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The correlation between the immunostains for p53 and Ki67 with bcl-2 expression and classical prognostic factors in colorectal carcinomas

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Abstract

The prognostic role of p53 and Ki67 in colorectal carcinomas (CRC) is very controversial in the literature. In our study, we tried to find if their immunostains are correlated with bcl-2 expression or other classical prognostic factors (sex, age, localization and size of tumor, the grade and staging of tumor). We studied 507 cases with CRC and chose 38 cases in which we realized these correlations. Fourteen cases were mucinous CRC, the other 24 cases being non-mucinous CRC (six well differentiated, 13 moderate and five poorly differentiated). For statistical analysis, we used the Statistical Program Graph Pad In Stat 3-Trial Version. We considered the significant association when $p<0.05$, with 95% confidence interval. *Results*. The median value was 75% for p53 expression, respectively 35% for Ki67 expression. Bcl-2 was positive in 47% of cases but not correlated with p53 or Ki67. We found a significantly statistical decrease p53 immunostain with grade of tumor (70% in well differentiated, respectively 40% in poorly differentiated CRC) and increase of Ki67 median expression (25% in well differentiated, respectively 60% in poorly differentiated CRC). Ki67 was correlated with age of patients, lymph node involvement, being more expressed in N2 (80%) than in N0 (22.5%) and with Dukes MAC staging (25% in B1, 60% in C2). P53 was correlated with age of patients and pT component, after pTNM staging (75% in pT2, 40% in pT4). P53 was not correlated with Ki67. *Conclusion.* The CCR prognostic is not determined only by proliferative capacity of tumoral cells.

***Keywords*:** colorectal carcinoma, prognostic factors, correlation.

Introduction

Classically, neoplasia has been considered a disturbance in the regulation of proliferation. The p53 gene mutation is related to carcinogenesis in most malignant diseases. The distribution of Ki67 and p53 as markers of cell proliferation is very studied in CRC. More articles reveal that their immunostain could predict the CRC prognostic.

The p53 protein is codificated by gene p53, located on the short arm of chromosome 17, a frequent site of allelic loss in many tumors. The wild p53 maintains the integrity of genes by detecting mutations and preventing the division of cells with damaged DNA. It blocks the cells in G_1 phase of cellular cycle. In CRC, the gene p53 may be rearranged and p53 protein may be altered. Therefore, the replication errors and deregulation of cells growth could appear.

The p53 protein is evidentiated by immunohistochemistry. The monoclonal antibody DO-7 reacts with both wild and mutant type of the p53 protein, being expressed intranuclear.

The Ki67 protein is another immunohistochemical marker utilized for identification of proliferative cells. It is expressed in all phases of cellular cycle, except the G_0 phase. The monoclonal antibody Ki67, clone Ki-S5, has the particular feature of recognizing the Ki67 antigen in formalin-fixed, paraffin-embedded material.

Bcl-2 oncoprotein inhibits apoptosis and it seems to be correlated with a good prognosis in CRC. However, its prognostic role is very controversial in literature.

Material and methods

We studied 507 cases with CRC and chose 38 cases in which we followed the classical prognostic factors: sex and age of patients, localization and size of tumor, the grade and staging of tumor.

Fourteen cases were mucinous CRC, the other 24 cases being non-mucinous CRC (six well differentiated, 13 moderate and five poorly differentiated).

Each classical parameter was correlated with p53 and Ki67, using the statistical programs.

For the immunohistochemical study, we used the following antibodies, provided by Lab Vision: p53 (clone DO-7), Ki67 (clone Ki-S5) and oncoprotein bcl-2 (clone 100/D5).

We used the immunoperoxidase method. Paraffin-embedded 5-micron tumor sections obtained from patients with CRC were utilized. Nuclear staining of 10% of the cells was the criteria for a positive p53 or Ki67 reaction.

For statistical analysis, we used the Statistical Program Graph Pad In Stat 3-Trial Version. First, we collected the data with the program Microsoft Excel.

