Int J Clin Exp Pathol 2016;9(2):2637-2642
www.ijcep.com /ISSN:1936-2625/IJCEP0018104

Original Article

Expression of mini chromosome maintenance protein 7 in esophageal carcinoma and clinical implications

Fumei Zhang[1], Dongxia Li[2], Fangheng Zhu[1], Sa Tang[1], Li Ye[1]

[1]Department of Pathology, Xinxiang Central Hospital, Xinxiang 453000, Henan, China; [2]Basic Medical College of Xinxiang Medical College, Xinxiang 453000, Henan, China

Received October 19, 2015; Accepted November 28, 2015; Epub February 1, 2016; Published February 15, 2016

Abstract: As one of the most common malignant tumors in digestive tract, esophageal carcinoma has an increasing trend of incidence and mortality rate. The effective method of early diagnosis, therefore, is of critical importance for improving patients' survival rate. Various biological molecules have been suggested to be related with pathogenesis of esophageal cancer. As one important modulatory factor for DNA replication, mini chromosome maintenance protein 7 (MCM7) has been studied in various tumors including colorectal, breast, pulmonary cancer, glioma, Hodgkin's lymphoma and prostate cancer. This study mainly investigated the diagnostic value of MCM7 in esophageal carcinoma. Immunohistochemical (IHC) staining was used to investigate the expressional profile and cellular localization of MCM7 in a total of 37 esophagus cancer tissue and adjacent tissues. RT-PCR was used to detect mRNA level of MCM7 gene in tumor and adjacent tissues. The correlation between MCM7 expression level and clinical indexes was analyzed. The expression of MCM7 mRNA and protein was significantly elevated in tumor tissues compared to adjacent tissues ($P < 0.05$ in both cases). MCM7 expression level was correlated with differentiation digress, distal metastasis and lymph node invasion, but was uncorrelated with other indexes including sex or age. MCM7 is closely correlated with pathogenesis and progression of esophageal carcinoma and is a potential biological marker for the tumor.

Keywords: Mini chromosome maintenance protein 7, esophageal cancer, RT-PCR

Introduction

As one of the most common malignant tumors in digestive tract, esophageal carcinoma is the sixth leading cause of mortality among all cancers worldwide, with a 5-year survival rate at 15%~25% [1]. The prognosis of esophageal cancer is unfavorable due to its insidious manifestation during the early stage. Therefore, timely and accurate diagnosis can significantly improve patients' survival rate, and decrease mortality. Common diagnostic approaches in clinics include X-ray barium meal examination, esophagus fleece-pulling, electronic endoscopy and tumor marker assay. The identification of novel tumor markers is of critical importance for early diagnosis of esophagus cancer.

Mini chromosome maintenance proteins (MCMs) are a group of protein markers reflecting cell proliferation. Firstly being identified in yeast cells, MCMs have highly conserved protein sequence, with pluripotent activities including DNA/RNA unwindase, proteinase and metal chelatase, for regulating initiation and elongation of DNA replication, making it one necessary factor for eukaryotic DNA replication [2]. As one family member, MCM7 is composed of 719 amino acids, with an average molecular weight at 80 kD [3]. MCM7 is one subunit of MCM protein polymer, and can affect meiosis activity of chromosome. Cells at late G1 stage or S stage but not latent stage may expression MCM7 mRNA [4]. Therefore, MCM7 may work as one reliable marker for cell proliferation. Various studies have shown the up-regulation of MCM7 in multiple malignant tumors including colorectal [5], breast [6], pulmonary carcinoma [7], glioblastoma [8], Hodgkin lymphoma [9] and prostate cancer [10]. This study utilized immunohistochemical (IHC) staining method, to quantify MCM7 expression level in both esophagus and adjacent tissues, for the further investigating the diagnostic impact of MCM-7.

