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Nuclear Aurora B and cytoplasmic Survivin expression is involved in lymph node metastasis of colorectal cancer

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Abstract. The chromosomal passenger complex (CPC) is a key regulator of chromosome segregation and cytokinesis, and consists of Aurora B kinase, INCENP, Survivin and Borealin. Aurora B is a member of a family of serine/threonine protein kinases, and Survivin belongs to the inhibitors of apoptosis (IAP) gene family, and is also a member of the CPC family. Aurora B and Survivin have also been reported to be overexpressed in various human cancers; however, as yet no studies have investigated the co-expression of Survivin and Aurora B in colorectal carcinoma. Therefore, in the present study, the correlation between Aurora B and Survivin expression was investigated using immunohistochemistry and the associated pathological features in colorectal carcinoma were analyzed. Our present findings showed that nuclear Aurora B and cytoplasmic Survivin expression are strongly associated with and involved in lymph node metastasis in colorectal cancer. Therefore, we suggest that nuclear Aurora B and cytoplasmic Survivin are useful diagnostic markers and therapeutic targets in colorectal carcinoma.

Introduction

Aurora B is a member of a family of serine/threonine protein kinases and phosphorylates a variety of substrate proteins, including histone H3 at ser10, to regulate numerous aspects of cell division, from chromosome condensation to cytokinesis (1,2). It is also a protein of the chromosomal passenger complex (CPC), localized on the centromeres from the prophase through to the metaphase-anaphase transition together with Survivin, inner centromere protein (INCENP) and Borealin to regulate segregation and cytokinesis (1-5). Aurora B is overexpressed in a various types of cancer including thyroid, prostate and endometrial carcinomas, and its upregulation is associated with cell proliferation, prognosis, metastasis and chromosomal number instability to produce multinuclearity (2-5). Previously, we also found that nuclear Aurora B expression is correlated with cell proliferation, multinuclearity and metastasis in oral cancer (6). However, the expression of Aurora B in colorectal cancer tissues is undetermined.

Survivin is a 142 amino acid protein containing a baculovirus inhibitor of apoptosis repeat protein domain, and thus is involved in inhibiting apoptosis (7,8). Additionally, it appears to function as a subunit of CPC to regulate cell division (1). Survivin is reported to be overexpressed in a variety of human cancers (7). At present, however, no studies that have investigated the co-expression of Survivin and Aurora B in colorectal carcinoma are available.

Therefore, the aim of this study was to investigate the correlation between Aurora B and Survivin expression and associated pathological features in colorectal carcinoma.

Materials and methods

Patients. Sixty-five patients who had undergone surgical resection for advanced colorectal carcinoma at Hiroshima Memorial Hospital, Japan, were enrolled in this study. In addition, 20 samples of normal colonic mucosa were examined as controls. Tumors from each patient were formalin-fixed and cut into parallel 4-5 mm sections, and the site of deepest tumor invasion was selected by microscopic examination in combination with hematoxylin and eosin staining. The study was approved by the ethics committee of the Hiroshima Memorial Hospital. Informed consent was obtained from all subjects.

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Table I. Aurora B and Survivin expression in normal colonic mucosa and colorectal cancer.

	Normal (%)	Cancer (%)	P-value
Number of cases	20	65	
Aurora B			
N+	0 (0)	38 (58.5)	<0.01
N-	20 (100)	27 (41.5)	
Survivin			
N+	0 (0)	48 (73.8)	<0.01
N-	20 (100)	17 (16.2)	
Survivin			
C+	0 (0)	27 (41.5)	<0.01
C-	20 (100)	38 (58.5)	

N+, positive nuclear expression; N-, negative nuclear expression; C+, positive cytoplasmic expression; C-, negative cytoplasmic expression.

