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• BRIEF REPORTS •

Mad2 and p27 expression profiles in colorectal cancer and its clinical significance

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Abstract

AIM: To investigate the expression of tumor suppressor gene *p27* and spindle checkpoint gene *Mad2* and to demonstrate their expression difference in colorectal cancer and normal mucosa and to evaluate its clinical significance.

METHODS: Immunohistochemical staining was used for detection of expression of Mad2 and *p27* in colorectal cancer and its corresponding normal mucosa.

RESULTS: *Mad2* was significantly overexpressed in colorectal cancer compared with corresponding normal mucosa ($P<0.01$, $\chi^2 = 7.5$), and it was related to the differentiation of adenocarcinoma, lymph node metastasis and survival period after excision ($P<0.05$, $\chi^2 = 7.72$, $\chi^2 = 4.302$, $\chi^2 = 6.234$). The rate of *p27* positive expression in adenocarcinomas and normal mucosa was 40% and 80% respectively. There was a significant difference in *p27* expression between adenocarcinomas and normal mucosa ($P<0.001$, $\chi^2 = 13.333$), which was related to the differentiation degree of adenoca rcinoma and lymph node metastasis ($P<0.05$, $\chi^2 = 8.901$ $\chi^2 = 4$). The positive expression of *p27* was not correlated with survival period after excision.

CONCLUSION: Defect of spindle checkpoint gene *Mad2* and mutation of *p27* gene are involved mainly in colorectal carcinogenesis and associated with prognosis of colorectal cancer.

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INTRODUCTION

Genomic instability is a hallmark of malignant cells and occurs either in the form of microsatellite instability or in the form of chromosomal instability (CIN)[1]. Microsatellite instability is reflected by alteration in polymorphic, short, tandem repeats sequences and is associated with a small fraction of colorectal carcinomas with germ-line or somatic mutations of DNA mismatch repair genes[2]. On the other hand, CIN, characterized by an alteration in chromosome number and commonly detected as aneuploidy, is likely to occur in most human malignancies. The fact suggests that CIN may contribute to tumorigenesis[3-5]. In yeast, loss of mitotic checkpoint frequently leads to abnormal chromosome number, resulting in aneuploidy or polyploidy[6]. Two major groups of mitotic checkpoint genes, budding uninhibited by benomyl (BUB) 1-3 and mitotic arrest defect (MAD) 1-3, have been identified in budding yeast[7]. Mammalian homologues of the yeast mitotic checkpoint protein have also been characterized[8-10]. To date, little information is available in literature about the expression of *Mad2* in carcinoma tissue. In this study, we used immunohistochemical technique to examine the expression of *Mad2* and *p27* in colorectal cancer to elucidate the relation of *Mad2* and *p27* to carcinogenesis and clinical pathological factors.

MATERIALS AND METHODS

Specimens

Cancer tissues and corresponding normal tissues were obtained from Chinese PLA 455 Hospital from January 2001 to May 2003. No patient was treated with anti-neoplasm therapy before tumor removal. Fourty patients (22 males, 18 females, aged 25 to 79 years, median age 52.5 years) were as follows: 21 cases of well differentiated adenocarcinoma, 11 cases of moderately differentiated adenocarcinoma, 8 cases of poorly differentiated adenocarcinoma. Twelve cases survived less than 18 mo, 28 cases surivived more than 18 mo after excision. All the tissues were fixed in 40 g/L formaldehyde, embedded in paraffin, and cut into 4 μm thick serial sections.

Reagents

Mad2 polyclonal rabbit antibodies directed against humans were provided by China Science and Technology University. *P27* and immunohistochemical kit were purchased from Beijing Zhongshan Biological Technology Ltd.

Immunohistochemistry

Immunohistochemical staining was performed following the manufacturer's instructions. Anti-*Mad2* antibody was diluted to 1:120. Anti-*p27* was ready to use reagent. For the negative control, the primary antibody was substituted by animal serum. *Mad2* positive expression was stained as brown-yellow mainly in cell plasma. *P27* positive expression was also stained in cell plasma. A semi-quantitative evaluation was used to determine positively expressed cells by viewing 10 vision fields at ×400 magnification as follows[11]: negative (-), <10% cells were stained; mild positive (+), 11-25% cells were stained; moderately positive (++), 26-50% cells were stained ; strong positive (+++), >50% cells were stained. The last three grades were all regarded as positive.