We used the t-test, chi square test and the contingency tables, Fischer's test, determining the values of p and chi. We considered the significant association when $p<0.05$, with 95% confidence interval.

To determine the number of positive nucleus we used a number of five pictures for each case by "hot-spot" regions, at 200 high power fields. The count was made using NIH's ImageJ program. Automatically, we numbered the positive and the negative nucleus and we determine the percent of the positive nucleus.

Results

Sex distribution

Out of the 38 cases studied, 16 were females and 22 were men. No statistical correlation was found between p53 or Ki67 immunostains and sex of patients ($p = 0.82$, respectively 0.27). Both men and women presented the p53 median stain by 75%.

The median immunostains for Ki67 was 45% for females and 30% for men.

Age distribution

The average age was 64.74 ± 8.49 years. The median age was 65.50 years, with minimum 39 and maximum 79 years. We compared the values for p53 and Ki67 immunostains for the age segment 39–65 years with the values obtained for 66–79 years group.

Median stain was 70% for p53, respectively 25% for Ki67 in the first group and 80% for p53, respectively 50% for Ki67 in second group. The age was strongly correlated with the expression of both antibodies ($p<0.0001$). So, at the older patients, both antibodies were more expressed.

Localization on the colon segments

76.31% of cases were placed on the left colon, others 23.69% being placed on the right colon.

The median stain for p53 was 90% in the right colon respectively 70% in the left colon.

For Ki67, the stain was 50% in right and 30% in the left colon. No statistical correlation was found ($p = 0.48$ for p53, respectively 0.23 for Ki67).

Size of tumor

The median size was 16.5 mm, with minimum 5 mm and maximum 36 mm.

No statistical correlation was found between p53 or Ki67 and size of tumor ($p = 0.79$ for p53, respectively 0.55 for Ki67).

Microscopic features

Fourteen cases (36.84%) were mucinous adenocarcinomas (MA), the other 24 cases (63.16%) being non-mucinous adenocarcinoma (NMA).

Out of the NMA, six were well-differentiated (grade I), 13 moderate (grade II) and five poorly differentiated (grade III). We observed that p53 was reversely correlated with grade of differentiation ($p<0.0001$).

The median stain was 70% in grade I, 65% in grade II, respectively 40% in grade III (Figures 1 and 3).

Ki67 immunostain increased with grade of differentiation but not statistically significant ($p = 0.21$). The median expression was 25%, 50%, respectively 60% in grade I, II and III (Figures 2 and 4).

In MA, the median stain was 75% for p53 and 25% for Ki67.

pTNM staging

A number of nine cases (23.70%) were in pT1 stage, 24 cases (63.16%) in pT2 and five cases (13.14%) in pT3 stage.

In 20 cases (52.63%) the lymph nodes were not involved (pN0), 14 cases (36.84%) were in pN1 and four cases (10.53%) in pN2.

P53 immunostain was reversely correlate with pT ($p = 0.02$), the median expression being 75% in pT1, 65% in pT2 respectively 40% in pT3.

The Ki67 stain was not correlated with pT ($p = 0.82$). The Ki67 stain was 30% in pT1, 45% in pT2, 40% in pT3. However, Ki67 was correlate with lymph node involvement ($p<0.0001$), the median stain being 22.5% in pN0, 50% in pN1 and 80% in pN2.

P53 was not correlated with pN ($p = 0.99$), the immunostain being 72.5% in pN0, respectively 80% in pN1 and pN2.

Dukes-MAC staging

A number of eight cases were in stage B1, 13 cases in B2, nine cases in C1 and eight cases in stage C. Only Ki67 was correlated with this classification ($p = 0.03$), the median stain being 25% in B1, 27.5% in B2, 40% in C1 and 60% in C2.

The median p53 stain was 90% in B1, 60% in B2, 80% in C1 and 60% in C2.

These values demonstrated again that the p53 was reversely correlated with depth of invasion but not also with lymph node involvement.

