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RAD50/MRE11/NBS1 proteins in relation to tumour development and prognosis in patients with microsatellite stable colorectal cancer

Jingfang Gao[1], Hong Zhang[2], Gunnar Arbman[3] and Xiao-Feng Sun[1]

[1]Department of Oncology, Institute of Clinical and Experimental Medicine, Linköping University, Linköping, Sweden, [2]Division of Biomedicine, School of Life Science, Skövde University, Skövde, Sweden and [3]Department of Surgery, Östergötland, Sweden

Summary. RAD50/MRE11/NBS1 complex is essential for DNA double-strand break repair and for maintaining genomic integrity. In this study, we immunohistochemically examined MRE11, NBS1 and RAD50 expression in primary CRCs (n=208), the corresponding distant (n=41) and adjacent normal mucosa (n=130), and lymph node metastases (n=26), and investigated their clinicopathological significance in colorectal cancers (CRCs). We found that the intensity and percentage of MRE11 and NBS1 in primary CRCs were positively correlated with each other and with RAD50 (P<0.0001). Strong expression of MRE11, NBS1 or combined RAD50/MRE11/NBS1 was related to MSS, positive hMLH1 expression, earlier tumour stage (TNM stage I and II) and favourable survival (P<0.05). A high percentage of MRE11 expression was associated with less local recurrence and high apoptotic activity (P<0.05). In MSS CRCs, the expression of MRE11 and NBS1 was stronger than that in normal mucosa (P<0.05), and strong expression of NBS1 in primary tumour was related to favourable survival of patients in TNM stage I and II (univariate analysis: P=0.03; multivariate analysis: P=0.07). In MSI CRCs, neither MRE11 nor NBS1 expression showed differences among normal mucosa, primary tumour and metastasis, or among clinicopathological variables. In conclusion, RAD50/MRE11/NBS1 proteins interacted with each other, which had different clinicopathological significance in MSS and MSI CRCs, and further, each component of the complex might have additional roles. NBS1 might be a prognostic factor for patients with MSS tumour in TNM stage I and II.

Offprint requests to: Xiao-Feng Sun, MD, PhD, Professor, Department of Oncology, Institute of Clinical and Experimental Medicine, Linköping University, 581 85 Linköping, Sweden. e-mail: xiasu@ibk.liu.se

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Introduction

Double strand break (DSB) is the most dangerous form of DNA damage in cells resulting from exposure to exogenous factors, such as ionizing radiation and chemical DNA-damaging agents, and from programmed developmental rearrangements, like meiosis, and immunoglobulin class-switch recombination. Cellular response to DNA DSB, including detecting and signalling DNA damage to a series of downstream molecules, activating cell cycle checkpoints, maintaining telomere stability, mediating DNA repair and initiating apoptosis, is essential for maintaining genomic integrity. Genetic defects that impair any aspect of the cellular response to DNA DSB may lead to gene mutation, translocation, rearrangement, amplification and deletion, which further provide predisposition to various types of cancers (Vamvakas et al., 1997).

The RAD50/MRE11/NBS1 complex plays a critical role in the cellular response to DSB (Assenmacher and Hopfner, 2004). It is also essential for cell growth and viability, for instance, null mutation in either of the RAD50, MRE11 or NBS1 gene leads to early embryonic lethality in mice (Xiao and Weaver, 1997; Luo et al., 1999; Zhu et al., 2001). Moreover, increasing evidence has shown that mutations in RAD50/MRE11/NBS1 genes are implicated in cancer development. Mice with hypomorphic *RAD50* mutation have exhibited growth defects and cancer predisposition (Bender et al., 2002). Further, hypomorphic mutations in *MRE11* and *NBS1* have been associated with the ataxia telangiectasia-like disorder (Stewart et al., 1999) and Nijmegen breakage syndrome (Carney et al., 1998), respectively, which are

characterized by neurological abnormalities, immune defects, chromosomal instability, radiation sensitivity and cancer predisposition. Recently, mutations in RAD50 and MRE11 have been found in human cancers with microsatellite instability (MSI), including colorectal cancer (CRC), but not in microsatellite stability (MSS) cases (Ikenoue et al., 2001; Kim et al., 2001; Giannini et al., 2002). These mutations have been associated with reduced mRNA and protein expression of all three members of the RAD50/MRE11/NBS1 complex (Giannini et al., 2002, 2004; Koh et al., 2005). These findings suggest that alterations in any members of the complex may destabilize its protein partners, and further, impairment of the complex expression may contribute to the development of MSI CRC.