Statistical analysis

The data were analyzed by SPSS version 10.0. χ^2 test was used for statistical analysis. $P<0.05$ was considered statistically significant.

RESULTS

Expression of Mad2 protein

The positive signals of *Mad2* protein were stained brown-yellow mainly in cell plasma and strength of color was directly proportional to positive percentage (Figure 1A). Positive

expression of *Mad2* protein was detected in 30 of 40 (75%) colorectal cancers, and 18 of 40 (45%) normal tissues. There was a significant difference in *Mad2* expression between colorectal cancer and normal tissue ($P<0.01$). Moreover, there were significant differences in *Mad2* expression among well, moderately, and poorly differentiated adenocarcinomas (Table 1). The expression of *Mad2* in colorectal cancer was related with lymph node metastasis and survival period after excision.

Table 1 Relationship between expression of *Mad2* protein and histological differentiation and lymph node metastasis

Groups	*n*	*Mad2*		Positive (%)	*P*
		+	-		
Normal tissue	40	18	22	45	
Adenocarcinoma	40	30	10	75	0.01
WD	21	12	9	61.9	
MD	11	10	1	99	
PD	8	8	0	100	0.041
Lymph node metastasis					
Absent	25	16	9	64	
Present	15	14	1	88	0.038
Survival period (months)					
<18	12	9	3	75	
⩾18	28	9	19	32	0.013

WD: well differentiated adenocarcinoma; MD: moderately differentiated adenocarcinoma; PD: poorly differentiated adenocarcinoma.

p27 protein expression in colorectal cancer and normal tissue

The positive signals of *p27* protein were stained brown-yellow mainly in cell plasma, weak nuclear staining was also observed. Immunoreactivity for *p27* was found in both normal and neoplastic tissues (Figure 1B). High expression of *p27* was observed in 32 normal tissues, and low expression was observed in remaining 8 normal tissues. Of 40 colorectal cancer samples, 16 (40%) had low expression of *p27*, and 24 (60%) had high expression. There was a significant difference in *p27* expression between colorectal cancer and normal tissue ($P<0.01$). Moreover, there were significant differences in *p27* expression among well, moderately, and poorly differentiated adenocarcinomas (Table 2). The expression of *p27* in colorectal cancer was related with lymph node metastasis. No relation of *p27* protein was found with survival period after excision.

Table 2 Relationship among expression of p27 protein and histological differentiation and lymph node metastasis

Groups	*n*	*p27*		Positive (%)	*P*
		+	-		
Normal tissue	40	32	8	80	
Adenocarcinoma	40	16	24	40	0.001
WD	21	13	8	61.9	
MD	11	2	9	18.2	
PD	8	1	7	12.5	0.012
Lymph node metastasis					
Absent	25	13	12	52	
Present	15	3	12	20	0.046
Survival period after excision					
<18	12	4	8	33.3	
⩾18	28	12	16	42.9	0.573

WD: well differentiated adenocarcinoma; MD: moderately differentiated adenocarcinoma; PD: poorly differentiated adenocarcinoma.

Correlation between Mad2 protein and p27

We analyzed the correlation between *Mad2* and *p27* protein expressions by χ^2 test. There was no significantly positive correlation between the expressions of *Mad2* and *p27*.

