RESEARCH ARTICLE

MCM3 as a Novel Diagnostic Marker in Benign and Malignant Salivary Gland Tumors

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Abstract

Background: Proliferation markers widely have been used to diagnose and determine the behaviour and prognosis of benign and malignant tumours. Minichromosome maintenance 3 (MCM3) is a novel proliferation marker. The aim of this study was to evaluate and compare MCM3 with Ki-67 in diagnosis of salivary gland tumours. Materials and Methods: In this retrospective study, immunohistochemical expression of MCM3 and Ki-67 was evaluated in 15 pleomorphic adenomas (PA), 17 mucoepidermoid carcinomas (MEC) and 18 adenoid cystic carcinomas (ADCC) . Labeling indices (LIs) for the two markers were calculated and compared. Results: MCM3 and Ki-67 LIs were significantly higher in MEC and ADCC compared to PA. The LI of MCM3 was significantly higher than that of Ki-67 in MEC and PA. There was no significant difference between the two markers in ADCC. A cut–off point of 8% with 74.3% sensitivity and 93.3% specificity for MCM3 was obtained to discern between benign and malignant tumors. Conclusions: These results suggest that MCM3 might be a useful proliferation marker for differential diagnosis and recognition of clinical behavior of salivary gland tumors.

Keywords: MCM3 - Ki-67 - immunohistochemistry - salivary gland tumors - benign - malignant

Asian Pacific J Cancer Prev, **14 (6)**, 3479-3482

Introduction

Salivary gland tumors (SGTs) comprise 5% of all head and neck neoplasms (Speight and Barrett, 2004). They show wide spectrum of clinical and histopathological characteristics. Therefore, diagnosis and treatment of SGTs is a challenge to the pathologists and surgeons (Ghazi et al., 2011). PA is the most common benign tumor and accounts for about 60% of SGTs. The malignant tumors that most commonly involve the salivary glands are MEC and ADCC (Ito et al., 2005). Final diagnosis is essentially based on the histopathological findings by hematoxylin-eosin (H&E)-stained sections. However, some tumors demonstrate common histopathological features and subsequently definite diagnosis is very difficult. Therefore, using other pathologic techniques such as Immunohistochemistry (IHC) seems to be useful to discern similar tumors and support histological assessment (Nagao et al.,2012). Proteins involved in cell cycle; play an important role in various biological events such as tumor formation and progression, therefore evaluation of proliferation markers are currently used to predict biological behavior and to differentiate benign from malignant tumors (Freeman et al., 1999). Some studies used cell proliferation markers to estimate the prognosis and outcome of salivary gland tumors. Ki-67 is the most commonly applied proliferation marker which has been used to study of several human malignancies such as soft tissue sarcoma, meningioma, breast cancer and non-Hodgkin's lymphoma (Brown and Gatter, 2002; Pich et al., 2004; Van Diest et al., 2004). Ki-67 is present in the nuclei of cells in the G1, S and G2 phases of the cell cycle of dividing cells, as well as in mitosis. This marker rapidly disappears after mitosis and the half life of the detectable antigen is one hour or less (Nurhan et al., 2001). Despite the biological roles of Ki-67 in cell cycle regulation, the function of this protein remains unclear since some reports suggest that Ki-67 have a role in ribosome biosynthesis instead of cell proliferation (Mac Callum and Hall, 2000). This protein may also be expressed in the cells undergoing apoptosis and also when the DNA-Synthesis is blocked (Van Oijen et al., 1998). Therefore assessment of cell proliferation needs other markers that directly regulate DNA replication.

The minichromosome maintenance (MCM) proteins play a crucial role in initiation and elongation of DNA replication and progression of cell cycle. MCM proteins which are consisted of six members, MCM2 to MCM7, interact with each other at the early stage of DNA synthesis and form a stable hetrohexamer with DNA helicase activity functioning in the DNA replication of eukaryotic cells (Forsburg, 2004; Junhui et al., 2011). Molecular evidence shows that MCM2-7 acts as a factor to ensure the genome is replicated only once in each

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cell cycle (Chevalier and Blow, 1996). The fundamental role of MCM proteins in regulation of DNA replication and its relation to clinicopathological characteristics and prognosis suggest that MCM proteins are a novel class of cellular proliferation markers (de Andrade et al., 2012). The value of MCM proteins as a diagnostic marker has been reported in other human neoplasms (Constantinos et al., 2010). The expression of MCM3 has not yet been studied in benign and malignant salivary gland tumors. As, we don't have any universally accepted other method to discern benign and malignant salivary gland tumors, we designed this study to evaluate MCM3 and Ki-67,as new and current proliferation markers in common salivary gland tumors and appraise their benefit for differential diagnosis.