Immunostain for bcl-2

Bcl-2 expression was observed in 18 cases (47.37%). Its expression was not correlated with p53 neither Ki67 immunostain ($p = 0.99$, respectively 0.97).

The p53 median stain was 72.5% in cases, which expressed bcl-2, and 80% in negative bcl-2 cases. The median Ki67 expression was 35% in both groups.

Immunostains for p53 and Ki67

Nuclear staining of 10% of the cells was the criteria for a positive p53 or Ki67 reaction. Three cases (8%) were negative for p53 and five cases (13%) were negative for Ki67.

The median immunostain was 75% for p53, respectively 35% for Ki67. The majority of cases (53%) were expressed p53 >70%. 50% of cases presented a Ki67 expression between 20–40%.

In nine cases (23.68%), we observed association of polyps. In these cases, the median p53 stain was 80% and Ki67 immunostain was 35%.

The p53 was not correlated with Ki67 immunostain ($p = 1$).

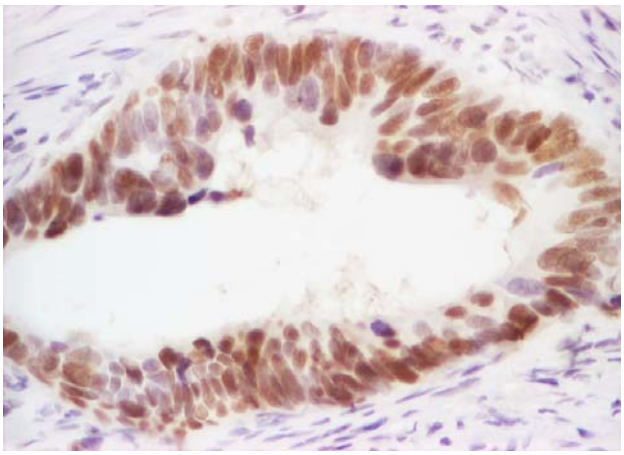

Figure 1 – ***High expression of p53 in a well-differentiated adenocarcinoma. The stage of tumor was pT1N0***

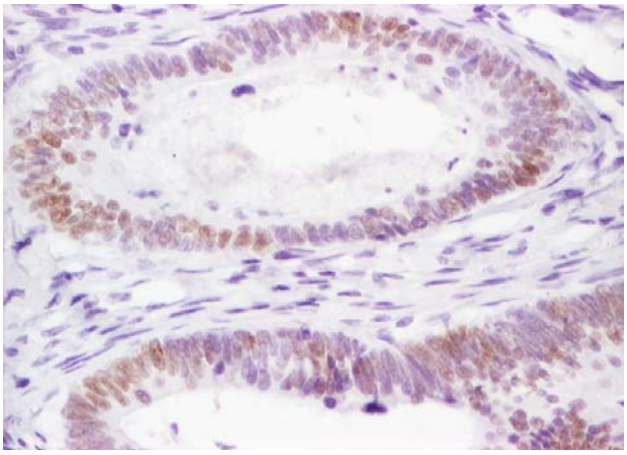

Figure 2 – ***Small expression of Ki67 in a well-differentiated adenocarcinoma. The stage of tumor was pT3N0***

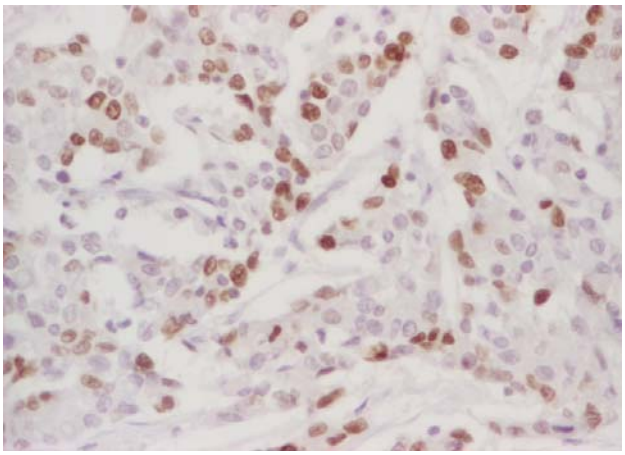

Figure 3 – ***Small expression of p53 in a poorly differentiated adenocarcinoma. The stage of tumor was pT3N1***