Immunohistochemical staining. Immunohistochemical staining for Aurora B and Survivin was performed with the LSAB system (Dako, Kyoto, Japan). In brief, paraffin blocks were cut into 3-4 μm sections. The sections were deparaffinized in xylene for 2 h, dehydrated with graded ethanol washes (100-70%) and treated with 3% hydrogen peroxide for 30 min. The sections were autoclaved three times in 10 mM citrate buffer (pH 6.0) for 5 min to allow antigen retrieval, and incubated overnight in anti-Aurora B primary monoclonal antibody (Transduction Laboratories, San Diego, CA, USA; 1:50 dilution) and anti-Survivin polyclonal antibody (NB500-201, Novus Biological, Littleton, CO, USA; 1:1000 dilution) at 4˚C. Aurora B and Survivin expression was semi-quantitatively estimated by determining nuclear and cytoplasmic localization. Immunoreactivity was graded according to the percentage of positive tumor cells, as follows: +++, strong (>60% of the tumor cells intensely stained); ++, moderate (20-60% intensely stained); +, mild (<20% intensely or weakly stained); or -, no staining. Survivin- and Aurora B-positive cases were graded ++ or +++, and negative cases were graded - or +.

Statistical analysis. The Statcel software package was used to statistically analyze the correlation between pathological factors, including lymph node metastasis, lymphatic invasion, venous invasion and distant metastasis, and the expression of Aurora B and Survivin in colorectal carcinomas. The χ^2 or Fisher's exact test were used for comparison of data between the groups. P<0.05 was considered to indicate a statistically significant difference.

Results

Aurora B and Survivin expression in normal colonic mucosa and colorectal carcinoma. We examined the expression of Aurora B and Survivin in 20 normal colonic mucosa and 65 colorectal carcinoma samples by immunohistochemistry. In normal colonic mucosa, cells exhibited extremely weak staining of Aurora B and Survivin (Fig. 1A-a and c, and Table I). Nuclear Aurora B and nuclear and/or cytoplasmic Survivin exhibited strong staining in colorectal carcinoma (Fig. 1A-b and d, and Table I). All cases showed a low nuclear Aurora B expression, and low nuclear and cytoplasmic Survivin in normal colonic mucosa. Although 38 of 65 cases (58.5%) showed nuclear

Table II. Correlation between the expression of Aurora B and Survivin and pathological factors in colorectal cancer.

	Aur B N		P-value	Sur N		P-value	Sur C		P-value
	+	-		+	-		+	-	
Number of cases	38	27		48	17		27	38	
Pathological features									
Histological differentiation									
Well/moderate	31	25	0.097	41	15	n.s.	22	34	n.s.
Poor/mucinous	7	2		7	2		5	4	
Lymph node metastasis									
Negative	23	24	<0.01	32	15	n.s.	13	34	<0.01
Positive	15	3		12	6		14	4	
Lymphatic invasion									
Negative	1	0	n.s.	1	0	n.s.	0	1	n.s.
Positive	37	27		47	17		27	37	
Venous invasion									
Negative	1	0	n.s.	0	1	n.s.	1	0	n.s.
Positive	37	27		48	16		26	38	

Sur C, Survivin cytoplasmic expression; Sur N, Survivin nuclear expression; Aur B N, Aurora B nuclear expression; n.s., not significant.

