UNIVERSITY OF MEDICINE AND PHARMACY CRAIOVA

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ABSTRACT

ENDOSCOPIC AND IMMUNOHISTOCHEMICAL CORRELATIONS IN COLORECTAL CANCER

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INTRODUCTION

Colorectal cancer (RCC) is an important cause of morbidity and mortality, with approximately 300,000 new cases and 200,000 deaths annually due to this disease in Europe and the United States. Some important facts are mentioned on CCR. Firstly its increasing incidence and prevalence of important and obvious, recorded around the globe. Then, affecting both genders, a situation that enables differentiated approach to screening and diagnostic processes.

Significant advances in the knowledge of carcinogenesis and natural history of colon cancer, especially establishing parentage polyp -cancer and the time required for this transition, important prerequisites for a new approach to diagnosis. Furthermore, the identification of a large proportion of cancers involving genetic transmission makes it possible to delimit the population at risk and the specific approach it.

At the moment the consensus was reached that the method of choice is colonoscopy applied to all subjects are in one of the categories of risk corresponding to this localization of cancer. However, in practice, the most widely used screening test is followed by that of the occult blood sigmoendoscopy flexible colonoscopy, and recently research DNA in faeces to hereditary forms (genetic) colon cancers.

This paper aims to study, using immunohistochemical methods, cellular markers involved in cell cycle control mechanism in the modulation of apoptosis, regulation of intercellular adhesion, cell mobility, and not least in angiogenesis.

We studied how these markers are involved in influencing the prognosis of patients with colon cancer trying to identify correlations between the degree of expression of these cell markers, extension and tumor aggressiveness and patient survival probability.

I also followed during the study if there is connection between the level of expression of cellular markers in the study and some clinical and laboratory characteristics of patients with colon cancer.

Following these studies and researches have found facts incidentally interesting dependencies and correlations are presented in the special part of the thesis.
GENERAL PART

Colorectal cancer (CCR) is currently considered the most common digestive tract cancer, representing 13% of all malignancies. In women it is the second most common cancer after breast cancer. In men ranked 3rd after lung and prostate cancer. Colorectal neoplasms has an equal incidence in both sexes with a steady increase between the second and ninth decade of life, the peak incidence occurring after the age of 55. The incident geographical variability, explained at least partly by the action of genetic factors, food, toxic etc. The role of these factors has been objectified by demographic studies on population migration from the low risk to high epidemiological risk areas [1, 2].

Mortality depends on the scope and possibility of early detection, in Romania is 13.25 deaths / 10000, with values above average in Arad, Timisoara, Bihor, Bucharest and below the national average in Gorj, Teleorman, Vaslui, Harghita. Regional differences observed in epidemiological studies in the world and the existence of different diets and lifestyles that correlate with the incidence of RCC in different geographical areas bordering suggest a strong determinism environmental factors in colonic carcinogenesis. Added to this is the observation on the incidence of RCC in population groups that emigrate from a low-incidence area in a geographic area with high incidence of RCC and in time would have within adoptive country.

Colorectal cancer develops as a result of genetic and epigenetic changes occurring within 10-15 years, leading to the transformation of normal colonic epithelium. Approximately 75% of patients with colorectal cancer are sporadic cases without presenting evidence that would have inherited the disease. The remaining 25% have a family history of colorectal cancer or suggesting the contribution of genetic or environmental factors common exposure favorable colorectal cancer, or a combination of both factors.

In terms of genetic colon cancer is a heterogeneous disease, research in recent years bringing into question numerous genetic changes important role in the occurrence of colon cancer, registering significant differences depending on the possibility of transmission of hereditary colon cancer association other malignancies in certain syndromes.

The possibility of hereditary transmission of genetic abnormalities underlying the induction of colon cancer creates opportunity for its classification: hereditary cancer representing 20-25% of all colon cancers and sporadic (non - hereditary) representing 75-80% of all colon cancers.
Whether occurs spontaneously in a single individual or manifest more people from the same family, the same location or different locations, cancer is a genetic disease, because the development of a tumor involving different genes controlling the major cellular physiological processes: cell proliferation, DNA repair cycle mitotic cell death. Colorectal cancer develops as a result of mutations in genes that control proliferation and cell death. There are changes in oncogenes and suppressor genes abnormalities of tumor growth (GST) and apoptosis [6] (Table I, Table II, Table III). Once formed malignant cells, primary tumor vascular components must invade vascular and lymphatic structures to form emboli in the blood stream to survive interaction with elements of the blood and immune system and be transported to distant sites organic [7 8]. At this level will be extravasated, join the target structures and realize secondary tumors [7-9].

