Australian Journal of Basic and Applied Sciences, 6(12): 445-452, 2012
ISSN 1991-8178

# Immunohistochemical Evaluation of The Proliferation Marker Mcm-2 In Oral Squamous Cell Carcinoma

[1]Heba N. Shalash, [2]Awatef I. Draz, [3]Dalia H. El-Rouby and [1]Reham A.A. Morsy

[1]Basic Dental Science Res., Dept, National Research Centre, Cairo, Egypt.
[2]Oral pathology Dept., Faculty of Dentistry, King Abdul Aziz University (KSA) and Cairo University (Egypt).
[3]Oral Pathology Dept., Faculty of Oral and Dental Medicine, Cairo University (Egypt).

**Abstract:** Background: The pathogenesis of oral squamous cell carcinoma is very complex and prognostic markers are difficult to find in these carcinomas of which the different subtypes have varying malignant potential. The study was conducted to examine the cellular distribution of MCM-2 in oral squamous cell carcinoma and their value to predict lymph node metastasis. Materials and methods: Thirty paraffin blocks of OSCC (10 well differentiated, 10 moderately differentiated and 10 poorly differentiated) were immunohistochemically stained with MCM-2 antibody. ANOVA and Tukey's tests were used for the statistical analysis, then correlation was performed between lymph node status and the mean percentage of MCM-2 expression. Results: All cases of OSCC express MCM-2 with variable cellular localization. There was a significant difference in the expression of MCM-2 between the different grades of OSCC cases. Using linear correlation analysis, a positive correlation was noted between the percentage of cases with positive lymph node metastasis and the mean area percentage of MCM-2 expression. Conclusions: Oral squamous cell carcinomas express MCM-2 with variable levels and cellular localization, consisting an important marker of biological behavior in these tumors. Further study with large sample size is recommended to assess their value in prediction of lymph node metastasis.

**Key words:** proliferation, MCM-2, oral squamous cell carcinoma

## INTRODUCTION

Cancer is a genetic disease. In order for cells to start dividing uncontrollably, genes that regulate cell growth must be damaged (Vogelstein and Kinzler, 2004). In fact, a series of several mutations to certain classes of genes is usually required before a normal cell will transform into a cancer cell. Only mutations in those certain types of genes that play vital roles in cell division, apoptosis (cell death), and DNA repair will cause a cell to lose control of its proliferation (Croce, 2008).

Around 95% of oral malignancies are oral squamous cell carcinomas (OSCC) (Montoro *et al*., 2008). OSCC is the sixth most common malignancy worldwide, which accounts for approximately 500,000 new cases of oral and pharyngeal cancer that are diagnosed annually and approximately 75% of these tumors occur in the developing world (Moore *et al*., 2000).

Regulation of the cell cycle is complex and involves a wide variety of genes and proteins, among which are the minichromosome maintenance (MCM) proteins that are essential replication initiation factors (Yang *et al*., 2006) playing a vital role in the licensing of the origin replication. The MCM protein family consists of six major isoforms (MCM 2-7), which have similar biochemical functions (Tye, 1999) and are equally important for continuous chromosome replication after the activation of early origins of DNA replication (Labib *et al*., 2000).

As cells exit mitosis, these newly synthesized MCM proteins accumulate in the nucleus (early G1 phase) and assemble into pre-replicative complexes (Labib *et al*., 2001). The nuclear localization of the MCM 2-7 complex is regulated by the cyclin-dependent kinases (CDKs) (Sclafani and Holzen, 2007). MCM 2-7 are imported into the nucleus when CDK activity is low in early G1 and exported from the nucleus during S phase when CDK activity is high (Labib *et al*., 2001).

Since MCM activity is essential for DNA replication in dividing cells and is lost in quiescence (Madine *et al*., 2000), MCMs are obvious markers for proliferation (Kodani *et al*., 2001; Stoeber *et al*., 2001; Forsburg, 2004). Molecular studies suggested that increased levels of MCMs mark not only proliferative malignant cells, but also precancerous cells and the potential for recurrence (Going *et al*., 2002).

In breast cancers, increasing neoplasm grade is associated with increased MCM-2 expression (Fanshawe *et al*., 2005). MCM-2 up-regulation was also observed in esophageal, renal, and lung cancers (Lau *et al*., 2007). MCM-2 and MCM-5 proteins demonstrated increased expression in ovarian adenocarcinomas (Gakiopoulou *et al*., 2007). Significant associations between MCM over-expression and high grade have also been described in prostate (Meng *et al*., 2001), urothelial (Korkolopoulou *et al*., 2005) and renal carcinomas (Rodins *et al*., 2002).