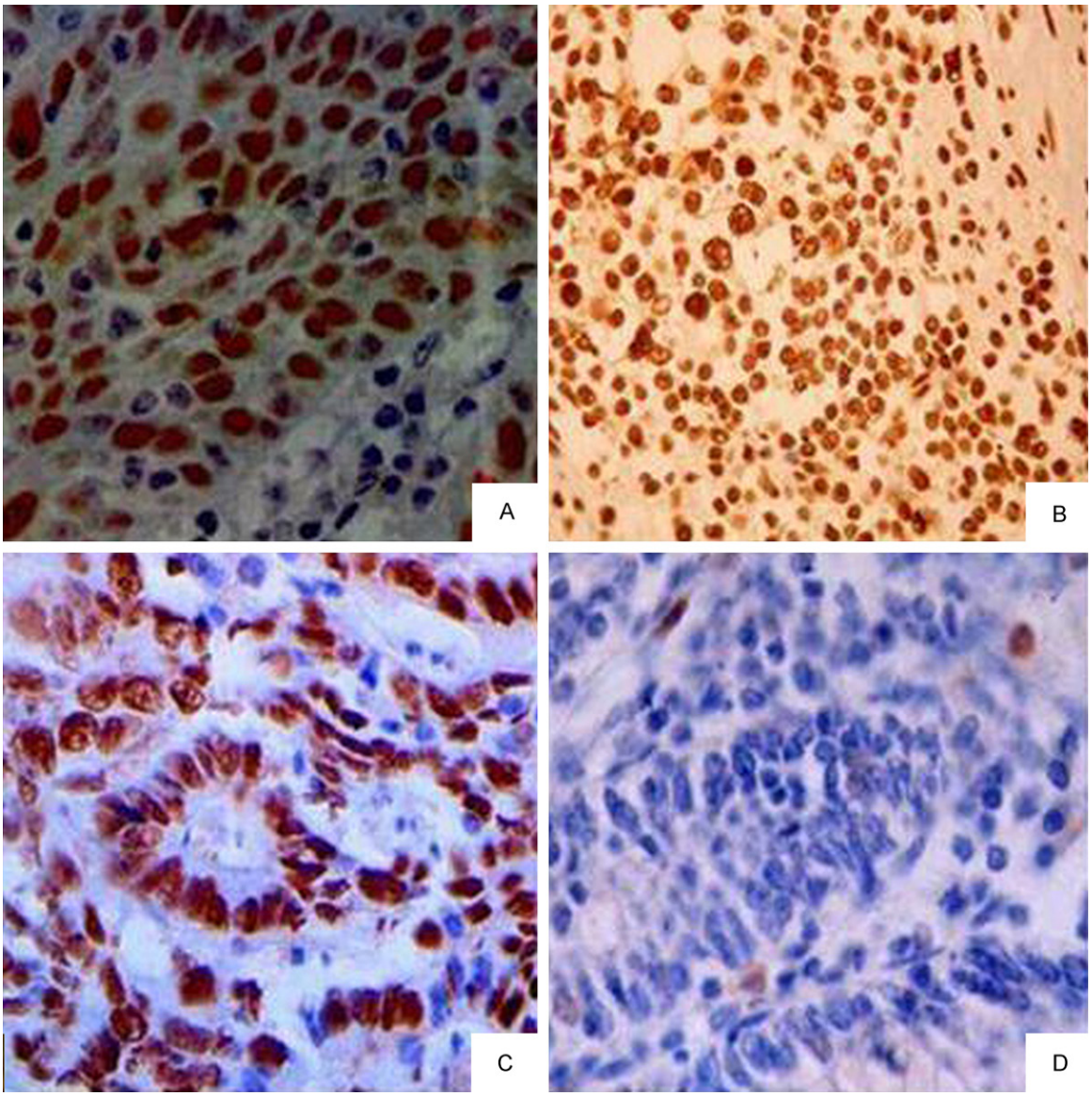

Figure 1. MCM protein expression (×400). A. High-differentiated esophageal carcinoma tissue; B. Moderate-low differentiated esophageal carcinoma tissue; C. Colorectal carcinoma tissue; D. Tumor adjacent tissue.

Materials and methods

Research subjects

For immunohistochemical (IHC) staining, a total of 37 cases of esophageal carcinoma patients (29 males and 8 females, average age = 60.72 years old) in Xinxiang central hospital from April 2014 to April 2015 were recruited. Pathological examinations revealed 17 of high differentiated and 20 of moderate to low differentiated tumors, and 20 patients having lymph node metastasis while 12 individuals had distal metastasis. Both esophageal tissue and adjacent tissues (> 1.5 cm) were collected and prepared for paraffin-based tissue samples by the department of pathology of Xinxiang central hospital. A total of 5 cases of colorectal carcinoma tissues were collected as the positive control group. All patients receive no chemo- or radio-therapy before the surgery. HE staining confirmed the malignancy nature of tumor tissues and absence of tumor cells in adjacent tissues.