Our previous study, analyzing expression of RAD50 in primary CRCs along with their normal mucosa specimens and lymph node metastases, has shown overexpression of RAD50 in MSS primary CRCs (Fig. 1A), but not in MSI ones, and that strong or high expression of RAD50 in primary MSS CRCs was related to earlier tumour stage, better differentiation, strong inflammatory infiltration and better survival, indicating that upregulation of RAD50 might be involved in the cellular response at the earlier tumour stage against MSS CRC from further progression (Gao et al., 2008). Since RAD50 is one component of the RAD50/MRE11/NBS1 complex, it is possible that the three proteins interact with each other, during CRC development. To test this hypothesis, we examined expression of MRE11 and NBS1 and analyzed association of the expression of MRE11, NBS1 and combined RAD50/MRE11/NBS1 with clinicopathological significance and patients' survival in the same series of CRCs.

Materials and methods

Materials

Expression of MRE11 and NBS1 proteins was examined in paraffin-embedded tissue by immunohistochemistry in 208 patients with primary CRC from Linköping University Hospital, Linköping, and Vrinnevi Hospital, Norrköping, Sweden, between 1982 and 2001. We also examined the protein expression in their corresponding distant normal mucosa (from the distant margin of resections, which was histologically free from pretumour and tumour, n=41), adjacent normal mucosa (normal mucosa adjacent to the primary tumour, n=130) and metastases in the regional lymph nodes (n=26). The clinicopathological characteristics of patients and tumours, including gender, age, tumour location (colon and rectum), TNM stage, growth pattern (expansive and infiltrative) and differentiation (good, moderate, poor and mucinous/signet-ring cell type) were obtained from surgical and pathological records, stratified by microsatellite status and summarised in Table 1. The good and moderate differentiation was considered as better, while poor differentiation and mucinous/signet-ring cell type were graded as worse differentiation. The patients were followed up until April 2006, and 76 patients died of CRC by that time. Microsatellite status determined by the microsatellite marker Bat 26 (Evertson et al., 2003; Emterling et al., 2004), apoptotic activity by terminal deoxynucleotidyl transferase-mediated dUTP-digoxigenin nick end labelling (Evertsson et al., 1999), RAD50 mutation by PCR-SSCP-DNA sequencing (Gao et al., 2008), expressions of hMLH1 (Jansson et al., 2003), proliferating cell nuclear antigen (PCNA) (Sun et al., 1996) and RAD50 (Gao et al., 2008) by immunohistochemistry were reviewed from our previous studies. There was no information available concerning tumour location in three patients, Dukes' stage in eight, growth pattern in 12, local recurrence in 116, microsatellite status in 49, apoptosis in 164, hMLH1 expression in 163, PCNA expression in 121 and RAD50 expression in five patients. The study was approved by the ethical committee at the Faculty of Health Sciences, Linköping University, Sweden.

Three colon cancer cell lines (KM12C, KM12SM and KM12L4a) were kindly provided by Prof. IJ. Fidler (M.D. Anderson Cancer Carter, Houston, TX), and used to confirm the specificity of the antibody against MRE11 or NBS1 protein by Western blotting.

Immunohistochemistry

Five-micrometer paraffin-embedded sections were

Table 1. The clinicopathological characteristics of patients with colorectal cancers.

Variables	MSS (n =128)	MSI (n = 31)
Gender		
Male	68	19
Female	60	12
Age (years)		
Mean	71	71
Range	42-89	44-95
Tumor location		
Colon	54	28
Rectum	72	3
Unknown	2	
TNM stage		
I	19	1
II	44	15
III	33	11
IV	27	3
Unknown	5	1
Growth pattern		
Expansive	70	17
Infiltrative	56	8
Unknown	2	6
Differentiation		
Good	10	1
Moderate	85	14
Poor	15	6
Mucinous/signet-ring	18	10

