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Correlation of tumor grading and cellular proliferation in colorectal adenocarcinomas

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Problem/Background : *Several values including patient status, tumor biomarkers and molecular aspect are used as predictors in the patient follow-up and management of colorectal cancer. Tumor grading or differentiation is a prognostic factor which is practically identifiable in routine histologic sections. However, decision making of histologic grade in some cases is difficult.*

Objective : *To study the correlation between cell proliferation, using Ki67 immunohistochemical staining, and grading of colorectal adenocarcinoma.*

Design : *Retrospective analytic study.*

Setting : *Department of Pathology, Faculty of Medicine, Chulalongkorn University*

Materials and Methods : *One hundred and sixty-two cases, first diagnosed as colorectal adenocarcinoma, were recruited in our study. Tumor grading was classified into well-differentiated, moderately-differentiated, poorly-differentiated and undifferentiated type, reviewing on hematoxylin and eosin stained slides in each case, according to the World Health Organization's criteria 2000. Neoplastic cell proliferation is determined by Ki67 immunostain. The correlation was analyzed by means of the Spearman's rank correlation coefficient.*

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Results : *Correlation between neoplastic cell proliferation and tumor differentiation of colonic carcinoma was found statistically significant (P = 0.014).*

Conclusions : *This study demonstrated a significant correlation between cell proliferation and differentiation of colorectal cancer. The poorer tumor differentiation is, the higher is the proliferation rate. The Ki67 may have a role in some tumors, which are difficult to grade in routine histologic sections.*

Keywords : *Cellular proliferation, Colorectal cancer, Tumor differentiation.*

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มานะ ทวีวิศิษฏ์, นฤดา จิรกาลวสาน, มุกดา ชัยพิพัฒน์, อุบล พุ่มสุข, นฤมล วิเศษโอภาส. ความสัมพันธ์ระหว่างเกรดและการแบ่งตัวของเซลล์ในมะเร็งลำไส้ใหญ่. จุฬาลงกรณ์เวชสาร 2549 ก.ค; 50(7): 461 – 70

เหตุผลของการทำวิจัย : *ค่าต่าง ๆ ไม่ว่าจะเป็นสภาวะของผู้ป่วย รวมถึงตัวชี้วัดทางชีวภาพและเชิงโมเลกุล ถูกนำมาใช้เพื่อติดตามผลการรักษาผู้ป่วยมะเร็งลำไส้ใหญ่เพื่อการดูแลผู้ป่วยอย่างมีประสิทธิภาพ เกรด หรือการเปลี่ยนแปลงของมะเร็งก็ถูกนำมาใช้ในการทำนายโรคด้วยเช่นกัน โดยอาศัยการตรวจจากสไลด์เนื้อเยื่อ ถึงกระนั้น ในเนื้อมะเร็งบางรายก็เป็นการยากที่จะตัดสินใจในการให้เกรดของมัน*

วัตถุประสงค์ : *เพื่อศึกษาความสัมพันธ์ระหว่างอัตราการแบ่งตัวกับการเปลี่ยนแปลงของเซลล์มะเร็งลำไส้ใหญ่*

รูปแบบการวิจัย : *การศึกษาเชิงวิเคราะห์ย้อนหลัง*

สถานที่ทำการวิจัย : *ภาควิชาพยาธิวิทยา คณะแพทยศาสตร์ จุฬาลงกรณ์มหาวิทยาลัย*

ตัวอย่างและวิธีการศึกษา : *อาศัยชิ้นเนื้อจากผู้ป่วยโรคมะเร็งลำไส้ใหญ่จำนวน 162 ราย โดยแบ่งเกรดหรือระดับการเปลี่ยนแปลงของเนื้องอกเป็นเจริญเปลี่ยนแปลงได้ดี ปานกลาง แย่ และแทบจะไม่เจริญเปลี่ยนแปลงเลย ซึ่งอ้างอิงตามเงื่อนไขการแบ่งขององค์การอนามัยโลกเมื่อปี พ.ศ.2543 สำหรับดัชนีบ่งชี้การเจริญของเซลล์มะเร็ง ศึกษาโดยอาศัยการย้อมชิ้นเนื้อทางกระบวนการอิมมิวโนฮีสโตเคมี แล้วคำนวณความสัมพันธ์ระหว่างระดับการเปลี่ยนแปลงของเนื้อมะเร็งกับอัตราการเจริญของเซลล์มะเร็งลำไส้ใหญ่ด้วยกระบวนการทางสถิติ*