For RT-PCR analysis, 32 esophageal cancer patients in Xinxiang central hospital from April 2014 to February 2015 were recruited. Both

Table 1. MCM7 expression

Tissue type	N	MCM7				Positive rate (%)	*P* value
		-	+	++	+++		
Esophageal carcinoma	37	7	10	12	8	81.1	0.000
Adjacent tissue	37	26	7	3	1	29.7	

Table 2. MCM7 expression and clinical parameters

Parameter	MCM7				N (positive)	Positive rate	*P* value
	-	+	++	+++			
Age							0.306
≥ 60 years	2	4	9	4	17	89.5%	
< 60 years	5	6	3	4	13	72.2%	
Sex							0.503
Male	6	6	9	8	23	79.3%	
Female	1	3	3	1	7	87.5%	
Tumor site							0.638
Upper	2	1	2	1	4	66.7%	
Middle	2	4	5	5	14	87.5%	
Lower	3	4	4	4	12	80.0%	
Type							0.960
Ulcer	2	1	3	3	7	77.8%	
Medulla	1	2	3	1	6	85.7%	
Fungating	2	4	4	1	9	81.8%	
Constrictive	1	1	1	1	3	75.0%	
Erosive	1	2	2	1	5	83.3%	
Differentiation							0.028
High	4	7	4	2	13	76.5%	
Low to moderate	3	3	8	6	17	85.0%	
Lymph node metastasis							0.014
Yes	2	3	8	7	18	90.0%	
No	5	6	3	3	12	70.6%	
Distal metastasis							0.013
Yes	1	2	4	5	11	91.7%	
No	6	9	6	4	19	76.0%	

tumor and adjacent tissues (> 1.5 cm) were collected and frozen at -80°C for further use. There were 19 males and 13 females in all patients, aging between 40 and 85 years old (average = 68.32 years).

All of the patients had signed the informed consent, and the study was approved by Xinxiang central hospital medical ethics committee.

IHC (SP) staining

Paraffin-based tissue blocks were serially sectioned. After de-wax and heat anti-retrieval, endogenous activity of peroxidase was blocked, followed by serum blocking. Mouse anti-human MCM7 monoclonal antibody (Santa Cruz, US) was added, followed by biotin-labeled secondary antibody. SP reaction and DAB chromogenic substrates were added to visualize the signal. Hematoxylin was used for counter-staining, followed by dehydration in gradient ethanol. The slide was immersed in xylene and mounted with coverslips.

In each slide, the staining was classified based on both staining intensity and positive cell percentage. In brief, a staining intensity score was given as 0 (no staining), 1 (light yellow), 2 (brown yellow) or 3 (dark brown). Meanwhile a cell percentage score was given as 0 (< 5% positive cells), 1 (5%~25% positive cells), 2 (26%~50% positive cells), 3 (51%~75% positive cells) or 4 (> 75% positive cells). The IHC score was calculated as the product of both intensity and percentage score and was deduced as negative (-, 0~2 scores), weak positive (+, 3~5 scores), positive (++, 6~8 scores) and strong positive (+++, > 8 scores).

RT-PCR

Total RNA was extracted by Trizol method and was quantified using ultraviolet spectrometer. cDNA was synthesized using mRNA as the template in reverse transcription kit (Baosheng, China) following manual instruction in a sequence of 37°C incubation, 95°C denature and 4°C icing. A fluorescent quantitative PCR cycler was used to amplify DNA samples under the following conditions: 95°C pre-denature for 5 min, followed by 40 cycles each containing 95°C denature (30 sec), 60°C annealing (30 sec) and 72°C elongation (40 sec). Ct value

Table 3. MCM7 mRNA level and clinical parameters

Parameter	N	Relative level	*t* value	*P* value
Sex			0.745	0.464
Male	19	0.2840±0.1922		
Female	13	0.2328±0.0161		
Age			-0.231	0.820
< 60 years	9	0.2527±0.0162		
≥ 60 years	23	0.2608±0.0775		
Differentiation grade			2.186	0.040
High	16	0.2261±0.0172		
Low to moderate	16	0.2438±0.0192		
Lymph node metastasis			2.174	0.041
Yes	11	0.2437±0.0173		
No	21	0.2173±0.0192		
Distal metastasis			2.386	0.027
Yes	6	0.2446±0.0183		
No	26	0.2263±0.0175		

was collected and analyzed for expression level.

Statistical analysis

SPSS 19.0 software package was used to analyze all data. The expression of MCM7 between tumor and adjacent tissues was compared by chi-square test. Rank-sum test was used to compare clinical parameters. mRNA relative expression level was expressed as mean ± standard deviation (SD). The comparison of clinical parameters was performed by independent two-sample t-test. A statistical significance was defined when $P < 0.05$.

Results

IHC staining

Under microscopic examination, MCM7 was localized in nucleus and is abundantly expressed in esophageal carcinoma tissues as brown-yellow or dark brown granules (**Figure 1A** and **1B**) but not in adjacent tissues (**Figure 1D**).

