THE CORRELATION BETWEEN THE CLASICAL AND MODERN PROGNOSTIC FACTORS WITH THE ANGIOGENESIS AND LYMPHANGIOGENESIS IN THE COLORECTAL CARCINOMAS

DOCTORAL THESIS

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In our paper we made a statistical study from 507 colorectal carcinomas (CRC) regarding the classical prognostic factors (age, sex of patients, localization on the colon segments, macro- and microscopical aspects, pTNM and Dukes-MAC staging, the association with adenomatous polyps, etc.). We choose more than 200 cases which made the immunohistochemical reactions and in 52 of cases we studied the molecular aspects of CRC.

The most interesting and complex results were observed when we made the multiple correlations between the classical and IHC prognostic factors. These results are the following:

- the MSI-H carcinomas were predominated p53 negative, independently by bcl-2 or c-erbB-2 expression
- the MSS carcinomas were predominated p53+/c-erbB-2-/E cadherin+, independently by bcl-2 expression and presented a higher intensity of the angio- and lymphangiogenesis counted with VEGF-A, respectively VEGF-C, compared with the MSI tumors
- independently by bcl-2 or c-erb-B2 expression, the CRC which presented positivity for p53 also presented more frequent angiolymphatic invasion that means a more aggressive behaviour
- the immunophenotype bcl-2-/p53+/c-erbB-2+ was associated with the poorer differentiated adenocarcinomas and the cases bcl-2+/p53-/c-erbB-2+ were well differentiated or presented mucinous component. To make an interpretation about the histological grade of differentiation and the microscopic type like prognostic factors attach to the immunohistochemical markers, we proved that the bcl-2 and p53 expression presented a prognostical value only in the grouped interpretation and c-erbB-2 expression had not prognostical value. The cases bcl-2+/p53-, independently by c-erbB-2 expression seems to have a better prognosis
- most of the cases diagnosed in pT4 stage were bcl-2+/p53+, independently by c-erbB-2 expression and they did not presented mucinous component. The p53+ cases although presented bcl-2 positivity seem to have an unfavorable prognosis. The carcinomas bcl-2+/p53+ were associated with the presence of mature neoformed vessels
- the immunophenotype bcl-2-/p53+/VEGF-A+/VEGF-C+ was associated more frequent with the presence of lymph node metastasis and with a high lymphhendotelial area counted with D2-40 antibody. So, although the angiolymphatic invasion and the D2-40 lymphhendotelial area were not correlated with pN stage, the multiple correlation between this immunophenotype, D2-40 and the presence of lymph node metastasis proved that the cases with angiolymphatic invasion present a higher risque for lymph node metastasis
- we proved that the presence of neuroendocrine differentiation seems to show a better prognosis and it was associated with the immunophenotype Ecad+/bcl2+/p53-/c-erbB-2+/VEGF-R3-
- the carcinomas E cadherin negative were predominated MSI tumors with positivity for VEGF-C and VEGF-R3 and the presence of immature and intermediary vessels.

The Mena immunoexpression was not observed in the normal colorectal mucosa neither in the polyps without dysplasia but it was overexpressed in the polyps with dysplasia and in the tumoral cells of CRC, especially in that cases with angiolymphatic invasion. In the polyps with severe dysplasia we also observed the predominance of intermediary vessels marked CD105+/SMA+ in double immunostain. The Mena intensity was directely correlated with p53 and c-erbB-2 expression and also with the values of EA.
It was not correlated with the histological grade of differentiation neither with lymph node metastasis, Ki67 index, VEGF-A or E cadherin expression. The Mena expression was higher in the MSS than MSI carcinomas and the higher intensity was associated with that factors which show an unfavorable prognosis. This antibody could help to evaluate the risque of malignant transformation for the adenomatous polyps.

