Histol Histopathol (2010) 25: 351-370

http://www.hh.um.es

Histology and Histopathology

Cellular and Molecular Biology

Review

MCM proteins as diagnostic and prognostic tumor markers in the clinical setting

Constantinos Giaginis[1], Stephanie Vgenopoulou[1], Philippe Vielh[2] and Stamatios Theocharis[1]

[1]Department of Forensic Medicine and Toxicology, Medical School, University of Athens, Athens, Greece and

[2]Department of Biology and Pathology, Institute Gustave Roussy, Cedex, Villejuif, France

Summary. Minichromosome maintenance (MCM) proteins are essential for the process of DNA replication, functioning as license components for the S-phase of cell-cycle initiation and further exerting weak helicase activity to unwind DNA from its supercoiled state at replication forks. The requirement for MCM proteins in cycling cells and their absence in quiescent ones supports evidence for their potential clinical application as cell proliferation markers. In the last few years, aside from their utility as cell proliferation markers, the assessment of MCM expression levels in diverse human malignancies has been the focus of extensive research in an aim to facilitate tumor diagnosis and prognosis in clinical settings. The present article aims to review the available data so far concerning the clinical significance of MCM protein expression in human neoplasia in comparison to conventional proliferative markers. A review of the literature revealed that MCM expression is associated with important clinicopathological parameters for patient management and also exhibits significant diagnostic and prognostic value in several malignancies. MCMs are characterized by higher specificity and sensitivity than the conventional proliferative markers, such as Ki-67 and PCNA, and are thus considered as diagnostic and prognostic tools of greater clinical significance in several types of human malignancy.

Key words: MCM proteins, Cell proliferation, Diagnosis, Prognosis, Cancer

Offprint requests to: Stamatios Theocharis, MD, PhD, Pathologist Assistant Professor of Forensic Medicine and Toxicology, Medical School, University of Athens, 75 M. Asias str., Goudi, GR11527, Athens, Greece. e-mail: theocharis@ath.forthnet.gr

Introduction

Minichromosome maintenance (MCM) proteins were first recognized in yeast *Saccharomyces cerevisiae* as temperature-sensitive mutants defective in the maintenance of minichromosomes, which play a crucial role in plasmid replication and cell cycle progression (Tye, 1999). To date, six main highly conserved DNA-binding members (MCM-2 to -7) have been well documented to interact with each other, forming a heterohexamer complex (Bell and Dutta, 2002). The MCM protein complex is associated with the origins of DNA replication to form part of the pre-replicative complex (preRC). Activation of MCM complex by cyclin-dependent kinases, such as Cdc6, Cdt1 and Dbf4/Cdc7, leads to initiation of DNA synthesis (Maiorano et al., 2006). In fact, MCM proteins are considered to function as licensing components for the S-phase of cell-cycle initiation (Takisawa et al., 2000; Laskey and Madine, 2003). They are tightly bound to chromatin in late mitosis and G1, while being removed in the S and G2 phases. Once DNA replication is completed and all MCM proteins have been displaced from chromatin, they remain as a soluble nuclear pool during the G2 phase and early mitosis (Tachibana et al., 2005). This regulation allows the control of replication origin firing in order to restrict the chromosome replication to only one round per cell-cycle (Romanowski and Madine, 1997). MCM proteins also have another crucial function during the replication process, as they exert weak helicase activity by binding to chromatin long before the initiation step of DNA synthesis in order to unwind DNA from its supercoiled state at replication forks. This specific action gives rise to the replication machinery to contribute to DNA synthesis (Tachibana et al., 2005).

Additional members of the MCM family have

recently been identified, although their role in DNA replication has not to date been fully clarified. Among them, MCM-1 belongs to the MADS box transcription factor family, which interacts with several cofactors to bind their cognate DNA sequences cooperatively. The direct involvement of MCM-1 in DNA replication includes its binding to multiple sites of yeast autonomously replicating sequences (ARSs), as well as stimulation of ARSs activity by MCM-1 binding (Chang et al., 2003, 2004). Another MCM member, MCM-8, seems to associate with chromatin at the onset of the S-phase and is considered to exert ATPase and DNA helicase activity, stimulating DNA synthesis independently of the MCM-2 to MCM-7 complex (Maiorano et al., 2005). Moreover, the MCM-9 member was identified by searching for MCM-2 to MCM-8 like proteins in the sequence databases. However, its role remains to be clarified (Lutzmann et al., 2005). The last member, MCM-10 may have a dual function, first to stabilize DNA polymerase-α-primase and second to target it to chromatin (Ricke and Bielinsky, 2004).

DNA replication is an essential process for the viability of cells, and mutations in any of the steps involved in such a process may prove to be lethal. Moreover, proteins involved in cellular responses to stalled replication forks are generally not essential for cell viability, however, their absence may result in the sensitivity of various fork blocking agents or may induce genomic instability due to loss of DNA replication checkpoint controls. In this aspect, it is well established that the proliferative capacity of tumor cells constitutes an essential feature of proliferating tumors that often renders clinical diagnosis and/or prognosis more objective and informatively relevant.

Many replication proteins have been shown to be overexpressed in transformed or cancer cell lines compared to normal cells, which render them potentially important biomarkers for routine clinical applications in cancer diagnosis and prognosis. Among them, two conventional proliferative indices, Ki-67 and PCNA, have been widely used as tumor cell proliferation markers. However, both have frequently proven to be limited with respect to their ability to ascertain patient diagnosis and/or prognosis. More to the point, a significant setback in the application of Ki-67 as a proliferation marker is that its precise function remains unknown despite the high number of speculations about its possible biological roles in cell-cycle regulation and protein biosynthesis (Brown and Gatter, 2002; Mehrotra et al., 2006). Another drawback of Ki-67 labeling index (LI) is that its immunostaining is affected by external factors such as nutrient deprivation, which could subsequently underestimate the number of cells in cycle (Bainsh and Gerdes, 1987; Mehrotra et al., 2006). Moreover, 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, but not in the G0 and early G1 phase of quiescent cells. Importantly, Ki-67 shows variations in its expression in the G1 phase and may not be expressed in cells entering the G1 from the G0 phase. It has also been suggested that Ki-67 plays a role in ribosome biosynthesis rather than being directly responsible for cell proliferation (MacCallum and Hall, 2000). Another widely used marker, PCNA, has been proven less specific to express the proliferation state of cells, as in addition to its role in DNA replication it is also required for DNA repair, being an auxiliary factor for DNA polymerase δ (Toshi and Bravo, 1988). Moreover, PCNA presents a long term t1/2 being expressed in post division sister cells (Theocharis et al., 1994). Recently, geminin, which functions as a protector of genome stability by preventing the untimely binding of MCM complex to chromatin during the S phase, the G2 phase and early mitosis, has been identified as a novel tumor cell proliferation marker. However, geminin expression was shown to be restricted to S, G2 and early M cell-cycle phase (Tachibana et al., 2005). In light of the above considerations, there is still strong demand for more specific and precise markers related to the cell-cycle 'state' of cells in different tissues, especially in the case of malignancy.

In this context, the assessment of MCM expression levels in diverse human malignancies has recently been the focus of extensive research in an attempt to facilitate tumor diagnosis and prognosis in clinical settings. The requirement for MCM proteins in cycling cells, but their absence in quiescent ones, supports strong evidence for their potential clinical application as cell proliferation markers. Moreover, MCM proteins were shown to be more frequently expressed in cells of several malignant tissues than those of normal ones. A similar observation was also reported in cells presenting dysplasia in normal and malignant tissues (Freeman et al., 1999). Substantial clinical evidence in several types of neoplasia also suggested that antibodies against MCM proteins identified a greater number of cells in cycle than those against PCNA and Ki-67 (Toshi and Bravo, 1988; Ha et al., 2004). Aside from their utility as cell proliferation markers, MCM proteins also constitute diagnostic and prognostic tools of great clinical significance in several human malignancies.

In the last few years, MCM protein expression has been widely studied in several types of neoplasia in relation to important clinicopathological characteristics for patient management and survival. In this aspect, we aimed to review the available data to date, concerning the clinical significance of MCM protein expression in different types of malignancy, in comparison to the conventional proliferative markers.

MCM expression in human malignancy

Based on the evidence that MCM proteins play a crucial role in the control of cell-cycle regulation, several studies were conducted to evaluate the significance of their expression *in vivo*, in relation to clinicopathological characteristics and patient prognosis. A significant number of existing studies also compared

the expression of MCM proteins to that of certain conventional proliferation indices, such as Ki-67 and PCNA with respect to their diagnostic and prognostic value. The available data concerning the clinical significance of MCMs protein expression in various human malignancies are discussed in the following sections and are summarized in Tables 1-3.

Head and neck neoplasia

Both MCM-2 and Ki-67 LIs showed increased expression from the transformation of normal laryngeal epithelium through dysplasia to squamous cell carcinoma (SCC) (Chatrath et al., 2003; Szelachowska et al., 2006). A significant correlation was noted between MCM-2 and Ki-67 LIs, but MCM-2 expression was consistently higher than Ki-67 in paraffin-embedded tissue sections of normal larynx, laryngeal dysplasia and laryngeal SCC (Chatrath et al., 2003, 2006; Kato et al., 2003b; Szelachowska et al., 2006). Thus, MCM-2 proved to be a more reliable and useful marker than Ki-67 in assessing the growth of normal and malignant cells and in evaluating tumor aggressiveness of esophageal SCC (Kato et al., 2003a). On the other hand, MCM-2 LI did not correlate with p53 expression, suggesting that MCM-2 may be regulated via a p53-independent pathway (Kodani et al., 2001). Kodani et al. also showed significantly higher MCM-2, Ki-67 and p53 LIs in oral SCC compared to dysplasia. Thirteen dysplasia cases which progressed to SCC were characterized by significantly higher MCM-2 levels than the other counterparts (Kodani et al., 2001). In another study, all

Table 1. Associations of MCM proteins expression with clinicopathological parameters and patients' survival in head and neck, thyroid, gastric, colon and liver malignant tumors.

Type of neoplasia	Sample Size	Age	gender	Grade	Stage	pT	pN	pM	Type	Proliferation markers	Survival	References
Head and Neck												
MCM-2	40			+						+(Ki-67)		Chatrath et al., 2003
	49			-	-		-			+(Ki-67)	+	Szelachowska et al., 2006
	38			+						+(Ki-67)		Chatrath et al., 2006
	93		-	+	+	+	+	+		+(Ki-67)	±	Kato et al., 2003a
	116											Kodani et al., 2001
	46			+				-			+	Kodani et al., 2003
	62			+								Sirieix et al., 2003
	62	-	-	-		-					-	Vargas et al., 2008
MCM-4	60	-	-	-		+	-					Huang et al., 2005
Thyroid												
MCM-2	128											Mehrotra et al., 2006
	42			-						-(Ki-67)		Cho Mar et al., 2006
MCM-5	52									+(PCNA)		Guida et al., 2005
	95	-	-		-	-	-					Kebebew et al., 2006
MCM-7	52									+(PCNA)		Guida et al., 2005
MCM-7	95	-	-		-	+	-					Kebebew et al., 2006
Gastric												
MCM-2	103	-	-			-	-			+(Ki-67)	+(diffuse)-(intestinal)	Tokuyasu et al., 2008
	277					+				+(Ki-67)	±	Huang et al., 2006
Colon												
MCM-2	75									+(Ki-67)+(cyclin A)		Scott et al., 2003
	55	-					+			+(Ki-67)+(PCNA)		Guzi*f*ska-Ustymowicz et al., 2008
	96	-	-	+	+	-	+			+(Ki-67)		Giaginis et al., 2009
MCM-5	96	-	-	-	-	-	-			+(Ki-67)		Giaginis et al., 2009
Liver												
MCM-2		-	-		+							Freeman et al., 2003
	95	-	-									Marshall et al., 2005b

cells expressing phase markers of cell-cycle progression, such as cyclin D1, A and B1, co-expressed MCM-2, whereas Ki-67 was not expressed in a proportion of these cells (Chatrath et al., 2006).

In addition, it was shown that clusters of MCM-2 and MCM-5-positive cells were present in cytological preparations from SCC, but not in those presenting atypical hyperplasia or inflammation in non-neoplastic tissues. These findings suggested that, based on the results for liquid-based cytology enhanced by immunohistochemistry for MCM-2 and -5, laryngeal SCC patients could be separated into those requiring further investigation and those who could be followed-up without resort to biopsy (Chatrath et al., 2003). Accordingly, Scott et al. showed that MCM-2 was more frequently expressed in the surface layers of oral moderate or severe dysplasia and SCC compared to benign keratosis/mild dysplasia (Scott et al., 2006). MCM-2 was found to be a more sensitive marker for both histological and cytological diagnosis of oral malignancy and dysplasia than Ki-67. According to this study, cytological data were fully consistent with histopathological findings, indicating that MCM-2 immunocytochemistry could be an important clinical practice in some settings, including their use in developing countries, for early detection of oral SCC and dysplasia (Scott et al., 2006).