deparaffinised in xylene, rehydrated by gradual ethanol to water. The sections were cooked at high pressure with Tris-EDTA buffer (pH 9.0) for 8 minutes. Following preincubation in methanol with 0.3% H_2O_2 for 20 minutes, the sections were incubated with protein block (Dako, Carpinteria, USA) for 10 minutes and then mouse monoclonal antibody 12D7 to MRE11 (1:350; Abcam, Cambridge, UK) or mouse monoclonal antibody 1D7 to p95 NBS1 (1:500; Abcam) at room temperature for 30 minutes. After washing in phosphate-buffered saline (PBS, pH 7.4), the sections were incubated with anti-mouse secondary antibody (Dako) at room temperature for 25 minutes. Subsequently, the sections were subjected to 3,3'-diaminobenzidine tetrahydrochloride for 8 minutes and then counterstained by haematoxylin. Sections known positive staining for MRE11 or NBS1 as positive control, and negative control replacing the primary antibodies by IgG1 were included in each run.

Staining specificity, intensity and percentage of MRE11 and NBS1 were independently scored by two of the authors (Zhang H., a pathologist, and Gao J.). Staining intensity was graded as negative, weak, moderate or strong expression. Percentage of the positive tumour cells with nuclear staining was classified as <25%, 25-49%, 50-75% or >75%. We did not evaluate the percentage of MRE11 and NBS1 expression in normal mucosa and metastases due to the small size of the sections. Based on the similarities of clinicopathological features in the tumours with negative, weak or moderate expression, we considered them as one group, called weak expression, and tumours with strong staining as a strong expression group. Similarly, the percentage of positive tumour cells was classified as ≤ 75% or >75% by setting 75% as a cut-off point, regardless of the expression intensity. There were 14 cases with discrepant scoring, and re-examination was performed by using a dual-headed microscope to reach agreement. In order to avoid artificial effect, cells in areas with necrosis, poor morphology and in the margins of sections were not taken into account.

Western blotting

Total proteins were extracted from KM12C, KM12SM and KM12L4a colon cancer cells, respectively, using RIPA buffer (1x PBS, 1% Nonidet P-40 (Amaresco), 0.5% sodium deoxycholate, 0.1% SDS) according to the manufacture's instruction (Santa Cruz Biotechnology). In brief, 20 µg proteins in loading buffer were heated at 98°C for 5 minutes in the presence of 2-mercaptoethanol. The proteins were separated by electrophoresis in gradient (4-15%) Tris-HCL gels (Bio-Rad, Hercules, CA) and transferred to nitrocellulose membranes (Hybond-P, Amersham) by electroblotting in 25mM Tris, 192mM glycine, and 20% methanol, pH 8.3. After blocking with 5% non-fat milk in Tris-buffered saline containing 0.1% Tween-20 (TBS-T), pH 7.4, at room temperature for 1 hour, the membranes were incubated with the antibody to MRE11 (1:2000; Abcam)