Our study proved that the angiogenesis in CRC is an earlier event which began in the premalignant stage of adenomatous polyps with severe dysplasia and had an oscilating feature. The double immunostain CD105-SMA showed that the neoformed vessels had the capacity to became mature and to transforme in other vessels. So, the intermediary vessels CD105+/SMA+ are the transition from immature to mature vessels. The number of the immature vessels and the diameter of neoformed vessels were smaller in the poorly differentiated CRC, especially in the cases with mucinous component. So, if the mature vessels are indeed resistant at the antiangiogenic drugs, the choice of the cases for the antiangiogenic treatment might to take into account the grade of vessels maturation but also the histological grade of differentiation, more than the presence of lymph node metastasis. The VEGF-A intensity was higher in the cases with lymph node metastasis, especially in that localized in the right colon segments.

These findings, statistically verified and correlated with the data from the literature, confer a better prognosis to the cases with the immunophenotype bcl-2+/p53-/E caderină+ which are diagnosed in earlier stages (pT1,2), without lymph node metastasis or angiolyphatic invasion, with the neuroendocrine differentiation. The Ki67 index and the intensity of angiogenesis are independently prognostic factors and the value of p53 like prognostic factor could be evaluated only in correlation with bcl-2 expression. The angiogenesis could be counted only making a correlation between the VEGF-A expression, the values of EA and the type of neoformed vessels determined by double immunostain CD105-SMA.

Regarding the localization on the colon segments, the DNA analysis, correlated with the statistical data about the classical and immunohistochemical prognostic factors showed that the carcinomas localized on the right colon segments had a better prognosis, especially the MSI tumors, with mucinous component, without lymph node metastasis, without p53 and MLH-1 positivity.

The carcinomas localized on the left colon segments were diagnosed in earlier stage, probably because the symptoms were earlier. The higher expression for p53 in these cases confer them an unfavorable prognosis. Regarding the left colon segments, we observed that the carcinomas localized on the recto-sigma seems to have a better prognosis than the cases localized on the descendent segment of colon. The features observed in the anal canal carcinomas showed an unfavorable prognosis and did not prove the efficacity of antiangiogenic drugs in these cases. The highest correlation with lymph node metastasis presented the cases with the immunophenotype bcl-2-/p53+/VEGF-A+/VEGF-C+ and the angiolyphatic invasion. The adenomatous polyps with severe dysplasia and a higher intensity of Mena protein seemed to present a high risque for malignant transformation, one reason why the therapeutical protocols might take into account this aspect.
ORIGINAL CONTRIBUTIONS

1. We made some correlations between the classical and modern prognostic factors which give us the possibility to establish some criteria to evaluate the prognosis and the treatment options of CRC. These criteria are especially based on the immunophenotype bcl-2/p53 and its correlations, like a first step for molecular classification of the CRC.

2. The study about Mena expression in CRC is the first study in the international literature.

3. The presence of intermediary vessels identified by double immunostain CD105/SMA and also their predominance in well differentiated carcinomas was not published yet in other PubMed citated studies.

4. Although the most of published studies regarded the microvascular density, we counted the angio- and lymphangiogenesis using a computer-assisted method which supposed the endothelial area determination and it was more objective.

5. The multiple correlations between the classical and modern immunohistochemical prognostic factors, the aspects of microsatellite instability and that of angio- and lymphangiogenesis were not effectuated in such big number of cases with CRC.

6. We elaborated a model of histological report which include the classical and modern parameters of CRC which are prognostic and predictive values in the daily diagnosis:
   - sex and age of the patients
   - tumor localization on the colon segments
   - the sporadic or ereditar nature of the carcinoma
   - the existence of the hepatic, lymph node or other metastasis (after the analysis of at least 12 lymph nodes)
   - with or without pre-operative radiotherapy
   - the pTNM and Dukes-MAC staging of tumor
   - the histological criteria which are necessary to appreciate if that tumors could be a MSI carcinoma: the microscopical type, the histological grade of differentiation, the aspects of the nucleus, type of tumor’s increase (infiltrating or nodular increase), the presence of lymphocytes infiltration intratumoral or in the tumoral stroma, the presence or absence of the dirty necrosis area, the expression of p53, MLH-1 and, if it is possible the expression of MSH-2, MSH-6, PMS-2
   - the presence or absence of the angiolymphatic invasion
   - the expression of p53, bcl-2, Ki67, Chromogranin A and, și, if it is possible the expression of E cadherin in the primary tumor are necessary for the molecular classification
   - the personal data of the patient are necessary to pursue the evolution