ผลการศึกษา : *พบความสัมพันธ์ระหว่างระดับการเปลี่ยนแปลงของเนื้อมะเร็งกับการเจริญของเซลล์อย่างมีนัยสำคัญทางสถิติ (P = 0.014)*

สรุป : *ถ้าอัตราการแบ่งตัวของมะเร็งยิ่งสูงขึ้น การเปลี่ยนแปลงของเซลล์มะเร็งลำไส้ใหญ่ก็ยิ่งแย่ลงตามไปด้วย ดังนั้นอาจนำอัตราการแบ่งตัวของมะเร็งมาใช้ทดแทนได้ในมะเร็งบางรายที่ยากต่อการให้เกรด*

คำสำคัญ : *การแบ่งตัวของเซลล์, มะเร็งลำไส้ใหญ่, การเปลี่ยนแปลงของเนื้องอก.*

The distribution of colorectal cancer is worldwide; between 20 % and 50 % of patients with colorectal cancer will pass away within five years after their diagnosis, generally because of extensive metastasis.[1] During the past decade this malignancy has been studied in various facets, e.g., epidemiology, etiology or pathogenesis.[2] One of the most significant influent factors for predicting the patient outcome is biological markers of neoplastic behavior.[3, 4] Apart from conventional stage and grading system, according to tumor differentiation, the proliferation factors at biomolecular levels have an important role in patient management and biological classification of tumors.[4]

Neoplastic cellular proliferation will act autonomically without control. Ki67 is a cell cycle associated antigen of nuclear protein which expresses in all cell growth phases except the resting phase (G_0).[5,6] The number of cells stained positively for monoclonal Ki67 antibody correlates with tissue proliferation rate and degree of tumor differentiation.[7] Our study is designed to prove this concept and to evaluate the utility of Ki67 as a prognostic biomarker of colorectal adenocarcinoma.

Materials and Methods

One or two paraffin blocks per case from 162 cases, first diagnosed as colorectal adenocarcinoma at the King Chulalongkorn Memorial Hospital from 2002 to 2003 were selected. Representative blocks always comprised the deep invasive portion and in the majority of cases superficial parts of the tumor were also included. Subjects who have had a history of polyposis syndrome, recurrent tumor and previous treatment, either chemotherapy or radiation, were excluded from the study. Four- micrometer-thick haematoxylin and eosin stained slides were reviewed and classified the tumor differentiation, according to the World Health Organization (WHO) histological classification of tumors of the colon and rectum. They consisted of well-differentiated (grade 1), moderately -differentiated (grade 2), poorly-differentiated (grade 3) and undifferentiated (grade 4) when glandular structures occurred >95 %, 50 – 95 %, 5 – 50 % and <5 %, respectively. In addition some variants, composed of mucinous adenocarcinoma and signet-ring cell carcinoma, were classified as poorly differentiated whereas medullary carcinoma was classified as undifferentiated. Those specimens were noted the depth of tumor penetration, number of regional lymph node metastasis and distant organ spreading, according to TNM staging as well as their location, including ascending, transverse, descending and rectosigmoid regions.

In the determination of neoplastic cell proliferation, formalin-fixed, paraffin wax-embedded blocks were deparaffinized in xyline, rehydrated through graded alcohols and employed Ki67 antibody (mouse antihuman, clone MIB1, 1/300 dilution; Dako), a biotin-conjugated secondary antibody, avidin-horseradish peroxidase, and the chromogen diaminobenzidine tetrahydrochloride (DAB) as a detection agent on the tumor tissue sections. Ki67 immunohistochemical stains were evaluated semiquantitatively and counted the number of positive cells expressing nuclear brown stain among the neoplastic cells. Field selection used highest Ki67 expression areas (hot spots) by lower power scanning and scored as: negative, <10 %; 1+, 10 - 25 %; 2+, 26 – 50 %; 3+, 51-75 %; and 4+, >75 %.

Data were analyzed by statistical program SPSS for Windows, Version 11.5. The correlation between Ki67 immunohistochemistry and tumor differentiation (histologic grade) together with some available patients' background such as age, tumor location, tumor size, regional lymph node status and staging was assessed by means of the Spearman's rank correlation coefficient. Confidence intervals were 95 %. A *P* value of <0.05 was considered statistically significant.