A comparison of MCM-2 LI and clinicopathological characteristics in 93 patients with esophageal SCC revealed significant associations between MCM-2 LI

Table 2. Associations of MCM proteins expression with clinicopathological parameters and patients' survival in lung, breast, ovarian, endometrial, cervical, renal, prostate and urothelial malignant tumors.

Type of neoplasia	Sample Size	Age	gender	Grade	Stage	pT	pN	pM	Type	Proliferation markers	Survival	References
Lung												
MCM-2	221		-	-	-				+	+(Ki-67)	+	Ramnath et al., 2001
	128		+	+	-	-	-		+		+	Yang et al., 2006
	145		+	+	+				+	+(Ki-67)	+	Hashimoto et al., 2004
Breast												
MCM-2	56	-		+		+	-	+		+(Ki-67)	+	Gonzalez et al., 2003
	120	-		+		-	-			+(Ki-67)		Shetty et al., 2005
Ovarian												
MCM-2	85			+	+				-	+(Ki-67)+(p27(Kip-1))	+	Gakiopoulou et al., 2007
	85			+								Scott et al., 2004
MCM-5	85			+	+				+	+(Ki-67) +(p27(Kip-1))	+	Gakiopoulou et al., 2007
Endometrial												
MCM-2	92									+(Ki-67)		Kato et al., 2003b
MCM-3	92									-(Ki-67)		Kato et al., 2003b
MCM-7	212	+		+	-		-	-	-	+(Ki-67)	+	Li et al., 2005
Cervival												
MCM-5	77			+								Murphy et al., 2005
RCC												
MCM-2	66	-	-	+	-	-				+(Ki-67)	-	Rodins et al., 2002
	176	-	-	+		+			-		+	Dudderidge et al., 2005
Prostate												
MCM-2	92			-	-		-				+	Meng et al., 2001
	58			+		-		+			+	Dudderidge et al., 2007
MCM-7	79			-	+					+(Ki-67)		Padmanabhan et al., 2004
	249	-				+				+(Ki-67)	+	Laitinen et al., 2008
Urothelial												
MCM-2	71										+	Burger et al., 2007
	44	-	±	+						±(Ki-67)	±	Krüger et al., 2003
	65			+	+				+	+(Ki-67)	+	Korkolopoulou et al., 2005
MCM-5	65			+	+				+	+(Ki-67)	+	Korkolopoulou et al., 2005

and tumor size, lymph node and organ metastatic status, as well as tumor histopathological stage and grade (Kato et al., 2003a). Kodani et al. further reported that MCM-2 LI was significantly higher in the moderately compared to well differentiated SCCs, being also associated with the mode of carcinoma invasion (Kodani et al., 2003). In contrast to the previous studies, Szelachowska et al. did not find significant associations between MCM-2 LI and tumor histopathological stage and grade, nor lymph node metastasis, which may be ascribed to the considerably lower number of cases and the different MCM-2 expression definition (Szelachowska et al., 2006).

Concerning the prognostic value of MCM-2 expression, the survival rates of patients with high MCM-2 LI tumors were significantly lower than those with low MCM-2 LI. However, MCM-2 expression was not identified as an independent prognostic factor in multivariate analysis (Kato et al., 2003a). Kodani at al. also reported that MCM-2, but not Ki-67, apoptotic index, p53, p27 and p21 LIs, was correlated with patient prognosis (Kodani et al., 2003). Accordingly, Szelachowska et al. reported a significant association with a decreased disease-free survival period in the group of patients presenting over 10% of cancer cells positive for MCM-2 protein, whereas no effects of Ki-67 on patient survival was noted. In contrast to the previous studies, MCM-2 expression was identified as an independent risk factor for poor patient survival (Szelachowska et al., 2006), which may be ascribed to the different cut-off point for MCM-2 expression definition compared to that proposed by Kato et al. (62.7%) and Kodani et al. (40%).

Williams et al. performed an immunofluorometric assay to detect MCM-5 levels in cells isolated from gastric aspirates of 40 patients undergoing gastroscopy for suspected or known esophageal carcinoma or symptoms of dyspepsia. The test discriminated, with high specificity and sensitivity, between patients with

Table 3. Associations of MCM proteins expression with clinicopathological parameters and patients' survival in neurological, skin, hematological and soft tissue malignant neoplasia.

Type of neoplasia	Sample Size	Age	gender	Grade	Stage	Type	Proliferation markers	Survival	References
Neurological									
Oligodendrogliomas									
MCM-2	32			+			+(Ki-67)	±	Whartonet al.,. 2001
Gliomas									
MCM-2	48			+			+(Ki-67), +(cyclin A, B1)	-	Scott et al., 2005
Meningioma									
MCM-2	30					-	-(Ki-67)	±	Hunt et al., 2002
Astocytomas									
MCM-2	66			+					Facoetti et al., 2006
MCM-3	169	+	-	+			+(Ki-67)	+	Söling et al., 2005
Craniopharyngiomas									
MCM-6	60	-	-				+(DNA Topo II alpha)	+	Xu et al., 2007
Skin									
MCM-5	110			+					Liu et al., 2007
MCM-7	51						+(Ki-67), -(PCNA), -(p21)		Gambichler et al., 2008
Hematological									
MCM-2	110	-	-		-			±	Obermann et al., 2005
MCM-6	70	-	-		-			+	Schrader et al., 2005
Soft tissue									
Histiocytomas									
MCM-2	38						+(Ki-67)		Osaki et al., 2002
Chondrosarcomas									
MCM-6	31			+				+	Helfenstein et al., 2004
Myxofibrosarcomas									
MCM-2	51			+			+(Ki-67)	+	Sington et al., 2004
Soft tissues/ bone tumors									
MCM-2	41					-	-(Ki-67)	+	Matsubara et al., 2008

and without esophageal cancer, suggesting that elevated MCM-5 levels in gastric aspirates presented high predictive value for esophageal cancer (Williams et al., 2004). Given the magnitude in the difference between MCM-5 levels in benign and malignant disease found in this study, it was assumed that even small lesions at an early stage would be detected. However, additional data involving a large number of unselected cases is warranted to determine whether this novel diagnostic approach can be exploited as a screening tool to detect early curable tumors (Williams et al., 2004).

In non-dysplastic squamous epithelium and Barrett's mucosa, high MCM-2, MCM-5, and Ki-67 protein expression was largely confined to the proliferative layers and down-regulated in differentiated areas. Expression persisted to the mucosal surface in dysplastic squamous epithelium and Barrett's mucosa. Thus, the persistent expression of MCM-2, MCM-5, and Ki-67 proteins in luminal compartments of dysplastic esophageal squamous epithelium and dysplastic Barrett's mucosa may be diagnostic markers, implying disruption of cell-cycle control and differentiation in these dysplastic epithelia (Going et al., 2002). In this context, Sirieix et al. also showed that MCM-2 was not expressed on the luminal surface of normal squamous esophageal, gastric antrum and duodenal epithelial cells (Sirieix et al., 2003). In Barrett's esophagus, the percentage of surface cells expressing MCM-2 was highly correlated with the degree of dysplasia. In patients who developed esophageal adenocarcinoma, biopsies with prior dysplasia presented higher MCM-2 expression than the matched control patients. In the prospective cohort, the histopathological diagnosis of dysplasia or esophageal adenocarcinoma and the cytological MCM-2-positive brushings were concordant in 91% of the patients, and the results correlated with the frequency of cases with surface expression of MCM-2. Thus, it was assumed that MCM-2 expression could be used to detect dysplasia and esophageal adenocarcinoma, as well as patients with Barrett's esophagus at risk of subsequent development of dysplasia and esophageal adenocarcinoma. Moreover, a cytological brushing technique combined with MCM-2 immunohistochemical staining may be potentially exploited in surveillance and screening protocols (Sirieix et al., 2003).

In another study, Vargas et al. immunohistochemically assessed the expression of MCM-2, Ki-67 and geminin in malignant salivary gland tumors in order to examine their usefulness in the diagnosis and prediction of tumor behavior (Vargas et al., 2008). MCM-2 expression was higher than Ki-67 and geminin in all cases. In addition, MCM-2 LI was higher in adenoid cystic carcinomas than in carcinoma ex pleomorphic adenomas, acinic cell and mucoepidermoid carcinomas, pleomorphic adenomas and polymorphous low-grade adenocarcinomas. MCM-2 LI was also higher in carcinoma ex pleomorphic than in pleomorphic adenomas. MCM-2 LI was not associated with tumor histopathological grade or patient outcome. These findings suggested that MCM-2 may be a sensitive proliferation marker in malignant salivary gland tumors and may prove useful in the differential diagnosis between pleomorphic adenomas and carcinoma ex pleomorphic adenomas, and adenoid cystic carcinomas and polymorphous low-grade adenocarcinomas. However, no proliferation marker was associated with patient age and gender, nor tumor size and site of involvement (Vargas et al., 2008).

Concerning other members of the MCM family, MCM-4 expression was assessed by RT-PCR in 60 esophageal cancer tissue samples obtained from Chinese patients (Huang et al., 2005). This study revealed increased MCM-4 expression in 65% of carcinoma cases when compared to normal esophageal epithelia where weak or no MCM-4 expression was detected. In 33% of the cases, increased MCM-4 expression was noted in the adjacent epithelia, but MCM-4 expression in esophageal carcinomas was significantly higher. Significantly different MCM-4 expression was found in patients with histopathological stage T3compared to T1, whereas no such associations with patient age and gender, tumor histopathological grade and lymph node metastasis were noted (Huang et al., 2005). In another study, Endl et al. also evaluated the expression pattern of MCM-3, p27 and Ki-67 proteins on germinal centers and oral mucosa, which display a well-defined spatio-temporal organization. The expression of p27 protein was closely related to differentiated cells, whereas MCM-3 and Ki-67 were predominantly localized in the regions of proliferating cells. Considerable numbers of cells that were growth-arrested, as confirmed by the absence of the Ki-67 protein, stained positive for MCM-3 protein. These results were confirmed in vitro using growth-arrested Swiss 3T3 cells. MCM-3 protein was expressed in cells that had abandoned the proliferation phase, but were not terminally differentiated, according to the absence of p27 protein expression. Thus, it was assumed that a combined analysis of Ki-67, MCM-3, and p27 protein expression could provide a more detailed insight into cell proliferation and differentiation processes that determine individual tumour growth (Endl et al., 2001).

Thyroid neoplasia

Both MCM-2 and Ki-67 expression was significantly higher in follicular and papillary thyroid carcinomas than in follicular adenomas or dominant nodules. However, Ki-67 LI better discriminated between follicular carcinomas and adenomas than MCM-2. In addition, both MCM-2 and Ki-67 LIs widely overlapped between the four histological groups, and the expression of these proteins was found to be heterogenous within these lesions (Mehrotra et al., 2006). On the other hand, Cho et al. reported that MCM-2, but not Ki-67 expression, was significantly higher in minimally invasive follicular carcinomas than in adenomas. Moreover, when follicular carcinomas were classified according to the presence or absence of

capsular and vascular invasion, a significant difference in MCM-2 LI, but not in Ki-67 LI, was noted (Cho et al., 2006).

Concerning other members of the MCM family, Guida et al. revealed, by immunohistochemistry, an up-regulation of MCM-5 and MCM-7 expression in anaplastic thyroid cancer, but not in normal thyroid tissue or papillary thyroid cancer (Guida et al., 2005). Both MCM-5 and MCM -7 LIs were significantly associated with PCNA LI in anaplastic thyroid carcinoma. In the same study, an analysis of human anaplastic thyroid cancer primary cell cultures and a transgenic mouse model of anaplastic thyroid carcinoma further confirmed these findings. In addition, an increased transcription rate was also established for MCM-7 up-regulation, as the activity of the MCM-7 promoter was more than 10-fold higher in anaplastic thyroid carcinoma compared to normal thyroid cells. Adoptive overexpression of wild-type p53, but not of its inactive (R248W and R273H) mutants, strongly down-regulated transcription from the MCM-7 promoter, suggesting that p53 knock-out may be implicated in MCM-7 up-regulation (Guida et al., 2005). In contrast to the previous study, Kebebew et al. demonstrated by RT-PCR that MCM-5 and MCM-7 were up-regulated in papillary thyroid carcinoma, the follicular variant of papillary thyroid carcinoma, and follicular thyroid cancers, but not in hyperplastic nodules and follicular adenomas (Kebebew et al., 2006). This discrepancy may be attributed to the use of RT-PCR to evaluate MCM-5 and MCM-7 mRNA expression instead of immunohistochemistry, which is a semi-quantitative method for protein expression assessment. In this study, it was also shown that MCM-7, but not MCM-5 mRNA expression was significantly higher in T4 than in T1-3 differentiated thyroid tumors, whereas neither MCM-5 nor MCM -7 were associated with patient age and gender or tumor histopathological stage and lymph node metastasis (Kebebew et al., 2006).

