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RESEARCH

MCM3 Protein Expression in Follicular and Classical Variants of Papillary Thyroid Carcinoma

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Abstract Minichromosome maintenance (MCM) proteins are needed as licensors in the DNA replication of eukaryotic cells and transcriptional control of MCM genes has critical role in the regulation of MCM functions. Different MCM protein family members are proposed as diagnostic or prognostic markers in various cancers due to their increased proliferative potential. Among MCM family members, minichromosome maintenance protein 3 (MCM3) expressions in both mRNA and protein levels were shown to be associated with papillary thyroid carcinoma (PTC). But, the usability of MCM3 in some histological variants of PTC might be controversial due to tissue specific molecular heterogeneities. In follicular variant of papillary thyroid carcinoma (FVPTC), a number of genes including MCM3 were shown to be differentially expressed which were specific to this kind of variant. Using immunohistochemistry method, MCM3 protein expression levels were compared in FVPTC, classic variant of papillary thyroid carcinoma (CVPTC), and multi-nodular goiter (MNG) tissues in a group of 32 cases. There was meaningful differences between MNG vs. FVPTC ($p = 0.016$) and MNG vs. CVPTC ($p = 0.019$) while there was no significant difference in the comparison FVPTC vs. CVPTC ($p = 0.15$). Four of the 5 CVPTC cases having surrounding tissue invasion had high expression values. For FVPTC and CVPTC, MCM3 protein expression results were parallel to our previous mRNA expression study while there was downregulation in protein expression despite the increased expression of MCM3 mRNA in MNG suggesting tissue-specific post-transcriptional events in benign thyroid neoplasms of which should be focused on. Moreover, the relatively lower MCM3 protein expression in FVPTC comparing to CVPTC could be due to a different tumorigenic pathway favored in this type of tissue.

Keywords Papillary thyroid carcinoma · Follicular variant of papillary thyroid carcinoma · Classic variant of papillary thyroid carcinoma · MCM · MCM3

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Introduction

Thyroid cancer, comprising up to 1 % of all cancer cases, is responsible 0.2 % of cancer deaths in USA [1]. Papillary thyroid carcinoma (PTC) is the most common histological type among the other types which accounts for up to 80 % of the total thyroid cancer cases [2]. Discrimination of histological variants of PTC is important because molecular features which define molecular behavior may differ from classic PTC (CVPTC) [3]. Follicular variant of papillary thyroid carcinoma (FVPTC) is the most common histological variant of PTC with a frequency of 9–22.5 % of all cases [4, 5]. The cytological diagnosis of FVPTC is often difficult because the nuclei of this variant rarely show all of the characteristics of papillary carcinoma [6, 7]. Differential expression of some genes in FVPTC which suggest a distinct molecular pattern for this variant was reported [7, 8].

The minichromosome maintenance (MCM) proteins, having at least six members, are the needed as licensors in the DNA replication of eukaryotic cells [9, 10]. In mammalian cells, transcriptional control of MCM genes has critical role in the regulation of MCM functions [11]. In this respect, MCM proteins are proposed as markers in terms of determination of cell cycle progress for proliferating cells [12]. Accordingly, usage of MCM proteins as proliferation

markers such as Ki-67 protein and proliferating cell nuclear antigen (PCNA) in clinical setting was suggested [12, 13]. The applicability of different MCM protein family members and some cell cycle regulators were tested by different researchers as diagnostic or prognostic markers in various cancers [13–15].

Minichromosome maintenance protein 3 (MCM3) encodes a nuclear protein of 808 amino acids and its expression is upregulated in proliferating cells [16]. Both MCM3 mRNA and protein expression levels were shown to be associated with PTC [8, 13]. However, usability of MCM3 for FVPTC as a proliferation marker might be controversial due to distinct molecular pattern of FVPTC [8].

The aim of this study was to compare the MCM3 protein expression levels in FVPTC and CVPTC where the control group was multi-nodular goiter (MNG) tissues.