Materials and Methods

Specimen selection

This retrospective study was performed using fifty formalin-fixed, paraffin embedded tissue blocks of salivary gland tumors which were collected from the Department of Oral Pathology, School of Dentistry, Shiraz University of Medical Sciences. H&E-stained sections were re-evaluated to confirm the diagnosis. There were 17 cases of MEC, 18 ADCC and 15 PA. The control group was 15 sections of the normal salivary gland. The baseline clinical data including the patient's age and gender as well as the site of the tumor were obtained from patients registered medical documents.

Immunohistochemistry

Immunostaining was performed on 4μm paraffin sections. Sections were de-paraffinized with xylene and rehydrated in graded ethyl alcohol. Before staining procedure, samples were immersed in citrate buffer solution (PH=6). Endogenous peroxidase activity was blocked with 3% hydrogen peroxide for 30 minutes. To evaluate the MCM3 and Ki-67 antigen expression, mouse monoclonal antibodies were used (respectively, clone CRCT2.1; 1:50; Novocastra Laboratories, UK and clone MIB-1; 1:100; DAKO, Denmark). Sections were incubated with primary antibody and then were washed in PBS. Finally, secondary antibody associated with Envision System was applied. Sections were washed in PBS again. 3,3diaminobenzidine hydrochloride (DAB) was applied as the chromogen for antibody detection. Sections were counterstained with Mayer's hematoxylin. Negative control was a section of the same sample that primary antibody was replaced by Tris-buffered Saline (TBS). The epithelial layer of normal oral mucosa that has previously shown immunostaining of both markers was considered as positive controls (de Andrade et al., 2012). The percentage of the positive tumor cells out of 1000 tumoral cells at high magnification (x400) was considered the labeling index (LI) of each marker. These cells were counted in five microscopic fields which illustrated more intense staining.

Statistical analysis

Statistical analysis was carried out using SPSS 11software. The data were analyzed by non-parametric Kruscal-Wallis and Mann-Whitney tests. P value<0.05 was considered significant.

Results

Base line data of all patients illustrated in Table 1. Out of 50 cases, 48 were positive for Ki-67 and 49 for MCM3 marker. Ki-67 and MCM3 positive cells were detected as a brown nucleus in epithelial cells of PA, MEC and ADCC. In control group ductal epithelium of only one case of the normal salivary gland was positive for MCM.

Pleomorphic adenoma

Out of 15 cases, one case was negative for MCM3 and two cases revealed no reaction with Ki-67 (Figure 1). MCM3 LI (mean±SD=3.73±2.46) ranged from 0-35%, Ki-67 LI (mean±SD=1.73±1.53) ranged from 0-15% with. The LI of MCM3 was significantly higher than Ki-67(p=0.07).

Mucoepidermoid carcinoma

All cases of MEC were positive for MCM3 and Ki-67 (Figure 2). MCM3 LI in MEC (mean±SD=33.52±4.73)

Table 1. Base Line Date of Patients

Tumors	No. of cases	Gender (Male:Female)	Age (mean)	Site
PA	15	7:08	40.2	8PG,7 MSG
MEC	17	6:11	52.9	8PG, 8MSG, 1 SMG
ADCC	18	6:12	54.5	11 MSG, 4SMG, 3PG,
Total	50	19:31	49.1	19PG, 26 MSG, 5 SMG

*PA:Pleomorphic adenoma, MEC: Mucoepidermoid carcinoma, ADCC:Adenoid cystic carcinoma, PG:Parotid gland, SMG: Sub mandibular gland, MSG:Minor salivary gland