Gastric neoplasia

Both MCM-2 and Ki-67 LIs were significantly higher in intestinal than in diffuse type stage III gastric carcinoma cases (Tokuyasu et al., 2008). Moreover, MCM-2 LI was significantly higher than Ki-67 LI in both histological types of gastric carcinoma. However, no significant associations of MCM-2 and Ki-67 LIs with clinicopathological parameters, such as patient age and gender, tumor size, lymph node metastasis, vascular and lymphatic invasion or p53 expression were noted. Diffuse-type carcinomas with high MCM-2 LI were significantly associated with shorter patient survival in comparison to those with low MCM-2 LI. Conversely, no such relationship was found for MCM-2 in intestinal-type or for Ki-67 in either intestinal- or diffuse-type gastric carcinoma patients (Tokuyasu et al., 2008).

In a tissue microarray (TMA) based immunohistochemical study of 277 gastrointestinal stromal tumors, the increment of American National Institutes of Health risk levels significantly correlated with increasing Ki-67 and MCM-2 LIs (Huang et al., 2006). The relationship between MCM-2 and Ki-67 LIs was modeled as linear, while MCM-2 LI was considerably higher with a stepwise escalation related to risk levels. Both MCM-2 and Ki-67 were found to correlate positively with tumor size and mitotic activity. In multivariate analysis, Ki-67 and MCM-2 LIs were strongly predictive of shorter disease-specific survival. However, MCM-2 was not as predictive as Ki-67 in multivariate analysis. Although Ki-67 LI was an independent prognostic indicator, simultaneous detection of MCM-2 was recommended as a prognostic adjunct for gastrointestinal stromal tumors, given its better sensitivity and stepwise escalation with increasing risk levels (Huang et al., 2006).

Colorectal neoplasia

MCM-2 protein was detected in 37 of 40 colorectal carcinoma patients, but in none of 25 healthy controls (Davies et al., 2002). MCM-2 protein was present in the cell nucleus, as it is essential for DNA replication during mitosis in the colonic epithelium (Davies et al., 2002). In addition, MCM-2 LI for entire glands in colon adenocarcinoma was found to be significantly higher than those in normal glands and adenomas (Scott et al., 2003). Moreover, it was shown that MCM-2 protein was expressed in more cells than Ki-67 in both normal and neoplastic colonic epithelia. Antibodies against Ki-67 failed to stain any cells labelled with phosphohistone H3 and occasional those labelling with cyclin D1. In contrast, MCM-2 identified every cell expressing any examined marker of the cell-cycle phase (Scott et al., 2003).

Concerning the diagnostic value of cell proliferation markers in colorectal cancer, MCM-2, Ki-67 and PCNA LIs were significantly associated with the presence of lymph node metastases, but not with patient age or tumor location (Guzińska-Ustymowicz et al., 2008). Moreover, the expression of these proteins in the primary tumor site correlated with each other. However, the data that Ki-67, PCNA and MCM-2 positivity may be indicative of lymph node involvement are restricted to primary tumors presenting pathological stage pT3 and degree of histological malignancy G2 (Guzińska-Ustymowicz et al., 2008). In an immunohistochemical study conducted by our group on a larger number of cases, MCM-2 and Ki-67, but not MCM-5 expression, were significantly associated with tumor histopathogical grade, lymph node metastasis, the presence of malignancy in adenomas and vascular invasion (Giaginis et al., 2009). MCM-2, but not Ki-67 expression was correlated with Dukes' stage, whereas no significant associations were noted with patient age and gender, tumor size and location, as well as coexistence of adenomas for both markers. Significant positive

correlations were found between the expression of MCM-2 or MCM-5 proteins and that of Ki-67, as well as between MCM-2 and MCM-5 proteins. Significant positive relationships between the expression of MCM-2 or MCM-5 proteins and p53 protein were also noted. However, they were consistently lower than those with Ki-67 protein (Giaginis et al., 2009). MCM-2 and Ki-67 expression was also examined in patients with active or inactive inflammatory bowel disease (IBD) (Davies et al., 2004). MCM-2 LI was significantly increased in the superficial one-third of glands in active compared to inactive/quiescent ulcerative colitis and active compared to inactive/quiescent Crohn's disease. MCM-2 LI was also significantly greater than Ki-67 LI in active IBD, both in entire glands and in the superficial one-third of the glands. For entire glands, MCM-2 LI was significantly higher in ulcerative colitis compared to Crohn's disease. There was also an increased cell-cycle entry, as indicated by expression of MCM-2, and to a lesser extent Ki-67, in the superficial one-third of colonic glands in active IBD compared to inactive/quiescent IBD cases. Thus, the detection of MCM-2 may contribute to improved histological assessment of small size colonic biopsies with IBD and may enable the development of a direct stool-based test for detection of active IBD and potentially for the assessment of disease activity (Davies et al., 2004). On the other hand, Gray et al. did not recommended Ki-67 or MCM-2 staining to differentiate serrated polyps with abnormal proliferation from conventional hyperplastic polyps, since staining characteristics were not significantly different between the two groups, and frequent variable crypt staining within a given polyp was difficult to interpret (Gray et al., 2006).

Liver diseases

In an attempt to differentiate hepatocellular carcinoma (HCC) from its precursor lesions, Quaglia et al. showed that the proportion of cells expressing MCM-2 was higher than that expressing Ki-67, which in turn was higher than that of cells expressing geminin (Quaglia et al., 2006). A significant trend of increasing Ki-67 expression was also found, from regenerative nodules to HCC, whereas this trend was not significant for geminin or MCM-2. Moreover, the combination of these markers identified four different cell kinetic patterns: resting, licensed, slowly growing and expanding nodules, supporting evidence that combining MCM-2, geminin and Ki-67 could represent a valuable tool in the understanding of HCC progression in cirrhosis (Quaglia et al., 2006).

In another study, the proportion of hepatocytes expressing MCM-2 always exceeded that expressing Ki-67 and positively correlated with increasing stage of fibrosis and viral replication in hepatitis C virus (HCV) - infected patients (Freeman et al., 2003). Weaker but significant associations between the proportion of hepatocytes expressing MCM-2 and inflammatory indices, including interface hepatitis, portal tract inflammation, lobular inflammation and steatosis were also noted. No association was found between the proportion of hepatocytes expressing MCM-2 and patients' age, gender or past alcohol consumption. On the other hand, the proportion of Ki-67 positive hepatocytes did not correlate with any clinical, laboratory or histological parameter, supporting evidence that MCM-2 is a more sensitive marker of hepatocyte proliferation than Ki-67 (Freeman et al., 2003). In this context, Marshall et al. showed that hepatocyte cell-cycle phase distribution was altered in chronic HCV infection compared to liver regeneration following reperfusion injury consistent with G1/S cell-cycle arrest (Marshall et al., 2005a). More to the point, hepatocyte MCM-2 expression was found to be elevated in chronic HCV and liver regeneration, but negligible in normal liver. In proportion to MCM-2, there was no difference in cyclin D1 between chronic HCV infection and liver regeneration. In contrast, there was a striking reduction in cyclin A, B1, and phosphorylated histone 3 protein in chronic HCV infection compared to liver regeneration. In chronic HCV infection, MCM-2 and p21 expression were associated with fibrosis stage and positive serum HCV RNA (Marshall et al., 2005a). The same authors also suggested that hepatocyte cell-cycle entry may be important in the pathogenesis of post-transplant HCV hepatitis and that assessment of MCM-expression by immunohistochemistry could contribute to the identification of patients at high risk for progressive fibrosis before it occurs (Marshall et al., 2005b).

Pancreatic diseases

Biliary brush cytology is the standard method of sampling a biliary lesion; however, it exhibits low sensitivity for the detection of malignancy. For this purpose, Ayaru et al. determined MCM-2 and MCM-5 expression by immunohistochemistry in 30 tissue specimens from patients with malignant/benign biliary lesions (Ayaru et al., 2008). MCM-5 bile sediments from 102 patients with biliary lesions of established or indeterminate aetiology were also assessed by an automated immunofluorometric assay. In benign pancreatobiliary structures, both MCM-2 and MCM-5 protein expression was confined to the basal proliferative epithelium, in contrast to malignant pancreatobiliary lesions where expression was seen in all tissue layers. Moreover, the percentage of MCM-2 and MCM-5-positive nuclei was higher in malignant tissues than in benign ones. MCM-5 levels in bile were found to be significantly more sensitive than brush cytology for the detection of malignancy in patients with an indeterminate lesion, with a comparable positive predictive value, supporting evidence that MCM-5 in bile, detected by a simple automated test, may prove to be a more sensitive indicator of pancreatobiliary

malignancy than routine brush cytology (Ayaru et al., 2008).

Lung neoplasia

In 41 bronchial biopsy specimens, including normal mucosa, metaplasia, dysplasia, and carcinoma *in situ,* the average frequency of MCM-2 LI was higher than that of Ki-67 (Tan et al., 2001). In metaplastic lesions, the antibody against MCM-2 was frequently detected in cells near the epithelial surface, whereas the antibody against Ki-67 was not. Thus, MCM-2 was detected 2-3 times more frequently in proliferating, pre-malignant lung cells than the Ki-67 antigen. Moreover, for both antibodies, the mean percentage of positive cells, as well as their staining intensity, was increased from normal mucosa to metaplasia and from metaplasia to dysplasia. The promise of MCM-2 as a sensitive marker for pre-malignant lung cell detection was enhanced by the fact that it was present in cells at the surface of metaplastic lung lesions, which were more likely to be exfoliated into the sputum (Tan et al., 2001).

The clinical significance of MCM-2 expression in non-small cell lung carcinoma (NSCLC) was comprehensively evaluated in two large cohort studies. Both studies revealed a significant association between MCM-2 expression and tumor histopathological type, as SCC cases more frequently expressed MCM-2 than adenocarcinomas (Ramnath et al., 2001; Yang et al., 2006). On the other hand, no significant associations between MCM-2 expression and tumor histopathological stage, as well as smoking habits were noted (Ramnath et al., 2001; Yang et al., 2006). Furthermore, the study of Ramnath et al. showed that MCM-2 LI was associated with Ki-67 LI (Ramnath et al., 2001), while Yang et al. documented no relationships with tumor size and lymph node metastasis (Yang et al., 2006). However, conflicting results were reported concerning the other clinicopathological characteristics of NSCLC patients. Specifically, the study of Ramnath et al. conducted on 221 NSCLC cases, revealed no significant association between MCM-2 expression and patient gender or tumor histopathological grade, whereas Yang et al. using 128 NSCLC cases documented that MCM-2 was more frequently expressed in men and poorly differentiated carcinomas compared to women and well or moderately differentiated carcinomas (Ramnath et al., 2001; Yang et al., 2006). These discrepancies may be attributed to the larger number of NSCLC cases evaluated in the study of Ramnath et al. Moreover, in the latter study, NSCLC cases were grouped into four categories based on the extent of MCM-2 expression (0-24%, 25-49%, 50-74% and 75-100%) to process the statistical analysis of the clinicopathological data, whereas Yang et al. used a cut-off point of 25% for the definition of MCM-2 expression. Concerning the prognostic significance of MCM-2, both studies showed that NSCLC patients with less than 25% MCM-2 immunoreactivity presented a longer median survival time than patients with ≥ 25%. On the other hand, no effect of Ki-67 expression on patient survival was noted, supporting evidence that MCM-2 was a clinically superior predictor of survival than Ki-67 in NSCLC patients (Ramnath et al., 2001; Yang et al., 2006). In another study conducted on 145 lung adenocarcinoma cases, MCM-2 LI significantly correlated with patient gender, tumor histopathological grade and stage (Hashimoto et al., 2004). MCM-2 LIs were also associated with those of Ki-67 and p53, while significantly higher MCM-2 and Ki-67 LIs were noted in non-pure rather than pure bronchioloalveolar carcinomas. In contrast to the previous studies, both MCM-2 and Ki-67 LIs were independent prognostic indicators in non-pure bronchioloalveolar carcinomas (Hashimoto et al., 2004).

