Expression of budding uninhibited by benzimidazoles - 1 and mitotic arrest deficient - 2 in endometrial carcinoma and its significance

Q. Zhao[1], A.P. Bian[1], Y. Zhang[2], L. Qin[2], H.R. Shi[2], K. Su[2]

[1]Department of Obstetrics & Gynecology, the First Affiliated Hospital of Zhengzhou University, Zhengzhou
[2]Zhengzhou Maternal and Child Health Hospital, Zhengzhou (China)

Summary

Objective: The aim of this study was to explore the expression of budding uninhibited by benzimidazoles-1 (Bub1) and mitotic arrest deficient-2 (Mad2) in endometrial carcinoma and its significance. *Materials and Methods:* The expression of Bub1 and Mad2 in 30 human normal endometrial tissues (group A), 30 complexly-hyperplastic endometrial tissues (group B), and 63 endometrial carcinoma tissues (group C) was observed using immunohistochemistry (the streptavidin-peroxidase method). *Results:* The positive expression rates of Bub1 in groups A, B, and C were 86.67%, 56.67%, and 28.57%, respectively. The positive rate of Bub1 protein was correlated with the differentiation degree and clinical stage of endometrial carcinoma ($p < 0.05$) other than lymph node metastasis ($p > 0.05$): A higher differentiation degree and a more advanced stage of endometrial carcinoma indicated a higher positive rate of Bub1 protein. The positive rates of Mad2 protein in groups A, B, and C were 23.33%, 56.67%, and 85.71%, respectively. The positive rate of Mad2 protein was correlated with the differentiation degree of endometrial carcinoma ($p < 0.05$) other than its clinical stage and lymph node metastasis ($p > 0.05$): A lower differentiation degree indicated a higher positive rate of Mad2 protein. Bub1 and Mad2 proteins were negatively correlated in the endometrial carcinoma tissues ($r = - 0.719$, $p < 0.001$). *Conclusion:* Bub1 and Mad2 proteins interact with each other. They may play an important role in the initiation and development of endometrial carcinoma.

Key words: Endometrial carcinoma; Budding uninhibited by benzimidazoles-1 (Bub1); Mitotic arrest deficient 2-like protein 1; Spindle checkpoint.

Introduction

Endometrial carcinoma is one of the three major gynecological malignant tumors with an increasing incidence in recent years. It greatly threatens the life quality of patients and their families. Its early diagnosis and timely treatment, particularly its precancerous diagnosis and prevention, have become a widespread concern of patients as well as of gynecologists. Budding uninhibited by benzimidazoles-1 (Bub1) and mitotic arrest deficient-2 (Mad2) are important components of spindle checkpoints [1]. They monitor and ensure the fidelity of cell mitosis and have a predictive value for the prognosis of some tumors [1, 2].

To explore the roles by Bub1 and Mad2 in the initiation and development of endometrial carcinoma and their clinical significance, the authors observed their expression in human normal endometrial, complexly-hyperplastic endometrial, and endometrial carcinoma tissues.

Materials and Methods

General data

A total of 63 paraffin-embedded specimens of endometrial carcinoma resected between January 2007 and January 2011 (group A) were collected. Meanwhile, 30 paraffin-embedded specimens of complex-hyperplastic endometrial tissues during the same period (group B), as well as of normal endometrial tissues (group C), were taken. The averages ages of the involved patients in the three groups were 49.43 ± 3.50, 49.27 ± 5.47, and 50.77 ± 4.82 years, respectively, showing no significant differences. All the patients did not receive hormonal therapy, radiotherapy, or chemotherapy before surgery. According to classification and staging of endometrial carcinoma by the International Federation of Gynecology and Obstetrics (FIGO) in 1980, 39 tissues were in Stage I, 17 in Stage II, 5 in Stage III, and 2 in Stage IV. Thirty-eight tissues were well differentiated, 17 were moderately differentiated, and eight were poorly differentiated. Eight patients had lymph node metastasis. This study was conducted in accordance with the Declaration of Helsinki and with approval from the Ethics Committee of the First Affiliated Hospital of Zhengzhou University. Written informed consent was also obtained from all participants.