**Corresponding Author:** Heba N. Shalash, Basic Dental Science Res., Dept, National Research Centre, Cairo, Egypt.
E-mail: heba.shalash@hotmail.com daliaelrouby@hotmail.com

Scott *et al*. (2006) stated that in OSCC, there was a very widespread expression of MCM-2. The highest labeling indices LI values were observed in the least differentiated OSCC. They added that high MCM LI values were seen in the surface layers in all the examined cases. Moreover, small clumps of sloughed immunopositive epithelial cells were frequently identified at the surface of OSCC (Scott *et al*., 2006). On the other hand, Szelachowska *et al*. (2006) demonstrated high prognostic value of MCM-2 in oral spinocellular cancer.

The present study aimed to evaluate the expression of MCM-2 in oral squamous cell carcinoma (with different grades) and their value to predict lymph node metastasis.

## MATERIALS AND METHODS

Thirty paraffin blocks of oral squamous cell carcinoma were included in this study, all specimens collected from the archives of the pathological files of Oral Pathology Department, Faculty of Oral and Dental Medicine, Cairo University, General Pathology Department, Faculty of Medicine, Cairo, University and National Cancer Institute, Cairo University. From all specimens, 4 micrometer thick sections were prepared from paraffin blocks and stained with hematoxylin and eosin to confirm the diagnosis. Ten of the cases were diagnosed as well differentiated OSCC, 10 were diagnosed as moderately differentiated and the last 10 cases were diagnosed as poorly differentiated OSCC. Clinical information about lymph node metastasis was obtained from patients' medical records (summary of cases is displayed in table 1).

**Table 1:** Correlation between lymph node status and mean percentage of MCM-2 expression.

| | Total number of cases | Number of cases with positive lymph node metastasis | Number of cases with negative lymph node metastasis | Mean value |
|---|---|---|---|---|
| Well differentiated OSCC group | 10 | 1 | 9 | 11.343 |
| Moderately differentiated OSCC group | 10 | 3 | 7 | 20.712 |
| Poorly differentiated OSCC group | 10 | 4 | 6 | 29.97 |

### *Immunohistochemical Staining:*

Tissues were deparaffinized in xylene and rehydrated, and were transferred for antigen retrieval based on the manufacturers' recommendation. The slides were washed with phosphate buffer and separately incubated with MCM-2 (Lab Vision Corporation, USA) diluted at a ratio of 1:50 over-night. The slides were then washed again in PBS and incubated with biotinylated antibody for 30 minutes, and then were washed in PBS. Finally, the slides were incubated with peroxidase labeled streptavidin for 30 minutes and washed in PBS. Subsequently, DAB chromogen was applied for antibody staining (brown). The samples were stained with Mayer's hematoxylin for 5 minutes, dehydrated and covered with cover glass.

### *Immunohistochemical Evaluation:*

Using the image analyzer computer system, the area percentage of MCM-2 immunoreactivity was evaluated in 5 high power fields (x400) in each slide, and then the mean value was calculated. The image analysis was performed using a computer system (software Leica Quin 500) consisting of color video camera, color monitor, CPU of IBM personal computer connected to the microscope. The image analyzer was first calibrated automatically to convert the measurement units (pixels) produced by the image analyzer program into actual micrometer units.

### *Statistical Analysis:*

All the obtained data from the computer image analysis were given as mean values ± standard deviation (SD) for statistical evaluation. The ANOVA test and Tukey's tests for pairwise comparison were used to compare between the normal control group and the different grades of OSCC. Then, correlation was performed between lymph node status and mean percentage of MCM-2 expression in the different groups of OSCC.

### *Results:*

All cases of OSCC demonstrated positive MCM-2 immunoreactivity with variable degree & site specificity of positivity. Most of the well differentiated cases showed a nuclear MCM-2 reaction, expressed along the periphery of the epithelial cell nests, and at the invasive fronts. On the other hand, the central cores of the cell nests mostly showed negative MCM-2 reaction (fig. 1a).

Few cases showed both nuclear and cytoplasmic MCM-2 reaction, expressed also around the periphery of the epithelial cell nests. A strictly cytoplasmic immunoreaction distributed over the malignant cells arranged in the cell nests was observed in some instances. A single case showed MCM-2 immunostaining along the cell surface membranes of the tumor cells (fig.1b).