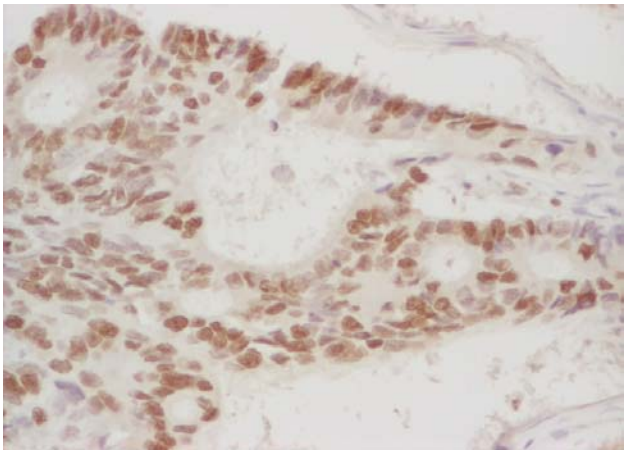

Figure 4 – ***High expression of Ki67 in a poorly differentiated adenocarcinoma. The stage of tumor was pT2N2***

Discussions

For the CRC, the following classical factors predict the prognosis: grade and stage of tumor, tumor size, lymph node involvement, blood vessel invasion, sex and age of patients.

The prognostic role of p53 and Ki67 in CRC is very controversial in the literature. Many studies reveal that the p53 immunostain is very high in CRC. The median stain is reported being between 43% [1] and 80% [2].

In the anal canal carcinomas the p53 expression seems to be smaller [3].

Some authors say that the p53 is directly correlated with prognosis, with vascular micro density and the stage of tumor but it reversely correlated to cytoplasm expression of bcl-2 [4, 5].

Other authors affirm that p53 is not correlated with bcl-2, stage and grade of tumor, age of patients or other classical parameters of tumors [6].

The same controversial results were found by different authors regarding Ki67 immunostain. Significant correlation was found between CD31 score, Ki-67 and parietal invasion [7, 8].

Other authors affirm that Ki67 is associated with better survival, but not associated with histopathological grade or bcl-2 expression [9, 10].

The median stain was reported being between 38% [10] and 59% [11].

In the majority of papers, p53 was not correlated with Ki67 but in some publications, p53 was reversely correlated with Ki67 and bcl-2 [10].

Many authors study the immunostains of these proteins and their expression but it is very difficult to establish a standard idea about their prognostic role. Our conclusions are similar with some papers regarding the correlations between p53, Ki67, bcl-2 and other classical prognostic factors. However, we found a significant correlation between p53 and grade of tumor, as well as p53 and pT component, after pTNM staging. Our correlations are different than the correlations reported in some papers.

We found that the p53 was more expressed in well-differentiated adenocarcinomas than in the poorly differentiated as well in pT1 than pT3. The results were accurately because were statistically verified. Other argument for the correctness of results was that the median value for B1 stage, after Dukes-MAC staging, was the same with that for C1, respectively the median value for B2 and C2.

These results prove that the p53 was reversely correlated with the depth of invasion but was not correlated with lymph node involvement. We studied more than 200 recent publications but we did not found other papers with similar results.

Conclusions

Several papers reveal the great value of study of CRC prognostic factors. In our study, we tried to find if the p53 and Ki67 immunostains are a very important prognostic factor and how they are correlated with other prognostic factors.

We found that the Ki67 immunostain was correlated with age of patients, lymph node involvement and it increased with grade of tumor.

P53 immunostain decreased with grade of tumor and was reversely correlated with depth of invasion but not with lymph node involvement. P53 was also correlated with the age of patients.

Neither p53 nor Ki67 were correlated with bcl-2 or other classical prognostic factors.