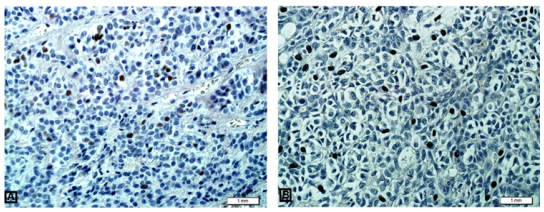

Figure 1. Expression of Ki-67(A) and MCM3 (B) in Cell Nuclie of PA

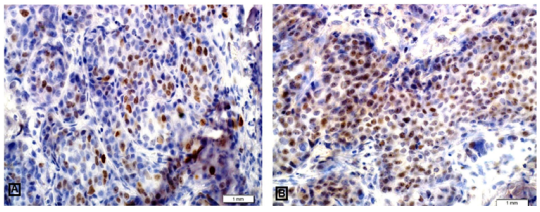

Figure 2. Expression of Ki-67(A) and MCM3 (B) in Cell Nuclie of MEC

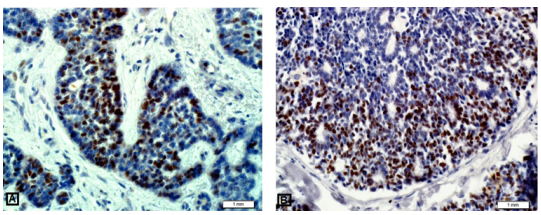

Figure 3. Expression of Ki-67(A) and MCM3 (B) in Cell Nuclie of ADCC

Table 2. LI of MCM3 and Ki-67 and Comparison between MEC, ADCC and PA

	Type of tumor	Mean±SD	Pair wise comparisons*
MCM3 LI	MEC	33.52±24.73	MEC/ADCC (0.126)
	ADCC	22.61±16.84	MEC/PA (0.000)
	PA	3.73±2.46	ADCC/PA (0.000)
Ki-67 LI	MEC	21.82±19.49	MEC/ADCC (0.66)
	ADCC	17.11±10.73	MEC/PA (0.000)
	PA	1.73±1.53	ADCC/PA (0.000)

*Mann-Whitney U test, PA:Pleomorphic adenoma, MEC: Mucoepidermoid carcinoma, ADCC:Adenoid cystic carcinoma

ranged from 1-75% Ki-67 LI (mean±SD=21.89±19.49) ranged from 1-50%. The LI of MCM3 was higher than Ki-67 significantly (p=0.036).

Adenoid cystic carcinoma

All cases of ADCC were positive for MCM3 and Ki-67 (Figure 3). MCM3 LI (mean±SD=22.61±16.84) ranged from 2-70% and Ki-67 LI (mean±SD=17.11±10.73) ranged from 1-40%. The LI of MCM3 in ADCC was higher than Ki-67, but there was no statistically significant difference between them (p=0.091).In solid and tubular pattern of ADCC the LI of MCM3 and Ki-67 was higher than cribriform pattern. We found that MCM3 and Ki-67 were significantly higher in MEC compared to PA (p=0.000). There was also a significantly higher expression in ADCC compared to PA (p=0.000).

Comparison between MEC and ADCC showed no significant difference in expression of MCM3 and Ki-67 (Table2). Cut off point for MCM3 marker was assessed by using the receiver operating characteristic (ROC) curve. We obtained a cut-off point of 8% for MCM3 which gave 74.3% sensitivity and 93.3% specificity to discern between malignant and benign salivary gland tumors.