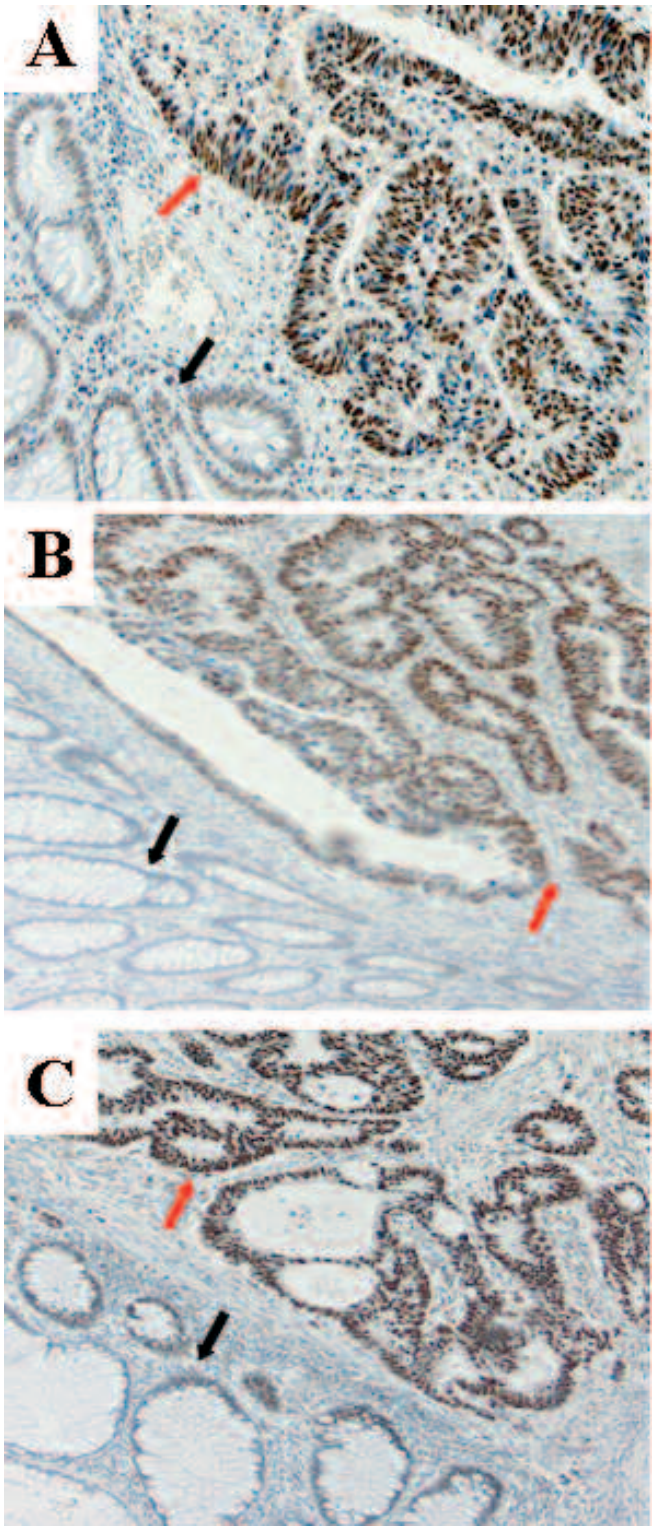

Fig. 1. Expression of RAD50 **(A)**, MRE11 **(B)** and NBS1 **(C)** was immunohistochemically examined in a microsatellite stable case. All three proteins were weak (black arrow) in the nucleus of adjacent normal mucosa and strong (red arrow) in primary colorectal cancer. x 100

or to p95 NBS1 (1:3000; Abcam) in TBS-T at 4°C overnight. After washing in TBS-T, the membranes were incubated with horseradish-peroxidase-conjugated anti-mouse Ig (Amersham) at 1:5000 at room temperature for 1 hour. Protein expression was detected by enhanced chemiluminescence (Amersham).

Statistical analysis

The χ^2 test and McNemar's method were used to determine the difference in expression of the proteins among normal mucosa, primary tumour and metastasis, as well as the relationship of the proteins' expression in primary CRC with clinicopathological features. Cox's Proportional Hazard Model was used to estimate the relationship between protein expression and patients' survival in univariate and multivariate analyses. The Kaplan-Meier method was used to calculate survival curves. Two-sided p-values of less than 5% were considered statistically significant.

Results

Expression of MRE11 and NBS1 in normal mucosa, primary CRCs and metastases

Expression of MRE11 and NBS1 proteins in KM12C, KM12SM and KM12L4a colon cancer cell lines was detected by Western blotting. The detected bands of approximately 79 and 95 kDa corresponded to MRE11 and NBS1, respectively (Fig. 2).

By immunohistochemistry, MRE11 was strongly expressed in the nucleus in 7 of 39 (18%) distant normal mucosa, 14 of 114 (12%) adjacent normal mucosa, 83 of 208 (40%) primary CRC and 8 of 21 (38%) lymph node metastasis specimens. NBS1 was strongly expressed in the nucleus in 14 of 41 (34%) distant normal mucosa, 17 of 116 (15%) adjacent normal mucosa, 84 of 207 (41%) primary CRC and 7 of 26 (27%) lymph node metastasis specimens (Table 2). The discrepancies of specimen numbers between MRE11 and NBS1 were due to available sections for the staining. MRE11 and NBS1 were also expressed in the cytoplasm of 59% and 3% primary CRCs, respectively (no cytoplasmic expression of MRE11 or NBS1 in normal mucosa and metastasis, data not shown). Cytoplasmic expression of either MRE11 or NBS1 in the primary CRCs was almost identical to its nuclear staining (P<0.0001). Therefore, in the further analyses, only nuclear staining, regardless of cytoplasmic staining, was considered as positive expression for MRE11 or NBS1. Moreover, there were 57% of 208 primary CRCs with >75% MRE11 expression, and 58% of 207 primary CRCs with >75% NBS1 expression. The expression percentage and intensity of MRE11 or NBS1 in primary CRCs was positively correlated with each other (P<0.0001, Table 3).