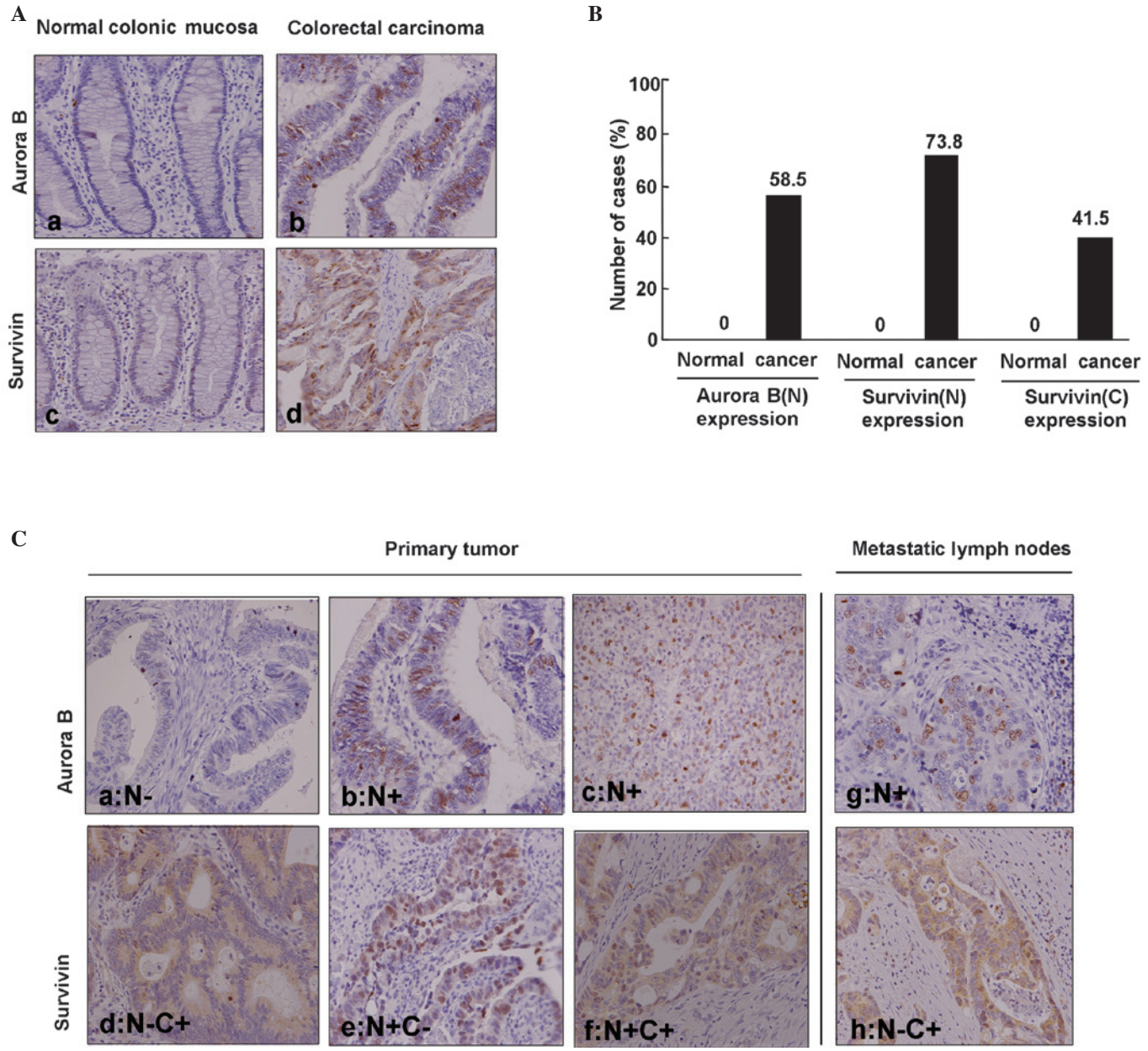

Figure 1. (A) In normal colonic mucosa, cells only exhibited very weak staining for Aurora B (a) and Survivin (c). Nuclear Aurora B (b), and nuclear and cytoplasmic Survivin (d) expression exhibited strong staining in colorectal carcinoma. (B) All cases demonstrated a low expression (0%) of nuclear Aurora B and nuclear and cytoplasmic Survivin in normal colonic mucosa, while 58.5% of cases demonstrated nuclear staining of Aurora B, 73.8% demonstrated exclusive nuclear staining of Survivin and 41.5% demonstrated cytoplasmic staining. (C) Expression of Aurora B and Survivin in primary colorectal carcinoma or metastatic lymph nodes by immunohistochemistry. Representative cases of low nuclear Aurora B expression (N-) (a) and high nuclear Aurora B expression (N+) (b and c), low nuclear Survivin expression and high cytoplasmic Survivin expression (N-C+) (d), high nuclear Survivin expression and low cytoplasmic Survivin expression (N+C-) (e), and high nuclear and cytoplasmic Survivin expression (N+C+) (f) in primary colorectal carcinoma. Representative cases of high nuclear Aurora B expression (N+) (g) and low nuclear Survivin expression and high cytoplasmic Survivin expression (N-C+) (h) are shown.

staining of Aurora B, 48 of 65 cases (73.8%) showed exclusive nuclear staining of Survivin, and 27 of 65 cases (41.5%) showed cytoplasmic staining (Fig. 1B and C, and Table I).