Discussion

In salivary gland tumors, the gold standard of diagnosis is histopathological examination of H&E stained sections (Speight and Barrett, 2004). However, sometimes IHC using appropriate biologic markers may be particularly useful for definitive diagnosis of similar tumors and predict clinical behavior. Proliferation markers such as Ki-67 have been used currently as a diagnostic and prognostic aid in several cancers. Some studies suggested that MCM proteins could be used as a novel marker for proliferating cells (Alison et al., 2002). Based on the evidence that MCM proteins have a crucial role in regulation of cell-cycle (Alison et al., 2002; Forsburg, 2004), we proposed that MCM3 proteins might represent a useful proliferative and diagnostic marker in salivary gland tumors .We analyzed MCM3 in comparison with Ki-67, in the most common benign and malignant SGTs. Our findings showed that the LI of MCM3 was significantly higher than Ki-67 LI. There was significantly different between MCM3 and Ki-67 in MEC and PA but not in ADCC. Youn et al. in 2010 showed that MCM3 has been expressed in a significantly greater number of cells than Ki-67 in papillary thyroid carcinoma which is in agreement with our results (Youn et al., 2010). In another study in 2008, Vargas et al. have demonstrated the higher expression of MCM2 in comparison with Ki-67 in malignant salivary gland tumors (Vargas et al., 2008). Studies that have been carried out regarding the cancer of bladder, prostate, breast and oral squamous cell carcinoma have also confirmed the higher expression of MCM2 than Ki-67 (Kodani et al., 2001; Meng et al., 2001; Gonzalez et al., 2003; Korkolopoulou et al., 2005). Szelachowska et al. (2006) demonstrated that MCM2 protein predicts better prognosis than Ki-67 in oral cavity squamous cell carcinoma (Szelachowska et al., 2006). Some studies have been reported that MCM2 can predict diagnosis and is a novel prognosis biomarker (Yang et al., 2012; Liu et al., 2013). Our study and previous findings show that MCM3 is a sensitive and reliable proliferative marker in salivary gland tumors. The difference between the expression of MCM3 and Ki-67 may be explained by the different expression of these markers in the cell cycle in which MCM3 expressed during a longer interval of the cell cycle. The expression of MCM3 has been found in the early G1 phase and throughout the whole cell cycle, whereas the expression of Ki-67 was during the late G1 to M phase (Constantinos et al., 2010). Therefore, our findings suggest that a proportion of epithelial tumoral cells which are in a primed replication state can be detected by MCM3. We showed that MEC and ADCC had a significantly greater proliferation activity compared to PA as determined by MCM3 and Ki-67 immunostaining. This result is comparable with a previous study which demonstrated that MCM2 might be a sensitive proliferation marker in malignant salivary gland tumors (Vargas et al., 2008) and another study which showed higher Ki-67 immunoreactivity in malignant salivary gland tumors (Azadeh et al., 2012). Our data revealed no significant difference between MEC and ADCC in expression of MCM3 and Ki-67. In contrast to this finding, Vargas et al. (2008) has reported that ADCC is a highly proliferative salivary gland neoplasm, with higher expression of Ki-67 and MCM2 in comparison with other SGTs. MECs can range from a low grade tumor to a high-grade carcinoma. Furthermore ADCC is a high grade neoplasm with various histopathological features including solid, tubular and cribriform patterns (Agarwal et al., 2008). These histopathological isoforms in a specific tumor affects the grading and clinical course of the tumor. In our study, due to the limited number of carcinomas, the grades of these tumors were not regarded and the absence of significant difference between MEC and ADCC was probably related to this issue. Therefore, a further study with a large sample size, with focusing on various histopathological isoforms of any explicit tumor is suggested. According to the most previous studies there was no different conclusion about the expression of MCM proteins in various tumors (Constantinos et al., 2010). Therefore MCM3 is useful to determine the clinical behavior and prognosis in several tumors and it can be a concern in the differential diagnosis of salivary gland tumors. The Vargas study demonstrated that ADCC had a higher proliferation rate compared to the other salivary gland tumors (Vargas et al., 2008). They obtained a 10% cut-off point to differentiate ADCC and Polymorphous low-grade adenocarcinoma. Their conclusion was almost

similar to our result which showed an 8% cut-off point between malignant and benign tumors. MCM3 and Ki-67 were not expressed in squamous metaplastic areas, keratin pearls and myoepithelial cells. This findings confirm that these markers were not expressed in well differentiated cells. In conclusion, our results suggest that MCM3 might be a novel proliferation marker in salivary gland tumors. Also, MCM3 can be used for differential diagnosis between malignant and benign salivary gland tumors with 8% cut-off point which showed 74.3% sensitivity and 93.3% specificity. Further studies are recommended to determine the usefulness of this marker in the prediction of tumor behavior and its relationship to clinical parameters such as lymph node metastasis.

Acknowledgements

The authors thank the Vice-Chancellery of Shiraz University of Medical Science for supporting this research (Grant#91-01-03-4477). The authors would like to thank Dr Shahram Hamedani (DDS, MSc) from Dental Research development center, who graciously helped us in editing and enhancing the English structure of the manuscript and Dr.Mehrdad Vossoughi for the statistical analysis.

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