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, χ² = 7.5), and it was related to the differentiation of adenocarcinoma, lymph node metastasis and survival period after excision (P<0.05, χ² = 7.72, χ² = 4.302, χ² = 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, χ² = 13.333), which was related to the differentiation degree of adenocarcinoma and lymph node metastasis (P<0.05, χ² = 8.901 χ² = 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)[11]. 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[21]. 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, 12 cases of poorly differentiated adenocarcinoma, 8 cases of well differentiated adenocarcinoma, 11 cases of moderately differentiated adenocarcinoma, 8 cases of poorly differentiated adenocarcinoma, 12 cases of well differentiated adenocarcinoma, 11 cases of moderately differentiated adenocarcinoma, 8 cases of poorly differentiated adenocarcinoma, 12 cases of well differentiated adenocarcinoma, 11 cases of moderately differentiated adenocarcinoma, 8 cases of poorly differentiated adenocarcinoma, 12 cases of well differentiated adenocarcinoma, 11 cases of moderately differentiated adenocarcinoma, 8 cases of poorly differentiated adenocarcinoma, 12 cases of well differentiated adenocarcinoma, 11 cases of moderately differentiated adenocarcinoma, 8 cases of poorly differentiated 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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     | 0.038  |
| Present            | 15 | 14   | 1            | 88     |
| Survival period (months) | |      |              |        |
| <18                | 12 | 9    | 3            | 75     |
| ≥18                | 28 | 29   | 9            | 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 high 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 | 16  | 24           | 40     | 0.001  |
| Adenocarcinoma     | 40 | 16  | 24           | 40     |
| 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 $\chi^2$ 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.[13]. 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[14]. 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 aneuploid, 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[25-31].

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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