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Original contribution

MCM4 and MCM7, potential novel proliferation markers, significantly correlated with Ki-67, Bmi1, and cyclin E expression in esophageal adenocarcinoma, squamous cell carcinoma, and precancerous lesions☆,☆☆

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Summary Minichromosomal maintenance (MCM) proteins are participants of DNA replication and may represent more accurate markers in determining the proliferative fraction within a tumor than proliferative marker Ki-67. Our study investigated the correlation between MCM4 and MCM7 expression and Ki-67, Bmi1, and cyclin E expression in esophageal adenocarcinoma, squamous cell carcinoma, and precancerous lesions. MCM4 and MCM7 expression had similar distribution as Ki-67 and Bmi1 expression in esophageal carcinoma and precancerous lesions. The mean percentage of MCM4, MCM7, and Ki-67 expression increased from squamous epithelium (5.5%, 7.3%, and 5.9%, respectively), to columnar cell metaplasia (11.2, 13.5%, and 3.4%), Barrett's esophagus (27.7%, 35.3%, and 8.3%), low-grade dysplasia (42.6%, 52.2%, and 12.9%), high-grade dysplasia (63.2%, 77.7%, and 29.6%), adenocarcinoma (61.3%, 75.5%, and 24.5%), and squamous cell carcinoma (74.1, 85.4%, and 36.3%). The percentages of MCM4 and MCM7 expression were significantly higher than Ki-67 expression. Using univariate analysis we found a high percentage of MCM4 expression (>70%) to be significantly associated with lymph node metastasis and shorter survival in the adenocarcinoma group. We also demonstrated the percentage of MCM4 and MCM7 expression to be significantly correlated with Ki-67, Bmi1, and cyclin E expression in esophageal carcinoma and precancerous lesions. MCM4 and MCM7 may serve as more sensitive proliferative markers for the evaluation of esophageal lesions.

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1. Introduction

The minichromosomal maintenance (MCM) protein family consists of six related proteins that have essential roles in the initiation of DNA replication [1]. MCM proteins are also involved in the elongation of DNA replication and other chromosome transactions including damage response, transcription, and chromatin structure [2,3]. Deregulation of the MCM proteins contributes to cell proliferation and tumorigenesis. Aberrant expressions of MCM proteins have been reported to be promising prognostic markers in a number of malignancies [4-17].

It has been claimed that MCM proteins are potentially more accurate in determining the proliferative fraction within a tumor than conventional proliferative markers such as Ki-67 [10]. The presence of MCM2, MCM5, and Ki-67 expression was previously reported in esophageal squamous dysplasia and Barrett's esophagus with glandular dysplasia [18,19]. Further, studies have observed MCM2 expression in esophageal squamous cell carcinoma and its positive correlation with Ki-67 expression [18,20]. MCM4 mRNA expression has also been observed in esophageal squamous cell carcinoma [21]. While limited studies on MCM4 and MCM7 expression in esophageal carcinoma have been reported, none of the studies to the best of our knowledge investigated MCM4 and MCM7 expression by immunohistochemistry.

Bmi1 is a member of the polycomb-group proteins and functions as a stem cell marker to regulate the proliferation of progenitor cells [22]. Our previous study demonstrated that Bmi1 expression was similar to Ki-67 expression in their distribution in the basal layer of normal squamous epithelium and extending to full thickness in esophageal carcinoma [23]. Cyclin E plays an important role in promoting G1 cell cycle transition to S-phase [24]. MCM7 has been reported to be the substrate of cyclin E/Cdk2 [25], and high level of MCM4 expression has been associated with cyclin E expression in non–small cell lung carcinoma [10]. In addition, we previously found aberrant expression and amplification of cyclin E significantly increased in dysplastic esophageal lesions [26].

In the current study, we first examined the immunohistochemical expression of MCM4 and MCM7 in comparison to the conventional proliferation marker Ki-67 in esophageal adenocarcinoma, squamous cell carcinoma, and precancerous lesions to determine the predictive value of these biomarkers for the progression of esophageal diseases. Next, we investigated the clinicopathologic association of MCM4, MCM7, and Ki-67 expression in esophageal adenocarcinoma, squamous cell carcinoma, and precancerous lesions. We also explored the correlation between MCM and Bmi1 as well as cyclin E expression.

2. Materials and methods

2.1. Construction of tissue microarrays

Tissue microarrays were constructed from representative areas of formalin-fixed specimens collected from 1997 to 2005 in the Department of Pathology and Laboratory Medicine, University of Rochester Medical Center, Rochester, NY. The tissue microarrays contained 82 squamous epithelium, 60 columnar cell metaplasia, 33 Barrett's esophagus, 38 low-grade dysplasia, 14 high-grade dysplasia, 108 esophageal adenocarcinoma, and 24 esophageal squamous cell carcinoma. Clinicopathologic data of the patients, including age, gender, TNM stage, histologic grade, and duration of survival, were obtained from the medical records. All patients' identifiers were removed. The study was approved by the institutional review board (Biomarkers of esophageal carcinoma, RSRB28546).

2.2. Immunohistochemistry

Immunohistochemical studies were performed on 5-μm thick sections of tissue microarrays. Briefly, after endogenous peroxidase activity was quenched and nonspecific binding was blocked, ready-to-use mouse monoclonal antibodies to MCM4 (1:50; Santa Cruz Biotechnology, Santa Cruz, CA) and MCM7 (1:50; Santa Cruz Biotechnology) were incubated at 4°C overnight, and Ki-67 (1:100; Dako, Carpinteria, CA) was incubated at room temperature for 30 minutes. The secondary antibody (Flex HRP, Dako) was incubated for 30 minutes. After washing, sections were incubated with Flex DAB chromogen for 10 minutes and counterstained with Flex hematoxylin for 5 minutes. Appropriate positive and negative controls were evaluated. Tissue microarrays were also stained with hematoxylin and eosin to be used for histologic comparison. The percentage of positive nuclear expression for MCM4, MCM7, and Ki-67 was reviewed by two pathologists. Various cut-offs were tested to establish high and low expression levels. The percentages close to mean expression levels of MCM4 (70%), MCM7 (70%) and Ki-67 (25%) expression correlated best with overall survival in esophageal carcinoma. The cut-offs were set at 70% for MCM4 and MCM7 and at 25% for Ki-67.

Bmi1 and cyclin E immunostaining were performed as previously described [23,26]. Mouse monoclonal antibodies to Bmi1 (1:100; Millipore, Bedford, MA) and cyclin E (1:100; Santa Cruz Biotechnology) were used for immunohistochemical studies.

2.3. Statistical analysis

Pairwise mean comparisons were used to analyze the percentages of immunostaining between the histologic groups: 1) adenocarcinoma, high-grade dysplasia, low-grade dysplasia, Barrett's esophagus, and columnar cell metaplasia, and squamous epithelium; 2) squamous cell carcinoma and squamous epithelium. Pearson's χ^2 tests, t tests, and Fisher exact tests were used as appropriate to assess the association between clinicopathologic characteristics and MCM4, MCM7, and Ki-67 expression. Univariate and multivariate regression models were generated. Probabilities of survival were