Mcm-2 Protein Expression Predicts Prognosis Better than Ki-67 Antigen in Oral Cavity Squamocellular Carcinoma

JOLANTA SZELACHOWSKA 1, PIOTR DZIEGIEL 2, JOANNA JELEN-KRZESZEWSKA 3, MICHAL JELEN 4, RAFAL MATKOWSKI 1, AGNIESZKA POMIECKO 5, BARBARA SPYTKOWSKA 5, MARIA JAGAS 5, IWONA GISTEREK 1 and JAN KORNAFEL 1

Departments of 1Oncology, 2Histology and Embryology, 3Maxillofacial Surgery and 4Pathology, Wroclaw Medical University, Wroclaw; 5Department of Radiotherapy, Lower Silesian Oncology Center, Wroclaw, Poland

Abstract. Background: The present study aimed at evaluating the expression intensities of the Mcm-2 protein and Ki-67 antigen in squamocellular carcinomas of the oral cavity and comparing their prognostic value. Materials and Methods: Forty-nine patients, operated on and treated with radiotherapy for carcinoma of the oral cavity floor and/or the mobile part of the tongue, were retrospectively analyzed. Results: A significant positive correlation was noted between the expression of Mcm-2 protein and that of the Ki-67 antigen, as well as an absence of such correlations with the remaining examined factors. A significant correlation with worse disease-specific survival period (DSS) in the group of patients demonstrating Mcm-2 protein expression in over 10% of cancer cells was detected (5-year cumulative DSS 50% vs. 76%). Conclusion: The results suggested that the expression of Mcm-2 protein may be used as a prognostic factor in patients with squamocellular carcinoma of the oral cavity.

Cancer of the oral cavity comprises less than 4% of total morbidity from malignant tumors in Europe. The squamocellular type accounts for over 90% of cancer diagnosed in the oral cavity. Most tumors develop multifocally and exhibit a marked tendency to relapse. The prognosis depends on the primary location of the tumor, its grade of malignancy (G), type of invasion and involvement of lymph nodes (1, 2). The proliferation markers currently applied, such as Ki-67, provide only a restricted picture of the involvement of the cells in the cell cycle (3-9). Moreover, the value of Ki-67 as a prognostic factor remains to be fully documented and the results of studies which evaluated the relationship between cell proliferation and the clinical course of the disease are contradictory (10-16). This can be attributed, to some extent, to the fact that the function of the Ki-67 protein remains unknown and the protein is not a key element in cell proliferation (17). The expression of Ki-67 may also appear when the synthesis of DNA is blocked or in cells undergoing apoptosis (7).

The Mcm-2 protein belongs to the family of six minichromosome maintenance 2-7 proteins (Mcm 2-7), engaged in recognition and control of DNA replication (18). In the early G1-phase of the cell cycle, Mcm proteins participated in the formation of the pre-replication complex (19-21). They bound to appropriate DNA sequences and, joined by Cdc6 proteins, formed the pre-replication complex (18, 20, 22). Mcm, representing a component of the pre-replication complex, exhibited the activity of helicase, which unwinds the DNA thread during replication (23). This allows for access to appropriate sites on the DNA and allows for replication. In the course of the S-phase, Mcm proteins became irreversibly detached from chromatin, assuring that DNA replication took place only once in the cell cycle (24). In the case of eukaryotic cells, Mcm proteins played a key role in DNA replication (18). In several studies, the expression of Mcm was demonstrated in cells remaining in the cell cycle, while loss of Mcm expression reflected the resting state of the cells (25-28). Thus, Mcm proteins are important controllers of DNA replication in eukaryotic cells and may potentially provide a useful marker of proliferation (20).

The present study aimed at evaluating the intensity of Mcm-2 expression as compared to that of Ki-67 antigen expression, as well as evaluating their usefulness as prognostic factors in patients with oral cavity carcinoma, treated surgically with subsequent radiotherapy. Our aims...
also included testing for a correlation between the Mcm-2 and Ki-67 indices compared to clinicopathological variables.

Materials and Methods

Patients. The studies were retrospectively performed on tumor samples from 49 patients with a diagnosis of squamouscellular carcinoma of the oral cavity floor or of the oral part of the tongue. All the patients were subjected to radical surgery, accompanied by removal of lymph nodes and post-operative radiotherapy, conducted in the Wroclaw Medical University (WMU) and in the Lower Silesian Centre of Oncology (LSCO), Poland, from 1996-2002. The mean age of the patients was 56 years, and the group included nine women and 40 men. In 30 patients the primary neoplastic lesion was located in the floor of the oral cavity, in 11 patients in the mobile part of the tongue, while in the remaining patients diffuse infiltration of both structures was observed.

The survival of the patients (DFS – disease-free survival, OS - overall survival; NS=not significant).

<table>
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<tr>
<th>Patient characteristics</th>
<th>Number of patients</th>
<th>5-year DFS</th>
<th>5-year DSS</th>
<th>5-year OS</th>
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<tr>
<td>Women</td>
<td>9</td>
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<tr>
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<td>56%</td>
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<td>76%</td>
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<tr>
<td>Positive lymph nodes</td>
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<td>55%</td>
<td>49%</td>
<td>NS</td>
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</table>

DFS: disease-free survival; DSS: disease-specific survival; OS: overall survival; NS=not significant.