Quantitative analysis of IHC results showed significantly elevated positive rate of MCM7 in esophageal cancer tissue compared to adjacent tissues (81.1% vs. 29.7%, $P < 0.05$, **Table 1**).

MCM7 expression was found to be correlated with differentiation grade, lymph node metastasis and distal metastasis ($P < 0.05$, **Table 2**) but not age, sex, location and pathological type. With lower differentiation grade, the positive rate of MCM7 expression was gradually increased.

RT-PCR

MCM7 mRNA expression level in esophageal cancer tissue was significantly higher than that in adjacent tissues (0.27±0.14 vs. 0.20±0.03, $P < 0.05$). mRNA level was correlated with differentiation grade, distal and lymph node metastasis but not with sex or age (**Table 3**). Higher differentiation grade in tumors occurred with lower MCM7 mRNA levels ($r=-0.634$, $P < 0.05$).

Discussion

Abnormal regulation of cell cycle is one important endogenous reason for tumor pathogenesis. The dysfunction of G1/S transition is crucial for tumor cell occurrence, proliferation and progression [11, 12]. As one group of protein marker reflecting cell proliferation, MCM family include eight members, namely, MCM2, MCM3, MCM4/Cdc21, MCM5/Cdc46, MCM6/Mis5, MCM7/Cdc47, MCM8 and MCM9, all of which are closely correlated with cell cycle regulation via initiating eukaryotic cell DNA replication and transition from G1 to S phase [13]. In latent cells, MCM expression level was relatively lower. In tumor cells, however, MCMs are abundantly expressed, making them a type of specific tumor markers with higher reliability than Ki-67 [14-16]. MCM7 can bind with other family members to form a complex with ATPase, ssDNA binding affinity and DNA helicase activity to open double strands of DNA during replication [17]. The zinc-finger and ATPase motif of MCM7 are necessary for DNA helicase and ATPase activity [4, 18]. As MCM7 participates in the whole process of DNA replication, it is one important regulatory factor for cellular DNA replication.

The expression of MCM7 in digestive tract tumors has been studied previously. Pillaire et al reported the correlation between MCM7 high-expression and shortening of survival time in colorectal cancer patients, suggesting the potency of MCM7 in prognostic prediction [19]. Zhou et al found elevated expression of MCM7 in hepatocellular carcinoma cells in contrast to normal liver tissues, in addition to the relationship between MCM7 and metastasis/invasion of liver cancer [20]. This study discovered higher expression of MCM7 in esophageal carcinoma tissue compared to adjacent tissues, suggesting that MCM7 high-expression may reflect proliferative activity of tumor cells and potency in tumor diagnosis. MCM7 expression level was correlated with differentiation grade, distal and lymph node metastasis, further suggesting the involvement of MCM7 in the pathogenesis, progression and invasion of esophageal cancer.

This study demonstrated the correlation between MCM7 expression in esophageal carcinoma tissues and clinical parameters, providing new insights of tumor pathogenesis. Larger samples size with multi-centered study should be pursued in future to enhance the early diagnosis and treatment of esophageal cancer.

Disclosure of conflict of interest

None.

Address correspondence to: Dr. Fangheng Zhu, Department of Pathology, Xinxiang Central Hospital, 56, Xinxiang Jin Sui Avenue, Xinxiang 453000, Henan, China. Tel: +86-373-2027117; Fax: +86-373-2027117; E-mail: zhufangheng@sina.cn

References

[1] Domper Arnal MJ, Ferrandez Arenas A and Lanas Arbeloa A. Esophageal cancer: Risk factors, screening and endoscopic treatment in Western and Eastern countries. World J Gastroenterol 2015; 21: 7933-43.

[2] Bailey R, Priego Moreno S and Gambus A. Termination of DNA replication forks: "Breaking up is hard to do". Nucleus 2015; 6: 187-96.

[3] Lengronne A and Pasero A. Closing the MCM cycle at replication termination sites. EMBO Rep 2014; 15: 1226-7.

[4] Wei Q, Li J, Liu T, Tong X, Ye XI. Phosphorylation of minichromosome maintenance protein 7 (MCM7) by cyclin/cyclin-dependent kinase affects its function in cell cycle regulation. J Biol Chem 2013; 288: 19715-25.

[5] Ishibashi Y, Kinugasa T, Akagi Y, Ohchi T, Gotanda Y, Tanaka N, Fujino S, Yuge K, Kibe S, Yoshida N, Mizobe T, Oka Y, Yoshida T, Shirouzu K. Minichromosome maintenance protein 7 is a risk factor for recurrence in patients with Dukes C colorectal cancer. Anticancer Res 2014; 34: 4569-75.