We compared the intensity of MRE11 or NBS1 expression among normal mucosa, primary CRCs and metastases in the whole series of cases (Table 2) and in the matched cases (the specimens from the same patients, data not shown). In the whole series of cases, the intensity of MRE11 or NBS1 expression was increased from adjacent normal mucosa to primary CRCs (P<0.0001), but there was no difference in the expression of MRE11 or NBS1 between primary CRCs

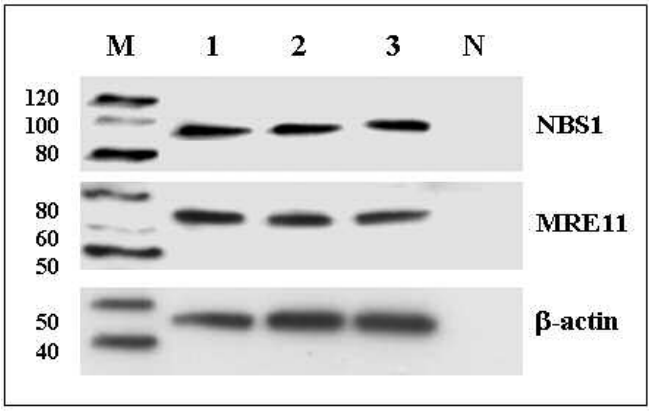

Fig. 2. Expression of MRE11 and NBS1 protein in KM12C (1), KM12SM (2) and KM12L4a (3) colon cancer cell lines was examined by Western blotting. The bands of approximately 79 and 95 kDa, compared to MagicMarkTMXP Western Protein Standard marker (M), corresponded to MRE11 and NBS1, respectively. ß-actin as protein loading control and replacement of total proteins by TBS as negative control (N) were run together with the protein samples.

Table 2. Intensity of MRE11 and NBS1 expression in distant normal mucosa, adjacent normal mucosa, primary colorectal cancers and lymph node metastases.

Category	MRE11 (%)		P*	NBS1 (%)		P*
	Weak	Strong		Weak	Strong	
Distant normal mucosa	32 (82)	7 (18)	0.01	27 (66)	14 (34)	0.44
Adjacent normal mucosa	100 (88)	14 (12)	<0.0001	99 (85)	17 (15)	<0.0001
Primary colorectal cancers	125 (60)	83 (40)		123 (59)	84 (41)	
Metastases	13 (62)	8 (38)	0.87	19 (73)	7 (27)	0.18

*Compared to primary colorectal cancers.

and metastases (P>0.05). Furthermore, the intensity of MRE11 expression (P=0.01), not NBS1 (P=0.44), was stronger in primary CRCs than distant normal mucosa. In the matched cases, similar phenomena were observed among the adjacent normal mucosa, primary CRCs and metastases, but there was no difference in the expression of MRE11 or NBS1 between distant normal mucosa and primary CRCs (P>0.05).

Expression of MRE11 and NBS1 in relation to clinicopathological features

As shown in Table 4, strong expression of either MRE11 or NBS1 in the primary CRCs was related, or tended to be related to, earlier tumour stage (TNM stage I and II) (P=0.02, P=0.07), MSS (P=0.01, P=0.002), positive hMLH1 expression (P=0.09, P=0.02), strong expression of PCNA (P=0.02 for both) and RAD50 (P<0.0001 for both), and favourable survival (P=0.01, P=0.05, data not shown). Further analysis showed that the strong expression of NBS1 protein, but not MRE11, was related to favourable survival in the patients with TNM stage I and II tumour (P=0.01, Fig. 3A), but not stage III and IV (P=0.23, Fig. 3B). Multivariate analysis revealed that NBS1 was a prognostic indicator in patients with stage I and II tumour, independent of gender, age, tumour location, growth pattern and differentiation (rate ratio 0.2, 95% CI 0.04-0.94, P=0.04, Table 5).

A high percentage of MRE11 or NBS1 expression was also associated with MSS (P=0.005, P=0.001) and strong expression of RAD50 protein (P<0.0001 for both proteins, data not shown). The percentage of MRE11 expression was higher in the patients with less local recurrence (P=0.04), high apoptotic activity (P=0.02), positive hMLH1 (P=0.002) and strong PCNA expression (P=0.01, data not shown). There was no association of MRE11 or NBS1 expression (both intensity and percentage) with gender, age, tumour location, growth pattern and differentiation (P>0 05, data not shown).