Immunostaining of the moderately differentiated cases revealed nuclear MCM-2 reaction with cytoplasmic expression distributed along the periphery of the epithelial cell nests, extending into the suprabasal cells and to the middle thirds of the cell nests, while the central cores showed negative reaction (fig.1c).

In addition, MCM-2 expression was noted at the invasive fronts of the tumor. Moreover, MCM-2 positive immunoreaction was also evident in the nuclei of the small nests of malignant epithelial cells invading the underlying C.T and surrounding numerous dilated blood vessels. Few cases showed strict cytoplasmic expression only distributed over the cells arranged in nests.

Most poorly differentiated cases of OSCC cases showed both positive nuclear and cytoplasmic MCM-2 immunoreaction, the expression was widespread all over the malignant squamous epithelial tissues (fig. 1d).

The highest value for MCM-2 immunoreactivity was recorded in the poorly differentiated squamous cell carcinoma (29.97± 5.677), while the lowest value was recorded in the control (5.009±1.398), (Fig.2, table1). ANOVA test revealed the difference between different grades of squamous cell carcinoma was statistically significant (p=0.000).

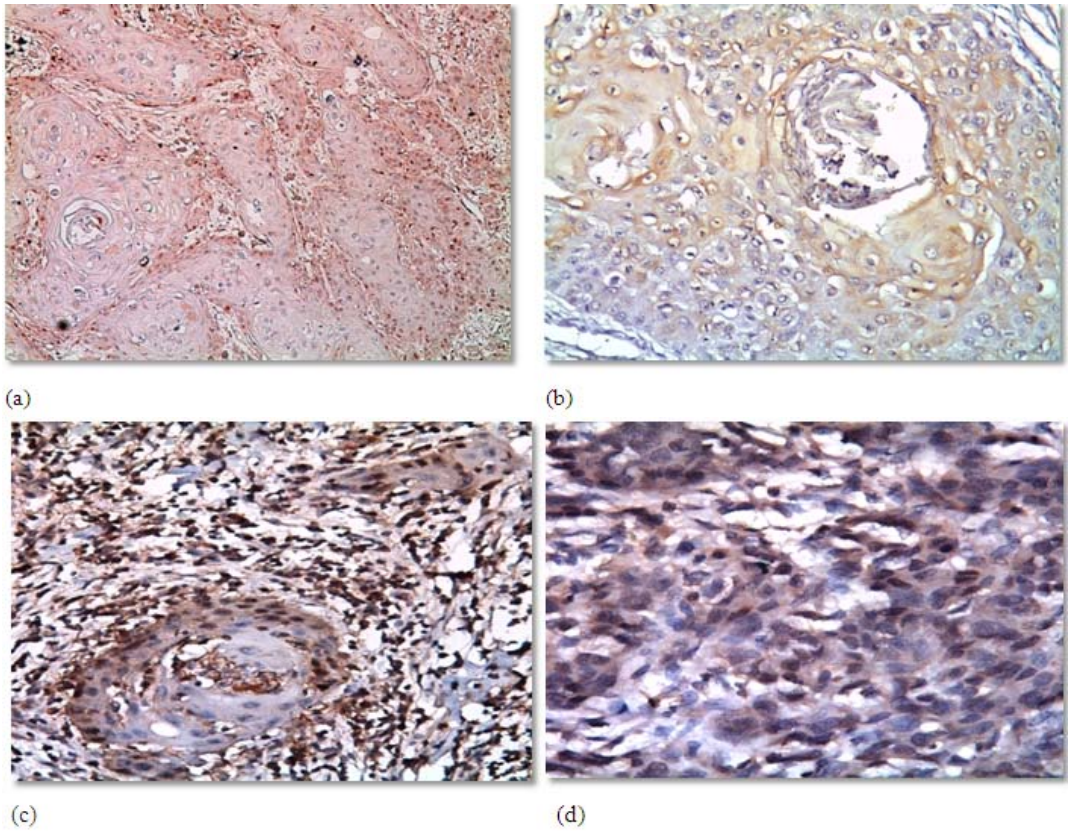


**Fig. 1:** MCM-2 expression patterns in oral squamous cell carcinoma. (a)Photomicrograph of a case of well differentiated OSCC showing positive nuclear MCM-2 reaction along the periphery of cell nests (MCM-2 X100). (b) Photomicrograph of a case of well differentiated OSCC showing positive cytoplasmic and membranous MCM-2 reaction along the borders of the epithelial cells in the cell nests (MCM-2 X200). (c) Photomicrograph of a case of moderately differentiated OSCC showing nuclear and cytoplasmic MCM-2 expression in the basal and some suprabasal cells of the cell nests (MCM-2 X200). (d) Photomicrograph of a poorly differentiated OSCC case showing positive nuclear MCM-2 immunoreaction distributed all over the malignant squamous epithelium. Positive cytoplasmic MCM-2 immunoreaction is also shown in some epithelial cells (MCM-2x400).