[6] Bhar A, Haubrock M, Mukhopadhyay A, Maulik U, Bandyopadhyay S, Wingender E. Coexpression and coregulation analysis of time-series gene expression data in estrogen-induced breast cancer cell. Algorithms Mol Biol 2013; 8: 9.

[7] Haruki T, Shomori K, Shiomi T, Taniguchi Y, Nakamura H, Ito H. Multiparameter analysis using cell cycle biomarkers for small-size lung adenocarcinoma: prognostic implications. Oncol Rep 2012; 28: 915-22.

[8] Erkan EP, Ströbel T, Lewandrowski G, Tannous B, Madlener S, Czech T, Saydam N, Saydam O. Depletion of minichromosome maintenance protein 7 inhibits glioblastoma multiforme tumor growth in vivo. Oncogene 2014; 33: 4778-85.

[9] Marnerides A, Vassilakopoulos TP, Boltetsou E, Levidou G, Angelopoulou MK, Thymara I, Kyrtsonis MC, Pappi V, Tsopra O, Panayiotidis P, Pangalis GA, Beris P, Patsouris E, Korkolopoulou P. Immunohistochemical expression and prognostic significance of CCND3, MCM2 and MCM7 in Hodgkin lymhoma. Anticancer Res 2011; 31: 3585-94.

[10] Shi YK, Yu YP, Tseng GC, Luo JH. Inhibition of prostate cancer growth and metastasis using small interference RNA specific for minichromosome complex maintenance component 7. Cancer Gene Ther 2010; 17: 694-9.

[11] Hesketh EL, Knight JR, Wilson RH, Chong JP, Coverley D. Transient association of MCM complex proteins with the nuclear matrix during initiation of mammalian DNA replication. Cell Cycle 2015; 14: 333-41.

[12] Powell SK, MacAlpine HK, Prinz JA, Li Y, Belsky JA, MacAlpine DM. Dynamic loading and redistribution of the Mcm2-7 helicase complex through the cell cycle. EMBO J 2015; 34: 531-43.

[13] Bruck I and Kaplan DL. The Dbf4-Cdc7 kinase promotes Mcm2-7 ring opening to allow for single-stranded DNA extrusion and helicase assembly. J Biol Chem 2015; 290: 1210-21.

[14] Kimura F, Okayasu I, Kakinuma H, Satoh Y, Kuwao S, Saegusa M, Watanabe J. Differential diagnosis of reactive mesothelial cells and malignant mesothelioma cells using the cell proliferation markers minichromosome mainte-

nance protein 7, geminin, topoisomerase II alpha and Ki-67. Acta Cytol 2013; 57: 384-90.
[15] Tolonen TT, Tammela TL, Kujala PM, Tuominen VJ, Isola JJ, Visakorpi T. Histopathological variables and biomarkers enhancer of zeste homologue 2, Ki-67 and minichromosome maintenance protein 7 as prognosticators in primarily endocrine-treated prostate cancer. BJU Int 2011; 108: 1430-8.
[16] Kimura F, Kawamura J, Watanabe J, Kamoshida S, Kawai K, Okayasu I, Kuwao S. Significance of cell proliferation markers (Minichromosome maintenance protein 7, topoisomerase IIalpha and Ki-67) in cavital fluid cytology: can we differentiate reactive mesothelial cells from malignant cells? Diagn Cytopathol 2010; 38: 161-7.
[17] Numata Y, Ishihara S, Hasegawa N, Nozaki N, Ishimi Y. Interaction of human MCM2-7 proteins with TIM, TIPIN and Rb. J Biochem 2010; 147: 917-27.
[18] Ramer MD, Suman ES, Richter H, Stanger K, Spranger M, Bieberstein N, Duncker BP. Dbf4 and Cdc7 proteins promote DNA replication through interactions with distinct Mcm2-7 protein subunits. J Biol Chem 2013; 288: 14926-35.
[19] Pillaire MJ, Selves J, Gordien K, Gourraud PA, Gentil C, Danjoux M, Do C, Negre V, Bieth A, Guimbaud R, Trouche D, Pasero P, Méchali M, Hoffmann JS, Cazaux C. A 'DNA replication' signature of progression and negative outcome in colorectal cancer. Oncogene 2010; 29: 876-87.
[20] Zhou YM, Zhang XF, Cao L, Li B, Sui CJ, Li YM, Yin ZF. MCM7 expression predicts post-operative prognosis for hepatocellular carcinoma. Liver Int 2012; 32: 1505-9.