Breast neoplasia

MCM-2 was more frequently expressed compared to the standard proliferation marker Ki-67 in breast cancer tissue sections (Gonzalez et al., 2003; Shetty et al., 2005), while geminin was present in only a minority of cells. Both MCM-2 and Ki-67 were detected in both lobules and ducts of normal breast tissue cases (Gonzalez et al., 2003; Shetty et al., 2005). MCM-2 expression was significantly associated with tumor histopathological grade and the Nottingham Prognostic Index (NPI) score, but not with patient age, lymph node status and vascular invasion (Gonzalez et al., 2003; Shetty et al., 2005). More to the point, it should be noted that increased tumor histopathological grade in breast cancer was associated with increased MCM-2, Ki-67 and geminin expression, which provides an estimate of the S-G2-M phase growth fraction in dynamic cell populations. On the other hand, the MCM-2/Ki-67 ratio decreased through the tumor histopathological grades, indicating a shift from a predominantly licensed state to an actively proliferating one (Shetty et al., 2005). Moreover, in the study of Gonzalez et al. a significant association between MCM-2 expression and tumor size was also noted, which however, was not confirmed by Shetty et al. Gonzalez et al. further demonstrated that MCM-2 LI was higher than that of Ki-67 in 221 invasive carcinoma TMA cores. In context, MCM-2 was further associated with the presence of distant metastases and with histopathological type when tumors were categorized according to excellent, good, moderate and poor prognostic groups (Gonzalez et al., 2003).

Concerning the prognostic value of MCM-2 in breast carcinoma, Gonzalez et al. showed that MCM-2 LI was associated with overall survival, disease-free interval and the development of regional recurrence and distant metastases. Importantly, MCM-2 was found to be a strong prognostic independent factor which was superior to histopathological grade, lymph node metastatic status, and Ki-67 LI, but not NPI score, providing evidence that it may be of utility as a

prognostic marker to redefine the prediction of outcome in breast cancer patients (Gonzalez et al., 2003). These findings were confirmed by the study of Shetty et al. in which MCM-2 was identified as the single most important predictor of the surrogate outcome measure NPI. However, only 12% in the variation of the NPI was ascribed to MCM-2, and therefore it was speculated that MCM-2 was a weak predictor of patient outcome due to its enhanced expression in poorly differentiated tumors (Shetty et al., 2005). In another study, a hypomorphic mutation of MCM-4 in a phenotype-based screen for chromosome instability in mice has recently been isolated (Shima et al., 2007). MCM-4 encoded a subunit of the MCM-2 to -7 complex, the replication-licensing factor and the replicative helicase. This mutation, named chromosome aberrations occurring spontaneously 3 (Chaos3), exclusively caused mammary adeno-carcinomas in approximately 80% of homozygous females. The MCM-4 (Chaos3) mutation appeared to destabilize MCM-2-7 complex, resulting in impaired DNA replication. These data revealed, for the first time, the causative role of an MCM mutation in cancer development, raising the possibility that hypomorphic mutations in MCM-2 to MCM-7 genes may increase breast cancer risk in humans (Shima et al., 2007).

Ovarian neoplasia

Both MCM-2 and MCM-5 LIs were significantly higher in ovarian adenocarcinomas compared to tumors of low malignant potential. In adenocarcinoma cases, the levels of MCM-2 and MCM-5 were significantly increased with advanced tumor histopathological stage and grade, as well as the presence of bulky residual disease (Gakiopoulou et al., 2007). A strong positive correlation was established between MCM-2 or MCM-5 and Ki-67 LI, as well as p53 protein expression. Both MCM-2 and MCM-5 were further associated with adverse patient outcome in both univariate and multivariate analyses. Subsequently, an adequately powered independent group of 45 patients was used to validate the results of the survival analysis. In this group, MCM-2 and MCM-5 expression retained their prognostic significance, reinforcing the assumption that both proteins could constitute promising prognostic markers in ovarian adenocarcinoma (Gakiopoulou et al., 2007). Scott et al. also showed that there was a significant increase in MCM-2, cyclin D1, A and B1, as well as phosphohistone H3 expression in the progression from normal ovary through serous cystadenoma and borderline tumors to cystadenocarcinomas, which was paralleled by an increase of cells in the S-phase fraction reflected by the cyclin A/MCM-2 ratio (Scott et al., 2004). Borderline tumors of increasing grade also showed increased MCM-2 and cyclin A expression, together with an increase in the S-phase fraction. In this study, 10 representative cases of serous cystadenocarcinoma were further examined by both flow cytometry and immunohistochemistry. Interestingly, there was a significant difference in the G0/G1 fractions determined by the two methods, with flow cytometry presenting a lower estimate of the number of cells in G0/G1, presumably due to nuclear fragmentation. Taken together, it was speculated that immunohistochemistry can be used to estimate cell cycle phase distribution in ovarian serous neoplasms, giving results similar to those of flow cytometric analysis and enabling direct assessment of tumour heterogeneity (Scott et al., 2004).

Endometrial neoplasia

In normal endometrial glands, the expression of MCM-2 and MCM-3 was significantly higher in the proliferative than in the secretory phase and was strongly correlated with Ki-67 expression (Kato et al., 2003b). Significant association between the expression of both MCMs and Ki-67 was also found in endometrial hyperplasia cases. In endometrial carcinomas, however, the expression of MCM-2 and MCM-3 was significantly lower than that in the non-malignant endometrium. There was only a weak correlation between MCM-2 and Ki-67 immunostainings and no significant correlation between MCM-3 and Ki-67 expression. These findings suggested that MCM-2 and MCM-3 expression may directly reflect cell proliferation in normal and hyperplastic endometrium, whereas the replication-licensing system may be aberrant in endometrial carcinomas (Kato et al., 2003b).

In an immunohistochemical study performed on TMA from paraffin blocks of endometrial carcinoma, MCM-7 and Ki-67 immunoreactivity was clearly evident in the nuclei of tumor cells (Li et al., 2005). MCM-7 and Ki-67 LIs were correlated with each other; however, MCM-7 LI was, in general, higher than Ki-67, suggesting that it labels more cells in the proliferative state. A significant association of MCM-7 LI with patient age and tumor histopathological grade was noted, as well-differentiated carcinomas and younger patients presented a lower MCM-7 expression. Poor survival was observed in endometrial carcinoma cases with high MCM-7 LI, while multivariate analysis rendered MCM-7 as an independent prognostic factor. On the other hand, Ki6-7 LI correlated with histopathological grade, but had no significant prognostic impact. Thus, it was speculated that MCM-7 may be a more reliable and useful marker than Ki-67 in assessing tumor proliferation and in the prognosis of endometrial carcinoma patients (Li et al., 2005). Niklaus et al. also reported that mid-reproductive age women exhibited significantly higher proliferative than secretory expression of Ki-67, PCNA and MCM-2 in the luminal and the glandular epithelium. In the latter, Ki-67, PCNA and MCM-2 had more positively stained nuclei in proliferative than in secretory endometrium. Moreover, both Ki-67 and MCM-2 LIs were significantly greater in the proliferative than in the secretory phase in luminal epithelium, whereas the wide

variation in secretory phase PCNA did not allow reliable comparisons (Niklaus et al., 2007).

Cervical neoplasia

In normal cervical epithelium MCMs were confined to the basal proliferative layer and were absent from terminally differentiated superficial keratinocytes (Freeman et al., 1999; Williams et al., 1998; Baldwin et al., 2003). On the other hand, in pre-malignant cervical intraepithelial neoplasia (CIN), the cellular proliferative layer expanded in proportion to histological grade, resulting in MCM-positive cells located at the epithelial surface (Williams et al., 1998; Freeman et al., 1999; Baldwin et al., 2003). In this context, an immunohistochemical study performed on uterine cervical cancer cases showed that the frequency of both MCM-3 and -4 expression was much higher in tumor cells than in normal proliferating cells of the uterine cervix and dysplastic cells, suggesting that MCM-3 and -4 can be used as markers to distinguish such cells (Ishimi et al. 2003). Williams et al. further stained cervical tissue smears for MCM-5 and CDC6 noting a "remarkably high specificity and sensitivity" in the expression of these proteins and the presence of atypia. In the same study, both Ki-67 and PCNA were much less effective (Williams et al., 1998). Murphy et al. also revealed a linear correlation between MCM-5 expression and the grade of dysplasia. Moreover, MCM-5 staining intensity was independent of high risk HPV infection, highlighting its potential as a biomarker in HPV related cervical dysplasia (Murphy et al., 2005). In this context, Davidson et al. also showed a lack of correlation between HPV positivity and MCM-5 staining intensity, indicating that MCM-5 protein expression was related to cell proliferation, being independent of HPV infection (Davidson et al., 2003). It should be noted that MCM-5 up-regulation was also detected in a variety of non-HPV related neoplasms (Murphy et al., 2005).

A comparison between the efficacy of immunocytochemistry for MCM-2 and -5 proteins and standard Pap testing in detecting disease in 455 cervical smears was recently processed in an Indian screening laboratory (Mukherjee et al., 2007). The MCM test was considered positive when immunolabelled cells were identified as dyskaryotic by the Pap counterstain. The MCM test was quicker than the Pap test and presented 100% inter-observer agreement compared with 85% for the Pap stain. Combining MCM staining and Pap counterstaining further detected 10 cervical cancer or pre-cancer cases, which were missed using the Pap test alone. Moreover, there was no evidence of reduced specificity with the MCM test which produced no false positive results. Thus, it was proposed that MCM immunocytochemistry may have considerable advantages and may be more cost effective and of greater benefit than the Pap test for cervical cancer screening in developing countries like India (Mukherjee et al., 2007).

Renal cell carcinoma (RCC)

In normal kidney tissues, MCM-2 nuclear immunostaining was identified in both glomeruli and renal tubules (Rodins et al., 2002). In renal tumors, MCM-2 expression was predominantly found at the periphery, being significantly greater than that of Ki-67. MCM-2 expression was also significantly higher in tumors derived from a labile (transitional cell carcinoma-TCC) than a stable epithelium (Rodins et al., 2002). A significant association was also demonstrated between MCM-2 expression and tumor histopathological grade, as well as angiogenic phenotype, but not with patient age and gender, tumor histopathological stage and tumor size. Furthermore, although not significant, survival analysis demonstrated that 100% of patients with low MCM-2 LI survived compared to 84% of those with a high MCM-2 LI presenting a follow-up period up to 53 months (Rodins et al., 2002). In 176 RCC cases, MCM-2 was also expressed at much higher levels than Ki-67 and geminin and was most closely linked to tumor histopathological grade (Dudderidge et al., 2005). For each marker, univariate analysis provided evidence that increased MCM-2 expression was associated with reduced disease-free survival time. Additionally, both MCM-2 and Ki-67 LI identified a unique licensed but non-proliferating population of tumor cells that increased significantly with tumor histopathological grade, also presenting prognostic significance. However, in multivariate analysis, Ki-67, but not MCM-2 was found to be an independent prognostic marker. In this context, it should be noted that although Ki-67 was identified as an independent prognostic marker, semi-quantitative assessment was difficult due to the very low proliferative fraction of cells identified by this marker. In contrast, MCM-2 identified an increased growth fraction that was closely linked to histopathological grade, providing prognostic information, and may be amenable to semi-quantitative analysis in routine pathologic assessment (Dudderidge et al., 2005).

Prostatic neoplasia

In a clinical study consisting of 92 prostate cancer patients, MCM-2 expression was consistently increased in malignant glands. In contrast, MCM-2 expression was low and limited to the basal cell layer in non-malignant prostate glands (Meng et al., 2001). MCM-2 expression was significantly associated with disease-free survival after definitive local therapy in both univariate and multivariate analyses, as patients with high MCM-2 expression exhibited shorter disease-free survival (Meng et al., 2001). Both Ki-67 and MCM-2 showed an upward trend from normal tissue through high grade prostatic intraepithelial neoplasia (PIN) and cancer, with a shift in proliferation from the basal to the luminal compartment

(Ananthanarayanan et al., 2006). In the vicinity of normal glands, higher MCM-2 LI was noted compared to distant glands, being associated with higher caspase-3 expression. These results demonstrated that proliferation and apoptosis may be altered not only in pre-neoplastic lesions, but also in apparently normal epithelium associated with cancer, while luminal cell expression of MCM-2 seems to be promising as a marker for the detection of normal epithelium with potential for malignancy (Ananthanarayanan et al., 2006). On the other hand, it should be noted that MCM-2 staining was completely negative in prostatic biopsies processed in Bouin's fixed tissues (Ananthanarayanan et al., 2005). It was also shown that induction of mitogen/extracellular-signal-regulated kinase kinase 5/extracellular signal-regulated kinase-5 (MEK5) expression resulted in increased levels of phosphorylated ERK5 and MCM-2, geminin and Ki-67 proteins in prostate cancer (Dudderidge et al., 2007). In 58 prostate cancer cases, MCM-2 expression was greater than Ki-67 and geminin expression. All three markers were significantly associated with Gleason score. However, there was no such relationship with tumor size or organ metastasis. In addition, there was a significant relationship between increasing ERK5 and either MCM-2 or Ki-67 expression. Both MCM-2 and Ki-67 were identified as prognostic factors in univariate analysis. However, only MCM-2 remained an independent prognostic marker on multivariate analysis. Taken together, these data showed that induction of MEK5/ERK5 signalling may be related to the activation of the DNA replication licensing pathway in prostate cancer, and that the strong prognostic value of MCM proteins may result from their function as relay stations coupling growth regulatory pathways to genome duplication (Dudderidge et al., 2007).