Using linear correlation, the percentage of cases with positive lymph nodes and the mean area percentage of MCM2 expression revealed a positive correlation (r=0.983, Fig.3)

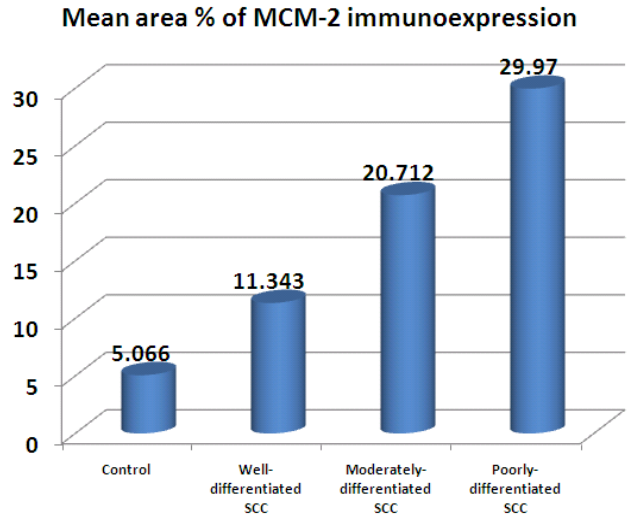


**Fig. 2:** Mean area % of MCM-2 immunoreactivity.

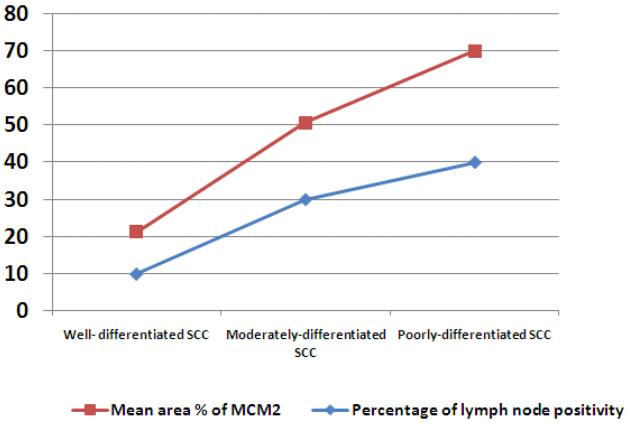


**Fig. 3:** Scatter plot showing correlation between lymph node status and mean percentage of MCM-2 expression.

***Discussion:***

Oral cancer is a major public health issue worldwide and it remains a highly lethal and disfiguring disease (Al-Rawi and Talabani, 2008). Despite considerable advances in the diagnostic and therapeutic techniques (Shaw *et al*., 2009), OSCC continues to present a poor prognosis with an estimated 5-year survival rate of only 56% in the United States and Western Europe (Moore *et al*., 2000).

Recent studies have proposed that MCM proteins may be sensitive proliferation markers and may serve as novel biomarkers for prognostication and diagnosis of various premalignant and malignant lesions (Stoeber *et al*., 2001; Fanshawe *et al*., 2005; Vargas *et al*., 2008).

The superior sensitivity of the MCM proteins over the standard proliferation markers such as Ki-67 resides in the fact that MCMs identify not only cycling cells, but also non-cycling cells with proliferative potential (Stoeber *et al*., 2001). Other proliferation markers in current use, such as proliferating cell nuclear antigen (PCNA) and Ki-67, provide only a limited assessment of cell cycle state (Daidone and Silvestrini, 2001).

In the current study, the immunohistochemical reactivity of MCM-2 in the normal control specimens was expressed mainly in the basal and suprabasal cells of the normal stratified squamous epithelium, with very few reactive cells in the middle third and a totally negative reaction in the superficial third. These results are in accordance with Chatrath *et al*. (2003), Chong-Jin *et al*. (2008). These findings indicate that cell division is confined to the basal and suprabasal cells, whereas the superficial cells have lost their proliferative ability.

In contradiction, Torres-Rendon *et al*. (2010) found that MCM-2 protein was located mainly in the suprabasal compartment only. The absence of MCM-2 expression in a significant proportion of basal cells was explained by assuming that these cells are in a temporary G0 state, as a part of a self-defense mechanism to maintain a controlled cell proliferation of the oral mucosa.

