1 (referent)	1 (referent)
AURKA (+)	75	19/569	1.07 (0.65-1.77)	1.13 (0.66-1.93)	0.89 (0.49-1.60)	31/569	0.99 (0.67-1.46)	1.00 (0.66-1.50)	0.99 (0.63-1.55)

The multivariate, stage-matched conditional Cox model included sex, age, year of diagnosis (continuous), BMI, family history of colorectal cancer, tumor location, stage, grade, CIMP, MSI, CIN, *BRAF*, *KRAS*, *PIK3CA*, LINE-1 methylation, p53, p21, cyclin D1, β-catenin, COX-2, and FASN.

activation in some cancers [65,66]. A study using human cancer cell lines [67] has shown that AKT is likely responsible for the up-regulation of AURKA for mitotic progression. In contrast, another study using mouse oocytes [68] has shown that the activation of AURKA on microtubule-organizing centers is independent of the PI3K-AKT pathway. As to the relationship between AURKA and the PI3K-AKT pathway, further studies are needed. We also demonstrated that AURKA overexpression was associated with nuclear cyclin D1 expression. A previous study using a mouse model [11] has reported that AURKA overexpression cause nuclear accumulation of cyclin D1, which is in agreement with our current data.

Recently, Aurora kinases have been targeted for cancer therapy, and a new class of drugs known as Aurora kinase inhibitors has been undergoing preclinical and clinical assessments [69,70]. Among them, VX-680 has shown promising results in animal studies, inhibiting tumor growth in a range of xenograft models and leading to regression of colon tumor [71]. VX-680 is already undergoing the clinical study, but there is no biomarker for selecting patients to benefit for clinical trials of this drug. Hereafter, AURKA expression in the resected specimens might attract increasing attention as a biomarker for patient selection. In this respect, our findings may have clinical implications.

In particular, a possible inverse relation between AURKA and family history of colorectal cancer merits discussion. An association between a molecular change and family history of colorectal cancer implies a genetic factor (and/or a shared environmental factor) that may contribute to the development of the given molecular change. The inverse association between AURKA and family history likely supports genetic predisposition to molecular changes (such as MSI) alternative to the AURKA-CIN pathway. Clarification of this issue by future research is important considering AURKA inhibitors as potential targeted therapy against colorectal cancers, including familial cases.

In conclusion, using a large number of colorectal cancers, we have shown that AURKA overexpression is independently associated with CIN. Our data support the hypothesis that AURKA may contribute to colorectal carcinogenesis through CIN.

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