Concerning another member of the MCM family, Padmanabhan et al. evaluated MCM-7 expression in 79 lymph node-negative prostate cancer cases. Interestingly, MCM-7 LI was significantly higher than Ki-67 LI in benign basal epithelial cells, PIN and epithelial tumor cells in adenocarcinoma, but not in benign luminal epithelial cells. MCM-7 was also a better discriminatory marker of proliferation between benign epithelium, PIN and invasive adenocarcinoma than Ki-67. The mean drop in MCM-7 basal cell proliferation index from benign to prostatic intraepithelial neoplasia and epithelial tumor cells in adenocarcinoma was significantly higher than Ki-67. MCM-7 LI was also significantly higher than Ki-67 LI at each risk level, suggesting that it may be a useful proliferation marker to stratify patients with lymph node-negative prostate cancer (Padmanabhan et al., 2004). In retrospective population-based material of 249 radical prostatectomy patients, Laitinen et al. further showed that increased MCM-7 and Ki-67 expression were significantly associated with a high Gleason score and poor progression-free survival. In multivariate analysis, both MCM-7 and Ki-67 were identified as independent prognostic factors in this type of cancer (Laitinen et al., 2008).

Urothelial neoplasia

Stage Ta/T1 urothelial carcinoma of the bladder (Ta/T1 BC) exhibits a marked tendency to recur. Besides histopathology, markers such as CK20 and Ki-67 were shown to predict its clinical course. In this aspect, Burger et al. evaluated, by using immunohistochemistry and TMA technology, the clinical significance of MCM-2 in 71 stage Ta/T1 carcinomas of the bladder in comparison to tumor histopathological stage and grade, CK20 and Ki-67 (Burger et al., 2007). CK20 was found to be non predictive, whereas histopathogical grade, as well as MCM-2 and Ki-67 LIs were significantly related to the recurrence rate in univariate analysis. Importantly, only histopathological grade and MCM-2 LI proved independent predictors of the recurrence rate in multivariate analysis (Burger et al., 2007). Accordingly, Kruger et al. showed that high MCM-2 expression levels were significantly associated with early tumor recurrence and early tumor progression in 44 cases of stage T1 bladder tumors analyzed by Biochip microarrays. There was also a borderline association of MCM-2 with Ki-67 LIs, but not with p53 LI. MCM-2, Ki-67 and p53 expression, as well as tumor histopathological grade and patient age were significantly associated with recurrence-free survival in univariate analysis. In contrast to the previous study, Ki-67, but not MCM-2 expression, was identified as an independent prognostic factor in multivariate analysis (Krüger et al., 2003). In another study conducted on 65 muscle-invasive urothelial cancer cases, the levels of MCM-2 and MCM-5 were significantly higher in high-grade, advanced-stage and non-papillary tumors (Korkolopoulou et al., 2005). Both MCM-2 and MCM-5 LIs were positively correlated with Ki-67 and p53 LIs. Moreover, increased MCM-2 and MCM-5 expression was significantly associated with poorer overall, but not disease-free survival in both univariate and multivariate analysis (Korkolopoulou et al., 2005).

Interestingly, an immunofluorometric assay was reported to measure MCM-5 levels in cells in 353 urine samples from patients with hematuria or lower urinary tract symptoms, or who were undergoing follow-up cystoscopy for urothelial neoplasia (Stoeber et al., 2002). At the assay cut-point where the false-negative and false-positive rates were the same, the MCM-5 test detected primary and recurrent bladder cancers with 87% sensitivity and specificity. At the cut-point where the specificities of urine cytology and the MCM-5 test were equal, the MCM-5 test proved more sensitive than urine cytology. At the lower detection limit of the MCM-5 test, sensitivity was highest, 92% and specificity was 78%. Importantly, patients with prostate cancer presented higher urine MCM-5 levels than men without

malignancy (Stoeber et al., 2002).

Neurological neoplasia

An immunohistochemical analysis conducted on 32 oligodendroglioma cases demonstrated significant associations between MCM-2 and Ki-67 LIs, as well as mitotic activity, while MCM-2 consistently identified an increased proportion of proliferative cells compared to Ki-67. MCM-2 LI was significantly higher in grade III than in grade II tumors. Oligodendroglioma cases with high MCM-2 LI presented a significantly poorer prognosis than those with low MCM-2 LI in univariate analysis (Wharton et al., 2001). Another immunohistochemical analysis of intracerebral gliomas, including diffuse and anaplastic astrocytomas, as well as glioblastomas, was performed using markers of cell cycle entry to investigate the estimation of prognosis and response to adjuvant chemotherapy in glial neoplasms, without the requirement for flow cytometric analysis. A significant increase of MCM-2, Ki-67, cyclin A and B1 expression with increasing grade from diffuse astrocytoma through anaplastic astrocytoma to glioblastoma was noted. In the subgroup of glioblastoma patients, the examined cell-cycle markers, including MCM-2, were not independent predictors of survival after radical radiotherapy (Scott et al., 2005). In another study conducted on 10 benign meningiomas which subsequently recurred within a 5-year period, together with 20 matched non-recurrent benign meningiomas, there was no significant correlation between MCM-2 LI and histopathological subtype, mitotic activity or Ki-67 LI and tumor recurrence (Hunt et al., 2002). However, MCM-2 LI in the area of highest proliferative activity within the tumor section was significantly increased in recurrent meningiomas. Moreover, 7 out of the 10 recurrent meningiomas displayed a MCM-2 LI greater than 30%, compared to 0 out of 20 for non-recurrent tumors. Thus, it was speculated that MCM-2 expression in meningioma may facilitate identification of patients presenting high risk of recurrence, for which adjuvant radiotherapy may be of benefit (Hunt et al., 2002). Concerning other members of the MCM family, MCM-3 was reported to be overexpressed in human astrocytic tumors, resulting in a tumor-restricted humoral immune response in 9.3% of patients with brain tumors and metastases, but not in healthy controls (Söling et al., 2005). MCM-3 expression in diffuse astrocytoma was significantly associated with Ki-67 expression, patient age, tumor histopathological grade and time to recurrence. Moreover, survival analysis rendered MCM-3 expression as an independent predictor of poor outcome in patients with astrocytoma (Söling et al., 2005). Facoetti et al. also revealed that MCM-7 showed higher expression in nuclei of primary astrocytomas compared to Ki-67, regardless of histopathological grade. In addition, a stronger increase of the MCM-7 LI in relation to tumor aggressiveness was noted (Facoetti et al., 2006a). In another study by the same research group, MCM-7 detected more cells in cycle than Ki-67 and PCNA in glioblastomas, while small cell glioblastoma, the most aggressive subset, displayed a significant increase of MCM-7-stained nuclei versus those stained with Ki-67 (Facoetti et al., 2006b).

Craniopharyngioma often recurs after resection, resulting in poor patient outcome. The reliable criteria for predicting tumor behavior are still lacking. However, it has been suggested that proliferative potential of the tumor cells is necessary for recurrence. In light of this view, tissue specimens from 32 patients with adamantine epithelioma and 31 patients with squamous papillary tumor were stained to evaluate the expression of MCM-6 and DNA topoisomerase II alpha (DNA Topo II alpha) (Xu et al., 2007). MCM-6 LI was significantly higher in adamantine epithelioma than in squamous papillary tumors. In the primary tumors of both subtypes, MCM-6 and DNA Topo II alpha LIs were higher in craniopharyngioma cases with recurrence than those without recurrence. There was also a strong linear positive correlation between MCM-6 and DNA Topo II alpha LIs. The median MCM-6 LI of the total 20 recurrent craniopharyngioma cases was not significantly different from that of their primary tumors. In contrast, the long term risk of tumor recurrence was higher in adamantine epithelioma than in squamous papillary tumors and was associated with MCM-6 and DNA Topo II alpha expression (Xu et al., 2007).

Skin neoplasia

Freeman et al. immunohistochemically evaluated the expression of MCM-2 and MCM-5 in biopsy specimens with normal-appearing skin, psoriasis, actinic keratosis, Bowen's disease, and SCC (Freeman et al., 1999). Cell counts were significantly higher in Bowen's disease and SCC, but not in psoriasis. Increasing expression of these proteins was noted in well, moderate, and poorly differentiated SCCs, while their staining was evaluated along the basal layer in normal-appearing skin, more than 50% of the epidermis in actinic keratosis, and greater than 90% of the epidermis in Bowen's disease (Freeman et al., 1999). Liu et al. further revealed that MCM-5 protein was expressed in the lower layers of the epidermis in psoriasis, while MCM-5 protein was also present throughout the tumor cells in bowenoid papulosis, Bowen's disease, and moderately/poorly differentiated SCC (Liu et al., 2007). In this study, MCM-5 protein was more frequently expressed in the periphery of well-differentiated SCC or larger nests of basal cell carcinoma. However, some small nests of basal cell carcinoma seemingly showed diffuse staining patterns. In agreement with the study of Freeman et al., well-differentiated SCC showed a significantly lower percentage of MCM-5 positive cells than did moderately differentiated SCC or poorly differentiated SCC. MCM-5 staining basically showed a similar staining pattern to that of PCNA, but more cells tended to be stained with MCM-5 than with PCNA (Liu et al., 2007). Hiraiwa et

al. also reported the expression of another member of the MCM family, MCM-7, in Bowen's disease and SCC, revealing a similar percentage of MCM-7 positive cells compared to the previous study (Hiraiwa et al., 1998). In this study, keratoacanthomas showed a peripheral pattern in which only the cells located in the basal cell layers were positive. Psoriasis vulgaris also showed this peripheral type of location, with the cells in the suprabasal layers also occasionally expressing MCM-7. Verruca vulgaris and basal cell carcinomas demonstrated a diffuse pattern, with positive epithelial cells distributed throughout the epithelial layers (Hiraiwa et al., 1998). Another immunohistochemical study was performed on skin specimens of 51 patients with parapsoriasis, mycosis fungoides, or lymphomatoid papulosis (Gambichler et al., 2008). Mycosis fungoides with stage IIB-IV and lymphomatoid papulosis presented a significantly increased percentage of Ki-67-positive cells than parapsoriasis and mycosis fungoides I-IIA, respectively. MCM-7 LI was significantly higher in mycosis fungoides IIB-IV and lymphomatoid papulosis when compared to parapsoriasis and mycosis fungoides I-IIA, respectively. Compared to parapsoriasis and mycosis fungoides I-IIA, mycosis fungoides IIB-IV was associated with significantly higher PCNA LI. Thus, it was speculated that Ki-67 and PCNA may be useful immunohistological parameters for clinical staging of mycosis fungoides, while MCM-7 may serve as a novel biomarker in the differentiation and prognostication of T cell lymphoproliferative skin disorders (Gambichler et al., 2008).

Although PCNA and Ki-67 have been extensively evaluated in melanocytic neoplasms, only a handful of investigations of MCM expression in benign and malignant mucocutaneous conditions have been conducted. In this regard, Boyd et al. revealed significant differences concerning the percentage of MCM-2 positively staining nuclei between benign or dysplastic nevi and melanoma metastases, as well as benign or dysplastic nevi and cutaneous melanoma metastases (Boyd et al., 2008). These data supported evidence that MCM protein expression may differ significantly in melanocytic neoplasms, thus providing an additional tool for distinguishing benign tumors from their malignant counterparts. However, there was no statistically significant difference between cutaneous melanoma metastases and primary cutaneous melanoma. In this context, the authors emphasized the fact that their findings were derived from a small pilot study without blinded evaluation of the tissue sections and lacking correlation with patient clinical outcome or accepted histologic prognostic factors (Boyd et al., 2008). In an elaborate study of cutaneous melanomas, Winnepenninckx et al. identified 254 genes involved in activating DNA replication, noting a significant difference in gene expression between patients with metastatic disease and those without metastases (Winnepenninckx et al., 2006). Included in this group were MCM-3, MCM-4 and MCM-6. Imunoperoxidase staining performed on tissue sections for these MCMs revealed that they were expressed in significantly greater amounts in the tumors from patients with distant metastases. However, grading of positively stained cells was performed on a fourth level scale and not by counting individual cells (Winnepenninckx et al., 2006).