Expression of MRE11 and NBS1 in MSS and MSI cases and their clinicopathological significance

In the MSS cases, the intensity of MRE11 or NBS1 expression in primary CRCs was increased compared to distant/adjacent normal mucosa, either in the whole series of cases or in the matched cases (Fig. 1B and 1C, P<0.05). There was no difference in the expression of MRE11 or NBS1 between distant and adjacent normal

Table 3. Correlation of staining percentage and intensity of MRE11 and NBS1 in primary colorectal cancers.

Category	Percentage					
	MRE11		P	NBS1		P
	≤75% (%)	>75% (%)		≤75% (%)	>75% (%)	
Intensity			<0.0001			<0.0001
Weak	82 (39)	43 (21)		77 (37)	46 (22)	
Strong	8 (4)	75 (36)		9 (5)	75 (36)	
Total	90 (43)	118 (57)		86 (42)	121 (58)	

Table 4. Intensity of MRE11and NBS1 expression in relation to clinicopathological and biological features in colorectal cancers.

Variable	MRE11 (%)		P	NBS1 (%)		P	RAD50/MRE11/NBS1 (%)		P
Category	Weak	Strong		Weak	Strong		Weak	Strong	
TNM stage			0.02			0.07			0.03
I+II	52 (53)	47 (47)		53 (54)	46 (46)		57 (59)	39 (41)	
III+IV	69 (68)	32 (32)		66 (66)	34 (34)		73 (74)	26 (26)	
Microsatellite status			0.01			0.002			0.002
MSS	68 (53)	60 (47)		68 (53)	60 (47)		74 (60)	50 (40)	
MSI	25 (81)	6 (19)		25 (83)	5 (17)		26 (90)	3 (10)	
hMLH1			0.09			0.02			
Negative	12(100)	0		10 (91)	1 (9)		11 (100)	0	0.08
Positive	24 (73)	9 (27)		16 (48)	17 (52)		24 (73)	9 (27)	
PCNA			0.02			0.02			0.001
Weak	40 (83)	8 (17)		33 (70)	14 (30)		42 (89)	5 (11)	
Strong	24 (62)	15 (38)		18 (46)	21 (54)		23 (59)	16 (41)	
RAD50			<0.0001			<0.0001			
Weak	111 (73)	42 (27)		105 (70)	45 (30)				
Strong	12 (24)	37 (76)		15 (30)	34 (70)				

mucosa, or between primary CRCs and metastases (P>0.05). Strong expression of MRE11 or NBS1 was also related to favourable survival (P=0.04, P=0.01, data not shown). Moreover, in the MSS cases with TNM stage I and II, strong expression of NBS1 was still related to favourable survival in univariate analysis (P=0.03, data not shown) and tended to be related to survival in multivariate analysis, independent of gender, age, tumour location, growth pattern and differentiation (rate ratio 0.1, 95% CI 0.01-1.20, P=0.07, Table 5).

In the MSI cases, there was no difference in the expression of the MRE11 and NBS1 among normal mucosa, primary CRCs and metastases, nor association between their protein expression and clinicopathological and biological variables. Among the MSI cases with available information of RAD50 mutation and MRE11 and NBS1 expression examined by immunohistochemistry (only two cases), one case showed an identical expression of MRE11 and NBS1, while another one presented increased expression of MRE11 and NBS1 in primary CRC, compared with the corresponding distant normal mucosa.

Expression of combined RAD50/MRE11/NBS1 in relation to clinicopathological features

We classified the combined expression of the RAD50/MRE11/NBS1 as strong or weak, based on the concordant expression of at least two out of the three proteins in the same case, a classification model used in a previous study (Angele et al., 2003). Strong expression of the combination was also related to earlier tumour stage (TNM stage I and II) (P=0.03), MSS (P=0.002), strong PCNA expression (P=0.001, Table 4) and