The phenomena of initiation and tumor progression occurring in new locations involves a series of dynamic interactions host - tumor [10]. Study intimate mechanisms of carcinogenesis and metastasis paved the understanding of the biological properties of tumor cells [7-29]. Following the work of different research groups focused on this area has been developed which involves pathophysiological model - Synthetic - the sequence of the following events: angiogenesis, impaired intercellular adhesion in the primary tumor, the destruction of the basement membrane and initiation of tumor invasion, arrest and adhesion of tumor cells in organs target.

From the microscopic point of view has colon cancer in 95% of cases of adenocarcinoma structure, the remaining 5% being the epidermoid carcinomas, melanomas, carcinomas adenoscoamoase, primitive colonic lymphoma or carcinoid tumors [3-5]. These types mucinous adenocarcinoma are associated with a poor prognosis and is more common in younger people, those with inflammatory bowel disease or in Lynch syndrome. Rarely adenocarcinomas may look schiros low density component fibrous glandular important.

Colonoscopy is theoretically the best screening test for colon cancer. It has high sensitivity 99%, specificity high. It has, as well as lighted instrument advantage that the detection of lesions, which may be removed (polipecomic endoscopic) or at least biopsied. However, presents a number of disadvantages. It is expensive, require special training of the patient (using colon cleansing preparations Fortrans type), it is relatively difficult to perform compliance was lower, requiring specialists to perform, present a moderate risk due to rare but possible complications.
PERSONAL CONTRIBUTIONS

Colon cancer study has drawn attention of researchers around the world due to the high incidence of this condition, the current opportunities to explore fully the colon imaging and biopsy sampling of material for the study of both histologic and genetic.

If the vast majority of European countries facing increased mortality in colon cancer (Germany, Austria, Norway, Hungary, Finland, UK) is a trend of decreasing mortality as a result of health policies applied in the case of Romania is alarming that colon cancer mortality a tendency constantly increasing in the last 22 years.

This situation of colon cancer incidence and mortality are recorded in our country, with opportunities for exploring imaging and histopathological analysis have created the necessary prerequisites led me to choose to study diseases as colon tumor research topic.

The research was conducted on a total of 120 patients diagnosed with colon cancer admitted to the Medical Clinic I of the Emergency County Hospital Craiova 2006-2011. The study was prospectively evaluated the clinical and paraclinal patients on admission and after surgery surveillance was performed at 1 year.

The diagnosis was based on imaging explorations available (barium enema, ultrasound, colonoscopy) and was confirmed by histopathological examination of colonic biopsy fragments and pieces of surgical resection. Later surgery, patients were treated and follow the protocols oncology therapeutic effect currently.

Parts of resection were initially examined and processed in the Laboratory of Pathology of the Emergency County Hospital Craiova. Anatomopathological examination consisted of evaluation of macroscopic colonic tumor and other tissue fragments resected (lymph nodes, peritoneum and other tissue fragments suspected neoplastic invasion) and then prepare the material to assess tissue histopathology and immunohistochemistry.

Histopathological examination involved include paraffin tissue fragments and subsequently obtaining histological sections stained hematoxylin - eosin that were examined microscopically for defining histological type, degree of differentiation, mucus production, the presence of intratumoral areas of necrosis and suppuration, the vascular and perineural extension, invasion of resection margins,
presence of synchronous neoplastic lesions and to assess the degree of extension locally. Immunohistochemical examination was performed at the Center for Microscopic Morphology and Immunology, University of Medicine and Pharmacy Craiova.

The results were analyzed using Microsoft Excel statistical analysis program that was used further winst program designed specifically for the statistical analysis in the medical field. Ultrasound imaging was performed the first scan routine in all patients. This was done in the Medical Clinic Emergency County Hospital Craiova using ALOKA ProSound ultrasound equipped with variable frequency convex transducer 2.5 - 6 MHz.

Colonoscopy was performed in the Laboratory of Digestive Endoscopy of the Emergency County Hospital Craiova endoscopy system using Olympus brand Exera and Evis, fitted with a flexible colonoscope length of 170 cm.

In the examination of serial colonoscopic biopsies were taken from both the tumor identified (minimum 4 biopsies) and in other pathological lesions revealed during examination (polyps, inflammatory lesions). If tumor formation stenosis that does not allow the passage of the endoscope, lumen remaining examination was indicated to be performed at 6 months postoperatively.

After examining colonoscopy, tumor formations were assessed in terms of macroscopic appearance as vegetable ulcerative or infiltrative infiltrative-stenosis, also stating as their exact location (check, colon ascedent, angle liver, transverse colon, splenic angle, descending colon, sigmoid colon, rectum).