Material and Methods

Subjects

A total of 32 cases who were admitted to University of Gaziantep, Sahinbey Research and Training Hospital between the years 2006 and 2009 were included in this study. Formalin-fixed paraffin embed (FFPE) archive materials

Table 1 Clinical and demographic features of the samples

No	Age	Sex	Tissue sample type	Size (cm)	Multiplicity count	Lymph node metastasis	Surrounding tissue invasion	MCM3 protein expression (%)
1	56	F	MNG	Various	−	−	−	3.00
2	16	F	MNG	Various	−	−	−	1.75
3	52	F	MNG	Various	−	−	−	18.00
4	52	F	MNG	Various	−	−	−	0.00
5	54	F	MNG	Various	−	−	−	4.00
6	33	F	MNG	Various	−	−	−	8.00
7	68	F	MNG	Various	−	−	−	5.67
8	49	F	MNG	Various	−	−	−	0.00
9	49	M	MNG	Various	−	−	−	4.75
10	46	M	MNG	Various	−	−	−	0.00
11	41	M	MNG	Various	−	−	−	0.00
12	28	F	FVPTC	1	−	−	−	5.71
13	45	F	FVPTC	3	2	−	−	5.50
14	50	F	FVPTC	4	2	−	−	18.25
15	47	F	FVPTC	5	−	−	−	9.38
16	61	F	FVPTC	1.2	4	−	−	0.50
17	46	F	FVPTC	1.2	−	−	−	15.00
18	24	F	FVPTC	4	−	−	−	4.14
19	29	F	FVPTC	2.8	−	−	−	17.00
20	27	F	FVPTC	2.4	−	−	−	17.50
21	28	F	FVPTC	6	3	−	−	11.00
22	80	F	CVPTC	3	−	+	+	28.75
23	53	M	CVPTC	4.2	2	−	+	28.67
24	77	F	CVPTC	4.5	Multiple	+	+	21.50
25	36	M	CVPTC	2	−	−	−	18.33
26	38	F	CVPTC	2.2	2	−	−	13.00
27	44	F	CVPTC	2.5	2	−	−	0.00
28	33	M	CVPTC	4.5	−	−	−	15.50
29	35	F	CVPTC	1.7	−	−	+	1.00
30	40	F	CVPTC	1.4	4	+	−	1.00
31	81	F	CVPTC	6.5	−	+	+	32.00
32	34	F	CVPTC	1.2	−	−	−	28.00

MNG multi nodular goiter, *FVPTC* follicular variant of papillary thyroid carcinoma, *CVPTC* classical variant of papillary thyroid carcinoma

consisting of 3 subgroups; MNG (n=11), FVPTC (n=10), and CVPTC (n=11) were used in this study (Table 1). Sex distribution of the study population was as 26 females and 6 males while the mean ages for females and males were 45.9 (range 16–81) and 43 (range 33–53), respectively. This study was approved by the local ethical committee in concordance with the declaration of Helsinki.

Immunohistochemical Staining

Immnunohistochemical (IHC) staining was performed by using 4-μm-thick sections on fully automated Leica BOND-MAX (Leica Biosystems, Nussloch, Germany) IHC system according to recommendations of the manufacturer. Pretreatment for antigen retrieval was performed in 10 mM (pH 6.0) citrate buffer. MCM3 antibody (Cell Signaling Technology, Danvers, MA, USA) was used in 1:25 dilution.

Interpretation of the Results

MCM3 protein expression evaluation was based on the percentage of the cells which were stained by antibody (Figure 1). Immunohistochemically stained samples were first visualized at x200 power and then transferred to computer as image files. Cell counting was established on computer by using image files out of 1000 cells in various locations on a slide.

Statistical Analysis

Percentage values of the cells having expression were used in statistics. Statistical analysis was performed using SPSS (v13.0, Chicago, Illinois, USA) statistical software. Study groups were analyzed in terms of differences by using Kruskal-Wallis and Mann–Whitney U-test at $p<0.05$ while p values were two-sided.

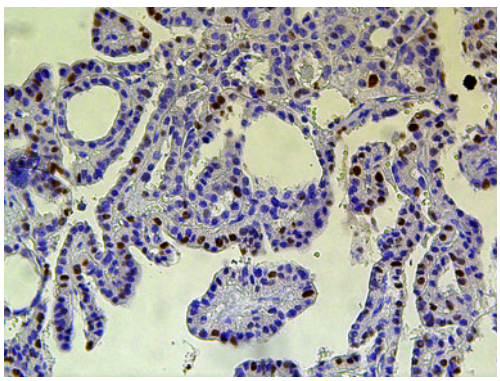

Fig. 1 Nuclear staining of MCM3 in PTC (×200)

Results

There was meaningful difference in the comparisons MNG vs. FVPTC ($p=0.016$) and MNG vs. CVPTC groups ($p=0.019$) while there was no significant difference in the comparison FVPTC vs. CVPTC ($p=0.15$) (Table 2). Although the difference between FVPTC and CVPTC was not statistically significant, the gradually increasing median values (MNG:3 %, FVPTC:10.18 %, CVPTC:18.33%) were prominent (Figure 2). Of the 5 CVPTC cases having surrounding tissue invasion, 4 had high expression values (Table 1).