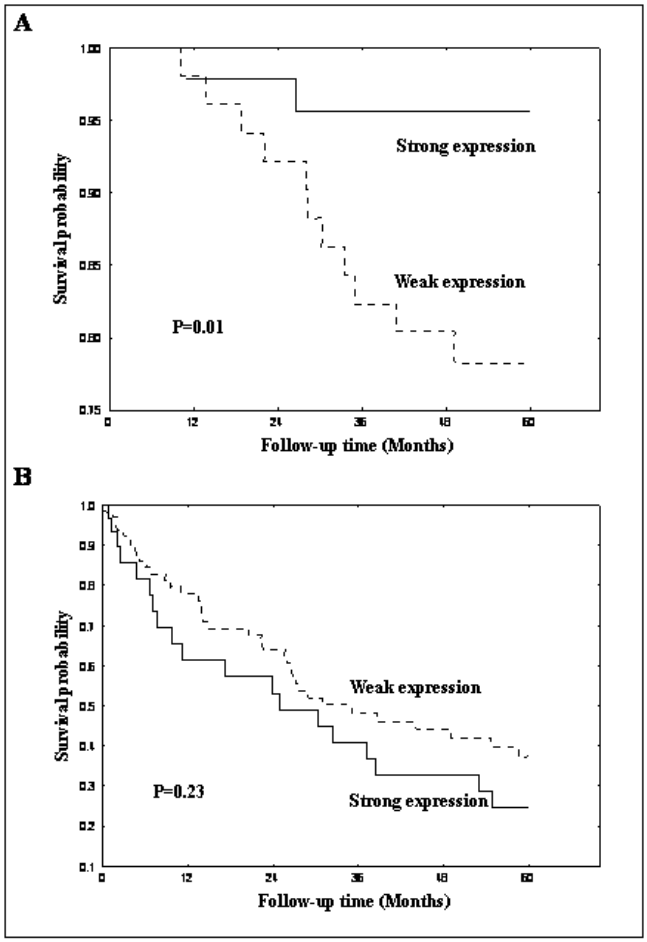

Fig. 3. Nuclear expression of NBS1 in tumour TNM stage I and II **(A)** and III and IV **(B)** of primary colorectal cancers in relation to patients' survival.

Table 5. Multivariate analysis of NBS1 expression, gender, age, tumour location, growth pattern and grade of differentiation in relation to survival of colorectal cancer patients in TNM stage I and II.

Variable	Total patients in TNM stage I/II				Patients with MSS in TNM stage I/II			
	No. (91)	Cancer death rate ratio	95% CI	P	No. (62)	Cancer death rate ratio	95% CI	P
NBS1				0.04				0.07
Weak	48	1.0	-		30	1.0	-	
Strong	43	0.2	0.04-0.94		32	0.1	0.01-1.20	
Gender				0.63				0.13
Male	46	1.0	-		30	1.0	-	
Female	45	0.7	0.23-2.44		32	4.6	0.65-33.16	
Age (year)				0.43				0.92
≤ 70	38	1.0	-		26	1.0	-	
>70	53	0.6	0.19-2.06		36	1.1	0.22-5.30	
Tumour location				0.84				0.58
Colon	45	1.0	-		24	1.0	-	
Rectum	46	0.9	0.24-3.17		38	1.7	0.24-12.70	
Growth pattern				0.30				0.85
Expansive	55	1.0	-		40	1.0	-	
Infiltration	36	1.9	0.56-6.71		22	0.9	0.15-4.83	
Differentiation				0.09				0.04
Better	65	1.0	-		46	1.0	-	
Worse	26	2.7	0.86-8.42		16	5.1	1.05-24.54	

favourable survival (P=0.03, data not shown). Further analysis stratified by microsatellite status and tumour stage showed that there was no association between the combined expression of RAD50/MRE11/NBS1 and gender, age, tumour location, growth pattern, tumour stage, differentiation or patients' survival, either in MSS or MSI cases with earlier or later tumour stage (P>0.05).