In the present study, MCM-2 was detected in all tissue sections 30/30 (100%) of OSCC (well, moderately and poorly differentiated tumors). Almost similar percentages of MCM-2 expression were previously detected in laryngeal squamous epithelial lesions (Gouvea *et al*., 2010), in epithelial ovarian tumors (Gakiopoulou *et al*., 2007), breast cancers (Gonzalez *et al*., 2003) and colorectal cancer (Guziñska-Ustymowicz *et al*. 2009a). The present finding suggests that MCM-2 may play a significant role in oral carcinogenesis.

In the current study, the positive MCM-2 immunoreaction was detected in the nuclei of the tumor cells in all the grades of OSCC, and this is in accordance with the findings obtained by Kodani *et al*. (2003), Chatrath *et al*. (2006), Torres-Rendon *et al*. (2009). This could be explained by the notion that when cells exit mitosis, these newly synthesized MCM proteins accumulate in the nucleus (early G1 phase) and assemble into pre-replicative complexes (Labib *et al*., 2001).

On the other hand, some cases in the current study showed cytoplasmic MCM-2 expression along with the nuclear one, a finding which is considered by Kodani *et al*. (2003), Chatrath *et al*. (2006), Torres-Rendon *et al*. (2009), Vargas *et al*., (2008), Forsburg, (2004), Ahn and Chang (2010) and Chong-Jin *et al*. (2008) to be non-specific. However, some recent studies noted cytoplasmic reaction, either along with a nuclear expression or exclusively in salivary gland carcinomas and benign and malignant adrenocortical tumors (Ghazy *et al*., 2011) and (Szajerka *et al*., 2008) respectively.

The cytoplasmic localization of MCM-2 could be explained by the fact that in S phase of the cell cycle, nearly the whole amount of MCM proteins dissociate from the chromatin, leaving only a small fraction bound to regions of un-replicated DNA (Labib *et al*., 2001). Subsequently, during G2/M phase, MCM proteins are absent on chromatin and are detectable predominantly in cytoplasm where they later undergo enzymatic degradation (Labib *et al*., 2001).

Few of the cases examined in the current study showed MCM-2 expression along the cell surface membrane of tumor cells, a result that might be explained by the possibility of presence of surface receptors on the cell membrane, however, MCM-2 protein exhibited cytoplasmic or nuclear localizations according to most studies (Forsburg, 2004; Vargas *et al*., 2008; Szajerka *et al*., 2008). However, a similar finding was reported by Going *et al*. (2002) who detected membranous staining of MCM-5 protein which is one member of MCMs protein family that shares many similar structural components with MCM-2 (Forsburg, 2004).

In the well differentiated cases of OSCC, positive nuclear immunoreactions was evident at the periphery of the epithelial cell nests and at the invasive fronts, while the core of the cell nests showed negative reaction. These observations are in accordance with Szelachowska *et al*. (2006), Scott *et al*. (2006) and Gouvea *et al*. (2010), which highlights the active proliferative state of these tumor cells.

Some well differentiated OSCC cases in this study showed both cytoplasmic and nuclear MCM-2 expression, a result that is considered by Kodani *et al*. (2003), Chatrath *et al*. (2006), Torres-Rendon *et al*. (2009), Vargas *et al*., (2008), Forsburg, (2004), Ahn and Chang (2010) and Chong-Jin *et al*. (2008) to be non-specific, which is not in accordance with the present study. Very few cases showed membranous staining of MCM-2 which is in accordance with Going *et al*. (2002) who detected membranous staining of MCM-5 protein in dysplastic squamous oesophageal epithelium and Barrett's mucosa.

As for the moderately differentiated OSCC cases, this study showed that MCM-2 immunoreaction was evident in the peripheral portions of the cancer nests, specifically in the basal and suprabasal cells and extended into the middle thirds of the epithelium, showing stronger intensities at the invasive fronts in some cases, while negative in the central core of keratin pearls. These findings are in accordance with Kodani *et al*. (2003), Chatrath *et al*. (2006), Torres-Rendon *et al*. (2009).

Some of the moderately differentiated OSCC cases included in this study expressed positive MCM-2 immunoreactivity in the cytoplasm only or along with a nuclear expression, and this is in contrast with the findings of Kodani *et al*. (2003), Chatrath *et al*. (2006), Torres-Rendon *et al*. (2009), whose findings revealed strictly nuclear MCM-2 expression. However, other cases in the current study showed strictly nuclear MCM-2 expression, scattered over the cell nests, which is in accordance with Kodani *et al*. (2003), Chatrath *et al*. (2006), Torres-Rendon *et al*. (2009).