Hematological malignancies

The percentage of MCM-6-expressing lymphoma cells was significantly higher than that of Ki-67-positive cells in lymph node biopsy specimens with mantle cell lymphoma (Schrader et al., 2005). The ratio of MCM-6-positive cells to Ki-67-positive cells was higher than in normal stimulated peripheral mononuclear blood cells, indicating an early G1-phase cell arrest in mantle cell lymphoma. High MCM-6 expression was associated with a significantly shorter overall survival time, while multivariate analysis rendered MCM-6 as an independent predictor of survival that was superior to the international prognostic factor and Ki-67 LI (Schrader et al., 2005). Oberman et al. also suggested that MCM-2 expression was capable of assessing tumor proliferation and may prove useful as an additional prognostic marker to redefine the prediction of outcome in diffuse large B-cell lymphomas (Obermann et al., 2005). More to the point, MCM-2 expression was clearly evident in the nuclei of proliferating non-neoplastic cells and malignant cells. A significant correlation between MCM-2 expression and the presence of bulky disease was noted. In univariate analysis, patients presenting MCM-2 positivity were characterized by poor disease specific survival, which, however, did not reach statistical significance in multivariate analysis (Obermann et al., 2005). In a cohort study of 79 CLL patients a large number of tumor cells exhibited proliferative potential, as expressed by MCM-2 detection, with a significant sub-population residing in early G1-phase (Obermann et al., 2007). Furthermore, DNA microarray in 22 patients with acute leukemia revealed genes which were differentially expressed (Wei et al., 2006). Ribosomal protein SA (RPSA), MCM-2 deficient and heterogeneous nuclear ribonucleoprotein A1 (HNRPA1) were significantly upregulated in refractory patients, suggesting that they may play a role in refractory acute leukemia and could be prognostic biomarkers (Wei et al., 2006). In another study, Lambert et al. immunohistochemically evaluated the expression of MCM-2 in megakaryocytes in trephine biopsies of myeloproliferative and myelodysplastic syndromes and compared them to megakaryocytes in marrows not involved in a primary hematological disorder (Lampert et al., 2005). In both normal and abnormal states, the proportion of megakaryocytes expressing MCM-2 was considerably higher than those expressing Ki-67. This was likely to be related to the process of endomitosis and endoreduplication. It was also demonstrated that a

significantly lower proportion of megakaryocytes expressed MCM-2 in myelodysplastic syndromes compared to myeloproliferative syndromes and marrows free of primary hematological disorders (Lampert et al., 2005).

Soft tissue neoplasia

Osaki et al. assessed the expression of MCM-2 by immunohistochemistry in 38 human malignant fibrous histiocytomas and 36 benign fibrohistiocytic tumors (Osaki et al., 2002). Nuclear expression of MCM-2 was noted in tumor cells, but not mitotic cells of all the malignant fibrous histiocytomas and 26 (72%) of the benign fibrohistiocytic tumors. Moreover, MCM-2 LI was significantly higher than Ki-67 LI in the malignant fibrous histiocytomas. No correlation was noted between the MCM-2 and p53 expression or apoptotic indices, which were significantly higher in the malignant fibrous histiocytomas than benign fibrohistiocytic tumors. These results indicated that MCM-2 was related with cell proliferation rather than apoptosis in malignant fibrous histiocytomas, and its expression was ubiquitous in proliferating cells, regardless of p53 expression (Osaki et al., 2002).

In comparison to other markers, such as Ki-67 and repp86, MCM-6 proved more effective in identifying proliferative activity in chondrosarcomas (Helfenstein et al., 2004). The MCM-6 LI was associated with tumor histopathological grade, as high grade chondrosarcomas (grade 2 and 3) displayed a significantly higher MCM-2 LI than low grade tumors (grade 1). Moreover, MCM-2 LI was significantly increased in grade 1 chondrosarcomas compared to enchondromas. Furthermore, by use of the MCM-6 LI, many cases of progressive disease were recognized among those of uncertain malignant potential, justifying their classification as low-grade chondrosarcomas. Moreover, MCM-6 LI with a cut-off point at 0.5% proved to be highly significant for the prediction of relapse or disease progression (Helfenstein et al., 2004). Sington et al. also revealed that MCM-2 LI was significantly higher than Ki-67 LI in 51 cases of myxofibrosarcomas (Sington et al., 2004). Both MCM-2 and Ki-67 LIs showed a significant association with mitotic activity, being also significantly increased with increasing grade of myxofibrosarcoma. MCM-2, but not Ki-67 LI, further showed a significant inverse exponential correlation with the time of the first recurrence. Myxoid and cellular areas showed no difference in the MCM-2 and Ki-67 LIs. It was therefore speculated that assessment of cell-cycle state by MCM-2 and Ki-67 may be a useful diagnostic adjunct in the histopathological assessment of myxofibrosarcoma, by enabling more accurate determination of grade and prediction of outcome (Sington et al., 2004). The same authors also revealed that MCM-2 was more frequently expressed than Ki-67 in 69 cases of malignant mesothelioma, including either reactive mesothelial hyperplasia or reactive pleural fibrosis. Counts in areas of maximum tumor staining showed significantly higher MCM-2 LIs in epithelioid and sarcomatoid mesotheliomas compared to reactive mesothelial hyperplasia and reactive pleural fibrosis. There was also a significant increase in MCM-2 LIs of epithelioid mesothelioma compared to reactive mesothelial hyperplasia (Sington et al., 2003). In a more recent study, Matsubara et al. immunohistochemically evaluated the expression of MCM-2 and caspase-3, as proliferation and apoptosis markers, respectively, in pre- and post- radio-hyperthermo-chemotherapy (RHC) specimens of 41 soft tissue and bone tumours (Matsubara et al., 2008). Response scores showed positive correlation with pre-RHC MCM-2 and post-RHC caspase 3 indices, inverse correlation with post-RHC MCM-2 and post-RHC growth indices and no correlation with prognosis. Multivariate analysis revealed high pre-RHC MCM-2 and high post-RHC MCM-2/caspase-3 indices as significant, unfavorable prognostic factors. Thus, it was supported that high proliferative activity in untreated sarcoma may predict good response to neoadjuvant therapy, but poor prognosis, whereas a high growth index, such as a high proliferation/apoptosis ratio (MCM-2/caspase-3) in a post-neoadjuvant therapy tumour specimen may be indicative of poor response and poor prognosis (Matsubara et al., 2008).

Conclusions

In the last few years, accumulative evidence has revealed that MCMs are characterized by higher specificity and sensitivity than other conventional proliferative markers. The most comprehensive data so far indicates that MCMs detect more cells in cycle than Ki-67 or PCNA in a range of normal and malignant tissue types from different organs and tissues, including larynx, stomach, liver, lung, breast, endometrium, cervix and brain, as well as soft tissue and lymphoma cells. The high specificity and sensitivity of MCMs are ascribed to the fact that PCNA and Ki-67 are required for the initiation of replication, representing the point of convergence of many signaling pathways involved in cell growth. On the other hand, MCMs are not associated with DNA repair like PCNA, while in the case of quiescence and resting cells MCMs still maintain replication competence in contrast to Ki-67. Moreover, MCMs mark all non-quiescent cells, whereas geminin identifies the sub-fraction that has entered the S-phase, but not exited mitosis. It was therefore speculated that MCMs could constitute better candidates for marking cells in cycle than Ki-67 and PCNA, as well as other transduction molecules or growth factor receptors.

It is certainly well-established that MCMs are overexpressed in various tumors and thus may affect many aspects of cancer biology. Elevated expression of several members of the MCM family was reported in the

malignant tumor stage of several organs, including larynx, thyroid, colon, pancreas, lung, ovary, cervix, kidney and prostate, as well as soft tissues and lymphomas. Despite the variety of methods used and the criteria of MCM expression definition, most studies suggested that MCMs play an important role in several types of human malignancy, being associated with important clinicopathological parameters for patient management. Among the members of the MCM family, MCM-2 has been studied in a wide range of human malignancies. In oral, colon, lung, breast, ovary, renal, urothelial and neurological carcinomas, MCM-2 expression was associated with tumor histopathological grade. MCM-2 expression was also associated with tumor histopathological stage in several malignancies, such as oral, colon, ovarian and urothelial carcinomas, but not in the cases of NSCLCs and RCCs. On the other hand, a lower number of studies evaluated the expression of MCM-5 and MCM-7 members, indicating significant associations with tumor histopathological grade in ovarian, cervical and urothelial carcinomas concerning MCM-5 and in endometrial and prostatic malignancies regarding MCM-7. For MCM-3, MCM-4 and MCM-6, the available data so far are extremely limited and no safe conclusions can be drawn. Thus, as the majority of studies so far are restricted to MCM-2, future research should be extended to the other members of the MCM family.

The most comprehensive data to date also documented that MCMs are promising prognostic markers in several types of cancer. MCM-2 expression was shown to correlate with the survival rate and prognosis in several human malignancies, such as oral, lung, ovarian, breast, renal, prostatic, urothelial and neurological carcinomas. Concerning the less studied members of the MCM family, a significant prognostic value for MCM-5 in ovarian and urothelial carcinomas was reported, while MCM-7 was associated with patient survival in endometrial and prostatic carcinomas. In most studies, multivariate analysis identified MCM expression as an independent negative predictor for patient survival. However, it should be noted that the available studies so far have not used a standard definition for MCM expression. Most studies evaluated MCM immunohistochemical expression based on the percentage of MCM positive malignant cells. However, they used different cut-off points for MCM expression definition. Thus, it is extremely important to define a standard criterion, as well as to establish precise cut-off points for MCM expression in order to consider MCM expression as a diagnostic and prognostic factor in routine clinical settings. Moreover, some of the studies were conducted on a limited number of cases, which further increases the probability of errors in the statistical analysis of the data, while the evidence obtained from studies performed on large clinical samples are far more reliable.

In this aspect, TMA technology could be useful in reliably evaluating the diagnostic and prognostic value of MCMs, since it can provide a time and cost-effective method to rapidly study a large number of samples in one slide without significant damage to the donor tissue block. However, attention should be taken in the selection of representative tissue to avoid missing important areas, especially in a heterogeneous tumor. It should also be kept in mind that the implementation of a novel molecular marker in clinical practice is justified only, if additional information is added to established parameters, while there is no major interest in purely molecular aspects relating to others unless basic insight or clinical guidance is provided. Thus, MCM proteins could be of interest in the management of malignancy only if it offers substantial diagnostic or prognostic value.

References

Ayaru L., Stoeber K., Webster G.J., Hatfield A.R., Wollenschlaeger A., Okoturo O., Rashid M., Williams G. and Pereira S.P. (2008). Diagnosis of pancreaticobiliary malignancy by detection of minichromosome maintenance protein 5 in bile aspirates. Br. J. Cancer 98, 1548-1554.

Ananthanarayanan V., Pins M.R., Meyer R.E. and Gann P.H. (2005). Immunohistochemical assays in prostatic biopsies processed in Bouin's fixative. J. Clin. Pathol. 58, 322-324.

Ananthanarayanan V., Deaton R.J., Yang X.J., Pins M.R. and Gann P.H. (2006). Alteration of proliferation and apoptotic markers in normal and premalignant tissue associated with prostate cancer. BMC Cancer 6, 73.

Baldwin P., Laskey R. and Coleman N. (2003). Translational approaches to improving cervical screening. Nat. Rev. Cancer 3, 217-226.

Bainsh H. and Gerdes J. (1987). Simultaneous staining of exponentially growing versus plateau phase cells with the proliferation-associated antibody Ki67 and propidium iodide: analysis by flow cytometry. Cell Tissue Kinet. 20, 387-391.

Bell S.P. and Dutta A. (2002). DNA replication in eukaryotic cells. Annu. Rev. Biochem. 71, 333-374.

Boyd A.S., Shakhtour B. and Shyr Y. (2008). Minichromosome maintenance protein expression in benign nevi, dysplastic nevi, melanoma, and cutaneous melanoma metastases. J. Am. Acad. Dermatol. 58, 750-754.