Discussion

In this study, we have demonstrated that MCM3 protein expression was significantly upregulated in FVPTC and CVPTC when compared to MNG. In our previous study [8], by using differential display-polymerase chain reaction method, we evaluated the gene expression differences between FVPTC, CVPTC, and benign thyroid nodules. In that study, a list of 21 differentially expressed genes were identified including MCM3 gene, and mRNA expression levels of MCM3 gene in FVPTC samples were lower than CVPTC samples while they were detected as upregulated in benign thyroid tissues. The results of our present study are compatible with our previous research regarding FVPTC and CVPTC tissues. However, when the data of our present and previous study for benign tissues were compared the conclusion is interesting; the upregulation of MCM3 mRNA expression detected in benign tissues [8] turned out not to be true for MCM3 protein expression. This difference could have been occurred due to tissue-specific post-transcriptional and/or post-translational events in benign thyroid neoplasms of which should be focused on. Additionally, this difference could be due to mRNA stability-effecting factors [11]. According to our current and previous studies, a partial deregulation for MCM3 in FVPTC comparing to CVPTC is prominent which may not be expected initially by reason of high proliferation rate in carcinomas. This deregulation, in terms of both mRNA and protein expressions, could be also due to the FVPTC-specific molecular mechanisms. This FVPTC-specific molecular biologic pattern and the heterogenic nature of this type of tissue was also pointed out by others [5, 7].

More precise and specific markers in the case of malignancy are strongly demanded [12]. On the other hand, widely-used conventional proliferative indices, Ki-67 and PCNA, may have limited potential in some extent [12]. However, MCM proteins are shown to be more effective than Ki-67 and PCNA in various tissues including larynx, stomach, liver, lung, breast, endometrium, cervix and brain, as well as soft tissue and lymphoma cells [12]. MCM3 is predominantly

Table 2 Statistical analysis results of the Mann–Whitney *U* test

	Total case count	*P*
MNG vs. FVPTC	21	0.016
MNG vs. CVPTC	22	0.019
FVPTC vs. CVPTC	21	0.15

MNG multi nodular goiter, *FVPTC* follicular variant of papillary thyroid carcinoma, *CVPTC* classical variant of papillary thyroid carcinoma

localized to the regions of proliferating cells as Ki-67 [17]. Additionally, MCM3 is found to be upregulated in PTC tissues and showed significant correlation with tumor size and the presence of extrathyroidal extension [13]. Therefore, MCM3 might be a more reliable and sensitive marker in terms of proliferation. However, the usefulness of MCM3 in some PTC subtypes such as FVPTC could be limited due its distinct molecular signature. Despite the small number of sample size, to the best of our knowledge, this is the first study comparing MCM3 protein expression in FVPTC and CVPTC. In our study, we determined relatively lower MCM3 expression in FVPTC comparing to CVPTC. This decrease specific for this type of tissue could be due to a different tumorigenic pathway favored. Although knowledge on molecular basis of thyroid tumorigenesis has considerably grown in recent years, the order of events in PTC tumorigenesis is not clear yet [18]. The signals entered through cell membrane which are directed to cytosolic or nuclear targets are related with proliferation, differentiation, stress response, apoptosis along with cell division. Any kind of functional disorders in this network could cause neoplastic transformation [19, 20]. The decrease in MCM expression is observed in quiescent or differentiating cells [21]. For a tumor, increased MCM expression may be expected. Apparently, this is not completely true for the FVPTC example. The course of FVPTC on the formation of thyroid cancer may be altered [8]. Moreover, MCM3 is not only the cell cycle related protein which was shown to be associated with PTC. In our previous study, SEPT7 gene product which belongs to septin family and which is responsible for kinethocore localization [22] was shown to be