The increased MCM-2 expression in the peripheral tumor cells and at the invasive fronts suggests a high rate of cellular proliferation and assists the subsequent invasion of the surrounding structures.

Concerning the poorly differentiated cases of OSCC, the positive immunoreaction of MCM-2 was distributed all over the malignant epithelial cells, indicating the considerable proliferative behavior of OSCC of higher grades in accordance with the findings of Kodani *et al*. (2003), Chatrath *et al*. (2006) and Torres-Rendon *et al*. (2009) who found that the positive immunoexpression of MCM-2 was increased and more distributed among the malignant epithelial cell layers in the lesser differentiated grades of OSCC..

Using computer image analysis, the present study revealed that the MCM-2 positive expression increased with the histological grade of the tumor, where the well differentiated cases showed the least expression (mean=11.343) followed by the moderate cases (mean=20.712), while the poorly differentiated cases showed the highest expression of MCM-2 (mean=29.97) with a highly statistically significant difference (p=0.000). This finding is in accordance with other studies in OSCC (Kodani *et al*., 2003; Chatrath *et al*., 2003; Scott *et al*.,

2006; Torres-Rendon *et al*., 2009), in salivary gland neoplasms (Vargas *et al*., 2008; Ghazy *et al*., 2011) and in non-benign epithelial ovarian tumors (Gakiopoulou *et al*., 2007).

The association between MCM-2 expression and higher tumor grades might be explained by the fact that in cancer, differentiated neoplastic cells tend to grow and spread at a slower rate than undifferentiated or poorly differentiated cells, which lack the structure and function of normal cells and grow uncontrollably (Evan and Vousden, 2001). Therefore, the withdrawal of cells from the cell cycle into differentiated state is coupled with down-regulation of MCM2 expression (Shetty *et al*., 2005). However, these findings are in contrast of those of Ahn and Chang (2010) who noted no significant relationship between MCM-7 expression and the histological grade of esophageal SCC.

This study also noted high LI of MCM-2 in cases with lymph node metastasis and a positive correlation was found between the percentage of cases with positive lymph nodes and the mean area percentage of MCM-2 expression (r=0.983), suggesting that high MCM-2 LIs might predict high rate of cellular proliferation and subsequent invasion of the surrounding structures leading to a poorer prognosis, which is in agreement with Chong-Jin *et al*. (2008), Szelachowska *et al*. (2006) using MCM-7, Vargas *et al*. (2008) and Guzińska-Ustymowicz *et al*. (2009a) in which MCM-2 over expression was associated with lymph node metastasis.

Given the above mentioned findings, it can be suggested that MCM-2 may serve as an important indicator to evaluate the biological behaviors of OSCC such as proliferation and invasion. Furthermore, MCM-2 may be used as potential therapeutic target.

***Conclusion:***

From the current study, it was concluded that MCM-2 protein plays a significant role in the development and progression of OSCC. Moreover, a correlation exists between the expression of MCM-2 and the histological grade of OSCC, with higher MCM-2 expression in poorly differentiated tumors, and a positive correlation exists between the positive lymph node metastasis and the mean area percentage of MCM2 expression. Hence, MCM-2 can be used as a proliferation marker, since its expression is elevated in rapidly dividing cells.

## REFERENCES

Ahn, J.H. and H.K. Chang, 2010. Expression of minichromosome maintenance protein 7 and Smad 4 in squamous cell carcinoma of the esophagus. The Korean Journal of Pathology, 44: 346-353.

Al-Rawi, N.H. and N.G. Talabani, 2008. Squamous cell carcinoma of the oral cavity: a case series analysis of clinical presentation and histological grading of 1,425 cases from Iraq. Clin Oral Invest., 12: 15-18.

Chatrath, P., I.S. Scott, L.S. Morris, R.J. Davies, S.M. Rushbrook, K. Bird, *et al*, 2003. Aberrant expression of minichromosome maintenance protein-2and Ki67 in laryngeal squamous epithelial lesions. Br. J. Cancer., 89: 1048-1054.

Chong-Jin, F., L. Hui-Jun, L. Jian-Na, L. Yong-Jun and L. Gui-Qing, 2008. Expression of Mcm7 and Cdc6 in Oral Squamous Cell Carcinoma and Precancerous Lesions. Anticancer Res., 28: 3763-3770.