In these conditions, it is very difficult to establish the prognostic role of p53 and Ki67. They could be independent prognostic factors but, if the classical prognostic factors are demonstrated to predict indeed the prognosis, we could say that only Ki67 is a really indicator of prognosis because it was correlated with the age and lymph node involvement. However, Ki67 did not correlate with the other classical factors, which indicate the prognosis.

The conclusion is that we need to study more attentively this subject because it seems to be very interesting and a much-argued opinion could help the patients in the future. There are necessarily to study these immunostains in more cases.

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References

[1] ZHAO D. P., DING X. W., PENG J. P., ZHENG Y. X., ZHANG S. Z., *Prognostic significance of bcl-2 and p53 expression in colorectal carcinoma*, J Zhejiang Univ Sci B, 2005, 6(12):1163–1169.

[2] TSAMADAS A. C., KARDAMAKIS D., PETSAS T., ZOLOTA V., VASSILIOU V., MATATSORIS T., KALOFONOS H., VAGIANOS C. E., SCOPA C. D., *Bcl-2, bax and p53 expression in rectal adenocarcinoma. Correlation with classic prognostic factors and patients' outcome*, In Vivo, 2007, 21(1):113–118.

[3] GURZU SIMONA, JUNG J., MEZEI T., *Immunohistochemically aspects of anal canal carcinomas*, Revista de Medicină şi Farmacie, 2000, 53:36–37.

[4] KONDO Y., ARII S., FURUTANI M., ISIGAMI S., MORI A., ONODERA H., CHIBA T., IMAMURA M., *Implication of vascular endothelial growth factor and p53 status for angiogenesis in noninvasive colorectal carcinoma*, Cancer, 2000, 88(8):1820–1827.

[5] GEORGIOU L., MINOPOULOS G., LIRANTZOPOULOS N., FISKA-DEMETRIOU A, MALTEZOS E, SIVRIDIS E., *Angiogenesis and p53 at the invading tumor edge: prognostic markers for colorectal cancer beyond stage*, J Surg Res, 2006, 131(1):118–123.

[6] GHAVAM-NASIRI M. R., REZAEI E., GHAFARZADEGAN K., SEILANIAN-TOOSI M., MALEKIFARD H., *Expression of p53 in colorectal carcinoma: correlation with clinicopathologic features*, Arch Iran Med, 2007, 10(1):38–42.

[7] INDINNIMEO M., CICCHINI C., STAZI A., LIMITI M. R., GHINI C., MINGAZZINI P., VECCHIONE A., *Immunohistochemical assessment of Ki-67 as prognostic cellular proliferation marker in anal canal carcinoma*, J Exp Clin Cancer Res, 2000, 19(4):471–475.

[8] INDINNIMEO M., CICCHINI C., STAZI A., LIMITI M. R., GHINI C., MINGAZZINI P. L., VECCHIONE A., *Prognostic impact of CD31 antigen expression in anal canal carcinoma*, Hepatogastroenterology, 2001, 48(41):1355–1358.

[9] SALMINEN E., PALMUS S., VAHLBERG T., ROBERTS P. J., SÖDERSTRÖM K. O., *Increased proliferation activity measured by immunoreactive Ki67 is associated with survival improvement in rectal/recto sigmoid cancer*, World J Gastroenterol, 2005, 11(21):3245–3249.

[10] SALEH H. A., JACKSON H., BANERJEE M., *Immunohistochemical expression of bcl-2 and p53 oncoproteins: correlation with Ki67 proliferation index and prognosis histopathologic parameters in colorectal neoplasia*, Appl Immunohistochem Mol Morphol, 2000, 8(3):175–182.

[11] DUCHROW M., ZIEMANN T., WINDHÖVEL U., BRUCH H. P., BROLL R., *Colorectal carcinomas with high MIB-1 labeling indices but low pKi67 mRNA levels correlate with better prognosis outcome*, Histopathology, 2003, 42(6):566–574.

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