Results

There were 162 subjects in this study, consisting of 80 males (49 %) and 82 females (51 %) with a mean age of 62 years and 59 years, respectively. Rectosigmoid colon was the preferentially involved site, accounting for 106 cases (65 %). For tumor depth, tumors involve submucosa (T1) in 1 cases (1 %), muscular propria (T2) 29 cases (18 %), pericolic fat (T3) 122 cases (75 %), and adjacent organs (T4) 10 cases (6 %). The tumors metastasize to regional lymph nodes as: less than 4 nodes (N1), 45 cases (28 %); 4 nodes or more (N2), 38 cases (23 %); and no lymph node metastasis (N0), 79 cases (49 %). Eighteen tumors were stage I (11 %), 56 tumors were stage II (35 %), 77 tumors were stage III (47 %) and 11 tumors were stage IV (7 %) (Figure 1). Regarding the tumor differentiation, 56 cases (35 %) were well-differentiated (grade 1), 84 cases (52 %) were moderately-differentiated (grade 2), 17 cases (10 %) were poorly-differentiated (grade 3) and 5 cases (3 %) were undifferentiated (grade 4).

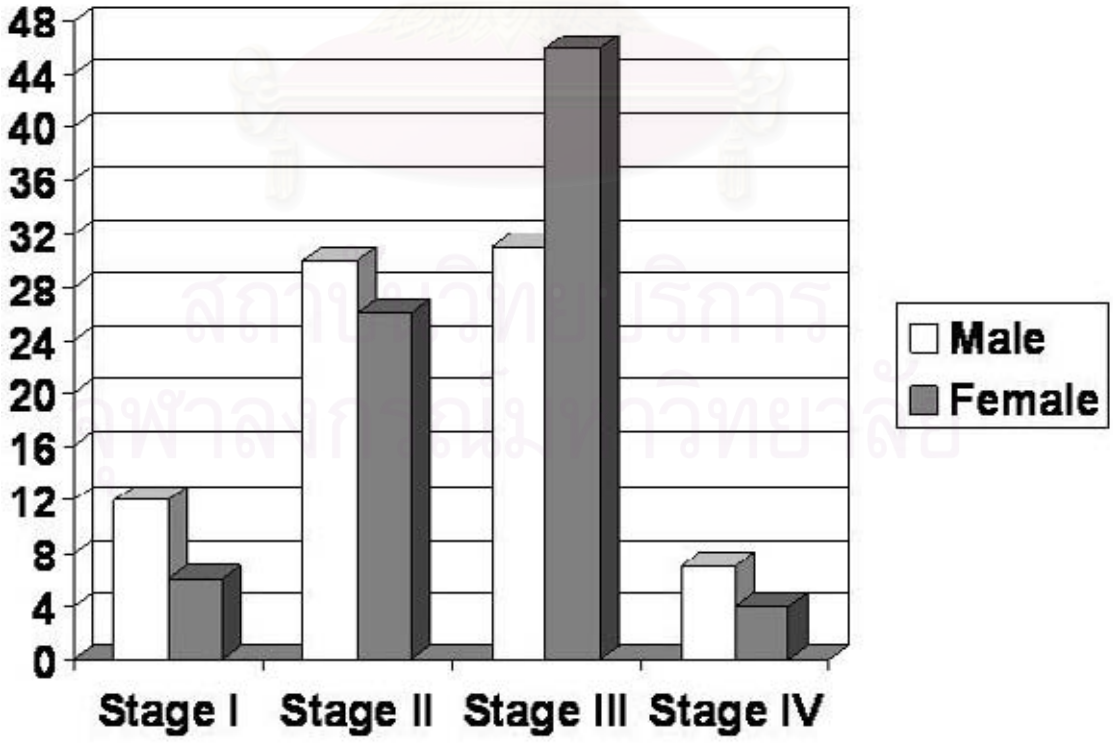

Figure 1. The tumor stage and sex distribution of 162 colorectal cancers in the study.