Croce, C.M., 2008. Oncogenes and cancer. N. Engl. J. Med., 358(5): 502-11.

Daidone, M.G. and R. Silvestrini, 2001. Prognostic and predictive role of proliferation indices in adjuvant therapy of breast cancer. J. Natl. Cancer Inst. Monogr., 27-35.

Evan, G. and K. Vousden, 2001. Proliferation, cell cycle and apoptosis in cancer. Nature, 17: 342-348.

Fanshawe, T., A. Prevost, R. Sainsbury, G. Williams and K. Stoeber, 2005. DNA replication licensing and cell cycle kinetics of normal and neoplastic breast. Br. J. Cancer, 93: 1295-1300.

Forsburg, S., 2004. Eukaryotic MCM Proteins: Beyond Replication Initiation. Microbiol. Mol. Biol. Rev., 68: 109-131.

Gakiopoulou, H., P. Korkolopoulou, G. Levidou, I. Thymara, A. Saetta, C. Piperi, N. Givalos, I. Vassilopoulos, *et al,* 2007. Minichromosome maintenance proteins 2 and 5 in non-benign epithelial ovarian tumours: relationship with cell cycle regulators and prognostic implications. Br. J. Cancer, 97: 1124-1134.

Ghazy, S.E., I.M. Helmy and H.M. Baghdadi, 2011. Maspin and MCM2 immunoprofiling in salivary gland carcinomas. Diag. Pathol., 6: 89.

Going, J.J., W.N. Keith, L. Neilson, K. Stoeber, R.C. Stuart and G.H. Williams, 2002. Aberrant expression of minichromosome maintenance proteins 2 and 5, and Ki-67 in dysplastic squamous oesophageal epithelium and Barrett's mucosa. Gut., 50: 373-377.

Gonzalez, M.A., S.E. Pinder, G. Callagy, S.L. Vowler, L.S. Morris, K. Bird, J.A. Bell, R.A. Laskey and N. Coleman, 2003. Minichromosome maintenance protein 2 is a strong independent prognostic marker in breast cancer. J. Clin. Oncol., 21: 4306-4313.

Gouvea, A.F., P.A. Vargas, R.D. Coletta, J. Jorge and M.A. Lopez, 2010. Clinicopathological features and immunohistochemical expression of p53, Ki-67, MCM-2 and MCM-5 in proliferative leukoplakia. J. Oral Pathol. Med., 39: 447-452.

Guzi?ska-Ustymowicz, K., A. Pryczynicz, A. Kemona and J. Czy?ewska, 2009a. Correlation between proliferation markers: PCNA, Ki-67, MCM-2 and antiapoptotic protein bcl-2 in colorectal cancer. Anticancer Res., 29: 3049-3052.

Kodani, I., K. Shomori, M. Osaki, I. Kuratate, K. Ryoke and H. Ito, 2001. Expression of minichromosome maintenance 2 (MCM2), Ki-67 and cell cycle- related molecules, and apoptosis in the normal-dysplasia-carcinoma sequence of the oral mucosa. Pathobiology, 69: 150-158.

Kodani, S., M. Osaki, K. Shomori, K. Araki, K. Ryoke *et al,* 2003. Minichromosome maintenance 2 is correlated with mode of invasion and prognosis in oral squamous carcinoma. J. Oral Pathol. Med., 32: 468-74.

Korkolopoulou, P., N. Givalos, A. Saetta, A. Goudopoulou, H. Gakiopoulou, I. Thymara, E. Thomas-Tsagli and E. Patsouris, 2005. Minichromosome maintenance proteins 2 and 5 expression in muscle-invasive urothelial cancer: A multivariate survival study including proliferation markers and cell cycle regulators. Hum. Pathol., 36: 899-907.

Labib, K., J.A. Tercero and J.F. Diffley, 2000. Uninterrupted MCM2-7 function required for DNA replication fork progression. Science, 288: 1643-1647.

Labib, K., S.E. Kearsey and J.F. Diffley, 2001. MCM2-7 proteins are essential components of prereplicative complexes that accumulate cooperatively in the nucleus during G1-phase and are required to establish, but not maintain, the S-phase checkpoint. Mol Biol Cell., 12: 3658-3667.

Lau, E., T. Toshiya, G. Liping, L. Shih-Hsin and J. Wei, 2007. The role of pre-replicative complex (pre-RC) components in oncogenesis. FASEB J., 21: 3786-3794.