Methods and result judgment

The expression of Bub1 and Mad2 in different groups was observed using immunohistochemistry (the streptavidin-peroxidase method). Endometrial glandular epithelial cells with yellow- or buffy-stained granules in cytoplasm and/or nucleus were considered positive. Results were observed using the semi-quantitative, two-observer double-blind method. Ten high power fields (10 × 40) were selected for each section. According to the percentage of the number of positive cells taken in that of the total cells in a field, four levels were assigned: (-) for no positive staining or no positive cells, (+) for 11% – 30%, (++) for 31% – 50%, and (+++) for ≥ 51%, in which (-) indicates negative expression, whereas (+)–(+++) indicate positive expression [2]. If a difference of ten

Revised manuscript accepted for publication January 28, 2013

Table 1. — *The positive expression rates of Bub1 and Mad2 proteins in different groups (n%).*

Group	n	Mad2 (+)	Bub1 (+)
A	30	7 (23.33)	26 (86.67)
B	30	17 (56.67)	17 (56.67)
C	63	54 (85.71)	18 (28.57)
x^2		6.944[1]	6.648[1]
		35.040[2]	27.515[2]
		9.495[3]	6.835[3]
p		0.008[1]	0.010[1]
		0.000[2]	0.000[2]
		0.002[3]	0.009[3]

Note: [1]group A vs. group B; [2]group A vs. group C; [3]group B vs. group C.

percent occurred between the observed results of the two observers, re-evaluation was performed.

Statistical analysis

Data were processed by SPSS13.0 software using Chi-square () test. Spearman rank correlation coefficient analysis was performed for the correlation between Bub1 and Mad2. A $p < 0.05$ was considered statistically significant.

Results

The expression of Bub1 and Mad2 proteins

The results of Bub1 and Mad2 protein by immunohistochemistry were summarized in Table 1. The positive expression rates of Bub1 in groups A, B, and C were 86.67%, 56.67%, and 28.57%, respectively, with the expression of group A significantly lower than that of any other group ($p < 0.01$). Furthermore, the less differentiated endometrial carcinoma and the more advanced the clinical stage, the lower the positive rate ($p < 0.05$). However, the positive rate was not associated with lymph node metastasis ($p > 0.05$, Table 2). The positive rates of Mad2 protein in groups A, B, and C were 23.33%, 56.67%, and 85.71%, respectively, with the expression of group A significantly higher than that of any other group ($p < 0.01$). Furthermore, the less differentiated endometrial carcinoma, the higher the positive rate ($p < 0.05$). However, the positive rate was not associated with the clinical stage or lymph node metastasis ($p > 0.05$, Table 3).

Table 4. — *Correlation between Mad2 and Bub1 in endometrial carcinoma tissues.*

Mad2 protein	Bub1 protein		Sum
	Positive	Negative	
Positive	11	43	54
Negative	7	2	9
Sum	18	45	63

Note: $r = -0.719$, $p < 0.001$.

The correlation between the expression of Bub1 and Mad2 proteins

The results of the correlation analysis of Bub1 and Mad2 in endometrial carcinoma are summarized in Table 4. Positive Bub1 and Mad2 existed concurrently in 11 patients and their negative concurrence in two. The result of the Spearman rank correlation coefficient analysis based on the percentage of the number of the positive cells in the number of the total cells in the same field showed a negative correlation between Bub1 and Mad2 ($r = -0.719$, $p < 0.001$).

Discussion

Endometrial carcinoma is one of the three major malignant tumors commonly occurring in female reproductive system. In recent years, its onset age shows a younger tendency and its incidence is on the increase. Disorders in cell cycle regulation greatly contribute to the initiation and development of tumors [3]. Bub1 and Mad2 are critical components of spindle checkpoints. During the process of cell mitosis, they monitor the morphology of the spindles, the connection between kinetochores and spindle microtubules, as well as the location and arrangement of chromosomes. Whenever abnormalities in the expression and/or function of Bub1 and Mad2 occur, spindle checkpoints will neglect chromosome damage and continue with their function in

Table 2. — *Correlations between Bub1 protein expression and endometrial carcinoma parameters.*

Group	Clinical Stage				Differentiation degree			Lymph node metastasis	
	I	II	III	IV	Well	Moderately	Poorly	Yes	No
n	39	17	5	2	38	17	8	8	55
The positive rate of Bub1 (n%)	16 (41.03)	2 (11.76)	0	0	15 (39.47)	3 (17.65)	0	0	18 (32.72)
x^2	8.125				6.409			3.665	
p	< 0.05				< 0.05			> 0.05	

Table 3. — *Correlations between Mad2 protein expression and endometrial carcinoma parameters.*