Discussion

The RAD50/MRE11/NBS1 complex plays an essential role in the cellular response to DSB, maintaining genetic stability and protecting cells from malignancy (Assenmacher and Hopfner, 2004). Our present study, along with our previous study, examined the expression of RAD50/MRE11/NBS1 proteins in MSS versus MSI CRCs, and analyzed the importance of their expression pattern in relation to clinicopathological features. In our previous study, we found increased expression of RAD50 protein in primary MSS CRCs compared with the corresponding normal mucosa, whereas there was no difference between primary CRCs and metastases. Moreover, the strong expression of RAD50 in MSS CRCs was correlated with earlier tumour stage and better survival. In contrast, in MSI CRCs, there was neither difference of RAD50 expression among normal mucosa, primary CRC and metastasis nor association of RAD50 expression with clinicopathological variables (Gao et al., 2008). In this study, we observed a similar expression pattern among normal mucosa, primary CRCs and metastases for MRE11 and NBS1 proteins and a similar prognostic significance for these two proteins, as well as the combined RAD50/MRE11/NBS1 in MSS versus MSI CRCs. In addition, expression levels of RAD50, MRE11 and NBS1 in primary CRCs were positively correlated with each other. These results were consistent with the observation that RAD50/MRE11/NBS1 proteins stabilize each other (Paull and Gellert, 1999), and indicated that RAD50/MRE11/NBS1 had different clinicopathological significance in MSS and MSI CRCs. In MSS cases, the increased expression of the three proteins in primary CRCs could be an earlier event of the CRC progression in response to a state of stress, which might prevent the tumour from further progression and be favourable for patients' prognosis. In MSI CRCs, several studies, including our previous study, have reported that MRE11 and RAD50 were frequently mutated (Ikenoue et al., 2001; Kim et al., 2001; Giannini et al., 2002; Gao et al., 2008). These mutations have been associated with reduced RNA and protein expression of all three proteins (Giannini et al., 2002, 2004; Koh et al., 2005). Although our present and previous studies could not find a correlation between RAD50 mutation and the three proteins in the limited cases (Gao et al., 2008), our results that MRE11 and NBS1 were less expressed in hMLH1-negative tumours might reflect the fact that MSI tumours had less expression of MRE11 and NBS1. We speculated that the lack of upregulation of RAD50/MRE11/NBS1 proteins in MSI CRCs could be attributed to the mutation of MRE11 or RAD50, resulting in impaired ability to repair DNA damage or induce apoptosis, which promotes cancer development.

Overexpression of the RAD50/MRE11/NBS1 proteins has been observed in gastric cancer (Matsutani et al., 2001). In contrast, reduced expression of the three proteins has been found in invasive ductal breast cancer, indicating that down-regulation of these DNA repair proteins is a common event in breast cancer (Angele et al., 2003). Although there were methodological differences, including various technique, stage of disease and inter-observer variation among these studies, this could not explain the different expression patterns of the RAD50/MRE11/NBS1 proteins among various cancers. These results indicate that the complex is important in cancer development and progression with cancer cell type specificity.

In the present study, together with our previous study, we also found a positive correlation between the individual and combined expression of RAD50/MRE11/NBS1 with PCNA expression. PCNA functions as a DNA sliding clamp for replicative DNA polymerases. Emerging evidence has shown that PCNA interacts with multiple proteins involved in DNA replication, repair, cell cycle control, and cellular differentiation (Maga and Hubscher, 2003). When DNA damage occurs, PCNA interacts with the proteins involved in cell cycle progression, and leads to cell cycle arrest and DNA damage repair. It is, however, still not understood exactly how PCNA interacts with RAD50/MRE11/NBS1 complex in the DNA DSB repair pathway, although our results suggested that PCNA was required for upregulation of the complex in the earlier stage of primary CRCs.

RAD50, MRE11 and NBS1 are the components of RAD50/MRE1/NBS1 complex, however, it has been demonstrated in vitro that the individual components are involved in different cellular responses to DNA damage induced by ionizing radiation or radiomimetic chemicals (Taylor et al., 2004). In our studies, despite the concordant association of the individual and combined expression of RAD50/MRE11/NBS1 with MSS, earlier tumour stage (I and II) and favourable survival in the same series of CRCs, each individual protein had additional clinicopathological significance. Strong or high RAD50 expression was further correlated with earlier tumour stage, better differentiation and higher inflammatory infiltration in MSS CRCs, while elevated MRE11 expression was related to less local recurrence and higher apoptotic activity in the whole series of CRCs, but not in MSS or MSI cases. Moreover, only NBS1 had prognostic significance in MSS patients with TNM stage I and II, with a tendency to be independent of gender, age, tumour location, growth pattern and differentiation. These findings suggested that the components of RAD50/MRE11/NBS1 might have additional roles despite being part of the complex.

In conclusion, RAD50/MRE11/NBS1 proteins interact with each other, which had different clinicopathological significance in MSS and MSI CRCs, and further, each component of the complex might have an individual role. NBS1 might be a biomarker for prognosis in patients with MSS CRC in TNM stage I and II.

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