For tumor cell proliferation, 88 tumors were negative for Ki67, while 74 tumors were positive (Table 1). In cases with positive for Ki67, the distribution was divided as <25 %, >25-50 %, >50 - 75 % and > 75 % was 24, 17, 17 and 16 cases, respectively (Figure 2-4). In addition, we found a significant correlation between Ki67 immunohistochemistry and neoplastic differentiation of the colorectum (correlation coefficient = 0.19, $P = 0.014$). The density of Ki67-positive neoplastic cells had a trend to be increased in poorer tumor differentiation. However, neoplastic grade and cellular proliferation were not statistically correlated with patients' age, tumor location, tumor size and stage.

Table 1. Distribution of Ki67 immunostains and differentiation of colonic carcinoma.

	Tumor Differentiation				
Ki67score	**Well**	**Moderate**	**Poor**	**Undifferentiated**	**Total**
Negative	37	42	8	1	88
1+	7	14	3	0	24
2+	5	8	3	1	17
3+	3	11	1	2	17
4+	4	9	2	1	16
Total	56	84	17	5	162

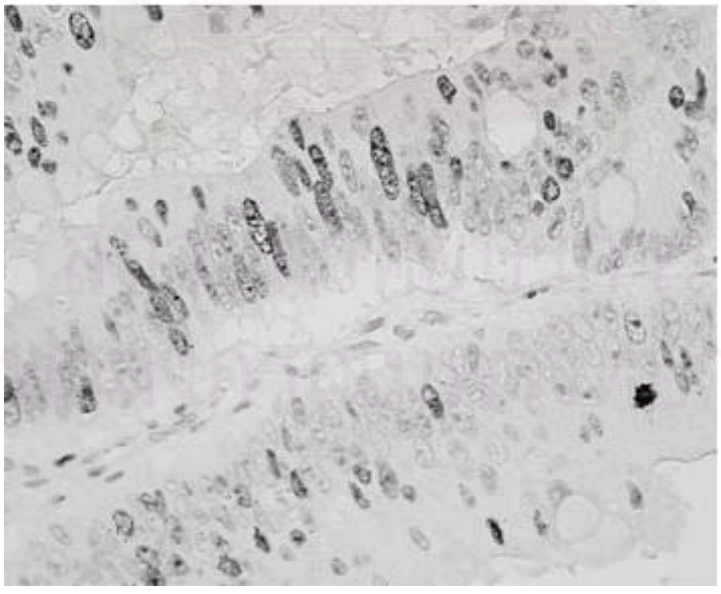

Figure 2. Ki67 is positive about 20 % (score = 1+) in well-differentiated adenocarcinoma (H&E x400).

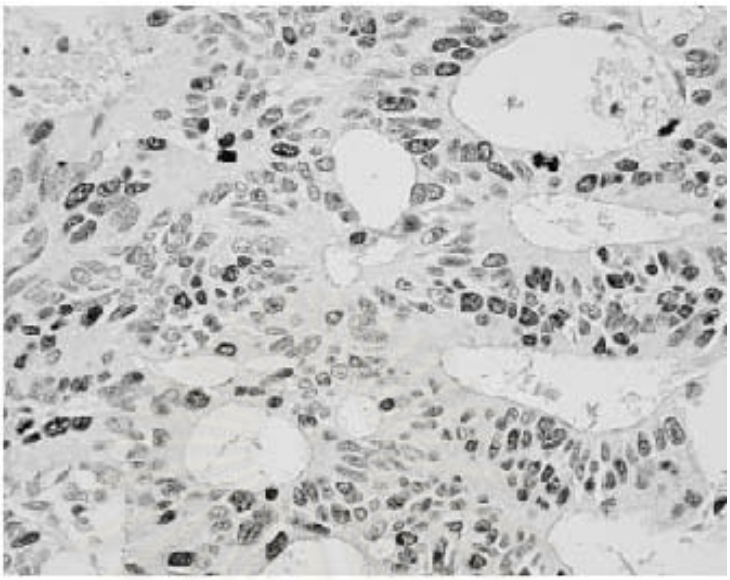

Figure 3. Ki67 is positive about 60 % (score = 3+) in moderately-differentiated adenocarcinoma (H&E x400).