Brown D.C. and Gatter K.C. (2002). Ki67 protein: the immaculate deception? Histopathology 40, 2-11.

Burger M., Denzinger S., Hartmann A., Wieland W.F., Stoehr R. and Obermann E.C. (2007). Mcm2 predicts recurrence hazard in stage Ta/T1 bladder cancer more accurately than CK20, Ki67 and histological grade. Br. J. Cancer 96, 1711-5171.

Chang V.K., Fitch M.J., Donato J.J., Christensen T.W., Merchant A.M. and Tye B.K. (2003). Mcm1 binds replication origins. J. Biol. Chem. 278, 6093-6100.

Chang V.K., Donato J.J., Chan C.S. and Tye B.K. (2004). Mcm1 promotes replication initiation by binding specific elements at replication origins. Mol. Cell. Biol. 24, 6514-6524.

Chatrath P., Scott I.S., Morris L.S., Davies R.J., Rushbrook S.M., Bird K., Vowler S.L., Grant J.W., Saeed I.T., Howard D., Laskey R.A. and Coleman N. (2003). Aberrant expression of minichromosome maintenance protein-2 and Ki67 in laryngeal squamous epithelial

lesions. Br. J. Cancer 89, 1048-1054.

Chatrath P., Scott I.S., Morris L.S., Davies R.J., Bird K., Vowler S.L. and Coleman N. (2006). Immunohistochemical estimation of cell cycle phase in laryngeal neoplasia. Br. J. Cancer 95, 314-321.

Cho M.K., Eimoto T., Nagaya S. and Tateyama H. (2006). Cell proliferation marker MCM2, but not Ki67, is helpful for distinguishing between minimally invasive follicular carcinoma and follicular adenoma of the thyroid. Histopathology 48, 801-807.

Davies R.J., Freeman A., Morris L.S., Bingham S., Dilworth S., Scott I., Laskey R.A., Miller R and Coleman N. (2002). Analysis of minichromosome maintenance proteins as a novel method for detection of colorectal cancer in stool. Lancet 359, 1917-1919.

Davies R.J., Scott I.S., Morris L.S., Rushbrook S.M., Bird K., Vowler S.L., Arends M., Miller R. and Coleman N. (2004). Increased expression of minichromosome maintenance protein 2 in active inflammatory bowel disease. Colorectal Dis. 6, 103-110.

Davidson E.J., Morris L.S., Scott I.S., Rushbrook S.M., Bird K., Laskey R.A., Wilson G.E., Kitchner H.C., Coleman N. and Stern P.L. (2003). Minichromosome maintenance (Mcm) proteins, cyclin B1 and D1, phosphohistone H3 and in situ DNA replication for functional analysis of vulval intraepithelial neoplasia. Br. J. Cancer 88:257-262.

Dudderidge T.J., Stoeber K., Loddo M., Atkinson G., Fanshawe T., Griffiths D.F., and Williams G.H. (2005). Mcm2, Geminin, and Ki67 define proliferative state and are prognostic markers in renal cell carcinoma. Clin. Cancer Res. 11, 2510-2517.

Dudderidge T.J., McCracken S.R., Loddo M., Fanshawe T.R., Kelly J.D., Neal D.E., Leung H.Y., Williams G.H. and Stoeber K. (2007). Mitogenic growth signalling, DNA replication licensing, and survival are linked in prostate cancer. Br. J. Cancer 96, 1384-1393.

Endl E., Kausch I., Baack M., Knippers R., Gerdes J. and 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.

Facoetti A., Ranza E., Grecchi I., Benericetti E., Ceroni M., Morbini P. and Nano R. (2006a). Immunohistochemical evaluation of minichromosome maintenance protein 7 in astrocytoma grading. Anticancer Res. 26, 3513-3516.

Facoetti A., Ranza E., Benericetti E., Ceroni M., Tedeschi F., Nano R. (2006b). Minichromosome maintenance protein 7: a reliable tool for glioblastoma proliferation index. Anticancer Res. 26, 1071-1075.

Freeman A., Morris L.S., Mills A.D., Stoeber K., Laskey R.A., Williams G.H. and Coleman N. (1999). Minichromosome maintenance proteins as biological markers of dysplasia and malignancy. Clin. Cancer Res. 5, 2121-2132.

Freeman A., Hamid S., Morris L., Vowler S., Rushbrook S., Wight D.G., Coleman N. and Alexander G.J. (2003). Improved detection of hepatocyte proliferation using antibody to the pre-replication complex: an association with hepatic fibrosis and viral replication in chronic hepatitis C virus infection. J. Viral Hepat. 10, 345-350.

Gakiopoulou H., Korkolopoulou P., Levidou G., Thymara I., Saetta A., Piperi C., Givalos N., Vassilopoulos I., Ventouri K., Tsenga A., Bamias A., Dimopoulos M.A., Agapitos E. and Patsouris E. (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.

Gambichler T., Bischoff S., Bechara F.G., Altmeyer P. and Kreuter A. (2008). Expression of proliferation markers and cell cycle regulators in T cell lymphoproliferative skin disorders. J. Dermatol. Sci. 49, 125-132.

Giaginis C., Georgiadou M., Dimakopoulou K., Tsourouflis G., Gatzidou E., Kouraklis G. and Theocharis S. (2009). Clinical significance of MCM-2 and MCM-5 expression in colon cancer: Association with clinicopathological parameters and tumor proliferative capacity. Dig. Dis. Sci. 54, 282-291.

Going J.J., Keith W.N., Neilson L., Stoeber K., Stuart R.C. and Williams G.H. (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., Pinder S.E., Callagy G., Vowler S.L., Morris L.S., Bird K., Bell J.A., Laskey R.A. and Coleman N. (2003). Minichromosome maintenance protein 2 is a strong independent prognostic marker in breast cancer. J. Clin. Oncol. 21, 4306-4313.

Gray D., Obermann E.C., Evans M., Hartmann A., Cooper K. and Blaszyk H. (2006). MIB-1 and MCM-2 immunohistochemical analysis does not aid in identification of serrated colorectal polyps with abnormal proliferation. Am. J. Clin. Pathol. 125, 407-412.

Guida T., Salvatore G., Faviana P., Ginnini R., Garcia-Rostan G., Provitera L., Basolo F., Fusco A., Carlomagno F. and Santoro M. (2005). Mitogenic effects of the up-regulation of minichromosome maintenance proteins in anaplastic thyroid carcinoma. J. Clin. Endocrinol. Metab. 90, 4703-4709.

Guzinska-Ustymowicz K., Stepien E. and Kemona A. (2008). MCM-2, Ki-67 and PCNA protein expressions in pT3G2 colorectal cancer indicated lymph node involvement. Anticancer Res. 28, 451-457.

Ha S.A., Shin S.M., Namkoong H., Lee H., Cho G.W., Hur S.Y., Kim T.E. and Kim J.W. (2004). Cancer-associated expression of minichromosome maintenance 3 gene in several human cancers and its involvement in tumorigenesis. Clin. Cancer Res. 10, 8386-8395.

Hashimoto K., Araki K., Osaki M., Nakamura H., Tomita K., Shimizu E. and Ito H. (2004). MCM2 and Ki-67 expression in human lung adenocarcinoma: prognostic implications. Pathobiology 71, 193-200.

Helfenstein A., Frahm S.O., Krams M., Drescher W., Parwaresch R. and Hassenpflug J. (2004). Minichromosome maintenance protein (MCM6) in low-grade chondrosarcoma: distinction from enchondroma and identification of progressive tumors. Am. J. Clin. Pathol. 122, 912-918.

Hiraiwa A., Fujita M., Adachi A., Ono H., Nagasaka T., Matsumoto Y., Ohashi M., Tomita Y. and Ishibashi M. (1998). Specific distribution patterns of hCDC47 expression in cutaneous diseases. J. Cutan. Pathol. 25, 285-290.

Huang H.Y., Huang W.W., Lin C.N., Eng H.L., Li S.H., Li C.F., Lu D., Yu S.C. and Hsiung C.Y. (2006). Immunohistochemical expression of p16INK4A, Ki-67, and Mcm2 proteins in gastrointestinal stromal tumors: prognostic implications and correlations with risk stratification of NIH consensus criteria. Ann. Surg. Oncol. 13, 1633-1644.

Huang X.P., Rong T.H., Wu Q.L., Fu J.H., Yang H., Zhao J.M. and Fang Y. (2005). MCM4 expression in esophageal cancer from southern China and its clinical significance. J. Cancer Res. Clin. Oncol. 131, 677-682.

Hunt D.P., Freeman A., Morris L.S., Burnet N.G., Bird K., Davies T.W., Laskey R.A. and Coleman N. (2002). Early recurrence of benign meningioma correlates with expression of mini-chromosome maintenance-2 protein. Br. J. Neurosurg. 16, 10-15.

Ishimi Y., Okayasu I., Kato C., Kwon H.J., Kimura H., Yamada K. and Song S.Y. (2003). Enhanced expression of Mcm proteins in cancer cells derived from uterine cervix. Eur. J. Biochem. 270, 1089-1101.

Kato H., Miyazaki T., Fukai Y., Nakajima M., Sohda M., Takita J., Masuda N., Fukuchi M., Manda R., Ojima H., Tsukada K., Asao T. and Kuwano H. (2003a). A new proliferation marker, minichromosome maintenance 2, is associated with tumor aggressiveness in esophageal squamous cell carcinoma. J. Surg. Oncol. 84, 24-30.

Kato K., Toki T., Shimizu M., Shiozawa T., Fujii S., Nikaido T. and Konishi I. (2003b). Expression of replication-licensing factors MCM2 and MCM3 in normal, hyperplastic, and carcinomatous endometrium: correlation with expression of Ki-67 and estrogen and progesterone receptors. Int. J .Gynecol. Pathol. 22, 334-340.

Kebebew E., Peng M., Reiff E., Duh Q-Y., Clark O.H. and McMillan A. (2006). Diagnostic and prognostic value of cell-cycle regulatory genes in malignant thyroid neoplasms. World J. Surg. 30, 767-774.

Kodani I., Shomori K., Osaki M., Kuratate I., Ryoke K. and Ito H. (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 I., Osaki M., Shomori K., Araki K., Goto E., Ryoke K. and Ito H. (2003). Minichromosome maintenance 2 expression is correlated with mode of invasion and prognosis in oral squamous cell carcinomas. J. Oral Pathol. Med. 32, 468-474.

Korkolopoulou P., Givalos N., Saetta A., Goudopoulou A., Gakiopoulou H., Thymara I., Thomas-Tsagli E. and Patsouris E. (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.

Krüger S., Thorns C., Stöcker W., Müller-Kunert E., Böhle A. and Feller A.C. (2003). Prognostic value of MCM2 immunoreactivity in stage T1 transitional cell carcinoma of the bladder. Eur. Urol. 43, 138-145.

Laitinen S., Martikainen P.M., Tolonen T., Isola J., Tammela T.L. and Visakorpi T. (2008). EZH2, Ki-67 and MCM7 are prognostic markers in prostatectomy treated patients. Int. J. Cancer 122, 595-602.

Lampert I.A., Horncastle D., Dilworth S., Roberts I., Alison M.R. and Naresh K.N. (2005). The expression of minichromosome maintenance protein-2 in normal and abnormal megakaryocytes and comparison with the proliferative marker Ki-67. Br. J. Haematol. 131, 490-494.

Laskey R.A. and Madine M.A. (2003). A rotary pumping model for helicase function of MCM proteins at a distance from replication forks. EMBO Rep. 4, 26-30.

Li S.S., Xue W.C., Khoo U.S., Ngan H.Y., Chan K.Y., Tam I.Y., Chiu P.M., Ip P.P., Tam KF. and Cheung A.N. (2005). Replicative MCM7 protein as a proliferation marker in endometrial carcinoma: a tissue microarray and clinicopathological analysis. Histopathology 46, 307-313.

Liu H., Takeuchi S., Moroi Y., Lin N., Urabe K., Kokuba H., Imafuku S., Dainichi T., Uchi H., Furue M. and Tu Y. (2007). Expression of minichromosome maintenance 5 protein in proliferative and malignant skin diseases. Int. J. Dermatol. 46, 1171-1176.

Lutzmann M., Maiorano D. and Mechali M. (2005). Identification of full genes and proteins of MCM9, a novel, vertebrate-specific member of the MCM2-8 protein family. Gene 362, 51-56.

MacCallum D.E. and Hall P.A. (2000). The location of pKi67 in the outer dense fibrillary compartment of the nucleolus points to a role in ribosome biogenesis during the cell division cycle. J. Pathol. 190, 537-544.