Group	Clinical Stage				Pathological grade			Lymph node metastasis	
	I	II	III	IV	G1	G2	G3	Yes	No
n	39	17	5	2	38	17	8	8	55
The positive rate of Mad2 (n%)	32 (82.05)	15 (88.24)	5 (100.00)	2 (100.00)	29 (76.32)	17 (100.00)	9 (100.00)	7 (87.50)	47 (85.45)
x^2	1.684				6.903			0.024	
p	> 0.05				< 0.05			> 0.05	

cell mitosis. This phenomenon is very likely to lead to errors in chromosome distribution, causing chromosome instability [4]. Bub1 is principally located in cytoplasm and closely relates with the proliferation and differentiation of cells [5]. Bub1 spindle checkpoint is a critical regulatory factor for chromosome mitosis and meiosis [6]. Its numerous functions in chromosome division are of great significance for predicting tumorigenesis and abnormal development of cells [7]. In this study, the positive expression rates of Bub1 in groups A, B, and C were 86.67%, 56.67%, and 28.57%, respectively, which is consistent with the result reported by Jeganathan K *et al* [8]. According to these authors, loss of Bub1 may lead to tumorigenesis. The positive rate of Bub1 protein in group C was significantly lower than that of any other group ($p < 0.01$). Furthermore, a lower positive rate was always indicated by a lower differentiation degree of endometrial carcinoma ($p < 0.05$). Bub1 has a very low probability of gene mutation. A decrease in Bub1 protein in endometrial carcinoma tissues may be caused by the mutation of the upstream genes of Bub1 in carcinoma tissues. Deficiency of Bub1 expression can lead to spindle checkpoint dysfunction in the early apoptosis pathway of damaged cells mediated by P53, causing the formation and accumulation of aneusomic chromosomes [9]. The rate of cells with aneusomic chromosomes in endometrial carcinoma tissues is noticeably higher than those in complex-hyperplastic and normal endometrial tissues [10]. Aneuploids can lead to carcinogenesis independently of gene mutation [11]. In addition, Bub1 overexpression leads to the formation of non-diploids and tumors, although the mechanism underlying its overexpression remains to be explored [12].

Mad2 is mainly expressed in cytoplasm. In this study, the positive rates of Mad2 in groups A, B, and C were 23.33%, 56.67%, and 85.71%, respectively, with the positive rate of group C higher than that of any other group ($p < 0.01$). Furthermore, a lower positive rate of Mad2 protein was always indicated by a lower differentiation level of endometrial carcinoma ($p < 0.05$). Mad2 regulates cyclins to make cellular chromosomes to separate [13]. In spindle checkpoints, it monitors and allows mitotic midanaphase transformation and normal fission [14]. Mad2 also has a very low probability of gene mutation. The overexpression of Mad2 in endometrial carcinoma may be caused by a certain or more than one factor. Such a phenomenon can damage the function of spindle checkpoints to cause their control functional abnormalities, thus increasing the instability of chromosomes and the probability of chromosome deletion [15]. Mad2 overexpression stimulates the onset and development of various types of tumors in mice [16] and has a predictive value for the prognosis of primary lung carcinoma [2].

Bub1 is located in the upstream of signal cascades, which is necessary for it to detect tension kinetochores. When chromosomes are damaged, the spindle checkpoint will be activated. In such a situation, Mad2 blocks cellular anaphase transformation by inhibiting the activity of anaphase promoting compounds [17]. A decrease in Bub1 decreases the kinetochore localizing capacity of Mad2; in such a situation, abnormalities in chromosomes are neglected and the process of mitosis is accelerated [18]. Accordingly, errors may occur to the distribution process of chromosomes, leading to their instability. Abnormalities in the expression of both Bub1 and Mad2 proteins may further aggravate such instability. Cells with instable chromosomes are prone to chromosomal aberrations, ultimately resulting in tumorigenesis. The blockage of the subaqueous increase in Mad2 during mitosis is an important signal component of mitotic checkpoints, but whether Mad2 serves as an important component of mitotic effect remains controversial [19].

In conclusion, abnormalities in the expression of Bub1 and Mad2 cause unstable chromosomes and aneuploid cells, ultimately leading to the proliferation and differentiation of cells [20] and tumorigenesis. Their interaction plays an important role in the initiation and development of endometrial carcinoma. The more advanced the clinical stage of endometrial carcinoma is, the lower the positive rate of Bub1 will be, which indicates Bub1 protein expression may be correlated with the prognosis of endometrial carcinoma. Further exploration of the functions of spindle checkpoint proteins is expected to provide a new idea for the treatment of endometrial carcinoma in clinical practice.

References

[1] Hannisdal K., Burum-Auensen E., Schjølberg A., De Angelis P.M., Clausen O.P.: "Correlation between reduced expression of the spindle checkpoint protein BubR1 and bad prognosis in tonsillar carcinomas". *Head Neck*, 2010, *32*, 1354.