Correlation between Aurora B and Survivin expression and pathological factors in colorectal cancer. We examined the correlation between pathological factors and Aurora B and/or Survivin expression in colorectal carcinoma. Aurora B nuclear-positive cases exhibited significantly increased lymph node metastasis (P<0.01), compared with Aurora B nuclear-negative cases. Survivin cytoplasmic-positive cases also demonstrated significantly increased lymph node metastasis (P<0.01) compared with Survivin cytoplasmic-negative cases, but Survivin nuclear-positive cases were not significantly different compared with negative cases. Nuclear Aurora B and cytoplasmic Survivin expression were significant factors of poor prognosis (Table II).

The correlation between nuclear Aurora B and cytoplasmic Survivin expression and pathological factors in colorectal carcinomas was examined. Notably, positive cytoplasmic Survivin

Table III. Correlation between the expression of nuclear Aurora B and cytoplasmic Survivin and pathological factors.

	N+C+	N+C-	N-C+	N-C-	P-value
Number of cases	17	23	13	18	
Pathological features					
Gender					
Male	8	11	5	8	
Female	9	12	8	10	
Histological differentiation					
Well/moderate	12	20	12	17	n.s
Poor/mucinous	5	3	1	1	
Lymph node metastasis					
Negative	4	19	12	17	(N+C- vs. N+C-) (N+C- vs. N-C+) (N+C- vs. N-C-) (p<0.01)
Positive	13	4	2	1	
Lymphatic invasion					
Negative	0	2	1	1	n.s
Positive	17	21	12	17	
Venous invasion					
Negative	1	0	1	1	n.s
Positive	16	23	12	17	

N+C+, positive nuclear Aurora B and positive cytoplasmic Survivin expression; N+C-, positive nuclear Aurora B and negative cytoplasmic Survivin expression; N-C+, negative nuclear Aurora B and positive cytoplasmic Survivin expression; N-C-, negative nuclear Aurora B and negative cytoplasmic Survivin expression; n.s., not significant.

Table IV. Correlation between the expression of Aurora B/ Survivin and primary tumor/ metastatic lymph nodes.

	Aur B N		P-value	Sur N		P-value	Sur C		P-value
	+	-		+	-		+	-	
Primary tumor	38	27	<0.01	48	17	<0.01	27	38	<0.01
Metastatic lymph node	13	1		1	13		12	2	

Sur C, cytoplasmic Survivin expression; Sur N, nuclear Survivin expression; Aur B N, nuclear Aurora B expression.

and positive nuclear Aurora B expression was significantly correlated with lymph node metastasis (P<0.001 and P<0.05, respectively), compared with positive cytoplasmic Survivin and negative nuclear Aurora B expression, and negative cytoplasmic Survivin and negative nuclear Aurora B expression, respectively (Table III). Both nuclear Aurora B and cytoplasmic Survivin cases revealed 76% lymph node metastasis.

Aurora B and Survivin expression and pathological factors in primary colorectal tumors and lymph node metastasis. The varying expression of Aurora B and Survivin in primary colorectal tumors and lymph node metastasis was examined.

Metastatic cases were found to exhibit a higher frequency of nuclear Aurora B and cytoplasmic Survivin expression, and a lower frequency of nuclear Survivin expression than primary tumor cases (Fig. 1C-g and h, and Table IV).