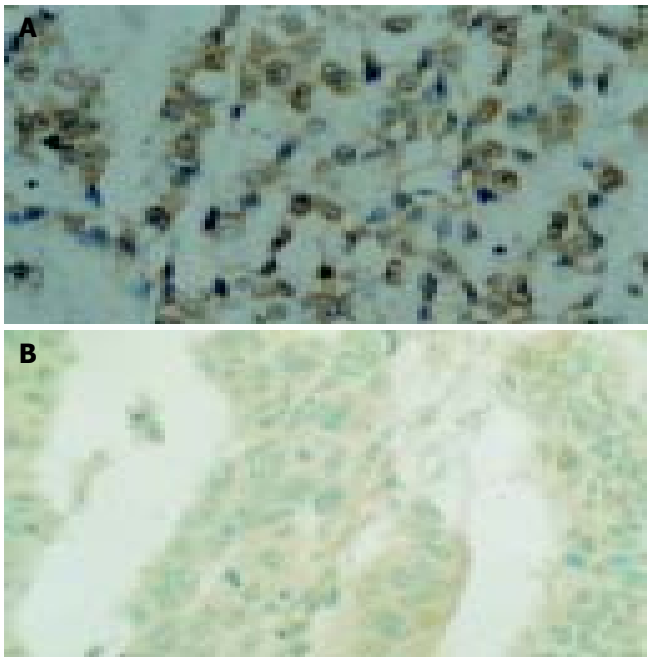

Figure 1 Strongly positive expression of *Mad2* and *p27* in poor differentiated and tubular adenocarcinomas. A: Strongly positive expression of *Mad2* in poor differentiated adenocarcinoma. B: Strongly positive expression of *p27* in tubular adenocarcinoma.

DISCUSSION

Mitotic checkpoints monitor the proper assembly of mitotic spindle and block the onset of anaphase unless all of the chromosomes are stably attached to a specialized region known as kinetochore[12]. It has been proposed that the mitotic checkpoint proteins, especially *Mad2*, may be crucial for generating the "wait" signal to prevent the onset of anaphase after microtubule disruption[13-15]. In the present study, the expressions of *Mad2* and *p27* proteins were examined in colorectal cancer and the corresponding normal tissue. *Mad2* expression in colorectal cancer was higher than that in the corresponding normal tissue. The expression of *Mad2* in colorectal cancer was related with histological differentiation, lymph node metastasis and survival period after excision. Our results regarding the expression of *Mad2* are not consistent with the finding that the reduced expression of *Mad2* in breast cancer cells reported by Li and Benezra[7], but it was similar to the study by Tanaka *et al*.[15]. The different expression might result from the surrounding in which cells lived. In an organ, cells could be influenced by nerves and endocrine hormones. Michel *et al.* showed that subtle differences in *Mad2* protein level markedly altered checkpoint function[16]. Therefore, inactivation of *Mad2* would be sufficient to lead to a haplo-insufficient effect and loss of mitotic checkpoint control. The most convincing evidence of the role of mitotic checkpoint defect in CIN in mammalian cells came from two recent studies in *Mad2*$^{-/-}$ mice, and in *Mad2*$^{-/-}$ human and mouse cells, showing that disruption of *Mad2* expression resulted in CIN[16,17]. It has been reported that CIN cells become aneuploidy, a hallmark of cancer that is associated with an aggressive tumor behavior and a poor prognosis[18]. Recent studies reported that the *Mad2* protein interacted with estrogen receptor β or the cytoplasmic domain of insulin receptors, which are thought to be regulators

of cellular growth[19-21]. Our study showed that *Mad2* protein overexpressed in cancer tissue was exclusively present in the cytoplasm of cancer cells. We speculate that cytoplasmic *Mad2* protein may enhance the positive regulatory action of estrogen receptor β and insulin receptor on cell proliferation.

P27 is a member of the Cip1/Kip1 family of cyclin-dependent kinase (CDK) inhibitors and a potential tumor suppressor gene[22]. Recent studies have demonstrated that targeted inactivation of *p27* could lead to development of multiple organ hyperplasia and malignancy *in vivo*[23,24]. In this study, we examined the expression of CDK inhibitor *p27* in colorectal adenocarcinomas and corresponding normal tissues. Low expression of *p27* was detected in cancer tissue compared with normal tissues. It was also related to histological differentiation and lymph node metastasis, but not related to survival period after excision. This evidence is similar to that previously reported in other tumors such as tumors of breast, stomach, prostate, lung, liver[25-32].

Orr-Weaver *et al*. thought that aneuploidy might increase the rate at which tumor suppressors are lost through the loss of heterozygosity. Our study showed that there was no significantly positive correlation between the expressions of *Mad2* and *p27*.

In conclusion, expressions of *Mad2* and *p27* are related to histological differentiation and lymph node metastasis of colorectal cancer. *Mad2* and *p27* proteins might be good markers for predicting histological differentiation and prognosis of colorectal cancer.

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