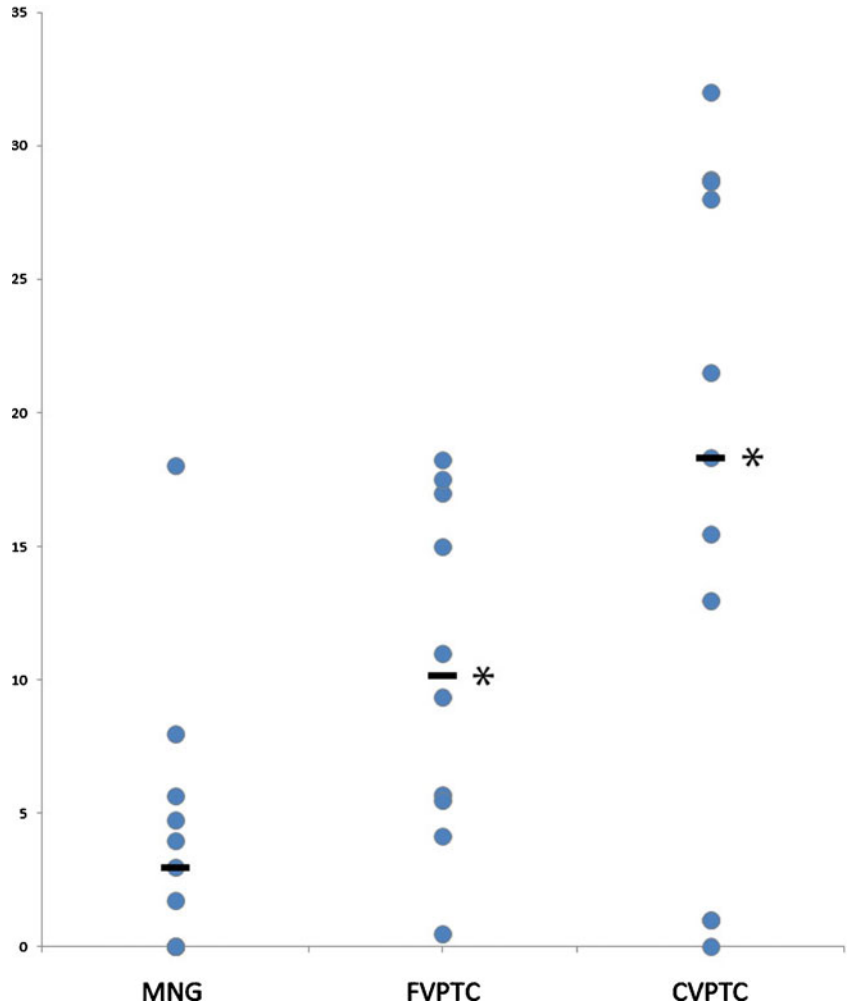

Fig. 2 Expression levels of MCM3 antibody based on percentage of cell count. *$P<0.05$ when compared to MNG

downregulated in FVPTC as MCM3 [8]. In this context, septin family genes may be involved in various steps in PTC tumorigenesis, especially in FVPTC.

Various cell replication-related gene products are shown to be upregulated in cancer cell lines [8, 15]. Among them, MCM proteins have been studied in several types of neoplasia increasingly probably due to its higher expression in malignant tissues other than normal tissues [9]. The main aim of the molecular marker studies for PTC in the literature seems to reduce number of unnecessary surgeries by developing an adjunct diagnostic procedure to conventional histological assessments [15]. Because the construction of study groups is based on conventional histopathological classification of tissues which may not be compatible with the molecular pattern of the tissues, an important percent of studies fail to succeed. Therefore, perfect match of the histopathological classification and molecular the pattern of tissues could be the key for this kind of studies. Further studies which especially include investigation of tissue specific post-transcriptional events for cell-cycle related gene products by using a larger cohort will be helpful in understanding of the roles of MCM3 in thyroid cancer tumorigenesis and its regulation.