[2] Kato T., Daigo Y., Aragaki M., Ishikawa K., Sato M., Kondo S. *et al*.: "Overexpression of MAD2 predicts clinical outcome in primary lung cancer patients". *Lung Cancer*, 2011, *74*, 124.

[3] Li Z.Q., Zhang J.B.: "Cell Cycle and Regulation". *Oncology Progress*, 2004, *2*, 146.

[4] Kadhiin M.A., Macdonald D.A., Goodhead D.T., Lorimore S.A., Maarsden S.J., Wright E.G.: "Transition of chromosome instability after plutonium-particle irradiation". *Nature*, 1992, *355*, 738

[5] Luo M., Weng Y., Tang J., Hu M., Liu Q., Jiang F. *et al*.: "MicroRNA-450a-3p Represses Cell Proliferation and Regulates Embryo Development by Regulating Bub1 Expression in Mouse". *PLoS On*e, 2012, *7*, e47914.

[6] Sun S.C., Kim N.H.: "Spindle assembly checkpoint and its regulators in meiosis". *Hum. Reprod. Updat*e, 2012, *18*, 60.

[7] Marchetti F., Venkatachalam S.: "The multiple roles of Bub1 in chromosome segregation during mitosis and meiosis". *Cell Cycle*, 2010, *9*, 58.

[8] Jeganathan K., Malureanu L., Baker D.J., Abraham S.C., van Deursen J.M.: "Bub1 mediates cell death in response to chromosome missegregation and acts to suppress spontaneous tumorigenesis". *J. Cell Biol*., 2007, *179*, 255.

[9] Gao F., Ponte J.F., Papageorgis P., Levy M., Ozturk S., Lambert A.W. *et al*.: "hBub1 deficiency triggers a novel p53 mediated early apoptotic checkpoint pathway in mitotic spindle damaged cells". *Cancer Biol. Ther*., 2009, *8*, 627.

[10] Ma Z., Liu L.: "The significance of DNA ploidy and S-phrase fraction (SPF) in endometrial carci. *Journal of Maternity and Child Care of China*, 2006, *21*, 3173.

[11] Duesberg P.: "Genetic instability of cancer cells is proportional to their degree of aneuploidy". *P. Natl. Acad. Sci. U. S. A*., 1998, *95*, 13692.
[12] Ricke R.M., Jeganathan K.B., van Deursen J.M.: "Bub1 overexpression induces aneuploidy and tumor formation through Aurora B kinase hyperactivation". *J. Cell Biol*., 2011, *193*, 1049.
[13] Pennisi E.: "Cell division gatekeepers identified". *Science*, 1998, *279*, 477.
[14] Orr B., Bousbaa H., Sunkel C.E.: "Mad2-independent spindle assembly checkpoint activation and controlled metaphase-anaphase transition in Drosophila S2 cells". *Mol. Biol. Cell*, 2007, *18*, 850.
[15] Michel L.S., Liberal V.: "Mad2 haplo-insuffciency causes premature anaphase and chromosome instability in mammalian cell". *Nature*, 2001, *409*, 355.
[16] Sotillo R., Hernando E., Díaz-Rodríguez E., Teruya-Feldstein J., Cordón-Cardo C., Lowe S.W. *et al*.: "Mad2 overexpression promotes aneuploidy and tumorigenesis in mice". *Cancer Cell*, 2007, *11*, 9.
[17] Ge S., Skaar JR., Pagano M.: "APC/C- and Mad2-mediated degradation of Cdc20 during spindle checkpoint activation". *Cell Cycle*, 2009, *8*, 167.
[18] Saitoh S., Kobayashi Y., Ogiyama Y., Takahashi K.: "Dual regulation of Mad2 localization on kinetochores by Bub1 and Dam1/DASH that ensure proper spindle interaction". *Mol. Biol. Cell*, 2008, *19*, 3885.
[19] Tipton A.R., Tipton M., Yen T., Liu S.T.: "Closed MAD2 (C-MAD2) is selectively incorporated into the mitotic checkpoint complex (MCC)". *Cell Cycle*, 2011, *10*, 3740.
[20] Brevini T.A., Pennarossa G., Maffei S., Tettamanti G., Vanelli A., Isaac S. *et al*.: "Centrosome amplification and chromosomal instability in human and animal parthenogenetic cell lines". *Stem. Cell Rev*., 2012, *8*, 1076.

Address reprint requests to:
H.R. SHI, M.D.
Department of Obstetrics & Gynecology,
The First Affiliated Hospital of Zhengzhou,
1 Jianshe Rd, Zhengzhou 450052 (China)
e-mail: cnqianzhao@126.com