Maiorano D., Cuvier O., Danis E. and Mechali M. (2005). MCM8 is an MCM2-7-related protein that functions as a DNA helicase during replication elongation and not initiation. Cell 120, 315-328

Maiorano D., Lutzmann M. and Merchali M. (2006). MCM proteins and DNA replication. Curr. Opin. Cell. Biol. 18, 130-136.

Marshall A., Rushbrook S., Davies S.E., Morris L.S., Scott I.S., Vowler S.L., Coleman N. and Alexander G. (2005a). Relation between hepatocyte G1 arrest, impaired hepatic regeneration, and fibrosis in chronic hepatitis C virus infection. Gastroenterology 128, 33-42.

Marshall A., Rushbrook S., Morris L.S., Scott I.S., Vowler S.L., Favies S.E., Coleman N. and Alexander G. (2005b). Hepatocyte expression of minichromosome maintenance protein-2 predicts fibrosis progression after transplantation for chronic hepatitis C virus: a pilot study. Liver Transpl. 11, 427-433.

Matsubara T., Eimoto T., Okabe M., Miyabe S., Fujiyoshi Y., Matsushita Y., Mizutani J., Yamada S. and Otsuka T. (2008). Proliferation and apoptosis of tumour cells before and after neoadjuvant therapy for high-grade extremity sarcomas: divergent associations with tumour response and prognosis. Histopathology 52, 706-716.

Mehrotra P., Gonzalez M.A., Johnson S.J., Coleman N., Wilson J.A., Davies B.R., and Lennard T.W.J. (2006). Mcm-2 and Ki-67 have limited potential in preoperative diagnosis of thyroid malignancy. Laryngoscope 116, 1434-1438.

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

Mukherjee G., Muralidhar B., Bafna U.D., Laskey R.A. and Coleman N. (2007). MCM immunocytochemistry as a first line cervical screening test in developing countries: a prospective cohort study in a regional cancer centre in India. Br. J. Cancer 96, 1107-1111.

Murphy N., Ring M., Heffron C.C., King B., Killalea A.G., Hughes C., Martin C.M., McGuinness E., Sheils O. and O' Leary J.J. (2005). p16INK4A, CDC6, and MCM5: predictive biomarkers in cervical preinvasive neoplasia and cervical cancer. J. Clin. Pathol. 58, 525-534.

Niklaus A.L., Aubuchon M., Zapantis G., Li P., Quian H., Isaac B., Kim M.Y., Adel G., Pollard J.W. and Santoro N.F. (2007). Assessment of the proliferative status of epithelial cell types in the endometrium of young and menopausal transition women. Hum. Reprod. 22, 1778-1788.

Obermann E.C., Went P., Zimpfer A., Tzankov A., Wild P.J., Stoehr R., Pileri S.A. and Dimhofer S. (2005). Expression of minichromosome maintenance protein 2 as a marker for proliferation and prognosis in diffuse large B-cell lymphoma: a tissue microarray and clinico-pathological analysis. BMC Cancer 5, 162.

Obermann E.C., Went P., Tzankov A., Pileri S.A., Hofstaedter F., marienhagen J., Stoehr R. and Dirnhofer S. (2007). Cell cycle phase distribution analysis in chronic lymphocytic leukaemia: a significant number of cells reside in early G1-phase. J. Clin. Pathol. 60, 794-797.

Osaki M., Osaki M., Yamashita H., Shomori K., Yoshida H. and Ito H. (2002). Expression of minichromosome maintenance-2 in human malignant fibrous histiocytomas: Correlations with Ki-67 and P53 expression, and apoptosis. Int. J. Mol. Med. 10, 161-168.

Padmanabhan V., Callas P., Philips G., Trainer T.D. and Beatty B.G. (2004). DNA replication regulation protein Mcm7 as a marker of

proliferation in prostate cancer. J. Clin. Pathol. 57, 1057-1062.

Quaglia A., McStay M., Stoeber K., Loddo M., Caplin M., Fanshawe T., Williams G. and Dhillon A. (2006). Novel markers of cell kinetics to evaluate progression from cirrhosis to hepatocellular carcinoma. Liver Int. 26, 424-432.

Ramnath N., Hernandez F.J., Tan D.F., Huberman J.A., Beck A.F., Hyland A., Todorov I.T., Brooks J.S. and Bepler G. (2001). MCM2 is an independent predictor of survival in patients with non-small-cell lung cancer. J. Clin. Oncol. 19, 4259-4266.

Ricke R.M. and Bielinsky A.K. (2004). Mcm10 regulates the stability and chromatin association of DNA polymerase-α. Mol. Cell. 16, 173-185.

Rodins K., Cheale M., Coleman N. and Fox S.B. (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.

Romanowski P. and Madine M.A.. (1997). Mechanisms restricting DNA replication to once per cell cycle: the role of Cdc6 and ORC. Trends Cell. Biol. 7, 9-10.

Schrader C., Janssen D., Klapper W., Siebmann J.U., Meusers P., Britinger G., Kneba M., Tiemann M. and Parwaresch R. (2005). Minichromosome maintenance protein 6, a proliferation marker superior to Ki-67 and independent predictor of survival in patients with mantle cell lymphoma. Br. J. Cancer 93, 939-945.

Scott I.S., Morris L.S., Bird K., Davies R.J., Vowler S.L, Rushbrook S.M., Marshall A.E., Laskey R.A., Miller R., Arends M.J. and Coleman N. (2003). A novel immunohistochemical method to estimate cell-cycle phase distribution in archival tissue: implications for the prediction of outcome in colorectal cancer. J. Pathol. 201, 187-197.

Scott I.S., Heath T.M., Morris L.S., Rushbrook S.M., Bird K., Vowler S.L., Arends M.J. and Coleman N. (2004). A novel immunohistochemical method for estimating cell cycle phase distribution in ovarian serous neoplasms: implications for the histopathological assessment of paraffin-embedded specimens. Br. J. Cancer 90, 1583-1590.

Scott I.S., Morris L.S., Rushbrook S.M., Bird K., Vowler S.L., Burnet N.G. and Coleman N. (2005). Immunohistochemical estimation of cell cycle entry and phase distribution in astrocytomas: applications in diagnostic neuropathology. Neuropathol. Appl. Neurobiol. 31, 455-466.

Scott I.S., Odell E., Chatrath P., Morris L.S., Davies R.J., Vowler S.L., Laskey R.A. and Coleman N. (2006). A minimally invasive immunocytochemical approach to early detection of oral squamous cell carcinoma and dysplasia. Br. J. Cancer 94, 1170-1175.

Sington J.D., Morris L.S., Nicholson A.G. and Coleman N. (2003). Assessment of cell cycle state may facilitate the histopathological diagnosis of malignant mesothelioma. Histopathology 42, 498-502.

Sington J.D., Freeman A., Morris L.S., Vowler S.L., Arch B.N. and Fisher C. (2004). Minichromosome maintenance protein in myxofibrosarcoma. Mod. Pathol. 17, 235-240.

Sirieix P.S., O'Donovan M., Brown J., Save V., Coleman N. and Fitzgerald R.C. (2003). Surface expression of minichromosome maintenance proteins provides a novel method for detecting patients at risk for developing adenocarcinoma in Barrett's esophagus. Clin. Cancer Res. 9, 2560-2566.

Söling A., Sackewitz M., Volkmar M., Schaarschmidt D., Jacob R., Holzhausen H.J. and Rainov N.G. (2005). Minichromosome maintenance protein 3 elicits a cancer-restricted immune response in patients with brain malignancies and is a strong independent predictor of survival in patients with anaplastic astrocytoma. Clin. Cancer Res. 11, 249-258.

Szelachowska J., Dziegiel P., Jelen-Krzeszewska J., Jelen M., Matkowski R., Pomiencko A., Spytkowska B., Jagas M., Gisterek I. and Kornafel J. (2006). Mcm-2 protein expression predicts prognosis better than Ki-67 antigen in oral cavity squamocellular carcinoma. Anticancer Res. 26, 2473-2478.

Shetty A., Loddo M., Fanshawe T., Prevost A.T., Sainsbury R., Williams G.H. and Stoeber K. (2005). DNA replication licensing and cell cycle kinetics of normal and neoplastic breast. Br. J. Cancer 93, 1295-300.

Shima N., Buske T.R. and Schimenti J.C. (2007). Genetic screen for chromosome instability in mice: Mcm4 and breast cancer. Cell Cycle 6, 1135-1140.

Stoeber K., Swinn R., Prevost A.T., de Clive-Lowe P., Halsall I., Dilwort S.M., Marr J., Turner W.H., Bullock N., Doble A., Hales C.N. and Williams G.H. (2002). Diagnosis of genito-urinary tract cancer by detection of minichromosome maintenance 5 protein in urine sediments. J. Natl. Cancer Inst. 94, 1071-1079.

Tachibana K.K., Gonzalez M.A. and Coleman N. (2005). Cell-cycle-dependent regulation of DNA replication and its relevance to cancer pathology. J. Pathol. 205, 123-129.

Takisawa H., Mimura S. and Kubota Y. (2000). Eukaryotic DNA replication: from-replication complex to initiation complex. Curr. Opin. Cell. Biol. 12, 690-696.

Tan D.F., Huberman J.A., Hyland A., Loewen G.M., Brooks J.S., Beck A.F., Todorov I.T. and Bepler G. (2001). MCM2-a promising marker for premalignant lesions of the lung: a cohort study. BMC Cancer 1, 6.

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

Theocharis S.E., Skopelitou A.S., Margeli A.P., Pavlaki K.J. and Kittas C. (1994). Proliferating cell nuclear antigen (PCNA) expression in regenerating rat liver after partial hepatectomy. Dig. Dis. Sci. 39, 245-252.

Tokuyasu N., Shomori K., Nishihara K., Kawaguchi H., Fujioka S., Yamaga K., Ikeguchi M. and Ito H. (2008). Minichromosome maintenance 2 (MCM2) immunoreactivity in stage III human gastric carcinoma: clinicopathological significance. Gastric Cancer 11, 37-46.

Toshi L. and Bravo R. (1988). Changes in cyclin/proliferating cell nuclear antigen distribution during DNA repair synthesis. J. Cell. Biol. 107, 1623-1628.

Vargas P.A., Cheng Y., Barrett A.W., Craig G.T. and Speight P.M. (2008). Expression of Mcm-2, Ki-67 and geminin in benign and malignant salivary gland tumours. J. Oral Pathol. Med. 37, 309-318.

Yang J., Ramnath N., Moysich K.B., Asch H.L., Swede H., Alrawi S.J., Huberman J., Geradts J., Brooks J.S. and Tan D. (2006). Prognostic significance of MCM2, Ki-67 and gelsolin in non-small cell lung cancer. BMC Cancer 6, 203.

Wei Q., Li Y., Chen L., Zhang L., He X., Fu X., Ying K., Huang J., Chen Q., Xie Y. and Mao Y. (2006). Genes differentially expressed in responsive and refractory acute leukemia. Front. Biosci. 11, 977-982.

Williams G.H., Romanowski P., Morris L., Madine M., Mills A.D., Stoeber K., Marr J., Laskey R.A. and Coleman N. (1998). Improved

cervical smear assessment using antibodies against proteins that regulate DNA replication. Prco. Natl. Acad. Sci. USA 5, 14932-14937.

Williams G.H., Swinn R., Prevost A.T., De Clive-Lowe P., Halsall I., Going J.J., Hales C.N., Stoeber K. and Middleton S.J. (2004). Diagnosis of oesophageal cancer by detection of minichromosome maintenance 5 protein in gastric aspirates. Br. J. Cancer 91, 714-719.

Wharton S.B., Chan K.K., Anderson J.R., Stoeber K. and Williams C.H. (2001). Replicative Mcm2 protein as a novel proliferation marker in oligodendrogliomas and its relationship to Ki-67 labeling index, histological grade and prognosis. Nephropathol. Appl. Neurobiol. 27, 305-313.

Xu J., Zhang S., You C., Huang S., Cai B. and Wang X. (2007). Expression of human MCM6 and DNA Topo II alpha in craniopharyngiomas and its correlation with recurrence of the tumor. J. Neurooncol. 83, 183-189.

Winnepenninckx V., Lazar V., Michiels S., Dessen P., Stas M., Alonso S.R., Avril M.F., Ortiz Romero P.L., Robert T., Balacescu O., Eggermont A.M., Lenoir G., Sarasin A., Tursz T., van den Oord J.J., Spatz A. and Melanoma Group of the European Organization for Research and Treatment of Cancer. (2006). Gene expression profiling of primary cutaneous melanoma and clinical outcome, J. Natl. Cancer Inst. 98, 472-482.

Accepted August 11, 2009