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References

1. Jemal A, Siegel R, Xu J, Ward E (2010) Cancer statistics, 2010. CA Cancer J Clin 60:277–300
2. Fagin JA, Mitsiades N (2008) Molecular pathology of thyroid cancer: diagnostic and clinical implications. Best Pract Res Clin Endocrinol Metab 22:955–969
3. Erickson L, Lloyd R (2010) Well-differentiated papillary thyroid carcinoma. In: Hunt J (ed) Molecular pathology of endocrine diseases. Springer, New York, pp 57–71
4. Ertek S, Yilmaz NC, Cicero AF, Vurupalmaz O, Demiroz AS, Erdogan G (2012) Increasing diagnosis of thyroid papillary carcinoma follicular variant in south-east Anatolian region: comparison of characteristics of classical papillary and follicular variant thyroid cancers. Endocr Pathol 23:157–160
5. Liu J, Singh B, Tallini G, Carlson DL, Katabi N, Shaha A, Tuttle RM, Ghossein RA (2006) Follicular variant of papillary thyroid carcinoma: a clinicopathologic study of a problematic entity. Cancer 107:1255–1264
6. LiVolsi VA (2011) Papillary thyroid carcinoma: an update. Mod Pathol 24(Suppl 2):S1–S9
7. Salajegheh A, Petcu EB, Smith RA, Lam AK (2008) Follicular variant of papillary thyroid carcinoma: a diagnostic challenge for clinicians and pathologists. Postgrad Med J 84:78–82
8. Igci YZ, Arslan A, Akarsu E, Erkilic S, Igci M, Oztuzcu S, Cengiz B, Gogebakan B, Cakmak EA, Demiryurek AT (2011) Differential expression of a set of genes in follicular and classic variants of papillary thyroid carcinoma. Endocr Pathol 22:86–96
9. Tye BK (1999) MCM proteins in DNA replication. Annu Rev Biochem 68:649–686
10. Maine GT, Sinha P, Tye BK (1984) Mutants of S. cerevisiae defective in the maintenance of minichromosomes. Genetics 106:365–385
11. Ha SA, Shin SM, Namkoong H, Lee H, Cho GW, Hur SY, Kim TE, Kim JW (2004) Cancer-associated expression of minichromosome maintenance 3 gene in several human cancers and its involvement in tumorigenesis. Clin Cancer Res 10:8386–8395
12. Giaginis C, Vgenopoulou S, Vielh P, Theocharis S (2010) MCM proteins as diagnostic and prognostic tumor markers in the clinical setting. Histol Histopathol 25:351–370
13. Lee YS, Ha SA, Kim HJ, Shin SM, Kim HK, Kim S, Kang CS, Lee KY, Hong OK, Lee SH, Kwon HS, Cha BY, Kim JW (2010) Minichromosome maintenance protein 3 is a candidate proliferation marker in papillary thyroid carcinoma. Exp Mol Pathol 88:138–142
14. Lei M (2005) The MCM complex: its role in DNA replication and implications for cancer therapy. Curr Cancer Drug Targets 5:365–380
15. Melck A, Masoudi H, Griffith OL, Rajput A, Wilkins G, Bugis S, Jones SJ, Wiseman SM (2007) Cell cycle regulators show diagnostic and prognostic utility for differentiated thyroid cancer. Ann Surg Oncol 14:3403–3411
16. Musahl C, Holthoff HP, Lesch R, Knippers R (1998) Stability of the replicative Mcm3 protein in proliferating and differentiating human cells. Exp Cell Res 241:260–264
17. Endl E, Kausch I, Baack M, Knippers R, Gerdes J, Scholzen T (2001) The expression of Ki-67, MCM3, and p27 defines distinct subsets of proliferating, resting, and differentiated cells. J Pathol 195:457–462
18. Zhang P, Zuo H, Ozaki T, Nakagomi N, Kakudo K (2006) Cancer stem cell hypothesis in thyroid cancer. Pathol Int 56:485–489
19. Shibru D, Chung KW, Kebebew E (2008) Recent developments in the clinical application of thyroid cancer biomarkers. Curr Opin Oncol 20:13–18
20. Salabe GB (2001) Pathogenesis of thyroid nodules: histological classification? Biomed Pharmacother 55:39–53
21. Madine MA, Swietlik M, Pelizon C, Romanowski P, Mills AD, Laskey RA (2000) The roles of the MCM, ORC, and Cdc6 proteins in determining the replication. J Struct Biol 129:198–210
22. Zhu M, Wang F, Yan F, Yao PY, Du J, Gao X, Wang X, Wu Q, Ward T, Li J, Kioko S, Hu R, Xie W, Ding X, Yao X (2008) Septin 7 interacts with centromere-associated protein E and is required for its kinetochore localization. J Biol Chem 283:18916–18925