After surgery, resection pieces were analyzed histologically in Pathology Clinical Emergency County Hospital Craiova. Initially, we performed macroscopic description of the piece resection, identifying anatomical segment of origin and its orientation. Then it was recorded appearance of macroscopic tumor formation: the shape, size, color, consistency, degree of colonic wall invasion, relationship between the tumor and resection of that segment ends, identifying local lymph nodes present in block resection or resection presented as separate parts if they were located away from the primary tumor. Later, tumor tissue fragments were obtained which were processed by paraffin inclusion. Blocks, thus treated were sectioned using semiautomatic microtome to obtain tissue material required prior microscopic examination after completing hematoxylin-eosin staining steps. Microscopic examination itself was performed using Zeiss research microscope Axioscop 2 Plus.
In the case of tumor formation which have been found to be inoperable during surgery were collected on this occasion some pathological tissue fragments are needed to confirm the diagnosis.

Histopathological examination was completed with the assessment stage pTNM tumor extension. Such were classified in stage I patients with T1 or T2 and N0M0, stage IIA patients with T3N0M0, stage IIB patients with T4N0M0, stage IIIA patients with T1 or T2 N1M0, stage IIIB patients with T3 or T4 and N1M0 in patients with stage IIIC any T N2M0 and stage IV patients with any T, any N and M1.

After examining pathologist tissue sections fixed on slides previously impregnated with polylysine, were analyzed by immunohistochemistry in the Center for the Study of Microscopic Morphology and Immunology, University of Medicine and Pharmacy Craiova. Were investigated following cell markers:


2 - BCL2 protein - highlighted with immunohistochemical reagent Mo Hu BCL2 Oncoprotein, Clone 124, Ready -to -Use, Autostainer containing monoclonal murine IgG1 kappa chains, anti - human BCL2 protein.

3 - E -cadherin (formerly known as ICAM) - highlighted with immunohistochemical E-cadherin MAb reagent mxh Clone NCH -38 RTU containing murine monoclonal antibody directed against human E -cadherin.

– CD44 - highlighted with immunohistochemical reagent Mo Hu CD44, phagocytic Glycoprotein -1 Clone DF1485 containing monoclonal murine IgG1 kappa chains, directed against human CD44.

– A1 - fetoprotein (AFP) - is a glycoprotein with a molecular weight of 70kD that can be highlighted using immunohistochemical reagent Rb Hu AFP Autostainer containing rabbit polyclonal antibodies directed against human AFP.

– The VEGF receptor (VEGF - R) - put out by means of immunohistochemical reagent Flt
– 4 (C -20), which contains a murine antibody directed against the receptor of the vascular endothelial growth factor (VEGF) human.

– TGFB -RI and TGFB -RII - put out by using reagents that V -22 L -21 containing murine antibodies directed against receptor type I and II of human TGFB.

– PCNA - revealed using immunohistochemical reagent Mo Hu PCNA, clone PC10, which contains murine monoclonal IgG2 kappa chains, directed against human PCNA. It is practical to study the tumors with high rate of cell division, in particular the radioresistant.

Immunohistochemical analysis revealed a moderate density increased PCNA and a moderate density of E- cadherin in cancer cells and other tumor markers showed a moderately low density (as in P53) or low (as in BCL2, CD44, AFP, VEGF, TGFBRI, TGFBRII).

VEGFR immunostaining is highly expressed in young patients and BCL2 was found to be more frequently expressed in patients from rural areas. Other markers have not been shown to be influenced by sex, age and origin of the patient.

The location of proximal colon cancer has been frequently associated with the expression of VEGFR, BCL2 or PCNA, while the distal location has been associated with the expression of TGFBRII. Marker E -cadherin was present predominantly in infiltrating tumors and VEGFR - stenosis showed a higher density infiltrative tumors.

Advanced TNM stages were associated with an increased expression of PCNA, CD44 and VEGFR. Markers PCNA, BCL2 and VEGFR had a higher density that E- cadherin was less expressed in poorly differentiated tumors. Markers PCNA and VEGFR were better expressed in advanced stages of primary tumor extension. The markers CD44 and VEGFR expression were increased in tumors or lymphatic extension in the presence of distant metastases. Marker BCL2 was better expressed if vascular invasion.

The level of expression of the marker PCNA was not found to have a statistically significant influence on survival of patients although its high level expression was correlated with a survival probability of 0.6. No markers E -cadherin expression or TGFBRII not been shown to have a statistically significant influence on survival of patients with all that if TGFBRII, higher density was associated with a lower probability of survival (0,4).
In conclusion, research immunohistochemical markers can be useful in the early detection of aggressive forms of colon cancer, although not all of the markers studied, and only a limited number of studies have shown the influence of the probability of survival of patients. However, it requires much larger population studies and research a bigger variety of tumor markers in order to obtain results with high statistical significance.

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