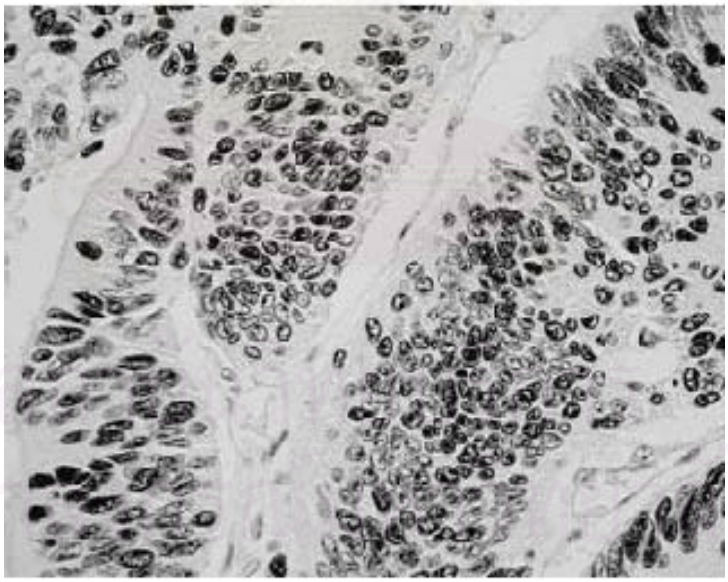

Figure 4. Ki67 is positive nearly 100 % (score = 4+) in mucinous carcinoma. The tumor is classified as poorly-differentiated malignancy (H&E x400).

Discussion

Colorectal carcinoma still remains a leading cause of cancer death in most countries without evidence of any decline in its incidence.[1,2] Regardless of surgical resection, which is the principle management, most oncologists need effective marker for patient follow-up and predicting the outcome. Recently, there is not only one ideal marker, but it is used as a panel and its reliability remains varied among different geographic areas.[3,4,8]

Similar to other malignancies, tumor grade or degree of differentiation is depended on tumor molecular kinetic and considered to be an important independent prognostic factor besides the staging system.[3] Differentiation of colorectal adenocarcinomas are usually graded into, namely: well- differentiated (grade 1), moderately- differentiated (grade 2) and poorly-differentiated (grade 3), when glandular architectures appear >95 %, 50 - 95 % and 5 - 50 %, respectively, and the colonic tumor is classified as undifferentiated type (grade 4) when glandular formation occurs <5 %, according to the WHO classification.[9]

However, histologic grading is subjective. Some authors have recommended a five-grade system, including well, well to moderately, moderately, moderately to poorly and poorly differentiated in order to complete the spectrum.[10] On the other hand, some authors have suggested that colonic tumor should be divided simply into low-grade, consisting of well and moderately-differentiated adenocarcinomas, and high-grade, including poorly-differentiated and undifferentiated carcinomas. The reason for this classification is that in multivariate analyses there is no difference between well and moderately differentiated tumors.[9]

Therefore, grading is problematic in some situations.[10] When a carcinoma possesses variegated differentiation, grading should be based on the least differentiated element and avoided the advancing edge.[10,11] Small foci of less differentiated are common at the deepest part, but this feature is insufficient to classify the tumor as poorly differentiated.[12] However, another way is to assess the growing tumor edge separately from the overall grade because this tumor fraction is considered to be most aggressive and more related tumor behavior.[13] In addition, in case of tiny biopsy specimen, gradation is difficult to report and tumor grades may not represent the whole tumor.

Ki67 is the antibody for detecting the intranuclear matrix protein, correlating with cell proliferation. In tumorigenesis turnover rate of malignant cells is significantly higher than normal because reversible G_1-G_0 pathway is mostly skipped.[5-7] Then Ki67 expression in malignancy is higher than in normal.[14-16] Backus HH and colleagues have found that the progression of primary colonic adenocarcinoma to liver metastasis is associated with an increased proliferation rate, using Ki67 measurement.[17]

In the present study, we found that the higher Ki67 is, the worse is the neoplastic differentiation. This result is statistically significant. Pathologists may add Ki67 as a prognostic biomarker, particularly in problematic cases such as heterogeneous differentiation of the tumor. For adenocarcinoma of the colon, there is a literature, that has prove it; according to which the result of Ki67 expression is similar between the central and peripheral parts of the tumor.[14]

In conclusion, Ki67 immunostain for determining cell proliferation is related to tumor differentiation or tumor grading in colorectal adenocarcinoma. This marker is sensitive and can probably be used for monitoring and predicting the stage of the disease and therefore helps planning the therapy.

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