Discussion

In the present study, we examined the expression of Aurora B and Survivin in 20 normal colonic mucosa and 65 colorectal carcinoma samples by immunohistochemistry. The results showed that nuclear Aurora B, and nuclear and cytoplasmic Survivin were highly and frequently expressed in colorectal

carcinoma cells. Moreover, the overexpression of nuclear Aurora B and cytoplasmic Survivin was significantly correlated with lymph node metastasis.

Aurora B kinase is a chromosomal passenger protein and it is essential for chromosome segregation through the phosphorylation of mitotic histone H3 (1,2). The phosphorylation status of histone H3 may be balanced by Aurora B kinase activity and PPI phosphatase activity (9). Overexpression of Aurora B produces multinuclearity and increases ploidy, which is important during human cancer development (2,10). In this study, we examined the expression of Aurora B in colorectal carcinoma. Aurora B expression levels were significantly higher in colorectal carcinoma than in normal colonic mucosa, and a high nuclear Aurora B expression was associated with lymph node metastasis. We previously identified that nuclear Aurora B expression is correlated with cell proliferation, multinuclearity and metastasis in oral cancer (6). Aurora B expression is also associated with a poor prognosis in prostate (4), endometrial (3) and thyroid carcinoma (5). Aurora B is also responsible for oncogenic Ras-mediated cell transformation, leading to the accelerated proliferation of tumor cells (11). These findings of these reports therefore confirm the findings of the present study. The detailed mechanism of metastasis promoted by Aurora B overexpression remains to be elucidated. Therefore, the correlation between Aurora B overexpression and migration or invasion of colorectal carcinoma cells should be investigated.

Survivin is a bifunctional protein that suppresses apoptosis and regulates cell division (12,13). Nuclear Survivin is suspected to control cell division, whereas cytoplasmic Survivin is considered to be cytoprotective (14). Survivin has a nuclear export signal (NES) in the linker region between the BIR domain and the COOH-terminal α helix (14-16). Results of certain studies have shown the nuclear translocation and accumulation of cytoplasmic Survivin following treatment with leptomycin B (15,16). In our study, cytoplasmic Survivin was associated with lymph node metastasis. Cytoplasmic Survivin is characterized as anti-apoptotic and is associated with microtubules, directly or indirectly interfering with the function of caspases (8). Our result is also supported by observations that the cytoplasmic expression of Survivin is associated with a poor outcome in breast cancer (17), lymphoma (18), liver cancer (19) and gastric carcinoma (20). In our previous study, the cytoplasmic Survivin overexpression was found to be associated with a poor prognosis in colorectal carcinoma (21).

Previous studies have shown that Survivin acts as a subunit of the chromosomal passenger complex (CPC) and interacts with other subunits of the CPC such as Aurora B, INCENP and Borealin to regulate cell division (12,13). We have found that the nuclear Survivin expression is significantly correlated with Ki-67 and Aurora B expression in oral squamous cell carcinoma (OSCC) (22). Notably, OSCC cases with nuclear Survivin and Aurora B expression exhibited marked malignant behavior. However, in this study, nuclear Survivin was not associated with Aurora B and pathological factors in colorectal carcinoma. The frequency of Survivin expression was extremely high in colorectal carcinomas compared with OSCC cases, thus nuclear Survivin may have varying roles in epithelial cancer and adenocarcinoma.

In this study, we found that the overexpression of nuclear Aurora B and cytoplasmic Survivin was significantly correlated with lymph node metastasis. Moreover, both nuclear Aurora B and cytoplasmic Survivin cases showed far poorer prognosis in colorectal carcinomas. Thus, nuclear Aurora B and cytoplasmic Survivin may be strong markers for the prediction of the malignancy of colorectal carcinomas.

In conclusion, our present findings showed that nuclear Aurora B and cytoplasmic Survivin expression are involved in lymph node metastasis, and both nuclear Aurora B and cytoplasmic Survivin are strongly associated with lymph node metastasis in colorectal cancer.

Therefore, we suggest that nuclear Aurora B and cytoplasmic Survivin are useful diagnostic markers and therapeutic targets in colorectal carcinoma.

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