Madine, M., M. Swietlik, C. Pelizon, P. Romanowski, A. Mills and R. Laskey, 2000. The roles of the MCM, ORC and Cdc6 proteins in determining the replication competence of chromatin in quiescent cells. J. Struct. Biol., 129: 198-210.

Meng, M.V., G.D. Grossfeld, G.H. Williams, S. Dilworth, K. Stoeber, T.W. Mulley, V. Weinberg, P.R. Carroll and T.D. Tlsty, 2001. Minichromosome maintenance protein 2 expression in prostate: characterization and association with outcome after therapy for cancer. Clin. Cancer Res., 7: 2712-2718.

Montoro, J., H. Hicz, L. De Souza, D. Livingstone, D.H. Melo, R.C. Tiveron and R.C. Mamede, 2008. Prognostic factors in squamous cell carcinoma of the oral cavity. Rev. Bras. Otorrinolaringol., 74(6): 861-6.

Moore, S.R., N.W. Johnson, A.M. Pierce and D.F. Wilson, 2000. The epidemiology of mouth cancer: a review of global incidences. Oral Dis., 6: 65-74.

Rodins, K., K. Cheale, N. Coleman and S.B. Fox, 2002. Minichromosome maintenance protein 2 expression in normal kidney and renal cell carcinomas: relationship to tumor dormancy and potential clinical utility. Clin Cancer Res., 8: 1075-1081.

Sclafani, R.A. and T.M. Holzen, 2007. Cell cycle regulation of DNA replication. Annu. Rev. Genet., 41: 237-280.

Scott, I.S., E. Odell, B. Chatrath, L.S. Morris, R.J. Davies, S.L. Vowler, *et al,* 2006. A minimally invasive immunocytochemical approach to early detection of oral squamous cell carcinoma and dysplasia. J. Path., 201: 187-197.

Shaw, R.J., G. McGlashan, J.A. Woolgar, D. Lowe, J.S. Brown, E.D. Vaughan, *et al,* 2009. Prognostic importance of site in squamous cell carcinoma of the buccal mucosa. Br. J. Oral Maxillofac. Surg., 47: 356-9.

Shetty, A., M. Loddo, T. Fanshawe, A. Prevost, R. Sainsbury, G. Williams and K. Stoeber, 2005. DNA replication licensing and cell cycle kinetics of normal and neoplastic breast. Br. J. Cancer., 93: 1295-1300.

Stoeber, K., T.D. Tlsty, L. Happerfield, G.A. Thomas, S. Romanov, L. Bobrow, E.D. Williams and G.H. Williams, 2001. DNA replication licensing and human cell proliferation. J. Cell Sci., 114: 2027-2041.

Szajerka, A., P. Dziegiel, T. Szajerka, M. Zabel, J. Winowski and Z. Grzebieniak, 2008. Immunohistochemical evaluation of Metallothionein, Mcm-2 and Ki-67 antigen expression in tumors of the adrenal cortex. AntiCancer Res., 28: 2959-2966.

Szelachowska, J., P. Dziegiel, J. Jelen-Krzeszewska, M. Jelen, R. Matkowski, A. Pomiecko, *et al,* 2006. Mcm-2 protein expression predicts prognosis better than Ki-67 antigen in oral cavity squamocellular carcinoma. AntiCancer Res., 26: 2473-2478.

Torres-Rendon, A., S. Roy, G.T. Craig and P.M. Speight, 2009. Expression of Mcm2, geminin and Ki67 in normal oral mucosa, oral epithelial dysplasias and their corresponding squamous-cell carcinomas. Br. J. Cancer., 100: 1128-1134.

Tye, B.K., 1999. MCM proteins in DNA replication. Annu. Rev. Biochem., 68: 649-686.

Vargas, P., W. Cheng, A. Barrett, G. Craig and P. Speight, 2001. Expression of Mcm-2, Ki- 67 and geminin in benign and malignant salivary gland tumors. J Oral Pathol Med (2008); 37: 309-318.Daidone MG and Silvestrini R. Prognostic and predictive role of proliferation indices in adjuvant therapy of breast cancer. J. Natl. Cancer Inst. Monogr., 27-35.

Vogelstein, B. and K.W. Kinzler, 2004. "Cancer genes and the pathways they control". Nature Medicine, 10(8): 789-99.

Yang, J., N. Ramnath, K.B. Moysich, H.L. Asch, H. Swede, S.I. Al-Rawi, I. Huberman, *et al,* 2006. Prognostic significance of MCM2, Ki-67 and gelsolin in non-small lung cancer. BMC Cancer, 6: 203.