Immunohistochemistry. The tumor samples were fixed in 10% buffered formalin, dehydrated and embedded in paraffin blocks. All immunohistochemical reactions were conducted in paraffin sections. For the estimation of Mcm-2 and Ki-67 antigen expressions, mouse monoclonal antibodies were used (clone CRCT2.1; 1:50; Novocastra Laboratories, UK, and clone MIB-1; 1:50; DAKO, Denmark, respectively). All the reactions were accompanied by negative controls, in which specific antibodies were substituted by the primary negative control. The studied paraffin sections were boiled in antigen retrieval solution in a microwave oven to unblock antigenic determinants. The investigated antigens were visualized using biotinylated antibodies, streptavidin-biotinylated peroxidase complex (LSAB2 kit) and diaminobenzidine (DAB). All the reagents employed were produced by DAKO.

The intensity of the immunohistochemical reactions was independently evaluated in coded preparations by two pathologists. The evaluation of the Ki-67 antigen and Mcm-2 protein expressions was conducted using a scale which took into account the percentage of cells manifesting nuclear color reactions: no reaction – 0 points, 1-10% – 1 point, 11-25% – 2 points, 26-50% – 3 points and over 50% – 4 points

Results

In the entire studied group, the 5-year cumulative DFS was 60%, the DSS was 63% and the OS was 54%. The 5-year survival results in the patient subgroups are provided in Table I. The staining results for both markers are summarized in Table II. The intensity of the reaction with anti-Mcm-2 antibodies was appraised to be no patients in eight cases, one patient in 14 cases (Figure 1A), two patients in eleven cases (Figure 1C), three patients in twelve cases (Figure 1E) and four patients in four cases. The intensity of the reaction with anti-Ki-67 antibodies was appraised, respectively, to be no patients in four cases, one patient in 14 cases (Figure 1B), two patients in 18 cases (Figure 1D),
three patients in nine cases (Figure 1F) and four patients in four cases. A significant correlation ($R=0.8; \ p<0.05$) between the expression of Mcm-2 and that of Ki-67 was detected (Figure 2). No significant correlation could be detected between the proportion of cells with Mcm-2 and Ki-67 expressions and the remaining clinicopathological factors studied. All the evaluated clinicopathological variables were examined with respect to their relationship.
to patient survival. A significantly shorter DSS was detected among patients with tumors where over 10% of the cells manifested Mcm-2 protein expression (Figure 3A). In this group of patients, the 5-year DSS was 50% vs. 76% in patients with tumors where ≤10% cells manifested Mcm-2 protein expression. The 5-year DFS was 50% vs. 75.8% (Figure 3B), and the OS was 48% vs. 66%, respectively. Despite marked differences in the shape of the survival curves, the differences in DFS and OS were not significant. No effects of the Ki-67 proliferation index on survival indices could be demonstrated (Figures 3C and 3D). In the groups of patients with a Ki-67 proliferation index of >10% vs. ≤10%, the 5-year DFS was 63% vs. 58.8%, the DSS was 63% vs. 58.8% and the OS was 57% vs. 55%, respectively. None of the remaining studied parameters could be shown to exert significant effects on DFS, DSS or OS.

Discussion

A significant positive correlation was detected between the expression of Ki-67 and that of Mcm-2, but only the latter was found to affect survival indices. In the case of the Ki-67 antigen, no relationship with survival was noted, in line with the results of an earlier report (16). The results indicated that the Mcm-2 protein may represent not only a proliferation marker, but may also serve as a useful prognostic factor in oral carcinoma. In recent years, the Mcm-2 protein has been confirmed to represent a strong prognostic index in tumors of different locations. Gonzalez et al. (29), in their study comparing the effect of Mcm-2 with that of Ki-67 on the survival of patients with mammary carcinoma, demonstrated higher Mcm-2 estimations. They found that the Mcm-2 proliferation index could serve as an

Figure 2. Correlation between Ki-67 antigen and Mcm-2 protein expressions in squamouscellular carcinomas of the oral cavity (R=0.8, p<0.001).

Figure 3. A. Disease-specific survival (DSS) as related to Mcm-2 protein expression (p<0.05). B. Disease-free survival (DFS) as related to Mcm-2 protein expression (p=0.089). C. DFS as related to Ki-67 antigen expression (p=0.67). D. DSS as related to Ki-67 antigen expression (p=0.88).
independent prognostic factor in mammary carcinoma and that it carried higher predictive value than evaluations of lymph node involvement or the histological grade of malignancy. In their analysis of the superiority of Mcm-2 estimations over those of Ki-67, this group reported that the Ki-67-based proliferation index might be significantly underestimated and that the difference in proliferation indices measured by estimation of Mcm-2 protein and Ki-67 expressions might reflect the fact that, in the early G1-phase of the cell cycle, the cells did not manifest Ki-67 expression (6). Moreover, they noted that, independently of the histological subtype of mammary carcinoma, Mcm-2 was expressed in a significantly larger number of cells in the cell cycle than Ki-67 (29). In their study on renal cancer, Rodins et al. (30) found that Mcm-2 represented a better proliferation marker than Ki-67 and that it could serve as an index of prognosis. They also noted that the fixation procedure for the material significantly affected the extent of Ki-67 antigen expression (30). In turn, Meng et al. (31) suggested that Mcm-2 is a significant, independent predictor of DFS in prostate cancer. In their study on non-small cell lung cancer, Rammath et al. (32) disclosed that the Mcm-2 proliferation index was an independent predictor of survival. The authors demonstrated a significant correlation between the proportion of cells manifesting expression of Mcm-2 or Ki-67. However, in contrast to Mcm-2, Ki-67 exerted no significant effect on patient survival (32). Studies on other types of tumors also confirmed the superiority of Mcm-2 protein expression over that of Ki-67 antigen as a marker of proliferation (33-35). The observations are consistent with the results presented in this study.

Conclusion

Mcm-2 protein expression can be used not only to estimate the proliferative index, but also as a prognostic factor for the survival of patients with oral cancer.

References


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